RESEARCH PROPOSAL

MODELING A CELLULAR TRANSMEMBRANE SIGNALING SYSTEM THROUGH INTERACTION WITH G-PROTEINS BY USING CONCURRENT CONSTRAINT PROCESS CALCULI

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ABSTRACT

At the cellular level of abstraction, molecular mechanisms to communicate with the environment involve many concurrent processes, objects, and relationships that dynamically drive the cell's function over time. The membrane the surface that acts as the boundary of a cell contains many receptors which are responsible for concurrently interacting with diverse signals molecules and sensing external information over time. Each receptor recognizes specific molecules that may bind to it. Binding activates signaling pathways that regulate molecular mechanisms and the flow of information in the cell. There is a special class of receptors which constitutes a common target of pharmaceutical drugs, the Guanine nucleotide-binding protein-coupled receptors (GPCRs). These receptors interact with their respective Guanine nucleotide-binding proteins (G proteins) to induce a intracellular signaling. We will develop a process calculi modeling by using concurrent constraint programming of the cellular transmembrane signaling mediated by receptors through interaction with G Proteins which in conjunction with quantitative and qualitative information will allow us to describe the behavior of this robust biological system.

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CHAPTER 1

Introduction

Progress in the life sciences, including genome sequencing and high-throughput experimentation, offers an opportunity for understanding biology and medicine from a systems perspective, that complements the more traditional component-based approach; this involves the integration of biological research with approaches from engineering discipline and computer science [11]. This recent research area, is known as computational and systems biology, an interdisciplinary field that applies the techniques of computer science, applied mathematics and statistics to address biological problems [2].

Molecular biologist use information and computer technology to process, analyze, understand, compare, and share scientific knowledge. With the recent progress of molecular biology, it is possible to describe the components that constitute living systems. The major effort is to scale up to systems biology, to take under consideration, how the individuals components of the cell interact to take part into complex systems. The challenge, is to use computer science techniques and its formalisms, for studying biological systems, such as metabolic networks, signaling pathways, regulatory circuits, or even an entire integrated cell [10]. The current context to model biological systems puts particular interest in three main domains of application and of reasoning: mathematics with ordinary and partial differential equations, statistics with stochastic simulation algorithms, and the field of computer science with processes calculi and multi-agent systems. These focuses are related to different layers of complexity, depending on the particular system under study, and the questions to be answered.

Complex biological processes such as tissue functions or immune responses; are orchestrated trough precise, dynamic regulation of cell behavior, primarily achieved through active dialogs between the cells and their environment; many of these activities are controlled by cell-surface receptors which in response to certain ligands trigger intracellular signaling reactions that elicit the cellular responses; this exchange of information, or signals, collectively named cellular signaling, is based on the cell's ability to ″read‟environmental cues, ″translate‟them into intracellular commands, and ″react‟with appropriate responses [1].

One of the most recent and exciting area to explore and understand the complexity of biological systems using computer science is the cellular signaling events or signal transduction pathways. This field studies the mechanisms that enable the transfer of biological information within and across cells [3]. Many diseases such as cancer involve malfunction of signaling events. Mathematical and computational modeling and simulation in this field, helps and guides molecular and computational systems biologists, to establish a conceptual framework in which to think and represent the ″reception‟, ″transfer‟and ″interpretation / translation / expression‟ of biological information. With this understanding it might be possible to make a contribution not only to theoretical biology, but even to experimental biologists by offering a fertile substrate to think and redesign experiments.

Most biological functions are mediated by cell surface receptors, i.e. a protein-protein interaction between the receptor and the respective ligand (signaling molecule). These molecules have three domains: an extracellular, a

membrane and an intracellular [4]. The association and interactions between these domains allow the transmition of the signal inside the cell. The interactions can be physical, such as when two proteins form a complex, or ″logical‶such as when one or more proteins control the behavior of one or more other proteins without physical interaction [*ibid*]. A large number of molecules and proteins take part in signal transduction events, starting from a relatively small and initial stimulus, resulting in a ″signal cascade‶that elicits a large response. Diverse signaling molecules, including neurotransmitters, hormones, phospholipids, photons, odorants, taste ligands and mitogen, bind in the membrane of the target cells to their specific Guanine nucleotide-binding protein-coupled receptors (GPCRs), also known as seven-transmebrane receptors (7TMRs) [5]. Subsequently the receptors interact with their respective G proteins to induce a cascade of downstream, i.e. intracellular signaling.

The modeling of cellular signaling processes is challenge due to the complexity of the organization and interactions of biological systems. It requires a deep understanding of the system in terms of its structure and its behavior. Considering that biological systems are complex and consist of a set of components interacting with each other in a (dynamic) environment [6], a conceptual framework from engineering, most specifically from computer sciences, the processes calculi, emerges as a formalism that can be used to represent and simulates the behavior of these systems. Process calculi involves the concurrent behavior of various processes, mechanisms, and parts. One of the most exciting recent applications based on process calculus is in the field of systems biology, in which a set of basic and general primitives for modeling biological systems is defined, inspired by actual biological processes, and these primitives are then used to develop an

executable model of an increasingly complex biological system [12].

In the context of this research proposal, we will use an approach that has been developing by researchers of the Research Group Avispa at the Pontificia Universidad Javeriana for several years, the concurrent constraint programming (CCP) as a possible computational model for biological phenomenas [8]. In particular, the use of non-deterministic temporal concurrent constraint programming (ntcc), a temporal CCP process calculus, has been used for modeling biological systems. The natural use of concurrent agents (i.e., processes) for modeling biological entities, the explicit notion of time for describing the evolution of the dynamic of a biological system, constraints as a formal mechanism for representing partial information in the state of the system, asynchronous and non-deterministic operators for modeling partial information about the behavior of the system and the possibility of including quantitative information for parameterizing models with experimental values, characterizes (ntcc) as a appropriate methodology for modeling in biology [7].

The complexity of the problem is reduced by decomposing the natural system into its basic elements, which are then reassembled and combined to form a comprehensive model of the real system. According to [3], a biological system is an assembly of biological components; hence to understand a biological system, it is not sufficient to describe its components in details, it is necessary to describe their behavior in relation to the characteristics of the system and to comprehend what happens when certain stimuli or disruptions ocurr. Concurrency theory and particularly process calculus, are emerging as a suitable tool to provide formal foundations to systems biology; the abstraction metaphor is to see biological

components as concurrent computations/programs (i.e. processes) running in parallel, in which the components can interact (i.e. communicate selectively based on its capabilities). To include quantitative aspects driving the behavior in a physico-chemical world, probabilistic/stochastic models are mandatory [13]. Most of the processes in the cellular signaling events involves chemical and enzymatic reactions. For this and other reasons considered in [13,14], it becomes necessary to take in to consideration the stochastic formulation of the process calculus developed in [9]. Process calculi based modeling has several advantages of its own. Our goal is to find useful abstractions that can improve our understanding of the cellular signaling system, and explore the opportunities of these representations in terms of analysis, simulation, and scalability.

This research proposal has two purposes: one purpose is to understand the interactions and the behavior of the cellular signaling processing information in the cell membrane and be able to compare it with other models from a biological point of view. The second one, it is a fundamental objective for the research group Avispa, we are interested in evaluate the uses of the concurrent constraint process calculi to explain and to model complex biological systems. Hence, for the purpose of the modeling process, we will consider for example, what kind of constructs of the calculus requires certain notions of stochastic choices, or if it is better to calculate a probability or if it is better a stochastic calculus. All these considerations taken from the biological point of view to be included in the notion of the calculus for modeling the evolution of the system toward a stable point in space and time.

CHAPTER 2

Overview

2.1 Research Problem Formulation

Why is needed predictive models of signal transduction systems?. The molecular changes that affect cell signaling cause/sustain diseases such as cancer. Over 200 drugs that target signaling proteins are currently in clinical trials with spectacular success in some cases, but the results are largely disappointing for most patients, there are too many combinations to consider in trials. We can use computational models to gain comprehension about this biological system and we can determined some useful predictions using a appropriate model specification of the system. A signaling protein is typically composed of multiple components (subunits, domains, and/or linear motifs) that mediate interactions with other proteins. The combinatorial complexity is a serious problem for the conventional modeling approach, because in the model the size tends to grow nonlinearly (exponentially) with the number of molecular interactions in a system when molecules are structured. The problem of combinatorial complexity necessitates a new modeling approach, in our case, the natural use of concurrent agents (i.e., processes) for modeling biological entities, the explicit notion of time for describing the evolution of the dynamic of the system, constraints as a formal mechanism for representing partial information in the state of the system, asynchronous and non-deterministic operators for modeling partial information about the behavior of the system and the possibility of including quantitative information for parameterizing models with experimental values, characterizes the concurrent constraint process calculi, particularly (ntcc) as a appropriate methodology [7] for modeling a cellular signaling transduction system mediated by receptor/ligand interactions.

2.1.1 Biological Problem Formulation

At the cellular level of abstraction, molecular mechanisms to communicate with the environment involve many concurrent processes, objects, and relationships that dynamically drive the cell's function over time. The membrane the surface that acts as the boundary of a cell contains many receptors which are responsible for concurrently interacting with diverse signals molecules and sensing external information over time. Each receptor recognizes specific molecules that may bind to it. Binding activates signaling pathways that regulate molecular mechanisms and the flow of information in the cell. A receptor is typically made up of three parts: the extracellular domain, the transmembrane domain and the intracellular domain. There is a special class of receptors which constitutes a common target of pharmaceutical drugs, the G-protein-coupled receptors (GPCR′s). These receptors interact with their respective G proteins to induce a intracellular signaling.

According to [24] when a ligand such as a hormone, neurotransmitter, or glycoprotein interacts with a GPCR′s on the surface of the cell, the ligand either stabilizes or induces a conformation in the receptor that activates a heterotrimeric G Protein (composed by α, β, and γ-subunits) on the inner membrane surface of the cell. In the inactive heterotrimeric state, GDP is bound to the Gα-subunit. Upon activation, GDP is released, GTP binds to Gα, and subsequently GαGTP dissociates from G$\beta\gamma$ and from the receptor (Fig. 1 and Fig. 2). Both GαGTP and G$\beta\gamma$ are then free to activate downstream effectors. The duration of the signal is determined by the intrinsic GTP hydrolysis rate of the Gα-subunit and the subsequently reassociation of GαGDP with G$\beta\gamma$.

The aim of this proposal is to develop a process model using concurrent

constraint programming about cellular signaling pathways trough G Proteins based on qualitative and quantitative information found in the literature [17-24]. Our modeling effort, will be directed towards describing the behavior of the system in terms of the ligand-receptor binding and the events associated with cellular signaling trough interactions with G Proteins.

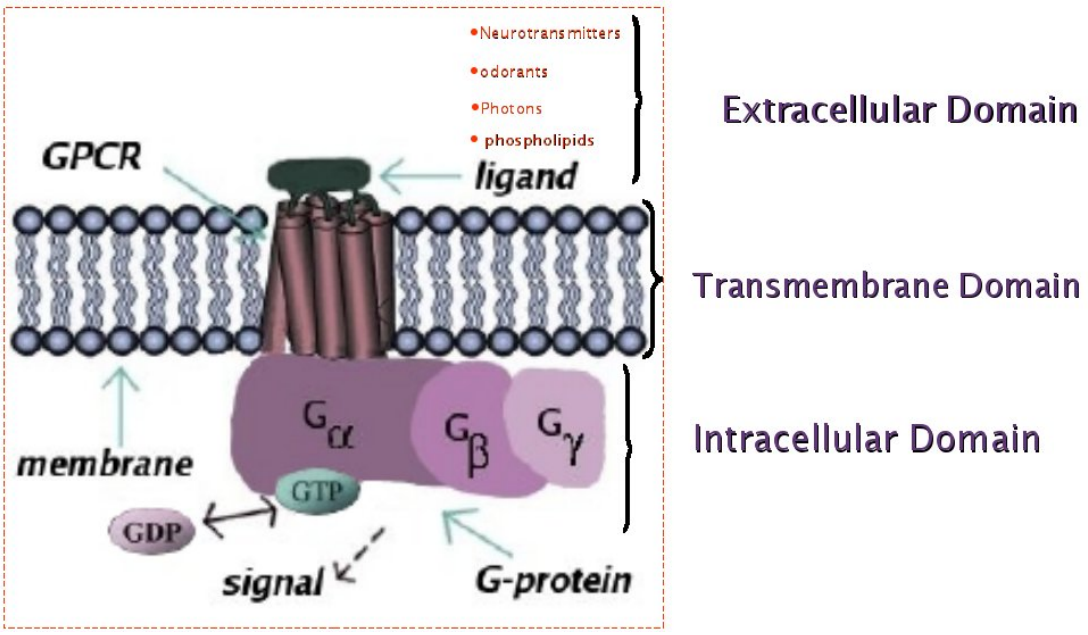

Figure 1. G-Protein-coupled receptor: first level of abstraction [54].

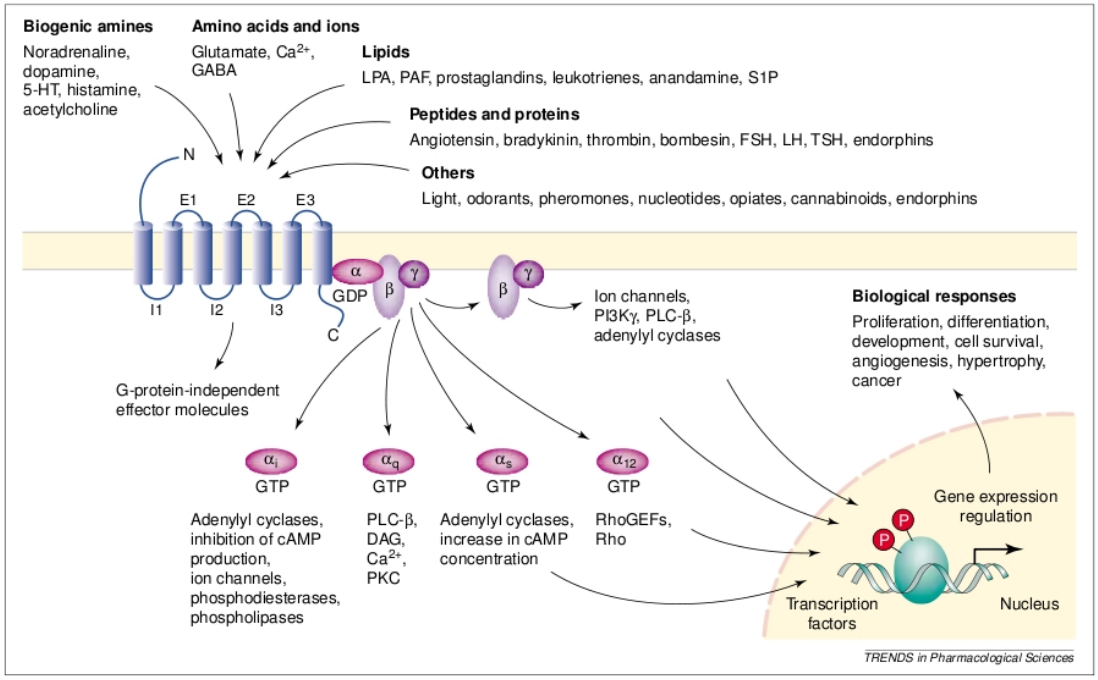

Figure 2. G-Protein-coupled receptor signaling pathway: second level of abstraction [22].

2.1.2 Mathematical Problem Formulation

The mathematical form for modeling of signaling transduction pathways depends on the properties of the studied system and the specific questions that are going to be answered. The ligand and receptor interaction is a common process that happens in most of signaling. With the binding of the ligand (L), the receptor (R) forms a ligandreceptor complex (LRC) with a characteristic kinetic constant k_{on}. On the other hand, the ligand-receptor complex can also dissociate to ligand and receptor with a kinetic constant k_{off}. The ratio of k_{off} to k_{on} is called dissociation constant k_d. If the receptor has other proteins associated in the inner part of the membrane, there are other kinetics constants that play a key role in the signaling process. This network of biochemical reactions that governs the signaling events to processing information in the cellular membrane trough interactions between receptors and ligands, are usually modeled using chemical equations of

the form $R_1 + ... + R_n \rightarrow_k P_1 + ... + P_m$, where the n reactants $R_i's$ (possibly in multiple copies) are transformed into m products $P_j's$. Either m or n can be equal to zero; the case $m = 0$ represents a degradation reaction, while the case $n = 0$ represents an external feeding of the products, performed by a biological reactive environment. Each reaction has an associated rate k, representing essentially its basic speed. The actual rate of the reaction is $k \bullet X_1...X_n$, where X_i denotes the number of molecules of type R_i present in the system.

2.2 Research Hypothesis

There is information available about kinetic models of cellular signaling pathway of G Protein [17-24]. Based on these models we propose to view the protein molecules involved in the signaling pathway as agents (i.e., processes), each capable of performing computations and exchanging information with others in order to achieve a common goal. According to [7], (ntcc) is a temporal concurrent constraint calculus suitable to model non-deterministic and asynchronous behavior and is particularly appropriate to model reactive systems that respond to stimuli from the environment. This particular feature of the (ntcc) process calculus, will allow us to build a preliminary model of the cellular signaling system mediated by the behavior of the G Proteins. We plan to follow an approach similar to that there are in [31], where a model of the Rho GTP-binding cycle is introduced. In particular, we plan to build an extended model based on a stochastic/probabilistic extension of (ntcc) process calculus to include quantitative information such as the rates of the different chemical reactions that control the processes of cellular signaling.

Another important part of the model development is characterizing the cell surface receptor. Receptors are proteins that act as the cell's sensors of outside

conditions relaying information to the inside of the cell. G-protein-coupled receptors constitute a common target of pharmaceutical drugs. As we mentioned before, this molecule has an extracellular domain, a membrane domain, and an intracellular domain. The cell surface receptor components will be accessed concurrently in each unit of time by different molecules (ligands and G Protein). In the modeling process will be necessary to establish new definitions for considering these domains as places for interaction that play a key role in the activation/deactivation of the cellular signaling.

To develop the model, we focus on questions such as, what components of the G Protein are needed to continue the cellular signaling cycle; the time and interaction between the ligand and the receptor during the period of the G protein cycle; the effect of enzymes that mediate the cycle, the interaction between the components of the system and their effectors, and other ones, that can be appear during the period of modeling; which we believe we can solve with the framework of the CCP-based process calculus developed by the Research Group Avispa.

CHAPTER 3

Objectives

This research proposal aims to meet the following objectives:

3.1 General objective

To analyze the advantages and possible limitations of using the concurrent constraint calculus model (CCP), to represent the biological system of the cellular transmembrane signaling mediated by receptors that trigger a chemical response to intracellular level through interaction with G Proteins.

3.2 Specific

1. To build a preliminary model based on (ntcc) process calculus for the cellular signaling transmembrane mediated by receptors that trigger a chemical response to intracellular level through interaction with G Proteins.

2. To extend the preliminary model using stochastic and/or probabilistic extension of (ntcc) process calculus including quantitative information about the chemical reactions for the cellular signaling transmembrane mediated by receptors through interaction with G Proteins.

3. To evaluate the preliminary and the extended model (probabilistic) of the cellular signaling transmembrane mediated by receptors through interaction with G Proteins by using the available tools (software) developed by the Research group Avispa.

4. To analyze the use of the process calculus in modeling cellular signaling transmembrane, focusing on computational systems biology.

CHAPTER 4

Justification

The signal transduction is a complex protein signaling process with a rich network of multifunctional interactions that occur in non-linear fashion. The computational systems biology as a recent research discipline promises a systemic approach to interpret and tackle the complexity in the dynamics of cellular signaling through the integration of biological information with a computational approach to shape a new biology [15].

Much effort has been made nowadays to find plausible mathematical and computational models for the description of G protein signaling dynamics in order to (i) analyze them as well as the efficacy of the ligand molecules, (ii) have a better understanding of how and which processes have control of the behavior of the machinery of the cellular signaling; and (iii) make a contribution at the level of molecular and pharmacological biology.

The main interactions and events are already well known [17-24] of the G-protein-coupled receptors (GPCR′s) and their interactions with their respective G proteins to induce an intracellular signaling, however, are not completely understood. This is especially true for aspects like cellular specificity, i.e., the way in which the different parts of the signaling pathway become active as well as time dependence of the activation. Cellular signaling -cellular information processing- is critical to the survival of all organisms and plays a critical role in human health and disease. The use of process calculus to represent biochemical systems has become a common effort to obtain compositional and scalable representations of

large biological systems, such as the ones found in systems biology [25-30]. Concurrent constraint programming based process calculus has shown to be convenient for modeling, simulating, and verifying several kinds of biological systems [7,8,16]. We will develop based on biochemical and computational information collected about cellular signaling pathways reviewed on the research literature and the experience of the research group Avispa, a formal model using a CCP-based process calculus for the cellular signaling mediated by G Proteins and thereby to make a contribution to the understanding of the biological foundations of these processes.

We believe CCP calculi offer at least two advantages to model this system. First, the ability to deal with various types of partial information, such as information about the state of the system (the exact values of the variables at each time), information about the temporal occurrence of events (e.g. the exact moment a binding occurs), information about particular interactions (whether an unexpected interaction actually happens) and, finally, information about the relative velocities of reactions (in the stochastic calculus). A second advantage is the declarative nature of the calculus that allows to formally verify properties of the model that might be difficult to infer from a simulation. All of these are important for the system we will address in this thesis.

CHAPTER 5

Scope

1. A discrete model based on (ntcc) process calculus for the cellular signaling transmembrane mediated by receptors that trigger a chemical response to intracellular level through interaction with G Proteins.

2. An extended model using stochastic and/or probabilistic extension of (ntcc) process calculus including quantitative information about the chemical reactions for the cellular signaling transmembrane mediated by receptors through interaction with G Proteins.

3. A report of results of the evaluation related with the application of the available tools (software) developed by the Research group Avispa for the discrete model and the extended model (probabilistic) of the cellular signaling transmembrane mediated by receptors through interaction with G Proteins.

4. A series of conclusions related with the advantages and possible limitations of using the concurrent constraint calculus model (CCP) for modeling cellular signaling transmembrane mediated by receptors that trigger a chemical response to intracellular level through interaction with G Proteins focusing on computational systems biology.

CHAPTER 6

Expected Results

The principal contributions that we expect from this research proposal are the following:

1. A process model in (ntcc) and a stochastic/probabilistic process model which allow us to represent the behavior of a biological cellular signaling system such as the cellular signaling transmembrane mediated by receptors that trigger a chemical response to intracellular level through interaction with G Proteins by using the characteristics of discrete time of the concurrent constraint process calculi.

2. A report of results from the simulation of the process model in (ntcc) and the extended version (probabilistic) of the process model for the cellular signaling transmembrane mediated by receptors through interaction with G Proteins using the available tools developed by the Research group Avispa.

3. A theoretical perspective to verify some biological cellular signaling properties to intracellular level through interaction with G Proteins by using the proof system of the (ntcc) process calculi.

4. A state-of-the-art about the modeling of these type of biological systems using concurrent constraint process calculi.

5. A research publication associate with the mentioned expected achievements.

CHAPTER 7

Theoretical Framework

7.1 Background

The field of Computational Sciences can provide to molecular and systems biologists the abstraction needed, in some cases much-needed, for consolidating knowledge of biological systems. Biology and Computer Sciences join as a field, where the overarching goal is to determine how the various parts of a biological system interact to produce the overall behavior of the system that we observe. One basic but critical example of such system is the cell. The typical cell has thousands upon thousands of molecular parts that interact in complex ways to produce a wide range of behavior. We need computational and mathematical models to integrate and interpret this information.

Many complex systems are difficult to describe and understand because they are composed of large numbers of elements interacting in a non-ordered way. A good example is cellular biology: diverse cellular components (genes, proteins, enzymes) participate in various reactions and regulatory interactions, forming a robust system. According to [57], a very useful representation of complex systems is given by graphs (or networks), in which it is denoted the components with nodes and their interactions by edges. The properties of these interaction graphs can then be analyzed by graph theoretical and statistical mechanics methods and this information can lead to important conclusions about the possible dynamical behaviors of the system. However, the study of quantitative information within languages for concurrency has recently gained a lot of momentum, specially because in many applications, quantitative information becomes crucial when refining models with experimental data, and it is the essence for verifications

purposes [31]. So, in this sense, for the motivation of this research proposal, we are interested in a novel approach for analyzing systems exhibiting complex stochastic and probabilistic behavior in the form of a discrete-timed concurrent constraint process calculus.

Some cellular signaling pathways have been studied using some formalisms of process calculi. For example, in [25] a particular signaling cascade involving MAP-Kinase proteins is developed using stochastic concurrent constraint process calculi, generically represented by enzymes that trigger a chain of enzymatic reactions inside cell's when an external stimulus occurs in the cell-surface receptor. The model basically was made with a list of reactions running in parallel by associating the corresponding basic rates of each reaction. Another model for the RTK-MAPK pathway was developed in [44] putting particular emphasis in biomolecular process of the protein-protein interactions using the pi process calculus. The authors developed the model based on three principles of correspondence, (i) the authors defined a primitive of the process to choosing the functional signaling domain to capture the functional and structural independence of domains in signaling molecules, (ii) the component residues of domains are modeled as communication channels, (iii) the molecular interaction and modification is modeled as communication and the subsequent change of channel names. The authors concluded, that the strength of this approach stems from treating molecular entities as a computational process which allow a representation at the level of molecular detail and dynamic behavior in a unified single description.

G-proteins represent a crucial family of signal transduction molecules that govern a variety of physiological functions. Moreover, GPCRs have traditionally

been (and continue to be) a major exploitable drug target, giving rise to a plethora of clinically relevant molecules. Thus, a more complete understanding of the fundamental properties of GPCRs and how they interact and activate their target G-proteins, is of utmost importance to future drug discovery [32]. How GPCRs operate is one of the most fundamental questions in the field of transmembrane signal transduction [33-38]. We identify that one of the most important features in the modeling of biological systems is the fact that in many cases, only partial information about their behavior at systems level is available. For this reason in [53] they argue explored the use of CCP as a possible computational model for representing this kind of information in biological systems, in particular using process calculi based on constraints. As was argued in [9], the explicit timed concurrent constraint programming language (ntcc) include a explicit notion of time to represent the processing information in a biological system, as too, a proof system to validate properties of this. They developed an extension to include quantitative information such as the rate that controls the frequency in which chemical reactions can occur in a cellular signaling pathway. Thereby we believe that this theoretical framework provides possibilities of application in the domain of molecular and cellular biology. Thus, in addition to our curiosity about the fascinating mechanism that cells use to respond to signals, there is a practical motivation to a better understanding of processes involved in cellular signaling in which the interactions of their components play a central role.

7.2 Literature Search Results

7.2.1 Mathematical Strategies: Equation-based Modeling

Mathematics with Ordinary and Partial Differential Equations In most biological models, the biochemical reactions system is described in a deterministic,

continuous manner by rate equations for the concentrations of reactants, products and complexes, based on mathematical equations as the most commonly used approach for modeling cellular signaling pathways [39]. Differential equations are a classical approach for biochemical systems modeling and have frequently been used to describe reactions of interest in cellular signaling pathways mediated by G Proteins [17-24].

To choose a mathematical formulation or a method of simulation of a biochemical reaction network (the pathway), models are based on the assumption that each species is uniformly distributed throughout the cell; this enables the construction of a set of equations that can might be algebraic or can capture variability over time (ordinary differential equations (ODEs)) or over time and space (partial differential equations (PDEs)). One equation is required for each species in the model, the solution of each of them provides the average concentration of each species as a function of time. The problem with mathematical modeling is that it requires a great amount of detailed (quantitative) information about every part of the system and is most naturally applied to systems that can be modeled centrally in which the dynamics are dominated by physical laws rather than information processing.

7.2.2 Statistical Approaches: Stochastic Simulation Algorithms

During the 1990s, these methods began to be applied widely to simulate biological systems. Recently, the efficiency of these methods has been increased significantly, so that the earlier simulations can now be solved on desktop machines instead of supercomputers, further reductions in computational cost will be relate to ″linking‟deterministic and stochastic regimes to produce new methods, and to

handle large numbers of coupled reactions, however, the promise of these methods still also depends on increases in computational power [33,50].

In the stochastic models [14,40-42] the events are described by probabilities and unlike the deterministic models given the same input, a stochastic model will give (somewhat) different results each time. This modeling is used when there are small numbers of molecules involved or when it is useful to track likely movements of individual molecules. The reaction rates are turned into reaction probabilities following the approach suggested by Gillespie [40]. A disadvantage of stochastic models is that they are more difficult to solve, and simulations must be run multiple times to gather statistics on the range of possible outcomes [20].

7.2.3 Computational Science Formalisms

Advanced computer science concepts are being used to investigate the ″molecule-as-computation‟abstraction [28], in which a system of interacting molecular entities is described and modeled, by a system of interacting computational entities. Computer formalisms, such as process calculus, were developed for the specification and study of systems of interacting computations; yet are now being used to represent biological systems, including e.g., regulatory metabolic networks, gene networks, signaling pathways and multicellular processes such as immune responses. These approaches enable simulation of the behavior of biological systems, as well as development of knowledge bases to supporting qualitative and quantitative reasoning about the properties of these systems.

The behavior of biological cellular signaling systems, depends on their dynamic interactions among its components. The combined effects of these

interactions are difficult to predict. A mathematical based equation model, is often useful for acquiring a quantitative and predictive understanding of a complex dynamical system, and has been used to studies of cellular signaling. However, current models are still far from capturing all of the relevant mechanisms and details that must be considered to provide realistic and complete pictures of how these systems behave. In particular, models based on process calculus can provide a better understanding at the level of interactions and emergent properties.

Rules Based Modeling Recent approaches are directed to seeking for explicit representation of the proteins interaction sites. The goal is to develop computational models consistent with a base of biological knowledge and having a resolution equal to that of experiments, ″we don't want to predict more than we can measure‟.

Recently, a new computational approach appears to provide a easy way to specify a model that incorporates details at the level of molecular interaction in the proteins actives sites. The rules-based modeling was developed by [21,47] as a hierarchical representation of molecules and their components (domains, motifs, etc). Roughly speaking, a rule such as ″ligand binds receptor with rate constant k whenever ligand and receptor have free binding sites‟, describes the features of reactants that are required for a particular type of chemical transformation to take place. Rules come from formal statements in biological literature that are testable and simplifies the specification of a model when the reactivity of a component in a system is determined by only a subset of its possible features.

Agent-Based Modeling According to [48] a system is made up of a set of interacting individuals, some of the observables of interest may be defined only at the system level, while others may be expressed either at the individual level or as an aggregate at the system level. The Agent-based modeling defines agent behaviors in terms of observables accessible to the individual agent, being most appropriate computational paradigm for domains characterized by a high degree of localization and distribution, dominated by discrete decisions; opposite to equation-based modeling, that it is most applied to systems that can be modeled centrally, and in which the dynamics are dominated by physical laws rather than **information processing** [*ibid*]. Agent-based modeling begins with behaviors through which individuals interacts with one another. These behaviors may involve multiple individuals directly or indirectly through a **shared environment**. The modeler pays close attention to the observables as the model runs, and may value the relations among those observables, as a result of the modeling and simulation activity.

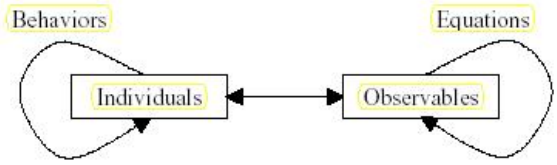

Figure 3. Unifying multiplicities: agent-based modeling [48].

This theory constitutes a useful approach for the modeling cellular signaling networks, in [49] was developed a agent-based model called Cellulat, in which a cell can be seen as an adaptive autonomous agent or as a society of such agents, where each one can exhibit a particular behavior depending on its cognitive capabilities, this model, takes into account two essential aspects of the cellular signaling networks: (*i*) cognitive capacities, which were modeled as the agent abilities to interact with the surrounding medium and (*ii*) a spatial organization,

obtained using a shared data structure through which the agents communicate between them. This methodology for modeling cellular signaling pathways can be used as a virtual laboratory.

Process Calculi: The Pi Calculus According to [43], the pi calculus was created as a theoretical framework by Milner and co-workers for the study of concurrent computation; now is widely accepted as a model for interacting systems with dynamically evolving communication topology. Its primitives are interactions and processes. It is based on names and uses a small set of operators to create terms that are referred to as process, the syntax is accompanied by a semantics that specifies the interpretation of processes, there is only one rule of action that allows two concurrent processes can interact using a channel, in which, one process acts as a sender, while the other acts as a receiver, in this message-passing shared environment, are described networks with evolving connectivity [28,51]. The concurrency paradigm and the pi calculus theory are uniquely suited to model and study biochemical processes in general and cellular signaling pathways in particular, that allow to fully represent complex molecular structures and events of cellular signaling [30].

In [13] is introduced a process model of the Rho GTP-binding protein, that is a molecular switch to produce a signaling physiochemical response in the cell, where the parts of the Rho GTP cycle were treated as components of a pi calculus process using its stochastic variant. They developed, a first basic model, and compositionally increased the complexity by including other components. In [30,44] is represented the complex RTK-MAPK cellular signal transduction pathway by using the pi calculus, unifying dynamic behavior and functions of

the pathway, with the molecular details that underly their behavior, with this framework the authors provide a comprehensive model mathematically well-define and biologically consistent.

As was proposed by Luca Cardelli and co-workers, ″the ultimate goal of systems biology it to predict the behavior of living matter. If we can devise process calculus that can predict behavior, then are on the right track. Ultimately, we want to understand the functioning of cells at useful levels of abstraction, and we want to be able to predict unknown behavior‶.

Process Calculi: The CCP Calculus Partial information arises naturally in the description of biological systems in absence of complete information of the whole system. In the modeling process, is possible to distinguish two kinds of these information: partial quantitative and partial behavioral. The first one involves incomplete information on the state of the system (e.g., the set of possible values that a variable can take). The second one refers to the uncertainty associated to behavior of interactions (e.g., the unknown relative speeds on which two systems interact). Notables efforts are made to find appropriate ways to represent these kind of partial information oriented to a better understanding of the complex pattern behavior frequent in biological systems.

According to [7,8,16] partial information is a central feature of concurrent constraint programming, a well-established formalism for concurrency. In CCP, process interact with each other by telling and asking partial information represented as constraints (e.g. a value of pH can be represent as $5 < pH < 7$). They argue, that the most appealing and distinctive feature of CCP, is that it combines

the traditional operational view of process calculus with a first order declarative logic; the process can be viewed at the same time, as computing agents and logic formulas, for these reasons CCP can be a convenient framework to describe and reason about biological systems. To achieve our first objective, will be used, the (ntcc) process calculus [45,46], a constraint-based calculus for modeling temporal non-deterministic and asynchronous behavior of processes, a suitable language for analyzing biological systems, where the above mentioned kinds of partial information are naturally captured.

In CCP [52] a fundamental issue is the specification of concurrent systems by means of constraints, a constraint represents partial information about certain variables, during the computation, the current state of the system is specified by a set of constraints called the store, in which processes can change the state of the system by telling information to the store (i.e., adding constraints), and synchronize by asking information to the store (i.e., determining whether a given constraint can be inferred from the store), a typical CCP process languages contains the following operators: (i) a tell operator adding a constraint to the store, (ii) an ask operator querying if a constraint can be deduced from the store, (iii) parallel composition combining processes concurrently, (iv) a hiding operator (also called restriction or locality) introducing local variables and thus, restricting the interface that a process can use to interact with others.

In the last few years a compositional modeling approach based on stochastic process calculus emerges [25] based on the parallel between molecules and reactions on one side and processes and communications on the other side. The stochastic modeling of a biological system implies be able to include in the process model

information about the frequency or propensity of interactions i.e., the rate associated to each active biochemical reaction in the biological system. In this sense, all active reactions are undergo in a (stochastic) race condition, and the fastest one is executed. In [9] is proposed an extended version of the CPP-based process calculus for modeling biological systems with the aim to include quantitative information about the biochemical reactions. This approach will be used for achieve our second objective, a more complete and enriched model of the cellular signaling pathway.

CHAPTER 8

Method

8.1 Description

8.1.1 The (ntcc) Process Calculus

It is a temporal extension of CCP [45] that captures the main features of timed and reactive systems that allows to model: unit-delays to explicitly model pauses in system execution, time-outs, to execute a process in the next time unit if in the current one a piece of information cannot be inferred, synchrony to control and coordinate the concurrent execution of multiple systems, infinite behavior to represent unbounded but finite delays in the execution of a system, non-determinism, to express the diverse execution alternatives for a system from the same initial conditions.

In succinctly speaking, modeling reactive computation, is parameterized on a constraint system, in which time is divided into discrete units and in each time unit, the environment supplies some information into a store, this information is represented by constraints, concurrent agents interacts to add new constraints to the store and the processes can be scheduled to run in future time units.

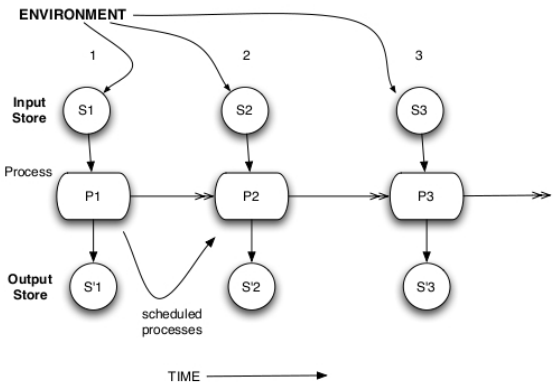

Figure 4. (ntcc) models reactive computation.

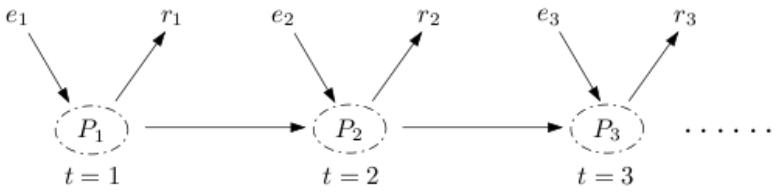

Figure 5. Discrete reactive computation in (ntcc).

In the process syntax, processes P, Q, ... ε *Proc* are built form constraint c ε C and variables x ε V in the underlying constraint system by:

$$\begin{array}{lcl} P, Q, \ldots & ::= & \mathbf{tell}(c) \quad | \sum_{i \in I} \mathbf{when}\ c_i\ \mathbf{do}\ P_i \quad | P \parallel Q \quad | \mathbf{local}\ x\ \mathbf{in}\ P \\ & | & \mathbf{next}\,(P) \quad | \mathbf{unless}\ c\ \mathbf{next}\ P \quad | \star\ P \quad | !\,P \end{array}$$

Figure 6. Process syntax in (ntcc).

Agent	Meaning
tell (c)	Adds c to the current store
when (c) **do** A	If c holds now run A
local (x) **in** P	Runs P with local variable x
$A \parallel B$	Parallel composition
next A	Runs A at the next time-unit
unless (c) **next** A	Unless c can be inferred now, run A
$\sum_{i \in I}$ **when** (c_i) **do** P_i	Chooses P_i s.t. (c_i) holds
$*P$	Delays P indefinitely (not forever)
$!P$	Executes P each time-unit

Figure 7. (ntcc) agents.

A more complete explanation of the process syntax, operational semantics and the logic approach for properties verification in the calculus (ntcc), can be found in [7,8,16,45,52,53].

To build a preliminary discrete model using (ntcc), for the cellular signaling transmembrane mediated by receptors that trigger a chemical response to intracellular level through interaction with G Proteins, we are going to use the chemical reactions that describes a first level of abstraction on the G Protein complex:

Notations in the basic model

Specie	Notation
$R(G\alpha\text{-}GDP)$	A
L	B
$R(G\alpha\text{-}GTP)L$	C_1
RL	D
$(G\alpha\text{-}GTP)$	E
$(G\alpha\text{-}GDP)$	F
R	G

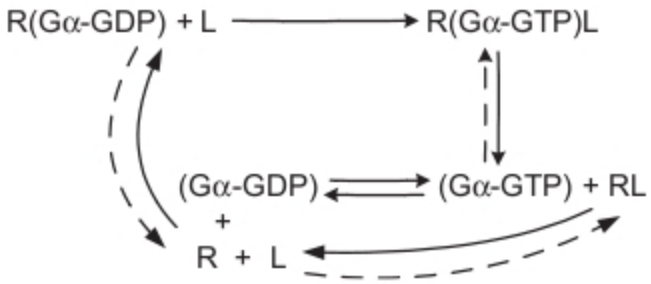

Figure 8. The reaction scheme of G Protein signaling in a first level of abstraction [5].

To construct the model each type of molecule will be represented as a variable whose value will be the number of occurrences present in the biochemical system. Each chemical reaction will be described by a recursive definition. When the reactants in the biochemical system are available, the reaction occurs to form products or complexes required in cellular signaling.

This first model will be extended to include more information (a second level of abstraction), for example, to represent the glycogen metabolism to produce glucose using the machinery of the G Proteins. Mammalian glycogen stores glucose in times of plenty (after feeding, a time of high glucose levels) and supplies glucose in times of need (during fasting or in fight-or-flight situations). In muscle, glycogen provides fuel for muscle contraction. In contrast, liver glycogen is largely converted to glucose that exits liver cells and enters the bloodstream for transport to other tissues that require it. Both the mobilization and synthesis of glycogen are regulated by hormones. The regulation of glycogen metabolism is a good way to introduce the idea of signal transduction. This is a very popular part of modern biochemistry. It's basically a way in which signals from outside the cell are transduced through a chain of molecules to affect a particular biochemical reaction. In this case, we are planning examine how the hormones such as glucagon, epinephrine, or insulin regulate glycogen synthesis and glycogen degradation.

The effect of the hormone on cAMP synthesis is the key part of any signaling pathway and it's best illustrated by using a general model based on cAMP production (there are other types of signaling pathways). Hormone binds to a cell surface receptor. The signal is transferred through the cell membrane to the inside part of the receptor molecule. This interacts with a G protein so that when hormone binds, the G protein is activated. G protein then diffuses to the membrane bound adenylyl cyclase molecule and, when the two proteins connect, the activity of adenylyl cyclase is stimulated and cAMP is produced. This leads to activation of protein kinase A. The stimulatory effect of the signal transduction pathway is transient because cAMP is rapidly degraded by phosphodiesterase. Thus, hormone

must usually be continuously present in order to get stimulation. There are other hormones that inhibit cAMP production by activating different G proteins that block adenylyl cyclase. This biochemical machinery include the following set of reactions:

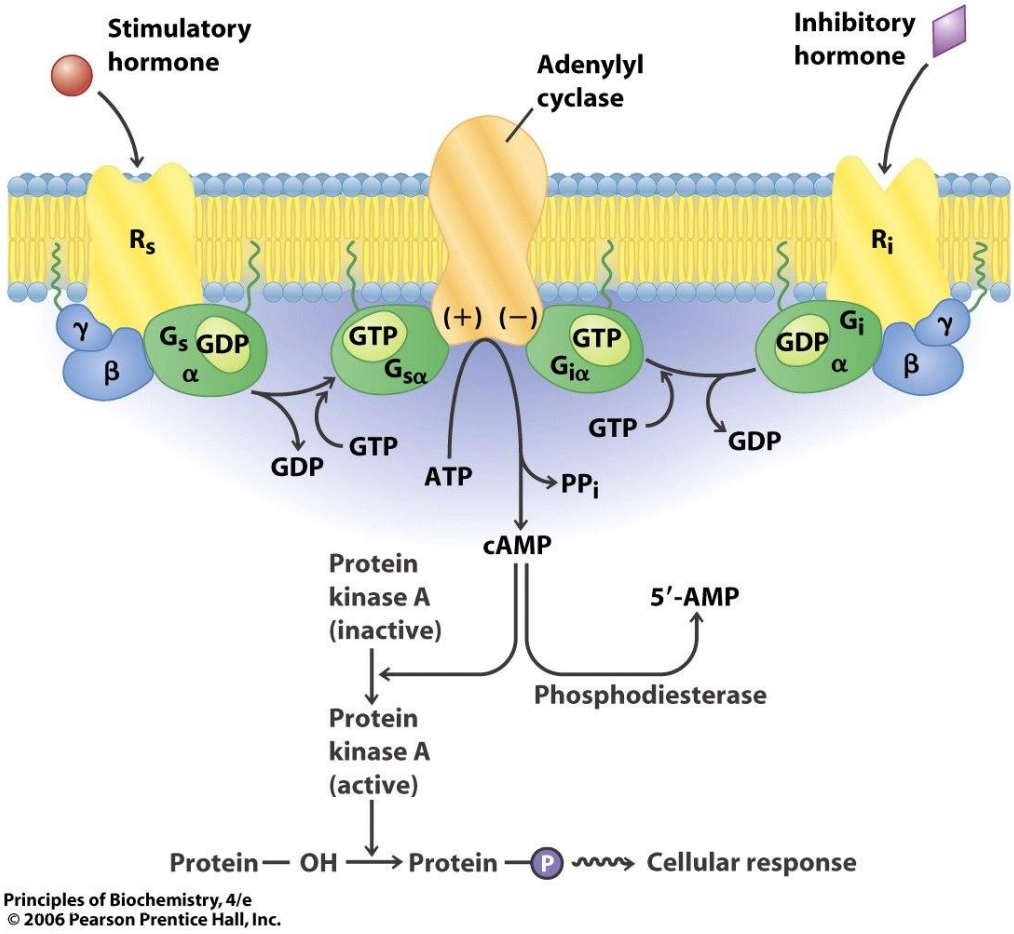

Figure 9. The reaction scheme of G Protein signaling in a second level of abstraction: the metabolism of glycogen.

Finally, we are planning to verify some properties of this biological system. The most important feature of process calculi is that their solid mathematical foundations allow to verify properties of the systems they model. In the case of (ntcc) such properties can be verified following a logic-based approach, expressing properties using a linear-temporal logic and deriving proofs using a proof system associated with the calculus [7,8,16,45,52,53].

8.1.2 The Stochastic and Probabilistic Timed Concurrent Constraint Process Calculus

According to [9,31] stochastic CCP-based process calculus, is an stochastic variant of CCP, by adding an exponentially distributed stochastic duration to all the instructions interacting with the store, this is achieved using rates, which are defined by functions mapping the current configuration of the store into a real number, this makes duration of processes explicitly dependent on context. In the stochastic model each action is related to a random variable which determines its duration (it is defined as function λ in the process syntax which represents the stochastic information in the language); given a set of competing actions (several reactions), the fastest action (i.e. the one with the shortest duration, e.g. in a chemical system, those reactions with the smallest rate) is executed, time is explicitly represented as discrete time units in which computation takes place. This theoretical framework provides constructs to control process execution along such units, a more detailed description can be found in [*ibid*].

$$P, Q ::= \mathsf{tell}_\lambda(c) \Big| \sum_{i \in I} \mathsf{when}\ c_i\ \mathsf{do}\ (P_i, \lambda_i) \Big| P \parallel Q \Big| \mathsf{local}\ x\ \mathsf{in}\ P \Big|$$
$$!P \Big| \mathsf{next}\ (P) \Big| \mathsf{unless}_\lambda\ c\ \mathsf{next}\ (P)$$

Figure 10. Process syntax in sthochastic timed concurrent constraint programming.

To extend the preliminary model of the cellular signaling system so as to account for quantitative information about chemical reactions, we will use a representation of the latter by a set of ODEs that describes a first level of abstraction on the G Protein complex (Fig. 11), once this information is included in the model, the model can provide the expected speed (duration) of the reaction and the probabilities about what reaction occurs first. For the second level of abstraction we did not find information about the rates constants for the chemical reactions, but the theoretical framework of (ntcc) supports modeling in absence of quantitative information in such a way that what is known in the abstract model can restrict the range of possible information in the second level.

$$\frac{d[A]}{dt} = -k_1^+[A][B] + k_1^-[C_1] + k_5^+[G][F] - k_5^-[A]$$

$$\frac{d[B]}{dt} = -k_1^+[A][B] + k_1^-[C_1] + k_4^+[D] - k_4^-[G][B] + k_s([L_{env}] - [B])$$

$$\frac{d[C_1]}{dt} = k_1^+[A][B] - k_1^-[C_1] - k_2^+[C_1] + k_2^-[D][E]$$

$$\frac{d[D]}{dt} = k_2^+[C_1] - k_2^-[D][E] - k_4^+[D] + k_4^-[G][B]$$

$$\frac{d[E]}{dt} = k_2^+[C_1] - k_2^-[D][E] - k_3^+[E] + k_3^-[F]$$

$$\frac{d[F]}{dt} = k_3^+[E] - k_3^-[F] - k_5^+[G][F] + k_5^-[A]$$

$$\frac{d[G]}{dt} = k_4^+[D] - k_4^-[G][B] - k_5^+[G][F] + k_5^-[A]$$

Figure 11. The state or differential equations in the model for the first level of abstraction [5].

The extended probabilistic preliminary (ntcc) processes model will be obtained by using the probabilistic choice operator. In [55] is introduced a probabilistic (ntcc) process calculus (pntcc), a discrete-timed CCP language that combines non-deterministic and probabilistic choices. The authors argue that a distinctive construct in (pntcc) is the one expressing probabilistic eventuality which describes the eventual execution of a process, possibly under the influence of complex patterns of behavior (which are representable as the parameters of the operator). The operational semantics for (pntcc) gives coherence to the two kinds of choices provided by the language, and is robust enough to support alternative definitions of (non-deterministic and probabilistic) choice operators.

8.1.3 Evaluation and Analysis of the Models

To evaluate the preliminary and the extended model (probabilistic) for the cellular signaling transmembrane mediated by receptors that trigger a chemical

response to intracellular level through interaction with G Proteins we will to use the available tools developed by the research group Avispa, such as ntccSim, a tool to run program specifications in (ntcc) and the Simulation and Verification tools developed for the probabilistic extension of the (ntcc) process calculus.

To analyze the use of the theoretical framework of process calculus (ntcc and its stochastic/probabilistic variant) in modeling cellular signaling transmembrane, under the focus of the Computational Systems Biology, we will be to compare the models achieved with available biological information in terms of concepts, definitions and mechanisms of cellular signaling processes.

8.2 Preliminary Results

There exists an agent-based model [56] of the cellular signaling transmembrane mediated by receptors that trigger a chemical response to intracellular level through interaction with G Proteins, by using NetLogo a multi-agent programming language and integrated modeling environment. The model captures the behavior of molecules in relation to the characteristics of the system to comprehend what happens when certain stimuli or disruptions occur. Two models were implemented, one for each level of abstraction.

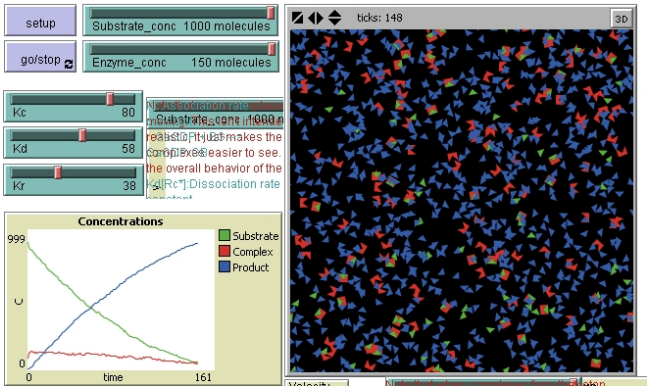

Figure 12. Agent-based model for the first level of abstraction.

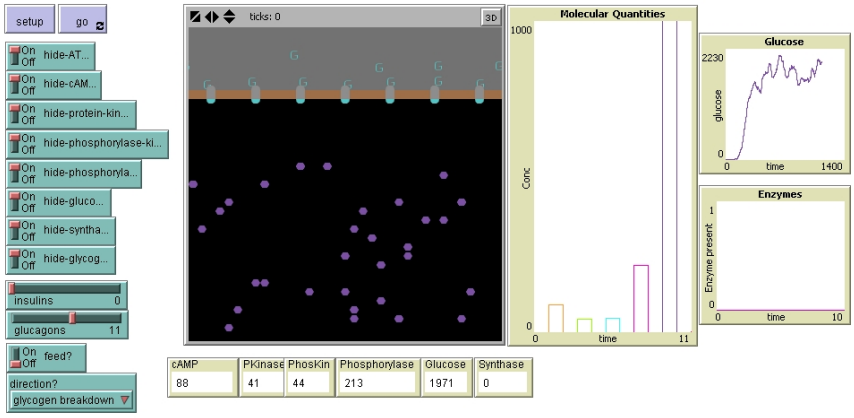

Figure 13. Agent-based model for the second level of abstraction.

8.3 Results Analysis

The ultimate goal of studying signal transduction is to understand how the components in a signaling cascade work together as a system to direct cellular responses to changes in the extracellular environment. This level of understanding requires: (*i*) quantitative/qualitative characterization of signaling components and their interactions (e.g., Measurement of concentrations and rate constants), and (*ii*) how a cell responds to an array of external signals over a range of intracellular operating conditions. This preliminary approach, showed a strong correlation between the production of GαGTP when the G Protein is disassociated in its two

components and in the second level of abstraction showed, that the production of glucose the final ouput in our model, depends on all reactions involved in the cellular signaling pathway. Therefore it is concluded, that the cellular signaling is a complex system in which its components interact concurrently, processing information over time from an external stimulus through the interaction of a ligand molecule with a cell-surface receptor to create a chemical signal cascade inside cell.

The fact that most of these relationships are non-linear implies a breakdown of the superposition principle (i.e. the whole is more than the sum of its parts). The behavior of a signal transduction system can depend qualitatively and nonlinearly on quantitative factors, such as the relative abundance of a signaling molecule or competition between concurrent processes that have counteracting effects. This biochemical reaction system has three distinctive characteristics: (*i*) consist of the species and their interactions, (*ii*) there is inherently concurrent, i.e. several interactions can usually happen independently and in parallel, and (*iii*) there is inherently stochastic, i.e. the timing behavior of the interactions is governed by stochastic laws.

CHAPTER 9

Resources

9.1 Human

The advisor of this research proposal is the Professor Camilo Rueda-Calderón, full Professor Faculty of Engineering, Chair of the Department of Science and Engineering of Computing and Director of the Research group AVISPA. The co-advisor is the Professor Frank-D. Valencia, CNRS Associate Research Scientist at LIX, École Polytechnique.

This research proposal will be a part of the active research line of the group AVISPA in Robust Theories for Emerging Applications in Concurrency Theory (REACT), a joint research effort between the Research Group AVISPA (Pontifica Universidad Javeriana at Cali, Colombia), the Musical Representations Team at IRCAM and the INRIA Team Comète (LIX, École Polytechnique, Paris, France). REACT is supported by COLCIENCIAS (The Colombian Agency for Science and Technology Development).

9.2 Schedule and Budget Summary

Schedule

Item	Date
Project Proposal by	June 17th 2009
Proposal Presentation by	July 3th 2009
Cellular signaling models in the Process Calculus (ntcc) by	August 30th 2009
Cellular signaling models in the Stochastic /Probabilistic Timed Concurrent Constraint Process Calculus by	October 30th 2009
Evaluation and Analysis of the Models by	January 30th 2010
Complete Analysis by	March 30th 2010
Complete Final Report by	April 30th 2010
Thesis Defense	June 2010

Budget Summary

Item	Budget in US Dollars
Supplies: paper, printed documents, photocopies.	200
Software	Those developed by the Research Group AVISPA.
Library materials	200
Travel support to a scientific meting national or/and international	1500
Financial Support for Academic Tuition	4000

Figure 14. Schedule and Budget Summary

CHAPTER 10

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