Mary Ann Blätke

Tutorial

Petri Nets in Systems Biology

1st Edition, August 2011

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CHAPTER 1

Introduction

What is the background of this tutorial? During the last decade the integrative research area of systems biology has been constantly gaining more importance. Experimental and computational approaches are combined to systematically investigate biological systems. To understand biology on its system level, the structural and dynamic properties of regulatory networks in biological systems have to be represented by a model describing the involved species and their interactions. Petri net theory offers the possibility to construct and analyse such models and to represent their structural and dynamic propertoies by various techniques.

Who should read this tutorial? This tutorial addresses scientists who are looking for an easy and intuitive way to translate a biological system into a Petri net model at arbitrarily chosen level of abstraction with the option of representing time and/or space-dependent processes. The tutorial is equally suitable for experimental and theory oriented bio-scientists. The examples given in the tutorial can be used by the interested reader to model her/his own biological system.

What can I learn? The tutorial offers an introduction to the Petri net formalism, how to construct a model of a biological system, analyse its structure and dynamic behaviour in terms of time-dependent behaviour, which is shown by several intuitive examples. At the end of the tutorial you will be able to model a biological system on your own using Petri nets. You will also know how to analyse the structure of your model, how to interpret the results and how to perform simulation studies to investigate the time-dependent dynamic behaviour. In addition, we also provide a chapter about model checking, which might be helpful to evaluate your model by verifying specified properties that you are interested in. We also show how to use the two Petri net tools *Snoopy* [19] and *Charlie* [8]. Based on these instruments you will be able to enhance your knowledge about the modelled biological system and to draw new conclusions from that.

Why should I use Petri nets? The graphical notation and construction of Petri nets allows you to easily and intuitively construct models of biological systems and to characterize the structure, behavioural properties related to the structure and time-dependent dynamic behaviour of a model by several related techniques. Petri nets can describe concurrent and parallel processes, as well as communication and synchronization in bipartite systems regardless of the abstraction level in a comprehensive and mathematically correct model [12]. Time as well as space aspects can be modelled by a Petri net. Several specialized Petri net classes are available to describe different scenarios and to consider different simulative approaches. Therefore, the kinetics of the qualitative Petri net model can be considered as stochastic, continuous or as a mixture of both (hybrid) [12].
In silico experiments with Petri net models permit to systematically analyse a biological system by

applying structural as well as dynamic analysing techniques to investigate perturbations. From the obtained results new insights can be achieved about the biological system. Thereby, you can increase your understanding, reveal gaps in knowledge, and detect missing and/or essential components. Based on a valid model it is possible to predict the system behaviour. This is might be helpful to investigate pathological states and their molecular basis aimed at identifying potential targets to develop therapeutical intervention strategies.
The Petri net formalism offers quite a few advantages over other and more broadly used modelling frameworks.. The different Petri net classes are interconvertible with each other without changing the qualitative structure. Due to the graphical visualisation of molecular networks by Petri nets, a bioscientist can intuitively understand the modelled mechanisms. The user does not have to deal with many different representations of a molecular network which do not obviously correspond to each other like a biological cartoon, the structure of the biological network, the mathematical equations (stochastic, continuous, etc.) and the implementation of the equations. Besides, the transformation of a molecular network represented by an Petri net into e.g. ODE equations is unique, but not vice versa [24]. Several reliable analysis tools have been develop to investigate qualitative and quantitative properties of Petri nets by structural analysis, simulation of the time-dependent dynamic behaviour and model checking.

What is the scheme of this tutorial? First of all, you will learn all the basics about the Petri net formalism motivated by small biological examples that are easy to understand. Next, you will see how to analyse the structure of a model and how to interpret the obtained results and their biological meaning. Afterwards, you will learn how to perform simulations with your model. We also offer a chapter about model checking, where you can learn how to verify specific properties of your model that you are interested in. Then, we introduce the two Petri net tools *Snoopy* [19] and *Charlie* [8].
All sections, where theoretical concepts are explained, are divided into an informal and a formal part. We start with an informal introduction, where we explain the basics and the biological relations. Subsequently and to be complete, we give the formal definitions and a small help on "how to read" the definitions at the end of the section.

What tools do I need? Several tools are available to model biological systems, simulate their time-dependent dynamic behaviour and analyse their structure. Here, we use the Petri net editor *Snoopy* [19] to model biological systems and simulate/animate their time-dependent dynamic behaviour. *Charlie* [8] is used to analyse the Petri net structure. Both software tools were developed at the chair of Data Structures and Software Dependability at the Brandenburg University of Technology Cottbus and are freely available for non-commercial use. You can download them at `http://www-dssz.informatik.tu-cottbus.de/DSSZ/Software/Software` [1].

CHAPTER 2

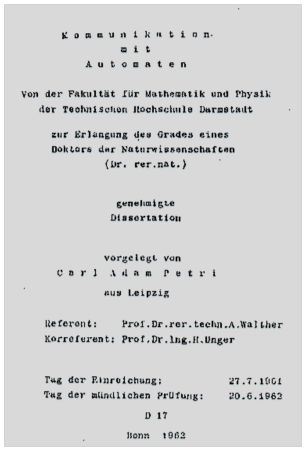
Petri Net Basics

In this chapter we give you all relevant information about Petri nets. We answer the questions:

What are Petri Nets?
Why are Petri nets useful and efficient in modelling biological systems?
How are Petri nets defined?

First of all, what does "Petri" mean?

> "Petri nets are used as a formal and graphically appealing language which is appropriate for modelling systems with concurrency and resource sharing. Petri net modelling has been under development since the beginning of the 60'ies, where Carl Adam Petri defined the language. It was the first time a general theory for discrete parallel systems was formulated. The language is a generalization of automata theory such that the concept of concurrently occurring events can be expressed." [2]



Kommunikation
mit
Automaten

Von der Fakultät für Mathematik und Physik
der Technischen Hochschule Darmstadt

zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer.nat.)

genehmigte
Dissertation

vorgelegt von
Carl Adam Petri
aus Leipzig

Referent: Prof.Dr.rer.techn.A.Walther
Korreferent: Prof.Dr.Ing.H.Unger

Tag der Einreichung: 27.7.1961
Tag der mündlichen Prüfung: 20.6.1962

D 17

Bonn 1962

Figure 2.1: ***Carl Adam Petri.*** *Carl Adam Petri (12 July 1926 – 2 July 2010) was a German mathematician and computer scientist. He was born in Leipzig. Petri nets were invented in August 1939 by Carl Adam Petri at at the age of 13 for the purpose of describing chemical processes. He documented the Petri net in 1962 as part of his dissertation, Kommunikation mit Automaten (communication with automata) [4].*

2.1 General Information about the use of Petri Nets in modeling biological processes

Petri nets were originally designed to represent discrete, concurrent processes of technical systems. They combine an intuitive, unambiguous, qualitative bipartite graphical representation of arbitrary processes with a formal semantics. Thus, the power of Petri nets is the explicit representation of concurrent processes, but they also offer a simple and flexible modelling language. Petri nets are also powerful and useful in modelling biological systems. Petri nets may unambiguously represent (bio-)chemical reactions in metabolism, signal transduction and gene expression and have been applied to neuronal processes as well. In the biological context, Petri nets are especially efficient in reconstructing complex molecular networks. A Petri net may represent:

- Stochastic (discrete) and kinetic (continuous) processes at arbitrary resolution of molecular detail within a single, coherent model;
- any chemical or biochemical reaction at any resolution of kinetic detail,
- the localization of molecules in different spatial compartments (cytoplasm, nucleus, etc.), as well as different localization in 1-, 2- or 3- dimensional space, and the translocation between different locations;
- the signalling states of single molecules, circuits or networks,
- the physiological state, behaviour or response of a cell.

Petri nets have already been applied to biological case studies like the regulation of the *lac* operon [21], Duchenné muscular dystrophy [10], the response of *S. cerevisiae* to copulatory hormones [7] and the yeast cycle [18]. Two examples for Petri nets applied to metabolic systems are the sucrose breakdown pathway in the potato tuber [13] and the iron homoeostasis process in human body [20]. There are a lot of other interesting articles dealing the application of Petri nets to biological systems, which can not all be cited. The abstraction degree of a model can vary from single molecules to cells to multicellular aggregations. Even a whole organism or population can be described by a Petri net. A Petri net can always be extended or edited by refining the components of the model by subnets. Advantageously, missing qualitative or kinetic information can be handled by Petri nets. The iterative process between wet-lab experiment and model-based predictions enables the researcher to close such gaps.

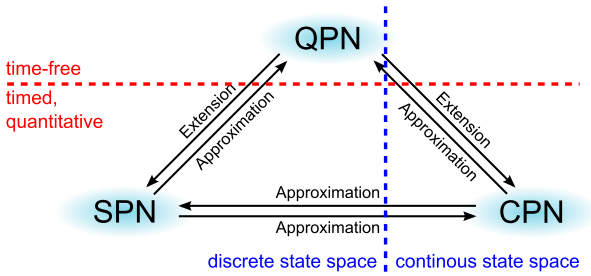


Figure 2.2: ***Conceptual Framework.*** *The Petri net formalism allows to switch between different network classes to describe qualitative (QPN), stochastic (SPN) and continuous (CPN) information in a cohesive Petri net model [12].*

Petri nets may serve as umbrella formalism to integrate qualitative and quantitative modelling, which allows to apply various analysis techniques. Several reliable software tools, which support the Petri net formalism are developed by the international community on computational methods [19]. Several specialized Petri net classes like qualitative, stochastic, continuous, or hybrid Petri nets and their coloured counterparts are available to describe different scenarios and to consider different simulative approaches. All network classes are interconvertible with each other without changing the network structure; see **Figure 2.2**. This allows the application of the same powerful analysis techniques to the underlying qualitative structure of all Petri net classes [12]. The wide range of network classes allows

the integration of qualitative, continuous and stochastic information. This allows the representation of different kinetic processes and different data types. Petri nets link structural and dynamic analysis techniques to investigate and validate a model such as graph theory, application of linear algebra to check a model and simulation methods. This facilitates the performance of simulation studies to explore the time-dependent dynamic behaviour, the in-depth analysis of structural criteria and the state space of a model.

2.2 Standard Petri Net

A Petri net is represented by a directed, finite, bipartite graph, typically without isolated nodes. The four main components of a general Petri net are: places, transitions, arcs and tokens; see **Figure 2.3, A**.

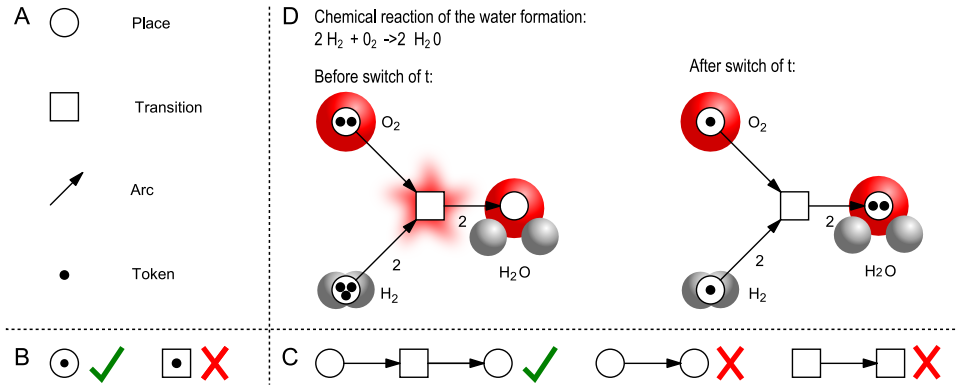


Figure 2.3: ***Petri Net Formalism.*** *Petri nets consist of places, transitions, arcs and tokens (A). Just places are allowed to carry tokens (B). Two nodes of the same type can not be connected with each other (C). The Petri net represents the chemical reaction of the water formation (D). A transition is enabled and may fire if its pre-places are sufficiently marked by tokens.*

Places are passive nodes. They are indicated by circles and refer to conditions or states. In a biological context, places may represent: populations, species, organisms, multicellular complexes, single cells, proteins (enzymes, receptors, transporters, etc.), molecules or ions. But places could also embody temperature, pH-value or membrane potential; see also **Section 3.3.1**. Only places are allowed to carry tokens; see **Figure 2.3, B**.

Tokens are variable elements of a Petri net. They are indicated as dots or numbers within a place and represent the discrete value of a condition. Tokens are consumed and produced by transitions; see **Figure 2.3, D**. In biological systems tokens refer to a concentration level or a discrete number of a species, e.g., proteins, ions, organic and inorganic molecules. Tokens might also represent the value of physical quantities like temperature, pH value or membrane voltage that effect biological systems. A Petri net without any tokens is called "empty". The initial marking affects many properties of a Petri net, which are considered in **Chapter 2**.

Transitions are active nodes and are depicted by squares. They describe state shifts, system events and activities in a network. In a biological context, transitions refer to (bio-)chemical reactions, molecular interactions or intramolecular changes; see also **Section 3.3.1**. If a place is connected by an arc with a transition, the place (transition) is called pre-place (post-transition). If a transition is connected by an arc with a place, the transition (place) is called pre-transition (post-place); see **Figure 2.4**. Transitions consume tokens from its pre-places and produce tokens within its post-places according to the arc weights; see **Figure 2.3, D**.

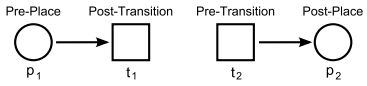


Figure 2.4: ***Places and Transitions.*** *Place p_1 is called pre-place of transition t_1, and transition t_1 is the post-transition of place p_1. Place p_2 is called post-place of transition t_2, and transition t_2 is the pre-transition of place p_2.*

Directed arcs are inactive elements and are visualised by arrows. They specify the causal relationships between transitions and places and indicate how the marking is changed by firing of a transition. Thus, arcs define reactants/substrates and products of a (bio-)chemical reaction. Arcs connect only nodes of different types; see **Figure 2.3, C**. Each arc is connected with an arc weight. The arc weight sets the number of tokens that are consumed or produced by a transition. The stoichiometry of a (bio-)chemical reaction can be represented by the arc weights.

The **Petri net Semantic** describes the behaviour of the net, which is defined by firing rules consisting of a precondition and the firing itself. The firing of a transition depends on the marking of its pre-places. A transition is enabled and may fire, if all pre-places are sufficiently marked. If a transition has no pre-places it is always enabled to fire. By firing a transition moves tokens from preplaces to postplaces and possibly changes the number of tokens. The firing of a transition changes the marking of the connected places. Thus, the marking of the net is changed to a new reachable marking, where some transitions are not any more enabled while others get enabled. The behaviour of a net is established by repeated firing of transitions. All possible ordered firing sequences result into the whole net behaviour, which is also called state space.

Formal Definitions:

Definition 2.1 (Standard Petri net) A standard Petri net *is a quadruple $N = (P, T, f, m_0)$, where:*

- *P, T are finite, non-empty, disjoint sets. P is the set of* places. *T is the set of* transitions.
- *f: $((P \times T) \cup (T \times P)) \to \mathbb{N}_0$ defines the set of directed* arcs, *weighted by non-negative integer values.*
- *m_0: $P \to \mathbb{N}_0$ gives the* initial marking.

How to read:
Assume the following small example of an enzymatic reaction; see **Figure 2.5**:

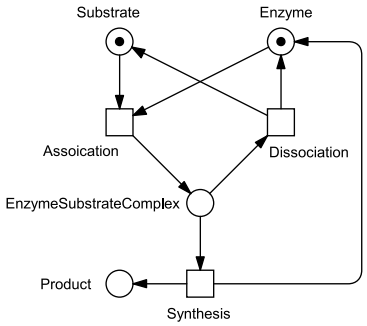


Figure 2.5: ***Running Example.*** *To illustrate the definition, we use the example of an enzymatic reaction $A + E \leftrightarrow AE \to E + B$.*

The Petri net $N = (P, T, f, m_0)$ in our running example; see **Figure 2.5**, consists of places P, transitions t, directed arcs f and the initial marking m_0:

- Set of places P: $P = \{Enzyme, Substrate, EnymeSubstrateComplex, Product\}$
- Set of places T: $T = \{Association, Dissociation, Synthesis\}$
- Set of directed arcs: $((P \times T) \cup (T \times P))$ is the combination of the following subsets
  - Places connected with $(\rightarrow)$ transitions:

$$\begin{aligned}(P \times T) = \{&Substrate \times Association,\\ &Enymze \times Assoication,\\ &EnymeSubstrateComplex \times Dissociation,\\ &EnymeSubstrateComplex \times Synthesis\}\end{aligned}$$

  - Transitions connected with $(\rightarrow)$ places:

$$\begin{aligned}(T \times P) = \{&Assoication \times EnymeSubstrateComplex,\\ &Dissociation \times Substrate,\\ &Dissociation \times Product,\\ &Synthesis \times Product,\\ &Synthesis \times Enzyme\}\end{aligned}$$

- Initial Marking m_0: $m_0 = \{Enzyme = 1, Substrate = 1, EnymeSubstrateComplex = 0, Product = 0\}$; the amount of tokens must be expressed as an integer variable.

At this point, we also like to introduce further notions and notations, which we use during the tutorial. The notation $m(p)$ refers to the number of tokens on place p in the marking m. Place p is clean (empty, unmarked) in m if $m(p) = 0$, otherwise place p is clean (empty, unmarked) in m. A set of places is called clean if all places are clean, otherwise the set is marked. The postset and preset of a node $x \in P \cup T$, is defined as:

- Preset: $\bullet x := \{y \in P \cup T \mid f(y, x) \neq 0\}$
- Postset: $x\bullet := \{y \in P \cup T \mid f(x, y) \neq 0\}$

For places and transitions, we get four types of sets:

- $\bullet t$ - preplaces of transition t (reaction's precursor)
- $t\bullet$ - postplaces of transition t (reaction's products)
- $\bullet p$ - pretransitions of place p (all producing reactions of a component)
- $p\bullet$ - posttransitions of place p (all consuming reactions of a component)

This definition can be extended and generalized for a set of nodes $X \subseteq\in P \cup T$. Now, the set of prenodes is given by $\bullet X := \bigcup_{x \in X} \bullet x$ and the set of postnodes refers to $X\bullet := \bigcup_{x \in X} \bullet x$

Definition 2.2 (Firing Rule) *Let N = (P, T, f, m_0) be a Petri net:*

- *A transition is* enabled *in marking m, written as $m\,[t\rangle$, if $\forall p \in \bullet t : m(p) \geq f(p, t)$, else disabled.*
- *A transition t, which is enabled in m, may fire.*
- *When t in m* fires*, a new marking m' is reached, written as $m\,[t\rangle\, m'$, with $\forall p \in P : m'(p) = m(p) - f(p, t) + f(t, p)$.*
- *The firing happens atomically and does not consume any time.*

How to read:
Assume the example above; see **Figure 2.5**:

- The transition *Association* is enabled in marking $m_0 = (1, 1, 0, 0)$, because the marking of both pre-places ($Enzyme = 1$, $Substrate = 1$) of the transition *Assoiciation* are equal to the respective arc weight (in both caes "1").

- Thus, the enabled transition *Association* may fire in m_0.
- Firing of the transition *Association* in m_0, leads to the marking $m_1 = (0, 0, 1, 0)$. The transition *Assoication* removes one token from the place *Enzyme* and one token from the places *Substrate*, both places do not get back any tokens. In consequence, both places are empty. The place *EnzymeSubstrateComplex* gains one new token. The place *Product* is not involved and thus, the number of tokens is not changed at all.

In addition, $m \in \mathbb{N}_0^{|P|}$ defined the marking of the given token situation, whereby $|P|$ denotes the number of places in a Petri net. All markings, which can be reached from a given marking m by any firing sequence, form the set of reachable markings $[m\rangle$. The set of markings $[m_0\rangle$ reachable from the initial marking m_0 constitutes the state space of a model.

2.3 Extended Standard Petri Net

2.3.1 Extended Representation

For representationals reason of large networks two more specific types of nodes (transitions, places) have been developed, called logical nodes and macro nodes. Both types allow to neatly arrange networks, but do not affect the properties of the Petri net. Indeed, this nodes are very useful to represent biological systems

- *Logical nodes*: A logical transition or places is indicated by a slight grey shade; see **Figure 2.6**. Logical nodes can be used to represent frequent elements in a network by identical copies of a node, e.g., reactions or components. Thus, logical nodes are a kind of connectors bringing together identical nodes that are repeated in the network structure. Logical places can be used to represent components with many cross links, like second messengers (cAMP, DAG, IP3 etc.), or energy equivalents (ATP, NADH etc.) that are linked to numerous reactions. Logical transition are able to represent reactions that are repeated all over the network or reaction, where a lot of components are involved. Such a reaction can be split in single parts using logical transitions.

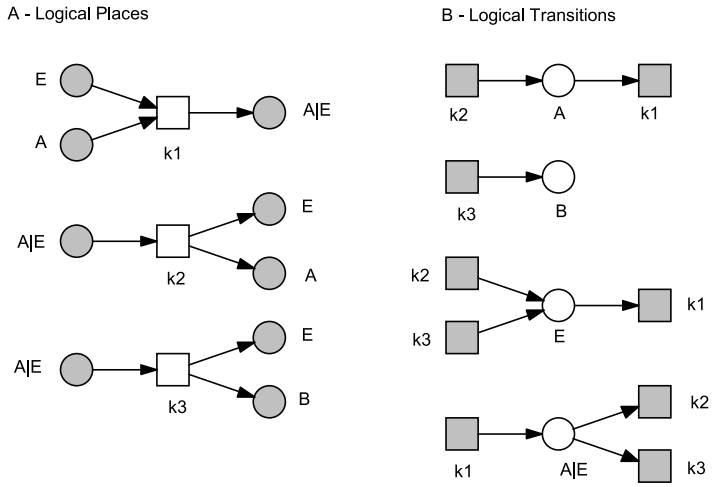


Figure 2.6: ***Logical Nodes.*** *Two representations of a reaction using logical places* ***(A)*** *and logical transitions* ***(B)****. The reaction $A + E \leftrightarrow AE \rightarrow E + B$ is split in single blocks. In* ***(A)*** *this blocks are chosen according the reaction, substrates and products are represented as logical places. In* ***(B)*** *each block represents all reactions that are related to the respective components. Here logical transitions are used.*

- *Macro nodes*: A macro node or a macro place are visualised as boxed nodes; see **Figure 2.7**. Macro nodes allow to hierarchically structure a network. Each macro node establish a new layer

of the network. Macro nodes can be arbitrarily nested. Advantegeously, macro nodes offer the possibility to refine the network structure on a new layer. In addition, macro nodes also allow to structure a model into meaningful parts of connected subnets, group species or reactions or adapted the hierarchical structure of biological networks, e.g., compartmentalization of cells or an entire organism. The boundary nodes of a macro place are transitions. Macro transitions have only boundary places.

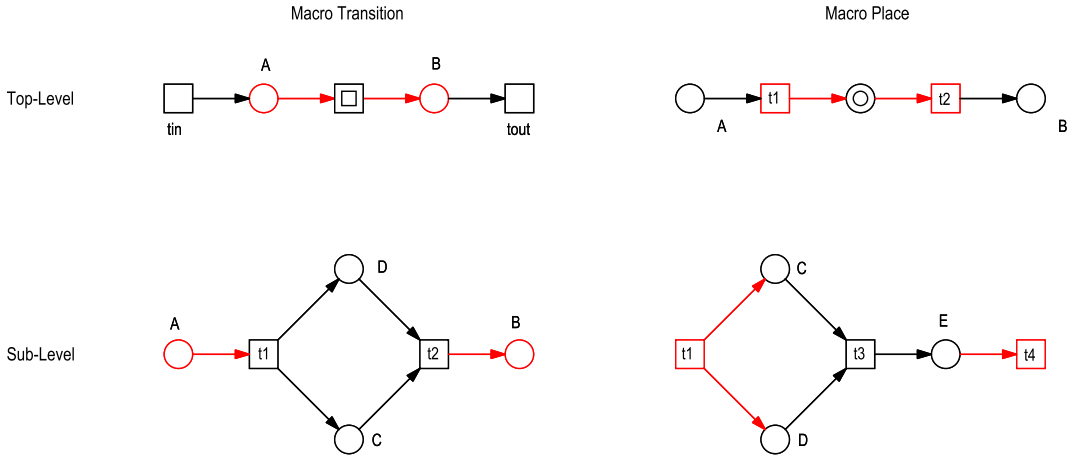


Figure 2.7: ***Macro Nodes.*** *Macro nodes allow refining of places or transitions by a detailed subnets on a deeper hierarchical level. The border nodes of a macro transition (place) are places (transitions).*

2.3.2 Extended Expressiveness

In order to reinforce the expressiveness of Petri nets, two arc types have been introduced, read (or test) edge and inhibitor edge; see **Figure 2.8**. Both types of arcs can be used to easier represent certain relationships in the network.

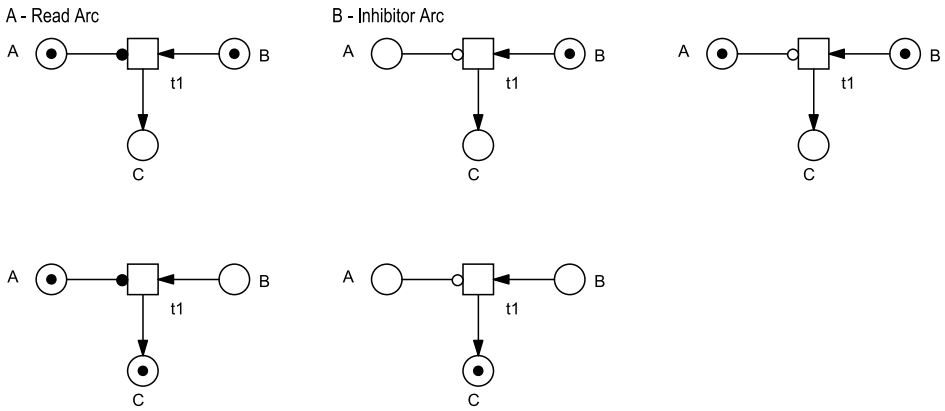


Figure 2.8: ***Inhibitor Edge and Read Edge.*** *(A) Read edge: Transition t_1 is enabled if place A and B are sufficiently marked. After firing, tokens are deleted from place B, but not from place A, which is connected with transition t_1 by a read edge. (B) Inhibitor edge: Transition t_1 is enabled if place B is sufficiently marked and place A is not sufficiently marked, which is connected with transition t_1 by an inhibitor edge. After firing tokens are deleted from place B, but not from A.*

- *Read edges* are represented by an edge and a filled dot at its end. Read edges only connect a place with a transition. If a place p is connected with a transition t via a read edge, the transition t is enabled if place p and all other places connected with transition t via the standard arc are sufficiently marked. By firing transition t, the amount of tokens on place p is not changed. Read edges can also be adapted by two opposed standard arcs. Thus, the qualitative analysis techniques of Petri nets can also be applied to models containing read edges.
- *Inhibitor edge* are represented by an edge and an empty dot at the end. Inhibitor edges only connect places with transitions. If a place p is connected with a transition by an inhibitor edge, the transition t is enabled if place p is *not* sufficiently marked, meaning the amount of tokens must be less than the respective arc weight, and all other places connected with transition t via the standard arc are sufficiently marked. Tokens are not deleted from the place p if the transition t fires. Inhibitor edges can not be reduced to the standard edges and thus they cause radical modification. All here introduced qualitative analysis techniques are not applicable to models containing inhibitor edges.

CHAPTER 3

Petri Net Modelling

After the theoretical excursion, we now address the modelling procedure itself. Here, we answer the following questions:

What is a model?
What can you expect from your model?
What points need to be considered before modelling?
How can you create your own Petri net model?

Modelling a biological system is similar to designing a game board. In both cases you need to define its structure and therewith the actions that are allowed and the different states that can be reached by each action.

First of all, you need to be aware that a model is only a simple, abstract representation of reality. A model can not explain every detail of the real biological system, but a model can help you to understand the structural relationships and the time-dependent dynamic behaviour. You can not expect new information about your biological system that you have not indirectly defined in the network structure and in the kinetics of the model. But do not be afraid if you do not have all detailed information of the biological system (components, reactions, kinetics) that you want to model. Even in this case, a model can be very helpful in studying the behaviour of the model or in revealing missing components and reactions. Even more, a model might also help to estimate kinetic parameters. This can be done by testing various modifications and perturbations, which would never be possible in your real system or would be too expensive. You may be able to test any hypotheses about the biological system by making model predictions. Additionally, in the case of Petri net models, there are several reliable analysis techniques available which might help you to better understand your model.
In the following sections, we talk about all steps that you need to consider while creating a qualitative model. After constructing your model, you can analyse its structure and the time-dependent dynamic behaviour. These two options are discussed in **Chapter 4 and 5**.

3.1 Analysing the System of Interest

Before starting the modelling procedure itself, you have to analyse your system very carefully and think about the points listed below.

1. Step: Make assumptions and think about abstractions to keep your model as simple as possible.
2. Step: Set boundaries to reasonably limit your model.
3. Step: Identify the involved components (and perhaps their different states).

4. Step: Identify all actions, (bio-)chemical reactions and any other changes occurring in your system.
5. Step: Define the relationships between components and reactions.
6. Step: Define the stoichiometry.
7. Step: Define the initial state.

Ask yourself what you know about the biological system. Perhaps, you have to go to the literature and collect more information about the components and reactions that are involved in your system. You should decide what the model should reflect and in how much detail you want to describe your system. Think of reasonable boundaries and of suitable assumptions to restrict the model of the biological system. A model should always be self-contained and as simple as possible. Often, it is not necessary to describe everything in detail. Start with a simple model. You can always refine your model and add further information if necessary.

3.2 Assumptions and Modelling Guidelines

Before modelling you should think of reasonable boundaries and of suitable assumptions to restrict the model of the biological system. But it is also important to agree on a conformable modelling procedure. All three points are important requirements to create a consistent model and to avoid errors, inconsistencies and contradictions while modelling.

Assumptions directly related to the biological context depend on the current issue and the chosen abstraction level. Therefore, you have to think about:

- System boundaries:include all relevant components and reactions;
- Abstraction level of processes: consider reactions in detail or merge a set of reactions into one bigger step;
- Abstraction level of the involved components: consider components as on unit, consider different states of a component or consider even functional domains of components;
- Handling of non-limiting components: “pseudo reactions” could be used to independently produce such components;
- Handling of products that have no more function in a model: “pseudo reactions” could be used to remove such components;
- Space: consider different compartments of the biological system and the translocation of components or neglect spatial information.

Additional scenarios are conceivable to model a biological system depending on the special context.

The decision on the modelling guideline goes along with the preassigned assumptions and boundaries. Here, we suggest some useful guidelines that you can consider while modelling. The strict observance depends also on the given issue, available information and chosen abstraction level.
The following guidelines are suitable for modelling biological systems:

- Reaction pathways (= Sequence of reactions reproducing its initial state, see also T-invariants, **Section 4.2.3**) like signal cascades or metabolic routes cover the entire model to restore the initial state of the model.
- The constant sum of molecules belonging to related components or different states of a component must be preserved (=Sum of tokens over a set of place is constant, see also P-invariants, **Section 4.2.3**). Mass conservation is assured if this property holds for each component (place) in the model.
- A Petri net model should be transition-bordered (all places have pre- and post-transitions), or place-bordered (all transitions have pre- and post-places), or not contain any border nodes at all (closed network structure).
  - Use input transitions to ensure that substrates do not limit the internal processes in a model.

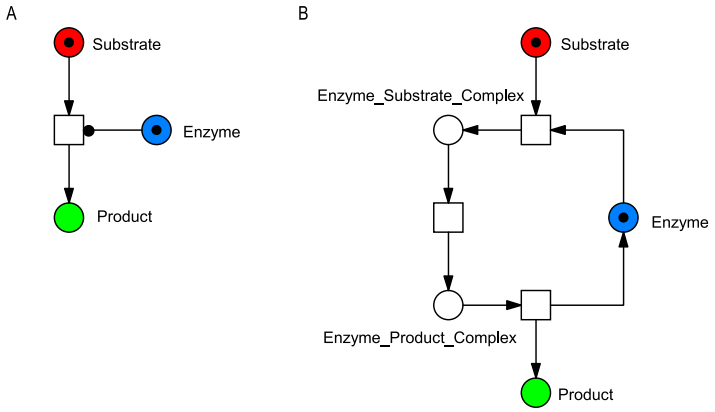


Figure 3.1: ***Representation of Enzymatic Reactions:*** *Petri net shown in (A) refers to a simple annotation of an enzymatic reaction $S \xrightarrow{E} P$. We couple the enzyme with the reaction by a read edge. Thereby the enzyme does not get consumed. The Petri net in (B) illustrates the single steps of an enzymatic reaction $S + E \rightarrow ES \rightarrow EP \rightarrow P + E$. The enzyme is temporarily consumed, but at the end released again.*

  - Use output transitions to ensure that products do not infinitely increase.
  - Use input places to ensure that initial substrates limit internal processes in the model.
  - Use output places to ensure that products accumulate.
- High abstraction level:
  - Reactions triggered by a component or a specific condition (temperature, pH value, membrane voltage) are considered as one single simplified reaction. Enzymes and similar triggers are not consumed by the reaction. They are connected with the corresponding transition via a read edge; see **Figure 3.1, A**.
  - A reaction sequence is reduced to one step.
- Low abstraction level:
  - Enzymatic reactions are refined into subreactions; see **Figure 3.1, B**.
  - Each reaction is split into elementary steps.

3.3 Creating a Petri Net Model

To construct a Petri net model of a biological system, the involved reactions and components must be identified according to the assumptions and the modelling guidelines in the step before. You need to represent all reactions as transitions. Places represent specific conditions (temperature, membrane voltage, pH-Value) or components. Afterwards you need to define the relationships between places and transitions with the help of arcs, and the stoichiometry by arc weights. **Section 3.6** gives several examples of how to translate chemical and enzymatic reactions into Petri nets.

3.3.1 Biological Interpretation of Places and Transition

In this section, we summarize biological interpretations of places and transitions. Both list are not complete and you can always add your own interpretation depending on your biological system.
A place could represent:

- molecular level:
  - atoms,
  - ions,

  - small inorganic molecules (oxygen, water, carbon dioxide, phosphates, acids, bases, etc.),
  - small organic molecules (hydrocarbons, carbohydrates, nucleic acid, amino acids, etc.),
  - second messengers (cAMP, DAP, IP3, PIP2, etc.),
  - energy equivalents (ATP/ADP, GTP/GDP, NAD^+/NADH, $NADP^+$/NADPH, etc.),
  - proteins (enzymes, transporter, ion-channels, protein complexes, protein domains, etc.),
  - molecules in certain localisations
- cellular level:
  - specific cell types (epidermal cells, neurons, germ cells, blood cells, etc.),
  - state of a single cell (healthy, infected, diseased, etc.),
  - single cell organism (bacteria, virus, etc.),
  - compartment of a cell (nucleus, endoplasmic reticulum, lysosome, mitochondria, etc.),
  - structural component of a cell (membrane, DNA, ribosome, cytoplasm, etc.)
- multicellular level:
  - cell complexes (layer of identical, different cells, etc.),
  - tissue (muscles tissue, nervous tissue, epithelial tissue, etc.),
  - organs (skin, muscles, liver, lunges, etc.),
  - multicellular organism (human, animal, plants, fungi, etc.),
  - populations (single-cell organisms, multicellular organisms, etc.),
  - state of the multicellular complex (healthy, infected, diseased, etc.),
- non-biological factors:
  - environmental factors (sun, wind, food, stress, etc.),
  - physical properties (pH-value, temperature, membrane voltage, pressure, red light, etc.),
  - abstract factors or conditions.

If you think of transitions, they can be interpreted as:

- (bio-)chemical reaction,
- dissociation/association,
- state shifts,
- actions,
- molecular changes,
- transport (active, inactive)/diffusion,
- phosphorylation/dephosphorylation,
- translation/transcription,
- degradation/synthesis,
- any event related to the above mentioned interpretation of places.

To keep this tutorial as simple as possible, we generalize the interpretation of places to components and the interpretation of transitions to reaction.

3.3.2 Petri Net Models of Biomolecular Reactions

Among the biological systems, a lot of similar processes are involved and in addition processes can be categorised by their functionality. Biological processes of one group can be simplified to a generalized mechanism. Here, we summarize such generalized mechanisms that you can find very frequently in biological systems. For each of those examples, we provide the Petri net structure of and small biological cartoon. You can reuse the Petri net models in your own model and connect different submodels with each other. The first **Figure 3.2** shows typical chemical reactions and their corresponding Petri net structure. **Figure 3.3**, we consider different transport mechanisms and how to represent them by a Petri net. Some Petri nets of general mechanisms in signal transduction are given in **Figure 3.4**. Posttranslational modifications (PTMs) regulate the functionality and activity of proteins and are thus, important in many biological mechanisms. **Figure 3.5** shows generalized Petri net models of the most common PTMs.

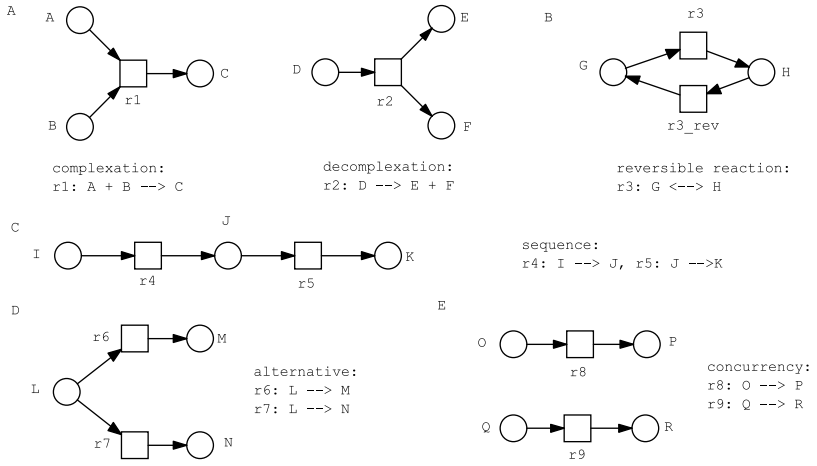


Figure 3.2: *Chemical Reactions.*

Here we depict Petri net structures of typical chemical reactions (adopted from [17].

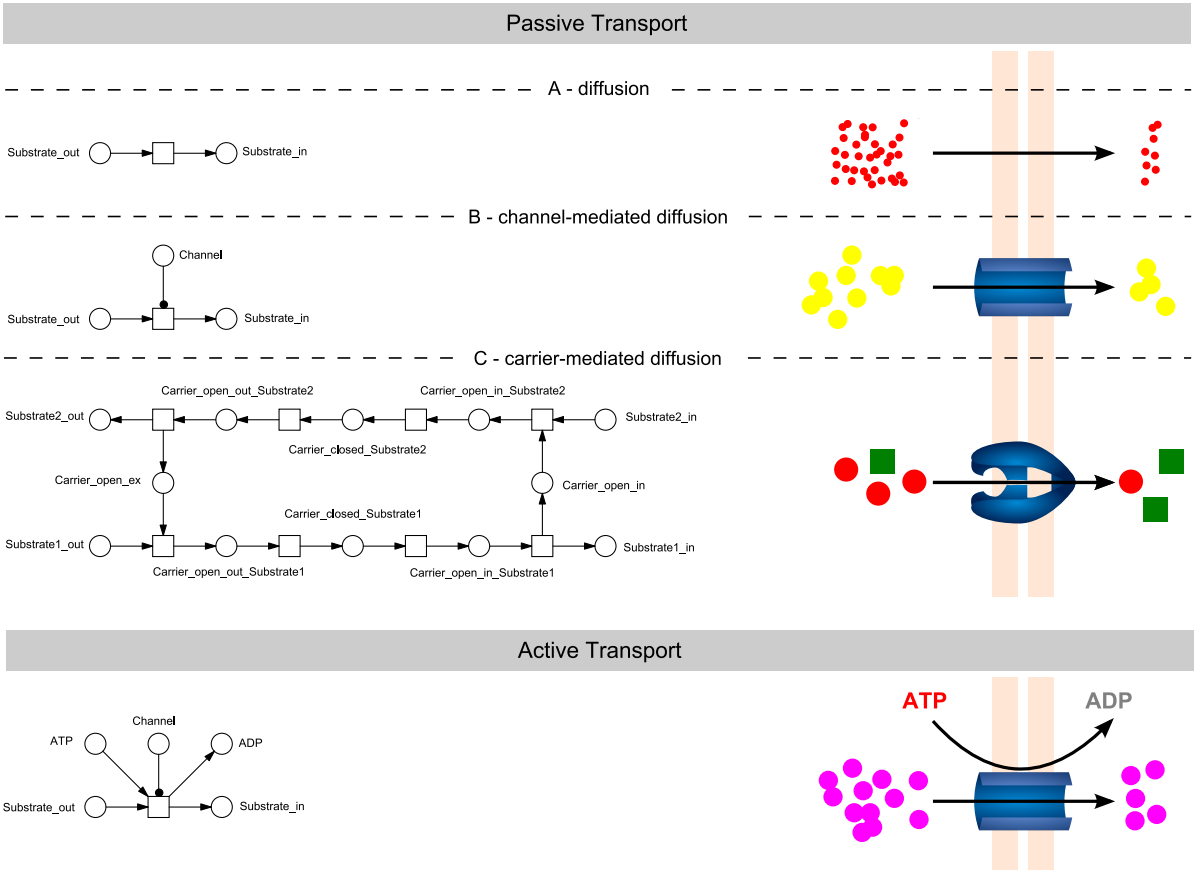


Figure 3.3: ***Molecular Transport Mechanisms.*** *Molecular Transport Mechanisms translated into Petri net models.*

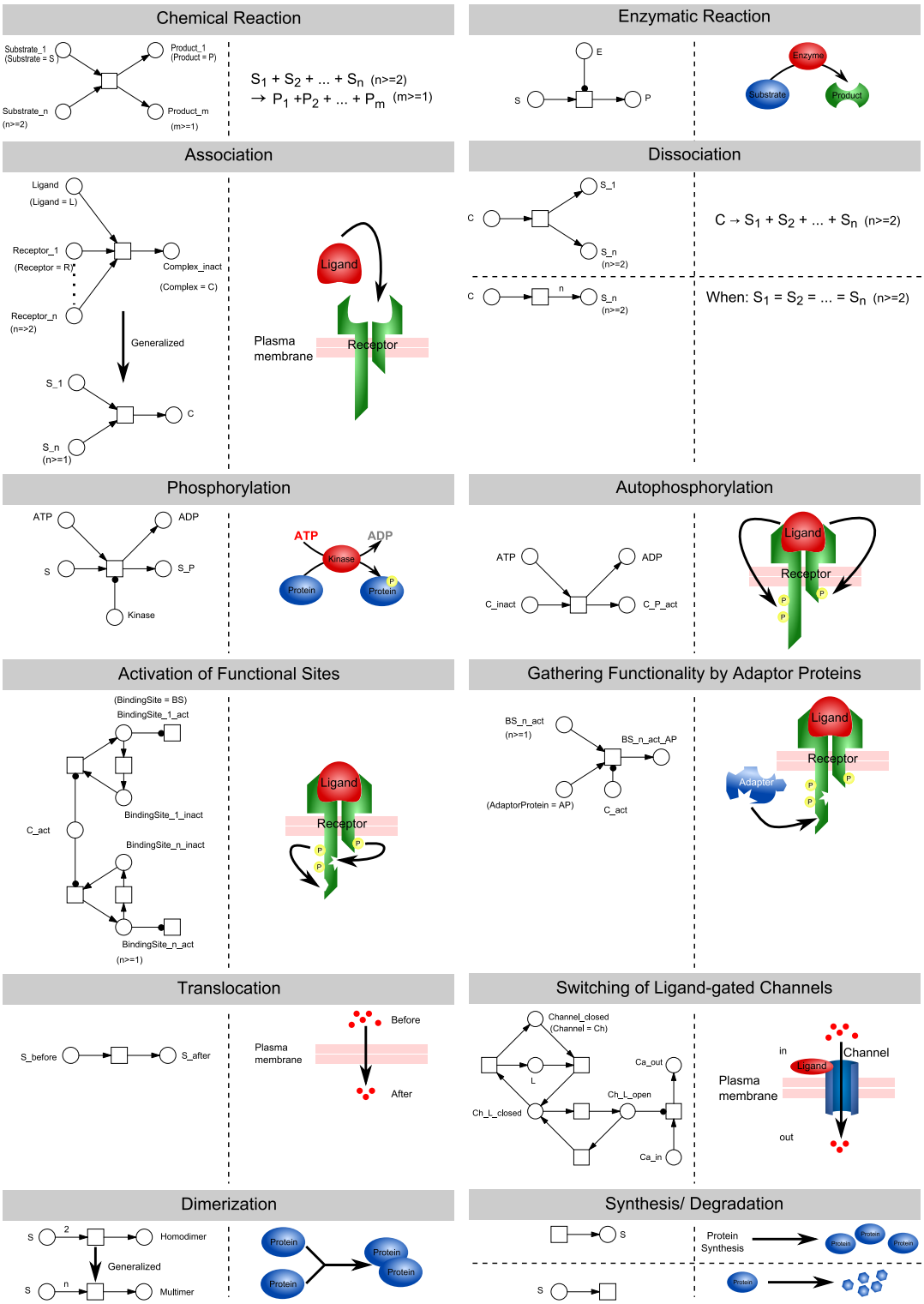


Figure 3.4: ***Signalling Mechanisms.*** *Most common signalling mechanisms translated into Petri net models (adopted from [15]).*

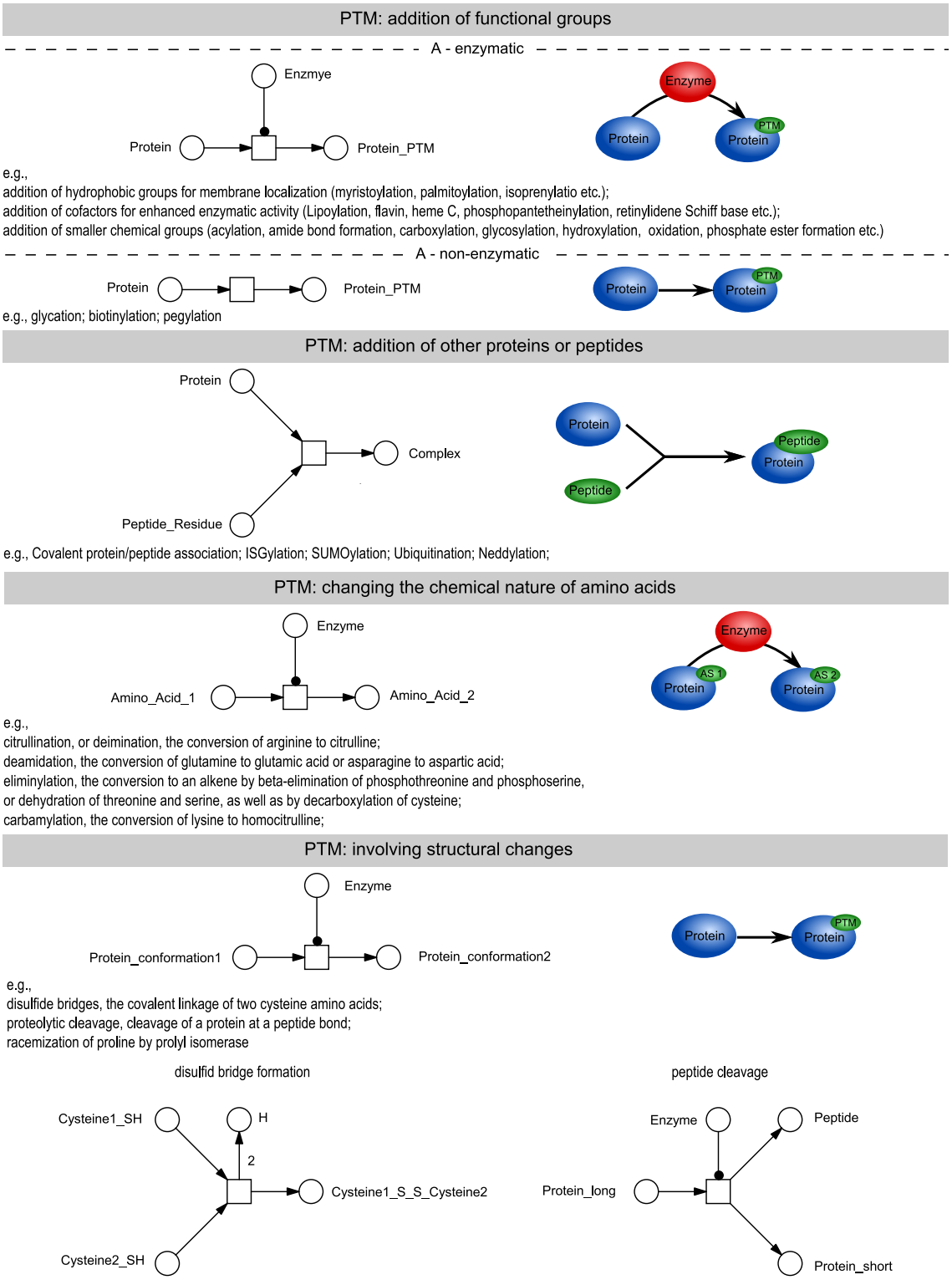


Figure 3.5: ***Posttranslational modification.*** *Posttranslational modifications(PTMs) of proteins translated into Petri net models.*

3.4 Initial State of a Petri Net Model

After setting the network structure of the Petri net, its initial state has to be defined, e.g., according to the initial state of the biological system. It is not necessary to put tokens on every single place. To set the initial marking you have to consider the following points:

- What is your input (substrates, signals)?
- Which internal components are available at the beginning?
- Which is the initial state of a component?
- Which are the structural components in your system (membranes, compartments, cell organells, DNA etc.)?

Another way to set an initial marking is to consider P-invariants; see **Section 4.2.3**. Every P-invariant should be initially marked by a token. The reactions in your model can only occur (transitions can only fire) if places are sufficiently marked. Thus, the initial marking determines the state spaces, meaning which states can be reached. With the help of the initial marking you can also simulate limitation, knock-downs or knock-outs and mutations or overexpression. You can test the influence of different manipulations and perturbations in your model on general and behavioural network properties, the state space and time-dependent dynamic behaviour.

3.5 Neat Arrangement of a Petri Net Model

Sometimes the network structure of a biological system grows very fast and the reflected processes are very complex. Additionally, in biological networks some components are highly interactive and act at several reactions, like second messenger and energy equivalents. Thus, the model might not be very readable, but confusing. To enhance the readability of your model you can use macro nodes and logical nodes if necessary; see **Section 2.2, Figure 2.7**.

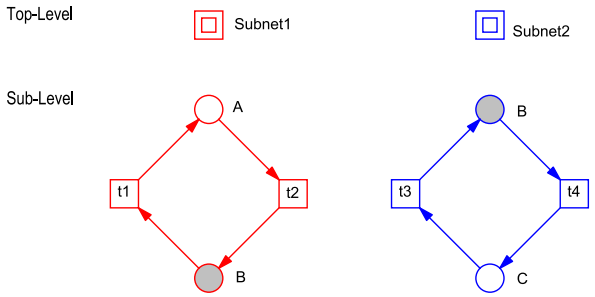


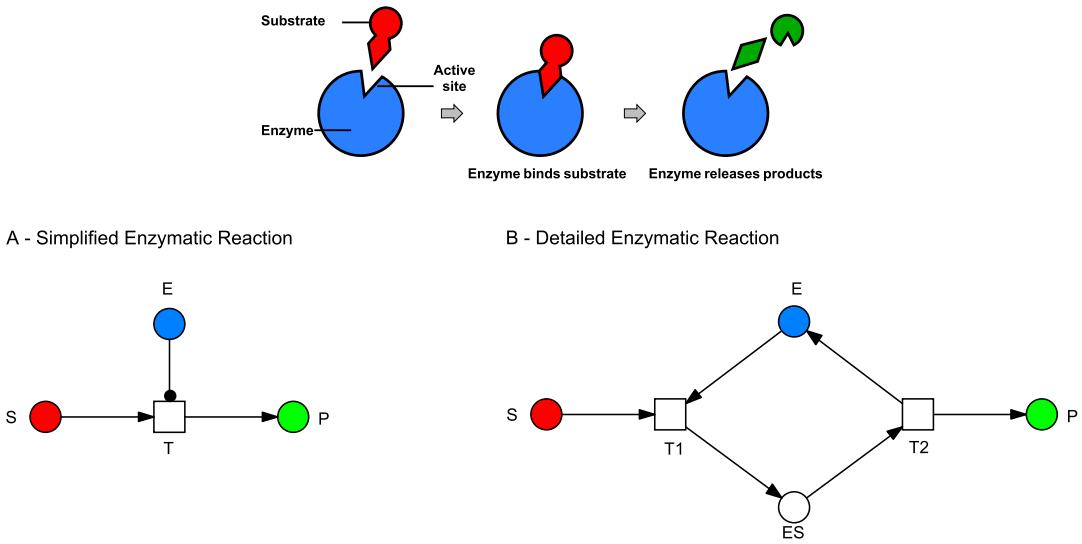
Figure 3.6: ***Joining Submodels****. Macro nodes can be used to build neatly arranged and hierarchically structured submodels. Separate submodels can be coupled by logical places that refer to the same component in all submodels.*

The application of macro nodes leads to a hierarchical model with different levels that are connected with each other. Hierarchical models allow you to neatly display the network structure. The hierarchical structure of a model does not change any properties of the flat model. With the help of macro nodes you can refine single places or transitions by a detailed subnet on a lower level and/or you can organize your model into functional units. A model could be subdivided into reaction sets, signal cascades or related components. To represent highly cross-linked components and transitions with multiple input and output, you should consider using logical places and transitions. By using macro nodes and logical places you can construct separate Petri net models and join them afterwards; see **Figure 3.6**. The submodels are connected via logical places, i.e., places of components that are shared among different submodels. The construction and analysis of separate Petri net models might also avoid errors and inconsistencies.

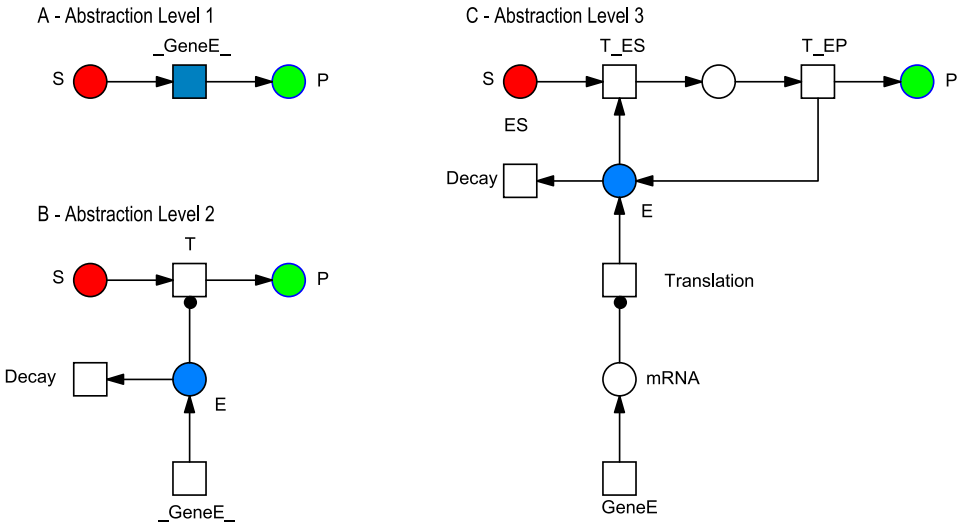
3.6 Examples

The next pages more examples of different enzymatic reactions like enzyme inhibition, signal amplification, feedback inhibition and gene regulation. As before in **Section 3.3.2**, these general structures can be reused in any biological system.

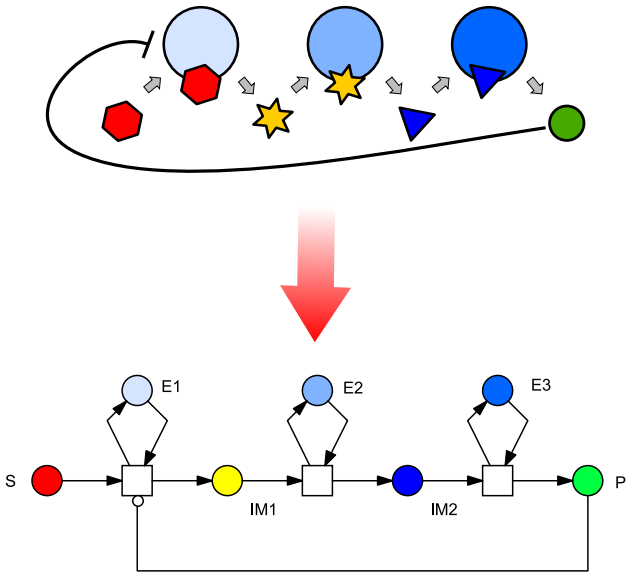
Example 3.1 (Enzymatic Reaction) *Here are two possibilities showing how to represent an enzymatic reaction using Petri nets. In **A**, the enzymatic reaction is simplified to one reaction. In **B**, we consider in addition the formation of an enzyme-substrate-complex. The enzymatic reaction is split into two steps.*



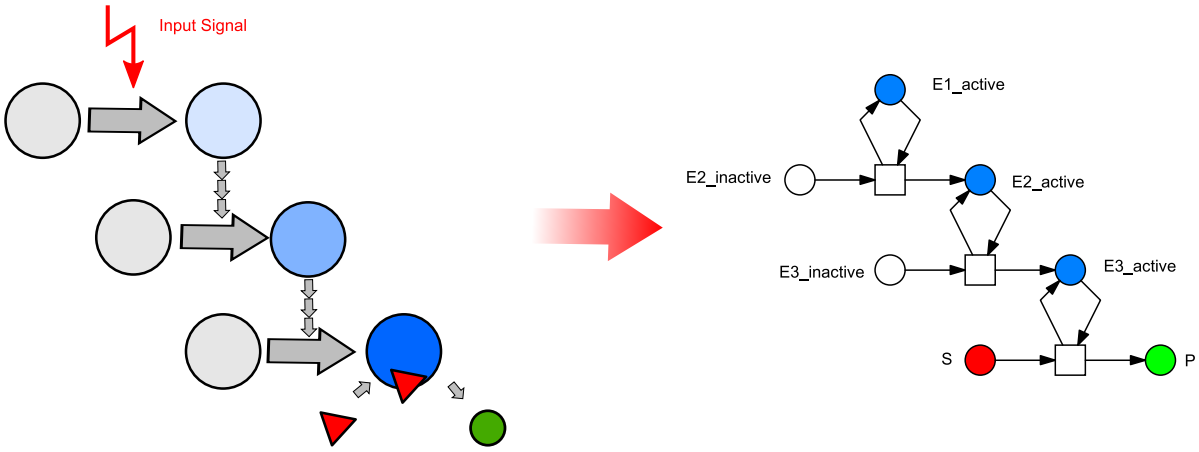
Example 3.2 (Enzymatic Reaction Coupled with Gene Expression) *The simple enzymatic reaction in **A** can be extended by adding more and more details about the gene expression, see **B** and **C**.*



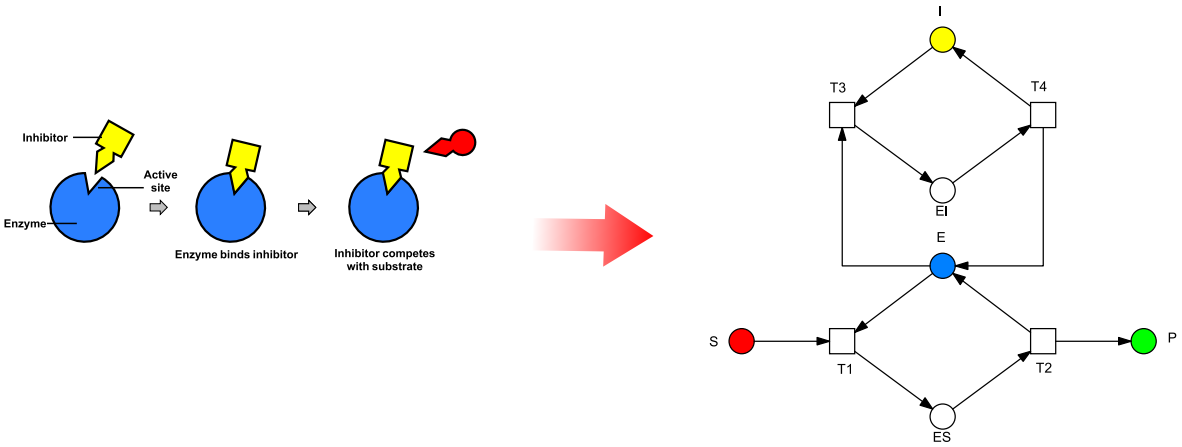
Example 3.3 (Feedback Inhibition) *In this example, the negative feedback is initiated by the product that inhibits the first enzyme of the reaction sequence. To realise the inhibition the product is connected with the first transition of the sequence via an inhibitor edge.*



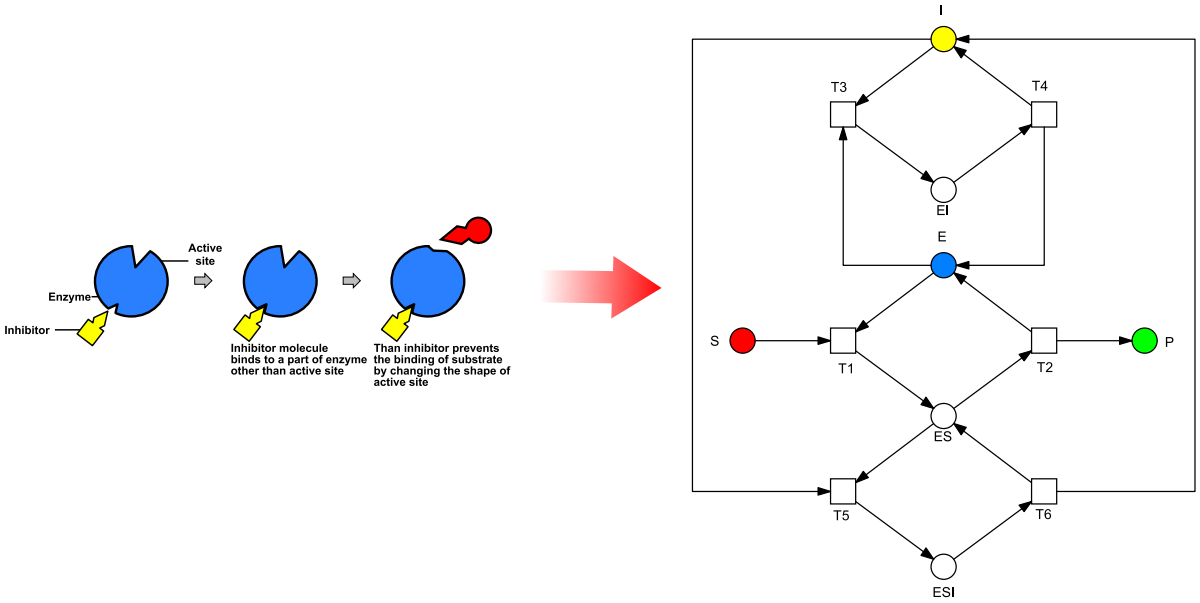
Example 3.4 (Signal Amplification) *In signal amplification multiple enzymes activate each other step by step. Signal amplification can be found in different signal pathway, e.g., in the mitogen-activated protein kinase (MAPK) cascade, where each enzyme can activate several enzymes in the next step of the signal pathway.*



Example 3.5 (Competitive Enzyme Inhibition) *The substrate and the inhibitor can both bind to the active site of the enzyme. The inhibitor and substrate can not bind at the same time to the enzyme, they exclude each other.*



Example 3.6 (Allosteric Enzyme Inhibition) *The inhibitor binds to a distinct site at the enzyme. Thus, the inhibitor does not compete with the substrate and can inhibit the enzyme independently whether the substrate is bound or not.*



CHAPTER 4

Qualitative Petri Net Analysis

In this chapter we talk about qualitative properties of the network structure and their meanings. All definitions and classifications are taken from reference [12]. Also, consider reference [12] if you are interested in more mathematical details. Here, we answer the questions:

Which properties can be determined for a Petri net model?
What does each property mean in general?
And what is the biological meaning of each property?
Which are important components?
How do I know, if my system can reach a certain state?

Again, the game board is a nice analogy to the modelling of a biological system. Each game has certain structures and rules. It is not possible to play chess on a merels boards, or vice versa. The same applies for biological systems. Each biological system has defined properties that should be reflected by its model.

The Petri net formalism allows you to characterize elementary system properties of the underlying Petri net model. Those properties can be determined without considering kinetic information and, therefore, without considering the the the time-dependent dynamic behaviour. It is not just possible to make statements about the pure qualitative properties of the network structure. The network structures also allows you to draw conclusions about behavioural properties independent of time of the model.
The fulfilment of certain properties is important for the consistency and strict observance of rules important for a biological system. These properties may vary among different case studies and depend on the specific issue, assumptions and modelling guideline. However, the properties of a Petri net can be used to validate the model and to check relevant system properties. Each property has a significant biological meaning.

4.1 Qualitative Properties

4.1.1 Structural Properties

The structural properties given in **Table 4.1** are elementary properties of a Petri net graph, which directly depend on the arrangement of places, transitions and arcs (including arc weights). Those properties characterize the network structure and are independent of the marking. They can be considered as an initial consistency check to prove that the model adheres to the assumptions and the modelling

guideline. Next to the informal description of each of those behavioural properties, we give the biological interpretation.

Table 4.1: *Qualitative Properties of a Petri net and their biological Meaning*

Property		**Informal Definition**	Biological Meaning
PUR	Pure	There are no two nodes, directly connected in both directions. This precludes read arcs and double arcs.	No component is produced and consumed by the same reaction. Thus, enzymatic or enzyme-like reactions are formulated in more detail.
ORD	Ordinary	All arc weights are equal to 1.	Every stoichiometric coefficient of each reaction is equal to one.
HOM	Homogeneous	All outgoing arcs of a given place have the same multiplicity.	Each consuming reaction associated with one component takes the same amount of molecules of this component.
CON	Connected	A Petri net is connected if it holds for every two nodes a and b that there is an undirected path between a and b. Disconnected parts of a Petri net can not influence each other, so they can be usually analysed separately. In the following we only consider connected Petri nets.	All components in a system are directly or indirectly connected with each other through a set of reactions, e.g., metabolic paths, signal flows.
SC	Strongly Connected	A Petri net is strongly connected if it holds for every two nodes a and b that there is a directed path from a to b, vice versa. Strong connectedness involves connectedness and the absence of boundary nodes. It is a necessary condition for a Petri net to be live and bounded at the same time.	All components in a system are directly connected with each other through a set of reactions, e.g., metabolic paths, signal flows.
NBM	Non-blocking Multiplicity	The minimum of the multiplicity of the incoming arcs for a place is not less than the maximum of the multiplicities of its outgoing arcs.	The amount of produced and consumed molecules of a certain component is always equal.
CSV	Conservative	All transitions add exactly as many tokens to their post-places as they subtract from their pre-places (token-preservingly firing). A conservative Petri net is structurally bounded.	The total amount of consumed and produced molecules by a certain reaction is always equal.
SCF	Static conflict free	There are no two transitions sharing a pre-place. Transitions involved in a dynamic conflict compete for the tokens on shared places.	For every reactant exist just one possible reaction or there are no two reactions sharing at least one reactant.
FT0	No input transition	There exist no transitions without pre-places.	Infinite source of a component.
TF0	No output transition	There exist no transitions without post-places.	Sink of a component.

FP0	No input place	There exist no places without pre-transitions.	The component can not be produced by any reaction. Thus, such components are limiting.
PF0	No output place	There exist no places without post-transitions	Components can infinitely accumulate in the system. Thus, they are not consumed by any reaction.

4.1.2 Behavioural Properties

Behavioural properties of a Petri net are listed in **Table 4.2 and 4.4**; they characterize the system behaviour of a model, which depend on the qualitative network structure and on the initial marking. The listed behavioural properties are independent of the time-dependent dynamic behaviour and thus, independent of kinetic information. Therefore, behavioural properties of a Petri net are determined by time-free decision about the systems behaviour, meaning time aspects are not considered. Next to the informal description of the behavioural properties, we give the biological interpretation.

4.1.2.1 General Behavioural Properties

General behavioural properties as shown in **Table 4.2** illustrate the properties of a Petri net model characterizing the boundedness, liveness and reversibility based on the underlying network structure. These properties are independent of the special functionality of the network itself. The informal definitions of the named properties are given below:

- *Boundedness*: For every place it holds that: Whatever happens, the maximum number of tokens on this place is bounded by a constant. This precludes overflow by unlimited increase of tokens; see 1-B, k-B, SB in **Table 4.2**.
- *Liveness*: For every transition it holds that: Whatever happens, it will always be possible to reach a state where this transition gets enabled. In a live net all transitions are able to contribute to the net behaviour forever, which precludes dead states, i.e., states where none of the transitions are enabled; see also Liv, DSt, DTr in **Table 4.2**.
- *Reversibility*: For every state it holds that: Whatever happens, the net will always be able to reach this state again. So the net has the capability of self-reinitialization; see also REV in **Table 4.2**.

The biological interpretation of those properties is given in **Table 4.2**.

Table 4.2: *General Behavioural Properties of a Petri net and their biological Meaning*

Property		**Informal Definition**	Biological Meaning
SB	Structurally bounded	A Petri is structurally bounded if it is bounded in any initial marking.	It is not possible that any component accumulates in the system independent of the initial conditions.
1-B	1-bounded	A Petri net is 1-bounded if all its places are 1-bounded.	Number of molecules or the concentration of every component is limited to one only.
k-B	k-bounded	A Petri net is k-bounded if all its places are k-bounded.	Number of molecules or the concentration level of each component is limited to a constant number k.
LIV	Liveness	Every transition of a Petri net contributes to the network behaviour forever.	All involved reaction will repeatedly occur and contribute to the time- (and spatial-) dependent development.

REV	Reversibility	The initial marking can be reached again from each reachable marking.	The initial state of a system can be reproduced by any possible state reached from the initial conditions.
DCF	Dynamically conflict free	A Petri net is has no dynamic conflicts if no state exists, in which two transitions are enabled, which could disable each other by firing.	The occurrence of a reaction inhibits another reaction which could also occur at the same time. The shared reactants are consumed by one of the reaction and no reactants are left or one reaction produces a component that directly inhibits the other reaction.
DSt	Dead states	A Petri net has a dead state if no transition can be enabled any more.	The system can run into a state, where no reaction can occur.
DTr	Dead transition	A transition in a Petri net is dead if it can not be enabled in any marking reachable from the initial marking.	The system can run from the initial state chosen initial state into at least one state, where at least one reaction can not occur any more.

Formal Definitions:

Definition 4.1 (Boundedness)

- *A place p is* k-bounded *if there exists a positive integer number k, which represents an upper bound for the number of tokens on this place in all reachable markings of the Petri net:* $\exists k \in \mathbb{N}_0 : \forall m \in [m_0\rangle : m(p) \leq k$.
- *A Petri net is* k-bounded *if all its places are* k-bounded.
- *A Petri net is* structurally bounded *if it is bounded in any initial marking.*

How to read:
Please consider the example given in **Figure 2.5**. First, we need to consider all marking m reachable from the initial marking m_0 by simply playing the token game. As result we get:

Table 4.3: *Reachable markings of the Petri net given in* ***Figure 2.5***

Place	m_0	m_1	m_2
Enzyme	1	0	1
Substrate	1	0	0
EnzymeSubstrateComplex	0	1	0
Product	0	0	1

Now, we can draw the following conclusion according to the definition

- Each component has an upper bound k. In addition, the upper bound for all components is equal to "1".
- All components are 1-bounded. Thus, the resulting Petri net is 1-bounded.
- You can think of any marking, the tokens will never infinitely accumulate in the Petri net. Thus, the Petri net is structurally bounded. The amount of *Product* molecules produced by *Synthesis* is limited by *Substrate*. The stoichiometry of the reaction *Association* and *Dissociation* is balanced. Thus, the number of molecules on place *Enzyme* or *Substrate* will not increase. The total number of *Enzyme*, $(Enzyme + EnzymeSubstrateComplex)$ and the total number of substrate and product $(Substrate + Product + EnzymeSubstrateComplex)$ is constant for each marking.

Definition 4.2 (Liveness)

- *A transition t is* dead in the marking *m if it is not enabled in any marking m' reachable from:* $\nexists m' \in [m\rangle : m'(t)$.
- *A transition t is* live*, if it is not dead in any marking reachable from* m_0.
- *A marking m is* dead*, if there is no transition which is enabled in m.*
- *A Petri net is* deadstate-free*, if there are no reachable dead markings.*
- *A Petri net is* live*, if each transition is live.*

How to read:
Please consider the example given in **Figure 2.5** and **Table 4.3**.

- In marking $m_0/m_1/m_2$ transition(s) *Synthesis* and *Dissociation*/*Association*/*Association* and *Dissociation* is (are) dead. Thus, transitions *Association*, *Dissociation* and *Synthesis* are dead.
- Transitions *Association*, *Dissociation* and *Synthesis* are not live, because they are dead.
- Marking m_2 is dead, non of the transitions *Association*, *Dissociation* and *Synthesis* can be enabled.
- Because of m_2 the Petri net has a deadstate.
- The Petri net is not live, because all transitions are not live.

Definition 4.3 (Reversibility) *A Petri net is* reversible *if the initial marking can be reached again from each reachable marking:* $\forall m \in [m_0\rangle : m_0 \in [m\rangle$.

How to read:
Please consider the example given in **Figure 2.5** and **Table 4.3**.

- The Petri net is not reversible, because the initial marking m_0 can not be reached from marking m_3.

4.1.2.2 Further Behavioural Properties

In order to consider the following properties, one need to consider important structural motifs of a Petri net: traps, siphons and invariants; see **Section 4.2**.

A Property related to siphon and traps is **STP**; see **Table 4.4**. This property depends on the initial marking. Consider also the information about traps and siphons given in **Sections 4.2.1 and 4.2.2**. The properties **CPI**, **CTI** and **SCTI** refer to the invariants of the model; see **Table 4.4**. They do not depend on the initial marking. Additional information about invariants can be found in **Section 4.2.3**.

Table 4.4: *Behavioural Properties of a Petri net related to Traps and Siphons and their biological Meaning*

Property		**Informal Definition**	Biological Meaning
STP	Siphon trap property	Every siphon includes an initially marked trap. This excludes input places.	The part of the system that represents an outflow of certain components by a siphon contains also an initial active trap. Thus, the outflow does not stop, because it gets new input from the trap.
CPI	Covered by place invariants	A Petri net is covered by P-invariants if every place belongs to a P-invariant.	Mass Conservation is given in the entire system.
CTI	Covered by transition invariants	A Petri net is covered by T-invariants if every transition belongs to a T-invariant.	The initial state of all sequences of reactions can be restored.

SCTI	Strongly covered by transition invariants	A Petri net is strongly covered by T-invariants, if it is covered by non-trivial T-invariants. Trivial T-invariants consist of only two reactions.	There are no two reactions that restore each other.

4.2 Structural Motifs

4.2.1 Trap

In general, a trap refers a device designed to catch something. In terms of Petri nets, a trap is subnet that catches tokens and retain at least one of them; see **Figure 4.1**. The number of tokens in a trap can decrease but never become zero. A trap can not become empty, if it has contained tokens. Post-transitions in a trap will always return tokens to the trap.
Cyclic structures in a biological system that are activated by an input should be represented in a model as trap. Another example is of molecules that can assume certain states, but in the end they are caught in a cellular compartment.

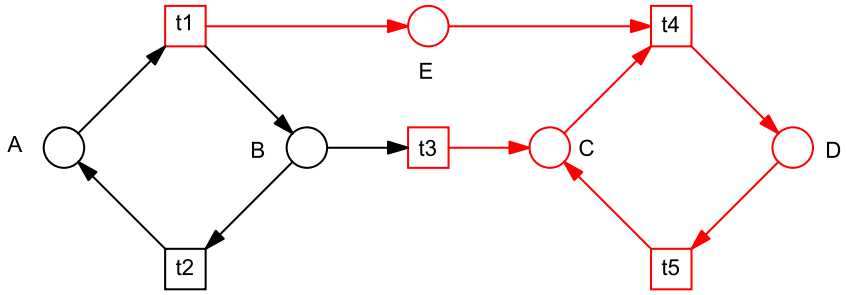


Figure 4.1: ***Trap.*** *The red coloured subnet of the Petri net indicates a trap. The place set {C, D, E} can not become empty once it has contained a token. The post-transitions of the set {t4, t5} are contained in set of pre-transitions {t1, t3, t4, t5}. The repeated firing of transition t4 and t5 will reduce the total token number in the trap, but can not remove all of them.*

Formal Definitions:

Definition 4.4 (Trap) *A set of places $Q \subseteq P$ is called* trap *if $Q\bullet \subseteq \bullet Q$ (the set of post-transitions is contained in 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.*

How to read:
Consider the example given in **Figure 4.1**.

- Set of all places $P = \{A, B, C, D, E\}$,
- Set of places constituting a trap $Q = \{C, D, E\}$
- $Q \subseteq P$: Q is a subset of P; meaning places C, D, E of set Q are contained in the set P
- $Q\bullet$: Set of post-transitions of places in set Q; $Q\bullet = \{t4, t5\}$
- $\bullet Q$: Set of pre-transitions of places in set Q; $\bullet Q = \{t1, t3, t4, t5\}$
- $Q\bullet \subseteq \bullet Q$: $Q\bullet$ is a subset of $\bullet Q$; meaning post-transitions $t4, t5$ of set $Q\bullet$ are contained in the set of pre-transitions $\bullet Q$.

4.2.2 Siphon

In the technical context, a siphon is a bent pipe or tube with one end lower than the other, in which hydrostatic pressure exerted due to the force of gravity moves liquid from one reservoir to another. Generally speaking, a siphon is a device that loses its content. In terms of Petri nets, a siphon refers to a subnet that releases all its tokens. **Figure 4.2** illustrates a possible structure of a siphon in a Petri net. A Petri net without siphons is live, while a system in a dead state has a clean siphon.
In biological terms, a siphon is a finite source of molecules or energy. It could also be a cyclic structure that might produce molecules by consuming itself.

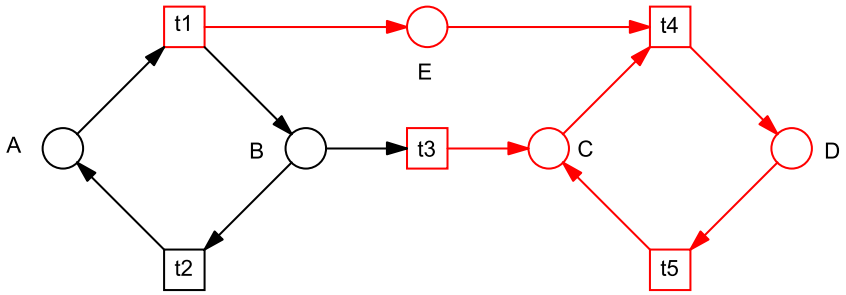


Figure 4.2: ***Siphon.*** *The red coloured subnet of the Petri net indicates a siphon. The place set {A, B} will become empty after a finite time. The pre-transitions of the set {t1, t2} are contained in set of post-transitions {t1, t2, t3}. The tokens can rotate by sequential firing of transition t1 and t2. Each cycle also produces tokens on place E. The cycle is cleaned up by firing of transition t3.*

Formal Definitions:

Definition 4.5 (Siphon) *A non-empty set of places $D \subseteq P$ is called* siphon *if $\bullet D \subseteq D\bullet$ the set of pre-transitions is contained in set of post-transitions, i.e., every transition which fires tokens onto a place in this siphon, also has a pre-place in this set.*

How to read:
Consider the example given in **Figure 4.2**.

- Set of all places $P = \{A, B, C, D, E\}$,
- Set of places constituting a trap $D = \{A, B\}$
- $D \subseteq P$: D is a subset of P; meaning places A, B of set D are contained in the set P
- $\bullet D$: Set of pre-transitions of places in set D; $\bullet D = \{t1, t2\}$
- $D\bullet$: Set of post-transitions of places in set D; $D\bullet = \{t1, t2, t3\}$
- $\bullet D \subseteq D\bullet$: $\bullet D$ is a subset of $D\bullet$; meaning pre-transitions $t1, t2$ of set $\bullet D$ are contained in the set of post-transitions $D\bullet$.

4.2.3 Invariants

In general, invariants are special features in a system. In mathematics, an invariant of a system is a predicate, which is not changed by the involved processes in the system. Meaning if the predicate is true at the initial state before the start of a sequence of processes, then it must be true at the end of the sequence ans always in between. In the Petri net context, invariants indicate states in the Petri net graph that are not changed after a transformation or a sequence of transformations. Petri net invariants can be split into place invariants and transition invariants; see **Figure 4.3**. Both types of invariants have a biological interpretation and thus, are meaningful to represent a biological system.

A *P-invariant*; see **Figure 4.3**, stands for a set of places over which the weighted sum of tokens is constant and independent of any firing. The total effect of each every transition on a P-invariant is

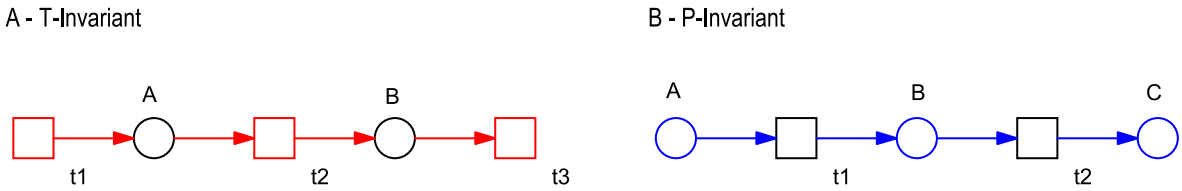


Figure 4.3: ***P- and T-invariants.*** *T-invariants restore an initial state, while P-invariants ensure the token preservation.*

zero. Thus, a P-invariant conserves the number of tokens. Obviously, each place in a P-invariant is bounded. In the biological context, with the help of P-invariants you can assure mass conservation and avoid an infinite increase of molecules in your model. P-invariants may describe components that are related with each other or the different states of a component.

A *T-invariant*; see **Figure 4.3**, stands for a sequence of transitions, which by their partially ordered firing reproduce an initial state, which enabled the firing of the transitions in the T-invariant. In a biological context, T-invariants describe a sequence of reactions that reproduce an initial state. T-invariants ensure that the model of the biological system can reinitialize a certain initial state. Also, the firing of transitions in a T-invariant leads to a steady state behaviour.

By looking at the P- and T-invariants, you can check their biological plausibility and thereby, prove the structural consistency of your model and/or gather new insights about the biological system itself.

Formal Definitions:

Definition 4.6 (P-invariants, T-invariants)

- *The* incidence matrix *of N is a matrix $\mathbb{C} : P \times T \to \mathbb{Z}$, indexed by P and T, such that $\mathbb{C}(p,t) = f(t,p) - f(p,t)$.*
- *A* place vector *(transition vector) is a vector $x : P \to \mathbb{Z}$, indexed by P ($y : T \to \mathbb{Z}$, indexed by T)*
- *A place vector (transition vector) is called* P-invariant (T-invariant) *if it is a non-trivial non-negative integer solution of the linear equation system $x \cdot \mathbb{C} = 0$ ($\mathbb{C} \cdot y = 0$).*
- *The set of nodes corresponding to an invariant's nonzero entries are called the* support *of this invariant x, written as $supp(x)$.*
- *An invariant x is called* minimal, *if $\nexists$ invariant z: $supp(z) \subset supp(x)$, i.e., its support does not contain the support of any other invariant z, and the greatest common divisor of all nonzero entries of x is 1.*
- *A net is* covered by P-invariants (T-invariants), *if every place (transition) belongs to a P-invariant (T-invariant).*

How to read:
Consider the example given in **Figure 2.5**.

- First of all, we have to write down the incidence matrix $\mathbb{C}$ of from the Petri net of our example. The incidence matrix is similar to the stoichiometric matrix of a reaction system.

$$\begin{array}{l} \\ \textit{Enzyme} \\ \textit{Substrate} \\ \textit{EnzymeSubstrateComplex} \\ \textit{Product} \end{array} \begin{array}{c} \begin{array}{ccc} \textit{Association} & \textit{Dissociation} & \textit{Synthesis} \end{array} \\ \begin{pmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{pmatrix} \end{array} = \mathbb{C}$$

P-Invariants	T-Invariants
• Place vector x: $x = \begin{pmatrix} x_1 & x_2 & x_3 & x_4 \end{pmatrix}$ Four places mean 4 entries in the vector.	• Transition vector y: $y = \begin{pmatrix} y_1 & y_2 & y_3 \end{pmatrix}'$ Three transition mean 3 entries in the
• Solution of $x \cdot \mathbb{C} = 0$: $\begin{pmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{pmatrix}' \cdot \begin{pmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{pmatrix} = 0$ We get two solutions: – P-invariant 1: $x = \begin{pmatrix} 1 & 0 & 1 & 0 \end{pmatrix}$ – P-invariant 2: $x = \begin{pmatrix} 0 & 1 & 1 & 1 \end{pmatrix}$	• Solution of $\mathbb{C} \cdot y = 0$: $\begin{pmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} y_1 \\ y_2 \\ y_3 \end{pmatrix} = 0$ We get only one solutions: – T-invariant 1: $x = \begin{pmatrix} 1 & 1 & 0 \end{pmatrix}$
• Support of the P-invariants (non-zero elements): – P-Invariant 1: $\{Enzyme, EnzymeSubstrateComplex\}$ – P-Invariant 2: $\{Substrate, Product, EnzymeSubstrateComplex\}$	• Support of the T-invariants (non-zero elements): – T-Invariant 1: $\{Association, Dissociation\}$
• The support of P-invariant 1 is not a subset of P-invariant 2, vice versa. The greatest divisor of the non-zero elements in both P-invariants is equal one. Thus, both P-invariants are minimal.	• The first condition is not relevant in the case of only one T-invariant. The greatest divisor of the non-zero elements in the T-invariant is equal one. Thus, the T-invariant is minimal.
• Each place is contained in at least one of the two P-invariants. Thus, the Petri net of our example is covered by P-invariants.	• Transition *Synthesis* is not contained in the T-invariant. Petri net of our example is not covered by T-invariants

4.3 State Space

In order to decide boundedness, liveness and reversibility it might be necessary to consider the state space; **Section 4.1.2**. The state space comprises all possible states that can be reached from the initial marking. It can also be visualised by a graph, where nodes correspond to a state (marking) of the Petri net and directed arcs indicate the firing of a single transition that leads to the next possible marking.

The state space is computed by determining all enabled transition at the initial marking and the follow-up marking reached by firing of a single enabled transition. This procedure must be repeated for each state. The state space represents all possible states and all firing sequences required to reach a certain state from the initial marking independent of time aspects. Nodes in the graph of the state

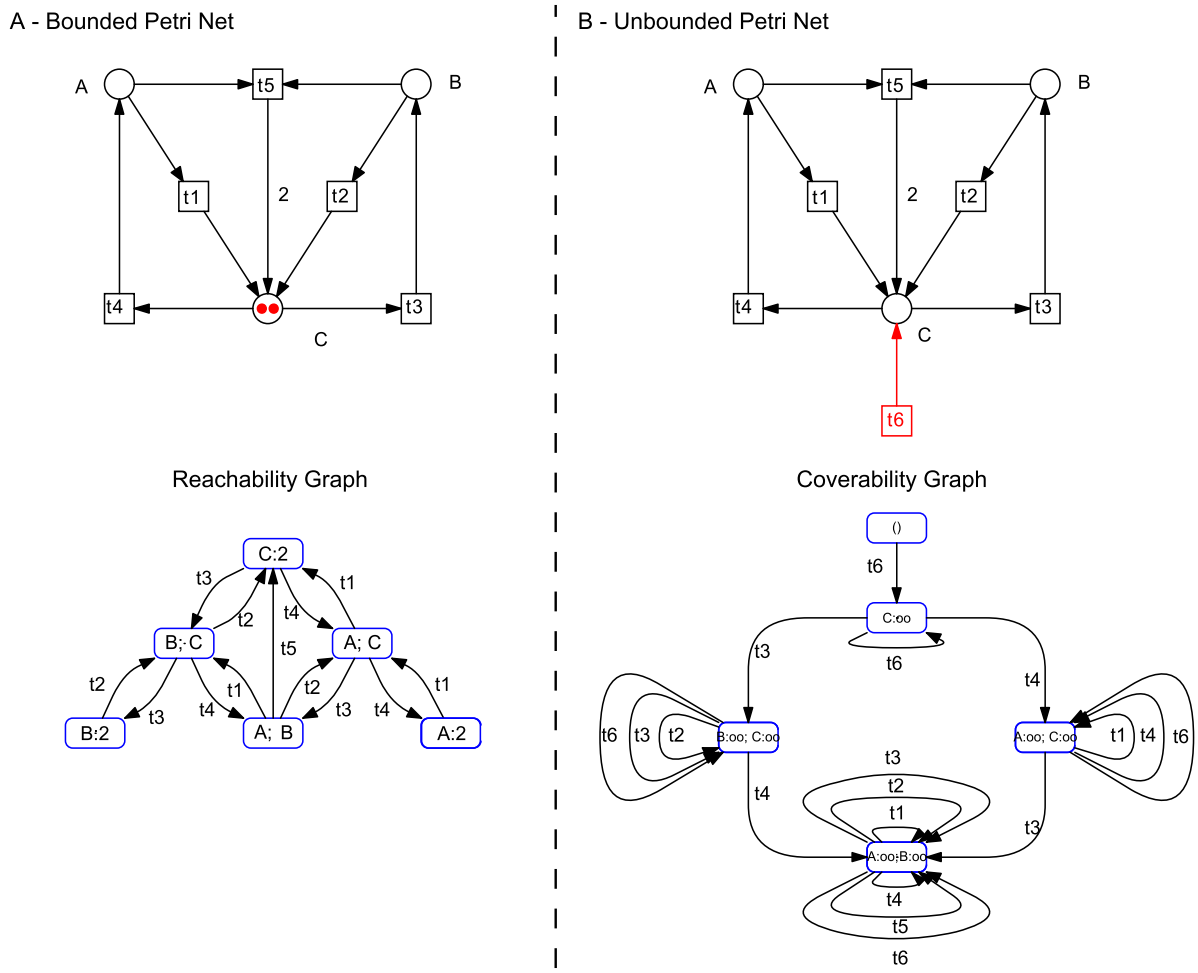


Figure 4.4: ***Reachability and Coverability Graph.*** *The reachability graph is constructed for bounded Petri nets (A). The initial marking of the bounded Petri net is depicted in red. The state space of an unbounded Petri net (B) is infinite. Thus, the coverability graph is constructed. The transition marked in red is always enabled. Therefore, the number of tokens infinitely increases in Petri net. In both graphs, the nodes display the marking of the current state. A place that is not marked, is not displayed. A marked place is followed the number of tokens. If a place carries only one token, the number is not displayed (trivial). Places that are unbounded, are indicated by "*inf*". Nodes with an empty marking are indicated by "()". Each arc is named by the transitions that induces the next state.*

space that have more than one successor (branching nodes), reflect either alternative or concurrent behaviour. Thus, branching nodes indicate dynamic conflicts, which have to be checked on the Petri net level. Nodes with out any successor represent dead states. Based on the state space graph, it is also possible to check for dead transitions.
The state space of a bounded Petri net can be visualised by a reachability graph. If the Petri net is not bounded, the coverability graph is constructed.
Based on the reachability graph we can decide the following behavioural properties of the underlying bounded Petri nets:

- k-bounded: The marking of each node in the state space is limited by a constant k.
- Reversibility: The reachability graph is strongly connected.
- Deadstate-free: The reachability graph has no terminal nodes, nodes without a successor.

- Liveness: The reachability graph is partitioned into strongly connected components (SCC), which refer to maximal sets of strongly connected nodes. A SCC is called terminal if no other SCC is reachable in the partitioned graph. A transition is live if and only if it is included in all terminal SCCs of the partitioned reachability graph. A Petri net is live if and only if this holds for all transitions.

Formal Definitions:

Definition 4.7 (State Space) *Let $N = (P, T, f, m_0)$ be a Petri net. The* state space *of N can be visualised by a graph $G(N) = (V_N, E_N)$, where*

- $V_N := [m_0\rangle$ *is the set of nodes,*
- $E_N := \{(m, t, m') \,|m, m' \in [m_0\rangle\,, t \in T : m\,[m_0\rangle\}$ *is the set of arcs.*

How to read:
Consider the example given in **Figure 2.5**.

- First, we have to construct the state space. We have already thought of all possible markings reachable from m_o in **Section 4.1.2.1**, see **Table 4.3**.
- $V_N = \{m_0, m_1, m_2\}$ contains the marking of all states that we have determined.
- $E_N = \{(m_0, Association, m_1)\,, (m_1, Dissociation, m_0)\,, (m_1, Synthesis, m_2)\}$ gives us the information of actual node, the enabled transition and the marking reached by the switching the respective transition.
- Based on V_N and E_N, we can now draw the reachability graph; see **Figure 4.5**:

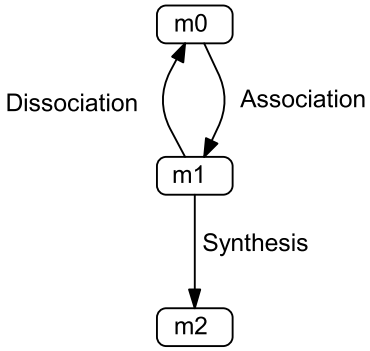
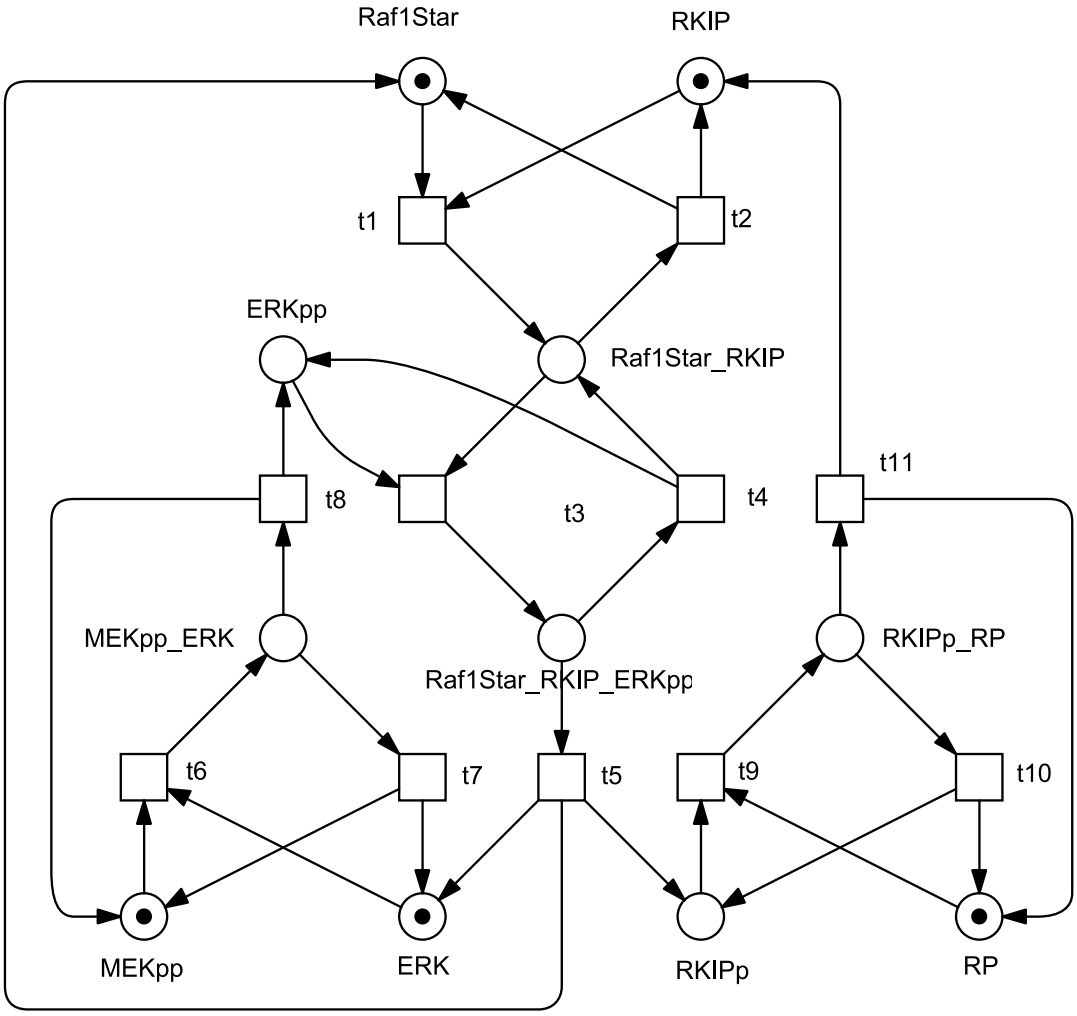


Figure 4.5: ***Reachability of the Running Example.*** *Reachability graph of the running example shown in **Figure 2.5**. The nodes are indicated by their marking; see also **Table 4.3**. The corresponding transition of each arc is written next to it.*

4.4 Examples

Example 4.1 (Analysis: RKIP Pathway [11]) *The Raf-1 interacting protein (RKIP) is an inhibitor of the MAP pathway [26]. RKIP binds to Raf-1 and thereby inhibits the phosphorylation of MEK by Raf-1. But RKIP is not a substrate of Raf-1. The interaction of the Raf-1-RKIP complex with ERK leads to the phosphorylation of RKIP and the dissociation of the complex. The Petri net model shown here is taken from [11] and refers to an ODE model given in [5].*

- *Petri net:*



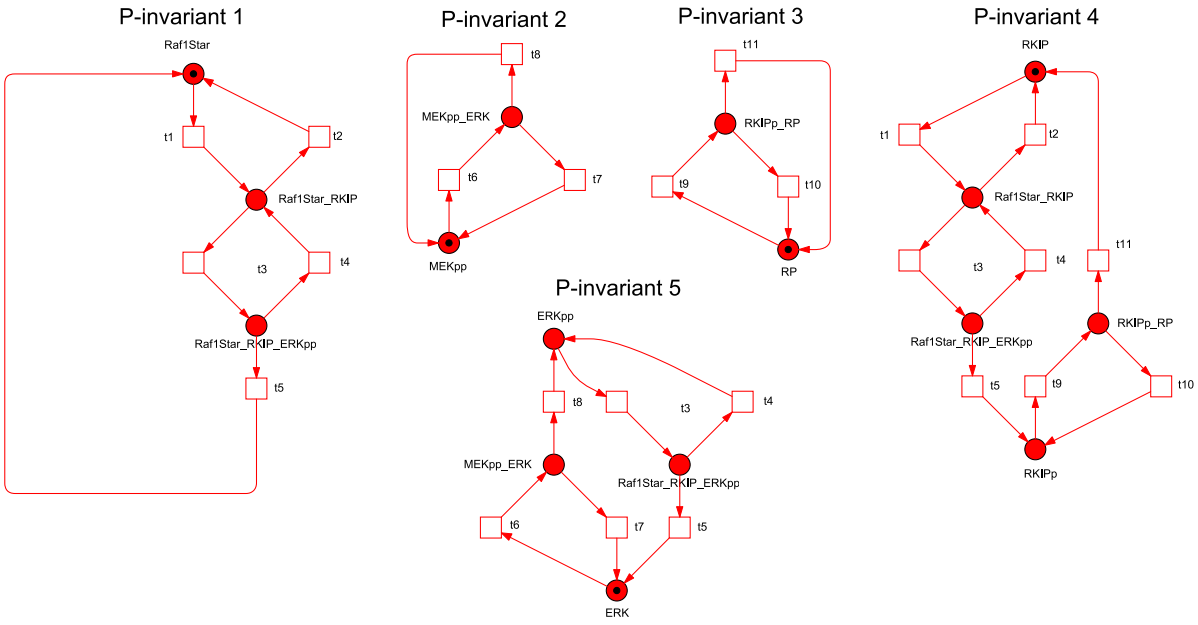
- *Assumptions:*
  - *Raf1 is always active (Raf1Star).*
  - *MEKpp is always phosphorylated.*
  - *Just a subset of ERK pathways that are regulated by RKIP are considered.*
- *Meaning of transitions:*
  - *t1: RKIP binds to Raf1Star (active state of Raf1) and thereby inhibits the binding of MEK to Raf-1 and consequently the phosphorylation of MEK.*

- *t2: Dissociation of the Raf1Star-RKIP complex.*
- *t3: Phosphorylated ERK (ERKpp) interacts binds to the Raf1Star-RKIP complex.*
- *t4: Dissociation of the Raf1Star-RKIP-ERKpp complex.*
- *t5: ERKpp phosphorylates RKIP and thereby causes the release of Raf1Star. ERK gets dephosphorylated by protein phosphatases (PP2A, MAPK phosphatases)*
- *t6: Binding of phosphorylated MEK (MEKpp) to ERK.*
- *t7: Dissociation of the MEKpp-ERK complex.*
- *t8: MEKpp phosphorylates ERK causing the release of ERKpp.*
- *t9: Binding of the RKIP phosphatase (RP) to phosphorylated RKIP (RKIPp).*
- *t10: Dissociation of the RKIPp-RP complex.*
- *t11: RP promotes dephosphorylation of RKIP causing the release of RKIP.*

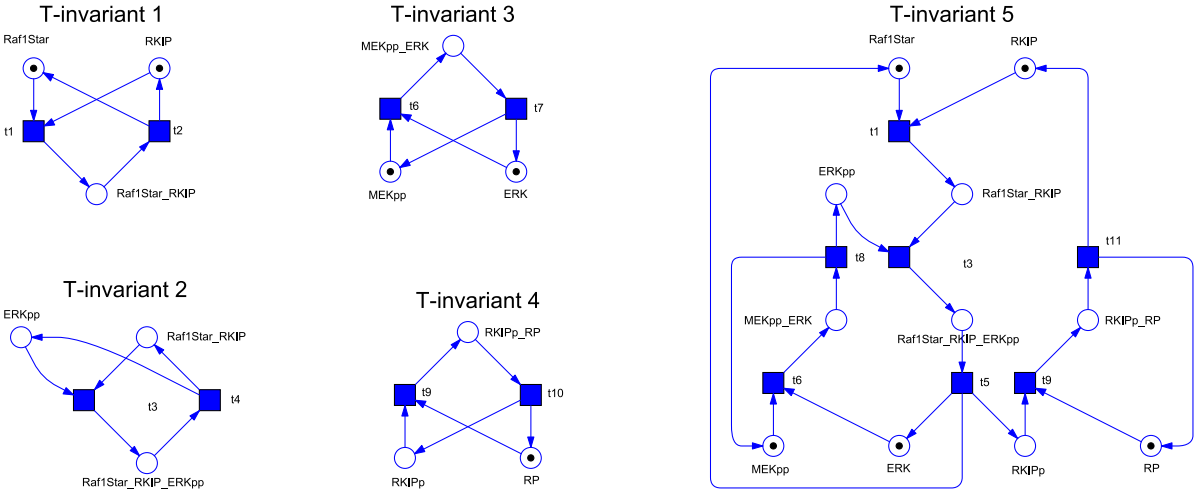
- *Properties:*

  - ***PUR = Y***
    The model contains no read arcs; side conditions for reactions. Enzymatic reactions are split into single steps.
  - ***ORD = Y***
    The stoichiometry in all reactions is "1".
  - ***HOM = Y***
    Each consuming reaction associated with one component takes the same amount of molecules of this component.
  - ***NBM = Y***
    The amount of molecules of a component being produced or consumed by each associated reaction is always equal.
  - ***CSV = N***
    The reactions in the model involve association/dissociation of different components.
  - ***SCF = N***
    There are reactions sharing the same components as reactants.
  - ***CON = Y***
    The involved components build a connected molecular network.
  - ***SC = Y***
    Moreover, each component has a direct path of reactions to all other components.
  - ***FT0 = Y***
    No external sources.
  - ***TF0 = Y***
    No external sinks.
  - ***FP0 = Y***
    No component acts only as a reactant.
  - ***PF0 = Y***
    No component acts only as a product.
  - ***STP = Y***
    The involved reactions form circles, the total amount of molecules in these circles will never become zero.
  - ***CPI = Y***
    Mass conservation is ensured. A P-invariant comprises all states of one component.
  - ***CTI = Y***
    All reactions in the model are contained in circles of reactions. Each circle can restore its initial state.
  - ***SCTI = N***
    Some of the circles make up by related reactions consist only of two steps.
  - ***SB = Y***
    Independently of the initial state, no component can infinitely accumulate.
  - ***k-B = Y***
    For each component exists an upper bound.
  - ***1-B = Y***
    In more detail, the upper bound for each component is restricted to one molecule.
  - ***DCF = N***
    If, e.g., the RafStar-RKIP complex dissociates it loses the possibility to bind phosphorylated ERK.
  - ***DSt = 0***
    At least one reaction can always take place.
  - ***DTr = N***
    For each reaction it is possible to reach a state, where the reaction can occur.
  - ***LIV = Y***
    Due to the cyclic reactions that restore each other, all reactions contribute forever to the signalling.
  - ***REV = Y***
    Due to the cyclic reactions that restore each other, the initial state can be reproduced.

- *P-invariants: Here, Here, each P-invariant describes all specific states of one component.*



- *T-invariants: Here, each T-invariant describes a circle of related reactions.*

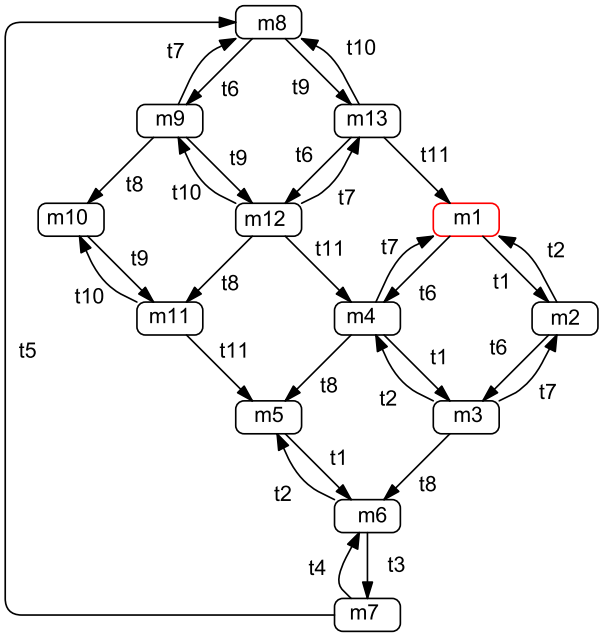


- *Siphons:*
  Here, each P-invariant makes up a siphon. The reactions involved in a P-invariant give at least one output to another P-invariant. Each P-invariant is connected with another P-invariant via at least one shared place. Thus, each P-invariant/siphon triggers the next reaction level and vice versa.

- *Traps:*
  Here, each P-invariant also constitutes a trap by definition. P-invariants can never run out of tokens. The reactions involved in a P-invariant give at least one input to another P-invariant. Each P-invariant is connected with another P-invariant via at least one shared place. Thus the corresponding traps in this model will always contain tokens. Meaning whatever happens, each protein will be available in at least one of its specific states.

- *Reachability Graph:*
  Due to the boundedness of the model, the reachability graph has been constructed. The initial marking is set to:
  Raf1Star, RKIP, MEKpp, ERK, RP = 1

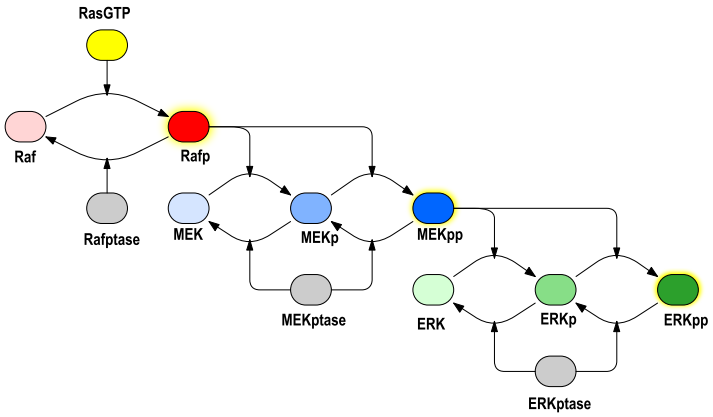
  - *Nodes:13*
  - *Edges: 30*
  - *SCC: 1*
  - *Terminal SCC: 1*



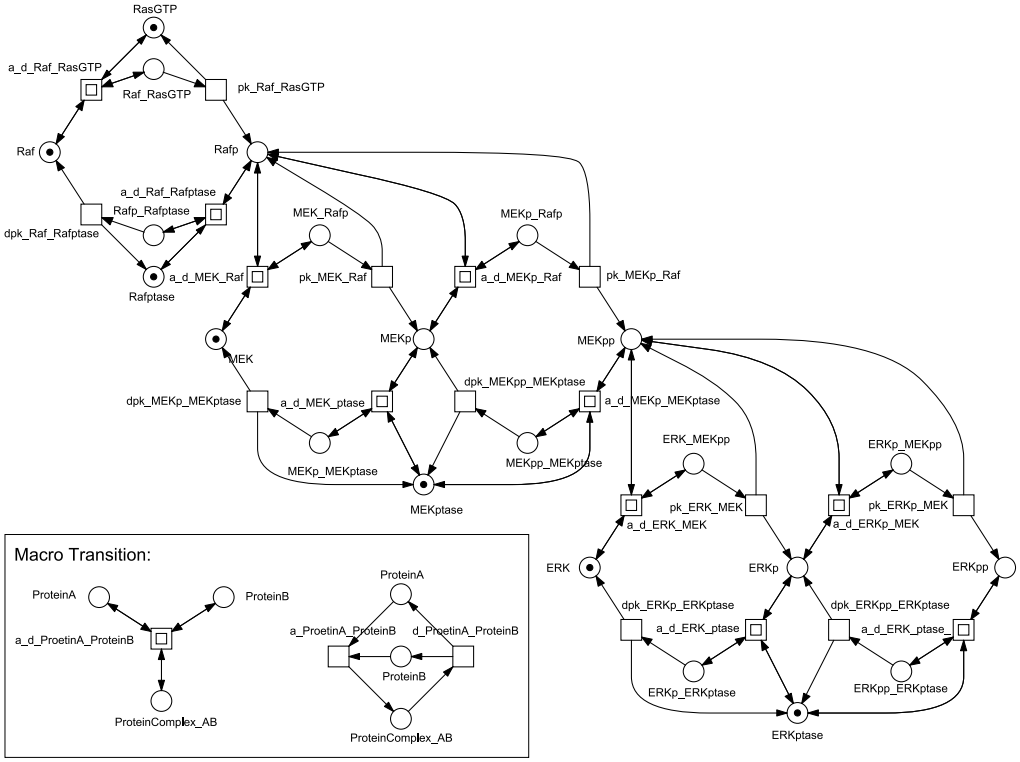
Place	*m1*	*m2*	*m3*	*m4*	*m5*	*m6*	*m7*	*m8*	*m9*	*m10*	*m11*	*m12*	*m13*
Raf1Star	*1*	*0*	*0*	*1*	*1*	*0*	*0*	*1*	*1*	*1*	*1*	*1*	*1*
RKIP	*1*	*0*	*0*	*1*	*1*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Raf1Star_RKIP	*0*	*1*	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Raf1Star_RKIP_ERKpp	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*0*	*0*
ERK	*1*	*1*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*1*
RKIPp	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*1*	*0*	*0*	*0*
MEKpp	*1*	*1*	*0*	*0*	*1*	*1*	*1*	*1*	*0*	*1*	*1*	*0*	*1*
MEKpp_ERK	*0*	*0*	*1*	*1*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*1*	*0*
ERKpp	*0*	*0*	*0*	*0*	*1*	*1*	*0*	*0*	*0*	*1*	*1*	*0*	*0*
RP	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*0*	*0*	*0*
RKIP_RP	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*1*

Example 4.2 (Analysis: MAP Kinase Cascade [11]) *The mitogen-activated protein kinase (MAPK) cascade is the core of the ubiquitous ERK-MAPK pathway regulating, e.g., cell division, cell differentiation. In this model (taken from [12]), we do not describe the signalling of the membrane receptors leading immediately to the activation of the intracellular MAPK pathway. Here, we consider the RasGTP complex as input which activates Raf by phosphorylation. Raf double phosphorylates MEK; and MEK in turn phosphorylates ERK twice. Double phosphorylated ERK serves as the output of our model. The dephosphorylation of the three signalling proteins is done by its corresponding phosphatase.*

- *Biological Cartoon:*



- *Petri net:*



- *Meaning of transitions:*
  - *a_ProteinA_ProteinB: Association of protein A and protein B*
  - *d_ProteinA_ProteinB: Dissociation of protein A and protein B*
  - *pk_ProteinA_ProteinB: Phosphorylation of protein A by protein B (protein kinase)*
  - *dpk_ProteinA_ProteinB: Dephosphorylation of protein A by protein B (protein phosphatase)*
- *Properties:*
  - ***PUR = Y***
    The model contains no read arcs; side conditions for reactions. Enzymatic reactions are split into single steps.
  - ***ORD = Y***
    The stoichiometry in all reactions is "1".
  - ***HOM = Y***
    Each consuming reaction associated with one component takes the same amount of molecules of this component.
  - ***NBM = Y***
    The amount of molecules of a component being produced or consumed by each associated reaction is always equal.
  - ***CSV = N***
    The reactions in the model involve association/dissociation of different components.
  - ***SCF = N***
    There are reactions sharing the same components as reactants.
  - ***CON = Y***
    The involved components build a connected molecular network.
  - ***SC = Y***
    Moreover, each component has a direct path of reactions to all other components.
  - ***FT0 = Y***
    No external sources.
  - ***TF0 = Y***
    No external sinks.
  - ***FP0 = Y***
    No component acts only as a reactant.
  - ***PF0 = Y***
    No component acts only as a product.
  - ***STP = Y***
    The involved reactions form circles, the total amount of molecules in these circles will never become zero.
  - ***CPI = Y***
    Mass conservation is ensured. A P-invariant comprises all states of one component.
  - ***CTI = Y***
    All reactions in the model are contained in circles of reactions. Each circle can restore its initial state.
  - ***SCTI = N***
    Some of the circles make up by related reactions consist only of two steps.
  - ***SB = Y***
    Independently of the initial state, no component can infinitely accumulate.
  - ***k-B = Y***
    For each component exists an upper bound.
  - ***1-B = Y***
    In more detail, the upper bound for each component is restricted to one molecule.
  - ***DCF = N***
    If, e.g., MEK gets dephosphorylated it loses the possibility to phosphorylate ERK.
  - ***DSt = 0***
    At least one reaction can always take place.
  - ***DTr = N***
    For each reaction it is possible to reach a state, where the reaction can occur.
  - ***LIV = Y***
    Due to the cyclic reactions that restore each other, all reactions contribute forever to the signalling.
  - ***REV = Y***
    Due to the cyclic reactions that restore each other, the initial state can be reproduced.

- *P-invariants:*

P-invariant	*Places*	*Meaning*
1	*ERKp_ERKptase, ERKpp_ERKptase, ERKptase*	*States of ERKptase*
2	*MEKp_MEKptase, MEKpp_MEKptase, MEKptase*	*States of MEKptase*
3	*RasGTP, Raf_RasGTP*	*States of RasGTP*
4	*Rafptase, Rafp_Rafptase*	*States of Rafptase*
5	*ERK, ERK_MEKpp, ERKp, ERKp_MEKpp , ERKp_ERKptase, ERKpp, ERKpp_ERKptase*	*States of ERK*
6	*MEK, MEK_Rafp, MEKp, MEKp_Rafp, MEKp_MEKptase, MEKpp, MEKpp_MEKptase, ERK_MEKpp, MEK, ERKp_MEKpp*	*States of MEK*
7	*MEK_Rafp, Raf, Raf_RasGTP, Rafp, Rafp_Rafptase, MEKp_Rafp*	*States of Raf*

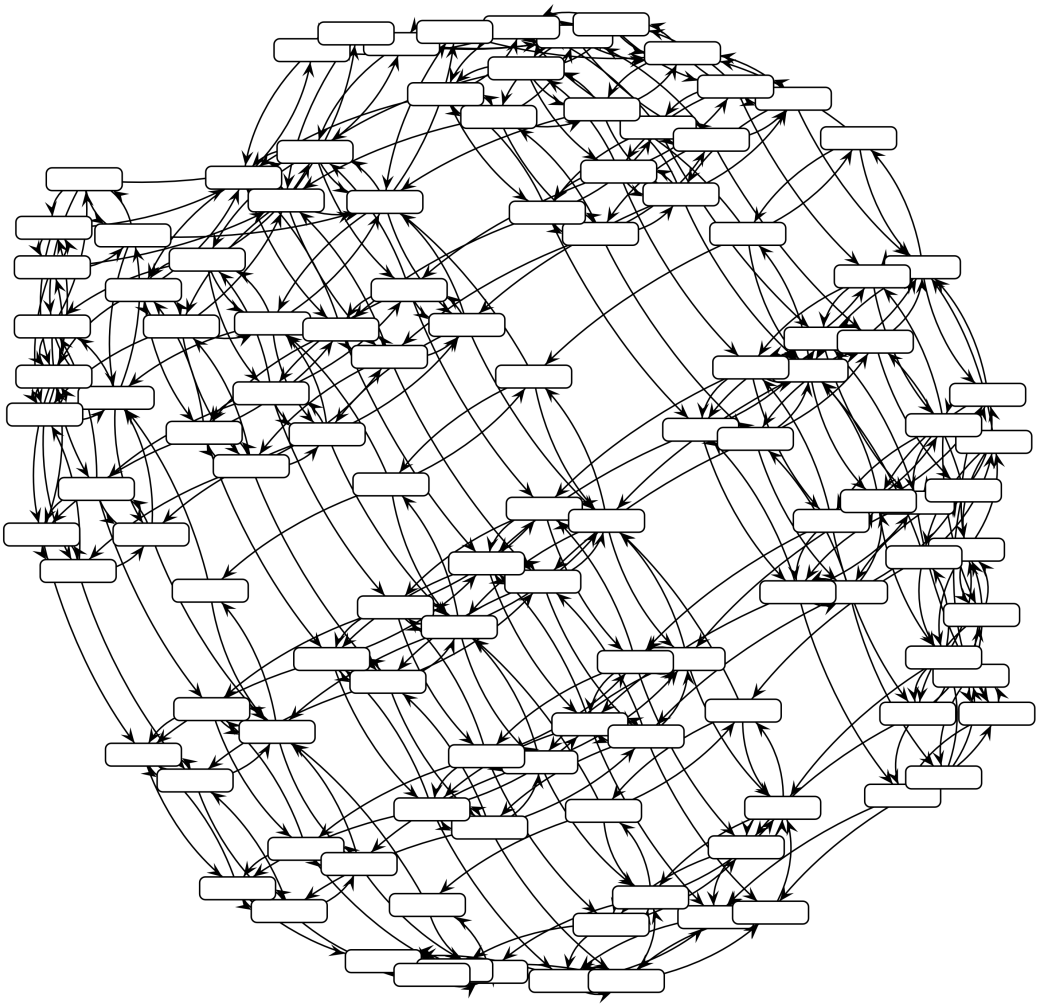
- *T-invariants:*

T-invariant	*Transitions*	*Meaning*
1	*a_Raf_RasGTP, d_Raf_RasGTP*	*Association and dissociation of Raf and RasGTP*
2	*a_Raf_Rafptase, d_Raf_Rafptase*	*Association and dissociation of Raf and Rafptase*
3	*a_k_ERK_MEK, d_k_ERK_MEK*	*Association and dissociation of ERK and double phosphorylated MEK*
4	*a_MEK_Raf, d_MEK_Raf*	*Association and dissociation of MEK and phosphorylated Raf*
5	*a_Raf_RasGTP, pk_Raf_RasGTP, a_Raf_Rafptase, dpk_Raf_Rafptase*	*Binding of Raf to RasGTP, phosphorylation of Raf and release, binding of phosphorylated Raf to Rafptase, dephosphorylation of phosphorylated Raf and relaese*
6	*a_MEKp_Raf, d_MEKp_Raf*	*Association and dissociation of phosphorylated MEK and phosphorylated Raf*
7	*a_MEKp_MEKptase, d_MEKp_MEKptase*	*Association and dissociation of phosphorylated MEK and MEKptase*
8	*a_MEK_Raf, pk_MEK_Raf, a_MEKp_MEKptase, dpk_MEKp_MEKptase*	*Binding of MEK to phosphorylated Raf, phosphorylation of MEK and release, binding of phosphorylated MEK to MEKptase, dephosphorylation of phosphorylated MEK and release*
9	*a_MEKpp_MEKptase, d_MEKpp_MEKptase*	*Association and dissociation of double phosphorylated MEK and MEKptase*
10	*a_MEKp_Raf, pk_MEKp_Raf, a_MEKpp_MEKptase, dpk_MEKpp_MEKptase*	*Binding of phosphorylated MEK to phosphorylated Raf, second phosphorylation of MEK and release, binding of double phosphorylated MEK to MEKptase, dephosphorylation of double phosphorylated MEK and release*
11	*a_k_ERKp_MEK, d_k_ERKp_MEK*	*Association and dissociation of phosphorylated ERK and double phsophorylated MEK*
12	*a_ERKp_ERKptase, d_ERKp_ERKptase*	*Association and dissociation of phosphorylated ERK and ERKptase*
13	*a_k_ERK_MEK, pk_ERK_MEK, a_ERK_ERKptase, dpk_ERKp_ERKptase*	*Binding of ERK to double phosphorylated MEK, phosphorylation of ERK and release, binding of phosphorylated ERK to ERKptase, dephosphorylation of phosphorylated ERK and release*
14	*a_ERKpp_ERKptase, d_ERKpp_ERKptase*	*Association and dissociation of double phosphorylated ERK and ERKptase*
15	*a_k_ERKp_MEK, pk_ERKp_MEK, a_ERKpp_ERKptase, pk_ERKpp_ERKptase*	*Binding of phosphorylated ERK to double phosphorylated MEK, second phosphorylation of ERK and release, binding of double phosphorylated ERK to ERKptase, dephosphorylation of double phosphorylated ERK and release*

- *Siphons:*
  Here, each P-invariant makes up a siphon. The reaction involved in a P-invariant give at least one output to another P-invariant. Each P-invariant is connected with another P-invariant via at least one shared place. Thus, each P-invariant/siphon triggers the next reaction level and vice versa.

- *Traps:*
  Here, each P-invariant also constitutes a trap by definition. P-invariants can never run out of tokens. The reactions involved in a P-invariant give at least one input to another P-invariant. Each P-invariant is connected with another P-invariant via at least one shared place. Thus the corresponding traps in this model will always contain tokens. Meaning whatever happens, each protein will be available in at least one of its specific states.

- *Reachability Graph:*
  Due to the boundedness of the model, the reachability graph has been constructed. The initial marking is set to:
  RasGTP, Rafptase, ERK, MEK, Raf, MEKptase, ERKptase = 1

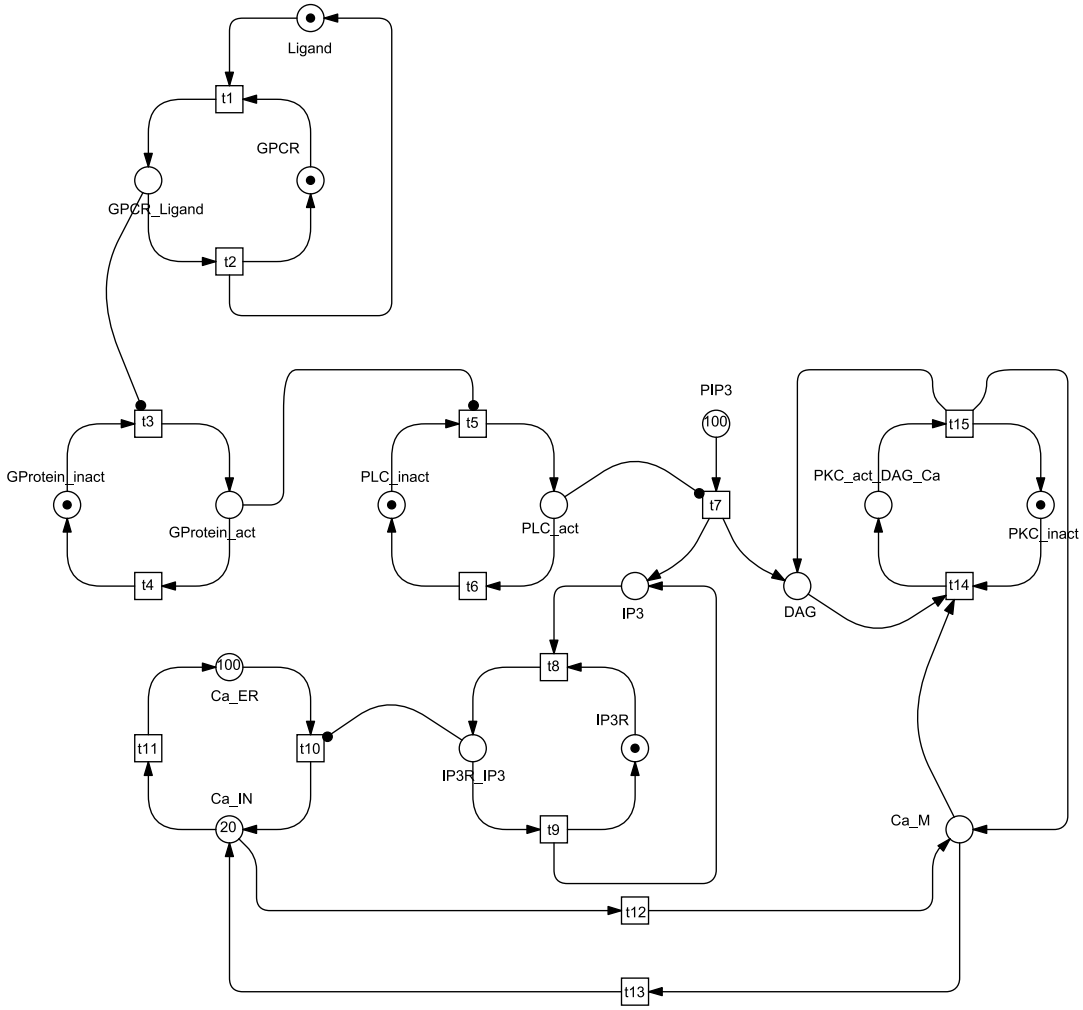


  - *Nodes:118*
  - *Edges: 468*
  - *SCC: 1*
  - *Terminal SCC: 1*

Note: The state space is to large to describe the different states in more detail.

Example 4.3 (Analysis: Phosphoslipase C Signalling) *The G-protein coupled receptor (GPCR) activates Phospolipase C (PLC) via its coupled G-protein. PLC itself activates two different signal pathways by catalyzing the hydrolysis of the membrane-bound PIP3 to Inositol-1,4,5-triphosphat (IP3) and Diacylglycerin (DAG), which are both important second messengers. IP3 diffuses into the cytoplasm and binds to the IP3 receptor (IP3R) at the endoplasmic reticulum (ER). As a result Ca^{2+} is released from the ER and diffuses across to the membrane. DAG remains at the membrane. Ca^{2+} and DAG recruit and activate PKC. PKC itself is now able to phosphorylate its target proteins.*

- *Petri net:*



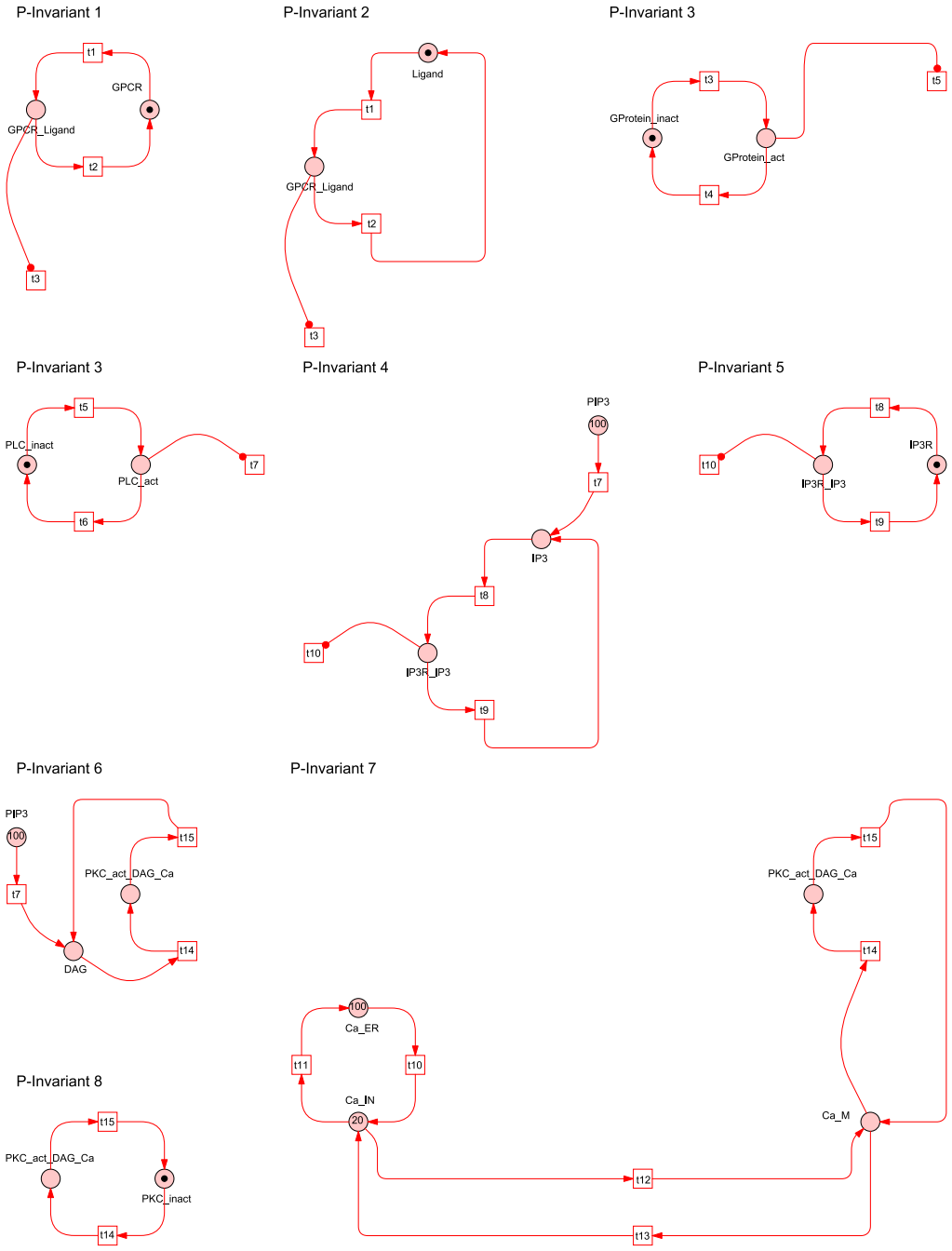
- *Meaning of transitions:*
  - *t1: Binding of the ligand to GPCR*
  - *t2: Dissociation of the ligand from the GPCR*
  - *t3: Activation of the G-Protein by the ligand bound GPCR*
  - *t4: Inactivation of the G-Protein*
  - *t5: Activation of PLC by the activated G-Protein*
  - *t6: Inactivation of PLC*
  - *t7: Splitting of PIP3 into IP3 and DAG catalysed by activated PLC*

- *t8: Binding of IP3 to IP3R at the ER*
- *t9: Dissociation of IP3 from IP3R at the ER*
- *t10: Release of Ca^{2+} from the ER into intracellular space*
- *t11: Influx of Ca^{2+} from the intracellular space into the ER*
- *t12: Diffusion of Ca^{2+} from the intracellular space to the membrane*
- *t13: Diffusion of Ca^{2+} from the membrane to the intracellular space*
- *t14: Binding of DAG and Ca^{2+} to inactive PKC causing activation of PKC*
- *t15: Dissociation of DAG and Ca^{2+} from active PKC causing inactivation of PKC*

- *Properties:*

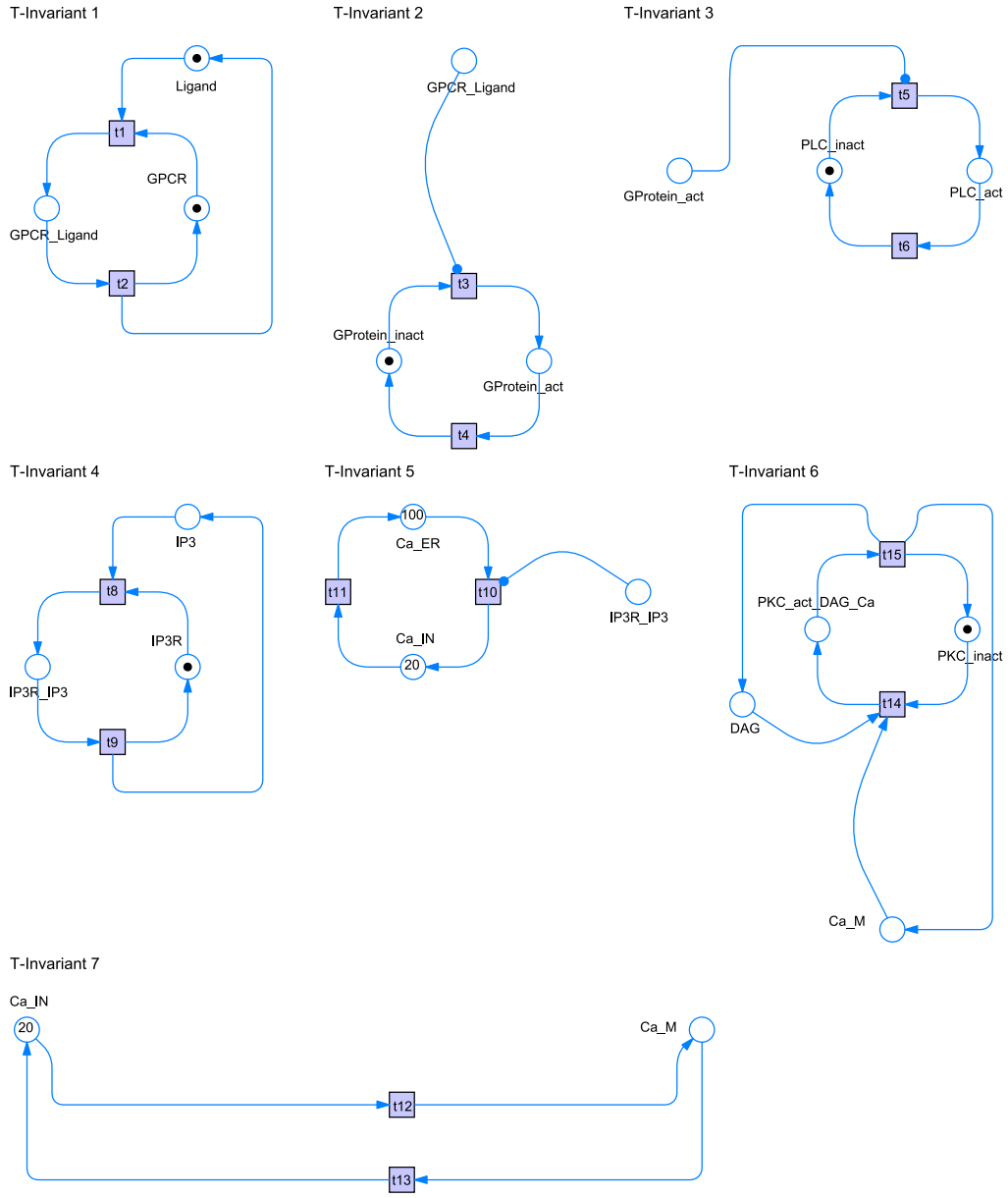
  - ***PUR = N***
    The model contains read arcs; side conditions for reactions, e.g., some components act as a direct trigger on the next reaction and get not consumed.
  - ***ORD = Y***
    The stoichiometry in all reactions is "1".
  - ***HOM = Y***
    Each consuming reaction associated with one component takes the same amount of molecules of this component.
  - ***NBM = N***
    The amount of molecules of a component being produced or consumed by each associated reaction is always equal expect for PIP3. PIP3 is only a reactant but never a product.
  - ***CSV = N***
    The reactions in the model involve association/dissociation of different components.
  - ***SCF = N***
    There are reactions sharing the same components as reactants.
  - ***CON = Y***
    The involved components build a connected molecular network.
  - ***SC = N***
    Not every component has a direct path of reactions to all other components, e.g., IP3 and DAG can not interact with components in the upper part of the signal pathway.
  - ***FT0 = Y***
    No external sources.
  - ***TF0 = Y***
    No external sinks.
  - ***FP0 = N***
    No component acts only as a reactant expect PIP3.
  - ***PF0 = Y***
    No component acts only as a product.
  - ***STP = N***
    Once all molecules of PIP3 are consumed, PIP3 is no longer available and the hydrolysis to IP3 and DAG can not take place any more.
  - ***CPI = Y***
    Mass conservation is ensured. A P-invariant comprises all states of one component.
  - ***CTI = N***
    The reactions build cycles that are able to restore their initial state. Just the degradation of PIP3 is not a part of an reaction cycle.
  - ***SCTI = N***
    Some of the circles made up by related reactions consist only of two steps.
  - ***SB = Y***
    Independently of the initial state, no component can infinitely accumulate.
  - ***k-B = Y***
    For each component exists an upper bound.
  - ***1-B = Y***
    In more detail, the upper bound for each component is restricted to one molecule.
  - ***DCF = N***
    If a component gets deactivated, it loses its function to trigger the next step in the signal pathway.
  - ***DSt = 0***
    At least one reaction can always take place.
  - ***DTr = N***
    For each reaction it is possible to reach a state, where the reaction can occur.
  - ***LIV = N***
    The hydrolysis of PIP3 stops, if PIP3 is used up. This reaction can not contribute to the dynamic behaviour forever.
  - ***REV = N***
    The initial state of the signal pathway can not be restored because of the limiting factor PIP3 and IP3/DAG that can not be withdrawn once produced.

- *P-invariants:*



Here, each P-invariant comprises the specific states of one protein (active (bound) or inactive (unbound) states of GPCR, G-protein, PLC, PKC, IP3R) or related states of a non-protein (different localizations of Ca^{2+}, bound or unbound state of the ligand, PIP3 its products (IP3, DAG) and their bound states). This ensures the mass conservation for each involved component.

- *T-invariants:*



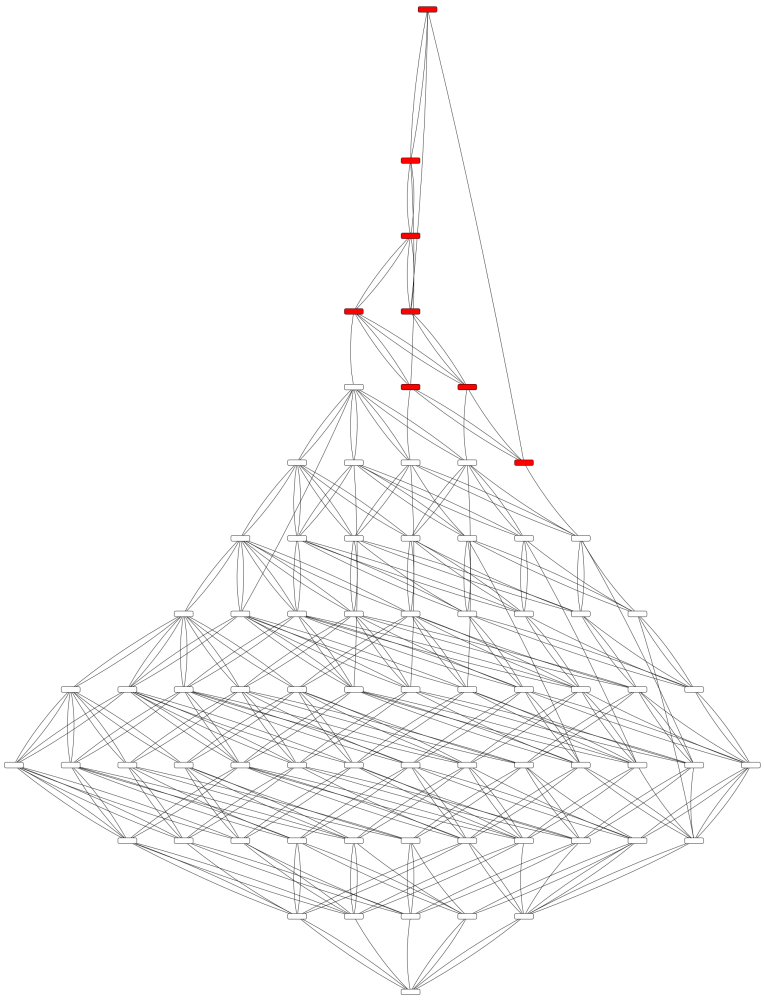
Each T-invariant describes reactions of a component that restore the initial state of the respective component. This includes activation/inactivation triggered by an another active protein (this is the case for G-protein, PKC) or by binding/dissociation of a non-protein (this is the case for GPCR, IP3R, PKC) and the binding/dissociation of the non-proteins themselves (DAG, IP3, ligand, Ca^{2+}). The model is not covered by P-invariants because PIP3 is a limiting factor that can not be recovered.

- *Siphons:*
  Here, each P-invariant (1-4, 6) that is connected with another P-invariant via a read edge also indicates a siphon. Those siphons trigger activation of other components without consuming themselves. Thus, the siphons will not run out of components. P-invariants 8, 9 constitute a

closed cycle and are therefore also siphons that can not run out of molecules. PIP3 is also a siphon, but it will be cleaned if PIP3 is completely degraded to IP3 and DAG.

- *Traps:*
  Here, each P-invariant also constitutes a trap by definition. P-invariants can never run out of tokens. Thus the corresponding traps in this model will always contain tokens. Meaning whatever happens, each component will be available in at least one of its specific states.

- *Reachability Graph:*
  Due to the boundedness of the model, the reachability graph has been constructed. The initial marking is set to: GPCR, Ligand, GProtein_inact, PLC_inact, PIP3, IP3R, Ca_ER, PKC_inact = 1



  - *Nodes:72*
  - *Edges: 336*
  - *SCC: 2*
  - *Terminal SCC: 1 (red nodes)*

Note: The state space is to large to describe the different states in more detail.

CHAPTER 5

Quantitative Petri Net Analysis

In this chapter, you learn, how to perform simulations with your model. Here, we will answer the questions:

Which kinetic considerations are possible?
What is the formal background of these considerations?
What you have to do with you qualitative model to get its time-dependent dynamic behaviour?

Several specialized Petri net classes are available to describe different scenarios and to consider different simulative approaches. Therefore, the kinetics of the qualitative Petri net model can be considered as stochastic, continuous or as a mixture of both (hybrid) [12]. Advantageously, the qualitative properties of a Petri net are independent of its kinetic consideration, meaning they do not change.

5.1 Stochastic Petri Nets

Since most bio-molecular processes are stochastic by their very nature, the applications of stochastic simulations are straightforward. The modelling of bio-molecular networks by stochastic Petri nets (SPN) was first proposed in [9], where they applied SPNs to a gene regulatory network. In the following years SPNs have been applied to several biological case studies discussed in [25], [17], [22], [23], [6].
The formal definition of a stochastic Petri net is given below. Here, we summarize the explanation of stochastic Petri nets given in [12].
The network structure of a quantitative time-dependent stochastic Petri net is given by the respective qualitative time-independent Petri net. Thus, the qualitative network structure and the discrete marking of the qualitative Petri net is maintained in the stochastic Petri net. In stochastic Petri nets, transitions become enabled as usual. A transitions gets enabled if pre-places are sufficiently marked. Before firing of an enabled transition $t \in T$, a waiting time has to elapse. The waiting time is an exponential distributed random variable $X_t \in [0, \infty)$ with the probability density function:

$$f_{x_t}(\tau) = \lambda_t(m) \cdot e^{-\lambda_t(m) \cdot \tau},\ \tau \geq 0.$$

The firing itself does not consume any time. The semantics of a stochastic Petri net is described by a continuous time Markov chain (CTMC). The CTMC of a stochastic Petri net without parallel transitions is isomorphic to the reachability graph. The arcs between the states are now labelled by the transition rates. Thus, in stochastic Petri nets all reactions defined in the network structure can still occur, but the likelihood depends on the probability distribution. Thus, the state space is also

maintained in the stochastic consideration, all sequences of events can still take place. Consequently, the same powerful analysis techniques can be applied to stochastic Petri nets as before to qualitative Petri nets.
The general procedure of a simulation run is not hard to understand. Each transition has its own local timer. The timer is set to an initial value if it is enabled (pre-places are sufficiently marked). Therefore, a waiting time is computed by the corresponding probability distribution at the respective time-point. The value will be different for each simulation run. The timer is decremented at a constant speed, and the transition will fire when the elapsed. If more than one transition is enabled at a certain time-point, a waiting time is computed for each of those transitions. Consequently, the transition with the smallest waiting time will fire and win the race. After firing, all waiting times will be set to zero and new waiting times will be computed for the enabled transitions at the reached state.

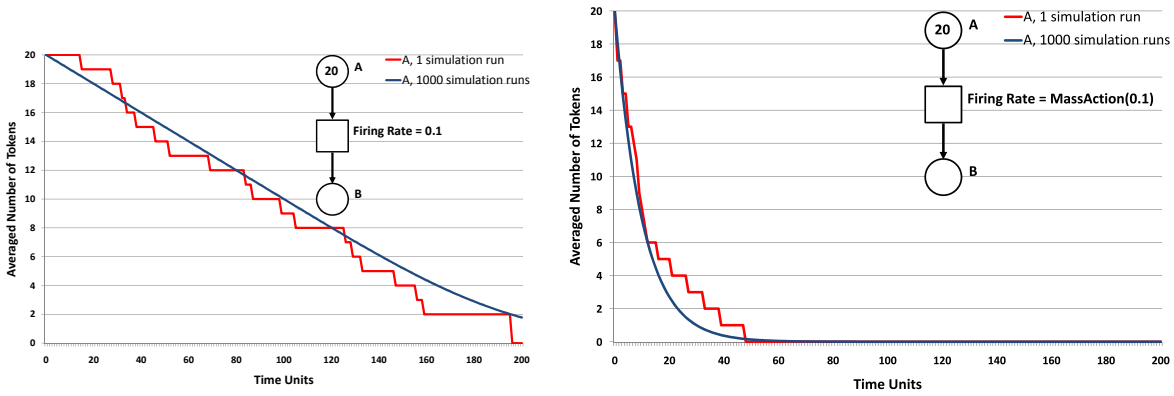


Figure 5.1: ***Stochastic Simulation Using Different Firing Rates.*** *The figure illustrates the stochastic simulation of an ordinary reaction given by the Petri net above using two different numbers of simulation runs and two different firing rates. In both cases, the time curves using just 1 simulation run (red) produced staircase-shaped in contrast to the blue curve, where 1000 simulation runs have been averaged. The edges of the single simulation runs have been smoothed out by averaging all simulations runs. In contrast to the firing rate defined by a single weighting parameter (left), the "MassAction" (right) considers the number of tokens on place A. Thus, the more tokens are on place A, the more tokens of A are consumed by firing of the transition. The firing rate in the left diagram results in a more or less linear function, where the firing rate on the right causes a curved line.*

Various probability functions can be chosen to define the random variables. Exponential distributions are suitable to describe (bio-)molecular systems. Meaning, the likelihood of a reaction (firing of a transition) follows an one-parametric exponential function with a marking-dependent parameter λ. Parameter λ specifies the local timer of each transition. However, the semantic of stochastic Petri nets with exponential distributed waiting times can be represented by a time Markov chain. The default firing rate for each transitions can be weighted by a second parameter; see **Figure 5.1, left**. Thus, you can weight the likelihood of two concurrent reaction to occur. In addition, the default firing rate of a transitions can be modified by defining specific mathematical and semantic function. Allowing the introduction of mass action functions, which consider the total number of tokens at the pre-places of an enabled transition; see **Figure 5.1, right**. Obviously, the likelihood of a reaction increases with an increase in its reactants.
The stochastic simulation of the time-dependent dynamic behaviour of a network indicates the time-dependent token flow for each place in the model, as well as firing frequency of each transition. One simulation run describes at least one path in the state space graph **Section 4.3**. It is also possible to perform multiple simulation runs and average the results of all runs. Thus, an averaged time course will be computed. The more simulation runs are performed, the more precise is the averaged time

course. All single simulation runs will fluctuate around the averaged time course.
To define the firing rates more precisely kinetic information of the biological system should be integrated. Ideally, the kinetic information are known from experiments. Experimental data could also be used to evaluate the time-dependent dynamic behaviour, time-dependent token flow, of the model. Nevertheless, stochastic simulations can be performed without knowing the exact kinetic parameters. To perform first test runs, a suitable set of kinetic parameters could also be estimated by trial and error to investigate the principle time-dependent dynamic behaviour of the model. However, more sophisticated methods to estimate parameters should also be considered. If it is not possible to reproduce the time-dependent dynamic behaviour of the biological system by the respective model, you may have to revise the network structure.

Formal Definitions:

Definition 5.1 (Stochastic Petri Net) *A biochemically interpreted stochastic Petri net is a quintuple* $SPN_{Bio} = (P, T, f, v, m_0)$*, where:*

- P, T *are finite, non empty, disjoint sets.* P *is the set of* places. T *is the set of* transitions.
- f: $((P \times T) \cup (T \times P)) \to \mathbb{N}_0$ *defines the set of directed* arcs*, weighted by non-negative integer values*
- v: $T \to H$ *is a function, which assigns a* stochastic hazard function h_t *to each transition* t*, whereby*
  $H := \bigcup_{t \in T} \left\{ h_t | \, h_t : \mathbb{N}_0^{|^\bullet t|} \to \mathbb{R}^+ \right\}$ *is the set of all stochastic hazard functions, and* $v(t) = h_t$ *for all transitions* $t \in T$*.*
- m_0: $P \to \mathbb{N}_0$ *gives the* initial marking.

How to read:
The definition for stochastic Petri nets is an extension of the definition for standard Petri nets; see **Definition 2.1**. Thus, the explanation of P, T and m_0 is similar. In addition, for stochastic Petri nets we need to define a hazard function h_t for each transitions, which is similar to the firing rate. The firing rate is expressed by an arbitrary equation using numerical constants, pre-defined parameters and pre-places, which produces results in $\mathbb{R}^+$. In our example; see **Figure 2.5**, we use the following firing rates: Figure 5.2 shows the averaged results of 10000 simulation runs.

- *Association*: $k_1 \cdot Enzyme \cdot Substrate$,
- *Dissociation*: $k_2 \cdot EnzymeSubstrateComplex$,
- *Synthesis*: $k_3 \cdot EnzymeSubstrateComplex$,
  with $k_1, k_2, k_3 = 0.1$

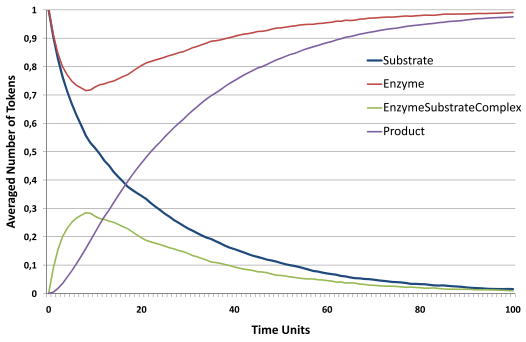
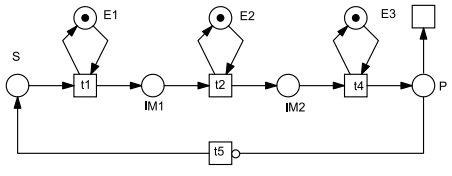


Figure 5.2: ***Stochastic Simulation of Running Example.*** *We simulate the stochastic time-dependent dynamic behaviour of the model shown in* ***Figure 2.5*** *using the firing rates, which we defined on the left.*

5.1.1 Examples

Example 5.1 (Stochastic Simulation: Feedback Inhibition) *In this example, we show the time-dependent dynamic behaviour of the feedback inhibition as shown before. For this purpose, we slightly modified the Petri net. The network structure and kinetic parameters result in a damped oscillation.At the end all components are in balanced steady state.*

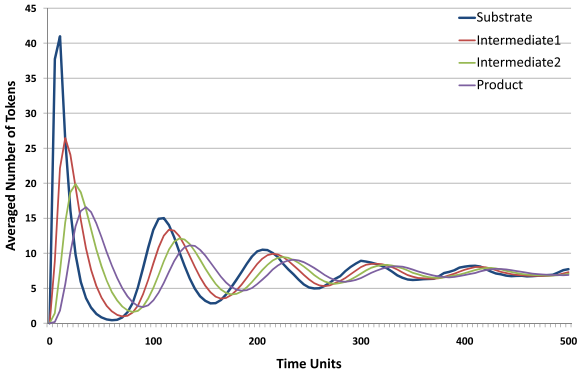
- Petri net:



Transitions:
t1 : MassAction(k1)
t2 : MassAction(k1)
t3 : MassAction(k1)
t4 : MassAction(k1)
t5 : MassAction(k2)

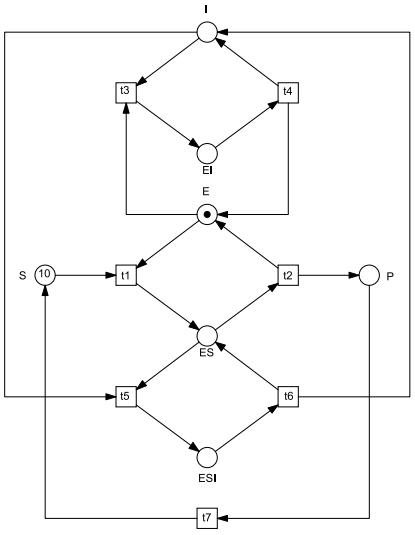
Parameters:
k1 = 0.1
k2 = 10

- Simulation:



Example 5.2 (Stochastic Simulation: Allosteric Enzyme Inhibition) *Here, we simulate the time-dependent dynamic behaviour of the allosteric enzyme inhibition shown before. For this purpose, we assume that there is a reverse reaction, where the product feeds into the substrate. The simulation graph shows, that the amount of product is reduced if the inhibitor is added to the systems.*

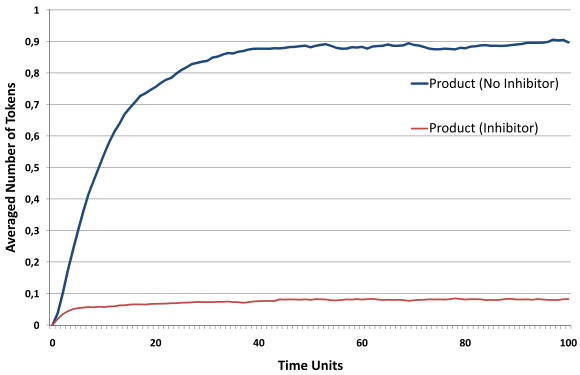
- Petri net:



Transitions:
t1 : MassAction(k1)
t2 : MassAction(k1)
t3 : MassAction(k2)
t4 : MassAction(k1)
t5 : MassAction(k1)
t6 : MassAction(k2)
t7 : MassAction(k1)

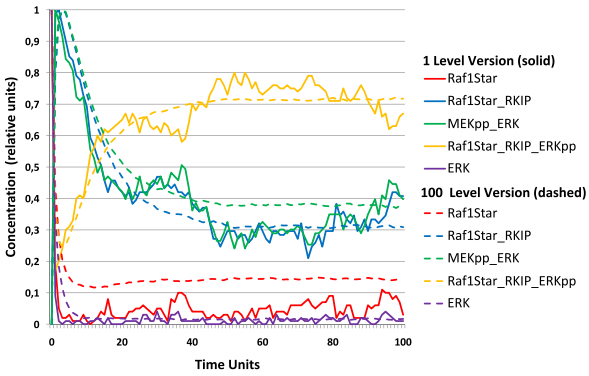
Parameters:
k1 = 0.1
k2 = 1.0

- Simulation:



Example 5.3 (Stochastic Simulation: RKIP Pathway [11] see Example 4.1) *For the simulation of the time-dependent dynamic behaviour of the RKIP pathway, we choose two different markings. In the first case, the amount of tokens on each initially marked place (see* ***Example 4.1****) is set to one and in the second case, we put 100 tokens each initially marked place. Anyway, the marking is related to the level semantic explained in the next example, see* ***Example 5.4****. In both cases, we averaged 100 simulation runs.*

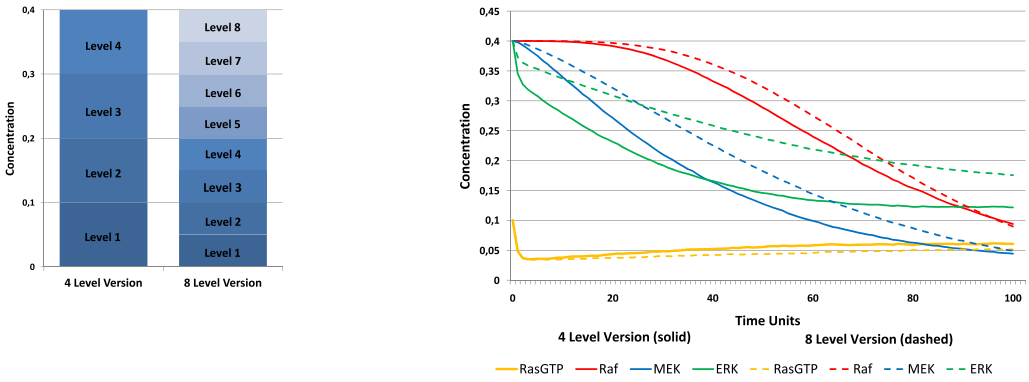
- *Simulation:*



The continuous behaviour can be very well adapted by employing the 100 level version to represent the concentration range.

Example 5.4 (Stochastic Simulation: MAP Kinase Cascade [12], see Example 4.2) *As you now from the sections before, tokens can also be interpreted as discrete concentration levels. For this reason the maximum molar concentration M can be split in $N+1$ different levels, ranging from $0, ..., N$. The discrete levels $0, (0, 1*M/N], (1*M/N, 2*M/N], ..., (N-1*M/N, N*M/N]$ stands for equivalent continuous states. In this example, all components have the same concentration range $(0.1...0.4)$, we employ 4 levels and 8 levels to represent the concentration range. Here, performed 10000 simulation runs.*

- *Simulation:*



To approximate the continuous behaviour it is sufficient to employ the 8 level version to represent the continuous concentration range.

5.2 Continuous Petri Nets

To be complete, we give a brief introduction into continuous Petri nets, we refer to the content given in [12]. Again, the network structure of a continuous Petri net is adopted from the structure of the underlying qualitative Petri net. Thus, we obtain the same qualitative state space from the network structure and can apply the same structural analysis techniques to continuous Petri nets. In continuous Petri nets, the marking is now given by a positive real number, called token value, instead of an integer as before in the case of qualitative and stochastic Petri nets. The token value can be considered as concentration. The formal definition of a continuous Petri net is given below. Here, we summarize the explanation of continuous Petri nets given in [12].
A transition is enabled in its current marking state if the token value of all pre-places is positive and greater than zero, $\forall p \in {}^\bullet t : m(p) > 0$. As in the case of stochastic Petri nets, several arbitrary firing rates can be defined by mathematical functions, like mass-action kinetic or the Michaelis-Menten kinetics, just to name two popular kinetics for biological systems. The firing rate may also be negative; in this case the reaction takes place in the reverse direction. In that way, reversible reactions are modelled using only one transition. The corresponding positive firing rate describes the forward direction.
The semantic of a continuous Petri net is given by the corresponding set of ODE equations, describing the continuous change over time on the token value of a given place. Where the pre-transition flow results in a continuous increase and post-transition flow results in a continuous decrease. A continuous Petri net is the structured description of an ODE-system. This description maybe less error prone than manually deduction of the ODE system.
In addition, it is also possible to convert stochastic and continuous Petri nets and thereby compare the obtained results of both simulative approaches. The qualitative structure is preserved.
Formal Definitions:

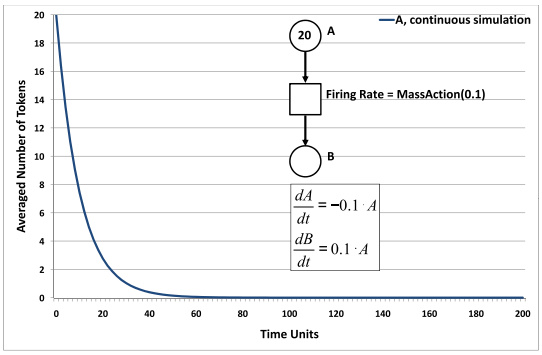


Figure 5.3: ***Continuous Simulation.*** *The figure illustrates the continuous simulation of an ordinary reaction given by the Petri net above using a mass action kinetic equation. The blue line corresponds to the decrease of the token value on place A. The results obtained from the continuous Petri net are equivalent to the results obtained from the respective ODE-system.*

Definition 5.2 (Continuous Petri Net) *A continuous Petri net is a quintuple CPN_{Bio} = (P, T, f, v, m_0), where:*

- *P, T are finite, non empty, disjoint sets. P is the set of* continuous places. *T is the set of* continuous transitions.
- *f: $((P \times T) \cup (T \times P)) \to \mathbb{R}_0^+$ defines the set of directed* arcs, *weighted by non-negative integer values*
- *v: $T \to H$ is a function, which assigns a* firing rate function h_t *to each transition t, whereby $H := \bigcup_{t \in T} \{ h_t | \, h_t : \mathbb{R}^{|{}^\bullet t|} \to \mathbb{R}^+ \}$ is the set of all firing rate functions, and v(t) = h_t for all transitions $t \in T$.*
- *m_0: $P \to \mathbb{R}_0^+$ gives the* initial marking.

How to read:
The definition for continuous Petri nets is an extension of the definition for standard Petri nets; see **Definition 2.1**. Thus, the explanation of P and T is similar. The The initial marking m_0 is now interpreted as real number. In addition, like in this case of stochastic Petri nets; see **Definition 5.2**, we need to define a firing rate for each transition. The firing rate is expressed by an arbitrary equation using numerical constants, pre-defined parameters and pre-places, which produces results in $\mathbb{R}^+$. In our example; see **Figure 2.5**, we use the same firing rates as before in the case of the stochastic interpretation:

- *Association*: $k_1 \cdot Enzyme \cdot Substrate$,
- *Dissociation*: $k_2 \cdot EnzymeSubstrateComplex$,
- *Synthesis*: $k_3 \cdot EnzymeSubstrateComplex$,
  with $k_1, k_2, k_3 = 0.1$

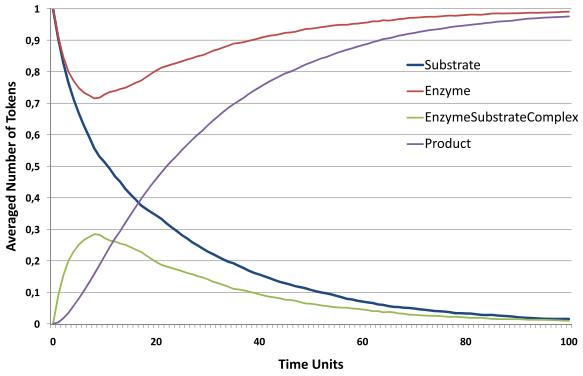


Figure 5.4: ***Continuous Simulation of Running Example.*** *We simulate the continuous time-dependent dynamic behaviour of the model shown in* ***Figure 2.5*** *using the firing rates, which we defined on the left.*

5.2.1 Examples

Example 5.5 (Continuous Simulation: Virus Infection) *The model shows the infection of healthy uninfected cells (UC) by a virus (V). After the virus enters the cell, the replication of the virus starts. Subsequently, the infected cell (IC) dies and a huge amount of the virus is released, which may infect other cells.*

- Reaction equations:

  $\oslash \overset{mu\_cell}{\rightarrow} UC$ (cell growth)
  $UC \overset{k\_cduc}{\rightarrow} \oslash$ (cell death)
  $UC + V \overset{k\_vi}{\rightarrow} IC$ (infection)
  $IC \overset{k\_cdic}{\rightarrow} \oslash$ (cell death)
  $IC \overset{mu\_virus}{\rightarrow} 10V$ (virus release)
  $V \overset{k\_vd}{\rightarrow} \oslash$ (virus degradation)

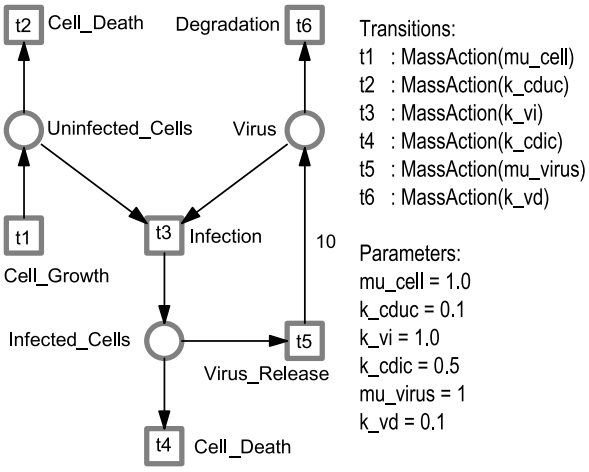
- ODEs:

  $\frac{dUC}{dt} = mu\_cell - k\_cduc \cdot UC - k\_vi \cdot UC \cdot V$

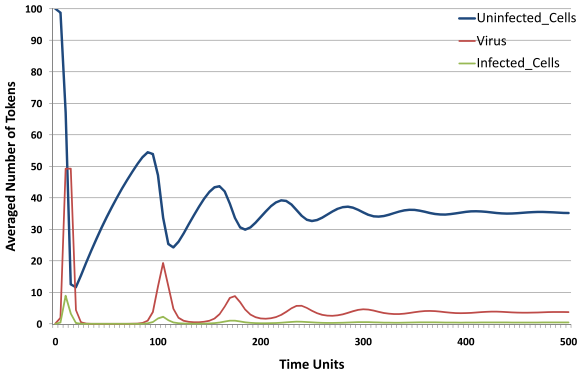
  $\frac{dIC}{dt} = k\_vi \cdot UC \cdot V - k\_cdic \cdot IC - mu\_virus \cdot IC$

  $\frac{dV}{dt} = 100 \cdot mu\_virus \cdot IC - k\_vi \cdot UC \cdot V - k\_vd \cdot V$

- Petri net:



- Simulation:



Example 5.6 (Continuous Simulation: Lotka-Voltera Model) *The Lotka-Voltera model (predator-prey model) is a famous model which describes the time-dependent oscillating behaviour of biological and ecological systems. The classic stability analysis of the model results in two fixed points. An unstable stable point, where the prey is eradicated, causing the predators to die of starvation. If predators are eradicated, the prey population grows infinitely. The second fixed point causes oscillation as shown here; the levels of the predator and the prey population cycle. A high number of predators leads to a low number of prey and vice versa.*

- Reaction equations:

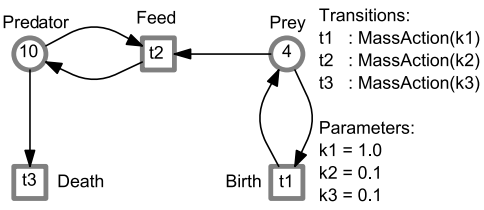
$$Prey \xrightarrow{k1} 2Prey$$
$$Prey + Predator \xrightarrow{k2} 2Predator$$
$$Predator \xrightarrow{k3}$$

- ODEs:

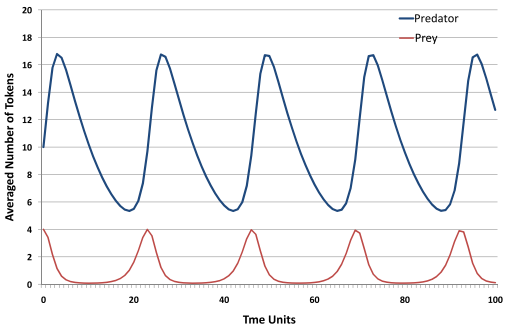
$$\frac{dPrey}{dt} = k1 \cdot Prey - k2 \cdot Prey \cdot Predator$$

$$\frac{dPredator}{dt} = k2 \cdot Prey \cdot Predator - k3 \cdot Predator$$

- Petri net:



- Simulation:



CHAPTER 6

Model Checking for Petri Nets

In this chapter we introduce some basics about model checking for Petri nets and the use of temporal logics. In the next sections, we address to the following questions:

What is model checking in general?
What are temporal logics?
How to apply model checking with temporal logics to your system?

Be reminded that we just explain very basic facts about model checking and temporal logics. We restrict ourself to an informal introduce of the here introduced concepts. Again we stick to the content given in references [12] and [11].

6.1 Introduction to Model Checking

Model checking is an automatic, model-based, property-verification approach. It is used to proof the correctness of a model and ensure specific systems properties, e.g., in time. Originally, model checking has been designed to automatically verify software, concurrent and reactive systems (systems interacting with heir environment). Since biological systems contain concurrent mechanisms and interact with environmental factors, model checking can also be used to verify models of biological system. In addition to general Petri net properties, model checking can be used to check special behavioural properties of the expected transient behaviour which reflect the intended functionality of the system. Properties are specified by mathematical equations using temporal logics; see **Section 6.2**. The truth of a specified property is determined by model checking.
The principle procedure of verifying a (biological) system via model checking is shown in **Figure 6.1**. The model checker gets an appropriate model of the system as input as well as system properties that should be verified. System properties are formulated using linear temporal logics. The actual verification entails proving the truth of the defined property. In the case of correctness the output of the model checker is "true", else the trace/path leading to the breach of the specified property maybe displayed as a counter example.

6.2 Temporal Logics

To specify properties ϕ of a model temporal logics are employed. Temporal logic has found an important application in formal verification, where it is used to state certain requirements of a system in a time line. Beside the classical propositional logic, temporal logics allow to specify temporal relations by

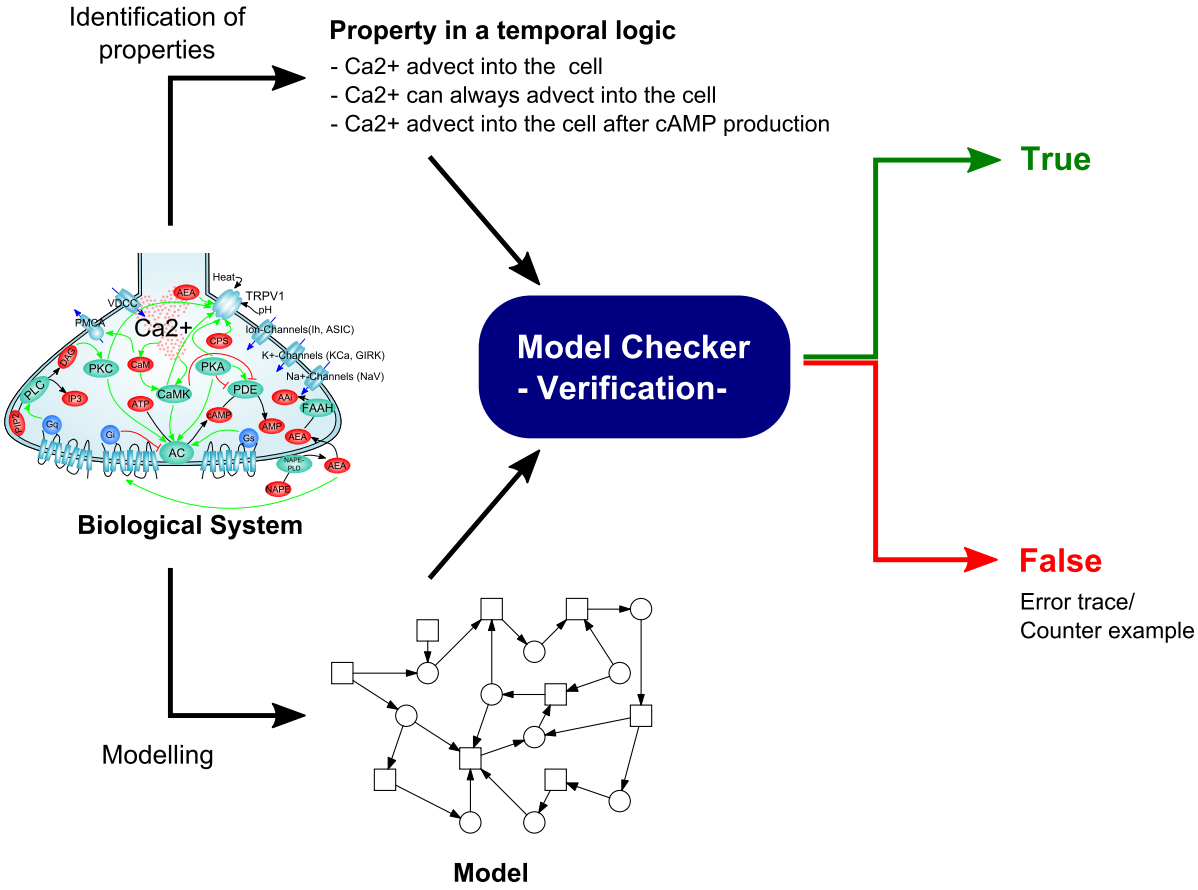
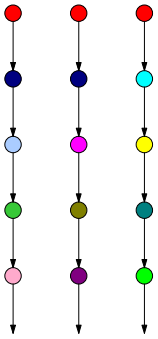


Figure 6.1: ***Verification via Model Checking.*** *Based on a (biological) system a model and properties of the system are derived. Properties that should be verified are formulated using temporal logics. Both, the specified properties and the model serve as input for the model checker. The model checker execute the verification step and proves the truth of the defined property. If the property is satisfied by the model, the output is "true", else "false" and a counter example.*

using additional operators (path quantifiers, temporal operators). Thereby, it is possible to describe, e.g., situation, where a certain state will be reached at the next time point, at some point, after another certain state or never. In a temporal logic you can express statements like "A certain signal molecule is always synthesized", "A certain signal molecule will eventually be synthesized", or "A certain signal molecule will be synthesized *until* the input signal is withdrawn". Consider the statement: "A molecule is produced". Though its meaning is constant in time, the truth value of the statement can vary in time. Sometimes the statement is true, ans sometimes it is false, but it is never true and false simultaneously. In temporal logics, specified properties can have a truth value which can vary in time, where atemporal logic can not.
Temporal logics always have the ability to reason about a time line. Temporal logics are distinguished into two classes: linear-time logics and branching-time logics; see **Figure 6.2**. Linear time logics think of time as a *set of paths*; where a path is a sequence of time instances. Branching time logics represent time as a tree. The tree is rooted at the present moment and branches out in the future. Thus, branching-time logics can reason about multiple time lines. This presupposes an environment that may act unpredictably. To continue the example above for branching-time logics, you can state "There is a possibility that a certain signal molecule will never be produced".

- Linear-time logics
  - Set of paths
  - A path is a sequence of states
  - Every sate has an unique successor
  - Infinite sequences
  - Linear Time Temporal Logic (LTL), Probabilistic Linear Time Temporal Logic (PLTL)



- Branching-time logics
  - Tree
  - A tree consists of a root and several branches in the future
  - Every sate has several successor
  - Infinite tree
  - Computation Tree Logic (CTL), Branching-time Continuous Stochastic Logic (CSL)

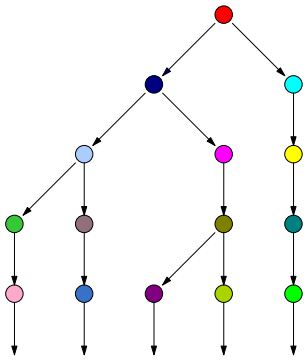


Figure 6.2: ***Linear-time and Branching-time Logics.*** *Temporal logics are used to specify properties of a Model. They can be categorized into linear-time logics (left) and branching-time logics (right) with distinct properties.*

In the case of Petri nets, the path/traces needed for linear-time logics can be gained from the entire state space (reachability graph) of the model or from simulation. The computation tree need for branching-time logics can only be constructed from the entire state space (reachability graph) of the model. Indeed, model checking requires boundedness.
Next, we will explain the most important temporal logics used for Petri nets: Computation Tree Logic (CTL) and Branching-time Continuous Stochastic Logic (CSL) represent branching-time logics. Linear Time Logic (LTL) and Probabilistic Linear Time Logic (PLTL) represent linear-time logics. Furthermore model checking can be divided into analytical model checking (CTL, CSL, LTL); see **Section 6.3**, and simulative model checking (PLTL/LTLc); see **Section 6.4**. Model checking for Petri nets with the here introduced temporal logics can been done with *Snoopy* [19], *Charlie* [8] and/or *Marcie* [16]; see **Table 6.1**.

Table 6.1: *Model Checking Tools and Temporal Logics*

	Snoopy [19]	*Charlie* [8]	*Marcie* [16]
CTL		+	+
CSL			+
LTL		+	
PLTL	+		

6.3 Analytical Model Checking

Analytical approaches used for model checking construct and analyse the entire state space of a model. Thus, the state space must be finite. Analytical model checking can not be applied to bounded models with an infeasible state space caused by the state space explosion problem. In such cases the computational effort will be to high. Computation Tree Logic (CTL) and Branching-time Continuous Stochastic Logic (CSL) represent branching-time logics and are used to state properties both operate on the reachability graph of the Petri net. But also Linear Time Logic (LTL) can used for analytical model checking operating on single paths.

6.3.1 Computation Tree Logic (CTL)

Computation Tree Logic (CTL) offers an impressive formalism to describe the properties of a computation tree. CTL represents a branching time logic with interleaving semantics. In the case of Petri nets, the computation tree is constructed by unwinding the reachability graph; see **Figure 6.3**.

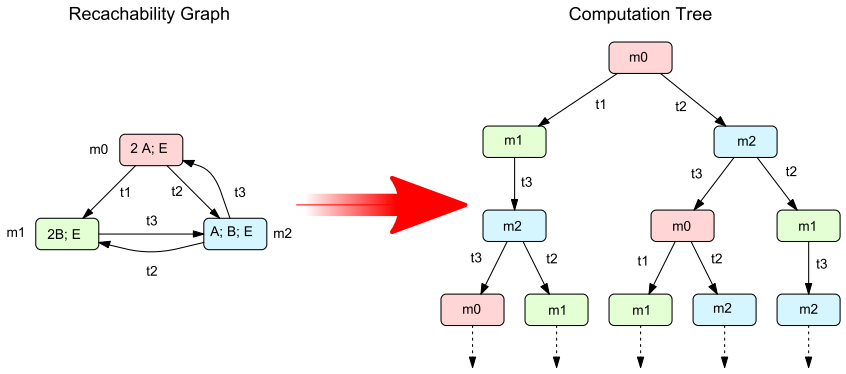


Figure 6.3: ***Unwinding the Reachability Graph.*** *Unwinding the reachability graph (left) into an infinite computation tree (right). The root of the computation tree is the initial state of the reachability graph.*

CTL is an extension of the classical propositional logic. In addition to standard logical operators, CTL also has path quantifiers and temporal operators. The single modules of CTL are:

- Atomic propositions:
  Each atomic proposition $\phi_1, \phi_2, ...\phi_n \in \Phi$ is a CTL formula. Atomic propositions consists of statements of the current token situation in a given place. Places are read as integer variables (Boolean variables) for k-bounded (1-bounded) Petri nets.
- Standard logical operators:
  $\neg\phi_1$ (negation), $\phi_1 \wedge \phi_2$ (conjunction), $\phi_1 \vee \phi_2$ (disjunction), $\phi_1 \rightarrow \phi_2$ (implication) are CTL formulas.
- Path quantifiers:
  $\boldsymbol{E}\phi$ *(**E**xistence*: The proposition ϕ is valid for at least one path.
  $\boldsymbol{A}\phi$ *(**A**ll)*: The proposition ϕ is valid for all computed paths.
- Temporal operators:
  $\boldsymbol{X}\phi$ *(Ne**X**t)*: The proposition ϕ is valid in the next, direct following state.
  $\boldsymbol{F}\phi$ *(**F**uture)*: The proposition ϕ is eventually valid at some time in the future.
  $\boldsymbol{G}\phi$ *(**G**lobally)*: The proposition ϕ is always globally valid forever.
  $\phi_1 \boldsymbol{U} \phi_2$ *(**U**ntil)*: The propositions ϕ_1 is valid until ϕ_2 is valid. At this position ϕ_1 does not have to be valid any more.

The combination of temporal operators and path quantifiers make up eight operators, which can be used to specify temporal properties of a model. Let $\phi_{[1,2]}$ be an arbitrary temporal-logic formulae. Than, the following formulae are valid in state m:

- **EX**ϕ: if there is a state reachable by one step where ϕ holds.
- **EF**ϕ: if there is a path where ϕ holds finally, i.e., at some point.
- **EG**ϕ: if there is a path where ϕ holds globally, i.e., at some point.
- **E**(ϕ_1**U**ϕ_2): if there is a path where ϕ_1 holds until ϕ_2 holds.

The other operators you can get by replacing the **E**xistence operator by the **A**ll operator. The formulation *"there is a path"* has than to be changed in *"for all paths"*; see also **Figure 6.4** for the graphical illustration of the eight temporal operators.

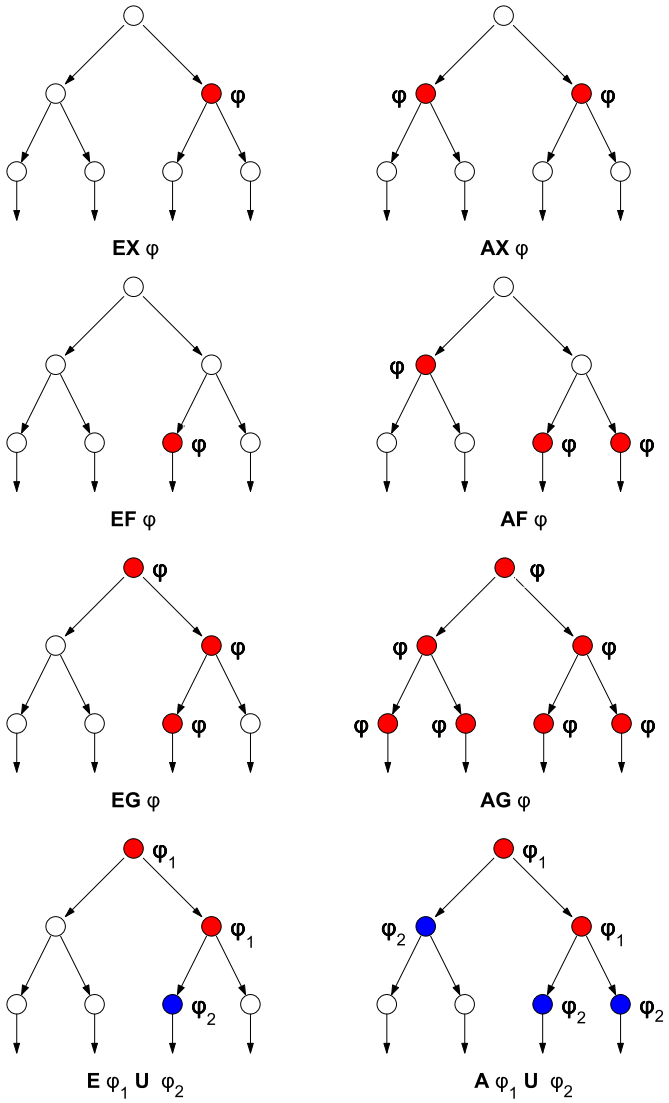


Figure 6.4: ***Temporal Logics for Branching-time Logics.*** *The eight CTL operators and their semantics in the computation tree, which we get by unwinding the reachability graph, compare* ***Figure 6.3****. The two path quantifier* ***E,A*** *relate to the branching structure in the computation tree:* ***E*** *- for some computation path (left column),* ***A*** *- for all computation paths (right column)*

Example 6.1 (CTL Model Checking: RKIP Pathway [11], see Example 4.1) *Places are interpreted as Boolean variables in the following formulae, in order to simplify the notations.*

- ***Property Q1:*** *There is a state where ERK is phosphorylated and RKIP is not phosphorylated:*

$$\boldsymbol{EF}[(ERKpp \vee Raf1Star\_RKIP\_ERKpp) \wedge (RKIP \vee Raf1Star\_RKIP \vee Raf1Star\_RKIP\_ERKpp)]$$

  Meaning: There is finally a state in one of the paths, where ERK is phosphorylated (ERKpp) or phosphorylated and bound to the Raf1Star-RKIP complex and RKIP, is in its initial state, in complex with Raf1Star or in complex with Raf1Star and ERK. This state corresponds to the marking m5 in the reachability graph. The shortest firing sequence is r6, r7.

- ***Property Q2:*** *The phosphorylation of ERK (to ERKpp) is independent of the phosphorylation of RKIP:*

$$\boldsymbol{AG}[(ERK \wedge \neg(RKIPp \vee RKIPp\_RP)) \rightarrow \boldsymbol{E}[\neg(RKIPp \vee RKIP\_RP)\ \boldsymbol{U} ERKpp]]$$

  Meaning: If we are in any state where ERK is phosphorylated and RKIP is not phosphorylated (RKIPp, RKIPp_RP), then there is a path where RKIP can not become phosphorylated as long as ERK is not phosphorylated.

- ***Property Q3:*** *A cyclic behaviour w.r.t. the presence/absence of RKIP is possible forever:*

$$\boldsymbol{AG}[(RKIP \rightarrow \boldsymbol{EF}(\neg RKIP)) \wedge (\neg RKIP \rightarrow \boldsymbol{EF}(RKIP))]$$

  Meaning: In any state where RKIP is marked exists a path to a state, where RKIP is not marked any more, and vice versa.

Example 6.2 (CTL Model Checking: MAP Kinase Cascade [12], see Example 4.2) *Places are interpreted as Boolean variables in the following formulae, in order to simplify the notations.*

- ***Property Q1:*** *There is just one possible pathway to fully activate ERK by passing through the states Rafp, MEKp, MEKpp and ERKp in order to reach ERKpp:*

$$\neg[\boldsymbol{E}(\neg RAFp\, \boldsymbol{U} MEKp) \vee \boldsymbol{E}(\neg MEKp\, \boldsymbol{U} MEKpp) \vee \boldsymbol{E}(\neg MEKpp\, \boldsymbol{U} ERKp) \vee \boldsymbol{E}(\neg ERKp\, \boldsymbol{U} ERKpp)]$$

  Meaning: it exists no path, where MEKp appears but RAFp has not been there; or where MEKpp appears but MEKp has not been there; or where ERKp appears but MEKpp has not been there; or where ERKp appears but ERKpp has not been there.

- ***Property Q2:*** *Dephosphorylation takes place independently. E.g., the duration of the phosphorylated state is independent of the duration of the phosphorylated states of MEK and Raf:*

$$(\boldsymbol{EF}[RAF \wedge (ERKp \vee ERKpp)] \wedge \boldsymbol{EF}[RAFp \wedge (ERKp \vee ERKpp)] \wedge \boldsymbol{EF}[MEK \wedge (ERKP \vee ERKPP)] \wedge \boldsymbol{EF}[(MEKp \vee MEKpp) \wedge (ERKP \vee ERKPP)])$$

  Meaning: There is a path that contains states, where Raf is not phosphorylated and ERK is phosphorylated (ERKp or ERKpp) and where Raf is phosphorylated (Rafp) and ERK is phosphorylated, where MEK is not phosphorylated and ERK is phosphorylated and where MEK (MEKp or MEKpp) is phosphorylated and ERK is phosphorylated.

6.3.2 Branching-time Continuous Stochastic Logic (CSL)

In the case of stochastic Petri nets (see also **Chapter 5.1**), we can now apply Branching-time Continuous Stochastic Logic (CSL). This allows to perform probabilistic analysis of the transient and steady state behaviour. CSL uses the CTMC for the verification of properties, which is isomorph to the reachability graph. In addition to the state space, the firing rates are also involved in CSL to consider the probability of a specified property. CSL replaces the path quantifiers (**E**, **A**) in CTL by the probability operators $\mathbf{P}_{\triangleright\triangleleft p}$ (transient analysis) and $\mathbf{S}_{\triangleright\triangleleft p}$ (steady state analysis) whereby $\triangleright\triangleleft p$ specifies the probability of the given formula (comparison operator $\bowtie$ can be replaced by $<, \leq, =, \neq, >, \geq$). The operator $\mathbf{P}_{=?}$ is used to return the probability (rather than compare probabilities).

- $\mathbf{P}_{\triangleright\triangleleft p}[\phi]$ - probability that paths fulfil ϕ is $\triangleright\triangleleft p$
- $\mathbf{S}_{\triangleright\triangleleft p}[\phi]$ - probability that ϕ holds in the steady state $\triangleright\triangleleft p$

In CSL, we can now use the following abbreviation:

- $true\mathbf{U}\phi \Rightarrow \mathbf{F}\phi$
- $\mathbf{EF}\phi \Rightarrow \mathbf{P}_{\geq 0}[\mathbf{F}\phi]$
- $\mathbf{AF}\phi \Rightarrow \mathbf{P}_{\geq 1}[\mathbf{F}\phi]$

Example 6.3 (CSL Model Checking: RKIP Pathway [11], see Example 4.1) *For the CSL model checking, we employ again a 7-level model. Places are now read as integer variables.*

- ***Property S1a:*** *There are reachable states where ERK is phosphorylated and RKIP is not phosphorylated.*

$$\boldsymbol{P}_{>0}[\boldsymbol{F}(ERKpp + Raf1Star\_RKIP\_ERKpp \geq 1 \wedge RKIP + Raf1Star\_RKIP\_ERKpp + Raf1Star\_RKIP = 7)]$$

- ***Property S2a:*** *The phosphorylation of ERK (to ERKpp) is independent of the phosphorylated state of RKIP*

$$\boldsymbol{P}_{>?}[\boldsymbol{G}((ERK = 7 \wedge RKIPp = 0 \wedge RKIPp_RP = 0) \rightarrow \boldsymbol{P}_{>0}[(RKIPp = 0 \wedge RKIPp\_RP = 0)\ \boldsymbol{U}(ERKpp \geq 1)])]$$

  As this property always holds true, the returned probability is 1.

- ***Property S3a:*** *A cyclic behaviour w.r.t. the presence/absence of RKIP is possible forever.*

$$\boldsymbol{S}_{>0}[RKIP = 0]$$
$$\boldsymbol{S}_{>0}[RKIP \geq = 1]$$

  Here the steady state operator is used to state the non-zero probabilities of RKIP being absent or present after long time, hence RKIP can cycle.

Example 6.4 (CSL Model Checking: MAP Kinase Cascade [12], see Example 4.2) *For the CSL model checking, we employ again the model with 4 and 8 Levels. Than, we compute the probability of the suggested properties by varying the initial level L, e.g., 0-N. For the sake of efficiency, we restrict the U operator to 100 time steps. Places are now read as integer variables. (Note: expression in curly bracket {...} indicate the initial state.)*

- ***Property S1a:*** *What is the probability of Rafp increasing, when starting in a state where the amount of Rafp is for the first time at N:*

$$\boldsymbol{P}_{=?}\left[(RafP = L)\ \boldsymbol{U}^{<100}\ (RafP > L)\ \{RafP = L\}\right]$$

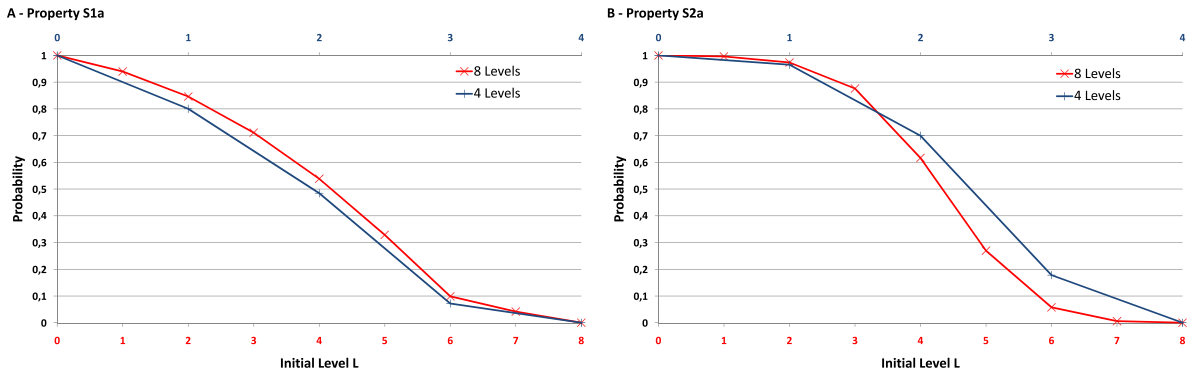


Figure 6.5: ***Probabilities of the Accumulation of Rafp.*** *The graphs show the probabilities of the accumulation of Rafp (A - Probability of property S1a, B - Probability of property S1b)as a function of the initial level. Here we compare the probabilities of two models with 4 and 8 total levels (reproduced from [12].*

Figure 6.5, A *indicates that it is absolutely certain that the concentration of RAfp increases from an initial level 0. The Rafp concentration is not increasing from a level N. More precisely, the increase of Rafp is very likely for low levels. At intermediate levels the increase and decrease of Rafp is equal. The likelihood of an Rafp increase is very low (but not impossible) for high levels. Therefore, the total amount of Rafp will not accumulate in the first layer.*

- ***Property S2a:*** *What is the probability that, given the initial concentrations of Rafp, MEKpp, ERKpp being zero, the concentration of Rafp rises above some Level L while the concentration of MEKpp and ERKpp remain at zero, i.e., Rafp is the first to react?*

$$\boldsymbol{P}_{=?}\left[((MEKpp = 0) \wedge (ERKpp = 0))\ \boldsymbol{U}^{<=100}\ (Rafp > L)\right.$$
$$\left.\{(MEKpp = 0) \wedge (ERKpp = 0) \wedge (Rafp = 0)\}\right]$$

Figure 6.5, B *points out, that it is very likely that Rafp rises, while MEKpp and ERKpp are zero for low levels of the upper half and even more likely for the bottom half if the levels. The decrease of the likelihood for higher level can be explained by property S1a. This property corresponds to properties Q1 of* ***Example 6.2*** *and C1 of* ***Example 6.9****.*

The analytical model checking approaches CTL and CSL have both high computational efforts. Analytical model checking constructs and analyses the entire state space, which might become infeasible due to state explosion problems. Thus, analytical model checking becomes more and more impractical with increasing state space. Additionally, CTL and CSL can not handle unbounded models with infinite state spaces. In order to avoid the enormous computational power required for large state spaces, time-dependent stochastic behaviour can be simulated by dedicated algorithms, and evaluated by simulative stochastic model checking or approximated by a deterministic continuous behaviour. Simulative model checking we be considered in the following sections.

6.3.3 Linear Time Logic (LTL)

As indicated before the Linear Temporal Logic (LTL) operates along paths. Paths are extracted from the reachability graph. In addition, paths could also be generated by simulating the time-dependent dynamic behaviour of model if stochastic and continuous Petri nets are used. In that case, the probabilistic extension of LTL (PLTL) should be considered; see **Section 6.4.1**. LTL allows to make statements about (all) paths staring in a state. In contrast to CTL, LTL uses no path quantifiers; see **Figure 6.6**. Thus, LTL implies the same modules as CTL despite of the path quantifiers; compare **Section 6.3.1**.

- Atomic propositions:
  Each atomic proposition $\phi_1, \phi_2, ...\phi_n \in \Phi$ is a CTL formula. Atomic propositions consists of statements of the current token situation in a given place. Places are read as integer variables (Boolean variables) for k-bounded (1-bounded) Petri nets.
- Standard logical operators:
  $\neg\phi_1$ (negation), $\phi_1 \wedge \phi_2$ (conjunction), $\phi_1 \vee \phi_2$ (disjunction), $\phi_1 \rightarrow \phi_2$ (implication) are CTL formulas
- Temporal operators:
  $\boldsymbol{X}\phi$ *(Ne**X**t)*: The proposition ϕ is valid in the next, direct following state.
  $\boldsymbol{F}\phi$ *(**F**uture)*: The proposition ϕ is eventually valid at some time in the future.
  $\boldsymbol{G}\phi$ *(**G**lobally)*: The proposition ϕ is always globally valid forever.
  $\phi_1 \boldsymbol{U} \phi_2$ *(**U**ntil)*: The propositions ϕ_1 is valid until ϕ_2 is valid. At this position ϕ_1 does not have to be valid any more.

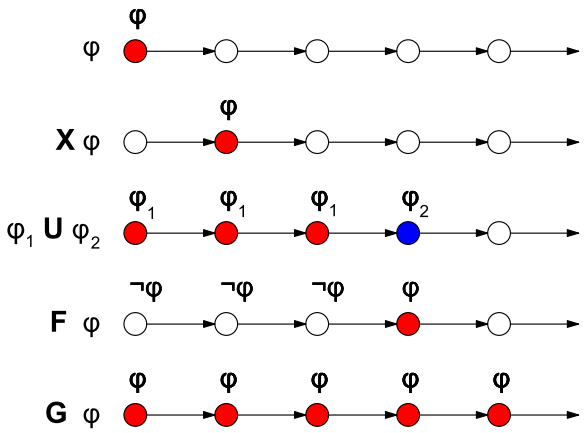


Figure 6.6: ***Temporal Logics for Linear-time Logics.*** *The four temporal operators for LTL/PLTL and their semantics applied to a path, which we get by simulating the time-dependent dynamic behaviour or by extracting path from the reachability graph.*

Example 6.5 (Traffic Light) *An easy example to understand LTL is the traffic light:*

- *Once red, the light can not become green immediately:* $\boldsymbol{G}(red \rightarrow \boldsymbol{X}\, green)$
- *The light becomes green eventually:* $\boldsymbol{F}\, green$
- *Once red, the light becomes green eventually:* $\boldsymbol{G}(red \rightarrow \boldsymbol{F}\, green)$
- *Once red, the light always becomes green eventually after being yellow for some time in between:* $\boldsymbol{G}(red \rightarrow \boldsymbol{X}(red\ \boldsymbol{U}\ \boldsymbol{X}\ (yellow\ \boldsymbol{U}\, green)))$

Please consider further examples with a biological background given in **Section 6.4.1**.

6.4 Simulative Model Checking

Simulative model checking approaches handle the state space through approximating results by analysing only a subset of the state space - a set of output from stochastic or continuous simulation algorithms. Thus, even a model with an infeasible or infinite state space can be subjected to model checking. But in consequence just linear time logics can be applied to state properties. Linear time logics operate in-turn over sets of linear paths through the state space, equivalent to operating on simulation traces. A given property holds if it holds in all paths. Consequently, there are no path quantifiers in temporal logics. In case of stochastic Petri nets, Probabilistic Linear Time Temporal Logic (PLTL) is used for simulative model checking; see **Section 6.4.1**. An extension of LTL is used

to specify properties of the model for simulative model checking. For continuous simulation LTLc, an modification of LTL is employed **Section 6.4.2**.

6.4.1 Probabilistic Linear Time Logic (PLTL)

The Probabilistic Linear Time Logic (PLTL) extends the standard LTL to a stochastic setting. In PLTL we also use the probability operators $\mathbf{P}_{\triangleright\triangleleft p}$ such as in CSL, and a filter construct, $\{\phi\}$, defining the initial state of the property. PLTL can be considered as a linear time counterpart to CSL. In PLTL embed probability operators are not allowed. PLTL can not be used to perform steady state analysis. The temporal operators in PLTL do not requires time bounds.
PLTL operates along sets of linear paths (traces) of temporal behaviour, which are generated by stochastic simulation runs. Each generated trace is evaluated to a Boolean truth value, and the probability of a property holding true is computed by the fraction of true values in the set over the whole set. Thus model checking with PLTL incorporates two approximations. The truth value of a single trace is approximated by operating over a finite sequence of states only, and the probability of the property is approximated through sampling a finite number of traces (a subset of the model's behaviour) only.
With the help of PLTL, you can easily evaluate the time-dependent behaviour of a model generated by stochastic simulation runs, as well as experimental time series data. Using PLTL allows to employ a greater number of tokens than in the case of analytical model checking. The efficiency of PLTL is much higher. In general, for very high numbers of molecules, the stochastic behaviour tends to the deterministic behaviour of the model.
Simulative model checking has its advantage when the range of behaviours that the model checker operates over becomes computationally infeasible. The simulative approach allows to check a property in a feasible time through approximating the state space of the model.

Example 6.6 (PLTL Model Checking: RKIP Pathway [11], see Example 4.1) *Places are read as integer variables. The properties are checked using 100 simulation traces from Gillespie's algorithm with a simulation time of 10, 000 s.*

- ***Property S1s:*** *There are reachable states where ERK is phosphorylated and RKIP is not phosphorylated.*

$$\boldsymbol{P}_{>0}\left[\boldsymbol{F}\left(ERKpp + Raf1Star\_RKIP\_ERKpp \geq 1 \wedge RKIP + Raf1Star\_RKIP\_ERKpp + Raf1Star\_RKIP = 7\right)\right]$$

- ***Property S2s:*** *The phosphorylation of ERK (to ERKpp) is independent of the phosphorylated state of RKIP*

$$\boldsymbol{P}_{\geq 1}\left[(RKIPp + RKIPp\_RP = 0)\ \boldsymbol{U}(ERKpp \geq 1)\right]$$

  Remember, that embed probability operators are not allowed in PLTL. Thus, this property is much weaker than in the case of CSL. The property says, from the initial state of the system, ERK can phosphorylate without RKIP being phosphorylated. In fact, this is always true from the initial state.

- ***Property S3s:*** *A cyclic behaviour w.r.t. the presence/absence of RKIP is possible forever.*

$$\boldsymbol{P}_{>0}\left[\boldsymbol{F}(RKIP \geq 1)\,\{time \geq 5000 \wedge RKIP = 0\}\right]$$
$$\boldsymbol{P}_{>0}\left[\boldsymbol{F}(RKIP \geq 0)\,\{time \geq 5000 \wedge RKIP \geq 1\}\right]$$

  Direct steady state analysis is not possible in PLTL. But steady state analysis can be approximated by very long simulation runs. Than, the property is checked at/near the end of the simulation. Here, the property of RKIP being absent and present. Here, the property of RKIP being absent and present in turns holds for long simulation runs.

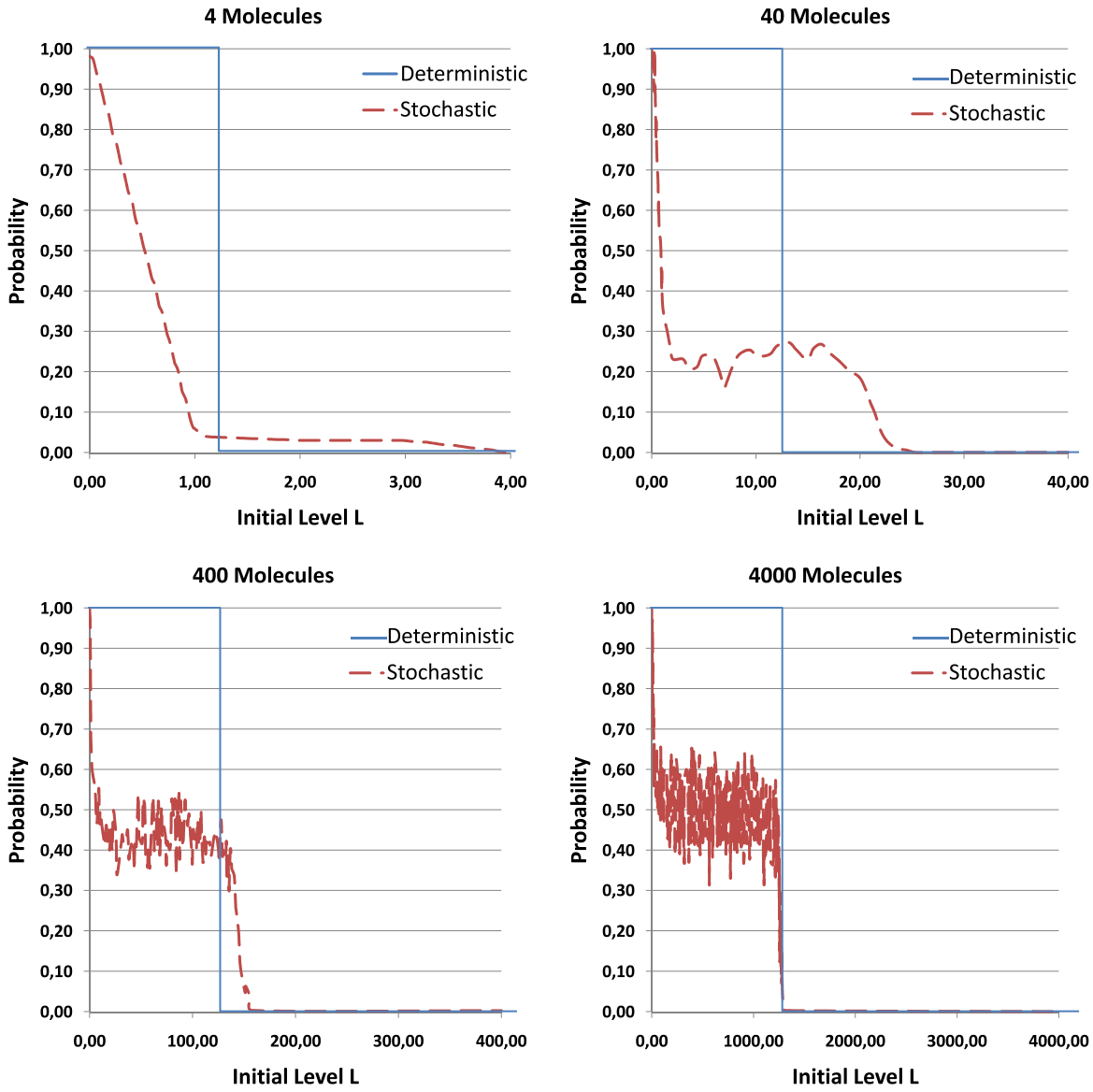


Figure 6.7: ***Simulative Model Checking of Property S1s.*** *Simulative model checking of property S1s at a varying number of molecules; 4, 40, 400, 4000. This shows a progression towards the deterministic behaviour as the number of molecules increases (reproduced from [12].*

Example 6.7 (PLTL Model Checking: MAP Kinase Cascade [12], see Example 4.2) *Places are read as integer variables. The properties are checked using 100 simulation traces from Gillespie's algorithm with a simulation time of 300 s.*

- ***Property S1s:*** *What is the probability of the concentration of Rafp increasing, when starting in a state where the level is for the first time at L:*

$$\boldsymbol{P}_{=?}\left[(RafP = L)\ \boldsymbol{U}(RafP > L)\,\{RafP = L\}\right]$$

Figure 6.7 *illustrates the probability of Rafp increasing for the stochastic and deterministic behaviour varying the amount of molecules from 4 up to 4000 molecules. From the deterministic*

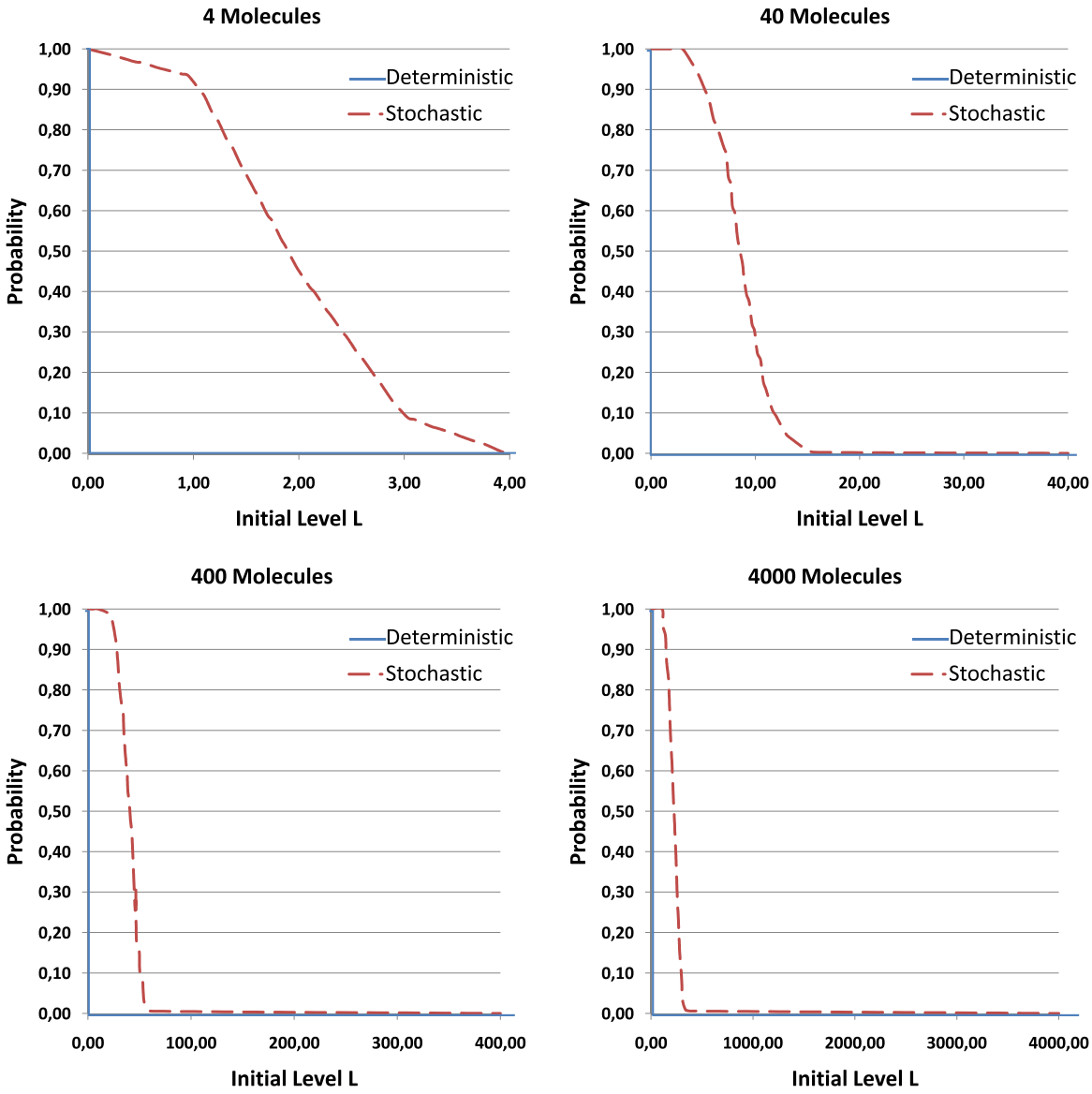


Figure 6.8: ***Simulative Model Checking of Property S2s.*** *Simulative model checking of property S2s at a varying number of molecules; 4, 40, 400, 4000. This shows a progression towards the deterministic behaviour as the number of molecules increases (reproduced from [12].*

behaviour it can be observed, that the Rafp will always increase until it reaches the maximum number, which corresponds to a concentration of around 0.1182 mMol. With increasing molecules, the maximum possible number of molecules in the stochastic behaviour of Rafp tends towards the deterministic behaviour. In more detail, the stochastic behaviour tends to a probability of 0.5. in its possible concentration range, due to the steady increase and decrease.

- ***Property S2s:*** *What is the probability that, given the initial concentrations of Rafp, MEKpp, ERKpp being zero, the concentration of Rafp rises abvoe some Level L while the concentration of*

MEKpp and ERKpp remain at zero, i.e., Rafp is the first to react?

$$\boldsymbol{P}_{=?}\,[((MEKpp = 0) \wedge (ERKpp = 0))\ \boldsymbol{U}(Rafp > L) \\ \{(MEKpp = 0) \wedge (ERKpp = 0) \wedge (Rafp = 0)\}]$$

Figure 6.8 *shows, that the stochastic behaviour drifts against the deterministic behaviour. In the deterministic scenario, the probability for ERKpp, MEKpp and Rafp being 0 is just given at the initial, else the probability is 1. The stochastic behaviour becomes less curved and more step-like shaped with an increasing amount of molecules and thereby tends towards the vertical line in the deterministic behaviour.*

6.4.2 Continuous (Probablistic) Linear Time Logic (LTLc/PLTLc)

In the case of continuous Petri nets, the discrete number of tokens is replaced by continuous value. Hence, the deterministic behaviour can not describe the behaviour of individual levels of molecules but their concentration. Repeated deterministic simulation will always yield into the same time-dependent behaviour. The state space of such models is continuous and linear. The natural choice to analysis continuous linear behaviour are natural linear time-dependent logics like LTL and PLTL. Here, LTL and PLTL are used in a deterministic setting and interpreted over the continuous simulation trace of ODEs. The deterministic model has only on (averaged) behaviour. Thus, the resulting probability for properties formalized in PLTL is 1 or 0.

Example 6.8 (LTLc Model Checking: RKIP Pathway [11], see Example 4.1) *Places are interpreted as real number variables. We used a simulation time of 10, 000 s to evaluate the deterministic behaviour. We use parameter $significant$ and noise to compare the concentration of spezies with certain thresholds. In this example, we applied PLTL to formalize the properties. All properties can be transferred into the LTL setting as well.*

- ***Property C1:*** *There are reachable states where ERK is phosphorylated and RKIP is not phosphorylated.*

$$\boldsymbol{P}_{\geq 1}\,[\boldsymbol{F}\,(ERKpp + Raf1Star\_RKIP\_ERKpp > noise \wedge \\ RKIP + Raf1Star\_RKIP\_ERKpp + Raf1Star\_RKIP \geq significant)]$$

- ***Property C2:*** *The phosphorylation of ERK (to ERKpp) is independent of the phosphorylated state of RKIP*

$$\boldsymbol{P}_{\geq 1}\,[(RKIPp + RKIPp\_RP \leq noise)\ \boldsymbol{U} \\ (ERKpp + Raf1Star\_RKIP\_ERKpp > noise)]$$

  Remember, that embed probability operators are not allowed in PLTL. Thus, this property is much weaker than in the case of CSL. The property says, from the initial state of the system, ERK can phosphorylate without RKIP being phosphorylated. In fact, this is always true from the initial state.

- ***Property C3:*** *A cyclic behaviour w.r.t. the presence/absence of RKIP is possible forever.*

$$\boldsymbol{P}_{\leq 0}\,[\boldsymbol{F}(RKIP \geq 1)\,\{time \geq 5000 \wedge RKIP \leq noise\}] \\ \boldsymbol{P}_{\leq 0}\,[\boldsymbol{F}(RKIP \leq noise)\,\{time \geq 5000 \wedge RKIP < noise\}]$$

  Direct steady state analysis is not possible in PLTL. But steady state analysis can be approximated by very long simulation runs. Than, the property is checked at/near the end of the simulation. Here, the property of RKIP being absent and present. Here, the property of RKIP being absent and present in turns holds for long simulation runs.

Example 6.9 (LTLc Model Checking: MAP Kinase Cascade [12], see Example 4.2) *Places are interpreted as real number variables. We used a simulation time of 400 s to evaluate the deterministic behaviour. We use parameter* $significant_{species}$ *and* $noise_{species}$ *to compare the concentration of a species (Rafp, MEKpp, ERKpp) with certain their thresholds. In this example, we applied LTL to formalize the properties. All properties can be transferred into the PLTL setting as well. Note, that properties C1, C2 and C3 correspond to the qualitative properties Q1; see* ***Example 6.2****., and that S2 of* ***Example 6.7*** *is the stochastic counterpart of C1.*

- ***Property C1:*** *The concentration of Rafp rises to a significant level, while the concentration of MEKpp and ERKpp remain close zero, i.e., Rafp is really the first species to ract:*

$$((MEKpp < noise_{MEKpp}) \wedge (ERKpp < noise_{ERK}))\ \boldsymbol{U}(Rafp > significant_{Rafp})$$

- ***Property C2:*** *If the concentration of Rafp is at a significant concentration level and that of ERKpp is close to zero, them both species remain in these state until the concentration of MEKpp becomes significant, i.e., MEKpp is the second species to react:*

$$((Rafp > significant_{Rafp}) \wedge (ERKpp < noise_{ERK})) \Rightarrow$$
$$((Rafp > significant_{Rafp}) \wedge (ERKpp < noise_{ERK}))\ \boldsymbol{U}(MEKpp > significant_{MEKpp})$$

- ***Property C3:*** *If the concentration of Rafp and MEKpp are significant, they remain so, until the concentration of ERKpp becomes significant, i.e., ERKpp is the third species to react:*

$$((Rafp > significant_{Rafp}) \wedge (MEKpp > significant_{MEKpp})) \Rightarrow$$
$$((Rafp > significant_{Rafp}) \wedge (MEKpp > significant_{MEKpp}))\ \boldsymbol{U}(ERKpp > significant_{ERKpp})$$

CHAPTER 7

Petri Net Editor: Snoopy

In this chapter, we introduce the Petri net editor *Snoopy* [19]. The following information about *Snoopy* can also be looked up in detail in references [19, 14]. *Snoopy* is a tool to design and animate hierarchical graphs, among others Petri nets. Thus, *Snoopy* allows constructing of Petri nets, as well as to animate and simulate the resulting token-flow. The tool has been developed - and is still under development - at the University of Technology in Cottbus [3], Dep. of Computer Science, "Data Structures and Software Dependability" [1]. The tool is in use for the verification of technical systems, especially software-based systems, as well as for the validation of natural systems, i.e., biochemical networks as metabolic, signal transduction, gene regulatory networks. Several Petri net classes are comprised in the unifying framework offered by *Snoopy*, like qualitative, stochastic, continuous and Hybrid Petri nets and in addition the coloured counterparts. *Snoopy* facilitates the consideration of the qualitative network structure of a model under specific kinetic aspects of the specialized Petri net class. The framework of *Snoopy* allows investigating Petri net models in various complementary ways. Therefore, Petri net models can be converted into each other. Different Petri net classes can be used simultaneously in *Snoopy*.

The three outstanding main characteristics of *Snoopy* are:

1. it is *extensible*; it's generic design facilitates the implementation of new Petri net classes
2. it is *adaptive*; several models can be used simultaneously, the graphical user interface adapts dynamically to the network class in the active window
3. it is *platform independent*; it is executable on all popular operating systems (linux, mac, windows)

Two specialized types of nodes support the design, systematic construction and neat arrangement of large Petri nets. Logical nodes act as connector or multiple used places or transitions sharing the same factor or function. Macro nodes allow to hierarchically design a Petri net.
All elements in each Petri net class can be individually edited and coloured. Computed node sets can be visualized on the network structure. The network layout can be changed manually or automatically. Syntactical errors in the network structure of a Petri net is prevented by the implementation of the graphical editor.
The tool design of *Snoopy* and its features facilitate the intuitive construction of Petri nets and the animation/simulation of the time-dependent dynamic behaviour. *Snoopy* is still under development and tries to cope with the needs of biological models. The constructed Petri net models can be exported and analysed in *Charlie*.
The next chapters are concentrated on the stochastic Petri net class. All other Petri net classes are similarly built.

7.1 Editor Mode

In this section, we explain the graphical user interface of the editing mode in *Snoopy* based on the stochastic Petri net class.
First, to open a new document, you have to start *Snoopy* and go to *File/New* or press the *new* button in the *tool bar*. A *template dialogue* opens, where you can now select the document template; see **Figure 7.1**.

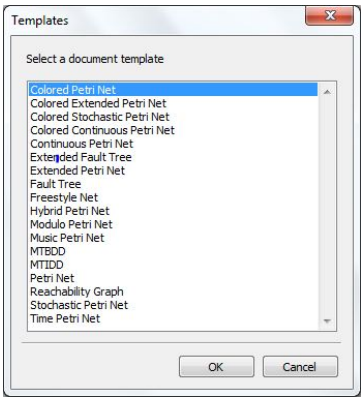


Figure 7.1: ***Select Template.*** *A new template of the chosen Petri net class can be opened in this dialogue.*

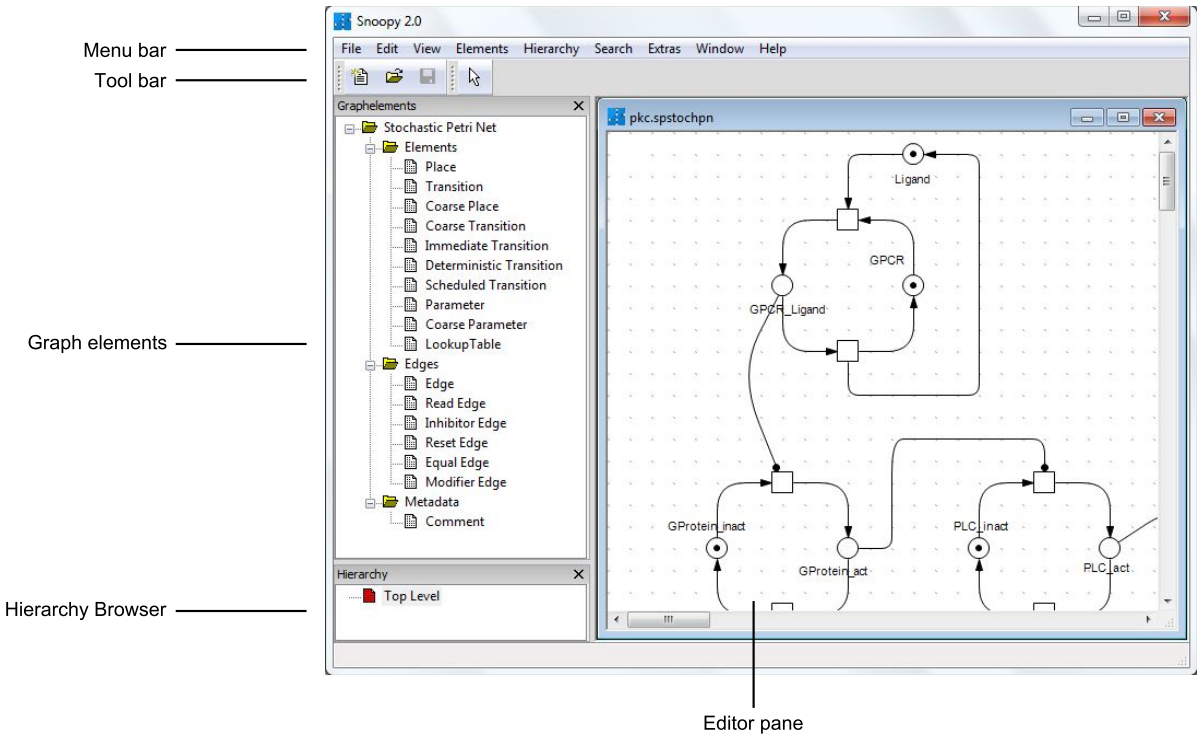


Figure 7.2: ***Graphical Editor User Interface in Snoopy.*** *The window is divided into four functional units: Menu bar (contains several functions), Tool bar (contains some short cuts), Graph elements (lists all available elements), Hierarchy browser (lists all levels) and Editor pane (canvas)*

Figure 7.2 shows the *graphical editor user interface* in *Snoopy* of the stochastic Petri net class. The drop down menus in the *Menu bar* offer the following commands:

- *File*: New/Open/Close Window/Save/Save as, Print, Export/Import, Preferences (change the default visualisation) and Exit
- *Edit*: Undo/Redo, Select All/Copy/Copy in new net/Paste/Cut, Clear/Clear all, Hide/Unhide, Edit selected elements/Transform Shapes, Layout (automatic layout function), Sort Nodes (by ID or name), Check Net (duplicate nodes, syntax, consistency) and Convert to
- *View*: Zoom 100%/Zoom In/Zoom Out, Net Information (numer of each element used in the model), Toogle Graphelements/Hierachy browser/Filebar/Log window, Show Attributes (choose for each elements which attributes to be shown in the model), Start Anim-Mode/Simulation-Mode/Steering-Mode
- *Elements* (list of all available elements): Select/ Place/Transition/ Coarse Place/Coarse Transition/ Immediate Transition/Deterministic Transition/Scheduled Transition/Parameter/Coarse Parameter/LookupTable, Edge/Read Edge/Inhibitor Edge/Reset Edge/Equal Edge/Modifier Edge and Comment
- *Hierarchy* (edit and browse hierarchy): Coarse (chosen elements are encapsulate in a macro node)/Flatten (chosen macro nodes are decapsulate) and Go Up in Hierarchy/Go To First Child in Hierarchy/Go To Next Sibling in Hierarchy/o To Previous Sibling in Hierarchy
- *Search*: Search nodes (by ID or name)
- *Extra*: Load node sets (visualise, e.g., T-, P-invariants, siphons and traps), Interaction and General Information (title, author, description, literature)
- *Window* (arrange all opened windows): Cascade/Tile Horizontally/Tile vertically, Arrange Icons/Next/Previous and Open Files
- *Help*: Help, About (current version), check update

The *tool bar* contains four short cuts to:

- *Open* a new document;
- *Load* a document;
- *Save* a document;
- *Select* an element.

The panel for the *Graph elements* displays all elements available in the current net class. To use one of them make a left-click on one of the elements. To edit or select all elements of the same class, use a right click on the respective element in the *Graph elements* panel.

The *Hierarchy Browser* lists all levels. A left-click the chosen hierarchical level opens the subnet in a new window.

The *Editor pane* is the canvas, on which to draw the network. Activate the element you want to use in *Graph elements* panel to use it on the canvas. A left-click on the *Editor pane* places the selected element on the canvas. To draw an arc between two nodes click left onto one node, hold the left-click and drag the line to the other node, now drop the left-click. To add edges to an arc push the CRTL key and click left on the arc. You can now drag the edge with another left-click. For a better orientation on the canvas, it is possible to use a grid which can be activated under *File/Preferences* in the *canvas* tab. You can also choose between to two edge styles in the *Preference dialogue* in the *elements* tab, choose among *line* or *spline*.

7.2 Elements of the Stochastic Petri Net Class

Next, we provide an overview of all elements available in the stochastic Petri net class in *Snoopy*, their visualisation and attributes; see **Table 7.1**. In the following subsections, all elements are explained in more detail.

Table 7.1: *bersicht zu den Petri-Netz-Elementen und ihren Attributesn*

Types of Nodes

Elements	Graphic	Attributes
Standard Place		ID, name, marking, comment, logic, graphic
Standard Transition		ID, name, function, comment, logic, graphic
Coarse Place		name, comment, graphic
Coarse Transition		name, comment, graphic
Immediate Transition		ID, name, weight function, comment, logic, graphic
Deterministic Transition		ID, name, delay, comment, logic, graphic
Scheduled Transition		ID, name, begin/repetition/end, comment, logic, graphic

Edge

Elements	Graphic	Attributes
Standrad Edge		ID, arc weight, comment, graphic
Read Edge		ID, arc weight, comment, graphic
Inhibitor Edge		ID, arc weight, comment, graphic
Reset Edge		ID, comment, graphic
Equal Edge		ID, arc weight, comment, graphic
Modifier Edge		ID, comment, graphic

Additional elements for modeling

Elements	Graphic	Attributes
Lookup Table		ID, name, (x, f(x))- values, graphic
Parameter		ID, name, parameter value, comment, graphic
Coarse Parameter		name, comment, graphic
Comment		comment, graphic

You can edit the attributes of each element. A double click on an element opens a *property dialogue* with different tabs where you can now modify the attributes of the chosen element. Here, we show as example the *place editing dialogue* in **Figure 7.3**. Advantageously, you can edit several elements at the same time by selecting all elements you like to edit.

7.2.1 Places

Snoopy offers two types of places, the *standard place* and the *coarse place*. *Coarse places* correspond to macro places and facilitate the hierarchical arrangement of Petri nets by opening a new layer to model a place in more detail; see **Section 2.3.1**.

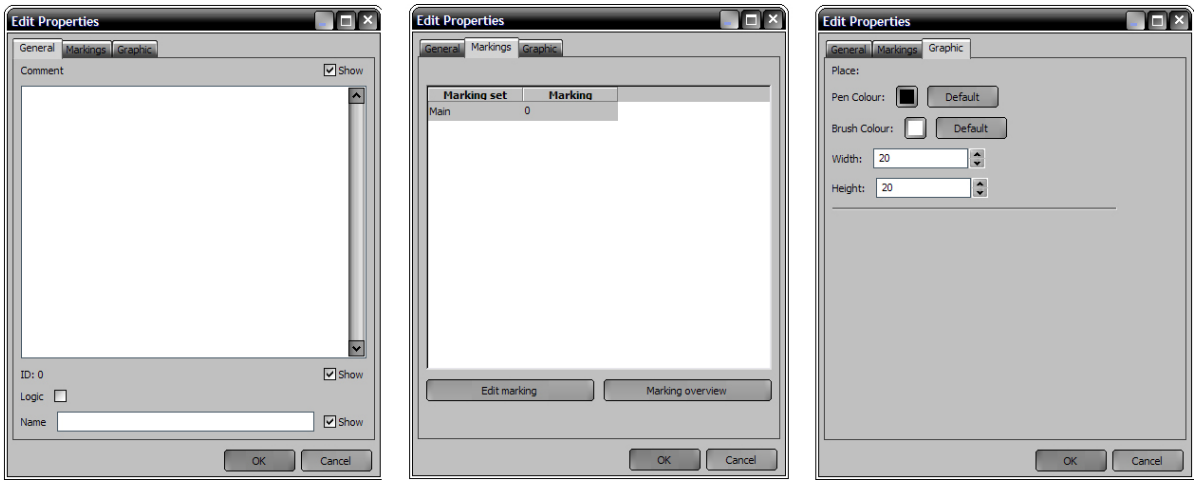


Figure 7.3: ***Place Dialogue.*** *In the first tab "General", the name of the place can be changed, comments can be provided and the place can be declared as logic. In the tab "Marking" (not available for coarse places), the number of tokens of the corresponding place can be specified (default is zero). The "Graphic" tab allows you to change the visualisation of the place.*

7.2.2 Transitions

Several types of transitions are available in *Snoopy*. Among them are *standard transitions* and *coarse transitions*. *Coarse transitions* correspond to macro transitions and facilitate the hierarchical arrangement of Petri nets by opening a new layer to model a transition in more detail; see **Section 2.3.1**. There are three more specific transitions available: *deterministic transitions*, *immediate transitions* and *scheduled transitions*. Those specific transitions are very useful to model timed input signals corresponding to the wet-lab experiment.

Figure 7.4 explains the differences between the different types of transition. The *rate function* of the *standard transitions* and the *weight function* of the *immediate transition* can be defined by various mathematical equations. *Snoopy* offers a *function/weight assistant* to define such mathematical expressions; see **Figure 7.5**. The *function/weight assistant* indicates which elements are allowed to be used in the equation; see **Figure 7.5**. In general, you can use pre-places of a transition and *parameters* to define the respective *rate/weight function*. Places that are not a pre-place of a transition can not be used in rate/weight functions. If you want to use such places in the firing rate, they must be connected with the respective transition via a *modifier edge*; see **Section 7.2.3**. Places connected via a *modifier arc* with a transition do affect the kinetic, but not the ability of the transition to be enabled. The *function/weight assistant* also offers several pre-defined mathematical functions that can be used to define individual equations; see **Table 7.3**.

Table 7.2: *Classification and Meaning of specific transitions.*

Transition Type	Informal Definition	Meaning
Immediate Transition	Immediate Transition	*Immediate transitions* fire as soon as they are enabled. The waiting time is equal to zero.
Standard Transition	Timed Transition	A waiting time is computed as soon as the transition is enabled. The transition fires if the timer elapsed zero and the transitions is still enabled.

Deterministic Transition	Fixed timed transitions with periodic firing trails	*Deterministic transitions* fire as soon as the fixed time interval elapses during the entire simulation run time. The respective deterministic transitions must be enabled at the end of each repeated interval.
Scheduled Transition	Fixed timed transitions with periodic firing trails between two time points	*Scheduled transitions* fire as soon as the fixed time interval elapsed during the given timepoints. The respective deterministic transitions must be enabled at the end of each repeated interval.

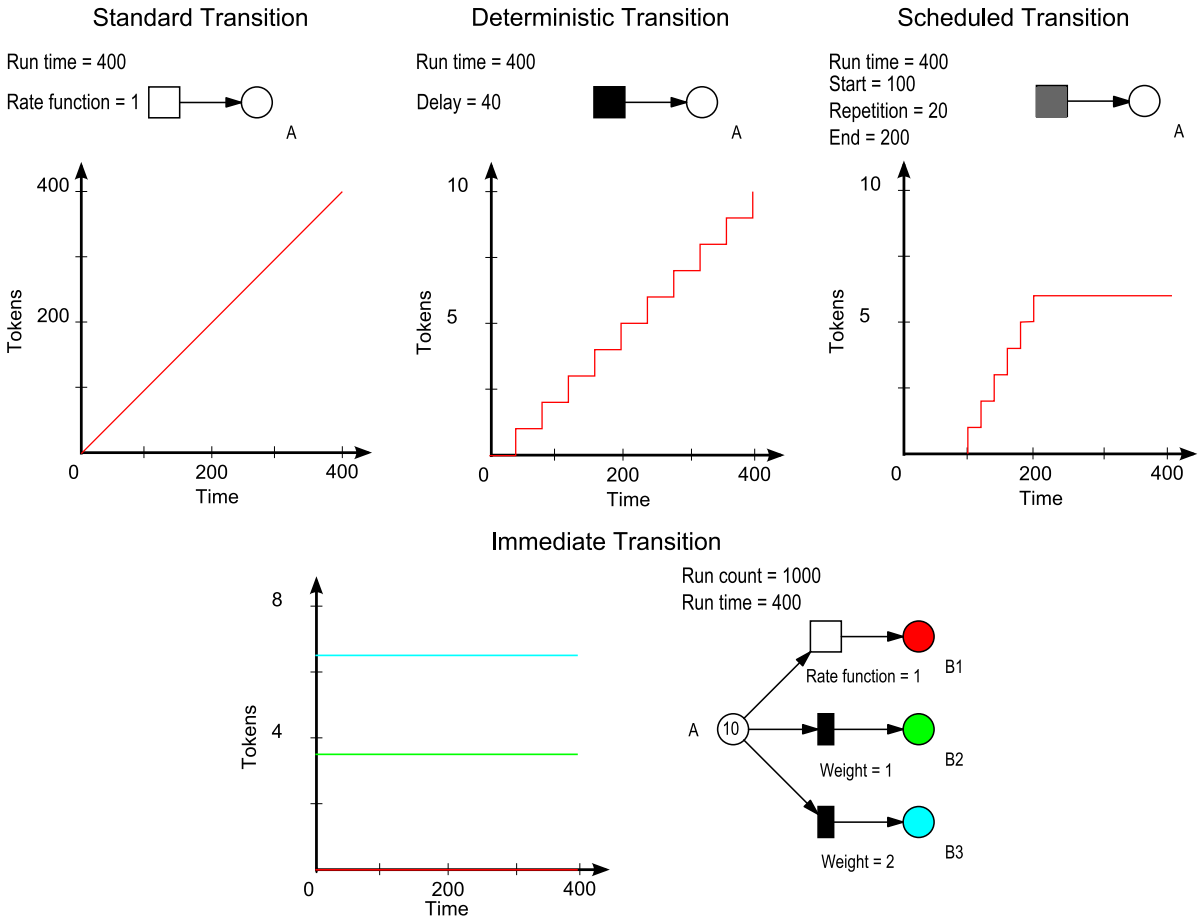


Figure 7.4: ***Comparison of the Standard Transitions and Specific Transitions.*** *For each transition, we show here its functionality by simulating their time-dependent dynamic behaviour. See also* ***Table 7.2*** *for further explanations.*

Table 7.3: *Pre-defined mathematical Functions.*

Name	Meaning of the Function
BioMassAction(.)	Stochastic law of mass action. Tokens are interpretated as single molecules.
BioLevelInterpretation(.)	Stochastic law of mass action. Tokens are interpretated as concentration.
ImmediateFiring(.)	Refers to immediate transitions.
TimedFiring(.)	Refers to deterministic transitions.
FixedTimedFiring_Single(.)	Refers to deterministic transitions that only fires once after a given time-point.
FixedTimedFiring(., ., .)	Refers to scheduled transitions.
abs(.)	Absolute value
acos(.)	Arc cosine function
asin(.)	Arc sine function
atan(.)	Arc tangent function
ceil(.)	Rounding up
cos(.)	Cosine function
exp(.)	exponential function
sin(.)	Sine function
sqrt(.)	Square root
tan(.)	Tangent function
floor(.)	Round off
log(.)	Natural logarithm with constant e as base
log10(.)	Common logarithm with constant 10 as base
pow(.)	Exponent

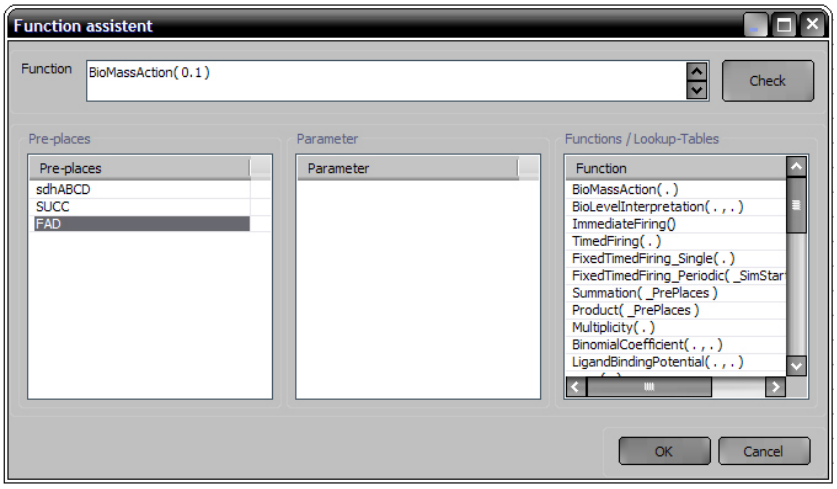


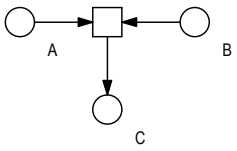
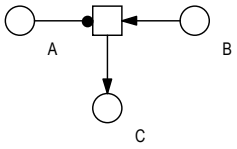
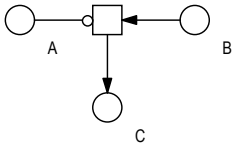
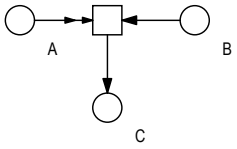
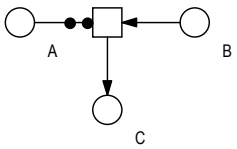
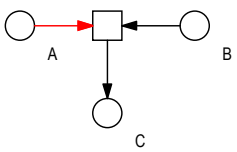
Figure 7.5: ***Rate/Wight Assistant.*** *The rate/weight assistant helps to define mathematical functions. All allowed elements (places, parameters) that can be used to define the rate/weight function of a transition are displayed. The assistant also offers several pre-defined mathematical functions that can be used to define rate/weight function. In addition, the assistant proofs whether or not the semantic of the equation is correct.*

7.2.3 Edges

In addition to the *standard edge*, *Snoopy* offers also several specific edge types: *read edge*, *inhibitor edge*, *reset edge*, *equal edge* and *modifier edges*. Those specific edge types can be very useful to model certain

scenarios, e.g., timed inputs, different stimulation patterns. The function of those specific edge types is explained in **Table 7.4**.

Table 7.4: *Edge types and their meanings.*

Edge	Illustration	Meaning
Standard Edge		The transition is enabled and may fire if both pre-places A and B are sufficiently marked by tokens. After firing of the transition, tokens are removed from the pre-places A and B; new tokens are produced on place C.
Read Edge		The transition is enabled and may fire if both pre-places A and B are sufficiently marked by tokens. After firing of the transition, tokens are removed from the pre-place B but not from pre-place A; new tokens are produced on place C. The firing of the transition does not change the amount of tokens on pre-place A.
Inhibitor Edge		The transition is enabled and may fire if pre-place B is sufficiently marked by tokens. The amount of tokens on pre-place A must be smaller than the given arc weight. After firing of the transition, tokens are removed from the pre-place B but not from pre-place A; new tokens are produced on place C. The firing of the transition does not change the amount of tokens on pre-place A.
Reset Edge		The transition is enabled and may fire if pre-place B is sufficiently marked by tokens. The amount of tokens on pre-place A has no effect on the ability to enable the transition and affects only the kinetics. After firing of the transition, tokens are removed from the pre-place B according the arc weight and all tokens on pre-places A are deleted; new tokens are produced on place C.
Equal Edge		The transition is enabled and may fire if number of tokens on pre-place A is equal to the correpsonding arc weight and place B is sufficiently marked. After firing of the transition, tokens are removed from the pre-place B but not from pre-place A; new tokens are produced on place C. The firing of the transition does not change the amount of tokens on pre-place A.
Modifier Edge		The transition is enabled and may fire if pre-place B is sufficiently marked with tokens. The amount of tokens on pre-place A has no effect on the ability to enable the transition and affects only the kinetics. After firing of the transition, tokens are removed from the pre-place B but not from pre-place A; new tokens are produced on place C. The firing of the transition does not change the amount of tokens on pre-place A.

7.2.4 Parameters

The element *parameter* can be used to define individual parameters. *Parameters* can be used to define *rate/weight functions* but not to define the number of tokens on a certain place. *Coarse parameters* are a third group of macro elements, which allow encapsulating *parameters*. Thereby, high numbers of *parameters* are not visible on the *top-level* or can also be categorized by the use of *coarse parameters*.

7.3 Configuration Sets

Snoopy supports the deposition of *configuration sets* for each type of node (e.g. *standard places*, *standard transitions*) and *parameters*; see **Figure 7.6**. *Configuration sets* offer a fast and convenient way to flexibly switch between, e.g., different initial markings, firing rates and parameter values at once.
The editing, adding and deletion of *configuration sets* is organized an *overview dialogue*. The button to display the *overview dialogue* can be found in *editor dialogue* of each type of node or *parameter*. For The *standard configuration set* for these elements can not be deleted or renamed. All *configuration sets* can be exported using *file/export/export to CSV*. The chosen *configuration sets* will be displayed on the Petri net itself and used for animation and simulation.

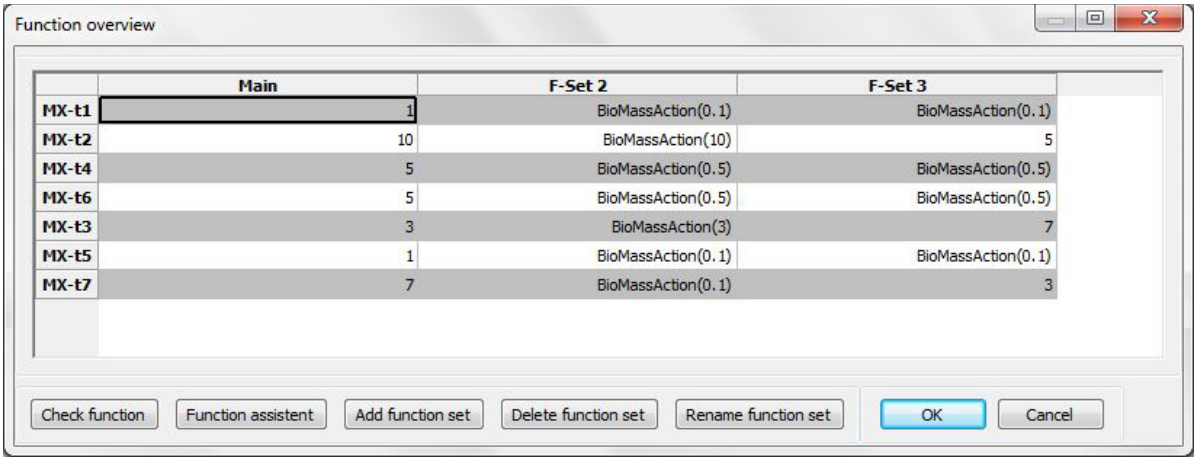


Figure 7.6: ***Configuration Set.*** *For each type of node and parameter exists an overview, where all elements are displayed and their values (marking, rate/weight function, parameter value). In the overview dialogue it is possible to edit single entries, to add new sets, rename set and delete sets.*

7.4 Animation Mode

The *animation mode* in *Snoopy* allows you to observe the token-flow; see **Figure 7.7**. To start the animation mode go to *View/Start Anim-Mode* or simply press *F5*. A new window opens, where you can now steer the animation. The *animation mode* is very useful to get a first expression of the causality of a model and how it works. Playing with the token-flow in the *animation mode* may help to understand the modelled mechanism.
You can animate the token-flow manually by clicking on the transition. If you want to fire a transition that is not enabled, you get the notification *"This transition is not enabled"*. You can quickly add tokens by clicking-left on a place and withdraw tokens by clicking-right on a place. You can also animate the token-flow by using the radio buttons on the *animation steering panel*. The animation can be controlled via the radio buttons step-wise forward and backward or sequentially as long as one transition can be enabled, otherwise you get the notification *"Dead State: There are no more enabled transitions."*. In the *animation steering panel* you find also information about the transition with the shortest waiting

time which fires next. You can also change further *animation properties* under *options*, like *refreshment*, *duration* and *stepping*.
In the *animation mode*, you have also access to the *configuration sets*. You can choose, which *configuration sets*, you want to use for the animation of the token-flow. The *"Stochastic Simulation"* button at the bottom of the dialogue starts the *stochastic simulation mode*.

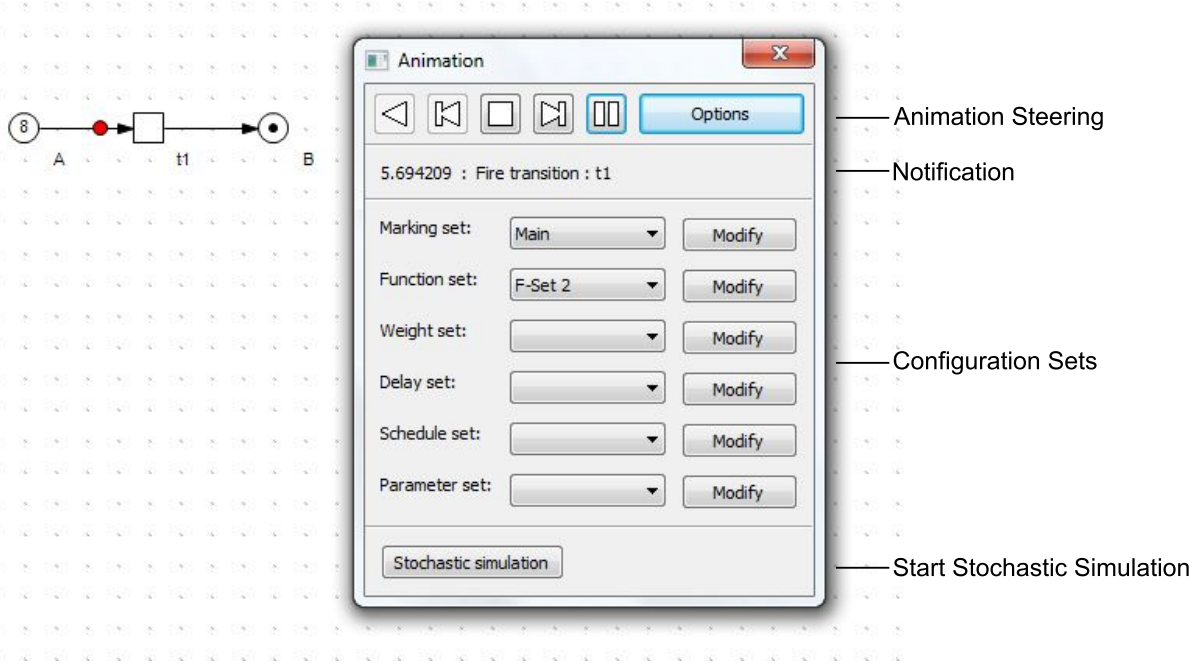


Figure 7.7: ***Animation Mode.*** *The token-flow can be animated by clicking on a transition or via the control keys in the animation steering panel.*

7.5 Simulation Mode

To perform stochastic simulations with the current model in the active window, you can start the *stochastic simulation mode*: *View/Start Simulation*, simply press *F6* or use the *stochastic simulation* button the *animation control panel*.
The *stochastic simulation mode* allows you to simulate the time-dependent dynamic behaviour of the model indicated by the token-flow or the firing frequency of the transitions. The token-flow indicates how the concentration levels or the discrete number of the components change over time. The firing frequency shows how often reactions are performed during the simulation time. Thus, you will get an impression of the time-dependent changes in your model, which might help to understand the wet-lab system. You can perform several simulation studies with your model by manipulating the structure and/or perturbing the initial state (test various stimuli, mutations, knock-outs/downs etc.) and kinetics. All results can be manually or automatically exported in the standard *.csv-format and thus, can be processed and analysed in other mathematical programs.

The graphical interface of the *stochastic simulation mode* can be divided in three functional units; see **Figure 7.8**:

- *Simulation Control*: The *simulation control* allows choosing the main settings and properties for the simulation. It can be further divided in 4 panels:

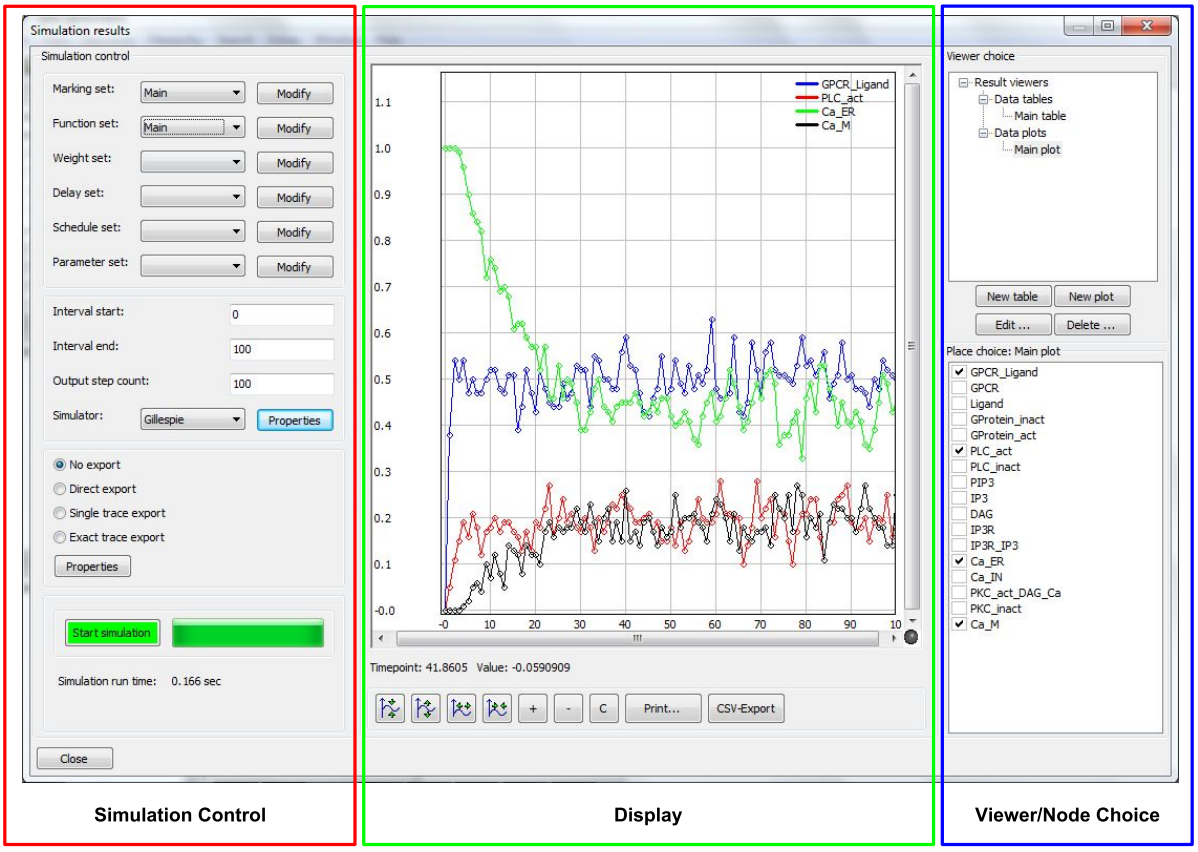


Figure 7.8: *Simulation Mode.* *The graphical simulation interface is tripartite, consisting of a panel for the simulation control to change the setting and simulation properties (red), display of simulation results as table or plot (green), viewer/node choice.*

  - *Configuration Sets*: Modify configuration sets by editing single entries or adding new sets and choosing the *configuration sets* that should be used for the simulation run.
  - *Simulation Properties*: Set *interval start* (time-point from which simulation results should be displayed), *interval end* (time-point where the simulation ends) and *output step count* (number of time-points that should be displayed in the given interval), choose *simulator* (Gillespie or FAU) and change other properties, e.g., *simulation run count* (number of simulation runs that should be performed to average the simulation results).
  - *Export Properties*: Different automatic export settings are available to the *.csv-format.
  - *Start Simulation*: Start simulation with the chosen settings and properties. The bar indicates the progress of the simulation and the required time is given below.
- *Viewer/Node Choice*: Choose which and how you want to display the simulation results.
  - *Viewer Choice*: You can choose between *data tables* and *data plots*. It is also possible to edit, add and delete the *data tables* and *data plots* with the buttons below. You can also choose if you want to display the token-flow (places) or the firing frequency (transitions) in a *data table* or *data plot*.
  - *Place Choice*: You can select the nodes that should be displayed in the *data table* or *data plot*.
- *Display*: In this panel the simulation results are displayed as *data table* or as *data plot* . If *data plot* is chosen, the x-axis shows the time-interval and the y-axis indicates the averaged number

of tokens. You can also edit the view of the plot via the buttons below: *compress/stretch x-axis*, *compress/stretch y-axis*, *zoom in/out*, *centre view*. There is also a button *csv export* which allows you to export the simulation results of the chosen places manually. With the *print...* button, you can also save an image of the current plot. The value of the averaged number of tokens or firing frequency is displayed in the plot by points and which are connected via interpolated lines. If you choose the *data table* the token-flow for the selected places is shown in a table. At the bottom of the window you find some options used for model checking, which are explained in **Section 7.6**.

7.6 Model Checking Mode

Based on the stochastic simulation, *Snoopy* is also enabled to perform model checking of linear-time properties; see **Section 6**. In more detail, a subset of probabilistic linear-time temporal logic (PLTL) can be employed to formulate and verify properties of the form:

$$P_{=?}\left[F_{[t_1,t_2]}\,[ap]\right]$$

The following grammar defines the atomic proposition *ap* of PLTL formulas:

```
ap =
'!' ap
| ap logicbinop ap
| term cmp term

logicbinop =
'&' | '|' | '->' | '<-' | '<->'

cmp =
'=' | '!=' | '>=' | '>' | '<=' | '<'

term =
term binop term | placename | number

binop =
'+' | '-' | '*' | '/'
```

Snoopy allows also to check several properties at the same time. Therefore, you have to separate the atomic proposition *ap* by ';'.

The following precedence rules apply to the logic operators:

- ! has higher precedence than &,
- & higher than |,
- | higher than $\rightarrow$,
- $\rightarrow$ higher than $\leftarrow$,
- $\leftarrow$ higher than $\leftrightarrow$.

So for example, $P \,|\, Q \,\&\, !R \rightarrow S$ is short for $(P \,|\, (Q \,\&\, (!R))) \rightarrow S$.

The binary logical connectives are given in **Table 7.5**.

The order of arithmetic operations, or precedence, is expressed here (from high to low):

- terms inside parentheses,
- multiplication and division (as they appear left to right),

Table 7.5: *Truth table for used logical operators.*

P	Q	$P \,\&\, Q$	$P \mid Q$	$P \rightarrow Q$	$P \leftarrow Q$	$P \leftrightarrow Q$
T	T	T	T	T	T	T
T	F	F	T	F	T	F
F	T	F	T	T	F	F
F	F	F	F	T	T	T

- addition and subtraction (as they appear left to right),

$$a + b * c \equiv a + (b * c) \not\equiv (a + b) * c$$

Operators of the same priority, or precedence, are evaluated as they appear from left to right.

To perform model checking in *Snoopy*, you have to open the simulation window and chose the table view; see **Figure 7.8**. At the bottom of the window, you find three more buttons; see **Figure 7.9**:

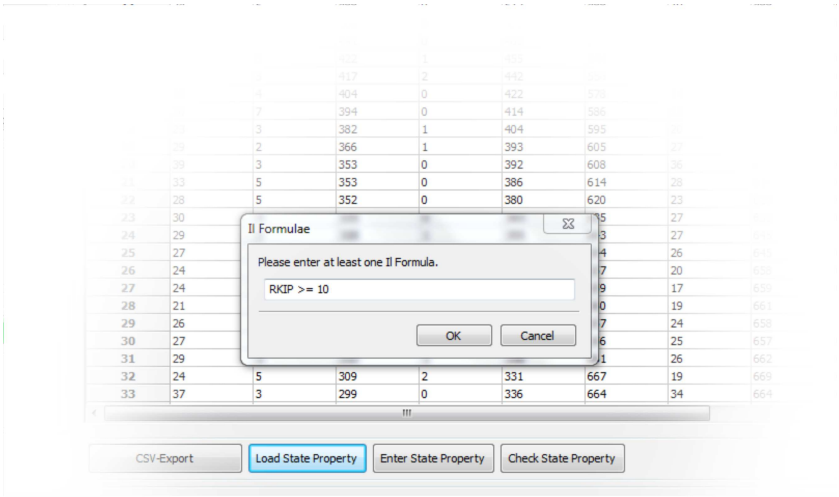


Figure 7.9: ***Model Checking in Snoopy.*** *Snoopy employs a basic model checker in the simulation mode. You can define or load properties that are checked by simulating the time-dependent dynamic behaviour. It is also possible to apply model checking on the averaged simulation results.*

- *Enter State Property*: Here, you can specify an *ap* directly in the dialogue. An empty text removes the current *ap* and no model checking is performed.
- *Load state property*: You can load an *ap*, that is defined in a text file.
- *Check state property*: The model checking is performed on the average behaviour of the previous simulation.

To perform model checking on all simulation traces, you have enter or load a property and run the simulation.

With the help of the *simulation run count*, you can state a number of simulation traces to which model checking should be applied:

- Default value “1” run: You only get the information if the defined property holds “true” or not “false”.

- Arbitrary number of runs: The probability of the defined properties is computed based on the number of simulation runs. In general, a high accuracy requires a high number of simulation runs. If you use the option *Check state property*, you will also just get an information if the defined properties is "true" or "false", because model checking is done on the averaged simulation results.

By *interval start* and *interval end*, you can set the time interval where model checking should be applied.

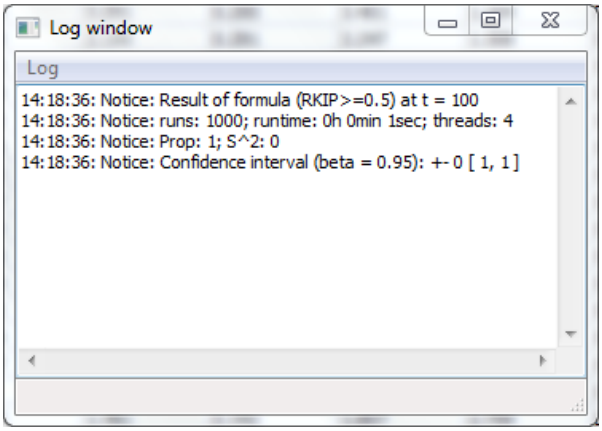


Figure 7.10: ***Model Checker Log Window.*** *The log window shows the results of the model checking. You get information about the checked formula, the number of simulation runs and threads used for the simulation, the probability of the defined property, its variance and the resulting confidence interval.*

The the model checking results are displayed in the log window; see **Figure 7.10**. The explanations are given below:

- Formula: shows the formula checked during simulation.
- Runs: shows the number of simulation runs performed.
- Runtime: shows the number of threads used for simulation.
- Threads: shows the number of threads used for simulation.
- Prop: shows the computed probability for the formula.
- S^2: shows the variance of the probability.
- Confidence Interval: shows the size of the confidence interval.
- $[a, b]$: shows the interval of the probability, calculated from the confidence interval

7.7 Get started

In this section, we give you a brief schedule and helpful hints on how to construct a Petri net in *Snoopy* and simulate its time-dependent dynamic behaviour. We will also pay attention to the hierarchically arrangement of a Petri net.

7.7.1 Modelling

We start with some useful points, you should consider while constructing a Petri net in *Snoopy*.

1. Start *Snoopy*
2. Open a new template for a stochastic Petri net: *File/New* - choose *Stochastic Petri Net*
3. Chose the class of node
   - Click on the canvas to set, e.g., *standrad transition* or *standard place*
   - Provide an unique name for each node or use the logic-functions if you want to use multiple identical copies of one node.

- Define the properties of the node (*initial marking*, *rate/weight function*, *delay*, etc.)

4. Choose the type of *edge*
   - Connect nodes via the chosen edge in the right way
   - Assign an *arc weight* corresponding to the stoichiometry if necessary
5. Choose *parameters* if you want to use them in *rate/weight functions* (strongly recommended)
   - Provide an unique name
   - Define parameter value
6. Provide any *comments* if you like

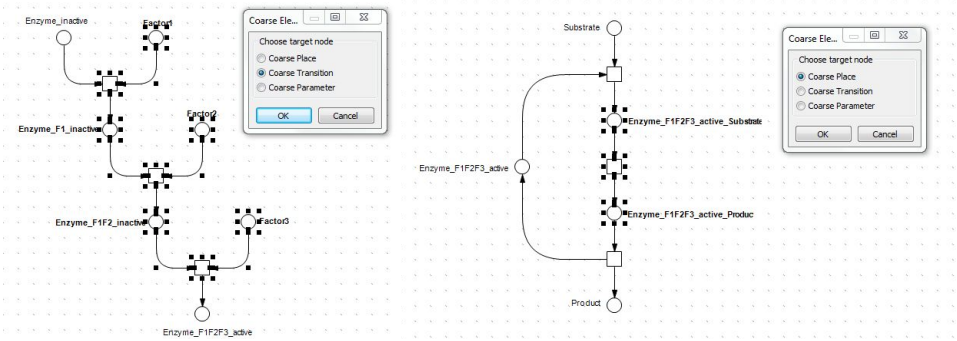


Figure 7.11: ***Application of Coarse Nodes.*** *Select the target nodes that you want to encapsulate by a coarse node. Use a coarse transition (place) if the target subnet is bounded by places (transitions).*

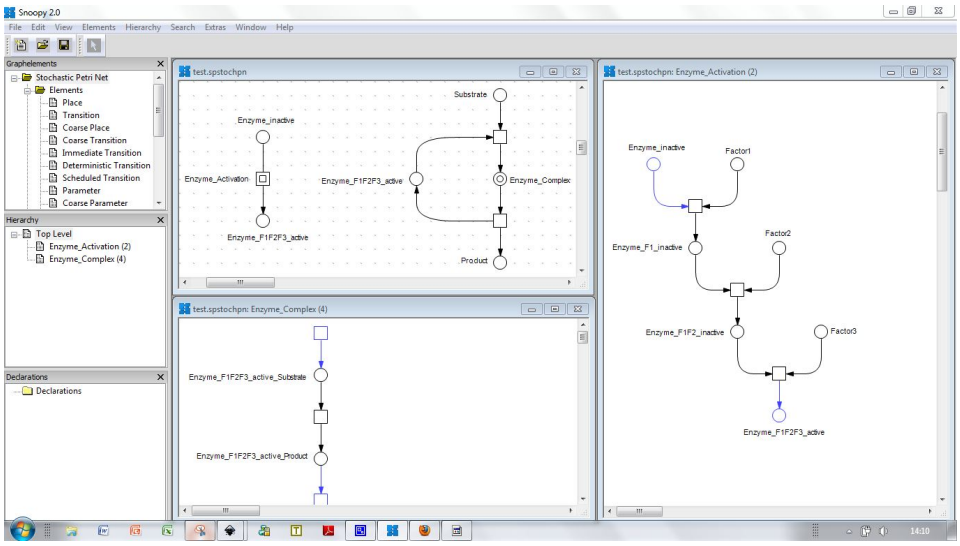


Figure 7.12: ***Hierarchical Structure of a Model.*** *The levels introduced by the coarse nodes of the top-level are displayed in the hierarchy browser. The selected submodels are displayed in separate windows corresponding to their levels. The top-level shows just the coarse nodes instead of the submodel.*

There are two possibilities to structure your model hierarchically and thus, provide a neat arrangement.

- Use coarse nodes from the beginning: Connect *coarse transitions (coarse places)* with all places (transitions), that you want to use on the new level. Construct the subnet on the separate level.

- Encapsulate parts of your model after construction via coarse nodes. To do so, consider the following steps:
  1. Select the elements that should be directed to a new layer; see **Figure 7.11**. Remember if you want to use *coarse transitions (coarse places)*, the subnet must be bounded by places (transitions) that are not selected.
  2. Go to *Hierarchy/Coarse*. A new dialogue opens. Choose the coarse node you want to use; see **Figure 7.11**. After the coarse function is applied to the selected subnet, a new level is displayed in the *Hierarchy Browser*; see **Figure 7.12**. The subnets are now hidden by the coarse node in the *top-level*.
  3. Name the coarse nodes.
  4. To view or edit a subnet, you can open it in a new window by clicking-right on the coarse node or clicking on the entry in the *Hierarchy Browser*.

Further, the coupling of two separate Petri nets with common nodes can be done in two ways:

- Use logical nodes: Indicate nodes that are contained in both models as logical nodes.
- Merge nodes: Drag the shared node of the first model on the respective node of the second model; see **Figure 7.13**. A dialogue opens, which allows to merge the two nodes and automatically adopt all connections to other nodes.

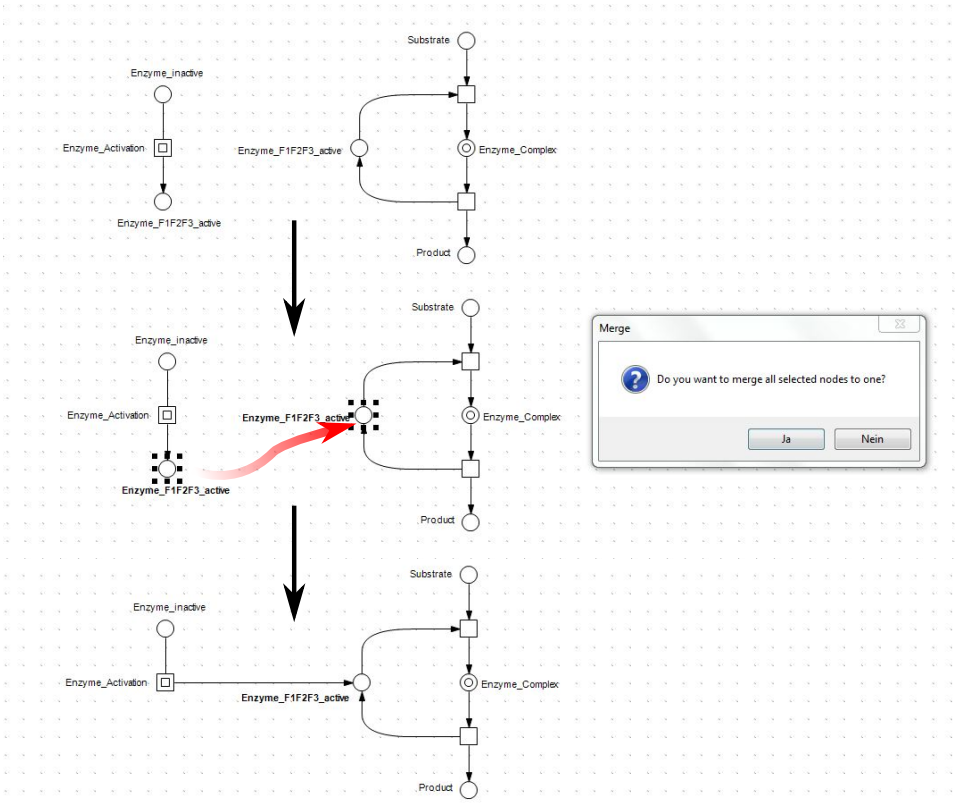


Figure 7.13: *Merge Nodes. Two separate models can be connected via common places that are merged by dragging one onto the other.*

If you want to undo any coarse node or decapsulate the subnet of a coarse node, you need to flatten the coarse node. To do so, go to *Hierarchy/Flatten*.

7.7.2 Simulation/Animation

In this section, we mention a few more words on how to perform a simulation.

1. Define your *rate/weight functions* and *delays* if you have not already done this before. The *rate/weight assistant* helps you to define mathematical equation and check whether the expression is correct or wrong.
2. Start the *simulation mode*: *View/Start Simulation* or simply press *F6*
3. Select the *configuration sets*, you want to use for the simulation run
4. Set simulation parameters: *interval start*, *interval end*, *output step count* and *simulation run count* (select the highest thread count number if you want to perform several simulation runs, this speeds up the computation).
5. Start the simulation
6. After the simulation has finished, choose the viewer, *data plot* or *data table*, and select the nodes you want to display.
7. Export the simulation results if you like to reuse them in other mathematical programs.

CHAPTER 8

Petri Net Analyser: Charlie

In this chapter, we introduce *Charlie* [8] a software tool to analyse Place/Transition nets. The tool has been developed - and is still under development - at the University of Technology in Cottbus [3], Dep. of Computer Science, "Data Structures and Software Dependability" [1]. The tool is in use for the verification of technical systems, especially software-based systems, as well as for the validation of natural systems, i.e., biochemical networks as metabolic, signal transduction, gene regulatory networks. The main features of *Charlie* are [1]:

- Structural properties (net classes, siphons/traps);
- Invariant based analysis (P- and T-invariants, dependent node sets);
- Reachability graph based analysis;
  - Behavioural properties (boundedness, liveness, reversibility);
  - Explicit CTL model checking;
  - Explicit LTL model checking;
  - Shortest path;
- Reachability/coverability graph visualisation using the JUNG library.

We use *Charlie* to determine the structural properties of a Petri net and use the analysis results to interpret the biological system. In the next sections, we show how to work with *Charlie* and explain the main features. See **Chapter 4** for a list of structural Petri net properties and their biological interpretation.

8.1 Graphical User Interface

In this section, we explain the graphical user interface and the offered analysis options. *Charlie* consists of three parts: the *main window*, a *protocol window* and the *analyser-thread manager*; see **Figure 8.1**. In the main window, you can find the *menu bar*, the *tool bar* and the *analysing dialogues*. The *menu bar* consists of four drop down menus:

- *File*:
  - *Open*: Open a Petri net, e.g., created in *Snoopy*. A file dialogue pops up and you can now select the Petri net file, you want to analyse. *Charlie* supports the file formats: spept, apnn, spmnet, sptpt, spm, spstochpn, spped, spcontped.
  - *Reload*: Reload the file that you already opened in *Charlie*. This is useful if you edit the Petri net while analysing and you now want to analyse the changes
  - *Save session*: Save all results that *Charlie* computed for the recent Petri net.

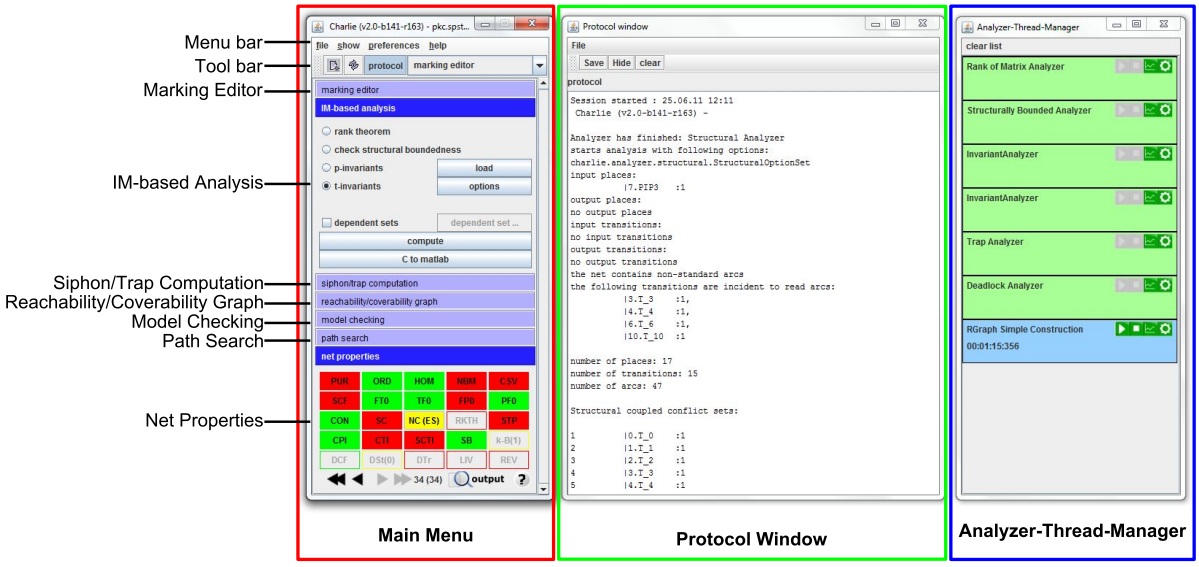


Figure 8.1: ***Charlie User Graphical Interface.*** *The user interface is divided into three parts: the main window (red), protocol window (green), analyser-thread-manager (blue). The main window contains the analysers which could be applied to the loaded Petri net.*

  - *Load Session*: Load results that have been computed for a Petri net that you have analysed before.
  - *Recent files*: List of the files, you used recently. After every start, *Charlie* checks if the files are still available.
  - *Exit*: Close *Charlie*
- *Show*:
  - *Show in Snoopy*: The currently loaded Petri net will be opened in *Snoopy*. Before using this option for the first time, you have to set up a path to the *Snoopy* executable. You can now specify the path in the file dialogue.
  - *Debug Petri net*: A new window opens with properties of the loaded Petri net, e.g., ids of places and transitions. This item is intended to be used by programmers of *Charlie*/*Charlie* plug-ins.
- *Preferences*:
  - *Always apply rules*: Based on some analysis results it is possible to deduce the fulfilment of other properties by certain rules. The rules are always applied automatically if you mark the check box.
  - *Write log files*: If you mark this check box, the analysis results are automatically written to a log-file.
  - *Filter*: The filter allows setting of some output filter options for the log file.
  - *Update*: Check for an updated version of *Charlie*.
- Help:
  - *Help*: A new window opens where you can find several facts about *Charlie*.
  - *About*: Gives you information about the current version, you are using.

The *tool bar* contains some useful short cuts to open a Petri net, to reload the currently opened Petri net, to show the *protocol* and a *fast access combo box* to open selected analysing dialogues.
In the *main menu*, you can also call an analyser. With the help of the analysers, you can investigate your model by computing several Petri net properties. *Charlie* incorporates several analysers/ functionalities:

- *Marking Editor*,
- *IM-based Analysis*,
- *Siphon/Trap Computation*,
- *Model Checking*,
- *Path Search* (not considered),
- *Net Properties*.

To open any of those analysers just click on the tab or use the *fast access combo box*. Each analyser has several options that you can chose, which we explain in the next sections.

In the *protocol window*, additional information about the Petri net structure and all analysis results of performed analysis are written down, as well as the fulfilment of the typical Petri net properties given in **Chapter 4**.

The *analyser-thread manager* shows which analyser have been performed (*green*), which are still running (*blue*), which have been paused (*orange*) and which have been cancelled (*pink*). Each analyser has four buttons. One to pause or restart an analyser and a second to cancel the analysis. A third button allows you to display some statistical information about the analysis and the fourth button displays information about the chosen analyser options. The computation time for each analyser is displayed in the corresponding panel. With the *clear* button on the top of the dialogue, you can clear the *analyser-thread manager*.

8.1.1 Marking Editor

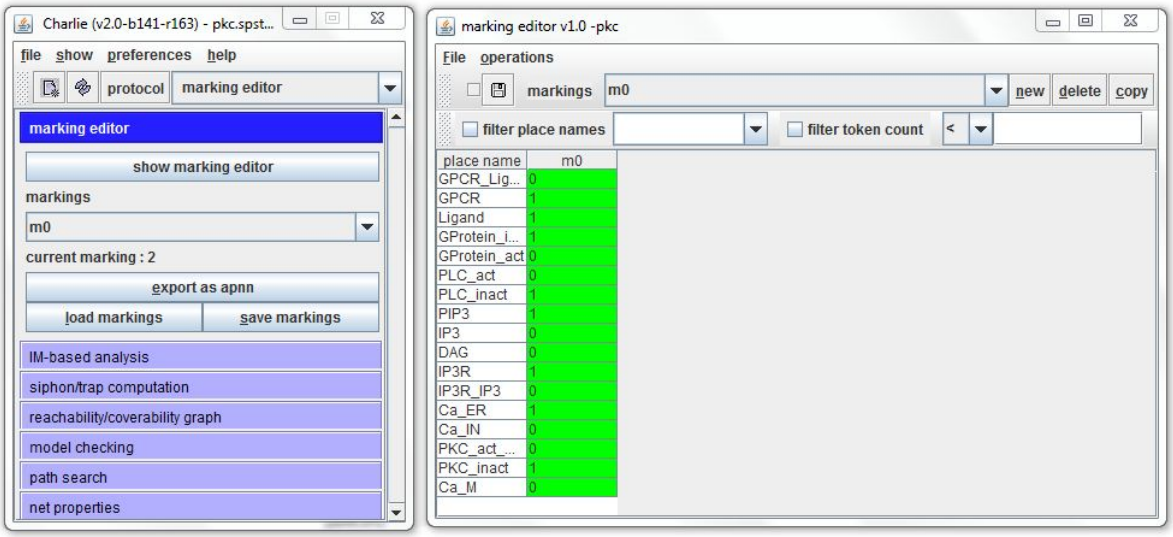


Figure 8.2: ***Marking Editor.*** *The marking editor allows adding and editing of marking sets which can be applied to the analysis.*

By opening a Petri net for structural analysis in *Charlie*, all main marking sets of the loaded Petri net are imported and the chosen marking is applied to the analysis. With the help of the *marking editor*, you can add new marking sets and edit them. To do so, click on *show marking editor* and a new window pops up. The marking editor displays the list of places and list of marking sets in a table. The main marking set can not be deleted or edited.
You can change the marking of the marking sets. Additionally, you can generate new marking sets. Click on *new* and an empty marking set (all entries equal to zero) appears in a new column. You can

also you copy a selected marking using the *copy* button. In both cases, you can afterwards edit the marking. It is also possible to remove marking sets using the *delete* button. However, the main marking set can not be removed, renamed or edited.
The marking editor also allows filtering of places by names or by the number of tokens. To search for a specific place, enable the check box *filter place names* and type the name of the place. Enable the check box *filter token count* if you want to filter places with a specific number of tokens, set the bound and choose a mathematical operation in the drop-down menu (< - less, <= -less-than-or-equal, = - equal, > - greater, >= - greater-than-or-equal-to, ! = - unequal). After applying on of a filter, only those places are shown, which fulfil the property.
You can also load marking sets form a file or save them to a file or save the Petri net and chosen marking to the Abstract Petri Net Notation format (*.APPN).
Use the drop-down menu to select a marking that you want to apply to the analysis. The *marking editor* is very helpful, if you want to investigate how a marking influences the properties of your Petri net model.

8.1.2 IM-based Analysis

All analysers that are contained in the tab *IM-Based Analysis*; see **Figure 8.3**; perform their computation based on the representation of a Petri net by its incidence matrix and linear algebra. The different analysers are:

- *Rank theorem*: Computes the rank of the incidence matrix. Based on the results some rules can be applied to deduce other properties, e.g., liveness.
- *Check structural boundedness*: See **Section 4.1.2.1** and **Table 4.2 B, SB**.
- *P-invariants*: See **Section 4.1.2.2** and **Table 4.4 D, CPI**.
- *T-invariants*: See **Section 4.1.2.2** and **Table 4.4 D, CTI, SCTI**.

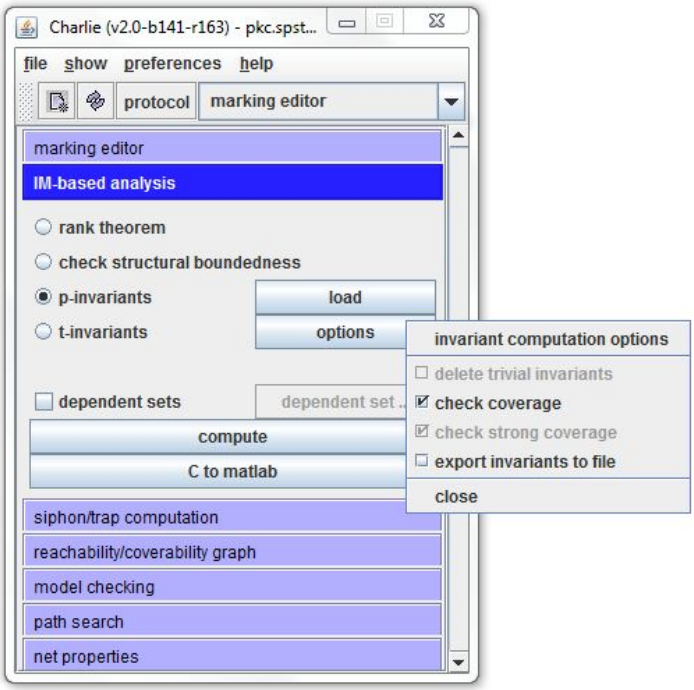


Figure 8.3: ***IM-based Analysis.*** *Based on the incidence matrix the integrated analysers compute the matrix rank, decide on structural boundedness and determine all P- and T-invariants of the model.*

To use one of the analysers mark the respective check box and click on *compute*. The *P-invariant analyser* and the *T-invariant analyser* have additional options, where the *rank theorem analyser* and the *check of structural boundedness analyser* have no further settings. All rules that can be applied to the results of the *rank theorem analyser* and the *check of structural boundedness analyser*, can be looked up in the rule set help; see **Section 8.1.6)**. For the computation of invariants you have the following options:

- *Delete trivial invariants* (only for T-invarinats): removes all trivial invariants from the list, i.e., only those invariants are listed that are not trivial.
- *Check coverage*: Checks if the Petri net is covered by the computed invariants
- *Check strong coverage* (only for T-invariants): Checks if the Petri net is covered by the computed invariants
- *Export invariants to file*: with this option you can set a path to a file, where all calculated invariants are written as text; this file can be imported by *Snoopy*. A file chooser opens to set the path.

To compute the coverage or strong coverage of the Petri net by invariants, you can also load invariants that have been computed before. To do so, click on the *load* button and a *file chooser* opens. By enabling the check box *dependent sets*, you can also calculate the dependent sets when invariants are computed. By default only the abstract depended sets are computed. Further calculations can be set by opening the options menu for dependent sets.

- *Strong dependent sets*: The strongly dependent sets of the invariants are computed.
- *Export sds to file*: Enable this check box in order to export the strong dependent sets to a file; a file chooser will be opened.
- *Abstract dependent sets*: The abstract dependent sets of the invariants are computed.
- *Export ads to file*: Enable this check box in order to export the abstract dependent sets to a file; a file chooser will be opened.
- *Connected ADS*: The connected abstract dependent sets of the invariant are computed.
- *Export cads to file*: Enable this check box in order to export the connected abstract dependent sets to a file; a file chooser will be opened.

Finally, you also have the opportunity to export the incidence matrix of the load Petri net in a several file formats using the *export incidence matrix* button. You can reuse the file for your own computations using, e.g., matlab to perform flux balance analysis.

8.1.3 Siphon/Trap Computation

In this tab, the *siphon/trap analyser*; see **Figure 8.4**, allows you to compute *traps* and *siphons*; see **Chapter 4.2.1 and 4.2.1**; by activating the corresponding check box and clicking on the *compute* button. You can choose between several options:

- *Export*: By selecting the check box *export siphons* (resp. *export traps*) one can choose a file to that the siphons (traps) shall be exported. The exported place sets can be visualised on the Petri net opened in *Snoopy*.
- *Proper Sets*: If the check box *proper sets* is selected then only the proper sets are computed and exported (if exporting is selected).
- When computing *siphons* is selected, one can additionally check the loaded Petri net for the siphon-trap-property by selecting the check box *stp*; see **Table 4.4, STP**. The analyser can also compute bad siphons and sound siphons if you check the respective boxes.

The *siphon/trap* tab offers also an integrated *place set analyser*. By clicking on the button *place set analyser* a new window opens, where all places are listed. One can click on the entries to manually check if the selected places constitute a *siphon* or a *trap* (check boxes at the bottom). In addition, it is shown, whether the set of places selected is a *bad siphon* or not. You can choose to display the names of the places, the IDs of the places or both in the menu *place settings*.

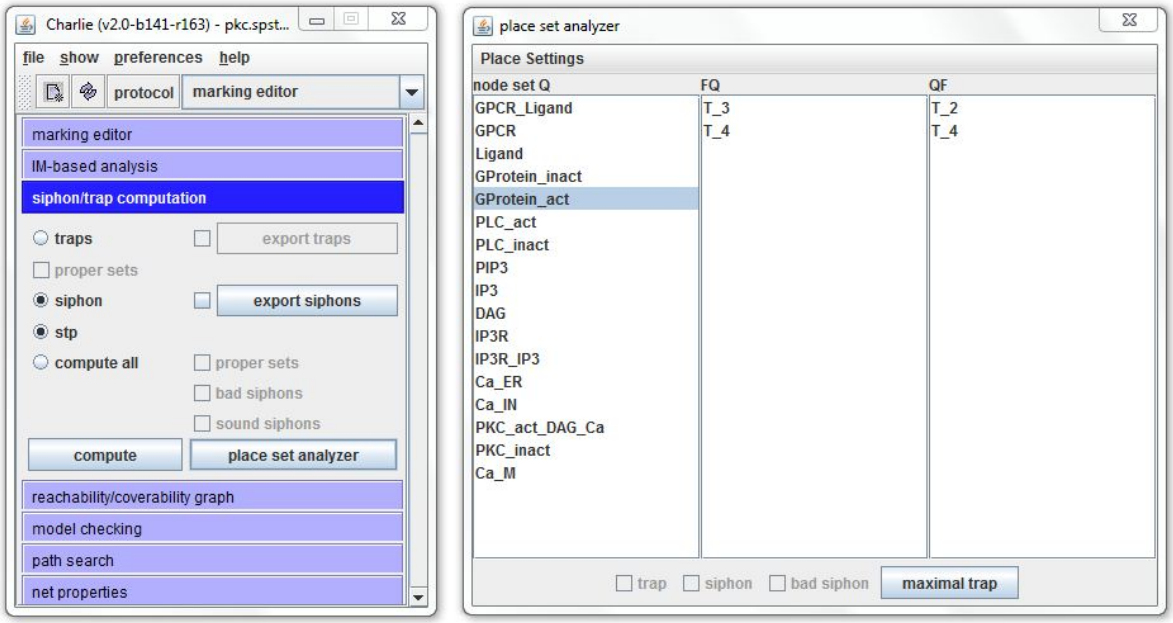


Figure 8.4: ***Siphon/Trap Analysis.*** *The analyser allows computing traps and siphons. It can also be checked if the siphon-trap properties hold. Additionally, a place set analyser is available.*

8.1.4 Reachability Graph/Coverability Graph

The *reachability/coverability graph* analyser computes the state space of the loaded Petri net and selected marking; see **Figure 8.5**. The reachability graph is constructed if the net is bounded, otherwise the coverability graph is constructed. Click on the *compute* button to start the analyser. After computing, the analyser gives some information about the constructed graph in the *rg info* dialogue about the number of *edges*, *states*, strong connected components (*ssc*) and the computation *time*. The analyser offers several options to define the computation of the state space:

- *Check boundedness*: Enable check box to check boundedness of the Petri net while computing the state space.
- *Stubborn reduction*: Enable check box to reduce the state space by stubborn sets. Stubborn sets are sets of transitions that are not affected by transitions that are not contained in the stubborn set. The state space is reduced but terminal states are persevered. The state space has no dead states if the reduced stubborn state space has no dead states.
- *Fire rules*: Choose a firing rule by enabling the respective check box.
  - *Single step*: Enable check box to compute all successor states of the current state by considering that just one enabled transition in the current state can fire at the same time.
  - *Max step*: Enable check box to compute the successor state of the current state that could be reached if all enabled transition in the current state fire at the same time.
  - *Max depth*: Define the number of firing events in a sequences that should be computed, meaning the depth of the graph, layers (*"0"* means that the entire state space is computed).

To visualise the constructed graph click on *view RG* or on *show window*. You can select the graph that should be displayed in the drop-down menu above. A new window pops-up, the *graph visualizer*, and shows the selected graph; see **Figure 8.5**. All strongly connected components are highlighted in a different colour. You have several options to change the visualization of the graph. You can open different graphs of the same Petri net (with variable markings) and/or different views of the graph in

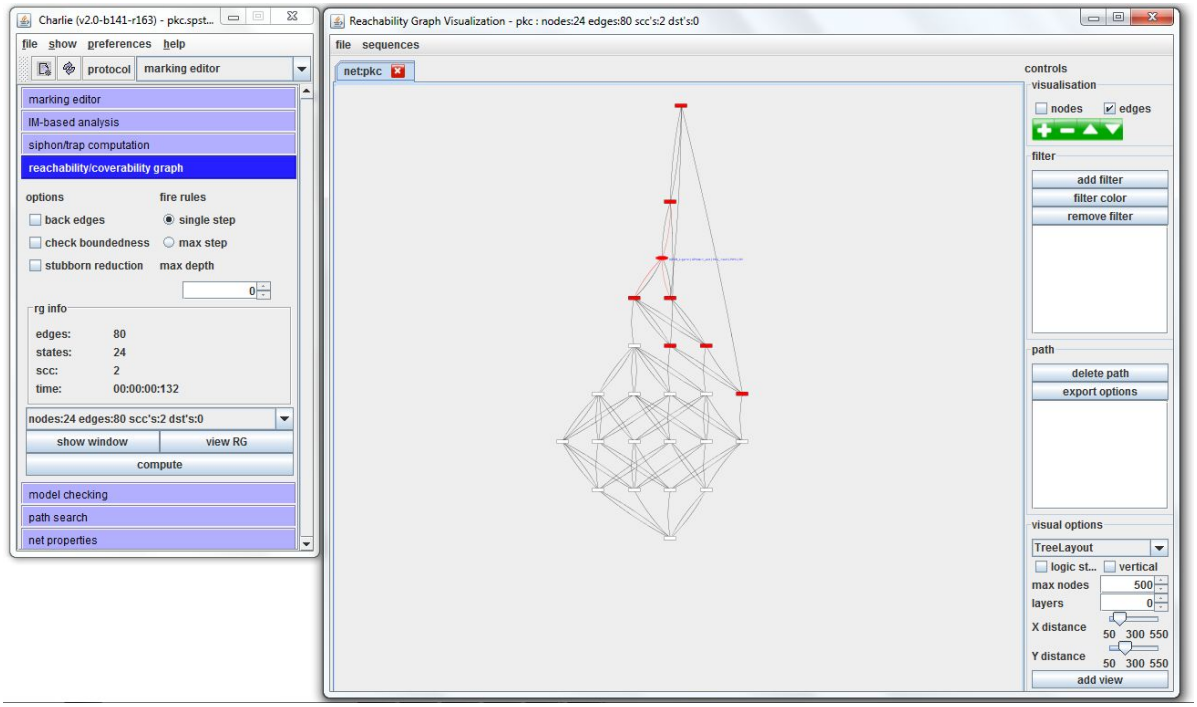


Figure 8.5: ***Reachability/Coverability Graph analyser.*** *Compute the state space of the loaded Petri net and visualise the computed state space as a graph. Several options allow to modify the visualization.*

separate tabs at the same time. The visualization of the graph can be changed in the *visualization control* at the right site of the window.

- *Visualisation*:
  - *Nodes*: Enable check box to display the state/marking of each node.
  - *Edges*: Enable check box to display the name of the transition at each arc.
  - *Buttons*: *zoom in*, *zoom out*, *go up*, *go down*.
- *Visual Options*
  - *Layouts*: Choose between different automatic layouts in the drop-down menu: *TreeLayout*, *ISOMLayout*, *FRLayout*, SpringLayout, *CycleLayout* to change the arrangement of the nodes and arcs in the graph.
  - *Logic sates*: Enable check box if you want to display states that can be reached from different states as logical nodes.
  - *Vertical*: Enable check box to vertically arrange the current view.
  - *Max nodes*: Choose a maximal number x of nodes that should be displayed. Just the first x nodes that can be reached from the initial marking are displayed.
  - Layers: Choose a number of layers that should be displayed meaning the depth of the graph, number of iteration for computing successor nodes.
  - *X distance*: Change the horizontal spaces between the nodes with the slider.
  - *Y distance*: Change the vertical spaces between the nodes with the slider.
  - *Add view*: Opens a new tab and apply the visual options to the graph.

- *Path* (not considered)
- *Filter* (not considered)

You can also manually manipulate the graph or the view:

- Move the entire graph by clicking on the canvas and dragging the graph.
- Rotate the entire graph by holding SHIFT and clicking on the canvas.
- Tilt the entire graph by holding CRTL and clicking on the canvas.
- Move single nodes by clicking and dragging the respective node.

By clicking on any node in the graph, the node will be highlighted and all outgoing arcs and the marking is displayed. A menu with the following options pops-up if you use a right-click:

- *Copy marking*: Copy the marking given as text next to current node. You can paste it in any text editor.
- *Create/Copy filter*: Creates an expression in the form of a boolean expression that can be used to create a filter.
- *Start path*: Selects the current node as start node of a path and stores the path.
- *Show next states*: All states that can be reached from the current state are displayed.
- *Show previous states*: All states that lead to the current state are displayed.
  In the *menu bar* of the *graph visualiser* are two more tab available with the following options:
  - *File*:
    - *Load Graph*: Load a graph that has been saved before.
    - *Save graph layout*: Save the actual graph layout to a file.
    - *Export graph layout*: Saves an image of the actual graph layout in a *.pdf file.
    - *Save session*: Save all results the *Charlie* computed for the recent Petri net.
    - *Load Session*: Load results that have been computed for a Petri net that you analysed before.
    - *Exit*: Close *graph visualiser*.
  - *Sequences*:
    - *Reduce sequence*: Aggregates nodes of repeated firing sequences.
    - *Expand sequence*: Expands aggregated nodes of repeated firing sequences.

8.1.5 Model Checking

Charlie offers also a *model checker*; see **Figure 8.6**; to perform explicit CTL and LTL model checking; see also **Sections 6.3.1 and 6.3.3** for further explanations. Before, we have a closer look at the options, we give you the CTL and LTL grammar used in *Charlie*.

- CTL grammar:

```
START  = ctl_formula ';'

ctl_formula = ap
| '(' ctl_formula ')'
| unop ctl_formula
| ctl_formula binop ctl_formula
| ae '(' ctl_formula 'U' ctl_formula ')'
| untemp ctl_formula

unop = '!' | '~'

binop = '&' | '|' | '->' | '<-' | '<->'
```

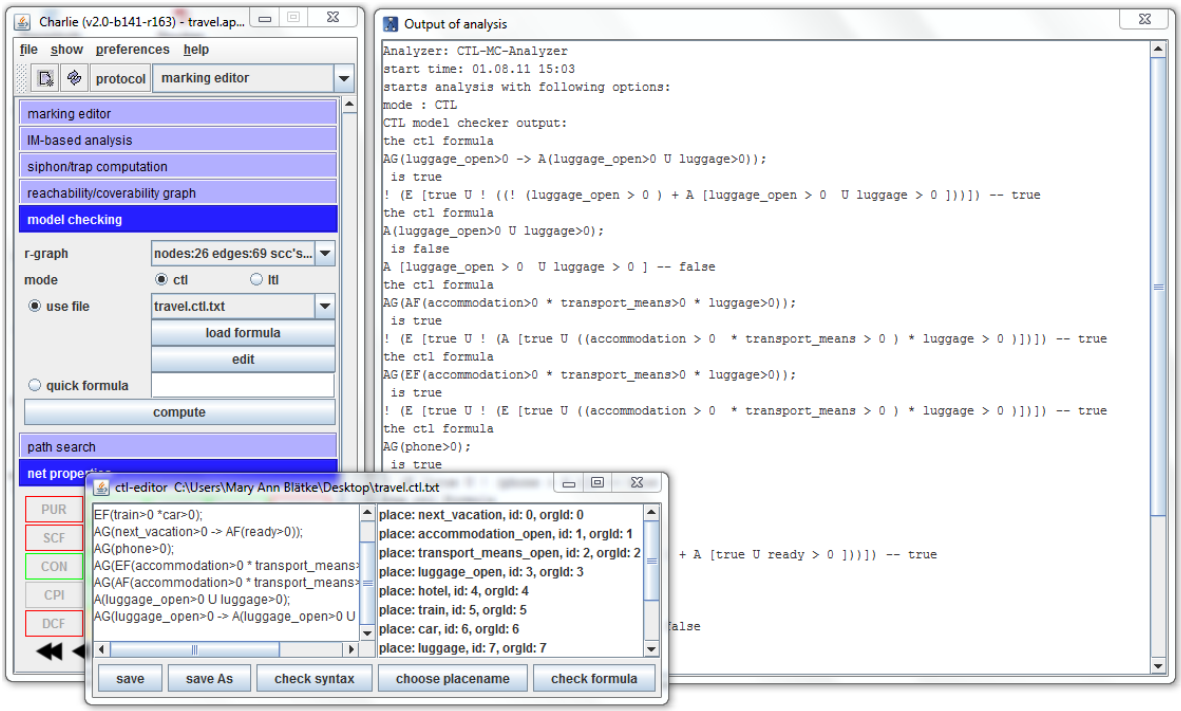


Figure 8.6: ***Model Checking in Charlie.*** *Charlie uses explicit CTL and LTL model checking to verify specific properties of the loaded model.*

```
ae = 'A' | 'E'

untemp = 'AX' | 'EX' | 'AF' | 'EF' | 'AG' | 'EG'

ap  =  PLACE cmp INT | 'true' | 'false'

cmp  = '==' | '!='| '>=' | '>' | '<=' | '<'
```

- LTL grammar:

```
START  = ltl_formula

ltl_formula = ap
| '(' ltl_formula ')'
| unop ltl_formula
| ltl_formula binop ltl_formula
| '(' ltl_formula 'U' ltl_formula ')'
| untemp ltl_formula

unop = '!' | '~'

binop = '&' | '|' | '->' | '<-' | '<->'

untemp = 'X'  | 'F' | 'G'

ap  =  PLACE cmp INT | 'true' | 'false'
```

```
cmp  = '=' | '!='| '>=' | '>' | '<=' | '<'
```

Before you can use the *model checker*, the reachability graph must be computed. The model checker thas the following options:

- *RG*: Choose a reachability graph from the drop-down menu.
- *Mode*: Choose between CTL and LTL.
- *Use file*: Choose a file that you already loaded in the drop-down menu.
- *Load formula*: Load an *ap*, that is defined in a text file.
- *Edit*: A small assistant opens, which helps you to define your CTL or LTL formula and checks the syntax.
- *Quick formula*: Enter an *ap*.
- *Compute*: Starts verification of the defined properties.

The *output window* shows the results, whether the property is true or false.

8.1.6 Net Properties

The dialogue *net properties* is not an analyser itself but shows the properties of the net that already have been analysed; see **Figure 8.7**. All properties and their short cuts as well as an informal description can be found in **Tables 4.1 - 4.4**.

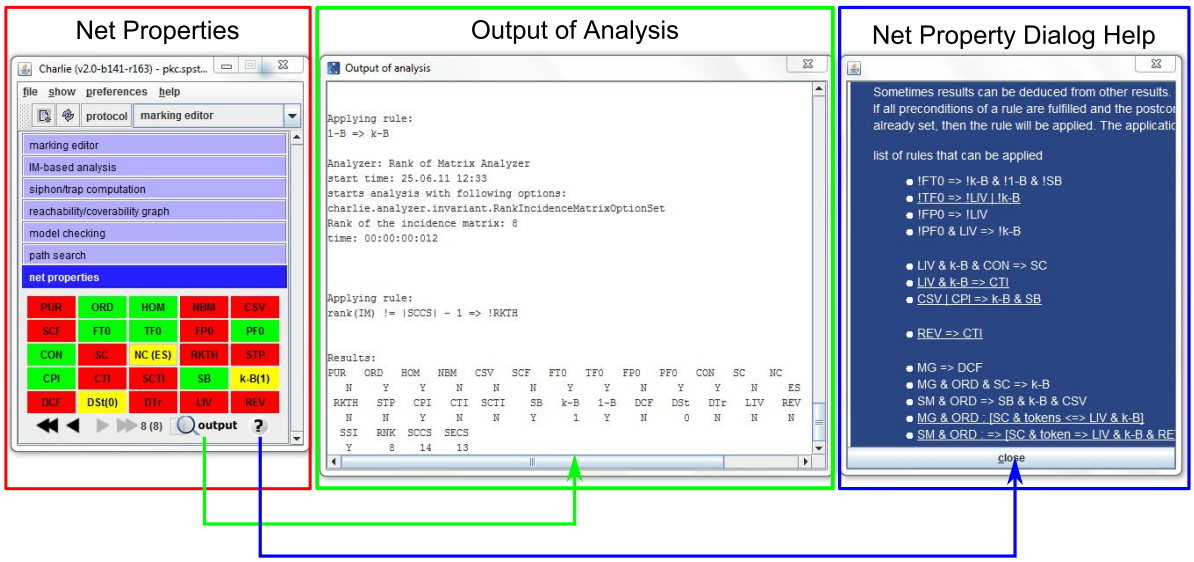


Figure 8.7: ***Net properties.*** *The dialogue net properties shows the computed properties of the loaded Petri net. In this dialogue, you can also open a new windows that gives additional information about the output and the rules that are implemented in Charlie.*

The qualitative properties given in **Table 4.1** are computed while loading a Petri net. Other properties are computed based on the results of the different analysers or are deduced from certain rules. A property with a *grey* background has not yet been analysed or it has not been found by a rule. Properties that either have been analysed or been implied by a rule have either a *red*, *green* or *yellow* background depending on the truth value. A *green* background always implies that the Petri net has the specific property, while a *red* background implies that the Petri net does not have the specific property. If the

background is *yellow* then an analyser found additional information for this property. Examples for this are the net class (NC) or the k-boundedness. Those colours can change from *green/red* to *yellow* but not vice versa and they will never change from *red* to *green* or vice versa.
Furthermore, you can go through the different results that either an analyser or a rule provided with the help of the radio buttons below; see **Figure 8.7**. The last result set always contains a summary of all results gained so far.
With the button *output*, one can open the *output dialogue*. In the *output dialogue* the analysers print out some additional information. Finally, you can get some additional information, by pressing the *help* button, about the rule set that is stored in the system. The rules that are listed in the rule set can be applied to the results that are either gained by an analyser or by another rule.

8.2 Visualisation of Analysis Results in Snoopy

All node sets, e.g., P-invariants, T-invariants, siphons, traps, that have been computed in *Charlie* can be opened in a text editor or be depicted on the respective Petri net in *Snoopy*. The visualisation of the place sets makes it much easier to understand their biological meaning. To do so, consider the next steps:

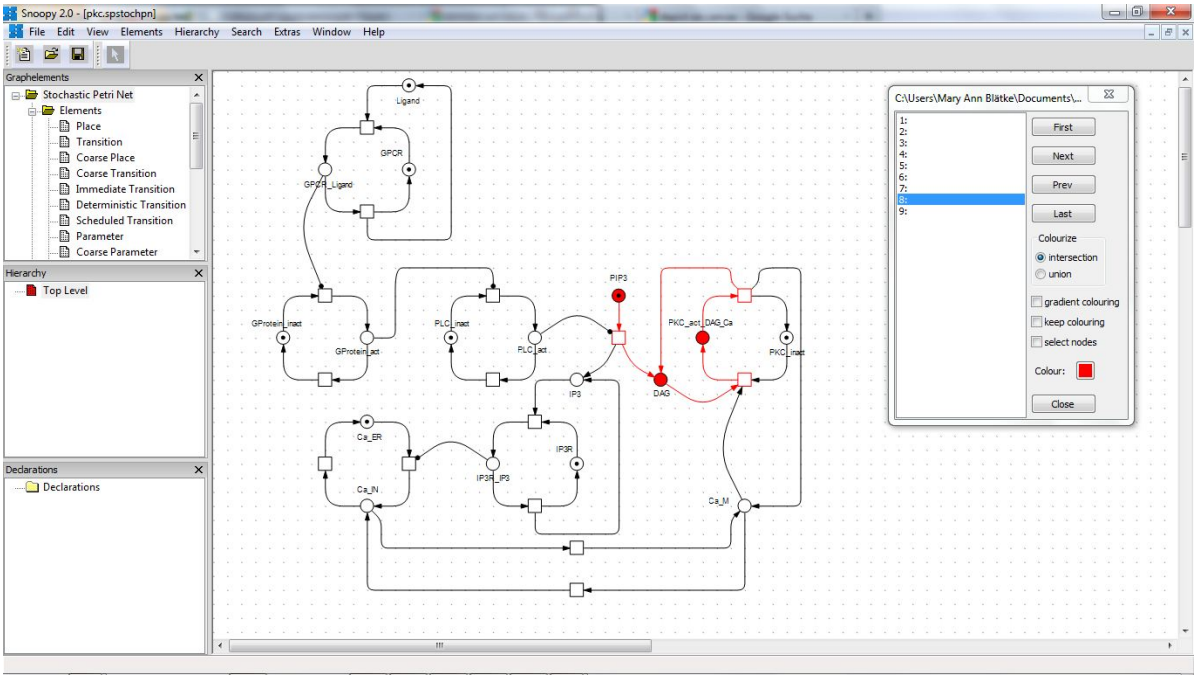


Figure 8.8: ***Visualise Node Sets in Snoopy.*** *All node sets computed in Snoopy can be imported in Snoopy and depicted on the respective Petri net.*

- Export the node set in *Charlie*.
- Open the respective Petri net in *Snoopy*.
- Load the node set: *Extras/Load node set file...* and browse to the file, you want to display.

After choosing the file path, a new window pops-up, which allows you to browse through the node sets. The node sets are depicted on the Petri net if you select one in the dialogue. You can select multiple node sets if you hold SHIFT. The buttons *First/Next/Prev/Last* can also be used to browse through the

node sets. The intersection/union of the selected node sets is shown if the check box *intersection/union* is enabled. You can also choose between different colours in the *colour* menu. There are three more options, you can choose:

- *Gradient Colouring*: Transitions/Places are shown in a lighter colour the edges.
- *Keep Colouring*: The selected elements of the displayed node sets still coloured after closing the dialogue.
- *Select Nodes*: The selected elements of the displayed node sets are selected to, e.g., copy or edit them.

Bibliography

[1] http://www-dssz.informatik.tu-cottbus.de.

[2] http://www.informatik.uni-hamburg.de/TGI/PetriNets/.

[3] http://www.tu-cottbus.de.

[4] W. Brauer and W. Reisig. Carl Adam Petri and 'Petri Nets'. *Informatik-Spektrum*, 29:369–374, 2006.

[5] K.-H. Cho et al. Mathematical Modelling of the Influence of RKIP on ERK Signalling Pathway. *Computational methods in systems biology*, 2602:127–141, 2003.

[6] E. Curry. Stochastic Simulation of entrained circadian rhythem. Master's thesis, School of Informatics, University of Edinburgh, 2006.

[7] A. Doi et al. Construction biological pathway models with hybrid functional Petri nets. *In Silico Biology*, 4:271–291, 2004.

[8] A. Franzke. Charlie 2.0 - A Multithreaded Petri Net Analyzer. Master's thesis, Brandenburg University of Technology Cottbus, 2009.

[9] P.J.E. Goss and J.Peccoud. Quantitative modeling of stochastic systems in molecular biology by using stochastic Petri nets. *Proceedings of the National Academy of Sciences USA*, 95:2340–2361, 1998.

[10] S. Grunwald et al. Petri net modeling of gene regulation of the Duchenne muscular dystrophy. *Biosystems*, 89:189–205, 2008.

[11] M. Heiner, R. Donaldson, and D. Gilbert. *Petri Nets for Systems Biology*, chapter 3, pages 61–97. Jones & Bartlett Learning, LCC, 2010.

[12] M. Heiner, D. Gilbert, and R. Donaldson. *Petri Nets for Systems and Synthetic Biology*, volume 5016 of *LNCS*, pages 215–264. Springer, 2008.

[13] I. Koch, B.H. Junker, and M. Heiner. Application of Petri Net Theory for Modelling and Validation of the Sucrose Breakdown Pathway in the Potato Tuber. In *Proc. 5th Workshop CPN, Univ. of Aarhus, October 2004*, volume 21 of *Bioinformatics, April 2005*, pages 1219 – 1226. (Advance Access published November 16, 2004), 2005.

[14] S. Lehrack. A Modelling and Simulation Tool for Stochastic Petri Nets Models of Biochemical Networks. Diploma thesis, Brandenburg University of Technology Cottbus, 2007.

[15] C. Li et al. Modelling and simulation of signal transductions in an apoptosis pathway by using timed Petri nets. *Journal of Biosciences*, 32(1):113–127, 2007.

[16] M. Heiner M. Schwarick, C. Rohr. MARCIE - Model checking And Reachability Analysis done effiCIEntly. *Proc. 8th International Conference on Quantitative Evaluation of SysTems (QEST 2011), IEEE Computer Society 2011, Achen*, 2011.

[17] W. Marwan, A. Sujatha, and C.Starostzik. Reconstructing the regulatory network controling commitment and sporulation in *Physarum polycephalum* based on hierarchical Petri net modeling and simulation. *Journal of Theoretical Biology*, 236:349–365, 2005.

[18] I. Mura and A. Csiksz-Nasy. Stochastic Petri net extension of a yeast cell cycle model. *Journal of Theoretical Biology*, 254:850–860, 2008.

[19] C. Rohr, W. Marwan, and M. Heiner. Snoopy - a unifying Petri net framework to investigate biomolecular networks. *Bioinformatics*, 26:974–975, 2010.

[20] A. Sackmann et al. An analysis of the Petri net based model of the human body iron homeostasis process. *Computational Biology and Chemistry*, 31(1):1–10, 2007.

[21] A. Sackmann, M. Heiner, and I. Koch. Application of Petri net based analysis techniques to signal transduction pathways. *BMC Bioinformatics*, 7:1–17, 2006.

[22] O. Schulz-Trieglaff. Modeling the randomness in biological systems. Master's thesis, School of Informatics, University of Edinburgh, 2005.

[23] O.J. Shaw, L.J. Steggles, and A. Wipat. Automatic parameterization of stochastic Petri net models of biological networks. Technical report, CS-TR-909, School of CS, University of Newcastle upon Tyne, 2005.

[24] S. Soliman and M. Heiner. A Unique Transformation from Ordinary Differential Equations to Reaction Networks. *PLoS ONE*, 5:1–6, 2010.

[25] R. Srivastava, M.S. Peterson, and W.E. Bentley. Stochastic kinetic analysis of the escherichia coli stress circuit using σ^{32}-targeted antisense. *Biotechnology and Bioengineering*, 75:120–129, 2001.

[26] K. Yeung et al. Suppression of the Raf-1 Kinase Activity and MAP Kinase Signalling by RKIP. *Nature*, 401:173 – 177, 1999.