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Romina Luciana Paturca

BIOMODELING WITH PETRI NETS

TURUN AMMATTIKORKEAKOULU
TURKU UNIVERSITY OF APPLIED SCIENCES

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Instructor: Al-Bermanei Hazem

Romina Luciana Paturca

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ABSTRACT

Petri nets are graphic-based modeling tools for systems where activity and information flows have an important role among physical components and activities. Since their introduction, about 50 years ago, Petri nets have been applied in various areas such as computer architecture, software design, workflow management, programming, databases, process modeling, diagnosis, simulation, discrete process control, communication protocols, and yet even further outside of computer science, for example, administration, theories of communication, natural sciences. At the same time, nets have been modified and theoretically investigated and they have increased the interest of computer scientists in net theory. Petri nets have also been adjusted in biology and used for modeling biological networks.

This thesis presents the basic notions of Petri nets and their use for modeling and verification of systems. The thesis starts with an introduction and a brief history of Petri nets. It continues with properties of Petri Nets, modeling and analysis techniques, and it concludes with the analysis of a biological case study with a Petri net model.

KEYWORDS:

Petri nets, mathematical model, model validation, heat shock response, P-invariants, T-invariants.

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LIST OF ABBREVIATIONS

PN	Petri Nets
P-invariants	Places invariants
T-invariants	Transitions invariants
HSR	Heat Shock Response
hsp	heat shock protein
mfp	misfolded protein
prot	protein
hsf	heat shock factor
hse	heat shock element

1 INTRODUCTION

A Petri net is a language of nets that is favorable for the representation, in a natural way, of logical interactions between activities and parts in a system that holds characteristics like concurrency, synchronization, sequentiality, and conflict. Petri nets can be used for graphical representation, as a visual communication tool like block diagrams, flow charts, logical trees.

Historically, the theory of Petri nets has its origins in the doctoral thesis of C.A. Petri submitted in 1962. It is stated in some sources that the theory was invented in 1939, for describing chemical processes, by the 13-year old Carl Adam Petri. Since then, Petri nets have been developed and used in both theory and practice, providing a strong means of communication between theoreticians and practitioners.

Petri nets have the ability to generalize the theory for process analysis by their high expression in competitive events.

A Petri net is identified as a bipartite directed graph whose nodes are places and transitions. Places represent conditions and transitions represent events. An event has a particular number of input and output conditions representing the pre-conditions and the post-conditions. In contrast with condition/event nets, place/transition nets can hold any number of conditions (tokens). Illustratively, places are depicted as circles, and transitions as boxes or bars. Each arc of this graph connects a place and a transition. If there is an oriented arc from a place towards a transition, then the place represents the point of entry for the transition; conversely, if the arc is oriented from the transition to the place, then the place represents the point of exit for the transition. Places may contain a variable number of tokens, and their entire distribution over the places of the net, represents the marking. Transitions occur by consuming the input tokens and producing them into the output tokens.

The execution of a Petri net is a nondeterministic process. During the execution, the active transitions are analyzed at each step. A transition is active at the moment when every entry point contains at least one token.

The execution of a transition occurs by eliminating one token from each input place and by adding one token to each output place. Only one transition can be executed at a certain step.

This thesis is organized as follows. Chapter two contains basic notions of graph theory and introduces Standard Petri nets. Chapter three presents the behavioral and structural properties of Petri nets. Chapter four discusses a couple modeling and analyzing methods of the formalism. Chapter five contains the basic information about the Heat Shock Response and the modeling of the Heat Shock Response with Petri nets.

2 BASIC NOTIONS AND FORMAL DEFINITIONS

2.1 Definitions of Graph Theory

Definition 2.1.1 A *graph* G = (V,E) consists of a set V of vertices (nodes) and a set E of edges (lines).

Definition 2.1.2 A *directed graph* is a graph with directed edges. An undirected graph is a graph in which edges have no direction.

Definition 2.1.3 A *simple graph* is an undirected graph which has no loops (edges which start and end on the same vertex). A multigraph is a graph with multiple edges.

Definition 2.1.4 A *weighted graph (network)* is a graph is which each edge has a weight (real number).

Definition 2.1.5 A *bipartite graph* is a graph in which vertices can be divided into two sets such that each edge connects one vertex in one set to one vertex in the other set.

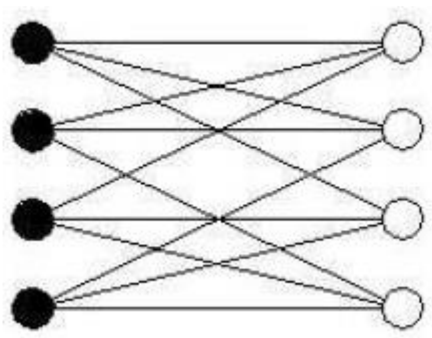

Figure 1. A bipartite graph

2.2 Description of Standard Petri Nets

A Petri net can be identified with a particular kind of bipartite directed graphs populated with four types of objects. These objects are places, transitions, directed arcs connecting places to transitions or transitions to places, and tokens. Graphically, places are represented by circles and transitions by bars or rectangular shapes. A place is an input to a transition if there is a directed arc from the place to the transition. A place is an output for a transition if there is a directed arc from the transition to the place. In its simplest form, a Petri net can be represented by a transition with its input and output places. This basic network can be used to represent different aspects of modeled systems. For example, input / output places may represent preconditions / postconditions and transitions may represent events. Inputs may signify the availability of resources, transition their use, and outputs may signify the release of resources.

Petri nets were designed to represent discrete and concurrent processes of technical systems, but they also offer a simple and flexible modeling language and they are useful in modeling biological systems being very efficient in reconstructing molecular networks.

Places are inactive nodes that refer to conditions or states. They are visualized by circles and in a biological context, they may signify: organisms, populations, species, cells, proteins, molecules, and they may also represent temperature or pH value as well as membrane potential.

Tokens are floating elements carried by places. Graphically, tokens are represented by dots and they indicate the discrete value of a condition, and in biological systems, they express discrete numbers of populations, species, concentration levels, pH values, and temperatures.

Transitions are active nodes that consume and produce tokens. They may represent different events and activities and, in a biological context, reactions, interactions, and intramolecular changes.

Directed arcs, represented by arrows, may define products of a reaction, reactants, determine the relationship between transitions and places, and show the changes that occur in the marking. (Blätke 2011, p. 5-6).

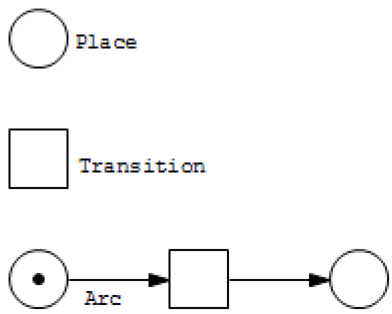

Figure 2. Graphical representation of places, transitions, and arcs

2.2.1 Formal Definition of a Petri Net (PN)

A **Petri Net** is a 5-tuple $PN = (P, T, F, W, M0)$, where:

$P = \{p1, p2, p3, \dots, pm\}$ is a finite set of places

$T = \{t1, t2, t3, \dots, tn\}$ is a finite set of transitions

$F \subseteq (P \times T) \cup (P \times T)$ is a set of arcs

$W: F \rightarrow \{1,2,3, \dots\}$ is a weight function

$M0: P \rightarrow \{0,1,2,3, \dots\}$ is the initial marking

$P \cap T = \emptyset$ and $P \cup T \neq \emptyset$

(Murata 1989)

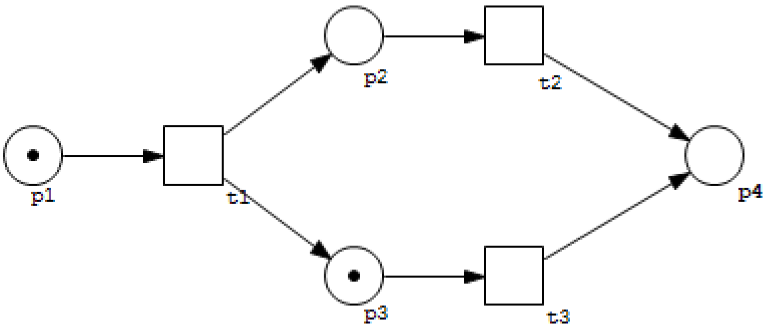

Figure 3. A Petri net with $P = \{p1, p2, p3, p4\}$, $T = \{t1, t2, t3\}$ and M_0= $(1\ 0\ 1\ 0)$

2.3 Enabling and firing rules

The dynamic evolution of the PN marking is ruled by transition firings which destroy and create tokens. Both the enabling and firing rules are specified through arcs and they are associated with transitions so that the enabling rule states the conditions under which transitions are allowed to fire and the firing rule defines the marking modification or the change of state produced by the transition.

Each place may contain a number of tokens. The transition is achieved only when all places situated on a higher position contain at least one token. At a certain moment, only one transition may occur by deleting and adding tokens. A transition can start when each of the places connected to it, has at least one token. When a transition is triggered, it removes the token from each input place and adds one to each place connected to it. Moving tokens between places takes place according to the firing rules imposed by transitions. Sometimes it is necessary for an input place to contain two or more tokens before the transition can start, and in this case, to avoid drawing more than one arc between place and transition, the multiplicity of arcs is denoted by a number next to the arc.

Definition 2.3.1 A transition is said to be *enabled* if each upstream place contains at least one token.

When transition t fires, it deletes from each place in its input set $\bullet t$ (preplaces of transition t) as many tokens as the multiplicity of the arc connecting that place to t, and adds to each place in its output set $t \bullet$ (postplaces of transition t) as many tokens as the multiplicity of the arc connecting t to that place.

Definition 2.3.2 A *firing* of an enabled transition removes one token from each of its upstream (input) places and adds one token to each of its downstream (output) places.

The firing of transition t, enabled in marking M produces marking M' such that

$$M' = M + O(t) - I(t)$$

This statement is indicated as $M\ [t > M'$ and is said that M' is directly reachable from M. (Blätke 2011, p. 7-8).

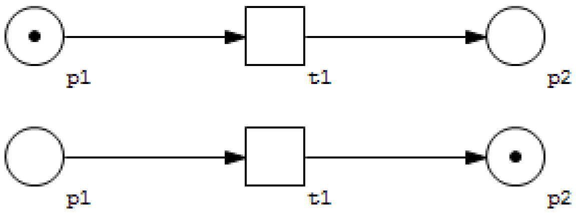

Figure 4. A firing transition. The marking before firing transition t_1. The marking after firing t_1.

3 BASIC PROPERTIES OF PETRI NETS

With Petri net models two types of properties can be studied: properties which depend on the initial marking and they are called behavioral properties, and properties which are independent of the initial marking and they are known as structural properties.

Before presenting the behavioral and structural properties, two features of Petri nets will be introduced: event graph and state machine.

Definition 3.1 A Petri net is called an *event graph* if each place has exactly one upstream and one downstream transition.

Definition 3.2 A Petri net is called a *state machine* if each transition has exactly one upstream and one downstream place.

In an event graph, a token can be consumed by only one transition and several places can come before a given transition. They can model synchronization and may be referred to as *marked graphs* or *decision free Petri nets*.

A state machine can model competition and does not allow synchronization. State machines have constant number of tokens and are referred to as *strictly conservative*. (Baccelli et al. 2001, p. 59)

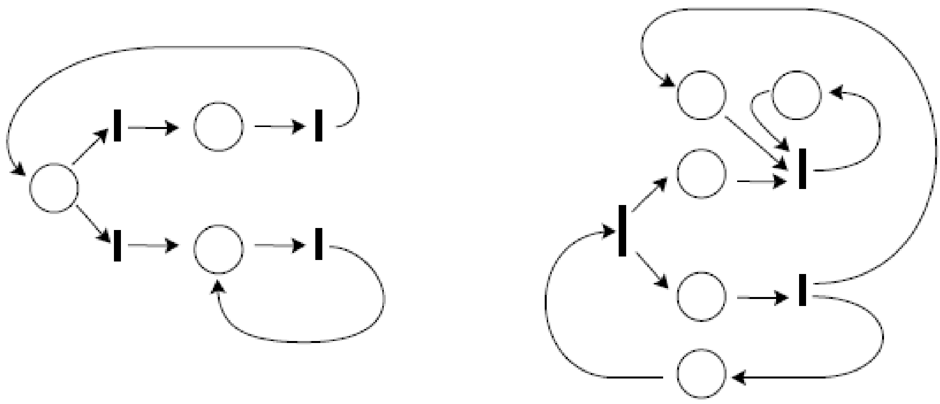

Figure 5. A state machine and an event graph
(Source: Synchronization and Linearity, Baccelli et al. 2001, pp.59)

3.1 Behavioral Properties

1. *Reachability* is the fundamental base for studying the dynamic properties of any system. The firing of the enabled transition will change the token distribution, and the sequence of firings issues the sequence of markings. A sequence of firings is denoted by

   $\sigma = M_0 \, t_1 \, M_1 \, t_2 \ldots t_n \, M_n \text{ or } \sigma = t_1 \, t_2 \ldots t_n$

   The marking Mn is reachable from the initial marking $M0$ if there exists a firing sequence that transforms $M0$ into Mn and in this case, is said that M_n is reachable from $M0$ by σ, and is indicated as $0\ [\sigma > Mn$. By $R(N, M0)$ or $R(M0)$ is denoted the set of all possible markings reachable from $M0$ in a net $(N, M0)$, and by $L(N, M0)$ or $L(M0)$ is denoted the set of all possible firing sequences from $M0$ in a net $(N, M0)$.

2. *Liveness* is related to potential fireability in all reachable markings. A transition is *live* if it is potentially firable in any marking, and it is *dead*, if it is not potentially firable. *Deadlock-freeness* is a weaker condition in which fireability of the net system is guaranteed, but some parts of it may not work at all.

3. *Safeness.* A Petri net is *safe* if the number of tokens in each place is not greater than 1 in any marking.

4. *Boundedness* characterizes the finiteness of the state space and it is a simple generalization of safeness. A Petri net is *bounded* if the token count in each place is less or equal to k (a finite number) for every marking reachable from the initial marking.

5. *Reversibility* characterizes recoverability of the initial marking from any reachable marking. In some cases, it is not necessary to get back to the initial marking as long as one can get back to some marking. In that case, the marking is called *Home State*.

6. *Conservation*. A Petri net is strictly *conservative* if the token count is constant in each marking.

7. *Coverability*. A marking M is *coverable* from $M0$ if there exists a marking $M' \in R(M0)$ such that $M(p) \leq M'(p)$ for all places p in the net.

8. *Persistance.* A Petri net is *persistent* if the firing of one transition from two enabled ones will not disable the other transition. In a persistent net, an enabled transition will remain enabled until it fires.

9. *Fairness*. The literature on Petri nets are proposes different notions of fairness. Here is presented one basic concept: *bounded-fairness*. A Petri net is a *bounded-fair (B-fair)* net if each pair of transitions in the net is in a B-fair relation; and for two transitions to be in a B-fair relation, the maximum number of times, that either one can fire, has to be bounded while the other transition is not firing.

3.2 Structural Properties

Whereas the behavioral properties of Petri nets are dependent on the initial marking and the firing rule, the structural properties depend only on the topological structure of Petri nets. These structural properties have a high significance in the designing of manufacturing systems, as they are dependent just on the layout, and not on the manner the system will be managed, a fact that is not known at the design level. The structural properties of Petri nets include *liveness*, *boundedness*, *consistency*, *repetitivity*, *conservativeness*, and *controllability* properties, and most of them can be verified by means of algebraic techniques.

1. *Structural Liveness*. A Petri net is *structurally live* if there exists a live initial marking.

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

3. *Consistency*. A Petri net is *consistent* if there exists an initial marking and a firing sequence from the initial marking back to the initial marking such that each transition occurs at least one in the firing sequence.

4. *Repetitivity*. A Petri net is *repetitive* if there exists an initial marking and a firing sequence from the initial marking such that each transition occurs unlimitedly in the firing sequence.

5. *Conservativeness*. A Petri net is *conservative* if all transitions add exactly as many tokens to their output places as they subtract from their input places.

6. *Controllability*. A Petri net is *completely controllable* if either marking is reachable from any other marking.

4 MODELING AND ANALYSING WITH PETRI NETS

A model represents a partial conception of the reality and the description of its main features and the connections among them. Many models may be given for a certain object of study and each of them may be focusing on different features of the given object. Computational modeling is a mathematical representation of the reality, and computational models simulate the reality by using the language of mathematics.

The starting point for modeling is represented by the following selection:

- Features whose effects are neglected and they will be ignored in the model.
- Features that affect the model but their behavior will not be studied in the model and they will be represented as inputs, parameters, external or independent variables of the model.
- Features that the model is focusing on, and they will represent the internal or dependent variables of the model.

The validation of models is checked by comparison to experimental data and as result models can be invalidated. Models are not certain, are not the reality, and their validation cannot be absolutely confirmed by experimental data. (Petre 2013).

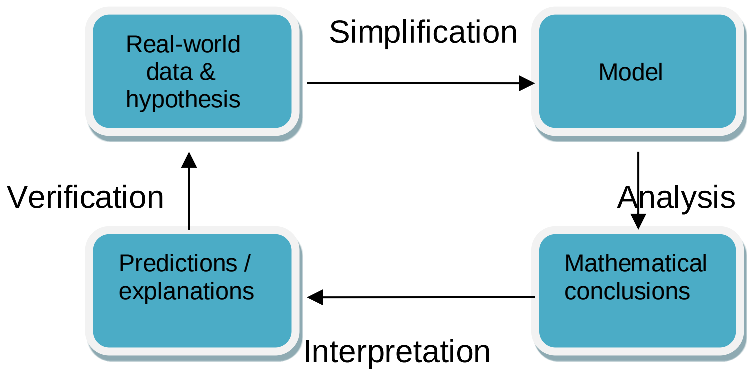

Figure 6. Modeling cycle (Petre 2013)

Petri nets were designed for and are used particularly for modeling. Petri nets are used to model systems with independent components, the occurrence of different activities and events in a system, and the flow of information or other resources within a system. The systems may be of numerous various kinds: computer hardware, computer software, computer networks, real-time computing systems, communication systems, logistic networks, manufacturing plants, physical systems, social systems, biological systems, and so on.

Petri nets compound a mathematical theory with a graphical representation of the dynamic behavior of a system.

Some of the methods used for modeling and analyzing systems with Petri nets are the reachability tree and incidence matrix.

Definition 4.1 ***(Reachability tree)*** The reachability tree of a Petri net (G, μ) is a tree with nodes in $N\ |P|$ which is obtained as follows: the initial marking μ is a node of this tree; for each q enabled in μ, the marking μ obtained by firing q is a new node of the reachability tree; arcs connect nodes which are reachable from one another in one step; this process is applied recursively from each such μ. (Baccelli et al. 2001, pp. 57)

Definition 4.2 *(Reachability graph*) The reachability graph is obtained from the reachability tree by merging all nodes corresponding to the same marking into a single node. (Baccelli et al. 2001, pp. 57)

The following example shows a Petri net with both transitions enabled and an initial marking $(1,1,1,1)$. If transition $q1$ is the first to fire, the next marking becomes $(1,1,0,2)$. In case that $q2$ fires first, the marking will be $(1,1,2,0)$. Starting from $(1,1,0,2)$, only $q2$ can fire and leads to the initial marking, and from $(1,1,2,0)$, by firing $q1$, only the initial marking can be reached. The reachability graph of $(1,1,1,1)$ has three different markings.

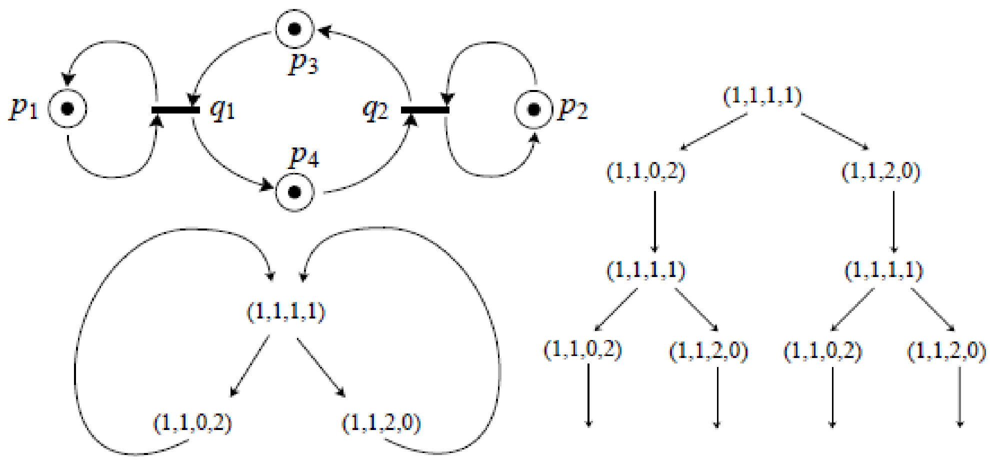

Figure 7. A Petri net with corresponding reachability graph and reachability tree (Source: Synchronization and Linearity, Baccelli et al. 2001, pp. 58)

An alternative method for representation and analysis of Petri nets is based on matrix equations used to represent the dynamic behavior of Petri nets. The method involves constructing the **incidence matrix** that defines all possible interconnections between places and transitions. The incidence matrix of a Petri net is an $n \times m$ matrix, where n is the number of transitions and m is the number of places.

4.1 Biomodeling with Petri Nets

Biological systems consist of species and the interactions among them. Some interactions can be independent and could fire in parallel. The characteristics of biological systems of being bipartite and having concurrent behavior make them suitable for modeling with Petri nets. The advantage of using Petri nets for modeling biological systems lies in the ability of analyzing both structural and behavioral properties of the system. Two important properties of PN met while modeling biological systems are the places and transitions invariants (P- and T-invariants). P-invariants are sets of places with constant weighted sum of tokens and they correspond to mass conservation relations. T-invariants are sets of transitions whose ordered firing reproduces the initial state and they correspond to elementary modes of a system.

A reaction-based biological network is formed out of a set of reactions with the following general form $S1 + S2 + \ldots + Sn \rightarrow P1 + P2 + \ldots + Pm$, where species $S1, \ldots, Sn$ represent the substrate of the reaction and species $P1, \ldots, Pm$ represent the products of the reaction.

Within the Petri nets formalism, species are modeled as places and reactions as transitions having substrates as pre-places and products as post-places. (Gratie & Petre 2013).

The following table presents some of the properties of Petri nets and their biological significance.

Table 1. Petri nets properties and their biological significance

Property	***Biological Significance***
Structurally bounded	*Components may not accumulate indefinitely, independently of the initial conditions.*
Liveness	*All reactions occur repeatedly.*
Reversibility	*The initial state can be reproduced by any possible state reachable from the initial conditions.*
Dead states	*States where no reaction can occur.*
Dead transitions	*Reactions that cannot occur in any state reachable from the initial state.*
Pure	*No component can be produced and consumed by the same reaction.*
Homogenous	*Each species is consumed in same amount by all reactions.*
Conservative	*Total amount of produced and consumed molecules by a reaction is equal.*

4.2 Snoopy and Charlie modeling tools

Snoopy is a software tool designed for modeling and running Petri nets. It supports standard PN as well as many extensions of the formalism. Snoopy provides different shapes for net elements, coloring of graph elements, animation and simulation of PN, exporting and importing to and from different file types.

For analyzing a model, Snoopy offers support for Charlie. Charlie is a software tool designed for analyzing structural and behavioral properties of PN and is used for the verification of different systems and the validation of natural systems.

For the case study presented in this thesis, the last versions of Snoopy (2013-07-30) and Charlie (2013-05-13) will be used.

5 MODELING OF THE HEAT SHOCK RESPONSE WITH PETRI NETS

5.1 The Heat Shock Response

The heat shock response (HSR) is a highly conserved regulatory mechanism that allows the cell to quickly react to elevated temperatures and stress conditions. A heat shock is produced by the exposure of a cell to a temperature greater than its optimum. The equilibrium in an over-heated cell is recovered by the heat shock response that diminishes the shock effects through the activity of specialized proteins. Exposed to high temperatures, proteins misfold and tend to form large aggregates with killing effects on the cell leading to programmed cell death. Opposing this, cells produce heat shock proteins (hsps) with the role of assisting misfolding proteins (mfps) in their correct refolding.

The heat shock response has been the subject of active research in the past decades, as the hsps play a major role in many biological processes. Thus, understanding the mechanism of HSR has fundamental importance for the biology of the cell as well as response to cellular affronts and treatment of certain diseases.

In this thesis, for our Petri net model, we consider the basic molecular model for the heat shock response proposed in the research paper *“A simple mass-action model for the eukaryotic heat shock response and its mathematical validation.”* (Petre et al. 2011).

5.2 Molecular Model for the Heat Shock Response

Through the course of the heat shock response, the heat shock factors (hsfs), also called monomers, form active components known as trimers, which bind to heat shock elements (hses). This bind leads to the appearance of heat shock proteins. The presence of sufficient amounts of hsp contributes to unbinding trimers from hse and separates them into monomers. Hsps facilitate the refolding of misfolding proteins.

The molecular model considered in this paper contains 10 species and 12 reactions, as proposed in the publication mentioned above.

The species are listed in Table 2, and following simplifications have been done: all types of heat shock proteins are treated as one species (hsp), all types of heat shock factors are also treated as one species (hsf), the aspect of proteins taken into account for the modeling process is the folding of proteins: correct folded (prot) or misfolded proteins (mfp).

The species of the basic molecular model for HSR are shown in Table 2.

Table 2. Species of HSR molecular model

$Species\ Number$	$Species$	$Significance$
(1)	hsf	$heat\ shock\ factor\ in\ monomeric\ state$
(2)	$hsf2$	$heat\ shock\ factor\ in\ dimeric\ state$
(3)	$hsf3$	$heat\ shock\ factor\ in\ trimeric\ state$
(4)	hse	$heat\ shock\ element$
(5)	$hsf3 : hse$	$a\ binding\ from\ hsf\ to\ hse$
(6)	hsp	$heat\ shock\ protein$
(7)	$hsp : hsf$	$a\ binding\ from\ hsp\ to\ hsf$
(8)	$prot$	$correctly\ folded\ protein$
(9)	mfp	$misfolded\ protein$
(10)	$hsp : mfp$	$a\ binding\ from\ hsp\ to\ mfp$

The reactions of the model for the heat shock response are presented in Table 3. Reactions 1 and 2 contain the dimerization and trimerization of heat shock factors; in reaction 3 trimers bind to heat shock elements; reaction 4 covers the generation of new heat shock proteins; in reactions 5-8, heat shock proteins contribute to the unbinding of heat shock factors, dimers (hsf2) and trimers (hsf3); with reaction 9 is represented the degradation of heat shock proteins; reactions 10-12 cover the protein misfolding and folding activities.

Table 3 presents the list of reactions in the molecular model for the heat shock response.

Table 3. Reactions of HSR molecular model

Reaction Number	***Reaction***
(1)	$2hsf \leftrightarrow hsf_2$
(2)	$hsf + hsf_2 \leftrightarrow hsf3$
(3)	$hsf3 + hse \leftrightarrow hsf3 : hse$
(4)	$hsf3 : hse \rightarrow hsf3 : hse + hsp$
(5)	$hsp + hsf \leftrightarrow hsp : hsf$
(6)	$hsp + hsf2 \rightarrow hsp : hsf + hsf$
(7)	$hsp + hsf3 \rightarrow hsp : hsf + 2hsf$
(8)	$hsp + hsf3 : hse \rightarrow hsp : hsf + hse + 2hsf$
(9)	$hsp \rightarrow \emptyset$
(10)	$prot \rightarrow mfp$
(11)	$hsp + mfp \leftrightarrow hsp : mfp$
(12)	$hsp : mfp \rightarrow hsp + prot$

5.3 Petri Net Model for the Heat Shock Response

The Petri net model of the heat shock response molecular model can be seen in Figures 9 and 10. In order to be able to validate our model, the numerical setup, the reaction parameters and the initial concentrations of species are consistent with the values reported in *"A simple mass-action model for the eukaryotic heat shock response and its mathematical validation"* publication and presented in the following tables:

Table 4. Reaction parameters

$\boldsymbol{Reaction}$	$\boldsymbol{Reaction\ Parameter}$
$R1\_fw$	3.49
$R1\_bw$	0.19
$R2\_fw$	1.07
$R2\_bw$	10^{-9}
$R3\_fw$	0.17
$R3\_bw$	$1.21 * 10^{-6}$
$R4$	$8.3 * 10^{-3}$
$R5\_fw$	9.74
$R5\_bw$	3.56
$R6$	2.33
$R7$	$4.31 * 10^{-5}$
$R8$	$2.73 * 10^{-7}$
$R9$	$3.2 * 10^{-5}$
$R10$	$7.77 * 10^{-5}$
$R11\_fw$	$3.32 * 10^{-3}$
$R11\_bw$	4.44
$R12$	13.94

Table 5. Initial concentrations of species

Species	***Initial Concentration***
hsf	0.67
$hsf2$	$8.7 * 10^{-4}$
$hsf3$	$1.2 * 10^{-4}$
hse	29.73
$hsf3 : hse$	2.96
hsp	766.88
$hsp : hsf$	1403.13
$prot$	$1.15 * 10^{8}$
mfp	517.352
$hsp : mfp$	71.65

To be able to compare the predictions of our study case with Snoopy implementation, the simulation time was 14400s, and the results are consistent as those reported in the publication. At 37° C, when no regulatory activities occur, the model is considered to be in a steady state. At 42° C, hsf3s bind to hses located on DNA strands and promote replication of heat shock genes. Hsps assist the correct folding of misfolding proteins and they react with hsf3's breaking bounds of hsf3:hse. Eventually, the concentration of DNA binding activity returns to basal level. Figure 8 illustrates the DNA binding activity of our model at 42° C.

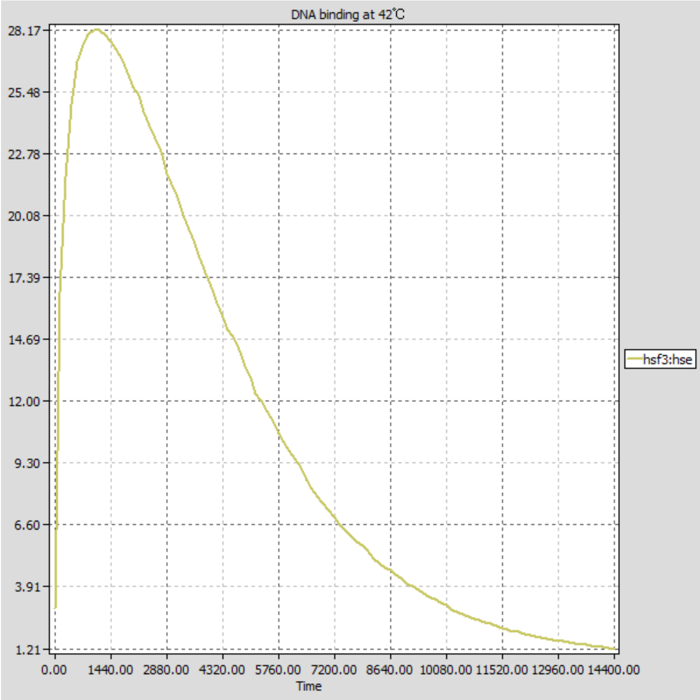

Figure 8. DNA binding activity at 42° C

To analyze our Petri net model, we used Charlie, the software tool that checks and computes several properties of the network. Two important properties of a Petri net for a biological system are the place invariants and transition invariants.

The P-invariants of our Petri net model are shown in Table 6 and they are consistent and encoded with the three mass conservation relations of the heat shock response molecular model reported in the *"A simple mass-action model for the eukaryotic heat shock response and its mathematical validation"* publication.

Mass conservation relations of the molecular model:

1. $[hse] + [hsf3{:}\,hse] = constant$
2. $[hsf] + 2 \times [hsf2] + 3 \times [hsf3] + 3 \times [hsf3{:}\,hse] + [hsp{:}\,hsf] = constant$
3. $[prot] + [mfp] + [hsp{:}\,mfp] = constant$

The first reaction is concerned with the total amount of heat shock elements; the second is concerned with the total amount of heat shock factors; the third reaction is concerned with the total amount of proteins. The amounts of heat shock elements, heat shock factors and proteins are constant in a cell.

Table 6. The P-invariants for the HSR reported by Charlie

Number	***Components***	***Multiplicity***
1	hse	1
	$hsf3{:}hse$	1
2	hsf	1
	$hsf2$	2
	$hsf3$	3
	$hsf3{:}hse$	2
	$hsp{:}hsf$	1
3	$prot$	1
	mfp	1
	$hsp{:}mfp$	1

The model being covered with P-invariants, also suggests that the Petri net is structurally bounded and conservative.

Table 7 illustrates the T-invariants of our model. They all validate the model in the sense that all successions of reactions that balance each other out, are present in a t-invariant. For example, the T-invariant 9 denotes the sequence of reactions needed in order to first produce and then consume one token of misfolded protein. Being covered by T-invariants, the net is repetitive and consistent.

The reachability graph property checked with Charlie, indicated that the reachability graph of the net contains one single place, meaning that all places are interconnected and from each place it can be reach any other one by following successive arcs.

Table 7. The T-invariants for the HSR reported by Charlie

Number	***Transition***	***Invariant***
1	$R3\_fw$	1
	$R3\_bw$	1
2	$R2\_fw$	1
	$R2\_bw$	1
3	$R1\_bw$	1
	$R1\_fw$	1
4	$R5\_fw$	1
	$R5\_bw$	1
5	$R1\_fw$	1
	$R5\_bw$	1
	$R6$	1
6	$R1\_fw$	1
	$R2\_fw$	1
	$R5\_bw$	1
	$R7$	1
7	$R1\_fw$	1
	$R2\_fw$	1
	$R3\_fw$	1
	$R5\_bw$	1
	$R8$	1
8	$R11\_fw$	1
	$R11\_bw$	1
9	$R10$	1
	$R11\_fw$	1
	$R12$	1
10	$R4$	1
	$R9$	1

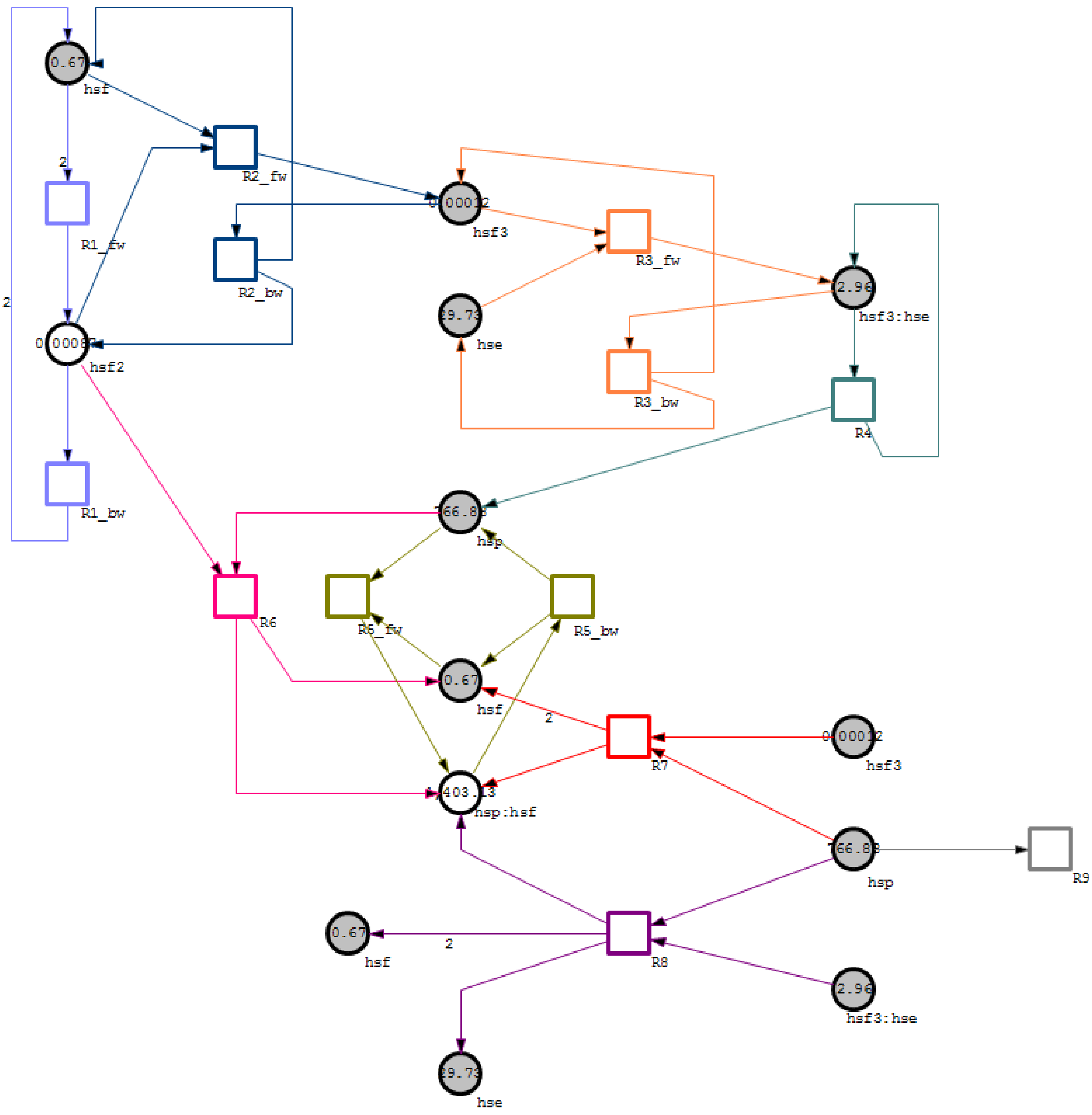

Figure 9. Petri net model for HSR containing the first 9 reactions of the molecular model

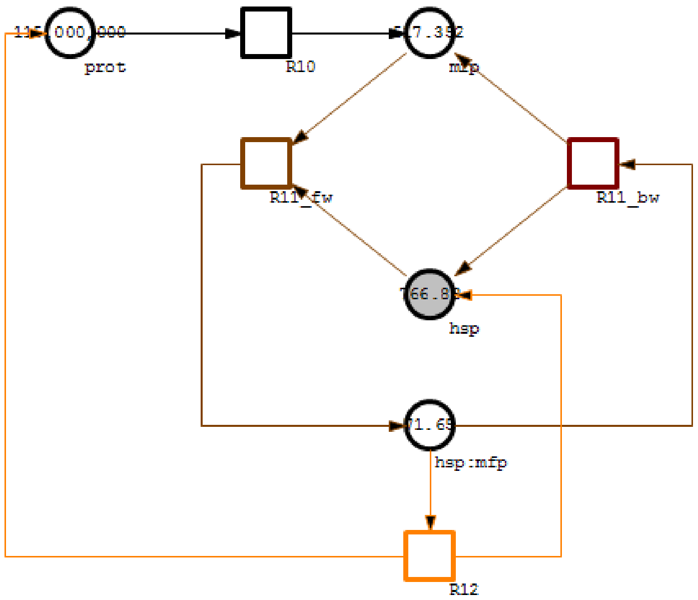

Figure 10. Petri net model for HSR containing reactions 10, 11, 12 of the molecular model

For a better visualization and clarity of the model, transitions (reactions in molecular model) and their corresponding arcs are depicted with different colors. The species of the molecular model represent the variables in the Petri net model, and graphically, they are indicated as circles containing their initial values (concentrations). To avoid the overlapping of arcs where possible, some species involved in more than one reaction, repeat in the model and they are indicated by grey filled places.

6 CONCLUSION

This thesis offered a brief introduction to Petri nets and their applications. Since their introduction, Petri nets have been modified and theoretically investigated and applied to a vast number of areas. Their research touched not only on various aspects of computer science and mathematics, but on natural sciences as well, representing a favorable mean for modeling and analyzing biological systems.

Modeling with Petri nets permits structural and behavioral analysis of modeled systems. In our Petri net model for the Heat Shock Response, we studied, in principle, the place invariants and transition invariants, and the model showed that they are similar with reported biological data.

Modeling and analyzing biology systems are significant for a better understanding of biology by reconstructing rules underlying biological systems, as well as for medical diagnosis and therapeutic suggestions.

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