Application of Coloured Petri Nets in Systems Biology

Thomas Runge[1,2]
[1]Brandenburg University of Technology Cottbus, Department of Computer Science,
chair of data structures and software dependability
Postbox 10 13 44, 03013 Cottbus, Germany
thomas.runge@informatik.tu-cottbus.de
phone: +49 355 69 3885
fax: +49 355 69 3830
[2]Technical University of Applied Sciences Berlin, Department of Bioinformatics,
Seestrasse 64, 13347 Berlin, Germany

Abstract

Computer aided analysis possibilities are necessary to improve the understanding of the complex biochemical processes. The often used kinetic models in biochemistry are based on differential equations. The results of such a kinetic model are often non-reliable on account of the lack of reliable data or of inconsistencies in the used model. Therefore, other supplementary methods are indispensable. A qualitative analysis should be done, before a quantitative (kinetic) analysis is made. This paper extends and refines the construction strategy of a coloured Petri net model of a metabolic network, previously introduced by Heiner et al. in [Heiner01]. To have the full range of Petri net analysis possibilities available, it is advisable to have a bounded and live Petri net model. A systematic half-automatic procedure, exploiting the place transition nets' T-invariants to construct an equivalent bounded and live coloured net, is presented to reach this aim.

Keywords: coloured Petri net, T-invariant, P-invariant, metabolic pathway, glycolysis pathway,

1. Introduction

Due to the rapidly growing amount of biologically experimental possibilities and the related amount of created experimental data, it is mandatory to transmit data in simple, analysable, and possibly validated models. Therefore, bioscientists need practicable, theoretically well-founded methods to construct, prove, analyse, and simulate a model, which is based on experimental data.

Today, there exist many quantitative models, which typically employ differential equations. Such models need kinetic parameters to describe and analyse a biochemical system. A restriction on hand of these models is often the imperfect and imprecise knowledge of the kinetic parameters, because up to now it is difficult to observe the processes in a cell at molecular level in vivo. Contrary, a qualitative analysis offers the possibilities of a structural analysis based only on information of the simple, atomic chemical reactions and their stoichiometric parameters. A qualitative analysis can be used for an intermediate validation of the given model structure.

Many different approaches for qualitative or quantitative analysis methods have been developed. For example, a graph theoretical approach is described in [Ehrentreich03]. A

mathematical approach is introduced in [Wiback02], computing a set of generating vectors that describe the conical steady-state solution space for flux distributions in a metabolic network, the so-called extreme pathways. But only Petri nets have been applied for both kinds of analysis. For example, quantitative Petri net models are introduced in [Chen03] and [Genrich01], qualitative Petri net models are described in [Reddy96], [Heiner03], [Heiner04], [Koch04], using place/transition nets, and in [Heiner01] and [Voss03], using coloured Petri nets.

In the latter papers the feasibility to construct a coloured Petri net model of the glycolysis and the pentose-phosphate pathways in an erythrocyte cell is examined. A deep understanding of the given network is used for a construction by hand of an environment for the modelled reaction chains. An experimental software package, called SY written by H. Genrich [GenrichSY], was used to construct the model stepwise. The work done by Voss et al. and Genrich was the starting point for the current paper. An extended, more general model of the glycolysis serves as case study. Starting from a simple, unbounded, and live place/transition net an equivalent bounded and live coloured Petri net will be constructed.

The contribution of this paper are the following: (1) An (half-) automatic way to construct a similar environment as the environment, constructed by Voss et al. by hand, is introduced exploiting the place transition nets' T-invariants to construct an equivalent bounded and live coloured net. (2) A more systematic modelling strategy is introduced.

It is assumed that the reader is familiar with place/transition (P/T) nets and coloured Petri nets (CPN). Otherwise, related literature is recommended [Starke90], [Jensen92], and [Jensen98].

The paper is organized as follows. In the next section, the essential biochemical and Petri net terms and concepts are introduced. In section three the motivation to combine place/transition nets and coloured Petri nets is described. The used case study – an extended glycolysis – is introduced in section four. Section five presents two modelling strategies to get a coloured Petri net base model and compares them shortly. Additionally, some possibly problems during the construction are explained. Section six introduces an (half-) automatic algorithm to compute an environment to extend the coloured Petri net base model and reach the aim of a bounded and live model. Finally, conclusions are given in section nine.

2. Biochemical and Petri Net Prerequisites

Metabolic networks are one of the main types of molecular biological networks. The term *metabolism* refers to the processes, which acquire and utilize energy (e.g. in form of ADP and ATP) and small building units (e.g. R5P). In general, a metabolic network consists of many interconnected atomic reactions. An atomic chemical reaction is described by its input compounds (also called educts), its output compounds, and the stoichiometric relations between them. A *metabolic pathway* is defined by its set of involved reactions and its input and output compounds. The educts, the intermediates, and the products are called *metabolites*. According to the applied abstraction level, different specifications of an atomic reaction can be used. An observed metabolic pathway is characterized by a set of *external* and *internal* metabolites. An external metabolite is a substance, which can be supplied and removed to/from the model/pathway. A supplied external metabolite is called s*ource* and a removable external metabolite is called s*ink*. All other metabolites are internal and can only be transformed into another internal or external metabolite(s). The so-called *ubiquitous* molecules are an exception. Those are the small molecules like *H2O; NADH; ADP; CO2* found in sufficiently large amounts in all organisms. These metabolites can be treated as external or as internal metabolites, depending on the desired environment behaviour. For ease of distinction, Voss et al. named the remaining substances *primary* [Voss03].

The regulation of the reaction rate of a reaction is controlled by one or more enzymes. In a qualitative model it is assumed that the system is in a *steady state*. A steady state is a special system state, in which all internal substance concentrations are constant. At a steady state the

total production rate of each internal metabolite is equal to its total consumption rate. Therefore, reaction rates are not considered explicitly in a qualitative analysis. For this reason, only the set of atomic reactions with their stoichiometric parameter are necessary to construct an analysable qualitative model and to perform a qualitative analysis.

Reaction Types

There exist three types of chemical reactions. The three types result in three different model components of an atomic reaction, shown in the glycolysis model later.

The classical, not reversible chemical reaction is named as *irreversible reaction*.

Two reactions are hidden behind the so-called *reversible reaction*. They are two complementary reactions catalyzed by the same enzymes, but often located in different compartments of a cell. The point is that, if they are in the same compartment, only one of them is active over a larger time period. The direction of the reversible reaction and the reaction rate is implicitly controlled by surrounding irreversible reactions. [BioWeb][1] contains an animation, which illustrates the thermodynamic behaviour of the glycolysis and gluconeogenesis, both controlled by three irreversible reactions.

Equilibrium reactions are similar to reversible reactions. The difference is that here both reactions may be active at the same time and in the same location. The aim of an equilibrium reaction is a stable state, concerning the directly involved compounds. The concentrations of the involved compounds at this stable state do not need to be the same.

These three reaction types are important for a correct quantitative analysis. The equilibrium and reversible reactions have different effects on the dynamic behaviour of the model. The behaviour of a reversible reaction is mostly similar to the behaviour of an irreversible reaction. But in biochemical context both reactions are often not distinguished. A reason may be the assumption that an equilibrium reaction can also be considered as irreversible reaction, if it is enclosed by irreversible reactions. A reversible reaction or an equilibrium reaction results in a trivial minimal T-invariant for place/transition nets. But for a coloured Petri net, a trivial minimal T-invariant exists only for an equilibrium reaction. A reason for this is the conflict avoidance principle, described later in this paper.

How to represent biochemical networks with Petri nets?

Metabolites are modelled as places and by convention the primary metabolites are represented by a larger place as the ubiquitous metabolites with a smaller one. Chemical reactions are modelled as transitions and stoichiometric relations as weighted arcs between places and transitions. The token of a P/T net represents an unit of the corresponding metabolite of the given place. Reddy, et al., Koch, et al., and Heiner, et al. have applied these simple transformation rules on biochemical systems to get P/T nets.

A direct modelling of the given set of reactions yields normally a bounded, not reversible and not live Petri net. So, it should be called "*base model*". The base model is place-bordered, because each pathway starts and ends with a set of metabolites. This model is not sufficient for a detailed analysis. So, more information are necessary.

Some Petri net properties and their biochemical interpretation

The invariant analysis of Petri nets plays a special role in biochemical context. Only positive invariants are considered. The positive *P-invariants*, defined by the solution vectors y (place vector) of the equation $y \cdot C = 0$, $y > 0$, whereby C is the (PxT) incidence matrix, represent in biochemical context a mass conservation law. The mass conservation law states that the mass of an isolated system will always remain constant, regardless of the processes acting inside the system.

[1] See: "Conceptual Insights" → "Chapter 16" → "Energetics of Glucose Metabolism"

Today, a greater attention in biochemistry lies on the minimal positive *T-invariants* in the Petri net theory, elementary modes in biochemistry, respectively. Positive T-invariants are defined by the positive solution vectors x (transition vector) of the equation $C \cdot x = 0$, $x > 0$. The place (P-) or transition (T-) invariant z is called *minimal*, if there exists no vector $w \geq 0$ with $supp(w) \subset supp(z)$, whereby supp(x) (read as support of x) describes the set of non-zero components in x, and the largest common divisor of all components of x is equal to one. A T-invariant gives structural insights in the represented pathway. The following definition of minimal biochemical pathways corresponds to the minimal T-invariant of Petri nets.

Elementary modes have been defined as the minimal set of enzymes that could operate at steady state. These modes can be calculated using the convex analysis with special conditions. A tool, which has no relation to Petri net theory, but is able to calculate elementary modes, is METATOOL, which is described by Pfeiffer et al. in [Pfeiffer99].

In the following, only minimal T-invariants are considered. Two groups and for each group two types of T-invariants (elementary modes) can be classified in metabolic Petri net models.

Trivial T-invariant

- **Trivial reaction T-invariant**
  A trivial T-invariant exists for each equilibrium reaction. These invariants are internal cycles. In a place/transition nets, a trivial T-invariant exists for a reversible reaction.
- **Trivial environment T-invariant**
  If the environment strategy of type I is used, explained below, a trivial T-invariant exists for each pair of supply and removal transitions of an ubiquitous compound.

Non-Trivial T-invariant

- **IO T-invariant**
  An IO T-invariant describes the exchange fluxes from one or more primary source metabolites to one or more primary sink metabolites.
- **Internal T-invariant**
  An internal T-invariant represents an internal cycle within the modelled system.

The following additionally introduced properties are essential for this paper.

XF are the set of post-nodes of the nodes, contained in the set X. FX are the set of pre-nodes of the nodes, contained in the set X. A non-empty set of places *H* is a *trap*, if the equation $HF \subseteq FH$ is valid. In other words, if a trap contains at least one token, it is then called marked, and then it always keeps its tokens. In biochemical context a marked trap is an indication of a disease, because a critical accumulation of molecules, represented by the marked trap, is reachable. Therefore, if no disease is modelled, then a marked trap should not exist. This fact can be interpreted as a consistency criterium of a model.

A non-empty set of places D is called a *co-trap*, if the equation $FD \subseteq DF$ is valid. If a co-trap is unmarked, then it will be always unmarked and all post-transitions of the co-trap will be never enabled. In biochemical context this indicates that the metabolites of the co-trap are essential for the corresponding pathway of the otherwise never enabled transitions. In the following, the name “co-trap” is shortly used for an unmarked co-trap and the name “trap” is shortly used for a marked trap.

Informally, a *structural conflict* is present, if at least two transitions exist, which have at least one common pre-place. A *dynamic conflict* is present, if a marking, reachable from the initial marking that realizes the structural conflict, exist. A *critical dynamic conflict* should be informally defined as a dynamic conflict, which results in an unmarked co-trap or in a marked trap. An example is shown in figure 3.

In [Lautenbach02] it has been shown that the net representation of a T-invariant, which reproduces the empty marking, does not contain neither a trap nor a co-trap. This is an important property in biochemical context and will be explained in detail in the next section.

Extending Analysis Feasibilities

With the knowledge about sources and sinks it is possible to extend the base model by an environment. Such an environment is only used to increase the Petri net model analysis possibilities, for example reachability graph/occurrence graph analysis and/or model checking techniques. Two useful types of environments are introduced.

Environment Type I

The simplest model of an environment is a transition bordered Petri net with an empty marking. This means that for each primary source a pre-transition without pre-places and for each primary sink a post-transition without post-places are added. For the ubiquitous molecules it is assumed that each of them is a source and a sink. A reason for this assumption is the aim to reproduce the empty marking of the net, which is equal to the assumption that all supplied molecules must be, possibly in another form, be removed. An unbounded and possibly live Petri net model is the result of this modelling. The liveness property depends on the source/sink specification of the primary metabolites and the modelled pathways. If a disease is modelled, then normally at least one co-trap or trap exists in the model and for this reason the net must not be live. If no diseases are modelled, which is currently done, then the net must be live for the empty initial marking, otherwise the selected reactions and source/sink specifications are unfavourably chosen. For example, no molecule can be transformed into another without a foregoing supply of them. The resulting model can be analysed by calculation of T-invariants, whereas the T-invariants can be classified in three types, which was previously described. If no disease is modelled, then the net representation of each minimal IO-T-invariant should not contain a trap or a co-trap, because each calculated minimal T-invariant reproduces the empty marking. A P-invariant and other extensive analysis methods can not be applied, because the resulting model is always unbounded. Some isolated case studies can be found in [Heiner03], [Heiner04] and [Runge04]. To fill the lack of P-invariant analysis the second type of environment was developed.

Environment Type II

The aim of the second environment type is to get a bounded and live Petri net model. To reach this aim, it is necessary to avoid co-traps and traps in the Petri net model, if no diseases have to be modelled. These traps and co-traps arise through the restriction of the amount of supplied source molecules and through an unfavourable occurrence sequence of transitions. An example is given in figure 3 in section 5. For this reason each critical dynamic conflict must be avoided in the model. To do this in a compact description, coloured Petri nets are used. The base model is extended with an environment, here named “extended model (II)”. Supplying and removing a bounded amount of metabolites from the base model in relation to each IO-T-invariant of environment type I is a task of the environment. The environment is used to conserve the steady state. Unfortunately, through this type of environment there exists always only one minimal IO-T-invariant within a coloured Petri net. This invariant is a summarized version of each minimal T-invariant of the environment of type I. The first attempt was made by Voss et al. [Voss003]. The construction of the environment was made stepwise and by hand with much knowledge about the modelled system. In this paper an automated construction of the environment of type II is shown by using knowledge from a P/T net model with environment of type I.

In summary, it may be said that the following prerequisites must be fulfilled to model a metabolic network with coloured Petri nets.

- A specification of a set of atomic reactions or pathways must be given.
- A sensible source/sink specification must be available.

It should be noticed, that the assumptions for each environment type from I to II get stronger.

3. Combination of place/transition nets and coloured Petri nets

In this paper a combination of analysis, simulation and modelling techniques of coloured and P/T Petri nets is used to get a new coloured model, which fulfils the requirements of quantitative and qualitative analysis. It is well known that an equivalence relation ((un-) folding) between coloured and P/T nets exists. On account of the equivalence of the P/T nets and coloured Petri nets, each of the described environment types can be expressed with both net classes and each described property can be directly used for both net classes. But the combination of the advantages of both classes makes a modelling and validation easier. It should be noticed that a structural conflict in a coloured Petri net differs slightly from a structural conflict in a P/T net. A structural conflict between two transitions in a CPN is only present, if the unfolded P/T net of both transitions of the CPN is not completely structural conflict free. The tool Design/CPN [Design/CPN] is used to construct the coloured Petri nets and the tool PED [PED] is used to construct the P/T nets. For this paper it is assumed that only properties or features of coloured Petri nets, especially of Design/CPN, are used, which enables an unfolding to a P/T net.

The place transition nets' T-invariants are exploited to construct an equivalent bounded and live coloured net. This equivalence type has no relation to an (un-) folding process. The coloured net is equivalent to a P/T net of the same system, if the T-invariants/elementary modes of the P/T net are also included in the coloured net, but on account of the environment in a summarized form.

Useful properties of both net classes

An advantage of the P/T nets is the possibility to calculate invariants in a simple way. INA, which can be found in [INA], is such a calculation tool. In [Runge04] it was shown that for place/transition nets not every possible P-invariant is biochemically interpretable, if a straightforward modelling is used. Each P-invariant of a biochemical coloured Petri net model must be biochemically interpretable, if additional knowledge about metabolite conservations are used during the modelling process. On account of the folding or the unfolding possibilities this knowledge can also be applied in P/T nets, but with a much larger effort. For example, the transport of the phosphate group can be expressed by a special arc inscription of the following example reaction, shown in figure 1. The constant value *P* and the other arc inscriptions are used to distinguish the transport of molecules. Hence, a P-invariant for ATP and ADP should be exists by this type of modelling.

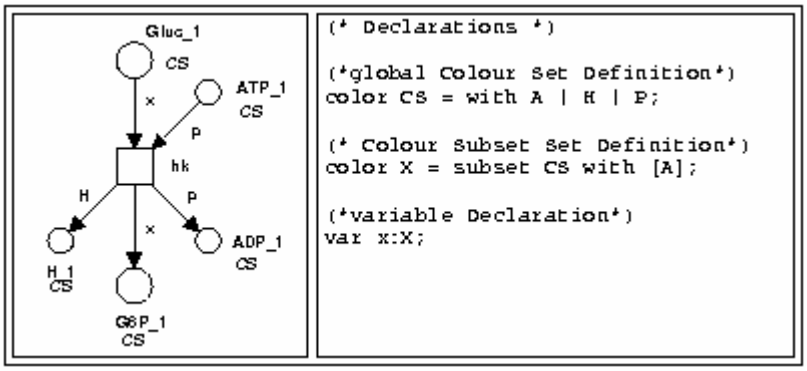

figure 1: CPN with irreversible reaction
Gluc + ATP → G6P + ADP + H^+

Until now, a disadvantage of coloured Petri nets is the absence of some analysis tools, which are able to calculate P- or T- invariants (CPN). First attempts to verify P- and T-invariants of coloured Petri nets was made by Genrich, with an experimental software package (called SY), and Voss et al. [Voss03]. It should be noticed that a T-invariant of a P/T net contains only a number of occurrences of transitions. A T-invariant of a coloured net contains additionally

information about binding elements (a concrete variable - value assignment) for a transition. To distinguish between them, a T-invariant (P/T) stands for a T-invariant in a P/T net and a T-invariant (CPN) stands for a T-invariant in a coloured net.

The great advantages of coloured Petri net models are the compactness of the model and the great simulation possibilities. Each of them will be shortly explained.

Compactness means that a smaller net for the same content as for P/T nets is reachable, if coloured Petri nets are used. Especially, for the later presented biochemical models it gets a clearer model. For example, after an unfolding of the coloured net, extra transitions or places in a P/T model may exist for each token colour. For this reason a coloured Petri net is longer human readable as a P/T net by increasing the net size.

The possibility to execute code, which is able to modify the state of the net, or to collect time data are examples of some extensions of coloured Petri nets, provided by Design/CPN. These possibilities will not be detailed explained now, but these are the reasons why a coloured net can be used for quantitative analysis and simulation. It is a great advantage, if the fundamental model/data structure must not be changed for quantitative and qualitative analysis.

Therefore, we will construct a bounded and live coloured Petri net by using the calculated minimal IO-T-invariants of a P/T net of the same biochemical system. This will be demonstrated in the next sections.

4. Case Study Glycolysis

The glycolysis is one of the main metabolic processes in human cells. In this paper we use the following selected pathways, which are all described in biochemistry books, for a case study.

GP
hk: Gluc + ATP → G6P + ADP + H
pgi: G6P → F6P
pfk: F6P + ATP → FBP + ADP + H
al: FBP → DHAP + GAP
tpi: DHAP ↔ GAP
gapA: GAP + Pi + NAD → NADH + H + BPS
pgk: BPS + ADP → PG3 + ATP
bpgm: BPS → DPG + H
bpgp: DPG + H2O → PG3 + Pi
gpm: PG3 → 2PG
eno: 2PG → H2O + PEP
pyk: PEP + ADP + H → ATP + Pyr
ldh: Pyr + H + NADH ↔ Lac + NAD
F1PP
scrK: Fruc + ATP → F1P + ADP + H
f1pa: F1P → DHAP + GA
tk: GA + ATP → GAP + ADP + H
F6PP
hk2: Fruc + ATP → F6P + ADP + H
GGIP
galK: Galac + ATP → Galac1P + ADP + H
gal: Galac1P → G1P
pgm: G1P ↔ G6P
PPP
g6pdh: G6P + NADP → 6PL + NADPH + H
6pgl: 6PL + H2O → 6GP + H
6pgd: 6GP + NADP → NADPH + CO2 + Ru5P
rpi: Ru5P ↔ R5P
rpe: Ru5P ↔ Xu5P
tkt: Xu5P + R5P ↔ GAP + S7P
tal: GAP + S7P ↔ F6P + E4P
tkt2: Xu5P + E4P ↔ GAP + F6P

figure 2: formulas of atomic reactions, the modelling basis

The considered pathways are the glycolysis pathway (GP), the pentose-phosphate-pathway (PPP), the fructose-1-phosphate-pathway (F1PP), the fructose-6-phosphate-pathway (F6PP), and the galactose-glucose interconversion pathway (GGIP). Fructose (Fruc), galactose (Galac), and their pathways interact with the glycolysis. All the individual reactions take place in the cytoplasm of a cell. Glucose-6-phosphate and/or fructose-6-phosphate are intermediate products of all described pathways. The described pathways start always with glucose (Gluc), fructose (Fruc) or galactose (Galac). Reaction products are lactate (Lac), pyruvate (Pyr), Ribose-5-phosphate (R5P), or/and NADPH. The gluconeogenesis — the nearly inversion of the glycolysis — is not modelled, because some reactions of them do not take place in the cytoplasm of a cell. Moreover, the gluconeogenesis is only active in liver cells. [Berg02] serves as biochemical reference for this paper. The figure 2 shows the considered set of atomic reactions. Some simple sequences will be later summarized.

5. Modelling Strategies of the Base Model

Two steps are necessary to get an extended model. The first one is to construct a base model with all biochemical information, which are available. The second step is to calculate automatically an environment for a base model. In this section two kinds of modelling metabolic networks to get a base model are discussed. A third verification step, using the ideas of effects and defects, can be additionally made to get a stronger confidence with the extended model (not shown). Two systematic methods to construct a metabolic coloured Petri net base model are described in this section.

To get a live model it is necessary to avoid each possible co-trap and trap under the prerequisites that the source metabolites are bounded. However, the general behaviour, represented by the T-invariants, must be conserved. Two strategies to construct a base model are introduced, but only for variant II a concrete general behaviour can be systematically defined. The general behaviour of variant I was indirectly obtained through a stepwise modelling.

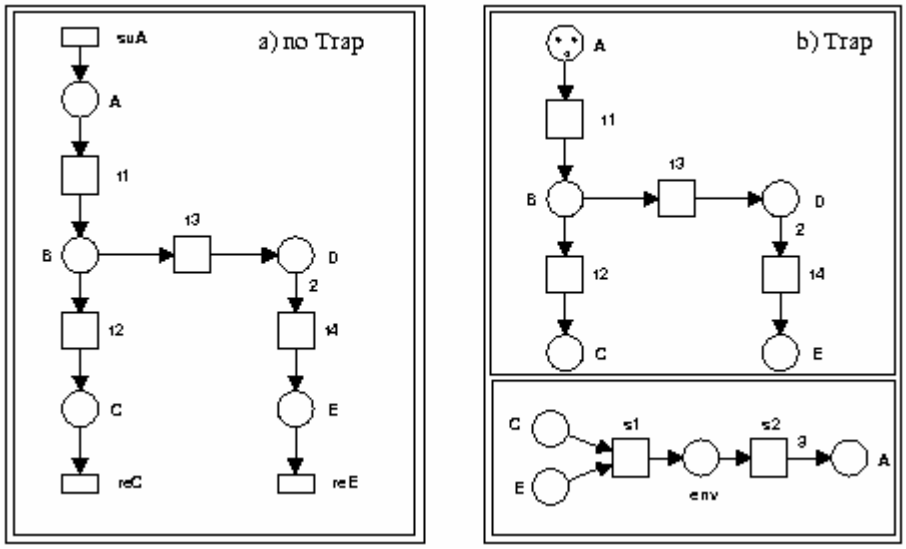

figure 3: P/T nets with different environment types
a)T-invariants: T1 = {suA, t1, t2, reC}; T2 = {2*suA, 2*t1, 2*t3, t4, reE}
b) T-invariant: T3 = {s2, 3*t1, t2, 2*t3, t4, s1}
critical dynamic conflict between t2 and t3 by marking (3*B)
occurence sequence o1 = (t1, t1, t1, t3, t2, t2) yields in an trap/co-trap

The figure 3 shows two short P/T nets with two different environment types to demonstrate the problem of unmeant traps and co-traps. The point is that the general behaviour of the net b) must be the same as the behaviour of the net a). But the P/T net b) contains a possible trap/co-trap. The transition occurrence sequence o1 = (t1, t1, t1, t3, t2, t2) produces a trap and a co-trap. The token on place D, which is not a sink place, can not be removed. For this reason the environment of the P/T net b) is not sufficient for an extended analysis, because it does not contain the general behaviour of the P/T net a). Another point is that the shown environment of P/T net b) merges the minimal T-invariants of the net a), but this is not necessarily a disadvantage.

There exist two techniques to solve the co-trap/trap problem. The *first technique* is to change the firing rule and to use information about the relative reaction rates. In other words, occurrence of transitions in conflict corresponds to the given relative reaction rates. A changing of the firing rule is a deep cut with a principle of Petri nets. The *second technique* is to avoid critical dynamic conflicts in the modelled Petri nets. Dynamic conflict detection is very expensive. For this reason, it is easier to resolve the corresponding structural conflict, because it is a necessary condition for a dynamic conflict. Voss et al. have constructed a CPN

model avoiding dynamic conflicts. A coloured Petri net with conflict avoidance has the same structure as the P/T net b) in figure 3, but the arc inscriptions are different. The CPN model is very compact and easy to read in contrast to a P/T net, which would be the result of an unfolding process of the CPN model. In this paper only the second technique is examined.

By using guards and different token colours, a structural conflict can be resolved. A token colour has to represent the information about the pathway on which the token has to go along. This strategy is not biochemically motivated, because there exist no difference of the same molecule. But in biochemical context alternative paths result often in different overall reactions, which allow us to discriminate molecules of the same type. Additionally, it may be said that only conflicts on primary metabolites must be resolved. These primary metabolites determine the main pathways. All other metabolites (ubiquitous molecules) are available in large amounts in the cell. Therefore, in this paper only conflicts on primary metabolites are considered. All arc inscriptions from and to ubiquitous places contain only constant values with a given multiplicity.

The following two base models are constructed by hand, but systematically. The first one is the application of the method used by Voss et al. in [Voss03]. The second is a method with another more systematically procedure. The structural conflict avoidance principle plays a large role during the construction. A calculation of the environment is performed, later in this paper.

Conventions

First, a reduction of each sequence is done. A sequence results in no significant structural information. See at abbreviations for the reduced sequences.

Secondly, the following naming conventions are used to obtain clarity. Each occurrence of a logical place (or fusion place) must have its own unique name (prerequisite of Design/CPN). Use the fusion set name with an appended "_x", whereby x is the x-th occurrence of a place in a fusion set, as the name for a place.

Thirdly, by a reversible or equilibrium reaction the transition, which represents the main reaction direction, becomes the enzyme name, which catalyzes the reaction. The other direction becomes the same name with the appendix "_rev". If more than one reaction (different educts, products) is catalyzed by one enzyme, then an additional identifier must be used. The different reaction rates are the biochemical interpretation of the different names.

Fourthly, Design/CPN does not allow place or transition names, which start with another character as a letter. Therefore, another abbreviation as regular must be sometimes used.

Fifthly, transitions, which have no relation to an IO T-invariant and which are a part of a reversible reaction, are removed. Those transitions have no contribution to the observed system. Therefore, the transitions "ldh_rev" and "rpi_rev" are removed.

Variant I

The main construction principle by this variant is the conflict avoidance principle, used by Voss et al. Much knowledge about the modelled system is necessary. It is only a principle and not a rule, because some non-critical dynamic conflicts must not be resolved. Look at figure 4 at place "GAP_1". The conflict between the transition "tpi_rev" and all other post-transitions of "GAP_1" must not resolved, because it exists an internal T-invariant ("tpi_rev" and "tpi"), which reproduces the same marking as it was before "tpi_rev" has been occurred.

A token colour by this variant of modelling represents the sink as target and the path, which has to go on the net. For each involved arc of a conflict a separate token colour should be used. To assure this, guards or variables with disjoint ranges must be used. In this context a combination of guards is equivalent to a combination of distinct variables. In this paper almost only variables are used to resolve the conflicts. This increases the clarity of the net by avoiding long guards on each transition. The figure 4 shows the resulting model, using only

the conflict avoidance principle, and the figure 6 shows the corresponding global declarations of the net.

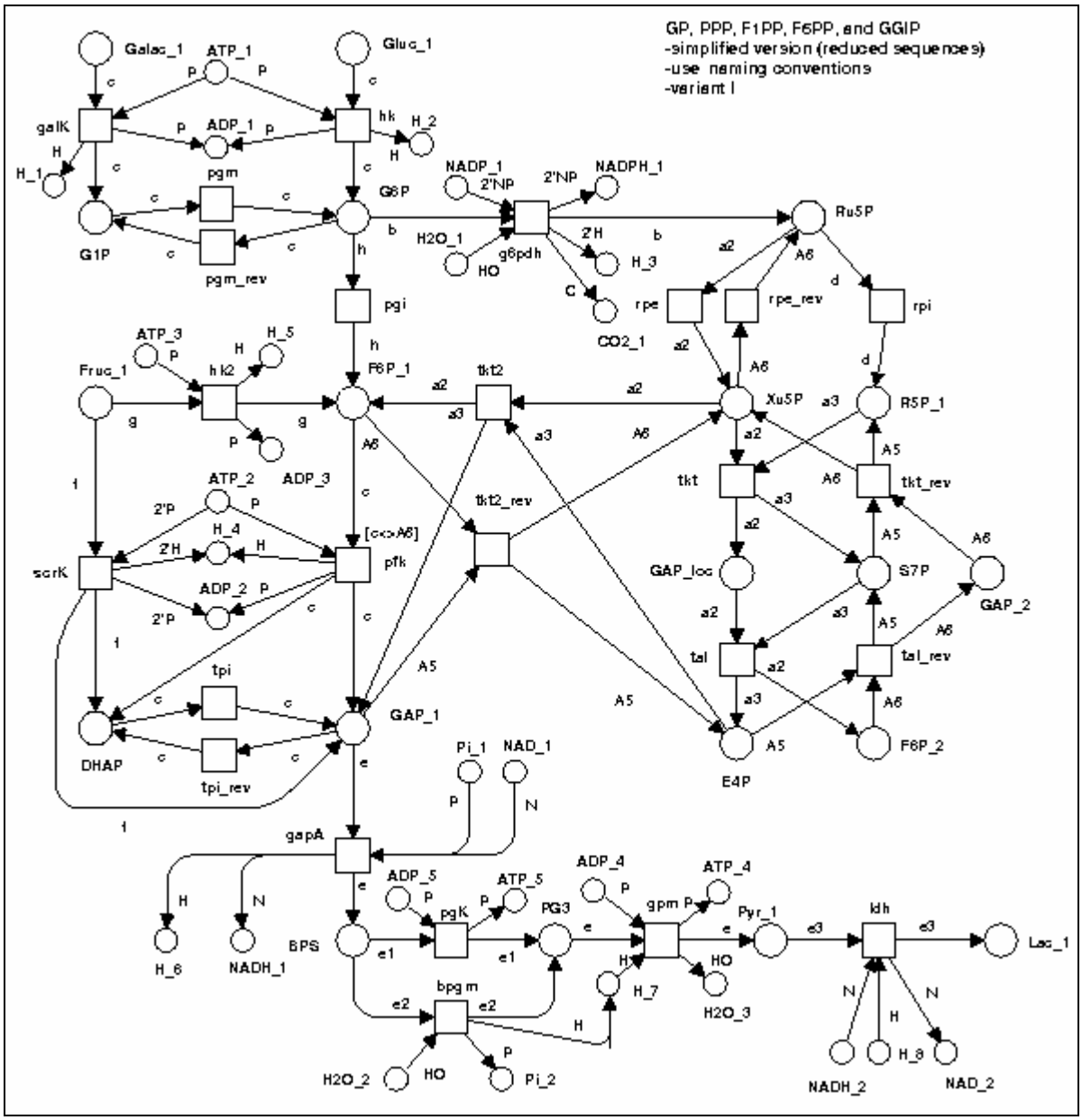

figure 4: base model, variant I

General Procedure

- First, each structural conflict must be determined.
- Secondly, starting from a source place through the net, each possible path must be distinguished by a token colour at a previously determined conflict. The knowledge about each path is obtained during a stepwise construction of the model or through additional literature.

Descriptions/Exceptions

An automatic construction is, if possible, very complex, because the used knowledge may be not systematic enough. 15 token colours are used to resolve all critical dynamic conflicts. An example of conflict solving follows. The lower part of the model from GAP to pyruvate and lactate is observed. It is easy to see that from GAP to Pyr two paths exist. Each Pyr can be transformed into Lac. For this reason there exist four possible paths in the lower part. Two paths transform GAP to Pyr and two paths transform GAP to Lac. Four colours are needed for

this part. Therefore, each token that reach GAP and should be transformed into Pyr or Lac must also represent one of the four paths in the lower part. The token colour “A2” and “A3” are extended with the information about the four sub-paths, represented by an appended constant. For example, “A2” represents the path through g6pdh, rpe, tkt, tal, tkt2, pfk and would be represented by one colour, if the lower part would not be exist.

A special operation for this model is done. The place “GAP_loc” corresponds to no fusion set, although all tokens on this place represent a “GAP” molecule like the other places in the fusion set “GAP”. If “GAP_loc” would be correspond to the fusion set “GAP”, it would be possible that the token on this place is consumed by “gapA”. If so, it is possible that not enough tokens (GAP) are available to transform all metabolites of the pentose-phosphate pathway (no occurrence of “tal”) into pyruvate or lactate. This problem can be classified. It exists, if an intermediate product occurs more than once in the partial net representation of an expected T-invariant. The figure 5 shows the pattern of this problem.

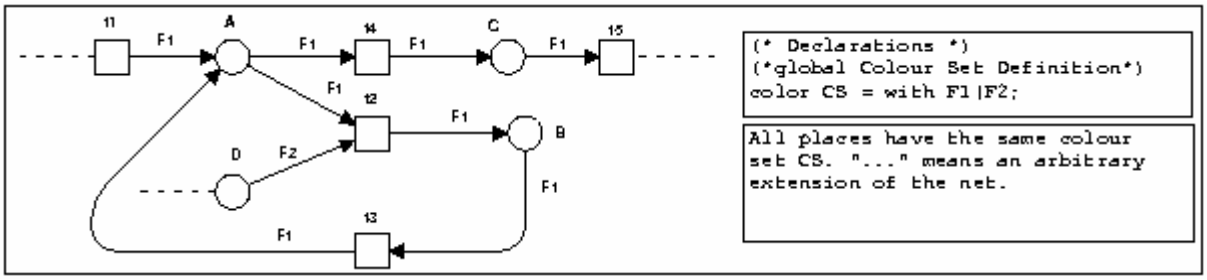

figure 5: Pattern of a conflict within a minimal T-invariant, which can not always be solved by different token colours. The transitions t1, t2, t3, t4 are a part of a minimal T-invariant. If place D contains a token F2 and the transition t1 occurs, then it exist a dynamic conflict between t2 and t4. If t4 occurs before t2 occurs and both belong to the same minimal T-invariant, then a trap is possible. Otherwise, the occurrence sequence t1, t2, t3, t4 is harmless.

The problem (the conflict) can be solved as shown with a special local place or with special guards. A transition in relation to their guard is able to transform a token from one colour to another colour. This can also be used to avoid such problems. But this solution is not adequate enough and much more complex as a solution with a local place. Voss et al. have an easier model, which does contain such a problem within the easiest form. It was solved by such a transformation from only one token colour to only one another token colour. But if more token colours of the place are possible, this solution is more complex as the other one.

```
 (*Declarations*)
color CS = with P|NP|C|H|HO|N|
               A1|A5|A6|A7|A8|A9|A10|
               A27|A28|A29|A210|
               A37|A38|A39|A310;
color A2 = subset CS with [A27,A28,A29,A210];
color A3 = subset CS with [A37,A38,A39,A310];
color B = subset CS with [A1, A27,A28,A29,A210, A37,A38,A39,A310];
color D = subset CS with [A37,A38,A39,A310, A1, A6];
color E = subset CS with [A27,A28,A29,A210, A37,A38,A39,A310, A7,A8,A9,A10];
color E1 = subset CS with [A29,A210,A39,A310,A9,A8];
color E2 = subset CS with [A27,A28,A37,A38,A7,A8];
color E3 = subset CS with [A27,A29,A37,A39,A7,A9];
color F = subset CS with [A7,A8,A9,A10, A5];
color G = subset CS with [A6, A7,A8,A9,A10 ,A5];
color I = subset CS with [A1,A5,A6,A7,A8,A9,A10,
                          A27,A28,A29,A210,A37,A38,A39, A310];
color J = subset CS with [A5,A6,A7,A8,A9,A10];

var b:B;var d:D;var c:I;var e:E;var f:F;var g:G;var h:J;
var a2:A2; var a3:A3;var e1:E1;var e2:E2; var e3:E3;
```

figure 6: Declarations of coloured Petri net model variant I

By this variant of systematic modelling no information of a possibly previously constructed P/T net is used. The construction depends only on the specification of the atomic reactions and much modeller's knowledge. A discrimination of the T-invariants by using the tokens is not possible. There exists no relation between them. Therefore, no direct selection of T-invariants in relation to simulation can be made.

Variant II

Another idea to construct a base model is now introduced. As previously suggested, minimal T-invariants of a P/T model can be used to construct a coloured Petri net base model. The construction of a P/T net is very easy and straightforward. It is a direct reflection of the atomic reactions with their stoichiometric parameter. The resulting P/T model must now be extended by the environment of type I. Hence, a T-invariant analysis is now possible. The calculated non-trivial minimal T-invariants (P/T) represent the general behaviour and the basic structure of the modelled system. These T-invariants are used to construct a coloured Petri net model without conflicts. It should be noticed that the T-invariant calculation depends on the source/sink specification. Therefore, for the given case study all ubiquitous molecules are not observed during the calculation of minimal T-invariants, because elsewhere much more invariants, but with no more new information, would be calculated. In other words a sensible selection of minimal T-invariants (elementary modes) in relation to the biochemical context is done. The tool INA [INA] calculates 40 minimal T-invariants, whereby 8 minimal T-invariants are trivial (reversible or equilibrium reactions). After inspection of these T-invariants it was realized that the transitions "ldh_rev" and "rpi_rev" only occur in a trivial T-invariant. For this reason they are removed.

The basic idea is that a direct relation between the token colour and a T-invariant must be exists. A token colour corresponds only to one minimal IO-T-invariant. For this reason almost each conflict can be resolved, because each token denotes its pathway from a source to a sink. The following selected example, the minimal IO-T-invariant t38 demonstrates an exception.

t38 = {3*hk, 3*g6pdh, 2*rpe, 1*rpi, 1*tkt, 1*tal, 1*tkt2, 2* pfk, 2*tpi, 5*gapA, 5*bpgm, 5*gpm}

After occurring of three times of hk and g6pdh, Ru5P contains three tokens. Now a critical dynamic conflict between rpi and rpe exists within the partial virtual net representation of the T-invariant t38, containing only nodes that correspond to the given T-invariant. To avoid such a conflict, more token colours must be used to set the path, on which token colour belongs to. This is done by using colour "C38A" and "C38B" for the given example. Both colours belong to the given T-invariant, but each of them corresponds to a specific path within the partial virtual net representation. Only such conflicts must be resolved under the assumption that a token colour exists for each minimal T-invariant.

The figure 7 shows the resulting model of the current modelling strategy. The corresponding declarations can be found in figure 8. Omit the arc inscriptions without multiplicity and we get the corresponding P/T net base model without an environment. In such a coloured Petri net an equilibrium reaction must result in the same arc inscriptions (same token colours) at both corresponding transitions (tpi, tpi_rev and pgm, pgm_rev). Otherwise no equilibrium of such a reaction can be reached. A reversible reaction must result into two transitions with different arc inscriptions (different token colours), because the transitions correspond to different T-invariants.

Conventions

At this model the following additional conventions are made.

First, each token colour contains the identifier (integer number) of the corresponding minimal T-invariant.

Secondly, if more than one token colour is necessary, then they will be discriminated by a non-numeric appendix.

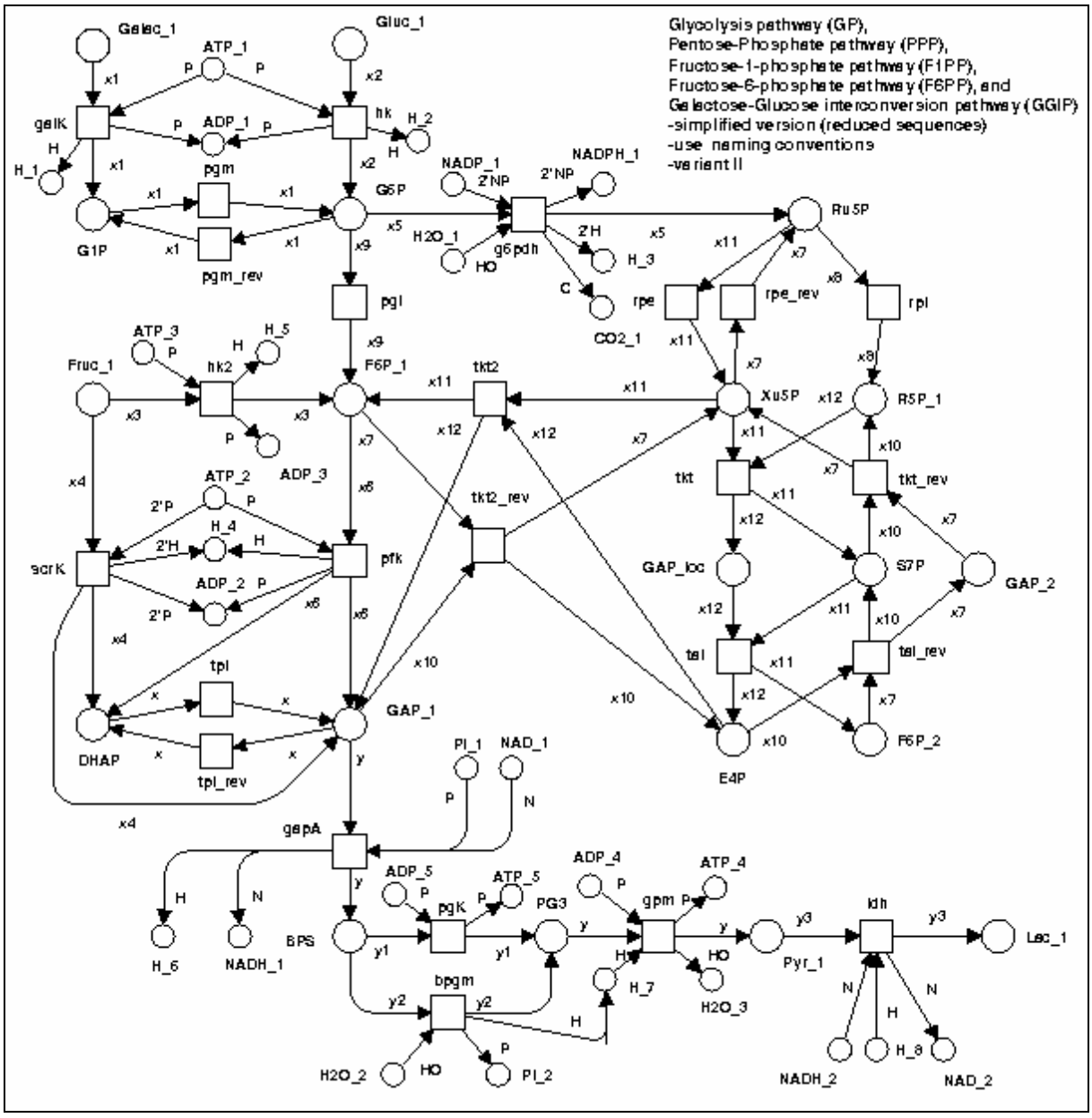

figure 7: base model, variant II

General Procedure

- First, a simple and straightforward construction of a P/T net with environment type I must be done. After them, a calculation of minimal T-invariants must be performed. This computation can also be done by using METATOOL [Pfeiffer99] to get the elementary modes, which are similar to minimal T-invariants.
- Secondly, for each transition/reaction collect the minimal T-invariants identifiers, which contains this transition. Create a variable on the corresponding arcs of the transition, which range represents the collected T-invariant identifiers. Examples of the resulting colours are shown in figure 8. On account of the restriction of variables no additional guards are necessary.
- Thirdly, it is necessary to compute structural conflicts for a P/T net representation of each minimal T-invariant. This can be easy done by using INA. For each detected conflict within a T-invariant resolve them by adding new token colours to the coloured base model. The T-invariants 17, 22, 27, 32, 33, 34, 35, 36, 37, 38, 39, and 40 are examples of such conflicts (to save paper size not shown). Much of the conflicts are at the same place,

whereby the effort is very small in difference to the conflict solving by variant I. A full automatic solution for this small problem is easy to imagine.

```
(*Declarations*)
color CS = with P|NP|C|H|HO|N|
               C9|C10|C11|C12|C13|C14|C15|C16|C17A|C17B|
               C18|C19|C20|C21|C22A|C22B|C23|C24|C25|C26|C27A|C27B|C28|C29|C30|
               C31|C32A|C32B|C33A|C33B|C34A|C34B|C35A|C35B|C36A|C36B|C37A|C37B|
               C38A|C38B|C39A|C39B|C40A|C40B;
color X1 = subset CS with[C9,C16,C23,C24,C25,C27A,C27B,C26,C33A,C33B,C34A,C34B,
                          C35A,C35B,C36A,C36B];(*galK**)
color X2 = subset CS with[C10,C15,C18,C19,C20,C21,C22A,C22B,C37A,C37B,C38A,C38B,
                          C39A,C39B,C40A,C40B];(*hk*)
color X3 = subset CS with[C17B,C28,C29,C30,C31,C32A,C32B];(*hk2*)
color X4 = subset CS with[C11,C12,C13,C14,C15,C16,C17A];(*scrK*)
color X5 = subset CS with[C9,C10,C33A,C33B,C34A,C34B,C35A,C35B,C36A,C36B,C37A,C37B,
                          C38A,C38B,C39A,C39B,C40A,C40B];(*g6pdh*)
color X6 = subset CS with[C18,C19,C20,C21,C22A,C23,C24,C25,C26,C27A,C28,C29,C30,
                          C31,C32A,C33A,C33B,C34A,C34B,C35A,C35B,C36A,C36B,C37A,
                          C37B, C38A,C38B,C39A,C39B,C40A,C40B];(*pfk*)
color X7 = subset CS with[C15,C16,C17B,C22B,C27B,C32B];(*tkt2_rev from F6P*)
color X8 = subset CS with[C9,C10,C15,C16,C17B,C22B,C27B,C32B,C33B,C34B,C35B,C36B,
                          C37B,C38B,C39B,C40B];(*rpi*)
color X9 = subset CS with[C15,C16,C18,C19,C20,C21,C22A,C22B,C23,C24,C25,
                          C26,C27A,C27B];(*pgi*)
color X10 = subset CS with[C15,C16,C17A,C22A,C27A,C32A];(*tkt2_rev from GAP*)
color X11 = subset CS with[C33A,C34A,C35A,C36A,C37A,C38A,C39A,C40A];(*rpe*)
color X12 = subset CS with[C33B,C34B,C35B ,C36B,C37B,C38B,C39B,C40B];(*tkt*)
color Y = subset CS with[C13,C14,C20,C21,C25,C26,C30,C31,C35A,C36A,C39A,C40A,
                         C35B,C36B,C39B,C40B,C11,C12,C18,C19,C23,C24,C28,C29,
                         C33A,C34A,C37A,C38A,C33B,C34B,C37B,C38B];
                        (*gapA union of Y1 and Y2*)
color Y1 = subset CS with[C13,C14,C20,C21,C25,C26,C30,C31,C35A,C36A,C39A,C40A,
                          C35B,C36B,C39B,C40B];(*pgK*)
color Y2 = subset CS with[C11,C12,C18,C19,C23,C24,C28,C29,C33A,C34A,C37A,C38A,
                          C33B,C34B,C37B,C38B];(*bpgm*)
color Y3 = subset CS with[C11,C13,C18,C20,C23,C25,C28,C30,C33A,C35A,C37A,C39A,
                          C33B,C35B,C37B,C39B];(*ldh*)

var x1:X1;var x2:X2;var x3:X3;var x4:X4;var x5:X5;var x6:X6;var x7:X7;
var x8:X8;var x9:X9;var x10:X10;var x11:X11;var x12:X12;
var y1:Y1;var y2:Y2;var y3:Y3;
var x:CS;
var y:Y;
```

figure 8: Declaration of coloured Petri net base model variant II

The direct mapping between the token colour and the corresponding minimal T-invariant is one of the great advantages of such a modelling. As shown later, it is possible to select a special linear combination of the minimal T-invariants for a simulation or analysis of an extended model.

Additionally, this modelling strategy in comparison to variant I is easy and straightforward. The model can be potentially with a very small exception full automated constructed, if the set of atomic reactions and the source/sink specification are given. The small exception lies on the same problem pattern as described and shown by variant I at the local place "GAP_loc".

A verification of the dynamic conflict solving in the given model can additionally be realized. Let us consider the simple conflict at the place BPS. There exist two post arcs. The potential dynamic conflict in relation to a P/T net is solved, if the following condition is fulfilled.

$$y1 \cap y2 \equiv \emptyset$$

Generalized it may be said, if the intersection of all colour sets of outgoing arcs of a place is equal to the empty set, then no conflict exists at this place.

Now, we have two base models, which are bounded, but not live. The next section introduces an algorithm, which results can be used to get an environment of type II for each of the base model.

6. (Half-)Automatic Computation of the Environment

To get a bounded model, it is necessary to limit the arbitrary supply of metabolites, which is not fulfilled by the environments of type I and II. This can be done by replacing the supplying and removing border transitions of environments I by two transitions, each for one task (supplying or removing). The figure 9 illustrates a transformation from a P/T net to a coloured net. The environment contains only two additional transitions, a start and a stop transition[2], and one additional place. The additional place "env" contains maximal one token. In relation with the start/stop transition it is used to get an empty base model within the extended model. If the place "env" contains a token, no other token is in the net. The "start" transition supplies all necessary metabolites and the "stop" transitions removes all produced or transformed metabolites.

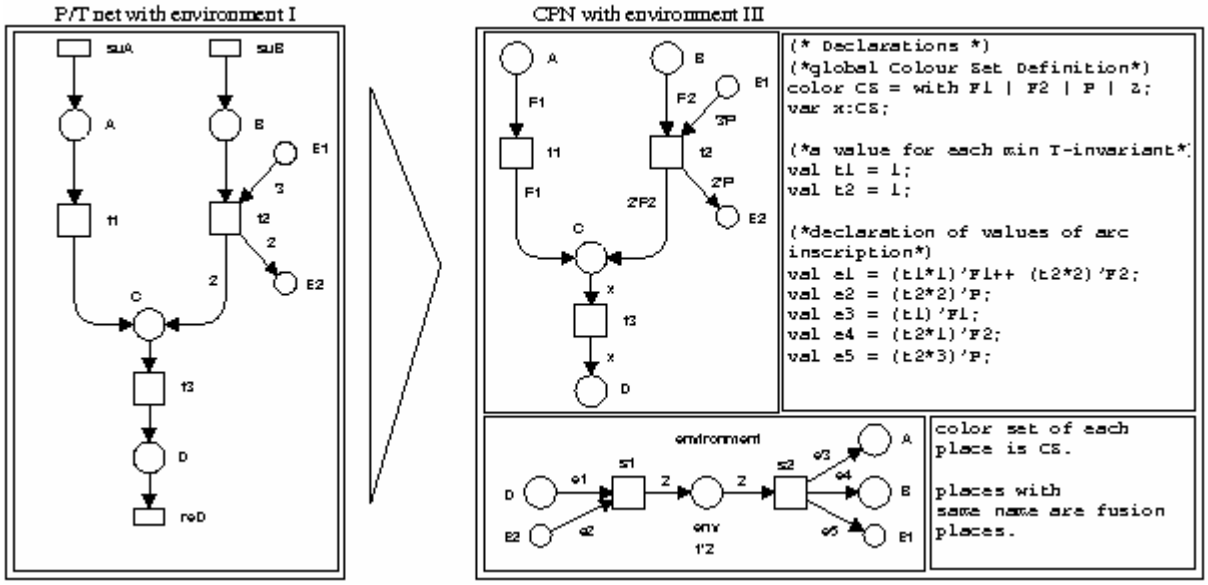

figure 9: example P/T net with environment I, which is transformed into a CPN with environment II
P/T net invariants: T1 = {suA, t1, t3, reD}; T2 = {suB, t2, t3, reD}
CPN invariant: T3 = {s2, t1, t2, 1*t3(x=F1), 2*t3(x=F2), s1}
init marking for t1 = A:1* F1; end marking for t1 = D: 1*F1
init marking for t2 = B:1* F2, E1:3*P; end marking for t2 = D: 2*F2, E2:2*P

Starting from a base model and the information about T-invariants (P/T), markings can be calculated, which contains the information how much metabolites and ubiquitous molecules must be supplied to realize an elementary mode. Such a marking should be called start marking. Each start marking corresponds to a so-called end marking. If all problematic conflicts are resolved in the base model, then each start marking is transformed by occurrence of the corresponding T-invariant (P/T) without border transitions into the end marking. These markings are used to determine the arc inscriptions of the "start" and "stop" transitions. Each computed marking, which realizes a T-invariant (P/T), can be straightforward transformed into the arc inscriptions on the arcs of the "start" and "stop" transition. An example is shown in figure 9. For a transformation it is necessary that only one start /end marking pair per T-invariant (P/T) is used. The calculation of such markings and the transformation into arc expressions are full automatic, but in special cases, depending on the modelling, for one T-

[2] In Design/CPN start and stop are not allowed as transition names. For this reason the names "s1" for stop and "s2" for start are used.

invariant more than one start/end marking pairs exist. Then a manual selection must be made or additional information, for example the token colour relation of variant II, must be used within the calculation algorithm to get only one marking.

With a modification of the constant values t1 and t2, each minimal IO-T-invariant can be separately activated. Or in other words, each integer linear combination of the minimal T-invariant can be explicitly selected and therefore simulated or analysed. A constant value exists for each minimal IO-T-invariant (P/T) (naming convention: “t” extended with T-invariant number). If only one T-invariant (P/T) is selected (ti = 1; tj = 0; j≠i), then the resulting T-invariant (CPN) is equivalent to the corresponding T-invariant of the P/T net.

Computation

To get a bounded model, which allows the selection of elementary modes, only the base model, the source/sink specification, and the previously calculated IO-T-invariants (P/T) are necessary. If the base model of variant II is used, these data are completely available. Under the assumption that each critical conflict is solved and no disease is modelled (co-trap risk), it is possible to calculate a start and end marking for each IO-T-invariant.

```
Input:
   tInv: TInvariant;
   cpn: coloured Petri net; //base model
   sources: set of places;
   sinks:set of places;
Output:
   (pre: marking; post:marking;)//pre => start marking;post => end marking
Initialisation:
   post = Ø; pre = Ø;
   tn:transition; //current considered transition
   prePostTN:PrePostMarkings;//set of marking pairs (pre, post)
   lastStep:PrePostMarkings; lastStep = Ø;//set of marking, before a tn occur
   currentStep:PrePostMarkings; currentStep = Ø;//markings, after a tn occured
   pet:list of Transitions;// possibly enabled transitions
   pet = possibleExtensions(Ø);
   pet = pet ∩ tInv;
   pet = removeNotSufficientMarkedTN(pet, post);
Main procedure without error/trap/deadlock detection/handling:
   while (tInv ≠ Ø) {
      lastStep = currentStep;
      currentStep = Ø;
      tn = selectTN(pet);
      prePostTN = tn.getPrePostMarkings();
      for (int i = 0; i < prePostTN.length; i++) {
         for (int j = 0; j < lastStep.length; j++) {
            if (lastStep[j].getPost().covers(prePostTN[i].getPre())) {
               pre:marking; post:marking;
               pre = lastStep[j].pre ∪ (prePostTN[i].pre \
                     (prePostTN[i].pre ∩ lastStep[j].post));
               post = prePostTn[i].post ∪ (lastStep[j].post \
                     (prePostTN[i].pre ∩ lastStep[j].post));
               //combination is allowed and new Marking is calculated
               currentStep.addPrePostMarking(pre, post);
            } //if
         } //for
      } //for
      tInv.occurOnce(tn);//modify T-invariant
      pet = pet ∪ possibleExtensions(tn);
      pet = pet ∩ tInv;
      pet = removeNotSufficientMarkedTN(pet, currentStep);
   }//while process one t-invariant
   (pre, post) = proveAndPossiblyRemoveMarkings(currentStep);
```

figure 10: short abstract algorithm to get environment type II

The given abstract algorithm, shown in figure 10, is similar to a construction algorithm of a run of a single T-invariant (P/T), but the current algorithm has no initial marking as prerequisite. Instead, the source/sink specification is used to know, on which place a token can be added. This specification can also be used to detect traps and co-traps within the net representation of the current considered T-invariant. Each transition of the given T-invariant (P/T) has to occur in relation to its partial order, whereby each possible binding element of the transition is considered. Additionally, all needed molecules of sources and all produced molecules of sinks are separated calculated to know, which tokens must be provided by the start transition and which tokens must be consumed by the stop transition. The calculation is finite, because the given T-invariant is also finite.

Results

Using the described algorithm for the computation of start and end markings for the base models (variant I and II), a transformation into arc inscriptions is also performed. For variant I this calculation was full automatic, but for the variant II a manual selection must be done for six T-invariants, because no additional information about the relation between token colour and T-invariant was used. Additionally, the graphical representation of the environment, shown in figure 11, must be constructed by hand.

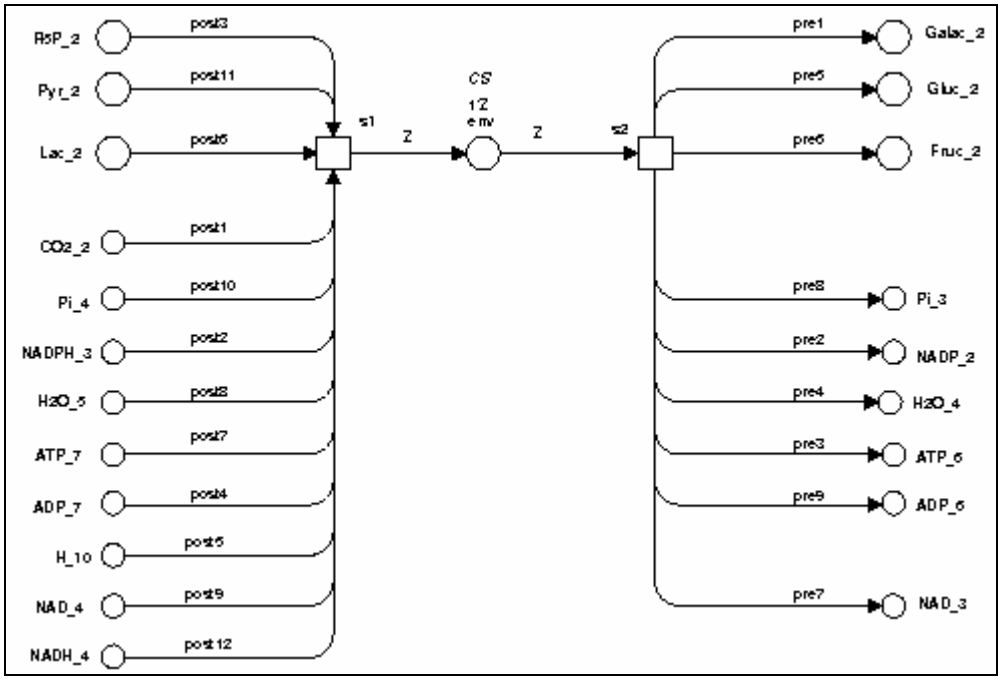

figure 11: environment of both extended base models
(the differences lie on the definition of the values on the arcs)

The base model must be only extended by the graphical representation of the environment and the declaration must be added to the global declaration node. To save paper size, only two example declarations for the model of variant II are shown in figure 12. Two different types are shown. “pre1” corresponds to a primary metabolite and “pre2” corresponds to a ubiquitous molecule, which have always the same token colour.

On account of the knowledge of the modelled system some new questions are recognized. The calculated markings realize a corresponding T-invariant, but an IO-T-invariant has often more than one interleaving sequence. Therefore, is it possible to calculate a start and end marking under the assumptions that no potentially concurrency is limited (*maximal concurrency*) or that the concurrency is maximal limited (*minimal concurrency*)? These markings can be biochemically useful. For the current case study only the concentrations of the ubiquitous molecules have an influence to minimal or maximal concurrency.

```
val pre1 = (1*t9)`C9++ (1*t23)`C23++ (1*t24)`C24++ (1*t25)`C25++ (1*t26)`C26++ (1*t33)`C33B++
           (2*t33)`C33A++ (1*t34)`C34B++ (2*t34)`C34A++ (1*t35)`C35B++ (2*t35)`C35A++
           (1*t36)`C36B++ (2*t36)`C36A++ (4*t16)`C16++ (1*t27)`C27A++ (4*t27)`C27B;
           (*Galac*)
val pre2 = (2*t9 + 2*t10 + 6*t33 + 6*t34 + 6*t35 + 6*t36 + 6*t37 + 6*t38 + 6*t39 + 6*t40)`NP;
           (*NADP*)
```

figure 12: example declaration for extended base model variant II

Existing Problem

On account of a bug of the used Design/CPN tool, a construction of the occurrence graph was up to now not possible. Therefore, the liveness property could not be proved. The problem, described by a smaller example, was reported to the "CPNTools-support". Using the effect and defect ideas would be another way to increase the confidence with the constructed model (not shown here).

7. Conclusions

After constructing a place/transition net and a calculation of minimal T-invariants a construction of a bounded and possibly live coloured Petri net was performed. The construction of the coloured net was divided into two separate steps. First, the base model was constructed by hand. After them an automatic calculation of an extended model with an environment was performed. The extended model of variant II represents the same T-invariants/elementary modes as the P/T model with environment type I.

It was shown that a combination of the analysis techniques of place/Transitions nets and coloured Petri nets can be used to get a more usefully and sensible model of a metabolic network. The constructed coloured Petri net models are bounded and possibly live. Therefore, we are now able to perform additional qualitative analysis techniques, for example model checking. After performing a qualitative analysis we are able, by using extensions of the coloured Petri net tool Design/CPN, to perform a quantitative analysis of a qualitative analysed model.

The construction of a base model must now be automated, for example by an extraction of pathways from a database. Moreover, the bottleneck is the calculation of sensible elementary modes/minimal T-invariants, which depends on the selected set of atomic reactions, the specification about source and sinks, and in relation to them the treatment of the ubiquitous molecules. It must be detailed observed, how the treatments of the ubiquitous molecules have an influence to the elementary modes. But this is a task for biochemists.

Additional case studies should be performed to increase the confidence with the application of Petri nets in biochemistry and this modelling technique.

Acknowledgement

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Abbreviations

Metabolites / Compounds

(chemical formulas from [Berg02] and [Wiback02])

2PG	2-Phosphoglycerate	C3H4O7P	**Galac**	Galactose	C6H12O6
6GP	6-Phosphogluconate	C6H10O10P	**GAP**	Glyceraldehyde-3-phosphate	
6PL	6-phosphoglucono-δ-lactone	C6H9O9P			C3H5O6P
BPS	1,3-Biphosphoglycerate	C3H4O10P2	**Gluc**	Glucose	C6H12O6
DHAP	Dihydroxyacetone phosphate	C3H5O6P	**Lac**	Lactate	C3H5O3
DPG	2,3-Biphosphoglycerate	C3H3O10P2	**PEP**	Phosphoenolpyruvate	C3H2O6P
E4P	Erythrose-4-phosphate	C4H7O7P	**PG3**	3-Phosphoglycerate	C3H4O7P
F1P	Fructose-1-phosphate	C6H11O9P	**Pi**	Orthophosphate, ionic form	
F6P	Fructose-6-phosphate	C6H11O9P			HO4P
FBP	Fructose-1,6-biphosphate	C6H10O12P2	**Pyr**	Pyruvate	C3H3O3
Fruc	Fructose	C6H12O6	**R5P**	Ribose-5-phosphate	C5H9O8P
G1P	Glucose-1-phosphate	C6H11O9P	**Ru5P**	Ribulose-5-phosphate	C5H9O8P
G6P	Glucose-6-phosphate	C6H11O9P	**S7P**	Sedoheptulose-5-phosphate	
GA	Glyceraldehyde	C3H6O3			C7H13O10P
Galac1P	Galactose-1-phosphate	C6H11O9P	**Xu5P**	Xylulose-5-phosphate	C5H9O8P
ADP	Adenosine diphosphate			C10H13N5O10P2	
ATP	Adenosine triphosphate			C10H13N5O13P3	
NAD	Nicotinamide adenine dinucleotide, oxidized form			C21H28N7O14P2	
NADH	Nicotinamide adenine dinucleotide, reduced form			C21H29N7O14P2	
NADP	Nicotinamide adenine dinucleotide phosphate, oxidized form			C21H29N7O17P3	
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form			C21H30N7O17P3	

Correspondence between Petri net transitions, abbreviations, and enzymatic reactions

Tn-name	enzyme name	reduced sequences	
hk	Hexokinase		
pgi	Phosphoglucose isomerase		
pfk	Phosphofructokinase	includes reaction:	al Aldolase
tpi	Triose phosphate isomerase		
gapA	GAP dehydrogenase		
pgK	Phosphoglycerate kinase		
bpgm	Bisphosphoglycerate mutase	includes reaction:	bpgp Bisphosphoglycerate phosphatase
gpm	Phosphoglycerate mutase	includes reactions:	eno Enolase pyk Pyruvate kinase
ldh	Lactate dehydrogenase		
scrK	Fructokinase	includes reaction:	f1pa Fructose 1-phosphate aldolase tk Triose kinase
hk2	Hexokinase		
galK	Galactokinase	includes reaction:	gal Galactose 1-phosphate uridyl transferase, UDP-Galactose 4-epimerase
pgm	Phosphoglucomutase		
g6pdh	Glucose 6-phosphate dehydrogenase	includes reactions:	6pgl Lactonase 6pgd 6-Phosphogluconate dehydrogenase
rpi	Phosphopentose isomerase		
rpe	Phosphopentose epimerase		
tkt	Transketolase		
tal	Transaldolase		
tkt2	Transketolase		