Qualitative Analysis of Cancer Pathways with Transition and Place Invariants

Guy Romaric Yemeli Ngandjoug

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Assoc. Prof. Dr. Ali Hakan Ulusoy
Acting Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science in Applied Mathematics and Computer Science.

Prof. Dr. Nazim Mahmudov
Chair, Department of Mathematics

We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Applied Mathematics and Computer Science.

Prof. Dr. Rza Bashirov
Supervisor

Examining Committee

1. Prof. Dr. Rza Bashirov

2. Prof. Dr. Benedek Nagy

3. Assoc. Prof. Dr. Enver Ever

ABSTRACT

It is of practical interest to know whether a biological network contains mass-preserving and state-preserving subnetworks. In a mass-preserving subnetwork, the total mass remains constant. In any state-preserving subnetwork, biological reactions bring the subnetwork back to an initial state. For instance, any reversible reaction forms a state-preserving subnetwork. In a large intricate biological network, it is rather cumbersome task to determine mass-preserving and state-preserving subnetworks. P-invariants and T-invariants are analysis methods that can be successfully used to determine mass-preserving and state-preserving subnetworks in a Petri net.

In this thesis, the information is derived from the biological databases Reactome and KEGG as well as from the existing literature to date, to create rather detailed Petri net model of cancer pathway, and perform its qualitative analysis with P-invariants and T-invariants.

Keywords: Biological network, Petri net, P-invariants, T-invariants.

ÖZ

Bir biyolojik ağın kitle-koruyucu ve durum-koruyucu alt ağlar içerip içermediğini bilmenin pratik önemi vardır. Bir kitle-koruyucu alt ağda, toplam kitle sabittir ve bu nedenle sınırlıdır. Herhangi bir durumu koruyan alt ağda, biyolojik reaksiyonlar alt ağı bu duruma geri getirir. Örneğin, geri-dönüşümlü herhangi bir reaksiyon, durum koruyucu bir alt ağ oluşturur. Büyük ve karmaşık bir biyolojik ağda, kitle ve durum koruyucu alt ağları belirlemek oldukça zorlu bir iştir. P-invariantlar ve T-invariantlar, kitle koruyucu ve durum koruyucu alt ağların belirlenmesinde başarılı olabilen Petri net analiz yöntemleridir.

Bu tezde, Reactome ve KEGG gibi biyolojik veri tabanlarından ve mevcut literatürden bilgi derleyerek kanser yollarının ayrıntılı Petri ağı modeli oluşturulur ve bu modelin P-invariantlar ve T-invariantlar metodları ile nitel analizi yapılmıştır.

Anahtar Kelimeler: Biyolojik ağda, Petri net, P-invariantlar, T-invariantlar.

DEDICATION

Dedicated to my lovely mother

Jacqueline JOUDA

As many are the sentences as expressions it exists, they cannot show the degree of love and affection that I feel for you. Thank you for all your love.

ACKNOWLEDGMENT

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Guy Romaric Yemeli Ngandjoug

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LIST OF ABBREVIATIONS

AKT	Protein Kinase B
AKTP	Phosphorylated AKT
AKTPP	Double Phosphorylated AKT
CPNs	Coloured Petri Nets
ERK	Extracellular-Signal-Regulated Kinases
ERKP	Phosphorylated ERK
ERKPP	Double Phosphorylated ERK
FPNs	Functional Petri Nets
HPNs	Hybrid Petri Nets
KEGG	Kyoto Encyclopedia of Genes and Genomes
KPNs	Continuous Petri Nets
MAKP	Mitogen-Activated Protein Kinase
MEK	Mitogen-activated protein kinase/ERK kinase
MEKP	Phosphorylated MEK
MEKPP	Double Phosphorylated MEK
PDK	Phosphoinositide-Dependent Kinase
PI	Phosphatidylinositol
PI3K	Phosphoinositide 3-Kinase
PIP	Phosphatidylinositol 3-bisphosphate
PIP2	Phosphatidylinositol 3,4-bisphosphate
PIP3	Phosphatidylinositol 3,4,5-bisphosphate
PN	Petri Net
PP2A	Protein Phosphatase 2

PTEN	Phosphatase and Tensin homolog
RAF	Rapidly Accelerated Fibrosarcoma
SPNs	Stochastic Petri Nets
TPNs	Time Petri Nets

Chapter 1

INTRODUCTION

There is a big challenge in understanding how complex molecular interactions control the cell behaviour in a large biological network. To deal with this challenge, one needs to find formal methods suitable enough to handle the task should be studied. Petri nets represent both formal method and practical tool which suits well to the nature of biological systems. The dynamical models can be viewed applying either quantitative analysis or qualitative analysis methods. Qualitative analysis of biological systems is aimed at creating the detailed description of the system's structure, its behaviour, and understanding the requirements and specifications of the biomolecular reactions. Meanwhile quantitative analysis requires accurate information on kinetics which are often lacking.

Petri nets represent a mathematical theory that was invented by Carl Adam Petri in the beginning of 60s. Over the past several decades, Petri nets have been used for modelling and analysis of various concurrent, distributed, asynchronous and dynamic systems [22]. Existence of effective software tools makes Petri nets a powerful modelling platform. Biological systems are bipartite consisting of substances/substrates and their interactions, referring respectively to places and transitions in Petri nets [19]. Because of similarities between biological systems and Petri nets, modelling with Petri nets has recently discovered to be advantageous for analysis of biological systems.

Protein Kinase B (AKT) and Mitogen Activated Kinase B (MAPK) pathways were investigated separately by several authors. This is the main motivation behind the present research to perform qualitative analysis of the whole network composed of these two important signalling pathways and their crosstalk. In this thesis, we perform qualitative analysis of the network composed of AKT and MAPK pathways aimed at identifying of state-preserving and mass-preserving fragments. Qualitative analysis is done in terms of P- and T-invariants, siphons and traps to predict structural and behavioural characteristics of underlying network.

This thesis is organized as follow. Chapter 2 is a brief introduction to Petri nets. Chapter, presents the properties of Petri nets and their analysis methods. Chapter 4 introduces the biological context behind of the present research. Chapter 5 deals with Petri net model of underlying biological network and its qualitative analysis. Finally, the thesis ends up with conclusions and remarks.

Chapter 2

PETRI NETS

The origin of Petri nets goes back to the beginning of 60s when Carl Adam Petri was working on his dissertation. Since then Petri nets has gained much attention. On one hand, Petri nets is increasingly demanded by practitioners for modelling scientific, engineering and industrial applications. On the other hand, it is rapidly developing research area in which hundreds of researchers contribute to the further development of the field [1].

Petri nets are suitable for modelling and simulation of scientific, engineering and industrial problems. Petri nets can be used to represent concurrent, distributed, uncertain and stochastic dynamic systems. Drawing and graphical abilities make Petri indispensable method for the modellers [2].

2.1 Regular Petri nets

There exist many extensions of Petri nets each employing additional characteristics such as colour, time, continuity, fuzziness, hierarchy, etc. Petri net can also be enhanced with multiple characteristics such as time and colour or hierarchy and colour. A Petri net with multiple extensions is more powerful and suits best to the structure of the system being modelled. The simplest Petri net is perhaps the one which has no extension or additional specification. Such a Petri net is usually called regular or classical Petri net, or simply Petri net, for short. Any regular Petri net is composed of three types of principal components: places, transitions and arcs. A **place** is denoted

by circle and a **transition** by a rectangular shape. **Arcs** are between places and transitions. An arc is incident from a place to the transition or vice versa. Arcs are labelled with positive integers called **weights**. Arcs establish direction of data flow over the net while arc weights determine its density. A Petri net can be formally defined as a 5-tuple $PN = <P, T, A, W, M_0>$ where:

- $P = \{p_1, p_2, \dots, p_n\}$ is a finite set of places,
- $T = \{t_1, t_2, \dots, t_m\}$ is a finite set of transitions such that $P \cap T = \emptyset$,
- $A \subseteq (P \times T) \cup (T \times P)$ is the set of arcs,
- $W: A \rightarrow \{1, 2, 3 \dots\}$ is a weight function,
- $M_0: P \rightarrow \{0, 1, 2, 3 \dots\}$ is the initial marking.

State of a Petri net or **marking** is recognised by distribution of the tokens among its places. A marking is represented by the vector $M_i = (m_1, m_2, \dots, m_n)$, and the number of tokens in place p_i is denoted either by $m(p_i)$ or m_i. The marking defines the state in a given Petri net or more precisely the state of the system described by the Petri net. So, the change of the state of the system corresponds to the change of the marking too. Several often-used applications of transitions, input and output places are shown in Table 1.

Table 1: Some interpretation of transitions and places

Area	**Input Places**	**Transition**	**Output Places**
Event processing	Precondition	Event	Postcondition
Data processing	Input data	Computation step	Output data
Signal processing	Input signals	Signal processor	Output signals
Resource allocation	Resource needed	Task or Job	Resources release
Propositional logic	Conditions	Clause in Logic	Conclusion
Buffer processing	Buffers	Processor	Buffer

Figure 1 represents an example of a regular Petri net $\mathrm{PN} = (\mathrm{P}, \mathrm{T}, \mathrm{A}, \mathrm{W}, \mathrm{M}_0)$ such that

- $P = \{p_1, p_2, p_3\}$
- $T = \{t_1\}$
- $A = \{(p_1, t_1), (p_2, t_1), (t_1, p_3)\}$
- $W = \{(p_1, t_1) = 2;\ (p_2, t_1) = 1; (t_1, p_3) = 1\}$
- $M_0 = (3,1,1)$

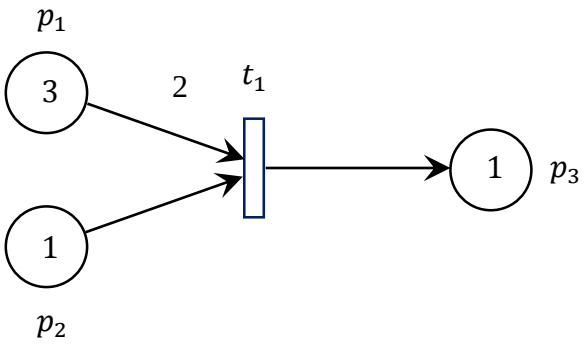


Figure 1: Graphical representation of the Petri net $PN = (P, T, A, W, M_0)$.

Let ${}^\bullet p$ and $p^\bullet$ represent, respectively, input place and output place of t. A transition t is said to be ***enabled*** if each ${}^\bullet p$ is marked with at least *w(* ${}^\bullet p, t$*)* tokens, where *w(* ${}^\bullet p, t$*)* is the weight of the arc from ${}^\bullet p$ to t. An enabled transition may or may not fire (or occur) depending on whether or not the event actually takes place. Firing of the transition t consists of withdrawing *w(* ${}^\bullet p, t$*)* tokens from ${}^\bullet p$ and adding *w(*$p^\bullet, t$*)* tokens in $p^\bullet$. Occurrence of a transition modifies marking accordingly by changing $m(p)$ into $m'(p)$ as follows $m'(p) = m(p) - w({}^\bullet p, t) + w(p^\bullet, t)$. For example, firing of $t_1$changes marking from $M_0 = (3,1,1)$ to $M_1 = (1,0,2)$.

A transition without input place is called **source** transition and the one without output place is called **sink** transition. The source transition is unconditionally enabled; thus,

it can fire an unlimited number of times, and the firing of a sink transition consumes or destroys tokens.

2.2 High-level Petri nets

Structural simplicity, existence of easy-to-use and user-friendly software tools have made Petri nets very popular among practitioners. Over the years Petri nets have been successfully applied for modelling and simulation in workflow management, business process organisation, performance evaluation, operating systems, resource allocation and many other areas. But one needs to use more advanced characteristics to model dynamic systems with complex properties. Unfortunately, modelling power of regular Petri net is somewhat limited and not enough for modelling many problems arising in scientific, engineering and industrial domains [4]. These limitations concern the qualitative and quantitative aspects. The extension of the qualitative aspect that has been discussed in the literature includes enhancing regular Petri nets with new features such as arcs with additional features, coloured tokens, etc. In what follows we below briefly describe existing Petri net extensions.

Petri nets with test and inhibitor arc. Test or read arcs are used to check the presence and absence of a token in the source place so that the token is not consumed at the end of action. Inhibitor arcs inhibit the firing action if specified place contains tokens [1,4]. It has been proved that the problem of producer and consumer cannot be modelled in terms of Petri nets without using inhibitor arc [23].

Self-modifying or functional Petri nets (FPNs). In FPNs it is possible to represent arc weights by functions of the tokens in places. This adds more dynamism to the system.

Coloured Petri nets (CPNs) In CPNs, the tokens are associated with a data type and arcs can be used together with conditions regarding these data types. In CPNs, one can distinguish between the tokens even in the same place. CPNs are very convenient for representing large Petri nets in a very compact form while keeping the main logic of the original net.

Time Petri nets (TPNs). Some problems include a quantifiable time parameter. Corresponding Petri net is expected to involve a time parameter. Such a Petri net is called time or timed Petri net. In TPNs a transition t is associated with time interval $[a_t, b_t]$ so the transition t can fire only after a_t time units elapsed but not later than b_t time units elapsed. It is assumed that the firing action takes no time. TPNs are used for quantitative modelling of dynamic deterministic systems.

Stochastic Petri nets (SPNs). In a system with high degree of randomness the time delay until next firing of a transition t is not fixed and, therefore, it is customary to associate firing delay of a transition t with a random variable. In SPNs, transition t is assigned an execution rate which equals to the value of random variable. Generalized stochastic Petri nets, generalized Markovian stochastic Petri nets, stochastic high-level Petri nets are all Petri nets which involve stochastic parameter.

Continuous Petri nets (KPNs) and Hybrid Petri nets (HPNs). KPNs are increasingly demanded just because in many dynamic systems processes flow smoothly so that discrete Petri nets are not sufficiently enough to model such problems. In KPNs, marks with non-negative real values are used instead of tokens. Switch to real numbers is requested by many applications. For example, concentration of the molecular species changes smoothly and can be naturally represented by real numbers.

In KPNs, firing rate of a transition expresses the ‘‘speed’’ of the transformation from input to output places. Hybrid Petri combine both abilities of discrete and continuous Petri nets [1] since they have both discrete and continuous places and transitions.

Chapter 3

PROPERTIES AND ANALYSIS METHODS OF PETRI NETS

After modelling with Petri nets, the question arises "What are the characteristics of the system being modelled?" The main advantage of the modelling with Petri net is that one can study characteristics of the system in terms of the properties that the Petri net model holds. Generally speaking, properties of Petri nets are classified into two classes. **Behavioural properties** of a Petri net depend on the initial marking while its structural properties deal with the net structure only [1,2].

3.1 Behavioural properties

The boundedness, safety, liveness, deadlock, reversibility, coverability, persistence and reachability are among behavioural properties.

Reachability. The marking M_1 is said to be reachable from its initial marking M_0 if there exists a firing sequence σ that will yield M_1.

Boundedness. A Petri net with an initial marking M_0 is said to be ***k-bounded*** if $m(p) \leq k, \forall p \in P$ and $\forall M \in R(M_0)$, where $m(p)$ is the number of tokens in place p in marking M. A Petri net is said to be **bounded** if it is k-bounded for $\exists k \in \mathbb{N}$.

Safety. A Petri net is said to be **safe** if it is k-bounded and $k = 1$.

Deadlock. A marking M' reachable from the initial marking M_0 is a **deadlock** if none of the transitions of the Petri net is enable in M'.

Liveness. A Petri net is said to be **live** if for all transitions there is a way to fire transition in $\forall M' \in R(M_0)$ and $\forall t \in T, \exists M \in R(M')$ such that t is enable in M'**.**

Reversibility. A Petri net is **reversible** means that for every state $M \in R(M_0)$ there is a way back to reach the initial marking. So, the net has the capacity of re-initialization. A Petri net is said to be **reversible** if for each marking $M \in R(M_0)$ M_0 is reachable from *M*.

Coverability. A marking M in a Petri net is **coverable** if $M' \epsilon R(M_0)$ such that $M' \geqslant M$, thus, $M'(p) \geq M(p)$ for $\forall p \in P$.

Persistency. A Petri net is **persistent** if for a pair of enabled transitions, occurrence of one of them does not disable another.

3.2 Structural properties

Some general structural properties are liveness, boundedness, and conservativeness, pure, ordinary.

Liveness. A Petri net is said to be **structurally live** if there exists an initial marking M_0 such that net is *live,* so a Petri net which is live is also structurally live.

Boundedness. A Petri net is **structurally bounded** if it is bounded for any initial marking M_0.

Conservativeness. A Petri net is **conservative**, if all transitions fire token-preserving, i.e. all transitions add exactly as many tokens to their post places (output places) as they subtract from their preplaces (input places).

Pureness. A Petri net is **pure** if there are no two nodes connected in both directions, meaning that the net contains no self-loop, i.e. $\forall x, y \in P \cup T: w(x, y) \neq o$ $\forall\, x, y \in P \cup T: W(x, y) \neq 0 \Rightarrow W(y, x) = 0$.

Ordinary. A Petri net is said to be **ordinary** if all of its arc weights are 1's.

3.3 Reachability and coverability tree methods

The basic idea behind of reachability tree method is to collect under tree structure all markings that are directly or indirectly reachable from the initial marking. The resulting tree is a rooted tree with the initial marking as the root and remaining markings as the nodes of the tree. In reachability tree each arc represents a transition occurrence. Reachability tree can be used to answer some questions, e.g., whether or not Petri net is bounded, live, reversible, safe, etc. Reachability tree method can be efficiently used for small or modest size Petri nets. However, the method does not work for Petri nets with infinite reachability set. For large Petri nets the method can result in continuously growing reachability tree leading to memory overflow.

Figure 3 represents the reachability tree of the Petri net in Figure 2. As can be seen, the initial marking is the vector (1 1 0 0 0) which is the root of the tree. There are two enable transitions t_2 and t_3 in the initial marking. Occurrence of t_2 changes the initial state to (0 1 1 0 1), in which t_3 becomes enabled. Then, occurrence of t_3 consequently sets the Petri net to marking (0 0 1 1 0), and so on.

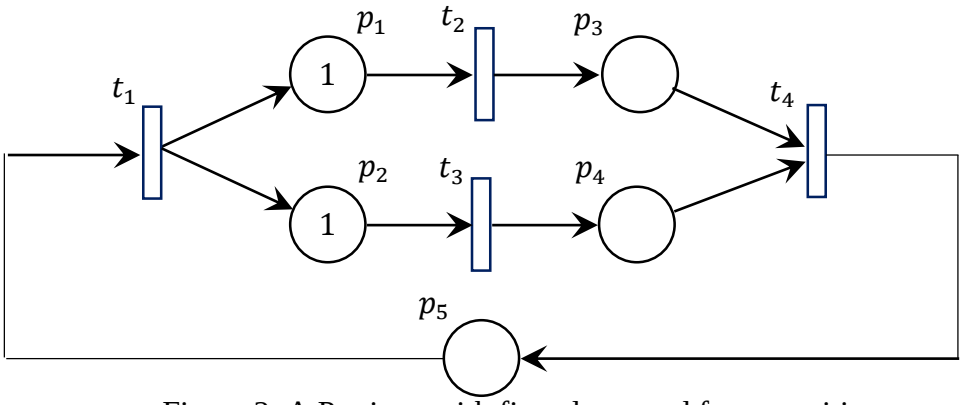


Figure 2: A Petri net with five places and four transitions.

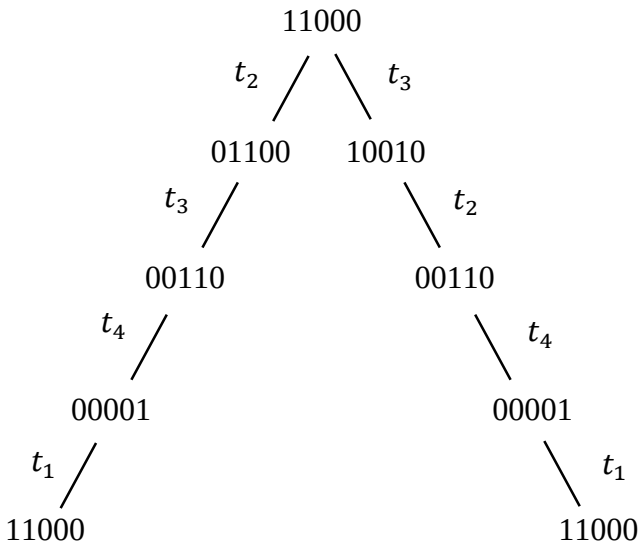


Figure 3: Reachability tree of Petri net illustrated in Figure 2.

It is impossible to draw all markings with the exact number of tokens in an unbounded and live Petri net, because number of the tokens in some places grows up to infinity. To keep the tree finite, a special symbol "ω" [1-3], which represents infinity, is used to indicate arbitrarily large number of tokens. For any positive integer $k > \omega$ the property $\omega \pm k = \omega$ holds. Use of the symbol ω allows us to define an abstract marking composed of positive integers and ω. Now reachability space of an unbounded and live Petri net can be represented by compact form using finite tree.

Related method is called the method of coverability tree. The coverability tree algorithms can be found in [2].

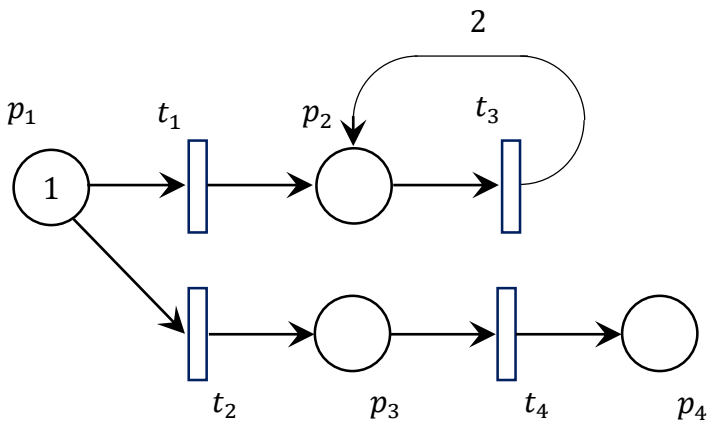


Figure 4: A regular Petri net.

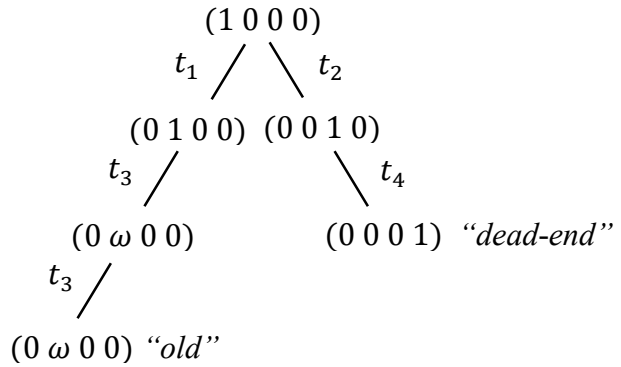


Figure 5: The coverability tree of the Petri net in Figure 4.

Here is example demonstrating use of the method. Consider the Petri net shown in Figure 4. Two transitions t_1 and t_2 are enable in the initial marking $M_0 = (1\ 0\ 0\ 0)$,. Occurrence of t_1 transforms M_0 to $M_1 = (0\ 1\ 0\ 0)$ and consequently enables t_3. Occurrence of t_3 in M_1 results in $M_3 = (0\ 2\ 0\ 0)$, which covers $M_1 = (0\ 1\ 0\ 0)$. Therefore, the new marking is $M_3 = (0\ \omega\ 0\ 0)$. Now, occurrence of t_2 in M_0 results in $M_2 = (0\ 0\ 1\ 0)$, which enables t_4. When t_4 occurs in M_2, this change marking to

$M_4 = (0\ 0\ 0\ 1)$, which is a dead-end node, since no transition is enabled in M_4. The coverability tree of this Petri net is shown in Figure 5.

The **coverability graph** of a Petri net PN is a labelled directed graph $G = (V, E)$, in which V is the set of distinct labelled nodes of the coverability tree, and E is the set of its arcs.

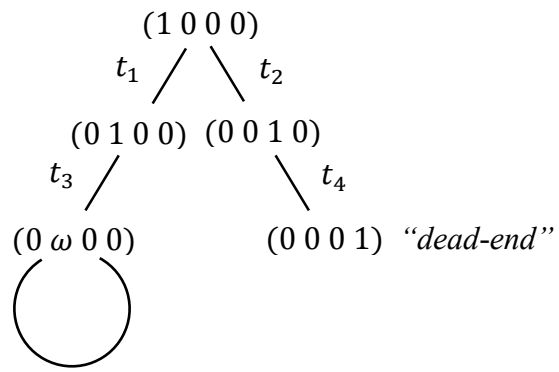


Figure 6: Coverability graph of Petri net in Figure 4.

As an example, consider the coverability graph of the Petri net shown in Figure 4 This graph is demonstrated in Figure 6. If a Petri net is bounded then its coverability and reachability graphs are same since the vertex set V becomes the same as the reachability set $R(M_0)$.

3.4 State equation method

The dynamic behaviour of many problems arising in engineering can be described in terms of differential or algebraic equations. It is a question of theoretical interest to know whether or not mathematical methods are applicable for studying properties in Petri nets. The method of state equation is an example of algebraic equation which is used to represent the structure of a Petri net and analyse the reachability property in Petri nets. So, the problem is posed as follows. Given regular Petri net check whether

a certain marking M_d is reachable from the initial marking M_0 or the sequence of transitions that fires to produce a reachable marking of $R(M_0)$.

In a Petri net with n transitions and m places, the incidences matrix A = $[a_{ij}]$ is an $n \times m$ matrix of integers and its typically entry is given by $a_{ij} = a_{ij}^{+} - a_{ij}^{-}$ where $a_{ij}^{+} = w(i,j)$ is the weight of the arc from the transition i to its output place j, and $a_{ij}^{-} = w(j,i)$ is the weight of the arc to transition i from its input place j. It is easy to see a_{ij}^{-} , a_{ij}^{+} and a_{ij}, respectively, represent the number of removed, added and remained tokens in place j when transition i fires.

If there exists a nonnegative integer solution x to the state equation then M_d is reachable from M_0, but reciprocal is not correct. Let $m \times 1$ be the vector representation of a marking M_k, where m_j denotes the number of tokens in place j after the k-th firing.

The state equation is expressed by $A^T \cdot x = \Delta M$, where $\Delta M = M_d - M_0$ and x is an $n \times 1$ column vector of nonnegative integer and is called the **firing count vector**. The ith entry of x denotes the number of times that the transition T_i must fire to transformM_0 to M_d.

Example: Consider the Petri net in Figure 4. Assuming that we want to check whether or not the destination marking $M_d = (0\ 10\ 0\ 0)$ is reachable from the initial marking $M_0 = (1\ 0\ 0\ 0)$, the state equation is given by:

$$\begin{pmatrix} -1 & -1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & -1 \\ 0 & 0 & 0 & 1 \end{pmatrix} \times \begin{pmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{pmatrix} = \begin{pmatrix} 0 \\ 10 \\ 0 \\ 0 \end{pmatrix} - \begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} = \begin{pmatrix} -1 \\ 10 \\ 0 \\ 0 \end{pmatrix}$$

The above matrix equation has a unique solution $x_1 = 1;\ x_2 = 0;\ x_3 = 9;\ x_4 = 0$ and, therefore, M_d is reachable from M_0 through occurrence of $\sigma = \{T_1, T_3\}$.

3.5 Place and transition invariants

In general, we say class of objects is invariant regarding specific transformation if certain property remains unchanged when the transformation is applied to that class. In context of Petri nets, invariants indicate states in the Petri net that remain invariant after firing or a sequence of transitions. The two known types of invariants are place invariants (or P-invariants) and transitions invariants (or T-invariants).

A P-invariant is a set of places over which regardless of firing sequence the weighted sum of tokens remains unchanged, so that occurrence of any transition has no effect on P-invariant. Indeed, this means that P-invariant conserves the number of tokens [5]. A T-invariant represents a set of transitions, whose firing returns Petri net to the initial state.

Below we provide brief introduction to the topic, but more detailed information the readers are directed to related literature [1,5].

Consider the incidence matrix $\boldsymbol{A}$, of a Petri net. A **place vector** is a vector $x: P \to \mathbb{Z}$, that is indexed by P. Similarly, a transition vector is a vector $y: T \to \mathbb{Z}$, that is indexed by T. Any non-trivial non-negative integer solution of the linear equation $x \cdot A = 0$ ($A \cdot y = 0$) is called P-invariant (T-invariant). **Support** of an invariant x, represented by $supp(x) = \{t_j: x_j > 0\}$, is a set of invariant's nonzero entries. An invariant x is called minimal, if $\nexists\ invariant\ z: supp(z) \subset supp(x)$, if the support of any other invariant z is not contained in its support. A Petri net is covered by P-

invariants (T-invariants), if every place (transition) belongs to a P-invariant (T-invariant).

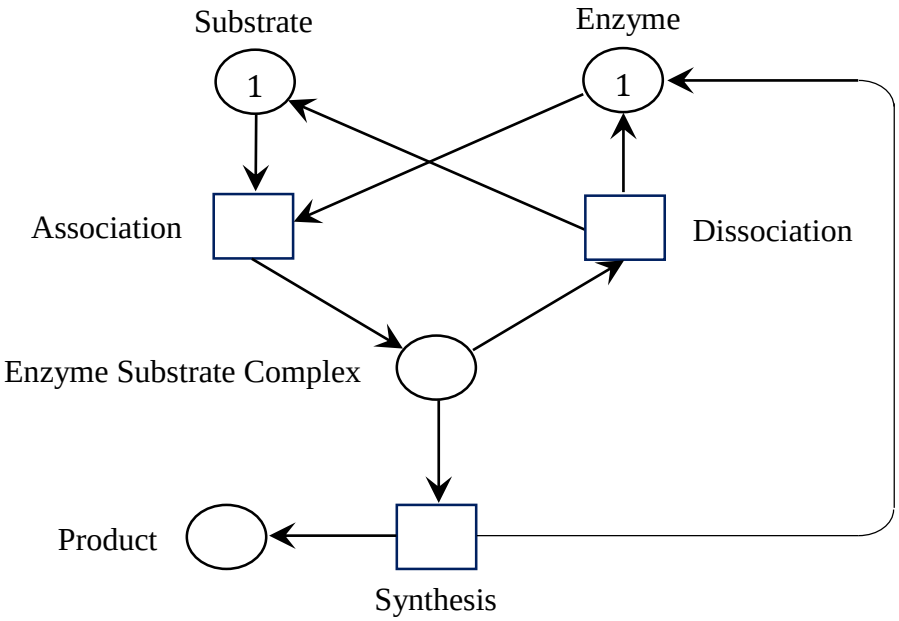


Figure 7: Petri net model of enzymatic reaction.

Let x and y be two P-invariants. If x and y have the same support, then x and y are linearly dependent, meaning that there exist two positive integers α and β such that $\alpha \cdot x = \beta \cdot y$. If α and β are nonnegative integers, then $\alpha \cdot x + \beta \cdot y$ is a P-invariant. If all components of vector $x - y$ are nonnegative integers, then $x - y$ is a P-invariant.

The incidence matrix of the Petri net in Figure 7 is as follows:

$$C = \begin{array}{r} \\ Enzyme \\ Substrate \\ EnzymeSubstrateComplex \\ Product \end{array} \begin{array}{c} \begin{array}{ccc} Association & Dissociation & Synthesis \end{array} \\ \begin{bmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 0 \end{bmatrix} \end{array}$$

P-invariants The state equation $x \cdot C = 0$, where $x = (x_1\ x_2\ x_3\ x_4)$ is the place vector, has two solutions $x = (1\ 0\ 1\ 0)$ and $x = (0\ 1\ 1\ 1)$. Support of the P-invariant

$(1\ 0\ 1\ 0)$ is $\{\text{Enzyme}, \text{EnzymeSubstrateComplex}\}$, while support of P-invariant $(0\ 1\ 1\ 1)$ is $\{\text{Substrate}, \text{Product}, \text{EnzymeSubstrateComplex}\}$. The two supports are not subset of one and another. The greatest common divisor of the non-zero elements in both P-invariants is 1 and, therefore, $(1\ 0\ 1\ 0)$ and $(0\ 1\ 1\ 1)$ are minimal P-invariants.

T-invariants The state equation $C \cdot y = 0$ with the transition vector $y = (y_1\ y_2\ y_3)$ has unique solution $(1\ 1\ 0)$. Support of the T-invariant is $\{Association, Dissociation\}$. The T-invariant is minimal.

3.6 Siphons and traps

By definition, a trap is devise designed to capture or restrain something. In Petri net, a **trap** is a subnet that caches tokens and retain at least of them; the number of tokens in a trap can decrease but never become zero [5] (see Figure 8).

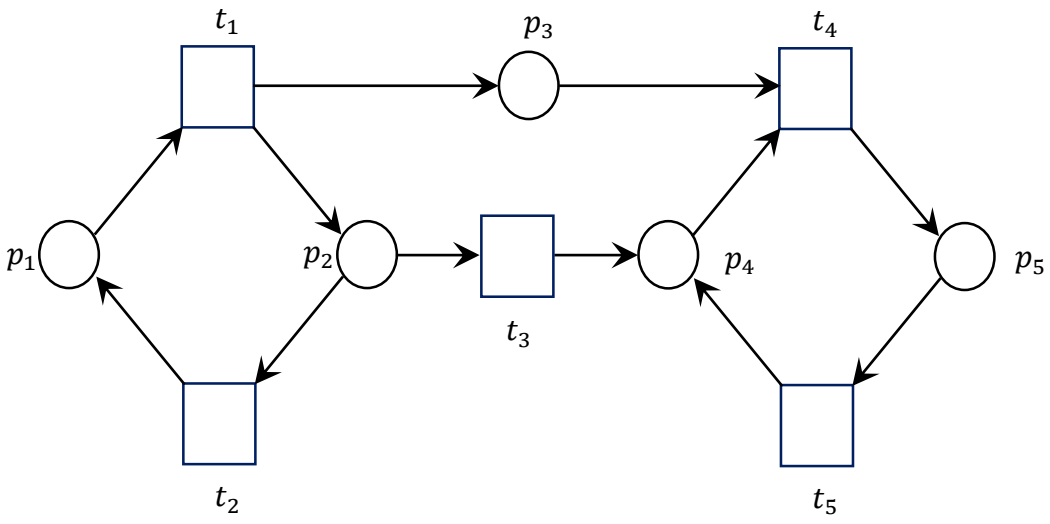


Figure 8: An example of trap.

A trap cannot become empty, if it has contained tokens, post-transitions in trap will always return tokens in the trap. The subnet in red colour indicates a trap.

Definition: A set of places $Q \subseteq P$ is called a trap if $Q \bullet \subseteq \bullet Q$ (the set of post-transitions is contained in the set of pre-transitions), i.e., every transition which subtracts tokens from a place of the trap, also has a post-place in this set.

Considering the example in Figure 8, we have:

- Set of all places $P = \{p_1, p_2, p_3, p_4, p_5\}$,
- Set of places constituting a trap $Q = \{p_3, p_4, p_5\}$,
- $Q \subseteq P$, meaning places p_3, p_4, p_5 of set of Q are contained in the set P,
- $Q \bullet$ is the set of post-transitions of places in set Q, thus, $Q \bullet = \{t_4, t_5\}$,
- $\bullet Q$ is the set of pre-transitions of places in set Q, thus, $\bullet Q = \{t_1, t_3, t_4, t_5\}$,
- $Q \bullet \subseteq \bullet Q$, thus, $Q \bullet$ is a subset of $\bullet Q$, meaning post-transitions t_4 and t_5 of set $Q \bullet$ are contained in the set of pre-transitions $\bullet Q$.

Considering the example in Figure 8, we have:

- Set of all places $P = \{p_1, p_2, p_3, p_4, p_5\}$,
- Set of places constituting a siphon $D = \{p_1, p_2, p_3\}$,
- $D \subseteq P$: D is a subset of P, meaning places A, B of set of D are contained in the set P,
- $D \bullet$: Set of post-transitions of places in set D, $D \bullet = \{t1, t2,\ t3\}$,
- $\bullet D$: Set of pre-transitions of places in set D, $\bullet Q = \{t1,\ t2\}$,
- $\bullet D \subseteq D \bullet$: $\bullet D$ Is a subset of $D \bullet$, meaning pre-transitions t1, t2 of set $\bullet D$ are contained in the set of post-transitions $D \bullet$.

Chapter 4

BIOLOGICAL CONTEXT

Protein Kinase B (AKT) and Mitogen Activated Kinase B (MAPK) signalling pathways play essential role in controlling cell survival, differentiation and its proliferation. Interaction between the AKT and MAPK pathways regulates growth. It is now becoming clear that some components of these pathways are mutated in human cancer [6], and, therefore, the crosstalk between AKT and MAPK is of utmost importance in cancer therapeutics. In this chapter, we use the biological databases together with tools and methods of bioinformatics to study the crosstalk between AKT and MAPK.

4.1 Biological databases

The collection of information related to metabolic, gene regulation and signal transduction networks in a computer readable form are compiled inside biological databases. Two important and popular biological databases are KEGG (Kyoto Encyclopedia of Genes and Genomes) and Reactome.

KEGG is a database of biological information used to understand structures and molecular mechanisms behind of organisms based on their genome information [7]. It is a collection of genome, biological pathway, disease, drug and chemical substance databases. KEGG is well-suited for bioinformatics research including data analysis. It accumulates genomic, chemical and network information under the same umbrella and provides software to handle the information. KEGG resources are

accessible at https://www.kegg.jp. KEGG COMPOUND, KEGG DRUG, KEGG REACTION, KEGG PATHWAY, KEGG ENZYME and other web services are available and accessible in KEGG.

Reactome is open access and peer-reviewed collection of human pathways and processes [8]. Reactome provides tools for the visualization, interpretation and analysis of biological pathways. Reactome is accessible to users on https://reactome.org.

4.2 MAPK pathway

Ras is an important upstream molecule of several signalling pathways including AKT and MAPK [9]. Cascade of phosphorylation and dephosphorylation reactions in MAPK pathway is schematically illustrated in Figure 9. Each phosphorylation/ dephosphorylation reaction is induced by dephosphatase/phosphatase. In this and preceding figures dephosphatases are explicitly named while phosphatases are shortly called “phase”.

MAPK signalling pathway is usually initiated by activation Ras protein. Ras transmits the signal to the plasma membrane and permit its activation [10]. Activated RAF (RAFP) induces a cascade of mitogen-activated protein kinase/ERK kinase (MEK) and extracellular signal regulated kinase (ERK). RAFP phosphorylates MEK to MEKP and then to MEKPP.

When activated, the extracellular-signal-regulated kinases (ERK) plays an important role in the induction of certain processes including cell proliferation, differentiation,

development [11]. ERK is regulated by phosphorylation mediated by MEKP and MEKPP.

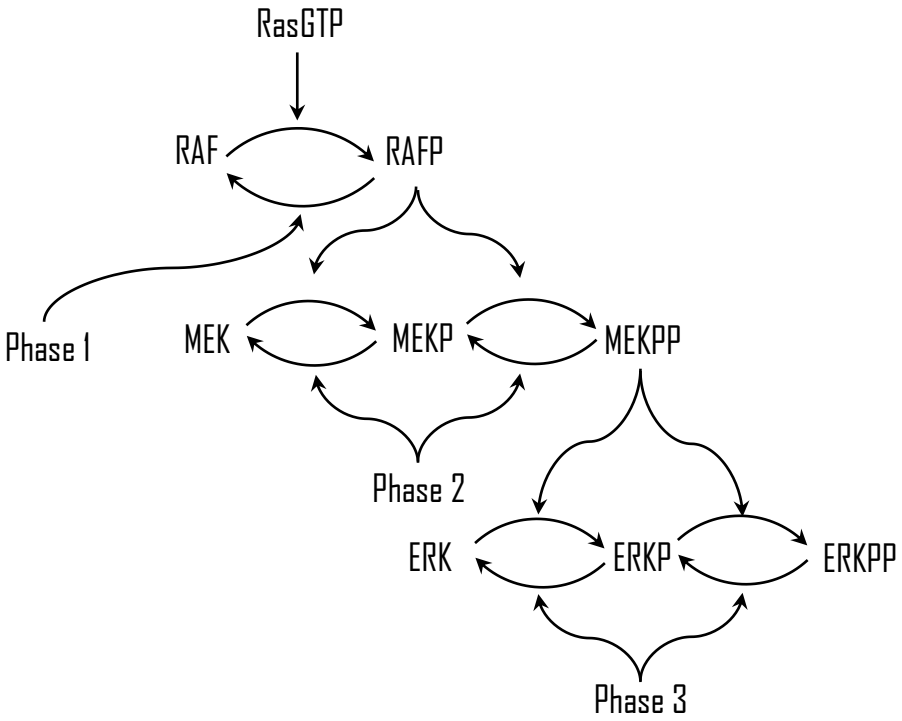


Figure 9: RAS/RAF/MEK/ERK cascade in MAPK pathway.

4.3 AKT pathway

AKT pathway is a signalling pathway that is responsible for cell survival, cell growth, cell proliferation, cell differentiation and other functions. Indeed, all these functions are important in cancer therapeutics.

The activation of the AKT pathway is a multi-step process. Phosphatidylinositol (PI) molecules are important players of intracellular signalling. In response to extracellular signals, these molecules generate second messengers including phosphatidylinositol 3,4-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3) [12,13]. Phosphoinositide 3-kinase (PI3K) phosphorylates PIP2 to PIP3 and PTEN

dephosphorylates PIP3 to PIP2 [12-17]. PIP3 in turn recruits AKT phosphorylation to AKTP and PDK induces dephosphorylation of AKTP to AKTPP. Cascade of phosphorylation/dephosphorylation reactions in AKT pathway is illustrated in Figure 10.

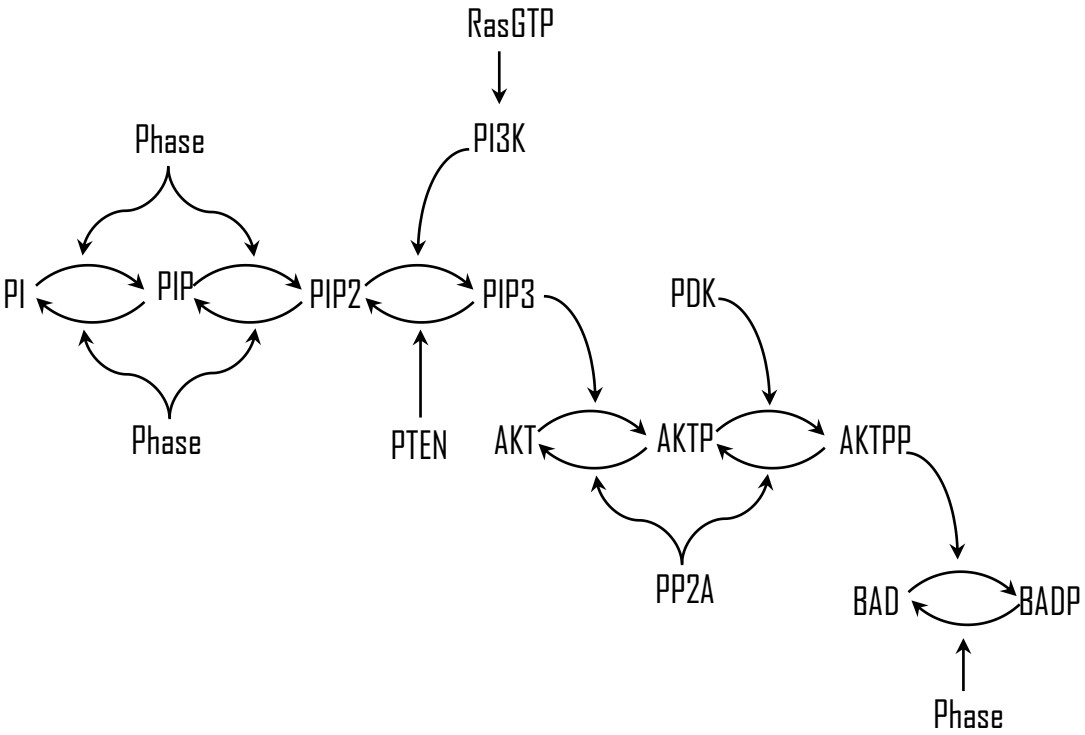


Figure 10: Cascade of phosphorylation reactions in AKT pathway.

Chapter 5

MODEL DESIGN AND ANALYSIS WITH PETRI NETS: CASE STUDY OF THE CROSSTALK BETWEEN AKT AND MAPK PATHWAYS

Biochemical reaction systems are composed of components of two types, species and their interactions. The interactions between species usually occur independently and concurrently [10]. This facilitates use of Petri nets for modelling reason, which has exactly same distinctive characteristics as underlying biochemical reaction systems. In this chapter, we discuss how to pass from signal transduction pathways to Petri nets and from Petri nets models to their qualitative analysis through performing simulations.

5.1 From a biological pathway to its Petri net model

Use of places and transitions in biological systems is straightforward. Places represent chemical compounds such as genes, and gene products, while transitions stand for chemical reactions that transform chemical compounds into one another [4,10,19]. Places in the preconditions represent substrates or reactants, meanwhile places in the postconditions represent reaction products. Arc weights are derived by the reaction stoichiometry.

The flow of tokens between places in a Petri net defines its dynamic behaviour. In the biochemical context, each firing of a transition in a Petri net is associated with

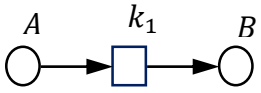
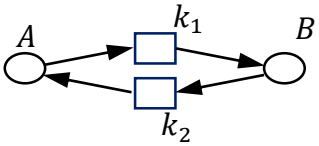
occurrence of a biochemical reaction which consumes substrates and creates products [19]. The correspondence between biological pathway elements and Petri net components is described by Table 2.

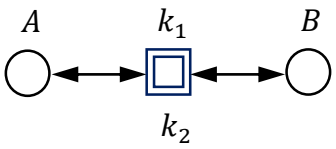
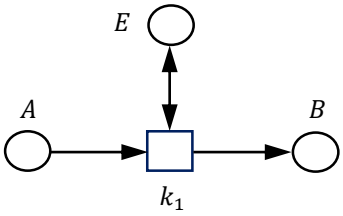
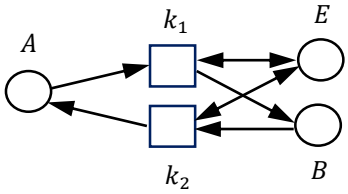
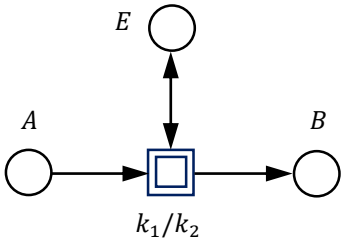
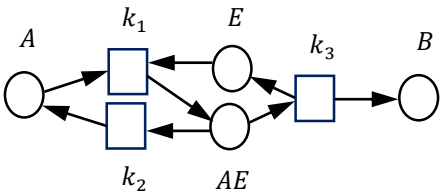
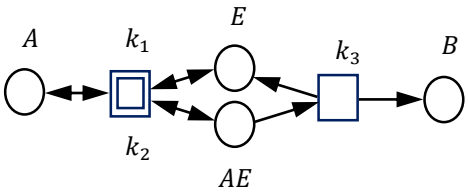
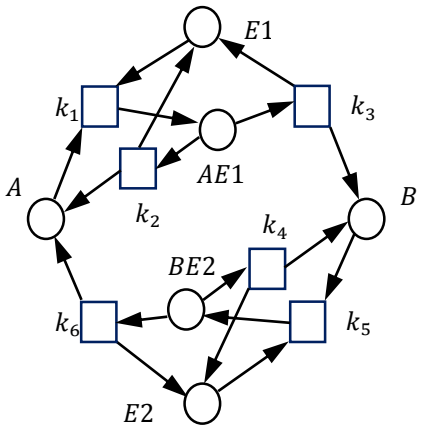
Table 2: Correspondence between pathway elements and Petri net components.

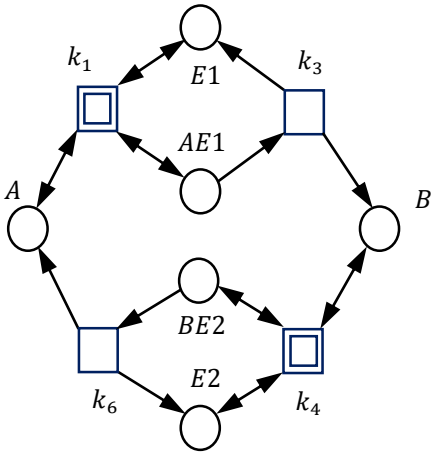
Pathway elements	**Petri net components**
metabolites, enzymes, compounds	places
Reactions	transitions
substrates, reactants	input places
reaction products	output places
stoichiometric coefficients	arc weights
quantity of metabolites, enzymes or compounds	tokens
kinetic laws of reaction	transitions rate

The basic net structures used to create Petri net model of MAPK pathway are discussed in [10]. These basic structures are represented in Table 3. In the present thesis, we use same basic net structures to create Petri net model of AKT and MAPK pathways and their crosstalk.

Table 3: Basic types of biochemical reactions and associated Petri net models.

Reaction		
symbolic representation	**name**	**Petri net model**
$A \rightarrow B$	Simple reaction	
$A \rightleftharpoons B$	Simple reversible reaction	

$A \rightleftharpoons B$	Hierarchical representation of reversible reaction	
$A + E \rightarrow B + E$	Enzymatic reaction	
$A + E \rightleftharpoons B + E$	Reversible enzymatic reaction	
$A + E \rightleftharpoons B + E$	Hierarchical representation of reversible enzymatic reaction	
$A + E \rightleftharpoons AE \rightarrow B + E$	Reversible enzymatic reaction with mass action kinetics	
$A + E \rightleftharpoons AE \rightarrow B + E$	Hierarchical representation of reversible enzymatic reaction with mass action kinetics	
$A + E1 \rightleftharpoons AE1 \rightarrow B + E1;$ $B + E2 \rightleftharpoons BE2 \rightarrow A + E2$	Cycle of reversible enzymatic reactions with mass action kinetics	

$A + E1 \rightleftharpoons AE1 \rightarrow B + E1;$ $B + E2 \rightleftharpoons BE2 \rightarrow A + E2$	Hierarchical representation of cycle of reversible enzymatic reactions with mass action kinetics	

Here is short explanation for the reactions represented in Table 3. A simple reaction $A \rightarrow B$ transforms the substrate A to the reactant B at the reaction rate r_1 while reversible reaction $A \rightleftharpoons B$ produces the reactant B from the substrate A and vice versa. Reversible reaction $A \rightleftharpoons B$ can be represented in hierarchical fashion. In a simple enzymatic reaction $A + E \rightarrow B + E$ the reactant B is obtained from the substrate A where enzyme E catalyses the reaction. In a reversible enzymatic reaction $A + E \rightleftharpoons B + E$, the substrate A produces the product B and vice versa where enzyme E catalyses both forward and reverse reactions. Reversible enzymatic reaction can also be represented in a hierarchical way. Reversible enzymatic reaction with mass action kinetics is a two-phase process. An intermediate product $AE1$ is obtained firstly from the substrate A, and then the product B is produced from $AE1$. All reactions proceed under catalytic activity of the enzyme E. A chain of enzymatic reactions can proceed in a circular way. The two enzymatic reversible reactions $A + E1 \rightleftharpoons AE1 \rightarrow B + E1$ and $B + E2 \rightleftharpoons BE2 \rightarrow A + E2$ can proceed in a circular fashion: product B is firstly obtained from the substrate A, which is then it is tuned into the product A. The

reactions are induced by the activity of the enzymes $E1$ and $E2$ whereever it is necessary.

5.2 Snoopy and Charlie software tools

Snoopy software tool supports discrete, continuous, hybrid, hierarchical, coloured and stochastic Petri nets. Snoopy provides a framework which has wide-spread applied in modelling biological processes [20]. Petri nets may easily serve as a convenient umbrella formalism integrating qualitative and quantitative modelling and analysis techniques. It is publicly available at http://www-dssz.informatik.tu-cottbus.de/DSSZ/Software/Snoopy.

A Snoopy screenshot with our case study on the screen is shown in the Figure 11.

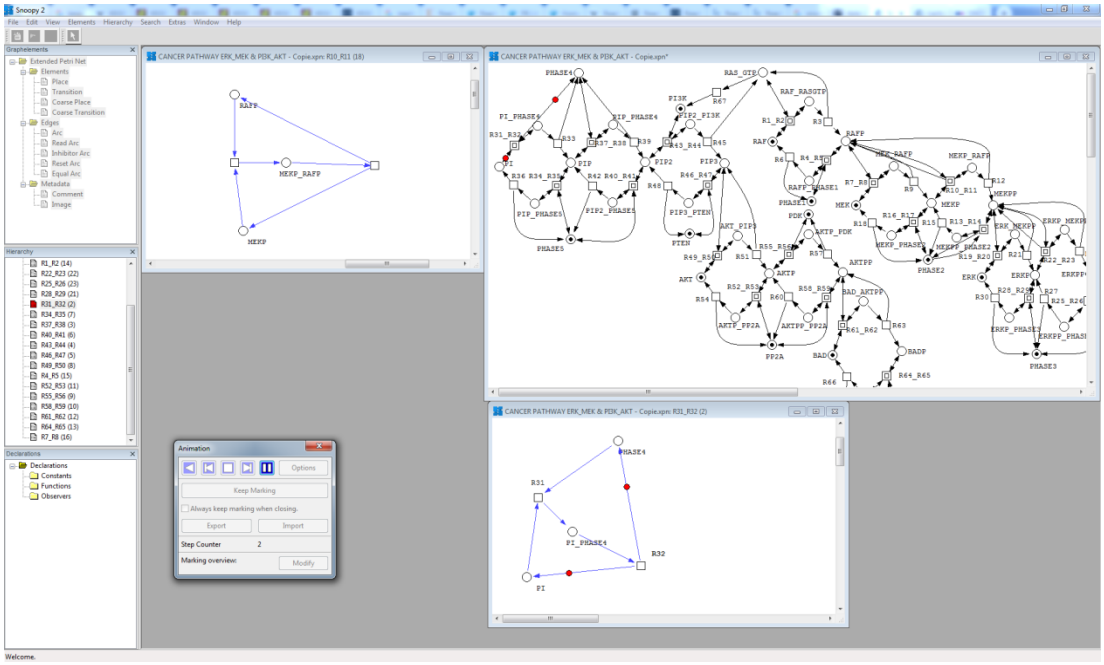


Figure 11: Snoopy screenshot of the case study.

Snoopy facilitates export and import of data among different software including Charlie and Marcie, which are also trademark of Brandenburg University of

Technology at Cottbus. Snoopy, Charlie and Marcie are indeed compatible software tools.

Charlie is a software tool with ability of use such analysis techniques as P-invariants, T-invariants, check for siphon and trap properties [21]. Charlie software toll is accessible at http://www-dssz.informatik.tu-cottbus.de/DSSZ/Software/Charlie.

5.3 Developing the Petri net model

We develop the Petri net model in accordance with the fragments represented in Table 3. Correspondence between Petri net objects (places and transitions) and the pathway components are detailed in Table 4 and Table 5. In these tables, proteins and their complexes are indicated by the sign "_" between the neighbouring proteins. The suffixes P and PP indicate phosphorylated and doubly phosphorylated forms, respectively. Petri net model of AKT and MAPK pathways and their crosstalk are schematically illustrated in Figure 12, while Petri net model developed using Snoopy software tool is demonstrated in Figure 13.

Table 4: Correspondence between metabolites and places.

Place mapping for Petri net model			
Index	Metabolite compound	Index	Metabolite compound
1	Activated RAS (RAS_GTP)	2	RAF
3	RAFP	4	RAF_RASGTP
5	PHASE1	6	RAFP_PHASE1
7	MEK	8	MEK_RAFP
9	MEKP	10	PHASE2
11	MEKP_PHASE2	12	MEKP_RAFP
13	MEKPP	14	MEKP_PHASE2
15	ERK	16	ERK_MEKPP
17	ERKP	18	PHASE3
19	ERKP_PHASE3	20	ERKP_MEKPP
21	ERKPP	22	ERKPP_PHASE3
23	PI3K	24	PHASE4
25	PI	26	PI_PHASE4
27	PIP	28	PHASE5
29	PIP_PHASE5	30	PIP_PHASE4
31	PIP2	32	PIP2_PHASE5
33	PIP2_PI3K	34	PIP3
35	PTEN	36	PIP3_PTEN

37	AKT	38	AKT_PIP3
39	PP2A	40	AKT_PP2A
41	AKTP	42	PDK
43	AKTP_PDK	44	AKTPP
45	AKTPP_PP2A	46	BAD
47	BAD_AKTPP	48	BADP
49	PHASE6	50	BADP_PHASE6

Table 5: Correspondence between reactions and transitions.

Transition mapping for Petri net model			
Name	Reaction description	Name	Reaction description
R1	Binding of RAS_GTP to RAF	R35	Dissociation of PIP_PHASE5 complex
R2	Dissociation of RAF_RAS_GTP complex	R36	Dephosphorylation of PIP by PHASE5
R3	Phosphorylation of RAF by RAS_GTP	R37	Binding PIP to PHASE4
R4	Binding RAFP to phase1	R38	Dissociation of PIP_PHASE4 complex
R5	Dissociation of RAFP_PHASE1 complex	R39	Phosphorylation of PIP by PHASE4
R6	Dephosphorylation of RAFP by PHASE1	R40	Binding PIP2 to PHASE5
R7	Binding RAFP to MEK	R41	Dissociation of PIP2_PHASE5 complex
R8	Dissociation of RAFP_MEK complex	R42	Dephosphorylation of PIP2 by PHASE5
R9	Phosphorylation of MEK by RAFP	R43	Binding PIP2 to PI3K
R10	Binding MEKP to RAFP	R44	Dissociation of PIP2_PI3K complex
R11	Dissociation of MEKP_RAFP complex	R45	Phosphorylation of PIP2 by PI3K
R12	Phosphorylation of MEKP by RAFP	R46	Binding PIP3 to PTEN
R13	Binding MEKPP to PHASE2	R47	Dissociation of PIP3_PTEN complex
R14	Dissociation of MEKPP_PHASE2 complex	R48	Dephosphorylation of PIP3 by PTEN
R15	Dephosphorylation of MEKPP by PHASE2	R49	Binding AKT to PIP3
R16	Binding MEKP to PHASE2	R50	Dissociation of AKT_PIP3 complex
R17	Dissociation of MEKP_PHASE2 complex	R51	Phosphorylation of AKT by PIP3
R18	Dephosphorylation of MEKP by PHASE2	R52	Binding AKTP to PP2A
R19	Binding MEKPP to ERK	R53	Dissociation of AKTP_PP2A complex
R20	Dissociation of ERK_MEKPP complex	R54	Dephosphorylation of AKTP by PP2A
R21	Phosphorylation of ERK by MEKPP	R55	Binding AKTP to PDK
R22	Binding MEKPP to ERKP	R56	Dissociation of AKTP_PDK complex
R23	Dissociation of MEKPP_ERKP complex	R57	Phosphorylation of AKTP by PDK
R24	Phosphorylation of ERKP by MEKPP	R58	Binding AKTPP to PP2A
R25	Binding ERKPP to PHASE3	R59	Dissociation of AKTPP_PP2A complex

R26	Dissociation of ERKPP_PHASE3 complex	R60	Dephosphorylation of AKTPP by PP2A
R27	Dephosphorylation of ERKPP by PHASE3	R61	Binding BAD to AKTPP
R28	Binding ERKP to PHASE3	R62	Dissociation of BAD_AKTPP complex
R29	Dissociation of ERKP_PHASE3 complex	R63	Phosphorylation of BAD by AKTPP
R30	Dephosphorylation of ERKP by PHASE3	R64	Binding BADP to PHASE6
R31	Binding PI to PHASE4	R65	Dissociation of BADP_PHASE6 complex
R32	Dissociation of PI_PHASE4 complex	R66	Dephosphorylation of BADP by PHASE6
R33	Phosphorylation of PI by PHASE4	R67	Activation of PI3K by RAS_GTP
R34	Binding PIP to PHASE5		

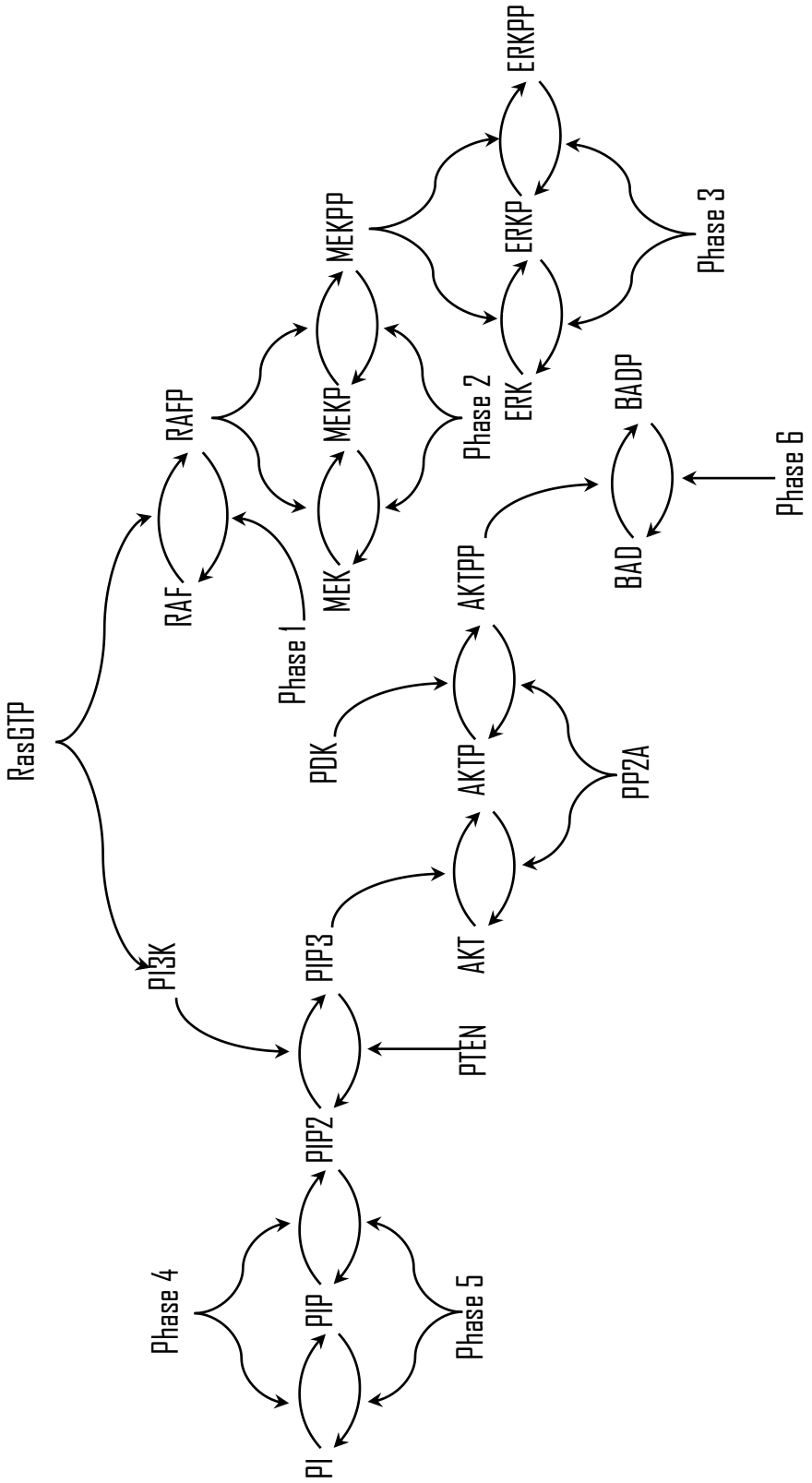


Figure 12: Schematic illustration of the crosstalk between AKT and MAPK pathways.

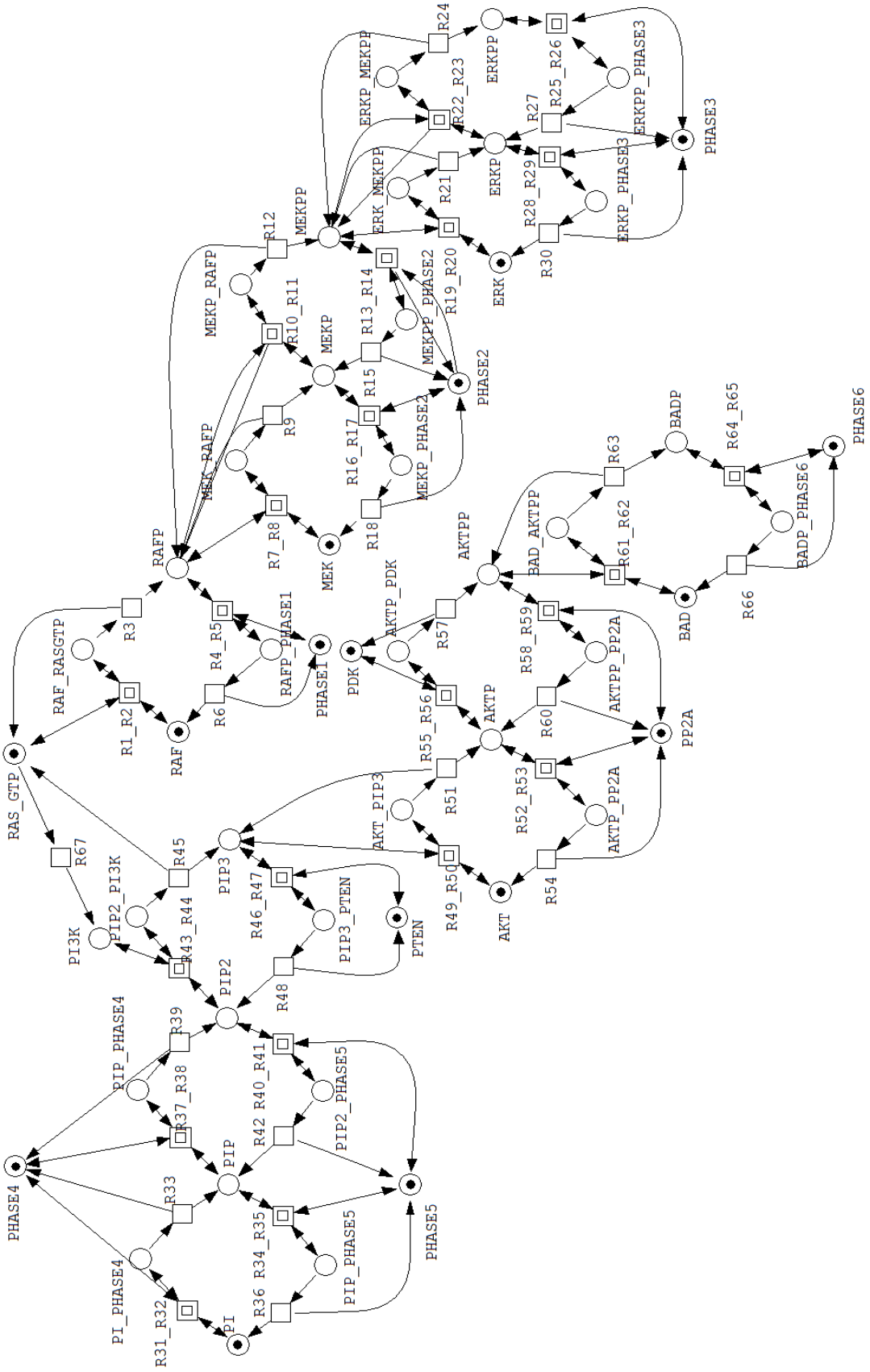


Figure 13: A snoopy snapshot of the Petri net model.

5.4 Simulations and qualitative analysis

5.4.1 Structural and behavioural properties

Below we describe the structural properties that depend directly on the places, transitions and arcs. These properties characterize the network structure. Structural properties are used to make sure whether net's characteristics are good enough to use it as modelling platform for the problem. Biological meaning of some structural properties are explained below [5].

Table 6: Structural properties and their biological interpretation.

Property	**Informal definition**	**Biological meaning**
Pure (**PUR**)	Net has loop-free structure. This excludes read arcs and double arcs.	A biological component cannot be produced and consumed by single reaction, that is, enzymatic reactions need to be formulated in more detail.
Ordinary (**ORD**)	All arcs have the same weight that is equal to 1.	Each stoichiometric parameter has the same numeric value equal to one.
Homogeneous (**HOM**)	Outgoing arcs of a place are assigned same weight.	Each consuming reaction that a component involved in consumes the same amount of this component (in terms of molecules).
Connected (**CON**)	A Petri net has connected structure.	All biological components are directly or indirectly connected with each other via biomolecular reactions.
Strongly connected (**SC**)	A Petri net is strongly connected.	All components are directly connected with each other via biomolecular reactions.
Non-blocking multiplicity (**NBM**)	The maximum of the weights of outgoing arcs is not greater than the minimum of the weights of the incoming arcs for a place.	There is a balance between amounts of produced and consumed molecules for a biological component.
Conservative (**CSV**)	A firing action adds as many tokens to post-places as the number of token removed from pre-places.	A reaction consumes as many molecules as the number of produced molecules.
Static conflict free (**SCF**)	None of the pre-places is shared by multiple transitions.	Biological reactions do not share reactants.

No input transitions (**FT0**)	All transitions have pre-places.	There is a finite source for a component.
No output transitions (**TF0**)	All transitions have post-places.	Sink of a component.
No input places (**FP0**)	All places have pre-transitions.	None of the reactions produces specified component.
No output places (**PF0**)	All places have post-transitions.	A component can be infinitely accumulated. This component is not consumed by any reaction.

Table 7: Behavioural properties and their biological meaning

Property	**Informal Definition**	**Biological meaning**
Structurally boundedness (**SB**)	A Petri net that is bounded for any initial marking is also structurally bounded.	Accumulation of a component depends on initial state of the system.
1-boundedness (**1-B**)	If all places are 1-bounded then the Petri net is 1-bounded.	Amount of any component is limited with one molecule.
k-boundedness (***k*-B**)	A Petri net is *k*-bounded if all its places are *k*-bounded at the most.	Amount of a component is limited with *k* molecules.
Liveness (**LIV**)	Every transition is enabled. If a transition is disabled there exists a firing sequence that enables it.	All reactions repeatedly occur contributing to the time-dependent and special-dependent development.
Reversibility (**REV**)	The initial marking can be reached from any marking.	Whatever the state reached there is a sequence of reactions that reproduces the initial state of biomolecular system.
Dynamically conflict free (**DCF**)	A Petri net is said to be free of dynamic conflicts if there does not exists a state, in which two enable transitions can disable each other in a circular way.	This a situation when two reactions can occur simultaneously, but occurrence of any of them inhibits another one. This is a typical situation when common reactants are fully consumed by one of the reactions.
Dead states (**DSt**)	A Petri net is dead if all transitions are disabled.	The system is deadlocked, no reaction can occur.
Dead transition (**DTr**)	A transition is dead if it is disable and cannot be enabled anymore.	A reaction cannot occur any more.
Siphon Trap Property (**STP**)	Every siphon contains a marked trap.	There is an outflow of the components induced by a siphon. The system has an initial active trap.

Covered by places invariants (**CPI**)	Every place of the Petri net belongs to some P-invariant.	The system has mass preserving nature.
Covered by transition invariants (**CTI**)	Every transition of the Petri net belongs to some T-invariant.	The initial state can be reproduced from any state of the biomolecular system.
Strongly connected by transition invariants (**SCTI**)	If Petri net is covered by non-trivial T-invariants then it is strongly covered by T-invariants. Any trivial T-invariant is a system of two reactions.	There does not exist a pair of reactions restoring each another.

5.4.2 Simulation results

We used Charlie simulation tools to conduct the simulations for the satisfiability of the properties represented in Table 6 and Table 7. The simulation results are provided in Table 8.

Table 8: Simulation results and their interpretations in biological context.

Structural properties	
PUR = YES:	The model does not use read arcs (side conditions for reactions). Enzymatic reactions are represented in detail as sequence of simple steps.
ORD = YES:	For all reactions the stoichiometry equal to 1.
HOM = YES:	If there exist multiple reactions consuming a component then these reactions consume equal number of molecules of this component.
CON = YES:	The molecular network has connected structure.
SC = YES:	In addition, each component is directly connected to all other components.
SB = YES:	Regardless of the initial marking none of the components can be infinitely accumulated.
STP = YES:	There is a chain of reactions occurred in a circular way. As a result, the total number of molecules in these chains of reactions will never be consumed.
CSV = NO:	Each reaction includes association and dissociation of components.
SCF = NO:	Some components are shared as reactants by multiple reactions.
FT0 = YES:	There does not exist any external source.
TF0 = YES:	There does not exist any external sink.
FP0 = YES:	None of the components is a reactant only.
PF0 = YES:	None of the components is a product only.

Behavioural properties

1-B = **YES**:	For each component there is one just one molecule.
***k*-B** = **YES**:	For each component the number of molecules is bounded.
LIV = **YES**:	Due to the cyclic nature of reactions each reaction occurs forever contributing to the signalling.
REV = **YES**:	Due to the cyclic nature of reactions there is a chain of reactions reproducing the initial state.
DCF = **NO**:	When a protein gets dephosphorylated it loses the ability to phosphorylate reactions.
DSt = **0**:	At least one of the reactions can always occur.
DTr = **NO**:	If a reaction is not active there still exits a way to activate it.
CTI = **YES**:	All reactions are involved in a circular chain of reactions. A chain of circular reactions can reproduce its initial state.
CPI = **YES**:	Mass conservation is indicated, meaning that a P-invariant covers all states of a specified component.
SCTI = **NO**:	Some of the chains of circular reactions make up by associated reactions consist only of two steps.

As a result of the analysis we determine the following P-invariants and T-invariants.

Minimal semi positive P-invariants and their meanings:

1. $\boldsymbol{X_1}$ = (PI_PHASE4, PIP_PHASE4, PHASE4) determines the states of PHASE4.
2. $\boldsymbol{X_2}$ = (PIP_PHASE5, PIP2_PHASE5, PHASE5) determines the states of PHASE5.
3. $\boldsymbol{X_3}$ = (PI, PIP, PIP2, PIP3, PIP_PHASE5, PIP2_PHASE5, PIP3_PTEN, PI_PHASE4, PIP_PHASE4, PIP2_PI3K, AKT_PIP3) determines the states of PI.
4. $\boldsymbol{X_4}$ = (AKTP_PP2A, AKTPP_PP2A, PP2A) determines the states of PP2A.
5. $\boldsymbol{X_5}$ = (AKT, AKT_PIP3, AKTP, AKTP_PP2A, AKTP_PDK, AKTPP, AKTPP_PP2A, BAD_AKTPP) determines the states of AKT.
6. $\boldsymbol{X_6}$ = (BAD, BAD_AKTPP, BADP, BADP_PHASE6) determines the states of BAD.
7. $\boldsymbol{X_7}$ = (RAS_GTP, PIP2_PI3K, PI3K, RAF_RAS_GTP) determines the states of RAS_GTP.

8. $\boldsymbol{X_8}$ = (RAF_RASGTP, RAF, RAFP, RAFP_PHASE1, MEK_RAFP, MEKP_RAFP) determines the states of RAF.
9. $\boldsymbol{X_9}$ = (MEKP_PHASE2, PHASE2, MEKPP_PHASE2) determines the states of PHASE2.
10. $\boldsymbol{X_{10}}$ = (MEK_RAFP, MEK, MEKP, MEKPP, MEKP_RAFP, MEKP_PHASE2, MEKPP_PHASE2, ERK_MEKPP, ERKP_MEKPP) determines the states of MEK.
11. $\boldsymbol{X_{11}}$ = (ERKP_PHASE3, ERKPP_PHASE3, PHASE3) determines the states of PHASE3.
12. $\boldsymbol{X_{12}}$ = (ERK, ERK_MEKPP, ERKP, ERKPP, ERKP_MEKPP, ERKP_PHASE3, ERKPP_PHASE3) determines the states of ERK.

Figures 14-16 show the examples of net structures representing P-invariants (invariants 1, 3 and 8 in the above list). All three P-invariants are automatically detected by the software and then we interpreted their meanings.

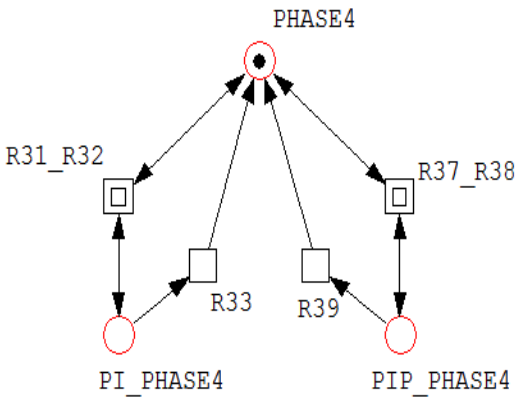


Figure 14: Place invariant X1 that determines the states of PHASE4.

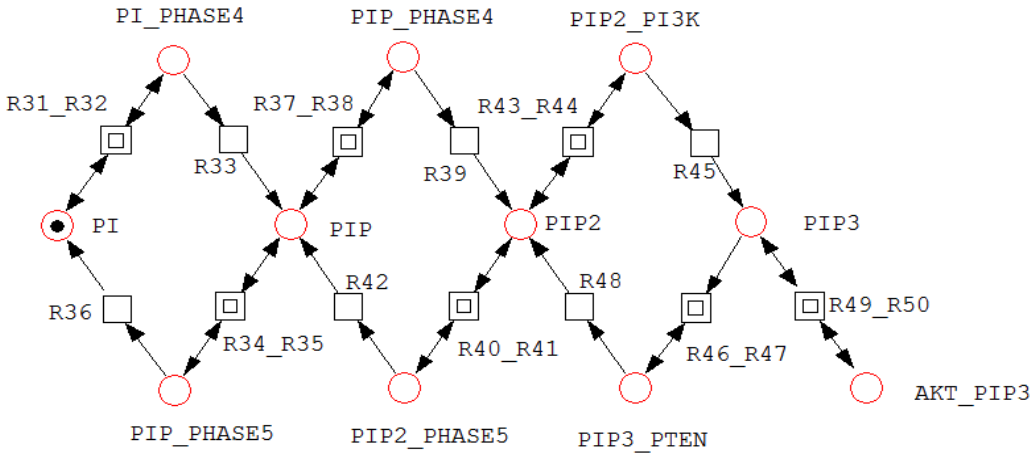


Figure 15: Place invariant X3 that determines the states of PI.

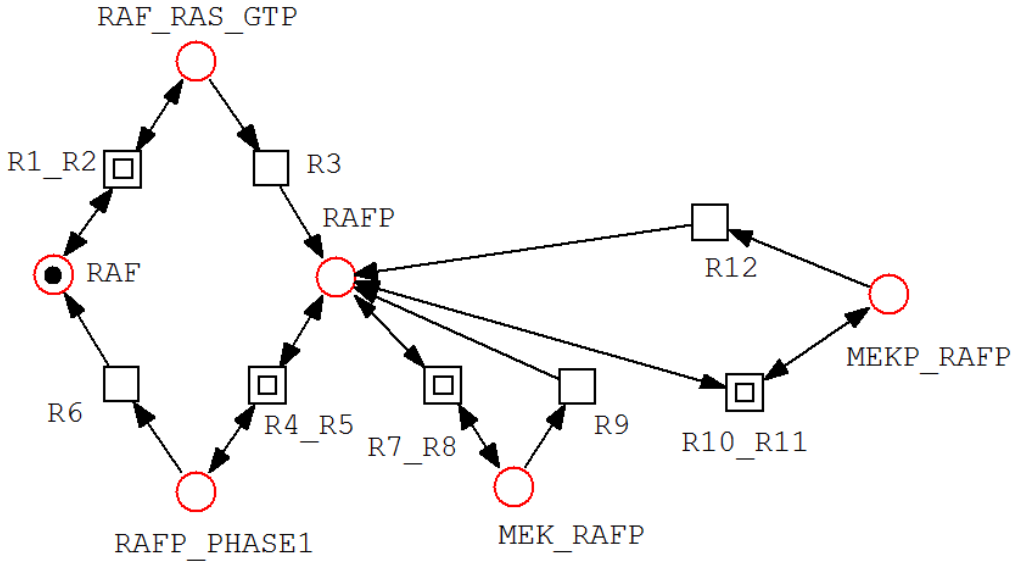


Figure 16: Place invariant X8 that determines the states of RAF.

Minimal semi positive T-invariants and the meanings:

1. $\boldsymbol{Y_1}$ = (R33, R36, R31, R34) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of PHASE4 to PI, phosphorylation of PI to PIP and its release, binding of PHASE5 to PIP and dephosphorylation of PIP and its release reactions.
2. $\boldsymbol{Y_2}$ = (R39, R42, R37, R40) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of PHASE4 to PIP, phosphorylation

of PIP to PIP2 and its release, binding of PHASE5 to PIP2 and dephosphorylation of PIP2 and its release reactions.

3. $\boldsymbol{Y_3}$ = (R45, R48, R43, R46, R67) determines a sequence of reactions reproducing the initial state. The sequence consists of activation of PI3K, binding of PI3K to PIP2, phosphorylation of PIP2 to PIP3 and its release, binding of PTEN to PIP3 and dephosphorylation of PIP3 and its release reactions.
4. $\boldsymbol{Y_4}$ = (R51, R54, R49, R52) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of PIP3 to AKT, phosphorylation of AKT to AKTP and its release, binding of PP2A to AKTP and dephosphorylation of AKTP and its release reactions.
5. $\boldsymbol{Y_5}$ = (R57, R60, R55, R58) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of PDK to AKTP, phosphorylation of AKTP to AKTPP and its release, binding of PP2A to AKTPP and dephosphorylation of AKTPP and its release reactions.
6. $\boldsymbol{Y_6}$ = (R63, R66, R61, R64) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of AKTPP to BAD, phosphorylation of BAD to BADP and its release, binding of PHASE6 to BADP and dephosphorylation of BADP and its release reactions.
7. $\boldsymbol{Y_7}$ = (R3, R6, R1, R4) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of RAS_GTP to RAF, phosphorylation of RAF to RAFP and its release, binding of PHASE1 to RAFP, dephosphorylation of RAFP and its release reactions.
8. $\boldsymbol{Y_8}$ = (R9, R18, R7, R16) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of RAFP to MEK, phosphorylation of MEK

to MEKP and its release, binding of PHASE2 to MEKP and dephosphorylation of MEKP and its release reactions.

9. $\boldsymbol{Y_9}$ = (R12, R15, R10, R13) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of RAFP to MEKP, phosphorylation of MEKP to MEKPP and its release, binding of PHASE2 to MEKPP and dephosphorylation of MEKPP and its release reactions.
10. $\boldsymbol{Y_{10}}$ = (R21, R30, R19, R28) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of MEKPP to ERK, phosphorylation of ERK to ERKP and its release, binding of PHASE3 to ERKP and dephosphorylation of ERKP and its release reactions.
11. $\boldsymbol{Y_{11}}$ = (R24, R27, R22, R25) determines a sequence of reactions reproducing the initial state. The sequence consists of binding of MEKPP to ERKP, phosphorylation of ERKP to ERKPP and its release, binding of PHASE3 to ERKPP and dephosphorylation of ERKPP and its release reactions.

Figure 17 shows two examples of T-invariants. Both T-invariants were automatically detected by the software and then we interpreted their meanings.

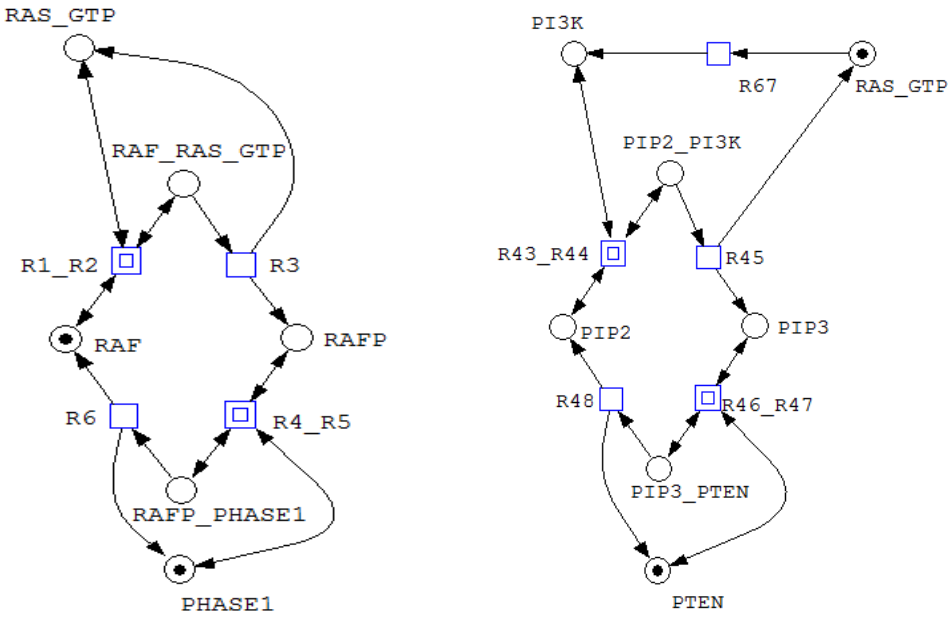


Figure 17: Transition invariants Y7 (in the left) and Y3 (in the right).

Trivial T-invariant present in our Petri net model, for example transition R1_R2 shows in Figure 18, consists of association and dissociation of proteins.

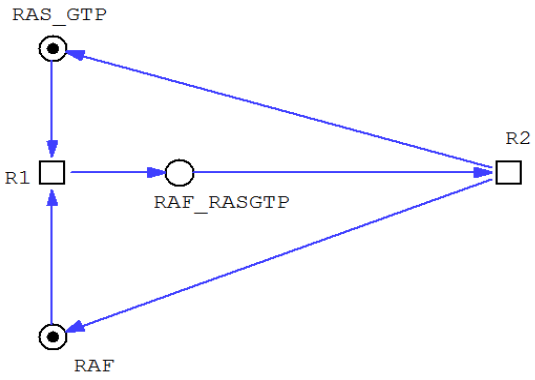


Figure 18: Example of trivial T-invariant constituting association and dissociation reactions between RAS_GTP and RAF.

Siphons and traps. The simulation results show that Petri net contains 16 siphons that are traps at the same time. All siphons (or traps) are listed below:

1. S_1 = (PI, PIP, PIP2, PIP3, PIP_PHASE5, PIP2_PHASE5, .PIP3_PTEN, PI_PHASE4, PIP_PHASE4, PIP2_PI3K, AKT_PIP3);

2. $\boldsymbol{S_2}$ = (AKTP_PP2A, AKTPP_PP2A, PP2A);
3. $\boldsymbol{S_3}$ = (MEK_RAFP, MEK, MEKP, MEKPP, MEKP_RAFP, MEKP_PHASE2, MEKPP_PHASE2, ERK_MEKPP, ERKP_MEKPP);
4. $\boldsymbol{S_4}$ = (AKT, AKT_PIP3, AKTP, AKTP_PP2A, AKTP_PDK, AKTPP, AKTPP_PP2A, BAD_AKTPP);
5. $\boldsymbol{S_5}$ = (PI_PHASE4, PIP_PHASE4, PHASE4);
6. $\boldsymbol{S_6}$ = (BADP_PHASE6, PHASE6);
7. $\boldsymbol{S_7}$ = (BAD, BAD_AKTPP, BADP, BADP_PHASE6);
8. $\boldsymbol{S_8}$ = (RAF_RASGTP, RAF, RAFP, RAFP_PHASE1, MEK_RAFP, MEKP_RAFP);
9. $\boldsymbol{S_9}$ = (ERKP_PHASE3, ERKPP_PHASE3, PHASE3);
10. $\boldsymbol{S_{10}}$ = (RAS_GTP, PIP2_PI3K, PI3K, RAF_RASGTP);
11. $\boldsymbol{S_{11}}$ = (RAFP_PHASE1, PHASE1);
12. $\boldsymbol{S_{12}}$ = (MEKP_PHASE2, PHASE2, MEKPP_PHASE2);
13. $\boldsymbol{S_{13}}$ = (ERK, ERK_MEKPP, ERKP, ERKPP, ERKP_MEKPP, ERKP_PHASE3, ERKPP_PHASE3);
14. $\boldsymbol{S_{14}}$ = (AKTP_PDK, PDK);
15. $\boldsymbol{S_{15}}$ = (PIP_PHASE5, PIP2_PHASE5, PHASE5);
16. $\boldsymbol{S_{16}}$ = (PIP3_PTEN, PTEN)

Chapter 6

CONCLUSION

In this thesis we show way of modelling and analysing signal transduction pathways or biochemical network using Petri net. This approach when through the translation of biological reaction into logical terms and then turn in net components. We have illustrated it by considering qualitative Petri net description of the MAPK and AKT signalling pathway.

The modelling of biochemical network using Petri net is appealing and easy because it is simple in its application, is visually comprehensible and allows computational manipulations. On the other hand, it can be further extended or modified to fit specific attributes required for modelling of a variety of systems (inhibitor arc, TPN, CPN, KPN, HPN are such extensions).

We mainly focus on transition and place invariants analysis, which play a role in model validation. Once we have a validated Petri net model, several application and extension can be applied. The net may be refined and extended to a quantitative model by including know or estimated kinetic parameters like concentration, reaction rates or time, by using hybrid or continuous Petri nets. In this way, the resulting quantitative Petri net model will maintain structural properties and some behavioural properties. In this case signal flows are given by simulation.

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