Modeling of Cancer Signaling Pathways

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Cancer is an ongoing problem all over the world. To find a cure to this disease, both clinicians and scientists are looking for a reasonable treatment method. According to Hanahan and Weinberg, one of the hallmarks of cancer is evasion of programmed cell death, referred to as *apoptosis*. Apoptosis is an important cellular process, and is regulated by many different pathways. Proteins in these pathways contribute to either cell death or cell survival depending on the cell stresses. Much research in systems biology has been devoted to understanding these pathways at the molecular level.

In this study a mathematical model is built to describe apoptosis, and the pathways involving the related proteins p53 and Akt. The primary purpose of the construction of the kinetic model is to verify that this network can exhibit bistability between cell survival and cell death. Sensitivity and bifurcation analysis are conducted to determine which parameters have the greatest effect on the system behavior.

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Dedication

To My Creator, Allah (c.c.)

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Chapter 1

Introduction

1.1 Cancer

Cancer is an ongoing worldwide problem for human health, despite intensive investigations by both clinicians and scientists who are trying to determine its underlying causes. As a disease, cancer is caused by accumulations of genetic mutations in the cell. The main characteristics of cancer were comprehensively described by Hanahan and Weinberg in "The Hallmarks of Cancer" [1]. According to their widely accepted classification, a cancer cell has the properties of: evading cell death, producing its own growth signals, disregarding antigrowth signals, replicating an unlimited number of times, promoting the formation of new blood cells, and invading through the surrounding tissues to induce secondary cancer.

In this study, the problem of cancer will be considered from a biomolecular basis by considering the means by which cancer cells are able to evade programmed cell death (called apoptosis). From this starting point, some important networks and their regulatory proteins introduced in Chapter 2 are examined in order to explore their relationship in the regulation of programmed cell death pathways. These proteins are involved in an intracellular signal transduction network that is responsible for regulating programmed cell death.

1.2 Systems Biology

Systems biology is a new biological field that focuses on a systems-level understanding of biological processes, including aspects of regulation. Building on the remarkable progress in

molecular biology, the systems biology approach can be used to develop predictive models based on an understanding of the structure and dynamics of molecular interactions within a network [2]. The goal of systems biology is to map complex biological pathways by considering the regulation of the components of the system, and to point out how these interactions affect the function and behavior of the system [3]. As a result, systems biology yields a holistic approach to biological research, which can be extended to cells, tissues and organisms [4].

1.3 Cell Signaling Networks

Our knowledge of cell signaling networks begins with interactions maps (sometimes called cartoon diagrams) that indicate protein-protein interactions and map their upstream and downstream reaction networks. These networks commonly have well-defined inputs and outputs, and are characterized by feedback loops, and multi-step regulatory controls. Due to the complexity of their structure and function, mathematical modeling has been needed to consider the system behavior of signaling networks [5].

1.4 Mathematical Models

Mathematical models complement experimental studies of real world problems in engineering, the life, social, and environmental sciences [6, 7]. Due to the complexity and nonlinearity of real world problem, it is often difficult to understand the role of each component in the behavior of a network. Mathematical modeling can be used to describe system dynamics, and better understand how network structure is related to system behavior [8]. Mathematical

models allow both the theoretical analysis of the system and the predictions of the results of experiments (see Figure 1.1).

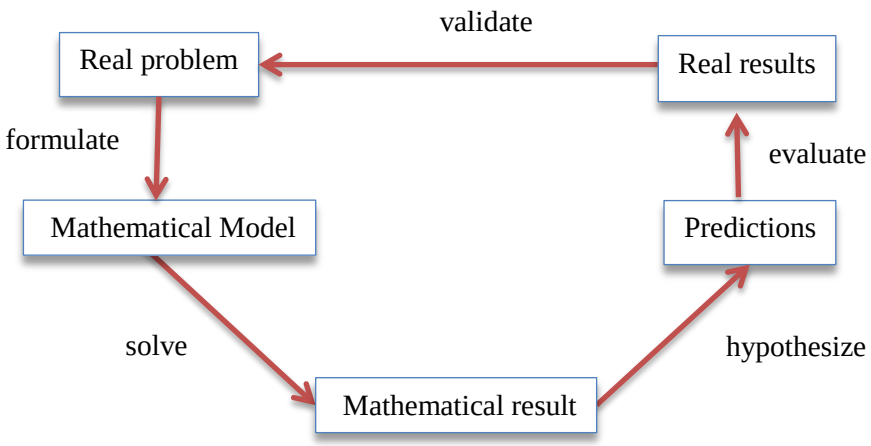


Figure 1.1: Representation of mathematical modeling

1.5 Bistability

A key feature of many cell signaling networks is bistability, which is the property of having two different stable steady states. In a cellular setting, bistability can arise from a strong positive feedback loop. For instance, if protein A activates protein B, and in turn protein B activates protein A (or likewise, if each inactivates the other) the system may show bistability [9]. In this study, bistability will correspond to two cell fates: cell survival or cell death.

1.6 The Objective of the Presented Study

Cancer develops from the molecular state of damaged cells, and one of the properties of the cancer is the evasion of cell death-signals. The aim of this research is to build a mathematical

model that describes the network responsible for regulating programmed cell death, and to verify conditions under which this network exhibits bistability.

To accomplish this goal, this thesis presents both biological and mathematical background on the mechanism and dynamics of cellular signaling pathways. For the biological background, related cell signaling pathways are examined in Chapter 2. Subsequently, mathematical background information is introduced in Chapter 3. This includes the techniques that are used to describe the pathways and the analysis that is used to investigate bistability. Chapter 4 describes a new model, which builds, in part, on previously published studies. Analysis of the model is presented in Chapter 4 with the discussion. Finally, concluding remarks and future directions are given in Chapter 5.

Chapter 2
Biological Background

2.1 Introduction

In this chapter, three intertwined signalling networks will be described. In describing these pathways, only the key proteins will be introduced. This description follows the network models presented by Legewie *et al.* [10] and Aguda *et al.* [11].

2.2 Apoptosis

Cells are the basic structural and functional unit of all living things. Cell death can be caused by injury, but can also be the result of programmed molecular interactions (the absence of growth factors or the presence of death signals on the cell surface). Because these interactions are systematically regulated by the organism, this mode of cell death is called programmed cell death [12]. Apoptosis is the best-understood programmed cell death process (and indeed, these two terms are sometimes used interchangeably) [13]. Apoptosis is the Greek word for the falling of leaves from trees; the term was introduced to biology in 1972 by Kerr, Wylie, and Curries [14].

2.2.1 The Importance of Apoptosis

Apoptosis is a homeostatic mechanism; it provides a means for organisms to eliminate abnormal or undesirable cells. For example, during the development of a human embryo, cells that link the fingers are killed via apoptosis to allow proper separation of the digits. Furthermore,

apoptosis functions as a quality control service or defense mechanism by eliminating damaged cells. For instance, when DNA damage occurs in the cell, the damaged cells normally undergo apoptosis to prevent their growth. Moreover, apoptosis occurs to maintain the cell population in developing tissues. Unlike the normal cells, cancer cells evade this cell death pathway by a number of mechanisms. From a clinical perspective, some chemotherapeutic drugs trigger the apoptotic pathway, allowing the targeting of neoplastic disorders such as cancer [15].

2.2.2 The Biochemistry of Apoptosis

2.2.2.1 Caspases

Caspases, which are enzymes, are key components of the apoptotic signaling pathway; cell death starts with the activation of caspases. Caspases lead to apoptosis by cleaving proteins into small peptide molecules. The classification of cell death is defined in terms of caspase activity [16].

Ten major caspases are classified as **initiators** (caspase-2, 8, 9, 10); which trigger caspase signaling cascade; **inflammatory** (caspase- 1, 4, 5); which trigger inflammation; and **effectors or executioners** (caspase-3, 6, 7); which are the agents of cell death, during apoptosis [16]. In this study, we will consider a network that includes two initiator caspases (8 and 9) and one effector caspase (3).

2.2.3 Apoptotic Pathways

Apoptosis is a highly regulated and controlled process. Apoptosis is fundamentally initiated through two distinct signaling networks: the extrinsic pathway and the intrinsic

pathway. Despite having an equivalent outcome (i.e., cell death), the initial points of these signaling pathways are different, as we next describe.

2.2.3.1 Extrinsic Apoptotic Pathway

The extrinsic pathway is induced from death receptors, which are placed on the cell surface. Death receptors are activated by extracellular death signals; when activated, they recruit and trigger initiator caspases 8 and 10. Subsequently, these activated initiator caspases activate the effector caspases 3 and 7 [15].

2.2.3.2 Intrinsic Apoptotic Pathway

The intrinsic pathway is triggered by cell stresses, such as DNA damage or loss of cell survival factors, such as oxygen or nutrients. The main characteristic of the intrinsic pathway is the deformation of the mitochondria, which causes release of the protein cytochrome-c from the mitochondria, triggering initiator and subsequently effector caspases [15].

2.3 p53 and Akt/PKB Pathways

Because of the importance of apoptosis in the regulation of cellular functions, scientists have been investigating other important cell signaling transduction network's impact on the apoptotic pathways. In this study, we will consider two other important pathways: p53 and Akt/PKB.

The p53 pathway involves a network of genes and their protein products. The activation of the p53 pathway is initiated by a variety of extrinsic and intrinsic stress signals, such as DNA damage, the lack of oxygen and nutrients. This pathway is regulated by positive and negative

autoregulatory feedback loops. The activity of the p53 pathways can lead to either cell cycle arrest or apoptosis [17].

Another important signal transduction pathway for the regulation of cell functions is the Akt/PKB signaling pathway. This pathway is activated in response to growth signals. The main contributions of the Akt/PKB pathway to the cellular functions involve nutrient metabolism, cell growth, transcriptional regulation and cell survival [18]. In this study, the Akt/PKB signaling pathway will be considered in not only regulating cell survival but also in blocking apoptosis.

Having mentioned the primary roles of these two pathways, we next consider proteins involved in the regulation of pathway behavior. In the p53 pathway, an autoregulatory feedback loop is provided via the interactions between p53 and the protein Mdm2, which inhibits p53 activity [19].

p53 is known as "the guardian of the genome" because it prevents the growth of cells that suffer DNA damage [20]. When DNA damage occurs, p53 induce cell growth arrest or apoptosis depending on the cell type and the stress, and the action of p53 co- activators [20]. Thus, p53 functions as a tumor suppressor gene, which functions to inhibit damaged cells from proliferation. As a result, p53 provides cell integrity; approximately 50% of human cancers involve a mutation in the p53 gene, and in particular 70% of colon cancers show p53 gene mutations [20].

Mdm2 is the main regulator oncoprotein of p53. The regulation of these two proteins is maintained by a negative feedback loop. After the phosphorylation of Mdm2, phosphorylated Mdm2, which is denoted as $Mdm2_p$ in this study, migrates from the cytosol to the nucleus from where it inhibits p53's activities. However, under cell stress, p53 activates either cell cycle repair

progression or a cell death signaling pathway. This cell cycle repair is initiated by mdm2 gene, which is activated by p53. Thus, the purpose of the negative feedback loops between Mdm2 and p53 is to keep p53 in the low concentration to prevent cell cycle arrest or apoptosis in the absence of cell stress. The direct regulation of p53 via Mdm2 suggests Mdm2 as a direct target of cancer treatment [21].

Unlike the p53-Mdm2 negative feedback loop, PTEN forms a positive feedback loop with p53 protein in the p53 pathway. PTEN is a tumor suppressor protein, and is activated via p53 protein after cell stresses. On the other hand, the role of PTEN on p53 activity is as follows. PTEN has a function to inhibit Akt/PKB signaling pathway. In normal conditions, Akt/PKB pathway provides the phosphorylation of Mdm2 via phosphorylated Akt (Akt_p), and translocates it from cytoplasm to nucleus. $Mdm2_p$ in the nucleus keeps p53 concentration level low to inhibit unwanted cell death or cell cycle arrest. Thus, the inhibition of Akt/PKB signaling pathways via PTEN results in the blockage of the entrance of the phosphorylated Mdm2 into the nucleus. As a result, PTEN indirectly modulates p53 protein level in the nucleus upwards in order to activate programmed cell death [for review 22, 23, 24, 25].

Thus, taking p53 and Akt/PKB signal transduction pathways together, we see that p53 and PTEN are pro-apoptotic (or tumor suppressor) proteins because they trigger the cell to undergo apoptosis, while, Mdm2 and Akt are anti-apoptotic (oncoproteins) because they prevent programmed cell death by inhibiting the activity of pro-apoptotic proteins.

In this study, we will address the interaction of p53-Akt/PKB network and apoptotic pathway.

The Akt/PKB signaling pathway affects the apoptotic pathways process in a number of ways. In this study, we will focus on the inhibitory effect of Akt/PKB on apoptosis via activation of phosphorylated XIAP ($XIAP_p$), which inhibits cell death, and inhibition of activated caspase3 ($casp3_a$) [26]. The p53 signaling pathway plays an opposite role in terms of the activation of apoptotic pathway. The detailed regulation of these pathways with apoptosis will be discussed in Chapter 4.

Chapter 3
Dynamical Networks

3.1 Introduction

The cell is the primary structural and functional unit of all living organisms. Cells use their structure to accomplish a range of functions, such as migration, and sending and receiving signals. The purpose of this chapter is to introduce a mathematical representation of biochemical reaction networks within the cell, based on descriptions of reaction rates. The law of mass action will be used as a starting point for describing enzyme kinetics, which will be used to describe protein degradation, phosphorylation, dephosphorylation, expression, activation, inactivation, complex formation and complex dissociation. A kinetic description of protein production will be provided as well.

Dynamical analysis of chemical networks will be considered as a second part of this chapter.

3.2 A Description of Chemical Reaction Networks

Before moving into the mathematical descriptions of chemical reactions, it is worthwhile to discuss the structure of chemical networks.

Chemical reaction networks consist of molecular species and reactions among them (specified in a figure as arrows). Molecular species could be ions, small molecules, or molecular complexes. Reactions among species include chemical production or degradation, reaction catalysis, and promotion or inhibition of activity. These processes cause production,

interconversion, and consumption in the network. Thus, the amount of each species changes over time [27].

As an illustration, consider the set of reactions shown below.

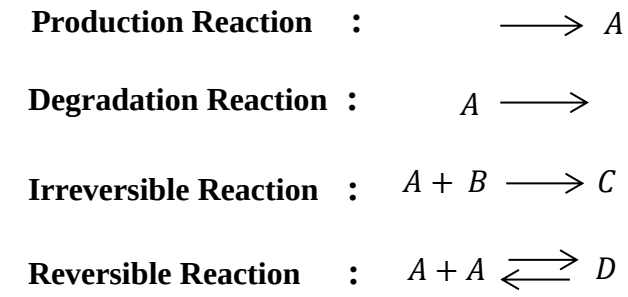


Figure 3.1: Chemical reaction networks

In Figure 3.1, A, B, C, and D are the molecular species. As an example, A and B is called the reactant, while, C is referred as product of the irreversible reaction

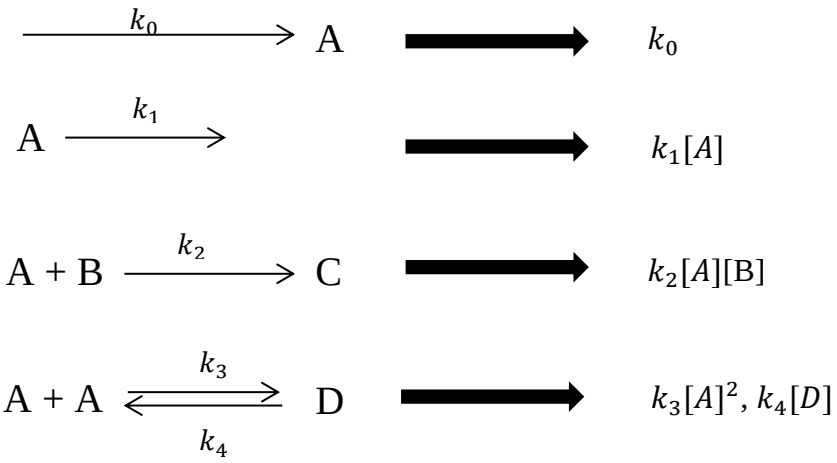
3.3 Dynamic Behavior of Chemical Reaction Networks

To describe the dynamics of the network, the reaction rates must be known. To arrive at reaction rates, it is firstly assumed that the reaction volume is well-mixed (i.e. molecules are present everywhere homogeneously). Secondly, it is assumed that there is a great many reactant molecules [27]. These assumptions were made to introduce the law of mass action in the following section:

3.3.1 Mass Action Kinetics

The law of mass action is derived by considering rate of collisions of the reactants in the reaction network [27]. Under the above assumptions, the law of mass action states that the rate of a chemical reaction is proportional to the abundance of the reactants. The abundance of molecules is defined as the concentration of the species. Generally, concentration is denoted as [.] [27].

By considering the networks in Figure 3.1, the reaction rates are given by the law of mass action as



where k_0, k_1, k_2, k_3 and k_4 are called the rate constants which are a concentration-independent measures of the velocity of a reaction [28]. The units of these rate constants are as follows: -the units of k_0 is (concentration) $(time)^{-1}$, the units of $k_1$is $(time)^{-1}$, the units of k_2 & k_3 are $(concentration)^{-1}(time)^{-1}$, and k_4 is $(time)^{-1}$.

Generally, the rate constants are depicted as

$$A \xrightarrow{k} B \qquad (3.1)$$

where k is a rate constant, A is reactant, and B is product.

As an example of a mathematical model, consider the irreversible reaction in (3.1). Using mass action kinetics, we have the following.

$$\underbrace{da(t)/dt}_{rate\ of\ change\ of\ [A]\ at\ time\ t} = \underbrace{-ka(t)}_{\text{rate of degradation of } [A] \text{ at time t}} \qquad (3.2)$$

$$\underbrace{db(t)/dt}_{rate\ of\ change\ of\ [B]\ at\ time\ t} = \underbrace{ka(t)}_{\text{rate of production of } [B] \text{ at time t}} \qquad (3.3)$$

where $a(t)$ and $b(t)$ denote the concentration of [A] and [B] at time t, respectively.

3.4 Dynamic Behavior of Biochemical Reactions

Individual chemical reactions as discussed above are referred to as elementary reactions. In contrast, some biochemical reactions, such as enzyme catalysis, are processes that involve small networks of individual elementary reactions. Unlike elementary reactions, mass action kinetics cannot be applied directly to biochemical reactions.

We begin with a discussion of enzyme catalysis. In the following, enzyme kinetics will be described with particular assumptions. Michaelis-Menten kinetics, which describes the rate of enzyme-catalyzed reactions, will be derived as a result of these assumptions.

The general enzyme-catalyzed reaction was firstly proposed in 1902 by Adrian Brown [28]

$$[S] + [E] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [C] \xrightarrow{k_3} [P] + [E] \tag{3.4}$$

where $[S], [E], [C]$, and $[P]$ denote the concentration of substrate, enzyme, complex, and product, respectively.

When mass action kinetics is applied to the individual reactions in this network, a set of differential equations is obtained as follows:

$$\frac{d[S]}{dt} = -k_1[S][E] + k_2[C] \tag{3.5}$$

$$\frac{d[E]}{dt} = -k_1[S][E] + (k_2 + k_3)[C] \tag{3.6}$$

$$\frac{d[C]}{dt} = k_1[S][E] - (k_2 + k_3)[C] \tag{3.7}$$

$$\frac{d[P]}{dt} = k_3[C] \tag{3.8}$$

where the last equation gives the reaction rate (velocity) of the network, and generally it is denoted v. Because the total enzyme concentration is conserved, the sum of $[C] + [E]$ is constant and is denoted E_T. Thus, Equ (3.6) will be eliminated by taking into consideration $[E] = [E_T] - [C]$.

3.4.1.1 Quasi Steady State Approximation

A separation of timescales can be applied to the network. The quasi-steady state assumption applies to species that reach equilibrium quickly compared to the rest of the network.

Following Briggs and Haldane [29], we assumed that the complex is in quasi-steady state. In quasi steady-state, the condition is $[C] = [C^{qss}]$, and C^{qss} is an independent variable. Thus;

$$k_1[S][E] - (k_2 + k_3)[C^{qss}] = 0 \qquad (3.9)$$

which is the quasi steady state assumption. Considering total enzyme concentration as a constant and this assumption together, we have

$$[C^{qss}] = \frac{[E_T][S]}{\frac{k_2 + k_3}{k_1} + [S]} \qquad (3.10)$$

Finally, (3.10) substituting into (3.8), we obtain

$$\frac{d[P]}{dt} = \frac{k_3[E_T][S]}{\frac{k_2 + k_3}{k_1} + [S]} \qquad (3.11)$$

writing $V_{max} = k_3[E_T]$ and $K_M = \frac{k_2+k_3}{k_1}$ to rewrite (3.11), the reaction rate (v) can be written as

$$v = \frac{V_{max}[S]}{K_M + [S]} \qquad (3.12)$$

This is called the Michaelis-Menten rate law. V_{max} is known as the maximal rate and K_M is referred to as the Michaelis constant, which is the substrate concentration at which the rate is half-maximal (see Figure 3.2). Also, this section could be review in Ref[30] and Ref[31].

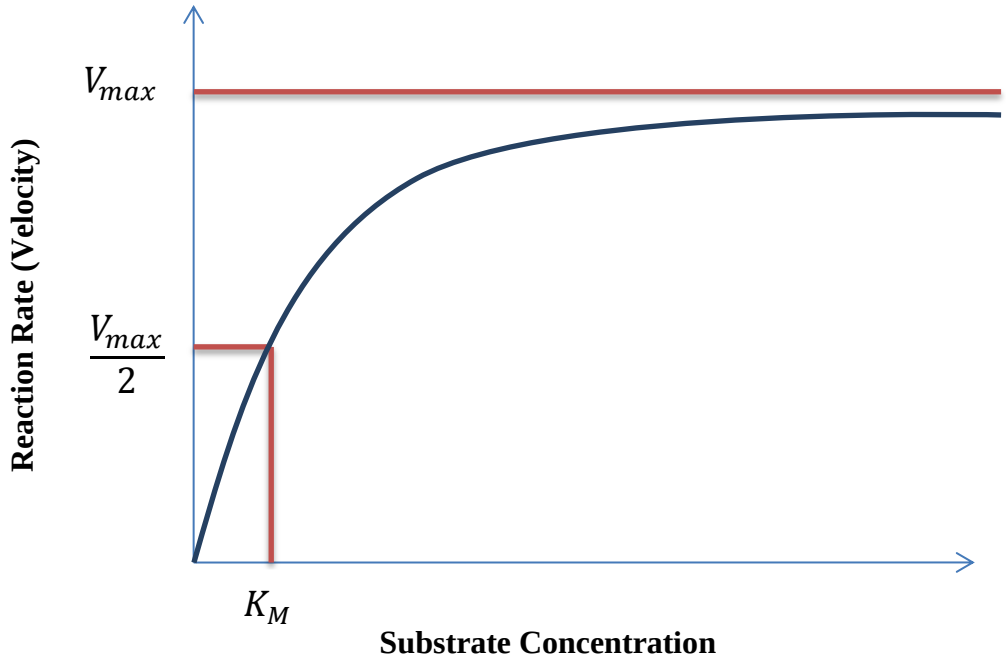


Figure 3.2: Michaelis-Menten Kinetics for single-substrate enzyme catalyzed reaction. As the substrate concentration increases, the reaction rate approaches the maximal velocity.

3.5 Cooperativity

Some enzymes bind to more than one substrate molecule, and in some cases a bound substrate molecule affects the affinity of binding of others. This is referred to as cooperativity [32]. Cooperativity causes nonlinear behaviors.

3.5.1 Hill function

When an enzyme has n binding sites, the reaction rate can be described by a Hill function:

$$v = \frac{V_{max} * [S]^n}{{K_d}^n + [S]^n} \tag{3.13}$$

K_d and n are empirical parameters.

Additionally, n is referred to as the Hill coefficient. This equation exactly reduces to Michaelis-Menten kinetics for $n = 1$.

Hill functions are commonly used to describe nonlinear and saturable behavior between species concentrations and their effects [32]. In this study, Hill functions are used to describe protein degradation.

3.6 Gene Regulatory Networks

Proteins are the essential ingredients for all cellular activities. Proteins are produced by gene expression, a process that occurs in two steps. Transcription, the first step, is copying of genetic information from one molecule (DNA) to the other (RNA). The resulting RNA molecule is called a messenger RNA (mRNA). The second step of gene expression is called translation. Through translation, the copied information is read along from the mRNA to synthesis a protein.

Gene expression events involve a large number of elementary reactions, are not easily decomposable.

Let us take a constitutive a gene expression model which is demonstrated in Figure 3.3.

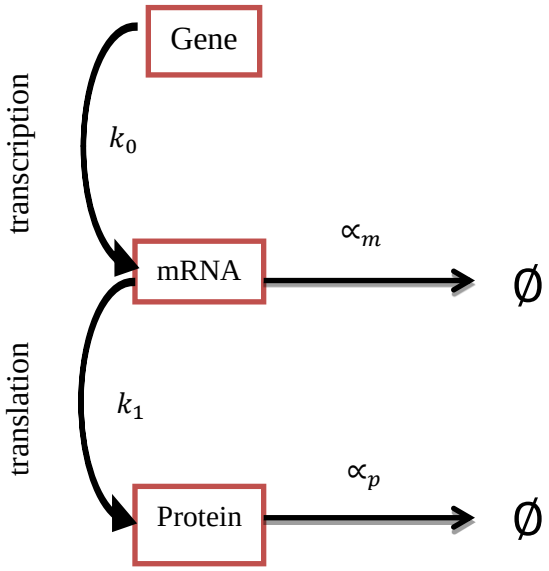


Figure 3.3: Gene expression model

$$\frac{dm(t)}{dt} = k_0 - \propto_m m(t) \quad (3.14)$$

$$\frac{dp(t)}{dt} = k_1 m(t) - \propto_p p(t) \quad (3.15)$$

where m is the concentration of mRNA molecules and p is the concentration of the gene's protein product. k_0 denotes the population-averaged transcription rate, and $k_1$is used to show the per-mRNA translation rate. $\propto_m$ and $\propto_p$ characterize the degradation rates of transcription and translation, respectively [27]. The degradation kinetics of the mRNA $(m(t))$ and protein $(p(t))$ are assumed to follow the first order mass action kinetics with rates $\propto_m$ and $\propto_p$ respectively [27].

3.6.1 Regulated Gene expression

Let us take a regulated gene expression model which is demonstrated in Figure 3.4.

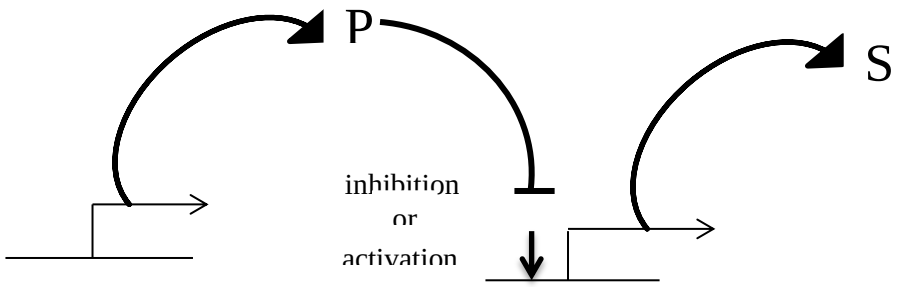


Figure 3.4: A simple representative of a gene regulatory network

A gene regulatory network consists of a group of genes' products which are called as proteins, and they regulate other genes' expression. If a gene protein product inhibits another gene's expression, the gene product is called inhibitor; if the product enhances another gene expression, it is called activator [27]. In this study, when the rate of the reaction which is the activation of another gene expression by a gene protein product is given simply linear term. However, the inhibitory regulation reaction's rate is described in the following:

$$\propto \frac{1}{1 + p(t)/K}$$

where $\propto$, the constant of the proportionality, is the maximal transcription rate.

3.7 Analysis of Dynamical Networks

In this section, phase plane portrait analysis, sensitivity analysis, bifurcation analysis, and stability will be addressed. The main purpose of using these techniques is to examine the behavior of the nonlinear dynamical systems since usually there is no analytical solution for these systems to interpret their results.

3.7.1 Stability Analysis

A reaction network's state is called stable when at long timescales the concentration of each of the chemicals in the reaction network remains constant. Alternatively, if the system settles into a pattern whereby the chemical levels oscillate predictably, it is called sustained oscillation. Stability analysis addresses, the long-time behavior of dynamical systems. This behavior could converge to either a stable state or a sustained oscillation [33]. Specifically, in this study, bistability will be considered.

Bistability means having two different stable behaviors depending on the initial conditions. The most necessary components in the bistability system are positive feedback and nonlinearity. However, there is no guarantee that the system will exhibit bistability if it has these two components. As always, model structure and also model parameters should be taken into consideration for bistability [33].

3.7.2 Phase Plane Portrait Analysis

Phase plane portraits were introduced by Henri Poincare in the 19^{th} century [34]. These provide a graphical description of the behavior of nonlinear systems without requiring any

analytical solutions. By mapping trajectories starting at a range of initial conditions, the behavior of a system can be interpreted in terms of its stable and unstable steady states. Even though a phase plot does not emphasize the concentration change with respect to time, it points out the time-varying relationship between the variables [35]. Normally, this analysis can only be used for second-order systems.

3.7.3 Bifurcation Analysis

Bifurcation analysis describes changes in the position of steady states as the values of the model parameters are changed [27]. A parameter interval over which the system exhibits two stable steady states is known as a bistable range. The size of this interval indicates the robustness of the bistable behavior (to changes in parameter values). That is, if the size of this interval is small, then small perturbation will significantly affect the system behavior. On the other hand, if the interval's size is large, system behavior will not be sensitive so much under the perturbations.

3.7.4 Sensitivity Analysis

An important method for the analysis of dynamical systems is sensitivity analysis. Sensitivity analysis describes how changes in the values of the parameters (which reflect the conditions of the system) affect the system behavior. Sensitivity analysis can be used to determine which parameters require additional research for strengthening the knowledge base, thereby reducing output uncertainty. Also, with the help of sensitivity analysis, insignificant parameters can be detected in order to eliminate them from the model. Moreover, sensitivity analysis can be used to identify which parameters have a significant impact on the output of a

model [36]. In this study, local sensitivity analysis will be used to address small variations around a nominal operating condition.

3.7.4.1 Local Sensitivity Analysis

In general, the dynamical system is described as:

$$\frac{\mathrm{dx}}{\mathrm{dt}} = \mathrm{f(x, t; k)}, \qquad \mathrm{x(0)} = \mathrm{x^0}, \tag{3.16}$$

where x is dependent variables, k is the m-vector of system parameters (which can include the initial conditions x^0), and t (time) is the independent variable [36]. In this thesis, k is considered to be a vector of parameter values.

Bifurcation analysis represents one approach to investigating system steady state behavior under changes in the values of the model parameters. An alternative is local sensitivity analysis. Local sensitivity analysis allows an easily calculation of the effect on the steady state as the parameter values are varied from their nominal values. When we consider Equ.3.16, the absolute local sensitivity of a steady state x^{ss} with respect to a variable k is given by the ratio of change of x^{ss} with respect to k, i.e. $\frac{dx^{ss}}{dk}$ which is called the absolute sensitivity coefficient. Sensitivity coefficients are computed via finite difference methods by discretization of differential equations. Using this approach, we use (3.16) to define the sensitivity to the j^{th} parameter k_j at the steady state of x^{ss} as

$$\frac{dx^{ss}}{dk_j} \cong \frac{x^{ss}\left(k_j + \Delta k_j\right) - x^{ss}(k_j)}{\Delta k_j} \tag{3.17}$$

with all other parameters held fixed [36]. This approach is using to predict the effect of small perturbations Δk_j at the parameter value $k = k_j$ [27].

To provide a measure of sensitivity that is independent of units and of the magnitudes of x^{ss} and k_j, the absolute sensitivity coefficient can be scaled as follows:

$$\frac{x^{ss}}{k_j} * \frac{dx^{ss}}{dk_j} \tag{3.18}$$

which is called the relative sensitivity coefficient; these provide a concise description of model behavior [27].

The relative sensitivity coefficient describes how local changes in parameter values affect system behavior. If the sensitivity coefficient is small, then system behavior is robust with respect to perturbations to that parameter [27].

Chapter 4
Model

4.1 Introduction

In this chapter, first of all, a few previously published mathematical models will be discussed to highlight where the idea of building a new cell signaling network for the regulation of apoptosis comes from. Then, specific proteins in the network will be described, and the signaling pathway of interest will be presented. Next, a kinetic model of the reaction network will be developed. Finally, the analysis of the model will be presented.

4.2 Bistability in the Apoptosis Pathway

Apoptosis is a key process in cell regulation, and a number of mathematical models have been developed to address different aspects of the apoptotic mechanism. As we discussed in Chapter 2, apoptosis is regulated in a number of ways: extrinsic and intrinsic pathways are well-known apoptotic regulatory pathways. Also, some proteins, such as p53 and Akt have important impacts on apoptotic pathways.

4.2.1 Bistability in caspase3 activation

Choi *et al.* [37] provided a mathematical model of caspase-3 activation describing regulation via three different pathways: activation via caspase8, inhibition via the protein XIAP, and activation via a complex of caspase3 and XIAP. The model was analyzed to assess the networks function in assuring the reliability and robustness of cell death. Moreover, Eissing *et al.* [38] developed a model of apoptosis exhibiting bistability. That model incorporated the extrinsic

apoptotic pathway: activation of caspase3, inhibition and degradation of caspase3 by XIAP, and activation of residual caspase8 by activated caspase3 in a feedback loop [10]. From these models, the current study draws its description of caspase3 activation, and XIAP inhibition of the caspase3.

The model of Bagci *et al.* [39] focuses on the formation of the apoptosome (a pro-apoptosis protein complex) and caspase3 activation. Bagci *et al.* [39] claimed that cooperativity provides robustness of bistability in comparison to the other regulatory mechanism. To prove the idea, Bagci *et al.* [39] developed a very large mathematical model with 32 ODEs. In our study, we did not examine specifically the regulation of the mitochondrial apoptotic pathway. Also, instead of using cooperativity for describing the formation of the apoptosome, we used cooperativity in the regulation of activated-caspase3 degradation via phosphorylated-XIAP.

4.2.2 Bistability in Akt-p53 pathway

Aguda and Wee studied the regulation of some proteins involve in the apoptotic signaling pathway [11]. The most important regulators of apoptosis are p53 and Akt, which mutually inhibit each other. Aguda and Wee assumed that the total Akt concentration was constant, and demonstrated bistability in a model that incorporates the proteins PTEN and Mdm2 into the p53-Akt network [11].

In this study, we present a novel network model that builds on these previous efforts. In particular, the previous models have not addressed the combination of the three signaling networks including apoptosis, p53, and Akt. We aimed to build such a model and verify that it could exhibit bistable behavior. We began the construction task by reviewing the signaling

pathways separately to understand their mechanisms on the regulation of cell metabolism. Then, we focused on the most important proteins on these networks: p53, Mdm2, PTEN, XIAP, caspase3, and Akt, which are heavily studied for cancer research. A detailed literature review was needed to build up a new signaling network model that describes the behavior of these proteins, and how they interact. These details will be given in the Model section.

4.3 Model

4.3.1 Experimental data for the pathways

The numbers below correspond to the interaction labels in Figure 4.1.

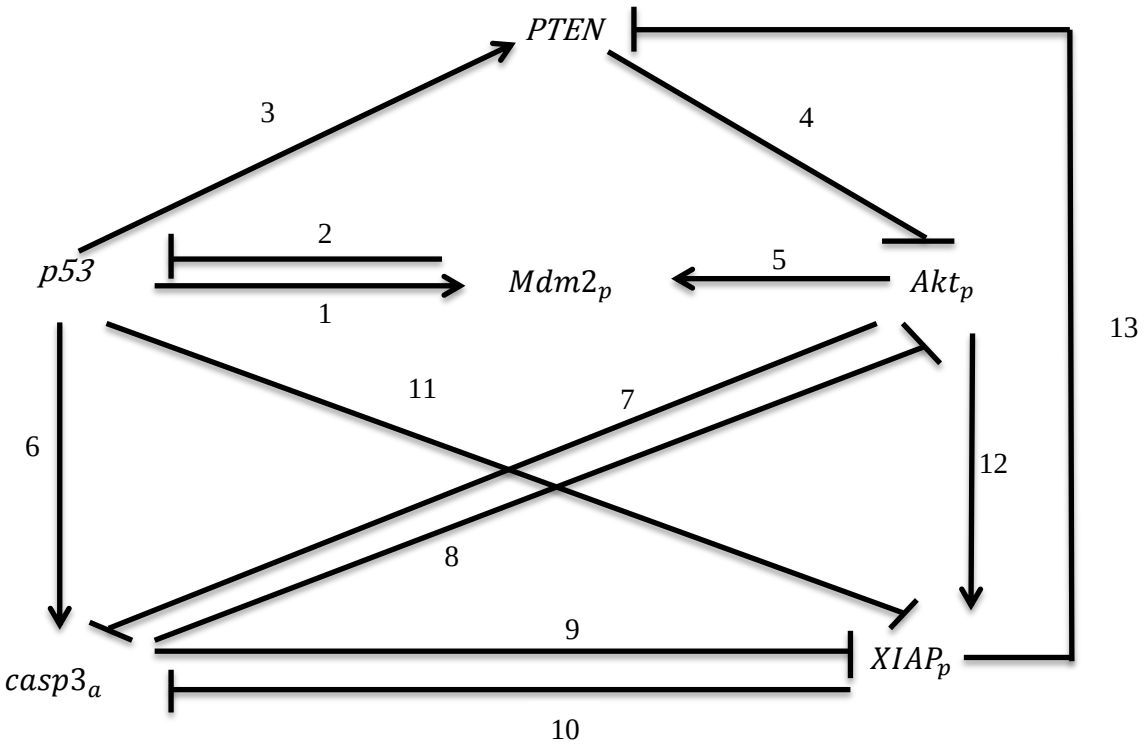


Figure 4.1: Simplified biological network that includes and pro- and anti-apoptotic proteins.

In Figure 4.1, arrows show activation; while, hammerheads denote inhibition.

p53 and Mdm2 (1, 2): Expression of the p53 protein is stimulated by cell damage, and p53 activates cell death and cell cycle arrest in damaged cells. When cells are not stressed, i.e. in the normal cell environment, p53 is inhibited by Mdm2, which keeps p53 at a low concentration. Mdm2 achieves this inhibition by forming a complex with p53 in the nucleus [40].

The Mdm2/p53 complex translocates from the nucleus into the cytoplasm, where Mdm2 facilitates the degradation of p53 [41]. On the other hand, expression of the mdm2 gene is induced by p53 when cell stresses occur [42]. As a result, an autoregulatory feedback loop exists between p53 and Mdm2 [43].

In the model presented in this study, the formation of the p53-Mdm2 complex will not be included explicitly. Rather, a term is included that describes the Mdm2-dependent degradation of p53.

PTEN and Akt_p (3, 4): p53 and PTEN form a positive feedback loop. p53 induces expression of, PTEN [44]. Moreover, PTEN indirectly increases p53 protein level by protecting p53 from Mdm2-induced degradation, by negatively regulating the Akt/PKB growth signaling, which activates Mdm2 [45]. In the model, we will assume that PTEN directly inhibits Akt_p (Figure 4.1).

Mdm2 and Akt_p (5): Phosphorylation of Mdm2 ($Mdm2_p$) by activated phosphorylated Akt (Akt_p) promotes the transport of Mdm2's from the cytoplasm to the nucleus [23].

p53 and caspase3 (6): p53 can induce cell death by activating genes on both the extrinsic and intrinsic apoptotic pathways [46, 47]. Although the role of p53 in the extrinsic pathway is poorly understood, p53 activates death receptors (TNF-R family) and caspase 8 [20]. For this report, we will assume that p53 directly activates caspase3, which interacts with caspase8 and caspase9 and executes apoptosis (Figure 4.1).

Akt_p and caspase3 (7, 8): Phosphorylated Akt and activated caspase3 form a positive feedback loop. Apoptotic pathways are negatively regulated by Akt at the pre-mitochondrial level by phosphorylation and modulation of proteins [23]. Akt also promotes cell survival at the post-mitochondrial level by directly phosphorylating and inactivating caspase9 [48]. Moreover, Akt inhibits caspase3's activation by phosphorylating XIAP [23]. In contrast, active caspase3 inhibits Akt phosphorylation [26]. Consequently, in this report, we will assume that Akt_p and caspase 3 mutually inhibit one another (see Figure 4.1).

XIAP and caspase3 (9, 10): Phosphorylated XIAP and activated caspase3 are involved in a mutual derepression positive feedback loop [49]. $caspase3_a$ decreases the $XIAP_p$ protein level, resulting in activation of caspase9 [50, 51]. In contrast, $XIAP_p$ is a direct inhibitor of caspase3 activation [47, 48].

p53 and XIAP (11): p53 inhibits XIAP activity indirectly [52, 53]. In the model (Figure 4.1), we will suppose that p53 directly inhibits XIAP, although this interaction is known to involve other cellular components (e.g. the protein SMAC, and the mitochondria).

Akt_p, $XIAP_p$, and PTEN (12, 13): Akt_p phosphorylates XIAP both directly [54] and indirectly [55] to inhibit the activation of caspase3. On the other hand, XIAP overexpression induces Akt phosphorylation by enhancing degradation of PTEN [56].

This research was conducted by utilizing both quantitative and qualitative data collection tools. As described in the previous section, qualitative data was used to establish the model network.

The second half of the study takes quantitative data into consideration to create a kinetic model. The kinetic model was developed by considering the regulation of each protein in the preceding figure in terms of production, degradation and regulatory reactions. With these additional details, we arrive at the network in Figure 4.2. The model was developed by assigning kinetic rates, taking into account enzyme and mass action kinetics introduced in Chapter 3. The ODEs are shown in Table1. For some kinetics, quasi steady state approximations and linearization of Michaelis-Menten kinetics were used.

The numerical simulations of the ODEs were done using the MATLAB 7.10.0 (R2010a) computing environment. The values of most of the model parameters were collected from two different papers [10, 11]. Parameters for which the values were unavailable were chosen to

ensure bistability of the system. The initial concentrations are not significant, since our analysis focuses on long-time behavior.

A schematic description of the above explanations is introduced in Figure 4.2. The model includes 7 species and 27 reactions. The total number of parameters in the model is 38, comprised of 6 production rates, 11 degradation rates, 2 Hill coefficients, 2 Michaelis-Menten constants, and 17 rate constants. The set of differential equations, their kinetic rates, and the kinetic rate constants are shown in Table 1, Table 2, and Table 3.

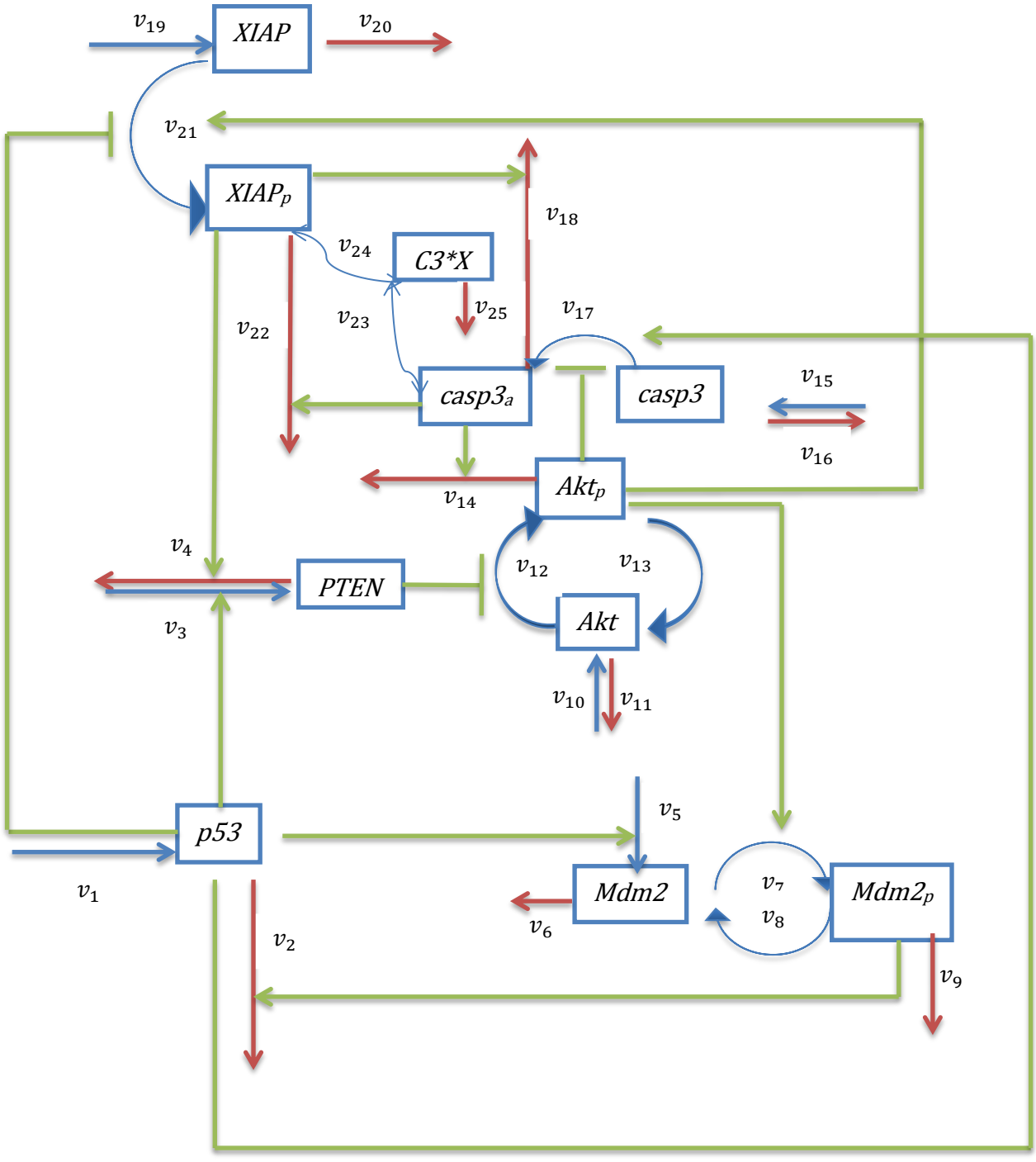


Figure 4.2: Kinetic scheme of the model

This kinetic scheme has four feedback loops (p53-Mdm2, p53-Akt, p53-apoptosis, and Akt-apoptosis) and three phosphorylation-dephosphorylation cycles that are Akt-Akt_p, XIAP-$XIAP_p$ and Mdm2-$Mdm2_p$; Akt_p, $XIAP_p$, and $Mdm2_p$ illustrate biochemically active Akt, XIAP, and Mdm2 proteins upon phosphorylation. The kinetic rates on the arrows will be given in the appendix as Table 2. Blue arrows denote the production rate, while red arrows show the degradation rate. Regulatory networks are shown with the green arrows. Cycles are used to describe the phosphorylation, dephosphorylation or activation depending on the proteins and their direction. Each parameter' units are given in Table 3 (in the last column, we have used A, B, and C to explain the estimation of parameters; see Table 5).

Table 1: Model equations

Differential Equations	
$d[p53]/dt$	$= v_1 - v_2$
$d[PTEN]/dt$	$= v_3 - v_4$
$d[Mdm2]/dt$	$= v_5 - v_6 - v_7 + v_8$
$d[Mdm2_p]/dt$	$= v_7 - v_8 - v_9$
$d[Akt]/dt$	$= v_{10} - v_{11} - v_{12} + v_{13}$
$d[Akt_p]/dt$	$= -v_{13} + v_{12} - v_{14}$
$d[casp3]/dt$	$= v_{15} - v_{16} - v_{17}$
$d[casp3_a]/dt$	$= v_{17} + v_{24} - v_{23} - v_{18}$
$d[XIAP]/dt$	$= v_{19} - v_{20} - v_{21}$
$d[XIAP_p]/dt$	$= v_{21} - v_{23} + v_{24} - v_{22}$
$d[complex]/dt$	$= v_{23} - v_{24} - v_{25}$

Table 2: Kinetic rates

Kinetic Rate	References
$v_1 = \mathbf{ksynp53}$	Mass action
$v_2 = (\mathbf{k_1} * [\mathbf{Mdm2_p}] + \mathbf{kdegp53}) * [\mathbf{p53}]$	Mass action
$v_3 = \mathbf{ksynPTEN} + \mathbf{k_2} * [\mathbf{p53}]$	Mass action
$v_4 = (\mathbf{kdegPTEN} + \mathbf{k_3} * [\mathbf{XIAP_p}]) * [\mathbf{PTEN}]$	Mass action
$v_5 = \mathbf{ksynMdm2} + \mathbf{k_4} * [\mathbf{p53}]$	Mass action
$v_6 = \mathbf{kdegMdm2} * [\mathbf{Mdm2}]$	Ref 11
$v_7 = \mathbf{k_5} * [\mathbf{Mdm2}] * [\mathbf{Akt_p}]$	Modify via linearization
$v_8 = \mathbf{k_6} * [\mathbf{Mdm2_p}]$	Modify via linearization
$v_9 = \mathbf{kdegMdm2_p} * [\mathbf{Mdm2_p}]$	Ref 11
$v_{10} = \mathbf{ksynAkt}$	Mass action
$v_{11} = \mathbf{kdegAkt} * [\mathbf{Akt}]$	Mass action
$v_{12} = k_8 * 0.25 * \left[\left(3212 * [PTEN] + 40 * \left(6448.09 * [PTEN]^2 - 33.945 * [PTEN] + 0.050625\right)^{\frac{1}{2}} - 3\right)/(1460 * [PTEN] - 3)\right] * [Akt]/(j_8 + [Akt])$	Modify from Ref 11 via QSSA explained in Appendix.
$v_{13} = \mathbf{k_9} * [\mathbf{Akt_p}]/(j_9 + [Akt_p])$	Ref 11
$v_{14} = (\mathbf{k_{10}} * [\mathbf{casp3_a}] + \mathbf{kdegAkt_p}) * [\mathbf{Akt_p}]$	Mass action
$v_{15} = \mathbf{ksyncasp3}$	Mass action
$v_{16} = \mathbf{kdegcasp3} * [\mathbf{casp3}]$	Mass action
$v_{17} = k_{11} * [casp3] * [p53] * 1/(k_{12} + [Akt_p])$	Mass action
$v_{18} = (\mathbf{k_{13}} * [\mathbf{XIAP_p}]^{\mathbf{n_1}} + \mathbf{kdegcasp3_a}) * [\mathbf{casp3_a}]$	Mass action
$v_{19} = \mathbf{ksynXIAP}$	Mass action
$v_{20} = \mathbf{kdegXIAP} * [\mathbf{XIAP}]$	Mass action
$v_{21} = k_{14} * [XIAP] * [Akt_p] * 1/(k_{15} + [p53])$	Mass action
$v_{22} = (\mathbf{kdegXIAP_p} + \mathbf{k_{16}} * [\mathbf{casp3a}]^{\mathbf{n_2}}) * [\mathbf{XIAP_p}]$	Ref 10
$v_{23} = k_{17} * [XIAP_p] * [casp3_a]$	Ref 10
$v_{24} = k_{18} * [complex]$	Mass action
$v_{25} = kdegcomplex * [complex]$	Mass action

Table 3: Kinetic rate constants

	Parameters	Description	Units	Value used	Bistable Ranges	References	Notes
1	*ksynp53*	production of p53	µM/min	0.02	80%-140% (0.016-0.028)	60	
2	*kdegp53*	degradation of p53	/min	0.02	20%-110% (0.004-0.022)	60	
3	*k1*	degradation of p53 via $Mdm2_p$	/(min*µM)	0.25	63.2%-105.28% (0.158-0.2632)	60	A
4	*ksynPTEN*	production of PTEN	µM/min	0.0018	-	60	B
5	*k2*	Increased production of PTEN via p53	/min	0.0012	-	11	A
6	*kdegPTEN*	degradation of PTEN	/min	0.0054	-	60	
7	*k3*	degradation of PTEN via $XIAP_p$	/(µM*min)	0.03	-	arbitrary	
8	*ksynMdm2*	production of Mdm2	µM/min	0.0018	12%-120% (0.0002-0.00216)	60	B
9	*k4*	Increased production of Mdm2 via p53	/min	0.01	40%-120% (0.004-0.012)	11	A
10	*k5*	Phosphorylation of Mdm2 via Akt_p	/(µM*min)	10	40%-120% (4-12)	11	A
11	*k6*	dephosphorylation of $Mdm2_p$	/min	0.8	80%-180% (0.64-1.44)	11	A
12	*kdegMdm2*	degradation of Mdm2	/min	0.005	80%-180% (0.004-0.009)	60	
13	*kdegMdm2p*	degradation of $Mdm2_p$	/min	0.005	80% (0.004-)	60	
14	*ksynAkt*	production of Akt	µM/min	0.09	40%-120% (0.036-0.108)	arbitrary	
15	*kdegAkt*	Degradation of Akt	/min	0.08	80% (0.064-)	arbitrary	
16	*k8*	Inhibition of phosphorylated-Akt via PTEN	µM/min	0.06	80%-120% (0.048-0.072)	arbitrary	
17	*j8*	Michaelis-Menten kinetics	µM	0.1	80% (0.08-)	-	
18	*k9*	dephosphorylation of Akt_p	µM/min	0.2	80%-120% (0.16-0.24)	11	
19	*j9*	Michaelis-Menten constant of dephosphorylation of Akt_p	µM	0.1	80%-120% (0.08-0.12)	11	
20	*k10*	Increased degradation of Akt_p via $casp3_a$	/(µM*min)	0.01	80% (0.008-)	arbitrary	

21	*kdegAkt$_p$*	degradation of Akt$_p$	/min	0.01	80% (0.008-)	arbitrary	
22	*ksyncasp3*	Production of caspase3	µM/min	0.012	80%-140% (0.0096-0.0168)	10	C
23	*kdegcasp3*	Degradation of caspase3	/min	0.06	40%-120% (0.024-0.072)	10	C
24	*k11*	Increased activation of caspase3 via p53	/min	0.03	80%-200% (0.024-0.06)	arbitrary	
25	*k12*	Inhibition of caspase3 activation via Akt$_p$	/min	0.05	80% (0.06)	arbitrary	
26	*k13*	Increased degradation of caspase3a via XIAP$_p$	/(µM*min)	0.01	60%-120% (0.006-0.012)	arbitrary	C
27	*kdegcasp3$_a$*	degradation of caspase3$_a$	/min	0.001	120% (0.0012)	10	C
28	*ksynXIAP*	production of XIAP	µM/min	0.0024	-	10	
29	*kdegXIAP*	degradation of XIAP	/min	0.06	80%-140% (0.048-0.084)	10	C
30	*k14*	Phosphorylation of XIAP via Akt$_p$	/min	0.3	60%-120% (0.18-0.36)	arbitrary	
31	*k15*	Inhibition of phosphorylated-XIAP via p53	µM	0.1	80%-200% (0.08-0.2)	arbitrary	
32	*k16*	Increased degradation of XIAP via caspase3$_a$	/(µM*min)	0.01	80%-160% (0.008-0.016)	arbitrary	C
33	*kdegXIAP$_p$*	degradation of XIAP$_p$	/min	0.0024	80%-160% (0.00192-0.00384)	arbitrary	
34	*k17*	caspase3$_a$ inhibits XIAP$_p$ through caspase3-XIAP complex(C3*X)	/(µM*min)	0.01	120% (0.012)	10	C
35	*kdegcomplex*	degradation of complex	/min	0.06	120% (0.072)	10	C
36	*k18*	activation of caspase3 by releasing caspase3 from the C3*X	/min	0.06	80% (0.048)	10	C
37	*n1*	Hill coefficient of XIAP$_p$ dependent degradation of caspase3$_a$	-	2	90%-270% (1.8-5.4)	arbitrary	
38	*n2*	Hill coefficient of caspase3$_a$ degradation of XIAP$_p$	-	1	60% (0.6)	arbitrary	

4.4 Results and Discussion

In this section, the results of the model will be presented using the analysis techniques introduced in Chapter 3.

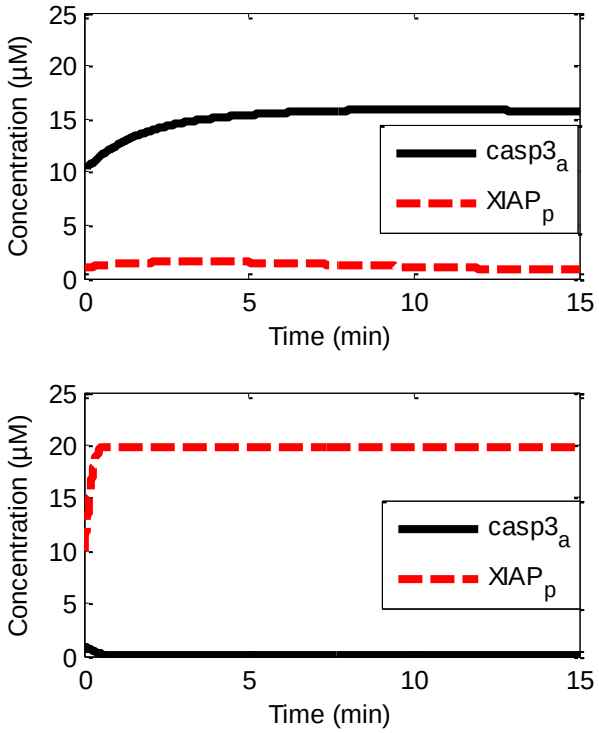


Figure 4.3: Bistability between $casp3_a$ and $XIAP_p$

Figure 4.3 shows that the systems long-time behavior depends on the initial conditions: the model is bistable. We refer to the steady state with high levels of active caspase3 ($casp3_a$) as the "death state"; low $caspase3_a$ corresponds to the "life state".

Table 4: Two stable points depending on the initial concentrations (μM) of the variables

	Cell death stable points	Cell survival stable points
p53	0.2235	0.2216
PTEN	0.2964	0.0497
Mdm2	0.5292	0.5220
$Mdm2_p$	0.2779	0.2811
Akt	1.1103	1.1192
Akt_p	0.0423	0.0433
caspase3	0.0904	0.0915
$caspase3_a$	1.7904	0.0693
XIAP	0.2419	0.2390
$XIAP_p$	0.3242	2.8092
caspase3-XIAP complex (C3*X)	0.0484	0.0162

Table 4 shows concentrations of the model species at the two stable steady states. The cell death state is reached by choosing pro-apoptotic proteins (p53, PTEN, caspase3, $caspase3_a$) at a high initial concentration compared with the anti-apoptotic proteins (Mdm2, $Mdm2_p$, Akt, Akt_p, XIAP, $XIAP_p$, C3*X). On the other hand, the life state is obtained from an initial condition in which the cell survival proteins have high concentrations. From the stability analysis, we can see that PTEN, $caspase3_a$, and $XIAP_p$ are the most distinctive stable steady states depending on the initial concentrations. The rest of the proteins are almost the same stable steady states even though the initial concentrations are different.

Activated caspase3 is characterized the effector of cell death: phosphorylated XIAP is known as the main inhibitor of activated caspase3. Thus, having two very different steady state values for these two proteins as shown in Table 4 is expected. The most interesting finding from the steady state concentrations is protein PTEN, which likewise exhibits two distinctive steady states. PTEN functions as an intermediary protein through a number of interactions in the signaling pathway. For instance, the formation of many cancers is associated with mutations in PTEN including breast, prostate, and brain cancers. Themsche et al. [56] pointed out that increased synthesis of $XIAP_p$ causes the increased PTEN degradation. Numerically, we showed that while PTEN is high, $XIAP_p$ is low; on the other hand, if $XIAP_p$ steady state value is high which means XIAP synthesis level is high, the steady state of PTEN is decreasing, as in Table 4. We also showed this result by using bifurcation analysis of ksynXIAP (production of XIAP) with respect to PTEN steady state value; however, the result will not be shown in the text.

An alternative demonstration of bistability is provided by a phase plane portrait. Figure 4.4 shows projections of the model's 11-dimensional trajectories onto the $caspase3_a$- and $XIAP_p$-plane.

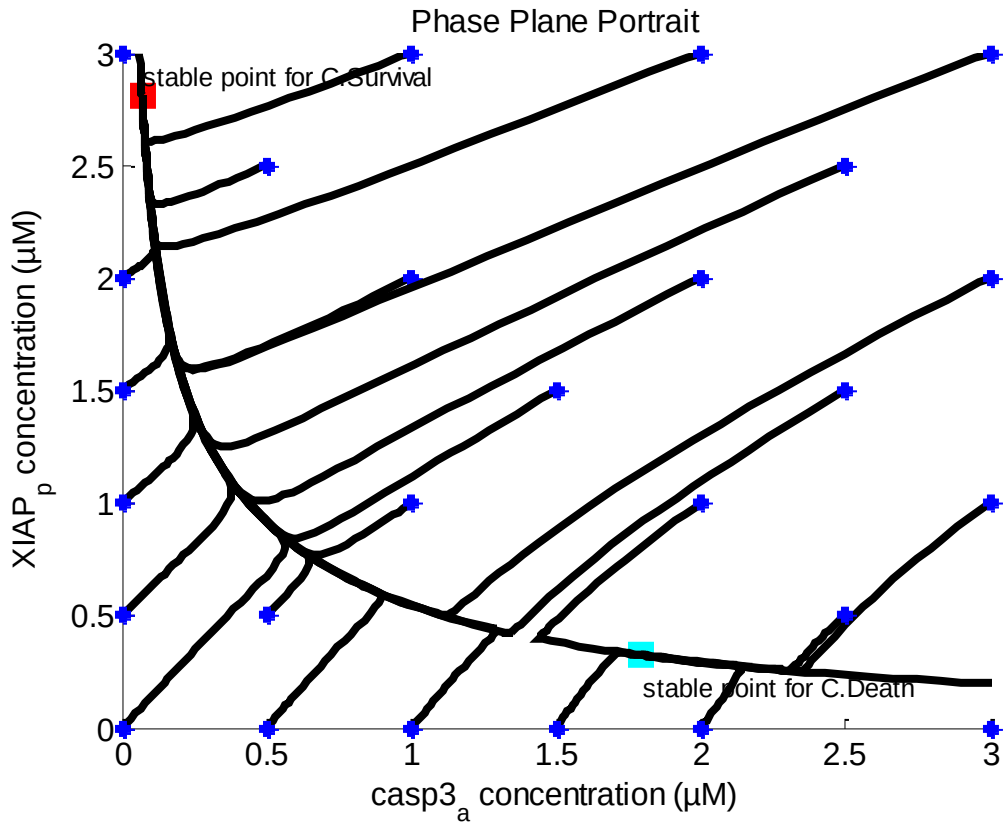


Figure 4.4: Projected phase plane portrait.

Next, we employed bifurcation analysis to explore the robustness of bistability. For each model parameter, we considered the range of parameter values over which bistability is maintained (while the other model parameters were fixed at their nominal values as given in Table 3).

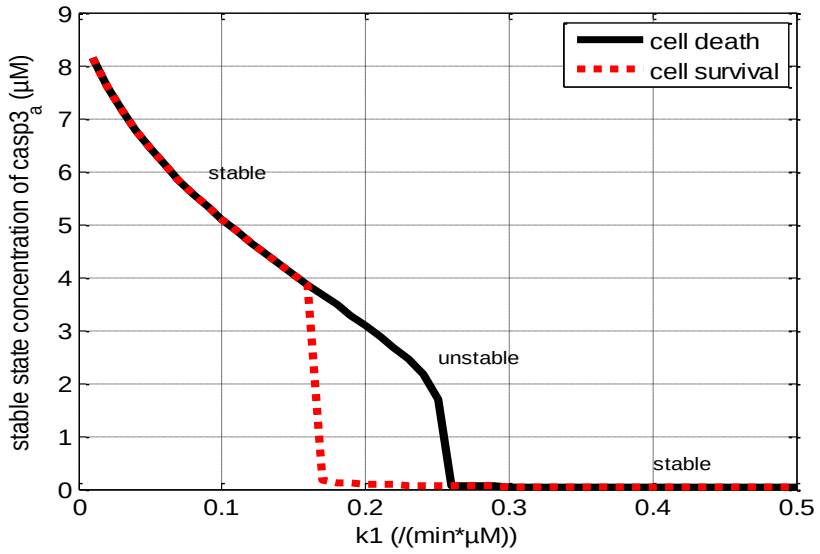


Figure 4.5: Bifurcation diagram for the bistable system.

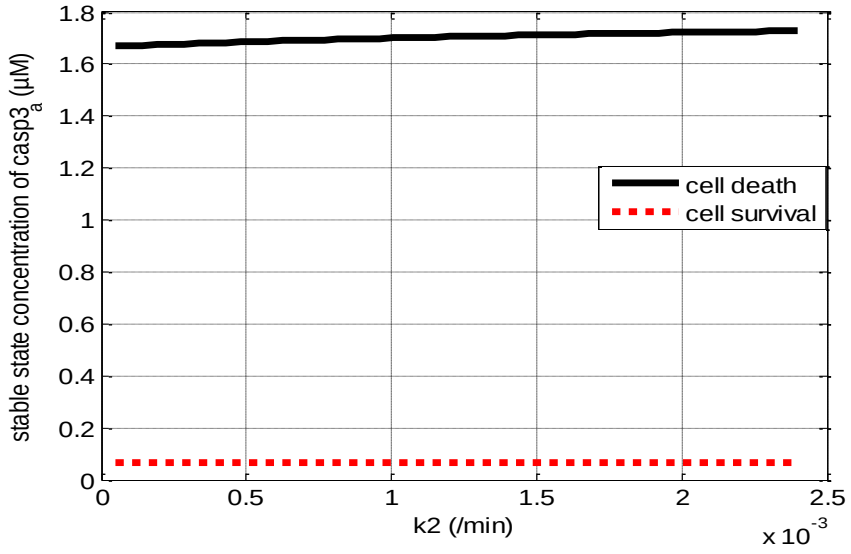


Figure 4.6: Continuation diagram for the bistable system

Figure 4.5 and Figure 4.6 illustrate this analysis. These both show the steady state concentration of active caspase3 as a function of parameter values. In Figure 4.5, we see that at low and high k1 values- the system is monostable. However, in the mid-range of k1 (degradation of p53 via $Mdm2_p$), two stable steady states appear. The size of this interval indicates the robustness of bistability with respect to this parameter. That is, when the size of this interval is small, system behavior will be fragile to any perturbation. However, if the size is large, system behavior will not be affected significantly by perturbations. On the other hand, in Figure 4.6, $caspase3_a$ has two stable steady state values for every value of parameter k2 (increased production of PTEN via p53). That is, the system is bistable for any value of k2 (with the other values in Table 3) is infinite. The range of the each parameter in the model is added in Table 3. These ranges indicate how robust the system's bistability is to changes in the values of the individual parameters. For example, parameter k3, which characterizes the rate of degradation of PTEN by $XIAP_p$, has an infinite bistable range, indicating that bistability will not be lost regardless of the value of this parameter (for the other parameters at their nominal values). Conversely, the parameter kdegMdm2, which characterizes the rate of degradation of Mdm2, has a narrow bistability range of only 0.004-0.009 /min, with a nominal value of 0.005 /min. Thus a change of as little as 20% in the value of this parameter can cause the system to become monostable, so that only one cell fate can be reached.

Finally, sensitivity analysis was employed to explore the parameters' effect on the model. We applied a local sensitivity analysis (as described in Chapter 3) at both the life and death states (Figure 4.7-4.17).

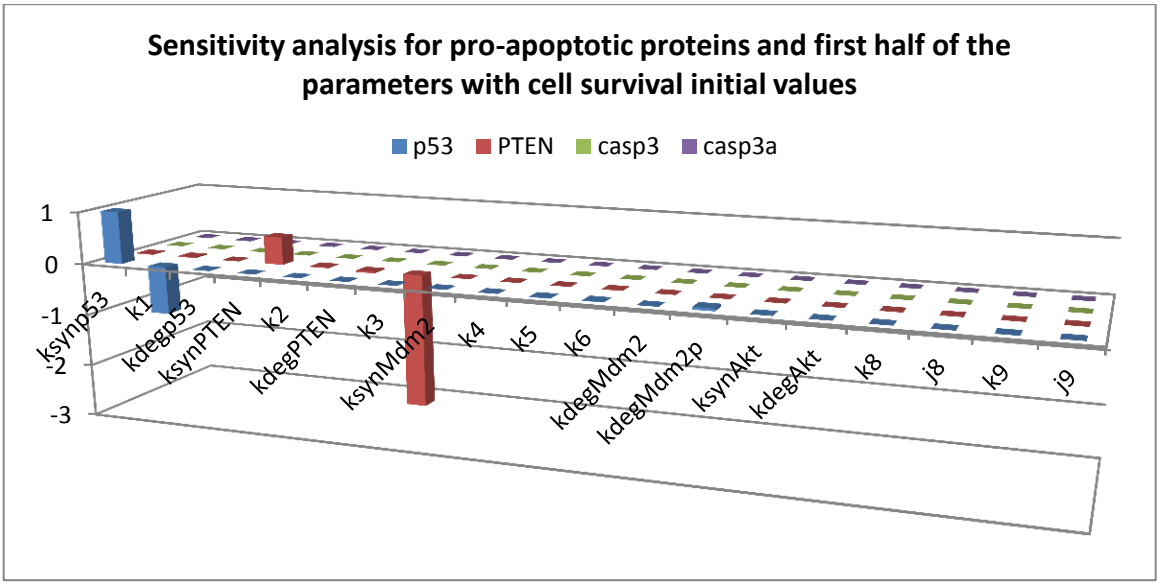


Figure 4.7: Sensitivity analysis at life state for pro-apoptotic proteins_1

ksynp53 (production of p53) has a positive impact on p53; while, k1 (degradation of p53 via $Mdm2_p$) has a negative impact. On the other hand, these two proteins cannot cause the change of bistable range of the protein p53 in the bifurcation analysis. ksynPTEN (production of PTEN) affects PTEN positively; ksynMdm2 (production of Mdm2) negatively affects the protein PTEN. However, from the bifurcation analysis, this effect cannot significantly cause the change of the range of the bistability.

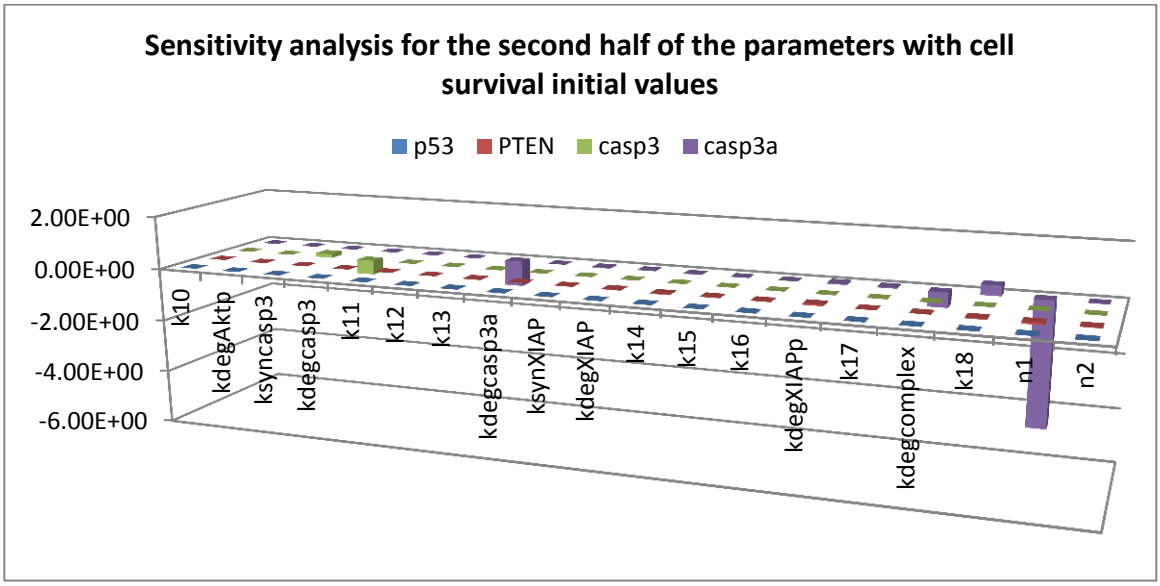


Figure 4.8: Sensitivity analysis at life state for pro-apoptotic proteins_2

kdegcasp3 (degradation of caspase3) affects protein caspase3. The bistable range of this protein via obtained kdegcasp3 has changed very little. The protein caspase3_a has negatively affected by k13 (increased degradation of caspase3_a via $XIAP_p$), kdegcomplex (degradation of C3*X), while, k18 (the activation of caspase3 by releasing caspase3 from C3*X) has a positive impact on activated caspase3. From the bifurcation anaylsis, only k13 has caused a big change of the range of bistability of caspase3_a.

Also, the hill coefficient *n1*(Hill coefficient of $XIAP_p$ dependent degradation of caspase3_a) affects the range of protein caspase3_a significantly. This means this high sensitivity corresponds to the change of the bistable range of caspase3_a depending on *n1*.

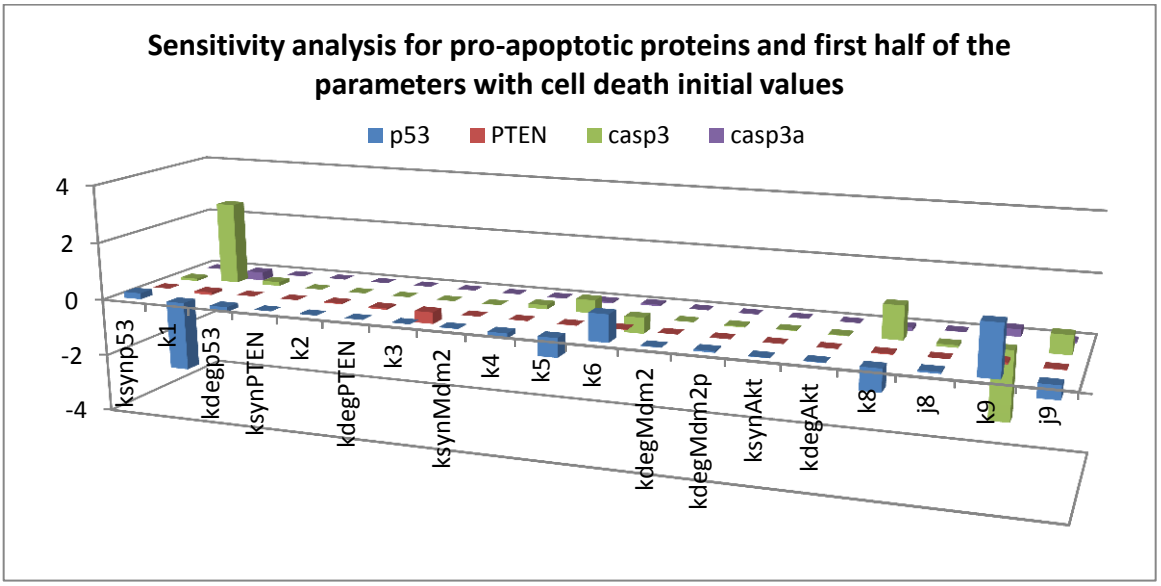


Figure 4.9: Sensitivity analysis at the death state for pro-apoptotic proteins_3

This graphs shows that k1(degradation of p53 via $Mdm2_p$), k5 (phosphorylation of Mdm2 via Akt_p), k8 (inhibiton of phosphorylated Akt via PTEN), and j9 (Michaelis-Menten constant of dephosphorylation of Akt_p) have negative impact on p53; while, k6 (dephosphorylation of $Mdm2_p$) and k9 (dephosphorylation of Akt_p) affect positively the protein p53. Also, k1, k4 (increased prodcution of Mdm2 via p53), k5, k8, and j9 have a positive effect on caspase3; while, k9 has impact on caspase3 negatively.

Moreover, k3 (degradation of PTEN via $XIAP_p$) seems effect PTEN value significantly. This result also match with the bifurcation analysis since k3 affects the range of PTEN bistability infinitely.

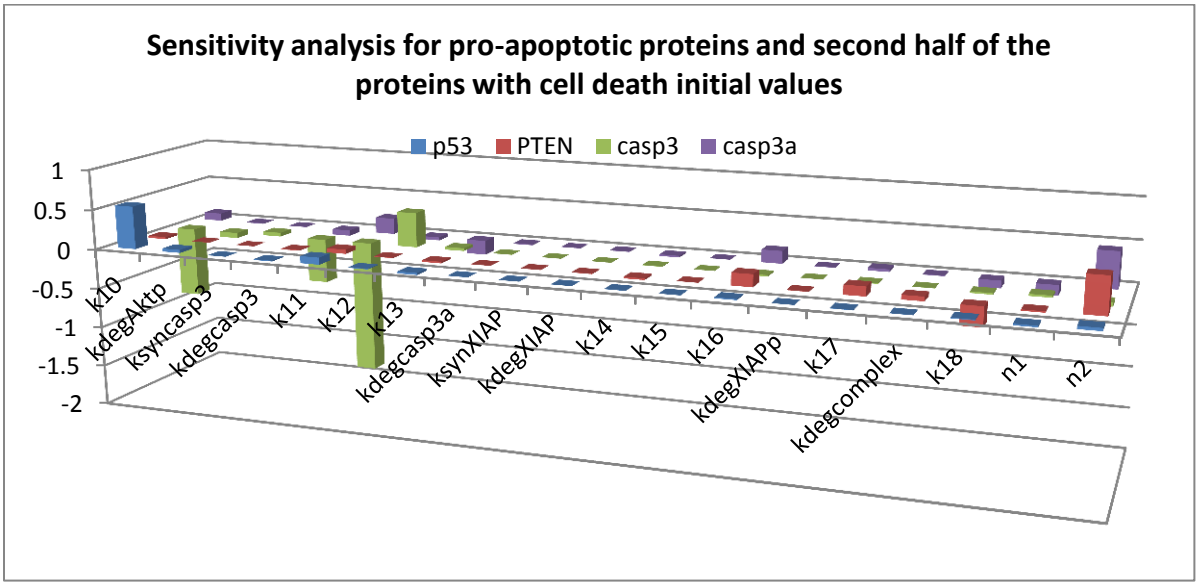


Figure 4.10: Sensitivity analysis at the death state for pro-apoptotic proteins_4

k10 (increased degradation Akt_p via $caspase3_a$), kdegcasp3, and k11 (increased activation of caspase3 via p53) have a significant negative impact on caspase3; whereas, k12 (inhibition of caspase3 activation via Akt_p) affects caspase3 positively. However, this high sensitivity of k12 on caspase3 is not a significant impact on the range of the bistable of caspase3.

In addition, k10 affects p53 positively. Also, while n2 (Hill coefficient of $caspase3_a$ degradation of $XIAP_p$) have most influential effect on the proteins PTEN and $caspase3_a$ positively, k18 (activation of caspase3 by releasing caspase3 from the C3*X) affects PTEN protein negatively.

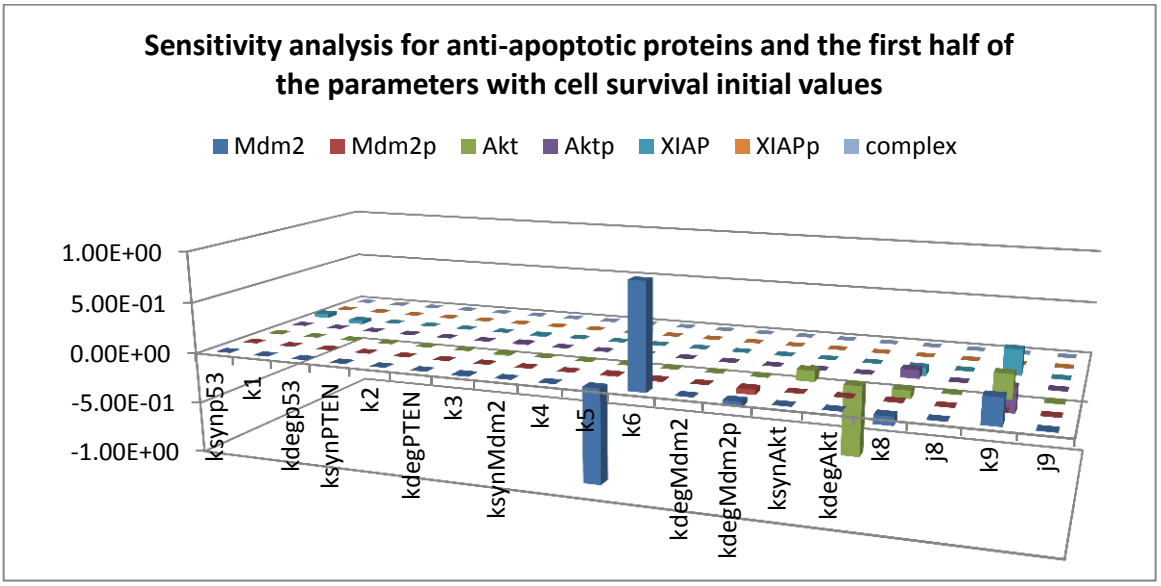


Figure 4.11: Sensitivity analysis at the life state for anti-apoptotic proteins_1

While k5 (phosphorylation of Mdm2 via Akt_p) has a negative effect on the protein Mdm2, k6 (dephosphorylation of $Mdm2_p$) affects positively. The high sensitivities of these two parameters on Mdm2 cannot cause the big change of the Mdm2 bistable range.

Another important parameter in this graph is kdegAkt (degradation of Akt). kdegAkt affects Akt negatively. However, this effect does not influence on the bistable range of Akt.

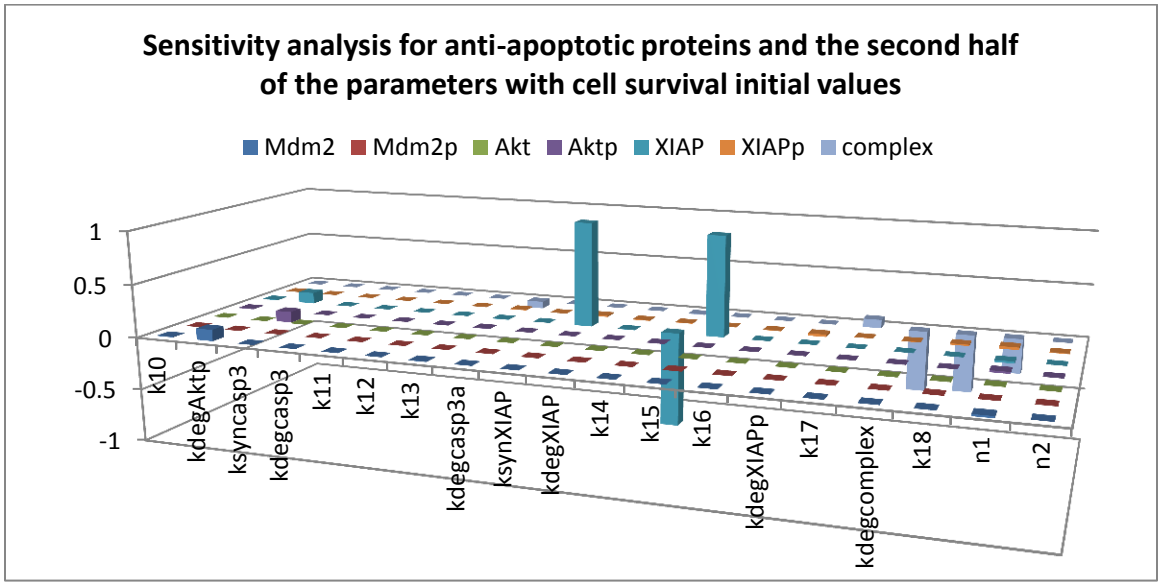


Figure 4.12: Sensitivity analysis at the life state for anti-apoptotic proteins_2

$kdegAkt_p$ (degaradation of Akt_p), ksynXIAP (production of XIAP), and k15 (inhibition of phosphorylated XIAP via p53) have a positive impact on the protein XIAP; while, k14 (phosphorylation of XIAP via Akt_p) has a negative impact on it. Also, the parameter $kdegAkt_p$ (degradation of phosphorylated Akt) affects the protein Mdm2 and Akt_p positively. Moreover, the protein C3*X (caspase3-XIAP complex) is negatively affected by kdegcomplex (C3*X degradation), k18 (activation of caspase3 by releasing caspase3 from the C3*X) and n1 (Hill coefficient of $XIAP_p$ dependent degradation of $caspase3_a$); whereas, k17 ($caspase3_a$ inhibits $XIAP_p$ through caspase3-XIAP complex (C3*X)) affects the complex positively.

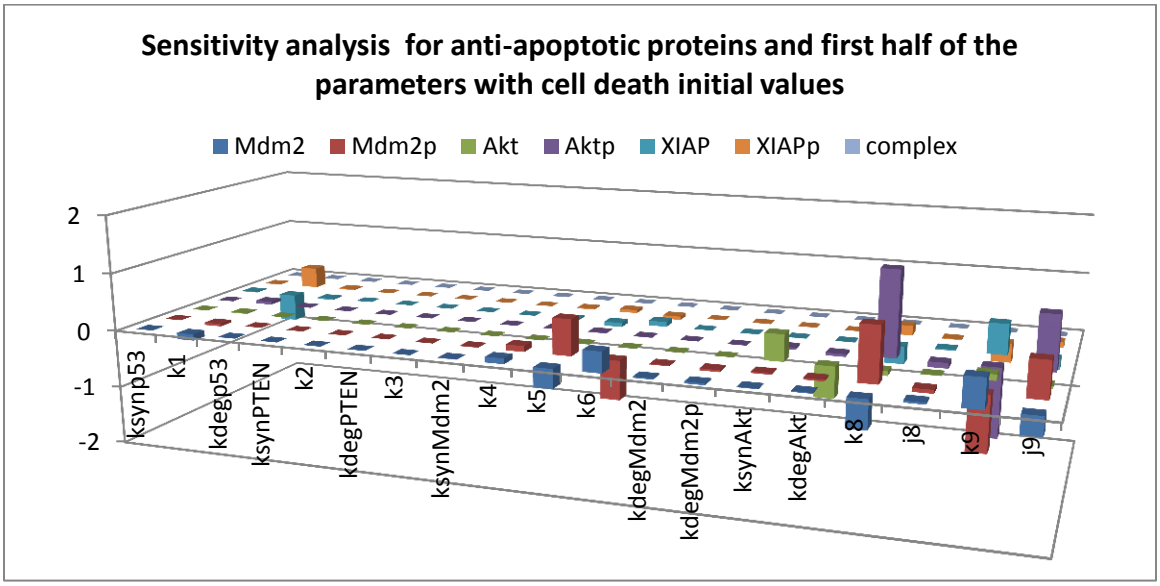


Figure 4.13: Sensitivity analysis at the death state for anti-apoptotic proteins_3

Most influential parameters on $Mdm2_p$ are k5 (Phosphorylation of Mdm2 via Akt_p), k6 (dephosphorylation of $Mdm2_p$), k8 (Inhibition of phosphorylated-Akt via PTEN), k9 (dephosphorylation of Akt_p), and j9 (Michaelis-Menten constant of dephosphorylation of Akt_p). Also, $XIAP_p$ is significantly affected the changes of k1 (degradation of p53 via $Mdm2_p$), k8, and k9.

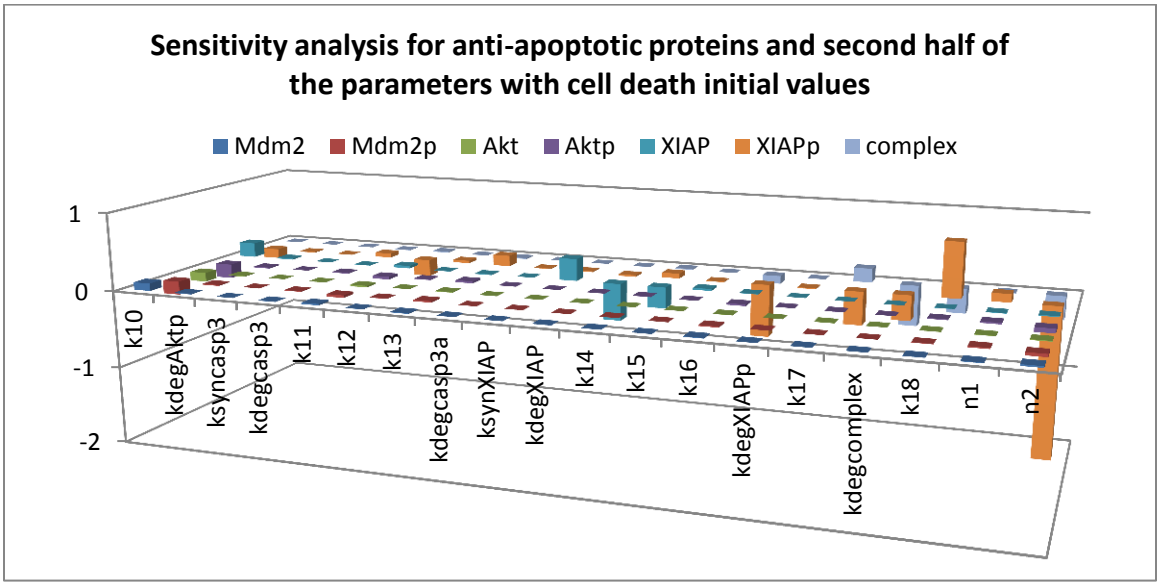


Figure 4.14: Sensitivity analysis at the death state for anti-apoptotic proteins_4

The most important outcome from this analysis is that k10 (increased degradation of Akt_p via $caspase3_a$), $kdegcasp3_a$ (degradation of activated caspase3), k11 (increased activation of caspase3 via p53), k13 (increased degradation of $caspase3_a$ via $XIAP_p$), k16 (increased degradation of XIAP via $caspase3_a$), k17 ($caspase3_a$ inhibits $XIAP_p$ through caspase3-XIAP complex (C3*X)), kdegcomplex (degradation of C3*X), k18 (activation of caspase3 by releasing caspase3 from the C3*X), n1 (Hill coefficient of $XIAP_p$ dependent degradation of $caspase3_a$), and n2 (Hill coefficient of $caspase3_a$ dependent degradation of $XIAP_p$) have an important effect on $XIAP_p$.

Overall from the each of the sensitivity analysis in the above, it was found that the values of the parameters kdegPTEN (degradation of PTEN), kdegp53 (degradation of p53), k2 (increased production of PTEN via p53), k3 (degradation of PTEN via $XIAP_p$) have little impact

on system behavior. Conversely, the most influential parameter on the model is k1(degradation of p53 via $Mdm2_p$). This has a negative impact on p53, and a positive effect on phosphorylated XIAP which triggers a cell to avoid apoptosis. The results of the sensitivity analysis are attached in the Appendix.

From the sensitivity analysis, it is shown that k13, which characterizes increased degradation of $caspase3_a$ via $XIAP_p$, has a significant impact on the protein concentration, and thus on the system behavior.

From the sensitivity analysis, k1 (the rate of degradation of p53 via $Mdm2_P$) is found to be another influential parameter. Biologically, this parameter is important in determining cell fate. Depending on the parameter value, the cell fate will change as follows: If the parameter value is high, cell goes to the cell survival; otherwise, cell undergoes the cell death since p53 protein has a positively significant effect on activated caspase3 [65].

Moreover, another important parameter from the sensitivity analysis is the Hill coefficient n1 (Hill coefficient of $XIAP_p$ dependent degradation of $caspase3_a$). Basically, our model is built on the cooperativity of $XIAP_p$ and $caspase3_a$. Thus, the change of the n1 value affects the system behavior very significantly since n1 represents the nonlinearity of the system and provides positive feedback loop with another parameter n2 (Hill coefficient of $caspase3_a$ dependent degradation of $XIAP_p$) between activated casapse3 and phosphorylated XIAP.

From the biological perspective, ksyncasp3 (production of caspase3) and $kdegcasp3_a$ (degradation of $caspase3_a$) parameters should have an influential effect on the system behavior since their values affect the activated caspase3 concentration, and implicitly the cell fate. Numerically, from the sensitivity analysis, their effects were found really to be very low.

Nevertheless, from the bifurcation analysis, their impacts on the system behavior were obtained differently. It is shown that the bistable range of ksyncasp3 is small; it influentially affects the system behavior with the any small perturbation. On the other hand, $kdegcasp3_a$ has wide bistability range since there is no lower bound for the bistable range.

Chapter 5
Conclusion and Future Directions

5.1 Conclusion

The development of cancer begins with the misregulation of normal cells at the molecular level. By considering this bio-molecular starting point of cancer, we investigated an important cell signaling network. In previous studies, Legewie *et al.* [10] discovered bistability in the intrinsic apoptotic pathway. On the other hand, Wee and Aguda [11] proposed bistability by considering the p53-Akt networks. Also, Bagci *et al.* [39] claimed that the most important feature for bistability of the system is to have a cooperative effect among the species. By taking into consideration these important findings, we developed a kinetic model consisting of three important cell signaling networks: apoptotic, p53 and Akt pathways. The purpose of building this kinetic model was to show bistability of the system since bistability is crucial for cell fate in terms of either cell death or survival.

To achieve this goal, first of all, we reviewed the literature to understand the mechanism of proteins in these pathways. Then, based on the biological data, we built up a kinetic model to analyze the behavior of the system. From stability and phase plane portrait analysis, we demonstrated that our kinetic network is bistable by considering pro- and anti-apoptotic proteins. From bifurcation and sensitivity analyses, we found that k1 (increased p53 degradation via $Mdm2_p$) is a key parameter for the control of the system; while, k2 (increased production of p53 via PTEN), k3 (degradation of PTEN via $XIAP_p$), kdegPTEN (constitutive degradation of PTEN) have little impact on system behavior.

The most challenging part of the research is to find appropriate values of the parameters. The weakness of this study is that we didn't compare our result with the previously published results because of not having enough time. And also, 15 parameters were chosen arbitrarily, but some of them were obtained by applying quasi steady state approximation to existence value in the previous study. The rest of them were chosen by considering maintaining the bistability. Even though these parameters do not show significant effect on the model from the sensitivity analysis, they make it difficult to have confidence in the model predictions. More numerical calculations are required to validate our results.

5.2 Future Works

Based on the present study, two other projects are being considered for the future. The first project is to extend the presented kinetic model by adding other proteins studied by Legewie *et al.* [10]. The pathways for his project are illustrated in Figure 5.1.

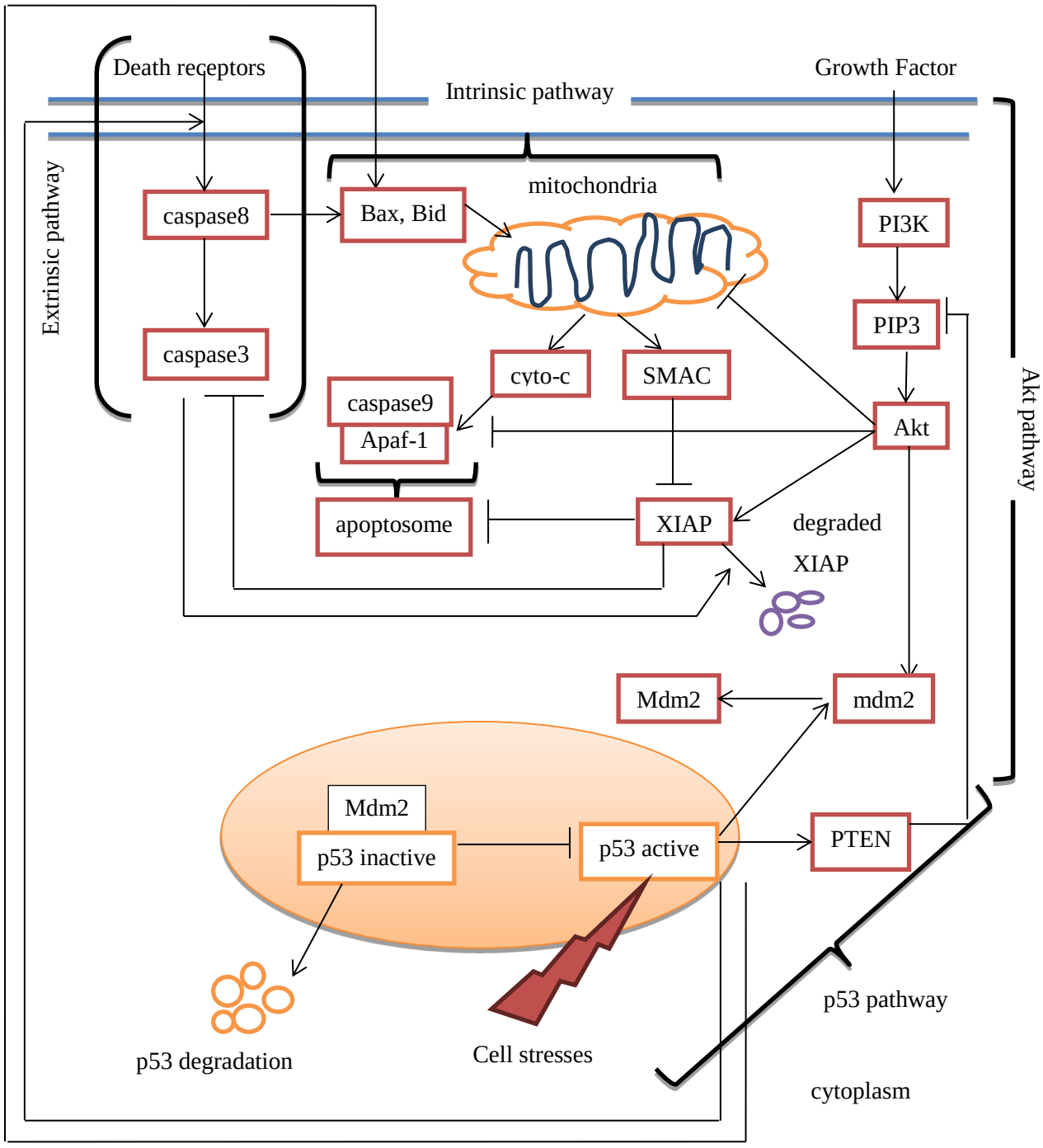


Figure 5.1: Proposed model to study apoptotic-p53-Akt pathways

Akt-p53-apoptotic pathways combine with specific proteins. In the Figure, apoptotic pathways are shown with the extrinsic and intrinsic pathways. The pink bubble shows the regulation of p53 via Mdm2, and also their negative feedback loop starting from the cytoplasm ending in the nucleus. The blue bubble illustrates the mitochondria. Cell stresses, growth factor, and death receptors are the external stimuli for these three important pathways. p53's effect on the apoptotic pathway is demonstrated with three different pathways including PTEN activation to inhibit Akt function, inducing TNF-R family in the extrinsic pathway, and initiating the activation of Bax in the mitochondria for the intrinsic pathway. On the other hand, the inhibition of the apoptotic pathway via Akt is demonstrated in three different ways. The inhibition of protein Bax via Akt causes the block of apoptosis. Also, Akt inhibits apoptosis via the activation of XIAP. In addition, Akt prevents the formation of the apoptosome consisting of APAF1 and caspase9 protein. It results in the inhibition of caspase3 activation.

The second project is to introduce a cancer drug to the present study. Cisplatin, a well-known and crucial chemotherapeutic drug, is widely used as a cancer treatment tool in breast, testicular, head and neck, bladder, lung, ovarian, cervical, prostate, and refractory non-Hodgkin's lymphomas. The usage of cisplatin in cancer treatment is generally associated with DNA damage, resulting in apoptosis, even though cisplatin can interact with the other cellular components. Thus, by considering cisplatin-induced DNA damage, our first objective would be to incorporate cisplatin into the mathematical model (Figure 5.2) [57]. When we are dealing with the cisplatin effect on the present model, we will consider the mechanism of cisplatin resistance,

which can cause secondary cancer formation [58] by switching the desirable effect of cisplatin during the treatment of malignancy [59].

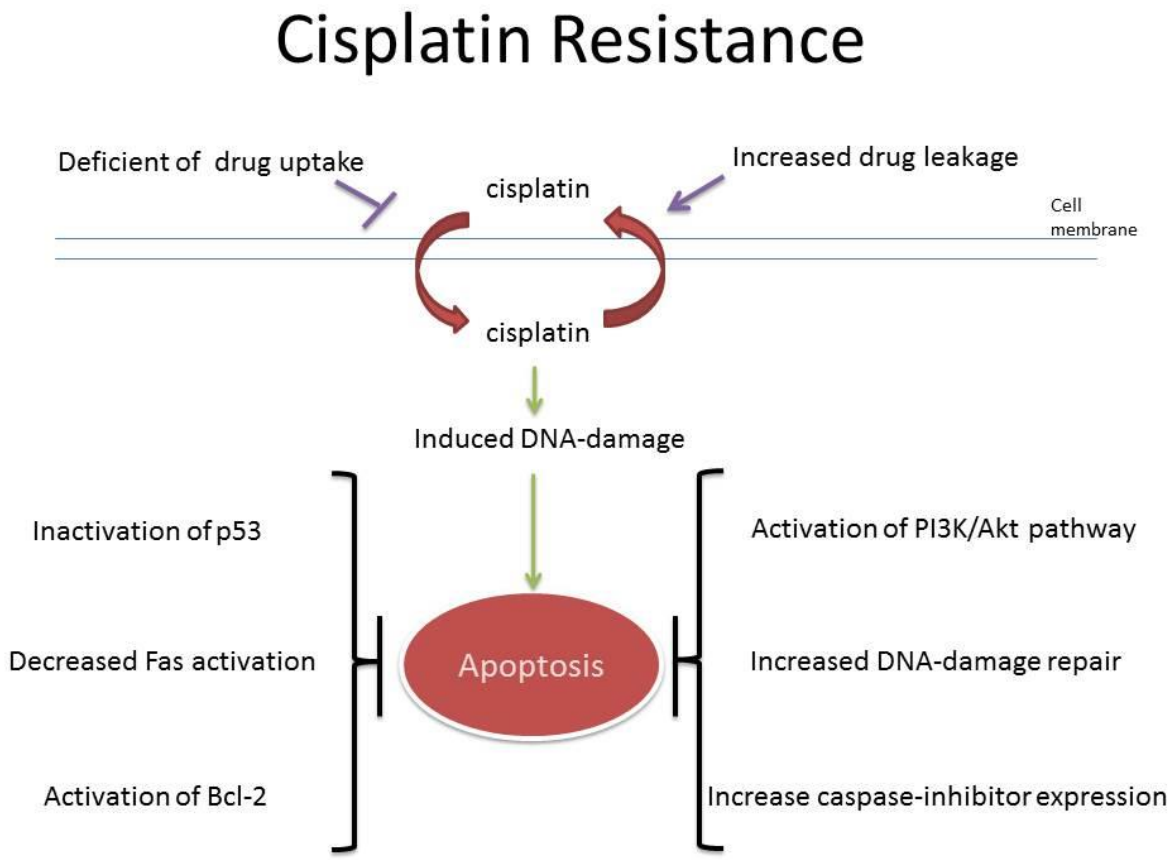


Figure 5.2: Cisplatin Resistance Pathways

In summary, the present study represents a step toward understanding the mechanisms of cancer signaling pathways. This knowledge will be useful in developing treatment for this condition, which is one of the most significant problems with human health worldwide. The proposed future work will extend this initial analysis to a more complete description of

intracellular behavior, and will address the ability of the drug Cisplatin to interfere with these pathways and thus improve health outcomes.

Appendix

Table 5: Modified Kinetics

	Explanation
A	Linearized Michaelis-Menten kinetics.
B	QSSA was applied to pten gene in Ref 60.
C	The same value used in Ref 10, but we only changed the units.

Table 6: Sensitivity analysis results for Cell Survival Initial values

	p53	PTEN	Mdm2	$Mdm2_p$	Akt	Akt_p	casp3	$casp3_a$	XIAP	$XIAP_p$	C3*X
ksynp53	0.999964	0.015248	4.71E-05	2.04E-05	2.64E-05	-2.48E-05	-0.00013	0.000571	0.040364	-2.02E-05	3.03E-05
k1	-0.90468	0.018167	-0.00056	-0.000105	-8.09E-05	0.000453	0.000593	-0.00718	-0.03691	0.002005	-0.0026284
kdegp53	-0.00421	-0.00015	-2.40E-06	-4.78E-07	-3.78E-07	1.94E-06	2.68E-06	-3.11E-05	-0.00017	8.80E-06	-1.18E-05
ksynPTEN	5.32E-06	0.528039	0.00067	-6.91E-06	0.000835	-0.00069	-9.66E-09	-3.91E-07	0.000652	-3.67E-08	-1.14E-08
k2	1.89E-07	0.01597	2.33E-05	-2.40E-07	2.89E-05	-2.39E-05	-3.60E-10	-6.33E-09	2.27E-05	-1.29E-09	-2.72E-10
kdegPTEN	-3.81E-08	-0.0295	-6.43E-05	6.63E-07	-7.75E-05	6.57E-05	1.74E-09	1.42E-06	-6.35E-05	3.41E-09	2.30E-08
k3	-0.00024	-2.5148	-0.00984	0.0001014	-0.01191	0.010044	3.40E-07	4.07E-07	-0.00964	4.93E-07	7.08E-06
ksynMdm2	-0.0009	-1.13E-05	0.001039	0.0009207	-1.78E-08	1.76E-08	7.01E-08	-5.13E-07	-3.60E-05	2.17E-08	-2.81E-08
k4	-0.00012	-2.50E-06	0.000126	0.0001235	-5.14E-09	1.62E-08	4.04E-08	-3.01E-07	-4.95E-06	7.40E-08	-9.28E-08
k5	-0.00923	0.00196	-0.90039	0.0092815	3.68E-06	1.52E-05	1.54E-05	-0.00041	-0.00033	8.25E-05	-0.0001243
k6	0.010004	0.005387	0.988652	-0.010194	-2.38E-05	1.65E-05	-5.01E-06	2.14E-06	0.000451	-1.82E-05	2.91E-05
kdegMdm2	0.000461	6.24E-06	-0.00054	-0.000474	1.04E-08	-1.82E-08	-4.78E-08	4.11E-07	1.85E-05	-5.58E-08	7.36E-08
$kdegMdm2_p$	0.04832	0.000617	-0.04931	-0.049379	9.75E-07	-9.83E-07	-3.83E-06	2.79E-05	0.001942	-1.26E-06	1.66E-06
ksynAkt	-5.55E-07	-7.64E-09	-5.76E-05	5.94E-07	0.102914	5.84E-05	2.42E-09	-3.51E-08	-5.75E-05	3.62E-09	-2.58E-10
kdegAkt	4.16E-06	7.06E-08	0.00043	-4.44E-06	-0.65989	-0.00044	-1.88E-08	2.63E-07	0.00043	-2.72E-08	2.07E-09
k8	-0.00079	-4.49E-05	-0.07947	0.0008195	-0.07592	0.081095	5.95E-06	-6.36E-05	-0.07971	9.22E-06	-5.48E-06
j8	1.03E-05	4.87E-07	0.001048	-1.08E-05	0.001022	-0.00106	-7.07E-08	7.90E-07	0.00105	-1.08E-07	5.55E-08
k9	0.002435	0.000138	0.244931	-0.002526	0.225698	-0.242	-1.83E-05	0.000196	0.245722	-2.82E-05	1.66E-05
j9	-2.67E-05	-1.40E-06	-0.00269	2.77E-05	-0.00258	0.002724	1.93E-07	-2.10E-06	-0.0027	2.96E-07	-1.66E-07
k10	5.21E-05	4.48E-05	0.005145	-5.30E-05	-1.16E-05	-0.0052	-1.35E-06	1.97E-05	0.005177	-5.31E-06	6.53E-06
$kdegAkt_p$	0.00107	6.66E-05	0.107308	-0.001107	-0.00016	-0.10745	-8.46E-06	8.86E-05	0.107701	-1.32E-05	8.30E-06
ksyncasp3	1.27E-10	6.09E-08	2.21E-08	-1.31E-10	1.05E-10	-2.23E-08	0.141289	7.11E-05	2.20E-08	-3.69E-08	7.87E-07
kdegcasp3	-1.02E-09	-2.73E-07	-9.60E-08	1.09E-09	-4.64E-10	9.72E-08	-0.53753	-0.00027	-9.62E-08	1.59E-07	-3.31E-06
k11	2.75E-08	9.49E-06	2.41E-06	-2.47E-08	1.93E-08	-2.43E-06	-0.00066	0.000559	2.42E-06	-3.13E-06	4.13E-05
k12	-2.10E-10	-4.80E-08	-1.22E-08	2.22E-10	-9.73E-11	1.23E-08	3.39E-06	-3.50E-06	-1.23E-08	1.59E-08	-2.14E-07

k13	-7.87E-05	-0.01181	-0.00356	3.67E-05	-2.92E-05	0.003599	5.84E-07	-0.96333	-0.00364	0.004952	-0.0690686
kdegcasp3$_a$	-1.74E-08	-5.73E-06	-1.45E-06	1.50E-08	-1.16E-08	1.46E-06	2.71E-10	-0.00028	-1.46E-06	1.94E-06	-2.60E-05
ksynXIAP	-0.00014	0.005149	-9.47E-05	9.76E-07	-9.65E-05	9.38E-05	2.04E-07	-0.02333	0.999977	0.011857	-0.0001659
kdegXIAP	3.17E-07	0.018047	6.87E-05	-7.08E-07	4.67E-05	-6.99E-05	-4.86E-09	0.01159	-0.0026	-0.00561	0.00031701
k14	-9.31E-05	-0.02602	-0.00081	8.34E-06	-9.46E-05	0.000822	1.79E-07	-0.01219	-0.90662	0.003656	-0.0049175
k15	-8.30E-05	0.010693	0.000133	-1.37E-06	6.50E-06	-0.00014	2.82E-08	0.002435	0.955636	-0.00079	0.00093302
k16	2.40E-07	0.010928	3.91E-05	-4.03E-07	2.77E-05	-3.98E-05	-2.67E-09	0.007617	3.89E-05	-0.00372	0.00017285
kdegXIAP$_p$	8.59E-07	0.042638	9.76E-05	-1.01E-06	9.30E-05	-9.96E-05	-2.87E-09	0.046449	9.62E-05	-0.02346	0.00034921
k17	-2.17E-06	0.007861	-0.00014	1.40E-06	2.10E-05	0.000137	3.18E-08	0.035295	-0.00014	-0.0023	0.0808143
kdegcomplex	-1.69E-06	0.006143	-0.00021	2.12E-06	1.29E-05	0.000208	1.16E-08	-0.55915	-0.00021	-0.00402	-0.5808464
k18	4.68E-06	-0.0288	0.000667	-6.88E-06	-6.96E-05	-0.00067	-6.66E-08	0.379156	0.000672	0.012557	-0.5539025
n1	-0.00021	-0.05958	-0.0174	0.0001794	-0.00022	0.017622	2.97E-06	-4.66009	-0.01753	0.023891	-0.33188
n2	-2.24E-07	-0.01255	-3.50E-05	3.61E-07	-3.04E-05	3.56E-05	1.51E-09	-0.01038	-3.46E-05	0.005158	-0.0001338

Table 7: Sensitivity analysis for Cell Death Initial values

	p53	PTEN	Mdm2	Mdm2$_p$	Akt	Akt$_p$	casp3	casp3$_a$	XIAP	XIAP$_p$	C3*X
ksynp53	0.210548	0.001603	0.002278	0.000973	-0.00015	-0.00095	-0.08733	0.007529	0.013014	-0.00871	6.56E-05
k1	-2.36577	-0.09335	-0.10866	-0.044318	0.013264	0.051123	2.838232	-0.29512	-0.46969	0.363695	-0.0023087
kdegp53	-0.16447	-0.00436	-0.00531	-0.002555	0.00061	0.002081	0.137896	-0.01405	-0.02194	0.016973	-0.0001295
ksynPTEN	2.98E-08	0.002235	3.14E-08	-6.17E-08	4.15E-09	-1.11E-07	-3.44E-08	3.03E-09	9.93E-09	-5.01E-09	-9.54E-11
k2	6.39E-07	0.028861	4.93E-07	-9.64E-07	6.77E-08	-1.56E-06	-7.96E-07	7.37E-08	2.13E-07	-1.12E-07	-1.99E-09
kdegPTEN	-7.81E-07	-0.05339	-7.83E-07	1.54E-06	-1.03E-07	2.71E-06	9.11E-07	-8.08E-08	-2.60E-07	1.32E-07	2.48E-09
k3	-6.25E-06	-0.40004	-6.22E-06	1.22E-05	-8.15E-07	2.13E-05	7.24E-06	-6.40E-07	-2.07E-06	1.04E-06	1.99E-08
ksynMdm2	-0.00919	-0.00014	0.00842	0.0074167	1.41E-05	7.09E-05	0.006044	-0.00056	-0.00093	0.000657	-4.91E-06
k4	-0.16473	-0.00432	0.093288	0.0991679	0.000408	0.005737	0.143134	-0.01423	-0.02279	0.016541	-1.08E-05
k5	-0.65196	-0.01273	-0.32894	0.6118493	0.001665	0.008567	0.45498	-0.04429	-0.07107	0.052308	-0.0002744
k6	0.921951	0.012791	0.350715	-0.652807	-0.00143	-0.00642	-0.55176	0.050945	0.083233	-0.06017	0.00059585
kdegMdm2	0.01075	0.000114	-0.01853	-0.014436	-1.12E-05	-6.62E-05	-0.00552	0.000492	0.000837	-0.00058	4.27E-06
kdegMdm2$_p$	0.048544	0.00077	-0.02884	-0.0337	-7.99E-05	-0.00041	-0.03295	0.003052	0.005072	-0.0036	2.69E-05
ksynAkt	-0.01342	-0.0003	-0.01107	0.0216872	0.448696	0.036551	0.016681	-0.00154	-0.00452	0.00238	4.20E-05
kdegAkt	0.015946	0.00033	0.013955	-0.027359	-0.52741	-0.04658	-0.01941	0.001774	0.005327	-0.00278	-4.99E-05
k8	-0.75059	-0.02621	-0.47472	0.9217082	-0.07677	1.448465	1.136326	-0.11339	-0.29884	0.166064	0.00294553
j8	0.057625	0.001929	0.031404	-0.060784	0.005075	-0.09179	-0.08196	0.008262	0.02027	-0.01173	-0.0001652
k9	1.70487	0.062882	0.483227	-0.908216	0.102337	-1.16485	-2.34651	0.248646	0.517223	-0.32906	-0.0028938
j9	-0.45262	-0.01309	-0.31094	0.6058618	-0.04571	0.920347	0.638034	-0.06133	-0.17081	0.090981	0.00184372
k10	0.535923	0.02628	0.095796	-0.171623	-0.11716	-0.18547	-0.87156	0.096389	0.19127	-0.12665	-0.0010109
kdegAkt$_p$	0.037323	0.002084	0.00695	-0.012403	-0.00886	-0.01293	-0.06648	0.007443	0.014848	-0.00981	-9.26E-05
ksyncasp3	0.000287	0.000265	0.000139	-0.000269	-7.61E-05	-0.00041	0.047798	0.003108	9.82E-05	-0.00217	0.00020042
kdegcasp3	-0.00938	-0.00795	-0.00367	0.0070437	0.002413	0.00983	-0.55096	-0.06983	-0.00327	0.055655	-0.0048103
k11	0.087077	0.05284	0.017985	-0.032774	-0.02081	-0.03545	-1.643	0.200742	0.032956	-0.21957	0.01306423
k12	-0.00695	-0.00523	-0.0023	0.0043509	0.001747	0.005794	0.433241	-0.03933	-0.00253	0.033079	-0.0027189
k13	-0.02405	-0.02043	-0.00938	0.0179757	0.006186	0.025037	0.034678	-0.17779	-0.00838	0.142645	-0.0123118
kdegcasp3$_a$	-0.00122	-0.001	-0.00047	0.0008897	0.000312	0.001266	0.001796	-0.0093	-0.00043	0.006983	-0.0006041

ksynXIAP	-0.00011	-0.00188	-7.72E-05	0.0001509	3.15E-05	0.000247	0.000136	-0.00199	0.288494	0.01132	0.00140334
kdegXIAP	0.000246	0.003908	0.000165	-0.000322	-6.95E-05	-0.00052	-0.00031	0.004127	-0.48606	-0.02144	-0.0028412
k14	-0.00215	-0.0197	-0.001	0.0019244	0.000568	0.002781	0.002926	-0.02026	-0.27465	0.063772	0.01169509
k15	6.22E-05	0.001114	4.40E-05	-8.61E-05	-1.78E-05	-0.00014	-7.76E-05	0.001178	0.025461	-0.00739	-0.0008515
k16	0.012353	0.15237	0.006765	-0.013141	-0.00335	-0.02024	-0.01626	0.156195	0.00411	-0.69047	-0.1037453
$kdegXIAP_p$	0.000229	0.00261	0.00012	-0.000233	-6.16E-05	-0.00036	-0.0003	0.002711	7.65E-05	-0.01149	-0.0017314
k17	-0.00383	0.11568	0.000477	-0.001099	0.000803	-0.00321	0.00731	0.032143	-0.00156	-0.43803	0.18265254
kdegcomplex	-0.00135	0.054552	-6.34E-05	8.28E-05	0.000306	-0.0004	0.002217	0.006122	-0.0005	-0.33122	-0.5332121
k18	0.008719	-0.23905	-0.00209	0.0045801	-0.00173	0.010211	-0.01904	-0.09195	0.003961	0.717198	-0.3184647
n1	-0.01772	-0.01566	-0.00704	0.013529	0.00457	0.018344	0.024751	-0.12544	-0.00602	0.107914	-0.0092506
n2	0.034798	0.445439	0.019083	-0.037072	-0.00943	-0.05704	-0.04569	0.441692	0.011536	-1.96764	-0.3065346

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