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Validation of Petri Net Apoptosis Models Using P-Invariant Analysis

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Abstract— **Apoptosis, the genetically programmed cell death, is essential to the physiology of most metazoan species, including the human. It involves a complex signal transduction pathway for the initiating of apoptotic signals, and the ultimate execution of apoptosis.**

This paper demonstrates the modeling of both the extrinsic and intrinsic human apoptotic pathways using Petri nets. This model is then validated using P-invariant analysis. Validation is required to systematically check the model and increase our confidence in it. While Heiner et al. has shown how T-invariants can be used for this purpose [1], this paper will explore the use of P-invariant analysis and ultimately establish it as an alternative way of performing validation for our qualitative model.

I. Motivation

Systems biology is a field of growing importance in biology research. It is concerned with the modeling, simulation and analysis of biological processes within a modeled biological system. By harnessing the vast calculative power of computer systems, a systems biological approach with a model at its center can break the bottleneck of wet-lab research to make new discoveries [2].

Naturally, a strong and accurate model is required before we can start to tap the calculative power of computers. There is thus a cyclic process in model development that requires the validation of the model, ensuring that the intended biological processes that have been modeled have some form of mathematical representation [3]. Methods of model validation vary between different types of models.

Petri net was chosen as a modeling approach because of their similarities with apoptotic and biological systems. 1) Biological systems are bipartite, consisting of the species/substrates and their interactions. 2) They are concurrent, allowing several interactions to take place simultaneously and independently of each other. 3) Finally, the interactions are stochastic and non-deterministic.

Petri nets have the same characteristics mentioned above. They provide an unambiguous as well as unifying representation of biological systems, allowing formal semantics with minimal disambiguity [4]. They are also intuitive and easily executable. Further, there are several software tools for processing Petri nets that are currently available for use [5].

In the paper by Heiner et al. [1], T-invariant analysis was performed on their model as a means of analysis and model validation. It was of interest to see what a related analytical calculation, the P-invariant analysis, would reveal if it was performed on the model.

This paper is organized as follows. In Section 2, we give a brief introduction of Petri nets. In Section 3, we indicate the improvement of model structure compared to Heiner's work. In Section 4, we present our Petri net model, which are followed by the P-invariant analysis and interpretations in Section 5. Finally, in Section 6, we end this paper with concluding remarks.

II. Introduction of Petri Nets

To keep this paper concise, only a quick introduction to Petri net will be provided. For a more formal description of Petri net, see for example [4][6], or the many other books and articles available on the subject.

Petri nets consist of four types of basic elements: places, transitions, edges/arcs, and tokens. Places, which are usually represented as circles, model the elements of the systems such as biological compounds or states. Edges indicate a causal relation between a place and a transition, usually marked with an arrowhead to indicate the direction of the relationship. They may be weighted to indicate various stoichiometric ratios of the required transition.

Tokens represent a form of quantization of a place, and can either be used to indicate a representation of concentrations, absolute count, or simply a state variable. Transitions, together with the edges connecting to it, enable tokens to flow between places indicated by the directional arrowheads.

Enabling a transition requires that all preplaces (places that have an edge pointing towards the transition) be filled with their relevant required weights. Firing the transition will then consume the respective tokens from the preplaces and put tokens in the postplaces.

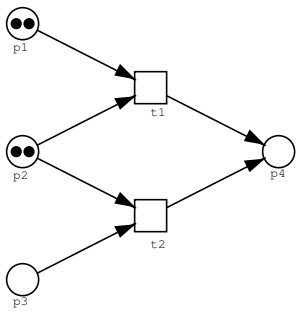

Fig. 1. A Petri net example.

A Petri net example is shown in Fig. 1. Transition t1 is enabled, and its firing results in the consumption of a token

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each from places p1 and p2, and a placement of token in p4. Transition t2 is disabled because its firing prerequisite condition is not satisfied by p3.

While this example shows the inclusion of tokens within the model, the model that is proposed in this paper is unmarked and non-quantitative and hence do not contain tokens. However the concept of tokens is still important for the understanding of the analytical techniques used.

III. Model Structure

While the model developed by Heiner et al. [1] was used as a starting point to build the model, for P-invariant analysis to be possible, it was necessary for a couple of structural differences to be put in place.

Firstly, input and output transitions must be excluded. Input transitions are transitions providing tokens to a place but do not consume tokens from other places. Conversely, output transitions remove tokens but do not provide tokens to any place. These "boundary" transitions are found in Heiner's model together with substrates that otherwise did not have any means to gain or lose tokens.

Secondly, inhibitory interactions must be modeled because many such interactions take place within the apoptotic pathways, such as between Bcl-2 and Bax [11]. Such interactions usually involve the consumption of both the inhibiting substrate and the inhibited substrate into an inactive complex.

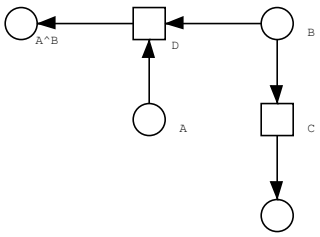

Fig. 2. Inhibiting transition D between place A and B.

Fig. 2 shows the representation of such an inhibiting interaction D between substrates A and B forming the inactive substrate complex $A^{\wedge}B$. This consumes tokens from B and downregulates the occurrence of the "forward" reaction C. Although conceptually substrate A is the inhibiting species and B the inhibited species, A and B are considered mutually inhibiting since both are consumed in the inhibitory process.

These two structural features of the model are necessary for P-invariant analysis to be carried out, which will be explained later.

IV. Petri net model

Once again, to keep this paper concise, a discussion of apoptosis and its pathways will not be provided in this paper. A number of sources [7]-[18] have been consulted to provide a consistent base of mechanisms and processes that has been used for the modeling.

A. Extrinsic Pathway

Fig. 3 shows the partial Petri net model of the extrinsic pathway as described by the above sources. The formation of the DISC (Death Inducing Signaling Complex) can be seen as a stepwise incorporation of such substrates as the Fas ligand (FasL), Fas receptor (Fas, activated by p53), Fas-associated protein with death domain (Fadd) and procaspase-8. DISC then releases the active caspase-8, which triggers the caspase cascade involving caspase-3. Caspase-8 itself is deregulated by cFLIP. All place names with a $^{\wedge}$ symbol represent an inactivated species.

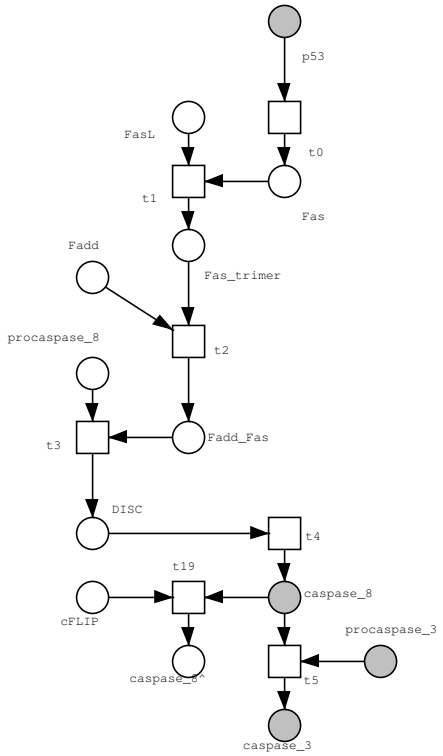

Fig. 3. Modeling the extrinsic pathway.

B. Intrinsic Pathway

Fig. 4 shows the model of the intrinsic pathway as described by the sources. Upregulation of NOXA and PUMA by p53 and their deactivation of Bcl-2 is modeled. Bax, which is upregulated by p53 and downregulated by Bcl-2, releases cytochrome c from the mitochondria. This stimulates the formation of the apoptosome, which cleaves and activates caspase-9 from its pro-form, initiating the caspase cascade just like in the extrinsic pathway.

Various inhibitory interactions are modeled, such as between SMAC/DIABLO and IAP, between IAP and executioner caspase-3 and procaspase-9, as well as between pro- and anti-apoptotic substrates Bax and Bcl-2.

It should be noted that for model simplicity and readability, several places in the model represent more than one substrate indicated by its place name. These substrates share similar functions and a fair extent of redundancy. Substrates

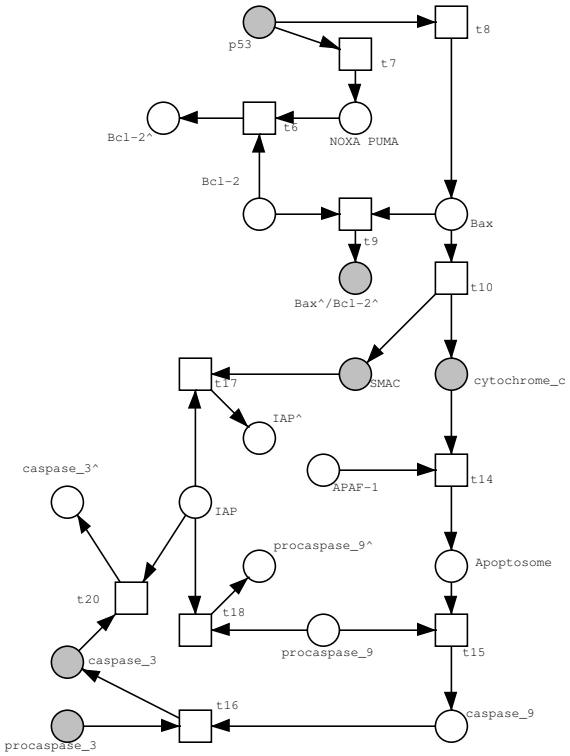

Fig. 4. Modeling the intrinsic pathway.

that represent multiple similar substrates are shown in Table I.

TABLE I

MULTIPLE SUBSTRATES REPRESENTED BY SINGLE SUBSTRATE

Name	Substrates Represented
Fas	Death receptors including Fas and TNFR1
FasL	Ligands of death receptors including FasL and TNF-α
Fas_trimer	Complex of 3 death receptors with ligands
FADD_Fas	Protein with death domain related to death receptor
procaspase_3	Procaspase-3, -6, -7
caspase_3	Active executioner caspases(-3, -6, -7)
Bax	Proapoptotic Bcl-2 family proteins (Bax, Bak, etc.)
Bcl-2	Antiapoptotic Bcl-2 family proteins (Bcl-2, Bcl-XL, etc.)

C. The Roles of Bid

Finally, Fig. 5 shows the partial model of the functions of Bid (tBid) as it is upregulated by caspase-8. This provides a link from the extrinsic pathway to the intrinsic pathway. By releasing SMAC/DIABLO, cytochrome c, as well as inhibiting Bcl-2, the role of tBid in the intrinsic pathway is similar to Bax. An intermediate caspase_8_i was introduced along with a transition t_x to characterize the enzymatic interaction caspase-8 has on Bid. In this way, this "crossing-over" phenomenon would not cause a breakdown in the extrinsic pathway in which Bid was always competing for caspase_8 tokens, preventing the extrinsic caspase cascade.

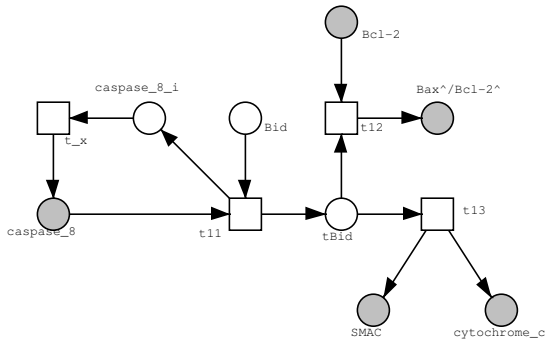

Fig. 5. Modeling the roles of Bid.

These three net components are intentionally displayed in this paper separately to promote readability and at the same time distinguish them as major pathways. The full net model can be obtained by joining together the three partial models at the place nodes indicated in grey.

V. P-INVARIANT ANALYSIS

Although the places and transitions are modeled based on the sources with the structural considerations described earlier, it is important to establish a method of verifying that the model indeed has some form of mathematical representation of the intended pathways. Heiner et al. have established this ability with T-invariant analysis [1]. It would be of interest to see if P-invariant analysis could be used to the same purpose. In this Section, P-invariant analysis will be performed on the Petri net model, and ultimately established as a means of validating the model.

A. Incidence Matrix

A Petri net with m places and n transitions has an incidence matrix $C = [c_{ij}]$, which is an $m \times n$ matrix of integers indexed by place i and transition j. The entries c_{ij} are given by

$$c_{ij} = c_{ij}^{+} - c_{ij}^{-}, \tag{1}$$

where c_{ij}^{+} is is the weight of the arc from transition j to its outplace place i, and c_{ij}^{-} is the weight of the arc from input place i to transition j [6]. In the case where a place is not connected to a transition, the corresponding c_{ij} value would be 0.

It is easy to deduce from (1), that the c_{ij} values represent the net changes in number of tokens in place i when transition j fires once. Because the Petri net model only involves arcs with weight values of 1, the incidence matrix C is expected to only contain entries that are 1, -1 or 0, representing token gain, loss, and no change, respectively.

B. P-invariant

The P-invariants of a Petri net are found by solving the system of linear equations given by

$$C^T \times x = 0, \tag{2}$$

which equivalently can be solved by

$$x \times C = 0, \tag{3}$$

with x being a horizontal integer m-vector and the RHS of the equation a horizontal null n-vector [6].

Each P-invariant, which is a horizontal integer m-vector of $R = [r_i]$, gives a series of weights r_i, such that the r-weighted sum of tokens in the places indexed by i will always remain the same regardless of any transition firing, assuming an initial non-zero marking.

C. P-invariants of Model

P-invariant analysis allows the identification of conservatory networks that always maintain a certain conservation of tokens. Applied to the model, it is hoped that networks containing substrates (places) that are related by a certain signal transduction pathway would emerge as the invariants. The conservation of tokens can be interpreted as a conservation of the same biological signal within the network. Hence places that are linked in a P-invariant are possibly linked by the same biological signal.

The structural changes as mentioned in Section IV were necessary so that there would not be any unregulated flow of tokens into/out of the net via input and output transitions. Otherwise, this would make P-invariants impossible since weighted sum of tokens would then be uncontrolled. Inactive species were also included as a way to provide conservation of tokens for every transition, in order for their firings to be tracked by P-invariant analysis.

Using the Snoopy software [19] to construct the Petri net and its companion software Charlie [20] to perform P-invariant analysis, 13 P-invariants were found. These invariants, and their constituent places including deactivated/inactive substrates are listed in Table II below.

TABLE II
P-INVARIANTS AND PLACES INVOLVED

No.	Places in P-Invariant
1	cFLIP, caspase_8^
2	Bcl-2, Bcl-2^, Bax^/Bcl-2^
3	procaspase_3, caspase_3, caspase_3^
4	IAP, IAP^, procaspase_9^, caspase_3^
5	p53, NOXA PUMA, Bax, Bcl-2^, Bax^/Bcl-2^, cytochrome c,Apoptosome, caspase_9, caspase_3, caspase_3^, Fas, Fas_trimer, FADD_Fas, DISC, caspase_8, caspase_8^, caspase_8_i, Bid, tBid
6	APAF-1, Apoptosome, caspase_9, caspase_3, caspase_3^, procaspase_8, DISC, caspase_8, caspase_8^, caspase_8_i
7	APAF-1, Apoptosome, caspase_9, caspase_3, caspase_3^, Fadd, FADD_Fas, DISC, caspase_8, caspase_8^, caspase_8_i
8	APAF-1, Apoptosome, caspase_9, caspase_3, caspase_3^, FasL, Fas_trimer, FADD_Fas, DISC, caspase_8, caspase_8^, caspase_8_i
9	p53, NOXA PUMA, Bax, Bcl-2^, Bax^/Bcl-2^, SMAC, IAP^,APAF-1, Apoptosome, caspase_9, caspase_3, caspase_3^, Fas, Fas_trimer, FADD_Fas, DISC, caspase_8, caspase_8^,caspase_8_i, Bid, tBid
10	procaspase_8, DISC, caspase_8, caspase_8^, caspase_3, caspase_3^, procaspase_9, procaspase_9^, caspase_9
11	Fadd, FADD_Fas, DISC, caspase_8, caspase_8^, caspase_3, caspase_3^, procaspase_9, procaspase_9^, caspase_9
12	FasL, Fas_trimer, FADD_Fas, DISC, caspase_8, caspase_8^, caspase_3, caspase_3^, procaspase_9, procaspase_9^, caspase_9
13	p53, NOXA PUMA, Bax, Bcl-2^, Bax^/Bcl-2^, SMAC, IAP^, procaspase_9, procaspase_9^, caspase_9, caspase_3, caspase_3^, Fas, Fas_trimer, FADD_Fas, DISC, caspase_8, caspase_8^, caspase_8_i, Bid, tBid

D. Interpreting P-invariant

To make sense of the invariants, critical places in the invariants need to be identified first. Two such critical places are places that do not have any incoming edges, and those that do not have any outgoing edges, which can aptly be termed start and stop places, respectively. For example, in the first invariant, cFLIP is a start place and caspase_8^ is a stop place (refer to Fig. 3). While there may be multiple stop places or start places per invariant, there must be at least one of each per invariant.

After these critical points have been identified, the rest of the places can be used to link the start place to the stop place using any combination of transitions and edges in between. In doing so, conservatory signaling pathways originating from the start place can be traced out to their final destination.

Identified P-invariants can thus be interpreted by considering them with respect to the start and stop places. Table III shows the list of P-invariants and their interpreted biological significance.

Invariants 6, 7, 8 and 9 are connected to each other because they all identify the same interactive pathway. The only difference lay in the starting places (procaspase_8, Fadd, Fas, and p53, respectively) that finally lead to the caspase cascade via the extrinsic pathway. In invariant 9, p53 was the starting place and its pro-apoptotic killing activity of anti-apoptotic signals originating from Bcl-2 and IAPs can be easily identified.

Invariants 10, 11, 12 and 13 are also connected because of the same four different starting points (as in invariants 6 to 9) mentioned above.

In fact, invariants 5, 9 and 13 are also connected and do not identify any unique interactive pathway. The only difference was the choice of the starting point for the "lower" segment of the intrinsic pathway. In invariant 5 it was Bax that started the propogation of signal via cytochrome c, leading to the caspase cascade; in invariant 9, APAF-1 was implicated for the same reaction, and in invariant 13 it was procaspase_9.

Hence, invariants 5 to 13 collectively represent the workings of both the extrinsic and intrinsic pathway, each identifying a different substrate that is important as a starting requirement for the pathways. Such substrates include (as represented by their places) p53, death receptor and ligand, proteins with death domains, procaspase-8, APAF-1 and procaspase-9.

The presence of multiple invariants that have the same biological significance is primarily due to places that only serve a single function of being a substrate for the next reaction. Such places, like APAF-1, and Fadd, do not affect the signaling pathways in more meaningful "secondary"

TABLE III

P-INVARIANTS AND PLACES INVOLVED

No.	P-Invariant Interpretation
1	cFLIP resulting in deactivation of caspase-8
2	Antiapoptotic Bcl-2 family used in inhibiting proapoptotic signals, or deactivated by NOXA and PUMA
3	procaspases being activated into caspase-3, -6, -7, and deactivated by IAP
4	IAPs deactivating procaspase-9 and caspase-3, -6, -7, or being deactivated by SMAC
5	p53 resulting in deactivation of Bcl-2, and activation of caspase -3, -6 and -7 via extrinsic and intrinsic pathways. Caspase-8 and tBid involvement into the intrinsic pathway is also shown.
6	same as 9
7	same as 9
8	same as 9
9	p53 resulting in deactivation of Bcl-2, deactivation of IAP, and activation of caspase-3, -6 and -7 via extrinsic pathway only. Caspase-8 and tBid activity into the intrinsic pathway is also shown. APAF-1 is shown as the stimulus for the intrinsic pathway.
10	same as 13
11	same as 13
12	same as 13
13	p53 resulting in deactivation of Bcl-2, deactivation of IAP, and activation of caspase-3, -6 and -7 via extrinsic pathway only. Caspase-8 and tBid activity into the intrinsic pathway is also shown. Procaspase-9 is shown as the stimulus for the intrinsic pathway.

ways other than simply being present for the next step to occur. An extended Petri net that involves these places in other dynamic ways will eliminate this problem of "repeated invariants".

It is noted that caspase_3^ was the stop point in invariants 5 to 13. This, however, does not mean that both the intrinsic and extrinsic pathways always end up in deactivated caspases-3, -6 and -7 (and hence termination of apoptosis). caspase_3^ was the stop point in this case because further pathways downstream of caspase_3, i.e. the execution pathway [11], were not included in the model.

In the model, caspase_3 was taken to be equivalent to the triggering of apoptosis itself, since it was assumed that high levels of caspases-3, -6 and -7 would always lead to the execution pathway. In this right, caspase_3^ should be viewed as a stop place while interpreting the P-invariants.

E. Model Validation Using P-invariant

The full list of biological activities involved in the Petri net model, as identified through P-invariant analysis is thus given as follows:

- Main Extrinsic Pathway leading to apoptosis
- Main Intrinsic Pathway leading to apoptosis
- Deactivation of caspase-8 by cFLIP
- NOXA and PUMA's upregulation by p53, deactivating anti-apoptotic Bcl-2 family members
- Mutual inhibition between pro- and anti-apoptotic Bcl-2 family members
- IAPs deactivating procaspase-9 and caspases-3 and -7
- SMAC/DIABLO deactivating IAPs
- Upregulation of tBid by caspase-8 to facilitate intrinsic pathway

This list fully includes all biological processes that have described in the sources [7]-[18], and which the author has set out to model. It is thus evident that P-invariants can be employed to analyze and validate extended versions of this Petri net model and similar signal transduction networks.

VI. CONCLUSION

The author has demonstrated how biological systems modeled with Petri nets can be analysed and validated using P-invariants, similar to how T-invariants have been used to the same purpose in [1]. The structural requirements that the model must have for P-invariants are also described.

P-invariant analysis has thus been established as an additional method of validating Petri net models of signaling transduction networks. It is recommended that additional validation techniques, such as T-invariant analysis, be used in conjunction with P-invariant analysis, described in this paper, when validating models, so as to further increase model confidence and prevent over-reliance on a single technique.

Besides validation via P-invariant analysis, the model used in this paper has also been validated using T-invariant analysis. The results of the T-invariant analysis are the same as those compiled in Section V, Part E. Common mechanisms can also be found in Heiner's work.

Compared to P-invariant analysis, T-invariant analysis also requires a set of structural requirements. P-invariant analysis will provide some overlapping pathways as shown in the last section. In this aspect, the results by P-invariant is not so intuitive as the ones by T-invariant. However, the P-invariant results reveal the complementary evidences to T-invariant.

The confidence of protein-protein interactions indicated in this paper is thus strong enough for it to be the subject for future developments and model extensions, so as to better study the intrinsic and extrinsic apoptotic pathways. Besides including more substrates as more discoveries are made by the scientific community, improvements such as a quantitative model can be considered.

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