Applying the Tuple Space-Based Approach to the Simulation of the Caspases, an Essential Signalling Pathway

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Summary

Apoptotic cell death plays a crucial role in development and homeostasis. This process is driven by mitochondrial permeabilization and activation of caspases. In this paper we adopt a tuple spaces-based modelling and simulation approach, and show how it can be applied to the simulation of this intracellular signalling pathway. Specifically, we are working to explore and to understand the complex interaction patterns of the caspases apoptotic and the mitochondrial role. As a first approximation, using the tuple spaces-based *in silico* approach, we model and simulate both the extrinsic and intrinsic apoptotic signalling pathways and the interactions between them. During apoptosis, mitochondrial proteins, released from mitochondria to cytosol are decisively involved in the process. If the decision is to die, from this point there is normally no return, cancer cells offer resistance to the mitochondrial induction.

1 Introduction

Apoptosis is a type of cell death to eliminate unnecessary or damaged cells in a perfectly controlled manner that minimizes damage to neighbouring cells. The resulting cell debris, which are always surrounded by plasma membrane are removed by phagocytosis, preventing inflammation in the area. The cell dying by apoptosis undergoes a series of morphological changes, reducing its volume. The membrane is altered and displays protuberances ("blebbing"), cytoplasm and cell organelles are condensed inside release factors, which promote mitochondrial death. When the mechanisms that regulate apoptosis fail, this balance is disturbed and may originate excess and defect, various pathologies. Resistance to apoptosis is one of the characteristics that contribute to the generation of a tumour and may also be the cause of some autoimmune diseases. Otherwise, excessive apoptosis could be related to neurodegenerative diseases. One of the most important features of apoptosis is the nucleus condensation and DNA fragmentation. Moreover, many proteins undergo cell rupture or proteolysis generally catalysed by caspases. All caspases (cysteine proteases) have a cysteine in its active centre and proteins produced cuts right behind the amino acid Asp. There are two major pathways of apoptosis activation and three pathways of activation of caspases and therefore apoptosis:

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doi:10.2390/biecoll-jib-2013-225

1. Receptor pathway or extrinsic cell death involving members of the receptor family of Tumour Necrosis Factor (TNFR) located on the cell surface.
2. The intrinsic mitochondrial pathway, controlled by members of the family of Bcl-2 proteins.
3. The Granzyme B pathway, which is a serine protease contained within the secretory granules of cytotoxic lymphocytes and natural killer cells. Granzyme B cleaves its protein substrates after Asp residues, and can promote caspase activation, and apoptosis.

During apoptosis, mitochondria release to cytosol proteins involved in the process decisively. One such protein is a cytochrome c released into the cytosol after the activation of some caspases. The release of mitochondrial proteins is accompanied by a loss of its function as organelle power generator, since it affects the process of electron transport. In this paper, by using "tuple spaces-based modelling and simulation" we denote the crucial role of interaction between mitochondria and caspases during apoptosis.

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: 1) Topology and locality, respectively employed a) to model complex topologies of signalling networks, which involve feed forward and feed backward relationships between signalling components placed into different intracellular compartments and structures, and b) to provide a global view of the signalling system as a composition of local interactions, and 2) Considering the amount of each component of the signalling pathways with respect to time, their location and status (active, inactive, free, bound, etc.).

As mentioned above, the simulation of intracellular signalling pathways requires a robust approach accounting for the crucial features that govern the activity, interaction and evolution of such a sort of complex distributed system.

In this paper we assume a simulation approach based on the notion of Biochemical Tuple Spaces for Self-Organizing Coordination (BTS-SOC) [1], where each tuple space works as a compartment where reactions take place, chemical reactants are represented as tuples, and biochemical laws are represented as coordination laws. 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. In particular, each biochemical tuple space is built around a ReSpecT chemical engine, whose core is an action selection mechanism based on Gillespie algorithm [4] – an algorithm typically used to simulate systems of chemical/biochemical reactions efficiently and accurately – to execute chemical reactions with the proper rate.

In this paper, we briefly present the main structural and functional characteristics of our BTS-SOC-based bioinformatics infrastructure [5] and then show how it can be applied to the simulation of the caspases signalling pathway [6], which plays a crucial role in the transduction and execution of the apoptotic signal induced by various stimuli.

2 The Simulation Infrastructure

The main components of our BTSSOC model for simulating intracellular signalling pathways are described in Table I. As can be seen from the Figure 1, the high level architecture of the BTSSOC-based *in silico* approach is defined in terms of the following two main modules:

- The BTS-SOC-based model;
- The GUI application.

Table I: Mapping cellular components.

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

The BTSSOC-based model represents our general model for intracellular signalling pathways based on the notion of Biochemical Tuple Spaces for Self-Organizing Coordination (BTSSOC). A detailed explanation of this model can be found at [5]. As shown in Figure 1, four main components define the structure of the BTSSOC-based model:

- Tuple centres;
- Chemical reaction sets;
- Reactants (R);
- Coordination laws.

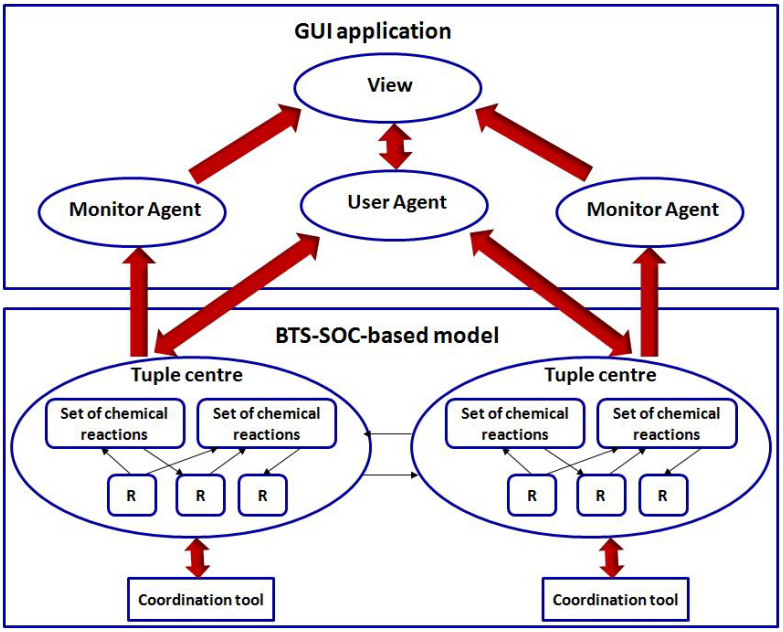

Figure 1: A high-level architecture for the BTS-SOC-based bioinformatics platform.

2.1 Chemical Reaction Sets

The components representing intracellular signalling elements must focus only on 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 (i.e., those modelling membrane receptors, proteins, enzymes and genes) is to perform chemical reactions for signal transduction, the most appropriate solution in the BTS-SOC-based approach is to model each signalling component as the set of the chemical reactions that defines its behaviour.

2.2 Reactants (R)

The elements recorded as tuples in a tuple centre (i.e., reactants and concentration) represent the information about the two main sorts of intracellular signal in the model: signalling molecules and activation/deactivation signals. Such elements are the inputs and outputs for the chemical reactions that belong to each tuple centre, and set their activation, duration, or deactivation either directly or indirectly. As a result, in the same way as in biological systems, evolution depends on the concentration and state of the reactants.

2.3 Coordination Laws

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, BTSSOC laws allow for tuple diffusion, making it possible for products to cross compartment boundaries as a result of biochemical reactions.

3 Methodological workflow

The methodological workflow of the major activities to be executed through BTS-SOC-based simulation platform during the modelling and simulation of the caspases apoptotic-signalling pathway is shown in Figure 2. The work was performed as follows:

1. Review of the literature involving the caspases pathway and experimental kinetic data of them in humans [7, 8].
2. Modelling the signalling components-*e.g.* chemical reactions-belonging to the caspases apoptotic signalling pathway. We start with a minimalist model where each signalling component is described by the following attributes: a) identity; b) concentration in each cellular compartment; c) free concentration; d) “bound” concentration; e) cellular compartment to which it belongs; f) chemical reactions involving the component and the order in which they occur according to the affinity of the components; and g) reaction temporality situation. Figure 3 and Table II summarize the models obtained from this phase.
3. Simulation of the caspases apoptotic-signalling pathway in the BTS-SOC-based bioinformatics infrastructure (see Figure 4 and Figure 5).
    a) Creating cellular compartments.
    b) Introducing reactants.
    c) Setting chemical reactions.

4. Getting simulation outcomes. After entering all required information and setting the initial parameters, the system is now ready to run the caspases apoptotic pathway simulation.
5. Analysis and parameter adjustment.

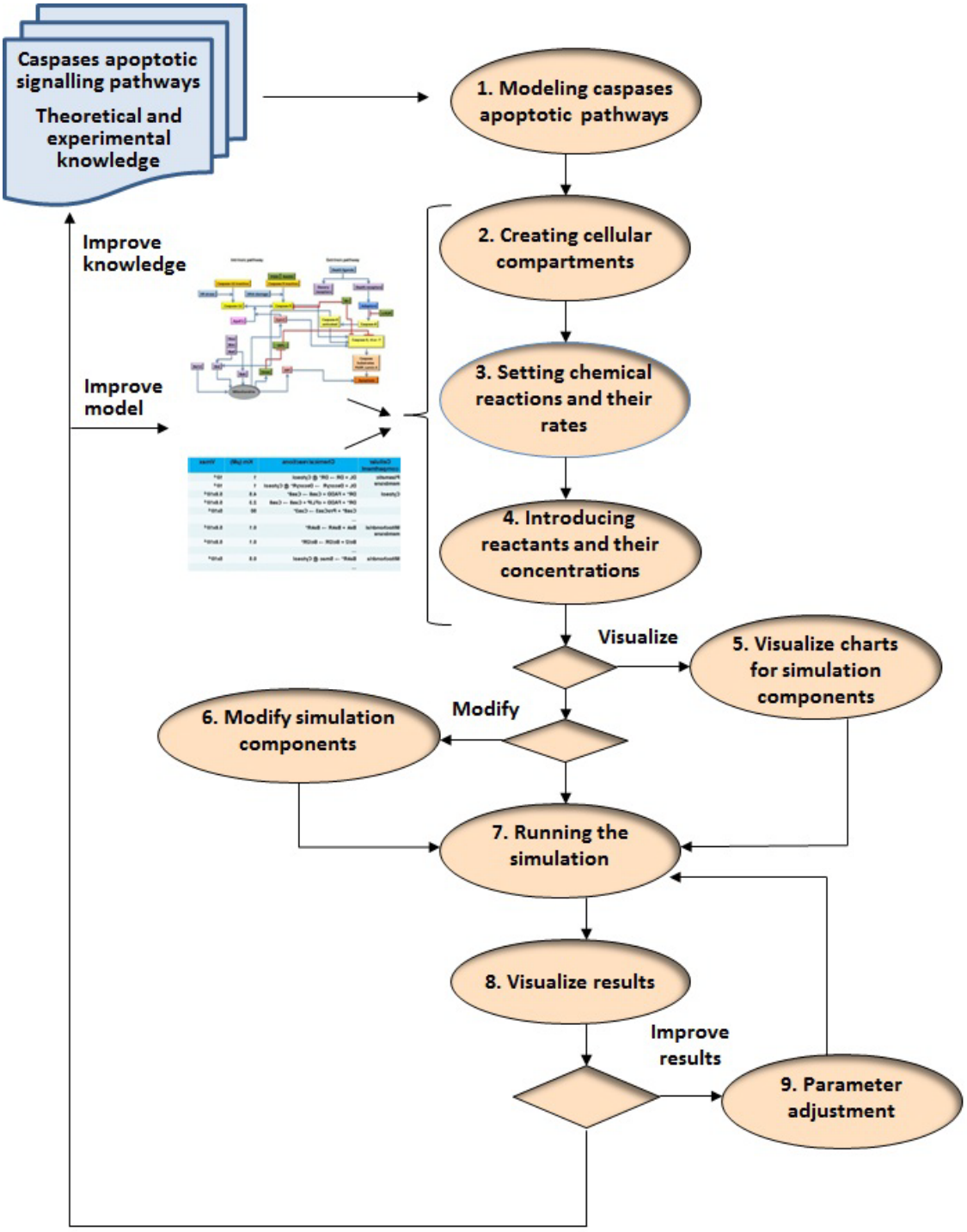

Figure 2: Methodological framework for modelling and simulation of caspases apoptotic signalling pathways.

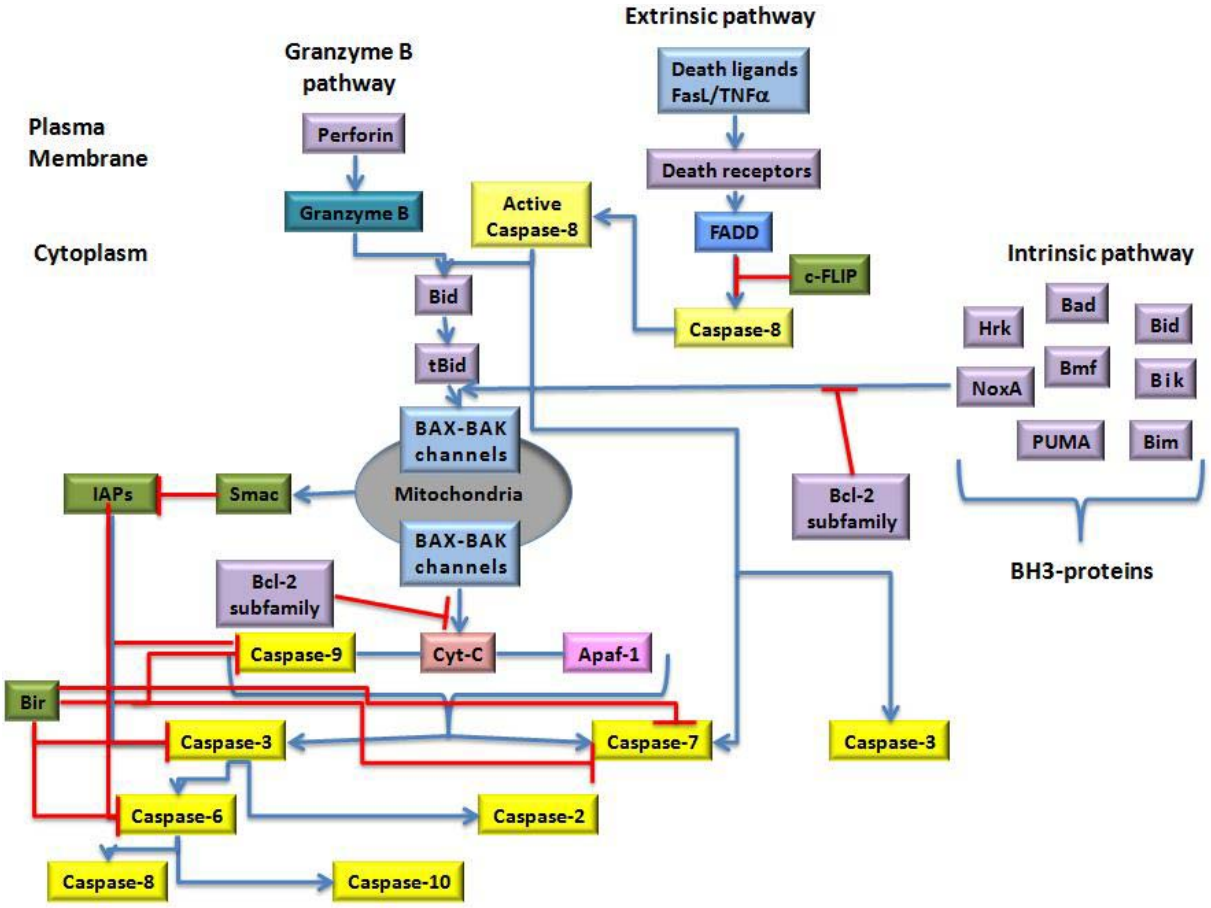

Figure 3: In this figure three activation pathways of caspases are shown: intrinsic (right), controlled by members of the family of Bcl-2 proteins; extrinsic (middle) activated by the members of the receptor family of Tumor Necrosis Factor; and left, granzyme serine protease family members, which are released by cytoplasmic granules of immune system cytotoxic T cells and natural killer cells. These three pathways lead to mitochondria, and after mitochondrial activation results in the release of proteins, there is normally no return, because caspases pathway starts.

4 Results and discussion

The role of mitochondria in apoptosis is essential in our modelling and simulation methodology; this allows us to include not only the intra-and extracellular space, but also the mitochondria, and all molecules contained in this energy producing organelle (see Figure 3). The route begins with death signals (hormones, growth factors, cytokines, stress, etc.). The modelling and setting up of these events are represented in Table II and Figures 4 and 5.

We take just one example for the simulation, as shown in Figure 3. The effectors caspases 3, 6, 7 are activated as a result of the activation of the extrinsic pathways, intrinsic or by Granzyme B. Once the cytochrome C escapes from mitochondria, the role of caspase-3 is critical. Caspase-3 is activated in the cytoplasm; however, two hours after being activated it may be localized in the plasma membrane, the cytoplasm and the core, the cancer cells offer resistance to the mitochondrial induction, preventing cell death. Figures 6 and 7 show the simulation of the connection among the three caspases pathways in the BTS-SOC-based bioinformatics infrastructure.

Table II: This table summarizes the biochemical interaction and kinetic parameters. The symbol "@" to the right of an equation indicates the cellular compartment in which the resultant reactant must be registered. Vmax is the maximum catalytic rate that can be achieved by a particular enzyme, Km is determined as the substrate concentration at which 1/2 Vmax is achieved.

Cellular compartments	Chemical reactions	Km (μM)	Vmax (μmol/mg/min)
Cytosol	PIDD/RAIDD + Cas9 + DNAdamage → Cas9*	0.2	1×10^{-5}
	Cas12 + ERstress → Cas12*	0.2	1×10^{-5}
	Cas9* + Apaf-1 + Cyt-C + dATP/ATP → Apoptosome	0.2	8×10^{-4}
	Cas9* + Apaf-1 + Cyt-C + Cas12* → 2Cas12*	1	1×10^{-5}
	Cas12* + Apaf-1 + Cyt-C + Cas9* → 2Cas9*	1	1×10^{-5}
	Smac + IAPs* → IAPs	1	1×10^{-4}
	IAPs* + Cas3* → Cas3	0.7	3×10^{-5}
	IAPs* + Cas6* → Cas6	0.2	3×10^{-5}
	IAPs* + Cas7* → Cas7	0.2	5×10^{-5}
Mitochondria/ Cytosol	BakR* + Smac → Smac @ Cytosol	0.5	5×10^{-6}
	BidR* + Smac → Smac @ Cytosol	0.5	5×10^{-6}
	Bcl2R* + Smac → Smac @ Cytosol	0.5	5×10^{-6}
	BakR* + AIF → AIF @ Cytosol	0.3	2×10^{-5}
	BidR* + AIF → AIF @ Cytosol	0.3	2×10^{-5}
	Bcl2R* + AIF → AIF @ Cytosol	0.3	2×10^{-5}
	BakR* + CytC→ Cyt-C @ Cytosol	0.1	8×10^{-7}
	BidR* + CytC → Cyt-C @ Cytosol	0.1	8×10^{-7}
	Bcl2R* + CytC→ Cyt-C @ Cytosol	0.1	8×10^{-7}
Mitochondrial membrane	Bak + BakR → BakR* @ mitochondria	0.1	5.8×10^{-5}
	Bid + BidR → BidR* @ mitochondria	0.1	5.8×10^{-5}
	Bcl2 + Bcl2R → Bcl2R* @ mitochondria	0.1	5.8×10^{-5}

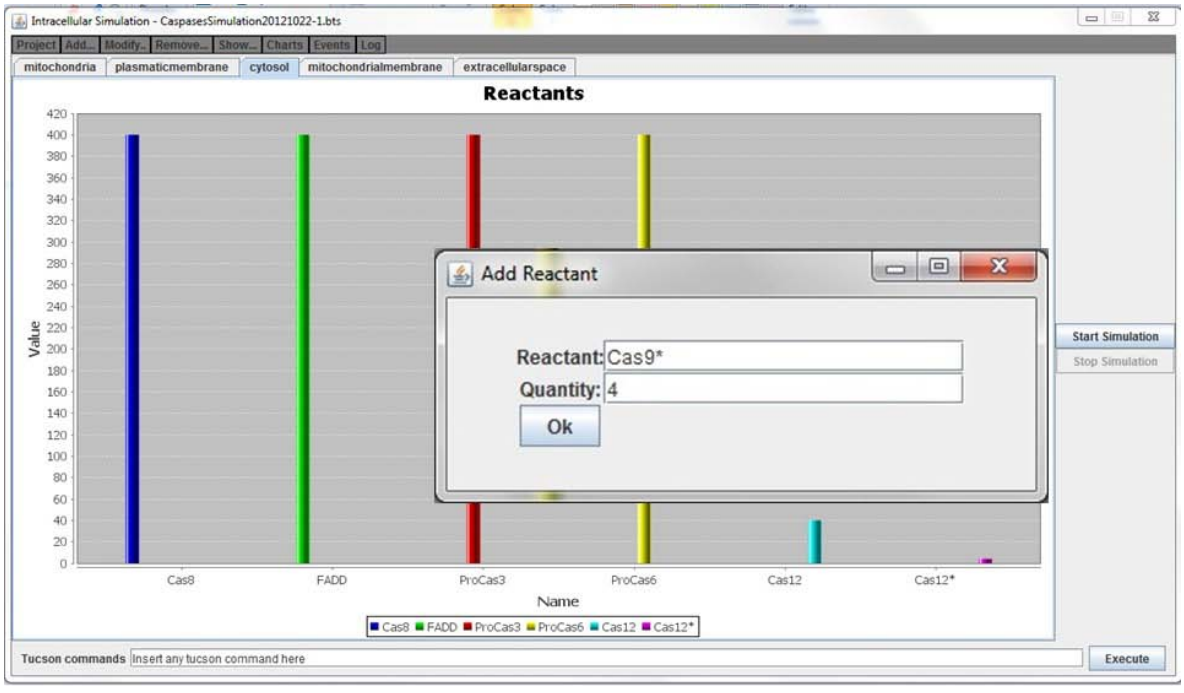

Figure 4: Introduction of reactants in the *cytosol* BTS.

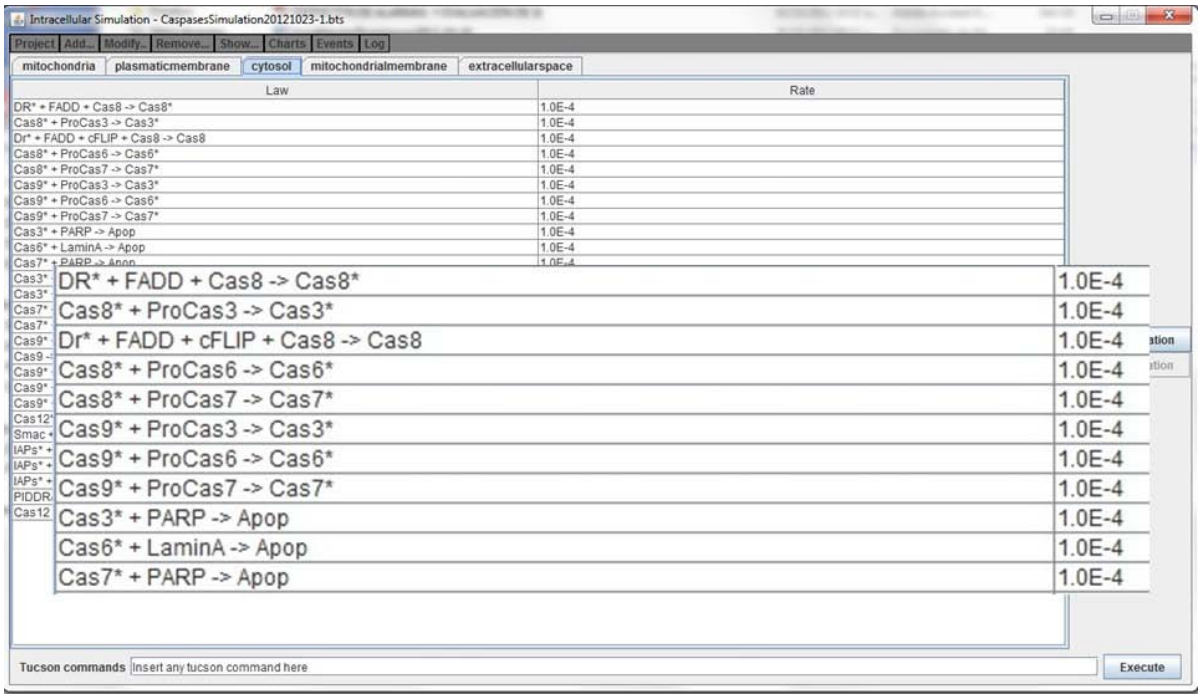

Figure 5: Setting chemical reactions.

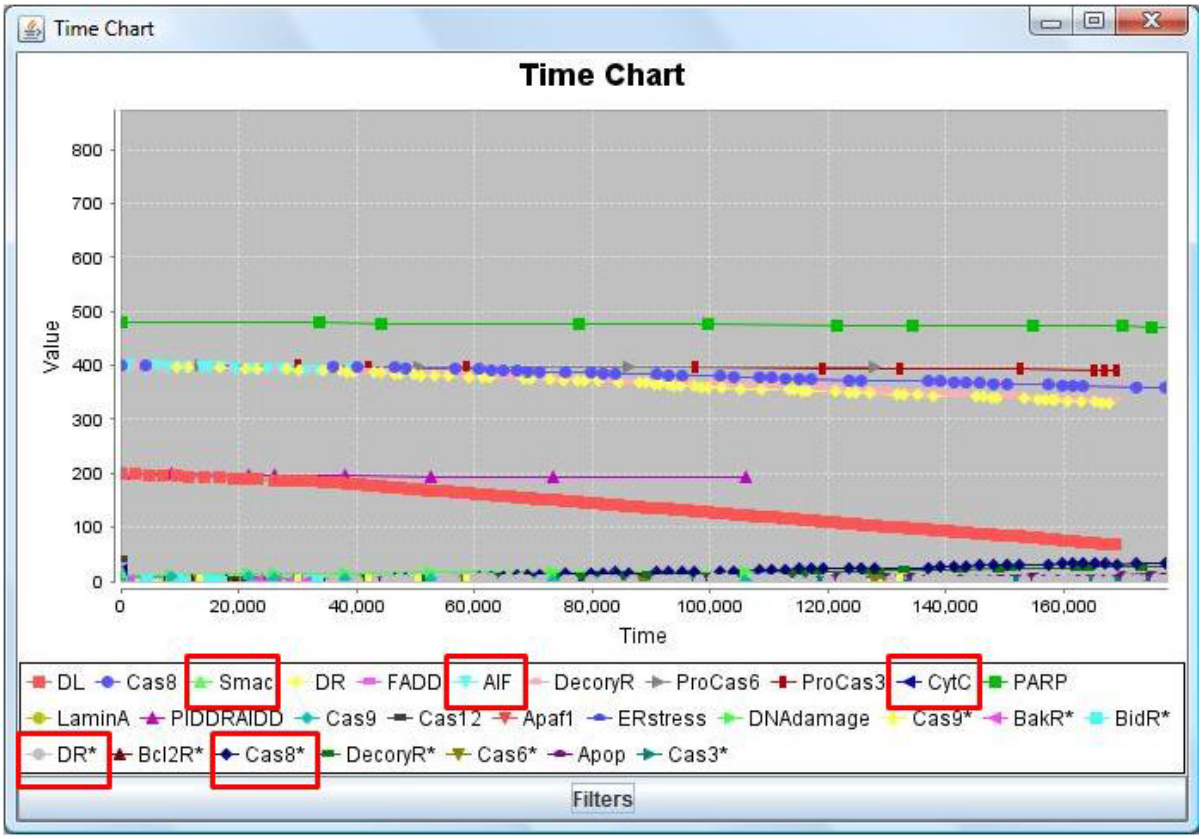

Figure 6: Concentration-time curves: connection between extrinsic and intrinsic pathways.

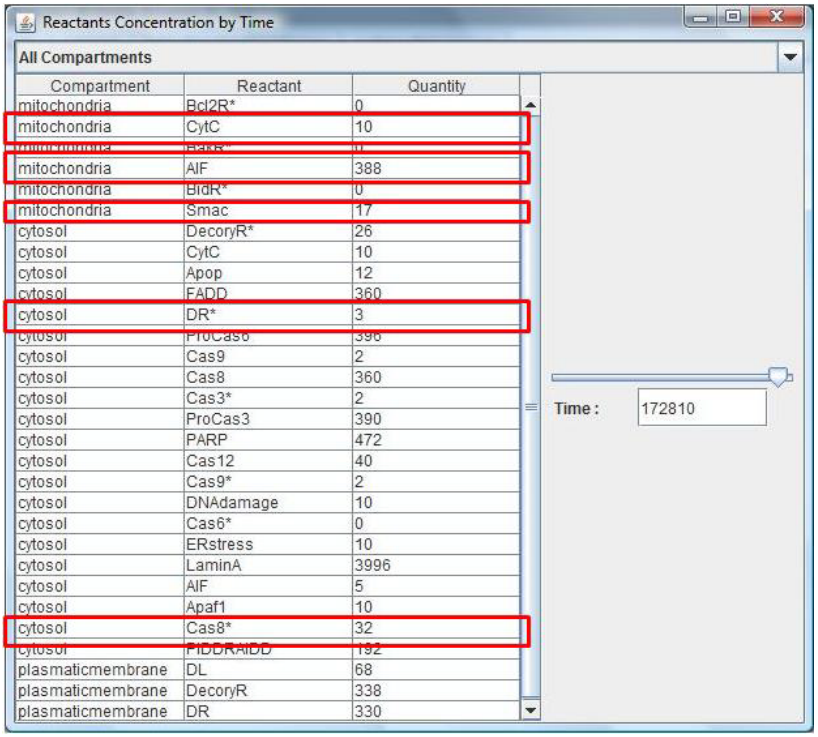

Figure 7: Concentration-time table: connection between extrinsic and intrinsic pathways.

5 Conclusion

When we run our simulation, we see how at a molecular level the cancer cells evade the caspases pathway, because cancer cells offer resistance to the mitochondrial induction. During apoptosis, mitochondrial proteins, released from mitochondria to cytosol are decisively involved in the process. If the decision is to die, from this point there is normally no return, cancer cells offer resistance to the mitochondrial induction. This platform is very useful because it allows designing experiments so that it is possible to perform different experiments in silico, before performing in vitro on the one hand, and on the other to visualize protein-protein interactions not easily detectable. An additional advantage of bioinformatics platform is that this allows experiments with each signaling pathway independently, which in experiments "in vivo" is not possible, because the elements belonging to the other tracks are present, which leads to crosslinking of the pathways. Thus, while in the experiments *in vivo* we can only appreciate the end result without knowing what signaling elements interacted among pathways, through experimentation *in silico* for each independent signaling pathways, one can view such interactions. The next step is to integrate PKC pathways, MAPK/ERK and PI3K/AKT, in order to achieve a true integration of apoptosis process in normal and cancer cells.

Acknowledgements

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

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