An in Silico Approach for Understanding the Complex Intercellular Interaction Patterns in Cancer Cells

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Abstract: Intercellular interaction allows cancer cells to preserve their malignance and through cell junctions to induce malignance in neighbouring cells and receive nutrients from them. The Wnt (wingless-related integration site) signalling pathway plays an important role in the formation of intercellular communications. In this work, we explore the complex interactions patterns of intercellular communication in cancer cells using an in silico modelling and simulation methodology developed by us. The proposed cellular signalling model, characterized by a multicompartmental nature, provides symbolic abstractions and accurate algorithms to model both intracellular and intercellular behaviours. In particular, in this work, we propose an in silico model and simulation of the formation of different communication channels, involving the Wnt signalling pathway. The final purpose of this study is to propose target molecules leading to break the communication between a cancer cell and surrounding normal cells. In this way, it is not necessary to carry out long series of different in vitro experiments, but only a few, because the focus should be only on the key molecules, which saves time and money. We observed, using in silico experiments, how the inhibition of Wnt signalling pathway prevents that the cells surrounding a cancerous cell are transformed.

1 INTRODUCTION

Intercellular communication allows the transfer of information from one cell to another. This type of interaction takes place by physical unions between the cells or through signalling molecules, released in the extracellular space by one cell and received by another cell through its receptors. By means of intercellular communication, the cells work in coordination, facilitating their survival. In the case of cancer cells, this communication allows them to continue growing, as well as inducing malignancy in neighbouring cells (Brücher and Jamall, 2014). Surrounding normal cells also send signals to cancer cells, thus these are destroyed, but not always successfully. The formation of junctions between normal and malignant cells plays an important role in the control of a cellular focus that will result in a tumour (Lum and Chen, 2015). The Wnt signalling pathway plays an important role in the formation of intercellular communications and regulates cell proliferation. The Wnt protein family includes a large number of cysteine-rich glycoproteins. Wnt proteins activate different signal transduction pathways. In this work, we model and simulate the canonical Wnt signalling pathway because this pathway is related to other signalling pathways in cancer.

The modelling and simulation of cellular signalling systems has found valuable support in a wide range of modelling approaches, which cover a wide spectrum ranging from mathematical models, e.g., ordinary differential equation systems, statistical methods and numerical methods, to computational models, e.g., cellular automata, Boolean networks, Petri nets, neural networks and multi-agent systems. Based on these models, different simulation tools have been developed from mathematical ones to computational ones (Alves et al., 2006). However, the majority of these former tools are based on abstractions to model only intracellular behaviour. Thus, they are not suitable to model intercellular communication. Consequently, in recent years, other major requirements in the simulation of cellular communication systems –such as multiple levels of compartmentalization, topology

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and locality– have emerged, guiding the development of new computational models and tools. Notable examples of computational simulation tools supporting such features are Bio-PEPA (Ciocchetta et al., 2009), MCell (Kerr et al., 2008), COPASI (Hoops et al., 2006), Virtual Cell (Cowan, 2012) and CompuCell 3D (Swat et al., 2012).

In this work, we simulate and explore the complex interaction patterns of intercellular communication in cancer cells using an in silico modelling and simulation approach developed by us, and motivated by two key elements: 1) a tuple space-based model (Gelernter, 1985) for the representation of the signalling elements (i.e., reactions with their own kinetic parameters, and reactants with their concentrations) and 2) a control mechanism for the selection and execution of reactions based on the Gillespie's algorithm (Gillespie, 1971) –a stochastic simulation algorithm typically used to mimic systems of chemical/biochemical reactions in an efficient and accurate way. A significant characteristic of this in silico approach is its multicompartmental nature. Specifically, it is suitable to model cells in their "social context", along with all those biological mechanisms that involve two or more cells, that is essential in the scenario discussed in this work. The main idea behind of this in silico modelling and simulation approach is to provide a computational experimentation environment that complements and guides in vitro experimentation in intra and intercellular signalling networks.

In this work, we showed and discussed how this in silico modelling and simulation approach can be used successfully to understand and explore the intercellular communication between a cancer cell and normal cells and, consequently, to propose key molecules, which can be targeted to allow us to break the communication between cancer cells and surrounding normal cells.

2 MATERIALS AND METHODS

2.1 The Multicompartmental Intercellular Signalling Model

Here we introduce the key elements of the intra and intercellular signalling model, characterized by its multicompartmental nature. The proposed model provides the logical abstractions for the representation and manipulation of the signalling elements, i.e., reactions, reactants and products. As mentioned earlier, the logical abstractions for the representation of the signalling elements are conceived from the notion of tuple and tuple spaces (Gelernter, 1985; González-Pérez et al, 2013), although the control mechanism for the selection and execution of the reactions is based in Gillespie's algorithm.

2.1.1 Tuple Space-based Model for Representation of Signalling Elements

Denote by C_i, $1 \leq i \leq m$, the *i-th* cell belonging to the cellular group *G*, which is represented by a set of *n* tuple spaces (*TS*) such that:

$$C_i = \{TS_{i1}, TS_{i2}, \ldots, TS_{in}\} \quad (1)$$

Each tuple space TS_{ij}, $1 \leq j \leq n$, is a set of tuples, where each individual tuple (*t*) represents a signalling element. Denote by *cr* a reaction, by *r* a reactant, and by *p* a product, therefore we have:

$$\forall t \in TS_{ij}, 1 \leq j \leq n, t = cr, t = r, or\ t = p \quad (2)$$

From (1) and (2) we have that any tuple *t* in any tuple space TS_{ij}, $1 \leq j \leq n$, and therefore in cell C_i, represents either a reaction (*cr*), a reactant (*r*) or a product (*p*). Note that each TS_{ij}, $1 \leq j \leq n$, represents a cell compartment, e.g., nucleus, mitochondria, cytoplasm, cell membrane, or even extracellular space, which guarantees the multicompartmental nature of the cell signalling model.

In order to simplify the notation, the subscripts corresponding to *cr*, *r* and *p* have not been considered in expression (2). However, it should be clear that each of these identifiers is accompanied by three sub-indices, the first refers to the set of spaces of tuples (cell), the second refers to the space of tuples (cellular compartment), and the third refers to the element itself (particular reaction, reactant o product).

Regarding representation of reactions, expression (3) provides the symbolic abstraction that allows to represent, and therefore manipulate, chemical reaction schemes commonly required when modelling cellular signalling systems, such as synthesis, decomposition, and standard equation for enzymatic reactions, as referred in expression (4) to (6), respectively.

$$cr([(r_1, reqMol_1), (r_2, reqMol_2)], K, [(p_1, pm_1), (p_2, pm_2)]) \quad (3)$$

where: r_1, r_2 are reactants and $reqMol_1$, $reqMol_2$ are the number of molecules involved of reactants r_1, r_2, respectively; *K* is the reaction rate constant; p_1, p_2 are products and pm_1, pm_2 are the number of molecules formed of products p_1, p_2, respectively.

$$A + B \rightarrow AB \quad (4)$$

$$AB \rightarrow A + B \quad (5)$$

$$S + E \rightarrow P + E \quad (6)$$

Let TS_{ij} and TS_{ik} be two neighbouring tuple spaces, which we represent by the tuple:

$$neighbouring(TS_{ij}, TS_{ik}), 1 \leq j, k \leq n, j \neq k \quad (7)$$

Consider also that a tuple space can have at most two neighbours, given the type of biological system that we are modelling. As already established, a tuple space (TS_{ij}) models a particular cellular compartment. Thus, an example of tuple space with more than one neighbour is given by the "cellular membrane" tuple space, which has as neighbours the "extracellular space" and the "cytosol" tuple spaces.

The notion of neighbouring tuple spaces (expression (7)) plays a key role in our signalling model, since it allows us to establish that the products formed by a reaction *cr* belonging to a tuple space TS_{ij}, are translocated to another tuple space TS_{ik}, if and only if TS_{ij} and TS_{ik} are neighbours. In this way, the continuity of the signal transduction is guaranteed through all tuple spaces (cell compartments) that make up the cell C_i.

Then, returning to expression (3), if we require that one of the products formed, for example p_1, be translocated to the tuple space TS_{ik}, being TS_{ij} and TS_{ik} neighbours, then the tuple (p_1, pm_1), located in the right part of the expression (3) will be replaced by the tuple (*translocate*(p_1, pm_1), TS_{ik}).

With regard to reactants and products involved in the reactions, both are also represented as tuples in the tuple space, through the symbolic abstraction provided in the expression (8).

$$r(r_i, Mol_i) \quad (8)$$

where r_i is the reactant and Mol_i is the number of available molecules.

2.1.2 The Algorithm for the Selection and Execution of Reactions

Once all the reactions and the reactants are modelled, then every reaction is explicitly simulated on the basis of the Gillespie algorithm. In detail, the main steps performed by the algorithm for the selection and execution of reactions, i.e., for starting and continuing cellular signal transduction, are summarized below:

1. Calculate the rate for each eligible reaction (*cr*) –see expression (3)– according to the expression:

$$Rate = K * \prod_{i=1}^{2} \binom{Mol_i}{reqMol_i} \quad (9)$$

where *K* is the reaction rate constant, Mol_i is the number of available molecules of reactant r_i, and $reqMol_i$ is the number of molecules required of reactant r_i.

The rate with which the reaction will be selected is equal to the rate of this reaction (*K*) –where *K* can be estimated as a measure of affinity or calculated from the maximum rate (V_{max}) and the Michaelis constant (K_m)– 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 reaction is not eligible for lack of any of the required reactants, then the rate (*Rate*) of this reactions will be zero.

2. Calculate the summation of the rates (*Rate*) of all eligible reactions, the resulting value is *RTot*.
3. Sort all eligible reactions by rate in a descending order.
4. Generate a random number ψ between 0 and 1.
5. From sorted list of eligible reactions, the *k-th* reaction is chosen if:

$$\psi \leq \frac{\sum_{i=1}^{k} Rate_i}{RTot} \quad (10)$$

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.

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

$$Stop_{time} = \frac{-ln(\tau)}{RTot} \quad (11)$$

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 (involving expressions (9), (10) and (11)) constitutes the simulation. The simulation concludes when there are no eligible reactions.

2.2 The Computational Simulation Tool Associated with the Proposed Model

The modelling approach proposed was integrated in Cellulat, an already existing computational simulation tool for signal transduction systems, developed by us (González-Pérez et al., 2013; Cárdenas-García et al., 2016). Cellulat establishes in itself an integrated virtual environment for in silico experimentation on cellular signalling pathways and networks, strongly dependent on characteristics such as multi-compartmentalization, location and topology.

As a highly interactive application, Cellulat provides the user with a wide range of visual and interactive tools, to follow and feedback at every moment the signal transduction that takes place in the simulated signalling network.

It is important to note that all the elements required by the simulation –cellular structures and compartments, reactions with their kinetic parameters, and reactants with their concentration or number of available molecules– are written or recorded in the same written language used when describing these elements; it is the simulation tool itself that will translate them into logical abstractions based on tuple spaces, previously introduced. Figure 1 shows the key phases in the creation and execution of a simulation using Cellulat.

Cellulat (in its Executable Jar File version) can be either executed or downloaded from the bioinformatics website of our research group at http://bioinformatics.cua.uam.mx/node/10. The instructions required for the download can be consulted on this website.

2.3 The Methodological Approach

The methodology followed in this work is based on a continuous bidirectional feedback between the in silico modelling and simulation approach and theoretical and experimental knowledge. That is, the proposed multicompartmental intercellular signalling model and the results of its associated computational simulation should provide valuable support to guide in vitro experimentation; while the results of theoretical and experimental research should lead to both the improvement of the model and the design of the most appropriate in silico experiments.

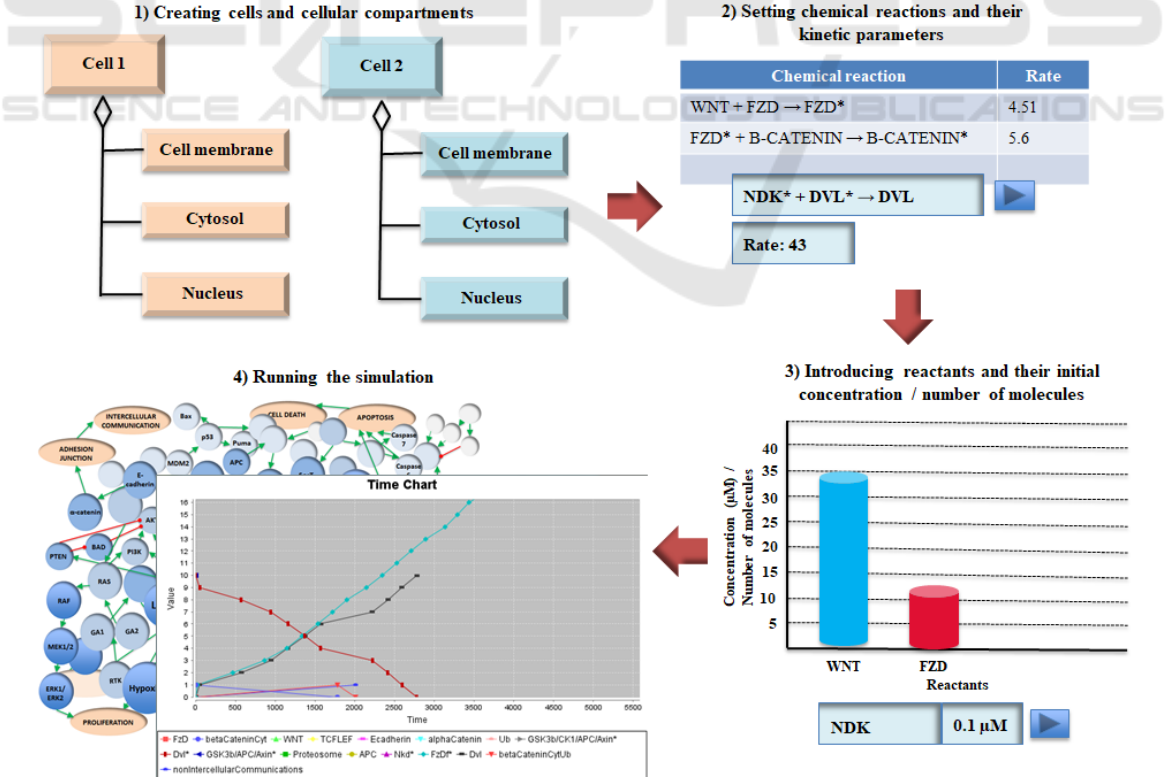

Figure 1: Sequence of the major activities carried out during the creation and execution of a cell signalling simulation using Cellulat.

The main phases involved in this methodology are summarized below:

Modelling phases:

1. Modelling the integrated Wnt signalling pathways.
2. Taking into account the model developed in phase 1, define the cellular structures and compartments involved in the signalling and, for each of these, the list of reactions and reactants located there.

Simulation phases:

3. Creating cellular compartments.
4. Setting reactions and their kinetic parameters.
5. Introducing reactants and their available concentration o number of molecules.
6. Design the in silico experiments.
7. Running the simulation.
8. Visualize simulation execution.

Analysis phases:

9. Analysis of simulation execution.
10. Feedback between the in silico modelling and simulation approach and theoretical and experimental knowledge.

3 RESULTS

3.1 The Integrated Model of Wnt Signalling Pathways

Figure 2 shows, as first result, a simplified version of Wnt signalling pathway model proposed by us, to be simulated using the in silico approach previously presented and following the methodology described above. As can be seen in Figure 2, β-catenin is the central part in this pathway, interacting with E-cadherin and α-catenin proteins. We have integrated into the model the canonical and non-canonical pathways as well as Notch (Borggrefe et al., 2016), Hedgehog (Shimizu and Nakagawa, 2015), and Hypoxias (Grunsven and Vlierberghe, 2014) ones. On the other hand, we will also consider, as the background of the model illustrated in Figure 2, other previously modelled signalling pathways with which the Wnt signalling path intersects, such as EGF/MAPK/JAK-STAT (González-Pérez et al., 2003), PI3K (González-Pérez et al., 2013) and caspases (Cárdenas-García and González-Pérez, 2013). In Table 1 can be observed some examples of signalling elements –reactions and reactants– defined as part of the integrated model of Wnt signalling pathways. Note that both types of cells (normal and cancer cells) exhibit the same signalling elements to form communication channels.

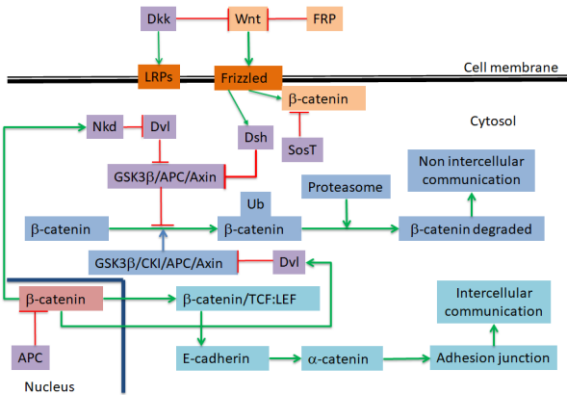

Figure 2: A simplified version of Wnt signalling pathway modelled in this study. Red arrows indicate inhibition relationships and green arrows indicate activation relationships.

3.2 The Simulation of the Wnt Signalling Pathways

As can be seen in Figure 3, the simulation is ready for execution. That is, cellular compartments, reactions and reactants have been created from the Wnt signalling pathway model, which has been conceived and verified as initial phase of our methodological approach.

The simulated Wnt signalling network is made up of 146 nodes representing reactants, 12 nodes representing cell processes, such as adhesion junction, proliferation, apoptosis and cell death, and 204 arcs representing reactions between the involved nodes. Table 1 shows some reaction examples. The overall signalling network extends across 5 cell compartments (i.e., extracellular space, cell membrane, cytosol, mitochondria and nucleus) comprising key cellular signalling pathways involved in growth and metabolism leading to survival, proliferation, tumour progression and cell death, as well as integration with the formation of intercellular interactions (i.e., EGF/MAPK/JAK-STAT, PI3K/AKT and caspases signalling pathways).

Table 1: Examples of signalling elements –reactions and reactants– defined as part of the integrated model of Wnt signalling pathways.

Cellular compartment	Reaction	Vmax	Reactant	Reactant Conc. (μM)	Reference
Extracellular space / membrane	WNT + FZD -> FZD*	4.51	FZD WNT	12 33	(Lee et al., 2003)
Cytosol	FZD* + β-CATENIN -> β-CATENIN*	5.6	β-CATENIN	0.001	(Hernández et al., 2012)
Cytosol	NDK* + DVL* -> DVL	43	NDK DVL	0.1 0.01	(Blaheta et al. 2005)
Cytosol	APC* + β-CATENIN* -> β-CATENIN	12	APC	0.4	(Blaheta et al. 2005)
Cytosol	β-CATENIN* + TCF-LEF -> β-CATENIN*/ TCF-LEF	33	TCF-LEF	8	(Hernández et al., 2012)

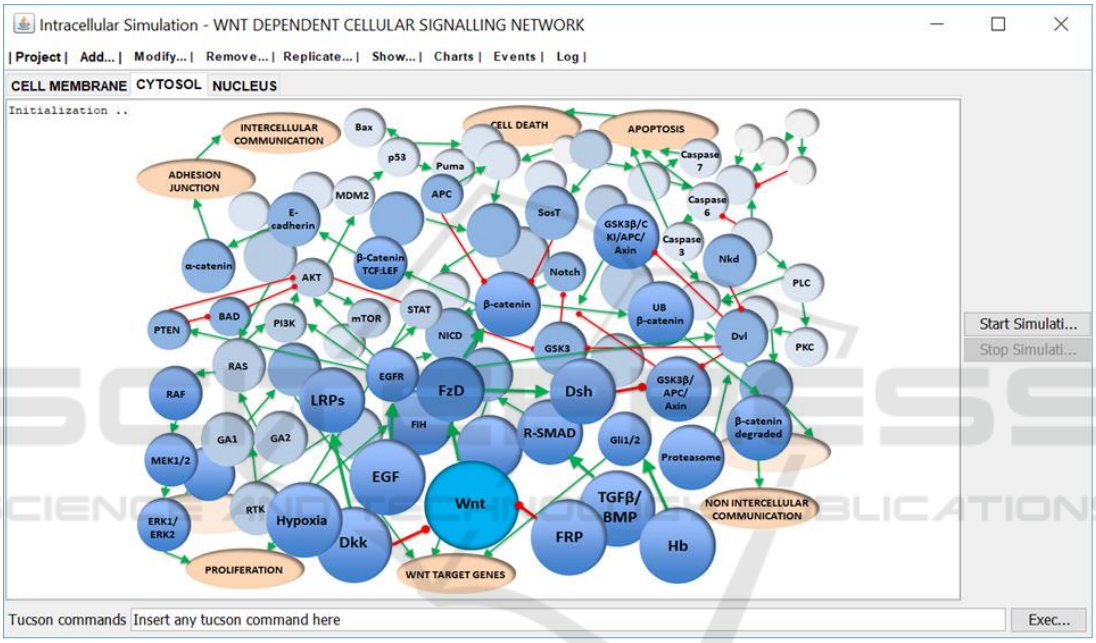

Figure 3: Cells, cell compartments, reactions and reactants have been created as the initial components required by the simulation of Wnt signalling pathways. Signalling elements, e.g., proteins and enzymes, are represented by solid blue spheres. Each signalling element is identified by its name (acronym). Red arrows indicate inhibition relationships and green arrows indicate activation relationships.

3.3 An Overview of the in Silico Experiments

The simulated intercellular communication scenario consisted of two cells: a cancer cell and a normal cell. The simulated cell junctions in cancer cell are used to share information with the normal cell. The goal of the designed in silico experiments was to find proteins that prevent the formation of gap junctions and leave the cancer cell uncommunicated.

The early in silico experiments carried out consisted of running the simulation through a series of combinations among the concentrations of several target proteins. e.g., FZD, NDK, DVL, DSH and APC. After a series of possible arrangements, i.e., increasing or decreasing the concentration, or even removing the protein, we selected two proteins, APC and NDK.

To determine the necessary concentration of APC or NKD to prevent the formation of intercellular junctions, experiments were designed using concentrations of APC or NDK with the lowest concentration of 0.001 μM and the highest concentration of 1000 μM, i.e., 0.001, 0.1, 1, 10, 100 and1000 μM.

We observed that the increase in the concentration of APC or NKD leads to degradation of β-catenin in early stages of simulated signal

transduction, thus preventing the formation of intercellular junctions. Regarding APC, at a concentration of 0.01 µM, it was observed that β-Catenin disappears at 1,000 milliseconds (ms) and DVL at 3,000 ms (see Figure 4). Concerning NKD, at a concentration of 0.001 µM, the disappearance of β-catenin and DVL is observed at 1000 ms, although their disappearance is even faster (500 ms) when using a concentration of 0.01 µM.

4 CONCLUSIONS

The in silico modelling and simulation approach allowed us to observe, that increasing the concentration of APC and NKD inhibits the Wnt signalling pathway, preventing the formation of intercellular junctions, since the β-CateninCyt is destroyed and disappears. In silico experimentation help us to determine the appropriate concentration of these target molecules.

To carry out the experiments in cancer cells, we use concentrations of 0.1 µM for both NKD2 and APC. Using this concentration, β-CateninCyt quickly disappears, and thus the formation of gap junctions is avoided. Thanks to in silico experiments we found two target proteins involved in the intercellular communication channels and we observed that their inhibition can stop cancer development. The next step is to use this result to guide the in vitro experiments, which in turn will feedback the in silico modelling and simulation approach proposed here.

The model of intercellular communication in cancer cells evolved meaningfully, from the first versions to later version, after multiple theoretical/experimental feedbacks which allowed to solve the following problems that emerged during the execution of the associated simulation:

1) the earliest models of intercellular communication in cancer cells did not include all the required reactions, particularly negative feedback (or balancing feedback), and 2) the estimated reaction rate constant of some reactions did not meet the required value, avoiding that such reactions were executed at the appropriate time by Gillespie's algorithm, resulting that some slower reactions were executed before the faster reactions.

As part of our future work, 1) we will increase the size and complexity of the Wnt signalling network, including other Wnt cross talk pathways, since if we are able to understand, explore and control this cellular signalling network, then tissue invasion and metastasis can be avoided, 2) larger intercellular communication systems (i.e., 3 o more cells) will be simulated for verifying how the proposed approach scales with the system size, and 3) we will use other related simulation tools, such as MCell (Kerr et al., 2008) and Virtual Cell (Cowan et al., 2012) for comparison with Cellulat.

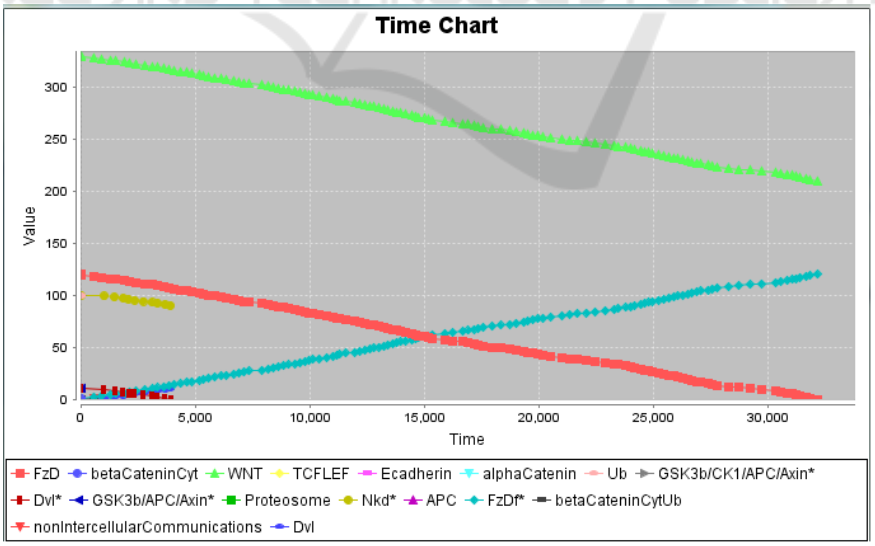

Figure 4: APC at a concentration of 0.01 µM. After a series of possible arrangements, we selected two proteins: APC and NKD. The APC protein is part of the complex that favors the ubiquitination of β-catenin, and therefore its destruction. If there is no β-catenin, there is no union formation. This is still observed using an APC concentration of 0.001 µM, β -catenin and DVL disappear around 2500 milliseconds. At this point only the Wnt receptor remains present. On the chart, the x-axis represents the time in millisecond, and the y-axis represents the concentration of reactants (scaled by 10) in micromolar.

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