Modelling and Analysis of Biochemical Networks with Time Petri Nets

Extended Abstract

Louchka Popova-Zeugmann[1], Monika Heiner[2], and Ina Koch[3]

[1] Humboldt-Universität zu Berlin, Department of Computer Science, Berlin, Germany, popova@informatik.hu-berlin.de
[2] Brandenburg University of Technology Cottbus, Department of Computer Science, Cottbus, Germany
monika.heiner@informatik.tu-cottbus.de
[3] Technical University of Applied Sciences Berlin, Department of Bioinformatics, Berlin, Germany, ina.koch@tfh-berlin.de

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 long-term objective of quantitative models is to predict the systems' dynamic behaviour. They are commonly used as soon as the necessary kinetic parameters are known, such as substance concentrations, equilibrium constants, and reaction rates. Related evaluation methods are typically based on solutions of systems of differential equations, see e. g. [9]. Corresponding tools are e. g. GEPASI [15] and E-CELL [25]. 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 not available or incomplete. Therefore, they 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, which is defined in case of stoichiometric models by the known stoichiometric equations. Then, the system topology - or structure - may be validated for self-consistency or sensible biochemical interpretation using approved graph theory results.

In this paper, we bridge the gap between quantitative and qualitative models and apply a timed version of bichromatic graphs, the time Petri nets [16], 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 one. For this purpose, we exploit the well-established 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 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. The crucial point of the presented approach is the total avoidance of any state space construction. Therefore, it may be applied also to infinite systems, i. e. unbounded Petri nets.

This extended abstract is organized as follows. The next two sections introduce into qualitative and quantitative modelling of biochemical networks using Petri nets, followed by a discussion of the quantitative analysis. Afterwards, the proposed approach is demonstrated using a representative case study, the sucrose breakdown pathway in the potato tuber [10]. Finally, we summarize some related work.

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, by which chemical compounds are converted into other chemical compounds, often catalysed by special enzymes. 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. 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. With other words, the concentrations of all compounds are constant.

Metabolic networks, often also called metabolic pathways, consist of numerous consecutive enzymatic reactions, transforming input compounds, the substrates, 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 controls. In metabolic pathways the chemical reactions of metabolites, given by their stoichiometric equations, are usually known, whereas the metabolite concentrations and other reaction constants are often unknown.

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

This straightforward modelling principle has been applied successfully to a variety of biological pathways, see [26] for a bibliography of related papers, and [6] for three representative case studies. The Petri net structure mirrors the biochemical topology, and the incidence matrix of the Petri net is identical to the stoichiometric matrix of the modelled metabolic system.

Following this line, we get place-bordered models, where the input compounds appear as source nodes (no predecessors) and the output compounds as sink nodes (no successors). To animate and analyse such a model, we need a model component to describe the environment behaviour 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). We get transition-bordered net models. Doing so, no assumptions about the quantitative relations between input/input, input/output, and output/output compounds are made. The expected Petri net behaviour consists of all partial order sequences of chemical reactions from the input to the output compounds respecting the given stoichiometric relations.

Transitions without preplaces, i. e. without preconditions, may fire infinitely often. So, they are obviously live and all their immediate postplaces are unbounded. Generally, the whole net is expected to be live and simultaneously unbounded in all places. Consequently, no analysis methods can be applied, which rely on a state space construction. Sometimes, the expected properties can be deduced by property-preserving structural reduction rules.

In the following section we demonstrate how to derive systematically timing parameters from a structural property of the qualitative model, which reflect the steady state. 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 Petri net property - the transition invariants, which are called in the following T-invariants for short.

T-invariants, introduced 1973 in [12], are multi-sets of transitions, able to reproduce a given marking, i. e. in the context of metabolic Petri nets - sets of chemical reactions, able to reproduce a given distribution of chemical compounds. Due to the fact of state reproduction, an observed behaviour, establishing a T-invariant, may happen infinitely often, resulting into cyclic system behaviour.

To describe all possible behaviour in a given cyclic system, it would be obviously of great help to have all system's basic (cyclic) behaviour, the so-called minimal T-invariants. In [24] they are called elementary modes. We get all minimal T-invariants by determining a generating system for all solutions of the following system of inequalities:

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

whereby $\mathcal{C}$ is the incidence matrix – a (card(P) $\times$ card(T)) - matrix with P for the set of places and T for the set of transitions – and x is the Parikh-vector of a transition sequence in the net. Then, any system behaviour may be described by a non-negative integer linear combination of minimal T-invariants.

Moreover, due to the steady state assumption, the components of a minimal T-invariant correspond to the relative firing rates of the involved transitions to maintain – while firing continuously – the given state. Relative firing rates may be simulated, using a timed transition model, by adjusting the transition times 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 integer linear programming problem, as given above, is known to be NP-complete.

4 Quantitative Analysis

Time Petri Nets (TPN) are classical Petri nets (PN), 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 last enabled. When t becomes enabled, it can not fire until a_t time units have elapsed, and it must fire not later than b_t time units, unless t is disabled by the firing of another transition. Firing a transition takes no time. The time is designed by real numbers, but the interval bounds are rational numbers. It is easy to see (cf. [20]) that w. l. o. g. the interval bounds can be considered as integers only.

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, is defined like the marking notion in classical PN. Thus, $m(p)$ gives the number of tokens in the place p in the net. The time marking, which is a transition vector, describes the time circumstance 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.

Of special interest are the so-called integer states. A state $z = (m, h)$ is an "integer" one, iff $h(t)$ is an integer or $\sharp$ for each t.

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. However, in [21] it is shown that the state space can be given parametrically and 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. In case of unbounded TPN, a lot of properties can be studied using the parametrical description.

In this work, metabolic systems are modelled by TPNs. In order to give time windows for the recurrent processes the shortest and longest time length have to

be computed. 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 verify deadlines by computing the longest time length of the sequence. In [8] it is shown that the shortest and the longest time length between two markings m and m' (exactly, between two states, the place markings of which are m and m') is (if finite) an integer one.

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. The major flux in the potato tuber carbon metabolism is the conversion of sucrose through hexose phosphates. 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 obviously of help.

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 pathway is characterised by 16 chemical stoichiometric equations, seven of them are reversible ones. For more details see [10]. The corresponding Petri net, see Figure 1, 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. Seven of them are trivial ones, corresponding to the reversible reactions. The remaining twelve non-trivial T-invariants are exploited for the calculation of the transitions firing rates, as sketched in section 3.

Using the parametric description approach, as sketched in section 4, it can be shown that all minimal T-invariants are still realizable in the steady state of the derived time Petri net model. Moreover, the time windows for the T-invariants' duration can be calculated.

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.

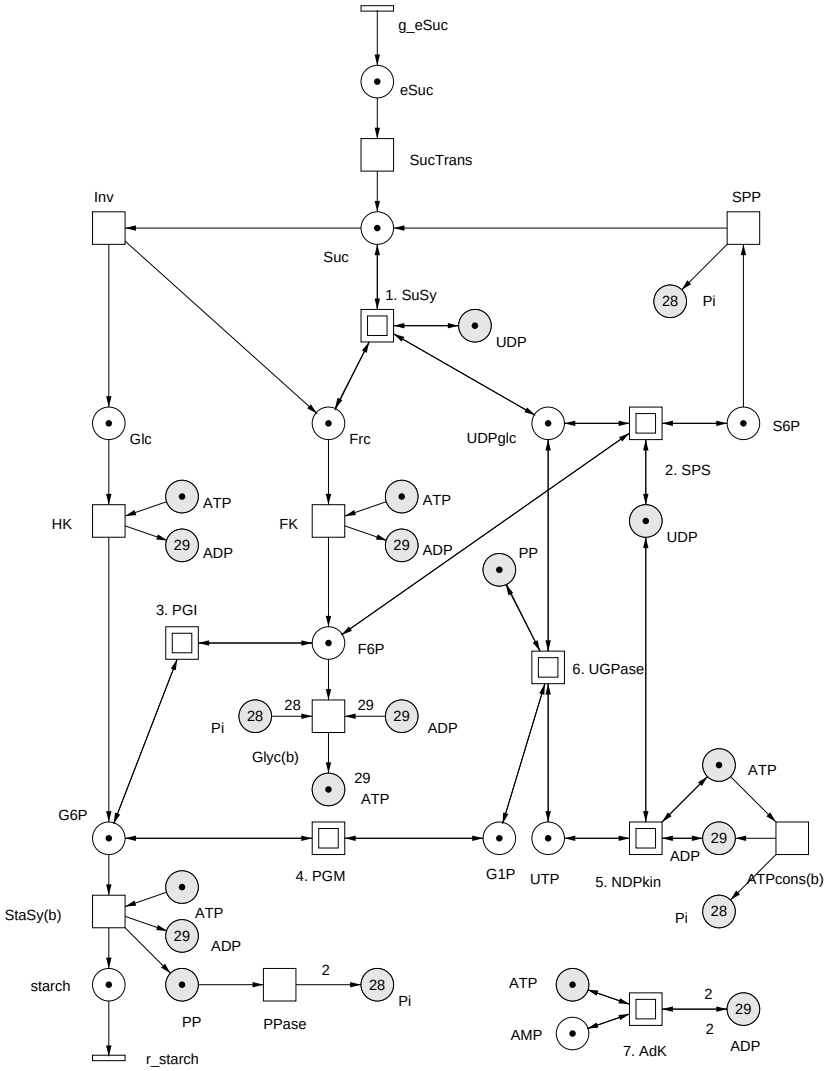

Fig. 1. The hierarchical Petri net model of the sucrose-to-starch pathway in the potato tuber. The macro transitions, given as two centered squares, hide each the two complementary transitions modelling reversible reactions. The flat transitions depict the generating input or consuming output transitions, respectively. Shadowed nodes stand for fusion nodes, modelling ubiquitous auxiliary substances. The given marking reflects a state, where all transitions are enabled.

Petri in his paper on interpretations of net theory [19]. The first paper, really demonstrating the modelling of metabolic processes by Petri nets, appeared 1993

[23]. In the meantime, several research groups followed this line. But a closer look on the literature (see [26] 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 [17], [18] and hybrid Petri nets [4], [13], [14], but also coloured Petri nets [5] as well as discrete time extensions [11] have been employed for that purpose. Contrary, qualitative aspects are discussed only in a few papers, see e.g. [23], [22], [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 TPN in [3]. The proofs there are based on the analysis method of TPN, introduced in [2] and further considered in [1].

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