Control of Metabolic Systems Modeled with Timed Continuous Petri Nets

Roberto Ross-León[1], Antonio Ramirez-Treviño[1], José Alejandro Morales[2], and Javier Ruiz-León[1]

[1] Centro de Investigaciones y Estudios Avanzados del I.P.N. Unidad Guadalajara
{rross,art,jruiz}@gdl.cinvestav.mx
[2] Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara
alejandro.morales@cucei.udg.mx

Abstract. This paper is concerned with the control problem of biological systems modeled with Timed Continuous Petri Nets under infinite server semantics. This work introduces two main contributions. The first one is a bottom-up modeling methodology that uses $TCPN$ to represent cell metabolism.
The second contribution is the control wich solves the Regulation Control Problem (RCP) (to reach a required state and maintain it). The control is based on a Lyapunov criterion that ensures reaching the required state.

Key words: Cell metabolome, Petri nets, Controllability, Stability.

This work was supported by project No. 23-2008-335, COETCYJAL-CEMUE, Mexico. R. Ross-León was supported by CONACYT, grant No. 13527.

1 Introduction

Petri nets PN [1], [2], [3] are a formal paradigm for modelling and analysis of systems that can be seen as discrete dynamical systems. Unfortunately, due to state explosion problem, most of the analysis techniques cannot be applied in heavy marked Petri nets. In order to overcome this problem, the Petri net community developed the Timed Continuous Petri Nets ($TCPN$) [4], [5], a relaxation of the Petri Nets where the marking becomes continuous and the state equation is represented by a positive, bounded set of linear differential equations.

The main $TCPN$ characteristics such as the nice pictorially representation, the mathematical background, the synchronization of several products to start an activity and the representation of causal relationship make $TCPN$ amenable to represent biochemical reactions and cell metabolism. In fact $TCPN$ marking captures the concentration of molecular species while differential equations together with the firing vectors represent the reaction velocity and the graph captures the metabolic pathways. The entire $TCPN$ captures the cell metabolome.

Several works model [6], [7], analyse [8], [9] and control [10], [11] metabolic pathways. Most of them deal with pseudo-steady states of the biochemical reaction dynamic. Nowadays, the scientific community is exploring the use of PN and

Recent Advances in Petri Nets and Concurrency, S. Donatelli, J. Kleijn, R.J. Machado, J.M. Fernandes (eds.), CEUR Workshop Proceedings, ISSN 1613-0073, Jan/2012, pp. 87–102.

their extensions [12], [13] to model biological systems since the former are able to capture the compounds flow, the reaction velocity, the enabling/inhibiting reactions and both the transitory and steady states of reaction dynamic into a single formalism.

This work is concerned on how to model the entire metabolome with $TCPN$. It proposes a bottom-up modeling methodology where biochemical reactions are modeled through elementary modules, and shows how these modules are merged to form metabolic pathways, and at the end the cell metabolism. The resulting model captures both, the transitory and steady state metabolome dynamics. It is worth noticing that the derived $TCPN$ model condenses several particular behaviors represented by the set of differential equations generated by the $TCPN$ itself. For instance, a single transition with four input places (a reaction needing four substrates) generates a set of four possible differential equations while two transitions with four input places each will generate a set of sixteen possible differential equations. Therefore highly complex behaviors emerging from few compounds interacting can be captured by $TCPN$.

This work also presents the control problem of reaching a required state (marking) representing a certain metabolite concentration. In order to solve this problem, an error equation is stated and stabilized using a Lyapunov approach. The solution is the reaction rate vector which is greater or equal to zero and lower or equal to the maximum settled by the kinetics of Michaelis-Menten for the current enzyme concentration. Thus, if a solution exists, it could be implemented *in vivo* by directed genetic mutation, knock-in (or knock-out) strategies or pharmacological effects.

Present paper is organized as follows. Section 2 gives $TCPN$ basic definitions, controllability and cell metabolic concepts. Next section introduces the proposed metabolome modeling methodology. Section 4 presents the problem of reaching a required state and synthesizes Lyapunov like transition flow for solving this problem. Following section presents an illustrative example to show the performance of the computed control law. In the last section the conclusions and future work are presented.

2 Basic Definitions

This section presents briefly the basic concepts related with PN, *Continuous* PN and $TCPN$. An interested reader can review [3], [14], [15] and [16] for further information. At the end of this section a useful form of the state equation for $TCPN$ under infinite server semantics is presented.

2.1 Petri Net concepts

Definition 1. *A Continuous Petri Net (ContPN) system is a pair* (N, m_0)*, where* $N = (P, T, Pre, Post)$ *is a Petri net structure (PN) and* $m_0 \in \{\mathbb{R}^+ \cup 0\}^{|P|}$ *is the initial marking.* $P = \{p_1, ..., p_n\}$ *and* $T = \{t_1, ..., t_k\}$ *are finite sets of elements named places and transitions, respectively.* $Pre, Post \in \{\mathbb{N} \cup 0\}^{|P| \times |T|}$

are the Pre and Post incidence matrices, respectively, where $Pre[i,j]$, $Post[i,j]$ *represent the weights of the arcs from* p_i *to* t_j *and from* t_j *to* p_i, *respectively. The Incidence matrix denoted by* C *is defined by* $C = Post - Pre$.

Each place p_i has a marking denoted by $m_i \in \{\mathbb{R}^+ \cup 0\}$. The set ${}^\bullet t_i = \{p_j \mid Pre[j,i] > 0\}$, $(t_i^\bullet = \{p_j \mid Post[j,i] > 0\})$ is the preset (postset) of t_i. Similarly the set ${}^\bullet p_i = \{t_j \mid Post[i,j] > 0\}$, $(p_i^\bullet = \{t_j \mid Post[i,j] > 0\})$ is the preset (postset) of p_i.

A transition $t_j \in T$ is enabled at marking m iff $\forall p_i \in {}^\bullet t_j$, $m_i > 0$. Its enabling degree is:

$$enab(t_j, m) = \min_{p_i \in {}^\bullet t_j} \frac{m_i}{Pre\,[i,j]} \tag{1}$$

and it is said that m_i constraints the firing of t_j. Equation (1) denotes the maximum amount that t_j can be fired at marking m; indeed t_j can fire in any real amount α, where $0 < \alpha < enab(t_j, m)$ leading to a new marking $m\prime = m + \alpha C[\bullet, j]$. If m is reachable from m_0 through a finite sequence σ of enabled transitions, then m can be computed with the equation:

$$m = m_0 + C\sigma \tag{2}$$

named the *ContPN* state equation, where $\sigma \in \{\mathbb{R}^+ \cup 0\}^{|T|}$ is the firing count vector, i.e., σ_j is the cumulative amount of firing of t_j in the sequence σ. The set of all reachable markings from m_0 is called the reachability space and it is denoted by $RS\,(N, m_0)$. In the case of a *ContPN* system, $RS\,(N, m_0)$ is a convex set [17].

Definition 2. *A contPN is bounded when every place is bounded* ($\forall p \in P, \exists b_p \in \mathbb{R}$ *with* $m\,[p] \leq b_p$ *at every reachable marking* m). *It is live when every transition is live (it can ultimately occur from every reachable marking). Liveness is extended to lim-live when infinitely long sequence can be fired. A transition* t *is non lim-live iff a sequence of successively reachable markings exists which converge to a marking such that none of its successors enables a transition* t.

2.2 Timed continuous Petri nets

Definition 3. *A timed ContPN is the 3-tuple* $TCPN = (N, \lambda, m_0)$, *where* N *is a ContPN,* $\lambda : T \to \{\mathbb{R}^+\}^{|T|}$ *is a function that associates a maximum firing rate to each transition, and* m_0 *is the initial marking of the net* N.

The state equation of a $TCPN$ is

$$\begin{aligned} \overset{\bullet}{m}(\tau) &= Cf(\tau) \\ \text{where } f(\tau) &= \overset{\bullet}{\sigma}(\tau) \end{aligned} \tag{3}$$

And under the infinite server semantics, the flow of transition t_j is given by

$$f_j(\tau) = \lambda_j enab(t_j, m(\tau)) \tag{4}$$

where λ_j represents the maximum firing rate of transition t_j. Notice that $TCPN$ under infinite server semantics is a piecewise linear system (a class of hybrid systems) due to the *minimum* operator that appears in the enabling function of the flow definition.

Definition 4. *A configuration of a $TCPN$ at m is a set of (p,t) arcs describing the effective flow of all transitions.*

$$\Pi(m)[i,j] = \begin{cases} \frac{1}{Pre[i,j]} & \text{if } p_i \text{ is constraining } t_j \\ 0 & \text{otherwise} \end{cases} \tag{5}$$

Definition 5. *The maximum firing rate matrix is denoted by*

$$\Lambda = diag\left(\lambda_1, \ldots, \lambda_{|T|}\right). \tag{6}$$

According to previous notation, the state equation and the flow vector are described by:

$$\begin{aligned} \dot{m} &= C\Lambda\Pi(m) \cdot m \\ f &= \Lambda\Pi(m) \cdot m \end{aligned} \tag{7}$$

The only action that can be applied to a $TCPN$ system is to slow down the firing flow. The forced flow of a controlled transition t_i becomes $f_i - u_i$ where f_i is the flow of the unforced system (i.e. without control) and u is the control action, with $0 \leq u_i \leq f_i$. The controlled state equation is:

$$\dot{m} = C\left[\Lambda\Pi(m) \cdot m - u\right] \tag{8}$$

$$0 \leq u_i \leq \left[\Lambda\Pi(m) \cdot m\right]_i \tag{9}$$

In order to obtain a simplified version of the state equation, the input vector u is rewritten as $u = I_u\Lambda\Pi(m) \cdot m$, where $I_u = diag\left(I_{u_1}, \ldots, I_{u_{|T|}}\right)$ and $0 \leq I_{u_i} \leq 1$. Then the matrix $I_c = I - I_u$ is constructed and the controlled state equation can be rewritten as:

$$\dot{m} = CI_c\Lambda\Pi(m) \cdot m \tag{10}$$

Notice that $0 \leq I_{c_i} \leq 1$.

2.3 Controllability

The classical linear systems definition of controllability cannot be applied to $TCPN$ systems because the required hypothesis are not fulfilled, that is, the input should be unbounded and the state space should be $\mathbb{R}^{|P|}$. The next definitions are taken from [18].

Definition 6. *Let N be net of a $TCPN$. The structural admissible states set is defined as $SASS(N) = \{\mathbb{R}^+ \cup \{0\}\}^{|P|}$ (all inital markings that can be imposed to a net). Let B be the base of the left annuller of the incidence matrix C. The equivalence relation $\beta : SASS(N) \to SASS(N)$ is defined as $m_1 \beta m_2$ iff $B^T m_1 = B^T m_2$, $\forall m_1, m_2 \in SASS(N)$. The system admissible states set is the equivalent class of the initial marking $Class(m_0)$ under β.*

In the sequel, let us denote by $int\left(Class\left(m_0\right)\right)$ the set of relative interior of $Class\left(m_0\right)$.

Definition 7. *Let (N, λ, m_0) be a TCPN system. It is fully controllable with bounded input $(BIFC)$ if there is an input such that for any two markings $m_1, m_2 \in Class\left(m_0\right)$, it is possible to transfer the marking from m_1 to m_2 in finite or infinite time, and the input fulfills (9) along the trajectory, and is controllable with bounded input (BIC) over $S \subseteq Class\left(m_0\right)$ if there is an input such that for any two markings $m_1, m_2 \in S$, it is possible to transfer the marking from m_1 to m_2 in finite or infinite time, and the input fulfills (9) along the trajectory.*

Definition 8. *Let (N, λ, m_0) be a TCPN system. Let $m_r \in RS\left(N, m_0\right)$ and $0 \leq I_{c_r}[i, i] \leq 1$. Then $\left(m_r, I_{c_r}\right)$ is an equilibrium point if $\dot{m}_r = CI_{c_r}\Lambda\Pi\left(m_r\right) \cdot m = 0$. Then, the steady state flow for $\left(m_r, I_{c_r}\right)$ is $f_{ss}\left(m_r, I_{c_r}\right) = I_{c_r}\Lambda\Pi\left(m_r\right) \cdot m_r$.*

An equilibrium point represents a state in which the system can be maintained using the defined control action. Given an initial marking m_0 and a required marking m_r, one control problem is to reach m_r and then keep it. For a further information about equilibrium points an interested reader can review [19].

2.4 Cell Metabolism

For the wellbeing of an given organism, each cell of that organism must transform the substances available in its surroundings to useful molecules. Such transformations take place as chemical reactions catalyzed by enzymes. In these reactions, a substrate tightly binds non-covalently to its enzyme active site to build an enzyme-substrate complex. At that moment, the enzyme chemically changes the substrate into one or more products and then releases it. The enzyme did not suffer any irreversible alterations in the process, and now is free to accept a new substrate [20].

There is no limit to the number of possible reactions occurring in nature. Nonetheless, after exhaustive analysis certain general patterns had emerged that became useful to describe several characteristics of biochemical reactions. In the case where a sole substrate becomes a single product, the reaction process is represented by the scheme:

$$E + S \rightleftharpoons ES \rightarrow EQ \rightleftharpoons E + Q \tag{11}$$

where E is the enzyme, S is the substrate, ES and EQ are the bound complexes and Q is the product.

Typically, the rate of these reactions is settled by the kinetics of Michaelis-Menten [21]. Under this kinetic model, the enzyme and substrate react rapidly to form an enzyme-substrate complex while $[S]$ and $[ES]$ are considered to be at concentration equilibrium (the same applies to $[EQ]$ and $[Q]$), that is, the rate

at which ES dissociates into $E + S$ is much faster than the rate at which ES brakes down to EQ.

Throughout the present work, we will consider a physiological cellular state where $[S] >> [E]$, which means that $[S] \rightleftharpoons [ES]$ equilibrium will always tend to complex formation. Therefore, ES dissociation rate is irrelevant and Scheme 11 can be abbreviated as follows:

$$E + S \rightarrow E + Q \tag{12}$$

where the association-dissociation is implicit.

In reactions with more than one substrate, binding can occur in different sequences; for instance, the following scheme represents an enzyme system with two substrates and all the possible sequences:

$$E + S_1 + S_2 \rightleftharpoons \begin{cases} ES_1 + S_2 \\ \quad \uparrow\downarrow \\ ES_1S_2 \rightarrow E + Q_1 + ... + Q_n \\ \quad \uparrow\downarrow \\ ES_2 + S_1 \end{cases} \tag{13}$$

Frequently the product of an enzyme is the substrate of another reaction and so on, to build a chain of reactions called metabolic pathways represented by $MP^j = \Gamma_1^j \Gamma_2^j \cdots \Gamma_n^j$ where Γ_i^j is a reaction (12) or (13) of a pathway j and Γ_k^j uses one or more products of Γ_i^m. Notice that j and m may represent different pathways.

Then a (Cell) Metabolome is $CM = \left\{ MP^i \,\middle|\, MP^i \text{ is a metabolic pathway} \right\}$, and the purpose of CM is to produce a particular set of metabolites in certain concentrations, essential to that cell.

3 Modelling the Metabolome

In order to model the metabolome using $TCPN$ it is necessary to identify how the elements involved in it will be represented. The next table relates the meaning of each element of the $TCPN$ with respect to metabolic reactions.

TCPN term	**Molecular interpretation**
Place	Molecular Species
Marking	Concentration
Transition	Reaction
Firing Rate	Rate of Reaction
Arc Weights	Stoichiometric Coefficients

The bottom-up approach herein proposed to model the metabolome consists of: a) representing reactions, the results of this stage are the elementary modules; b) merging elementary modules, where places of elementary modules representing the same molecular species on the same physical space in the cell will merge

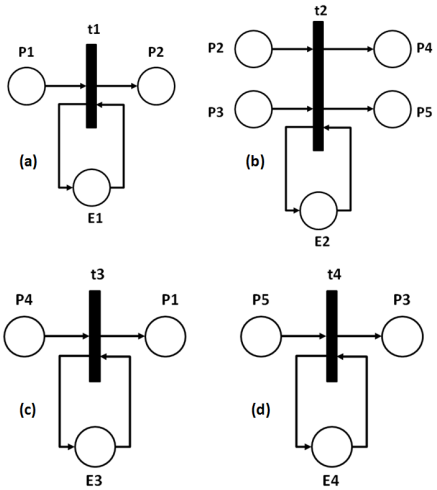

Fig. 1. Four elementary modules representing four diferent reactions.

into a single place. The results of this stage are pathway modules; and c) merging pathways modules, where places of pathways modules representing the same molecular species on the same cellular space will merge into a single place; the result of this stage is the metabolome model. For stages b and c, any specie being protein-mediated transported into a different organelle shall be modeled through the same elementary module, representing instead of substrate and product the same molecule in different spaces.

Next section describes these stages.

3.1 Representing Reactions

In order to represent each reaction Γ_i with $TCPN$ elementary modules representing the Scheme (12) or (13) are constructed. There exists one place p_j for each molecular species at the same physical space ms_j and one transition t_i to represent the reaction Γ_i. There exists one arc (p_s, t_i) if p_s represents a substrate. There exists one arc (t_i, p_q) if p_q represents a product. Finally, there exists a self-loop around p_e and t_i if p_e represents an enzyme. The initial marking $m_0\,[p_j]$ is the concentration of the molecular species ms_j at time $\tau = 0$.

Associated to transition t_i is λ_i representing the rate of reaction.

Example 1. Let $P1 + E1 \rightarrow P2 + E1$ be the Γ_1 reaction. There is one place for each molecular species ($P1$, $P2$ and $E1$), and one transition t_1 representing Γ_1. Finally, arcs are fixed in the way depicted in Figure 1a.

Assuming that the substrate concentration will remain higher than the enzyme concentration (this is an expected behavior of the system), the conflict

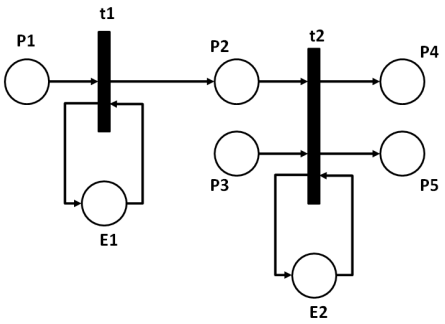

Fig. 2. Example of a Pathway Module.

between substrate and enzyme can be ignored. Hence, if a system has 2^n configurations originated by the n number of enzymes in conflict with substrates, all those configurations are eliminated because $\min([E],[S]) = [E]$ for all $\tau \geq 0$.

3.2 Merging Elementary Modules

Let N^1 and N^2 be two elementary modules, then the merging N is such that $N = (P, T, Pre, Post)$ where $P = P^1 \cup P^2$, $T = T^1 \cup T^2$, $Pre = Pre^1 \cup Pre^2$ and $Post = Post^1 \cup Post^2$. Notice that places representing the same molecular species in the same physical space are merged into a single place.

After a merging of elementary modules is made, pathway modules are obtained.

Example 2. Let $N^1 = \left(P^1, T^1, Pre^1, Post^1\right)$ and $N^2 = \left(P^2, T^2, Pre^2, Post^2\right)$ be two elementary modules showed in Figure 1a and Figure 1b respectively. Then, the merging is $N = (P, T, Pre, Post)$ where $P = P^1 \cup P^2 = \{P1, ..., P5, E1, E2\}$, $T = T^1 \cup T^2 = \{t_1, t_2\}$ and arcs are fixed in the way depicted in Figure 2, where the merging is showed.

3.3 Merging Pathway Modules

Let N^1 be a pathway module and N^2 be a pathway or an elementary module, then the merging N is such that $N = (P, T, Pre, Post)$ where $P = P^1 \cup P^2$, $T = T^1 \cup T^2$, $Pre = Pre^1 \cup Pre^2$ and $Post = Post^1 \cup Post^2$. Notice that places representing the same molecular species are merged into a single place.

After a merging of pathway modules is made, a metabolic model is obtained.

Example 3. Let $N^1 = \left(P^1, T^1, Pre^1, Post^1\right)$ be the pathway module showed in Figure 2. Let $N^2 = \left(P^2, T^2, Pre^2, Post^2\right)$ and $N^3 = \left(P^3, T^3, Pre^3, Post^3\right)$ be two elementary modules showed in Figure 1c and Figure 1d respectively. Then

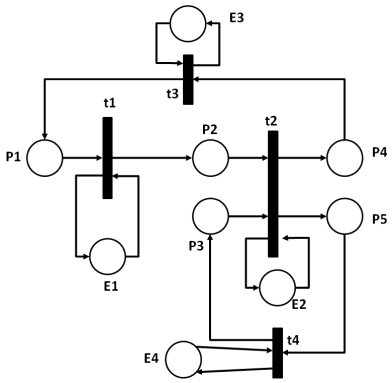

Fig. 3. Metabolic Model.

the merging is $N = (P, T, Pre, Post)$, where $P = \cup P^i = \{P1, ..., P5, E_1, ..., E_4\}$, $T = \cup T^i = \{t_1, t_2, t_3, t_4\}$ for $i = 1, ..., 4$. Arcs are fixed in Figure 3, where the merging is showed.

Although obtained metabolic models could be not live, the addition of a virtual transition and arcs going from the last place representing final products to the virtual transition and from virtual transition to the places representing initial products with an appropriate virtual reaction velocity will make the metabolic model live. For instance, consider the net of Figure 1a, it is a non-live net, but if we add a virtual input transition t_v to the place S and a virtual output transition t_v to the place Q the system will gain liveness, see Figure 4. Notice that t_v must to be the same transition added to the inital and final metabolites, this is because it is necessary to maintain the conservativeness of the matter of the system. This notion is based assuming that each module belongs to a bigger system, therefore, although the real input and output transitions could be not the same, they must have the same firing ratio.

4 Control Law

An important control problem in the metabolic engineering area is to reach a certain metabolome state such that the production of selected metabolites is regulated or particular processes are limited or favored. This problem is captured in $TCPN$ as the reachability problem, i.e. to reach a required state m_r from an initial state m_0 by means of an appropriate control action. This is formalized as follows.

Definition 9. *Let $TCPN$ be a metabolic model. Then the* `Regulation Control Problem` *in (m_r, I_{c_r}) ($RCP(m_r, I_{c_r})$) deals with the computation of a control law*

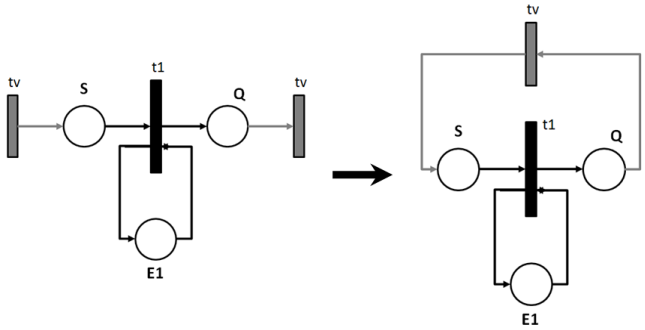

Fig. 4. Module forced to be live with the addition of a virtual transition t_v (in gray).

$I_c(\tau)$, $0 \leq \tau < \tau_f$ *feasible in the TCPN such that* $m(\tau_{ss}) = m_r$ *and* $I_c(\tau_{ss}) = I_{c_r}$, $\forall \tau_{ss} \geq \tau_f$.

In order to solve this problem, some extra places are added to the $TCPN$ metabolic model to detect the material passing through transitions. The following definition shows how these places are added to the $TCPN$.

Definition 10. *Let* (N, m_0, λ) *be a metabolic model* $TCPN$, *where* $N = (P, T, F)$. *Its extension is defined by* $xTCPN = (N_x, m_{0_x}, \lambda)$, *where* $N_x = (P \cup P_a, T, F \cup F_a)$, $|P_a| = |T|$, $m_{0_x} = \left[m_0 \; 0_{|T|} \right]^T$, $F_a = \{(t_i, p_{a_i}) \,|\forall t_i \in T \text{ and } \forall p_{a_i} \in P_a\}$. *Then the incidence matrix of* $xTCPN$ *is* $C_x = \left[C \; I_{|T|} \right]^T$.

Since $\Pi_x(m_x) = \left[\Pi(m) \; 0_{|T| \times |T|} \right]$, then the state equation of $xTCPN$ is:

$$\dot{m}_x = \begin{bmatrix} \dot{m} \\ \dot{m}_a \end{bmatrix} = \begin{bmatrix} CI_c \Lambda \Pi(m) \cdot m \\ I_c \Lambda \Pi(m) \cdot m \end{bmatrix} \tag{14}$$

$$m(0) = m_0, \; m_a(0) = 0 \tag{15}$$

Remark 1. Notice that the extension has the same dynamic over the metabolic model places and the extra places can only increase its marking. In fact, due to the $TCPN$ is live, then by construction the $xTCPN$ is also live. Then there exists at least one enabled transition. Hence $\Pi(m) \cdot m > \mathbf{0}$ (or equivalently $\dot{m}_a \geq \mathbf{0}$, the zero could be forced by an appropriate control law I_c).

Example 4. An example of an extended net is presented in Figure 5.

4.1 Solution to the RCP (m_r, I_{c_r})

Theorem 1. *Let* (N, m_0, λ) *be a metabolic model* $TCPN$ *and let* $xTCPN = (N_x, m_{0_x}, \lambda)$ *be its extension. If* (N, m_0, λ) *is BIC over int* $(Class(m_0))$ *(notice*

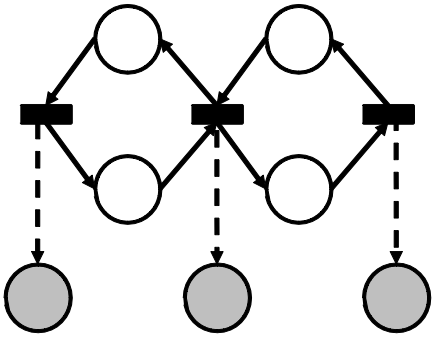

Fig. 5. Example of an extended net. The gray places are the set of added places P_a and the intermittent arrows are the set of added arcs F_a.

that neither the initial marking nor the required marking could be zero components) and (I_{c_r}, m_r) *is an arbitrary equilibrium point, then there exists* $I_c(\tau)$, $0 \leq \tau \leq \tau_f$ *feasible in the TCPN such that* $m(\tau_{ss}) = m_r$, $I_c(\tau_{ss}) = I_{c_r}$, $\forall \tau_{ss} \geq \tau_f$.

Proof. If the system is *BIC* over $int\,(Class\,(m_0))$, then there exists a positive solution $\sigma_r(\tau)$ feasible such that

$$m_r = m_0 + C\sigma_r \tag{16}$$

This result was taken from [18]. Thus there exists $f(\tau)$ such that:

$$\int_0^{\tau_f} f(\tau)d\tau = \int_0^{\tau_f} I_c \Lambda \Pi\,(m) \cdot m d\tau = \sigma_r \tag{17}$$

From (14):

$$m_a(\tau_f) = \sigma_r \tag{18}$$

Now, let

$$\begin{gathered} e_x(\tau) = \left[e(\tau)\ e_a(\tau)\right]^T,\ 0 \leq \tau \leq \tau_f \\ \left[e(\tau)\ e_a(\tau)\right]^T = \left[m_r - m(\tau)\ \sigma_r - m_a(\tau)\right]^T \end{gathered} \tag{19}$$

and

$$V\,(e_x) = e_x^T P_L e_x \tag{20}$$

where

$$P_L = \begin{bmatrix} 0 & 0 \\ 0 & I_{|T|} \end{bmatrix} \tag{21}$$

and $I_{|T|}$ is an identity matrix of order $|T| \times |T|$. We claim that $V\,(e_x)$ is a Lyapunov function, i.e it is positive definite and its derivative is negative definite.

Since Equation (20) is clearly non-negative definite, then we assume that (22) is positive semidefinite, then there exists $e_x(\tau') \neq 0$ such that:

$$V\left(e_x(\tau')\right) = \left[\, e^T \; e_a^T \,\right] \begin{bmatrix} 0 & 0 \\ 0 & I_{|T|} \end{bmatrix} \begin{bmatrix} e \\ e_a \end{bmatrix} = 0 \tag{22}$$

From (22), it is clear that $e_a(\tau') = 0$, then from (19) $m_a(\tau') = \sigma_r$. Thus, from (14) and (17) and letting $\tau_f = \tau'$:

$$\begin{aligned} \int_0^{\tau_f} \overset{\bullet}{m}(\tau) d\tau &= C\sigma_r \\ m(\tau_f) - m(0) &= C\sigma_r \end{aligned} \tag{23}$$

Thus, from Equation 16 $m(\tau_f) = m_r$, then $e_x = 0$, a contradiction. Hence $V\left(e_x\right)$ is positive definite.

Now , we prove that $\overset{\bullet}{V}\left(e_x\right)$ is negative definite. The differentiate of $V\left(e_x\right)$ is:

$$\overset{\bullet}{V}\left(e_x\right) = 2e_a^T \overset{\bullet}{e}_a = -2\left[\sigma_r - m_a\right]^T \overset{\bullet}{m}_a \tag{24}$$

Then, choosing I_c such that:

$$I_{c_i} = \begin{cases} 1 \text{ if } m_a\left[i\right] < \sigma_r\left[i\right] \\ 0 \quad otherwhise \end{cases} \tag{25}$$

we obtain:

$$\left[\sigma_r - m_a\right]^T I_c > \mathbf{0} \tag{26}$$

and

$$\left[\sigma_r - m_a\right]^T I_c = 0 \text{ iff } \left[\sigma_r - m_a\right]^T = \mathbf{0}$$

thus $\overset{\bullet}{V}\left(e_x\right) < 0$ and $\overset{\bullet}{V}\left(0\right) = 0$.

Since $m_a(0) = 0$ and it only increase its value, then I_{c_i} is feasible leading from $m_a(0) = 0$ to $m_a(\tau_f) = \sigma_r$, i.e. from m_0 to m_r. Moreover, assuming $m_r \in int\left(Class\left(m_0\right)\right)$ it is reached in finite time because $\overset{\bullet}{m}_a\left[i\right] = m\left(\min\left({}^\bullet t_i\right)\right) e^{\lambda\tau}$ and $m\left(\min\left({}^\bullet t\right)\right) \neq 0 \; \forall\tau$. At τ_f the control law must be switched from $I_c(\tau_f)$ to $I_c(\tau_{ss}) = I_{c_r}$ and the regulation control problem is solved.

■

The solution to the $RCP\ (m_r, I_{c_r})$ include both, the transitory and steady state control of metabolic systems. It is an improvement to current control solutions, where the biologist and metabolic engineers use stoichiometric non-dynamical approaches such as FBA (Flux Balance Analysis) [22], [23], [24] for the control of metabolic systems. Those are based on a pseudo-stationary state model, represented by the equation:

$$Sv = 0 \tag{27}$$

where S is the matrix of stoichiometry coefficients and the solution v gives the balance of mass for a single equilibrium point at that state (v is the reaction rates vector in a steady state).

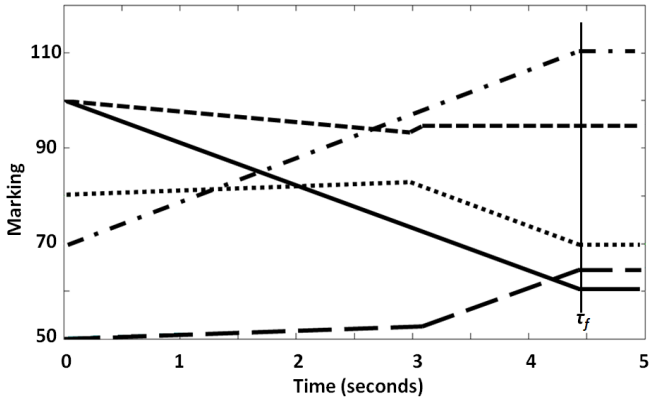

Fig. 6. Marking evolution of the net of Figure 3 applying $RCP\ (m_r, I_{c_r})$.

5 Illustrative Controlling Metabolic System Example

In order to illustrate the $RCP\ (m_r, I_{c_r})$ applied to a metabolic system, suppose the pathway module of Figure 2 together with modules c and d of Figure 1 comprise a cell metabolome. The initial marking used for this example is an arbitrary but physiologically possible initial state for the alleged metabolic model.

Example 5. Let the metabolome model of the Figure 3 be the system $TCPN = (N, \lambda, m_0)$ with $\Lambda = diag\,(2, 3, 4, 1)$ and $m_0 = \left[100\ 80\ 100\ 50\ 70\ 5\ 3\ 2\ 4\right]^T$. Let $m_r = \left[95\ 70\ 60\ 65\ 110\ 5\ 3\ 2\ 4\right]^T$ be a required marking. We make the extended system like the procedure showed in the Figure 5. We need the solution of σ_r from $m_r = m_0 + C\sigma_r$. Notice that there are a lot of solutions for σ_r but we only focus on the smallest solution of σ_r. For this example the solution is:

$$\sigma_r = \left[30\ 40\ 25\ 0\right]^T$$

Solving the $RCP\ (m_r, I_{c_r})$ and applying the control (25) to the $TCPN = (N, \lambda, m_0)$, the metabolite concentrations are depicted in Figure 6. The reaction velocities (transition flux) is depicted in Figure 7. Notice that from $\tau = 0$ to $\tau = \tau_f \approx 4.5$ occurs the transitory dynamics, and for $\tau > \tau_f$ the steady state is reached.

Example 6. In Figure 8 the evolution of marking m_a is depicted. When occurs $m_a[i] = \sigma_r[i]$ the control $I_{c_i} = 0$ makes $f_i = 0$ and $m_a[i]$ is maintained until $\tau = \tau_f\ (m_a = \sigma_r)$. Then I_c switches to I_{c_r} for the steady state control.

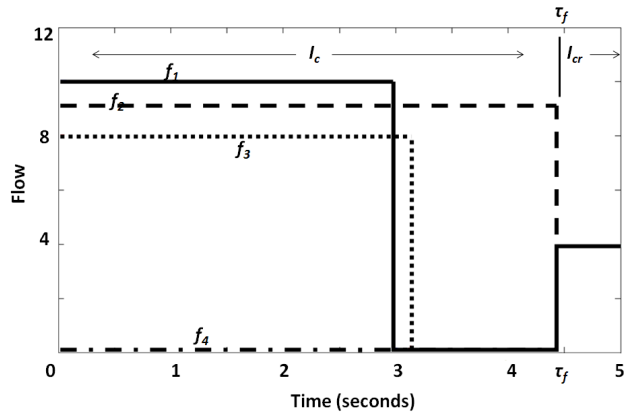

Fig. 7. Reaction velocities (transition flows) of the controlled metabolic model of the Example 5. Notice that $I_c(\tau)$ is applied for $0 \leq \tau < \tau_f$ and $I_{cr}(\tau)$ for $\tau > \tau_f$.

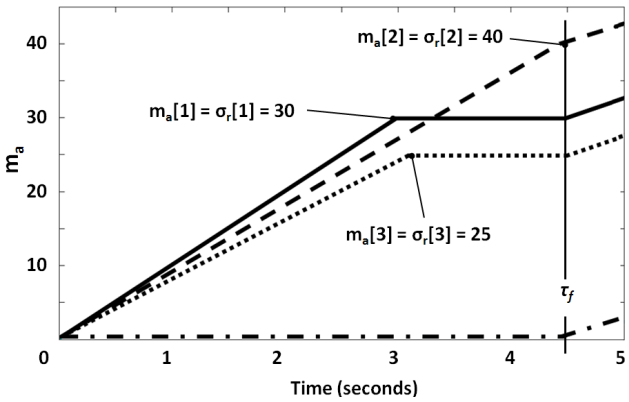

Fig. 8. Marking m_a of the Example 5.

6 Conclusions

This work presented a model methodology to capture the metabolome behavior. It uses a bottom-up approach where each individual biochemical reaction is modeled by elementary $TCPN$ modules and, afterwards, all the modules are merged into a single one to capture the whole metabolome behavior. Such characteristic of the methodology makes it simple and easy to use while the complex cell metabolic behavior is captured. This work also presented the problem of reaching a required metabolome state. The solution to this problem are the instantaneous reaction velocities that are realizable in biological system.

Present results are being applied to optimize metabolome fermentation in the production of tequila and to biofuels generation.

Future perspective involves introduction of stochastic modelling and merging the metabolome with the signaling and genetic networks.

References

1. Murata, T.: Petri nets: Properties, analysis and applications. In: Proceedings of IEEE. Volume 77. (1989) 541–580
2. Javier, Esparza; Manuel, S.: Compositional synthesis of live and bounded free choice petri nets. LNCS **571** (1991) 172–187
3. Jorg Dessel, J.E.: Free Choice Petri Nets. Cambridge University Press (1995)
4. Manuel Silva, L.R.: Continuization of timed petri nets: From performance evaluation to observation and control. 26th International Conference On Application and Theory of Petri Nets and Other Models of Concurrency (2006)
5. R. David, H.A.: Continuous petri nets. In: In Proc. Of the 8th European Workshop on Application and Theory of Petri Nets. (1987) 275–294
6. Peter J. E. Goss, J.P.: Quantitative modeling of stochastic systems in molecular biology by using stochastic petr nets. In: Natl. Acad. Sci. USA. Volume 95. (1998) 6750–6755
7. Masao Nagasaki, Atsushi Doi, H.M.S.M.: Petri net based description and modeling of biological pathways. Algebraic Biology (2005) 19–31
8. Monika Heiner, David Gilbert, R.D.: Petri nets for systems and syntethic biology. (2008) 215–264
9. David Angeli, Patrick De Leenheer, E.S.: A petri net approach to persistance analysis in chemical reaction networks. Biology and Control Theory: Current Challenges (2007) 181–216
10. Riel, N.A.W.V.: Dynamic modelling and analysis of biochemical networks: Mechanism-based models and model-based experiments. Briefings In Bioinformatics **7** (2006) 364–374
11. Nevoigt, E.: Progress in metabolic engineering of saccharomyces cerevisiae. Microbiology And Molecular Biology Reviews **72** (2008) 379–412
12. Mor Peleg, Daniel Rubin, R.B.A.: Using petri net tools to study properties and dynamics of biological systems. Journal of the American Medical Informatics Association **12** (2005) 181–199
13. Hiroshi Matsuno, Atsushi Doi, M.N.S.M.: Hybrid petri net representation of gene regulatory network. Pacific Symposium on Biocomputing **5** (2000) 338–349

14. Cassandras, C.G.: Discrete Event Systems. Modelling and Performance Analysis. Asken Associates (1993)
15. René David, H.A.: Continuous and hybrid petri nets. Journal of Circuites, Systems and Computers **8** (1998) 159–188
16. R. David, H.A.: Continuous petri nets. Proceedings of the 8th European Workshop on Application and Theory of Petri Nets (1987) 275–294
17. Manuel Silva, L.R.: On fluidification of petri net models: From discrete to hybrid and continuous model. Annual Reviews in Control 28 (2004) 253–266
18. C. R. Vázquez, A. Ramírez-Treviño, L.R.M.S.: On controllability of timed continuous petri nets. 11th Int. Workshop Hybrid Systems: Computational and Control **4981** (2008) 528–541
19. Cristian Mahulea, Antonio Ramirez, L.R.M.S.: Steady state control, zero valued poles and token conservation law in continuous net systems. Proceedings of the International Workshop on Control of Hybrid and Discrete Event Systems (2005)
20. Laszlo Kurti, B.C.: Strategic Applications of Named Reactions in Organic Synthesis. Elsevier Academic Press. USA (2005)
21. Segel, I.H.: Enzyme Kinetics. New York: Wiley-Interscience (1975)
22. J. M. Lee, E. P. Gianchandani, J.A.P.: Flux balance analysis in the era of metabolomics. Briefs in Bioinformatics **7** (2006) 140–150
23. J. S. Edwards, M. Covert, B.P.: Metabolic modelling of microbes: The flux-balance approach. Environmental Microbiology **4** (2002) 133–140
24. Francisco Llaneras, J.P.: Stoichiometric modelling of cell metabolism. Journal of Bioscience and Bioengineering **105** (2008) 1–11