SIMULATION AND VALIDATION OF MOLECULAR GENETIC TRIGGERS NETWORKS WITH TIMED HYBRID PETRI NETS

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Abstract. *In this paper a bistable self-organizing genetic structure with two alternative stable states (true and false) – one-dimensional molecular-genetic trigger (1D MGT) – is modeled with Timed Hybrid Petri Nets. It has been proved that the gene expression regulation process of lexA recA genetic systems with regulatory enzymes is controlled by 1D MGT. 1D MGT can be switched from one stable state to another one as a response to internal and external factors (i.e., temperature, UV radiation, pH, concentrations of specific molecules etc.) considered as input signals. These two stable states correspond to activated and to repressed states of recA gene, respectively. It is shown that 1D MGT being a self-organizing nanostructures occurred in living cells can fulfill the logical function NOT. The simulation of 1D MGT networks is implemented in the Visual Petri Nets tool using Timed Hybrid Petri Nets. Networks of two sequentially connected 1D MGTs with repressor and activator links are modeled. They can fulfill the logical functions OR and NAND.*
Keywords*: self-organizing nanostructures, repressor, activator, regulatory enzymes, promoter, molecular-genetic triggers, Timed Hybrid Petri Nets.*

Introduction

It is known that many kinds of systems in the field of computer sciences and engineering, including genetic processes in living systems can be simulated by *Timed Hybrid Petri Nets* (*THPN*) [1]. We show that the regulation of gene expression controlled by one-dimensional molecular genetic trigger (*1D MGT*) adequately can be modeled using *THPN*. Better understanding of molecular mechanisms of gene regulation process can contribute to solving problems relating to medicine or to *DNA* based molecular computing [5].

It has been proved that the regulation process of gene expression of *lexA recA* genetic system are controlled by *1D MGT* [3,4]. *MGT* is a self-organizing nanostructures with two stable states (alternative functioning regimes) [2]. These two stable states (*true* and *false*) of *MGT* correspond to the activated and inactivated states of genes. The switch from one stable state to another one is influenced by input signals such as temperature, UV radiation, pH, concentration of repressors or activators, etc. As a cell reacts to input signals we can control and direct the intercellular processes such as gene expression process [6].

The discrete-continuous simulation method of *MGT* proposed in this paper is based on the use of a special class of Petri nets (*PN*) – *Timed Hybrid Petri Nets* (*THPN*) [1]. *THPN* is based on the following primitives: a class of discrete/continuous places, discrete/continuous transitions and guard functions, discrete direct arcs, discrete/continuous test and inhibitory arcs, flow arcs, and re-formulation of the enabling and firing rules of transitions.

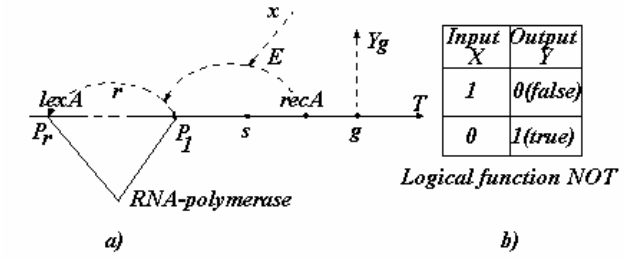
Most tools connected to the PN simulation provide a graphical user interface for the convenient drawing and editing of models. They usually include analysis of components for one or sev-

eral classes of Petri nets models. But still there is not such a program that would satisfy every one's needs, both commercial and educational, so we developed our own software, called it Visual Petri Nets + (VPNP) [1], for the discrete/continuous simulation, validation, and performance evaluation of distributed hybrid systems using *PN* and *THPN*.

The paper is organized as follows. Section 2 describes the molecular mechanism of *1D MGT*. In Section 3 we examined networks aspects of two sequential connected *1D MGT*s. The *THPN* based model and simulation of molecular-genetic trigger is presented in Section 4. Conclusions are drawn in Section 5.

Molecular mechanism of *1D MGT*

In this section we shortly describe the molecular mechanism of the lexA recA regulatory system of *E.coli* bacterium cells [4,5] (Figure1). It has been proved that this genetic system is controlled by *1D MGT* [4]. Prokaryotes are single-celled organisms without a nucleus, such as bacteria. In prokaryotes a gene is activated whenever the gene transcription process occurs. This state is denoted by *true* value. Otherwise, genes are considered inactivated (*false* state).



Input X	Output Y
1	0(false)
0	1(true)

Figure 1. The scheme of the transcriptional regulation process of *recA* gene. *Pr* - promoter of the regulatory gene *lexA*; *r* - repressors; P_1 - promoter of *recA, s* and *g* genes*; lexA* - regulatory gene encoding *LexA* protein, i.e., repressors; *recA* - gene encoding *RecA* enzyme denoted by *E* which can destroy repressor molecules *r*; *T* - transcription terminator, *x* denotes the input signals, output signals Y_g are encoded by *g* genes.

Figure 1 shows the scheme of a molecular-genetic system controlled by regulatory enzymes [4]. The promoter is the place where the process of gene transcription begins. *RNA* polymerase (*p*) bound to the promoter (P_1) starts to move along the genes (*s* and *g*) synthesizing *mRNA* copies of these genes. The protein molecules are synthesized as a result of the *mRNA* gene copies translation process by ribosomes. By *r* we denote repressor molecules (i.e., *LexA* protein) encoded by the regulatory *lexA* gene. If a repressor molecule *r* binds to the promoter P_1 then the genes being under this promoter control are repressed. The gene *recA* encodes the regulatory enzyme *RecA*. Due to a specific proteolytic activity the *RecA* protein can destroy repressor molecules *r*. As a result, all genes being under this promoter control become activated. Thus, *RecA* protein derepressing the own *mRNA* synthesis is a positive self–regulator.

The regulatory enzymes can be activated or inactivated in dependence with the values of input signals denoted by *x*. Let us denote by E^+ the activated state of the regulatory enzyme when enzymatic reaction rate is high. The inactivated state denoted by E^- corresponds to the low rate of enzymatic reaction. The value $x=0$ designates the optimal value of input signals (closed to 37^0C), $x=1$ corresponds to a high value of input signals (39^0C). The rate of regulatory enzymes reaction can be proportional or inverse proportional to the input signals, such as temperature, pH, UV radiation, concentration of molecules, etc. In the case of inverse proportional dependence on input signals a *1D MGT* can fulfill the logical function *NOT*. Thus, one of the two alternative functioning regimes, denoted by the *true* and *false* values, can be established in *1D MGT* system as a result of *in vivo* action of input signals *x* on regulatory enzymes *E* (Figure 1).

Networks of two *1D MGT*s

As it is mentioned above, the rate of regulatory enzymes can be characterized by proportional or inverse proportional dependence on different factors, considered as input signals. We examine examples of networks of sequentially connected *1D MGTs* (Figure 2). The linking of two *1D MGTs* can be realized by the gene s_r encoding

repressors r or by the gene s_a – activators a.

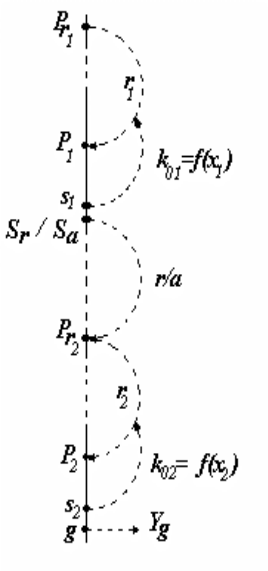


Figure 2. Sequential connected two *1D MGT*.

Let us denote by k_{01}, k_{02} the regulatory enzymatic reaction rates of enzymes E_1, E_2, respectively. We analyze all possible cases:

1. $k_{01}\sim x_1$, $k_{02}\sim x_2$. The output signals of *MGT* are denoted by Y_1 (Table 1).
2. $k_{01}\sim x_1$, $k_{02}\sim x_2^{-1}$. The output signals – Y_2.
3. $k_{01}\sim x_1^{-1}$, $k_{02}\sim x_2^{-1}$. The output signals – Y_3.
4. $k_{01}\sim x_1^{-1}$, $k_{02}\sim x_2$. The output signals – Y_4.

Table1. The output values Y_1, Y_2, Y_3, Y_4 for case of repressor and activator links.

Input		*Output*			
		repressor link			
x_1	x_2	Y_1	Y_2	Y_3	Y_4
0	0	0	1	1	1
0	1	1	0	1	1
1	0	1	1	1	0
1	1	1	1	0	1
		OR		***NOT AND***	

Input		*Output*			
		activator link			
x_1	x_2	Y_1	Y_2	Y_3	Y_4
0	0	1	1	1	0
0	1	1	1	0	1
1	0	0	1	1	1
1	1	1	0	1	1
			NOT AND		***OR***

4. Modeling of *MGT*

For sophisticated dynamic systems in which control mechanisms of genes and chemical reactions with enzymes are concurrently performed, it is more reasonable to use actual values for representing some objects (promoters, genes etc.) and concentrations, e.g., protein, *mRNA* and enzymes concentrations etc. They are described by both of discrete and continuous variables which are usually referred to as hybrid systems.

Figure 3 shows a *THPN* model of *1D MGT* implemented in *VPNP* tool. Significations of continuous b_i places and of continuous q_i transitions is follow: b_1 – concentration of repressor molecules r; b_2 – concentration of *recA_mRNA* molecules; b_3 – concentration of enzymes E^+; q_1 – constant rate of repressor molecules r synthesis; q_2 – transcription rate of *lexA* regulatiry gene; q_3 – translation rate of *recA_mRNA*; q_4 – degradation rate of repressor molecules r; q_5 – degradation rate *recA_mRNA* molecules; q_6 – degradation rate of enzymes E; q_7 – specific proteolytic activity of enzymes E^+ which destroy repressor molecules r.

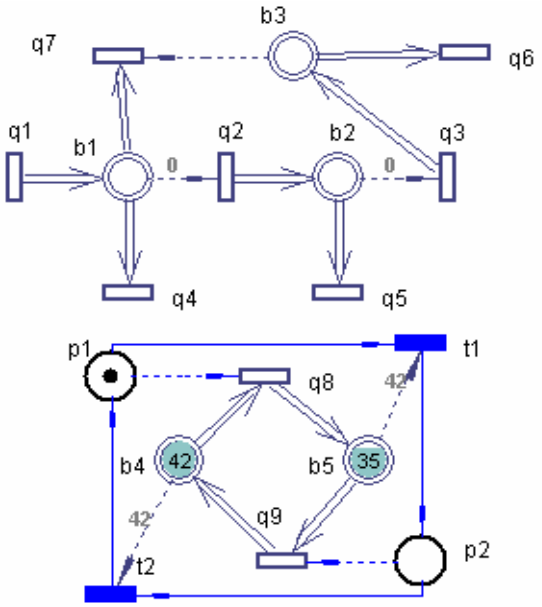


Figure 3. *THPN* model of *1D MGT*.

The molecular mechanism of recombination bistability on the basis of sigmoid kinetics of regulatory enzymes (i.e., *MGT*) is simulated using *VPNP* tool (Figure 4).

The continuous transition q_1 is fired with a constant rate. Thus, the concentration of repressor molecules r (b_1) increases. The degradation rate of repressor molecules is designed by the continuous transition q_4. The transcription processes of *recA* gene begins if the concentration of repressor molecules r does not exceed a threshold.

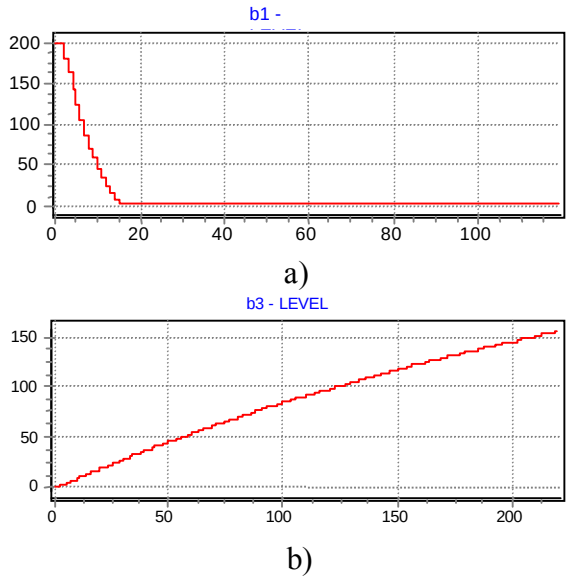


Figure 4. The *true* state of *MGT* corresponds to activated state of *recA* gene, i.e., a) the concentration of repressor molecules *r* is low (#b_1_LEVEL = 0) and b) the gene *recA* is activated, i.e., the value of #b_3_LEVEL is high.

The weights of all arcs are functions of system parameters. The continuous transition q_3 represents the *recA* gene transcription process rate. The degradation rate of recA_mRNA molecules is modelled by transition q_5. The concentration of enzymes E^+ is designed by b_3 and it occurs in biosystem due to translation process (b_3). In dependence of values of input signals (x) the enzymes pass into the inactivated state, denoted by E^- or into the activated state, denoted by E^+. The activated enzymes E^+ destroy repressor molecules r (feedback). This specific proteolytic activity of enzymes E^+ is modeled by continuous transition q_7.

Thus, as a result of input signals action on regulatory enzymes in molecular - genetic system one of two alternative regimes is established. Note, that the *true* state corresponds to activated state of *recA* gene (the concentration of repressor molecules *r* is low and the gene transcription process holds). The *false* state denotes the repressed state of *recA* gene, i.e., the repressor molecules *r* concentration is high. Networks of two *1D MGT* is modeled in similar way. It was shows that such networks can fulfill the logical functions *OR* or *NAND*.

Conclusions

A molecular mechanism of recombination bistability of *lexA recA* genetic system controlled by regulatory enzymes is modeled in VPNP tool. It is shown that due to feed-back in molecular-genetic system a self-organizing genetic nano-structure with two alternative functioning regimes (*true* and *false*) can appear. *THPN* simulation of *1D MGTs*, i.e., input/output signals processing by living cells at DNA level is proposed. It is shown that *MGT* networks being analogous of transistors can fulfill the logical functions *OR* or *NAND*.

VPNP tool exploits the graphical description of the model to show the dynamic evolution that takes place during its simulation directly on the Graphical User Interface representation which facilities are used to describe the structure of the our models. Dynamic graphical facilities are used to visualize the movement of the tokens or fluid, to provide snapshots of the model, and to represent the evaluation of computed (or measured) parameters values [1].

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