An Efficient Approach for the Hybrid Simulation of Intracellular Calcium Dynamics

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***Abstract*—**
The dynamics of intracellular calcium plays a vital role in organising many processes of the cell. However, creating and simulating an in-silico model to understand this phenomenon is not a straightforward task. One reason for such complexity is the requirement to employ deterministic and stochastic processes in one model. Therefore, hybrid simulation is of paramount importance in this context. Although many hybrid models have been proposed to study the dynamics of intracellular calcium, the speed of the underlying simulation algorithms is still slow as many of such algorithms do not take advantage of the structure properties of the intracellular calcium models. In this paper, we propose an efficient hybrid simulation that takes advantage of the model structure to boost the simulation performance. Our solution is based on the idea of coloured hybrid Petri nets to detect similar structures. We also compare the result of our simulation to the one produced by standard hybrid simulation.

***Index Terms*—Dynamics of Intracellular Calcium; Direct Simulation Approach; Colored Hybrid Petri Nets; Snoopy**

I. Introduction

The hybrid modelling of biochemical reaction networks has attracted many applications in the biological context due to the limitation of the stochastic simulation approach when executing large and complex models [1], [2], [3], [4]. When hybrid simulation is employed to execute biological models, the set of reactions is first partitioned into two subsets: the slow and fast reaction sets. Fast reactions are simulated deterministically (e.g., via numerical integration of a system of ordinary differential equations (ODEs) [5]), while the slow set is simulated stochastically (e.g., via a stochastic simulation algorithm (SSA) [6]). Afterwards, a synchronisation mechanism is utilised to switch between the deterministic and the stochastic regimes. The save in terms of runtime is obtained by assigning more reactions to the fast group. Hence, the numerical integration of the corresponding system of ODEs will require much less time than the individual and discrete firing of reactions via the SSA.

There are many applications where hybrid simulation is a necessary tool to study biological models. One of these applications is the simulation of intracellular calcium dynamics where the dynamics of the calcium affect many functions of the cell, such as gene expression, memory and learning [7].

The nature of the intracellular calcium model renders it intuitive to be simulated using the hybrid simulation approach. In other words, the continuous processes describe the movements between the cytosol, Endoplasmic Reticulum (ER) and the transfer into buffers as well as the diffusion process. ER releases the calcium into the cytosol under the condition of opening the inositol 1,4,5-triphosphate receptor channel (IP3R) [8], while the calcium channel opens and closes randomly [7] so the stochastic simulation has to capture the random behaviour. Moreover, continuous processes of the calcium dynamics affect the stochastic ones and vice versa (i.e., they are not isolated). Therefore, many hybrid models were introduced to simulate the dynamics of calcium (e.g., see [7], [9], [10], [11]). However, all these models do not take advantage of the structure of the calcium model to improve the simulation performance.

Colored Hybrid Petri Nets ($\mathcal{HPN}^{\mathcal{C}}$) are an excellent formalism to model and simulate large hybrid biological models such as the ones in [4], [2]. $\mathcal{HPN}^{\mathcal{C}}$ can easily be used to represent models with repeated components. However, currently $\mathcal{HPN}^{\mathcal{C}}$ is simulated by unfolding the hybrid model into a low-level version. Hence the current approach does not take advantage of the repeated components exposed by the $\mathcal{HPN}^{\mathcal{C}}$ to improve the simulation performance.

In this paper, we introduce an efficient approach to simulate the dynamics of hybrid calcium models via $\mathcal{HPN}^{\mathcal{C}}$. We use an $\mathcal{HPN}^{\mathcal{C}}$ model previously constructed in [12] to illustrate our proposal. We first extract the repeated components of the calcium model, represented by $\mathcal{HPN}^{\mathcal{C}}$, then we simulate only one copy for these components. The remaining ones should expose the same behaviour.

This paper is organised as follows: first we briefly discuss the dynamics of intracellular calcium and coloured hybrid Petri nets. Next, we propose the direct simulation algorithms to simulate the intracellular calcium model. After that, we present the model result as well as comparing our results with the result obtained via standard hybrid simulation algorithm.

II. Background

In this section we provide the necessary background about the dynamics of intracellular calcium dynamics as well as the main components of colored hybrid Petri nets.

A. Dynamics of Calcium

Many cell functions are regulated as a result of calcium activation events. One of these events is the export of calcium from the cell membrane or from an internal store such as the Endoplasmic Reticulum (ER) (also called calcium releasing) [13] to cytosol. The releasing of calcium from ER into cytosol causes an increase in the cytosolic calcium concentration and occurs rapidly in the scale of a millisecond. Calcium releasing is controlled by opening and closing a special type of channels such as the inositol 1,4,5-triphosphate (IP_3) receptor channel. These channels are found inside groups called clusters. The process of channel opening and closing occurs randomly based on the number of active subunits (see below).

The inositol 1,4,5-triphosphate receptor consists of four identical subunits where each subunit can be one of eight states. Opening the IP_3R channel is related with the activity of the subunits, the channel is open when there exist at least three subunits in the active state. The channel closes when the number of active subunits is less than three. Therefore, the channel opening and closing occurs randomly thus it requires a stochastic approach for representing this semantics. However, the transfer of calcium from ER to cytosol after a channel opened takes place in a scale of milliseconds and requires a continuous framework to model.

Moreover, the dynamics of intracellular calcium dynamics require the modelling of space and time [9], [11] introducing a spatio-temporal behaviour. The hybrid calcium model which contains the stochastic and deterministic processes were presented to study the behaviour of the calcium dynamics with only one channel and extended with more than one channel. But all these models did not simulate taking the advantage of the structure properties of the intracellular calcium dynamics.

B. Petri Nets

Petri nets can be considered as a type of directed and weighted graphs consisting of two main nodes: places (represented with circles) and transitions (represented with bars or boxes). Places and transitions are connected with arcs. A Petri net arc is directed from a place to a transition or vice versa. Places can be used to model passive elements of a system, while transitions denote active system components. Places contain positive integer values representing the place marking (tokens), while arcs have positive integer values denoting the arc weight.

Petri nets have been originally designed as place/transition nets. However, they have been extensively extended over the years to support different types of model semantics. In this paper, we are specifically interested in a special class of Petri nets called coloured hybrid Petri nets [2]

C. Colored Hybrid Petri Nets

Colored Hybrid Petri Nets ($\mathcal{HPN}^{\mathcal{C}}$) is a high level extension of standard Petri nets. $\mathcal{HPN}^{\mathcal{C}}$ were generalised in [14] to integrate colours with the elements of Generalised Hybrid Petri Nets $\mathcal{GHPN}$ [1]. In [1], Generalised Hybrid Petri Nets ($\mathcal{GHPN}$) have been proposed to extend hybrid Petri nets with more features of time, including several transition firing modes. The simulation algorithm of $\mathcal{GHPN}$ synchronises the firing between the stochastic biological process and the continuous biological process (see [1], [3]). $\mathcal{GHPN}$ elements can be grouped into three main sets: set of places (discrete and continuous), set of transitions (stochastic, continuous, deterministic delayed, immediate and scheduled) and set of arcs (standard, read, inhibitor, modifier, equal and reset. For more details about the using of these tools please see [1]).

The semantics of continuous transitions is related to a system of ODEs, they fire continuously. Therefore, continuous places which are connected with the continuous transitions are used to describe the deterministic process of the biological behaviour. In the opposite direction, the stochastic transitions fire randomly by generating two random numbers according to exponential distributed firing rates.

III. Materials and Methods

In this section, we present an efficient simulation approach to execute the dynamics of intracellular calcium dynamic via a coloured hybrid Petri net model.

The calcium model consists of two main parts. The first part describes the inflow of the calcium from ER into cytosol (occurs in a millisecond) as well as the diffusion processes. Therefore, this part requires a continuous component for modelling and simulating it. The second part of the calcium model describes the channel events (occur randomly) and it requires stochastic components for describing it. Next, we derive the semantics of the continuous part. After that, we derive the semantics of the stochastic part. Finally, we introduce an efficient hybrid simulation approach for simulating the whole model.

A. Direct Generation of Continuous Semantics

The continuous semantics of the calcium dynamics is described by a system of ODEs to model calcium diffusion among the different components of the cell. Therefore, we generate in this section a reduced system of ODEs based on the structure of the calcium model.

The continuous components of the $\mathcal{HPN}^{\mathcal{C}}$ calcium model are a set of coloured continuous places, a set of coloured continuous transitions and a set of directed arcs which connect the places with transitions and vice versa. The calcium concentration is described by a marking for each colour in the

continuous place. The change in the marking of each coloured continuous place is determined by the rate function of its pre and post continuous transitions. The pre transitions of a place increase its marking (increase the calcium concentration), while the post transitions decrease the place marking (decrease the calcium concentration). It is worth mentioning that the arc weight plays a vital role in calculating the rate of change for each place marking. Please see [5] for a detailed discussion of how the system of ODEs is constructed from continuous Petri nets using the bio-semantics. Please note that we do not consider the case of extended arcs (read, inhibitor, equal arc) as they are not required for the case study in this paper. In what follows, we propose the steps of calculating the change of the coloured place marking by deriving a general equation.

For each coloured place p_i^c:

1) Calculate the continuous inflow of its pre-transitions determined by Equation (1).

$$\frac{d(p_i^c)}{dt}(inf) = \sum_{t_j \in {}^\bullet p_i^c} \left(F(t_j, p_i^c) * v(t_j) \prod_{p_i \in t_j} \#(p_i) \right) \quad (1)$$

2) Calculate the continuous outflow of its post-transitions determined by Equation (2).

$$\frac{d(p_i^c)}{dt}(outf) = \sum_{t_j \in p_i^{c\bullet}} \left(F(p_i^c, t_j) * v(t_j) \prod_{p_i \in t_j} \#(p_i) \right) \quad (2)$$

3) Calculate the total change by Equation (3).

$$\frac{d(p_i^c)}{dt} = \frac{d(p_i^c)}{dt}(inf) - \frac{d(p_i^c)}{dt}(outf) \quad (3)$$

where
${}^\bullet p_i^c$ is the set of pre transitions of the place p_i^c,
$F(t_j, p_i^c)$ gives the arc weight from t_j to p_i^c,
$v(t_j)$ the rate function of the transition t_j,
$\#(p_i)$ is the number of colours in p_i,
$p_i^{c\bullet}$ is the set of post transitions of the place p_i^c and
$F(t_j, p_i^c)$ gives the arc weight from p_i^c to t_j.

Algorithm 1 summarises the steps of generating the ODEs from the coloured continuous places and continuous transitions. The main idea behind Algorithm 1 is generating a reduced system of ODEs based on the symmetric structure of the calcium model. It generate only one equation for the colours that evaluate the guard of the transition and the arc expression to true. Please note that we assume, for the purpose of this case study, that all the places belong to the same colour the same in-and outflow.

B. Direct Generation of the Stochastic semantics

The stochastic part of the intracellular calcium model can be described as stochastic chemical dynamics. It captures the behaviour of discrete events for the intracellular calcium dynamics. The stochastic chemical dynamics consists of a set of objects (chemical species) and their relations (chemical reactions). Our goal in this subsection is generating the chemical species and their corresponding chemical reactions from the $\mathcal{HPN}^{\mathcal{C}}$ calcium model.

Algorithm 1 Generating the system of ODEs for coloured continuous places

```
1: Input: Colored hybrid Petri net model of calcium;
2: Output: system of ODEs;
3: for each colored continuous place p do
4:    for each pre arc (t, p) of p do
5:       if The arc expression and the Guard of a transition
         evaluated to true then
6:          calculate the inflow of p^c from Equation (1);
7:       end if
8:    end for
9:    for each post arc (p, t) of p do
10:      if The arc expression and the Guard of a transition
         evaluated to true then
11:         calculate the inflow of p^c from Equation (2);
12:      end if
13:   end for
14:   calculate the total change for p^c from Equation (3);
15: end for
```

The stochastic components of the $\mathcal{HPN}^{\mathcal{C}}$ calcium model describe the activity of the intracellular calcium channels subunits. These components consist of a set of coloured stochastic places and coloured stochastic transitions. Additionally, a set of directed arcs link the coloured places with the coloured transitions and vice versa. In the following, we introduce the steps of generating the stochastic chemical dynamic system.

A group of objects together with their relations are called a system. The change in the state of each object over time is based reactions. As discussed previously, the stochastic model contains two main nodes: coloured stochastic places and coloured stochastic transitions where the places are the passive components, while the transitions are the active components.

To obtain the system reactions we should unfold every coloured stochastic place to a set of uncoloured places and unfold each coloured stochastic transition to a set of transition instances. Therefore, every transition instance has a set of uncoloured pre places and a set of uncoloured post places. Every uncoloured stochastic place contains a number of tokens. Therefore, we can derive the chemical species from the uncoloured stochastic places where the molecule number of species is denoted by the token numbers of the place and the chemical reaction can be generated from the transition instance. Algorithm 2 summarises the steps of generating the reaction from the stochastic part.

The main objective of generating the stochastic chemical dynamic system from the stochastic part of the calcium model is using a stochastic simulation algorithm as Gillespie [15] for calculating the total change in the marking of each coloured stochastic place by calculating the change in the molecules of the species.

The change in the molecules of a specie (change in the marking of a place) related with occurring the chemical reactions (firing a transition). Each reaction has two characterised mathematical quantities. One of these two quantities is its

Algorithm 2 System of Stochastic Reactions

Input: Colored hybrid Petri Net model of calcium;
Output: System of stochastic reactions ;
Define: R as reaction;
Define: $Rtants$ for the reactants of R;
Define: $Prod$ for the products of R;
for each stochastic transitions t **do**
  for each pre arc (p, t) of t **do**
    Group the pre places;
    Set pre places to $Rtants$;
  end for
  for each post arc (t, p) of t **do**
    Group the post places;
    Set post places to $Prod$;
  end for
  Generate the reaction R for t;
  Reinitialize $Rtants$ and $prod$;
end for

vector of state change $v_j = (v_{1j}, ..., v_{Nj})$, where v_ij gives the change in the molecules of the species S_i caused by a one reaction R_j, so if x is the system state and one reaction R_j occurs, the state of the system converts into $x + v_j$. The second quantity of R_j is its propensity function p_i, which is defined with Equation4.

C. Direct Simulation Algorithm

The simulation procedures of simulating the dynamics of intracellular calcium via the $\mathcal{HPN}^{\mathcal{C}}$ model are summerisd in the Algorithm 3. The algorithm starts by initialising the simulation start time to zero and setting the simulation end time. The main idea behind the working of this algorithm is calculating the rate of change in the marking for each coloured continuous place based on the derived ODEs, by Algorithm 1 and for each coloured stochastic place depending on the reaction system generated by Algorithm 2 over the time.

The rate of change in the colored continuous places is obtained by solving the system of ODEs. The ODEs system is initialised with the initial marking of the colors in the continuous place then integrating the system of ODEs until the firing a stochastic transition or firing an immediate transition.

Firing of immediate transitions converts the channel state from the closing state into the opening state and vice versa. Opening and closing a channel does not only depend on the firing of immediate transition but also depend on the number of active subunits.

Every channel consists of four identical subunits and every subunit has eight states. The transfer from one state to another occurs randomly by firing a stochastic transition. Selecting the stochastic transition leads to generating a system of reactions by calling Algorithm 2.

Simulation of the stochastic reactions is based on a stochastic simulation algorithm (e.g., via Gillespie direct method). The stochastic simulation involves producing two random numbers from uniform distribution$(0, 1)$, calculating the propensity function for each reaction from equation (4), calculating the total propensity using (5), calculating the next time of occurring the reaction by (6), determining the occurring reaction with (7) and fire it. After that, update the marking for each effected place. If the process was enabling an immediate transition then we fire it and check for another enabling immediate transition.

$$\text{propensity function} \qquad p_j(r) = c_j \prod_{i=1}^{N_j} \binom{r_i}{a_{ji}} \tag{4}$$

$$\text{total propensity} \qquad TP_0(r) = \sum_{j=1}^{m} p_j(r) \tag{5}$$

$$\text{next time} \qquad N_t = -\frac{1}{a_0(x)} lnr_1 \tag{6}$$

Reaction to occur R_μ

$$\sum_{j=1}^{\mu-1} p_j(r) < r_2 TP_0(r) \leq \sum_{j=1}^{\mu} p_j(r) \tag{7}$$

Algorithm 3 Calcium Dynamics Simulation (caDysim)

Initialize simulation time $t = 0$;
Set simulation end time t_{end};
Initialize the continuous Simulator by marking of colors in continuou placse $C_{cont}(m) = IN_{cont}(m)$;
repeat
  Start the continuous simulation by solving the ODEs generated by Algorithm 1 until need to a nother process N_p;
  Set $t = solvingtime$;
  Update the current marking ($C_{cont}(m)$);
  if $t_{end} \leq t$ **then**
    stop simulation;
  else if N_p is need to fire a stochastic transition **then**
    Get the reactions generated by Algorithm2;
    Initialize the stochastic Simulator by marking of colors in stochastic placse $C_{stoch}(m) = IN_{stoch}(m)$;
    for each reaction R_j **do**
      calculate the propensity function $P_j(r)$ from (4);
      Calculate TP_0 using (5);
      produce two random numbers r_1, r_2 according to uniform distribution (0,1);
      Calculate N_t from(6);
      Calculate occurring reaction using (7);
      Fire the reaction R_μ;
      Update the $C_{stoch}(m)$;
      Set $t = t + N_t$;
    end for
  else if (N_p is IM_t) & IM_t Enabled **then**
    Checkanotherimmedatetransition();
  end if
until $t < t_{end}$

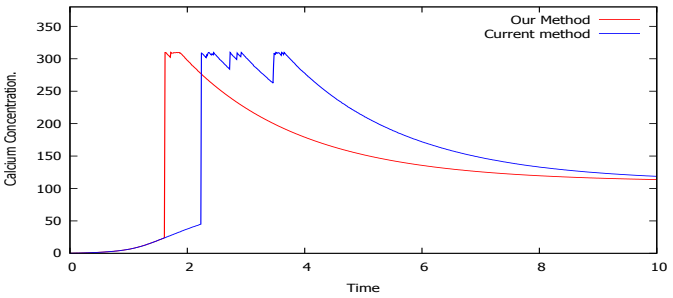

Fig. 1. Total calcium concentration in the cytosol.

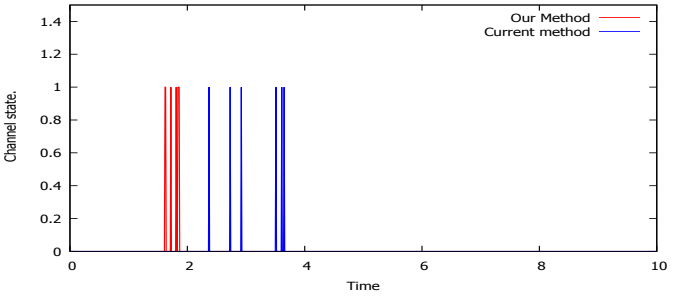

Fig. 2. Opening and closing the channel

The simulation procedures were implemented in Snoopy [16] – a graphical tool to construct and simulate different Petri net classes..

IV. Results and Discussion

In this section, we illustrate results of our simulation approach for the intracellular calcium dynamics via the $\mathcal{HPN}^{\mathcal{C}}$ model as well as the simulation results of the typical hybrid simulation algorithm. We also discuss the efficiency of our simulation procedures.

A. Algorithm Performance

Calcium releasing from ER into the cytosol occurs after the channel opening. To do that, we run the experiment with only one channel and we have noticed that the calcium flows into the cytosol as depicted in Fig. 1 when the channel is opened (cf., Fig. 2).

Fig. 1 has two curves: the red curve shows the calcium concentration in the cytosol from our direct simulation approach, while the other curve provides the calcium concentration in cytosol from the current simulation algorithm. Please note that although the two curves are not identical to each other, they have a similar behaviour. The reason for this difference is due to the effect of random opening and closing of the channel.

Fig. 2 illustrates the channel states (open/close) over the time with our method and the current method.

After transferring the calcium into the cytosol it affects the diffusion process as shown in Fig. 3. Fig. 3 shows the calcium diffusion on a grid of two- dimensional space with four clusters where every cluster contains a few of channels.

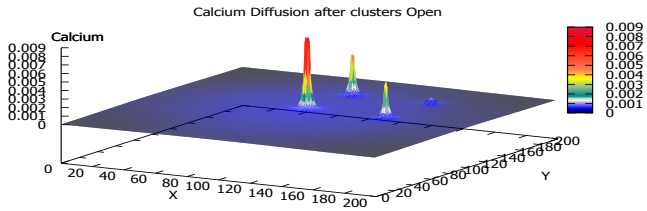

Fig. 3. Diffusion of calcium in two dimensional space of size 200x200

TABLE I
THE GRID SIZE AND THE SIMULATION RUN TIME* FOR DIFFERENT RUNS.

Grid Size	Time(second)		
	Unfolding**	unfolded Simulation	Direct simulation
(10x10)	2.821	2192	285
(50x50)	15.181	54800	7829
(100x100)	49.584	214816	26852
(200x200)	183.46	751856	83539

* performed on PC, Intel(R) Core(TM) i5-3230M CPU @ 2.60 GHz 2.60 GHz , RAM 4.0GB.
** Engine Gecode(intern)

B. Run Time Comparison

To measure the efficiency of our algorithm, we have conducted a number of experiments by varying the model size. The simulation run times are reported in Table I. We found that our extensions to the simulation algorithm play a vital role in the simulation run time compared to the current simulation algorithm. Obviously, the reduced simulation time is due to the reduction of the system of ODEs.

V. Conclusions and Future Work

In this paper, we presented a direct method to simulate the intracellular calcium dynamics via coloured hybrid Petri nets. Our simulation method is based on the direct generation of the model semantics from the coloured hybrid Petri nets. The method is applied on both deterministic and stochastic semantics of the model. The direct generation method of the deterministic part is based a system of ODEs, while the semantics of the stochastic part is based on the firing of the individual reactions. The proposed direct approach captures completely the behaviour of the intracellular calcium dynamics between cytosol and endoplasmic reticulum as well as describes the states of calcium channel subunits. The presented direct simulation algorithms has been implemented in Snoopy.

Although we have considered the model symmetry in the deterministic part, we are still working to take advantage of this merit in the context of the stochastic one. For the purpose of this paper, there are only a few stochastic transitions to represent the channel semantics. Thus we consider this extension as future work.

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