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An Overview Methodology for Writing Suitable Boolean Rules for Protein Signaling Pathways

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ABSTRACT

Boolean model elaborates discrete modelling of any biological system with the purpose to study its dynamical evolution. The representative network has been composed of nodes and edges that show the way of interactions between these nodes. The modelling consists of a set of logical functions, known as Boolean functions that represent the interactions between nodes, and are simulated to determine all attractors of the system, and consequently, its stable states are stated as fixed points. In this paper, we give a description of the methodology followed to write Boolean functions. We present two different Boolean models constructed by these two methods and the differences shown in the results they simulate. In a situation where experimental data are missing, the functions have been usually written under prediction and assumptions made for this occasion, because the path followed by the information to jump from one node to another was considered mandatory for the first Boolean model. Differently, in the second Boolean model activators and inhibitors are considered separately without any restriction, as in the first method. Here, the type of interactions was considered important, because we are interested to know only what flows in and out from any target node. The methodology has been applied firstly in a hypothetical representative system and then in four real signalling pathways. We have identified many differences in the simulated fixed points and concluded that the second model offers more results for further analysis. Consequently, there is a higher probability that we find, through second Boolean modelling, more suitable stable states that correspond to the biology.

Keywords: Boolean modelling, fixed point, stable state, attractor, logical functions.

1. INTRODUCTION

The study's main purpose of biological systems is the understanding of the complex interactions that exist between the elements of the network and leading to the understanding of how the genetic information flows through the network, and how it responds to specific stimuli happening in a cell or tissue. Due to this complex nature of the interactions our study is often impossible to be done by using only biological or biochemical knowledge. For this reason, as well as from a practical point of view, the combination of mathematical models, biological knowledge, and computer programming tools is turning to be mandatory for a better profound study [1, 2]. Depending on the type of the biological systems, regulatory system, signalling pathways, etc, and depending on the question whose answer is required, the network analysis may vary from a statistical method to a theoretical one, or sometimes it will request the combination of both ways. Here we focus on the theoretical study of an illustrative model representing a potential protein-protein interaction network (PPI), based on the use of Boolean modelling [3]. We have tried to present a methodology followed to study the stable states reached by the system. We describe the use of the

Boolean model, where each element of the system is specified with 0 and 1, meaning an inactive and an active state, respectively. We write the interactions' descriptive functions, based on the logical rules AND, OR, and NOT, in two different ways to identify the differences that may exist in the steady states reached by the system. The Boolean model indicates the evolution of the system to happen in short discrete time steps and provides to us the potential steady states reached by the system, which are mathematically stated by fixed points. Furthermore, our study is based on theoretical assumptions where we emphasize that not all the fixed points should be considered as suitable steady states for any specific biological system. We suggest that, despite the fact what the theory provides to us, we should always face the experimental evidence, if there is any available, or at least we should compare these theoretical results to the theoretical thought that exists in this field.

In the end, we follow the same methodology on real biological signalling pathways, focusing on the fixed points reached in any case. Simulations have been performed by BooleanNet [4] or BoolNet [5], for small and big systems, respectively. We have tried to give a conclusion about the most appropriate Boolean model to use, even though they are always established according to predictions and assumptions. Moreover, the construction of a Boolean model, i.e., writing its logical functions, is, in general, the most important and difficult part of the study [6], as they should provide to the readers the proper information aimed by the study even though the experimental evidence is often missing.

Finally, from the fixed points simulated by the model, many other data and information's can be derived from which the researchers can perform further studies on the target system [7].

2. METHODOLOGY: BOOLEAN MODEL

The network constructed by Boolean models is composed of nodes and edges (links) that show the interactions that exist between these nodes. All nodes can be described by one of the two qualitative states: ON (active state) or OFF (inactive state) corresponding to the binary numbers 1 and 0, respectively. The biological meaning related to these two states can be assumed according to the purpose of the study; however, the general idea is that when a node is in an active state (ON) means that it can perform correctly, whereas the node in an inactive sate (OFF) is not performing correctly. In other words, we give to the binary numbers 1 and 0 those attributes related to the ability of the node to affect the other node. Meanwhile, the edges between the elements may appear as activation or inhibition [8, 9] showing the regulating effect that one node has on another one. Biological relationships between components of the network (nodes) can be translated into mathematical equations using Boolean logical operators OR, AND, and NOT [3, 6]. These regulatory functions reflect the behaviour of regulators (components) toward each other.

Boolean modelling of biological systems, which are usually very big, generates transition graphs composted of 2^N transition states, where N is the number of elements. The transition states graph is used to describe how the system evolves in time until it reaches its final stable states. The system evolution is simulated by using two different update methods, synchronous and asynchronous updates. In the synchronous update method, all nodes are being updated at precisely the same time whereas, in an asynchronous update method, a single randomly chosen node is updated at any instant moment no matter if other nodes are being updated or not [1]. In both cases, the stable

states show the attractor basins of the system whose final attractors, where the system converges after several time-steps, are mathematically represented by fixed points [10, 11]. These fixed points are some binary vectors composed of numbers 0 and 1 that show in which state each of the elements is when reaching the stable state of the system. Fixed points are independent of the updating methods and they show the same attractors of the system, no matter what updating method is chosen to simulate the system. For this reason, here we simulate the Boolean model through the synchronous update method because we are focused only on the fixed points of the system, i.e., in the final stable states and not in the intermediate transition states through each system flows.

2.1 Model Construction

Model construction, in general, is a mathematical process that is based on different factors, but on this occasion, we believe that the type of interactions between the elements is the most important factor affecting the model. Depending on this idea, we may conclude into two types of biological systems: a system that evolves by changing just its state but not its size, changing the elements' states from active to inactive or vice versa; and a system that evolves by changing its size because of the death of elements (for exp., proteins, genes, cells, etc.) or the birth of them. In the first case, the interactions between elements show either up-regulating or down-regulating effects whereas, in the second type system, interactions are considered to be either constructive or destructive. Here, the first type of the system is considered, i.e. the system on focus is that one composed of the same elements all the time of its dynamic evolution, that change only their states (affecting each other but not destroying/creating any) until the system converges in one of the final stable states (if there is more than one).

Boolean modelling is firstly performed on a small illustrative network (Figure 1) created especially for this occasion only, and then it is applied on real biological PPI networks on Figure 4 and Figure 5. The illustrative model is a system composed of five elements (A, B, C, D, and E) and the interactions between them are occasionally defined (a, b, c, d, e, f, and g).

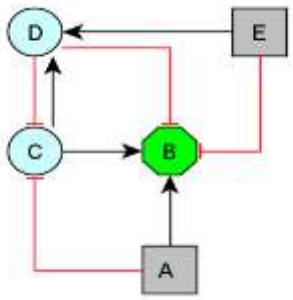

Figure 1. Illustrative model of a presupposed PPI system composed of five nodes and eight links, that represent the eight probably pathways. All nodes are divided by colours according to the role they "play" in the system. Nodes A and E are considered as input nodes, representing the initial conditions; D and C are intermediate nodes which represent the proteins of the system and B is the last node of the network which represents the output as it seems that all the pathways converge in it. The edges also are given in two colours showing that the red links show inhibition (down-regulating effect) whereas the black ones show activation (up-regulating effect).

Our main focus is to make proper dynamical modelling of a biological system that can provide the suitable final stable states that correspond to the biology behind the system on focus. In other words, we want to build a mathematical model that

qualitatively describes the changes over time of the state of each component of the system that finally will generate exactly the states that each element has when being in the final stable states. For this purpose, beyond the difficulty that might exist, we use discrete models instead of continuous ones because in this manner we can simplify the complexity of the target system and, particularly, when the experimental data is missing, we jump to a simulation model which is easier to perform compared to the simulation of a continuous model. The solution of the problem, in this case, i.e., the determination of the fixed points of the system, is given by the numerical simulations that are performed based on BooleanNet [4] or BoolNet [5], the use of each depends on the size of the target system. Small systems, composed of less than ten elements, are easily simulated with BooleanNet, a package written in python, whereas biological systems composed of more than ten elements are simulated with BoolNet, an R language package.

The dynamic of the system is described by observing the way that the system follows to go from one state to another every single time step, and finally, we focus on the stable states defined by the fixed point generated by the numerical simulations. We make the dynamical analysis of the same system but simulating two different models constructed. Both these models are based on Boolean functions but the way we chose to interpret the relationships between elements and consequently the mathematical equation written for this purpose is different. In the following sections, we present both models constructed, which are first applied to the illustrative system presented in the Figure 1.

2.1.1 Boolean Modelling: First model

Here we present how a Boolean model is constructed by writing Boolean functions following the interactions that exist between elements of the system. For this, we observe the path that information follows to flow from one node to another one. It is important to emphasize that when experimental evidence is missing then it is necessary to raise some assumptions before writing the logical equations [3, 12]. To be more specific, the operator OR is used when an element affects the future state of the target element from different paths (directly and not directly). The AND operator indicates a more conditional action because it is used when the future state of the target element is regulated by more than one element, at the same time. Following these rules, it is clear to understand that when a node is in an active state (1/ON) means that at least one of its neighbours is active (ON), when the operator OR is used, and all its neighbours are active when the operator AND is used. Operator NOT is used when an inhibition action happens, i.e. when the target element is down-regulated by at least one of its neighbours [6]. In all other cases, we consider the interactions between nodes as up-regulated actions.

Applying these assumptions and rules on the mentioned system (Figure 1), we get the functions given in Table 1, and the transition graph simulated on this occasion is given in Figure 2.

Table 1. Boolean functions for the system represented in Figure 1. This Boolean model is constructed based on the Boolean assumption explained in Section 2.1.1.

Node	Boolean Functions
A	$A(t+1) = A(t)$
E	$E(t+1) = E(t)$
C	$C(t+1) =$ **NOT** $[(A(t)$ **AND** $D(t)]$
D	$D(t+1) = E(t)$ **AND** $C(t)$
B	$B(t+1) = A(t)$ **OR** $C(t)$ **OR** (**NOT** $[D(t)$ **OR** $E(t)])$

2.1.2 Boolean Modelling: Second model

Differently from the first model, here we present a Boolean model of the same system (Figure 1) following a different approach toward the fixed points. Writing a differential equation to describe the change in the state during a period *t* we have to consider that each element, in this case, changes according to the information that flows in and out the target element and not the path it follows from one point to the other one. This approach is mathematically presented by the following equation:

$$\frac{dx}{dt} = inflows - outflows \tag{1}$$

The left side shows the rate of change of any element of the network while the right side shows the way how this change occurs. Precisely speaking, we consider that *inflows* represent the information or material coming in indicating a *positive effect* on the target element ($inflows > 0$), while the *outflows* represent the information or material coming out of the same target element indicating a *negative effect* on it ($outflows < 0$).

Based on this idea and on the suggestions given in [13], any system can be generally described by this set of Boolean equations:

$$x_i(t+1) = \begin{cases} x_1^a(t) \vee x_2^a(t) \dots \vee x_j^a(t) & (1) \\ \neg(x_1^i(t) \vee x_2^i(t) \dots \vee x_k^i(t)) & (2) \\ (x_1^a(t) \vee x_2^a(t) \dots \vee x_j^a(t)) \wedge \neg(x_1^i(t) \vee x_2^i(t) \dots \vee x_k^i(t)) & (3) \end{cases} \tag{2}$$

Equation (2) is a set of three equations showing in each case the way how an element is regulated by other elements of the system. As shown, Equation (2.1) is used when a specific element of the system x_i is only up-regulated by a number of j – activators; Equation (2.2) is used when x_i is only down-regulated by a number of k – inhibitors, and Equation (2.3) is used when x_i is regulated by both j – activators and k – inhibitors at the same time. Notice that here, equations are written by using ∧, ∨, and ¬ instead of logical operators AND, OR, and NOT.

Considering this model, we write Boolean equations for the illustrative system given in Figure 1 as shown in Table 2, and then the simulation of this model gives us the state transition graph, presented in Figure 3.

Table 2. Boolean functions for the system represented in Figure 1. This Boolean model is constructed based on Equation (2), as explained in Section 2.1.2.

Node	Boolean Functions – 2
A	A(*t* +1) = A(*t*)
E	E(*t* +1) = E(*t*)
C	C(*t* +1) = ¬[A(*t*) ∨ D(*t*)]
D	D(*t* +1) = E(*t*) ∨ C(*t*)
B	B(*t* +1) = [A(*t*) ∨ C(*t*)] ∧ ¬[D(*t*) ∨ E(*t*)]

2.2 Attractor Analysis and Further Discussions

Boolean functions in both tables (Table 1 and Table 2) have noticeable differences between them as well as they have similarities such as the future state of the inputs, which are supposed to be constant over time. Other equations are written following an independent assumption made in each case. The differences in equations are transmitted

and observed even in the results received from the simulation. Figure 2 and Figure 3 show the state transition graph received by simulating the first model and the second one, respectively. Both models produce the same number of fixed points, precisely four fixed points, and as a consequence, the system has four possible stable states to reach.

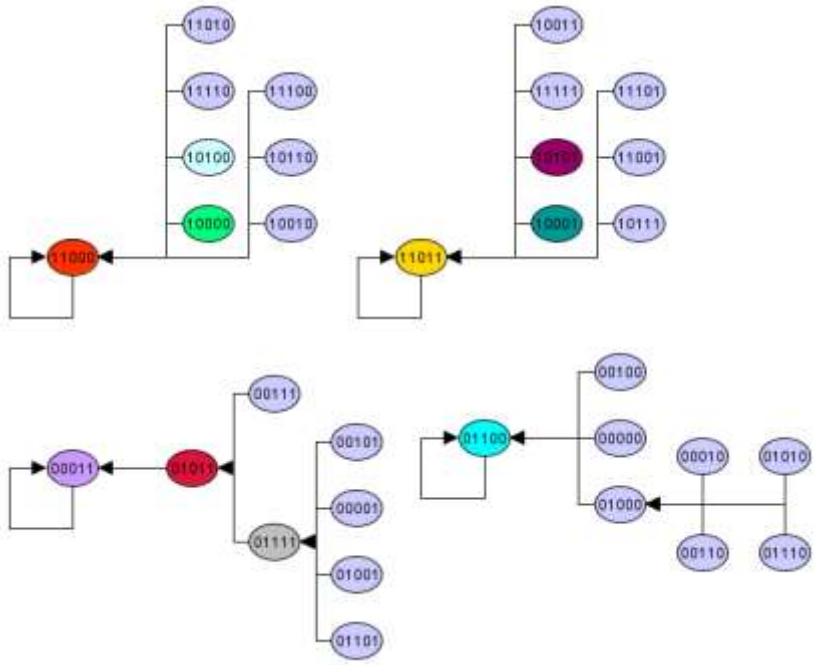

Figure 2. State transition graph created by the simulation of the first Boolean model. Fours attractor basins meaning that there are four fixed points which represent four possible stable states: {(11000), (11011), (00011), and (01100)}.

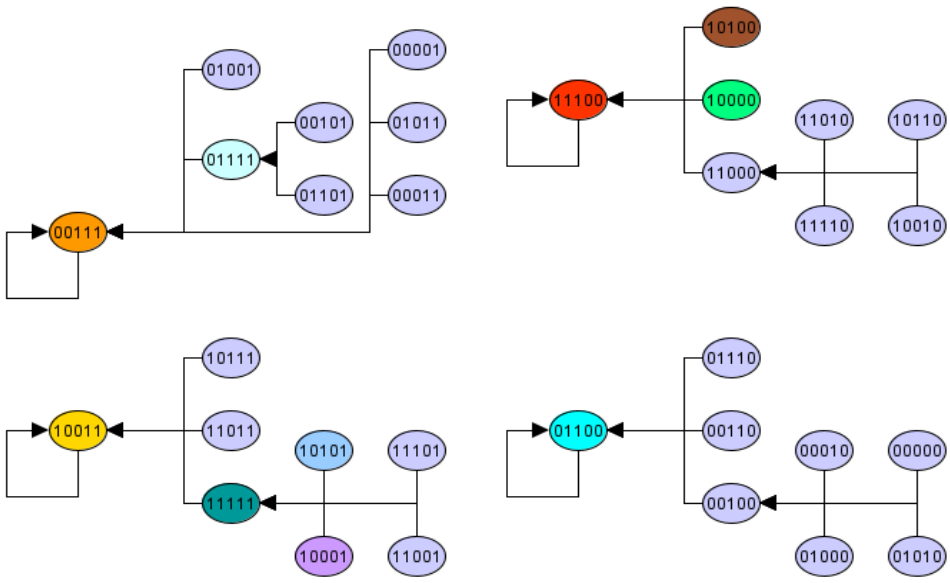

Figure 3. State transition graph created by the simulation of the second Boolean model. Fours attractor basins meaning that there are four fixed points which represent four possible stable states: {(00111), (11100), (10011), and (01100)}.

As shown in both figures, the difference between these two models lies in the fact that the type of fixed points, i.e., the elements of the system show a different state in the stable states simulated by two models. To be more specific, let us observe all fixed points and the state of all elements in these fixed points. To check the state of each node in any fixed point we should take into account that the order of the binary numbers corresponds to the order of elements running by the program, i.e., AECDB (Table 3).

Table 3. Fixed points of the system produced by simulating two different Boolean models. There are four fixed points in both models and each of them is shown the state of each element of the system given in Figure 1.

Node	Model – 1				Model – 2			
A	1	1	0	**0**	0	1	1	**0**
E	1	1	0	**1**	0	1	0	**1**
C	0	0	0	**1**	1	1	0	**1**
D	0	1	1	**0**	1	0	1	**0**
B	0	1	1	**0**	1	0	1	**0**

As shown in Table 3, only one fixed point is identically generated by two models. The other three fixed points are different and this fact suggests that the same system can converge in different stable states depending on the Boolean functions written in each case. In these circumstances, attention is shifted from the purpose to determine the stable states of the system to the question whose answer should be found: "Which is the best model to use, i.e., what are the most suitable equations to write to generate the most appropriate stable states of the system?" Obviously, as previously mentioned, nothing of these doubts would be considered as issues if we had experimental evidence about the system because in that case the appropriate equations would have been adjusted. However, because these data are usually missing, then the model is constructed completely theoretically by hypothesis and assumptions, which in the end generates some states that correspond or not to the biology. Accordingly, we suggest that before coming to a final conclusion, several tested should be done in accordance with the analysis of the system which should be done simultaneously.

Furthermore, it is very important to emphasize that in both models it is applied one universal rule that states that the material or energy that flows through two different stations (two different elements of the system), from one hand it is lost from the outflows, and on the other hand the same amount is gained from the inflows. This is related to the conservation law of mass or energy, which we consider as true when modelling a biological system [14]. In more details, let us consider that element A interacts only with element B in a way that what comes out from A goes directly to B, without any loss. Consequently, element A loses mass (or energy) at a rate $-k_1A$ whereas the mass (or energy) gained through this pathway by element B with being $+k_1A$. Although the relationships between elements (for exp., proteins) may be quite complex, we consider them simplified and that obey Boolean equations (in both cases). For this purpose, three assumptions are considered: 1 – we exclude any loss that might happen during the time of evolution; 2 – we admit that all flows happen in discrete short steps of time with the same constant rate, and 3 – we consider that the system is in temporal equilibrium and after each time step, the system enters again in a temporal equilibrium and this process happens continuously until the system reaches the final permanent equilibrium (stable state), represented by the fixed point. Depending on the pathway that the flow follows, the system may have the opportunity to reach more than one permanent equilibrium [15]. However, once that we know which all possible stable states are, we can then analyse them to conclude in the most suitable ones that

correspond to the biology deduced. This means that we are allowed to consider only those stable states, and consequently only those attractor basins, that enable the system to transit through states that are relevant to the biology conclusions.

2.3 Application on protein signaling pathways

As previously mentioned, establishing Boolean functions for a signaling pathway or a PPI network is required the recognition of the network and the biochemical reactions that bridge one protein to another. Experimental data is usually missing but in some other cases, there might be contradictory information [8, 16]. In such a situation the Boolean functions can be built under the known facts or network analysis. Because we are considering a biological system composed of proteins and interactions between them, what we extract from the system includes the pathways followed and the state (active or inactive) that each element is in these pathways.

Thus, to reach the best approach to the solution we firstly have to predict the way of the interactions between elements and then decide which are the most suitable set of equations for the inflows and outflows of the system. All assumptions made should be clear and in accordance with the biology that lies after what seems visible from the system. Furthermore, time is considered to be a discrete measurement. This implies that during the dynamical evolution no processes or events occur between the changes of time and for this reason we can consider time to be a physical time unit that can be as small as we need it to be in a specific situation (exp., second, minute, hour, day, year, etc.) [14]. Physical units are not always easy to understand so in a signaling pathway network the signal is the general information that flows through time from one element to another one, but in a specific meaning, the signal may be mass, different chemical material, energy, etc. It is always very difficult to predict the future of system evolution, and this happens especially in system biology.

In this section, we show the method described above applied on real biological systems identified and constructed upon experimental evidence discovered so far. The first system considered is that presented in Figure 4.

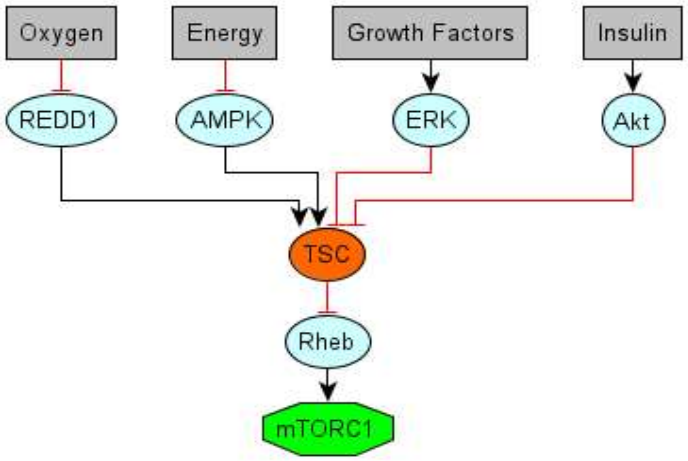

Figure 4. A partial schematic showing some of the components of the nutrient-sensing pathway upstream of mTORC1, originally presented in [17]. All nodes are divided by colors according to the role they "play" in the system. The edges also are given in two colors showing that the red links show inhibition (down-regulating effect) whereas the black ones show activation (up-regulating effect).

From the above Figure 4, the signalling pathway system is part of a bigger and more complex system, given in [17], while here is a sample composed of eleven nodes and ten links that represent the eleven pathways. Oxygen, energy, growth factor, and insulin are considered as input nodes that determine the initial conditions of the system; REDD1, AMPK, ERK, and Akt are proteins affected directly by the inputs. These proteins, together with TSC and RHEB are intermediate nodes but differently, from others, TSC is the most important node because its degree is higher compared with others. mTORC1 is the last node of the network which represents the output because it is the last affected protein since that all the pathways finally converge in it.

Following the same methodology and logic, as it is expressed and applied in the illustrative system, presented in Section 2.1, Boolean functions for both models are given in Table 4 and Table 5.

Table 4. Boolean functions for the system represented in Figure 4. This Boolean model is constructed based on the Boolean assumption explained in Section 2.1.1.

Node	Boolean Functions – 1
Oxygen **Energy** **Growth Factor** **Insulin**	Oxygen (t +1) = Oxygen (t) Energy(t +1) = Energy(t) Growth Factor (t +1) = Growth Factor (t) Insulin(t +1) = Insulin(t)
REDD1 **AMPK** **ERK** **Akt** **TSC**	REDD1(t +1) = **NOT** Oxygen (t) AMPK(t +1) = **NOT** Energy (t) ERK(t +1) = Growth Factor (t) Akt (t +1) = Insulin (t) TSC(t +1) = REDD1(t) **AND** AMPK(t) **AND NOT** ERK(t) **AND NOT** Akt(t)
RHEB **mTORC1**	RHEB(t +1) = **NOT** TSC(t) mTORC1(t +1) = RHEB(t)

Table 5. Boolean functions for the system represented in Figure 4. This Boolean model is constructed based on Eq. (3), as explained in Section 2.1.2.

Node	Boolean Functions – 2
Oxygen **Energy** **Growth Factor** **Insulin**	Oxygen (t +1) = Oxygen (t) Energy(t +1) = Energy(t) Growth Factor (t +1) = Growth Factor (t) Insulin(t +1) = Insulin(t)
REDD1 **AMPK** **ERK** **Akt** **TSC**	REDD1(t +1) = ¬ Oxygen (t) AMPK(t +1) = ¬Energy (t) ERK(t +1) = Growth Factor (t) Akt (t +1) = Insulin (t) TSC(t +1) = (REDD1(t) ∨ AMPK(t)) ∧ ¬ (ERK(t) ∨ Akt(t))
RHEB **mTORC1**	RHEB(t +1) = ¬ TSC(t) mTORC1(t +1) = RHEB(t)

As it is shown in the above tables, there is only one rule that is different between the two models. This rule is related to the most important node of the system TSC, as it has the highest degree coefficient among all other elements of the system [18], and for this reason, it is possible to see the difference that exists. Another element, especially the

target one mTORC1, i.e., the output of the system, is regulated only by one other element, so there is no clear change between rules. Even though, the final stable states are not all identical for both simulated models. In Figure 5 it is shown that for both models there are sixteen fixed points generated, indicating that there are sixteen permanent stable states (equilibriums).

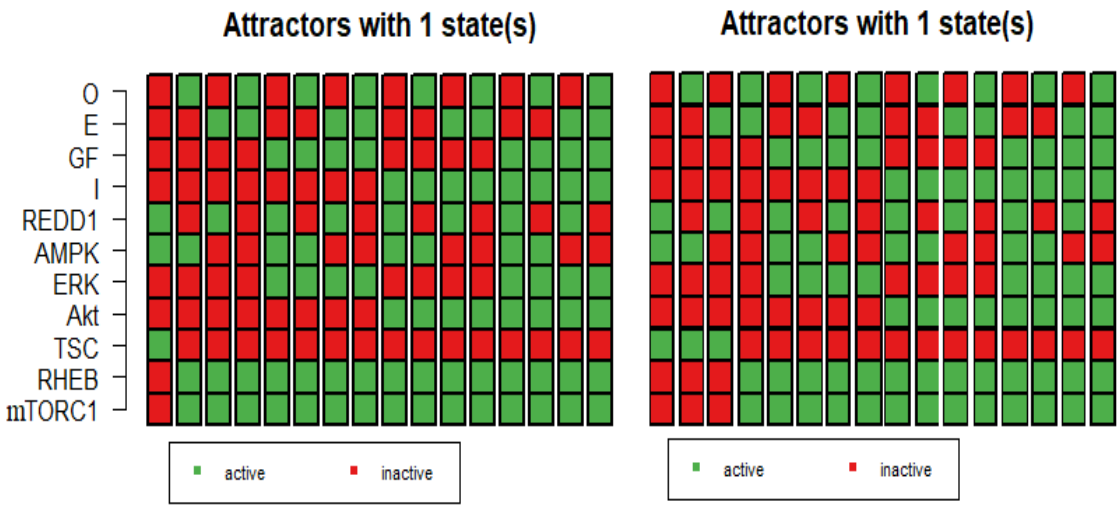

Figure 5. Attractors graph, showing the fixed points of the system presented in Figure 4. The left side and right side show the attractors of the system simulated by the first model and second model, respectively. Each square presents the state of each element according to the colour it has: red squares show an inactive state while green squares show an active state. In both models, there is the same number of stable states and the difference between them is identified to be in only two fix points, where the state of RHEB and mTORC1vary from one model to the second one.

2.3.1 More protein signalling pathways

To deduce the most suitable model for further research on the dynamical evolution of biological systems more tests are needed. Since that this is a theoretical study based on numerical simulations there would be a moment when the results will be compared with those limited experimental data or biological information that exist. Regardless, before arriving at this point, we should be convinced which model gives the most suitable approach to the calculation of the fixed points. For this, to be secure for the differences that exist between two models we make Boolean modelling of three more signalling pathways that are composed of more than ten elements, and which are presented in SIGNOR [19]. In these cases, the complexity of the systems is increased [20] as well as the differences between the regulatory functions written according to both models are more visible, as shown in Figure 7. As previously mentioned, numerical simulations are performed with BoolNet. These numerical simulations correspond to the biological systems presented in Figure 6. All these systems have on their focus the signalling pathway of mTORC1, which represents the protein complex of protein kinases mTOR. We have been particularly interested in performing a dynamical analysis of signalling pathways where this protein is included because it is found that this protein plays a crucial role and is of great importance in several diseases related to cancer.

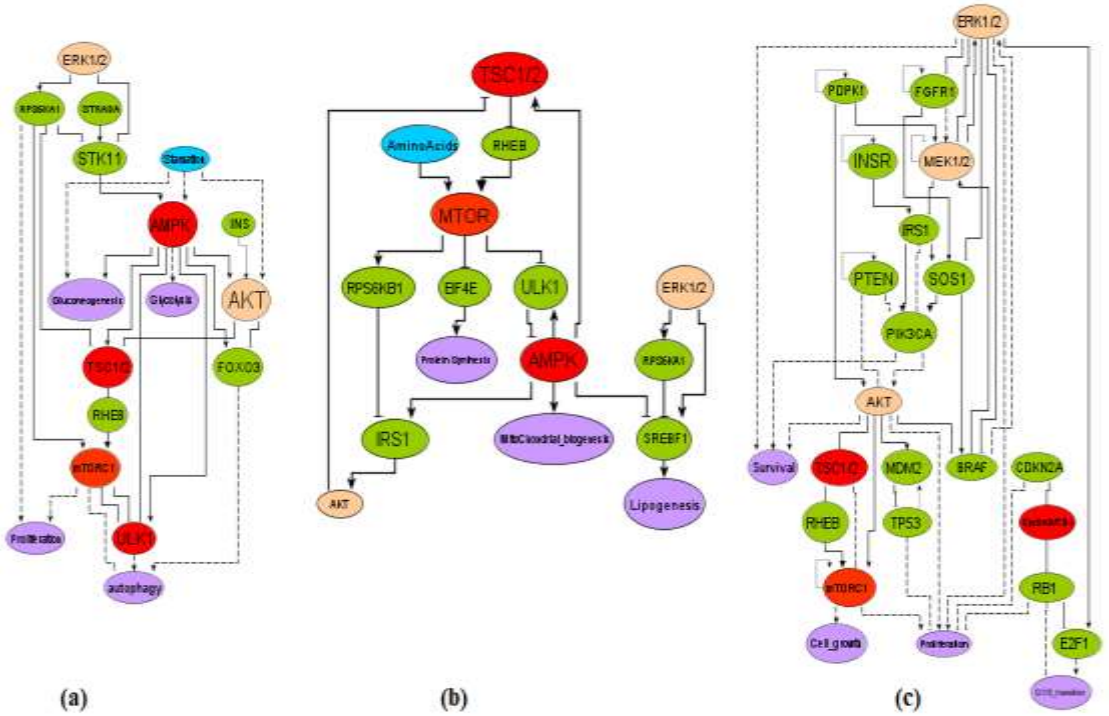

Figure 6. Protein signalling pathways. (a) AMPK – signalling pathway; (b) mTOR – signalling pathway; and (c) protein signalling pathway corresponding to Luminal Breast Cancer. All three systems are extracted by the original ones presented in [19].

After analysing each of these systems we simulate their attractors (fixed point) according to the rules written, in both models presented above, following the same logic. In Figure 7 we give attractors simulated for the AMPK-signaling pathway (Figure 7.a and Figure 7.b), for the mTOR-signaling pathway (Figure 7.c and Figure 7.d), and for the luminal breast cancer signaling pathway (Figure 7.e and Figure 7.f)

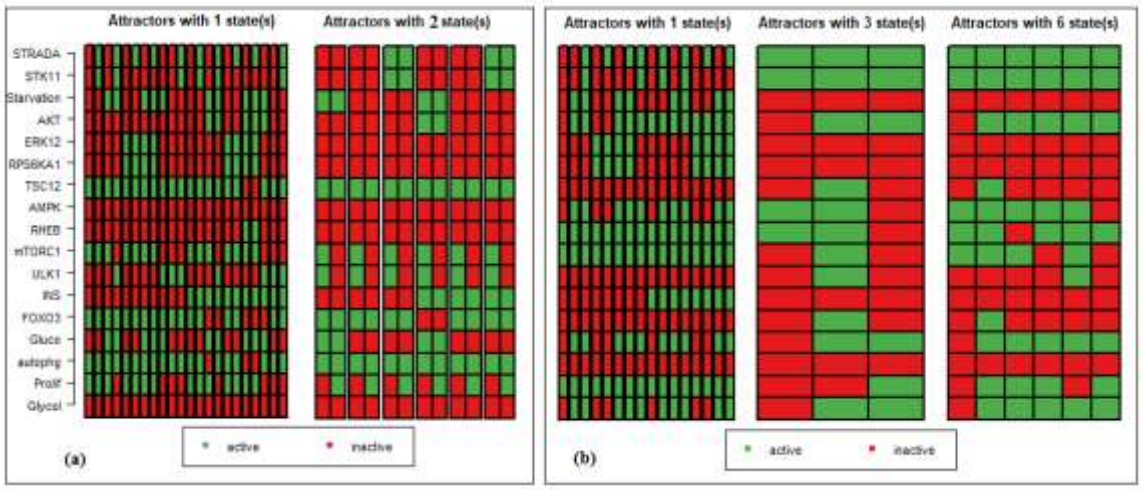

Figure 7. Attractors graph created by the simulation of the first Boolean model applied on the system presented in Figure 4. This shows the presence of sixteen fixed points and the state of each node in each stable state.

As shown in Figure 7, there are many differences between the attractors calculated through simulation of the two Boolean models. Interestingly, we see now the presence of limit cycles which are increased in number and size when we simulate under the second Boolean model. Moreover, we see that this increase is proportional to the size of the system. The more elements are included in the system, the bigger is the number of cycle limits generated as well as the bigger they are in size. We recall that the size of a cycle limit is measured from the number of states that are covered by one cycle limit [21].

In these conditions, arriving at this point, the fixed-point analysis is needed [7, 22]. This analysis should be based either on analytical or biological analysis. In other words, all fixed points should be analysed in detail and the correspondence to the biology should be found for further analysis and researches [23]. Although this is a very important step of the research to follow, this is out of the scope of this paper. Here, we are interested to conclude which is the most suitable model to use in the next dynamical analysis. Definitely, the connection to the biology or experimental evidence would be helpful but even though we don't have them we can still arrange a conclusion. As shown from the above figures we can easily understand that both models, even though are discrete models, give different fixed points, and when the system gets bigger the number of fixed-point get bigger as well, and moreover, the system reaches more limit cycles and more big limit cycles. All of these results are received in the second model, i.e., the model constructed with rules that separate inhibitors and activators seem to give more results. From the logical point of view, doubtless, we can say that the more results (fixed points or limit cycles) are calculated, the more opportunities we have to find suitable stable states that may correspond to biology. And because the second model generates more results this is a reason to believe that the second method of Boolean modelling offers a better approach to reality. On the other hand, as we previously mentioned, Boolean rules can be just a straightforward procedure if the experimental data would have been present, but because they are usually missing then we have to predict and assume the way the signalling follows to flow. This is only for the first method, whereas for the second one we do not need to make any assumptions because this model is based on the logic rules that consider inhibitors and activators separately. In this way, by using the second Boolean model, we give both types of interactions the same weight in the network, without causing any restriction. This is one more reason why we suggest that the dynamical evolution of a biological system is easier and more approachable to reality if we use the second Boolean model.

3. CONCLUSION

In this paper we have given a full description of the methodology followed by using Boolean functions. We perform numerical simulations to observe the dynamical evolution of the system and by establishing its stable states which have been identified as fixed points. We follow two different ways of Boolean modelling to identify all possible differences that may show up during dynamical analysis. The First Boolean model was the most performed among this type of analysis, i.e., this is the model where logical functions have been written according to the predictions and assumptions related to the way the information flows through the system, that is made ahead. This is a restricted model as we permit ourselves to assume a pathway excluding other pathways without having experimental evidence. Obviously, this will affect the results which seem to be very different from the result achieved by applying the second Boolean

model. This second model follows another strategy. Accordingly, the logical equations have been written while considering all activators and inhibitors that affect a node separately. In this way, we haven't excluded any factor but on the contrary we have given the same weight to all regulating factors. In the end of this research work we have suggested that the second model was more suitable for Boolean dynamical analysis for biological systems. Our future research work will be focused in more deeply analysis of the biological science by using Boolean dynamical analysis.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interests associated with this publication and there is no financial fund for this work that can affect the research outcomes.

REFERENCES

[1] Newman M. (2010) Networks: an introduction, Oxford University Press.

[2] Zhang X., Moore, C. and Newman M. Random graph models for dynamic networks, *The European Physical Journal B,* 2017; 90(10); 1-14.

[3] Helikar T., Kochi N., Konvalina J. and Rogers, J.A. Boolean Modeling of Biochemical Networks, *The Open Bioinformatics Journal,* 2011; 5; 16-25.

[4] Albert I., Thakar J., Li S., Zhang R. and Albert R. Boolean network simulations for life scientists, *Source Code for Biology and Medicine,* 2008; 3(16); 1-8.

[5] Christoph M., Martin H. and Hans A. K. BoolNet -- an R package for generation, reconstruction and, *Bioinformatics,* 2010; 26(10); 1378-1380.

[6] Nikolajewa S., Friedel M. and Wilhelm T. Boolean networks with biologically relevant rules show ordered behavior, *Biosystems*, 2007; 90; 40-47.

[7] Radde N. Analyzing fixed points of intracellular regulation networks with interrelated feedback topology, *BMC System Biology,* 2012; 6(57); 1-16.

[8] Albert R. and Othmer H.G. The topology of the regulatory interactions predict the expression pattern of the segment polarity genes in Drosophila melanogaster, *J. Theor. Biol.,* 2003; 223; 1-18.

[9] Saadatpour A. and Albert R. Boolean modeling of biological regulatory networks: A methodology tutorial, *Methods,* 2013; 62*;* 3-12.

[10] Kauffman S. Metabolic stability and epigenesis in randomly constructed genetic nets, *J. Theor. Biol.,* 1969; 22; 437-467.

[11] Ghoshal G. and Barabasi A.L. "Ranking stability and super-stable nodes in complex networks, *Nature Communications,* 2011; 2; 1-7.

[12] Kociaj I. An Attractor Analysis of mTOR Signaling Pathway Synchronous Update of One Specific Initial State., *International Journal of Innovative Science and*

Research Technology, 2021; 6(1); 35-39.

[13] Mendoza L. and Xenarios I. A method for the generation of standardized qualitative dynamical systems of regulatory networks, *Theoretical Biology and Medical Modelling*, 2006; 3(1); 1-18.

[14] Haefner J.W. (2005) Modeling Biological Systems: Principles and Applications, 2 ed., Springer US, pp. XI, 475.

[15] Joo J.I., Zhou J. X., Huang S. and Cho K. Determining relative dynamic stability of cell states using boolean network model, *Scientific reports*, 2018; 8(1); 1-14.

[16] Kauffman S. A proposal for using the ensemble approach to understand genetic regulatory networks, *J. Theor. Biol.*, 2004; 230; 581-590.

[17] Sabatini D.M. Twenty-five years of mTOR: Uncovering the link from nutrients to growth, *Proceedings of the National Academy of Sciences*, 2017; 114(45); 11818-11825.

[18] Scardoni G. and Laudanna C. Centralities based analysis of complex networks, *New Frontiers in Graph Theory*, 2012;323-348.

[19] Licata L., Surdo L.P., Iannuccelli M., Palma A., Micarelli E., Perfetto L., Peluso D., Calderone A., Castagnoli L. and Cesareni G., SIGNOR 2.0, the SIGnaling Network Open Resource 2.0, *Nucleic Acids Research*, 2020; 48; 504-510.

[20] Rao V. (2012) Critical Phenomena in Complex Networks.

[21] Bridoux, F., Gaze-Maillot, C., Perrot, K., and Sene, S., Complexity of limit-cycle problems in Boolean networks, in *International Conference on Current Trends in Theory and Practice of Informatics*, 2021.

[22] Pathak H.K. (2018) An introduction to nonlinear analysis and fixed point theory, Springer.

[23] Bressloff P.C. Stochastic switching in biology: from genotype to phenotype, *Journal of Physics A: Mathematical and Theoretical*, 2017*;* 50(13); 133001.