Petri Net Based Model Validation in Systems Biology

Monika Heiner[1] and Ina Koch[2]

[1] Brandenburg University of Technology Cottbus, Department of Computer Science, Postbox 10 13 44, 03013 Cottbus, Germany
monika.heiner@informatik.tu-cottbus.de
http://www.informatik.tu-cottbus.de/~wwwdssz
[2] Technical University of Applied Sciences Berlin, Department of Bioinformatics, Seestrasse 64, 13347 Berlin, Germany
ina.koch@tfh-berlin.de
http://www.tfh-berlin.de/bi/

Abstract. This paper describes the thriving application of Petri net theory for model validation of different types of molecular biological systems. After a short introduction into systems biology we demonstrate how to develop and validate qualitative models of biological pathways in a systematic manner using the well-established Petri net analysis technique of place and transition invariants. We discuss special properties, which are characteristic ones for biological pathways, and give three representative case studies, which we model and analyse in more detail. The examples used in this paper cover signal transduction pathways as well as metabolic pathways.

1 Introduction

The interest in biological systems is as old as mankind. It was always of great interest to understand the complex interactions and thus the behaviour in an organism, in a cell, or in a special metabolism. These questions are still a major challenge in systems biology. *Escherichia coli* – a famous bacterium often used as typical model organism (see e.g. [19]), because it is rather simple in comparison to higher organisms – contains already more than thousands of biochemical components. A human is composed of some five octillion atoms [17]. The human brain is estimated to be built of about 100 billion neural components with hundreds of trillions interconnections [13]. Just the description, not to speak of analysis or simulation, of such huge systems requires computational techniques. Biological molecular systems are characterized by a large number of components and interactions between components, which are manifold, strongly coupled, and associative rather than additive. There is almost no real understanding of the design principles that govern intact biological systems [30].

A recent goal in systems biology is to understand the processes in a living cell. Similar to computer scientists, biologists use "divide and conquer"-techniques to investigate subsystems experimentally. Processes in the cell are divided into

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special metabolisms or pathways, which often correspond to special main functions of the cell, e.g. carbon metabolism, energy metabolism, purine metabolism, glycolytic pathway, and many others. Due to newly developed techniques in experimental biology, e.g. microarray analysis, a lot of data of biological processes have been produced over the last years. With the huge and rapidly increasing amount of data of biological systems, the following difficulties arise:

1. Data Collection Data of biochemical pathways are basically stored in the literature. But typically, special subsystems in special organisms under very special aspects are investigated, such that the entire data publication is widespread over various scientific fields. E. g., results of pharmaceutical research on special human pathways with respect to a special disease are published in other journals than data resulting from fundamental research on the same human pathways or data of the same pathway in other organisms. Cross references are the exception. Intelligent data mining techniques might be of help to search for known data of special biological processes. Up to now, much effort must be spent for gathering and evaluating relevant biological literature to get the appropriate data which one is interested in.

2. Data Representation To handle the arising amount of data it is necessary to represent and store them computationally using a unique description of biochemical pathways. There are several special databases of pathway or interaction data, e.g. *EC Enzyme Database* [1], *EMP Enzymology Database* [33], *MPW Metabolic Pathway Database* [34]. All of them use different description techniques. The most commonly used database, containing many pathways of different species, is the *KEGG Database* [11], but its representation of concurrently behaving pathways by monochromatic graphs is not free of ambiguities, as discussed e. g. in [7].

Biochemical pathways are modelled at different abstraction levels. It must be distinguished between quantitative (kinetic) models and qualitative (stoichiometric or even purely causal) models. The first ones represent the actual objective and real purpose in the long-term. They are used as soon as kinetic parameters, such as substance concentration, equilibrium constants, and reaction rates of a pathway are known. The aim of these models is to predict the system's dynamics. Related evaluation methods are typically based on solutions of systems of differential equations [8], [10], [28], [29]. Related tools for simulation were developed, such as GEPASI [20], and E-CELL [36]. Contrary, qualitative models are commonly used only, if kinetic parameters are not available or incomplete. All these qualitative models are based on more or less graphtheoretical descriptions of the system topology, which are defined in case of stoichiometric models by the known stoichiometric equations.

3. Data Validation Models are widely used for biotechnological questions, e.g. for the optimization of pathways in order to maximize the yield of special products. With the increasing number of known metabolites and known interactions between them, a validation of the interaction network becomes more and more important. The net behaviour is not understandable and predictable anymore by using merely human skills.

But, available evaluation packages for quantitative models are not able to check the model for validity. There is a strong demand for mathematical methods to validate a model for consistency and to answer questions on general structural and dynamic properties like liveness, dead states, traps, structural deadlocks, and invariant properties.

Moreover, existing methods are dedicated to a certain system type or a certain pathway represented by special graphs, see [15], [42], and many others.

Hence, a crucial point seems to be the concise and unambiguous representation of biological networks to handle computationally these highly integrated networks in an efficient manner. It is necessary to get a consistent view of the entire current state of knowledge about a particular pathway. For that purpose, a readable language with a formal, and hence unambiguous semantics would be obviously of great help as a common intermediate language in order to avoid the production of just larger patchwork, exposed to even more interpretation choices. Independently of the given description level and the particular view extension, all pathways exhibit inherently very complex structures, exploiting all the patterns well-known in software engineering, like sequence, branching, repetition, and concurrency, in any combination. But, opposite to technical networks, natural networks tend to be much more complex and apparently unstructured, making the understandability of the full network of interactions extremely difficult and therefore error-prone.

Petri nets could play an integrating role by serving as a common intermediate representation. They are able to provide a mathematically unique representation of biochemical pathways, whereby different biochemical processes may be depicted hierarchically at different abstraction levels. Moreover, established Petri net analysis techniques may be used for the validation of qualitative biochemical models, before they are extended to quantitative ones. Altogether, Petri nets enjoy the following features which might be of great help for systems biology: (1) readability – to support understanding, and therefore enable fault avoidance in the model construction process, (2) executability (animation techniques) – to experience a model in order to get really familiar with it, (3) validation techniques – for consistency checks to ensure the model integrity and correspondence to reality, and (4) analysis techniques – for qualitative as well as quantitative behaviour prediction.

In this paper, we focus on model validation by means of qualitative models, because it is obviously necessary to check at first a model for consistency and correctness of its biological interpretation before starting further analyses, aiming in the long-term at behaviour prediction by means of quantitative models. The expected results, justifying the additional expense of a preliminary model validation, consist in concise, formal and therefore unambiguous models, which are provably self-consistent and correspond to the modelled reality.

This paper is organized as follows. In the next section we describe shortly the main modelling principles for building Petri net models of biological systems. In section three we discuss and motivate the basic principles of model validation we used. Three representative case studies are presented in section four, the apoptosis in mammalian cells, the sucrose breakdown pathway in the potato

tuber, and the combined glycolysis and pentosephosphate pathway in erythrocytes. To model and validate them, we use (low-level) place/transition nets as well as (high-level) coloured nets. Section five gives an overview on related work, and the final section provides a summary with an outlook on future work.

2 Modelling

There are three main types of molecular biological networks – metabolic pathways, signal transduction pathways, and gene expression networks.

Living organisms require a continuous influx of free energy to carry out their various functions. The term *metabolism* refers to the overall process, through which living systems acquire and utilize the free energy they need. During this process many chemical reactions take place, by which chemical compounds are converted into other chemical compounds, often catalysed by special enzymes. Despite of the complexity of their internal processes, living systems maintain – under normal conditions – a *steady state*, which means that the concentrations of the inner compounds are constant. This steady state is maintained by a sophisticated mesh of metabolic controls. Metabolic pathways are series of consecutive enzymatic reactions producing specific products. Their reactants, intermediates, and products are called *metabolites*. In metabolic pathways the chemical reactions of metabolites, given by their stoichiometric equations, the metabolite concentration, and the enzyme concentrations are usually known. We have here a flux of chemical substances. Two of the presented case studies are metabolic pathways – case study two, the sucrose breakdown pathway, and case study three, the combined glycolysis and pentosephosphate pathway.

Signal transduction pathways, also called *information metabolism*, have molecular on/off switches that transmit information when “on”; i. e. there is a signal, which will be passed on to the next substance. They describe how the cell receives, processes, and responds to information from the environment. These information processing circuits are widespread and diverse, e.g. half of the 25 largest protein families, encoded by the human genome, deal primarily with information processing [2]. Often signal-transduction cascades mediate the sensing and processing of stimuli. They detect, amplify, and integrate diverse external signals to generate responses such as changes in enzyme activity, gene expression, or ion-channel activity. Apoptosis, described in the first case study, is a typical signal transduction pathway.

Gene expression is the combined process of transcription of a gene into mRNA and its translation into protein. The level of gene expression can be measured by microarray experiments, giving information about expressed genes and protein-protein interactions. Based on these data, biological networks have been constructed to analyse the underlying processes. Special databases have been built for these data, e.g. [40], [41], and many others are emerging. Corresponding case studies may be found in [18], [19], [43].

Popular models used for all three types of biopathways are schematic and informal ones, usually they need additional verbose explanations how to read them. To get a unifying as well as unambiguous knowledge representation, allowing at the same time some consistency checks to get the unification approved,

we have to apply representation techniques enjoying a formal and therefore unambiguous semantics. To model the different biopathway types as a Petri net[1], possibly consisting of several components, each biochemical compound (metabolite) is assigned to a place. The relations between some biochemical compounds, established by chemical reactions, are represented by transitions, modelling a biochemical atomic event. The corresponding arcs reflect the given stoichiometric relations. However, while the modelling of the biochemical compounds is quite straightforward, the elaboration of the transition structures tends to be rather time-consuming, requiring a lot of reading and interpretation of various verbose or graphical statements. Figure 1 shows a simple Petri net, modelling just one chemical reaction, given by its stoichiometric equation.

This easy-to-use modelling principle has been applied successfully to a variety of biological pathways, see [39] for a bibliography of related papers. The Petri net structure then truly reflects the biochemical topology, and the incidence matrix of the net is identical to the stoichiometric matrix of the modelled metabolic system. The Petri net behaviour gives the set of all partial order sequences of chemical reactions from the input to the output compounds respecting the given stoichiometric relations.

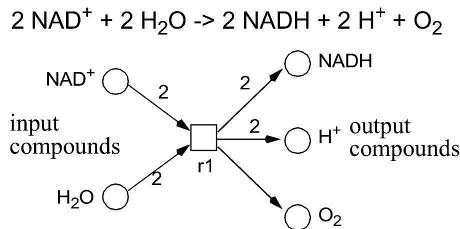

Fig. 1. Petri net model of a single chemical reaction (light-induced phosphorylation), given by its stoichiometric equation.

Moreover, the same modelling idea may be applied on a more abstract level, where stoichiometric details are not known or do not matter, resulting into a partial order description of binary causal relations of the basic (re-) actions involved.

To get readable representations we utilize three widely used short-hand notations: (1) Test arcs, represented as bidirectional arcs, stand shortly for two unidirectional arcs. Usually, signal transduction does not involve the immediate resetting of the triggering signal(s). Therefore, such events are modelled by test arcs. Similarly, enzyme reactions are catalytic reactions, i.e. there is no consumption of the biochemical compound, so they are modelled by test arcs, too. (2) Fusion nodes, given in grey, serve as connectors to glue together distributed net components. They are often used to highlight special molecules like ADP, ATP,

[1] We consider the reader to be familiar with the basic Petri net terminology, otherwise please take a look in appropriate literature, e.g. [27].

NAD^+, Pi etc., which play a slightly particular role in metabolic networks. They are called ubiquitous because they are found in sufficiently large amounts in the cell. For ease of distinction, the remaining substances are named primary. (3) When appropriate, we exploit hierarchical structuring techniques. Transition-bordered subnets are abstracted by so-called macro transitions, represented by two centrically nested squares. We are going to apply this notation to abstract from the two directions of reversible reactions and to abstract from purely linear reaction sequences.

Implementing these principles, we usually get place-bordered models, where the input compounds appear as source nodes (no predecessors) and the output compounds as sink nodes (no successors). To animate and analyse such a model, we need an environment to produce the input compounds and to remove the output compounds. There are basically two styles, how such an environment behaviour can be described.

(1) The tokens for all input compounds are generated by auxiliary input transitions (source nodes, having no predecessor), while the tokens of all output compounds are consumed by auxiliary output transitions (sink nodes, having no successor). To high-light the special meaning of these transitions for the whole model and in order to distinguish them from the ordinary ones, they are drawn as flat hollow bars. Doing so, no assumptions about the quantitative relations of input/output compounds are made. Because there are transitions without preplaces, we get automatically unbounded Petri nets (at least the postplaces of the input transitions are unbounded).

(2) The tokens for all input compounds are generated by one auxiliary transition *generate*, while the tokens of all output compounds are consumed by one auxiliary transition *remove*. Both transitions are connected by an auxiliary place *cycle*, compare Figure 2. The arc weights represent the stoichiometric relations of the sum equation of the whole network. This kind of environment behaviour reflects explicit assumptions about the quantitative relations of input/output compounds. Because now there are no transitions without preplaces, we have a chance to get bounded models.

3 Model Validation

The transformation from an informal to a formal model involves the resolution of any ambiguities, which must not happen necessarily in the right way. Therefore, the next step in a sound model-based technology for behaviour prediction should be devoted to model validation.

Model validation aims basically at increasing our confidence in the constructed model. There is no doubt that this should be a prerequisite before raising more sophisticated questions, where the answers are supposed to be found by help of the model and where we are usually ready to trust the answers we get. So, before thinking about model analysis, we are concerned with model validation.

To accomplish model validation, we need validation criteria, establishing consistency checks for the model. Looking for such criteria, we should take into

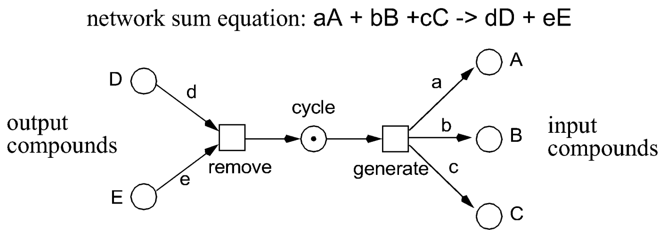

Fig. 2. Petri net environment component, style 2.

account that our models are usually the outcome of a quite heuristic procedure assembling together separate pieces, perhaps with different possible interpretations, into a larger picture.

Thus, a first and very evident question concerning a model of increasing size is the one, whether all the former basic behaviour of the smaller pieces, i. e. model components, are still maintained in the larger model, and that there are no unwanted additional ones. Due to the model's size and inherent complexity, such a property can hardly be decided without computational support.

For that purpose, we exploit two of the fundamental behavioural properties, a Petri net may exhibit – the transition invariants (T-invariants for short), and the place invariants (P-invariants for short), which have been introduced 1973 in [16].

T-invariants are multi-sets of transitions, reproducing a given marking, i.e. in the context of metabolic Petri nets – sets of chemical reactions, reproducing a given distribution of chemical compounds, or more generally spoken in the context of arbitrary biological Petri nets – sets of actions, reproducing a given system state. Due to the fact of state reproduction, a behaviour, establishing a T-invariant, may happen infinitely often, resulting into cyclic system behaviour.

To describe all possible behaviour in a given cyclic system, it would be obviously of great help to have all system's basic (cyclic) behaviour. In [31], [32] they are called elementary modes of pathways, where compounds have reached a dynamic concentration equilibrium, i. e. steady state. Then, any system behaviour may be decomposed into a positive linear combination of basic behaviour. Having this, model validation means to compare the calculated basic behaviour with the expected one.

To implement these considerations, we use – opposite to [31], [32] – standard Petri net analysis techniques and tools. To make life easy, we take the empty Petri net (no tokens at all), whereby all input and output nodes are transitions (environment style 1, compare chapter 2). An input transition may fire forever, each time generating tokens on all its postplaces. Consequently, such a net structure represents an unbounded net (there is no finite upper bound for the total token number in the net), which are generally harder to handle than bounded ones. Contrary, an output transition consumes by each firing the tokens of its preplaces, therefore decreasing the total number of tokens.

So, if we now take the empty net, we are able to look for all T-invariants, i.e. for all multi-sets of transitions reproducing the empty marking. That seems to

be – at least currently – the best way to handle inherently unbounded systems, without assuming anything about the system environment. But, to give the net a real chance to reproduce the empty marking, any read arcs in the model under discussion have to be transformed into unidirectional ones, reflecting the main flow direction.

As it is well-known, we get all minimal semi-positive T-invariants by solving the following integer linear programming problem by determining the generating system:

$\mathcal{C} \cdot x = 0,$	whereby	$\mathcal{C}$	–	$(P \times T)$-incidence matrix
$x \neq 0, x \geq 0$		x	–	transition vector

Similarly, we can calculate all minimal semi-positive P-invariants by solving the following integer linear programming problem by determining the generating system:

$y \cdot \mathcal{C},$	whereby	$\mathcal{C}$	–	$(P \times T)$-incidence matrix
$y \neq 0, y \geq 0$		y	–	place vector

and interpret them as substance preservation rules in the given biological system. To be able to apply this kind of validation rules to the whole network, we need the second style of environment behaviour introduced in the former modelling section.

The calculation of T-/P-invariants requires only structural reasoning, the state space need not to be generated. Therefore, the danger of the famous state space explosion problem does not apply here. However, solving integer linear programming problems is known to be NP-complete.

Because of the given application, we are interested only in the minimal semi-positive T-/P-invariants. Therefore, they are called T-/P-invariants for short in the following.

4 Case Studies

In the following we sketch three case studies, demonstrating the general principles established above. They are ordered according the modelling convenience of the used Petri net class. See the appendix for all the acronyms used throughout this section.

4.1 Apoptosis

4.1.1 Biological Background. To demonstrate that even incomplete and uncertain knowledge may be subject of our technology, we start with apoptosis. This term refers to the regulated cell suicide program, which is of central importance in the cells' life cycle. It allows the organism to control cell numbers and tissue sizes and to protect itself from morbid cells. Neurodegenerative diseases, e.g. Alzheimer's, Huntington's, and Parkinson's disease, and other diseases as AIDS and cancer, exhibit often disturbances in apoptosis or its regulation.

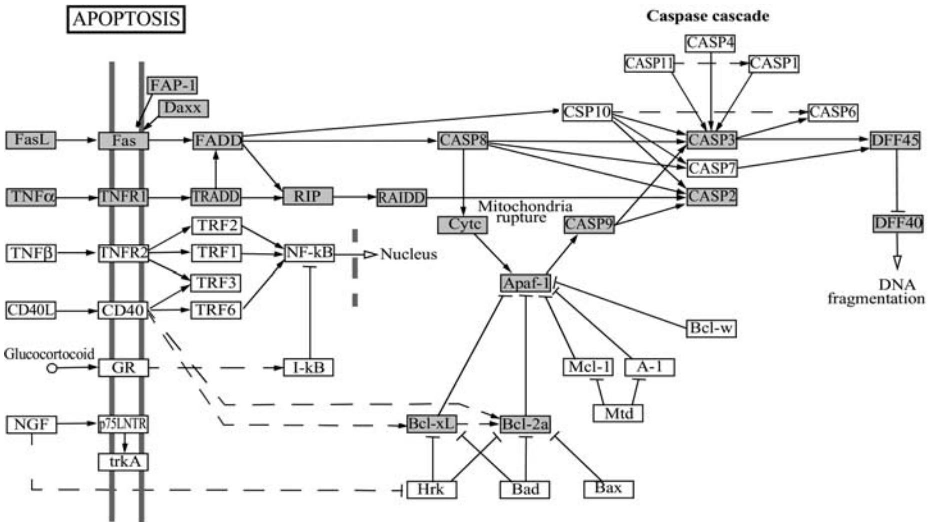

Fig. 3. The KEGG [11] representation of apoptosis. Crossbar arrowheads indicate inhibition. Branching arcs go to alternative as well as to concurrent successors. For further ambiguities see [7]. The fragment considered here is highlighted in grey.

A variety of different cellular signals initiate activation of apoptosis in distinctive ways, depending on the various cell types and their biological states, compare Figure 3. Caspases (cysteinyl-aspartate-specific proteinases) play a crucial role in the apoptotic signal transduction pathways. In living cells, caspases exist as inactive zymogens, which are activated by proteolytic cleavage. The caspases convey the apoptotic signal in a proteolytic cascade, whereby caspases cleave and activate other caspases which then degrade other cellular targets [9].

Getting a survey on the current state of the art, comprising numerous assumptions, requires a lot of reading, including the creative interpretation of various graphical representations. Figure 3 gives one of them, which is commonly used.

4.1.2 Petri Net Model. Due to the accuracy of available knowledge, which lacks particularly any stoichiometric relations of the chemical reactions involved, ordinary place/transition nets (no arc weights) are sufficient here, see Figure 4. Two further modelling aspects are worth mentioning. Usually, signal transduction does not involve the resetting of triggering signal(s). Hence, such circumstances are modelled by test arcs. Biological systems are typically full of inhibitors, i.e. compounds, preventing by their presence a certain reaction. To reflect these situations in a qualitative model adequately, we need inhibitor arcs.

4.1.3 Model Validation. Before analysing the model, two adaptations are done: (1) Test arcs are replaced by normal ones, corresponding to the main flow.

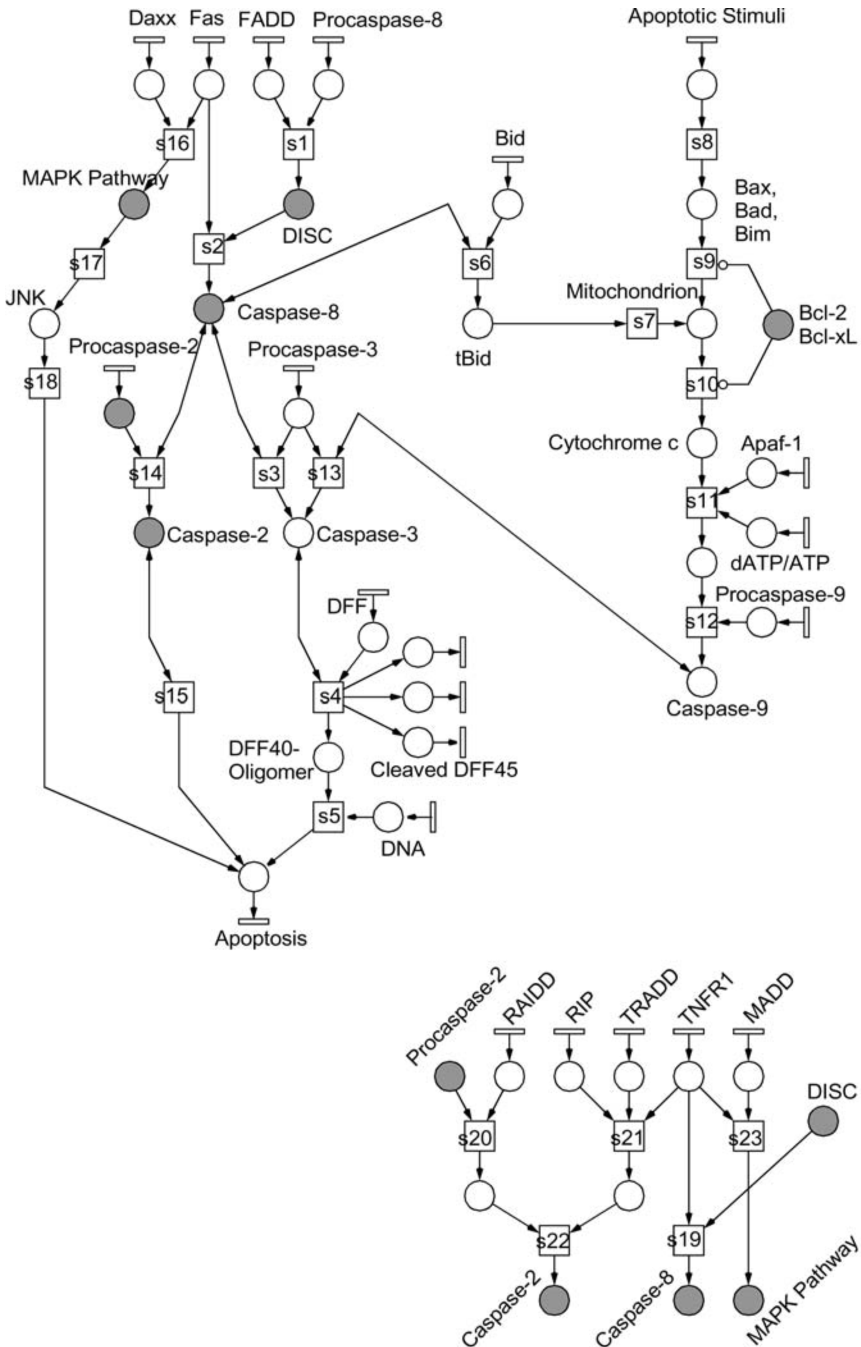

Fig. 4. Petri net of apoptosis in two net components. Please note, input and output transitions are drawn as flat bars and have the same name as the place, they are related to. Grey nodes represent fusion nodes and realizes the connection between different net components. Read arcs reflect the signal transduction principle.

(2) Apoptosis inhibitors are not taken into account. All inhibiting substances are only input compounds for the considered system model. Input compounds are not produced dynamically by the system behaviour, i. e. they come from the system environment. That's why their presence would just exclude modelled system behaviour without adding new functionality.

When computing all T-invariants by help of the Integrated Net Analyser tool INA [35] we get the following results. There are two receptors (Fas, TNFR1) and three basic apoptotic pathways per receptor (caspase-8, JNK, caspase-2) as well as an apoptotic stimuli-induced pathway in our model. Altogether, there are ten T-invariants. In the transition vectors given below the generating input and the consuming output transitions have been skipped for sake of simplicity.

Fas-induced:

s1, s2, s3, s4, s5: Fas/caspase-8/caspase-3 – Fas-induced direct caspase-8/caspase-3 pathway
s1, s2, s6, s7, s10, s11, s12, s13, s4, s5: Fas/caspase-8/mitochondrion/cytochrome c/caspase-9/caspase-3 – Fas-induced Bid-controlled cross-talk
s16, s17, s18: Fas/MAPK-Pathway/JNK – Fas-induced JNK pathway
s1, s2, s14, s15: Fas/caspase-8/caspase-2 – Fas-induced caspase-8/caspase-2 pathway

Apoptotic stimuli-induced:

s8, s9, s10, s11, s12, s13, s4, s5: apoptotic stimuli/Bax,Bad,Bim/mitochondrion /cytochrome c/Apaf-1/caspase-9/caspase-3 - apoptotic stimuli-induced mitochondrial pathway

TNFR1-induced:

s1, s19, s3, s4, s5: TNFR1/caspase-8/caspase-3 – TNFR1-induced direct caspase-8/caspase-3 pathway
s1, s19, s6, s7, s10, s11, s12, s13, s4, s5: TNFR1/caspase-8/mitochondrion/cytochrome c/ Apaf-1/caspase-9/caspase-3 - TNFR1-induced Bid-controlled cross-talk
s1,s19,s14,s15: TNFR1/caspase-8/caspase – TNFR1-induced caspase-8/caspase-2 pathway
s23, s17, s18: TNFR1/MAPK-Pathway/JNK – TNFR1-induced JNK pathway
s20, s21, s22, s15: TNFR1/caspase-2 – TNFR1-induced direct caspase-2 pathway

The minimal semi-positive T-invariants describe the basic system behaviour, because they represent the linearly independent semi-positive integer solutions of the system of linear equations resulting from the incidence matrix C. All possible system behaviour can be described by a positive linear combinations of these T-invariants. All known pathways in the modelled apoptosis fragment are reflected in a corresponding T-invariant, and there is no computed T-invariant without an apoptosis-related interpretation. Due to the given environment style, there are no P-invariants here.

4.2 Carbon Metabolism in Potato Tuber

4.2.1 Biological Background. The accumulation of starch in the Solanum tuberosum (potato) tuber is a crucial point in biotechnology. The conversion of sucrose through hexose phosphates is the major flux in the potato tuber carbon metabolism. Nearly all genes, believed to be directly involved in the sucrose breakdown transition, have been cloned by transgenic approaches. However, some fundamental questions are still open.

Figure 5 gives an overview of the sucrose breakdown pathway in potato tuber, for details see [5]. Sucrose delivered to the tuber can be cleaved in the cytosol by *invertase* to yield glucose and fructose, or by *sucrose synthase* to yield fructose and UDP-glucose. By *hexokinase*, *fructokinase*, and *UDPglucose pyrophosphorylase* hexosephosphates are produced, which are equilibrated by the action of *phosphoglucose isomerase* and *phosphoglucomutase*, and could lead either to starch synthesis, to glycolysis, or to sucrose synthesis through *sucrose phospahate synthase* and *sucrose phosphate phosphatase*. The following 16 chemical stoichiometric equations characterise the pathway.

R1. SuSy:	*sucrose synthase*	Suc + UDP <—> UDPglc + Frc
R2. UGPase:	*UDPglucose pyrophosphorylase*	UDPglc + PP <—> G1P + UTP
R3. PGM:	*phosphoglucomutase*	G6P <—> G1P
R4. FK:	*fructokinase*	Frc + ATP —> F6P + ADP
R5. PGI:	*phosphoglucose isomerase*	G6P <—> F6P
R6. HK:	*hexokinase*	Glc + ATP —> G6P +ADP
R7. Inv:	*invertase*	Suc —> Glc + Frc
R8. Glyc(b):	*glycolysis*	F6P + 29 ADP + 28 P_i —> 29 ATP
R9. SPS:	*sucrose phospahate synthase*	F6P + UDPglc <—> S6P + UDP
R10. SPP:	*sucrose phosphate phosphatase*	S6P —> Suc + P_i
R11. NDPkin:	*NDP kinase*	UDP + ATP <—> UTP +ADP
R12. SucTrans:	*sucrose transporter*	eSuc —> Suc
R13. ATPcons(b):	*ATP consumption*	ATP —> ADP + P_i
R14. StaSy(b):	*starch synthesis*	G6P + ATP —> starch + ADP + PP
R15. AdK:	*adenylate kinase*	ATP + AMP <—> 2 ADP
R16: PPase:	*pyrophosphatase*	PP —> 2 P_i

4.2.2 Petri Net Model. We have validated the pathway using the Petri net given in Figure 6, reflecting the stoichiometric equations by a non-ordinary place/transition net. Transitions are named by the enzyme catalysing the chemical reaction or by summarized processes (R8, R13, R14). Reversible reactions are modelled by macro transitions, i.e. by hierarchical nodes, hiding the forward and backward reactions. The places for external sucrose (eSuc) and starch represent the source and sink, respectively. Correspondingly, the interface to the environment consists of one transition generating tokens for eSuc and of one transition consuming the tokens from starch, both drawn as flat bars.

4.2.3 Model Validation. The following set of 19 minimal T-invariants has been calculated using INA [35], all enjoy a sensible biological interpretation. The reversible reactions yield seven T-invariants, consisting of only two transitions.

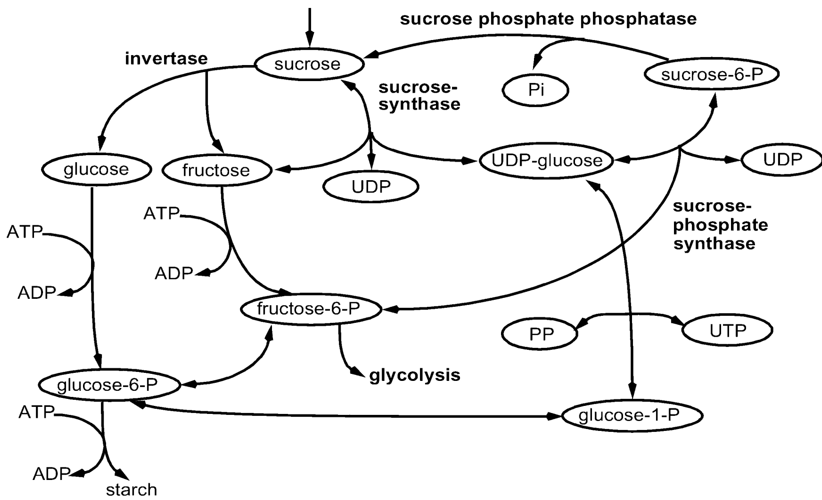

Fig. 5. Schematic overview of the central carbon metabolism in potato tuber. All equations, except R11, R13, R15 and R16, are represented.

The remaining 12 invariants are given below by the transition names, followed by their amount in brackets (if unequal to one). The net is covered by T-invariants, consequently all reactions contribute to the pathway.

T-Invariants with sucrose cleavage by sucrose synthase:

TI-8: geSuc, SucTrans, SuSy(29), UGPase, PGM_rev, FK(29), Glyc(b), StaSy(b), rStarch, SPS(28), SPP(28), NDPkin_rev.

TI-9: geSuc, SucTrans, SuSy, UGPase, PGM_rev, FK, Glyc(b), StaSy(b), rStarch, ATPcons(28), NDPkin_rev.

TI-10: geSuc(15), SucTrans(15), SuSy(15), PGI_rev(14), UGPase(15), PGM_rev(15), FK(15), Glyc(b), StaSy(b)(29), rStarch(29), NDPkin_rev(15), PPase(14).

T-Invariants with sucrose cleavage by invertase

TI-11: geSuc, SucTrans, Inv(14), UGPase_rev(13), PGM(13), HK(14), FK, Glyc(b), StaSy(b), rStarch, SuSy_rev(13), NDPkin(13), PPase(14).

TI-12: geSuc(3), SucTrans(3), Inv(29), UGPase_rev(26), PGM(26), HK(29), FK(29), Glyc(b)(3), StaSy(b)(3), rStarch(3), SPS(26), SPP(26), NDPkin(26), PPase(29).

TI-13: geSuc, SucTrans, Inv, HK, FK(27), Glyc(b), StaSy(b), rStarch, SuSy(26), SPS(26), SPP(26), PPase.

TI-14: geSuc, SucTrans, Inv, HK, FK, Glyc(b), StaSy(b), rStarch, ATPcons(26), PPase.

TI-15: geSuc(15), SucTrans(15), Inv(15), HK(15), FK(15), PGI_rev(13), Glyc(b)(2), StaSy(b)(28), rStarch(28), PPase(28).

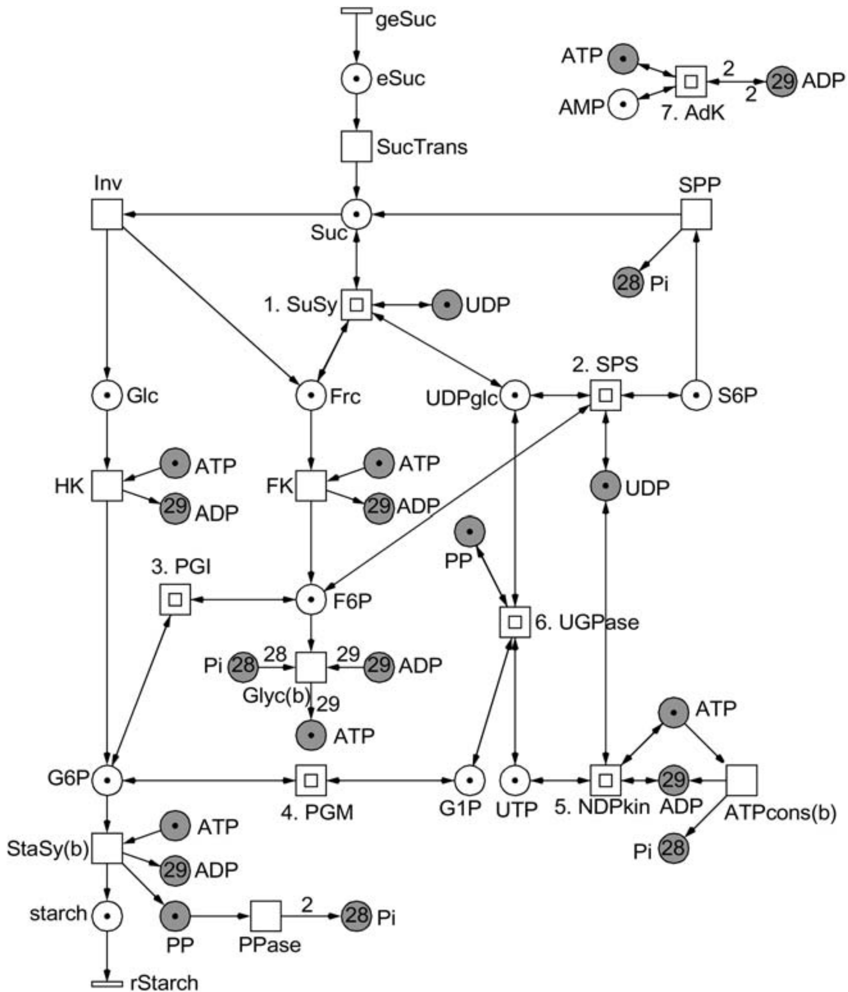

Fig. 6. Petri net of the central carbon metabolism in potato tuber, see Figure 5 and the stoichiometric reaction equations given in subsection 4.2.1.

TI-16: geSuc, SucTrans, Inv(29), HK(29), FK, PGI, UGPase_rev(28), PGM(28), Glyc(b)(2), SuSy_rev(28), NDPkin(28), PPase(28).
TI-17: geSuc(3), SucTrans(3), Inv(59), HK(59), FK(59), UGPase_rev(56), PGM(56), PGI(3), Glyc(b)(6), SPS(56), SPP(56), NDPkin(56), PPase(56).
TI-18: geSuc, SucTrans, Inv, HK, FK(57), PGI, Glyc(b)(2), SuSy(56), SPS(56), SPP(56).
TI-19: geSuc, SucTrans, Inv, HK, FK, PGI, Glyc(b)(2), ATPcons(56).

There are two possibilities for sucrose cleavage, two for hexose phosphate utilisation, and four for ATP utilisation. Both possibilities for hexose utilisation (starch synthesis and glycolysis) can occur together in one invariant (TI-11 –

TI-14), while starch synthesis cannot happen without glycolysis, because no cellular process can take place without available energy in form of ATP or other similar cofactors. Apparently, the incoming sucrose, needed to generate ATP in glycolysis, can either be cleaved by Inv or by SuSy, but not by both at the same time, even if more than one sucrose after cleavage is used in glycolysis (TI-12, TI-17). The cleavage of sucrose by Inv and SuSy can only occur together in one invariant, if sucrose cycling is involved (TI-13, TI-18).

Moreover, we obtained the following three P-invariants: PI-1: UDPglc, UTP, UDP.PI-2: ATP, AMP, ADP. PI-3: G6P, F6P, G1P, UTP, ATP(2), ADP, S6P, Pi, PP(2). Invariants PI-1 and PI-2 comprise the metabolites containing uridine or adenosine residues, respectively. Invariant PI-3 represents the sum of all compounds, which can directly or indirectly provide a phosphate group. The sum of the phosphorylated metabolites is unchanged.

Due to the kind of environment description, the model is obviously unbounded. To get a bounded Petri net, we changed to the other style of environment description discussed in section 2. When we calculated the arc weights for the environment model, we get 75 and 144, respectively. That was done for a simplified network by summarizing the *hexophosphatases (G6P, G1P, F6P)* into one place. But even for this simplified version the state space (more than 1010 states) cannot be handled by the tools available for the analysis of bounded non-ordinary place/transition nets. For more details see [14].

4.3 Glycolysis and Pentose Phosphate Metabolism

4.3.1 Biological Background. The glycolysis pathway (GP) is a sequence of reactions that converts glucose into pyruvate with the concomitant production of a relatively small amount of ATP. Then, pyruvate can be converted into lactate. The version chosen here is that one for erythrocytes [2]. In the graphical representation of Figure 7, the GP consists of the reactions 9 to 20. The pentose phosphate pathway (PPP), also called hexose monophosphate pathway, again starts with glucose and produces NADPH and ribose-5-phosphate (R5P) which then is transformed into glyceraldehyde-3-phosphate (GAP) and fructose-6-phosphate (F6P) and thus flows into the GP. In Figure 7, the PPP consists of the reactions 9, 1 to 8, and 15 to 20.

Whereas the GP generates primarily ATP with glucose as a fuel, the PPP generates NADPH, which serves as electron donor for biosyntheses in cells. The interplay of the glycolytic and pentose phosphate pathways enables the levels of NADPH, ATP, and building blocks for biosyntheses, such as R5P and Pyr, to be continuously adjusted to meet cellular needs. This interplay is quite complex, even in its somewhat simplified version discussed here.

4.3.2 Petri Net Model. The application of Petri nets to this field began in the nineties with the publications of Reddy et al. [25], [26], presenting a place/transition net to model the structure of the combined GP/PPP of erythrocytes. They compute also S- and T-invariants, but provide neither a full P-invariant nor a non-trivial T-invariant. A thorough analysis of an extended

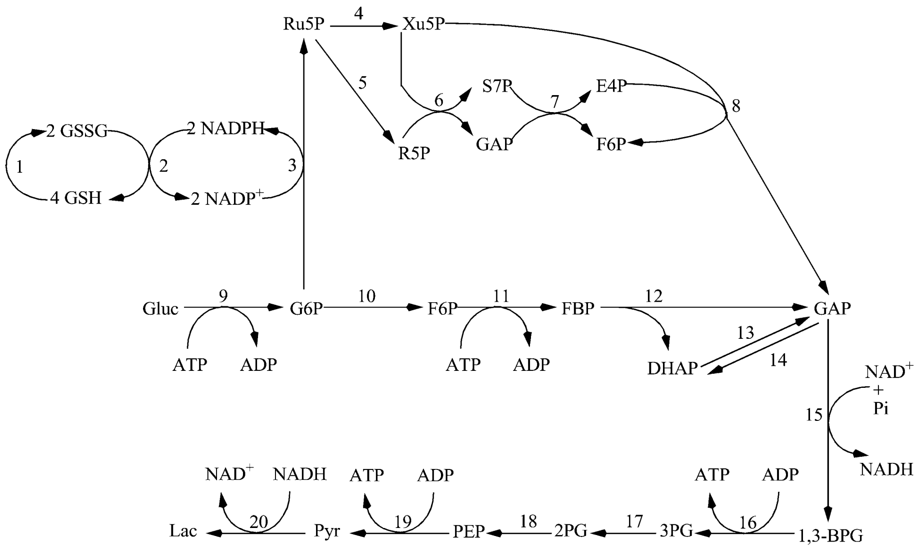

Fig. 7. Typical representation of the glycolysis and pentose phosphate metabolism according to [26]. The arc numbers identify the chemical reactions. Prefix numbers in node names stand for multiplicities. It's open for interpretation what the meaning of equally named nodes might be.

form of this pathway was performed in [12], which is presented and analysed in [38] as a coloured Petri net. In the Petri net of Figure 8, the GP consists of the reactions l1 to l8, while the PPP consists of the reactions l1, m1 to m3, r1 to r5, and l3 to l8.

In this section, we use Design/CPN [4] to edit and execute models. The calculations reuse an experimental software package SY, written by H. Genrich in Standard ML, for the symbolic analysis of coloured Petri nets, for details see [38].

4.3.3 Model Validation. The crucial point of using coloured instead of low-level Petri nets is the possibility to discriminate between different molecules of the same metabolite via their identifiers (colours). This allows to separate different branches of a composite pathway and to distinguish among molecules on the same place according to their origin and destination reaction. Alternative metabolic paths (splitting at conflict places, here G6P, GAP, Ru5P) most often result in different metabolic overall reactions. Therefore, they have to be discriminated, in order to combine them afterwards deliberately. This discrimination is performed by attributing different identifiers to the molecules and by additionally blocking certain transitions for particular molecules (using guards).

Using the software package SY, we were able to calculate an environment net component (not shown in this paper) to get a bounded model, which already had been found (but just by guessing) in [12] for a low-level model version of this metabolic pathway.

There are four pairs of ubiquitous substrates which, if produced or consumed by a reaction, are transformed into each other, namely (ADP, ATP), (NAD^+, NADH), (2 GSSG, GSH), and (NAD^+, NADH). This is validated by corresponding P-invariants. The attempt to calculate P-invariants covering primary substances failed (at the beginning), and it turned out that essential products (CO_2, H2O) were missing in the PPP model. Correcting the model by replacing two equations (compare Figure 7 and Figure 8)

reaction 3: G6P + 2 $NADP^+$ + H2O → Ru5P + 2 NADPH + 2 H^+ + CO_2,
reaction 18: DPG → PEP + H2O

leads to the following P-invariant:

p-inv_C = [(Gluc, 6), (G6P, 6), (F6P, 6), (FBP, 6), (CO_2, 1), (Ru5P, 5), (R5P, 5), (Xu5P, 5), (S7P, 7), (E4P, 4), (DHAP, 3), (GAP, 3), (BPS, 3), (Lac, 3)].

An inspection of C reveals that the integer weight factor of any substance equals the number of C-atoms bound in it. Thus the P-invariant p-inv_C expresses the conservation rule that the sum of C-atoms bound by all involved substances is constant. This represents obviously a sensible biochemical interpretation. Similarly, the conservation of Oand P-atoms could be shown in the improved model by help of P-invariants [38].

There are three T-invariants, enjoying sensible interpretation and covering the net, given in short-hand notation:

glycolysis pathway (l1, C), (l2, D), (l3, C), (l4, D), (l5, D), (l7, 2*D), (l8, 2*D), (s1, D), (s2, D)
pentose phosphate pathway (l1, G + 2*H), (l3, 2*H), (l4, 2*H), (l5, 2*H), (l7, 5*H), (l8, 5*H), (m1, G + 2*H), (m2, 6*D), (m3, 6*D), (r1, G), (r2, 2*H), (r3, (G,H)), (r4, (G,H)), (5, H), (s1, D), (s2, D)
pathway including the reverse reaction l2′ (l1, G'), (l2', 2*H'), (l7, H'), (l8, H'), (m1, G' + 2*H'), (m2, 6*D), (m3, 6*D), (r1, G'), (r2, 2*H'), (r3, (G',H')), (r4, (G',H')), (5, H'), (s1, D), (s2, D)

5 Related Work

The idea to represent chemical systems, consisting of chemical compounds and chemical reactions, by net models has already been mentioned 1976 by C. A. Petri in his paper on interpretations of net theory [23]. The first paper really demonstrating the modelling of metabolic processes by Petri nets appeared 1993 [25]. In the meantime, several research groups followed this line, and Petri nets have been applied to all three types of molecular biological networks [18], [43]. But a closer look on the literature (see [39] for a bibliography) reveals that the majority of papers, applying Petri nets for modelling and analysis of biological systems, concentrate on quantitative aspects – despite the severe restriction, often encountered in the construction of these models, the imperfect knowledge of the kinetic parameters. Typical examples of used Petri net extensions

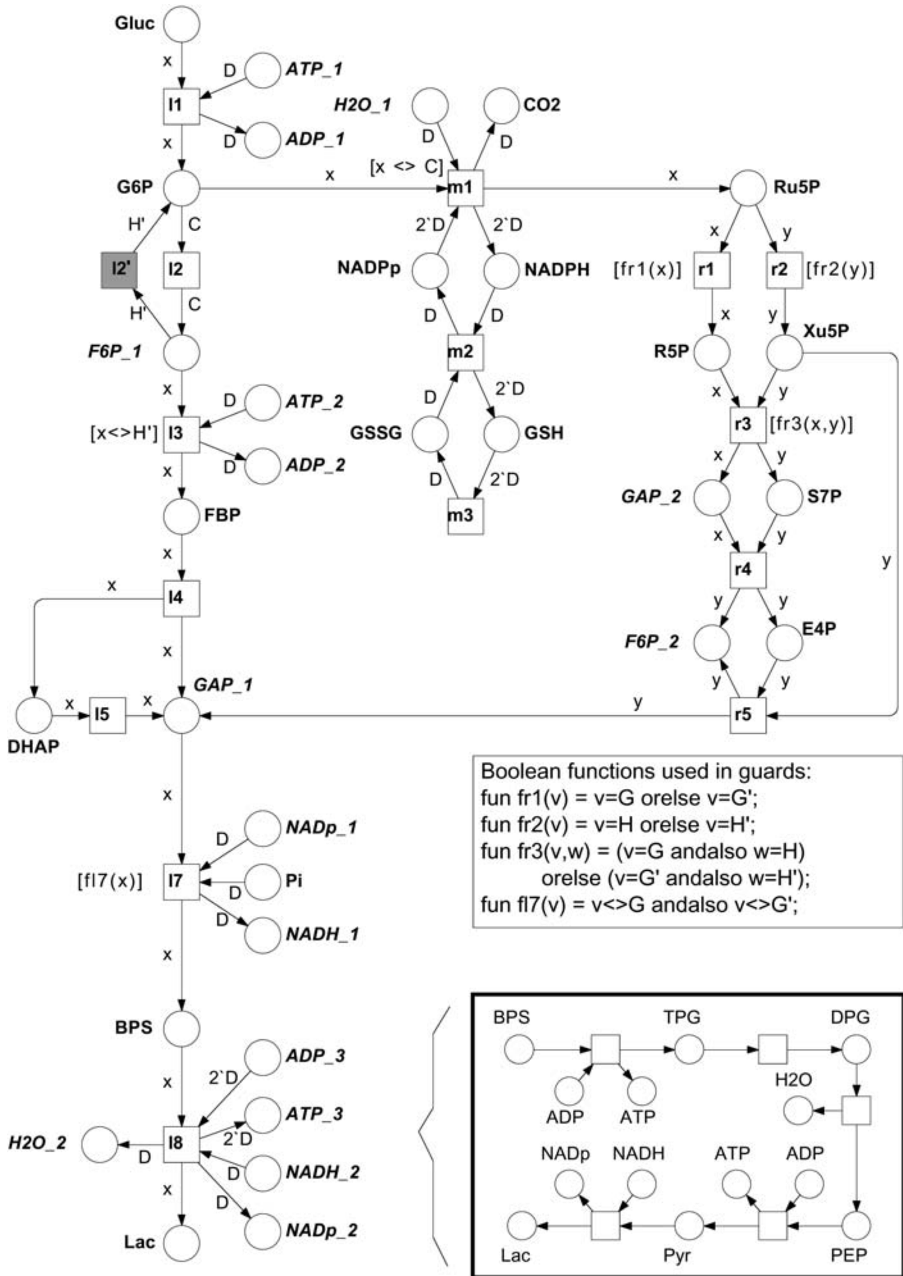

Fig. 8. The metabolism of Figure 7 as Coloured Petri net. All places have the colour set CS = {C, D, F, G, H, G′, H′}. A term in brackets [] is a transition guard. A transition t′, highlighted in grey, denotes the reverse counterpart of the reversible reaction t. The (substitution) transition l8 summarizes the reaction sequence going from BPS to Lac. Names of fusion nodes are given in italic, numbered consecutively.

are stochastic Petri nets [21], [22] and hybrid Petri nets [3], [18], [19], but also coloured Petri nets [6] as well as discrete time extensions [12] have been employed for that purpose. Contrary, qualitative aspects are discussed only in a few papers, see e.g. [25], [26].

For computing conservation relations (P-invariants) and elementary modes (T-invariants) of metabolic pathways, the software package METATOOL [24] has been developed by biologists and applied successfully in a number of cases. However, merely the integer weighted P-invariants are detected. Moreover, only the overall reaction equations, i. e. the net effects of a pathway execution, can be computed, and any consideration of its dynamics, in particular of the partial order of the reaction occurrences, is missing. No other possibilities to validate a model are provided.

6 Summary and Outlook

Petri nets represent a concise formal representation, allowing a unifying view on knowledge stemming from wide-spread different sources, usually represented in various, sometimes even ambiguous styles. The derived models can be validated by checking their P- and T-invariants for suitable biological interpretations. This approach has been demonstrated by three examples covering different abstraction levels of molecular biological networks represented by different Petri net classes.

These results encourage us to continue our work on this rapidly developing field and to extend Petri net modelling and analysis tools by features which are supportive for biological molecular systems. [37] gives an overview of symbols and reaction types which have to be considered in pathway modelling, among them the extension, which represents an essential property of biochemical networks – the inhibitor arc.

In the given context, major challenges for Petri net analysis techniques are: (1) Analysis of bounded, but not safe nets, with inhibitor arcs. Even if the model is bounded, the state space tends to be very huge due to stoichiometric relations, see case study two. (2) Analysis of unbounded models. In any case, model checking of temporal formulae seems to be the next step for more fine grained questions the model has to pass.

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Appendix: Abbreviations

Case Study 1: Apoptosis, Biochemical Compounds

Apaf-1:	apoplectic protease activating factor 1
Ask1:	apoptosis signal-regulating kinase-1
ATP:	adenosine triphosphate
Bad:	Bcl-x_L/Bcl-2 associated death promoter
Bax:	Bcl-2 associated X protein
Bcl-2:	B-Cell lymphoma 2
Bid:	Bcl-2 interacting domain
CASP:	caspase
Caspase:	cysteinyl aspartate-specific protease
CrmA:	cytokine response modifier A
Cyt c:	cytochrome c
dATP:	desoxyadenosine triphosphate
Daxx:	Fas death domain associated protein xx
DFF:	DNA fragmentation factor
DFF40:	40 kDa unit of DFF
DFF45:	45 kDa unit of DFF
DISC:	death inducing signaling complex
FADD:	Fas associating protein with death domain
FAP-1:	Fas associated phosphatase-1
Fas:	Fas receptor
Fas-L, FasL:	Fas ligand
FLIP:	FLICE inhibitory protein
JNK:	c-Jun amino-terminal kinase
MADD:	mitogen activated kinase activating death domain
MAPK:	mitogen activated protein kinase
RAIDD:	RIP associated Ich-1/CED homologous protein with death domain
RIP:	receptor interacting protein
tBid:	truncated Bid
TNF, TNFα:	tumor necrosis factor
TNFα-R, TNFR1:	tumor necrosis factor receptor
TRADD:	TNF receptor 1 associated death domain

Case Study 2: Potato Tuber, Metabolites

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
eSuc	extern sucrose
F6P	fructose-6-phosphate
Frc	fructose
G1P	glucose-1-phosphate
G6P	glucose-6-phosphate
Glc	glucose
Pi	phosphate ionized
PP	pyrophosphate
S6P	sucrose-6-phosphate
Suc	sucrose
UDP	uridine diphosphate
UDPglc	uridine diphosphate glucose
UTP	uridine triphosphate

Case Study 3: Glycolysis and Pentose Phosphate Metabolism

Biochemical Compounds

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BPS	1,3-Biphosphoglycerate
DHAP	Dihydroxyacetone phosphate
DPG	2-Phosphoglycerate
E4P	Erythose-4-phosphate
FBP	Fructose biphosphate
F6P	Fructose-6-phosphate
GAP	Glyceraldehyde-3-phosphate
Gluc	Glucose
GSH	Glutathione
GSSG	Glutathionedisulfide
G6P	Glucose-6-phosphate
Lac	Lactate
NADH	Nicotinamide adenine dinucleotide, reduced form
NAD+/NADp	Nicotinamide adenine dinucleotide, oxidized form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NADP+/NADPp,	Nicotinamide adenine dinucleotide phosphate, oxidized form
PEP	Phosphoenolpyruvate
Pi	Orthophosphate, ionic form
Pyr	Pyruvate
Ru5P	Ribulose-5-phosphate
R5P	Ribose-5-phosphate
S7P	Sedoheptulose-5-phosphate
TPG	3-Phosphoglycerate
Xu5P	Xylulose-5-phosphate

Correspondence between Petri net transitions/reaction numbers and enzymatic reactions

l1/9	Hexokinase
l2/10	Phosphoglucose isomerase
l3/11	Phosphofructokinase
l4/12	Aldolase
l5/13	Triosephosphate isomerase (forw.)
l6/-	Triosephosphate isomerase (backw.)
l7/15	GAP dehydrogenase
l8/16-20	Reaction path consisting of: phosphoglycerate kinase, phosphoglycerate mutase, enolase, pyruvate kinase, and lactate dehydrogenase
m1/3	G6P oxidation reactions
m2/2	Glutathione reductase
m3/1	Glutathione oxidation reaction
r1/5	Ribulose-5-phosphate isomerase
r2/4	Ribulose-5-phosphate epimerase
r3/6	Transketolase
r4/7	Transaldolase
r5/8	Transketolase