Delay Time Estimation for Petri Net Models of Signaling Pathways Based on Experimental Data

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Abstract: In this paper, we propose a delay time estimation algorithm for Petri net models of signaling pathways based on experimental data. Firstly, we model signaling pathways (we use the example of ErbB4 receptor signaling pathway) with discrete Petri nets. Then, we propose a delay time estimation algorithm for Petri net models with experimental data, and assign estimated delay times to a model to be run on the simulation tool Cell Illustrator 3.0. Finally, we verify the simulation result by comparing with the experimental data and evaluate the performance of the estimation algorithm.

1 Introduction

Signaling pathways regulate elaborate cell communication mechanisms by controlling various alteration procedures of cell behavior, such as cell growth, survival, proliferation, and apoptosis. Under such cellular communication mechanism, cell activities could be preciously governed and maintained in a good condition along with other biochemical interactions and processes. Till now, such signaling pathways have been investigated by many biological experiments as well as computer experiments.

Recently, Li *et al.* [1] proposed a qualitative modeling method by paying attention to the molecular interactions and mechanisms using discrete Petri nets. Further, they proposed a timed Petri net based method of determining the delay times of transitions, i.e., the time taken in firing each transition. Their basic consideration is that the number of tokens flowed into a place is equivalent to the number of tokens flowed out. They also performed computational simulations of apoptosis as a running example along with obtained delay times [2]. However, the estimation of a delay time in their proposal did not consider biological experimental data. Therefore, in order to carry out more precise simulation that could reproduce the facts described in biological experiments and/or scientific literature, we propose a new simulation method that estimates a delay time based on given experimental data.

In this paper, we first model signaling pathways with discrete Petri nets using the modeling method proposed by Li *et al.* [1]. Then, we propose a delay time estimation algorithm for Petri net models with provided concentration behaviors of proteins depicted by Hatakeyama *et al.* [3]. Finally, the availability of proposed algorithm is confirmed through simulation experiments on ErbB4 receptor signaling pathways with a simulation tool Cell Illustrator 3.0..

2 Model Decomposition

In this paper, we use the example of ErbB4 receptor (reside in ErbB receptor family) signaling pathway to explain our proposed method. ErbB tyrosine kinase receptors mediate mitogenic signal cascade by binding a variety of ligands such as EGF and recruiting the different cassettes of adaptor proteins. The binding of ligands to ErbB receptors results in diverse biological outputs, such as cellular proliferation and differentiation, and their deregulated expression or mutation highly correlates with the incidence of certain types of human cancer. First of all, we modeled ErbB4 signaling pathways with discrete Petri nets using the modeling method proposed by Li *et al* . We make no reference to detail of the modeling method because of space limitations, please refer to Ref. [1].

Before the procedure of a delay time estimation, we decompose a model into smaller components. The procedure of pathway decomposition is intended to independently determine a delay time within each component that is a subgraph of Petri net model. This is expected to improve computational efficiencies because of the reduction in the dimensionality of the search space and in the number of local minimma [5].

It is easy to decompose a Petri net models of signaling pathways into smaller components. Here, we regard enzyme places possessing experimental data which are called *data places* hereafter. Before the explanation of model decomposition method, we discuss some properties of enzyme places as follows: (i) if a data place $dp_n(n{\in}N)$ connects to a output transition via a test arc, dp_n must exist in component C_n; (ii) if a place exists in the component C_n, all the transitions connected from the place via normal arcs must exit in C_n. However, (i) and (ii) do not hold in case that the place is already exists in other component, therefore (iii) if a transition is in C_n, all the places connected from the transition must appear in C_n. These properties are based on the assumption (a) the reactions denoted by these transitions determine the concen-

tration levels of protein of places connected with a transition via normal arc(s), and (b) a transition connected with a place via an inhibitory arc or a test arc does not affect the concentration level of the place.

To explain the method of decomposing a Petri net model, we give a simple example (Fig. 1). Firstly, we choose the most upstream data place as dp_1 and search the component C_1 by following the above rules. After that, we choose the next upstream data place as dp_2, and search the component C_2. In this way, we decompose the model to construct components starting from more upstream data place. By following above method of constructing component, we decompose a Petri net model of ErbB4 receptor signaling pathway into smaller components (Fig. 2).

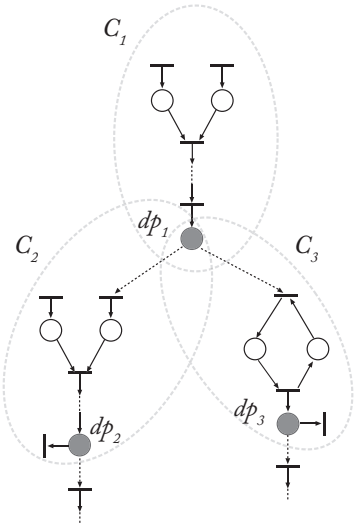

Figure 1: Example of model decomposition. Black colored places are data places (dp_n).

Experimental data of ErbB4 receptor signaling pathway is illustrated in a graph and corresponding tables depicting a variation of activated enzyme concentration over time. In the present study, when we estimate a delay time, we used experimental data which EGF concentration is at 10 nM. In this paper, we propose a new estimation algorithm to determine a delay time of each transition based on these data. Note that, the time point where the activated enzyme concentration reaches the peak of concentration curve on the graph could be considered as the time point D of which the activation of enzyme is the highest after receiving extracellular stimuli. The time point D is used for a delay time estimation.

3 Algorithm to Estimate Delay Time

In this section, we show an algorithm to estimate delay time of each transition to evaluate dynamic behaviors and confirm the validity the model. To do simulation of systems, Petri net model is usually extended by assigning firing delay time to transitions. Such extended Petri net is called timed Petri net $\overline{N} = (\overline{T}, \overline{P}, \overline{E}, \alpha, \beta)$. The firing rule of a timed Petri net is

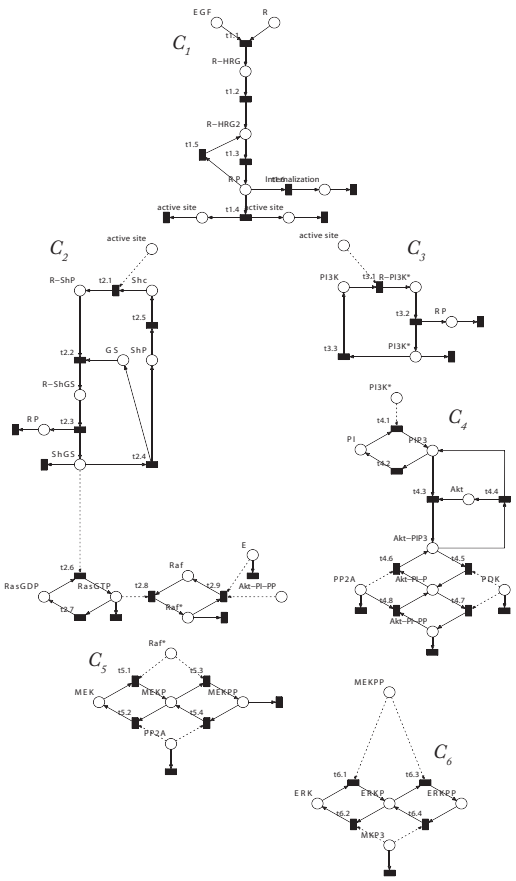

Figure 2: Components of a discrete Petri net model of ErbB4 receptor signaling pathway.

extended as discussed below. For the details of basic Petri net theory, the readers are suggested to refer to Ref. [4].

[**Firing rule of timed Petri nets** $\overline{N}$] (1) If the firing of a transition t_i is decided, tokens required for the firing are reserved. (2) When the delay time d_i of transition t_i passed, t_i fires to remove the reserved tokens from the input place of t_i and put tokens into the output places of t_i.

In a timed Petri net, the firing times per unit time f_i, called firing frequency, of a transition t_i is constrained by its delay time d_i, and the maximum of firing frequency is the reciprocal of d_i.

In the following, we will give our delay time estimation algorithm for Petri net models of signaling pathways.

Notations and functions used in the algorithm are as follows:

(1) Let PN_c=(PN, DC) denote a Petri net model that is decomposed according to the decomposition method stated in section 2, where DC=$\{C_1, C_2, \cdots, C_k\}$ and C_i∈$DC(i$∈$N)$ denotes each smaller component.

(2) Let T_u denote the set of transitions that are not estimated.

(3) Let function of $sizeof()$ be an operation that calculates the element number of the set.

≪ **Experimental Data-based Delay Time Estimation**≫

For a given decomposed Petri net PN_c,
MainEstimate(PN_c)
1° $a \leftarrow 1$
2° **repeat**
$MakePath(C_i)$
do if (sizeof ($Path$) >= 2)
then $Select1(Path)$
else if (sizeof ($Path$) == 0)
then break
do if (sizeof ($Path$) >= 2)
then $Select2(Path)$
$EstimateDelay1(Path)$
until sizeof ($Path$) <= 0)
3° **do if** ($|T_u| \neq 0 \wedge t_i \in C_i \wedge t_i \in T_u$)
then $EstimateDelay2(C_i)$
4° $a \leftarrow a + 1$
do if ($a <= i$)
then goto 2°
else stop

In algorithm ≪ **Experimental Data-based Delay Time Estimation**≫, the function of **MakePath** (C_i) is designed to compute the shortest paths $path_i (i=1, 2, \cdots)$ between any two data places in C_i, and $Path=\{path_1, path_2, \cdots\}$. It is satisfied that $path_i \in Path$ must include at least one transition without estimation in C_i. After that, one $path_i \in Path$ is selected to be estimated according to following cases:

(i) if $|Path| \geq 2$ holds, the function of **Select1** ($Path$) is invoked to compute and return the result of $path_i$ that exists in the shortest path between most-upstream data place and most-downstream data place in C_i;

(ii) if no element exists in $Path$, no operation is executed.

Then, if $|Path| \geq 2$ holds, **Select2** ($Path$) is used to compute and return the result of $path_i \in Path$ satisfying the condition that the number of transition of $path_i$ is the least. In step 2°, **EstimateDelay1** ($Path$) is considered to compute the delay time $d_{(m,n),i} (i \in N)$ of un-estimated transitions in $path_i$ that is selected through the function of $Select1$ or $Select2$. In the function of **EstimateDelay1** ($Path$), two equations are used to calculate the delay time till the estimation of all the transitions in $path_i$ finished.

$$d_{(m,n),1} + d_{(m,n),2} + \cdots + d_{(m,n),x} = D_n - D_m \tag{1}$$

$$\sum_{i=1}^{m} \frac{\beta_i}{d_{I_i}} = \sum_{j=1}^{m} \frac{\alpha_j}{d_{O_j}} \tag{2}$$

The left-hand member of (1) shows the sum of the delay time of all the transitions involved in $path_i$ between two selected data places. The right-hand member of (1) shows elapsed time between selected data places that they reach at the peak of the amount respectively, i.e., the difference between downstream data (D_n) and upstream data (D_m). Equation (2) is designed to calculate a delay time of transitions between back and forth places that exist between selected data places (Fig. 3), and to guarantee the total token amounts flowed in and flowed out per unit time are equivalent.

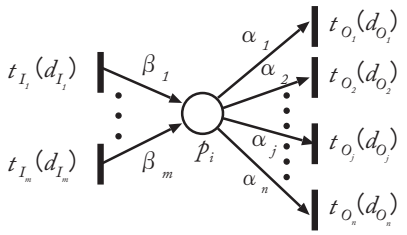

Figure 3: The illustration of equation (2). Place and transition are denoted as p_i and t_i.

In step 3°, **EstimateDelay2** (C_i) is considered to compute the delay time of left transitions in C_i without doing estimation. The delay time of such transition t_{O_j} (see Fig. 3) is obtained based on equation (2) in the context of its net structure.

Using this algorihtm, a timed Petri net model with delay time of transitions can be constructed based on real experimental data. With this estimation, the performance variation along with time change may probably provide a number of valuable insights. Further, it can be considered that the simulation result is more precious to give new presumption and closed to the actual environment rather than the simple estimation according to topological structure proposed by Li *et al.* [2].

In the next section, we will do simulation to evaluate our estimation alogirhtm.

4 Simulation Result

Cell Illustrator 3.0 (CI) is a software tool that enables biologists to draw, model, elucidate and simulate complex biological processes and systems. It has outstanding drawing capabilities, moreover it allows researchers to model metabolic pathways, signal transduction cascades, gene regulatory pathways as well as dynamic interactions of various biological entities such as genomic DNA, mRNA and proteins. CI models are used to visualize biological pathways, interpret experimental data and test hypotheses. In addition, it provides researchers with model diagrams of publication quality and simulation result charts [6].

To confirm the availability of proposed algorithm, we estimated the delay times of the model. And then, we checked the validity of estimated delay times by conducting simulation experiments on the model with estimated delay times. To simulate the model, we must determine the initial marking which is assignment of tokens to each place. We determined the number of initial tokens so as to flow the tokens only once from most upstream place through most downstream place

by $trial\,and\,error$. With the estimated delay times and initial marking, we simulate the model by CI. As the result of simulation, we obtained the same simulation results with experimental data at each data places; the rising time of enzyme activation is equals to the experimental data (Fig. 4), i.e., we succeed to carry out time series simulation of signaling pathways based on experimental data.

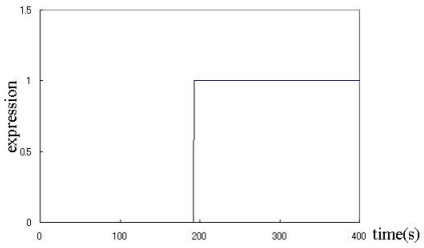

Figure 4: One of simulation result charts. This simulation result is activation of MEK (mitogen-activated protein kinase kinase).

5 Concluding Remarks

We have proposed a delay time estimation algorithm for Petri net model of signaling pathways based on biological experimental data. We have modeled a discrete Petri nets model of ErbB4 receptor signaling pathway using modeling method proposed by Li *et al.*, in order to evaluate proposed algorithm. Then we have assigned the delay time of each transition by using proposed estimation algorithm and further carried out the simulation experiment on Cell Illustrator. By comparing the behaviors of simulation results with Hatakeyama *et al.* [3], finally, we have confirmed the appropriateness and validity of estimated timed Petri net model of ErbB4 that the estimation method may probably provide a number of precious valuable insights.

However, the estimation algorithm proposed in this paper has some points which must be improved. In proposed estimation method, if there is a place whose output transitions are in conflict, we regard firing frequencies of each output transitions as equal. But, if we model biochemical reactions more precise, it is supposed to be different at each transition. As the future work, we try to improve the estimation algorithm to run more precise simulation. Additionally, we modeled a signaling pathway in a discrete Petri net model and analyzed it to see token movement by using a timed Petri net, We should find efficient method for converting a discrete Petri net model to a continuous one.

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