Selected aspects of essential hypertension and cardiovascular disease – modeled and analyzed using timed Petri nets

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The main steps of the Timed Petri net modeling the role of inflammation and immunity in essential hypertension and cardiovascular disease analysis

- Informal description of the Petri net modeling the role of inflammation and immunity in essential hypertension and cardiovascular disease.
- Time assesment - determining the time each transition takes to fire
- Analysis of the model without time:
  - Analysis of the network structural properties
  - Invariant analysis
  - MCT (Maximal Common Transitions) sets computation and analysis (based on the t-invariants set)
  - T-clusters analysis
  - *In silico* knockout analysis
- Analysis of the model with time:
  - Analysis supported by the state simulator of the net

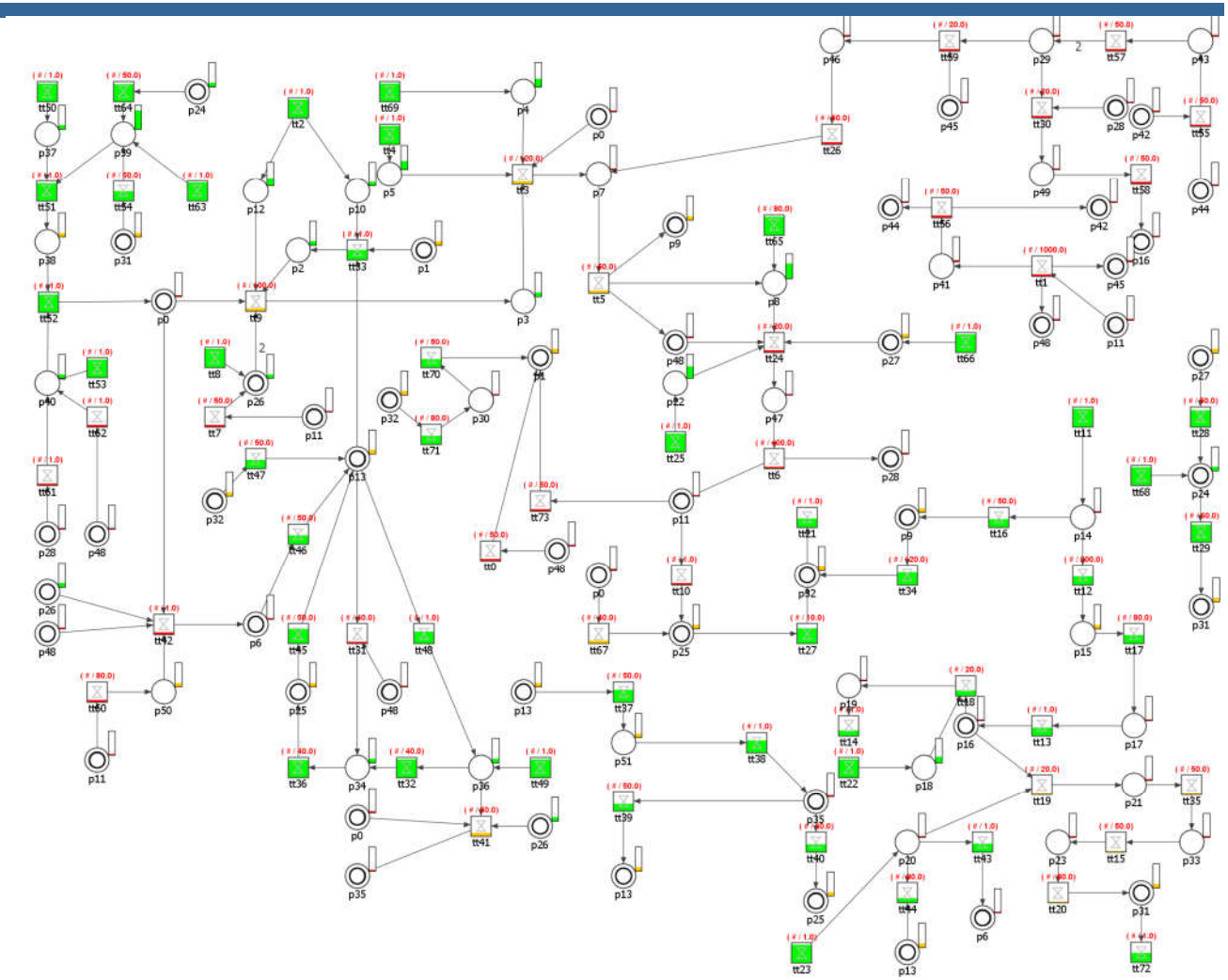
A role of inflammation and immunity in essential hypertension and cardiovascular disease – informal description

- Hypertension promotes atherosclerosis and is a major source of morbidity and mortality.
- The most common type of hypertension is essential hypertension, whose exact etiology remains unknown.
- Recently, inflammatory process has been proposed to play a key role in the pathogenesis of this phenomenon.
- The activation of the immune system in cardiovascular disease depends on neoantigens generation.
- Many factors common to the hypertensive milieu, including angiotensin II, aldosterone, cytokines and altered mechanical forces like shear stress stimulate enzyme sources such as the NADPH oxidases, uncoupled nitric oxide synthase (eNOS) and the mitochondria to produce reactive oxygen species (ROS) which contribute to hypertension in many ways.

A role of inflammation and immunity in essential hypertension and cardiovascular disease – informal description

- ROS can affect T cell polarization and cytokine secretion.
- Inflammatory cells such as macrophages and granulocytes can release ROS, further promoting an oxidative environment.
- There is substantial evidence to show that ROS modulate T cells function.
- Moreover, several recent studies have supported the concept that cytokines produced by T cells and other inflammatory cells contribute to hypertension.
- The novel, proinflammatory cytokine IL-17, that has a pivotal role in autoimmune diseases, was found to be one of them.
- This cytokine is produced by TH17 cells, a subset of CD4+ cells.
- Thus, T-lymphocyte-dependent inflammation accompanied by an oxidative stress play a key-role in human hypertension.

The Timed Petri net modeling role of inflammation and immunity in essential hypertension and cardiovascular disease



Determining the delay time of transitions

- The durations of biochemical reactions composing the process have been taken into account (present in the real process and may be crucial for understanding its nature)
- Lack of the precise time data coming from the experiments.
- Our approach:
  - We have identified within the model the processes of the longest and shortest time duration.
  - Next, basing on the data gathered from the literature and expert knowledge (e. g. synthesis takes more time than binding of the molecule to the receptor or the complex formation; binding is faster than dissociation), we have developed a time scale (J. Scheidel et al., 2015, Metabolites 5: 766–793).
  - We have used the state simulator to check whether each transition have a chance to fire.

The duration of transitions firing

Process	Duration	Time assessment
binding, influence, activation	1 (20)	Seconds (up to minute for larger complexes)
vasoconstriction	10	Seconds to minutes
decrease, reduction	40	About twice shorter than synthesis and expression
generation, formation, increase, production	50	Few minutes
synthesis, expression, muscles relaxation	80	Minutes
T lymphocytes activation and proliferation via AT1R	100	>half an hour
migration Th lymphocytes into the blood vessels	120	>half an hour
The immune system activation in interstitium under local hypertonic state	200	>hour
acute phase reaction in the liver	400	Hours
chronic inflammatory process	1000	Days

The method of determining the delay time of transitions

Modelling and simulation of signal transductions in an apoptosis pathway by using timed Petri nets

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This paper first presents basic Petri net components representing molecular interactions and mechanisms of signalling pathways, and introduces a method to construct a Petri net model of a signalling pathway with these components. Then a simulation method of determining the delay time of transitions, by using timed Petri nets – i.e. the time taken in firing of each transition – is proposed based on some simple principles that the number of tokens flowed into a place is equivalent to the number of tokens flowed out. Finally, the availability of proposed method is confirmed by observing signalling transductions in biological pathways through simulation experiments of the apoptosis signalling pathways as an example.

[Li C, Ge Q-W, Nakata M, Matsuno H and Miyano S 2006 Modelling and simulation of signal transductions in an apoptosis pathway by using timed Petri nets; *J. Biosci.* **32** 113–127]

- Based on the assumption that, for any reaction, a total amount of consumed substrates is equal to a total amount of products of the reaction.
- The Petri net should be acyclic and without inhibitory arcs.

The method of determining the delay time of transitions

- In the conflict situation at a certain place, the same delay should be assigned to the transitions going out from that place.
- It does not reflect real reactions in a cell.
- We have used this method to assign the delay time to all transitions in our model.
- We have used the state simulator to check whether each transition have a chance to fire.
- Unfortunatelly, many of the delay time values were not consistent with the data gathered from the literature and expert knowledge.
- The Petri net we developed has cycles.

Analysis of the network structural properties

- The model contains 51 places and 73 transitions
- The net is **pure**, i.e. there are no two nodes, connected in both directions (this precludes read arcs).
- The model is also **not ordinary**, because not every stoichiometric coefficient of each reaction is equal to 1.
- The net is **connected but not in a strong sense**. It means, that there exists an undirected path between any two places but there may not be a directed path between them, which implies that there are no independent processes within the analyzed model.

Analysis of the network structural properties

- The net is **not structurally conflict-free**, because it contains places with two or more outgoing arcs. This entails that there are reactions sharing at least one substrate.
- The model contains neither in-places nor out-places (i.e., without pre-transitions or post-transitions, respectively), but there exist a few input and output transitions (i.e., without pre-places or post-places, respectively).
- The net is also **unbounded**, since there are no upper bounds of the number of tokens.

Invariant analysis

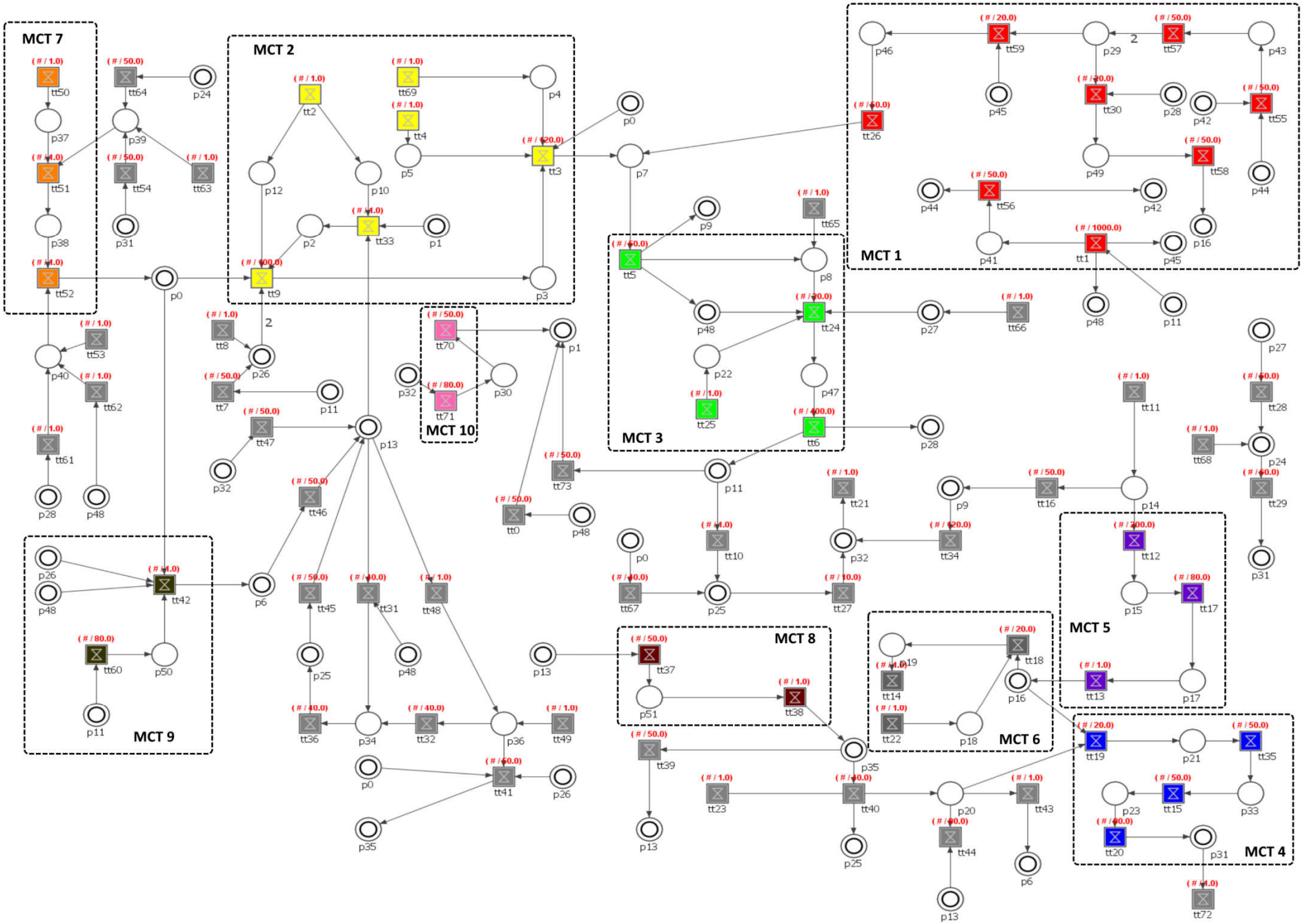
- The model has 2588 minimal t -invariants covering all transitions and no p-invariant.
- The smallest t-invariants consist of three transitions, while the five largest t-invariants contain each 44 transitions.
- Since there are no read arcs within the model, all minimal t-invariants are also the feasible ones.

MCT (Maximal Common Transitions) sets

- Calculated to check the feasible t-invariants for their biological meaning.
- The transitions are grouped into the so-called maximal common transition sets (MCT-sets), if and only if they participate in the same t -invariants.
- They correspond to some functional modules of the biological system.
- We yield 10 MCT-sets consisting of more than one transition being non-trivial ones.
- All of them represent connected subnetworks.

Identified MCT sets and their meaning

MCT-set	Contained transitions	Biological meaning
1	1, 26, 30, 55, 56, 57, 58, 59	The initiation of blood coagulation, and the generation of bradykinin via the kallikrein-kinin system
2	2, 3, 4, 9, 33, 69	Lymphocytes T activation in hypertension as a part of immune system defense
3	5, 6, 24, 25	Impact of VEGFC-VEGFR2 axis on the nitric oxide synthesis and relaxation of vascular smooth muscles
4	15, 19, 20, 35	The participation TNF alpha in the activation of the acute phase response in the course of hypertension
5	12, 13, 17	Local activation of the immune system due to changes in the local hypertonia associated with an activation of VEGFC
6	14, 18, 22	Impact of VEGFC-VEGFR3 axis on the lymphatic endothelium
7	50, 51, 52	Angiotesinogen-Angiotensin axix activation leading to AngiotensinII formation
8	37, 38	Peroxynitrite formation as a part of an oxidative stress signaling pathway
9	42, 60	Activation of a key enzymes of an oxidative stress (NADPH oxidases) through AT1 receptor
10	70, 71	The influence of shear stress on the formation of neoantigens and increased arterial blood pressure


MCT 1
MCT 2
MCT 3
MCT 4
MCT 5
MCT 6
MCT 7
MCT 8
MCT 9
MCT 10

T-clusters analysis

- An analysis of similarities between t-invariants which may lead to finding some dependencies between the corresponding biological sub-processes and discovering previously unknown properties of the modeled system.
- We have applied UPGMA (Unweighted Pair Group Method with Arithmetic means) clustering algorithm and Pearson's correlation similarity measure and we have used the Mean Split Silhouette (MSS) evaluation to find the optimal number of t-clusters (E. Grafahrend-Belau et al., 2008, BMC Bioinformatics 9:90)
- Unfortunately, we have obtained one huge cluster covering almost the whole net (about 99% of t-invariants) and several ones containing small number of t-invariants.

T-clusters analysis

Cluster no.	Biological interpretation	No. of t-invariants	Contained processes	
			MCT-sets	Single transitions
c_1	Formation and effects of reactive oxygen species on hypertension	4	$m_8(13)$	$t_{21}(0.06)$, $t_{23}(0.05)$, $t_{27}(0.20)$, $t_{39}(0.24)$, $t_{40}(0.60)$, $t_{43}(0.39)$, $t_{45}(0.11)$, $t_{46}(0.13)$, $t_{47}(0.11)$
c_2	Processes mediated by PGI2, resulting in the reduction of blood pressure and maintenance of health state	2		$t_{28}(0.13)$, $t_{29}(0.33)$, $t_{66}(0.04)$, $t_{68}(0.13)$, $t_{72}(0.33)$
c_3	Processes leading to hypertension, with particular emphasis on the effect of nitric oxide, the role of ADMA and VEGFR2	7		$t_{21}(0.11)$, $t_{23}(0.21)$, $t_{27}(0.39)$, $t_{32}(4.11)$, $t_{36}(1.53)$, $t_{43}(0.77)$, $t_{44}(0.37)$, $t_{45}(0.21)$, $t_{46}(0.27)$, $t_{47}(0.21)$, $t_{48}(0.90)$, $t_{49}(0.43)$
c_4	Processes leading to hypertension, with particular emphasis on the role of IL-17	2		$t_{11}(1.72)$, $t_{16}(25.00)$, $t_{21}(0.06)$, $t_{23}(0.05)$, $t_{34}(0.08)$, $t_{44}(0.12)$, $t_{47}(0.11)$
c_5	The local changes in the interstitium due to fluctuations in the local independent osmotic sodium concentration	1	$m_5(0.9)$, $m_6(0.1)$	$t_{11}(0.86)$
c_6	The influence of oxidative stress and inflammation on vascular endothelium in the course of arterial hypertension without changes regarding the lymphatic endothelium	2572	m_1, m_2, m_3, m_4, $m_5(99)$, $m_6(99.9)$, m_7, $m_8(87)$, m_9, m_{10}	t_0, t_7, t_8, t_{10}, $t_{11}(97.41)$, $t_{16}(75.00)$, $t_{21}(99.77)$, $t_{23}(99.69)$, $t_{27}(99.41)$, $t_{28}(99.87)$, $t_{29}(99.67)$, t_{31}, $t_{32}(95.89)$, $t_{34}(99.92)$, $t_{36}(98.47)$, $t_{39}(99.76)$, $t_{40}(99.40)$, t_{41}, $t_{43}(98.84)$, $t_{44}(99.50)$, $t_{45}(99.68)$, $t_{46}(99.60)$, $t_{47}(99.58)$, $t_{48}(99.10)$, $t_{49}(99.57)$, t_{53}, t_{54}, t_{61}, t_{62}, t_{63}, t_{64}, t_{65}, $t_{66}(99.96)$, t_{67}, $t_{68}(99.87)$, $t_{72}(99.67)$, t_{73}

- The 2588 feasible t-invariants clustered by UPGMA algorithm.
- The processes contained in the clusters are listed.
- Processes are divided into non-trivial MCT-sets and single transitions.
- The integer values presented in the brackets indicate to how extent a given process is contained in the cluster of t-invariants (no value – 100%).
- The total number of t-invariants in the cluster, together with its biological interpretation is listed.

In silico knockout analysis

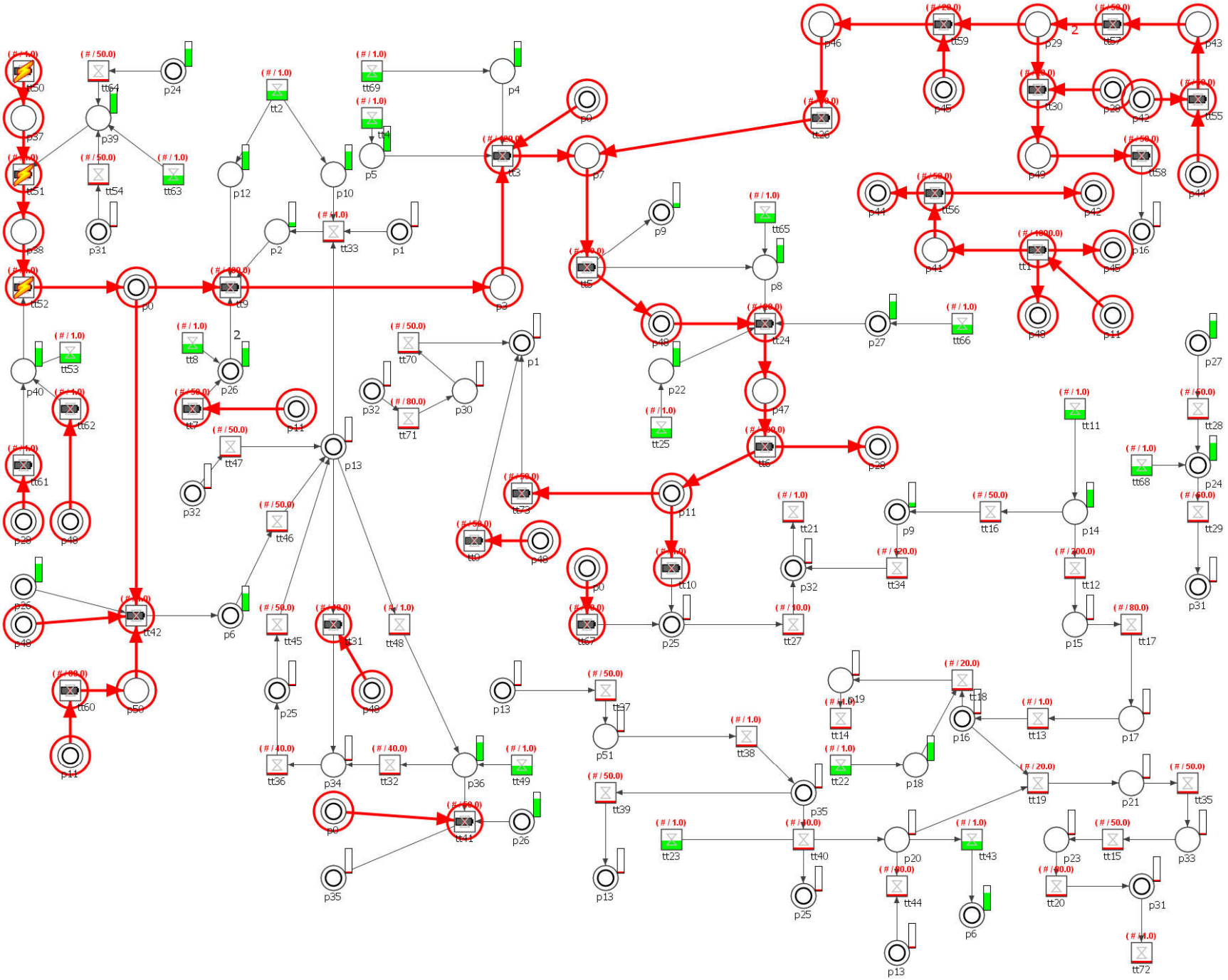
- Approach based on t-invariants (S. Grunwald et al., 2008, Biosystems 92(2): 189-205)
- Selected transitions can be excluded from the model and the remaining t-invariants examined.
- It is interesting, from biological point of view to detect which parts of the modeled system will be affected by the knockout of the selected transitions.
- It can also be investigated, which transitions should be knocked out to achieve a desired model behavior.
- The knockout of a transition segments a set of t-invariants into a subset of t-invariants containing this transition and its relative complement.

In silico knockout analysis

- According to the authors, the other transitions in the model that are affected by such a single MCT knockout are only those that belong to its affected t-invariants and any other.
- Basing on the analysis supported by the state simulator of the net, we've noticed, that in many cases, the knockout of a selected MCT-set causes the inactivity of the transitions that do not belong to the affected (destroyed) t-invariants (as described in S. Grunwald et al., 2008, Biosystems 92(2): 189-205).

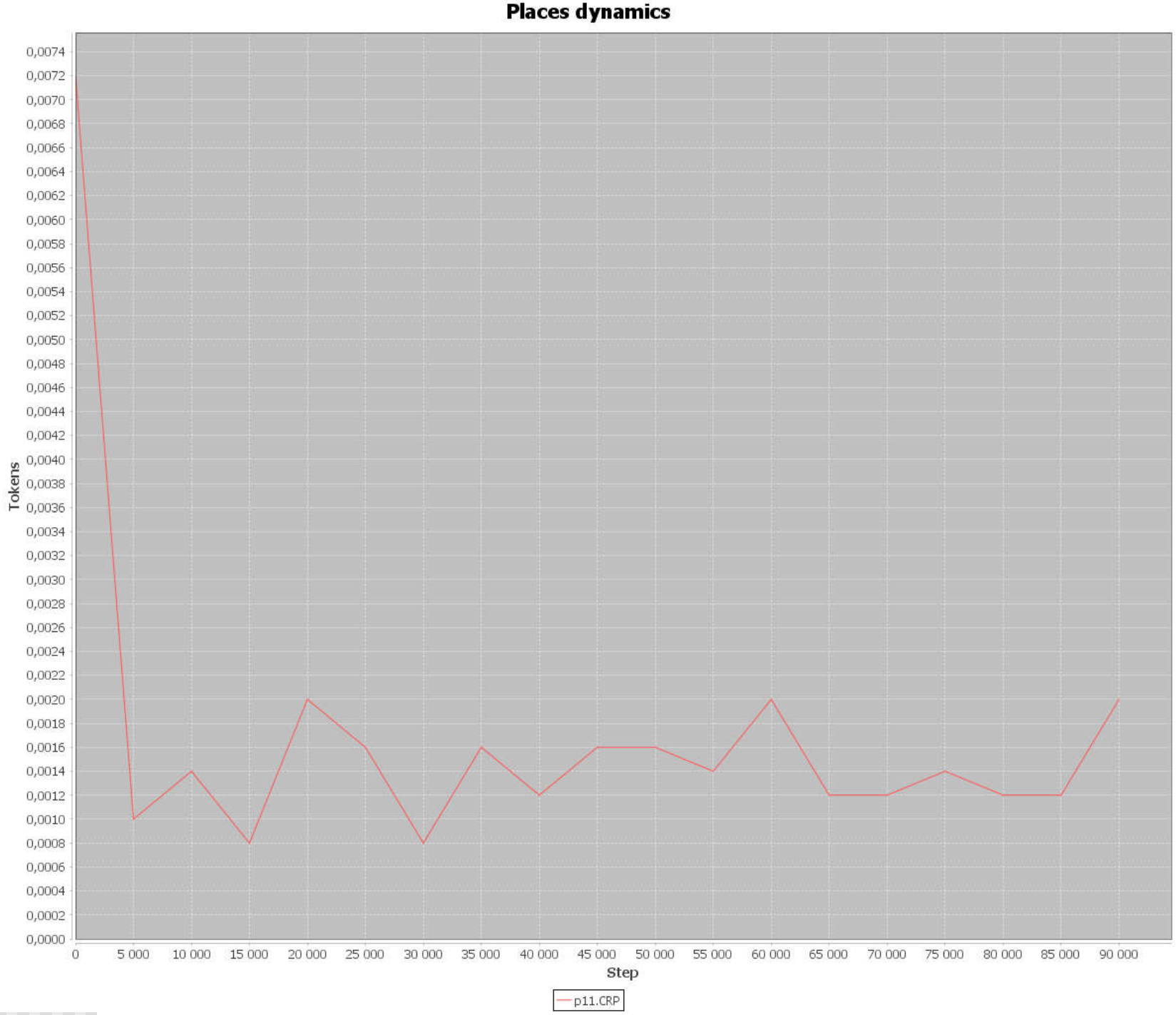
In silico knockout analysis

- Approach based on t-invariants (S. Grunwald et al., 2008, Biosystems 92(2): 189-205), supported by the state simulator of the net.
- In the first step, selected transitions are excluded from the network and its behavior is simulated.
- Next, all inactive transitions (identified by state simulator) are also removed from the network and the remaining t-invariants are further investigated.
- Example: Knockout of the transitions belonging to m_7, connected with the emergence of an essential hypertension activation through angiotensinogen-angiotensin axis in the human body.
  - We have noticed, that m_7 knockout indirectly influences CRP (place p_{11}) through the inactivation of the transition tt_6 (acute phase reaction in the liver).



Analysis supported by the state simulator of the net

- Next, in the course of simulating the DPN model, we adjusted the duration (ranging from 1 to 50) of tt_{50}, tt_{51}, tt_{52} transitions (RAA) to reflect high and low concentrations of the angiotensin II (place p_0).
- We have also observed that the level of the CRP (place p_{11}) was indirectly influenced.


Places dynamics
Tokens
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0,0070
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0,0062
0,0060
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0,0056
0,0054
0,0052
0,0050
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0,0046
0,0044
0,0042
0,0040
0,0038
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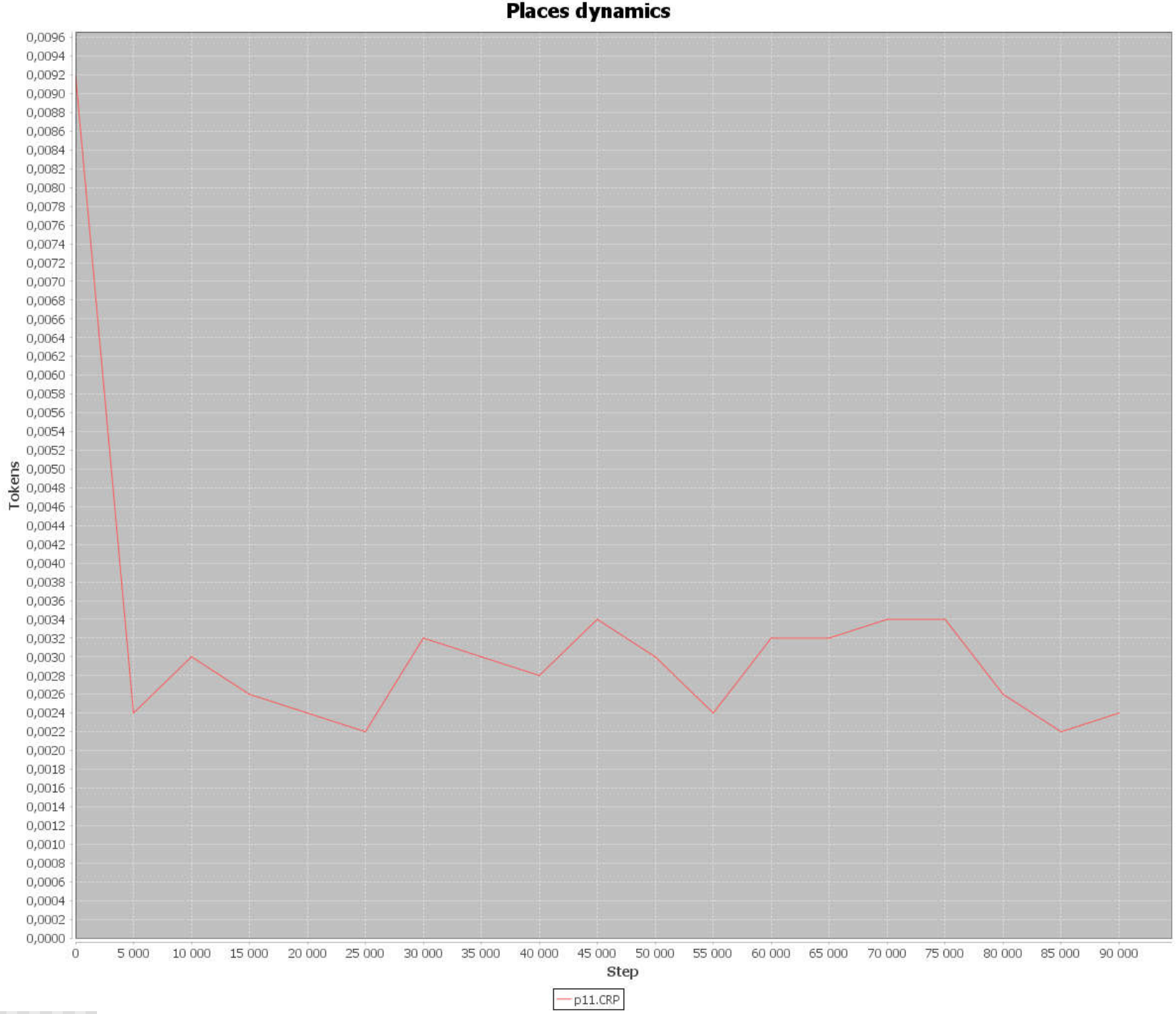
90 000
Step
p11.CRP


Places dynamics
Tokens
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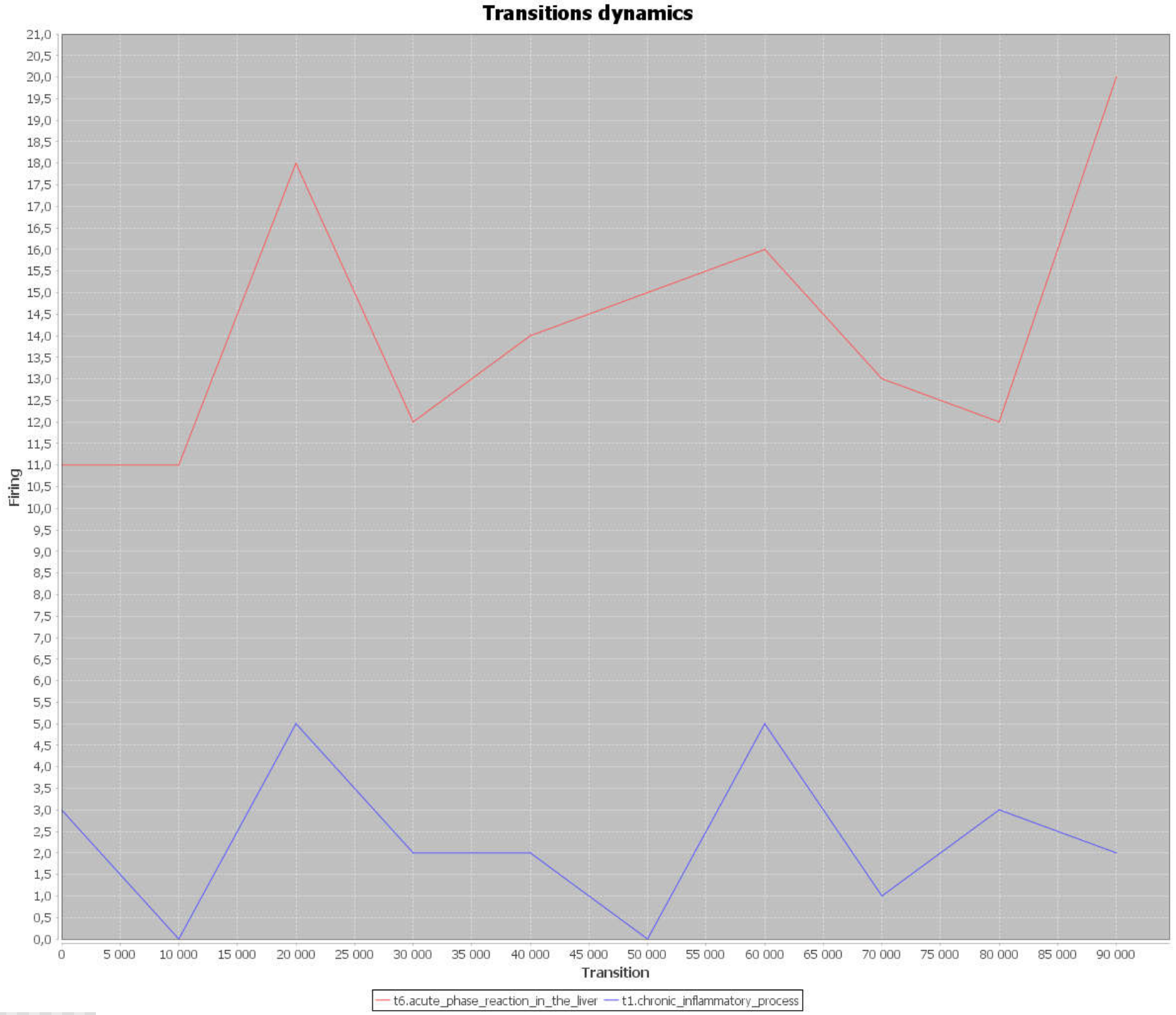
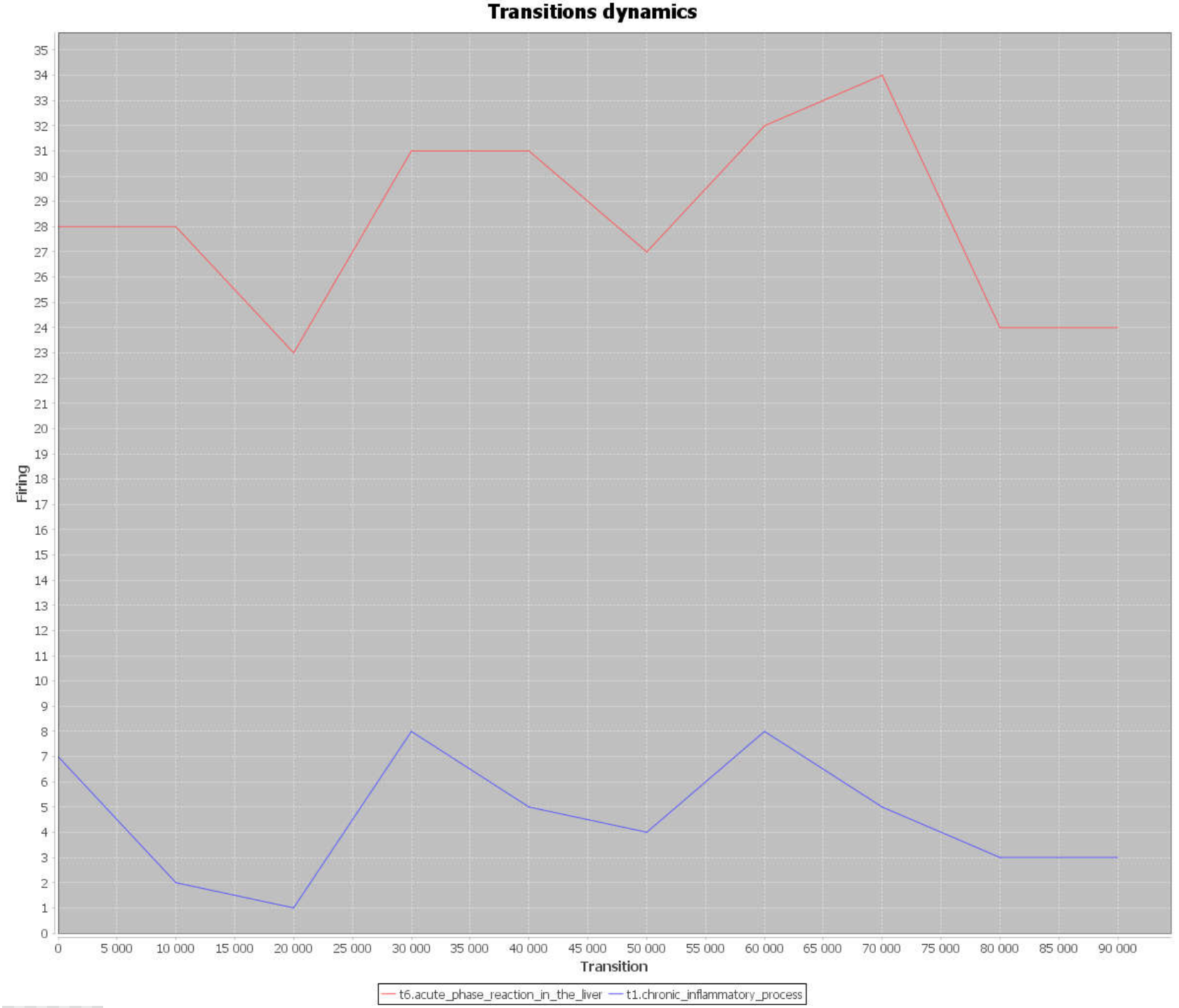

Transitions dynamics
Firing
Transition
t6.acute_phase_reaction_in_the_liver
t1.chronic_inflammatory_process


Transitions dynamics
Firing
Transition
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t6.acute_phase_reaction_in_the_liver
t1.chronic_inflammatory_process

Conclusions

- The analysis of the essential hypertension timed Petri net based model, the knockout of selected transitions and t-clusters has allowed to draw valuable biological conclusions:
- The CRP level is indirectly influenced by the renin-angiotensin-aldosterone system. It is a very interesting observation, since it has been previously reported that high level of CRP correlates with higher prevalence of cardiovascular complications and risk of developing it. Hence, it suggests that a treatment of essential hypertension with RAAS blockers can have potentially anti-inflammatory effect.
- Oxidative stress and inflammation, both in the form of acute-phase response and chronic inflammation, appear to play a key role for an endothelial dysfunction and an emergence of an essential hypertension in the human body.

Conclusions

- For the emergence of essential hypertension activation of angiotensinogen-angiotensin axis is very important, and as a result angiotensin II is formed, which through its receptors, mainly leads to vasoconstriction.
- The ADMA, VEGFR2 and IL-17 as key components of the hypertension-inflammation axis, can lead to hypertension in the absence of the angiotensinogen-angiotensin axis activation.
- VEGF-C takes part in salt homeostasis and regulation of blood pressure (the VEGF-C-macrophage-lymphangiogenesis pathway is proved to play a role in the protection against developing hypertension in response to a HS (high sodium) intake in animals). This mechanism probably plays a very similar role in humans.