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POZIOMY ANALIZY T-NIEZMIENNIKÓW W CZASOWYCH SIECIACH PETRIEGO*

Streszczenie. t-niezmienniki są powszechnie stosowane w analizie modeli systemów biologicznych opartych na sieciach Petriego, gdzie często interpretuje się je jako pewne podprocesy. Jeżeli klasyczną sieć Petriego rozszerzy się do czasowej, zbiór niezmienników pozostaje ten sam, jednak ich interpretacja może ulec zmianie ze względu na dodatkowe ograniczenia czasowe. W niniejszym artykule wyróżniono kilka poziomów analizy t-niezmienników. Proponowaną klasyfikację zastosowano do modelu ekspresji genów opartego na czasowych sieciach Petriego. Analiza pokazuje, że niektóre t-niezmienniki można interpretować jako autonomiczne procesy, podczas gdy inne są możliwe do zrealizowania jedynie jako wybrane sekwencje w ramach większych przebiegów czasowych. Wyniki wskazują, że analiza czasowa stanowi dodatkowy i wartościowy poziom oceny t-niezmienników w sieci czasowej.

LEVELS OF ANALYSIS OF T-INVARIANTS IN TIME PETRI NETS

Summary. t-invariants are commonly used in Petri net-based analysis of models of biological systems, where they are often interpreted as subprocesses. However, when a classical Petri net is extended to a time Petri net, the set of t-invariants remains unchanged, while their interpretation may change due to additional time constraints. In this paper, several levels of interpretation of t-invariants are distinguished. The proposed classification is applied to a time Petri net model of gene expression. The case study shows that some t-invariants can be interpreted as autonomous timed processes, whereas others are feasible only as selected sequences within larger sequence of firings. The results indicate that time analysis provides an additional validation level for t-invariants in a time net.

1. Introduction

Petri nets are a discrete formalism widely used for modeling complex systems, including biological ones, especially when concurrency, causal dependencies and structural analysis are important [1, 6, 8]. One of their advantages is that the same net struc-

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ture can be analyzed qualitatively first and then extended with additional quantitative information, such as time constraints or stochastic parameters [4].

An important part of the Petri net-based analysis is the study of t-invariants. In biological applications, they are often interpreted as subprocesses or fragments of steady-state behavior [2, 3, 14]. However, this interpretation is based mainly on the structure of the net. As a consequence, a correct t-invariant does not necessarily correspond to a feasible or biologically meaningful behavior. This problem is especially relevant in models that contain read arcs, since such arcs are not represented in the incidence matrix. In Petri net models, a read arc requires a specified number of tokens to be present in a place for a transition to fire, but these tokens are not consumed. It can also be represented by an input and an output arc of equal weight whose contributions to the incidence matrix cancel, so the corresponding matrix entry is zero although the arc still affects transition activation [4, 14].

Adding time to a Petri net model introduces an additional interpretative layer. In time Petri nets (TPN), each transition is assigned a time interval and only within it, transition can fire and produce tokens [11, 12]. This temporalization does not modify the incidence matrix of the classical Petri net and therefore does not change the set of t-invariants computed for this structure. Nevertheless, it may change the answer to the question whether a given t-invariant can be realized as an actual process in the time net. Earlier studies have shown that t-invariants can be used as a starting point to move from qualitative to timed models and to study the time feasibility of transition sequences [9, 10]. A similar distinction between the timeless net structure and the timed behavior also appears in recent TPN studies, where t-invariants are used in the analysis of time-dependent properties [13]. Different extensions of Petri nets, including timed, stochastic, colored or hybrid have been used to represent complex aspects of biological systems [4, 7].

The research problem considered in this paper is therefore not whether t-invariants exist in a TPN, since they are still obtained from the unchanged incidence matrix of the underlying classical net. The problem is whether they preserve their interpretation as feasible processes when the read arcs, conflicts between transitions and time constraints are taken into account. In particular, a t-invariant specifies only how many times particular transitions should be fired. It does not determine the order of these firings, and it does not guaranty that a corresponding firing sequence exists for a given initial marking. In a TPN, even such a sequence may fail to have a valid timed realization satisfying the assumed time constraints.

The aim of this paper is to analyze how the addition of TPN semantics affects the interpretation of t-invariants computed for the underlying net structure. For this purpose, several levels of interpretation are proposed. The proposed approach is intended to support the assessment of whether a t-invariant can be treated as an autonomous timed process, only as a fragment of a broader timed behavior or merely as a mathematical object whose biological interpretation requires additional attention.

2. Classical and time Petri nets

Definition 1. *Petri net* [8]

A Petri net is a tuple $\mathcal{N} = (P, T, F, W, m_0)$, where $P = \{p_1, \dots, p_n\}$ is a finite

set of places, $T = \{t_1, \dots, t_m\}$ is a finite set of transitions, $P \cap T = \emptyset$, $F \subseteq (P \times T) \cup (T \times P)$ is a set of arcs, $W : F \rightarrow \mathbb{N}_+$ is a function assigning positive integer weights to arcs and $m_0 : P \rightarrow \mathbb{N}$ defines the initial marking.

A marking m assigns to each place a number representing tokens. Transition t_j is active in a given marking m if each of its input places contains at least as many tokens as required by the weight of the corresponding input arc. Firing an active transition removes tokens from its input places and adds tokens to its output places according to the arc weights. In some models read arcs can be used to represent conditions that require the presence of a token without consuming it. Since such an arc does not change the marking, its effect is not visible in the incidence matrix and later must be taken into account when analyzing, e.g., the feasibility of t-invariants. In models of biological systems, places usually represent chemical compounds, complexes or states of the system, whereas transitions correspond to reactions or regulatory events.

Definition 2. *Time Petri net (TPN)* [12]

A time Petri net is a pair $\mathcal{T} = (\mathcal{N}, I)$, where $\mathcal{N} = (P, T, F, W, m_0)$ is a classical Petri net and $I : T \rightarrow \mathbb{Q}_0^+ \times (\mathbb{Q}_0^+ \cup \{+\infty\})$ assigns to each transition a time interval, and for each $t \in T$, in $I(t) = (I_1(t), I_2(t))$ it holds that $I_1(t) \leq I_2(t)$.

When transition t becomes active, the time elapsed since this activation starts to be measured. The transition cannot fire before time $I_1(t)$ has elapsed (called the *earliest firing time* or *eft*), and must fire no later than after $I_2(t)$ has elapsed. This second value is called *latest firing time* or *lft* for short. Throughout this paper, strong TPN semantics is assumed, i.e., an active transition cannot be postponed beyond its latest firing time.

3. Levels of interpretation of t-invariants in a TPN

In this section, several levels of interpretation of t-invariants are introduced. This classification does not concern the way in which t-invariants are computed. However, it concerns the question of whether a given t-invariant can be associated with an actual behavior of the net, first without considering time and then in a TPN.

Let $C = [c_{ij}]_{n \times m}$ be an incidence matrix of net $\mathcal{N}$. In such a matrix, c_{ij} denotes the change in the number of tokens in place p_i caused by firing transition t_j . A t-invariant is given in the following Definition 3.

Definition 3. *t-invariant* [14]

Let C denote the incidence matrix of $\mathcal{N}$. A t-invariant x is a vector $x \in \mathbb{N}^m$ satisfying the equation $C \cdot x = 0$. The set of all such t-invariants is denoted by $\mathcal{X}$.

The support of t-invariant x is set $s(x) = \{t_j \in T : x_j > 0; j = 1, 2, \dots, m\}$. Firing the transitions from this set x_j number of times returns the net to the same marking as before the first transition from $s(x)$ fired. In practice, t-invariants are usually the basic units of analysis representing some subprocesses within the modeled system. In the case of a TPN, equation $C \cdot x = 0$ remains unchanged for the underlying net structure (i.e., a *skeleton*). However, the information contained in the vector x alone does not determine whether there exists a firing sequence and a corresponding assignment of firing times that satisfy the constraints *eft* and *lft*. For this reason, we distinguish between

a (structural) t-invariant, its support, a possible firing sequence and a timed realization of this sequence.

A t-invariant is a mathematical object. Its existence means that a certain combination of transition firings does not change the marking. However, it does not imply that there exists a firing sequence executable from the initial marking m_0 (or any given starting marking m from which a given t-invariant starts). In the following definitions, when we say that a firing sequence corresponds to a t-invariant x , we mean that every transition t_j occurs in this sequence exactly x_j times. A *transition sequence* $\sigma = t_{i_1} t_{i_2} \dots t_{i_k}$ specifies the assumed order of firings of transitions present in σ .

Definition 4. *Feasible t-invariant* [14]

A t-invariant x is called feasible if there exists a transition sequence σ executable in net $\mathcal{N}$ from a given marking m , such that every transition t_j occurs in σ exactly x_j times. The set of such t-invariants is denoted by $\mathcal{X}_{\text{feas}}$.

If such a sequence σ exists, then $m + C \cdot x = m$, which we can denote as $m \xrightarrow{\sigma} m$, since $C \cdot x = 0$. However, this equality does not express all the enabling conditions. If the net contains read arcs, some transitions of σ may require the presence of tokens in places, and one must remember that neither tokens nor read arcs are represented in the incidence matrix. Therefore, t-invariant may be valid as a solution of $C \cdot x = 0$, while no executable transition sequence corresponding to it exists from the starting marking m . Therefore, the distinction between $\mathcal{X}$ and $\mathcal{X}_{\text{feas}}$ is an important stage in the analysis.

The next level of distinction concerns time feasibility. It requires that firing times can be assigned to a transition sequence in a way consistent with time function I . Based on the TPN semantics introduced above, in this paper we propose three new definitions. By timed realization (or *timed run*) of a sequence $\sigma = t_{i_1} t_{i_2} \dots t_{i_k}$ we understand a sequence of pairs $\sigma^\tau = ((t_{i_1}, \tau_1), (t_{i_2}, \tau_2), \dots, (t_{i_k}, \tau_k))$, where τ_1 is the time elapsed from the beginning of the run to the first firing, and for $\ell > 1$, τ_ℓ is the time elapsed between the firing of $t_{i_{\ell-1}}$ and the firing of t_{i_ℓ} . Thus, the total firing time is $\Theta_\ell = \tau_1 + \tau_2 + \dots + \tau_\ell$. A timed realization is valid if every firing satisfies the activation rule and the time constraints of the TPN. In particular, if transition t_{i_ℓ} becomes active at time τ_ℓ^{act} and fires as the ℓ -th transition of the sequence, then $I_1(t_{i_\ell}) \leq \Theta_\ell - \tau_\ell^{\text{act}} \leq I_2(t_{i_\ell})$.

Definition 5. *Strong TPN-feasible t-invariant*

A t-invariant x is called strong TPN-feasible in net $\mathcal{T} = (\mathcal{N}, I)$ if there exists a transition sequence σ such that every transition t_j occurs in σ exactly x_j times, no transition outside $s(x)$ occurs in σ , and σ has a valid timed realization in $\mathcal{T}$. The set of such t-invariants is denoted by $\mathcal{X}_s^T$.

The term strong refers here to the fact that the realization is restricted to the support of the t-invariant. A strong TPN-feasible t-invariant can be interpreted as an autonomous timed process. This means that the transitions belonging to the support of x can be fired (a required number of times) without firing any transition outside of $s(x)$. However, in practice, transitions outside $s(x)$ may still become active during the run of σ . If their I_2 constraint forces them to fire before σ can be completed, then such a t-invariant is not strong TPN-feasible, since σ does not have a valid timed realization. A good example is a case when a time sequence σ^τ consisting of ℓ transitions has a total firing time Θ_ℓ

that is longer than $I_2(t)$ of some input (always active) transition t . Therefore, t must fire before σ^τ is finished, making such a sequence invalid from the definition 5. perspective. Therefore, t-invariant x with its firing sequence σ^τ cannot be classified as strong TPN-feasible. The next two definitions provide an additional classification level for such a case.

Definition 6. *Strict weak TPN-feasible t-invariant*

A t-invariant x is called *strict weak TPN-feasible* in $\mathcal{T} = (\mathcal{N}, I)$ if there exists a valid timed run σ^τ in $\mathcal{T}$ such that, after removing from this run all firings of transitions not belonging to $s(x)$, every transition $t_j \in s(x)$ occurs exactly x_j times in the remaining transitions sequence. The set of such t-invariants is denoted by $\mathcal{X}_{\text{sw}}^\tau$.

Strict weak TPN-feasibility allows the process represented by a t-invariant to occur inside a broader timed run. In such a run, transitions outside $s(x)$ may fire. However, additional firings of transitions from $s(x)$ are not allowed, because after ignoring all firings outside $s(x)$, the remaining sequence must still correspond exactly to t-invariant x .

Definition 7. *Weak TPN-feasible t-invariant*

A t-invariant x is called *weak TPN-feasible* in $\mathcal{T} = (\mathcal{N}, I)$ if there exists a valid timed run σ^τ in $\mathcal{T}$ and a selected subsequence ρ^τ of σ^τ (preserving the order of firings in σ^τ) such that all transitions from ρ^τ belong to $s(x)$, and for every $t_j \in s(x)$, subsequence ρ^τ contains exactly x_j firings of t_j . The set of such t-invariants is denoted by $\mathcal{X}_w^\tau$.

The remaining firings in σ^τ (not belonging to ρ^τ), including additional firings of transitions from $s(x)$, are treated as additional timed events. Previously defined strict weak TPN-feasibility does not allow additional firings of transitions from the support of the t-invariant, while weak TPN-feasibility allows them, provided that one firing sequence corresponding to x can still be selected from the run.

Some relations hold between the introduced classes. Every strong TPN-feasible t-invariant must also be feasible in the classical net, i.e.,

$$\mathcal{X}_s^\tau \subseteq \mathcal{X}_{\text{feas}} \subseteq \mathcal{X}.$$

Moreover, every strong TPN-feasible t-invariant is also strict weak TPN-feasible, and every strict weak TPN-feasible t-invariant is weak TPN-feasible:

$$\mathcal{X}_s^\tau \subseteq \mathcal{X}_{\text{sw}}^\tau \subseteq \mathcal{X}_w^\tau.$$

The first inclusion follows from the fact that a run containing no transitions outside $s(x)$ is a special case of a run in which such transitions are allowed. The second inclusion follows because if all firings of transitions from $s(x)$ in a run correspond exactly to x , then this part of the run can also be treated as the selected subsequence required in the weak case. No general inclusion between $\mathcal{X}_{\text{feas}}$ and $\mathcal{X}_{\text{sw}}^\tau$ or $\mathcal{X}_w^\tau$ is assumed. A t-invariant may be feasible in the untimed net but infeasible under time constraints.

From the biological interpretation point of view, the distinction between the above classes is very important. A t-invariant only describes a vector x for which $C \cdot x = 0$. A feasible t-invariant additionally corresponds to a possible untimed behavior of the model. A strong TPN-feasible t-invariant can be interpreted as an autonomous timed

process. A strict weak TPN-feasible t-invariant may occur in a broader timed run but without additional firings of transitions from its support, while a weak TPN-feasible t-invariant describes one recognizable firing sequence selected from a larger timed behavior.

4. Time-dependent analysis of t-invariants

The purpose of the analysis is to specify how the interpretation of t-invariant changes when time Petri nets are considered. The first stage is the classical structural analysis, i.e., for net $\mathcal{N}$ the set of t-invariants is computed. The second stage is the feasibility analysis, in which, for each t-invariant x , transition sequences σ are searched for, in which every transition t_j occurs exactly x_j times. These sequences must be executable from the starting marking m , taking into account all transition activation conditions, including those resulting from read arcs. This stage makes it possible to identify t-invariants that do not correspond to any behavior possible from the considered initial state of the net.

The third stage is the analysis of time feasibility in net $\mathcal{T}$. For each sequence σ corresponding to some t-invariant, it is checked whether there exists an assignment of firing times satisfying the constraints $I_1(t)$ and $I_2(t)$ for all transitions. In particular, it is necessary to take into account the fact that, under strong TPN semantics, an active transition cannot be postponed indefinitely. If its $I_2(t)$ time is reached, the transition has to fire immediately. Therefore, the feasibility of a sequence may depend not only on the transitions belonging to the support of the analyzed t-invariant, but also on other or even competing transitions that are active in the same markings.

The fourth stage assigns a time-dependent assessment to each analyzed t-invariant. For a strong TPN-feasible t-invariant, the evaluation is based on a pair (σ, σ^τ) , where σ is a transition sequence in which every transition t_j occurs exactly x_j times, no transition outside $s(x)$ occurs in σ and σ^τ is a valid timed realization of this sequence. The search for a pair (σ, σ^τ) is restricted to sequences whose transitions belong to $s(x)$ and occur in the multiplicities specified by x . Therefore, σ^τ can be obtained (if it exists) by simulating the net dynamics, where at each step, only transitions that are active in the current marking and still need to be fired according to x are considered. If, at some moment, any transition outside of $s(x)$ must fire because of the $I_2(t)$ constraint, and transitions from $s(x)$ in σ^τ have already the fastest possible firing times (equal to $I_1(t_j)$) then pair (σ, σ^τ) is not valid and x is not strong TPN-feasible.

For a strict weak TPN-feasible t-invariant x , the evaluation is based on a valid timed run that may include transitions outside of $s(x)$. However, after removing all such firings of transition $t_i \notin s(x)$, the remaining sequence must contain each transition $t_j \in s(x)$ exactly x_j times. For a weak TPN-feasible t-invariant, the evaluation is based on a valid timed run σ^τ that may include any transition from T . In this case, however, it is sufficient to select a subsequence ρ^τ of this run, preserving the original order of firings. Each transition $t_j \in \rho^\tau$ also belongs to $s(x)$ and each transition $t_j \in s(x)$ occurs in the selected subsequence exactly x_j times. Other firings in the run, including additional firings of transitions from $s(x)$, are treated as additional timed events.

For strict weak and weak TPN-feasibility, the analysis is performed on timed runs of the whole model. In the strict weak case, additional firings of transitions outside

$s(x)$ are allowed, but additional firings of transitions from $s(x)$ are not. In the weak case, additional firings from both outside and inside $s(x)$ are allowed, provided that one selected subsequence corresponding to x can still be identified in the run.

For a fixed candidate transition sequence σ , checking time feasibility does not require enumerating all possible firing times from the intervals $[I_1(t), I_2(t)]$. Instead, one has to check whether there exists σ^τ that satisfies the activation and firing constraints of the TPN. One option to consider when checking for such a run is assigning the fastest firing times $I_1(t)$ for transitions in $s(x)$. It should also be noted that the realization time of a t-invariant is not, in general, equal to the sum of $I_1(t)$ or $I_2(t)$ values of transitions from its support. Time intervals in a TPN are measured from the activation time of each transition, and several transitions may be active simultaneously.

5. Case study

5.1. Analysed model

The gene expression model is shown in Figure 1 as a time Petri net. It represents selected stages of gene expression with negative feedback regulation [5]. The net contains 24 places, 31 transitions and its structure has 9 t-invariants.

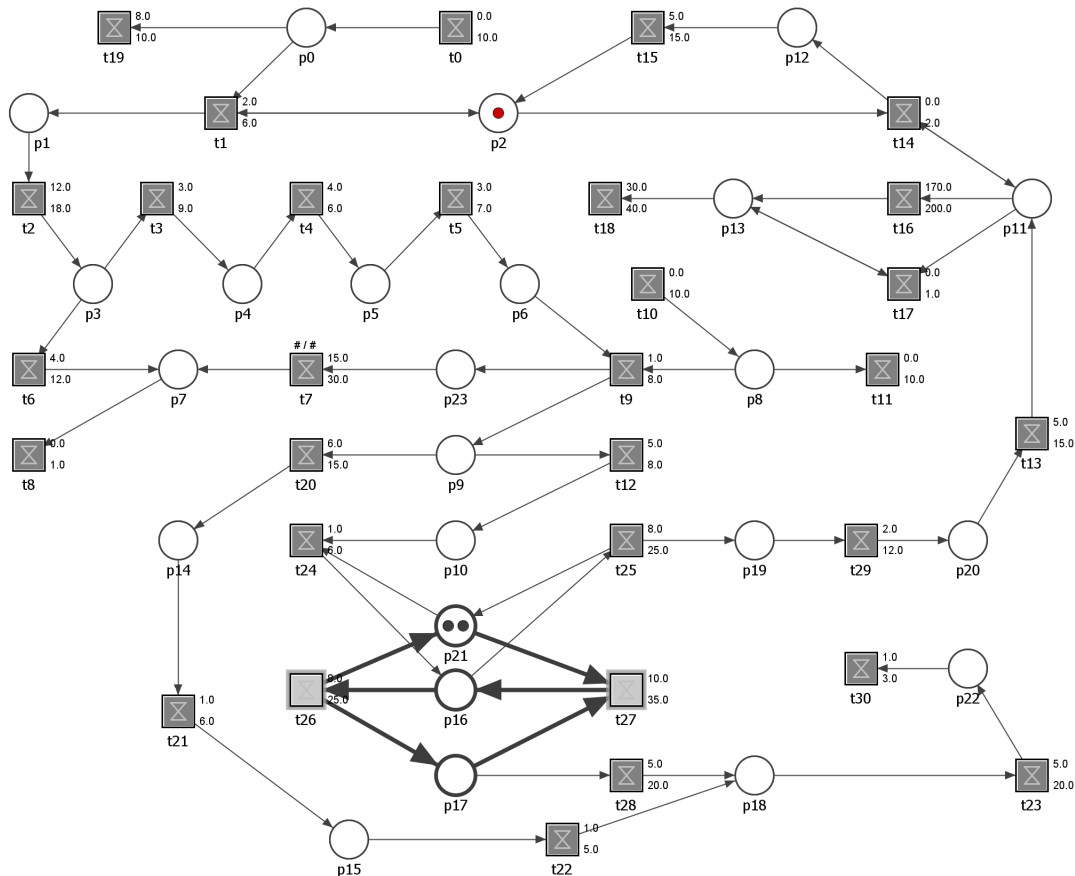


Fig. 1. Case study net of gene expression. A structure of t-invariant x_5 is marked with thicker arcs and light gray, $[I_1(t), I_2(t)]$ values are close to each transition.

Each transition is assigned a time interval $[I_1(t), I_2(t)]$. The initial marking con-

tains one token in place p_2 and two tokens in place p_{21} . The model also contains three read arcs: between p_2 and t_1 , between p_{11} and t_{14} , and between p_{13} and t_{17} . These arcs are important for analysis because they do not appear in the incidence matrix but still influence transition activation and further t-invariants analysis.

Tabela 1

Transitions with eft/lft times and places (tokens if any)

Transitions:			
#	Name	#	Name
t_0	DNA region identification [0,10]	t_{16}	TF protein decay started [170,200]
t_1	transcription start [2,6]	t_{17}	TF decay process [0,1]
t_2	transcription process [12,18]	t_{18}	decay ended [30,40]
t_3	splicing start [3,9]	t_{19}	transcription failed [8,10]
t_4	splicing process [4,6]	t_{20}	translation stalling [6,15]
t_5	export from nucleus [3,7]	t_{21}	RQC recognition [1,6]
t_6	RNA correctness check [4,12]	t_{22}	truncated chain ubiquitination [1,5]
t_7	cytoplasmic mRNA decay [15,30]	t_{23}	proteasomal degradation [5,20]
t_8	RNA disposal [0,1]	t_{24}	chaperone binding and folding start [1,6]
t_9	translation start [1,8]	t_{25}	productive folding [8,25]
t_{10}	ribosome ready [0,10]	t_{26}	folding failure [8,25]
t_{11}	ribosome busy [0,10]	t_{27}	chaperone refolding retry [10,35]
t_{12}	translation process [5,8]	t_{28}	misfolded TF ubiquitination [5,20]
t_{13}	TF activation [5,15]	t_{29}	TF dimerization or partner binding [2,12]
t_{14}	TF binding [0,2]	t_{30}	protein waste disp [1,3]
t_{15}	TF unbinding [5,15]		
Places:			
#	Name	#	Name
p_0	gene	p_{12}	promoted bound
p_1	trans trigger	p_{13}	decaying started
p_2	promoter free (1)	p_{14}	translation stalled complex
p_3	pre mRNA	p_{15}	truncated TF chain
p_4	splicing trigger	p_{16}	folding intermediate
p_5	mRNA in nucleus	p_{17}	misfolded TF
p_6	mRNA in cytoplasm	p_{18}	ubiquitinated TF
p_7	RNA waste	p_{19}	folded TF monomer
p_8	ribosome pool	p_{20}	TF dimer cytoplasm
p_9	translation triggered	p_{21}	chaperone pool (2)
p_{10}	nascent TF protein	p_{22}	protein waste
p_{11}	active TF	p_{23}	mRNA back in cytoplasm

5.2. Structural t-invariants

In the presented net, 9 minimal t-invariants were obtained. Table 2 shows their support and provides a short biological or process-oriented interpretation.

For each t-invariant, a corresponding firing sequence has been selected. These sequences are used as candidates for the feasibility analysis. In particular, for t-invariants with larger support, the order of some transitions may be changed. Table 3 lists the

Tabela 2

Minimal t-invariants of the analyzed model

#	Support	Interpretation
x_1	t_{14}, t_{15}	TF binding and unbinding cycle
x_2	t_0, t_{19}	DNA region identification followed by transcription failure
x_3	t_0, t_1, t_2, t_6, t_8	Transcription followed by RNA correctness check and disposal
x_4	t_{10}, t_{11}	Local ribosome-ready/ribosome-busy cycle
x_5	t_{26}, t_{27}	Folding failure followed by refolding retry
x_6	$t_0, t_1, t_2, t_3, t_4, t_5, t_7, t_8, t_9, t_{10}, t_{12}, t_{13}, t_{17}, t_{24}, t_{25}, t_{29}$	Productive expression path with TF decay process
x_7	$t_0, t_1, t_2, t_3, t_4, t_5, t_7, t_8, t_9, t_{10}, t_{12}, t_{13}, t_{16}, t_{18}, t_{24}, t_{25}, t_{29}$	Productive expression path with delayed TF decay
x_8	$t_0, t_1, t_2, t_3, t_4, t_5, t_7, t_8, t_9, t_{10}, t_{20}, t_{21}, t_{22}, t_{23}, t_{30}$	Translation stalling, RQC recognition and degradation
x_9	$t_0, t_1, t_2, t_3, t_4, t_5, t_7, t_8, t_9, t_{10}, t_{12}, t_{23}, t_{24}, t_{26}, t_{28}, t_{30}$	Misfolding followed by ubiquitination and degradation

sequences used in the analysis. For weak TPN-feasibility, a proposed sequence is treated as an example selected from a larger timed run.

Tabela 3

Example transition sequences for the t-invariants

t-invariant	Representative sequence
x_1	t_{14}, t_{15}
x_2	t_0, t_{19}
x_3	t_0, t_1, t_2, t_6, t_8
x_4	t_{10}, t_{11}
x_5	t_{26}, t_{27}
x_6	$t_0, t_1, t_2, t_3, t_4, t_5, t_{10}, t_9, t_{12}, t_{24}, t_7, t_8, t_{25}, t_{29}, t_{13}, t_{17}$
x_7	$t_0, t_1, t_2, t_3, t_4, t_5, t_{10}, t_9, t_{12}, t_{24}, t_7, t_8, t_{25}, t_{29}, t_{13}, t_{16}, t_{18}$
x_8	$t_0, t_1, t_2, t_3, t_4, t_5, t_{10}, t_9, t_{20}, t_{21}, t_7, t_8, t_{22}, t_{23}, t_{30}$
x_9	$t_0, t_1, t_2, t_3, t_4, t_5, t_{10}, t_9, t_{12}, t_{24}, t_7, t_8, t_{26}, t_{28}, t_{23}, t_{30}$

5.3. Feasibility and TPN-feasibility results

The first classification step concerns feasibility. A t-invariant is feasible if its corresponding transition sequence σ is executable from the starting marking m . Not all structurally correct t-invariants satisfy this condition. For example, transition $t_{14} \in s(x_1)$ requires the presence of a token in p_{11} by a read arc. Since no such token is present in the initial marking, x_1 is not feasible in m_0 . Similar problems concern x_5 and x_6 , and they are not feasible in m_0 . The remaining t-invariants are feasible in the classical, non-timed net. However, their timed interpretation becomes more restrictive; as a result, a feasible sequence in the classical net may become infeasible as a (strict) weak timed TPN-invariant because it will require firings from outside of support.

The results are summarized in table 4. The column $\mathcal{X}_{\text{feas}}$ denotes feasibility in the classical net, $\mathcal{X}_s^T$ denotes strong TPN-feasibility, i.e., realization without firing transitions outside the support, $\mathcal{X}_{\text{sw}}^T$ denotes strict weak TPN-feasibility. Finally, $\mathcal{X}_w^T$ denotes weak TPN-feasibility.

Tabela 4

Classification of t-invariants according to feasibility levels

#	$\mathcal{X}_{\text{feas}}$	$\mathcal{X}_s^T$	$\mathcal{X}_{\text{sw}}^T$	$\mathcal{X}_w^T$	Explanation
x_1	no	no	yes	yes	Transition t_{14} depends on read arc from p_{11} .
x_2	yes	no	no	yes	In the initial marking, t_1 is forced to fire before t_{19} can. Weak realization requires execution of x_1 firing sequence.
x_3	yes	no	no	yes	The execution is longer than the <i>lft</i> of transition t_0 . Additional firings of t_0 , beyond the single firing counted in x_3 , are treated as background events.
x_4	yes	yes	yes	yes	Short cycle t_{10}, t_{11} can be completed before other deadlines.
x_5	no	no	yes	yes	Requires token in p_{16} to start.
x_6	no	no	no	yes	Transition t_{17} requires both p_{11} and p_{13} ; production of p_{13} requires contextual use of t_{16} and an additional active TF token.
x_7	yes	no	no	yes	Long productive path; repeated firings of t_0 and t_{10} transition are forced by their <i>lft</i> values.
x_8	yes	no	no	yes	Long stalling path. The selected firing sequence is feasible only in a larger timed run with additional firings, including repeated firings of t_0 and t_{10} .
x_9	yes	no	no	yes	Long degradation path; additional timed context is needed because of repeated t_0 and t_{10} firings and folding conflicts.

The table shows that only one t-invariant, x_4 , is strong TPN-feasible. This invariant has small support, so its sequence can be completed immediately or after a short delay, before other deadlines interfere with the realization. The strict weak class contains x_4 , x_1 and x_5 . These t-invariants are not feasible in m_0 as isolated sequences. In the case of x_1 , its firing sequence requires a token in p_{11} , which makes transition t_{14} active. In the case of x_5 , a token is required in p_{16} .

The weak TPN-feasible class can be the most relevant for long processes. Invariants x_3 , x_7 , x_8 and x_9 contain input transitions t_0 and t_{10} that are always active. Because they have finite latest firing times, they cannot remain active indefinitely during a long process without firing again. Therefore, a long timed run may contain additional firings of transitions that also belong to the support of the analyzed t-invariant (Definition 7.)

t-invariant x_2 shows a different type of problem. The sequence $\sigma_{x_2} = t_0, t_{19}$ is feasible in m_0 , however, after t_0 fires, both t_1 and t_{19} become active. Since $I_2(t_1) < I_1(t_{19})$, so t_1 is forced to fire before t_{19} . Thus, x_2 is not strong TPN-feasible since no valid time run $\sigma_{x_2}^\tau$ exists. One of the t-invariants, namely x_6 , is the most restrictive case. It contains t_{17} , which requires a token in p_{13} (connection by a read arc). However, a token in p_{13} can be produced only by t_{16} , which is not part of $s(x_6)$. Moreover, t_{16} and t_{17} are in conflict over tokens in p_{11} . Therefore, a valid time run for x_6 (for weak TPN-feasibility) requires a broader context in which an additional token is produced and a contextual firing of t_{16} creates the token in p_{13} .

5.4. Influence of time parameters

The classification of t-invariants depends on both the structure of the net and the values of the time intervals assigned to transitions. The most important time effects observed in the analyzed model are summarized in table 5. The first observation concerns x_2 . In the classical net, the sequence t_0, t_{19} is possible. However, in the TPN, this sequence cannot be realized in m_0 , if t_1 remains active. If t_0 fires at time τ , transition t_1 can fire between $\tau + 2$ and $\tau + 6$, while t_{19} can fire only after $\tau + 8$. Since t_1 and t_{19} compete for the token in p_0 , the earlier deadline of t_1 prevents the firing of t_{19} . This shows that a t-invariant may be feasible in the classical net but lose its direct timed interpretation.

Tabela 5

Main timing effects influencing t-invariant interpretation

Transitions	Time relation	Effect on t-invariants
t_1 and t_{19} after t_0	$t_1 : [2, 6]$, $t_{19} : [8, 10]$	In the initial context, t_1 must fire before t_{19} can fire. This prevents a strong timed realization of x_2 .
Input transition t_0	$t_0 : [0, 10]$	In long occurrences, t_0 is forced to fire repeatedly. This prevents strict weak interpretation of long t-invariants containing t_0 .
Input transition t_{10}	$t_{10} : [0, 10]$	The ribosome pool (p_8) may need to be regenerated repeatedly before t_9 fires. This affects x_6, x_7, x_8 and x_9 .
t_3 and t_6 after t_2	$t_3 : [3, 9]$, $t_6 : [4, 12]$	RNA correctness check can be selected before splicing is forced, which makes the occurrence of x_3 possible in a timed context.
t_{12} and t_{20} after t_9	$t_{12} : [5, 8]$, $t_{20} : [6, 15]$	The stalling branch can be selected by firing t_{20} before the deadline of t_{12} , which is important for x_8 .
t_{16} and t_{18}	$t_{16} : [170, 200]$, $t_{18} : [30, 40]$	The TF decay branch is much slower than the expression and mRNA decay branches. This strongly affects x_7 and contextual realization of x_6 .

The second observation concerns input transitions, such as t_0 and t_{10} with finite $I_2(t)$ values. For long sequences corresponding to t-invariants with larger support, the time needed to complete them is usually much longer than the *lft* deadlines of such input transitions. Therefore, such transitions may be forced to fire repeatedly. This is the main reason why long t-invariants x_3, x_7, x_8 and x_9 are not strict weak TPN-feasible, although they are weak TPN-feasible. The additional firings of t_0 or t_{10} (which are present in the supports of these four t-invariants) are treated as contextual events rather than as part of the selected firing sequence. The next observation concerns conflicts that are resolved by time. For example, after t_2 produces a token in p_3 , both t_3 and t_6 may become active. Transition t_3 represents splicing branch, while t_6 represents correctness check of the RNA. Since t_6 can fire before the latest firing time of t_3 , the firing sequence corresponding to x_3 can still be selected in a valid timed run. A similar situation occurs after firing t_9 , where t_{12} and t_{20} compete for a token in p_9 . Another observation concerns very slow transitions. Invariant x_7 contains transition t_{16} , whose earliest firing time is much larger than the times assigned to any other transitions and even longer than the total time of most of the transition sequences. Consequently, many other processes can occur before t_{16} is fired. t-invariant x_6 is even more dependent on this background firing

(outside of its support) because its support contains t_{17} but not t_{16} , while the token required by the read arc of t_{17} can only be prepared through the contextual firing of t_{16} .

This shows that the time-dependent classification of t-invariants is not a simple extension of structural analysis. The same t-invariant may be feasible in the classical net, infeasible as an autonomous timed subprocess and still meaningful as a selected firing sequence in some larger timed behavior. This is important in biological models, where long pathways usually coexist with input transitions that are always active.

6. Conclusion

In this paper, the influence of TPN semantics on the interpretation of t-invariants was analyzed. The main point of the proposed approach is that adding time intervals to transitions may significantly change their interpretation. A t-invariant does not necessarily correspond to a transition sequence executable from the starting marking and even such a sequence may fail to have a valid timed realization. To make this distinction, several classes of t-invariants were introduced. This classification makes it possible to separate t-invariants that can be interpreted as relatively autonomous timed processes from those that can only be recognized as selected firing sequences in larger timed runs.

The case study showed that these distinctions are useful in practice. In the analyzed model all minimal t-invariants differ in their feasibility status. For example, only a small number of t-invariants can be treated as strong TPN-feasible, while longer biologically meaningful paths usually require weak TPN-feasible classification because additional firings of input transitions may occur during a longer timed run. This observation is particularly important, e.g., for biological models, where processes rarely occur in complete isolation. Long pathways may coexist with the degradation and regeneration of resources, repeated activation of input transitions, and other background reactions. Therefore, the weak TPN-feasibility class is a practical way to preserve a biological interpretation of a t-invariant when it appears as part of a broader timed behavior. At the same time, the strict weak class remains useful as an intermediate case where transitions outside of t-invariant support may fire in the sequence, but additional firings of transitions from the support are not allowed.

More specifically, the time-dependent analysis changes the biological interpretation of several subnets that may appear self-contained at the structural level. The cycle x_1 and x_5 require previously prepared states, while the pathway x_2 cannot proceed autonomously because the timing forces t_1 to fire before t_{19} . Pathway x_3 and three longer ones: x_7, x_8, x_9 require repeated firings of source or resource-related transitions, whereas x_6 additionally depends on a contextual firing of t_{16} outside its support. Consequently, for the considered initial marking and time intervals, only the local ribosome cycle x_4 retains the interpretation of an autonomous timed module. The remaining t-invariants should be interpreted as condition-dependent or embedded biological subprocesses.

The presented approach also indicates which aspects of a model should be checked when t-invariants are interpreted. In particular, read arcs or conflicts may change the status of a t-invariant. Thus, the classification can help identify which structural processes remain meaningful after temporalization and which require additional assumptions. In the current study, the analysis is based on some example transition sequences and selected timed runs rather than on an exploration of the timed state space. For larger mo-

dels, a more systematic or automated search procedure would be needed, especially for weak TPN-feasibility, where the selected firing sequence may be embedded in a much larger timed run. The case study still demonstrates that even this level of analysis can reveal important differences between structural and timed interpretations of t-invariants.

A natural extension of the proposed approach would be to study how perturbations of time intervals $[I_1(t), I_2(t)]$ influence t-invariants classification. In other words, how a given t-invariant, belonging to a specific class (e.g., strict weak-TPN feasible), is susceptible to losing such a classification when the intervals change or to what degree. In such an analysis, the intervals $[I_1(t), I_2(t)]$ could be narrowed, widened, or modified within biologically plausible ranges, and the membership of t-invariants in the introduced classes could be checked again. This would make it possible to distinguish t-invariants whose timed interpretation is stable from those that depend on a narrow choice of time parameters. Such analysis requires a separate study and is left for future work.

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