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Time Petri Nets for Modelling and Analysis of Biochemical Networks

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Abstract. Biochemical networks are modelled at different abstraction levels. Basically, qualitative and quantitative models can be distinguished, which are typically treated as separate ones. In this paper, we bridge the gap between qualitative and quantitative models and apply time Petri nets for modelling and analysis of molecular biological systems. We demonstrate how to develop quantitative models of biochemical networks in a systematic manner, starting from the underlying qualitative ones. For this purpose we exploit the well-established structural Petri net analysis technique of transition invariants, which may be interpreted as a characterisation of the system's steady state behaviour. For the analysis of the derived quantitative model, given as time Petri net, we present structural techniques to decide the time-dependent realisability of a transition sequence and to calculate its shortest and longest time length. All steps of the demonstrated approach consider systems of integer linear inequalities. The crucial point is the total avoidance of any state space construction. Therefore, the presented technology may be applied also to infinite systems, i.e. unbounded Petri nets.

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1. Introduction

Biochemical networks are modelled at different abstraction levels. It is common sense to differentiate between quantitative (kinetic) models and qualitative (stoichiometric or even purely causal) models. The fast majority of published biochemical case studies employs quantitative models; so they seem to be the favourite choice. The reason lies probably in the long-term objective to predict the systems' dynamic behaviour by continuous quantitative measures. Quantitative models are prevalently used as soon as a "critical mass" of the necessary kinetic parameters is known - such as substance concentrations, equilibrium constants, or reaction rates. Then, the missing kinetic parameters are usually taken from the taxonomically nearest neighbour species, for which they are available, or they are estimated. Related evaluation methods have typically to deal with systems of ordinary differential equations (ODEs), see e.g. [9]. Corresponding tools are e.g. GEPASI [16] and E-CELL [26]. But, available evaluation packages for quantitative models do not support any model validation techniques.

Contrary, qualitative models are generally used only, if kinetic parameters are indeed deficient or not available at all. Moreover, they are accepted tentatively as intermediate step for larger models, if the solution of ODEs is not feasible due to the problem complexity. Qualitative models consider the steady state of a biochemical network, where the kinetic parameters are supposed to be constant. All these qualitative models are based on some graph-theoretical description of the system topology, or structure, which is defined in case of stoichiometric models by the known stoichiometric equations. In biology, the qualitative analysis is basically used to derive all possible pathways through a network, especially the minimal ones, but without any explicit validation objective in mind. In [7] we have proposed to take advantage of the explicitly given system structure to validate the model for self-consistency or sensible biochemical interpretation, using approved graph theory results.

Typically, quantitative and qualitative models are handled as separate ones. In this paper, we bridge the gap between quantitative and qualitative models and apply a timed version of directed bichromatic multigraphs, the time Petri nets [17], for modelling and analysis of molecular biological systems. So, we introduce another intermediate step by way of a first quantitative model, which is still discretely treatable. We demonstrate, how to develop quantitative models of biochemical networks in a systematic manner, starting from the underlying qualitative one. For this purpose, we exploit the well-established structural Petri net analysis technique of transition invariants [13], which may be interpreted as a characterisation of the system's steady state behaviour. For the analysis of the derived quantitative model, given as time Petri net, we present a structural technique to decide the time-dependent realisability of a transition sequence, esp. of a transition invariant, given by its Parikh vector. Moreover, the shortest and longest time length for a transition sequence can be calculated. All steps of the demonstrated approach consider systems of integer linear inequalities. The techniques employed consist in their solution or the solution of a related linear program. The crucial point is the total avoidance of any state space construction in the whole approach. Therefore, the presented technology may be applied also to infinite systems, i.e. unbounded Petri nets.

This paper is organised as follows. The next two sections give an introduction into qualitative and quantitative modelling of biochemical networks using Petri nets, followed by a discussion of the quantitative analysis techniques applied. Afterwards, it is sketched how the proposed approach can be applied to a representative case study, the sucrose breakdown pathway in the potato tuber [10], [11]. Finally, we summarise some related work and give conclusions, comprising also an outlook.

2. Qualitative Modelling

Living organisms require a continuous influx of free energy to carry out their various functions. The term metabolism alludes to the overall process, through which living systems acquire and utilise the free energy they need. During this process many chemical reactions take place, usually catalysed by special enzymes, by which chemical compounds are converted into other chemical compounds. Refering to the processes' purpose, these involved primary chemical compounds are called metabolites. Additionally, there exist auxiliary compounds, which are generally supposed to be ubiquitous ones and available in sufficient quantities. Despite of the complexity of their internal processes, living systems maintain - under normal conditions - a steady state, where all primary and auxiliary compounds have reached a dynamic concentration equilibrium, i.e. the concentrations of all compounds are permanently reproduced in a constant amount due to the constant reaction rates.

Obviously, the steady state and the steady state behaviour are fundamental characteristics of any network. This explains why network evaluation typically starts with the steady state assumption, before taking into account also transient state behaviour, which might be caused, e.g., by a change of the living conditions. In this paper, the steady state behaviour will be considered only.

Metabolic networks, often also called metabolic pathways, consist of numerous networked enzymatic reactions, transforming input compounds, the educts, via several intermediate compounds into output compounds, the products. We have here an infinite continuous flux of chemical compounds. The steady state is maintained by a sophisticated mesh of metabolic control mechanisms. In metabolic pathways the chemical reactions of metabolites, given by their stoichiometric equations, are usually known, whereas the metabolite concentrations and other reaction-specific quantitative parameters are often unknown. Therefore it makes obviously sense to start with a qualitative model.

To derive a qualitative Petri net model of the biochemical network behaviour under the steady state assumption, each biochemical compound (metabolites, auxiliary compounds) is assigned to a place. The relations between biochemical compounds are established by chemical reactions. They are represented by transitions, modelling the biochemically atomic events. The corresponding arc multiplicities reflect the given stoichiometric numbers of the reactions' stoichiometric equations, compare Figure 1.

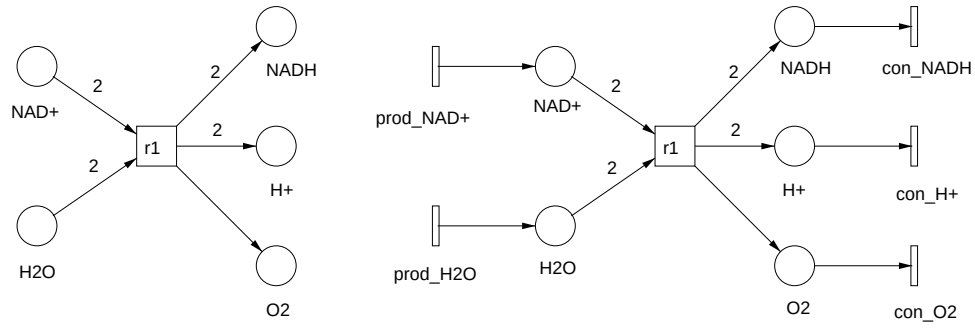

Figure 1. Example light-induced phosphorylation - 2 NAD^+ + 2 $H_2O \rightarrow$ 2 NADH + 2 H^+ + O_2. Left - the core model without environment behaviour, right - the core model enriched by the producing and consuming boundary transitions. Boundary transitions are given as flat ones to highlight their special meaning.

This straightforward modelling principle has been applied in a prosperous manner to a variety of biological networks, see [27] for a bibliography of related papers, and [6] for three representative case studies. The Petri net structure mirrors the biochemical network topology, and the incidence matrix of the Petri net coincides with the stoichiometric matrix of the modelled metabolic system.

Following this construction principle, we get place-bordered models (see Figure 1, left side), where the input compounds appear as source nodes (no predecessors) and the output compounds as sink nodes (no successors). Obviously, there is no initial marking, where each (re-) action may happen infinitely often to make the model live. To animate permanently such a core model, opening the doors to standard analysis techniques, we need an additional model component to describe the environment behaviour of the network under consideration, producing the input compounds and consuming the output compounds. There are basically three styles, how such an environment behaviour can be described, compare [6].

We use here a quite simple one, where the tokens for all input compounds are generated by supplemental input transitions (which are now the source nodes of the net), while the tokens of all output compounds are consumed by supplemental output transitions (becoming the new sink nodes). Doing so, the place-bordered models are transformed into transition-bordered ones (see Figure 1, right side). All these input and output transitions work independently. Therefore, no assumptions about the quantitative relations between the single input/input, input/output, and output/output compounds are made. Now, the expected Petri net behaviour reflects all partial order sequences of chemical reactions from the input to the output compounds respecting the given stoichiometric relations. To be precise, the qualitative (i.e. time-less) Petri net behaviour consists of all behaviour possible under any timing conditions.

Transitions without preplaces, i.e. without preconditions, may fire infinitely often (or with other words, infinitely fast). Thus, they are obviously live and all their immediate postplaces are unbounded. Generally, the whole Petri net model is expected to be live and simultaneously unbounded in all places. Consequently, no analysis methods can be applied, which rely on state space construction. Sometimes, the two expected properties (liveness, unboundedness) can be deduced by property-preserving structural reduction rules.

In the following section we demonstrate, how to derive systematically timing parameters from a structurally decidable property of the qualitative model, which reflects the steady state behaviour. The imposed time restrictions might make the model bounded.

3. Quantitative Modelling

To transform a qualitative model into a quantitative one, still representing the steady state behaviour, we exploit a fundamental behavioural, but structurally decidable Petri net property - the transition invariants, which are called in the following T-invariants for short.

T-invariants, introduced 1973 in [13], are multi-sets of transitions with a total zero effect on the marking; with other words, if such a multi-set fires (in an appropriate order), a given (appropriate) marking is reproduced. Therefore, in the context of metabolic Petri nets, T-invariants stand for multi-sets of chemical reactions, which are able to reproduce a given distribution of chemical compounds, e.g. the steady state, and they will do so, if the token situation allows the firing of all transitions involved. Then, they are called to be realizable ones. Due to the fact of state reproduction, an observed behaviour, establishing a T-invariant, may happen infinitely often, resulting into cyclic system behaviour.

A T-invariant corresponds to a possible pathway through the network, which is defined by the net representation of the T-invariant, i.e. a subnet of the whole network, consisting of the transitions belonging to the T-invariant, all their pre- and post-places and all arcs in between with their given multiplicities. If all transitions belong to a T-invariant, we call the network covered by T-invariants and all reactions may contribute to some pathway.

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, the so-called minimal T-invariants.[1] Minimal T-invariants correspond to minimal multi-sets of transitions with the state reproduction property, i.e. they do not include any subset of transitions with the property on hand. To calculate T-invariants, we need the incidence matrix $\mathcal{C}$ of the net, which is a (card(P) $\times$ card(T)) - matrix with P for the set of places and T for the set of transitions. An entry (i, j) in the incidence matrix gives the token change on the place i by firing of the transition j. We get all minimal T-invariants by determining the uniquely defined basis for all integer solutions of the following system of integer linear inequalities:

$$\begin{cases} \mathcal{C} \cdot x = 0 \\ x \geq 0 \\ x \neq 0 \end{cases},$$

whereby $\mathcal{C}$ is the incidence matrix, and x is the Parikh vector (counting vector) of a transition firing sequence in the net. Then, any cyclic system behaviour in the steady state may be described by a non-negative integer linear combination of minimal T-invariants.

In the given application setting, we distinguish two particular kinds of T-invariants. First, the two transitions, modelling a reversible reaction, establish always a trivial T-invariant. Second, among the non-trivial T-invariants the so-called I/O-T-invariants are often of special interest, which include boundary transitions.

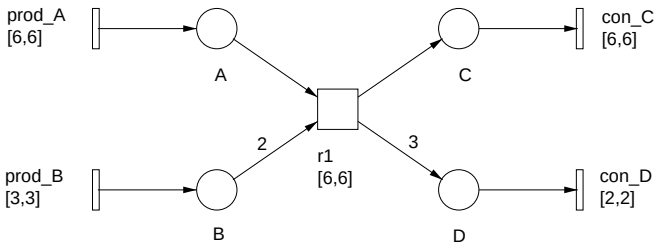

Figure 2. A transformation of a qualitative model into a quantitative one. Let's assume the following transition order: $prod\_A$, $prod\_B$, $r1$, con_C, con_D. The firing durations, given in brackets, are normalised to the time unit 6, the least common multiple of the minimal T-invariant's entries.

Let us consider the example given in Figure 2, at first as qualitative Petri net. The initial marking is empty. So, we look for T-invariants, reproducing the empty marking. Obviously, there is just one minimal T-invariant, given as Parikh vector (1, 2, 1, 1, 3), which is an I/O-T-invariant, covering the net.

[1] In [25] they are called elementary modes.

With other words, the given empty marking is reproduced, if the transitions $prod\_A$, $r1$, and con_C fire each ones, the transition $prod\_B$ twice, and the transition con_D three times, of course in accordance with the partial order defined by the net structure.

Moreover, due to the steady state assumption, the entries of a minimal T-invariant's Parikh vector correspond to the relative firing rates of the involved transitions to maintain - while firing continuously - the given stable state. Refering again to the example in Figure 2, the steady state is preserved as long as $prod\_B$ fires two times and con_D fires three times as often as the other three transitions, per any time unit. Using a timed-transition model, relative firing rates may be simulated by adjusting the transition times appropriately, as given in Figure 2 as interval times (compare next section). To get integers, the transition times are normalised to the time unit, which is given by the least common multiple of all entries in the minimal T-invariant (in the example, this time unit is 6).

Generally, minimal T-invariants may overlap. If a given transition is involved in several minimal T-invariants, the corresponding firing rate of this transition has to satisfy the steady state demands of all state-reproducing processes (pathways). Therefore, the appropriate firing rates of all transitions are given by the vector sum of all minimal non-trivial T-invariants, reduced by the greatest common divisor of all vector entries. Then, to get integer interval boundaries (firing durations), which reflect accurately all relative firing rates, the normalization time unit has to be chosen as the least common multiple of all entries in the vector sum. This calculation procedure may exceed the computer representation accuracy. In this case, the firing durations have to be approximated appropriately.

The calculation of T-invariants requires only structural reasoning. The state space does not have to be generated. Therefore, the danger of the famous state space explosion problem does not apply here. However, solving the given system of linear inequalities, as given above, is known to be of exponential complexity.

Due to the transformation procedure, we expect a kind of behavioural equivalence between the qualitative and the derived quantitative model. A natural equivalence notion seems to be that the basic behaviour, i.e. all minimal non-trivial T-invariants, of the qualitative model is preserved in the quantitative model. For this purpose, all minimal non-trivial T-invariants, which are realizable in the qualitative model, have to be still realizable in the quantitative model. To be precise, they have to occur in the steady state part(s) of the whole behaviour of the quantitative model, e.g. given by the terminal strongly connected component(s) of its reachability graph. However, in the next section, a promising analysis technique is introduced to check the time-dependent realizability of a given T-invariant, which does not construct the reachability graph. Especially, if the firing durations had to be approximated, this analysis technique might be used to get the approximation approved.

4. Quantitative Analysis

To make the paper self-contained, we refresh here the main technical results used for the approach presented in this paper.

Time Petri Nets (TPN) are classical Petri nets, where a time interval $[a_t, b_t]$ is associated to each transition t, whereby a_t and b_t are relative to the time, when t was enabled last. When t becomes enabled, it can not fire before a_t time units have elapsed, and it has to fire not later than b_t time units, unless t got disabled in between by the firing of another transition. The firing itself of a transition takes no time. The time interval is designed by real numbers, but the interval bounds are non-negative rational

numbers. It is easy to see (cf. [21]) that w.l.o.g. the interval bounds can be considered as integers only. Thus, the interval bounds a_t and b_t of any transition t are natural numbers, including zero, and $a_t \leq b_t$ or $b_t = \infty$.

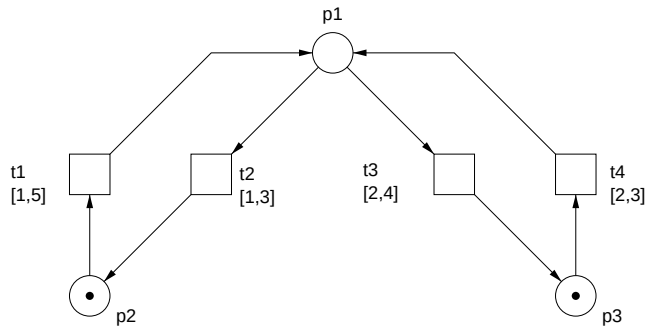

Figure 3. Z_1 - a Time Petri Net

Every possible situation in a given TPN can be described completely by a state $z = (m, h)$, consisting of a (place) marking m and a time marking h. The (place) marking, which is a place vector (i.e. the vector has as many components as places in the considered TPN), is defined as the marking notion in classical Petri nets. Thus, $m(p)$ gives the number of tokens in the place p in the net. The time marking, which is a transition vector (i.e. the vector has as many components as transitions in the considered TPN), describes the time circumstances in the considered situation. The value $h(t)$ shows the time elapsed since the transition t became most recently enabled, if t is enabled at the marking m, and $h(t) = \sharp$ otherwise. As initial state we consider the state $z_0 = (m_0, h_0)$ with $h(t) = 0$ for all transitions t, enabled at m_0.

In the net Z_1, compare Figure 3, the initial state is $z_0 = ((0, 1, 1), (0, \sharp, \sharp, 0))$. In the initial state, the transitions t_1 and t_4 are enabled, but neither t_1 nor t_4 may fire because of their time restrictions. Thus, z_0 can change into another state only as time elapses. For example, the change of states $z_0 \xrightarrow{1.3} z_1$ is feasible, where z_1 is given by $m_1 = m_0$ and $h_1 = (1.3, \sharp, \sharp, 1.3)$. Furthermore, z_1 can change into the state z_2 with $z_1 \xrightarrow{1.0} z_2$, where the state z_2 is given by $m_2 = m_1$ and $h_2 = (2.3, \sharp, \sharp, 2.3)$. In z_2 the transition t_4 can fire, yielding the state z_3 with: $m_3 = (1, 1, 0)$ and $h_3 = (2.3, 0, 0, \sharp)$. Now, as time progresses by 2, state z_3 changes into the state z_4, with $m_4 = m_3$, $h_4 = (4.3, 2.0, 2.0, \sharp)$. Subsequently, t_1 can fire and z_4 is changed into a state z_5 with $m_5 = (2, 0, 0)$ and $h_5 = (\sharp, 2.0, 2.0, \sharp)$. Thus, the sequence $z_o \xrightarrow{1.3} z_1 \xrightarrow{1.0} z_2 \xrightarrow{t_4} z_3 \xrightarrow{2.0} z_4 \xrightarrow{t_1} z_5$ is executable in Z_1. For more details and for formal definitions cf. [21].

Out of all possible states, the so-called integer states will be of special interest. A state $z = (m, h)$ is an "integer" one, iff $h(t)$ is an integer or $\sharp$ for each t. Considering the TPN Z_1 again, the initial state and the state z_5 are integer states, whereas the states z_1, z_2, z_3 and z_4 are not.

The set of all reachable states for a certain TPN, i.e. the state space of the net, is in general infinite (and dense), of course. The state space can be defined as the union of all sets C, defined below recursively:

Basis: $C_0 := \{z \mid \exists\tau(\tau \in \mathbb{R}_0^+ \wedge z_0 \xrightarrow{\tau} z)\}$

Step: Let C be already defined. Then C' is derived from C by firing t

(formally $C \xrightarrow{t} C'$), iff

$C' := \{z \mid \exists z_1 \exists z_2 \exists\tau(z_1 \in C \wedge \tau \in \mathbb{R}_0^+ \wedge z_1 \xrightarrow{t} z_2 \xrightarrow{\tau} z)\}$.

However, in [22] it is shown that the state space can be given parametrically. For this purpose, each set C is given parametrically by one state, whereby the time marking is defined parametrically. For example, considering the TPN Z_1 again, the set C_0 has the parametric form

$$C_0 = \{((0,1,1),(x_1,\sharp,\sharp,x_1)) \mid 0 \le x_1 \le 3\}.$$

After firing t_4 from an arbitrary state, belonging to C_0, the set C_1 will be achieved, and C_1 has the parametric form

$$C_1 = \{((1,1,0),(x_1+x_2,\sharp,x_2,\sharp)) \mid 2 \le x_1 \le 3, x_1+x_2 \le 5, 0 \le x_2 \le 4\}.$$

The parametric state $((0,1,1),(x_1,\sharp,\sharp,x_1))$ defines the set C_0, and the parametric state $((1,1,0),(x_1+x_2,\sharp,x_2,\sharp))$ defines the set C_1. The parameter x_1 in C_0 has to satisfy the constraint $0 \le x_1 \le 3$, and the parameters x_1, x_2 in C_1 have to satisfy the three constraints $2 \le x_1 \le 3, x_1+x_2 \le 5, 0 \le x_2 \le 4$. At the same time, C_0 is the parametric description of the empty transition sequence, and C_1 is the parametric description of the transition sequence t_4. Thus, the parametric description for the transition sequence t_4, t_3 is given by the following system of linear inequalities (SLI)

$$\{((0,1,1),(x_1+x_2+x_3,\sharp,\sharp,x_3)) \mid \underbrace{\begin{array}{l} 2 \le x_1 \le 3 \\ x_1+x_2 \le 5 \\ 2 \le x_2 \le 4 \\ x_1+x_2+x_3 \le 5 \\ 0 \le x_3 \le 3 \end{array}}_{(SLI-1)} \}.$$

Accordingly, for t_4, t_3, t_4 the parametric description is

$$\{((1,1,0),(x_1+x_2+x_3+x_4),\sharp,x_4,\sharp)) \mid \underbrace{\begin{array}{l} 2 \le x_1 \le 3 \\ x_1+x_2 \le 5 \\ 2 \le x_2 \le 4 \\ x_1+x_2+x_3 \le 5 \\ 2 \le x_3 \le 3 \\ x_1+x_2+x_3+x_4 \le 5 \\ 0 \le x_4 \le 4 \end{array}}_{(SLI-2)} \}.$$

For exact definition cf. [22].

Moreover, in [21] it is proven that the knowledge of the net behaviour in the reachable integer states is sufficient to determine the entire behaviour of the net at every time. The set of the integer states is finite, if and only if the time net is bounded. Thus, when a TPN is bounded, qualitative and quantitative analyses can be done using the integer states only. However, in case of unbounded TPN or if the state space exceeds the available memory capacity, a lot of properties can be studied using the parametric description only.

Till now it was assumed that all values $h_0(t)$ are 0 or $\sharp$. It is easy to see that similar considerations can be done starting with an arbitrary initial state. In this case, the states belonging to the state class C_0 have to satisfy the following two conditions: The "old" condition $x_1 \leq \min\{lft(t) \mid t \in T \wedge t^- \leq m_0\}$ is kept and the inequality $x_1 \geq 0$ is replaced by the condition $x_1 \geq \max\{h_0(t) \mid t \in T \wedge t^- \leq m_0\}$. Further it is clear that, because of the free choice of the initial state, now a path can be studied starting at an arbitrary state. Of course it makes sense to start at a reachable one, but this is no restriction at all.

As already introduced in [8] we use the parametric description of a given transition sequence in two ways: first, in order to decide, if the sequence can fire in the TPN; and second, applying linear optimisation, to compute the shortest and longest time length of the sequence. For example, in order to verify that the transition sequence t_4t_3 can fire in the TPN Z_1 we have to prove that the system of linear inequalities (SLI-1) is solvable. Analogously, the unsolvability of the linear systems of inequalities (SLI-2) means that the transition sequence t_4, t_3, t_4 can not fire in the TPN Z_1. For computing the longest time length for the sequence t_4, t_3 we have to solve the *Linear Program*

$$x_1 + x_2 + x_3 \longrightarrow \max$$

$$\text{subject to (SLI-1)} .$$

In [8] it is shown that the shortest and the longest time length between two markings m and m' is an integer one, if finite. This result is based on the algorithm, given in [22]:

Let $\sigma = (t_1, \tau_1, t_2, \tau_2, ..., \tau_{n-1}, t_n)$ be a sequence between two given states in a TPN, where all the times $\tau_i, i = 1, ..., n-1$ are *nonnegative real numbers*. Then, the algorithm finds two further sequences $\sigma_1 = (t_1, \tau_1^{(1)}, t_2, \tau_2^{(1)}, ..., \tau_{n-1}^{(1)}, t_n)$ and $\sigma_2 = (t_1, \tau_1^{(2)}, t_2, \tau_2^{(2)}, ..., \tau_{n-1}^{(2)}, t_n)$, where all the times $\tau_i^{(j)},\ j = 1, ..., n-1$ are *integers*, and it holds for the time lengths of the three sequences:

$$\textit{the length of } \sigma_1 \leq \textit{the length of } \sigma \leq \textit{the length of } \sigma_2$$

The running time of this algorithm is $\mathcal{O}(n^2)$, whereas finding an integer solution of an inequality system is a NP-hard problem, in general.

In this paper, TPNs are used to model metabolic systems. In order to give time windows for certain pathways - not necessarily minimal ones, in the steady state, their shortest and longest time length are of interest. These time windows characterize the duration of the network's transformation of the input into the output compounds (network response duration). Moreover, the duration range of the transient state, i.e. the shortest and longest time length of reaction sequences to enter the steady state, might be worth being communicated. Please note, all the considered time notions are relative ones.

5. Case Study - Central Carbon Metabolism in the Potato Tuber

The accumulation of starch in the *Solanum tuberosum* (potato) tuber is a crucial point in biotechnology [10]. The major flux in the potato tuber carbon metabolism is the conversion of sucrose through hexose phosphates into starch. Nearly all genes, believed to be directly involved in the sucrose breakdown transformation, have been cloned by transgenic approaches. However, some fundamental questions are still open. A deeper understanding of the network behaviour, underlaying the whole metabolism, might be beneficial.

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.

Altogether, this metabolic network is characterised by 16 chemical stoichiometric equations, seven of them are reversible ones. For more details see [10]. The corresponding Petri net, compare Figure 4, consists of 17 places (10 primary compounds, among them one input compound $eSuc$, and one output compound $starch$, and 7 ubiquitous substances) and 25 transitions (9 for the non-reversible reactions, 2·7 for the reversible reactions, one input transition g_eSuc, and one output transition r_starch). There are 19 minimal T-invariants covering the net, for details see [11]. Seven of them are trivial ones, corresponding to the seven reversible reactions. The remaining twelve non-trivial T-invariants go into the calculation of the transition firing rates, following the approach sketched in section 3. The entries in the vector sum of all minimal T-invariants vary between 6 and 236. The least common multiple of all entries in the vector sum exceeds the standard computer accuracy. Thus, we have to skip this last normalization step and the firing times have to be approximated by rational numbers. Doing so, we abandon the chance to construct the integer state space, but our original objective - to get structural methods based on the state space's parametric description - still works.

Using the parametric description approach, as summarized in section 4, it can be shown that all minimal T-invariants are still realisable in the steady state of the derived time Petri net model. Doing so, we can be sure that the derived timed model is self-consistent. Moreover, the time windows for the durations of pathways of special interest can be calculated using linear programming.

As a next step it is planned to compare this quantitative TPN model and the calculated measures with the quantitative model, given in [10] as a system of ordinary differential equations.

6. 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 [20]. The first paper, really demonstrating the modelling of metabolic processes by Petri nets, appeared 1993 [24]. In the meantime, several research groups followed this line. But a closer look on the literature (see [27] for a bibliography) reveals that the majority of papers, applying Petri nets for modelling and analysis of biological systems, concentrate on quantitative aspects. Typical examples of used Petri net extensions are stochastic Petri nets [18], [19] and hybrid Petri nets [4], [14], [15], but also coloured Petri nets [5] as well as discrete time extensions [12] have been employed for that purpose. Contrary,

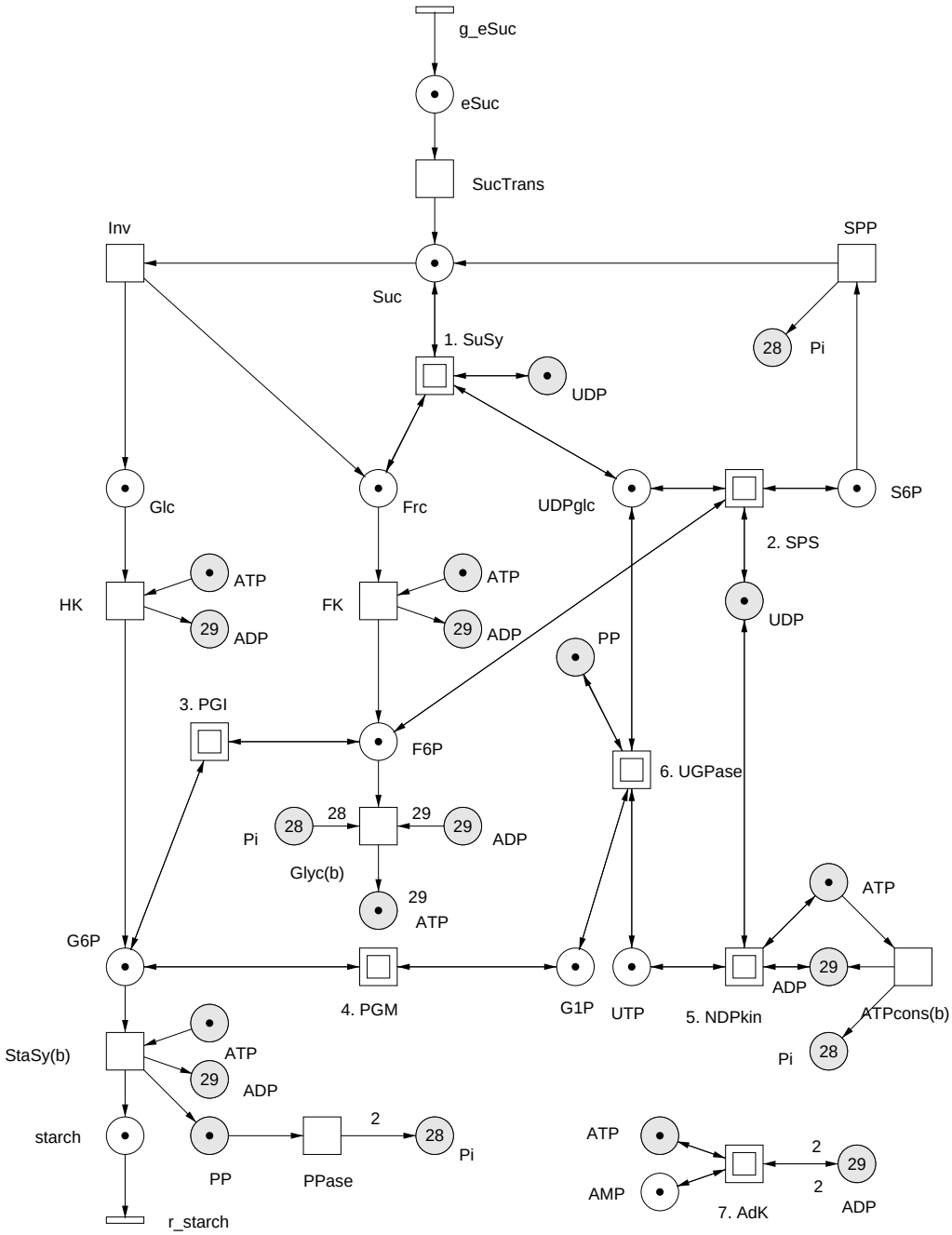

Figure 4. The hierarchical Petri net model of the sucrose-to-starch pathway in the potato tuber. The macro transitions, given as two centered squares, hide each the two complementary transitions modelling reversible reactions. The flat transitions depict the generating input or consuming output transitions, respectively. Shadowed nodes stand for fusion nodes, modelling ubiquitous auxiliary substances. The given marking reflects a state, where all transitions are enabled.

qualitative aspects are discussed only in a few papers, see e.g. [24], [23], [6], [7]. No paper is known to discuss and present an approach how to derive the quantitative model in a systematic manner from the qualitative one.

Computations, similar to the ones discussed in section 4, have also been made for a slightly modified time Petri net in [3]. There, the proofs are based on the analysis method of time Petri nets, introduced in [2] and further considered in [1].

7. Conclusions

In this paper we use time Petri nets to develop a discretely treatable quantitative model for biochemical networks. Our approach starts with the qualitative model and the well-established structural analysis method to computate the minimal transition invariants. After converting the qualitative Petri net into a quantitative one, we give a structural technique to prove the time-dependent realisability of a given transition sequence, and by this means of a transition invariant. Moreover, we are able to calculate the shortest and longest time length of transition sequences using linear programming. The crucial point of the whole approach is the total avoidance of any state space construction. Therefore, it may be applied also to infinite systems, i.e. unbounded Petri nets.

Up to now, only such interval times are used, where lower and upper bounds are equal. This special case is a result of the calculation procedure relying entirely on the qualitative model. As soon as timing parameters are also derived experimentally, interval times are recommendable to cope with unavoidable measurement inaccuracies. That's why we deal with the more general case right from the beginning. It might be of interest whether the analysis techniques would enjoy severe simplification, if the given application does indeed restrict itself to the special case only.

The objective of our work is a general integrative approach to model and analyse biochemical networks qualitatively as well as quantitatively. Each kind of model contributes a different perspective by providing different analysis techniques and related conclusions. Thus, they are not competing, but complementing each other.

In this paper, we explain our approach for a special metabolic network of the central carbon metabolism in the potato tuber. But it may be applied to other biochemical networks of different types, e.g. signal transduction pathways, too.

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