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Reaction network analysis in biochemical signaling pathways

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Reaction network analysis in biochemical signaling pathways

Submitted by **Ivan Martínez Forero** in partial fulfillment of the requirements for the Doctoral Degree of the University of Navarra

This dissertation has been written under our supervision at the Department of Physics and Applied Mathematics, and we approve its submission to the Defense Committee.

Signed on October 15, 2009

Dr. Antonio Peláez López   Dr. Pablo Villoslada Díaz

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Signed on October 15, 2009

Ivan Martínez Forero

A María. A Laura. A mi familia

Acknowledgements

ES: What is your background?
IM: I am a physician
ES: Do you mean a physicist?
IM: No, I am doctor in medicine
ES: So, you are a *real doctor*. Where are your patients?

Eduardo Sontag, *Focus Group Meeting October 2008. Mathematical Biosciences Institute*

Contents

Preface

What are the factors that promote the appearance of diseases and determine the response to a specific treatment? During the last fifty years significant advances in biological research have revealed the basic components of living organisms including DNA, RNA and proteins. Several scientific efforts have elucidated the main processes by which these building blocks are produced and degraded. The origin of once unexplained fatal diseases like cystic fibrosis is now taught to first year medical students. The complete human genome is available for anyone with an Internet connection and enough time to learn the meaning of contig, expressed sequence tag, messenger RNA or alternative splicing

Biologists have the capacity to measure the expression of thousands of genes in one single experiment and to compare how this gene expression changes in terms of time or specific perturbations. Similar approaches are available for proteins or even whole cells. However, has this spectacular revolution in biological research changed the way in which medicine is practiced today? The answer is a clear yes. But there are still several questions that remain unsolved for the most common group of diseases, the ones responsible for the higher burden of human suffer: cancer, neurodegenerative diseases, cardiovascular diseases and autoimmune diseases.

Biology and medicine are gradually becoming quantitative sciences. High throughput quantitative techniques provide researchers with large amounts of biological data that is difficult to analyze with intuition alone. Computational and mathematical methods are in this case urgently needed as well as collaboration between mathematicians and biologists. This is not an easy task. There are several hurdles, beginning with the language used in both fields. Mathematics is a formal science based on theorems and proofs. Biology is an experimental science rooted in hypothesis testing through experiments. Mathematics likes generalization but biology is interested in reductionism, one gene, one protein, one disease. A frontier area where shared efforts are giving continous success is the physicochemical modeling of biochemical reactions, the subject of this work

Signaling pathways are networks of chemical reaction involved in the control of key cellular processes. They are composed of repeating structures denominated signaling motifs that show interesting dynamical properties (oscillations, bistability) susceptible of mathematical modeling. Deregulation of the control mechanism of signaling pathways is a common theme in complex diseases.

The aim of this thesis is to improve the understanding of signaling pathways through a theoretical study of chemical reaction networks. The equilibrium solution to the equations derived from chemical networks will be analytically resolved using tools from algebraic geometry.

The chapters are organized as follows:

1. An introduction to chemical dynamics in biological systems with a special emphasis on steady state analysis

2. Complete description of the chemical reaction network theory explaining the new results applied in this thesis We also cover the inverse problem in reaction kinetics whereby if the differential equations could be derived from a chemical system, there is the possibility to apply the theorems covered in the chapter

3. Signaling pathways are constituted by signaling motifs. In this chapter some of the most common motifs are resolved

4. Apoptosis or programmed cell death displays an interesting dynamical mark: bistability, thoroughly analyzed

5. A mathematical model for the JAK-STAT signaling pathway

6. A state of the art discussion of systems biology and autoimmune diseases

7. General conclusions and outlook of this work.

Chapter 1

Introduction

Biological organisms are conformed by different types of cells. Each group of cells has a well defined organization and function to constitute a tissue and then an organ. However, the correct activity of tissues and organs depends on the adequate communication between each of its cells. A communication systems is required.

Cells interact among them using chemical signals broadly classified as autocrine, paracrine and endocrine. Autocrine signals are produced and sensed by the same cells. Paracrine signals are secreted to nearby medium, where cells recognize them using specific receptors to the chemical signal. Examples of paracrine signals are cytokines and neurotransmitters. In the case of endocrine communication, the signal (an hormone) is released to the bloodstream and the effect is mediated in distant organs, after binding to the correct receptor.

Signal transduction pathways share some common features:

1. Specificity. Ligand receptor binding is controled in precise detail by accurate complementarity between the signal and the receptor. In multicellular organisms specificity is also attained due to the selective expression of receptors in the cells performing the function indicated by the ligand

2. Adaptation. Receptor protein production or activity promotes a negative feedbak loop that abrogates the signal. The mechanims involved are receptor internalization, receptor degradation and receptor protein messenger RNA downregulation

3. Amplification. Ligand binding activates an enzyme cascade that produces a geometrically increase in the signal strength. This is accomplished through the simultaneous activation of several proteins downstream ligand-receptor binding

4. Integration. The physiological effect of signals is not produced in isolation. The cell integrates incoming signals to generate an adequate output according to the cell status. There is a crosstalk between signaling pathways that ultimately provides the cell with instructions to perform a determined biological process

A common theme in cellular communication is the need of a receptor and a signal (a ligand). But, What happens after receptor binding to its ligand? How an external chemical signal regulates the

activity of intracellular components? What are the requirements to turn off a response elicited to an hormone? Cells are equipped with reaction networks responsible of transforming the upcoming chemical signals in information that helps the cell to take decisions about key cellular processes. The aforementioned chemical networks are known as signaling pathways.
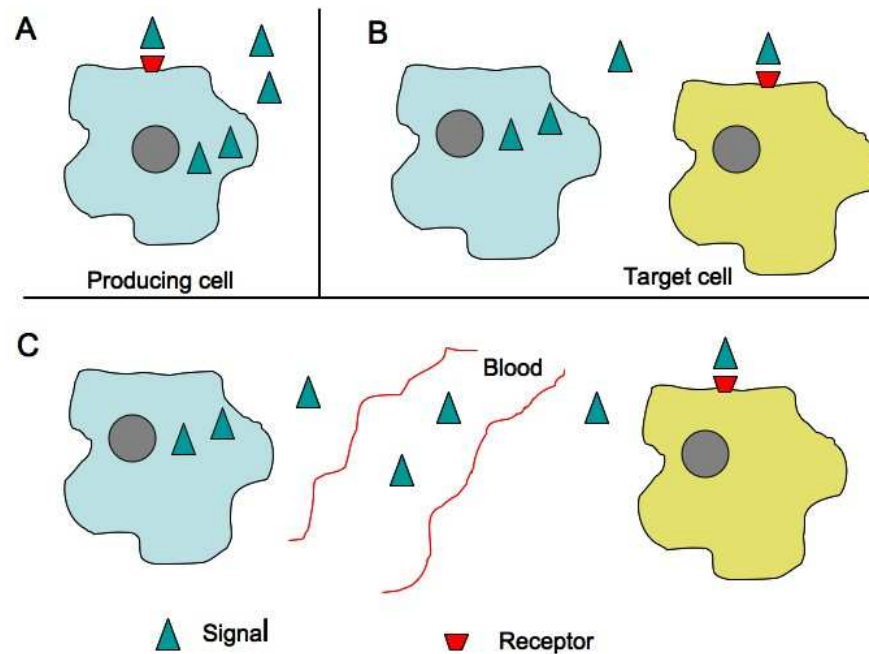
Figure 1.1: *A. Autocrine signal. The cell responds to its own product B. Paracrine signal. A cell senses a signal produced by a neighbour cell. C Endocrine signal. A distant cell reacts to a hormone transported in the blood.*

1.1. Signaling pathways

A signaling pathway (SP) is a chemical reaction network conformed by a set of proteins that mediate the transduction of biochemical signals (in terms of cytokines, hormones) to specific changes in cellular function. In order to complete its role, signals must be first sinthesized and secreted from the signaling cells. After traveling in the blood or in the extracellular medium, signals bind to specific receptors in target cells. Many receptors are transmembrane proteins and serve as a bridge between the extracellular medium and the cytoplasm. Upon signal perception receptors change their conformation to an active state. During the time of receptor activation, adaptor proteins initiate a sequence of reactions that ultimately bring about chemical signal physiological effects[1].

Ligand-receptor interactions take place through reversible non-covalent modifications. Ligand receptor binding displays effector specificity meaning that each ligand receptor complex generates a clearly defined cellular response. For example insulin in liver cells promotes glycogen

synthesis but no glucogen degradation. The kinetics of ligand-receptor binding could be modeled using the following equation

$$R + L \leftrightarrows LR \tag{1.1}$$

where the forward reaction occurs at a rate k_{on} and the backward reaction at a rate k_{off}. The dissociation (or affinity constant) can be calculated using this formula

$$k_d = \frac{[LR]}{[L][R]} \tag{1.2}$$

[L],[R] and [LR] represent the equilibrium concentrations of ligand, receptor and ligand-receptor complex. k_d values are in the range of $10^{-12}M...10^{-6}M$. The lower k_d, the higher affinity between ligand and receptor. We will discuss in more detail receptor kinetics in another section of this introduction[2].

After the receptor in the target cell is activated by its ligand on the surface of the cell membrane, a group of molecules takes the message to the nucleus where they induce changes on cell behavior. Chemical signals can augment or decrease the production of a protein or collection of proteins. Following the insulin example, this hormone increases gene expression of the hexokinase protein, an essential mediator in glycogen synthesis. In another way, extracellular signals often induce changes in the activity of proteins. Insulin inhibits the action of glycogen phosphorylase, a protein that interrupts the action of glycogen synthase, the key enzyme involved in glycogen synthesis.

The types of biochemical reactions performed by SP are diverse. Production and degradation, molecular modifications (methylation, acetylation) and activation and inhibition reactions are among the most common. A repeating process is the phosporylation and deposphorylation of protein cascades mediated by kinases and dephosphatases, respectively. This type of reaction gives rise to non-trivial dynamic properties susceptible of mathematical modeling[3].

There is no clear way to classify signaling pathways. Using the terminology proposed in a well known book of the field, SP could be divided in two groups: Classical and non-classical. Classical pathways include the ones activated by G-protein coupled receptors, receptors with enzymatic activity or steroid hormones. The mediators (or second messengers) of this group are metabolites as cyclic AMP or dyacilglycerol. Pathways responsive to cytokines or growth factors represent non-classical pathways. This group has been extensively studied during the last fifteen years. The prototypes comprise NF-κ B and JAK/STAT signaling pathways. This classification is only academical since it has been demostrated that signaling pathways usually don't work in isolation. Cells respond to a myriad of perturbations using common relay devices capable of integrating diverse inputs into a well defined cellular output. SP have combinatorial capacity[4][5].

Signaling pathways are tightly regulated. Cells have several control mechanisms to interrupt the action of chemical signals and terminate in this way the cellular response promoted by them. Cells regulate the number of receptors and their activity as well as the production of signaling components (kinases, adaptors). Regulatory circuits are a hallmark in SP biology. Therefore it is not of surprise that common human diseases such as cancer and type II diabetes could be

originated in the abnormal activity of transduction pathways.

Protein interaction networks and signaling pathways are composed by a large number of interacting entities. In order to understand the underlying complexity both in structure and dynamics it is required some kind of mathematical analysis. Terms like steady state, bifurcation point and limit cycle acquire a biological meaning. This thesis is about the mathematical analysis of signaling pathways. Mathematics starts in the next section.

1.2. Mathematical modeling of biochemical reactions

High efficiency technologies (such as DNA microarray and mass spectrometry) provide researchers with a great amount of data to analyze. Gene expression experiments and proteomic assays are static, taking an instantaneous picture of the cellular state. Static data is of relevance to reconstruct the normal components of protein and gene interactions networks. But we are interested in the dynamical behavior arising from these interactions, often elucidated through development of a mathematical model

The steps required to produce a mathematical model for a biochemical reaction network are the following[6]:

1. Available data (components,interactions,parameter values) is translated into a mathematical model

2. The model structure and dynamical properties are extensively analyzed

3. Using the model some predictions can be made

4. Predictions are tested with experiments

5. Conclusions and data can be incorporated to the model

6. The cycle repeats until the model is nearly complete and accurate

In this section the first two steps of model building are explained using as an example receptor ligand dynamics.

1.2.1. In search of good data. Network reconstruction

Mathematical model building relies on previous information about the components and interactions among the components. There exist a growing number of databases that provide curated knowledge to identify the proteins participating in the chemical reaction of interest. Among the most widely used databases are KEGG (Kyoto Encyclopedia of Genes and Genomes), STRING and REACTOME. In these repositories it is possible to find cartoons that indicate the elements of the network and a sketch of the connections between them. Of great interest is the experimental evidence that relates perturbations in the components to observable phenotypic variations[7][8][9].

Once a complete part list is available it is time to make sense of such a list. The scope of

the model can be broad and include a detailed description of all proteins, proteins modifications and control mechanism affecting the chemical network. In another way, the model can take into account only the most important components and interactions. For example in the case of the receptor ligand system we can include all the known states of the receptor (resting, activated, sensitized, desensitized) or only distinguish between an inactive and an active form.

Models in biology are phenomenological or mechanistic. Phenomenological models describe the data and try to infer casualty from statistical correlations. Mechanistic models seek to understand the phenotype trough the study of the emergent properties of molecules interacting inside a cell. Mechanistic models can be viewed as a form of dynamical system, susceptible to be handled with mathematical reasoning. A dynamical system describes how its components change over time. Dynamical systems depend on paramaters, but biological parameters (such as reaction rate constants) are difficult to obtain. This has led some authors to advocate for a biology with no parameters, emphasizing the need to obtain general conclusions about the dynamics of a biochemical system relying only on the structure of the chemical reactions. However there are databases that include parameter information, see for example DOQCS (Database of Quantitative Cellular Signaling)[10].

1.2.2.   One-dimensional systems

In this section of mathematical analysis we will follow a classical presentation of dynamical systems. For the dimension of a system we mean the number of variables that conform it. One dimensional systems have one variable, two-dimensional systems, two variables, and so on. A system is linear if all the terms are linear. We will discuss first the characteristics of one-dimensional systems using as an example a production and degradation reaction for a single chemical specie.

In the previous subsection we describe the information needed to develop a mechanistic dynamical system. Now is time to introduce the dynamic. Here the state of a particular component represents its concentration over time. In this regard the temporal evolution of the species participating in the reactions can be described by a system of ordinary differential equations. The terms for each component represent the net production and degradation of the species taking place in the chemical network. The time evolution derives from the equation

$$\frac{dx}{dt} = f(x, k), \tag{1.3}$$

where $x \in \mathbb{R}^n$ is a vector of variable representing chemical species concentrations, $k \in \mathbb{R}^m$ is a vector of reaction parameters and $f : \mathbb{R}^{m+n} \to \mathbb{R}^n$ is the vector function that describe the balance between production and consumption for each x_i. In the case of a single chemical specie, the ODE for the production and degradation example using mass action kinetics is:

$$\dot{x} = k_1 - k_2 x \tag{1.4}$$

The steady state (or equilibrium) of the system can be obtained solving the algebraic equation $f(x, k) = 0$. In our example the equilibrium can be calculated using the expression $x = \frac{k_1}{k_2}$. It is clear that for each choice of reaction parameters the system will converge to an isolated steady

state. In figure 1.2 there is the qualitative evolution of the system depending on the initial condition x_0. For $x_0 > \frac{k_1}{k_2}$, the trajectory (the solution) slows down to the equlibrium point. If $x_0 > \frac{k_1}{k_2}$ then x goes up until reaching the steady state. It is worth to mention the impossibility of other
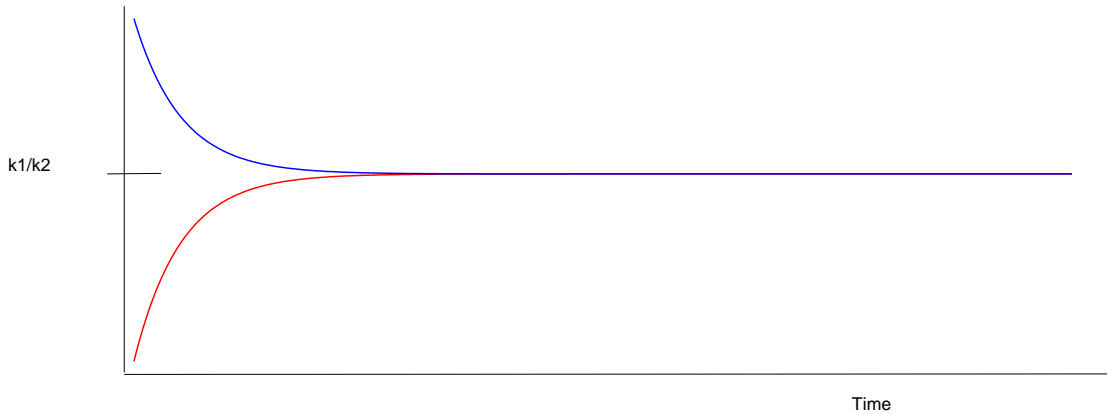

Figure 1.2: *Qualitative evolution of the production-degradation system. In blue a trajectory with initial condition* $x_0 > \frac{k_1}{k_2}$. *In red a trajectory where* $x_0 < \frac{k_1}{k_2}$

types of dynamical behavior (i.e. oscillations) in one dimensional systems. Hence, there are no periodic solutions to $\dot{x} = f(x)$

Other types of kinetics are commonly implemented in biochemical simulations. We will briely cover Michaelis-Menten kinetics and the Hill equation. In enzymatic reactions, the enzyme is not produced or degraded and forms a transient complex with the reactant. In this case a quasi steady state approximation is valid only if the enzyme is in much lower concentration than the reactant concentration. Michaelis-Menten kinetics can be formulated in the following form

$$\dot{x} = \frac{V_{max}x}{K_m + x}$$

(1.5)

where V_{max} is the maximal reaction rate and K_m is the substrate concentracion where half the maximal reaction velocity is reached. Michaelis-Menten kinetics has been widely used in biochemical reaction network analysis. However, in signaling pathways where postranslational modifications are common (i.e phosphorylation) the quasy steady state approximation does not hold because a chemical specie can be at the same time substrate and enzyme. In such case mass action kinetics seems the appropriate choice[11].

In some reaction networks involving multiunit proteins or dimers of the same protein, the binding of the ligand (or substrate) produces a conformational change allowing an easier recruiting for another ligand molecule. This is known as cooperativity and is found in ligand-receptor systems, transcription factor binding to DNA and in the hemoglobin when it is in the presence of oxygen.

Cooperativiy is mathematically formulated as the Hill equation:

$$\dot{x} = \frac{V_{max}x^n}{K_m + x^n}$$

(1.6)

V_{max} and K_m have the same interpreation as in the Michaelis-Menten kinetics and n designates the Hill coefficient or the degree of cooperativity of the reaction. For example in the hemoglobin reaction $n = 2.8 - 3$.

1.2.3. Two dimensional systems

Here we will use three examples, all having two variables:

1. Ligand-receptor dynamics

2. Glycolytic oscillations

3. The Bruselator

Ligand receptor dynamics

The initial process in a SP is ligand-receptor binding. Figure 1.3 displays the chemical reactions involved in this system. The chemical species are
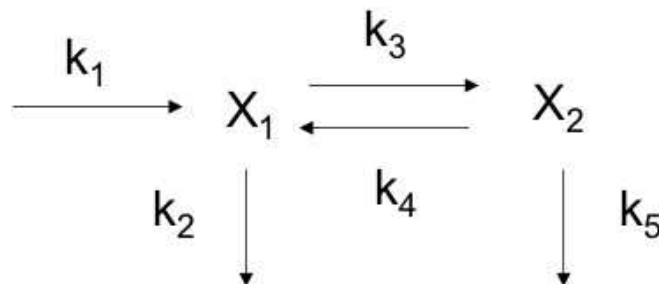

Figure 1.3: *Receptor ligand dynamics. X_1 is the inactive receptor, X_2 is the active receptor. k_1, k_2, k_3, k_4, k_5 represent reaction rates.*

$x_1=$ inactive receptor

$x_2=$ active receptor

The network is composed of five reactions with the following reaction rates assuming mass action kinetics.

Inactive receptor production k_1

Inactive receptor degradation $k_2 x_1$

Receptor activation $k_3 x_1$

Receptor deactivation $k_4 x_2$

Activated receptor degradation $k_5 x_2$

This leads to a system of ODE

$$\dot{x}_1 = k_1 + k_4 x_2 - (k_2 + k_3)x_1 \tag{1.7}$$

$$\dot{x}_2 = k_3 x_1 - (k_4 + k_5)x_2 \tag{1.8}$$

After ligand binding, inactive receptor decays rapidly and the active receptor becomes fully operative in just a fraction of time. The steady state (or equilibrium) of the system can be calculated solving the algebraic equation $f(x,k) = 0$. For the running example this is

$$x_1 = \frac{(k_4 + k_5)k_1}{k_2 k_4 + k_2 k_5 + k_3 k_5}, \; x_2 = \frac{k_3 k_1}{k_2 k_4 + k_2 k_5 + k_3 k_5} \tag{1.9}$$

Another way to observe the steady state of the systems is using a phase plane. A nullcline is the set of all points where $\frac{dx_i}{dt} = 0$. We can identify an equilibrium in the intersection of the nullclines. In the example there is only one steady state. However, more interesting dynamics have been observed in biochemical reaction networks. This is the topic of the next part of this work.

Glycolytic Oscillations

Periodic variation in chemical species concentration is a prevalent property of living organisms. Examples include calcium waves, oscillations in glucose metabolism and the control of the cell cycle, all of them important processes in cell physiology. The origin of periodicity is an intense area of research. Some authors propose two general requirements for biochemical oscillations: negative feedback and time delay. In this part we will deal with oscillations trough an example of glycolytic oscillations[12]

Phosphofructokinase I (PFK 1) is an enzyme that catalyzes the transfer of a phosphate to fructose-6-phosphate to yield fructose-6-biphosphate. The phosphate donor is ATP. In the reaction, ATP is transformed to ADP. ADP is an activator of PFK 1 while ATP is an inhibitor. Fructose-6-biphosphate (the product of the enzymatic reaction) is also an activator of PFK 1. The system could be modeled using the following ODEs[13]

$$\dot{x}_1 = k_1 - k_2 x_1 x_2^2$$
$$\dot{x}_2 = k_2 x_1 x_2^2 - k_3 x_2 \tag{1.10}$$

where x_1 , x_2 represent substrate (fructose-6-phosphate) and product (fructose-6-biphosphate) respectively. Figure 1.4 displays the temporal evolution of metabolite concentration. In Figure 1.5 appears the phase plane of the system. There is an oscillatory trajectory for the parameters values specified in the legend of the figure. In biochemistry, deregulation in oscillating systems can be the basis of the so-called dynamic diseases. Examples include cyclical neutropenia and the relapsing remitting variant of multiple sclerosis.
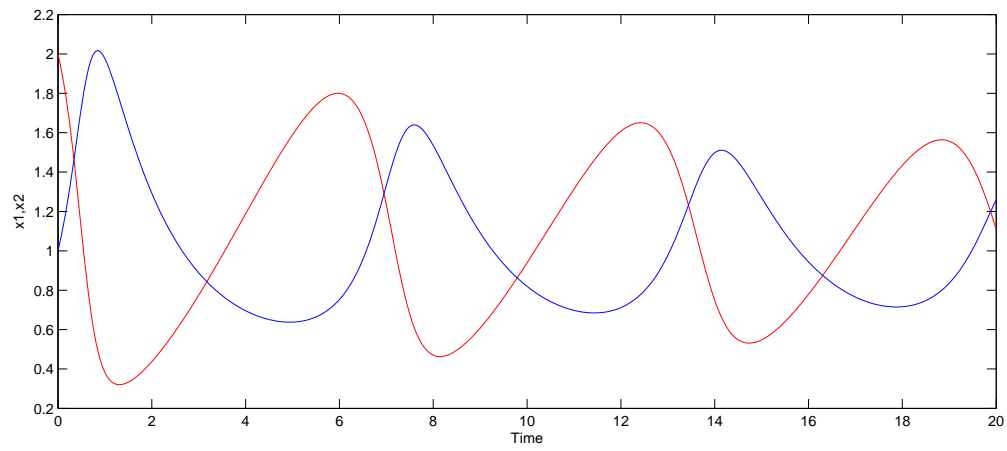
Figure 1.4: *Time course for the glycolytic system. Red line represents x_1 and blue line x_2. The parameter values used in the simulation were $k_1 = 1, k_2 = 1, k_3 = 1.0001$ Initial conditions are $x_1 = 2, x_2 = 1$*
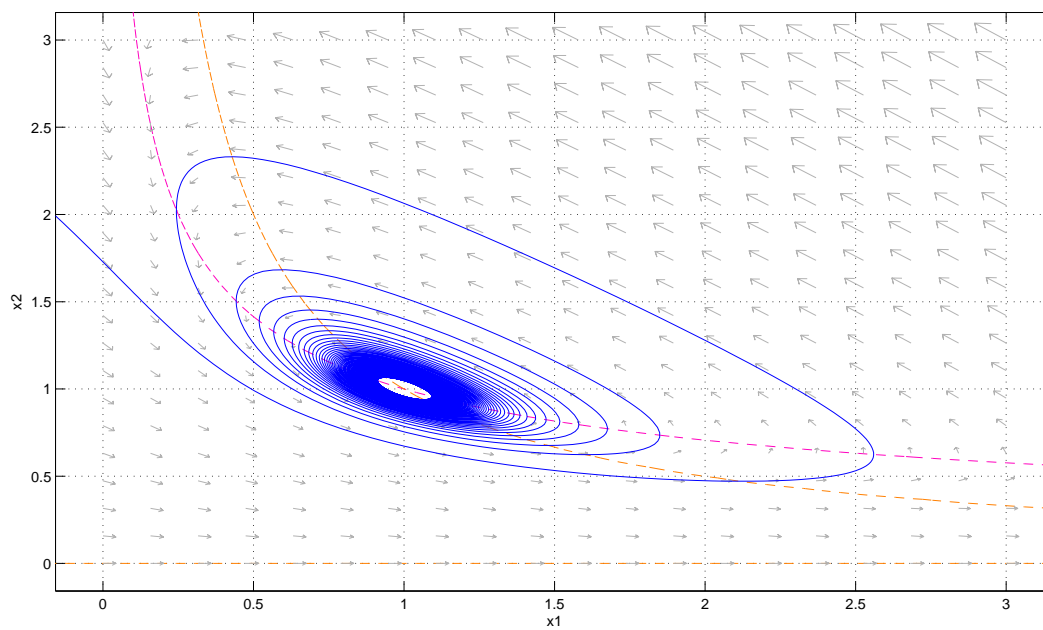
Figure 1.5: *Phase plane of the glycolytic system. Parameter values are the same as in Figure 1.4*

The Bruselator

In 1968 Prigogine and Lefever [14] reported a two-dimensional chemical mechanism displaying atypical dynamicss such as oscillation or bistability. The ODE system is

$$\dot{x} = a - bx + cx^2y - dx$$
$$\dot{y} = bx - cx^2y - ey \tag{1.11}$$

where a, b, c, d, e are reaction rate parameters. For the case $e = 0$, the brusselator as a unique steady state at $x = \frac{a}{d}, y = bd/ac$. This steady state is unstable when $d^3 - bd^2 + ca^2 < 0$. As shown in Figure 1.6 the unstable equilibrium point is surrounded by a limit cycle. A limit cycle is an isolated closed trajectory. If all the solutions converge to the limit cycle, it is called stable,
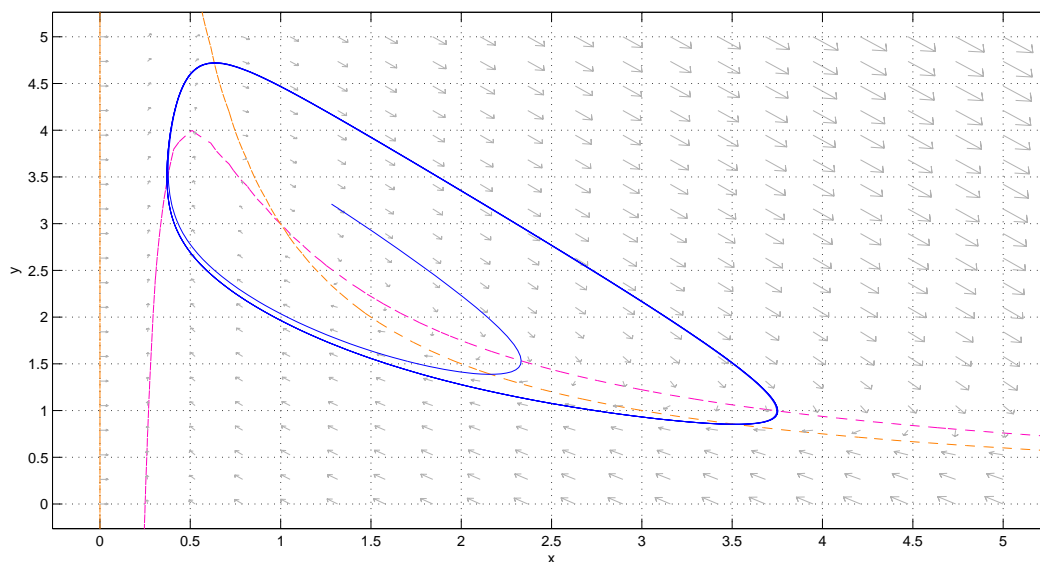
Figure 1.6: *Phase plane of the Bruselator. Parameter values are $a = 1, b = 3, c = 1, d = 1, e = 0$*

otherwise unstable. In Figure 1.7 appears the temporal evolution of the system, where x and y oscillate without interruption .

Limit cycles are common in science. They describe sytems that display self sustained oscillations. Examples include the heart beat, periodic neuron firing and intracelular calcium oscillations. Until now we have explained two qualitative solutions for a two-dimensional system:

1. Converge to a steady state

2. Converge to a limit cycle

The well known Poincare-Bendixson theorem stays that there are no more posibilities in two-dimensional systems. According to this theorem any continous planar system approaches either a fixed point or a periodic orbit. There is no place for chaos.
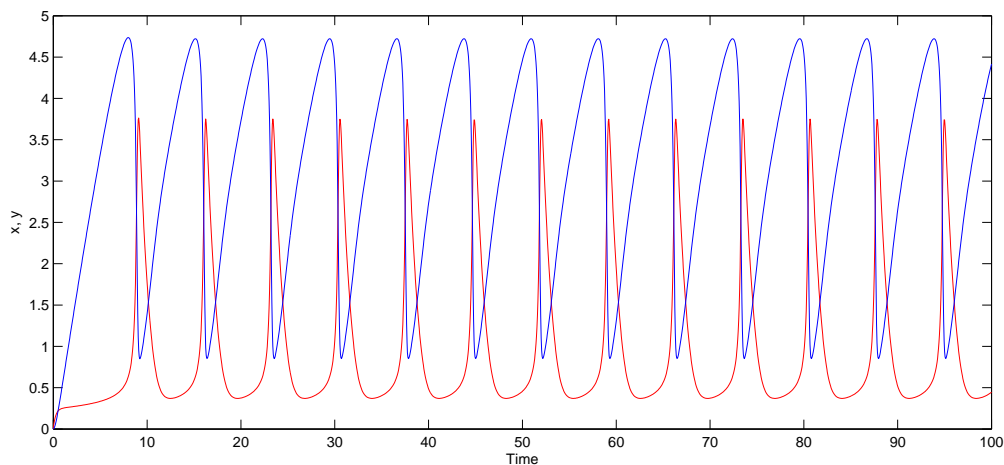
Figure 1.7: *Temporal evolution of the Bruselator. In red x solution, in blue y solution*

1.2.4.   Three-dimensional systems

Two dimensional systems are capable of self-sustained oscillations and as 1D systems of setting down to a steady state. In systems of three variables (3D) it was thought that the qualitative behavior would include limit cycles and steady states. It was a great susrpise when in 1963, Edward Lorenz reported a system with three variables that exhibited a dynamical pattern never oberved before. The ODE system is

$$\dot{x} = -\sigma(x - y)$$
$$\dot{y} = rx - y - xz$$
$$\dot{z} = xy - bz \tag{1.12}$$

This system lead to the development of a new field of mathematical research known as chaos theory. But, what is chaos? We will follow a classical definition from Steve Strogatz[15]. **Chaos** is aperiodic long-term behavior in a deterministic systems that exhibit sensitive dependence on initial conditions. Aperiodic means that solutions do not converge to fixed points or periodic orbits. Deterministic means that the system has no random or noisy inputs or parameters. The form of the solutions depends entirely of the non-linearities present in the system. Sensitivity to initial conditions means that close solutions separate exponentially. This can be observed by changing y initial conditions as displayed in Figure 1.8.   The phase space of the Lorenz system is the classic butterfly attractor.   Another famous chaotic system is the Rössler equation. Otto Rössler is a german physician interested in chemical kinetics where non-linear terms naturally occur. The Rössler system is

$$\dot{x} = -y - z$$
$$\dot{y} = x + ay$$
$$\dot{z} = b + z(x - c) \tag{1.13}$$

A phase space plot of this ODE system is shown in Figure 1.10. It is remarkable how simple mathematics (one or two non-linear terms) gives rise to such complicated dynamics.
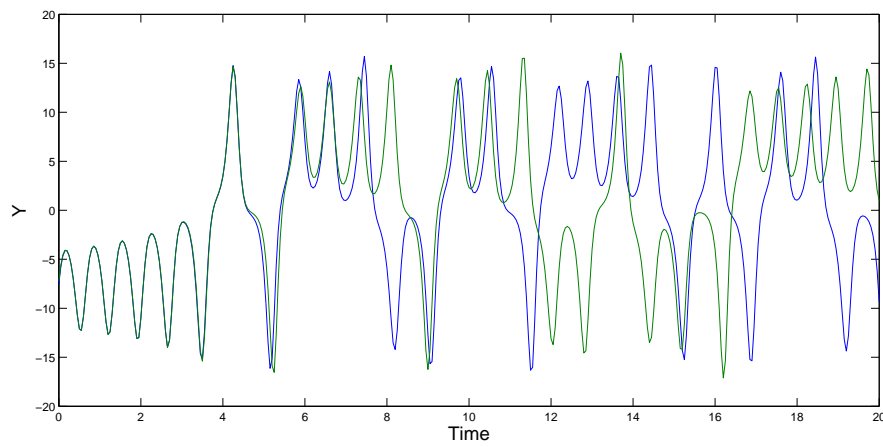
Figure 1.8: *Time series for the Lorenz system. Parameter values are $\sigma = 10, b = \frac{8}{3}, r = 28$. Initial condition are $x = -7.5, y = -3.6, z = 30$. In green a time series with the original initial condition. In blue changing $x_1 = -7.4$*
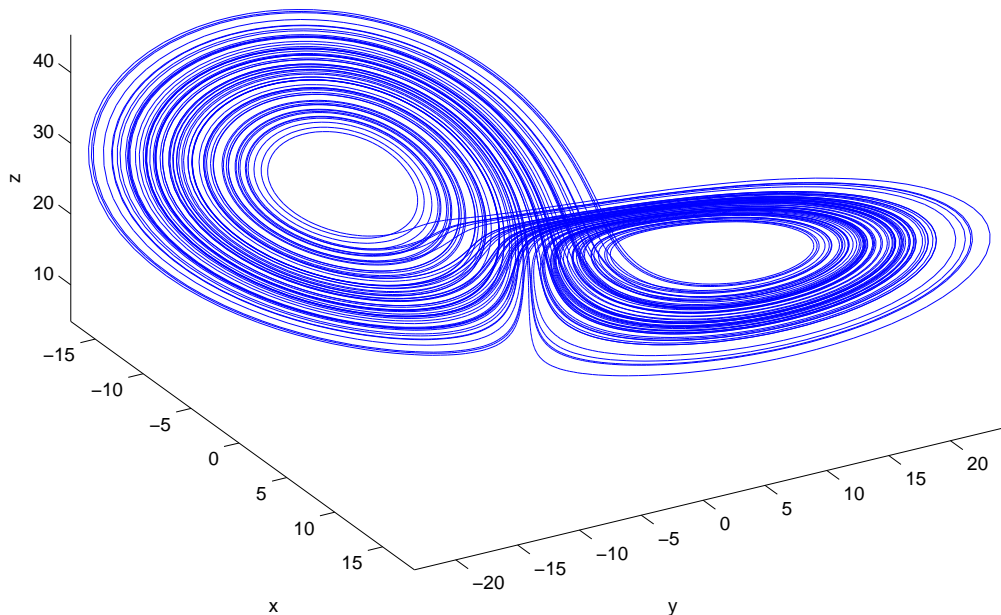
Figure 1.9: *The Lorenz attractor. Parameter values are equal to Figure 1.8*
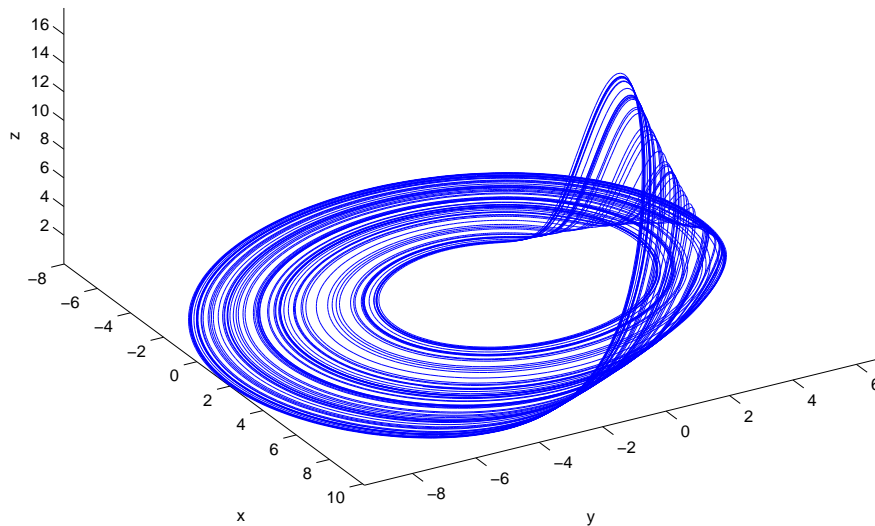
Figure 1.10: *The Rössler attractor. Parameter values are $a = 0.2, b = 0.2, c = 5.0$*

1.2.5.   Bifurcations

The qualitative solutions of dynamical systems can vary if the parameters are changed. In the previous sections we have explained the possible outcomes for fixed parameters values. But, if one or several of the parameters are perturbed, new phenomena can come into sight. New steady states can appear, the stability can change or in some systems chaos can be present. The qualitative changes in system dynamics observed after modifying parameters are known as bifurcations. The values at which bifurcations occur are bifurcation points.

Bifurcations have received special attention in mathematical modeling of SP. Bifurcations could be the origin of life and death switches in the apoptosis network, or the biochemical background for cell cycle progression. As usual we will cover this topic with an example: the *lac* operon

Lac Operon

Signaling pathways are composed by chemical reactions. Chemical kinetics analysis often gives rise to non linear dynamical systems. In particular simple reaction mechanisms, such as bimolecular reactions i.e $x_1 + x_2 \rightarrow x_3$ produce non linear terms in ODE representation. If we observe the "trivial" mathematical object derived from this simple interaction

$$\dot{x}_1 = -k_1 x_1 x_2 \tag{1.14}$$

we can see the appearance of a non linear term. Simple chemical interactions could be the source of interesting non linear dynamics. This would be clarified using a classical example : the lactose

utilization network of *Escherichia coli*.

Bacteria consume glucose in their metabolism. The glucose is obtained from the external medium and imported to the cytoplasm where it can be processed in order to obtain energy. Several enzymes participate in this process. One mean of regulation of glucose metabolism is the transcriptional control of the enzymes involved in glucose homeostasis. In prokaryotic cells (i.e. bacteria) groups of genes participating in the same function, are transcribed together conforming an operon[16].

Escherichia coli depends on glucose. When there is no glucose in the medium to use, *Escherichia coli* takes on lactose and transform it into glucose. The enzymes responsible to perform this function are LacY and LacZ. LacY or β-galactoside permease transports lactose into the cell. LacZ or β-galactosidase cleave lactose into glucose and galactose. It also converts lactose into allolactose. LacY and LacZ (along with LacA, not involved in lactose metabolism) constitute the *lac* operon. The *lac* operon is tightly regulated. In the absence of lactose an inhibitor protein LacI binds to the operator region of the *lac* operon and inhibits gene expression. In the presence of lactose, its metabolite allolactose produces a conformational change in LacI preventing LacI binding to the operator an allowing *lac* operon expression.[17] The *lac* operon could be modeled with the ODE system

$$\dot{x}_1 = c_0 + c(1 - \frac{1}{1 + x_2^n}) - \gamma x_1 \tag{1.15}$$

$$\dot{x}_2 = lx_2 - \delta x_2 - \frac{vx_1 x_2}{h + x_2} \tag{1.16}$$

where x_1 is the *lac* operon mRNA concentration and x_2 is allolactose concentration. c_0 is mRNA basal production, c is the mRNA growth rate, γ and δ are decay constants. v is the maximum rate of the reaction and h is a saturation term. l denotes lactose concentration. For the parameters values represented in Figure 1.11 there are three steady states, two stable and one unstable. The bifurcation diagram shows how changes in the l parameter controls *lac* operon gene expression. The plot was obtained using the MAPLE system implementing methods presented in Chapter 2.This type of behavior (bistability) is common in biochemical networks both in eukaryote and prokaryote cells. It could be the origin of differentiation and memory. Perhaps could be the cause of lack of response to a lowering cholesterol drugs, as discussed in section 1.3 section[18].

1.2.6.   Network motifs

Chemical species are not isolated, but linked and cells are thus reaction networks. In the last decade there has been a growing interest in the structural properties of biological networks. Several topological measures (includind degree, cluster coefficient) have been defined and applied to the elucidation of how genes and proteins are interconnected. Genes and protein-protein interaction networks seem to follow certain design patterns, repeatedly converging to similar architectures during evolution. This patterns are currently known as network motifs. Most of the work done in this field has covered transcription networks. In this part of the introduction we will briefly review some well characterized network motifs along with some dynamical properties they exhibit. Several software implementations are designed to detect network motifs in transcription
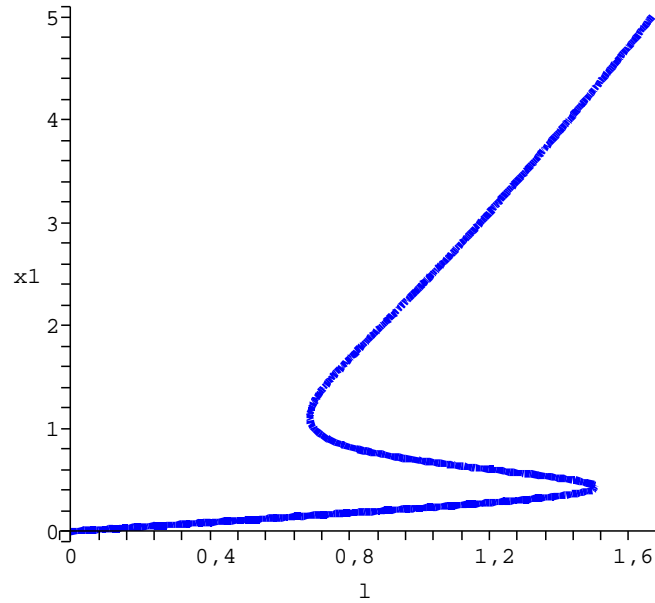
Figure 1.11: *Bifurcation diagram for the lac operon system. l is lactose concentration. x_1 represents lac operon mRNA. Parameter values used were $c = \gamma = v = 1, c_0 = \frac{1}{20}, h = 2, m = 5, \delta = \frac{1}{5}$ and $n = 5$*

networks and in protein-protein interaction networks. In Figure 1.12 there is a schematic representation of the networks motifs presented[19]. **SIM (Single input motif)** a signal S controls the production of x and y with reaction constants r_1 and r_3 respectively. This motif can generate an ordered expression program for each of the components under regulation of S. The equation for this motif is

$$\dot{x} = r_1 S - r_2 x$$
$$\dot{y} = r_3 S - r_4 y$$

The terms $r_1 S$ and $r_3 S$ could be transformed to k_1 and k_3 and the degration rates r_2 and r_4 to k_2 and k_4. Now the ODE sytem is

$$\dot{x} = k_1 - k_2 x$$
$$\dot{y} = k_3 - k_4 y$$

X could be considered as a master regulator of the target gene. The SIM components perform an orchestrated biological function. In the right upper panel of Figure 1.13 appears a time series simulation for the SIM motif.

   Regulatory chain motif. In this architecture a signal regulates the production of x which in turn controls the production of y. The model for this system is

$$\dot{x} = k_1 - k_2 x$$
$$\dot{y} = k_3 x - k_4 y$$
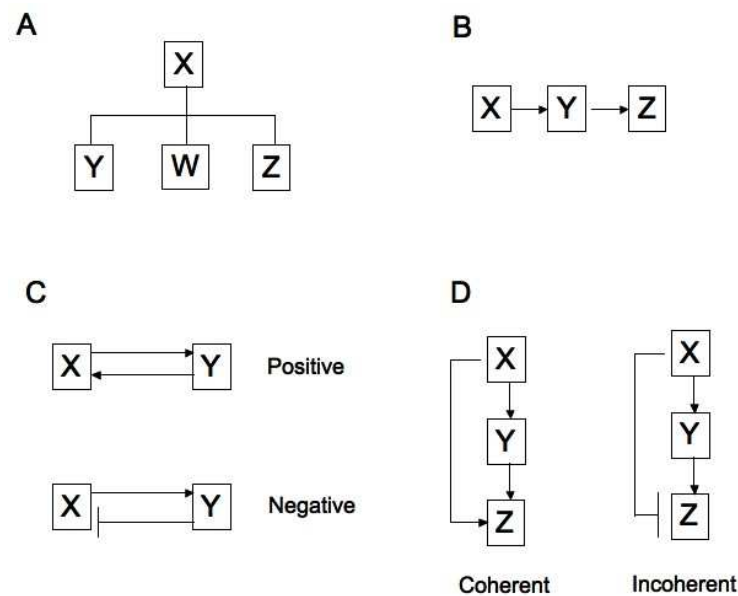
Figure 1.12: *Network motifs. A Single input motif B. Regulatory chain motif C. Feedback loop (positive and negative) D. Feedforward loop (coherent and incoherent)*

As observed in the left upper panel of Figure 1.13, there is a delay in the activation of y in relation to x, because y variations depends on x which is zero at the start of the simulation.

Feedback motif. Feedback motis are postive or negative. In positive FB the product of a regulation cascade augments the production of the initial chemical specie. This motif occurs in more than 50 % of repressor promoters in bacteria and eukaryotic cells The ODE system for a positive FB is

$$\dot{x} = k_1 + k_5 y - k_2 x$$
$$\dot{y} = k_3 x - k_4 y$$

When levels of species X increase, expression of Y is induced, which represses expression of X (and its own expression). When levels of X fall below a threshold, its production is stimulated because of the absence of the repressor Y. This control mechanism yields oscillations around a mean value, which, in turn, depends on other parameters of the system such as synthesis and degradation rates. A negative feedback could be model by the ODE

$$\dot{x} = k_1 + k_5 \left(\frac{1}{1+y}\right) - k_2 x$$
$$\dot{y} = k_3 x - k_4 y$$

Feed-forward loops (FFL) consist of three components: A, B, and C. A is a regulator of B and C (left). FFL is coherent if the sign (activation or suppression) of the path A-B-C is the same as the sign of the path A-C. If the signs do not match, the FFL is incoherent. FFL can reject

transient inputs and activate only after persistent stimulation. An ODE system for a coherent FFL is

$$\dot{x} = k_1 - k_2 x$$
$$\dot{y} = k_5 + k_3 x - k_4 y$$

FFL loops are the most extensive studied both trough analytical and experimental approaches. FFL dynamics resembles filters for protecting systems from rapidly changing signals that otherwise will trigger unwanted cellular responses.

The Bifan motif is composed of two source nodes directly cross-regulating two target nodes. This motif is able to act as signal sorter, a synchronizer, or a filter. It also provides temporal regulation of signal propagation. Network motifs function and dynamics have been tested experimentally. There is data available for the RC motif and the coherent FFL. In Figure 1.13 appears time series plots for the SIM, RC, negative feedback loop and coherent feedforward loop motifs. Network motifs are
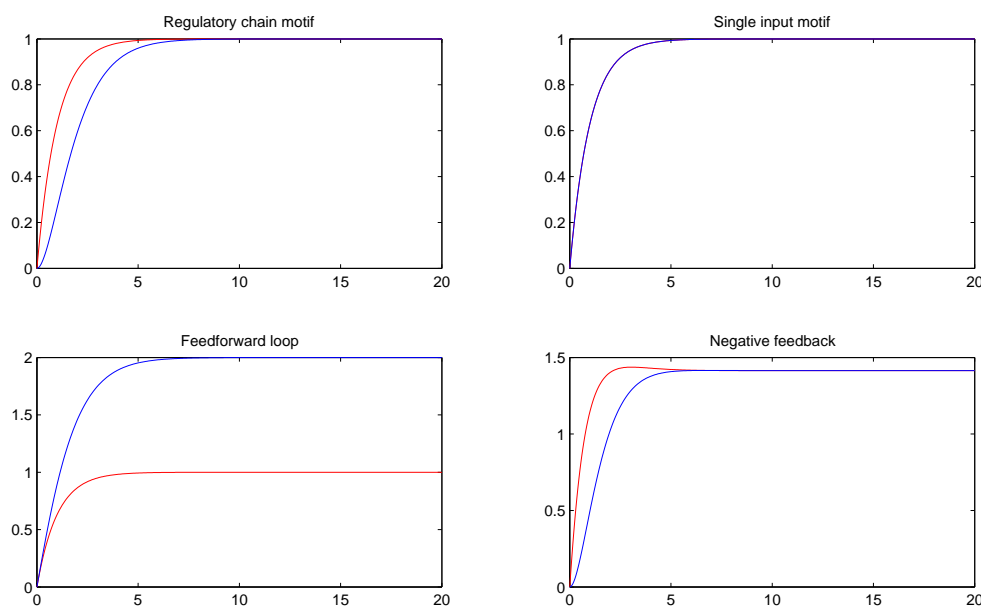
Figure 1.13: *Time series for the motifs described in the text. In red x concentration and in blue y concentration.Parameter values are $k_i = 1$ for all i*

1.3.   Signaling pathways in human disease

Cancer, neurodegenerative diseases, cardiovascular disease and autoimmune diseases are the most common cause of death and suffering in the world. This group of maladies can be classified as complex diseases in contraposition to monogenic diseases (Mendelian heredity) such as cystic fibrosis or sickle cell anemia, that result in modifications of a single gene affecting all the cells of

the organism. The cause is usually unknown, resulting from a combination of genetic and environmental factors. Complex disease treatment is most of the time supportive and lagging behind the natural course of the disease. This means that when a therapeutic regimen is started, control devices in normal functioning have been subsumed. Take the example of a malignant tumor. A mass of 1 mm^3 size contents nearly 1x10^9 cells and has grown for almost ten years[20].

SP are important for the appropriate physiology of the cell. They are involved in cell growth, cell proliferation, apoptosis, control of gene expression an protein transduction among other relevant biological processes. Aberrations in receptor number, protein phosporylation or dephosporylation or cytoplasm transport can have profound effects in cells and organisms well being. Therefore it is not surprising that SP participate in the pathogenesis of complex diseases. SP are in this sense, ideal drug target candidates. Understanding how SP interact among each other and produce a phenotype is fundamental to design effective drug interventions to treat complex diseases. The first step is to gather as much information as possible about the composition and connectivity of SP both in health and disease[21].

Several approaches (both genomic and proteomic) are available to understand the molecular details behind SP organization. Here, it is worth to mention two genomic tools commonly used to dilucidate SP biology: gene expression microarrays and single nucleotide polymorphism (SNP) identification. From the proteomics point of view sources of data are mass spectrometry (MS) and two dimensional polyacrylamide gel electrophoresis (PAGE)[22].

All cells in the body have the same composition of genes, but not all genes are expressed (or turned on) in every cell. At the same time, a particular gene can be expressed under very specific conditions and stay completely silent in others. The study of these changes can now be addressed through the use of a technology called DNA microarrays. A microarray is a tool for analyzing gene expression that consists of a small membrane or glass slide containing samples of many genes arranged in a regular pattern. Microarrays have proven useful in stratifying the prognosis of several types of cancer and in proposing new drug targets based on differential gene expression between patients and healthy persons. Dynamical studies of gene expression after different perturbations (drugs,infections) have also be accomplished.

A SNP is a change in one nucleotide inside the DNA sequence. An example is ATTG to AATG, where the second T has been replaced for an A. SNPs occur in the human population more than one percent of the time. Most SNPs are located in non-coding regions, that is, in parts of the genome not directly involved in protein codification. However SNPs could be present in regulatory regions where they can produce subtle changes in gene expression. Sets of SNPs could be associated with the development of complex diseases. SNPs can also be involved in the grade of response to a drug. SNPs in signaling pathways components have been reported in the case of multiple sclerosis, breast cancer, etc. The functional role of SNPs is not well understood. In order to assess how SNPs impinge on SP physiology it is necessary to obtain data regarding the effect of SNPs upon protein components of the SP[23].

Proteomic experiments (MS, PAGE) can be used in two ways. First, changes in protein ex-

pression (as well as gene expression) serve as a mean to compare protein abundances in response to perturbations. This pattern of response can be translated to a marker of a specific condition. Second, proteomic data is taken as the source for network reconstruction of protein interactions, the building blocks of SP.

Now, we will like to illustrate how mathematical models of signaling pahtways are interesting tools to discover properties of reaction networks (bistability) that could be the hidden origin of treatment lack of response in a common complex disease. For this, we will first briefly review the cholesterol pathway.

1.3.1.   Cholesterol metabolism pathway

Coronary heart disease (CHD) is the leading mortality cause in developed countries. Several risk factors are associated with CHD appearance including increasing age, male gender, hereditary predisposition, tobacco smoke, high blood pressure and high blood cholesterol. High blood cholesterol is a modifiable risk factor for CHD through diet, exercise or drug therapy (i.e. statins)[24].

Cholesterol metabolism is intricate. Several types of components and interactions participate to maintain cholesterol concentration in the normal range. Lipoproteins are molecules responsible of transporting lipids (including cholesterol) between the tissues. In table 1.1 there is an overview of lipoprotein classification and composition[25]. Overwhelming clinical evidence suggest

Lipoprotein class	Density (g/mL)	Protein (%)	Cholesterol(%)
HDL	1.063-1.021	33	30
LDL	1.019-1.063	25	50
IDL	1.006-1.019	18	29
VLDL	0.95-1.006	10	22
Chylomicrons	< 0.95	1-2	8

Table 1.1: *Composition of lipoproteins. High-density liporoteins (HDL), Low-density liporoteins (LDL), Intermediate-density lipoproteins (IDL) and Very low-density lipoproteins (VLDL)*

a direct connection linking LDL high plasma concentration and CHD. Statins are a group of drugs indicated to reduce blood cholesterol and CHD risk. Statins inhibit HMG-CoA reductase, the principal enzyme in cholesterol synthesis pathway. In the complex system regulating cholesterol metabolism it is necessary to understand which are the control mechanisms with the goal to manipulate the components and its interactions and in this way reduce CHD risk. A mathematical model is an interesting tool because it allows to propose quantitative predictions susceptible to be confirmed with experiments. Mathematical models also provide insight to identify the species most susceptible to interventions with pharmacological agents.

1.3.2.   A dynamical model of lipoprotein metabolism

In this section we analyze a model for lipoprotein dynamics proposed by Barahona et al[26]. The model has five variables representing VLDL, IDL, LDL, intracellular cholesterol concentration

(IC) and LDL receptor (LR) fraction. The ODE system is:

$$V\dot{L}DL = -k_v VLDL + u_v$$
$$I\dot{D}L = k_v VLDL - k_i IDL - d_i IDLLR$$
$$L\dot{D}L = k_i IDL - d_l LDLLR - dLDL$$
$$\dot{L}R = -b(d_i IDL + d_l LDL)LR + c\frac{1 - LR}{IC}$$
$$\dot{I}C = (\chi_i d_i IDL + \chi_l d_l LDL)LR + \chi_l dLDL - d_{ic}IC \qquad (1.17)$$

The parameters $k_v, k_i, d_i, d, d_l, b, c, \chi_i, \chi_l, d_{ic}$, represent production and degradation rates. Two parameters, u_v and d_{ic}, are affected by pharmacological intervention. u_v and d_{ic} constitute secretion rate of VLDL from the liver and depletion rate of intracellular cholesterol respectively. For example u_v value is reduced by statins. In figure 1.14 appear bifurcation diagrams for equilibrium solutions versus u_v . Increasing VLDL production rate produces a steady rise in blood IDL,LDL,
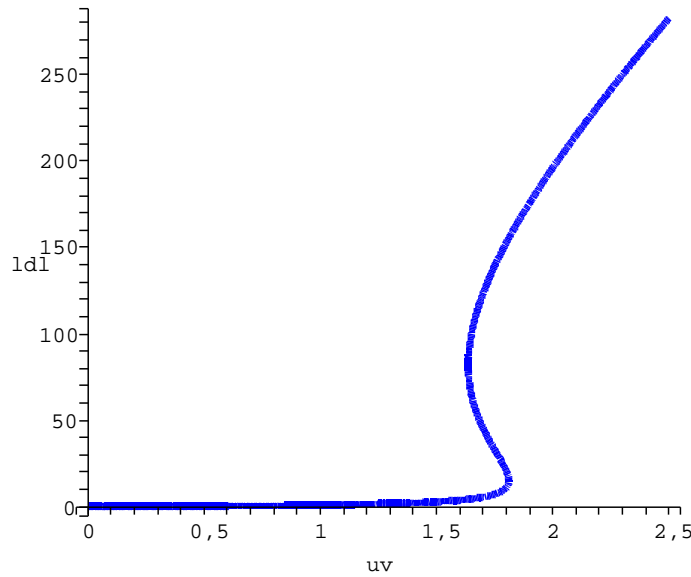
Figure 1.14: *LDL equilibrium solution vs u_v. Parameters values $k_v = 0.3, k_i = 0.025, d_i = 2, d_l = 0.01, d = 0.0075, \chi_i = 0.1, \chi_l = 0.6, b = 0.1, c = 0.05, d_{ic} = 1$.*

IC and a decline in LR levels. After reaching a threshold there are two possible solutions: one with low cholesterol levels and another with high cholesterol levels. The same threshold changes the LR equilibrium concentration allowing high and low LR levels. Some clinical observations can be explained with this model. Statins are effective in nearly 70 percent of patients taking the medication. However nearly 5 percent of treated subjects maintain cholesterol concentration over 600 mg/dL. As observed in figure the presence of bistability suggests that if a patient is already in the upper branch of the diagram is almost impossible to return to the branch of low cholesterol concentration.

A simple example as the one described in this part of the work highlight the role of mathematical

modeling in signaling pathways research. Intervention aimed at restoring normal signaling function could be tested first *in silico* and then if appropriate *in vivo*. This is the tenet of systems biology.

1.4. Systems biology

Systems biology is a growing area that aims to understand biological systems at a holistic level. It has applications in various areas related to medicine including biomarker discovery, improvement of drug target identification and prognosis assessment[27][28] . For example, using network analysis, one of the most pursued approaches in systems biology, we were able to identify Jagged-1-Notch as a therapeutic target for MS [29]. Systems biology is well positioned to respond to the increasing need of personalized medicine in complex diseases like multiple sclerosis and cancer by integrating theoretical and experimental research. From the theoretical side, it takes computational methods to study the structure and dynamics of biological networks. From the experimental side, it uses tools that permit the measure of genes, proteins and metabolites at a large scale, the omics revolution. The end-point is, by combining all pieces of information in computational models, to generate new knowledge about how the system works (i.e. the immune system) in health and disease and which are the best suitable approaches for therapy.

In an interesting case, biomarkers of the response to therapy in complex diseases, systems biology can be helpful at different levels. First it can provide new candidate genes and proteins coming from network and pathway analysis, to be assessed as biomarkers of the response to disease modifying drugs. Second, once a set of biomarkers have been validated, we can create network or dynamical models where to assess their functional implications. Finally, computational models can provide predictions or therapeutic strategies for restoring non-responder to the responder status.

1.5. Summary

In the previous sections there is an overview of signaling pathways, the possible dynamic behavior engendered, and some mathematical methods of analysis. Table 1.2 summarizes the explanation according to the dimension of the system. We also mentioned the importance of

Dimension	Qualitative behavior allowed
1D	Steady state
2D	Steady state or periodic behavior
nD ($n \geq 3$)	Chaos, steady state, periodic behavior . . .

Table 1.2: *Dynamic possibilities according to system dimension*

signaling pathways in biology and medicine. Finally a simple example showed the power of mathematical modeling in the elucidation of drug mechanism and lack of response.

Chapter 2

Mathematical methods

2.1. Introduction

Signaling pathways (SP) are chemical reaction networks susceptible of mathematical modeling. In this chapter we present the theory required to understand the dynamic properties of SP. To this end throughout the text we will follow a classic biochemical reaction network. In 1970, Edelstein proposed a reaction scheme which has multiple steady states and a hysteresis loop.[30] The structure of the model is displayed in Figure 2.1. The network is composed by three species
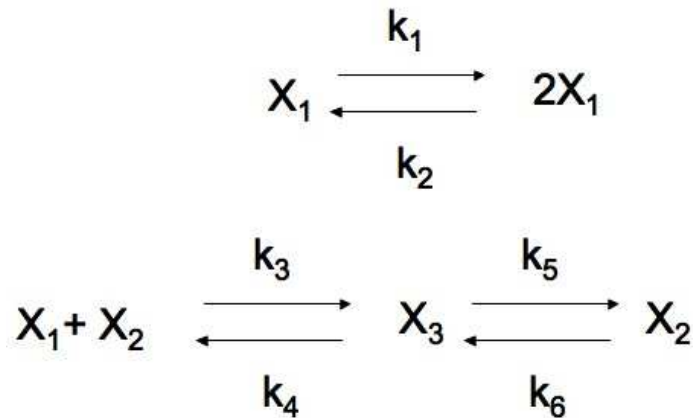
Figure 2.1: *Edelstein chemical reeaction network scheme.* $X_1 = A, X_2 = B, X_3 = C$

(A,B,C) and six reactions. The chemical mechanism represented is the specie A autocatalytic production and posterior enzymatic degradation. During the explanation we will assume that the chemical reactions occur in a well stirred chemical reactor with constant temperature. The following sets are used frequently in this chapter:

- $\mathbb{R}^n$ for the n Euclidean space

- $\mathbb{R}^n_{>0}$ for the positive orthant of $\mathbb{R}^n$

- $\mathbb{R}^n_{\geq 0}$ for the non-negative orthant of $\mathbb{R}^n$

The questions we will like to answer with the theory are these:

1. Does the system admit a positive equilibrium ?

2. Does the system admit multiple positive equlibria ?

3. In what range of parameters does equlibria or multiple equilibria occur?

This questions are not easy to solve and a lot of mathematics is needed in order to scratch the surface of the problem defined.

2.2. Definitions

A reaction network is composed by three sets[31]:

- Species, the chemical components of the network, S

- Complexes, the formal combinations of species that appear before and after of reaction arrows, C

- Reactions, specify how complexes are joined by arrows, R

There are some restrictions in reaction networks. Each element of S must appear in at least one complex. In the same way a complex must participate in at least one reaction and no complex is at the same time a reactant and a product. That is reactions of the form A→A are not allowed. In the Edelstein model $S=\{$ A,B,C $\}$, $C=\{$ A, 2A, A+B, C, B $\}$ and $R=\{$ A→2A,2A→A,A+B→C, C→A+B, C→B,B→C$\}$. We denote the number of species m, the number of complexes n and the number of reactions r. In the outgoing example $m = 3, n = 5$ and $r = 6$.

Each chemical entity is associated with a continous variable representing its concentration, measured in moles per litre, M, or in another appropriate unit. Only non-negative concentration are biologically realistic. We will use x_i to identify species concentrations. In this way A=x_1, B=x_2 and C=x_3. Complexes are denoted y and can be reactant complexes y and product complexes y'.Complexes can be reactants in a reaction an products in another reaction. Reactions are represented as $y \rightarrow y'$. A complex vector contains the stoichiometric coefficient of species y_i in complex y. Stoichiometries are usually integers. In open systems we use a special complex, known as the zero complex **0** whose all entries are 0 and has as many entries as the number of species of the system under study. As an example, the complex vector for complex 2A is $\begin{bmatrix} 2 \\ 0 \\ 0 \end{bmatrix}$.

The complex matrix Y is a m x n matrix that contains as columns the complex vectors. A reaction vector is the vector resulting after substracting the product complex from the reactant complex, $y' - y$. For the reaction A+B→C the reaction vector is $\begin{bmatrix} -1 \\ -1 \\ 1 \end{bmatrix}$.

The stoichiometric matrix, N, has m x r size and its columns represent the reaction vectors of the chemical network. For the Edelstein model we obtain

$$Y = \begin{bmatrix} 1 & 2 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix} \qquad N = \begin{bmatrix} 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 & 1 & -1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{bmatrix} \tag{2.1}$$

Chemical networks in general have conservation relations that can be identified calculating the left null space of N. If s is the rank of N, there are $m - s$ conservation relations. In our working example $s = 2$, therefore there is a conservation relation $x_2 + x_3 = c$. Conservation relations are also known as stoichiometric compatibility classes and have important consequences in the study of chemical reaction network equilibrium solution as outlined in the next section. Conservation relations are useful for reducing the dimension of the system under consideration.

A kinetics for a reaction network $\{S, C, R\}$ is a function that describes the rate at which chemical species interact to form products. The most common kinetics implemented so far is mass action kinetics (MA). In MA, the rate of the reaction is proportional to the product of the concentration of the reactant species and a kinetic constant k_i. The general form of MA is

$$K_{y \to y'}(x) = k_{y \to y'} \prod_{s \in S} x_s^{y_s} \tag{2.2}$$

where x is the concentration vector. Reaction parameters are positive and estimated using chemical principles or deduced from experiments. It is worth to mention that accurate parameters values are hardly known for complex chemical networks. The reaction rates form a vector $\mathbf{v} \in \mathbb{R}^r$. In the Edelstein case $v = (k_1 x_1, k_2 x_1{}^2, k_3 x_1 x_2, k_4 x_3, k_5 x_3, k_6 x_2)^t$

The matrix N can be viewed as the multiplication of two matrices $Y I_a$ where Y is the complex matrix and I_a is an n x r incidence matrix[31]. Each column of I_a represents a reaction and has an entry -1 for the reactant complex and 1 for the product complex. In the same way the reaction vector $\mathbf{v}$ is the product of $I_k \Psi(x)$. I_k is a r x n matrix containing in the columns the complexes and using as entries the kinetic constants for each reaction, k_i for reactants. $\Psi(x)$ is a monomial vector for the species participating in each complex. For the Edelstein example

$$I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix} \qquad I_k = \begin{bmatrix} k_1 & 0 & 0 & 0 & 0 \\ 0 & k_2 & 0 & 0 & 0 \\ 0 & 0 & k_3 & 0 & 0 \\ 0 & 0 & 0 & k_4 & 0 \\ 0 & 0 & 0 & k_5 & 0 \\ 0 & 0 & 0 & 0 & k_6 \end{bmatrix} \qquad \Psi(x) = \begin{pmatrix} x_1 \\ x_1^2 \\ x_1 x_2 \\ x_3 \\ x_2 \end{pmatrix} \tag{2.3}$$

An often cited matrix is the kinetic matrix A, which is the product of $I_a I_k$. For our example

$$A = \begin{bmatrix} -k_1 & k_2 & 0 & 0 & 0 \\ k_1 & -k_2 & 0 & 0 & 0 \\ 0 & 0 & -k_3 & k_4 & 0 \\ 0 & 0 & k_3 & -k_4 - k_5 & k_6 \\ 0 & 0 & 0 & k_5 & -k_6 \end{bmatrix} \tag{2.4}$$

Another useful matrix is α. This is an m x r matrix whose entries are the stoichiometries of the reactant species in the corresponding reaction:

$$\alpha = \begin{bmatrix} 1 & 2 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 & 0 \end{bmatrix} \tag{2.5}$$

The ODE system for a chemical network is of the form

$$\dot{x} = Nv(k, x) \tag{2.6}$$

where N is the stoichiometric matrix and **v** is the reaction vector. Using the decompositions previously explained the ODE system is also presented in the following form

$$\dot{x} = Y I_a I_k \Psi(x) \qquad or \qquad \dot{x} = Y A \Psi(x) \tag{2.7}$$

According to these considerations, the differential equations for the Edelstein network are

$$\dot{x}_1 = k_1 x_1 - k_2 x_1^2 - k_3 x_1 x_2 + k_4 x_3$$
$$\dot{x}_2 = -k_3 x_1 x_2 + k_4 x_3 + k_5 x_3 - k_6 x_2$$
$$\dot{x}_3 = k_3 x_1 x_2 - k_4 x_3 - k_5 x_3 + k_6 x_2 \tag{2.8}$$

A final definition is needed. The stoichiometric subspace for a reaction network is the linear subspace defined by

$$T = span(y' - y \in \mathbb{R}^r : y \to y' \in R) \tag{2.9}$$

In our example, the stoichiometric subspace is conformed by the reaction vectors {C-B,A}.The significance of T is that the concentration of each chemical is constrained to evolve in an affine subspace which is a parallel translate of T.

2.3. Equilibrium solutions

In the previous section we explained a framework for chemical reaction networks. Starting from the structure of chemical reactions, it is possible to derive in a unique and orderly way an ODE system for the dynamical study of CRN. Differential equation from a CRN are tied to the network structure. From this point on, if we know the reaction parameters (with appropriate units) and initial conditions , we could begin with a numerical analysis of the systems to observe how species concentrations change in time (Figure 2.2). That is, we are able to numerically integrate the system taking advantage of software implementation (MATLAB, Mathematica, XPP). Using numerical methods it is also customary the evaluation of the CRN response to parameter variation, in other words a sensitivity analysis. Two approaches are available for sensitivity analysis: local and global. In local analysis the value of a parameter is changed in small amounts and variation of a defined output is used as a reporter quantity. The field of metabolic control analysis (MCA) deals with this type of CRN examination . The methods for the global approach include Monte-Carlo simulations and Latin-Hypercube algorithm. The idea is to modify all the parameters at once and quantify the observed difference in a previously defined output.The hurdle
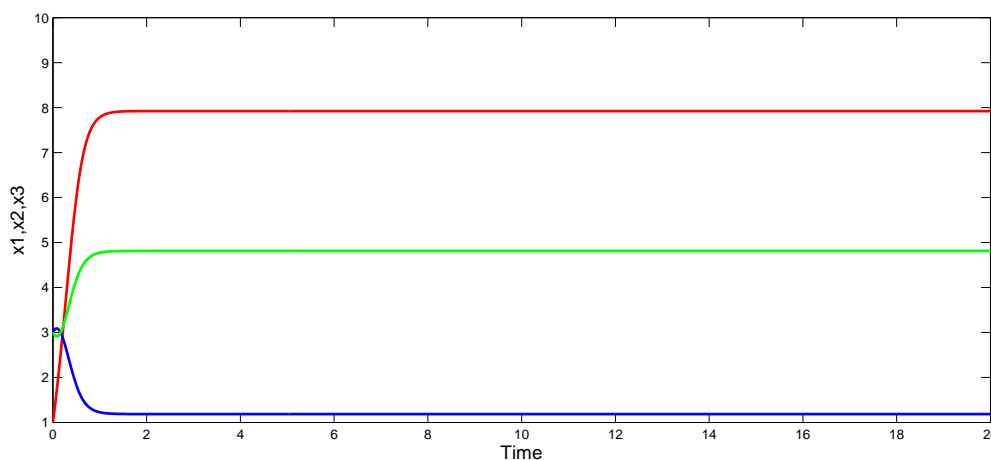
Figure 2.2: *Time series for the Edelstein system. Parameter values are $k_1 = 8.5, k_2 = k_3 = k_4 = k_5 = 1, k_6 = 0.2$. Initial condition are $x_1 = 1, x_2 = 3, x_3 = 3$ In red x_1, in blue x_2, in green x_3*

(as we mention before) is to obtain accurate parameters from scientific literature or experiments.

If parameters are difficult to obtain it is desirable to gain some insight of CRN dynamical capabilities using reaction structure alone. Some authors promote this approach and called it "complex biology with no parameters"[32]. In order to understand CRN we would like to solve the vectorial equation $N\mathbf{v}(k, x) = 0$ to find the stationary states where the system converges. But the point here is that even moderately large polynomials (as the ones derived from CRN) are poorly understood. We are facing to resolve a non-linear polynomial system in several variables. Two general theories have adressed this issue : Feinberg's Chemical Reaction Network Theory (CRNT) and Clarke's Stoichiometric Network Analysis (SNA).[33][34][35][31][36] It is not our objective to completely describe CRNT or SNA,but we believe it is essential to have a broad knowledge of CRNT and SNA underpinnings. We will discuss first CRNT and then SNA.

Chemical Reaction Network Theory - CRNT

CRNT is a theory proposed and devoloped by Martin Feinberg during the last 35 years[37]. The remarkable characteristic of CRNT is its capacity to discard multistability (the presence of two or more steady states) using only the network structure. The theory has produced two theorems based on a positive number easily calculated for any CRN. This number is called deficiency δ. To calculate δ we need to define what a linkage class means. Sets of complexes joined by reactions are called linkage classes. Linkage classes are known in graph theory as the weak components of a directed graph. Now, deficiency $\delta = n - l - s$ where n is the number of complexes, l is the number of linkage classes and s is the rank of the stoichiometric matrix. For the Edelstein network $\delta = 1$ because $n = 5, l = 2$ and $s = 2$. Another way to calculate $\delta = n - l - s$ is with the formula $\delta = rank(I_a) - rank(YI_a)$ where I_a and YI_a are used as defined in the previous section. In our example $rank(I_a) = 3$ and $rank(YI_a) = 2$, so $\delta = 1$.

One key consideration in CRNT is that solution trajectories for ODE systems derived from CRN are not free to move in a random path across the positive orthant. Stoichiometric compatibility classes (conservation relations) impose a great restriction in the possible dynamic behavior since all trajectories starting from different initial conditions must stay inside the stoichiometric compatibility class defined by the CRN under study. For this reason when CRNT ask a question about multistability it is presented in the following form : The question of real interest is whether the differential equations for a reaction system can admit multiple positive equilibria **within a stoichiometric compatibility class**. A graphic is useful to illustrate how for different values of the conservation relation it is possible to have one, two or three steady states (Figure 2.3).
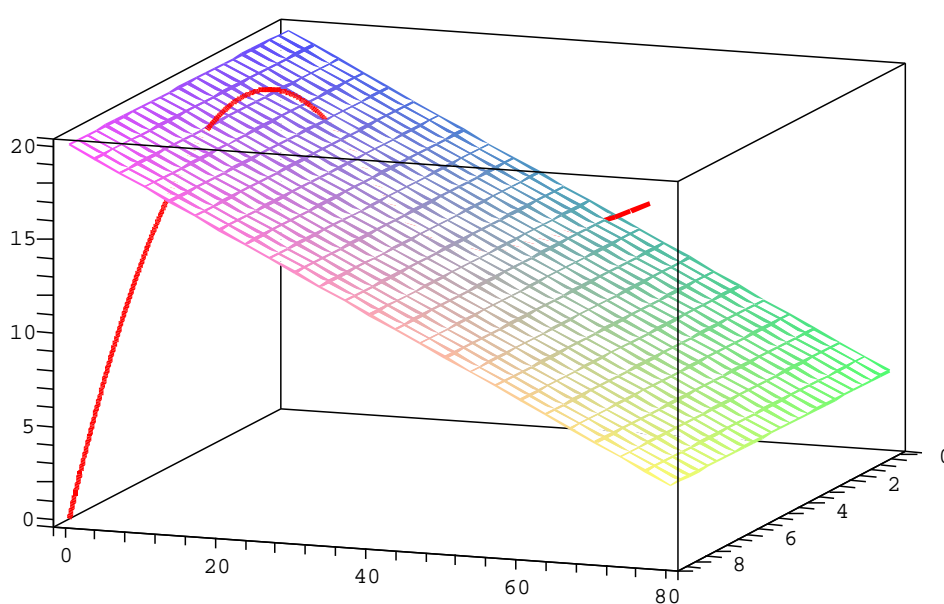
Figure 2.3: *Number of equilibrium solutions of the Edelstein system changing the value of conservation relation. Figure kindly provided by Dr Sergio Ardanza-Trevijano, Department of Physics and Applied Mathematics, University of Navarra.*

The zero deficiency theorem stays that for a CRN that is weakly reversible with $\delta = 0$ there exists within each compatibility class only one stable asymptotic equilibrium and there cannot exist cyclic trajectories. Multistability is discarded. We have not explained what weakly reversible means. A network is reversible if all its reactions are reversible. We will use Feinberg's words to define weak reversibility. A network is weakly reversible if whenever there is a directed arrow path leading from complex y to complex y', there is also a path leading from y' back to y. The deficiency zero theorem also states that if the CRN is not weakly reversible, the ODE system cannot admit a positive equilibrium or a cyclic trajectory with a positive composition. Deficiency zero theorem is proved in [31][38] not published until 1995 even it was originally stated in 1972

For networks of $\delta \geq 1$ the deficiency one theorem and the deficiency one algorithm could be applied to discard multistability. We will consider reaction networks with l linkage classes. Let δ denote the deficiency of the network; let δ_θ denote the deficiency of of the θth linkage class and suppose that both of the following conditions are satisfied:

1. $\delta_\theta \leq 1$

2. $\delta = \sum_{\delta=1}^{l} \delta_\theta$

If the network is weakly reversible, then for any mass action kinetics, the ODE system admits precisely one equilibrium in each positive stoichiometric class. There are some networks with $\delta = 1$ that violate part 2 of the deficiency one theorem and can display multistability. This is the case of the Edelstein network. The deficiency for the overall network is 1, but the deficiency for each of the two linkage classes is 0. The deficiency one theorem is proved in [38][36].

CRNT has gain broad attention because with little knowledge can give answers to difficult questions as the ones presented in the introduction. However, CRNT must be viewed as a negative theory valid to discard hypothesis. This approach has been followed in recent CRNT applications in the MAPK pathway and in the apoptosis model analyzed in chapter 4[39].

Stoichiometric Network Analysis - SNA

The reaction rate vector $v(x,k)$ of the system $\dot{x} = N\mathbf{v}(k,x) = 0$ lies in the kernel (null space) of N. The work of Bruce L Clarke investigates in detail this solution set and a wealth of literature uses his ideas to the study of (mainly) metabolic networks. SNA tries to get insight to CRN network dynamics using the reaction space instead of the concentrations space. SNA has also been applied to the analysis of CRN stability. The vector of reaction rates can be decomposed in $diag(k)\phi(x)$. For the Edelstein network:

$$v(x,k) = \begin{pmatrix} k_1 x_1 \\ k_2 x_1^2 \\ k_3 x_1 x_2 \\ k_4 x_3 \\ k_5 x_3 \\ k_6 x_2 \end{pmatrix} = \begin{pmatrix} k_1 & 0 & 0 & 0 & 0 & 0 \\ 0 & k_2 & 0 & 0 & 0 & 0 \\ 0 & 0 & k_3 & 0 & 0 & 0 \\ 0 & 0 & 0 & k_4 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_5 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_6 \end{pmatrix} \begin{pmatrix} x_1 \\ x_1^2 \\ x_1 x_2 \\ x_3 \\ x_3 \\ x_2 \end{pmatrix} = diag(k)\phi(x)$$

In this way $\dot{x} = N diag(k)\phi(x)$ and since the matrix $N diag(k)$ is constant when we calculate the jacobian of $f(x,k) = N diag(k)\phi(x)$, we only have to find the derivative of the monomial $\phi(x)$. This is:

$$D_x f(x,k) = N diag(k) D_x \phi(x) = N diag(k) \begin{pmatrix} 1 & 0 & 0 \\ 2x_1 & 0 & 0 \\ x_2 & x_1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix}$$

In biology, there is interest in positive equilibria, $x_i^0 > 0$ for $i = 1 \ldots m$ in which $f(x^0, k) = 0$. The equilibrium is stable only if all the eigenvalues of the jacobian $D_x f(x^0, k)$ have negative real

parts. If parameter values are known, it is possible to calculate the equilibrium points numerically or in the other case symbolically. Symbolic solution, if feasible, is superior because allows us to study the dynamic of the system in relation to the reaction rates and analyze the existence of multistability and bifurcations. We will follow an analytic point of view using the Edelstein example.

Let us suppose that a equilibrium is known, $x^0 = (x_1^0, x_2^0, x_3^0) > 0$. The vector h_0 contains the inverse of x_1^0, x_2^0, x_3^0, $h^0 = (h_1^0, h_2^0, h_3^0) = (\frac{1}{x_1^0}, \frac{1}{x_2^0}, \frac{1}{x_3^0}) > 0$. The matrices $diag(x^0)$ and $diag(h^0)$ are inverse among them. A variable change $w = diag(h^0)x$ is useful to express the system $x = f(x, k)$ in w terms . The relation between the w space and the x is displayed in the next diagram.

$$
\begin{array}{ccc}
x & \xrightarrow{\;f(,k)\;} & f(x, k) \\
{\scriptstyle diag(x^0)}\big\uparrow & & \big\downarrow{\scriptstyle diag(h^0)} \\
w & \xrightarrow[\;\tilde{f}(,k)\;]{} & \tilde{f}(, k)
\end{array}
$$

To the vector $\mathbf{1} = \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}$ of w corresponds the steady state x^0 of the x space according to the scheme:

$$
\begin{array}{ccc}
x^0 & \xrightarrow{\;f(,k)\;} & 0 \\
{\scriptstyle diag(x^0)}\big\uparrow & & \big\downarrow{\scriptstyle diag(h^0)} \\
1 & \xrightarrow[\;\tilde{f}(,k)\;]{} & 0
\end{array}
$$

The system $\dot{x} = f(x, k)$ is transformed into the system $\dot{w} = diag(h^0)x = diag(h^0)f(x, k) = \check{f}(w, k)$. The next relations hold:

$$\check{f}(w, k) = diag(h^0)f(x, k) = diag(h^0)Nv(x, k) = diag(h^0)Ndiag(k)\phi(x)$$

$$D_w\check{f}(w, k) = diag(h^0)Ndiag(k)D_w\phi(x) = diag(h^0)Ndiag(k)D_x\phi(x)D_w x =$$

$$= diag(h^0)Ndiag(k)D_x\phi(x)diag(x_0)$$

From the last formula we can deduce that $D_w\check{f}(w, k) = diag(h^0)D_x f(x, k)diag(x^0)$. As the jacobian matrices are similar, the eigenvalues are the same for the two systems. It is possible to study x^0 stability of the systems $\dot{x} = f(x, k)$ taking into account the stability of the $\mathbf{1}$ steady state of $\dot{w} = \check{f}(w, k)$. A link between the matrix α (pg 25) and the monomial vector ϕ is stated in the following equation:

$$D_x\phi(x)diag(k) = diag(\phi(x))\alpha^t$$

$$(2.10)$$

In the Edelstein CRN this is:

$$
D_x\phi(x)diag(x) = \begin{pmatrix} 1 & 0 & 0 \\ 2x_1 & 0 & 0 \\ x_2 & x_1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix} \begin{pmatrix} x_1 & 0 & 0 \\ 0 & x_2 & 0 \\ 0 & 0 & x_3 \end{pmatrix} = \begin{pmatrix} x_1 & 0 & 0 \\ 2x_1^2 & 0 & 0 \\ x_1x_2 & x_1x_2 & 0 \\ 0 & 0 & x_3 \\ 0 & 0 & x_3 \\ 0 & x_2 & 0 \end{pmatrix}
$$

$$
diag(\phi(x))\alpha^t = \begin{pmatrix} x_1 & 0 & 0 & 0 & 0 & 0 \\ 0 & x_1^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & x_1x_2 & 0 & 0 & 0 \\ 0 & 0 & 0 & x_3 & 0 & 0 \\ 0 & 0 & 0 & 0 & x_3 & 0 \\ 0 & 0 & 0 & 0 & 0 & x_2 \end{pmatrix} \begin{pmatrix} 1 & 0 & 0 \\ 2 & 0 & 0 \\ 1 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix} = \begin{pmatrix} x_1 & 0 & 0 \\ 2x_1^2 & 0 & 0 \\ x_1x_2 & x_1x_2 & 0 \\ 0 & 0 & x_3 \\ 0 & 0 & x_3 \\ 0 & x_2 & 0 \end{pmatrix}
$$

We can deduce that

$$
D_w\check{f}(1,k) = diag(h^0)Ndiag(k)D_x\phi(x^0)diag(x^0)
$$

$$
= diag(h^0)Ndiag(k)diag(\phi(x^0))\alpha^t
$$

and with this formula it is straightforward to calculate the jacobian as long as the equilibrium points x^0 are known. The reaction vector $v(x,k)$ has been presented as $v(x,k) = diag(k)\phi(x)$, if the reaction rates in the equilibrium $v(x^0,k) = diag(\phi(x^0))$, it is convenient to write $diag(v(x^o,k) = diag(k)diag(\phi(x^0))$ and substituting in the jacobian $D_w\check{f}(1,k) = diag(h^0)Ndiag(v(x^0,k))\alpha^t$. If we return to the original jacobian

$$
D_xf(x^0,k) = diag(x^0)D_w\check{f}(1,k)diag(h^0)
$$

$$
= diag(x^0)diag(h^0)Ndiag(v(x^0,k))\alpha^t diag(h^0) = Ndiag(v(x^0,k))\alpha^t diag(h^0)
$$

In the Edelstein network using $v^0 = (x^0,k)$ we have

$$
D_xf(x^0,k) = \begin{pmatrix} 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 & 1 & -1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{pmatrix} \begin{pmatrix} v_1^0 & 0 & 0 & 0 & 0 & 0 \\ 0 & v_2^0 & 0 & 0 & 0 & 0 \\ 0 & 0 & v_3^0 & 0 & 0 & 0 \\ 0 & 0 & 0 & v_4^0 & 0 & 0 \\ 0 & 0 & 0 & 0 & v_5^0 & 0 \\ 0 & 0 & 0 & 0 & 0 & v_6^0 \end{pmatrix} \begin{pmatrix} 1 & 0 & 0 \\ 2 & 0 & 0 \\ 1 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix} diag(h^0) =
$$

$$
\begin{pmatrix} v_1^0 & -v_2^0 & -v_3^0 & v_4^0 & 0 & 0 \\ 0 & 0 & -v_3^0 & v_4^0 & v_5^0 & -v_6^0 \\ 0 & 0 & v_3^0 & -v_4^0 & -v_5^0 & v_6^0 \end{pmatrix} \begin{pmatrix} 1 & 0 & 0 \\ 2 & 0 & 0 \\ 1 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix} diag(h^0) =
$$

$$\begin{pmatrix} v_1^0 - 2v_2^0 - v - 3^0 & -v_3^0 & v_0^4 \\ -v_3^0 & -v_3^0 - v_6^0 & v_4^0 + v_5^0 \\ v_3^0 & v_3^0 + v_6^0 & -v_4^0 - v_5^0 \end{pmatrix} diag(h^0)$$

and this the jacobian in terms of the equilibria reaction rates.

A vector v^0 is an equilibrium vector only if it fulfills the following conditions:

1. $Nv^0 = 0$ for $v^0 \geq 0$

2. $v^0 = v(x^0, k)$ for at least one $x^0 \in \mathbb{R}_{\geq 0}^m$

Now, we will try to determine the set $K_v = Null(N) \cap \mathbb{R}_{\geq 0}^m$. In the running example, this is to find $v > 0$ such that

$$Nv = \begin{pmatrix} 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 & 1 & -1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{pmatrix} =$$

$$\begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix} v_1 + \begin{pmatrix} -1 \\ 0 \\ 0 \end{pmatrix} v_2 + \begin{pmatrix} -1 \\ -1 \\ 1 \end{pmatrix} v_3 + \begin{pmatrix} 1 \\ 1 \\ -1 \end{pmatrix} v_4 + \begin{pmatrix} 0 \\ 1 \\ -1 \end{pmatrix} v_5 + \begin{pmatrix} 0 \\ -1 \\ -1 \end{pmatrix} v_6 = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}$$

After the manipulation we have the same CRN with constant velocities. The net production of chemical species will be:

$$\begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \end{pmatrix} = \begin{pmatrix} 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 & 1 & -1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{pmatrix} = \begin{pmatrix} v_1 - v_2 - v_3 + v_4 \\ v_4 - v_3 + v_3 - v_6 \\ v_3 - v_4 - v_5 + v_6 \end{pmatrix}$$

We are asking for the constant velocities distribution that maintain species concentration invariable in time. This is the solution to the system:

$$v_1 - v_2 - v_3 + v_4 = 0$$

$$v_4 - v_3 + v_5 - v_6 = 0$$

$$v_3 - v_4 - v_5 + v_6 = 0$$

$$v_1 \geq 0, v_2 \geq 0, v_3 \geq 0, v_4 \geq 0, v_5 \geq 0, v_6 \geq 0$$

It looks like a linear algebra problem, but the real task is to find solutions for a system of equalities and inequalities. It turns out that the solution set K_v of $Nv = 0, v \geq 0$ is the intersection of 12 semispaces in $\mathbb{R}^6$, a pointed polyhedral cone. There is an equivalent representation of the pointed polyhedral cone as the set of non-negative linear combinations of a finite vector set

known as *extreme rays*[40].Several software implementations are available to calculate extreme rays.(CellNetAnalyzer, Metatool to name a few)[41]. In our case the extreme rays of K_v have been obtained with the program Metatool:

$$
E = \begin{pmatrix}
1 & 0 & 1 & 0 & 0 \\
1 & 0 & 0 & 1 & 0 \\
0 & 1 & 1 & 0 & 0 \\
0 & 1 & 0 & 1 & 0 \\
0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 1 & 1
\end{pmatrix}
$$

The columns of E are linear independent and for this reason each vector of K_v has a unique defined coordinate, termed *convex coordinate*. Defining $E_1 \ldots E_5$ the extreme rays and $j = (j_1, j_2, j_3, j_4, j_5)^t \geq 0$, K_v is of the form $v = Ej = \sum_{i=1}^{5} j_i E_i$. In the CRN under study this is:

$$
v = \begin{pmatrix}
1 & 0 & 1 & 0 & 0 \\
1 & 0 & 0 & 1 & 0 \\
0 & 1 & 1 & 0 & 0 \\
0 & 1 & 0 & 1 & 0 \\
0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 1 & 1
\end{pmatrix}
\begin{pmatrix}
j_1 \\ j_2 \\ j_3 \\ j_4 \\ j_5
\end{pmatrix}
= \begin{pmatrix}
j_1 + j_3 \\
j_1 + j_4 \\
j_2 + j_3 \\
j_2 + j_4 \\
j_3 + j_5 \\
j_4 + j_5
\end{pmatrix}
$$

As stated before, the jacobian of the system $\dot{x} = f(x, k) = Nv(x, k)$ in an equilibrium point x^0 is $D_x f(x^0, k) = N diag(v(x^0, k)) \alpha^t diag(h^0)$. If $v^0 = v(x^0, k)$ and $D_x f(x^0, k) = N diag(v^0) \alpha^t diag(h^0)$, then the next formula holds

$$
N diag(v^0) \alpha^t diag(h^0) = N diag(Ej) \alpha^t diag(h^0)
$$

For our case

$$
\begin{pmatrix}
1 & -1 & -1 & 1 & 0 & 0 \\
0 & 0 & -1 & 1 & 1 & -1 \\
0 & 0 & 1 & -1 & -1 & 1
\end{pmatrix}
\begin{pmatrix}
j_1 + j_3 & 0 & 0 & 0 & 0 & 0 \\
0 & j_1 + j_4 & 0 & 0 & 0 & 0 \\
0 & 0 & j_2 + j_3 & 0 & 0 & 0 \\
0 & 0 & 0 & j_2 + j_4 & 0 & 0 \\
0 & 0 & 0 & 0 & j_3 + j_5 & 0 \\
0 & 0 & 0 & 0 & 0 & j_4 + j_5
\end{pmatrix}
\begin{pmatrix}
1 & 0 & 0 \\
2 & 0 & 0 \\
1 & 1 & 0 \\
0 & 0 & 1 \\
0 & 0 & 1 \\
0 & 1 & 0
\end{pmatrix}
diag(h^0)
$$

$$
= \begin{pmatrix}
-j_1 - j_2 - 2j_4 & -j_2 - j_3 & j_2 + j_4 \\
-j_2 - j_3 & -j_2 - j_3 - j_4 - j_5 & j_2 + j_3 + j_4 + j_4 \\
j_2 + j_3 & j_2 + j_3 + j_4 + j_5 & -j_2 - j_3 - j_4 - j_5
\end{pmatrix} diag(h^0)
$$

Some mathematical theory links the sign of the matrix components with the eigenvalues. This type of theory allows to perform a qualitative study of the stability of the system based on the matrix structure without parameter values difficult to obtain in several scientific disciplines[42]. As the terms in the diagonal of $diag(h^0)$ are positive, the sign distribution of $D_x f(x^0, k)$ is the same of js matrix. BL Clarke put a great emphasis on the study of the sign distribution of the matrix in convex coordinates.

BL Clarke associates to extreme rays the corresponding extreme currents (or elementary modes-EM) defined as subnetworks determined by the non-null elements of the extreme rays. The biochemical interpretation is that non zero entries in EM are active reactions in steady state while zero entries are inactive in equilibrium. The basic consideration is to decompose the CRN in subnetworks more easy to resolve using the reaction space instead of the concentration space. The subnetworks are the minimal group of reactions for which the CRN admits steady states. In the example this is[6]:

$$E_1 = \begin{bmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} = A \leftrightarrows 2A \quad E_2 = \begin{bmatrix} 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \end{bmatrix} = A + B \leftrightarrows C \quad E_3 = \begin{bmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \end{bmatrix} = A \to 2A \quad A + B \to C \to B$$

$$E_4 = \begin{bmatrix} 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 1 \end{bmatrix} A \leftarrow 2A \quad A + B \leftarrow C \leftarrow D \quad E_5 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \end{bmatrix} = C \leftrightarrows B$$
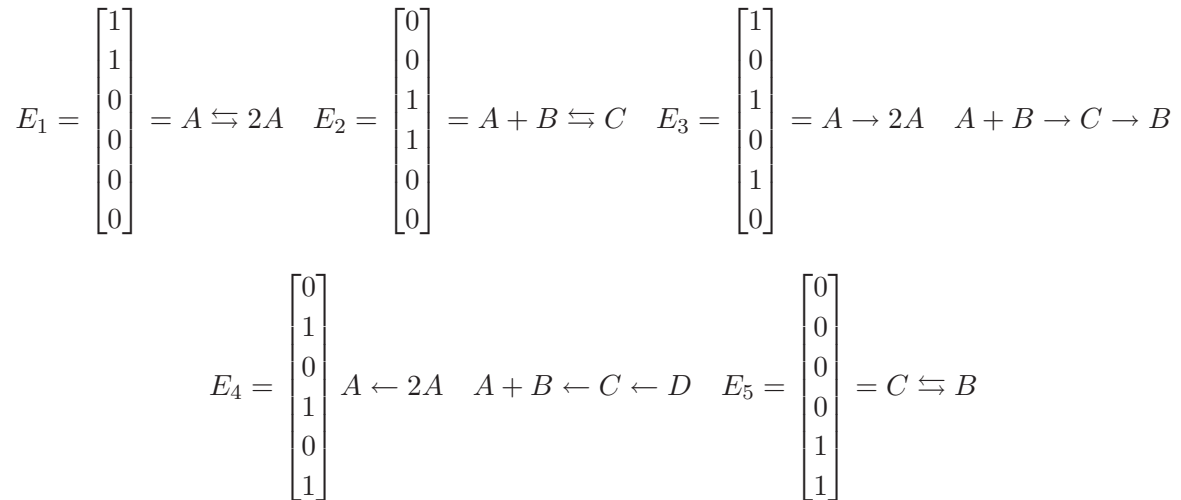
EM are classified in stoichiometric generators or positive circuits according to the result of the product $I_a E_i$. If $I_a E_i = 0$ the E_i is a positive circuit, otherwise E_i is a stoichiometric generator. Recently C. Conradi based on research by K Gaterman has demonstrated that a network capable of multistability must hold at minimum one stoichiometric generator[43][44][45]. The idea behind this statement is that in networks with positive circuits only, $rank(I_a) = rank(YI_a)$. Taking in consideration the alternative deficiency definition $\delta = rank(I_a) - rank(YI_a)$ it is clear that $\delta = 0$ and applying the deficiency zero theorem there is no possibility for multistability in this group of CRN. In the Edelstein system, elementary modes E_3 and E_4 are stoichiomatric generators while E_1, E_2 and E_5 are positive circuits[46].

A complementary approach - algebraic geometry

The ODE system derived from a CRN endowed with mass action kinetics is a polynomial system in several variables. Most of the time these polynomials are non-linear making them difficult to solve. During the last years there has been a growing interest in applying algebraic geometry methods to the study of CRN in equilibrium. As in the case for SNA and CRNT we are not interested in a deep presentation of algebraic geometry, but in order to exploit its capabilities we will make a brief overview of the main concepts required to deal with CRN. We refer the interested reader to an excellent treatise on this topic.[47]

We will first define in a broad sense what is a ring. A ring is a set where the addition, subtraction, multiplication and division operations can be defined with the usual properties (commutative,distributive,etc). If the non-null elements have an inverse, the ring now is a field. In

this context the set of real numbers $\mathbb{R}$ is a field while the integers $\mathbb{Z}$ are a ring. A monomial in $x_1, \ldots x_n$ is a product of the form $x_1^{b_1} x_2^{b_2} \ldots x_n^{b_n}$, where $b_1, b_2 \ldots b_n$ are nonnegative integers. For example, $x^a = x_1^2 x_2 x_3^4$ is a monomial and $|a| = |(2, 1, 4)| = 2 + 1 + 4 = 7$ is the grade of the monomial. A polynomial is a combination of monomials that can be represented in the following form $g = \sum_a a_a x^b$ where a_a are coefficients. Taking a coefficient field k, $k[x_1, x_2, \ldots, x_n]$ denotes the ring of all polynomials in $x_1, x_2, \ldots, x_n$ with coefficients in k. We now require another definition. An ideal I is a subset of $k[x_1, x_2, \ldots, x_n]$ if satisfies the following conditions[47]:

- $0 \in I$

- If $f, g \in I$ then $f + g \in I$

- If $f \in I$ and $h \in k[x_1, x_2, \ldots, x_n]$ then $hf \in I$

This definition is used to understand the Hilbert Basis Theorem that states that every ideal in $k[x_1, x_2, \ldots, x_n]$ is finitely generated. A set of generators of an ideal is called a basis. That is, there exists $f_1 \ldots f_m \in I$ such that $I = < f_1 \ldots f_m > = \{g_1 f_1 + \ldots g_m f_m; g_1 \ldots g_m \in k[x_1, x_2, \ldots x_n]\}$

A variety is the set of solutions of a polynomial system. We can consider the system $f_1(x_1, \ldots x_n) = \cdots = f_m(x_1, \ldots x_n) = 0$ and the variety $V(f_1 \ldots f_m) = \{x \in \mathbb{R}^n \quad ; f_1(x) = \cdots = f_m(x) = 0\}$ The ideal $I = < f_1, \ldots f_m >$ contains infinite polynomials, but $V(I) = \{x \in \mathbb{R}^n; f(x) = 0 \text{ for all } f \in I\} = V(f_1, \ldots f_m)$. For this reason, in order to find the solutions of the system we are interested in obtaining an adequate basis of $I = < f_1, \ldots f_m >$. If we are willing to solve the equation $Nv(x, k) = 0$ we would like to get a basis that permit us to eliminate some variables and back-substitute to obtain the value of the remainder variables. One type of generator or basis that permits applying elimination theory for an ideal is the Gröbner basis with lexicographic order. The definitions of Gröbner basis and lexicographic order lie ahead, but first it is important to define what an order means. As stated before a polynomial is a combination of monomials. An order is a procedure to exactly rearrange the terms of a polynomial in ascending or descending way. Several monomial orderings have been described including lexicographic (lex), graded lexicographic (grlex) and graded reverse lexicographic order (grevlex). Let us take two monomial x^b and x^a. The vector difference $b - a$ is used to define the lex order or any other order presented so far. The definition for the most common polynomial orders are:

1. Lex order: $x^b >_{lex} x^a$ iff the left-most nonzero entry in b-a is positive. An example will clarify this term. $x^3 y^2 z$ and $x^2 y z^2$ are monomials in $[x, y, z]$. The exponent vector for the first monomial is (3,2,1) and (2,1,2) for the second monomial. The difference vector is (1,1,-1) and the left-most nonzero entry is positive, so $x^3 y^2 z >_{lex} x^2 y z^2$.

2. Graded order (grlex) : $x^b >_{grlex} x^a$ iff $|b| > |a|$ or $|b| = |a|$ and $b >_{lex} a$. In the example $x^3 y^2 z >_{grlex} x^2 y z^2$.

3. Graded reverse lexicographic order (grevlex): $x^b >_{grevlex} x^a$ iff $|b| > |a| or |b| = |a|$ and the right-most nonzero entry in b-a is negative. In the running example this is $x^3 y^2 z >_{grevlex} x^2 y z^2$.

A Gröbner basis for an ideal I is one in which the polynomial remainder with respect to the basis determines membership of I. It is a basic result that a Gröbner basis always exist for any ideal and

any monomial order, but according to the monomial order of choice the result can be different. Some standard methods for Gröbner basis calculation include Buchberger's algoritms, Gröbner walk and F4 algorithm. General use mathematical software such as MAPLE and Mathematica have implementations of algorithms for Gröbner basis calculation. Buchberger algorithm relies in the use of S-polynomial that for two polynomial f_1 and f_2 is of the form:

$$S(f_1, f_2) = \frac{s}{LT(f_1)} f_1 - \frac{s}{LT(f_2)} f_2$$

where $s = LCM(LM(f_1), LM(f_2))$. LM stands for leading monomial, LCM for least common multiple and LT for leading term. An example will clarify how S-polynomial is calculated. $f_1 = x^2 y + 2xy^2$, $f_2 = 3y^2 + 2$ and using the lex order, the terms to obtain $S(f_1, f_2)$ are:

1. Leading monomial (LM): For f_1 the leading monomial is $x^2 y$ and for f_2 is y^2

2. Leading term (LT): $LT(f_1) = x^2 y$ and $LT(f_2) = 3y^2$

3. $s = x^2 y^2$

Replacing in the formula $S(f_1, f_2) = \frac{x^2 y^2}{x^2 y} x^2 y + 2xy^2 - \frac{x^2 y^2}{3y^2} 3y^2 + 2$. This is $S(f_1, f_2) = 6xy^3 - 2x^2$. The Gröbner basis obtained in this work were determined using the Groebner package in MAPLE. The computational cost of calculating a Gröbner basis is hard and some problems are almost never solved in realistic time, even that theoretically it is always possible to obtain a Gröbner basis for an ideal. The main use of this type of calculations is to find the solutions of a polynomial system. Let us show how this can be done. Our example is:

$$x^2 - y - z = 0$$

$$x + y^2 + z - 12 = 0$$

$$x + y + z - 6 = 0$$

The Gröbner basis with respect the lex order is given by the three polynomials:

$$h_1 = 83z - 66 - 18z^2 + z^3$$

$$h_2 = -12y - 13z + 2yz + z^2 + 42$$

$$h_3 = x + y + z - 6$$

h_1 only involves the variable z, so the solution to $83z - 66 - 18z^2 + z^3$ gives the possible values of z for the system under study. In this case $z = (1, 6, 11)$. It is easy to find the solutions of y and x just by replacing in h_2 and h_3. This procedure can be performed through an elimination step (eliminate x and y) and then applying an extension step.

The idea behind applying algebraic geometry to CRN is that the ODE system derived from CRN endowed with mass action kinetics is a polynomial set conformed by monomials representing the production and elimination rates of chemical species. If SNA and CRNT allow to identify the possibility of certain dynamical behavior, algebraic geometry is an essential tool to find where this behavior can appear.

2.4.   Region of multistability

In the last section there was an overview of the current methodologies to infer CRN qualitative dynamics from network structure alone. SNA and CRNT were presented as means of discarding the capacity of a specified CRN to display multistability. However, there is still an open question to localize the region where this property can be shown up. Algebraic geometry methods are a natural choice to adress that need. To ilustrate how to use algebraic geometry to uncover the place of multistability, we will continue dissecting the Edelstein network. As already mentioned this network displays multistability for certain reaction parameter values. In Figure 2.3 appears how according to different location of equilibrium curve and stoichiometric compatibility class intersection there is one, two or even three steady states. To identify the exact points of intersection we follow this procedure

1. Reduce N to its row reduced echelon form, RD

2. Identify stoichiomatric compatibility classes

3. Based on RD construct new equations multyplying RD to the vector of reaction rates $\mathbf{v}$(k,x).

4. Add to the previous system the equation representing stoichiomatric compatibility classes (conservation relations). We will call this system AD

5. Calculate the Gröbner basis of AD using an elimination order (i.e. lexicographic order)

6. Normally this basis will content only one variable and the conservation parameter

The procedure for the Edelstein system yield this result

$$RD = \begin{bmatrix} 1 & -1 & 0 & 0 & -1 & 1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{bmatrix} \qquad v(k,x) = \begin{bmatrix} k_1 x_1 \\ k_2 x_1^2 \\ k_3 x_1 x_2 \\ k_4 x_3 \\ k_5 x_3 \\ k_6 x_2 \end{bmatrix} \qquad x_2 + x_3 = c \qquad (2.11)$$

The new system AD is

$$k_1 x_1 - k_2 x_1^2 - k_5 x_3 + k_6 x_2 = 0$$
$$k_3 x_1 x_2 - k_4 x_3 - k_5 x_3 + k_6 x_2 = 0$$
$$x_2 + x_3 - c = 0 \qquad (2.12)$$

Now it is time to calculate the Gröbner basis for the new system. x_1 represents the product in the Edelstein network, so it is of interest to represent equilibrium solutions of x_1 in terms of different c values. The MAPLE command to obtain the basis is gbasis([f,g,h],plex(x_2, x_3, x_1, c) where f,g,h are each of the elements of the polynomial system AD. The complete basis is a huge polynomial system and therefore we don't reproduce it here. Using the parameters described in [31], the first element of the basis is $10x_1 c - 2c + 10x_1^3 - 63x_1^2 - 187x_1$. This is a third grade polynomial in x_1. In Figure 2.4 appears a bifurcation diagram for the equilibrium solution of x_1 in terms of c. It is evident that only for a small range of c there is the possibility of multistability. Algebraic
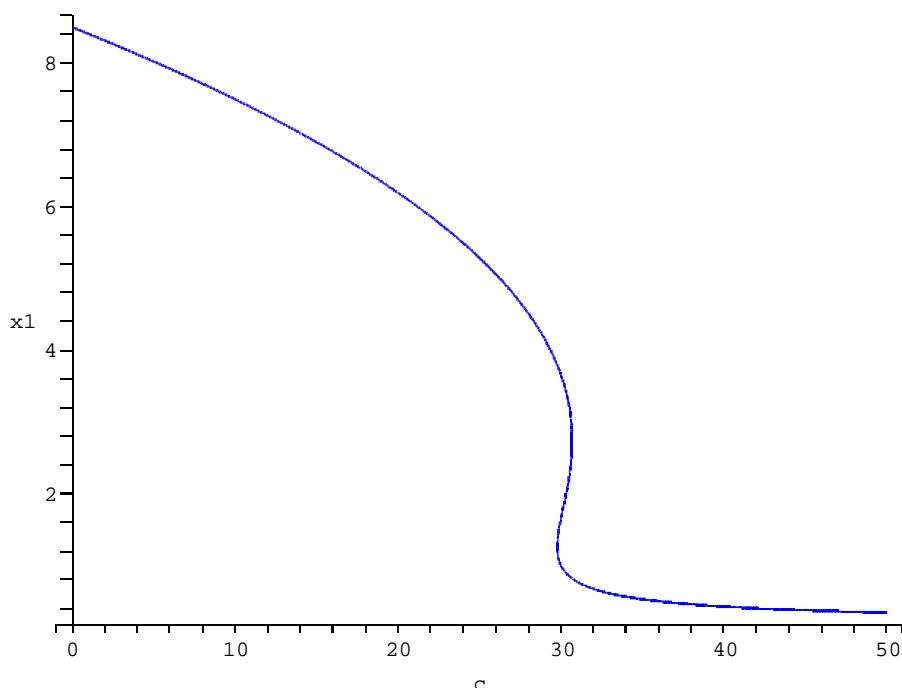
Figure 2.4: *Bifurcation diagram x_1 vs c where $c = x_2 + x_3$*

geometry methods allowed us to identify which interval of stoichiometric compatibility class there exist multiple steady states. CRNT gives a clear answer of whether or not a CRN has the capacity of multistability but tells nothing about the exact place where this occurs. The method developed in this section when correctly applied is able to identify the region of multistability.

2.5. The inverse problem

An ODE system could be derived from a CRN. When the CRN is analysed using mass action kinetics the ODE systems is a polynomial differential equation. Powerful methodologies are available to classify CRN according to the capacity of exhibiting certain dynamic behavior (CRNT, SNA). It would be of great help if this kind of approaches could be incorporated to the study of polynomial differential equations not derived a priori from CRN. In fact, the first step is to identify which polynomial systems are originated from CRN. It turns out that the sufficient and necessary condition for a polynomial differential equation to be represented as a CRN is very simple. This is best understood with an example [48]. Let us consider the Lorenz system

$$
\begin{aligned}
\dot{x} &= -\sigma(x - y) \\
\dot{y} &= rx - y - \boxed{xz} \\
\dot{z} &= xy - bz
\end{aligned}
\tag{2.13}
$$

The term $\boxed{xz}$ in the second polynomial implies that x and z associate and degrade y but y does not appear in the monomial xz. The terms similar to xz in the y equation are known as negative

cross-effect terms and are used to identify which polynomial differential equations are derived from CRN [48]. The following statement can be applied to classify polynomial differential equations in two categories: derived or not derived from CRN. *A polynomial differential equation system can be considered as the mass action type deterministic model of a chemical reaction if and only if it does not contain terms expressing negative cross-effect.* In other words, every time a negative term appears in the right hand side of an equation, the variable that we are differentiating must appear as a factor in the negative term

Therefore the Lorenz system is not obtained from a CRN. Let us now consider the Rössler system

$$
\begin{aligned}
\dot{x} &= -y - z \\
\dot{y} &= x + ay \\
\dot{z} &= b + z(x - c)
\end{aligned}
\tag{2.14}
$$

In the Rössler system there are is also a negative cross-effect term ($-z$ in the x equation). For equations with no cross-effect terms is possible to obtain the underlying chemical network using an algorithm.

An algorithm to obtain a CRN from an ODE system

In this part, the entire analysis will be performed taking as an example a polynomial system proposed by J Toth in 1979.[48]

$$
\begin{aligned}
\dot{x} &= -2\alpha x^2 v + 2\gamma z^4 \\
\dot{y} &= 3\alpha x^2 v - 3\beta y^3 v^2 \\
\dot{z} &= 4\beta y^3 v^2 - 4\gamma z^4 \\
\dot{v} &= \alpha x^2 v - 2\beta y^3 v^2 + \gamma z^4
\end{aligned}
\tag{2.15}
$$

The steps in the algorithm and how are they applied to the Toth networks are

1. Discard negative cross-effect terms. No this kind of terms in Toth network.

2. Count the number of species. This is easy, is the same number of variables in the system. in the example the set of species $S = \{x, y, z, v\}$

3. Identifiy the reactant complexes and construct the appropriate complex vector. Reactant complexes constitute the reaction rate monomials. In the Toth network there are three monomials $x^2 v, y^3 v^2, z^4$ corresponding to the reactant complexes $2X + V, 3Y + 2V, 4Z$ respectively. The complex vector using as a row order x, y, z, v are

$$
2X + V = \begin{bmatrix} 2 \\ 0 \\ 0 \\ 1 \end{bmatrix} \qquad 3Y + 2V = \begin{bmatrix} 0 \\ 3 \\ 0 \\ 2 \end{bmatrix} \qquad 4Z = \begin{bmatrix} 0 \\ 0 \\ 4 \\ 0 \end{bmatrix}
\tag{2.16}
$$

4. Identify the number of reactions.

5. Construct the stoichiometric matrix N. The coefficients in the equations represent the value of the reaction vector in each reaction. In the Toth network there are three reactions and as a column order we will take the reactant complexes arranged in this form $2X + V, 3Y + 2V, 4Z$. The matrix for the running network is

$$
N = \begin{bmatrix} -2 & 0 & 2 \\ 3 & -3 & 0 \\ 0 & 4 & -4 \\ 1 & -2 & 1 \end{bmatrix} \tag{2.17}
$$

6. Reconstruct the product complexes from the reactant complexes and the stoichiometric matrix. Remember that each column in N represents a reaction vector and a reaction vector is the difference between product complex and reactant complex. Therefore the product complex is just the sum of the respective reaction vector and the reactant complex. The product complexes identified according to the rate constant are

$$
P_1 = \begin{bmatrix} 0 \\ 3 \\ 0 \\ 2 \end{bmatrix} \qquad P_2 = \begin{bmatrix} 0 \\ 0 \\ 4 \\ 0 \end{bmatrix} \qquad P_3 = \begin{bmatrix} 2 \\ 0 \\ 0 \\ 1 \end{bmatrix} \tag{2.18}
$$

Therefore the product complexes are $3Y + 2V, 4Z, 2X + V$ respectively.

7. With the reactant complexes and product complexes specified the process is over.

The CRN for the Toth differential equations is

$$
2X + V \xrightarrow{\ \alpha\ } 3Y + 2V
$$
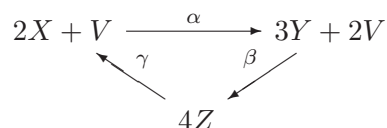
Now we can apply some of the concepts developed in previous sections. The number of species for the CRN is 3, the number of complexes is 3, there is only one linkage class and the rank(N) is 2. The deficiency of this network $\delta = 0$. This result indicates that no matter what values reaction parameters take there is only one asymptotic not periodic steady state. It is remarkable how a highly non-linear polynomial differential equation as the one described by Toth has a simple dynamic behavior identified using only the structure of the CRN inferred from the ODE system.

2.6.   Summary

In this chapter we covered the mathematical background neccesary for biochemical reaction network analysis. The emphasis was steady analysis using a mixture of parametric and non-parametric methods. We also decribe two algorithms. The first one can find the steady states of a CRN from a formula derived through algebraic geometry methods. The second algorithm allows the reverse-engineering of an ODE system to a CRN. This reconstruction makes possible to implement the robust methods explained to elucidate CRN dynamics in cases not related to chemistry or biochemistry.

Chapter 3

Signaling motifs

In this chapter we cover signaling pathway motifs. In particular we are interested not in structural analysis but in the dynamic behavior of some reaction motifs. For this, the theory explained in chapter 2 is widely applied. The first section addresses in detail chemical reaction networks of ligand-receptor interactions. Section 3.2 deals with the capability of reactions schemes to engender multistability and in finding the exact place in concentration space where this occurs.

3.1. Ligand receptor interaction

The first step in the signal transduction process is ligand receptor binding. Several drugs bind to membrane receptors and for this reason solid uderstanding of ligand receptor dynamics is fundamental to the identification and improvement of new drug targets. In this section we will analyze a model for ligand receptor interaction interested in elucidate the qualitative dynamical properties of receptor function. The receptor has two possible states, active and inactive. The model is composed by five chemical species representing inactive receptor ($R_1 = x_1$), active receptor ($R_2 = x_3$), ligand ($L = x_2$) and the complex of the ligand with the two possible receptor conformations ($C_1 = x_4, C_2 = x_5$) . Figure 3.1 depicts the reaction scheme [49]. There are four complexes and eight reactions. The stoichiometric matrix and the reaction rate vector are

$$
N = \begin{pmatrix}
-1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 \\
0 & 0 & -1 & 1 & 1 & 0 & 1 & -1 \\
1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 \\
0 & 0 & 1 & -1 & -1 & 1 & 0 & 0
\end{pmatrix}
\qquad
v(k,x) = \begin{bmatrix}
k_1 x_1 x_2 \\
k_2 x_2 x_3 \\
k_3 x_2 x_3 \\
k_4 x_5 \\
k_5 x_5 \\
k_6 x_4 \\
k_7 x_4 \\
k_8 x_1 x_2
\end{bmatrix}
\qquad (3.1)
$$
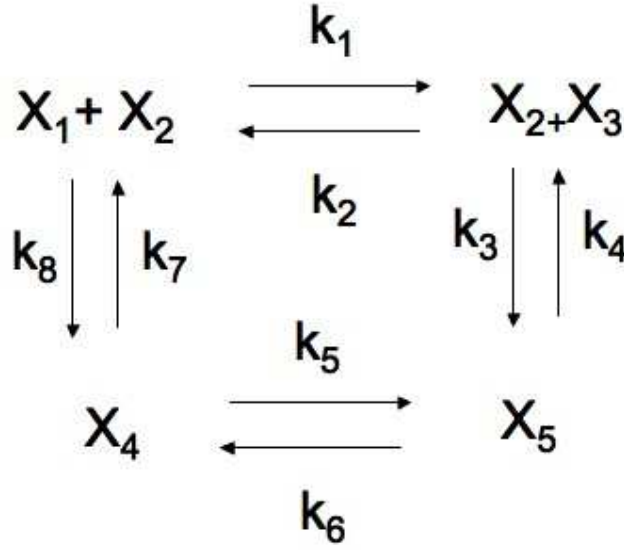
Figure 3.1: *Ligand receptor chemical reaction network*

with this information, the ODE systems for this CRN is

$$\dot{x}_1 = -k_1 x_1 x_2 + k_2 x_2 x_3 + k_7 x_4 - k_8 x_1 x_2$$
$$\dot{x}_2 = -k_3 x_2 x_3 + k_4 x_5 + k_7 x_4 - k_8 x_1 x_2$$
$$\dot{x}_3 = k_1 x_1 x_2 - k_2 x_2 x_3 - k_3 x_2 x_3 + k_4 x_5$$
$$\dot{x}_4 = k_5 x_5 - k_6 x_4 - k_7 x_4 + k_8 x_1 x_2$$
$$\dot{x}_5 = k_3 x_2 x_3 - k_4 x_5 - k_5 x_5 + k_6 x_4 \tag{3.2}$$

The rank of N is 3, so there are two conservation relations that can be calculated using the left null space of N. In this CRN

$$C_1 = \begin{pmatrix} 1 \\ 0 \\ 1 \\ 1 \\ 1 \end{pmatrix} \qquad C_2 = \begin{pmatrix} 0 \\ 1 \\ 0 \\ 1 \\ 1 \end{pmatrix}$$

are a basis for the left null space of N corresponding to $x_1 + x_3 + x_4 + x_5 = \alpha$ and $x_2 + x_4 + x_5 = \beta$, where α and β represent total receptor amount and total ligand amount respectively. These are the stoichiometric compatibility classes. For each pair of positive α and β the stoichiometric compatibility classes describe a $\mathbb{R}^5$ affine subspace where the solutions of the ODE system stay according to the initial conditions that satisfy α and β. Now we can study the equilibrium behavior of the model. The approach is to implement the knowledge developed in chapter 2. We will identify using two methods (SNA and CRNT) the posssibility or not of this network to exhibit multistability. Trying to solve the ODE system is a daunting task. The problem is to

find the solution of a 5 dimensional non-linear system. As explained in the previous chapter this could be accomplished using Gröbner basis. In this example we do not take advantage of this methodology. Instead, to characterize receptor ligand qualitative behavior, we first calculate the elementary modes (EM) of the receptor system, that is the extreme rays of the polyhedral cone that constitute solutions of the equation $Nv(k,x) = 0$. The EM are

$$E_1 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} \quad E_2 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} \quad E_3 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \end{pmatrix} \quad E_4 = \begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \end{pmatrix} \quad E_5 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \end{pmatrix} \quad E_6 = \begin{pmatrix} 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 1 \end{pmatrix}$$

A neccesary condition for a CRN to display multistability is that $I_a E_i \neq 0$. Remember that I_a is a n x r matrix containing as entry -1 for the reactant complex and 1 for the product complex in the respective column. I_a matrix for the system under study is

$$\begin{pmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 \\ 1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 \\ 0 & 0 & 1 & -1 & -1 & 1 & 0 & 0 \end{pmatrix}$$

The result of the product $I_a E$ where E is the matrix of EM column vectors is a matrix with all its entries equal to 0 which means receptor ligand binding model has not the capablity of multistability. The same result can be obtained calculating the deficiency of this CRN. The number of complexes n is 4, rank(N) (s) is 3 and there is only one linkage class. If $\delta = n - l - s$, the deficiency for this CRN is 0 and according to the deficiency zero theorem there is only one asymptotically stable equilibrium for each stoichiometric compatibility class. The last statement holds for any choice of reaction parameters.

Receptor trafficking

Receptor proteins are tightly controlled by different cellular processes including receptor internalization, degradation and recycling. The objective is to regulate how the signal (in form of ligand) is transduced in gene expression changes. It has been demonstrated how receptor trafficking is an important mechanism for the adequate function of signaling pathways. In Figure 3.2 appears a representation of a CRN that takes into account receptor internalization and renovation. The chemical species are:

1. x_1 free membrane receptor

2. x_2 ligand

3. x_3 ligand receptor complex

4. x_4 internalized receptor
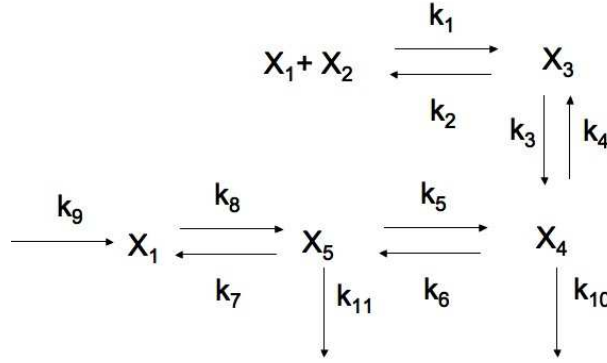
Figure 3.2: *Ligand receptor trafficking chemical reaction network*

5. x_5 free cytoplasmatic receptor

Free receptor binds to the ligand and produces a receptor ligand complex that after activating an scaffold protein is internalized in endosomes. Some amount of internalized receptor is released to the cytoplasm where it has the cappacity to travel to cell membrane and in this way re-initiate the cycle. There are degradation terms for the internalized and free cytoplasmatic receptor. There is also a production term for the membrane bound receptor. The stoichiometric matrix N and the reaction vector $v(k,x)$ are:

$$
N = \begin{pmatrix}
-1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 \\
-1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & -1 & 1 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 & 0 & 0 & -1
\end{pmatrix}
\qquad
v(k,x) = \begin{pmatrix}
k_1 x_1 x_2 \\
k_2 x_3 \\
k_3 x_3 \\
k_4 x_4 \\
k_5 x_4 \\
k_6 x_5 \\
k_7 x_5 \\
k_8 x_1 \\
k_9 \\
k_{10} x_4 \\
k_{11} x_5
\end{pmatrix}
$$

The ODE system is

$$
\begin{aligned}
\dot{x}_1 &= -k_1 x_1 x_2 + k_2 x_3 + k_7 x_5 - k_8 x_1 + k_9 \\
\dot{x}_2 &= -k_1 x_1 x_2 + k_2 x_3 \\
\dot{x}_3 &= k_1 x_1 x_2 - k_2 x_3 - k_3 x_3 + k_4 x_4 \\
\dot{x}_4 &= k_3 x_3 - k_4 x_4 - k_5 x_4 - k_{10} x_4 \\
\dot{x}_5 &= k_5 x_4 - k_6 x_5 - k_7 x_5 + k_8 x_1 - k_{11} x_5
\end{aligned}
\tag{3.3}
$$

Now we will like to analyze the qualitative behavior of the system. There are six complexes (n, we must not forget the zero complex) one linkage class (l) and the rank (s) of N is five. So, deficiency

$\delta = n - l - s = 0$. As the previous example, according to the deficiency zero theorem there is no possibility for multistability no matter the value of reaction parameters. It is interesting to compare this model with a previous study published by Zi et al[50]. This group proposes a similar model in which all the reactions are reversible except the $x_4 \leftrightarrows x_5$ that is considered irreversible. Now, the new CRN is not weakly reversible. In this scenario there is only a minor change in the system. However, for the model developed by Zi et al. it is impossible that in the equilibrium all chemical species have a non-negative concentration.

3.2. Reaction motifs

Signaling pathways are composed of repetitive elements known as signaling motifs. A more elaborate definition proposed by Uri Alon is "signaling motifs are patterns of interconnections that recur in many different parts of a network at frequencies much higher than those found in randomized networks" Signaling motifs have not only structural importance but also functional relevance as information processing devices[51][52][53]. Most of the research undertaken in this area has been devoted to understand network motifs in transcription networks. However, some specific motifs appear more frequently in SP than in transcription networks. In this section we review three signaling motifs found in biochemical reaction networks.

Two substrate enzyme catalysis

In this scheme an enzyme binds two substrates to catalyze the formation of a product[37]. The substrate binding could be in an ordered or in an unordered fashion. The CRN are displayed in Figure 3.3. We are interested in the possibility or not of multistability. Ordered substrate
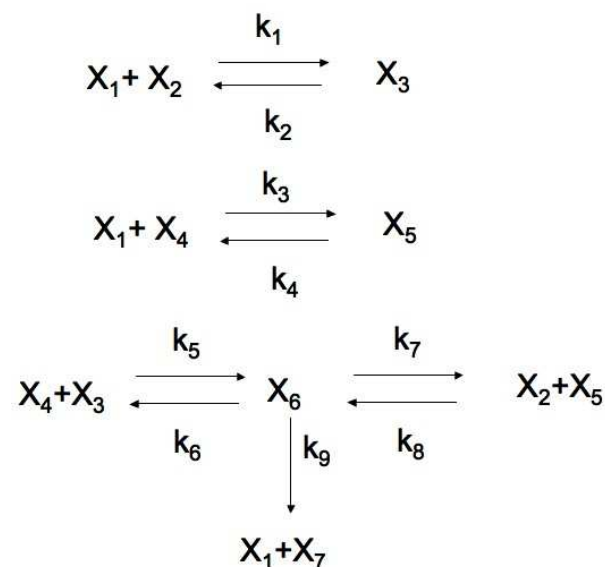

Figure 3.3: *Unordered two substrate enzyme catalysis CRN*

binding has zero deficiency and thus has no capability to exhibit bistability. In contrast, unordered binding CRN has deficiency 1 but the deficiency of each of the two linkage classes is 0, suggesting the plausibility of multistability. Here we apply the algorithm explained in chapter 2 to find the region of multistability. The chemical species are:

- x_1=free enzyme

- x_2= substrate 1

- x_3= enzyme-substrate one complex ES1

- x_4= substrate 2

- x_5= enzyme-substrate two complex ES2

- x_6= enzyme-substrate 2-substrate 2 complex ES1S2

- x_7= product

The enzyme binds substrate 1 and substrate 2 in an unordered mechanism to conform enzyme-substrate complexes. The complex with the two substrates and the enzyme (ES1S2) is the final step to generate the product. There are turnover reactions for the substrates and a degradation term for the product. N and $v(k,x)$ are of the form:

$$
N = \begin{pmatrix}
-1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
-1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 1 & 0 & -1 & 0 & 0 \\
1 & -1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 \\
0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & -1
\end{pmatrix}, \quad
v(k,x) = \begin{pmatrix}
k_1 x_1 x_2 \\
k_2 x_3 \\
k_3 x_1 x_4 \\
k_4 x_5 \\
k_5 x_4 x_3 \\
k_6 x_6 \\
k_7 x_6 \\
k_8 x_2 x_5 \\
k_9 x_6 \\
k_{10} \\
k_{11} \\
k_{12} x_2 \\
k_{13} x_4 \\
k_{14} x_7
\end{pmatrix}
$$

The ODE system for this CRN is:

$$
\begin{aligned}
\dot{x}_1 &= -k_1 x_1 x_2 + k_2 x_3 - k_3 x_1 x_4 + k_4 x_5 + k_9 x_6 \\
\dot{x}_2 &= -k_1 x_1 x_2 + k_2 x_3 + k_7 x_6 - k_8 x_2 x_5 + k_{10} - k_{12} x_2 \\
\dot{x}_3 &= k_1 x_1 x_2 - k_2 x_3 - k_5 x_3 x_4 + k_6 x_6 \\
\dot{x}_4 &= -k_3 x_1 x_4 + k_4 x_5 - k_5 x_3 x_4 + k_6 x_6 + k_{11} - k_{13} x_4 \\
\dot{x}_5 &= k_3 x_1 x_4 - k_4 x_5 + k_7 x_6 - k_8 x_2 x_5 \\
\dot{x}_6 &= k_5 x_3 x_4 - k_6 x_6 - k_7 x_6 + k_8 x_2 x_5 - k_9 x_6 \\
\dot{x}_7 &= k_9 x_6 - k_{14} x_7
\end{aligned} \tag{3.4}
$$

The system has one conservation relation representing the total enzyme concentration $c = x_1 + x_3 + x_5 + x_6$. The reduced form of N along with a basis of the left null space C are:

$$R = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & -1 \\ -1 & 0 & 1 & -1 & 1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & -1 \end{pmatrix}, \quad C = \begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 1 \\ 1 \\ 0 \end{pmatrix}$$

In order to find the equilibrium solution of the CRN we change the system for the equivalent:

$$Rv(k, x) = 0$$

$$x_1 + x_3 + x_5 + x_6 - c = 0$$

Now the algebraic system is:

$$\begin{aligned} k_1 x_1 x_2 - k_2 x_3 - k_7 x_6 + k_8 x_2 x_5 - k_{14} x_7 &= 0 \\ k_3 x_1 x_4 - k_4 x_5 + k_7 x_6 - k_8 x_2 x_5 &= 0 \\ k_5 x_3 x_4 - k_6 x_6 - k_7 x_6 + k_8 x_2 x_5 - k_{14} x_7 &= 0 \\ k_9 x_6 - k_{14} x_7 &= 0 \\ k_{10} - k_{12} x_2 - k_{14} x_7 &= 0 \\ k_{11} - k_{13} x_4 - k_{14} x_7 &= 0 \\ x_1 + x_3 + x_5 + x_6 - c &= 0 \end{aligned} \tag{3.5}$$

With the ideal composed by the last six polynomials it is feasible to calculate a Gröbner basis with the lexicographic order $(x_1, x_2, x_3, x_4, x_5, x_6, x_7, c)$. Using the Maple Gröbner package we can calculate a basis for the system. c serves as a bifurcation parameter(Figure 3.4). The first polynomial of the basis can be taken as a mean to construct a bifurcation diagram of x_7 vs c because it only contains terms with x_7 and c. This diagram indicates how the x_7 steady state changes as the total enzyme varies. It can be seen that for c values between 0.3 and 1.3 the system has three steady states. The upper and lower branches are stable while the intermediate is unstable. There is evidence that two-substrate binding is the mechanims behind some cyclin-dependent kinase catalyzed reactions in the cell cycle machinery[54][55].

Enzyme catalysis with mixed inhibition

Enzyme inhibitors are molecules with the ability to interfere the catalytic activity of an enzyme. In the mixed inihibition reaction, the inhibitor binds to the free enzyme and also to the complexes conformed by the enzyme and the substrate. Mixed inhibitors have a different binding site to the enzyme that the one used for the substrate. Figure 3.5 shows the CRN scheme[56]. The chemical species participating in this CRN are:
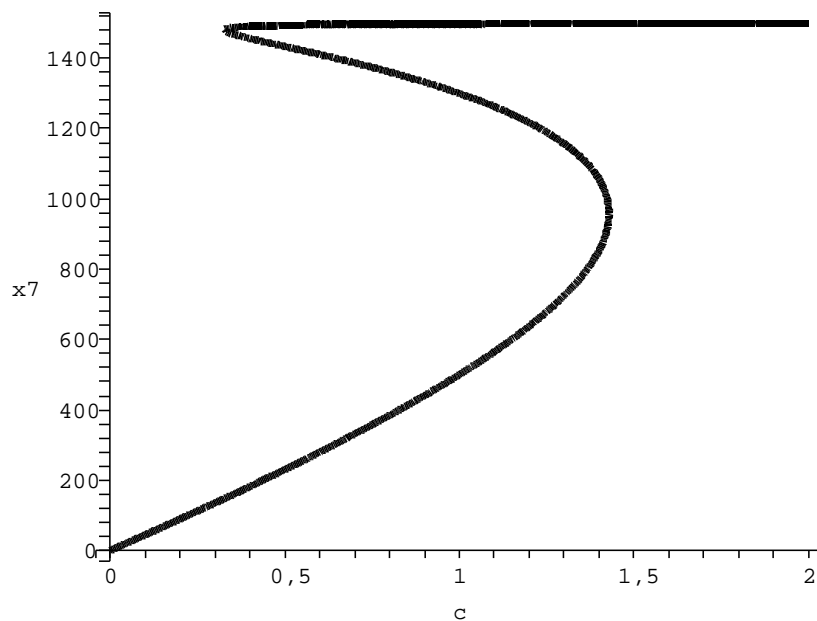
1. x_1= Enzyme

Figure 3.4: *Bifurcation diagram x_7 vs c. Parameter values are:* $k_1 = 93.43, k_2 = 2539, k_3 = 481.6, k_4 = 1183, k_5 = 1555, k_6 = 121192, k_7 = 1688, k_8 = 0.02213, k_9 = 85842, k_{10} = 2500, k_{11} = 1500, k_{12} = 1, k_{13} = 1, k_{14} = 1$
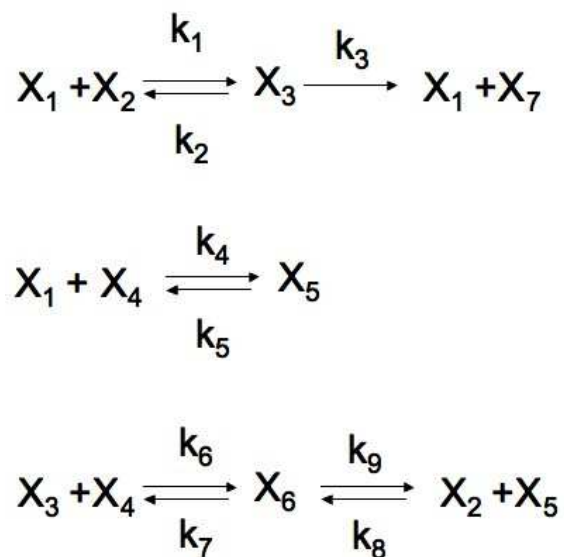

Figure 3.5: *Enzyme catalysis with mixed inhibition chemical reaction network*

2. x_2= Substrate

3. x_3= Enzyme-substrate complex

4. x_4= Inhibitor

5. x_5= Enzyme-inhibitor complex

6. x_6= Enzyme-substrate inhibitor complex

7. x_7= Product

The CRN has seven chemical species and 13 reactions. The stoichiometric matrix N along with the reaction vector v are:

$$
\begin{pmatrix}
-1 & 1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 1 & 0 \\
1 & -1 & -1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 1 & -1 & 1 & 0 & 0 & 0 & -1 & -1 & 0 & 1 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0
\end{pmatrix},
\quad
v(k,x) =
\begin{pmatrix}
k_1 x_1 x_2 \\
k_2 x_3 \\
k_3 x_3 \\
k_4 x_1 x_4 \\
k_5 x_5 \\
k_6 x_3 x_4 \\
k_7 x_6 \\
k_8 x_6 \\
k_9 x_2 x_5 \\
k_{10} x_2 \\
k_{11} x_4 \\
k_{12} x_7 \\
k_{13} \\
k_{14}
\end{pmatrix}
$$

We will like to implement again the algorithm described in chapter 2 to elucidate whether or not the CRN can exhibit multistability. The first step is row reduced the stoichiometric matrix. In this case rank(N)=6 and there is a conservation relation for the total amount of enzyme $x_1 + x_3 + x_5 + x_6 = c$:

$$
R =
\begin{pmatrix}
1 & -1 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1
\end{pmatrix},
\quad
C =
\begin{pmatrix}
1 \\
0 \\
1 \\
0 \\
1 \\
1 \\
0
\end{pmatrix}
$$

The algebraic system to solve is:

$$Rv(k,x) = 0$$

$$x_1 + x_3 + x_5 + x_6 - c = 0$$

and this is:

$$k_1 x_1 x_2 - k_2 x_3 - k_8 x_6 + k_9 x_2 x_5 - k_{12} x_7 = 0$$
$$k_3 x_3 - k_{12} x_7 = 0$$
$$k_4 x_1 x_4 - k_5 x_5 + k_8 x_6 - k_9 x_2 x_5 = 0$$
$$k_6 x_3 x_4 - k_7 x_6 - k_8 x_6 + k_9 x_2 x_5 = 0$$
$$k_{10} x_2 + k_{12} x_7 - k_{13} = 0$$
$$k_{11} x_4 - k_{14} = 0$$
$$x_1 + x_3 + x_5 + x_6 - c = 0 \tag{3.6}$$

We now calculate the Gröbner basis of the system with the lexicographic order $(x_1, x_2, x_3, x_4, x_5, x_6, x_7, c)$ The first polynomial of the basis has only x_7 and c terms and serves as an analytic solution of the bifurcation diagram represented in Figure 3.6. The bifurcation diagram allow us to observe the
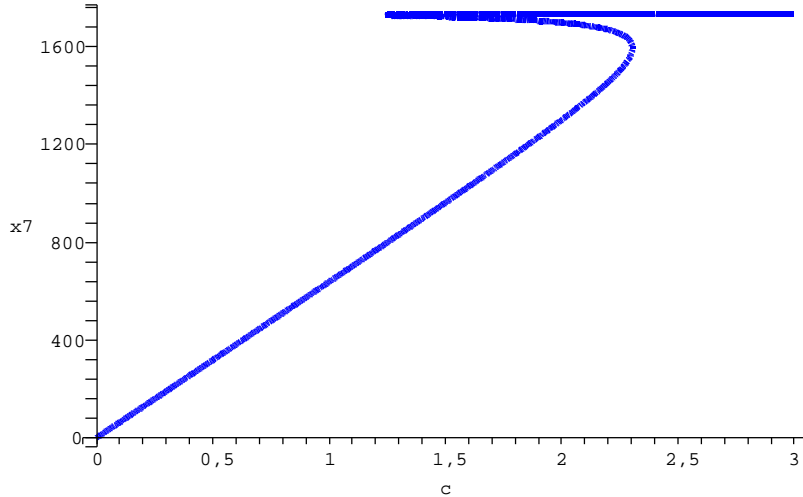
Figure 3.6: *Bifurcation diagram x_7 vs c. Parameter values are: $k_1 = 25979.537, k_2 = 3.3722455, k_3 = 5844.999, k_4 := 5.334155, k_5 = 16623.325, k_6 = 12200.836, k_7 = 1472.3849, k_8 = 15145.809, k_9 = 9647.324, k_{10} = 1, k_{11} = 1, k_{12} = 1, k_{13} = 1734.2661, k_{14} = 1$*

place of multistability in the mixed enzyme inhibitor system. This simple CRN is a well known component of signaling pathways and metabolic networks. Mixed inhibition is a key regulatory module in protein metabolism and cleavage modulated pathways as the coagulation cascade [57].

Autophosphorylating motif

Autophosporylation kinases are involved in important cellular processes. For example ATM (ataxia telangiectasia mutated) protein is a DNA damage sensor implicated in the activation of p53 a tumor supressor protein mutated in almost 50 % of human cancers. In the same way calcium/calmodulin-dependent protein kinase II is another autophosporylating molecule responsible of glutamate receptor phosporylation and therefore of changing electric properties of neurons[58]. In this motif an active kinase (K*) can activate itself to produce two activated
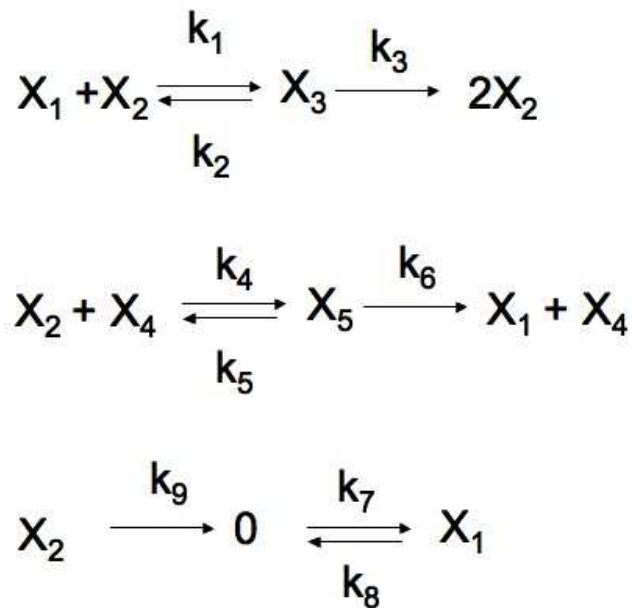
Figure 3.7: *Autophosphorylation motif chemical reaction network*

K* molecules. Phosphatases (P) de-phosphorylate K* proteins. The components of this reaction network are :

- Inactive kinase x_1

- Active kinase x_2

- Complex inactive-active kinase x_3

- Phosphatase x_4

- Complex phosphatase-active kinase x_5

The reaction scheme is depicted in Figure 3.7. There is turnover reaction for the inactive kinase x_1 and a degradation reaction for the active kinase x_2. As usual the stoichiometric network N and the reaction vector $v(x, k)$ are:

$$
N = \begin{pmatrix}
-1 & 1 & 0 & 0 & 0 & 1 & 1 & -1 & 0 \\
-1 & 1 & 2 & -1 & 1 & 0 & 0 & 0 & -1 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0
\end{pmatrix}, \quad
v(k, x) = \begin{pmatrix}
k_1 x_1 x_2 \\
k_2 x_3 \\
k_3 x_3 \\
k_4 x_2 x_4 \\
k_5 x_5 \\
k_6 x_5 \\
k_7 \\
k_8 x_1 \\
k_9 x_2
\end{pmatrix}
$$

There is a conservation relation $x_4 + x_5 = ptot$ representing the total amount of phosphatase. The ODE system for this motif is:

$$\dot{x}_1 = -k_1 x_1 x_2 + k_2 x_3 + k_6 x_5 + k_7 - k_8 x_1$$
$$\dot{x}_2 = -k_1 x_1 x_2 + k_2 x_3 + 2k_3 x_3 - k_4 x_2 x_4 + k_5 x_5 - k_9 x_2$$
$$\dot{x}_3 = k_1 x_1 x_2 + k_2 x_3 - k_3 x_3$$
$$\dot{x}_4 = -k_4 x_2 x_4 + k_5 x_5 + k_6 x_5$$
$$\dot{x}_5 = k_4 x_2 x_4 - k_5 x_5 - k_6 x_5 \tag{3.7}$$

If we employ the method described in chapter 2 it is possible to identify an interval where the system displays bistability in the stoichiometric compatibility class(Figure 3.8). Now, it
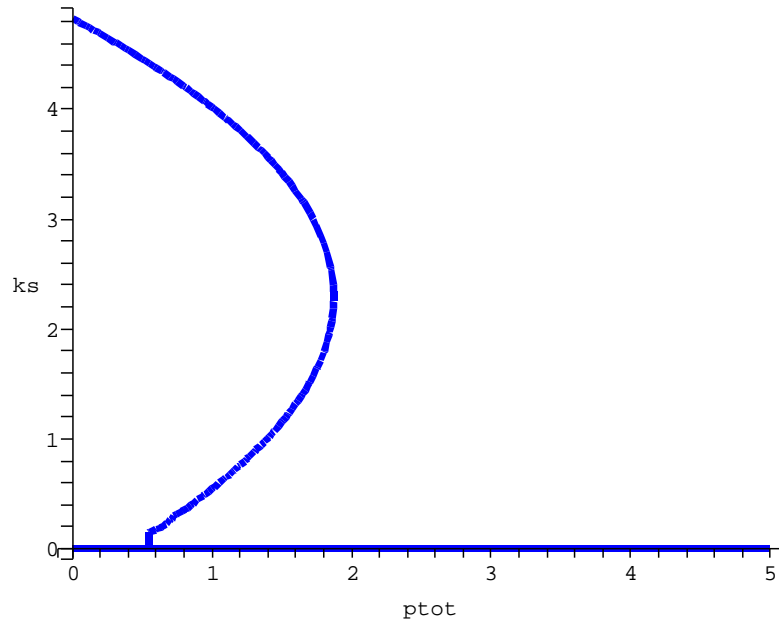
Figure 3.8: *Bifurcation diagram active kinase (k) vs total phosphatase (p). Parameter values are* $k_1 = 3, k_2 = 0.1, k_3 - 3, k_4 = 40, k_5 = 0.1, k_6 = 10, k_7 = 2.5, k_8 = 0.5, k_9 = 0.5$

is interesting to study what is the dynamic behavior of this system if there is no turnover or degradation reactions. In this case the terms $k_7, k_8 x_1, k_9 x_2$ of equation 3.7 are eliminated from the ODEs. Another conservation relation appears $x_1 + x_2 + 2x_3 + x_5 = ktot$. $ktot$ is the total kinase available. As observed in Figure 3.9 bistability is still possible in the closed case. This point us to interrogate which signaling motifs are robust against subtle modification in their structure to serve as reliable switches. In table appears such classification. If the system dynamics depends on the turnover rate it will be useful to delineate how the autophosporylating switch varies according to k_8 and k_9 parameter change. The bifurcation diagram displayed in Figure 3.10 allows to conclude that for a certain interval of these degradation $(k_8, k_9 = d)$ parameters, there exist mulitistability.
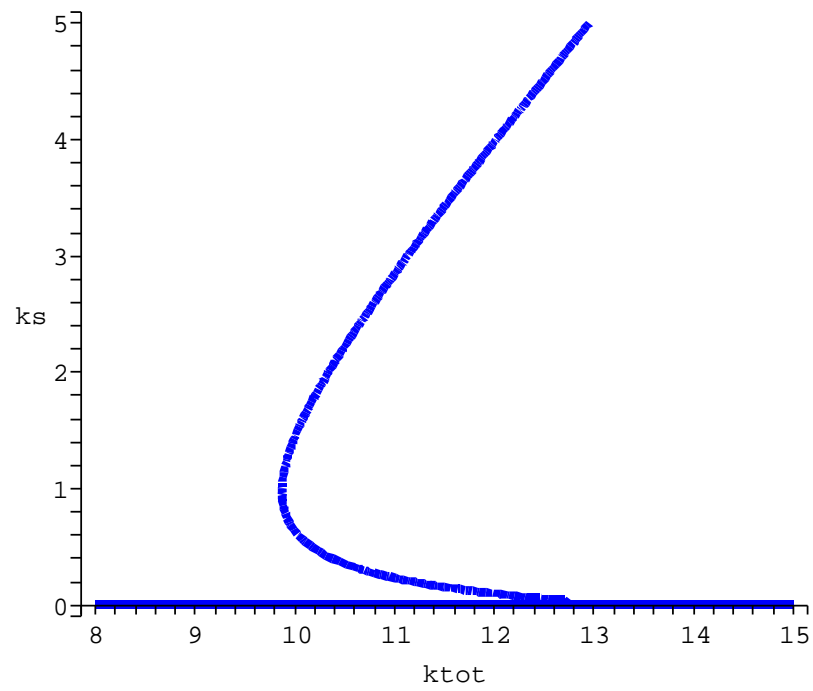
Figure 3.9: *Bifurcation diagram active kinase (k) vs total phosphatase kinase (ktot) in the closed system*
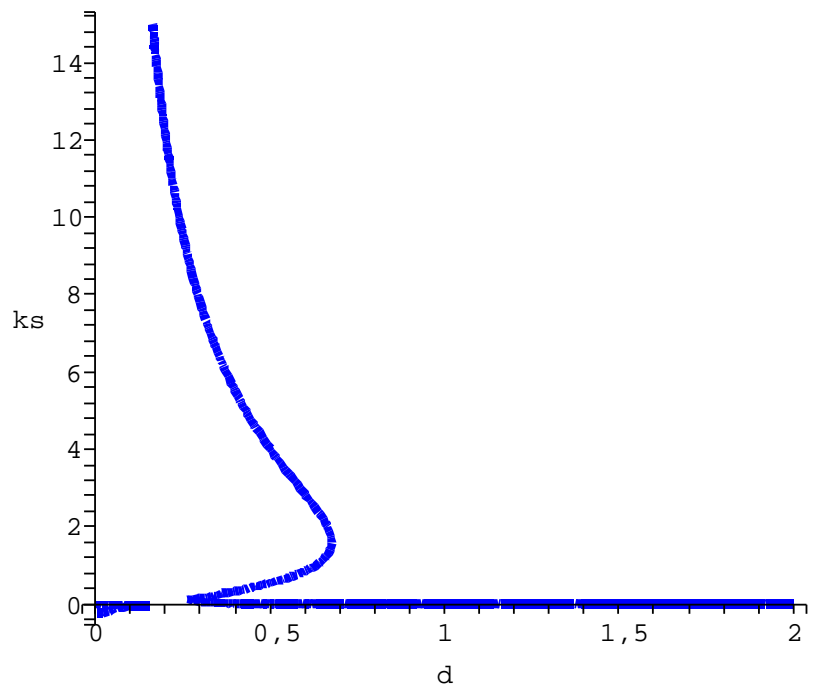
Figure 3.10: *Bifurcation diagram active kinase (k) vs $d = k_8 = k_9$ in the open system*

Reaction mechanism	Bistability open system	Bistability closed system
Two substrate binding	Yes	No
Mixed inhibition	Yes	No
Competitive inhibition	No	No
Uncompetitive inhibition	No	No
Autophosphorylation	Yes	Yes

Table 3.1: *Capacitiy of bistability for the reaction schemes treated*

3.3. Summary

Signaling pathways are composed of repetitive units or reaction modules. In this chapter we have classified reaction modules according to its capacity for displaying or not multistability and for some of them resolved analitically the locus of equilibria. We also evaluated how reaction motifs respond to the introduction or elimination of turnover terms in the possibility or not of multistability.

Chapter 4

Bistability analysis of a caspase activation model for receptor-induced apoptosis

Apoptosis is an essential process for organism homeostasis. Several abnormalities in apoptosis control can promote the development of autoimmune diseases, neurodegenerative diseases or cancer. Thus, understanding the apoptosis machinery is of considerable biological and medical interest. The molecular mechanisms underlying programmed cell death are complex and yet susceptible of mathematical analysis.

In 2004, Eissing et al. proposed a mathematical model for receptor-induced apoptosis[59]. In their work they build a chemical reaction network for the core-signaling pathway responsible for apoptosis initiation. They reported that translating the current knowledge related to apoptosis into a system of differential equations, they were able to reproduce one of the qualitative requirements for an apoptosis model: bistability.

In this chapter we re-evaluate this model from another perspective. Using stoichiometric network analysis (SNA) over the reactions reported in [59] we show that in the stationary state some of the reactions are blocked. It means that these groups of reactions are prohibited of carrying flux and do not participate in elementary modes (steady state reaction generators). Chemical reaction network analysis (CRNT) applied to an apoptosis reduced network (without blocked reactions) states that bistability is impossible for any set of reaction parameters. Finally we propose an improved model for receptor induced apoptosis In summary our results illustrate the power of SNA and CRNT in evaluating the feasibility of a chemical reaction network.

4.1. Introduction

Apoptosis is one of the essential cellular processes including cell division, metabolism and DNA synthesis[60][61]. It is triggered by different external and internal stimuli. Dying cells from acute damage such as hypoxia activate an inflammatory response capable of inducing a deleterious effect over the affected tissues. Apoptotic cells do not induce inflammation and in this way do not

interfere with the proper function of adjacent cells. Some morphological indicators of apoptosis include:

1. Cell volume shrinkage

2. Loss of cell adhesion

3. Chromatin fragmentation

4. Appereance of blebs at the cell surface

5. Cytoskeleton disassembly

The core of the apoptosis machinery is composed by a group of proteases (the caspases) that after some input signal begin a cascade of chemical reactions that terminates in cell death. Caspases are controlled by several mechanisms, among others, inhibitors like XIAP or BAR[62]. The ultimate goal of apoptosis machinery is to distinguish whether or not a stimulus is capable of irreversible activating the reaction cascade. Apoptosis can be viewed as a bistable system that changes between a "life "state and a "death "state according to the conditions sensed by the cell[63].

Death by apoptosis is a well orchestrated process.Apoptosis can be initiated by internal (DNA irreverible damage) or external stimuli in the form of cytokine induced cell death. Different signaling pathways are implicated and for this reason apoptosis signaling is classified in the intrinsic pathway and in the extrinsic pathway. Figure 4.1 and 4.2 describe in detail each of these reaction networks.

During the last years there has been an increasing interest in the mathematical modeling of biochemical reactions[1]. In this approach cartoons of biological processes are transformed into mathematical entities, often systems of ordinary differential equations (ODE)[64]. The objective behind these types of models is to reproduce an experimentally observed behavior and then be able to predict unobserved characteristics of the systems under study.

Apoptosis is a suitable system for mathematical modeling. First, it is complex. By complex we mean that its collective properties cannot be explained from the study of each component in isolation. Second it displays a qualitative property (bistability) useful to model validation. Third the central mechanism of apoptosis is well known and parameters for ODE simulation are available in the literature.

In this regard various attempts to model apoptosis have been published[65][66][67]. In 2004 Eissing et al. proposed a model for receptor induced apoptosis. Based on the current literature they build a network of chemical reactions and using mass action kinetics developed a system of ODE for the apoptosis central core. According to [59] this model displays bistability as required.

Dynamical simulation needs the detailed knowledge of reaction parameters. When there is no certainty regarding the value of each parameter, an approach that allows drawing information of the system with only the structure of the chemical reaction network is of great help[37]. This is the case of stoichiometric network analysis (SNA) and chemical reaction network theory (CRNT)[37].
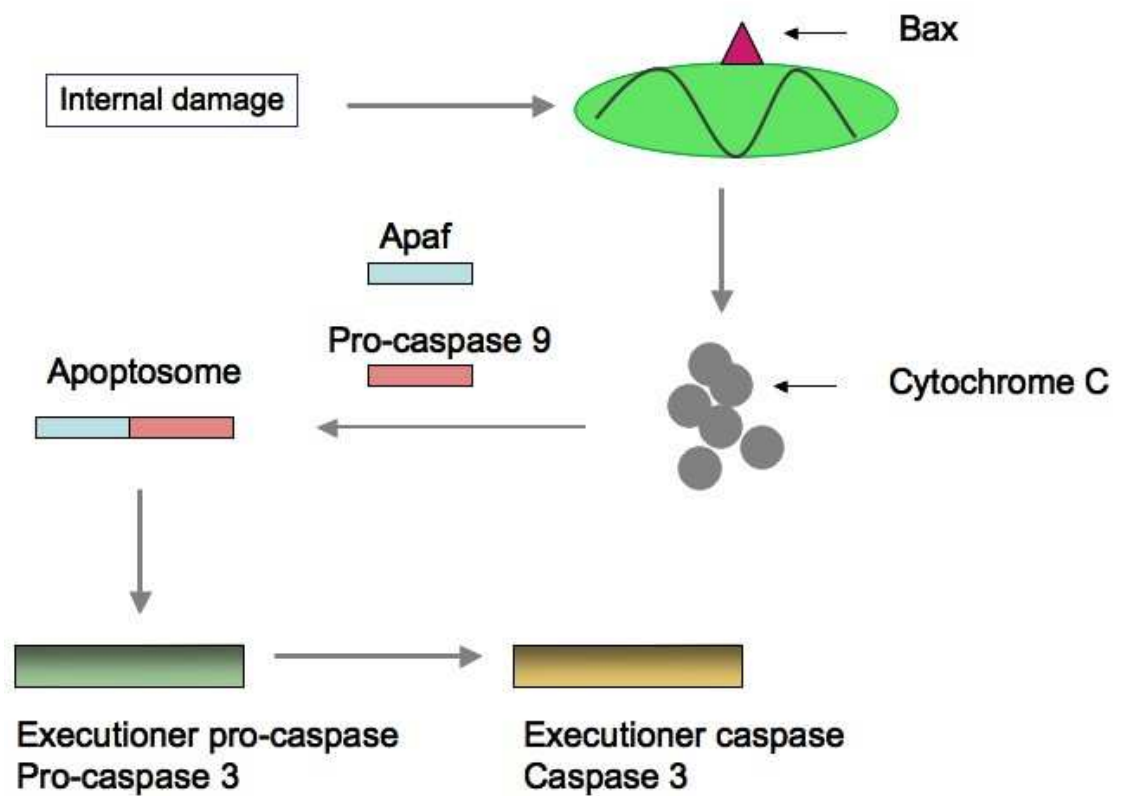
Figure 4.1: *Intrinsic apoptotic pathway. An internal damage (irreparable DNA error, hign calcium concentration) activates a sensor located in the mitochondria (Bax). Bax activation promotes cytochrome C release to the cytoplasm. In the cytoplasm cytochrome C along with Pro-caspase 9 and Apaf-1 conform a complex known as the apoptosome. The apoptosome is able to cleave executioner pro-caspase 9 and in this form initiate apoptosis.*
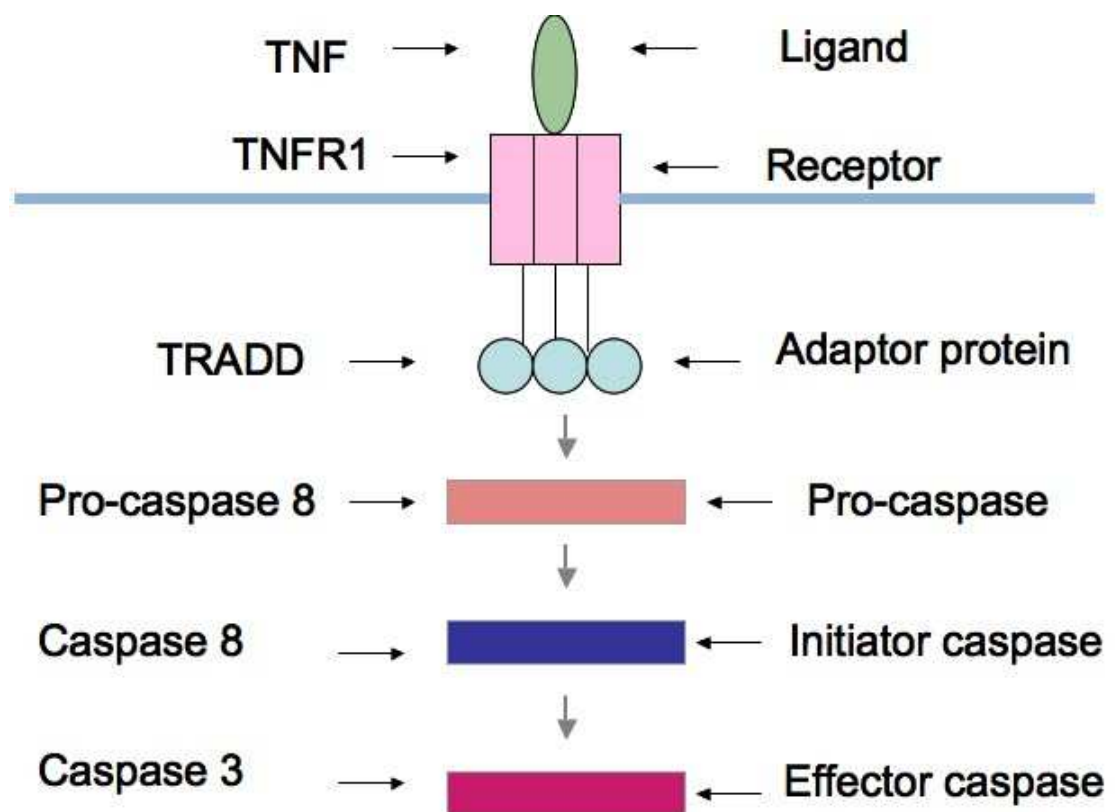
Figure 4.2: *Cell respond to external signals in a myriad of ways.As an example we will use Tumor Necrosis Factor α (TNF-α) signaling pathway Some cytokines (i.e. TNF) can induce cells to commit suicide. In this extrinsic apoptosis pathway a cytokine binds to its receptor (TNFR1) and unleashes a cascade of events that terminates in apoptosis induction. The first of these events is the activation of adaptor proteins (TRADD) that form a complex with initiator pro-caspases (Pro-caspase 8)and cleave them to produce functional caspases (Caspase 8). The cytoplasmic domains of TRADD and TNFR1 interact using death domains present in each protein. Death domains amplify the pro-apoptotic signal. The initiator caspase in turns cleaves downstream executioner pro-caspase to undergo apoptosis*

SNA and CRNT are methodologies for the study of the qualitative dynamical behavior of chemical networks[33]. CRNT has received special attention in the last years as a reliable method to discard hypothesis about the mechanism of a particular chemical reaction network[68].

In this work we evaluate the model in [59] under the perspective of SNA and CRNT. In particular we show that the apoptosis mechanism modeled by Eissing et al. has some stoichiometric anomalies (e,g blocked reactions). In addition when we reduced the network eliminating blocked reactions, it is unable to exhibit bistability for any set of positive reaction parameters. Based in SNA and CRNT the model of Eissing et al. can be discarded as an appropriate representation of receptor induced apoptosis.

4.2. Experimental procedures

The model developed by Eissing et al. is a system of ODE representing the kinetic behavior of eight species in the apoptosis core. The chemical species are:

x_1= Caspase 8 (C8)

x_2= Caspase 8 cleaved (C8*)

x_3= Caspase 3 (C3)

x_4 = Caspase 3 cleaved (C3*)

x_5= Inhibitor of apoptosis (IAP)

x_6= Complex IAPC3*

x_7= BAR protein

x_8= Complex BARC8

The chemical reaction network that represents the apoptosis mechanism is depicted in Figure 4.1.

0 represents the zero complex, meaning that the system is open. This network has 19 reactions. There are five degradation reactions for the species x_3, x_2, x_4, x_6, x_8 and one for the complex $x_4 + x_5$. We will discuss in more detail this atypical chemical reaction in the results section. In addition of degradation terms, the chemical network has turnover reactions for the species x_1, x_3, x_5, x_7. The model contains several simplifications. For example intermediary cleavage products are not taken into account.

The ODE system arising from this network according to the article (1) is:
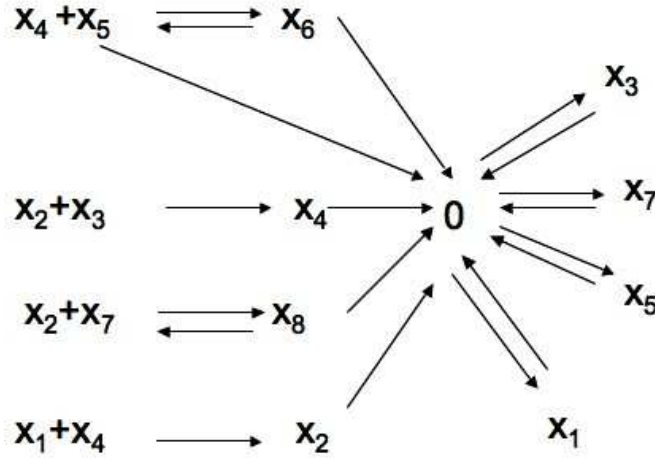
Figure 4.3: *Reaction network for receptor induced apoptosis*

$$\dot{x}_1 = -k_2 x_1 x_4 - k_9 x_1 + k_9$$
$$\dot{x}_2 = k_2 x_1 x_4 - k_5 x_2 - k_{11} x_2 x_7 + k_{11} x_8$$
$$\dot{x}_3 = -k_1 x_2 x_3 - k_{10} x_3 + k_{10}$$
$$\dot{x}_4 = k_1 x_2 x_3 - k_3 x_4 x_5 + k_3 x_6 - k_6 x_4$$
$$\dot{x}_5 = -k_3 x_4 x_5 + k_3 x_6 - k_4 x_4 x_5 - k_8 x_5 + k_8$$
$$\dot{x}_6 = k_3 x_4 x_5 - k_3 x_6 - k_7 x_6$$
$$\dot{x}_7 = -k_{11} x_2 x_7 + k_{11} x_8 - k_{12} x_7 + k_{12}$$
$$\dot{x}_8 = k_{11} x_2 x_7 - k_{11} x_8 - k_{13} x_8 \tag{4.1}$$

It is interesting to observe a cleaved caspase 3 (x_4) time series plot with low caspase 8 activated levels compared with a time series plot for high caspase 8 levels as proposed in the paper by Eising et al. Surprisingly, for low and high caspase 8 concentrations the caspase 3 peak and steady state is exactly the same. This move us to study this model with analytical tools. First,we build the reaction network in the software Cellnetanalyzer (version 8) and calculated the stoichiometric matrix (N) and other topological properties[41]. This matrix is a succinct description of a chemical system. N is q x r matrix, where q represents the number of species and r the number of reactions(15). The n_{ij} element of N is the stoichiometric coefficient of metabolite i in reaction j. The sign convention is negative for reactants and positive for products in each reaction. N is also used in the construction of the mass balance equation

$$\dot{\mathbf{x}} = N\mathbf{v}(x) \tag{4.2}$$

where x is the vector of the concentrations of the species and $\mathbf{v}$(x) is the vector of reaction rates.
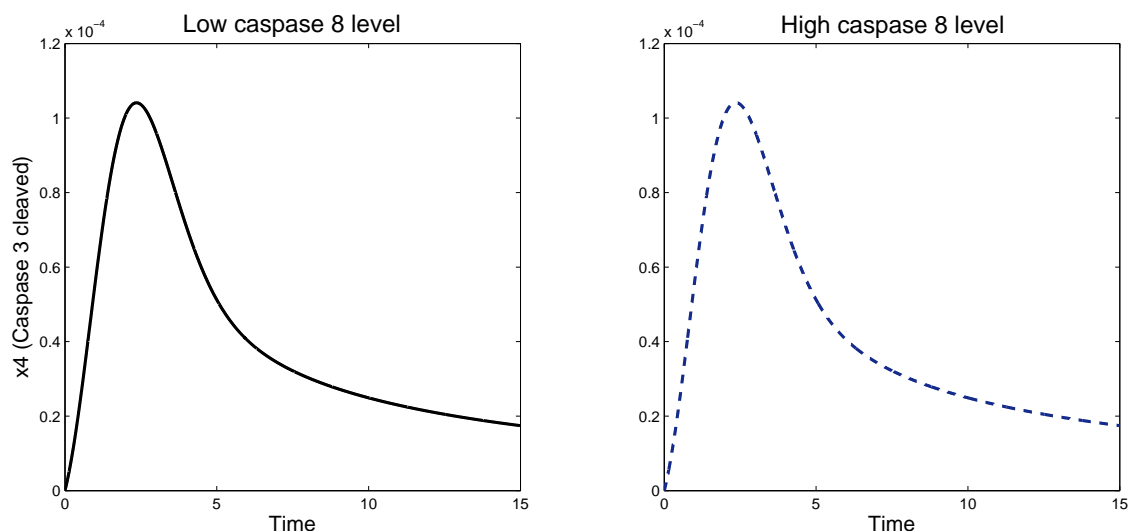
Figure 4.4: x_4 (Caspase 3 cleaved) time series for low (left) and high (right) active caspase 8 levels. Parameter values and initial conditions are in table 1 of [59]

In the analysis of a biochemical network we are interested in identifying the reaction vectors that generate all possible steady states of the system. That is, to find the non negative solutions to the equation:

$$N\mathbf{v} = 0 \tag{4.3}$$

This solutions represent the nonnegative linear combinations of a set of vectors known in the biochemical literature as elementary modes (EM), extreme currents or extreme pathways[69]. EM form a pointed polyhedral cone in the reaction space. We calculated EM using Cellnetanalyzer.

Another methodology used in this work is CRNT. CRNT connects the structure of a chemical reaction network with the qualitative characteristics of the ODE system arising from it[35]. The conclusions derived through CRNT only require the assumption of mass action kinetics. Some definitions are needed to understand CRNT. A complex is an object that appears before or after of a reaction arrow e.g. $x_4 + x_5, x_6, x_3$. The set of complexes that are internally connected by reactions are called linkage classes.

For each network CRNT assigns a non negative integer number δ called deficiency. δ is equal to $m - l - s$ where m is the number of complexes, l is the number of linkage classes and s is the rank of the stoichiometric matrix. If δ is zero the networks does not have the capacity for multiple steady sates(18). If δ is one and l is one, we can apply the deficiency one algorithm to decide whether or not the network can exhibit multiple steady states. In order to perform the CRNT analysis we employed the CRNT Toolbox 1.1 available from http://www.che.eng.ohio-state.edu/ feinberg/crnt/.

4.3. Results

4.3.1. Topological properties

The chemical network has 8 species and 19 reactions. This means that the stoichiometric matrix (N) has 8 rows and 19 columns. The rank of N is 8. Thus there are not linearly dependent
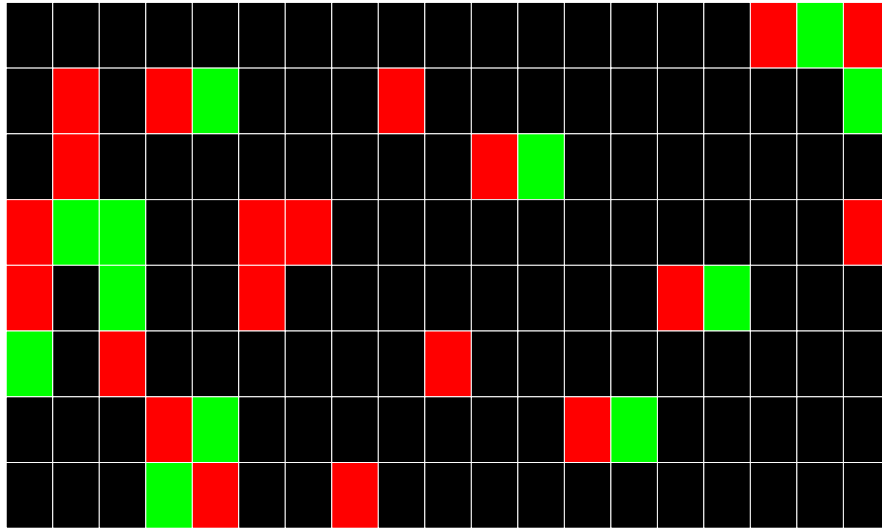
Figure 4.5: *Stoichiometric matrix for the apoptosis system. The rows are the chemical species and the columns the reactions. In green production terms, in red degradation terms.*

rows in N. In other words this network does not have conservation relations. Conservation relations are linear combinations of species concentrations that stay constant. Conservation relations form the left null space of N. In our case we will expect that the total quantity of caspase 3 (C3+C3*) and caspase 8 (C8+C8*) were the origin of conservation relations. According to our analysis this is not possible in the apoptosis network proposed by Eissing et al.

4.3.2. Blocked reactions

From the SNA we identify five blocked reactions:

1. $x_4 \rightarrow 0$

2. $x_4 + x_5 \rightarrow 0$

3. $x_2 \rightarrow 0$

4. $x_6 \rightarrow 0$

5. $x_8 \rightarrow 0$

Blocked reactions (BR) are incapable of carrying flux under certain conditions due to stoichiometric restrictions and can be eliminated in further analysis. BR do not participate in EM, as

we will explain in the next section.

Now, we want to discuss the meaning of one of these reactions, $x_4 + x_5 \rightarrow 0$. Here x_4 interacts with x_5 and both get degraded. This is highly unusual in chemical reactions. In addition this degradation term $(k_4 x_4 x_5)$ is only taken into account in the x_5 dynamical equation. If the kinetic law used in the elaboration of the ODE system is mass-action, a similar term must appear in the corresponding x_4 equation. In the model by Eissing et al. this term is missing.

4.3.3. Elementary modes

Elementary modes (EM) are a unique set of vectors that determine the flux distribution of the network in the steady state. Each EM is composed by r entries, where r is the number of reactions in the network. The number of entries is reduced if there appear blocked reactions. In the apoptosis network we identified seven EM with 14 entries. The five blocked reactions do not participate in EM and thus could be eliminated from the network. In the subsequent study blocked reactions were deleted from the chemical network to produce a reduced network.

4.3.4. CRNT

We applied CRNT analysis to this reduced network. The deficiency in this case is 1. The result of the deficiency one algorithm states that taken with mass action kinetics, the network cannot admit multiple positive steady states no matter what positive values the rate constants might have. Therefore the reduced network does not fulfils the essential requirement for an apoptosis model: bistability. In summary our results indicate that the model proposed by Eissing et al. has topological flaws highlighted with SNA and CRNT and for instance is unable to represent apoptosis adequately.

4.3.5. A new model

Based on the current knowledge of apoptosis regulation, here we describe a new model for receptor induced cell death. In Figure appears a diagram of the proposed model. The model has seven species and fourteen reactions. The species are:

x_1= Caspase 8 activated (C8*)
x_2= Caspase 3 (C3)
x_3= Complex C8*C3
x_4= Caspase 3 activated (C3*)
x_5= Inhibitor of apoptosis (BAR)
x_6= Complex C8*BAR
x_7= Complex C8*C3BAR

The reaction rates conform the vector
$v = (k_1 x_1 x_2, k_2 x_3, k_3 x_3, k_4 x_1 x_5, k_5 x_1, k_6 x_3 x_5, k_7 x_7, k_8 x_7, k_9 x_2 x_6, k_{10} x_2, k_{11} x_5, k_{12} x_4, k_{13}, k_{14}).$
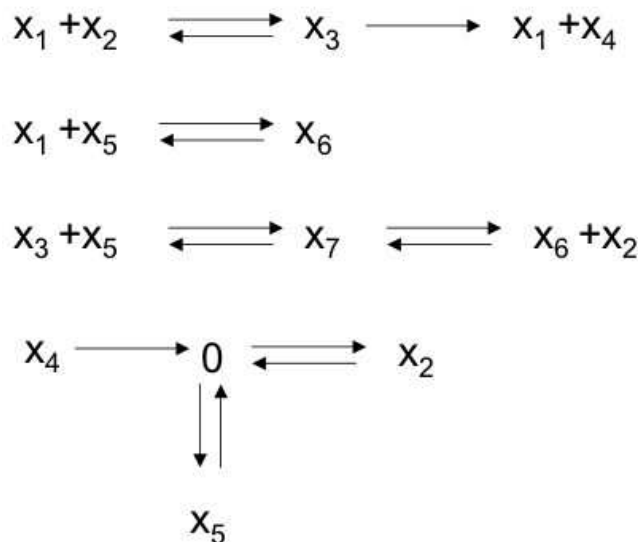
Figure 4.6: *A new model for receptor induced apoptosis*

Figure 4.7 shows the stoichiometric matrix, N for the system.   The ODE system for this network is

$$\dot{x}_1 = -k_1 x_1 x_2 + k_2 x_3 + k_3 x_3 - k_4 x_1 x_5 + k_5 x_6$$
$$\dot{x}_2 = -k_1 x_1 x_2 + k_2 x_3 - k_9 x_2 x_6 - k_{10} x_2 + k_{13}$$
$$\dot{x}_3 = k_1 x_1 x_2 - k_2 x_3 - k_3 x_3 - k_6 x_3 x_5 + k_7 x_7$$
$$\dot{x}_4 = k_3 x_3 - k_{12} x_4$$
$$\dot{x}_5 = -k_4 x_1 x_5 + k_5 x_6 - k_6 x_3 x_5 + k_7 x_7 - k_{11} x_5 + k_{14}$$
$$\dot{x}_6 = k_4 x_1 x_5 - k_5 x_6 + k_8 x_7 - k_9 x_2 x_6$$
$$\dot{x}_7 = k_6 x_3 x_5 - k_7 x_7 - k_8 x_7 + k_9 x_2 x_6 \tag{4.4}$$

There is a conservation relation for the total C8*, that is $x_1 + x_3 + x_6 + x_7 = et$. The deficiency for this network is 2 and CRNT analysis stays that taken with mass action kinetics there is the possiblity to admit multiple steady states. In order to verify this statement, parameters reported for some of the reactions in the apoptosis network were used in numerical analysis [70].

If we use this conservation relation as a bifurcation parameter, the model now proposed has the possibility to admit three stady states in a range of total C8*, two stable and one unstable. In Figure 4.8 appears the bifurcation diagram for the apoptosis system. In chapter 3 we analyzed how changes in turnover rates have influence in the dynamic behavior of chemical reaction networks. Now, we would like to perform a similar procedure for the apoptosis model proposed. The parameter k_{11} controls the degradation of BAR (x_5) an inhibitor of caspase activation. The
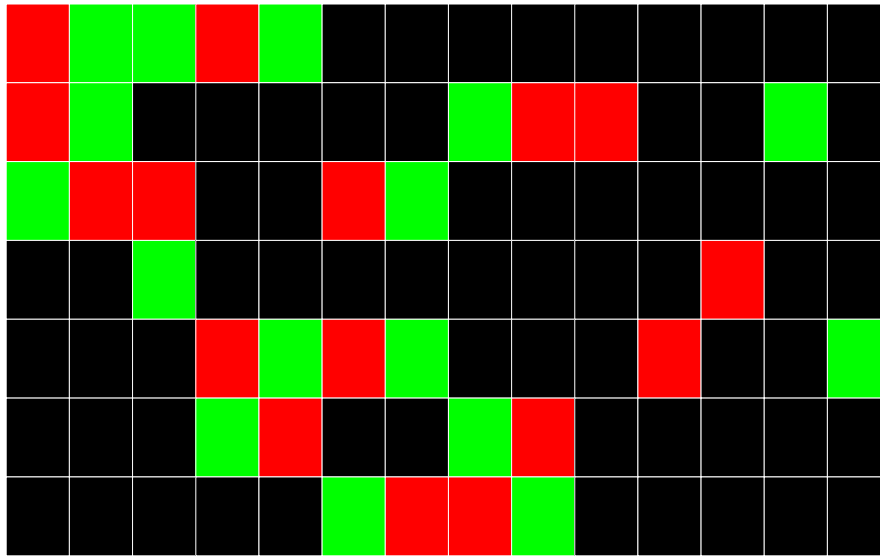
Figure 4.7: *Stoichiometric matrix for the new system. The rows are the chemical species and the columns the reactions. In green production terms, in red degradation terms.*
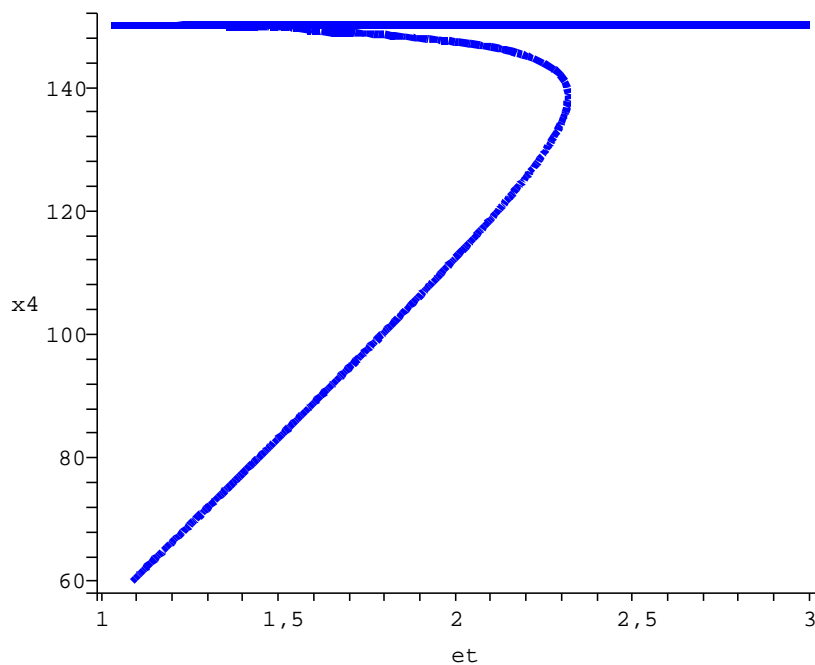
Figure 4.8: *Bifurcation diagram X_4 vs et. The parameters used are $k_1 = 62846.678, k_2 = 0.70598597, k_3 = 1223.6617, k_4 = 12.903767, k_5 = 603.65743, k_6 = 29514.848, k_7 = 119.08971, k_8 = 1225.0265, k_9 = 4048.1216, k_{10} = 1, k_{11} = 1, k_{12} = 1, k_{13} = 150.08654, k_{14} = 8.6541.10^{-2}$*

bifurcation diagram in Figure 4.9 shows how even for a region in which is supposed to be multi-stability ($et = 2$ according to Figure 4.8) slight variations in k_{11} allows the system to commute between low and high executioner caspase x_4 (cleaved caspase 3).   This is interesting because
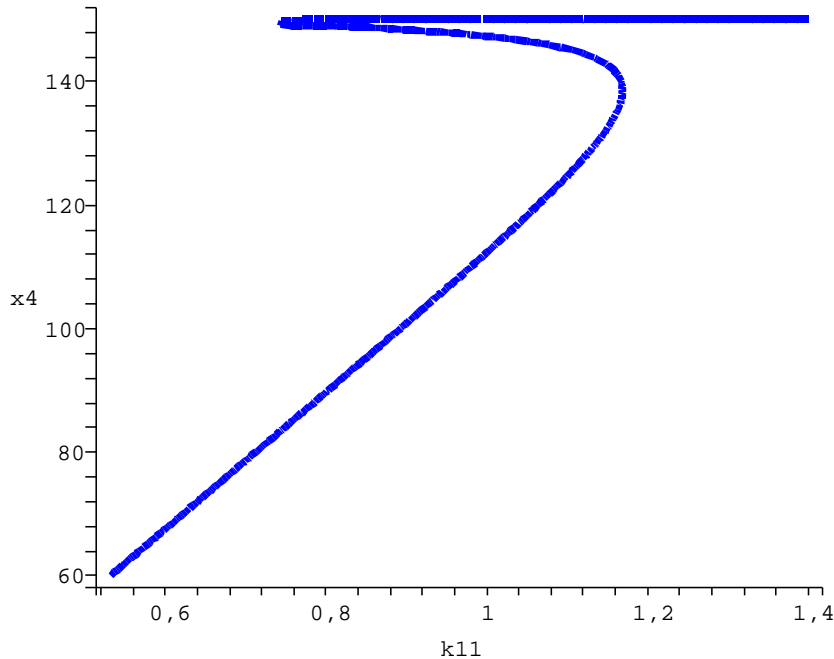
Figure 4.9: *Bifurcation diagram X_4 vs k_{11}. The remaining parameters are the same as in Figure 4.8 and et = 2*

if a pharmacological perturbation is not suitable to interfere with the total amount of initiator caspase (Total C8=et), the system can be controled with drugs that promote or inhibit x_5 degradation. The clinical implication is if the physician wants to promote apoptosis (i.e cancer cells) the procedure is to augment k_{11} and if he wants to inhibit apoptosis just prescribe k_{11} reduction.

4.4.   Conclusion

We evaluated a model for the apoptosis mechanisms core. We used instead of a simulation approach, an analysis based only in the structure of the reaction network. This parameter free approximation has gained considerably attention in the field of systems biology[46][32]. In particular the relation between the structure of the network and the qualitative properties (like bistability) inherent to the system is of great importance because the identification of reliable reaction parameters is a hard task[39].

Chemical reactions are usually modeled by lumping together reactions and ignoring the behavior of intermediary products. This can lead to the different dynamical properties if one compares the behavior of complete mechanisms and their lumped counterpart. For example Tyson et al. using CRNT, recently showed that a simple model of enzyme catalysis that exhibits multistability, lost

this property by neglecting enzyme-substrate intermediates [11]. Representing chemical reactions as accurate as possible is an essential requirement to develop appropriate mathematical models of cellular processes. We follow this statement in the analysis of the model proposed by Eissing et al.

Using SNA we showed that in the system proposed by Eissing et al. some of the reactions are blocked and therefore unable to participate in any flux distribution in the steady state. Furthermore, when CRNT was applied to the reduced network without blocked reactions, bistability is discarded. This highlights the need of a theory that allows designing biochemical networks with certain properties from simple mechanisms. A starting point will be the elucidation of the minimal multistationary reaction network.

We also were capable of developig a new model based on caspase regulation that has the required property of bistability in an adequate paramater range. In summary our results illustrate the power of SNA and CRNT in evaluating the feasibility of a chemical reaction network. We believe that systems biology will benefit from the continuous improvement of these pair of theories.

Chapter 5

Type 1 interferon signaling pathway in multiple sclerosis

5.1.   Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous systems (CNS) with unknown etiology that occurs more frequently in the northern areas of the world, and is particularly frequent in Europe and North America. No definitive cure is available for MS, and disease-modifying therapies such as interferon-β (IFNB), Glatiramer acetate (GA) or Natalizumab are only partially effective and induce side-effects that limits patient's quality of life [71] [72]. Although two of these drugs were developed based in a well know therapeutic target (the T-cell receptor specific for Myelin Basic Protein for GA, VLA4 integrin for natalizumab), the mechanisms of action of these drugs are only partially understood. The economical and social burden of the disease is considerable as it affects predominantly young professional adults. The number of persons affected in the EU-25 is estimated to be between 400.000 and 455.000 (http://ec.europa.eu/health) and similar numbers could be applied to the US. A Canadian study reported a substantial increase over the last 50 years in the female to male sex ratio, now exceeding >3.2 females per male contracting the disease[73].

The average lifetime cost of the disease is estimated at > 1.5 million euros per MS patient in the UK, which is likely to be roughly representative for EU-25 countries. Currently, MS therapies are prescribed based in disease activity and not in the predicted response to a specific drug. This is due to the lack of biomarkers for identifying responders and non-responders to therapy. For example, it has been estimated that up to 40 % of patients do not respond to IFNB [74] [75] which implies that many patients are exposed to the side-effects of a drug which is no beneficial to them and that the health systems are expending money without providing a social benefit. There is therefore a pressing and timely need both for new therapies with higher efficacy and good safety profile and as well as for improving the efficacy of existing treatments. The improvement of both the efficacy and safety of existing drugs is a good strategy that would pay benefits both for the society as well as for the pharmaceutical industry.

The search for personalized medicine aims to identify the best therapy for a given patient or

more practically the subgroup of patients with better efficacy and less side effect for a given drug (stratified medicine), which is going to have a big impact in chronic diseases [76]. Successful identification of treatment response-predictive genetic/biomarkers would support early treatment of those patients most likely to respond to it, while allowing treatment of non- responders with alternative medication or at least to prevent them suffering side-effects [77] [78] [79]. The success of specialty medicine drugs, mainly for neurological diseases, is based in a good safety profile as well as in enhancing efficacy through selecting good responders more than in a new mechanism of action [80].

Interferon beta (IFNB) is the most common treatment choice in patients affected by multiple sclerosis (MS). IFNB reduces by a third the number of relapses, and also delays the progression of the disease [81]. Currently, there are three commercial forms of IFNB available in the market, Betaferon (IFNB1b), Avonex and Rebif (both IFNB1a). INFB belong to the Type I interferon cytokine family. Type I interferons (IFNs) are cytokines that have antiviral, antiproliferative and immunomodulatory effects. There are many type I IFNs including interferon α (with 13 different subtypes), interferon β interferon κ and interferon ω. All type I IFNs bind to the same receptor. These molecules are widely used in the treatment of hepatitis C, multiple sclerosis and various types of cancer. The mechanism of action is not well understood, although considerable evidence suggests a combination of immunomodulatory and anti-inflammatory effects [82].

The efficacy of IFNs is limited, with up to 40 % of the MS patients not responding to the therapy and similar rates of efficacy have been observed in hepatitis C patients [83]. The cause of this lack of efficacy is not known, but it could be related with its pleiotropic activity and the generation of common adverse events that limit dosage. However, individuals differences in the genes activated by IFNB as well as differences in the pathogenic mechanisms at work in each individual might account for the lack to response to therapy. It will be of great clinical value to find markers of response to this drug in order to begin an early intervention in the responders and to avoid its use in non-responders.

5.2. Interferon beta: Mechanism of action involved in the treatment of MS

IFNB is a regulator of the immune systems acting over a broad range of immune cells including dendritic cells, T cell, and B cells. IFNB also influences the activity of astrocytes, microglia and neurons [84]. The immunomodulatory effects of INFB are diverse. IFNB downregulates the expression of type II MHC molecules and inhibits T cell proliferation. IFNB promotes the expression of Th2 cytokines and the immunosuppressive cytokine IL-10. In the blood-brain barrier, IFNB inhibits the traffic of T cells, improving the integrity of endothelial cells, a mechanism that implies reducing the activity of adhesion molecules and decreasing the production of metalloproteinases [85] [86]. Interestingly, two different groups have reported the involvement of IFNB in the control of inflammation inside the CNS, in addition to its better known effects in peripheral

immune system. Dendritic cells in the brain respond to IFNB diminishing IL-23 production and favoring IL-27 secretion [87]. IL-27 is actually known to inhibit Th17 cells differentiation. Also, microglia in the presence of IFNB decrease the release of inflammatory chemokines, halting lymphocyte infiltration into the CNS [88] [89] . In summary, IFNB has a protector profile of the CNS during inflammation.

5.3.   Type I interferon signaling pathway

The type I IFN signalling pathway is composed by a receptor (IFNAR) and three signalling pathways: MAPkinase, JAK-STAT and PI3k pathways [90] [91] (Figure 5.1).   The components
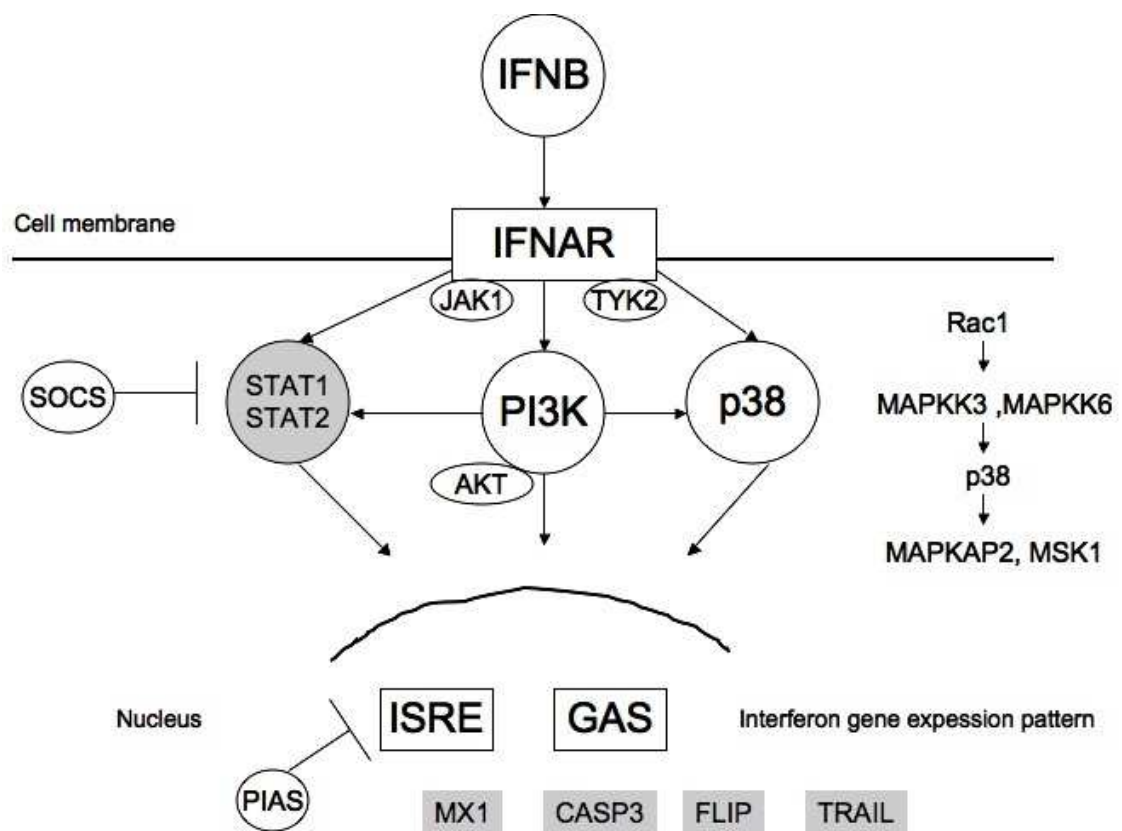
Figure 5.1: *After the binding to its receptor (IFNAR), interferon β (IFNB) activates a cascade that controls the gene expression of hundreds of genes. The signaling pathways involved in this process are JAK-STAT, Phosphoinositide 3-kinase (PI3K) and MAPK. SOCS (Suppressors of cytokine signaling) in the cytoplasm and PIAS in the nucleus (protein inhibitors of activated STAT) regulate the amplitude of the signal initiated by IFNB. After activation of several transcription factors through the three signalling cascades, they migrate to the nucleus where they promote the expression of hundred of genes including ISRE (interferon stimulated response element) and GAS (IFN-γ activated site) responding genes, which are responsible for the biological effects of IFNB.*

of the type 1 IFN signaling pathway are the ideal candidates to look for markers of response to IFNB therapy.

The first step in the IFNB transduction pathway is the binding of IFNB to the interferon type I receptor (IFNAR). IFNAR is composed of two subunits IFNAR1 and IFNAR2 [92]. IFNB activates the JAK-STAT signaling pathway. In the case of IFNB, JAK1 and TYK2 are the components of the JAK family of proteins that participate in IFNB transduction [93]. The complex formed by JAK1 and TYK2 phosporylates the STAT1 and STAT2 proteins. STATs are transcription factors capable of traveling back and forth the nucleus where they regulate the expression of hundreds of genes that mediate INFB biological effects. For the appropriate control of transcription, the STAT1/STAT2 complex bind to IRF9 protein (interferon regulatory factor) [94].

Albeit all subtypes of type I IFN interact with the same receptor and activate similar pathways, they produce different cellular responses. The origin of this discrepancy is, perhaps, a change in the regulatory dynamic of each of the signaling events according to the IFN that initiates the signal transduction process. The elaborate crosstalk between these signaling cascades guarantees IFNB activity specificity. Several mechanisms control type I IFN signaling pathway. Suppressors of cytokine signaling proteins (SOCS) inhibit the type I IFN pathway preventing the phosporylation of STATs proteins (Figure 5.1). At the nuclear level protein inhibitors of activated STAT (PIAS) block STAT1 binding to the DNA [95] [96]. Although not yet studied, SOCS and PIAS genes are interesting candidate biomarkers.

In this chapter we develop a mathematical model of the core module of the JAK-STAT signaling pathway based on experimental data from patients with MS.

5.4. Experimental procedures

We studied 10 patients with MS all of whom provided their informed consent. This work was approved by the Ethical Committee of the University of Navarra and informed consent was obtained from all participating subjects.

To quantify the response of CD4 T cells to interferon beta we used multicolor flow cytometry. First, peripheral blood mononuclear cells (PBMC) were isolated with Ficoll-Paque (Pharmacia Biotech) and incubated at 37 C in RPMI 1640 at 10^6 cells/mL. Then PBMC were stimulated with increasing doses of recombinant interferon beta (0,100,200,300,500,1000,2000,4000,5000,7000, 10000,12000,14000,16000,18000,2000 U/mL) for one hour. Phosphorylated Stat-1 was detected by flow cytometry as described by He et al[83]. Briefly, the cells were fixed with parafomaldehyde, permeabilized with methanol and stained with PE-conjugated anti-pStat-1, FITC-conjugated anti-CD3, PERCP-conjugated anti-CD45 and APC-conjugated anti-CD4 all from BDBiosciences. Stained samples where then passed through a FACSAria cytometer and analyzed with FlowJo software (Treestar). As a measure of activation we used the mean fluorescense intensity of pStat-1 signal gated over CD4+CD45+ cells.

Mathematical model

The chemical reaction network scheme is depicted in Figure 5.2. The components of the
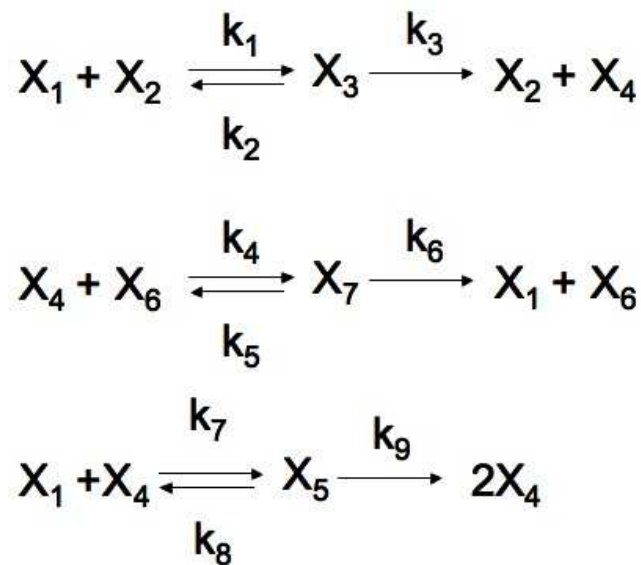
Figure 5.2: *JAK-STAT chemical reaction network*

model are:

1. JAK protein $= x_1$

2. STAT 1 $= x_2$

3. JAK-STAT1 complex $= x_3$

4. Phosphorylated STAT1 $= x_4$

5. Unphosphorylated STA1-Phosphorylated STAT1 complex $= x_5$

6. Phosphatase $= x_6$

7. Phosphorylated STAT1-Phosphatase complex $= x_7$

The principal process described in this model is STAT1 phosporylation and dimerization.There is also a negative regulation represented by STAT1 dephosporylation. The model is simple but represents the current knowledge about JAK-STAT signaling pathway. We follow the methods developed in Chapter 2 to analyze the proposed model. The stoichiometric matrix N and the

reaction vector $v(k, x)$ are:

$$
N = \begin{pmatrix}
-1 & 1 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\
-1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & -1 & 1 & 2 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\
0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0
\end{pmatrix}, \quad
v(k, x) \begin{pmatrix}
k_1 x_1 x_2 \\
k_2 x_3 \\
k_3 x_3 \\
k_4 x_4 x_6 \\
k_5 x_7 \\
k_6 x_7 \\
k_7 x_1 x_4 \\
k_8 x_5 \\
k_9 x_5
\end{pmatrix}
$$

The ODE system is:

$$
\begin{aligned}
\dot{x}_1 &= -k_1 x_1 x_2 + k_2 x_3 + k_6 x_7 - k_7 x_1 x_4 + k_8 x_5 \\
\dot{x}_2 &= -k_1 x_1 x_2 + k_2 x_3 + k_3 x_3 \\
\dot{x}_3 &= k_1 x_1 x_2 - k_2 x_3 - k_3 x_3 \\
\dot{x}_4 &= k_3 x_3 - k_4 x_4 x_6 + k_5 x_7 - k_7 x_1 x_4 + k_8 x_5 + 2 k_9 x_5 \\
\dot{x}_5 &= k_7 x_1 x_4 - k_8 x_5 - k_9 x_5 \\
\dot{x}_6 &= -k_4 x_4 x_6 + k_5 x_7 + k_6 x_7 \\
\dot{x}_7 &= k_4 x_4 x_6 - k_5 x_7 - k_6 x_7
\end{aligned}
$$

$$(5.1)$$

The reduced system along with the conservation relations are:

$$
R = \begin{pmatrix}
1 & -1 & 0 & 0 & 0 & -1 & 0 & 0 & 1 \\
0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 \\
0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1
\end{pmatrix} \quad
C_1 = \begin{pmatrix} 1 \\ 0 \\ 1 \\ 1 \\ 2 \\ 0 \\ 1 \end{pmatrix} \quad
C_2 = \begin{pmatrix} 0 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} \quad
C_3 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \end{pmatrix}
$$

In order to find the equilibrium solution of the CRN we change the system for the equivalent:

$$Rv(k, x) = 0$$

$$x_1 + x_3 + x_4 + 2x_5 + x_7 - c_1 = 0$$

$$x_2 + x_3 - c_2 = 0$$

$$x_6 + x_7 - c_3 = 0$$

c_1, c_2 and c_3 represent total STAT, total JAK and total phosphatase respectively.
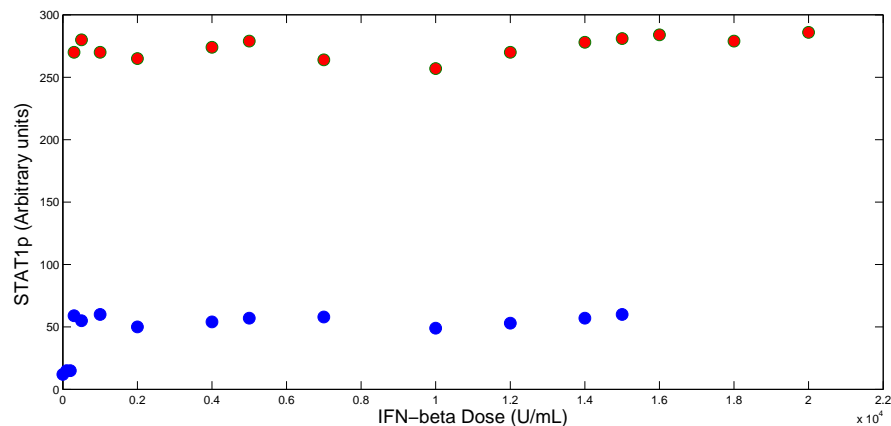
Figure 5.3: *Bistability in inteferon stimulated JAK-STAT pathway. In blue the low steady state and in red the high steady state*

5.5.   Results

Figure 5.3 displays a representative dose response curve of phosporylated STAT1 against increasing recombinant interferon beta administration, clearly illustrating a hysteresis loop. For low interferon doses there is only one steady state, for an interval of interferon availability there are two possible steady states and finally with high interferon doses there is a high stationary branch. This also can be observed in cytometry histograms as shown in Figure 5.4. Now we evaluate if the model proposed in the Methods section is capable of reproducing the dynamic behavior suggested by the experimental data. To this end we calculate first the deficiency of the network δ. The chemical reaction network under study has deficiency 2. Applying the advanced deficiency algorithm, the JAK-STAT chemical network has the possibility to exhibit multistability. Using parameter values obtained from the literature, Figure 5.5 demonstrates that the chemical reaction network proposed can reproduce the qualitative behavior of the experimental data.

5.6.   Conclusion

Current therapies for MS are partially effective and with common side effects, limiting their usefulness. This situation is due in part to the lack of biomarkers of response to therapy that will allow identifying the best responders, increasing drug efficacy, and will avoid treating non-responders with these drugs, preventing the appearance of adverse effects. This situation is going to be even more complicated with the appearance of new immunomoduladors already in the final states of phase III clinical trials (i.e. Fingolimod, Laquinimod, Rituximab, Daclizumab, among others) and with the development of combination therapy. The discovery of biomarkers of the response to therapy can help in the process of identifying the best therapy for the most appropriate patient, with the ultimate goal of developing personalized medicine.

Pharmacogenomics has been the most useful approach for discovering drug biomarkers. The power of genome-wide association studies in combination with high through-put techniques such as DNA

Figure 5.4: *Representative flow cytometry histograms for low (upper-left), intermediate (upper-right) and high interferon beta dose*

Figure 5.5: *Bifurcation diagram for x_4 (STAT1p) vs c_1. Parameter values taken from [5]*

arrays, proteomics or metabolomics, can provide the tools for the discovery of such biomarkers. However, due to the complexity of multifactorial diseases such as MS and the pleiotropic activity of the disease modifying drugs, we believe that systems biology will become a useful approach for integrati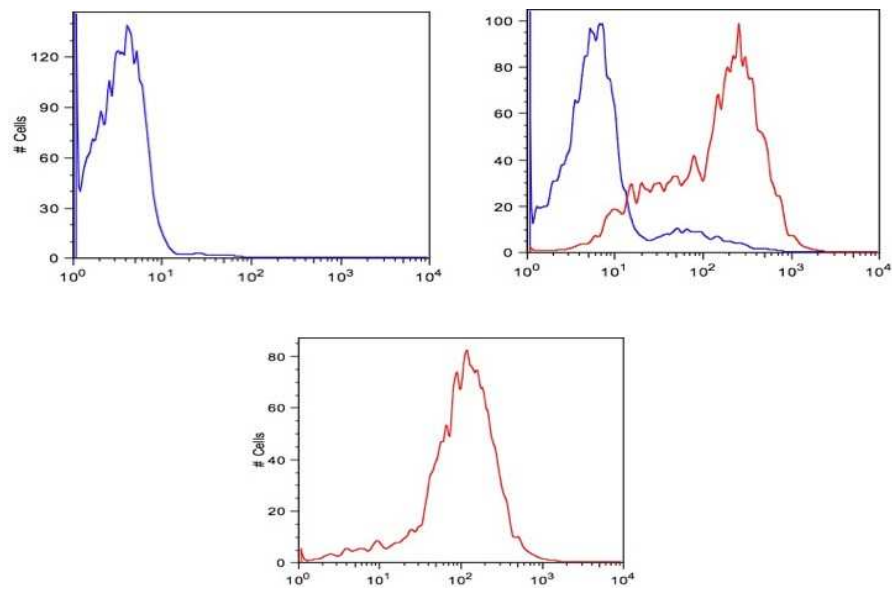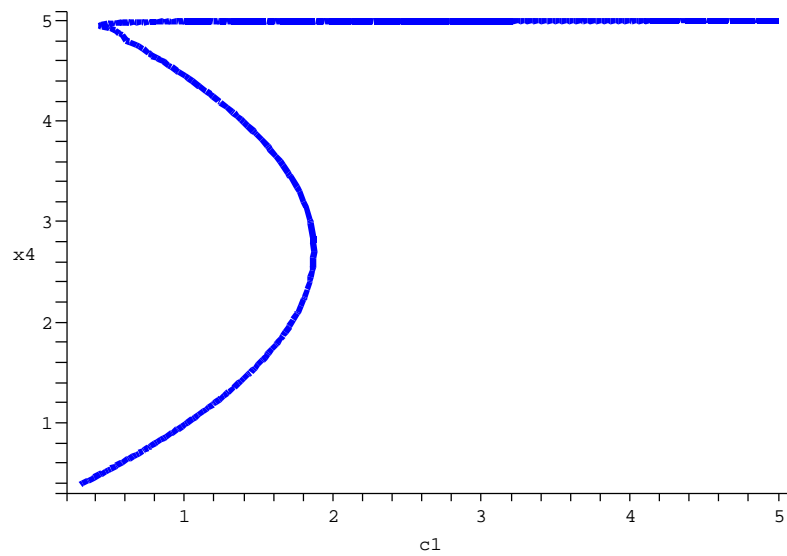ng biological, clinical and imaging data and obtaining meaningful information for making decisions about care and therapy [97] [98]

Discovery of biomarkers for the response to disease modifying therapy in Multiple Sclerosis is a high priority because it will have a profound impact in how we prescribe and monitor such drugs. Patients will benefit because by selecting the best responders, the efficacy is going to be enhanced and by removing non-responders, we can prevent them for suffering inconvenient side effects and even to move them to other therapies. Overall, patients adherence to therapy will be increase and the economic cost of these therapies will be reimbursed with an improved quality of life and in the reduction of cost due to disability in the long-term. However, this ideal scenario, the promise of personalized medicine, is not straightforward and research done to date as discussed in this review reveals that the task is enormous, complex and still far from direct clinical applications.

One of the greatest challenges in this area is how to get the maximum advantage from apparently unconnected scientific fields to benefit MS patients. Personalized medicine requires the integration of data and techniques from different disciplines including population genetics, immunology, bioinformatics, clinical research, neuroimaging and systems biology. The current efforts to find predictors of efficacy for the most extensively studied therapy IFNB have provide promising preliminary results that requires further validation. However, the efforts conducted to date reveal the complexity of the topic. No single SNP or gene expression marker accurately classifies INFB treated patients as responders or non-responders. This suggests that the combination of different types of information such as SNPs, gene expression patterns, proteins and clinical variables, in addition to well-defined and powerful cohorts are necessary to reach clinical utility. Moreover, more validation studies with larger patients cohorts are urgently needed.[99]

In this chapter we demostrated the existece of bistability in the response to interferon beta in patients with MS. We also developed a mathematical model resembling the qualitative behavior of the experimental data. It is interesting to note that apparently similar cells respond in a remarkable different fashion to the same stimuli. As already mentioned, biomarkers for evaluating the response to IFNB are actively being investigated. Perhaps ahead of looking for SNPs, changes in gene or protein expression, a useful strategy will be to study in detail the underlying dynamics of interferon beta signaling pathway. It is tempting to speculate that the different steady states observed in the STAT1P phosphorylation curve could represent responder or not responder status. If this is the case approaches oriented to transform one state into other will promote the advance of MS treatment.

Chapter 6

Treatment of autoimmune diseases: A systems biology approach

Autoimmune diseases are a major health problem. In the last years we have seen a considerably increase in therapeutic tools available for this devastating group of diseases. Treatment efficacy has improved, but still is not curative. In this final chapter we review current strategies in autoimmune disease treatment and highlight the opportunity that systems biology represents for the development of better and safer treatments.

6.1. Introduction

Autoimmune diseases are a group of more than 70 chronic pathologies characterized by an inappropriate immune system response against the own tissues. Their prevalence is approximately 5 percent of the population on western countries and for unknown reasons the incidence is steadily rising. Women in the young and middle ages are the most affected[100]. The aetiology is not well understood. Epidemiologic studies are clear to demonstrate a genetic susceptibility to autoimmune diseases. In addition research in genetically similar populations living in different environments shows that environmental factors have a particular significance in the origin of these diseases. Although they are classified according to the principal mediator of the damage (antibodies or cells), autoimmune diseases share common pathological mechanisms than can be exploited therapeutically.[101][102]

Systems biology is a new scientific field that integrates mathematical modeling, computational simulation and high throughput experiments with the aim to understand organisms structure, dynamics, control mechanisms at a system level[103].

In this work we will focus on strategies directed to alter the threshold of immune activation, modulate antigen-specific responses, reconstitute the immune system and spare target organs. Finally we will take into consideration the opportunity that represents systems biology for the development of useful and safe treatments for autoimmune diseases.

6.2. Key strategy. Regulation of immune system activation

The immune system is designed to respond against dangerous signals[104][105]. For the correct activatio of an immune response, the antigen (signal 1) must first bind to the specific receptor. Another requirement is the accurate ensemble of costimulatory molecules (signal 2). This group of molecules are ideal candidates to regulate (augment or decrease) T cell activation and in therefore control the strength of the immune system response.

T cell signaling and costimulatory molecules

Inappropriate T cell activation plays a central role in pathogenic immune responses. For the proper initiation of T cell function it is required that several co-stimulatory molecules get involved after T cell receptor binding to major histocompatibility complex in antigen presenting cells. One group of such costimulatory molecules is the CD80-CD86/CD28-CTLA4 complex. CD80 (B7-1) and CD86 (B7-2) bind to CD28. They are activators of T cell signaling. CTLA4, a homologue of CD28, binds to B7-1/B7-2 and unleashes an inhibitory signal over T cell response. Various therapeutic molecules interfere with this costimulatory signal. Abatacept is a fusion protein between CTLA-4 and immunoglobulin that blocks CD28-B7 costimulation. Abatacept is now approved for use in rheumatoid arthritis (RA)[106]. Another drug that targets B7/CD28 pathway is RuDex. RuDex is an oral B7-1 antagonist that has successfully completed two Phase I clinical trials and is now being tested in RA patients[107].

T cell receptor (TCR) mediated signaling has been the subject of intense research searching for inhibitors or regulators that can alter lymphocyte activation. One of the most successful examples are inhibitors of NFAT calcineurin dependent dephosporylation such as cyclosporin or tacrolimus. NFAT inhibitors exhibit toxicity related to the wide distribution of calcineurin. For this reason molecules that inhibit nodes in TCR signaling pathway only in T lymphocytes will target immune system activation more specifically. However is not easy to find a target in the myriad of components that constitute TCR signaling pathway. It is in this context where systems biology looks promising (Figure 6.1). Using mathematical techniques integrated with experimental data it is possible to identify which genes and proteins are relevant to a particular state such as T cell activation or anergy[108]. In this context the concept of robustness adquires great importance. Robustness is a property of biological systems that enables them to sustain their function against various external or internal perturbations. Autoimmune diseases can be viewed as a failure of robustness of normal self tolerance mechanisms. Manipulation of TCR signalling pathway robustness with interventions that exploit the fragility of this pathway in the disease state is an interesting area of research. As an example a model of the metabolic syndrome robustness suggests that multiple therapies with multiple targets are required to cure or at least to control metabolic syndrome.[109]
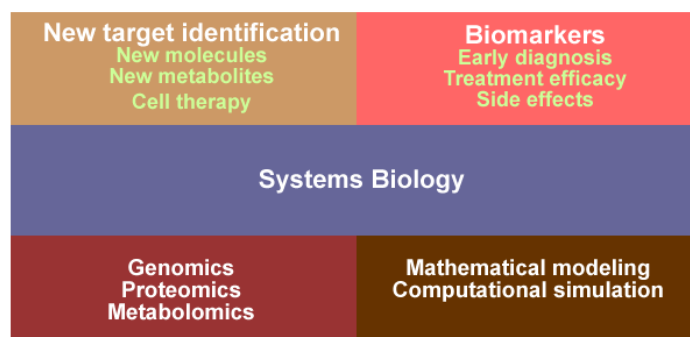
Figure 6.1: *Systems biology approach. Genomics, proteomics and metabolomics linked with mathematical modeling form the core of systems biology. In autoimmune diseases a systems biology approach will favour the development of new therapies and biomarkers as highlighted in the text.*

Cytokines and cytokine signaling

Cytokines are extracellular proteins that have pleiotropic effects in the immune system. They participate in the control of T cell growth, inflammation and tissue migration of activated leukocytes. The most successful therapies for autoimmune diseases are capable of blocking inflammatory cytokines. Anti-TNF-α (Infliximab) and soluble TNF-α receptors (Etanercept) are widely used in the treatment for rheumatoid arthritis, Chron's disease and ankylosing spondylitis[110].

Interfering with cytokines that are produced by T cells or that act on T cells is another strategy to treat autoimmune diseases. Interleukin 1 (IL-1) and interleukin 6 (IL-6) have pathological roles in RA. Antibodies that inhibit IL-1(anakinra) and IL-6 (tocilizumab) are well tolerated and show efficacy in slowing disease activity in RA patients[111]. Interleukin-2 belongs to a family of cytokines, which includes IL-4, IL-7, IL-9, IL-15 and IL-21. IL-2 is an autocrine factor for T cells. Recently monoclonal antibodies that bind to IL-2 receptor B alpha (CD25) subunit (daclixumab) have yielded promising results in the treatment of multiple sclerosis. After binding to IL-2 receptor IL-2 activates PI3K, Jak/STAT and Ras/MAPK signaling pathways. Inhibitors of IL-2 signaling are attractive targets(10). In this way CP690, 550 a selective inhibitor of janus kinase 3 (JAK3) has demonstrated clinical efficacy in rheumatoid arthritis[112]. However JAK3 is also activated by erythropoietin resulting in anaemia.

IL-15 belongs to the same group of IL-2 and promotes lymphocyte survival as well as activation of natural killer cells. Clinical data suggest that an anti-IL-15 monoclonal antibody is safe and

produces significant improvement in disease active in rheumatoid arthritis patients[113]. IL-12 and IL-23 are cytokines produced by macrophages, dendritic cells and B lymphocytes in response to antigen activation. They mediate T cell differentiation towards TH1 and TH17. IL-2 and IL-23 share a common subunit p40. A monoclonal antibody against p40 induces beneficial clinical responses in patients with Chron's disease and psoriasis[114][115].

Understanding cytokine signalling and TCR signalling requires a detailed knowledge of the structure (molecular interactions) and dynamics of the signalling network in the face of various perturbations and in different steady states (disease state or healthy state). Succesful applications of this strategy related to drug discovery are the experimentally validated models of EGFR signalling pathway and cytokine induced apoptosis[116][117]. Cytokine blocking has the potential to favour the appearance of opportunistic infections. How to predict in which patients and how severe will be the infection is not intuitive. Computational models of cytokine signaling have been developed to understand the role of these molecules in the pathogenesis and treatment of septic shock. Using a similar strategy for cytokine actions in autoimmune diseases will be beneficial in order to predict side effects and develop combined therapies with the contemporary agents.

Metabolites

Generating an appropriate (or inappropiate) immune response requires considerable amounts of energy. The ability of a lymphocyte to proliferate or get activated is regulated by its capacity to uptake essential sources of energy such as glucose or aminoacids. In fact, there are differences in the metabolic profile between resting and proliferating lymphocytes. For example the mitogenic stimulation of naive T cells induces a 20 fold increase in glucose uptake and metabolism [118]. Energetic metabolism modulators oriented to induce a beneficial (tolerant) metabolic profile in T cells could be exploited as a new therapeutic strategy. This is the case of statins, peroxisome proliferator activated receptor alpha agonist (PPAR-a) and methylthioadenosine (MTA). Statins block the enzyme 3-hydroxi-3-methylglutaryl-coenzime A (HMG-CoA) essential for cholesterol metabolism. In T cell statins are capable to induce shifts from TH1 cytokine production to TH2 type cytokine secretion. PPAR-a agonists seem to do the same action. Statins have shown moderate efficacy in RA and multiple sclerosis [119]. Another example is MTA. MTA is a component of methionine metabolism. In an animal model of multiple sclerosis MTA prevents acute relapses and reverses the chronic phase of the disease. MTA suppressed T-cell activation in vivo and in vitro, likely through a blockade in T-cell signaling [120].

Combination therapy has proven useful in some chronic and complex diseases such as HIV (AZT-3TC) and dyslipidemia (nicotinic acid-lovastatin). Each drug of a multicomponent intervention is directed toward a different molecular target so that the synergistic effect gives rise to a better outcome than with an individual intervention. In autoimmune diseases there seems to be several possible drug targets, but no one is a magic bullet. It will be of great utility to test in a systematic way the benefit or harm that the combinatorial use of current therapies in autoimmune diseases will have in halting disease progression. In our opinion computational models that simulate the action of multiple interventions in the disease state will change the way drug discovery research is made in the present days.

6.3.   Key Strategy. Modulation of antigen-specific response

The antigen that initiates the autoimmune response is not known for the majority of autoimmune diseases. As the disease progresses to the chronic state new antigens in target tissues are recognized by T cells and antibodies, a phenomenon called epitope spreading. For these reasons developing drugs that modulate antigen-specfic response is a very difficult endeavour. However some attempts have proven to be useful. This is the case of glatiramer acetate (GA) a small peptide designed to be similar to the myelin basic protein (MBP) a main antigen in multiple sclerosis. GA induces a TH2 type response to myelin antigens and is now approved for clinical use in MS. Its efficacy is nearly 30 percent in diminishing acute relapses[121].

Based on the success of GA, a new altered peptide ligand directed to aminoacids 82-98 (MBP8389) is now being tested in clinical trials. MBP8298 is the antigen carried by patients with HLA DR2 present in 50-70 percent MS patients. When used in low doses reduces brain lesions but can exacerbate the disease at high doses [122]. However previous attempts for treating MS patients with altered peptide ligands (APL) showed mixed results with several cases of reactivation of their disease [123] .

Proteomic analysis has proven to be useful in detecting candidate antigens in MS. Using these antigens in DNA constructs with immunosuppressive motifs it has been possible to reduce the number of relapses in 50 % in an animal model of MS [124]. Similar approaches are now being tested in type 1 diabetes mellitus. Heat shock protein 60 (HSP60) is an autoantigen implicated in type-1 diabetes. DiaPep277 is a peptide derived from HSP60 that during a period of two years maintained insulin secretion in patients with new onset type 1 diabetes [125]. Specific antigen vaccination has resulted in significant reductions of disease severity in animal models of autoimmune diseases such as type 1 diabetes, RA and MS. The purpose of this strategy is to induce anergy or apoptosis of autoreactive T cells, promote regulatory T cells proliferation or favour a shift to a TH2 phenotype [126]. This approach looks promising if theoretical work regarding TCR-MHC interaction is joined with proteomic data derived from antigens banks [127].

6.4.   Key strategy. Cell therapy

Almost all current therapies in autoimmune diseases are based on systemic suppression of the immune system by means of monoclonal antibodies or small molecules. No one is curative. Most autoimmune diseases are originated from functional defects of immune system cells. Therefore it seems reasonable to eliminate or modulate the cells responsible for the damage instead of blocking aberrant cytokine production or dismal cell activation. This is the quest of cell therapy.

In the past 15 years there has been a renewed enthusiasm in regulatory T cells (Tregs). Tregs are classified in three groups according to their cytokine production profile and surface marker properties. One group belongs to the CD4 T cell pool. Regulatory T cells type 1 (Tr1) secrete interleukin 10 (IL-10) while T helper type 3 cells (Th3) produce transforming growth factor-β

(TGF-β). There is another group of Tregs that express high levels of CD25 and the transcription factor forkhead box P3 (CD25, FOXP3+)[128][129]. The mechanisms by which Tregs suppress the immune system are not fully understood. Deficient number or altered function of Treg may be involved in the pathogenesis of autoimmune diseases. There is evidence in animal models of RA that depletion of Tregs favour disease progression and that early injection of ex vivo proliferated Tregs can revert inflammation and joint destruction. Similar results have been observed in animal models of MS and inflammatory bowel disease [130]. Another approaches include the use of cytokines (TGF-β for example) that enhance Treg function and number or transfection of nonregulatory T cells with FOXP3 oriented to develop antigen-specific Tregs with increased suppressive activity. However there are concerns related to cell therapy with Treg. Large numbers of Treg can increase the risk of developing cancer and an excessive suppression could promote the appearance of opportunist infections or even induce rebounds of the autoimmune disease .

B cells are one of the pathogenic agents in autoimmune diseases. B cells can produce autoantibodies towards local tissues, act as antigen presenting cells with stimulatory properties over T cells and even produce cytokines in a way similar to T cell. These actions taken altogether can induce inflammation and tissue destruction. Therefore B cells constitute an interesting target in autoimmune diseases. Various strategies are available that range from B cell depletion to manipulation of B cell survival [131]. Rituximab, is an anti CD-20 monoclonal antibody that was initially approved for the treatment of B cell non-Hodgkin lymphoma. The CD-20 molecule is a cell surface marker present only in B cells. Interrupting B cell function is a therapeutic option in a group of autoimmune diseases. Rituximab has proven to be useful in patients with SLE and RA. This monoclonal antibody initiates B cell death trough antibody-dependent cellular cytotoxicity [132]. Clinical trials using rituximab in other autoimmune disease are currently underway [133]. Promising targets in B cell biology are B-cell activating factor (BAFF) and toll like receptors (TLR) especially TLR7 and TLR9 [134][135].

6.5.   Key strategy. Sparing of target organs

Autoreactive T cells and antibodies directed to molecules in local tissues can promote immune damage. T cells, especially type 1 and type 17 helper cells, are essential to the appearance of autoimmune diseases through the production of chemokines that can recruit inflammatory cells which mediated tissue destruction. In a similar way autoantibodies can favour the formation of immune complexes and complement activation, both known mediators of immune damage.

Chronic inflammation is a hallmark of autoimmune diseases, therefore any intervention oriented to reduce the number and activity of inflammatory cells in disease sites will be beneficial. Lymphocytes enter tissues from circulation through the binding to specific adhesion molecules that can be blocked with monoclonal antibodies. In the central nervous system integrin $\alpha_4\beta_1$ is the principal adhesion molecule. An anti- $\alpha_4\beta_1$ monoclonal antibody (natalizumab) is now approved for use in MS [136]. Clinical trials using natalizumab in Crohn's disease show encouraging results

[137]. As a selective blocker of adhesion molecules, natalizumab abrogates T cell migration across biological barriers. This effect can increase the risk of infections. In particular, two patients taking natalizumab in combination with interferon beta developed progressive multifocal leucoencephalopathy caused by JC virus infection. At the moment this drug is sold with a warning label.

Chemokines are chemotactic cytokines that exert their biological actions through binding to G protein receptors in target cells. Chemokines guide lymphocytes to sites of active inflammation. Interfering with chemokines or chemokines receptors is an interesting strategy to reduce inflammation in disease tissues. FTY720 (fingolimod) is a modulator of sphingosine 1-phosphate receptor (SP1). S1P regulates lymphocyte cell trafficking between the lymphatic system and the blood. Therefore FTY720 decreases lymphocyte number in blood interrupting lymphocyte migration from secondary lymphoid tissues. FTY720 has proven safe and effective in clinical trials of transplant rejection and MS [138].

Induction of reactive oxygen and nitrogen species (RONS) is essential in innate immune system response. However, excess in RONS could mediate kidney damage in systemic lupus erythematosus (SLE) and neurodegeneration in MS and Alzheimer disease. Inducible nitric oxide synthase (iNOS) is one nitric oxide synthase (NOS) isoform in charge of generating nitric oxide. Nitric oxide (NO) has a dual role in the promotion of tissue damage. It has the capacity to produce lipid peroxides that favor neuronal loss in models of MS, but in the other hand, NO can regulate important signaling pathways implicated in inflammation such as NF-κ B pathway. There is agreement that the role observed in NO is context dependent. For this reason, several groups are now developing new iNOS modulators that can explode NO virtues and inhibit undesirable side effects.

Receptor activator of nuclear NF-κ B ligand (RANKL) is involved in osteoclast activation, a prominent mediator of bone and joint destruction in RA. Denosumab is a monoclonal antibody against RANKL that binds with high affinity and suppresses RANKL mediated actions. In a phase II clinical trial denosumab reduce bone resorption and promote bone mineralization in postmenopausal women [139]. A similar approach can be used in RA. Matrix metaloproteinases (MMP) are a group of proteases involved in leukocyte migration through endothelial cells. There are at least 25 MMP that can degrade almost all components of extracellular matrix. MPP are thought to be mediators of cellular infiltration in the pathogenesis of multiple sclerosis and RA. Interferon beta acts partially blocking MMP-7 and MMP-9. Finding specific MMP in affected tissues is a good therapeutic strategy that needs to be explored, although side effects due to widespread distribution of MMP seem to be the main limitation for their use in clinical setting [140].

Finally, ahead of interfering with cell migration to reduce inflammatory burden, there is a clear request to develop new interventions that can promote tissue restoration in chronic autoimmune diseases. In this setting cell therapy and nanotechnology can play an outstanding role as shown by recent reports of neural precursor cells-neurospheres in the treatment of EAE [141]

6.6. Summary

Autoimmune diseases are complex diseases. With complex we mean that they are the result of multiple interacting components whose collective properties can not be explained from the study of each component in isolation. Defect in various levels, from genes to cells can lead to autoimmune diseases. It is important to consider that interventions at the gene level can produce T or B cell dysfunction and vice versa. But, how can you accurately predict the benefits or drawbacks of such intervention? To our opinion systems biology (SB) is part of the response. SB integrates theoretical and experimental research. From the theoretical side, SB takes methods to study the structure, dynamics and control devices present in life organisms. In particular SB is interested in the topology and behaviour over time of biological networks. From the experimental side SB uses tools that permit the measure of genes, proteins and metabolites at a large scale. For SB genomics, transcriptomics and proteomics are its principal sources of data [142]

In particular SB is well positioned to address these questions in autoimmune diseases:

- How combination therapy will be more beneficial than single interventions and which side effects are expected with combination therapy?

- How to develop reliable and easy to use biomarkers to follow treatment efficacy?

- What are the rate-limiting steps in autoimmune diseases pathogenesis?

- What is the difference between silent autoimmunity and autoimmune diseases?

The goal for the coming years is to develop therapies that can halt disease progression and ameliorate patients suffering in a more significant way. Truly interdisciplinary research will be of great help.

Conclusions and Outlook

Conclusions

This thesis was about the mathematical analysis of reaction networks implicated in signaling pathways. As a physician the signaling pathways covered are related to human diseases specially autoimmune diseases.

In Chapter 2 we make a review of the current methods available for the understanding of reaction networks based on reaction network structure alone. We propose an algorithm for CRN solution using a mixture of M Feinberg chemical reaction network theory and algebraic geometry. In particular with CRNT multistability is discarded or not. If there is place to multistability, Gröbner basis methods indicate the place and conditions for multistability to occur.

In Chapter 3 we apply the methods developed in Chapter 2 to solve and classify the qualitative dynamics of reaction motifs. We show that considering open and closed versions of reaction modules has a profound influence in the possible outcome of chemical network function. The motifs covered have important medical implications because novel drug targets are being produced to control the activity of key components in these reaction modules

Chapter 4 deals with an apoptosis model. We first discard a published model or receptor induced apoptosis due to intrinsic structural errors. It is demonstrated that when the topological inaccuracies are corrected the systems does not exhibit an essential qualitative property of an apoptosis model: bistability. Finally we elaborate a new apoptosis model capable of bistabilty using parameters reported in the literature. We analyze means to modify the behavior of this model introducing variations in the degration terms of a susceptible drug target

In Chapter 5 we test experimentally the dynamics of a signaling pathway in human patients affected by a complex disease, multiple sclerosis. In a dose response curve we observed a hysteresis loop. A mathematical model for the JAK-STAT signaling pathway accounts for this hysteresis loop. We discuss the clinical implications of such findings.

Chapter 6 is a state of the art discussion of the key strategies that will benefit patients affected by autoimmune diseases. We advocate for the combination of experiments and mathematical models to adress the complex nature of this devastating group of disases.

Outlook

In biomedical research there is a gap between high efficiency technologies and the knowledge derived from the data produced by them [116][78]. The methods described in this works are susceptilbe to automotation and direct linking to experimental data. Some initiatives are oriented in this way.[143]. Another future improvement is the adaptation of the methods discussed in chapter 2 to large systems (>20 components). Currently algebraic geometry methods have a high computational cost and new algorithms are needed to grasp the complexity inherent to biological systems.

Cells are open systems that interchange information and material with the environment. Some of the reaction networks explored in this work are closed and when a structural change is introduced in the form of a turnover reaction, the qualitative dynamic of the system can change abruptly. It is of clinical interest to experimentally validate this mathematical observation because there are pharmacological strategies oriented to inhibit or augment each of the procedures in the turnover reaction: production and degradation. Let us suppose that after the administration of a treatment there are two groups of cells that respond in different ways to the drug, one with high efficacy and the other with low efficacy. If the reaction module affected by the pharmacological compound is the same and is closed (due to the disease for example), the dynamic behavior of the system can be altered promoting the return to the efficacy state by means of turnover manipulation.

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Summary

The objective of this thesis is to improve the understanding of chemical reaction networks derived from signaling pathways of biological interest. To this end, we make a study of the ordinary differential equations that model the dynamics of the chemical species participating in the network. We put a special emphasis in equilibrium solutions of the system applying methods from algebraic geometry and convex analysis. The techniques developed are used in the study of biological systems of interest such as apoptosis and interferon β signaling pathway which are involved in human complex diseases (cancer and multiple sclerosis).

Resumen

El objetivo de esta tesis es mejorar la comprensión de los sistemas de redes reacciones químicas derivados de vías de señalización en biología. Para esto, realizamos un estudio de los tipos de ecuaciones diferenciales que modelan la dinámica de las especies químicas que participan en la red. Hacemos especial énfasis en las soluciones de equilibrio del sistema con la aplicación de técnicas de geometría algebraica y análisis convexo. Los métodos desarrollados son utilizados en el estudio de sistemas biológicos de interés como la apoptosis y la vía de señalización del inteferon β implicados en enfermedades humanas complejas como cáncer y esclerosis múltiple.