When Does the Repressilator Oscillate? Hopf Bifurcations and Stability of Differential Systems

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Abstract

The repressilator is a genetic network in which three genes code for proteins that, upon their presence, induce cellular repression of the next gene, which forms a negative feedback loop with nonlinear interactions. Elowitz and Leibler [8] describe this network based on six nonlinear first-order differential equations. In such a system, oscillations may occur, which is valuable for studying since this model can be tested experimentally via artificial regulatory networks. This project investigates the mathematical conditions of exhibiting oscillatory behaviours in repressilators. We study the differential stability using both mathematical and computational approaches and find that significant cooperative binding effects, efficient repressions, and high transcription rates promote oscillations. We confirm the existence of Hopf bifurcation by demonstrating the bifurcation diagram and the formation of the limit cycle numerically. We also present the numerical simulations of the dynamical behavior of the repressilator with respect to different parameters. Finally, we find more complex behaviours of the genetic network by extending the repressilator in two ways: 1) adding spatial effects, leading to synchronization property. 2) adding more genes in the loop, leading to the change of frequency and amplitude of the oscillation associated with different parameters.

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Chapter 1

Introduction

> *"Biology is the study of complicated things that give the appearance of having been designed for a purpose."*
>
> Richard Dawkins [1]

The highly interdisciplinary nature of mathematical biology determines the diversity of the discipline's research methods. Scientists have developed many theoretical tools to study the complexity of biological systems, and mathematics also benefits from this development. However, it seems to be a long way to go to understand and predict the behaviour of biological systems accurately.

1.1 Background

1.1.1 Gene Network

Network processes reflect the complexity of biology, and many primary functions of living organisms could be performed through the networks of interacting biomolecules at the cell level [2]. A gene regulatory network is a collection of molecular regulators that interact with each other and with other substances in the cell to govern the gene expression levels of mRNA and proteins, which, in turn, determine the function of the cell [1]. This process consists of two main steps: transcription and translation. Figure 1.1 shows the

[1] Richard Dawkins (2015). "The Blind Watchmaker: Why the Evidence of Evolution Reveals a Universe without Design", p.13, W. W. Norton & Company

primary components of this regulation: genetic information which is coded in DNA, is copied to RNA, called mRNA, which is used to construct protein. However, a special regulatory protein, called transcription factors coded by DNA, leads to either the activation or repression of gene expression. In other word, this might result in promoting or inhibiting the expression of gene, forming a positive or negative feedback loop. In a negative feedback loop, increased output from the system inhibits future production by the system [22]. Over the past decades, a large amount of qualitatively work has been done to study the gene regulatory network, e.g. Tyson (1975) [26] studied the existence of a periodic solution in negative feedback cellular control processes; Glass and Pasternaek (1978) [12] considered the oscillations in the piecewise linear equation used to model biological control systems; Thomas (1981) conjectures two main theorems about the positive and negative feedback circuits , among others [25] which are proven by JL.Gouzé in 1998 [13]. However, such systems were not well understood. In 2000, Elowitz and Leibler [8] proposed a transcriptional repressor system called repressilator, and implemented it within an *Escherichia coli*, producing anticipated functions, which is a mile stone in synthetic biology.

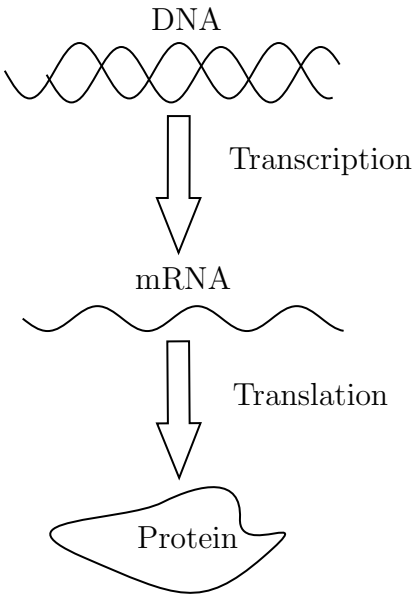

Figure 1.1: The central dogma of molecular biology: transcription and translation.

1.1.2 Repressilator

The repressilator is a genetic network where three genes code for proteins whose presence results in a repression of the next gene in a circular way, which forms a negative feedback loop with nonlinear interactions. Figure 1.2 is the original network from Elowitz and Leibler, where the first protein *LacI*, suppresses the transcription of the second repressor gene, *tetR* through the expression of the protein. Finally, λ-*cI* protein suppresses the expression of *lacI*, bringing the cycle to a close. Elowitz and Leibler describe this network in six nonlinear first-order differential equations, which could exhibit oscillations, and the network diagram is shown in Figure 1.3. Later on, Buse *et al.* (2009) proposed a simplified version (see Figure 1.4) of the original repressilator model, in which three of the six equations are considered to be in quasi-equilibrium and algebraic relations are used to replace them [3]. They prove this reduced model undergoes a supercritical Hopf bifurcation. Moreover, Buzzi and Llibre (2015) prove that the full repressilator equations also undergo a supercritical Hopf bifurcation [5]. Overall, these studies outline a critical role of Hopf bifurcation and the stability of differential systems. The question is that, given a system known to present oscillations for some parameter values, can we determine these parameter values?

1.2 Aim and Scope

This paper aims to use mathematical and computational approaches to investigate the conditions to exhibit oscillations of repressilators. In particular, we identify three primary objectives:

1. To investigate whether we can determine the parameter values for a given system that presents oscillations.

2. To confirm the existence of Hopf bifurcation and identify its type in the repressilator.

3. To extend repressilator to more complex gene regulatory networks and investigate their dynamical properties.

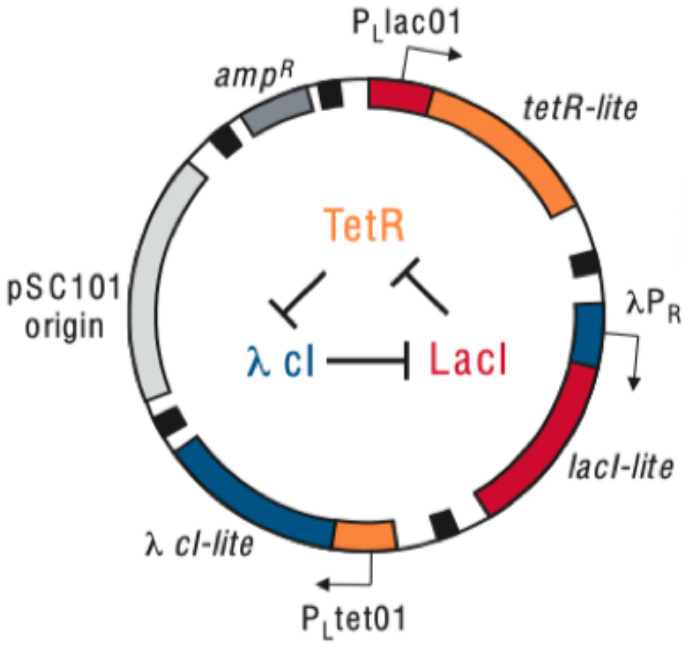

Figure 1.2: Repressilator in *Escherichia coli* [8]

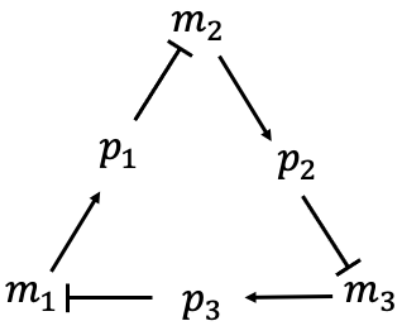

Figure 1.3: The full repressilator diagram.

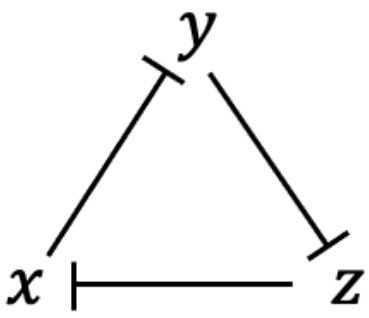

Figure 1.4: The reduced repressilator diagram.

We limit our scope to the six nonlinear first-order differential equations proposed by Elowitz and Leibler and thus study the stability of this differential system. Specifically, we study this system together with its reduced one comparatively, listing some essential properties. Moreover, we numerically demonstrate their extensions to more complex gene regulatory networks and associated behaviours.

1.3 Overview

This paper consists of theoretical modelling and numerical simulations, including six chapters. We now briefly outline each chapter in more detail.

Chapter 2 (*An Introduction to Biological Modelling*) presents the schematic and related definitions of mathematical modelling for biological systems in the context of using differential equations to model molecule interactions.

Chapter 3 (*Differential Systems: Linear and Nonlinear*) provides a brief account of the theory behind the qualitative methodology of linear and nonlinear differential systems. We investigate the stability theory for both linear and nonlinear differential systems, leading up to the bifurcation analysis of the repressilator model.

Chapter 4 (*Mathematical Modelling and Analysis of Repressilators*) presents the whole mathematical analysis process for both full and reduced repressilator model, including the uniqueness of the equilibrium point, linearization, and bifurcation analysis.

Chapter 5 (*Extension of Repressilator*) demonstrates two ways to extend the repressilator model.

Chapter 6 (*Numerical Simulations of Oscillations*) explains the computer simulations of previous theoretical studies, including a comparative study for both full and reduced models of repressilator. We also show the numerical simulations of dynamical behaviours of repressilator on different parameters. Moreover, we demonstrate more complex behaviours of the genetic network by extending the repressilator in two ways: 1) adding spatial effect, leading to synchronization property. 2) adding more genes in the loop, leading to the change of frequency and amplitude of the oscillation associated with different parameters.

We end this paper with Chapter 7 (*Conclusions and Further Directions*), which presents

discussions of the main results, as well as some suggestions for further research.

Chapter 2

An Introduction to Biological Modelling

To study biological systems mathematically, we represent them in terms of differential equations. Mathematicians have developed many tools and techniques, such as phase line and plane analysis, linear stability analysis, bifurcation analysis, asymptotic analysis, etc. This chapter will discuss some important definitions and results of applied dynamical system theory and its application in biological processes. Firstly, in section 2.1, we describe the process of mathematical modelling of biological systems. In section 2.2, we show how we model the molecule dynamics as well as derive the Hill functions by the law of mass action. We also present some essential properties of using Hill functions to model negative feedback.

2.1 Schematic of Mathematical Modelling

Mathematical modelling plays an essential role in synthetic biology as a crucial connection between the concept and realisation of a biological circuit [28]. Generally, Figure 2.1 illustrates the crucial role of how mathematical modelling could be applied to biology.
To solve a biological problem, firstly, we have to formulate the problem as a mathematical representation in terms of variables, functions, parameters, and equations [17]. While variables change as part of the solution, parameters remain fixed, and equations are repre-

sented in terms of mathematical objects that relate to physical quantities. The equations can be solved **analytically**, which entails framing the problem in a well-understood form and manually calculating the exact solution, or **asymptotically**, which approximates the solution of models with small parameters, or **numerically**, which entails computing approximate exact solutions using computational methods. Furthermore, models need to be refined continuously, and once a mathematical model has been validated against experiment, a computer could simulate those modelling and solving processes, allowing it to forecast future behaviour of the biological system (e.g. predict the evolution of pandemic), optimise parameters for improved performance (e.g. minimise or maximise the metabolic process), or regulate the biological system (e.g. regulate structure and function of molecule). Finally, biological interpretation brings the whole process to an end, which involves interpreting the results based on biological significance and further insights.

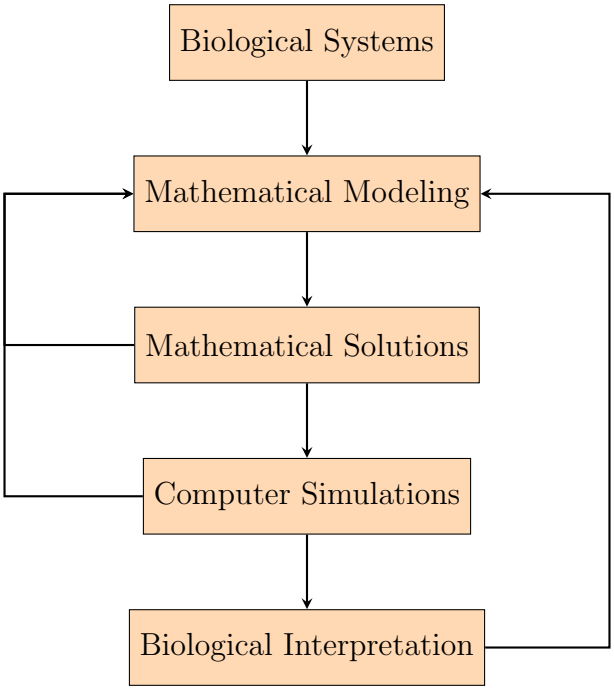

Figure 2.1: Application of Mathematical Modelling of biology

Biological systems, on the other hand, are complex systems, with increasing levels of complexity arising from collective behaviour and emergent features at several levels. Therefore not all scientific models are expressed in a precise, numerical, and quantitative manner [19]. A mathematical model is similar to a road map in that it depicts a geographical area. Despite the fact that the map does not depict every element of the environment, it

contains enough information to allow users to plan a trip [28].

2.2 Rate Equations and the Hill Functions

The model mentioned previously is described in terms of the evolution of state variables for various system properties for a given initial state, which can describe the rate of change of the state variables as a function of time. If we ignore the spatial effect like the diffusion, the system can be written as:

$$\text{Rate of change of } x = \text{production of } x - \text{degradation of } x$$

However, the situation is even more complex because biological processes are accompanied by so many chemical reactions, often resulting from interactions between molecules. Thus, we introduce the law of mass action which gives us a precise rule of how to model the chemical reactions quantitatively.

Theorem 2.2.1 (Law of Mass Action [9]) *The law of mass action is the proposition that the rate of the chemical reaction is directly proportional to the product of the activities or concentrations of the reactants.*

We follow Santillá's paper [23] and consider the following chemical reaction, which describes that n ligand molecules x bind simultaneously the receptor X

$$X + nx \underset{}{\overset{K_d}{\rightleftharpoons}} X_{nx} \tag{2.1}$$

and the chemical equilibrium equation is obtained by using the law of mass action

$$[X][x]^n = K_d[X_{nx}] \tag{2.2}$$

where K_d is called the dissociation constant, and $[\,\cdot\,]$ denotes the concentration of each chemical. Assuming that the receptor is a constant number, we have

$$[X] + [X_{nx}] = [X_T] \tag{2.3}$$

Solving 2.2 and 2.3 we could get the the fractions of occupied and free molecules of X, which is given by

$$H = \frac{\text{Occupied Receptor}}{\text{Total Receptor}} = \frac{[X_{nx}]}{[X_T]} = \frac{[x]^n}{K_d + [x]^n} = \frac{[x]^n}{\theta^n + [x]^n} \tag{2.4}$$

$$\overline{H} = \frac{\text{Free Receptor}}{\text{Total Receptor}} = \frac{[X]}{[X_T]} = \frac{K_d^n}{K_d + [x]^n} = \frac{\theta^n}{\theta^n + [x]^n} \tag{2.5}$$

where $\theta^n = K_d$. H and $\overline{H}$ are known as Hill function.

Let's rewrite the Hill functions in the following way,

$$H(x) = \frac{x^n}{\theta^n + x^n} \tag{2.6}$$

or:

$$\overline{H}(x) = 1 - H(x) = \frac{\theta^n}{\theta^n + x^n} \tag{2.7}$$

The Hill function could model the feedback loop, which is a framework the output of this loop can promote or inhibit itself or another process. Let's explain the properties of the Hill functions.

$$\frac{dH}{dx} = \frac{nx^n\theta^n}{(\theta^2 + x^n)^2} > 0 \tag{2.8}$$

$$\frac{d\overline{H}}{dx} = \frac{-nx^n}{(\theta^2 + x^n)^2} < 0 \tag{2.9}$$

As the concentration of transcription factor (x) varies, the Hill functions either increase or decrease between 0 and 1 sigmoidally, which presents the activation (Figure 2.2) and repression (Figure 2.3) respectively. The parameter θ represents the threshold where the function has value $\frac{1}{2}$ [7]. This function could describe the dynamical behaviours that increase (or decrease) to some extent and finally saturate to a constant, and the Hill coefficient n itself is an accurate approximation of the molecule binding; thus, it is also feasible to model the cooperativity in gene regulatory networks (M. Santillán, 2008) [23]. Another important property of the Hill function is that when $n \to \infty$, the function exhibits

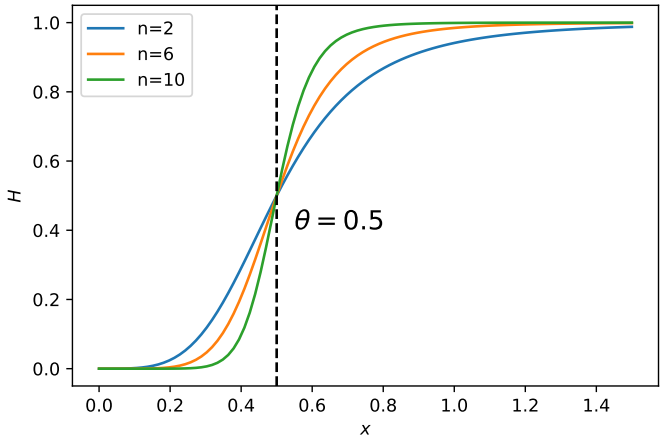

Figure 2.2: The activation modeled by the Hill function

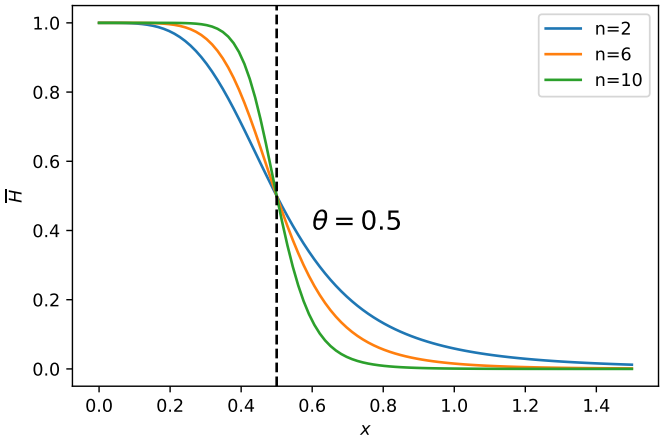

Figure 2.3: The repression modeled by the Hill function

switch-like behaviour, i.e., near the threshold θ, the function crosses the threshold line ($x = \theta$) and changes discontinuously from 0 to 1 (or 1 to 0).

In summary, although genes regulate cellular functions through complex chemical reactions, we could use rate equations together with the law of mass action to model the dynamics. The model is called a deterministic model since it consists of a system of ordinary differential equations, making it possible to employ analytical and computational techniques for further research.

Chapter 3

Differential Systems: Linear and Nonlinear

3.1 Linear System of Differential Equations

Consider an autonomous linear system in the form of

$$\frac{d\mathbf{x}}{dt} = \mathbf{M}\mathbf{x}, \mathbf{x} \in \mathbb{R}^n \tag{3.1}$$

where $\mathbf{M}$ is a matrix in the form of

$$\begin{pmatrix} a_{11} & a_{12} & \dots & a_{1n} \\ a_{21} & a_{22} & \dots & a_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{n1} & a_{n2} & \dots & a_{nn} \end{pmatrix} \tag{3.2}$$

3.1.1 Equilibrium Point

Definition 3.1.1 (Equilibrium point [21]) *An equilibrium point of a dynamical system represented by an autonomous system of ordinary differential equations is a solution that does not change with time.*

3.1.2 Stability

Considering the linear system 3.1, we have the following theorems to determine the stability.

Theorem 3.1.1 (Asymptotic stable [15]) *Let* $\mathbf{x}^*$ *be a stable solution for* $t \geq t_0$*. If there exists* $\zeta(t_0) \geq 0$ *such that*

$$||\mathbf{x}(t_0) - \mathbf{x}^*(t_0)|| < \zeta \implies \lim_{t\to\infty} ||\mathbf{x}(t) - \mathbf{x}^*(t)|| = 0 \tag{3.3}$$

then the solution is said to be asymptotically stable (or uniformly and asymptotically stable).

Theorem 3.1.2 (Stability of Differential System [15]) *Considering a system* $\dot{\mathbf{x}} = \mathbf{M}\mathbf{x}$*, where* $\mathbf{M}$ *is a constant matrix, with eigenvalues* λ_i*.*

- If the system is stable, then $\mathbb{R}(\lambda_i) \leq 0$.
- If either $\mathbb{R}(\lambda_i) < 0$; or if $\mathbb{R} \leq 0$ and there is no zero repeated eigenvalue; then the system is uniformly stable.
- If $\mathbb{R}(\lambda_i) < 0$, then the system is asymptotically stable.
- If $\mathbb{R}(\lambda_i) > 0$, for any i, the system is unstable.

3.1.3 Classification of Equilibrium Point

Considering a two dimensional linear system as following

$$\frac{dx_1}{dt} = a_{11}x_1 + a_{12}x_2 \tag{3.4}$$
$$\frac{dx_2}{dt} = a_{21}x_1 + a_{22}x_2 \tag{3.5}$$

Generally, the equation has solution in a form of $x_1 = X_1 e^{\lambda_1 t}, x_2 = X_2 e^{\lambda_2 t}$. Substitute

them into above equation, the solution is the same as solving the following equation

$$\begin{vmatrix} a_{11} - \lambda & a_{12} \\ a_{21} & a_{22} - \lambda \end{vmatrix} = 0 \tag{3.6}$$

which is also equivalent to

$$\lambda^2 - T\lambda + \Delta = 0 \tag{3.7}$$

where $T = (a_{11} + a_{22}), \Delta = a_{11}a_{22} - a_{12}a_{21}$. Therefore, two eigenvalues are

$$\lambda_{1,2} = \frac{T \pm \sqrt{T^2 - 4\Delta}}{2} \tag{3.8}$$

and two corresponding eigenvectors are: $(v_1, w_1)^T, (v_2, w_2)^T$. Thus, the linear system has a general solution

$$x_1 = c_1 v_1 e^{\lambda_1 t} + c_2 v_2 e^{\lambda_2 t} \tag{3.9}$$

$$x_2 = c_1 w_1 e^{\lambda_1 t} + c_2 w_2 e^{\lambda_2 t} \tag{3.10}$$

and the constants c_1, c_2 are determined by initial conditions.

There are general three cases of stability.

1. If $\mathbb{R}(\lambda_1) < 0$,and $\mathbb{R}(\lambda_2) < 0$, then $\lim_{t\to\infty} x_i = 0$. Thus the solution is asymptotically stable.

2. If $\mathbb{R}(\lambda_i) > 0$, for some i, then $\lim_{t\to\infty} = \infty$. Thus the solution is unstable.

3. If $\mathbb{R}(\lambda_i) \leq 0$, then the solution is stable but not asymptotically stable. More detail need to be discussed.

Specifically, the type of solutions is determined by T and Δ. Figure 3.1 summarizes the classification of equilibrium.

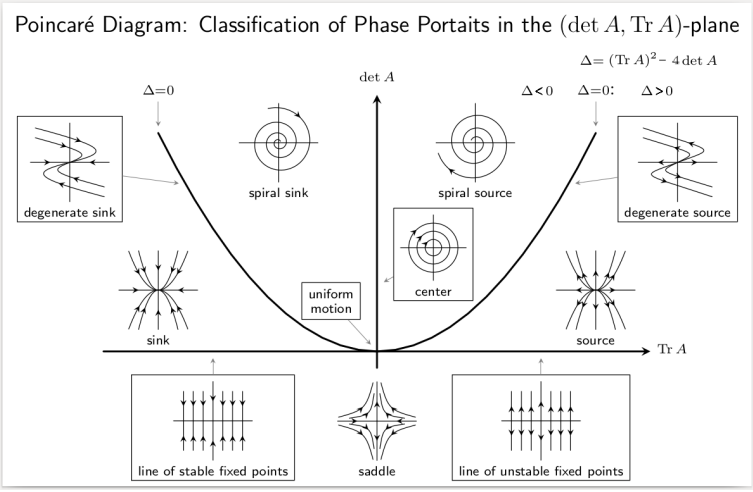

Figure 3.1: The Trace-determinant plane. [10]

3.2 Nonlinear System of Differential Equations

Linear equations are easy to solve and to analyse the nature of their solutions, so the solutions of linear equations can be used to analyse how nonlinear equations behave when solved near equilibrium points.

3.2.1 Linearization

Considering a system described by an autonomous ODE system:

$$\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x}), \mathbf{x} \in \mathbb{R}^n \tag{3.11}$$

where $\mathbf{F}$ is a vector of functions and an equilibrium point $\mathbf{x}^*$ satisfies:

$$\mathbf{F}(\mathbf{x}^*) = 0 \tag{3.12}$$

To study the stability of the system, we are interested in how small perturbations to the exact equilibrium points are propagated as time varies.
Considering a small perturbation around the equilibrium points: $\mathbf{x} = \mathbf{x}^* + \delta\mathbf{x}$, $0 < \delta \ll 1$.
Substituting it into the ODE system:

$$\frac{d}{dt}(\mathbf{x}^* + \delta\mathbf{x}) = \mathbf{F}(\mathbf{x}^* + \delta\mathbf{x}) \tag{3.13}$$

using Taylor expansion to expand right hand side up to $\mathcal{O}(\delta^2)$

$$\frac{d\delta\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x}^*) + \nabla\mathbf{F}(\mathbf{x}^*)\delta\mathbf{x} + \mathcal{O}(\delta^2) \tag{3.14}$$

If we only keep the linear term and cancel out the δ, we could get a linearised system:

$$\frac{d\mathbf{x}}{dt} = \nabla\mathbf{F}(\mathbf{x}^*)\mathbf{x} \tag{3.15}$$

where $\nabla\mathbf{F}(\mathbf{x}^*) \in \mathbb{R}^{n\times n}$ is the Jacobian matrix, which reflects the stability of the system. Hartman-Grobman theorem [18] states that there is a homeomorphism defined in the neighbourhood of the hyperbolic equilibrium point. In other word, for a non-hyperbolic equilibrium point, the linearised system can not determine the stability. Thus, for hyperbolic equilibrium, if we calculate the eigenvalues of Jacobian and follow the below theorem, we can know the stability of the equilibrium point.

3.2.2 Limit Cycle

Theorem 3.2.1 (Limit Cycle [24]) *A limit cycle is a closed trajectory, where the neighboring trajectories are not closed and they either spiral towards or away from the limit cycle.*

There are some characteristics of limit cycle.

1. A limit cycle is said to be stable if all neighboring trajectories approach it.
2. Limit cycle can be semi-stable and it is possible to have either stable or unstable equilibrium point inside itself.
3. There cannot be another limit cycle in the neighborhood of one limit cycle. The limit cycle is isolated.
4. Limit cycle is independent on initial conditions and perturbations.

The Limit cycle can only exist in a nonlinear system; even though we can find orbit solutions in a linear system, they are not isolated. The existence of the limit cycle could be determined by Poincaré-Bendixson Theorem.

Theorem 3.2.2 (The Poincaré-Bendixson Theorem [18])

$$\begin{cases} \dot{x} = f(x, y) \\ \dot{y} = g(x, y) \end{cases} \tag{3.16}$$

Consider above two dimensional system, let R be a subspace region of phase plane bounded by closed curves C_1 and C_2.

1. *R does not contain any equilibrium point*

2. *At each point of C_1 and C_2, the direction field points to the interior of R*

then the system contains at least one stable limit cycle.

3.2.3 Bifurcation

In biology, we usually investigate how a system depends upon its parameters, which make the system exhibits different behaviours. In synthetic biology, we intend to detect different dynamic behaviour types and determine which experimental settings should be adjusted to get the desired functions, which is crucial to mimicking a complex biological system. In particular, the threshold at which the behaviour changes attracts much attention, which is called bifurcation in applied mathematics. Let's present formal definitions.

Definition 3.2.1 (Bifurcation [18]) *A qualitative change in dynamics occurring upon a small change in a parameter.*

Definition 3.2.2 (Hopf Bifurcation [24]) *In dynamical systems, Hopf bifurcation occurs when the stability of the system changes and there is born of limit cycles, or periodic solutions. For a linearised system, Hopf bifurcation occurs when at least one of the eigenvalues of the Jacobian crosses into the right half-plane as the parameter varies.*

Generally, bifurcation theory is the study of how the behaviour of dynamical systems such as ODE changes when parameters are varied. Although the behaviour of a dynamic system could be interpreted by the classification of the equilibrium point, the equilibrium

point can be created or destroyed, or its stability can be changed as the parameters of the system varied. The parameter values at which they occur are called bifurcation points, which are the thresholds when the qualitative structure of a system changes [24]. Hopf bifurcation is mostly used to study oscillations and can identify the critical parameter values that allow the stability of a system to change from a steady-state to a periodic solution [14] [11] [27]. In this paper, we focus on the threshold at which the repressilator exhibits oscillations.

Chapter 4

Mathematical Modelling and Analysis of Repressilators

In this chapter, we will use mathematical techniques mentioned previously to study the dynamical behaviours of repressilators. We start from the full model, including the differential equations of the repressilator model (section 4.1), the proof of the uniqueness of equilibrium point (section 4.2), linear stability analysis (section 4.3), and bifurcation analysis (section 4.4). The mathematical analysis of the reduced model follows the same structure. Finally, we close this chapter by comparing the parameter paces of both models.

4.1 Deterministic Differential Equations of Repressilator Model

Elowitz and Leibler use the following differential system to model the repressilator network:

$$\frac{dm_1}{dt} = \alpha_0 - m_1 + \frac{\alpha}{1 + p_3^n} \tag{4.1}$$

$$\frac{dm_2}{dt} = \alpha_0 - m_2 + \frac{\alpha}{1 + p_1^n} \tag{4.2}$$

$$\frac{dm_3}{dt} = \alpha_0 - m_3 + \frac{\alpha}{1 + p_2^n} \tag{4.3}$$

$$\frac{dp_1}{dt} = -\beta(p_1 - m_1) \tag{4.4}$$

$$\frac{dp_2}{dt} = -\beta(p_2 - m_2) \tag{4.5}$$

$$\frac{dp_3}{dt} = -\beta(p_3 - m_3) \tag{4.6}$$

where α_0 represents the production rate of protein, α is the transcription rate in the absence of repressions, β stands for the proportion of the decay rate between protein and mRNA, m and p are the concentration of mRNA and repressor-protein. For both equations describing the dynamics of the concentration of mRNA and protein. The left hand side of these equations is the rate of change of mRNA and protein, respectively. The term $\frac{\alpha}{1+p^n}$ is of the form of the Hill function, which describes the interactions between two components.

If we rewrite these equations in a more compact form to make it more generalized:

$$\frac{dm_i}{dt} = \alpha_0 - m_i + \frac{\alpha}{1 + p_{i-1}^n} \tag{4.7}$$

$$\frac{dp_i}{dt} = \beta(p_i - m_i) \tag{4.8}$$

where $i = 1, 2, 3, ..., N, N + 1 = 1$. N stands for the number of genes. For instance, if $N = 3$, then $i = 3$ and $i - 1 = 2$, where we can get equations 4.3 and 4.6; Similarly, $i = 2$ so $i - 1 = 1$, where we get equations 4.2 and 4.5; Setting $i = 1, i = 1 + 3 = 4, i - 1 = 3$, where we get equations 4.3 and 4.6. Thus, following above recipes, these differential system could model networks with N genes (see section 5).

4.2 Existence of the Uniqueness of Equilibrium Point

To conduct qualitative analysis for an ODE systems:

$$\frac{d\mathbf{x}}{dt} = f(\mathbf{x}),\ \mathbf{x} \in \mathbb{R}^n_{\geq 0} \tag{4.9}$$

We need to figure out the equilibrium point first, which is defined by the point $\mathbf{x}^*$ such that $f(\mathbf{x}^*) = 0$. Simply solving the equations:

$$\frac{dp_i}{dt} = \frac{dm_i}{dt} = 0 \tag{4.10}$$

denote equilibrium points $p^* = m^* = R$ and we will get:

$$(R)^{n+1} - \alpha_0(R)^n + R - (\alpha + \alpha_0) = 0 \tag{4.11}$$

and

$$\alpha_0 - m_1 + \frac{\alpha}{1 + m_3^n} = 0 \tag{4.12}$$

$$\alpha_0 - m_2 + \frac{\alpha}{1 + m_1^n} = 0 \tag{4.13}$$

$$\alpha_0 - m_3 + \frac{\alpha}{1 + m_2^n} = 0 \tag{4.14}$$

If we substitute repeatedly, we could get an equation containing only one variable:

$$\alpha_0 + \frac{\alpha}{1 + \left(\alpha_0 + \frac{\alpha}{1+\left(\alpha_0 + \frac{\alpha}{1+m_i^n}\right)^n}\right)^n} = m_i \tag{4.15}$$

This equation could be simplified by introducing two functions, f and g:

$$f \circ f \circ f \circ (m_i) = g(m_i) \tag{4.16}$$

where $f(\eta) = \alpha_0 + \frac{\alpha}{1+\eta^n}$, $g(\eta) = \eta$, $t \geq 0$.

The equilibrium point is determined by the solution to equation 4.16 and the number of intersections of the two functions determines the number of equilibrium point.

In the first quadrant, the right hand side is an increasing linear function passing through the original point, while the behaviour of the left hand side function could be found by taking the derivatives.

We denote a new function: $F = f \circ f \circ f$, take the derivatives by chain rule:

$$F' = (f \circ f \circ f) = f'(f \circ f)(f \circ f)' = \underbrace{f'(f \circ f)}_{<0}\underbrace{f'(f)}_{<0}\underbrace{f'}_{<0} \tag{4.17}$$

Since $f(\eta)' = -\frac{\alpha n \eta^{n-1}}{(1+\eta^n)^2} < 0$, then $(f(f(\eta)))' < 0$, as well as $(f(f(f(\eta))))' < 0$. Thus g is a monotonically increasing function, the equilibrium point is unique since F is monotonically decreasing. From Figure 4.1, we could see that as n increases, there is always an equilibrium point and thus uniqueness has been proven. The general case of the uniqueness is discussed in section 5.2.

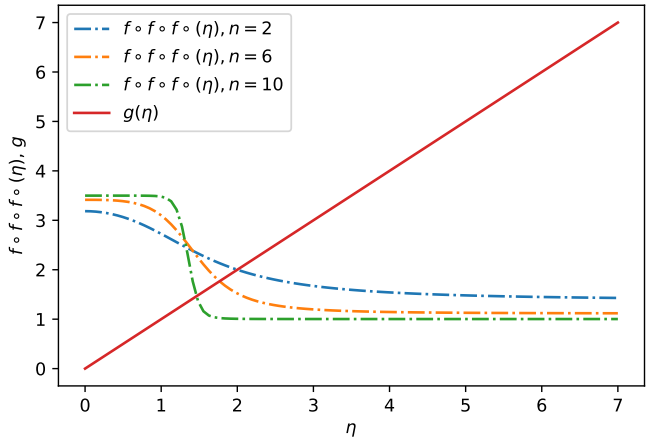

Figure 4.1: The Uniqueness of the Equilibrium Point

4.3 Linear Stability Analysis for the Full Repressilator Equations

In section 4.2, the uniqueness of equilibrium point has been proved. Next, we consider a small perturbation near the equilibrium point for three-gene network model: $\mathbf{x} = \mathbf{x}^* + \delta\mathbf{x}$, $0 < \delta \ll 1$, where $\mathbf{x} = (m_1, m_2, m_3, p_1, p_2, p_3)$, $\mathbf{x}^* = (m_1^*, m_2^*, m_3^*, p_1^*, p_2^*, p_3^*)$. Substitute into system for m_1 and p_1 and others can be calculated by symmetry:

$$\frac{d}{dt}(m_1^* + m_1) = \alpha_0 - (m_1^* + \delta m_1) + \frac{\alpha}{1 + (p_3^* + \delta p_3)^n} \tag{4.18}$$

$$\frac{d\delta m_1}{dt} = \alpha_0 - (m_1^* + \delta m_1) + \alpha\left(\frac{1}{1+p_3^{*n}} - \frac{np_3^{*n-1}\delta p_3}{(1+p_3^n)^2}\right) + \mathcal{O}(\delta^2) \tag{4.19}$$

Rearrange this equation:

$$\frac{d\delta m_1}{dt} = \underbrace{\alpha_0 - m_1^* + \frac{\alpha}{1+p_3^*}}_{\text{Equilibrium point}} - \delta m_1 - \frac{\alpha n p_3^{*n-1}}{(1+p_3^*)^2}\delta p_3 + \mathcal{O}(\delta^2) \tag{4.20}$$

As the first three terms on the right hand side satisfy the 4.10, thus they vanish. Then, we cancel out the δ on both side and conduct the same process, we can get the linearised system:

$$\frac{dm_1}{dt} = -m_1 - \frac{\alpha n p_3^{*n-1}}{(1+p_3^{*n})^2}p_3 \tag{4.21}$$

$$\frac{dm_2}{dt} = -m_2 - \frac{\alpha n p_1^{*n-1}}{(1+p_1^{*n})^2}p_1 \tag{4.22}$$

$$\frac{dm_3}{dt} = -m_3 - \frac{\alpha n p_2^{*n-1}}{(1+p_2^{*n})^2}p_2 \tag{4.23}$$

$$\frac{dp_1}{dt} = -\beta(p_1 - m_1) \tag{4.24}$$

$$\frac{dp_2}{dt} = -\beta(p_2 - m_2) \tag{4.25}$$

$$\frac{d_3}{dt} = -\beta(p_3 - m_3) \tag{4.26}$$

The coefficient could be simplified by eliminating α using 4.11 Therefore, we denote $\Gamma = -\frac{\alpha n R^{n-1}}{(1+R^n)^2} = -\frac{nR^{n-1}(R-\alpha_0)}{(1+R^n)^2}$, and the Jacobian of the system is as following

$$\begin{pmatrix} -1 & 0 & 0 & 0 & 0 & \Gamma \\ 0 & -1 & 0 & \Gamma & 0 & 0 \\ 0 & 0 & -1 & 0 & \Gamma & 0 \\ \beta & 0 & 0 & -\beta & 0 & 0 \\ 0 & \beta & 0 & 0 & -\beta & 0 \\ 0 & 0 & \beta & 0 & 0 & -\beta \end{pmatrix} \tag{4.27}$$

The characteristic equation is:

$$(\lambda+1)^3(\lambda+\beta)^3-\Gamma^3\beta^3=0 \tag{4.28}$$

In order to make the system exhibit oscillations, we need to destabilize the equilibrium points. Thus we need at least one eigenvalue to cross the right half-plane as the parameter varies.

4.4 Bifurcation Analysis for the Full Repressilator Equations

Rewrite the characteristic equation by doing some algebraic work [1]:

$$[(\lambda+1)(\lambda+\beta)-\Gamma\beta][(\lambda+1)^2(\lambda+\beta)^2+(\lambda+1)(\lambda+\beta)\Gamma\beta+\Gamma^2\beta^2]=0 \tag{4.29}$$

Which is equivalent to:

$$\begin{cases} \lambda^2+(1+\beta)\lambda+\beta(1-\Gamma)=0 \\ \text{OR} \\ \underbrace{(\lambda+1)^2(\lambda+\beta)^2+(\lambda+1)(\lambda+\beta)\Gamma\beta+\Gamma^2\beta^2=0}_{\text{of the form } x^2+xy+y^2=0} \end{cases} \tag{4.30}$$

Remark 1 *By the fundamental theory of algebra, the characteristic equation has six roots. However, although symbolic computation software could solve this equation directly, each solution might have a particular format. In the following, rearrange the equation to spot patterns and re-express the characteristic equation in complex number form, which consists of three quadratic equations. Therefore, it is easy to solve them analytically by the symmetric form.*

Theorem 4.4.1 *Equation $x^2+xy+y^2=0$, $x,y\neq 0$, is equivalent to $x=\frac{-1\pm i\sqrt{3}}{2}y$.*

[1] Formula: $x^3-y^3=(x-y)(x^2+xy+y^2)$

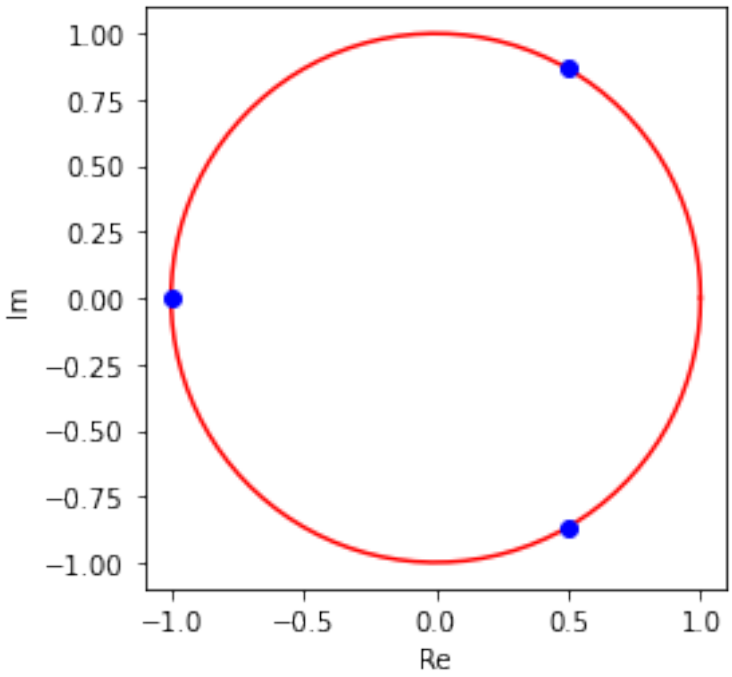

Figure 4.2: Three quadratic equations in a complex plane.

Based on this theorem (see proof in section .2), we could identify a similar form, where $x = (\lambda + 1)(\lambda + \beta), y = \Gamma\beta$. Thus we could get:

$$\begin{cases} \lambda^2 + (1+\beta)\lambda + \beta(1-\Gamma) = 0 \\ \text{OR} \\ (\lambda+1)(\lambda+\beta) = \dfrac{-1 \pm \sqrt{3}i}{2}\Gamma\beta \end{cases} \tag{4.31}$$

By the fact that: $e^{\pm\frac{\pi}{3}i} = \frac{1}{2} \pm \frac{\sqrt{3}}{2}$, $e^{\pi i} = -1$, we could simplify further.

$$\begin{cases} \lambda^2 + (1+\beta)\lambda + \beta(1+e^{\pi i}\Gamma) = 0 \\ \text{OR} \\ \lambda^2 + (1+\beta)\lambda + \beta(1+e^{\pm\frac{\pi}{3}i}\Gamma) = 0 \end{cases} \tag{4.32}$$

In a complex plane, which is divided equally into three part, and each boundary corresponds to a quadratic equation, we can rewrite the characteristic equation in a compact form:

$$\lambda^2 + (\beta+1)\lambda + \beta(1+e^{\theta i}\Gamma) = 0, \theta = -\frac{\pi}{3},\ \frac{\pi}{3},\ \pi \tag{4.33}$$

Since this is a quadratic equation with three different complex numbers as parameters, the whole equation will give us six roots in total. For each complex parameter, the roots are conjugate since we only care about the real part of the root, so the conjugate roots share the same stability property. Let's denote the roots as pairs:

$$\begin{cases} \theta = \pi \implies \lambda_{1,2} \\ \theta = -\dfrac{\pi}{3} \implies \lambda_{3,4} \\ \theta = \dfrac{\pi}{3} \implies \lambda_{5,6} \end{cases} \tag{4.34}$$

Solve the equation:

$$\lambda_{1,2} = \frac{-(\beta+1) \pm \sqrt{(1+\beta)^2 - 4\beta(1-\Gamma)}}{2} \tag{4.35}$$

Setting $\Re(\lambda_{1,2}) = 0$, we will get $\Gamma = 1$, however, since $\Gamma < 1$, so the eigenvalues are always negative, thus stable.

Similarly:

$$\lambda_{3,4} = \frac{-(\beta+1) \pm \sqrt{(1+\beta)^2 - 4\beta\left(1 - \Gamma\left(\frac{1}{2} - \frac{\sqrt{3}}{2}i\right)\right)}}{2} \tag{4.36}$$

Setting $\Re(\lambda_{3,4}) = 0$ and we obtain:

$$\Re\left(-(\beta+1) \pm \sqrt{(\beta-1)^2 - 2\beta\Gamma + 2\sqrt{3}\beta\Gamma i}\right) = 0 \tag{4.37}$$

Here, we encounter the square root of a complex number, by setting $\rho\cos(\phi) = (\beta-1)^2 - 2\beta\Gamma, \rho\sin(\phi) = 2\sqrt{3}\beta\Gamma$, where $\phi \in [0, 2\pi)$, we can eliminate the square root:

$$\Re\left(-(\beta+1) \pm \sqrt{\rho e^{i\phi}}\right) = 0 \tag{4.38}$$

Extract the real part and we will get:

$$-(\beta+1) \pm \sqrt{p}\cos\left(\frac{\phi}{2}\right) = 0 \tag{4.39}$$

Using the trigonometric formula [2], we can get:

$$\frac{\rho + \rho\cos(\phi)}{2} = (\beta + 1)^2 \tag{4.40}$$

Rewrite this equation by substituting back for ρ and $\rho\cos(\phi)$:

$$\underbrace{\rho^2}_{LHS} = \underbrace{\left(2(\beta+1)^2 - \rho\cos(\phi)\right)^2}_{RHS} \tag{4.41}$$

Expand both sides:

$$\begin{aligned} LHS &= ((1-\beta)^2 - 2\beta\Gamma)^2 + 12\beta^2\Gamma^2 \\ &= (1-\beta)^4 - 4\beta\Gamma(1-\beta)^2 + 4\beta^2\Gamma^2 + 12\beta^2\Gamma^2 \\ &= 16\Gamma^2\beta^2 - 4\Gamma\beta^3 + 8\Gamma\beta^2 - 4\Gamma\beta + \beta^4 - 4\beta^3 + 6\beta^2 - 4\beta + 1 \end{aligned} \tag{4.42}$$

$$\begin{aligned} RHS &= \left(2(\beta+1)^2 - (\beta-1)^2 + 2\beta\Gamma\right)^2 \\ &= 4\Gamma^2\beta^2 + 4\Gamma\beta^3 + 24\Gamma\beta^2 + 4\Gamma\beta + \beta^4 + 12\beta^3 + 38\beta^2 + 12\beta + 1 \end{aligned} \tag{4.43}$$

Cancelling the understanding terms:

$$\beta\left(12\Gamma^2\beta - 8\Gamma(\beta+1)^2 - 16(\beta+1)^2\right) = 0 \tag{4.44}$$

The threshold relationship is:

$$\frac{(\beta+1)^2}{\beta} = \frac{3\Gamma^2}{4+2\Gamma} \tag{4.45}$$

The system is stable when $\frac{(\beta+1)^2}{\beta} > \frac{3\Gamma^2}{4+2\Gamma}$, and unstable when $\frac{(\beta+1)^2}{\beta} < \frac{3\Gamma^2}{4+2\Gamma}$.

The critical value β_{bif} can be obtained by solving $\frac{(\beta_{bif}+1)^2}{\beta_{bif}} = \frac{3\Gamma^2}{4+2\Gamma}$.

Rewrite it as below and this is a quadratic equation:

$$\beta_{bif}^2 + (2 - \frac{3\Gamma^2}{4+2\Gamma})\beta_{bif} + 1 = 0 \tag{4.46}$$

Solve this equation:

[2] $\cos(\frac{\phi}{2})^2 = \frac{1+\cos(\phi)}{2}$

$$\beta_{bif\pm} = \frac{3\Gamma^2 - 4\Gamma - 8}{8 + 4\Gamma} \pm \frac{\Gamma\sqrt{9\Gamma^2 - 24\Gamma - 48}}{8 + 4\Gamma} \tag{4.47}$$

We denote the critical value of equilibrium point as R_0, recall the equilibrium equation 4.11 and rewrite it as:

$$\alpha = (R_0^n + 1)(R_0 - \alpha_0) \tag{4.48}$$

The above two equations could provide us two critical values and they are also the boundaries of bifurcation diagram in parameter space (α, β).

4.5 Linear Stability Analysis for the Reduced Repressilator Equations

The oscillatory mechanism of the repressilator could be reduced to a three dimensional ODE system. In the reduced model, the differential equations of mRNA concentration can be assumed to be near equilibrium, and by the following substitution:

$$m_1 = \frac{\alpha}{1 + p_3^n} + \alpha_0,\ m_2 = \frac{\alpha}{1 + p_1^n} + \alpha_0,\ m_3 = \frac{\alpha}{1 + p_2^n} + \alpha_0, \tag{4.49}$$

A three dimensional differential system is obtained and it can capture the behavior of oscillations like the full model does (O. Buse, *et al.*,2010) [4].

The new reduced system is as follows:

$$\frac{dx}{dt} = \frac{\alpha}{1 + z^n} - x \tag{4.50}$$

$$\frac{dy}{dt} = \frac{\alpha}{1 + x^n} - y \tag{4.51}$$

$$\frac{dz}{dt} = \frac{\alpha}{1 + y^n} - z \tag{4.52}$$

By using the same idea, we could prove the uniqueness of equilibrium point, denoted by (r, r, r), where r satisfies:

$$\frac{\alpha}{1 + r^n} = r \tag{4.53}$$

which is same as:

$$\alpha = r^{n+1} + r \tag{4.54}$$

The Jacobian matrix of the linearized system is as follows:

$$\begin{pmatrix} -1 & 0 & \gamma \\ \gamma & -1 & 0 \\ 0 & \gamma & -1 \end{pmatrix} \tag{4.55}$$

where $\gamma = \frac{-nr^n}{1+r^n}$.

The characteristic equation is:

$$[\gamma - (\lambda + 1)][\gamma^2 + \gamma(\lambda + 1) + (\lambda + 1)^2] = 0 \tag{4.56}$$

4.6 Bifurcation Analysis for the Reduced Repressilator Equations

Like what we have done for the full model, by solving above equation and we expect to find three roots:

$$\lambda = -\gamma e^{i\theta} - 1,\ \theta = -\frac{\pi}{3},\ \pi,\ \frac{\pi}{3} \tag{4.57}$$

The real parts of the eigenvalues are:

$$\mathbb{R}(\lambda) = -\gamma \cos(\theta) - 1,\ \theta = -\frac{\pi}{3},\ \pi,\ \frac{\pi}{3} \tag{4.58}$$

There are two cases:

- If $\theta = \pi$, $\mathbb{R}(\lambda_1) = \gamma - 1 = \frac{-nr^n}{1+r^n} - 1 < 0$.

- If $\theta = \pm\frac{\pi}{3}$, $\mathbb{R}(\lambda_{2,3}) = -\gamma \cos\left(\pm\frac{\pi}{3}\right) - 1 = \frac{n}{2}\frac{r^n}{1+r^n} - 1$

The threshold r_0 of where $\mathbb{R}(\lambda_{2,3}) = 0$ is

$$r_0 = \left(\frac{2}{n-2}\right)^{\frac{1}{n}}, \ n > 2 \tag{4.59}$$

Since the first eigenvalue is always negative, the stability of this system depends on the remaining two eigenvalues according to the theorem 3.1.2. We can see that $\mathbb{R}(\lambda_{2,3}) < 0$ if $r < r_0$ thus stable and $\mathbb{R}(\lambda_{2,3}) > 0$ if $r > r_0$ thus unstable. However, if we choose $n \leq 2$, $\mathbb{R}(\lambda_{2,3})$ is always negative.

If we substitute r_0, we could get the parameter space.

$$\begin{aligned}
\alpha &= r_0^{n+1} + r_0 \\
&= \left(\frac{2}{n-2}\right)^{\frac{1}{n}} \left(\frac{2}{n-2} + 1\right) \\
&= \left(\frac{2}{n-2}\right)^{\frac{1}{n}} \left(\frac{n}{n-2}\right) \\
&= \frac{n}{2} \left(\frac{2}{n-2}\right)^{\frac{1}{n}} \left(\frac{2}{n-2}\right) \\
&= \frac{n}{2} \left(\frac{2}{n-2}\right)^{\frac{1}{n}+1} \\
&= \frac{n}{2} \left(\frac{n}{2} - 1\right)^{-\frac{n+1}{n}}
\end{aligned} \tag{4.60}$$

The parameter space (α, n) is constructed by the below equation.

$$\alpha_{bif} = \frac{n}{2} \left(\frac{n}{2} - 1\right)^{-\frac{n+1}{n}} \tag{4.61}$$

4.7 Comparison of Models: The Full and Reduced Model

Based on the previous analysis, we summarize some essential properties of full and reduced repressilator models. These results are the foundation of the numerical results in the further section on numerical simulations.

	Full model	Reduced Model
Dimension of Differential System	6	3
Stable roots of characteristic equations	2	1
Number of activation	3	0
Number of repression	3	3
Equlibrium point	$p^* = m^* = R$	$x = y = z = r$
Equlibrium equation when $\alpha_0 = 0$	$\alpha = R^{n+1} + R$	$\alpha = r^{n+1} + r$
Threshold to destablize the system	$\frac{(\beta+1)^2}{\beta} = \frac{3\Gamma^2}{4+2\Gamma}$	$\alpha = \frac{n}{2}(\frac{n}{2} - 1)^{-\frac{n+1}{n}}$
Conditions to exhibit oscillations	$\frac{(\beta+1)^2}{\beta} < \frac{3\Gamma^2}{4+2\Gamma}$	$\alpha > \frac{n}{2}(\frac{n}{2} - 1)^{-\frac{n+1}{n}}$
Parameter spaces	(α, β)	(α, n)

Table 4.1: A brief comparison of both full and reduced model

Chapter 5

Extension of Repressilator

This section extends the repressilator in two ways: 1) adding spatial effects 2) adding more genes in the loop. We also present one case study of this extension. In the end, we present a summary of studying biological circuits using differential equations.

5.1 Adding the Spatial Effect

Based on the reduced repressilator model, if we consider two identical repressilators, following Buse and Pérez [4], we could construct a six dimensional differential system with spatial effect in cells.

$$\frac{dx}{dt} = \frac{\alpha}{1+z^n} - x + d(u-x) \tag{5.1}$$

$$\frac{dy}{dt} = \frac{\alpha}{1+x^n} - y \tag{5.2}$$

$$\frac{dz}{dt} = \frac{\alpha}{1+y^n} - z \tag{5.3}$$

$$\frac{du}{dt} = \frac{\alpha}{1+w^n} - u + d(x-u) \tag{5.4}$$

$$\frac{dv}{dt} = \frac{\alpha}{1+u^n} - v \tag{5.5}$$

$$\frac{dw}{dt} = \frac{\alpha}{1+v^n} - w \tag{5.6}$$

Where d is the diffusion effect, coupling the cells could be achieved *in vivo* by using either intercell signalling via quorum sensing mechanism or the addition of a subnetwork that provides coupling to each of the cells [4]. Within two repressilators, this system is nothing but the reduced model adding diffusion terms for each one of their components. If $d > 0$, x tends to move towards u; If $d < 0$, u tends to move towards x; If $d = 0$, there is no diffusion effect.

5.2 Increasing More Genes in the Loop

Let's propose a general model with N genes in the loop,

$$\frac{dx_i}{dt} = f(x_{i-1}) - x_i, \ i = 1, 2, ..., N, N+1 = 1 \tag{5.7}$$

$$f(x) = \begin{cases} \dfrac{\alpha}{1+x^n}, & \text{for repression} \\ \dfrac{\alpha x^n}{1+x^n}, & \text{for activation} \end{cases} \tag{5.8}$$

Solving for equilibrium point:

$$f \circ f \circ \cdots f \circ (x_i) = x_i \tag{5.9}$$

Based on previous analysis, we know that if the left hand side has a negative sign thus has a decreasing function, the system has a unique equilibrium point. The below function could determine the sign.

$$F = \prod_{i=1}^{N} \text{sign}(f'(x_i)) \tag{5.10}$$

For the repressilator, if there are an odd number of components in the loop connected by the repressed Hill function, the system has a unique equilibrium since $F < 0$. Otherwise, there would be multiple equilibrium points.

Rigorously, Let's introduce the theorems proposed by Gouzé (1998), which were initially conjectured by Thomas (1981). We state the theorem and its corollaries. Readers can

refer more detail in [25] [13].

Definition 5.2.1 *A circuit will be positive (negative) if F is positive (negative).*

This implies that the loop is negative if there are odd number of repressed function, thus the product of their signs will be negative.

Theorem 5.2.1 *Considering a differential system:*

$$\dot{\mathbf{x}} = f(\mathbf{x}) \tag{5.11}$$

where $\mathbf{x} \in D$, D is an open convex set of $\mathbb{R}^n$, and f is at least C^1 on D. If all circuits of the interaction graph are nonpositive, and if there is at least one non-zero term in the expansion of the determinant of the signed Jacobian matrix (matrix of sign), then the function f is injective on D.

This theorem brings to two specific corollaries, which provide analytical tools for matrix analysis.

Corollary 5.2.1 *If all circuits of the interaction graph are nonpositive, and if there is at least one non-zero term in the expansion of the determinant of the signed Jacobian matrix, then there is at most one equilibrium in D.*

Corollary 5.2.2 *If all circuits of the interaction graph are nonpositive, and if all the main diagonal elements are negative, then there is at most one equilibrium in D.*

If we recall the matrices of full model and reduced models, we find that all of the main diagonal elements are negative given the fact of both of these repressilators have odd number of repressed functions. This proves the uniqueness of equilibrium point of both rigorously.

5.3 Case Study

The simplest negative feedback N loop contains 1 repression and $N-1$ activation, which gives the system below.

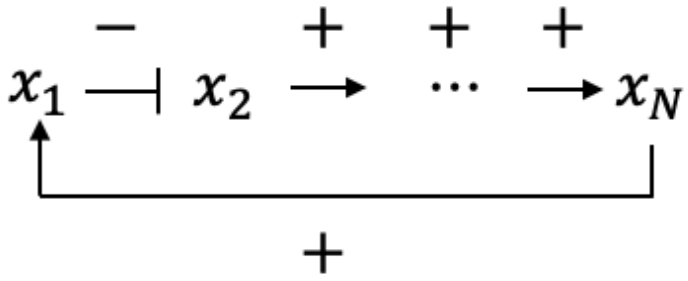

Figure 5.1: Negative Feedback loop with One Repression.

$$\frac{dx_1}{dt} = \overline{f}(x_N) - x_1 \tag{5.12}$$
$$\frac{dx_2}{dt} = f(x_1) - x_2 \tag{5.13}$$
$$\frac{dx_3}{dt} = \overline{f}(x_2) - x_3 \tag{5.14}$$
$$\vdots \tag{5.15}$$
$$\frac{dx_N}{dt} = \overline{f}(x_{N-1}) - x_N \tag{5.16}$$

where $f(x_i) = \frac{\alpha}{1+x^n}$, representing repression, $\overline{f}(x_i) = 1 - f(x_i) = \frac{\alpha x^n}{1+x^n}$, representing activation, respectively.

And the Jacobian of linearized system is as following:

$$\begin{pmatrix} -1 & 0 & 0 & \dots & 0 & -\gamma \\ \gamma & -1 & 0 & \dots & 0 & 0 \\ 0 & -\gamma & -1 & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \ddots & 0 & 0 \\ 0 & \dots & 0 & -\gamma & -1 & 0 \\ 0 & \dots & 0 & 0 & -\gamma & -1 \end{pmatrix} \tag{5.17}$$

where $\gamma = -\frac{\alpha n r^{n-1}}{(1+r^n)^2}$ and r is the equilibrium point. For simplicity, we denote this system as $(+,-,+,...,+)$. This can represent the relationship between the current component and its previous component, where $+(-)$ is activation (repression), respectively. However,

although the matrix could reflect lots of local dynamical properties of the circuit, it is difficult to evaluate this large matrix. Thus, we will only present the numerical simulations of this case.

5.4 Summary of Studying Biological Circuits

Based on previous discussions, we hereby list some essential tips of constructing biological circuits aiming to exhibit oscillatory behaviors.

1. A loop is negative if the number of repression connecting each component is odd.
2. Hill functions are a nice way to represent activation and repression because they share many of the experimentally observed essential qualities.
3. It it necessary to use linear stability analysis to confirm whether a system is robust to small perturbation.
4. Computational approaches provide a better understanding of the properties of equilibrium points.
5. A combination of mathematical and computational methods might bridge the gap between concept and realization in constructing biological circuits.

Chapter 6

Numerical Simulations of the Oscillations

In this section, we use computer simulations to verify our theoretical work for the repressilator and further extension of this system, aiming to investigate the dynamical properties of these differential systems. In section 6.1, and 6.3, we present the parameter spaces and associated dynamical behaviours of the full and reduced model on different parameters. We mainly focus on the (α, β) space for the full model, while for the reduced model, we focus on the (α, n) space. Each space consists of two different regions, the stable and unstable (oscillatory) regions. Thus, we show that we can indeed determine the parameter values for a given system that presents oscillations. Moreover, section 6.2 shows the existence of supercritical Hopf bifurcation in the repressilator by demonstrating the bifurcation diagram and the formation of the limit cycle. Section 6.5 and 6.6 present more complex behaviours of the extension of the repressilator by increasing more genes and adding spatial effect in the loop. We provide a case study of the extension of increasing more genes, i.e. a negative circuit with one repression for $N = 3$ and $N = 6$. Finally, the biological significance of the different behaviours under varying parameters is discussed.
Technically, the repressilator system could be solved by using **scipy.integrate.odeint**. This is a nice way to solve the initial value problem of the first order ODE systems. Besides, we use **ipywidgets.interact** to create user interface (UI) controls (see Figure

6.1) for our model, which allows us to see how the dynamical behaviours change with different parameters. Figure 6.2 shows an example with parameters and initial values of this UI controls. Readers could refer .1 for the python code and run it in their environment. We choose $(m_1, m_2, m_3, p_1, p_2, p_3) = (1.5, 1.0, 1.3, 0.5, 0.5, 0.5)$ for full model and $(x_0, y_0, z_0) = (1.5, 1.0, 1.3)$ for the reduced model to demonstrate the solutions clearly. It is noticeable that we all choose $\alpha_0 = 0$ for plots of dynamical behaviours, but the effect of this parameter will also be shown when plotting the equilibrium point varied with parameters. For the generalized model, we choose initial values all 0. Plotting the bifurcation diagram requires solving for the equilibrium points first, where we use the bisection method to solve this nonlinear equation, and the algorithm is provided in the appendix .3.

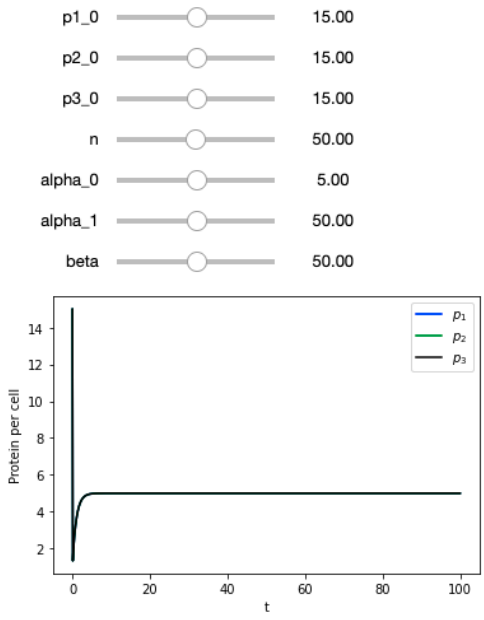

Figure 6.1: UI controls by using IPython's widgets.

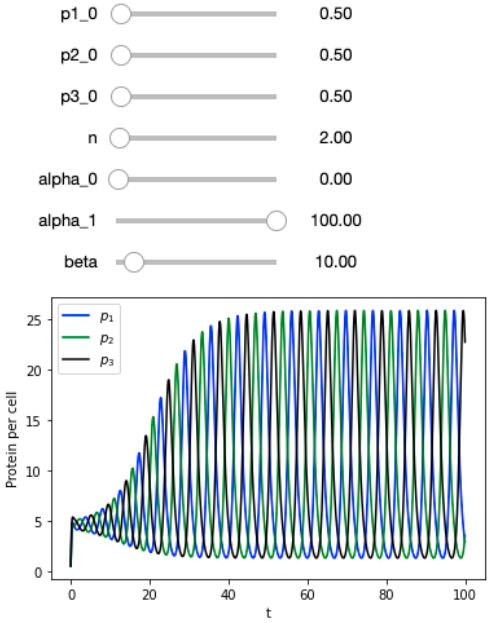

Figure 6.2: UI controls: an example.

6.1 Parameter Space and Oscillations of the Full Model

We use bisection method to solve the below equation

$$\alpha = (R_0^n + 1)(R_0 - \alpha_0) \tag{6.1}$$

The solution plot shows that as n increases, equilibrium point moves higher and increasing

α_0 will shift the plot vertically (see Figure 6.3).

Recall the equation:

$$\beta_{bif\pm} = \frac{3\Gamma^2 - 4\Gamma - 8}{8 + 4\Gamma} \pm \frac{\Gamma\sqrt{9\Gamma^2 - 24\Gamma - 48}}{8 + 4\Gamma} \tag{6.2}$$

which constitutes the parameter space (see Figure 6.4).

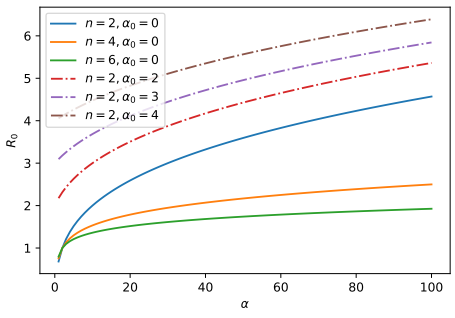

Figure 6.3: Equilibrium point varied with parameters.

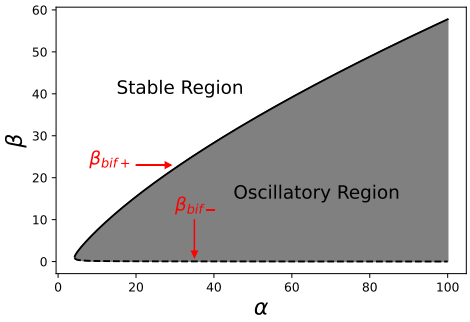

Figure 6.4: Parameter space when $n = 2$.

Equation 4.47 and 4.11 are used to construct a bifurcation diagram. Two branches (solid and dash line) represent the two roots of β_{bif}. The space consists of two different regions, and as we vary n the sections of the domain will change. Verdugo [27] shows that a small increase in the value of the Hill coefficient changes the behaviours dramatically. This is due to the fact that the boundary of bifurcation swings from one side to another side for a fixed parameter point. Following his idea, we choose $(\alpha, \beta) = (100, 100)$ and $n = 2.0, 2.1$. Figure 6.5 demonstrates that the system becomes unstable and exhibits oscillation when there is a small increase in the Hill coefficient.

Moreover, if we increase the Hill coefficient to $n = 5$, the unstable domain becomes much larger, and there is no limitation on β considering large α, which is consistent with the results found by Elowitz and Leiber. Figure 6.6 shows similar behaviours regardless of how much β is.

If we decrease the repression rate to $\alpha = 10$ and varying β could make the parameters cross the bifurcation boundary, thus causing a change of behaviours, shown in Figure 6.7.

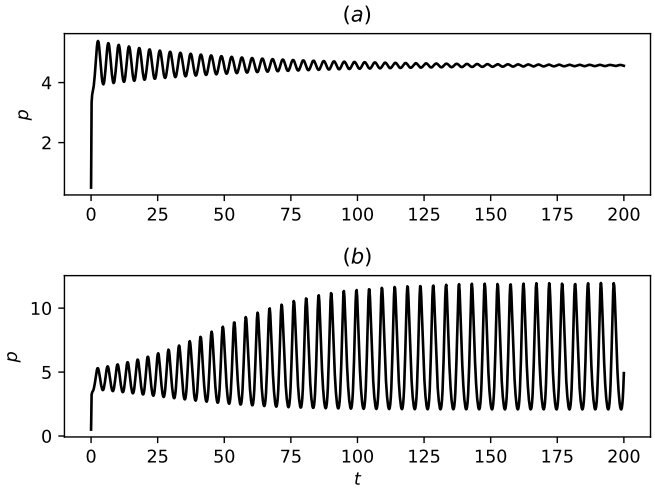

Figure 6.5: Behaviors near the branch of bifurcation diagram.(a): damped oscillation. $(\alpha, \beta, n) = (100, 100, 2.0)$, (b) sustainable oscillation. $(\alpha, \beta, n) = (100, 100, 2.1)$. A small perturbation makes the system exhibit a clear transient from stable to oscillatory steady state.

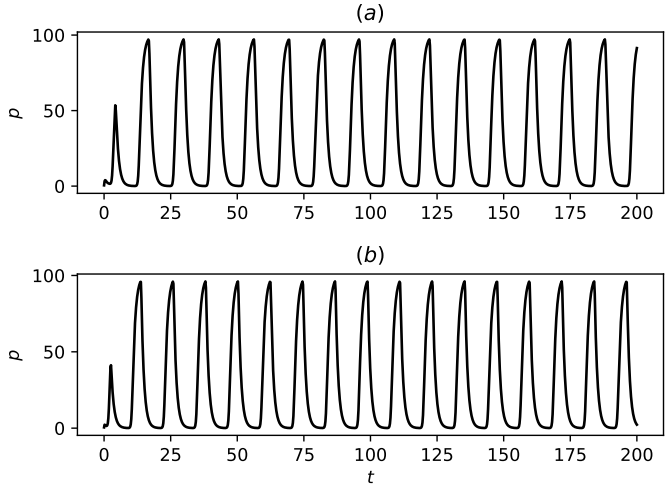

Figure 6.6: Behaviors with higher α. (a): $(\alpha, \beta, n) = (100, 5, 5)$. (b) $(\alpha, \beta, n) = (100, 50, 5)$. The unstable domain becomes larger and limitation on β under high repression rate α decreases.

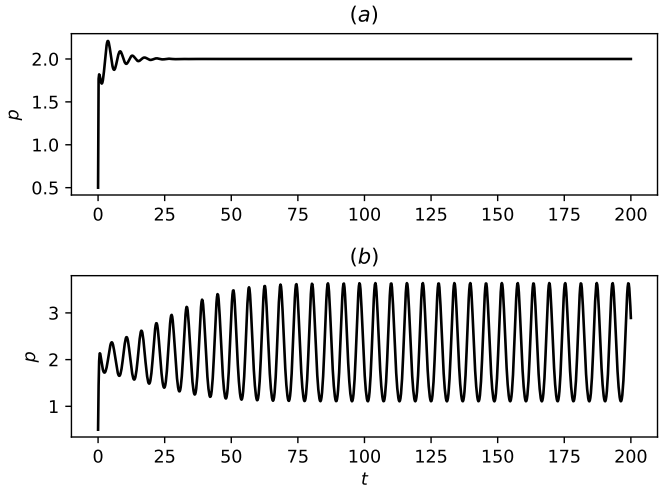

Figure 6.7: Behaviors with lower α. (a): damped oscillation. $(\alpha, \beta, n) = (10, 100, 2)$. (b): sustainable oscillation. $(\alpha, \beta, n) = (10, 5, 2)$. Changing degradation will cross the boundary of the parameter space thus causes different behaviors.

6.2 Hopf Bifurcation in the Repressilator

Theoretical studies [3] [5] [4] show that the repressilator system undergoes a supercritical Hopf bifurcation. Proofs start from solving equilibrium equations and substitute them into linearized systems. By some analysis of the eigenvalues of matrices, the first Lyapunov coefficient $l_1(p_0)$ is calculated, where p_0 is the equilibrium point for the limit cycle to branch. When $l_1(p_0) < 0$, the Hopf bifurcation is supercritical while $l_1(p_0) > 0$ subcritical Hopf bifurcation occurs and the limit cycle that branch from p_0 is unstable [5]. However, computing the Lyapunov coefficient is tedious. Thus, we will not show the precise work here. Readers could refer [3] (the reduced model) and [5] (the full model) for further details. In this section, we will confirm the existence of supercritical Hopf bifurcation by demonstrating the bifurcation diagram and the formation of the limit cycle numerically. For the convenience of demonstrating the full formation of limit cycle in this system, we choose initial values $(m_i, p_1, p_2, p_3) = (0, 1, 2, 3)$, setting parameters as $(\alpha, \beta) = (100, 5)$, and vary n as $1, 1.5, 5$, respectively. In theory, when the Hill coefficient increases, the oscillations occurs easier since the unstable domain (oscillatory region) grows, removing limitation of other parameters to make the system exhibit oscillations.

Figure 6.8 demonstrates the trajectories in phase plane for (m_1, p_1), other pairs such as (m_2, p_2) or (m_3, p_3) shows the same behavior thus we ignore them. When $n = 1.0$, the

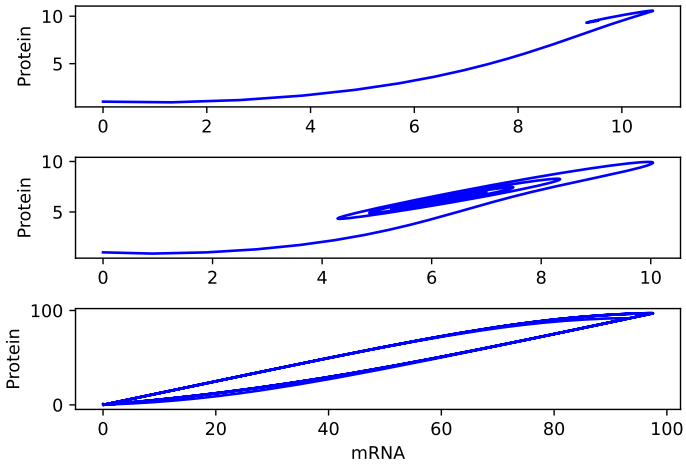

Figure 6.8: Formation of the limit cycle as n increases. Top: $n = 1.0$. Middle: $n = 1.5$. Bottom: $n = 5.0$.

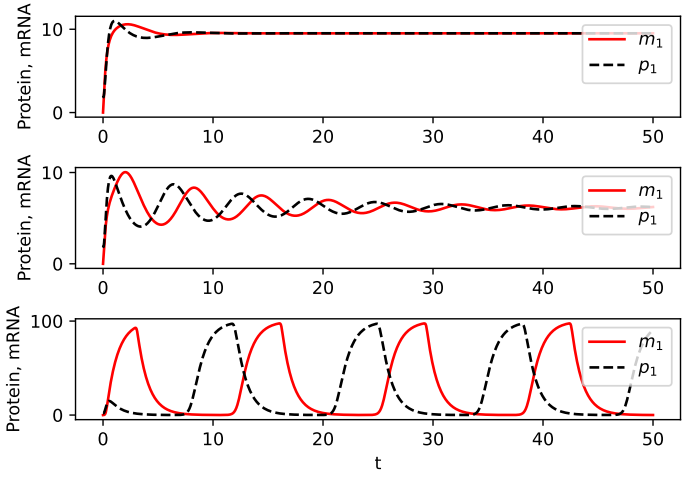

Figure 6.9: Dynamical behaviors as n increases. Top: $n = 1.0$. Middle: $n = 1.5$. Bottom: $n = 5.0$.

trajectory tends to the upper right of the space and turns back in a quick way and then stops, which means that the concentration of both mRNA and protein increases at the beginning and then saturates to a constant value. This value is a stable equilibrium point, and there is no oscillation in this case. This system is not strong enough to exhibit oscillations under a low Hill coefficient. When $n = 1.5$, the trajectory moves towards to upper right of the space but starts to spiral after a turning point. The spiral is called a stable spiral since it moves nearer and nearer to a point in a circular way. In this case, we could expect to see a few oscillations at the beginning. However, this is not a sustainable oscillation. Under a higher Hill coefficient $n = 5$, we could find that the trajectory remains on the limit cycle, and the system exhibits sustainable oscillations. Behaviours of each component in a full view could be seen in Figure 6.9.

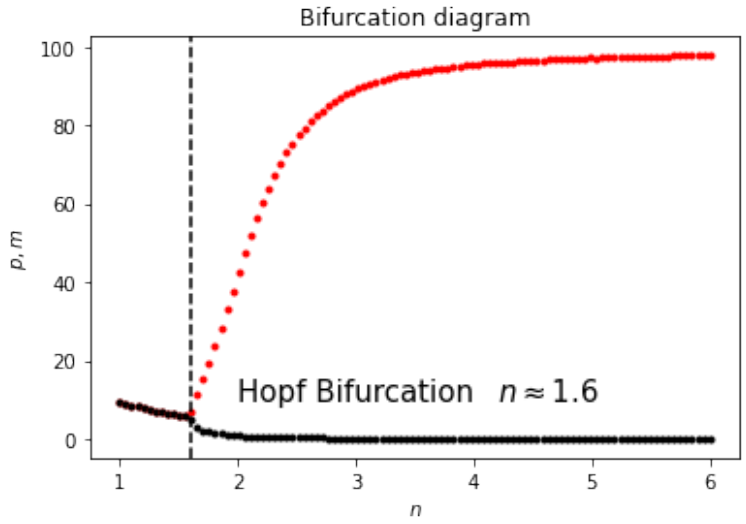

Figure 6.10: Supercritical Hopf bifurcation with approximate threshold $n \approx 1.6$.

Figure 6.10 demonstrates the supercritical Hopf bifurcation and the approximate value of Hill coefficient to make the system exhibit sustainable oscillations.

6.3 Parameter Space and Oscillations of the Reduced Model

We also use bisection method to solve the below equation, shown in Figure 6.11.

$$\alpha = (r_0^n + 1)r_0 \tag{6.3}$$

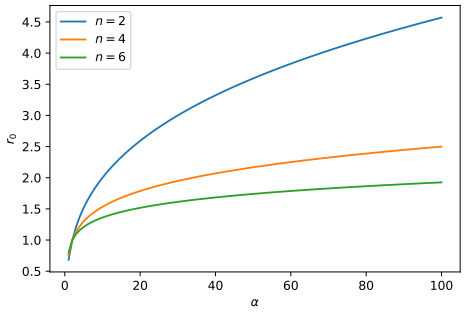

Figure 6.11: Equilibrium point varied with parameters of reduced model.

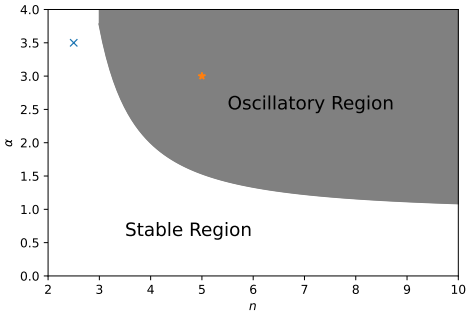

Figure 6.12: Parameter space of reduced model.

The equilibrium protein concentration increases as the transcription rate promote, while it is repressed by increasing Hill coefficients. Topologically, it demonstrates that the position of the equilibrium point shifts from 0 to ∞ as α varies [4]. This solution is similar to the full model while there is no α_0 that could shift the plot vertically.

Recall the equation:

$$\alpha_{bif} = \frac{n}{2}\left(\frac{n}{2} - 1\right)^{-\frac{n+1}{n}} \tag{6.4}$$

The oscillatory region is the 2-D space where the parameters satisfy $\alpha > \alpha_{bif}$. We can plot the parameter space based on the above analysis. The dark shading region is the unstable steady state that leads to oscillations, and the equation 4.61 constructs the boundary.

Choose points in each region, $(2.5, 3.5), (5, 3)$ and solve the system numerically:

In Figure 6.13, we can see that the solution corresponding to the stable region shows decay behaviour while the solution corresponding to the unstable region exhibits sustainable oscillation. Thus, our theoretical work makes sense.

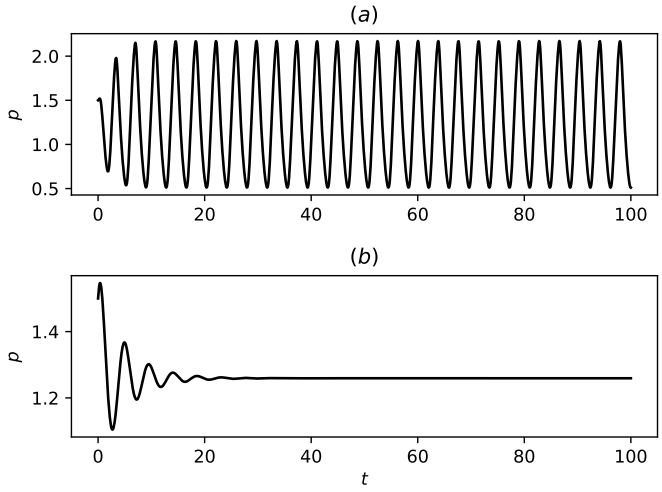

Figure 6.13: Behaviors corresponding to two picked point of reduced model. (a): Oscillatory region. (b): Stable region.

6.4 Summary of the Repressilator

Based on the numerical results, we hereby list some essential points of the differential system of repressilator after our computational analysis. These results suggest that we can determine the parameter values for a given system that presents oscillations.

1. Significant effects of cooperative binidng , efficient repressions and high transcription rate promote oscillations.

2. The repressilator indeed undergoes supercritical Hopf bifurcation

3. Behaviours from decay, then damped oscillation to sustainable oscillations indicate a transition from stable region to unstable region.

4. Increasing the Hill coefficient will increase the unstable region, thus making the system easy to oscillate.

6.5 Case Study: Negative Circuit with One Repression

In this section, we investigate the dynamical behaviours of the simplest negative feedback loop (with only one repression) mentioned in section 5.3, especially for the cases when $N = 3$ and $N = 6$. We start by introducing each loop and its plots of numerical solutions. Then we change parameters, aiming to find the threshold for the system to exhibit sustainable oscillations.

Recall the linearised system

$$\frac{d\mathbf{x}}{dt} = \mathbf{J}\mathbf{x} \tag{6.5}$$

where $\mathbf{J} \in \mathbb{R}^{N\times N}$ is

$$\begin{pmatrix} -1 & 0 & 0 & \dots & 0 & -\gamma \\ \gamma & -1 & 0 & \dots & 0 & 0 \\ 0 & -\gamma & -1 & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \ddots & 0 & 0 \\ 0 & \dots & 0 & -\gamma & -1 & 0 \\ 0 & \dots & 0 & 0 & -\gamma & -1 \end{pmatrix} \tag{6.6}$$

6.5.1 Negative Feedback Loop with Three Components

Considering a loop with $N = 3$ and thus the system is $(+, -, +)$. The first component represses the second one and is activated by the third one, while the second component always activates the third one, which forms a closed loop. We solve the system numerically given the parameters $n = 1.5, \alpha = 10$ and plot the solutions for each component, shown in Figure 6.14. We can see that all solutions show damped oscillations. Although they all start from $(0, 0, 0)$, the constant values they finally achieve differ, following the relationship: $x_1 > x_3 > x_2$. This is important since, in either the full repressilator model or reduced repressilator model, all concentrations have an equal steady state.

Then, we increase the Hill coefficient, keeping $\alpha = 10$, we can see the changing behaviours, from damped oscillations to sustainable oscillations, as shown in Figure 6.15. We could

conclude that the threshold for the system to exhibit sustainable oscillations must lie between $n = 2.0$ to $n = 2.5$. Bifurcation diagram (see Figure 6.16) illustrates that the approximate threshold of Hill coefficient is 0.24.

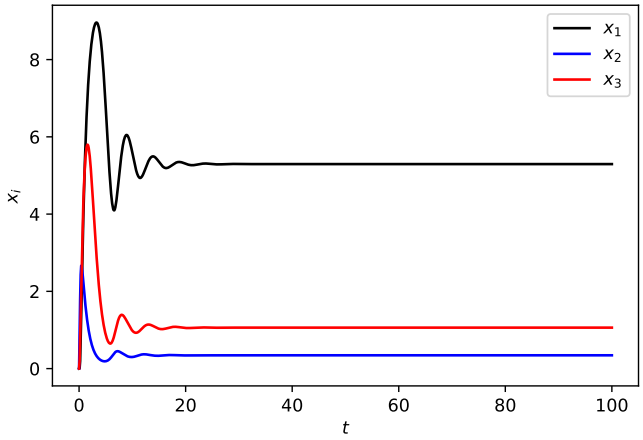

Figure 6.14: Damped oscillations of each component. $n = 2, \alpha = 10, N = 3$.

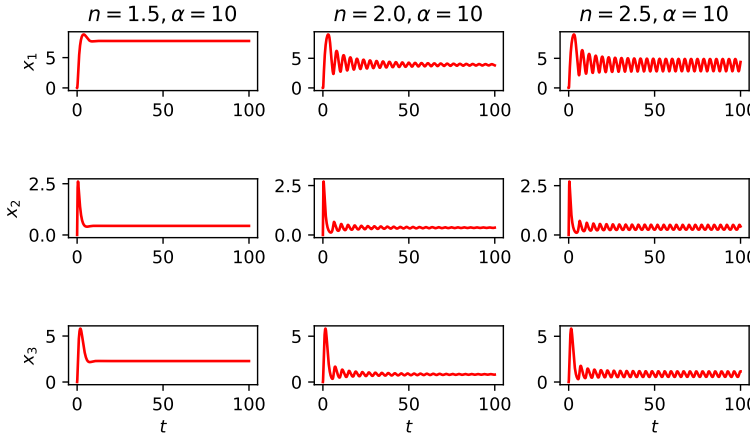

Figure 6.15: Threshold for sustainable oscillations occur when $N = 3$.

6.5.2 Negative Feedback Loop with Six Components

Now, considering a longer loop, $N = 6$. Thus the system is $(+, -, +, +, +, +)$, repression occurs between the first and second component, others are all activations. We also choose $\alpha = 10$ and Figure 6.17 shows the behaviors of each component. In the $N = 3$ case, all oscillations are bounded by the first component and the second component remains at

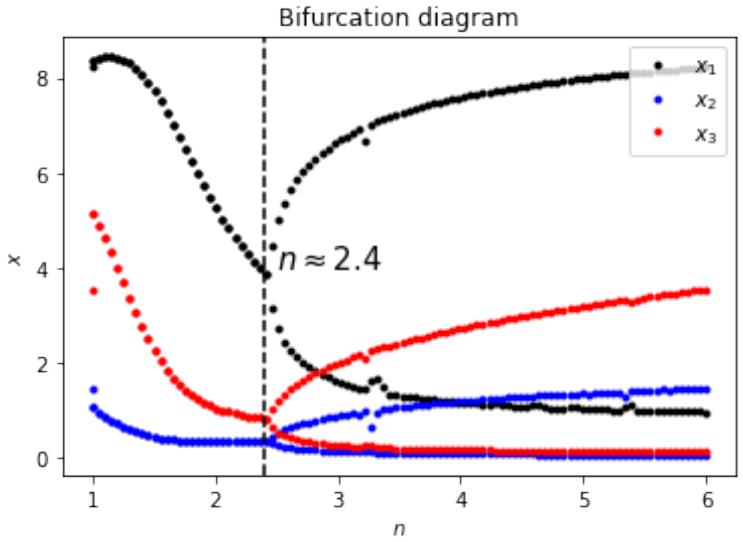

Figure 6.16: Bifurcation diagram with approximate threshold $n \approx 2.4$.

the bottom, although they all start from the same values $(0, 0, 0, 0, 0, 0)$.

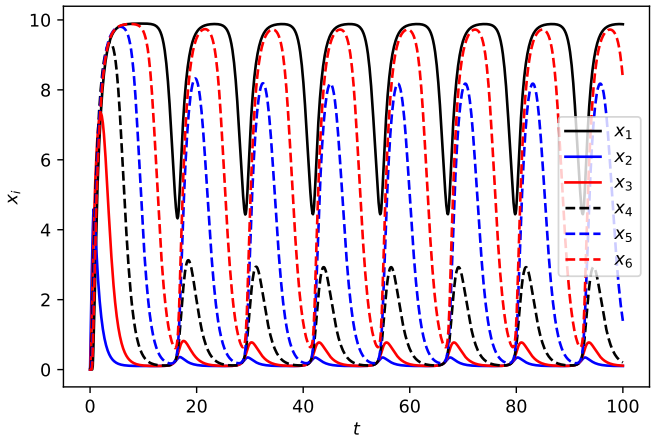

Figure 6.17: Sustainable oscillations of each component. $n = 2, \alpha = 10, N = 6$.

Figure 6.18 demonstrates how a small change in the Hill coefficient affects the behaviours of each component. When $n = 1.9$, the system is stable, and all components tend to have steady solutions. However, when $n = 2.0$, all components start to oscillate permanently. Roughly speaking, apart from the behaviour of the first component, it seems that there is a sharp peak at the beginning, which increases rapidly and then decreases dramatically, consequently forming a peak. This peak becomes wider and wider from x_2 to x_6. This phenomenon also occurs in the previous case when $N = 3$. Therefore, to investigate this pattern, we try different parameters to how they affect the oscillatory patterns. Figure

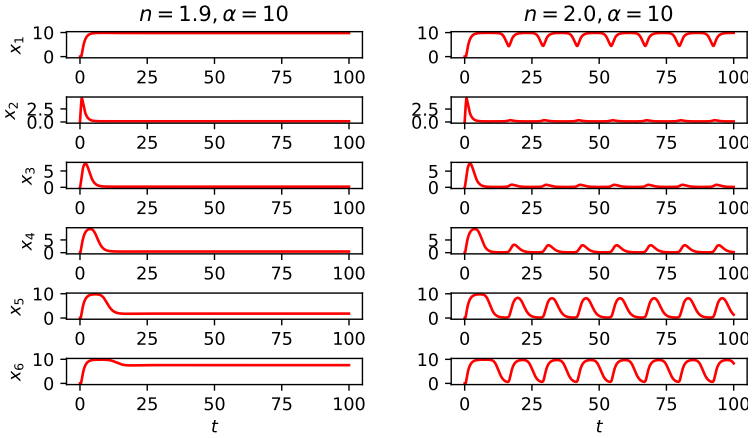

Figure 6.18: Threshold for oscillations when $N = 6$.

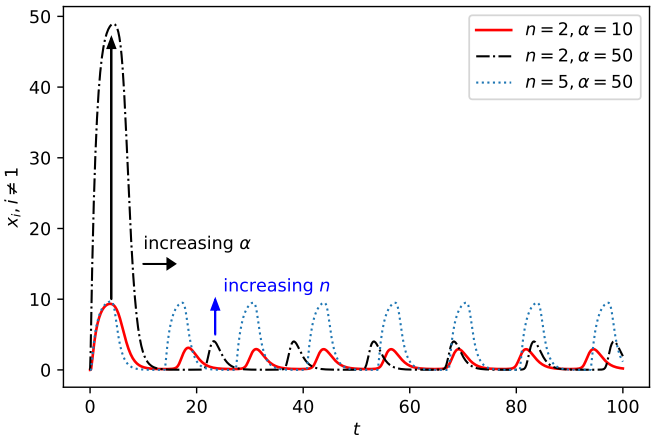

Figure 6.19: Oscillatory with a change of frequency and amplitude on different parameters.

6.18 shows a general behaviour of each component except the first one under the effect of different parameters. As we increase the transcriptional rate α, the peaks of the oscillations arise. In particular, the first peak rises most, and the whole oscillation shifts from left to right (see the solid red line and the black dash-dot line). If we increase the Hill coefficient n, the peaks of oscillation rise but are bounded by the first peak (see the solid red line and blue dot line).

However, we can not present an explanation for this change of frequency and amplitude, and further mathematical analysis for this loop needs to be done. Future research to study these oscillatory behaviours analytically would include analyzing the nonlinear higher-order terms and the corresponding Hill coefficient.

6.6 Synchronization Properties of Repressilator

We present dynamical behaviours called the synchronization properties for repressilator with spatial effect, which are highly related to both diffusion rate and repression rate. Buse and Pérez (2010) suggest that as the transcription rate α increases, the shape of the limit cycle changes qualitatively.

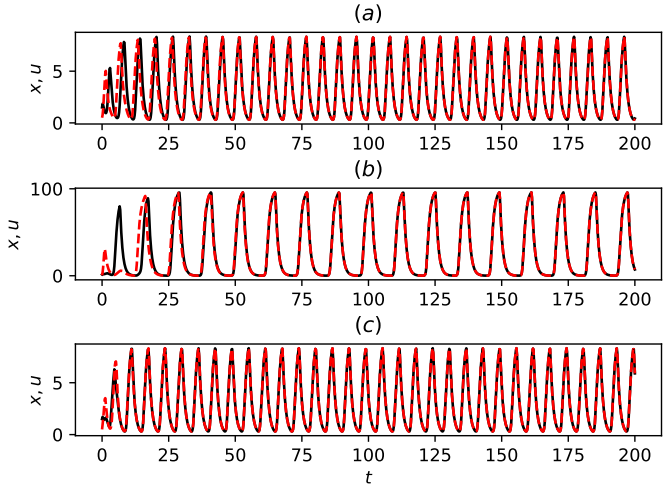

Figure 6.20: Diffusion effect with spontaneous oscillation. (a): Low repression rate $(\alpha, n, d) = (10, 5, 0.1)$. (b):High transcription rate $(\alpha, n, d) = (100, 5, 0.1)$. (c): High diffusion rate $(\alpha, n, d) = (10, 5, 1)$. Both higher transcription and diffusion rate cause faster synchronization.

This behaviour suggests that spatial effect is crucial to understanding the gene network.

Computational bifurcation analysis might help investigate how the diffusion rate affects the coupled repressilator. Readers also could refer [11] [16] for more oscillatory properties and biological significance of synchronization of repressilator at the cell level.

6.7 Biological Significance

From computer simulations, we know that both full and reduced models have periodic solutions under certain parameter conditions, which is also a verification of the theoretical work regarding the existence of supercritical Hopf bifurcation in the repressilator equations, and a clear formation of the limit cycle could be seen by increasing Hill coefficient n, keeping other parameters as constants. We could also see a qualitative change from steady state (Damped oscillations) to unstable behaviour (Sustainable oscillations) by a small change of n.

From a biological perspective, we recall the biological meanings of three main parameters in repressilator equations: α, β and n, which represent the transcription rate in the absence of repressions, the proportion of the decay rate between protein and mRNA, and cooperative binding effects, respectively. Overall, high α, β, and n promote the oscillations, which leads to the general principle of designing and constructing biological circuits for specific behaviours, and adding spatial effects and increasing the number of gene in the loop can exhibit more complex behaviours.

Chapter 7

Conclusions and Further Directions

In summary, we have studied the mathematical conditions of exhibiting oscillations for repressilators using mathematical and computational approaches. The foundation of our theoretical work is provided by the stability theory of linear and nonlinear differential systems. Our mathematical analysis section has also derived the equations used to construct the parameter spaces for both full and reduced models. We thus confirmed that repressilator undergoes a supercritical Hopf bifurcation. Finally, we find more complex behaviours by adding spatial effects and increasing more genes in the loop.

Overall, we have met all goals of this project. Firstly, we can determine the parameters for a given system that present oscillations and we have also identified that a high transcription rates, significant effects of cooperative bindings, and efficient repressions would promote oscillations, which might provide general principles for designing and constructing biological circuits, such as cell division [20] and circadian clocks [6]. Secondly, we have numerically confirmed that the steady-state of the repressilator system undergoes a supercritical Hopf bifurcation, with the born of a stable limit cycle. Finally, extensions of the repressilator brings our attention to more complex behaviours like synchronization properties and the change of the frequency and amplitude of the oscillations.

Future research can focus on studying more complex gene networks and associated behaviours. Firstly, we could develop computational bifurcation analysis tools to study the synchronization properties of coupled repressilators, which might lead to the findings of chaotic behaviours. Moreover, since it is challenging to study a nonlinear system with

a significantly extensive gene network analytically, advanced analytical approaches can focus on studying higher order nonlinear terms in the differential systems, which might enhance our understanding of nonlinear systems.

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.1 Code Accessibility

The Python codes applied to generate the results presented in all figures can be found at:

`https://github.com/Homingdung/repressilator`

.2 Proof of Theorem 4.4.1

Divided by y^2 on the both sides:

$$\left(\frac{x}{y}\right)^2 + \left(\frac{x}{y}\right) + 1 = 0 \tag{1}$$

Setting $t = \frac{x}{y}$, we will get a quadratic equation:

$$t^2 + t + 1 = 0 \tag{2}$$

where solutions are $t = \frac{x}{y} = \frac{-1 \pm i\sqrt{3}}{2}$,therefore we get the following equation:

$$x = \frac{-1 \pm i\sqrt{3}}{2} y \tag{3}$$

.3 Root-Finding Method

Bisection method was applied to solve $\alpha_{bif} = r_0^{n+1} + r_0$. Since the uniqueness of fixed point was proven in 4.2, rewrite the equation as $F(\alpha, r) = \alpha - r^{n+1} - r$, where $F(\alpha, 0) = \alpha > 0$ and $F(\alpha, \alpha) = -\alpha^{n+1} < 0$, thus the solution of $F(\alpha, r) = 0$ lies between $(0, \alpha)$.