

Understanding the PI3K/AKT Anti-Apoptotic Signalling Pathway: a Tuple Space-Based Computational Framework for Simulating the Signal Transduction

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Abstract

The PI3K/AKT pathway is one of the main processes involved in cancer development since it primarily controls cellular proliferation and apoptosis. Understanding its behaviour and how it interacts with other pathways or how it is influenced by the presence of specific molecules, is a crucial task in cancer therapy. In this paper we propose a model developed according to the abstractions provided by the Biochemical Tuple Spaces for Self-Organising Coordination framework and simulated on top of TuCSon. The model and simulation procedure is fully described, demonstrating how much flexible and robust the computational framework is. Simulation results show critical points in the overall cascade, where activations or inhibitions can change the fate of the cell, turning it into apoptosis or proliferation.

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Mathematics Subject Classification: Signal Processing Systems

Keywords: Signal transduction, Anti-apoptotic signalling pathways, Biochemical Tuple Spaces for Self-Organising Coordination, Tuple space-based computational framework

1 Introduction

A crucial role in the control of functions related to cancer biology, including cellular proliferation, survival, migration and angiogenesis, is played by the PI3K/AKT signalling pathway. A deep understanding of its constituting and interacting mechanisms is required to subsequently analyse and predict the role of anticancer agents acting over the PI3K/AKT signalling pathway.

For this purpose, an adoption of modelling and simulating techniques seems promising. Over the last three decades, different computational and mathematical tools that enable modelling and simulation of intracellular signalling pathways have been used. However, in recent years, other major requirements in the simulation of intracellular systems have emerged, guiding the development of new computational models and tools. In particular, the notion of topology and locality emerged as key feature with a purpose of *(i)* to model complex topologies of signalling networks, which involve feed forward and feed backward relationships between signalling components placed into different intracellular compartments, and *(ii)* to provide a global view of the signalling system as a composition of local interactions.

Given these requirements, in this paper we adopt a simulation approach based on biochemical tuple spaces, as introduced in [1] for pervasive services ecosystems. More precisely, the approach is based on the notion of Biochemical Tuple Spaces for Self-Organising Coordination (BTS-SOC), where each tuple space works as a compartment where biochemical reactions take place, chemical

reactants are represented as tuples, and biochemical laws are represented as coordination laws by the coordination abstraction. Technically, biochemical tuple spaces are built as ReSpecT tuple centres [2], running upon a TuCSoN coordination infrastructure [3]. Tuples are logic-based tuples, while biochemical laws are implemented as ReSpecT specifications for each tuple—so they can be inserted, modified and removed from the compartment (the type centre) via simple Linda-based coordination primitives.

In this paper, we show how our BTS-SOC-based simulation platform can be applied to the simulation of complex interaction patterns of anti-apoptotic intracellular signalling pathways. Accordingly, initially we present a review of computational and mathematical tools commonly used for modelling and simulation of intracellular signal transduction (section 2). In section 3 we present and discuss the general model and infrastructure for simulating intracellular signalling systems, along with a high-level architecture. Then in section 4 we develop and discuss a study case—the simulation of the anti-apoptotic PI3K/AKT signalling pathway. Finally, section 5 concludes the paper with the discussion of the results obtained.

2 Computational and Mathematical Tools for Modelling and Simulation of Intracellular Signalling Pathways

Historically, different modelling approaches have been developed to deal with intracellular signalling pathways, from mathematical models – mainly ODEs – to computational models — process algebra, such as stochastic π -calculus [4] and κ -calculus [5]. Accordingly, different simulation tools have been developed, from mathematical ones – see a survey in [6] – to computational ones, such as SPiM [7]. While they typically address scenarios with a single compartment, in recent years a new trend has emerged which moves from the

single global approach to mechanisms and constructs tackling the multi-compartment scenario.

On the side of mathematical models, PDEs have been used. They introduce the notion of space, but they still do not model bounded compartments dealing with a continuous space; also, they are not stochastic.

On the side of computational models, we could cite the following works. In [8] the $S\pi@$ process calculus is introduced to deal with the notion of compartments (possibly with variable volumes), by adding to the stochastic π calculus the idea that process-molecules are situated inside a location. In [9] Bio-PEPA has been extended for expressing hierarchies of locations with different sizes, which permits to model compartments, membrane and cell intra-compartment and inter-compartment reactions. In Beta-binders and its extension called BlenX, systems are modelled as a set of boxes representing biological entities at different levels – proteins, cells, etc – and are simulated on top of BetaWB [10]. However, in this field few simulators make it possible to flexibly define network topologies – they mostly deal with a small number of chemical compartments – and to the best of our knowledge no one provides features of network mobility and fine-tuning control over different behaviour in different nodes—for this typically falls out of the context of biological systems.

Many agent-based models [11] are available for simulation of complex computational systems. An agent-based model is built using autonomous and possibly heterogeneous agents, which can be situated in an environment. Agent behaviour is typically modelled through a set of rules describing how the agent behaves in some given environmental conditions. Rules can be of different types, according to the specific model/architecture: from simple reactive rules, specifying how the agent must react to environmental stimuli/perceptions, to pro-active ones, specifying how the agent must behave with respect to its goals and tasks.

In order to develop and execute agent-based simulations, many simulation

frameworks were developed, such as MASON [12], Repast [13], NetLogo [14], and Swarm [15]. There, the environment is also typically a first-class abstraction whose structure, topology, and dynamics can be explicitly modelled—as required by our case study and by simulation of biochemical systems in general. However, the above approaches do not provide tools for sophisticated design of behavioural rules in the environment, at least up to the point of supporting an efficient simulation of chemical-like reactions and, more generally, of stochastic behaviours.

3 The Tuple Space-Based Framework for Simulation of Intracellular Signalling Pathways

Modelling intracellular signal transduction requires a robust approach accounting for the crucial features that govern the activity, interaction, selectivity, inhibition and evolution of such a sort of complex distributed system, such as situatedness, adaptation, diversity, eternity, topology and locality. Such features have found a suitable support in the concept of Biochemical Tuple Spaces for Self-Organising Coordination (BTS-SOC) introduced in [1]. In fact, the cell detects the spatial context (the cellular compartments) of signalling elements, enabling or disabling actions and interactions accordingly (situations); dynamically adapts to external changes (adaptively); can change its function over time and then the activities being executed (presumption and diversity); is able to tolerate runtime changes in terms of structure, elements and function (eternity). Also, BTS-SOC supports topology and locality, respectively to model the intracellular compartments, and to provide a view of the signalling system as a composition of local interactions. Finally, and more generally, its biochemical inspiration makes a BTS-SOC infrastructure particularly suited for simulating systems where the very same concepts BTS-SOC is based on – such as chemical

reaction and concentration – are among the most relevant ones.

As a result, the simulation approach adopted in this work is based on the notion of BTS-SOC, where each tuple space works as a compartment where biochemical reactions take place, chemical reactants are represented as tuples, and biochemical laws are represented as coordination laws by the coordination abstraction. Technically, biochemical tuple spaces are built as ReSpecT tuple centres [2], running upon a TuCSoN coordination infrastructure [3]. Tuples are logic-based tuples, while biochemical laws are implemented as ReSpecT specification tuples—so they can be inserted, modified and removed from the compartment (the type centre) via simple Linda-based coordination primitives [17]. Tuples in BTS-SOC are associated with an activity/pertinency value, resembling chemical concentration, and allowing chemical reactants to be represented as tuples. Biochemical laws are represented as coordination laws by the coordination abstraction, evolving tuple concentration over time according to a rate in the same way as chemical substances in a solution. Also, BTS-SOC laws allow for tuple diffusion, making it possible for products to cross compartment boundaries as a result of biochemical reactions.

3.1 The representation of the structures and components involved in intracellular signalling

The high-level architecture of our BTS-SOC-based simulation platform is depicted in Figure 1. Table 1 shows how the cellular components and structures involved in intracellular signalling map onto the BTS-SOC computational abstractions.

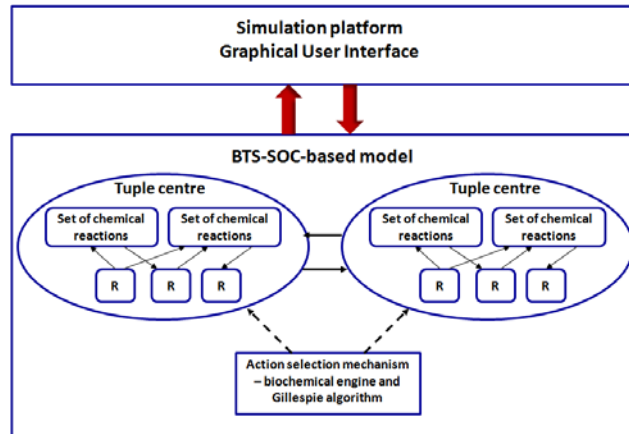


Figure 1: A high-level architecture for the BTS-SOC-based bioinformatics platform

Table 1: A high-level architecture for the BTS-SOC-based bioinformatics platform

Cellular components and structures involved in intracellular signalling	Computational abstractions of the BTS-SOC model
Extracellular milieu and intracellular compartments, i.e., extracellular space, membrane, cytosol, nucleus and mitochondria.	Tuple centres.
Signalling components, i.e. proteins (membrane receptors, enzymes, regulators, adapters, etc.) and genes.	Chemical reactions set.
Signalling molecules, i.e., ATP, inorganic phosphate, second messengers, etc.	Reactants and concentrations recorded as tuples in the tuple centre.

As can be seen from Figure 1 and Table 1, the intracellular compartments involved in signalling pathways are represented as biochemical tuple spaces—that is, TuCSoN tuple centres suitably programmed with ReSpecT. Modelling the intracellular space as a set of tuple centres allows for the explicit representation of the intracellular compartments – as shown in Figure 1 - and makes it possible to distinguish chemical reactions based on the intracellular segment where they actually occur—an essential spatial feature when modelling cellular systems.

The task of the components representing intracellular signalling elements (i.e., those modelling membrane receptors, proteins, enzymes and genes) is only the transformation of input signals into output signals, according to the behaviour of the corresponding signalling element. So, if the main task of the signalling components is to perform chemical reactions for signal transduction, the most appropriate solution in the BTS-SOC approach is to model each signalling component as the set of the chemical reactions that defines its behaviour – see Table 1. As one could see from Table 2, different sorts of chemical reactions are considered for signal transduction.

Table 2: Different types of chemical reactions are involved in the intracellular signalling model

Type of chemical reaction	Expression
Collaborative activation	$aA^* + bB^* + cC \rightarrow cC^*$
Alternative activation	$aA^* + bB \rightarrow bB^*$
Complex formation	$aA + bB + cC^* \rightarrow mABC^*$
Decomposition reactions	$AB \rightarrow A + B$
Standard equation for enzymatic reactions	$E + S \leftrightarrow ES \rightarrow E + P$
Interaction from and to extracellular space	$\rightarrow bB$ $bB \rightarrow$

Another important component involved in intracellular signalling are the signalling molecules – i.e., the inputs and outputs for the chemical reactions that belong to each tuple centre, and it is necessary to set their activation, duration or deactivation either directly or indirectly.

As shown in Table 1, the signalling molecules are recorded as tuples in a tuple centre. As a result, like in biological systems, the evolution of the model depends on the concentration and state of the reactants.

3.2 The action selection mechanism

In the BTS-SOC model, each biochemical tuple space – i.e., tuple centre - is built around a ReSpecT chemical engine, whose core is an action selection mechanism based on Gillespie algorithm [16] – an algorithm typically used to simulate systems of chemical/biochemical reactions efficiently and accurately – to execute chemical reactions with the proper rate. In the Gillespie's model, a chemical system is seen as a unique well-mixed solution. Every molecule is explicitly modelled and every reaction, in which they can participate, is explicitly simulated on the basis of a stochastic algorithm, the Gillespie's *stochastic simulation algorithm* (SSA). Once the system has been initialised, i.e., molecules, reactions and reaction rates are defined, the simulation proceeds choosing the next reaction to occur on the basis of a random number and its *propensity function* that is calculated based on the reaction rate and on the number of reactants. The time interval to update the simulation time is also computed step by step depending on a random number and on the sum of propensity functions of all reactions. The iteration of these steps constitutes the simulation. In detail, the main steps performed by the action selection mechanism for signal transduction are summarized below:

- i. Calculate the rate for each eligible chemical reaction, according to the expression:

$$Rate = ConstantRate * \prod_{i=1}^{num-reactants} \binom{Mol_i}{requiredMol_i}$$

The rate with which the reaction will be selected is equal to the rate of this reaction (*ConstantRate*) multiplied by the product of the binomial coefficients of the available moles of each reactant involved in the reaction and the number of moles of this required by the reaction. If a chemical reaction is not eligible for lack of any of the reactants required, then the rate of this reaction will be zero.

- ii. Calculate the summation of the rates of all eligible reactions, the resulting value is *RTot*.
- iii. Sort all eligible reaction by rate in a descending order.
- iv. Generate a random number ψ between 0 and 1.
- v. From sorted list of eligible reactions, the *i*-th reaction is chosen if:

$$\psi \leq \frac{\sum_{i=1}^n Rate_i}{RTot}$$

Note, that the value of the summation is equal to 1 for the last reaction in the sorted list, so, if there are eligible reactions, then one of them will always be executed.

- vi. Generate a random number τ between 0 and 1. Stop execution of the reactions for a time given by:

$$Stop_time = \frac{-\ln(\tau)}{RTot}$$

3.3 The graphical user interface of the tuple spaces-based simulation platform

The Graphical User Interface (GUI) – see Figure 2 - provides the tools for users to perform the activities listed below.

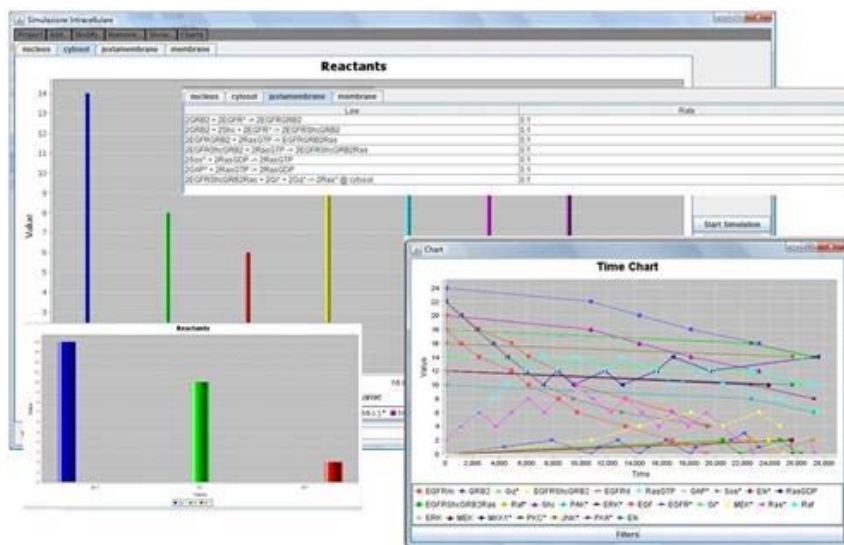


Figure 2: The GUI of the tuple space-based simulation platform. Different charts showing the state of the simulation components and its interactions can be supported by the drawing canvas

1. Creation, modification and visualization of the simulation components – e.g., cellular compartments, signalling components, signalling molecules and cellular processes – either during the implementation of the simulation or during the execution of it.
2. Feedback on the results provided by the simulation run - e.g., the behaviour of the simulated system over time, and the current state of each component (i.e., signalling elements) of the simulation.

4 A Case Study: Modelling and Simulation of an Anti-Apoptotic PI3K/AKT Signalling Pathway

The cell has a self-destruction system, which starts and operates in a regulated manner. It is called apoptosis and includes the decision to start self-destruction as well as the proper execution of the apoptotic program. As such, it requires the coordinated activation and execution of multiple sub programmes. On the other hand, the cancer cells initiate anti-apoptotic programs, since their goal is to survive. Tumorigenesis and tumour progression are the result of imbalance in cell proliferation, differentiation and apoptosis. Thus, in tumour cells, activation of the anti-apoptotic signalling pathway associated with phosphatidyl inositol-3-kinase (PI3K), mitogen-activated protein kinases (MAPKs) and protein kinase C (PKC) occurs. The tumour cells survive after exposure to stress and these proteins are only expressed at high levels in transformed cells [18].

4.1 Phosphatidyl inositol-3-kinase (PI3K) / AKT (protein kinase B) anti-apoptotic signalling pathway

The phosphatidyl inositol 3-kinases (PI3K) is a conserved family of lipid kinases that catalyze the phosphorylation of phosphatidyl inositol (PtdIns) membrane in the D3 position of the inositol ring, of which the phosphatidyl inositol- (3,4,5)-triphosphate (PIP3) is the most common [19, 20]. These kinases are heterodimers composed of a catalytic subunit, p110, and a regulatory subunit, p85 being the most common.

The PI3Ks are classified depending on the substrate selectivity, and they are divided into five classes (IA, IB, II, III and IV), although only the class IA PI3K, whose function is associated with signal transduction of receptor tyrosine kinases, has been linked to cancer in humans [21]. Meanwhile, the class IB kinases transmit signals of membrane receptors coupled to G proteins, and can also

activate PI3K-IA through the recruitment of intracellular tyrosine kinases [21]. PI3K controls several key functions related to cancer biology, among which are proliferation, cell survival, migration and angiogenesis [19-21] - for this work, we only consider proliferation and cell survival.

The activity of PI3K is regulated by two phosphatases: PTEN (Phosphatases homology to tensin), which is a tumour suppressor, and SHIP2 [19, 20, 22]. If PTEN (SHIP 1) or SHIP 2 inhibit PI3K, the cancer cells stop growing and die; however if Ras is active, it allows the cell to survive even in the presence of regulators of the PI3K/AKT pathway – see Figure 3.

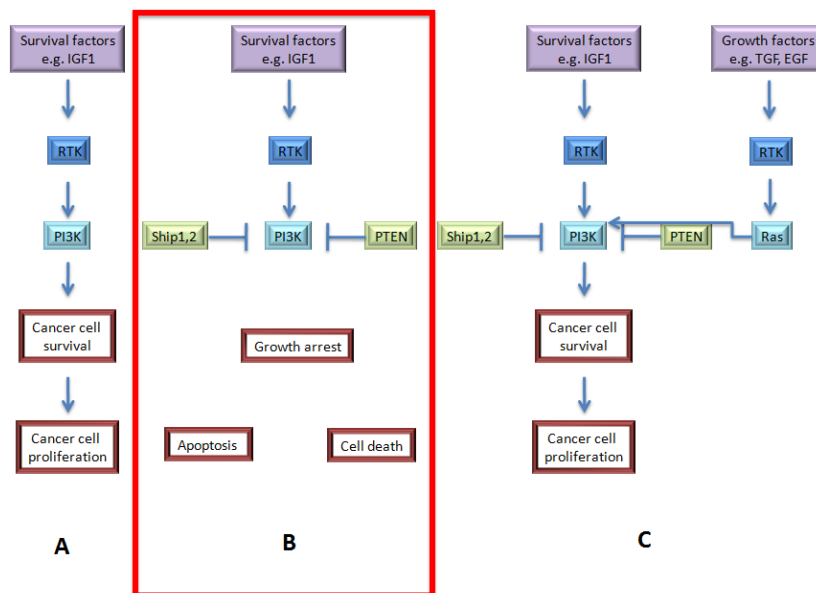


Figure 3: This image shows three different possibilities in the PI3K/AKT pathway.

In panel “A”, PI3K activation allows the survival of the cancer cell. In panel “B”, PTEN and/or Ship1,2 inhibit PI3K and allow the cell to stop growing and eventually die. In panel “C”, if Ras activates PI3K, even in the presence of PTEN and Ship 1,2, the cancer cell survives.

The serine / threonine kinase AKT (also known as PKB) is a major target of PIP3, and in humans three different isoforms (AKT1, AKT2 and Akt3) have been

described. The binding of AKT to PIP3 causes a conformational change which makes AKT phosphorylated and thus, activated by phosphatidylinositol-dependent kinases (PDKs) [23].

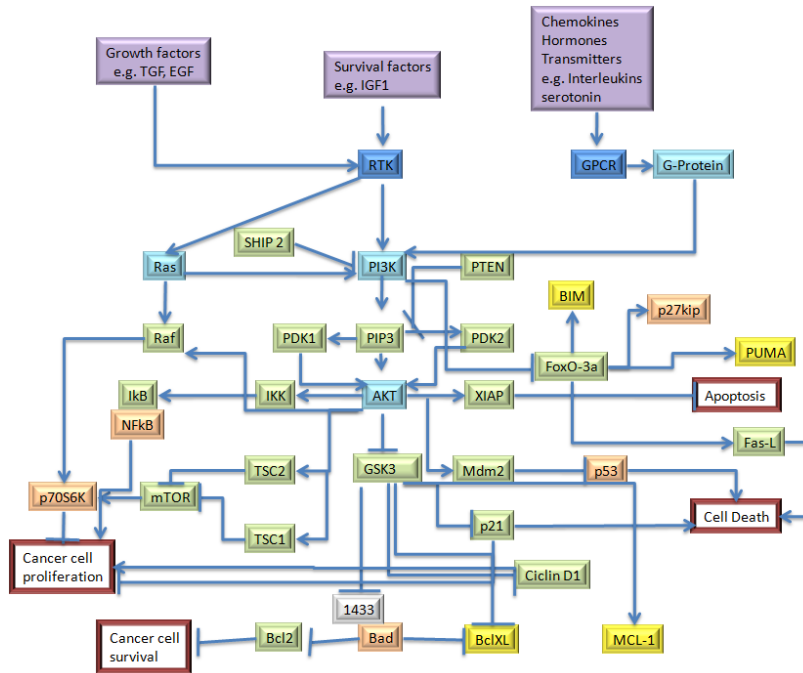


Figure 4: Once the PI3K/AKT pathway is started, the cell may have different final destinations depending upon the activation status and type of molecules that are at that moment within the cell, and if it is a cancer cell or a normal cell.

AKT targets include multiple transcription factors and signalling proteins, thus activation of AKT results in decreased levels of expression or activity of several pro-apoptotic proteins such as FasL and Bim [21, 23] and in increase of anti-apoptotic proteins Bcl-2, Bcl-xL, XIAPs [21, 23] and survival proteins such as AKT itself [21, 23]. AKT also promotes cell cycle by inhibiting the regulation of control points, such as phosphorylation and inhibition of the inhibitors of cyclin-dependent kinases and p27Kip1 p21Cip/WAF1 [21, 23] and glucogenosynthase (GSK3- α and β), promoting glycolysis in the cell and therefore

cell growth [21, 24]. The role of AKT in cancer is well established and described, its activation directly contributes to the survival and proliferation of cancer cells and their growth [19, 23].

As described above, once the PI3K/AKT pathway starts, the cell may have different final destinations depending upon the activation status and type of molecules that are at that moment within the cell. Figure 4 summarizes these possibilities.

4.2 Modelling PI3K/AKT anti-apoptotic signalling pathway

The PI3K/AKT pathways influence either directly or indirectly whether a cell will undergo apoptosis. These events are probably essential for the survival of both cancer and normal cell.

Modelling and simulation an intracellular signalling network with the BTSSOC-based approach can be handled as an incremental process of definition and refinement of the signalling pathways and components in the network. The modelling process begins by considering a single signalling pathway from the complex network of the anti-apoptotic signalling pathways. The PI3K/AKT pathway has been selected for this purpose. At the beginning of the modelling process it is appropriate to consider only the main features of the components involved in the anti-apoptotic signal transduction, i.e. those needed for achieving a comprehensible and functional simulation.

In this paper, we start with a minimalist model where each signalling component is described by the following attributes:

- Identity;
- Concentration in each cellular compartment;
- Free concentration;
- “Bound” concentration;

- Cellular compartment to which it belongs;
- Chemical reactions involving the component and the order in which they occur according to the affinity of the components;
- Reaction temporality situation.

Such a coarse-grained modelling strategy leads to an initial BTSSOC-based model of the PI3K/AKT signalling pathways, whose main features are summarised in Table 3 and Table 4.

Table 3: Modelling the signalling components belonging to anti-apoptotic PI3K/AKT signalling pathway. The symbol “@” on the right side of an equation indicates the cellular compartment in which the resultant reactant must be registered.

Cellular compartment	Reaction number	Reaction expression
Extracellular space and membrane	(1)	$RTK + SF \rightarrow RTK^* @cytosol$
	(2)	$GPCR + GF \rightarrow GPCR^* @cytosol$
Cytosol	(3)	$RTK^* + PI3K \rightarrow PI3K^* + RTK^*$
	(4)	$RTK^* + Ras \rightarrow Ras^* + RTK^*$
	(5)	$GPCR^* + Gp + AC \rightarrow GPCR^* + GBetaGGamma + ACGAlfa$
	(6)	$ACGAlfa + ADP \rightarrow cAMP + ACGAlfa$
	(7)	$ACGAlfa + ATP \rightarrow cAMP + ACGAlfa$
	(8)	$cAMP + PI3K \rightarrow PI3K^*$
	(9)	$PI3K^* + PIP3 \rightarrow PIP3^* + PI3K^*$
	(10)	$PI3K^* + AKT \rightarrow AKT^* + PI3K^*$
	(11)	$PIP3^* + AKT \rightarrow AKT^*$
	(12)	$PIP3^* + PDK1 \rightarrow PDK1^*$

- (13) $PIP3^* + PDK2 \rightarrow PDK2^*$
- (14) $PDK1^* + AKT \rightarrow AKT^* + PDK1$
- (15) $PDK2^* + AKT \rightarrow AKT^* + PDK2$
- (16) $AKT^* + AKT \rightarrow 2AKT^*$
- (17) $AKT^* + IKK \rightarrow IKK^* + AKT$
- (18) $AKT^* + XIAP \rightarrow XIAP^* @nucleus + AKT^*$
- (19) $AKT^* + Raf1 \rightarrow Raf1^* @nucleus + AKT^*$
- (20) $AKT^* + GSK3^* \rightarrow GSK3 + AKT$
- (21) $AKT^* + Mdm2 \rightarrow Mdm2^* + AKT$
- (22) $PTEN^* + PIP3^* \rightarrow PIP3 + PTEN$
- (23) $SHIP2^* + PI3K^* \rightarrow PI3K + SHIP2$
- (24) $IKK^* + I\kappa B/NF\kappa B \rightarrow I\kappa B/NF\kappa B^* + IKK$
- (25) $Ras^* + PI3K \rightarrow PI3K^* + Ras$
- (26) $AKT^* \rightarrow AKT^* @nucleus$
- (27) $Mdm2 + p53^* \rightarrow Mdm2 + p53$
- (28) $4p53^* \rightarrow 2p53^* @nucleus$
- (29) $GSK3^* + BAD/1433^* \rightarrow BAD/1433 + GSK3$
- (30) $BAD/1433^* + BclXL^* \rightarrow BclXL @nucleus + BAD1433^*$
- (31) $BAD/1433^* + Bcl2^* \rightarrow Bcl2 @nucleus + BAD1433^*$
- (32) $4GSK3^* \rightarrow 2GSK3^* @cytosol + 2GSK3^* @nucleus$
- (33) $4BclXL^* \rightarrow 2BclXL^* @cytosol + 2BclXL^*$
- Nucleus
- (34) $AKT^* + TSC1 \rightarrow TSC1^* + AKT$
- (35) $AKT^* + TSC2 \rightarrow TSC2^* + AKT$
- (36) $AKT^* + AKT \rightarrow 2AKT^*$
- (37) $TSC1/TSC2^* + mTOR^* \rightarrow mTOR + TSC1/TSC2$

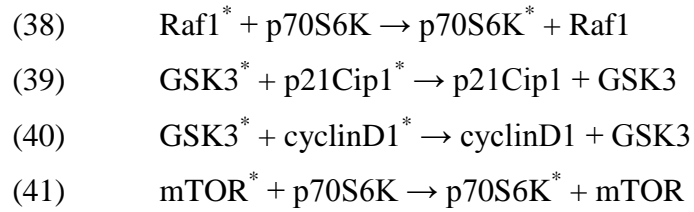


Table 4: Initial concentrations of the reactants belonging to anti-apoptotic PI3K/AKT signalling pathway

Component	Identity	Concentration
SF	Survival factor	0.1 nM
GF	Growth factor	0.1nM
RTK	Receptor tyrosine kinase	0.25 μM
GPCR	G protein-coupled receptor	0.25 μM
G Protein	signal transmitter	0.1 μM
AC	Adenylate cyclase	0.25 μM
ADP	Adenosine diphosphate	260.0 pM
ATP	Adenosine triphosphate	2.5 mM
PTEN (SHIP 1)	Phosphatase that regulates the PI3K/AKT pathway	0.27 μM
Raf	serine/threonine-protein kinase	0.07 μM
PDK1	3-phosphoinositide dependent protein kinase-1	1 μM
PDK2	pyruvate dehydrogenase	1 μM
PIP3	Phosphatidylinositol (3,4,5)-triphosphate	7 μM
IKK	I κ B kinase	0.1 μM
AKT	(PKB) Serine/Threonine specific protein kinase	0.2 μM

XIAP	X-linked inhibitor of apoptosis protein	0.1 μ M
p70S6K	Serine/threonine kinase	0.17 μ M
mTOR	Serine/threonine kinase	0.6 μ M
TSC1	Tumor suppressor 1	1 μ M
TSC2	Tumor suppressor 2	0.1 μ M
GSK3	Serine/threonine kinase	0.1 μ M
Mdm2	Negative regulator of the p53 tumor suppressor	0.1 μ M
p53	Tumor suppressor	0.1 μ M
Cyclin D1	G1/S specific cyclin	0.1 μ M
p21	Cyclin-dependent kinase inhibitor 1	0.1 mM
Bcl2	Regulator protein	0.1 μ M
1433	Regulator protein	0.1 μ M
Bad	Bcl-2 associated death promoter	0.1 μ M
BclXL	Transmembrane molecule	0.1 μ M

4.3 Setting the simulation framework

Figure 5 shows the workflow of the major activities to be executed through BTS-SOC-based simulation platform while simulating the PI3K/AKT anti-apoptotic signalling pathway. As depicted in this figure, the following activities should be completed before running the simulation:

1. Creating cellular compartments.
2. Setting chemical reactions and their rates.
3. Introducing reactants and their concentrations.

Following this workflow, the first activity to be carried out is the creation of cellular compartments (step 1 in Figure 5). In our BTS-SOC model, a tuple centre (BTS) is required for each cellular compartment involved in the signalling pathway to be simulated. The PI3K/AKT anti-apoptotic signalling pathway begins

in the extracellular space, continues in the membrane, it goes through the cytosol, some signalling components in and out of the mitochondria, finally ending in the nucleus. In our first simulation the extracellular space and membrane are represented in a single compartment. Therefore, four tuple centres (*membrane*, *cytosol*, *mitochondria* and *nucleus*) are required to model four intracellular compartments.

After the cellular compartments are created, the next step is the setting of chemical reactions which define the evolution of the system (step 2 in Figure 5), i.e. the biochemical laws modelling the behaviour of the signalling elements down the signalling pathway – see Table 3. The whole set of reactions needs to be clearly associated to the different compartments before proceeding, it is also necessary to insert them in the proper BTS. In our BTS-SOC model, based on the Gillespie algorithm, every chemical reaction has a rate that expresses (along with the concentration of the input elements) the probability of the transformation. Like almost every other component of the system, the reactions are also represented as tuples in a tuple centre.

The last step to set up the simulation is the registration of the reactants listed in Table 4 (step 3 in Figure 5). First of all, each reactant belongs to a specific cellular compartment—so, it has to be put in the appropriate BTS. Initially, only the pre-existing reactants – i.e., those required in the compartments before the signalling pathway is activated – have to be put in the BTS.

As can be seen from Figure 5, the simulation platform is now ready to run the PI3K/AKT simulation.

4.4 PI3K/AKT simulations and results

After entering all required information and setting the initial parameters, the system is now ready to run the PI3K/AKT pathway simulation. Biologically, apoptosis is initiated when normal cells are transformed into abnormal cells,

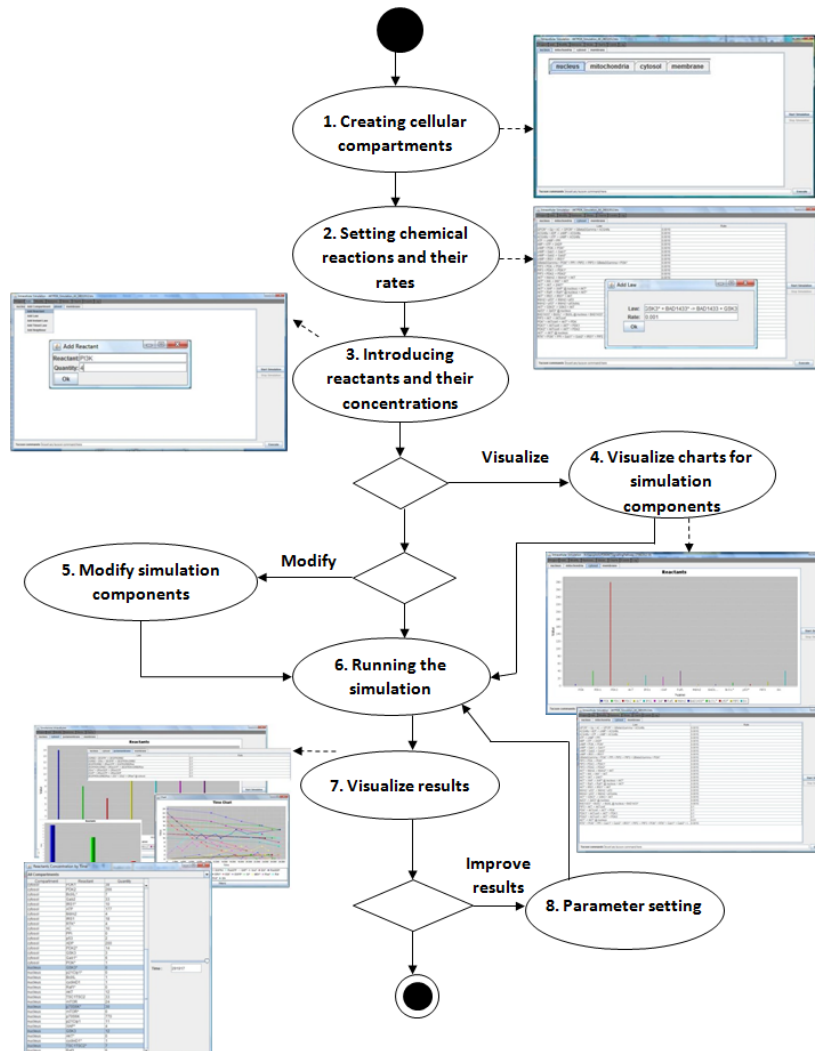


Figure 5: Workflow of the BTS-SOC-based simulation platform. The diagram describes the behaviour of the platform during a run, including setting parameters.

which starts a self-destruction mechanism. However, the cancer cell survives by producing survival factors that initiate anti-apoptotic pathway.

4.4.1 From RTK activation to PI3K activation

PI3K/AKT signalling pathway is initiated when a survival factor (SF), binds

to its receptor (RTK). This event triggers the first reaction of PI3K/AKT signalling pathway, leading the signal to the final activation of transcription factors in the nucleus and, consequently, the activation of transcription of proteins that allow the cell to survive and proliferate - see Figure 4. The existence of the reactant *SF* in the *membrane* BTS is the main triggering event for our simulation. Once the reactant *SF* is available in the *membrane* BTS, the biochemical engine chooses the only eligible reaction for execution – i.e., equation (1) in Table 3.

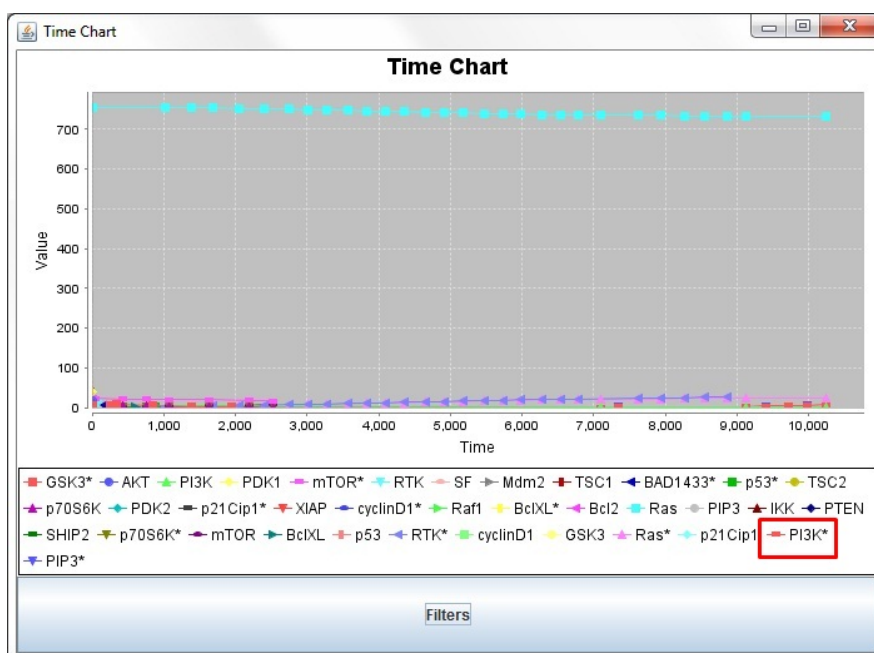


Figure 6: Execution of the reactions involved in the first segment of the simulation: from *SF* binding to PI3K activation. At the bottom of the graph all the reactants implicated in these reactions can be seen. The symbol “*” refers to active/phosphorylated state of the reactant.

Once RTK is active – i.e., RTK* - and after a short, non-deterministic period of time (defined by Gillespie algorithm and ruled by probability), the chemical engine chooses and performs one of two eligible chemical reactions of the *cytosol*

BTS - i.e., equations (3) and (4) in Table 3. Which one among the two reactions is picked for execution by the biochemical engine (given both the identical initial rates and the interaction 1:1 between reactants) depends on the free concentration for each reagent involved in the reaction. Figure 6 shows the execution of the simulation for the first PI3K/AKT anti-apoptotic pathway segment, from RTK activation to PI3K activation.

4.4.2 From PI3K activation to conformational change and activation of AKT

The production of PI3K* and, consequently, the incremental availability of it in the *cytosol* BTS leads to PIP3 activation and thus to the amplification of the original signal, as shown in the equations (9) to (15) of Table 3. Figure 7 shows the phase of the simulation where the activation of AKT (AKT*) has taken place.

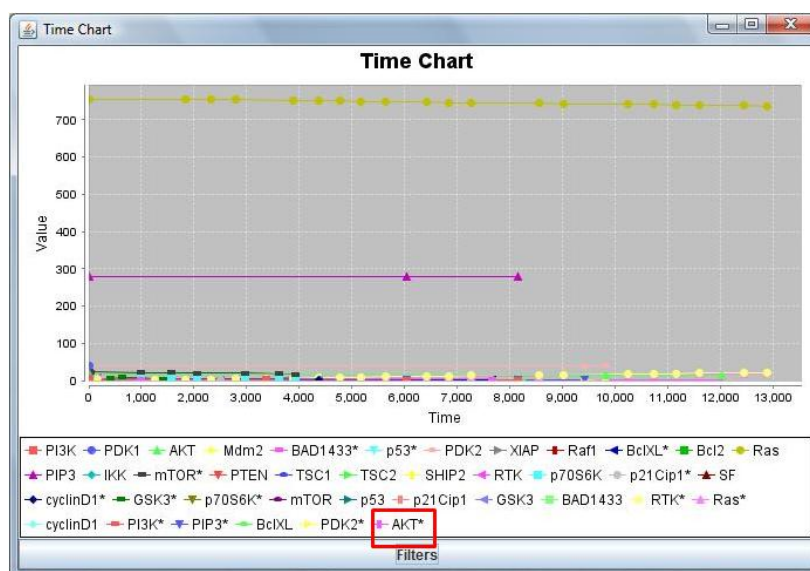


Figure 7: Execution of the reactions involved in the second segment of the simulation: from PI3K activation to AKT activation. At the bottom of the graph all the reactants implicated in these reactions can be seen. The symbol “*” refers to active/phosphorylated state of the reactant.

4.4.3 From active AKT to activation of downstream effector proteins

AKT activation allows the sequential activation of a series of reagents and hence cell survival and proliferation. AKT target proteins can be classified into three distinct groups: anti-apoptotic proteins, anti-p53 and proteins promoting cell proliferation. In the PI3K/AKT simulation the phosphorylation of these target proteins is represented by the reactions (17) to (21), (34) and (35) in Table 3. Figures 8 and 9 show the variation in levels of reactants and products during signal transduction to achieve the activation of many downstream effector proteins – e.g., IKK, XIAP, Raf1 and Mdm2.

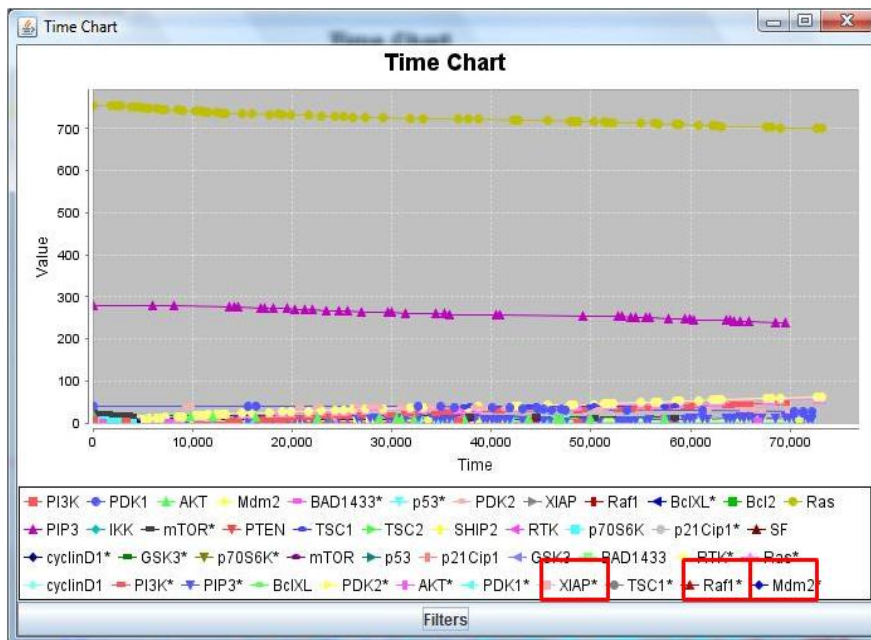


Figure 8: Execution of the reactions involved in the third segment of the simulation: from activation of AKT to activation of many downstream effector proteins. At the bottom of the graph the reactants implicated in these reactions are shown.

The symbol “*” refers to active/phosphorylated state of the reactant.

4.4.4 From activation of downstream effector proteins to activation of transcription factors

The activation of the AKT effector proteins results in the following cellular processes: 1) a decrease of expression or activity levels of several pro-apoptotic proteins, 2) an increase of anti-apoptotic proteins Bcl-2, Bcl-xL and XIAPs and 3) a positive feedback on the AKT protein itself. In the PI3K/AKT simulation these phenomena are represented by the reactions (27), (29) and (38) to (41) in Table 3.

The selection and execution of these reactions leads to the conclusion of the signal transduction, which ends exactly with the blocks of the inhibition of the transcriptional factors in the *nucleus* BTS (since GSK3 is inhibited by AKT, see reaction (20) in Table 3). GSK3 is also a target of AKT phosphorylation, which determines its inactivity, blocking its transcriptional activity and regulation of metabolism. The inhibition of GSK3 protects cells from apoptosis, but the exact mechanism is not known.

4.4 Final remarks

As explained in section 3.2, in each execution cycle the action selection mechanism – through the Gillespie algorithm – determines: 1) how long to wait before becomes active again and 2) which reaction execute among all eligible chemical reactions.

Upon simulating this anti-apoptotic pathway, we observed that a cancer cell escapes death, but if components PTEN and SHIP2 are present in the signalling system – equations (22) and (23) in Table 3 - then de cell stops growing and dies (see Figure 10).

However, if simultaneously with the activation of PI3K, Ras is active, Ras also activates PI3K, although PTEN and SHIP2 are present, according to the equation (25) in Table 3. Figure 11 shows how the mere presence of active Ras allows the cell to survive even in the presence of regulators of the PI3K/AKT pathway.

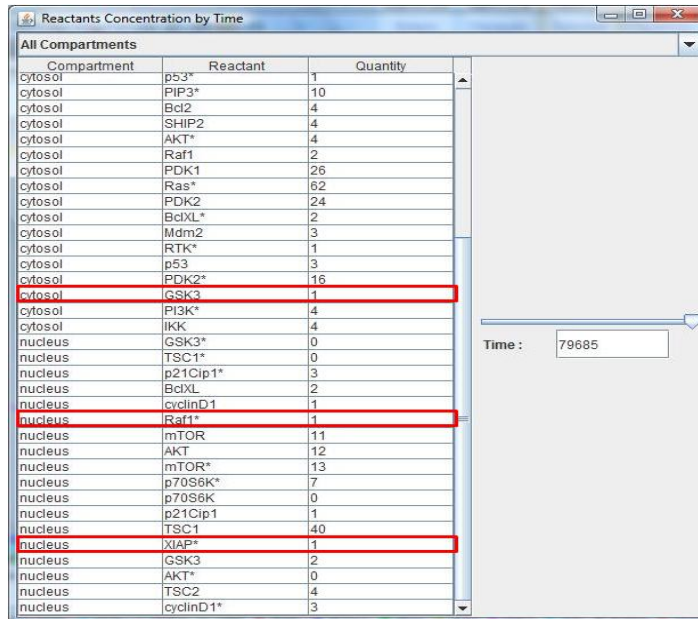


Figure 9: Variations of reactant concentrations in time. Third segment of the simulation: from activation of AKT to activation of many downstream effector proteins.

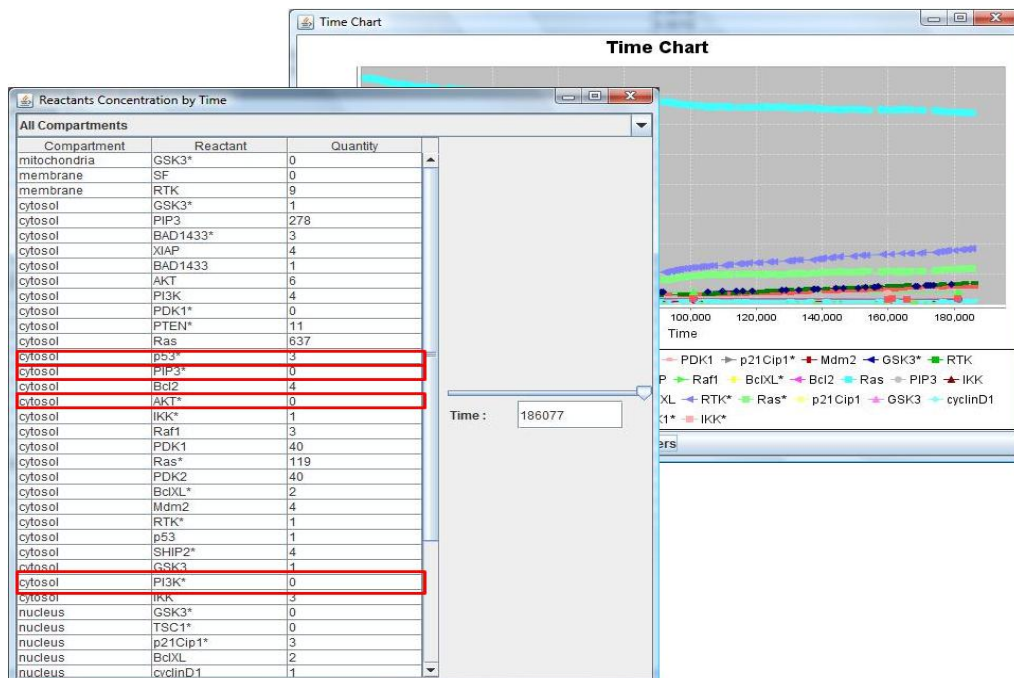


Figure 10: The presence of PTEN and SHIP 2, as well as the activation of p53, cause the cell to stop growing and die.

As shown above, the proposed tool successfully simulated the complex interaction patterns of the anti-apoptotic PI3K/AKT pathway, which is a great first step in predicting the molecular mechanism explaining how dietary polyphenols can inhibit cancer cell growth.

5 Conclusion

As a first approximation, using the tuple space-based simulation platform, we modelled and simulated the complex interaction patterns of the anti-apoptotic PI3K/AKT pathway, in order to determine the effectiveness of our platform, and to employ it in future prediction of experimentally non visualized interactions between the pathway components and polyphenols, as well as to represent the molecular action mechanism.

The platform presented here is a robust and flexible tool that meets all the requirements needed for simulating a signalling pathway. In particular, our goal is to employ it to predict the mechanism of action, at the molecular level, of dietary polyphenols.

As one could see in Figure 12, the incremental development of the modelling and simulation proceeds by incorporating other signalling components and pathways, defining the interactions among them (set of chemical reactions), and including the other features of the biochemical elements that are required for the accurate modelling of the signalling system. Clearly, the more the modelling process preserves the essential features of signal transduction, the more the intracellular signalling model becomes significant.

The next step of our work is to identify the potential targets of these polyphenols and to perform experiments *in silico* and then in cell cultures. This platform will allow us also to model the reversible reactions, it will be developed incrementally and will allow us to model increasingly complex reactions and interactions, which are difficult to detect experimentally.

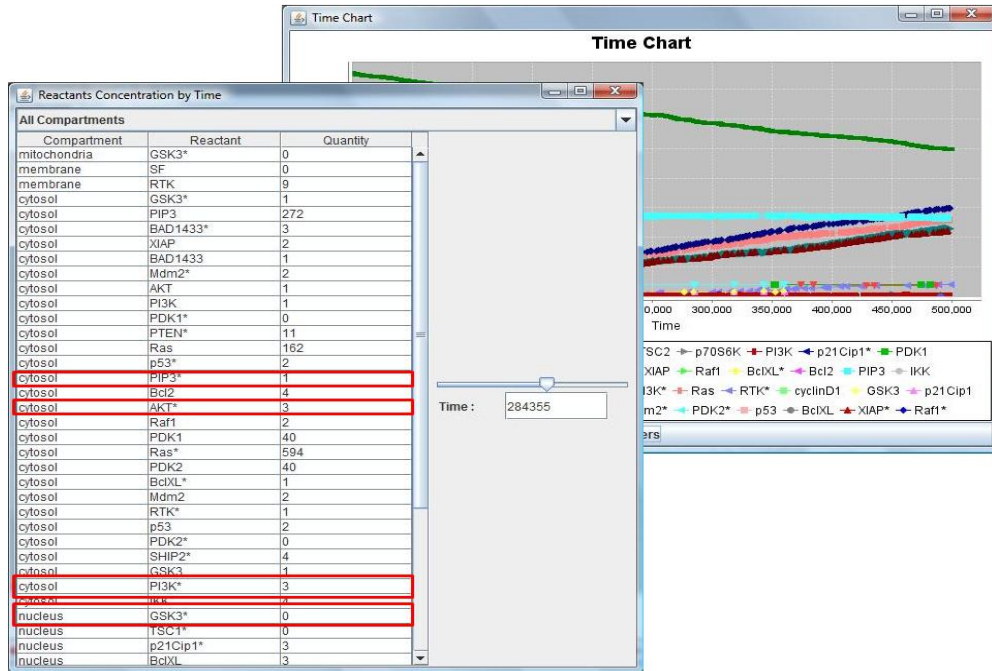


Figure 11: Active Ras allows the cell to survive even in the presence of PTEN and SHIP 2.

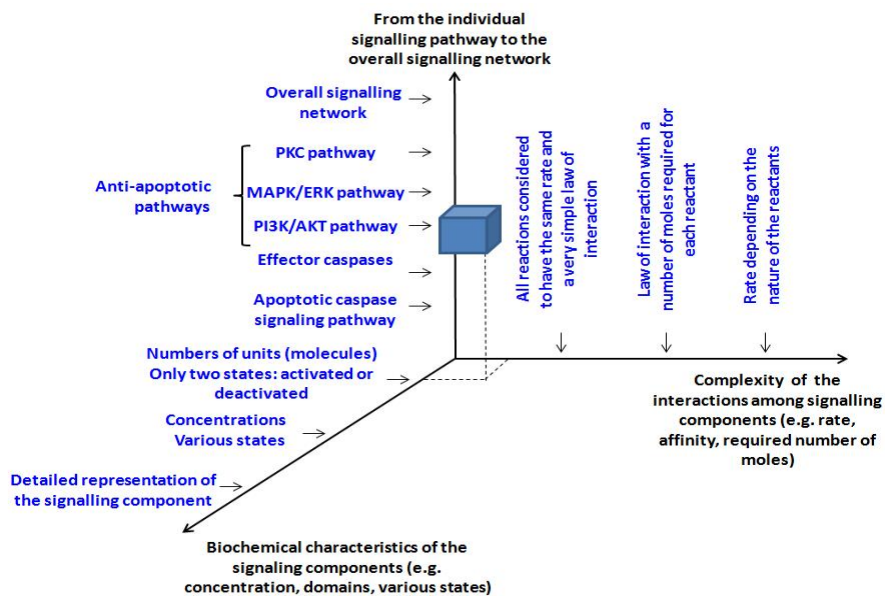


Figure 12: Incremental modelling process of anti-apoptotic signalling pathway PI3K/AKT, the cube represents the characteristics of our current work and moves it according to our needs.

ACKNOWLEDGEMENTS. The authors would like to thank Andrea Boccacci for making a valuable contribution to this project.

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