Modeling a Biological Transmembrane Signaling System by Using Concurrent Constraint Process Calculi

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Abstract

One of the most recent and exciting area using computer science formalisms to explore and understand the complexity of biological systems and its further applications in biological engineering or drug design are the cellular signaling events or signal transduction pathways. At the level of cellular abstraction, molecular mechanisms to communicate with the environment involve many concurrent processes and relationships interacting with diverse signals molecules sensing external information over time to control the flow of information in the cell. To address these concerns, this research proposal has two purposes: the first one 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 is to analyze the advantages and possible limitations of using the concurrent constraint calculus (CCP) model to represent a biological transmembrane signaling process.

***Key Words*—Concurrent constraint process calculi, cell-signaling pathways, molecular and computational systems biology modeling.**

I. Introduction

The transmembrane cellular signaling system is a complex process with a rich network of multi-functional interactions that occurs in non-linear fashion. The molecular and computational systems biology promises a systemic approach to interpret and tackle the complexity of cellular signaling through the integration of biological information, applied mathematics and statistics with approaches and techniques of computer science [1]. Much effort has been made nowadays to find plausible mathematical and computational models for the description of the cellular 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.

Most of the biological functions are mediated by cell surface receptors, i.e. a protein-protein interaction between the receptor and its respective ligand (signaling molecule). The association and the interaction between them 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 [2]. A large number of molecules and proteins take part in transmembrane signaling events, starting from a relatively small and initial stimulus, resulting in a "signal cascade" or a "signal transduction pathway" 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-transmembrane receptors (7TMRs) [3]. Subsequently the receptors interact with their respective guanine proteins (G-proteins) to induce a cascade of downstream, i.e. transmembrane signaling. The main components and events are already well known [4]-[5]-[6]-[7]-[8]-[9]-[10]-[11] but the interactions with their respective G-proteins to induce an intracellular signaling, however, are still 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 dependencies 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 [12]-[13]-[14]-[15]-[16]-[17]. Concurrent constraint programming (CCP) based

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process calculi has shown to be convenient for modeling, simulating, and verifying several kinds of biological systems [18]-[19]-[20]-[21]. It is considered that (CCP) process calculi offers at least two advantages to model this biological system. The first one, 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 that a binding occurs), information about particular interactions (whether and unexpected interaction actually happens) and, finally, information about the relative velocities of reactions (in a stochastic calculus). The second advantage is the declarative nature of the calculus that allows to formally verifying properties of the model that might be difficult to infer from a simulation.

The motivation in this sense is to develop a model based on biochemical and computational information available and to profit from the experience of the research group Avispa of the Pontificia Universidad Javeriana-Cali to devise a (CCP) calculus model for the transmembrane cell signaling process and thereby, to make a contribution in terms of the understanding of the biological foundations and the processing information of these processes.

II. Research Problem Formulation

The field of computational science can provide to molecular and systems biologists the abstraction needed for consolidating knowledge of biological systems. Biology and computer science with its techniques and formalisms 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 is observed. One basic but critical example of such systems is the cell. The typical cell has thousands upon thousands of molecular parts that interact in complex ways to produce a wide range of behaviors. Computational and mathematical models are needed to integrate and interpret this information and mechanisms.

Many complex systems are diffcult to describe and understand because they are composed of large numbers of elements interacting in a non-ordered way. A good example is the cellular biology: diverse cellular components (genes, proteins, enzymes) participate in various reactions and regulatory interactions, forming a robust system. According to [22], a very useful representation of complex systems is given by graphs (or networks) in which are 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 in languages for concurrency has recently gained a lot of momentum, specially because in many applications, quantitative information becomes crucial when models are refined with experimental data, and it is the essence for verifications purposes [23]. So, in this sense, for the motivation of this research proposal, exists a particular interest in a novel approach for analyzing systems that exhibit complex stochastic and probabilistic behaviors 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 [24] 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 [25] 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 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 unifed 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 [26]. How (GPCRs) operate is one of the most fundamental questions in the field of transmembrane signal transduction [27]-[28]-[29]-[30]-[31]-[32]. It has been identified 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 [33] explores the use of (CCP)-based process calculus as a possible computational model for representing this kind of information in biological systems, in particular using process

calculi based on constraints. As was argumented in [34], the explicit timed concurrent constraint programming language (ntcc) includes a explicit notion of time to represent the processing information in a biological system, as well as a proof system to validate properties. The autors 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 it is consider that this theoretical framework provides possibilities of application in the domain of molecular and cellular biology. Thus, in addition to the 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 transmembrane signaling in which the interactions of their components play a central role.

Why it is needed computational predictive models of transmembrane signaling 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 since there are too many combinations to consider in trials. We can use computational models to gain comprehension about biological systems and to determine some useful predictions using an appropriate model specification of the system.

There is a special class of receptors that constitutes a common target of pharmaceutical drugs, the G-protein-coupled receptors (GPCRs). These receptors interact with their respective G-proteins to induce an intracellular signaling. According to [35] when a ligand such as a hormone, neurotransmitter, or glycoprotein interacts with (GPCRs) 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 G_α-subunit. Upon activation, GDP is released, GTP binds to G_α, and subsequently G_αGTP dissociates from $G_{\beta\gamma}$ and from the receptor (Fig. 1 and Fig. 2).

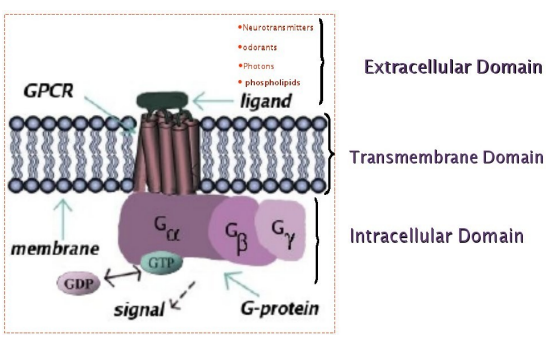

Fig. 1. G-Protein-coupled receptor: first level of abstraction [36].

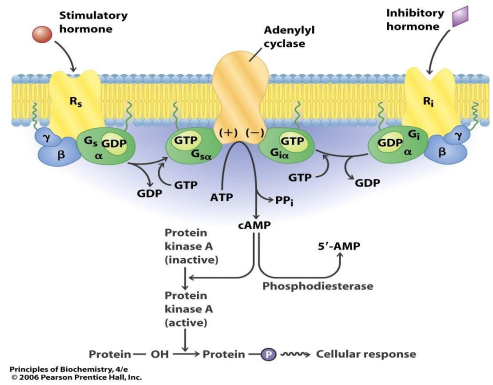

Fig. 2. The reaction scheme of G Protein signaling in a second level of abstraction: the metabolism of glycogen.

Both G_αGTP 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 G_α-subunit and the subsequently reassociation of G_αGDP with $G_{\beta\gamma}$.

The mathematical form of the model of transmembrane signaling events depends on the properties and the specific questions that are going to be answered and studied. The ligand and receptor interaction is a common process that happens in most of signaling. With the binding of the ligand (L) and the receptor (R) is formed a ligand-receptor 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 n or m 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 a simple reaction can be represent as $k \bullet X_1..X_n$, where X_i denotes the number of molecules of type R_i present in the system.

There exists an agent-based model [37] of the transmembrane cell signaling 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 (Fig. 1 and fig. 2). This preliminary approach, showed a strong correlation between the production of $G_{\alpha}GTP$ 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 output in the 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 modeling effort in the process calculi methodology will be directed towards describing the behavior of the system in terms of ligand-receptor binding and the events associated with cellular signaling trough interactions with G-proteins by using the useful abstractions of the concurrent constraint process calculi, that resolves the problem of modeling biological systems in which only partial information about their behavior at system level is available using a time discrete formulation. For this reason, it is consider that (CCP) is a computational model for representing this kind of information in the biological context, based on constraints to improve the understanding of transmembrane signaling systems in terms of its interactions, and explore the opportunities of these representations in terms of analysis, simulation, and scalability.

III. Research Hypothesis

There is information available about kinetic models of cellular signaling pathway of G-proteins as was mentioned above. Based on these models we propose to view the protein molecules involved in the signaling pathway as agents (i.e., processes) capable of performing computations and exchanging information with others in order to achieve a common goal. According to [18], (ntcc) is a temporal concurrent constraint calculus suitable to model non-deterministic and asynchronous behavior and it is particularly appropriate to model reactive systems that respond to stimuli from the environment. This particular feature of the (ntcc) process calculus, will allow building a preliminary model of the transmembrane cell signaling system mediated by the behavior of the G-proteins. We plan to follow an approach similar to that in [22] where a model of the Rho GTP-binding cycle is introduced. In particular, it is planned 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. An important aspect to be considered as a crucial part of the model development is the characterization of 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 with an extracellular domain, a membrane domain, and an intracellular domain. (GPCRs) constitute a common target of pharmaceutical drugs. Receptors 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 will be consider (i) what components of the G-protein are needed to continue the cellular signaling cycle; (ii) the time and interactions between the ligand and the receptor during the period of the G-protein cycle; (iii) the effect of enzymes that mediate the cycle, (iv) the interactions between the components of the system and their effectors, and other ones, that can be appear during the period of modeling and that can be solve it with the framework of the CCP-based process calculus developed by the research group Avispa.

IV. Methodology

The reactivity feature of the calculus will be used to procedurally 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. Succinctly speaking, in a reactive computation time is divided in discrete time units. In each of these, a state of the system is computed. This state is represented as the information supplied to a global store. This information takes the form of a set of constraints. Based on constraints in the store, each time unit also schedules some processes to be launched at subsequent time units. This sets the context to the evolution of biological interactions as time goes by.

The computational formalism to be used is the (ntcc) process calculus, a temporal extension of (CCP) [38] that captures the main features of timed and reactive systems that allows to model (i) unit-delays to explicitly model pauses in system execution, (ii) time-outs to execute a process in the next time unit if in the current one a piece of information can not be inferred, (ii) synchrony to control and coordinate the concurrent execution of multiple systems, (iv) infinite behavior to represent unbounded but finite delays in the execution of a system, and (v) non-determinism to express the diverse execution alternatives for a system from the same initial conditions. 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 [18]-[19]-[20]-[32]-[38]-[39]. To build a preliminary discrete model using (ntcc) for the biological system of interest it is going to use a set of chemical reactions [3] that describes a first level of abstraction on the G-protein complex (Fig 1.).

To construct the model each type of molecule will be represented as a variable whose value will be the number of occurrences of that molecule present in the biochemical system. Each chemical reaction will be described by by a (ntcc) process. The amounts of reactants in the biological system are conditions guarding the triggering of the (ntcc) process modeling the resultant products or complexes, as required in cellular signaling.

This model will be extended to include more information (a second level of abstraction, Fig. 2), 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. Hormones regulate both the mobilization and synthesis of glycogen. The regulation of glycogen metabolism is a good way to introduce the idea of signal transduction. In this case, it is interesting to examine how hormones such as glucagons, epinephrine, or insulin regulate glycogen synthesis and glycogen degradation and its effect on the cAMP receptor protein synthesis as a key part of any signaling pathway.

The compositional nature of the calculus allows the seamless integration of this extra detail into the abstract model. Furthermore, it will also explore using the linear logic associated to the calculus to formally verify some properties of this biological system. This is possible because the (ntcc) model is supplied with a proof system, so that biological properties expressed in the linear logic can be proved to hold (or not hold) in a given calculus model of the biological system [18]-[19]-[20]-[32]-[38]-[39].

According to [9]-[31] a 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 a function of 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. To include quantitative information about chemical reactions of the biological systems of interest will be going to use the kinetic constants (rates) associated to the set of chemical reactions that describe the first level of abstraction of the G-protein complex (Fig. 1), once this information is included, 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 (Fig. 2) even if information about the rates of the chemical reactions involved could not be found, the theoretical framework of (ntcc) supports computing in the absence of complete information, so what is known in the abstract model restricts the range of possible behavior in the second level.

For the observation of the behavior of models generated in the transmembrane cell signaling events mediated by receptors that trigger a chemical response to intracellular level through interaction with G-proteins will be going to use the available software developed by the research group Avispa, a simulator of the model in (ntcc) and its probabilistic extension and also some formal verification tools specific to processes calculi. To analyze the use of the theoretical framework of process calculus (ntcc and its stochastic/probabilistic variant) in modeling transmembrane cell signaling events, under the focus of the computational systems biology, will be by comparing the models achieved with available biological information in terms of concepts, definitions and mechanisms of cellular signaling processes.

V. Expected Results

It is interesting from a theoretical perspective to propose a process model which allows to represent the behavior and the verification of some properties of the biological transmembrane cell signaling to intracellular level through interaction with G-proteins using the characteristics of discrete time of the concurrent constraint process calculi and the proof system of this and its probabilistic variant to evaluate the advantages and possible limitations of using the concurrent constraint calculus model (CCP) for modeling cellular signaling events with the focus of the computational systems biology. The ultimate goal of studying cell signaling transduction events is to understand how the components in a cascade of processing information work together as a system to direct cellular responses to changes in the extracellular/intracellular 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. 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 molecules 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. As was proposed by Luca Cardelli and co-workers in a research event on systems biology, "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".

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