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Colored Stochastic Petri Nets for Modeling Complex Biological Systems

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Abstract

The stochastic modeling of biological systems is gaining more and more attention when stochastic fluctuations need to be described. On the other hand, the biological systems under investigation become much more complex than before. Traditional modeling methods like stochastic Petri nets do not easily scale and thus become difficult to tackle this situation. This paper aims to present a colored stochastic Petri net approach for systems biology to modeling complex biological systems. Specifically, this paper describes a type of biochemically-interpreted colored stochastic Petri nets and their extensions and analysis techniques for systems biology and illustrates their applications using Ca^{2+} release sites that consist of a set of coupled Ca^{2+} channels.

Keywords: *colored stochastic Petri nets; systems biology; complex biological systems; Ca^{2+} channels; Ca^{2+} release sites*

1. Introduction

There has been a long and successful history in computational biology and systems biology of using rate kinetic ordinary differential equations (ODEs) to model biochemical reactions. However, the evolution of biological systems is an inherently stochastic process. When a biological system contains small numbers of molecules, the law of mass action is not adequate as it only describes the average behavior. In this situation, stochastic modeling and analysis methods become necessary in order to gain quantitative and accurate understanding of the underlying dynamics of biological phenomena that depend on stochastic fluctuations [1].

Stochastic Petri nets (*SPN*) have recently become a popular modeling paradigm for capturing the dynamics of complex biological systems [2], which then can help to understand the behavior by integrating detailed biochemical data and providing quantitative analysis results, see *e.g.*, [3]. *SPN* provide a natural, graphical way for modeling biological systems and powerful, mathematically-founded techniques for analyzing them [4].

However, like other standard Petri nets, *SPN* do not easily scale. So *SPN* have been mainly restricted so far to describe relatively small biological systems. *SPN* tend to grow quickly for modeling complex systems, and the built models are difficult to be managed and understood. In order to model complex biological systems, colored Petri nets promise to be a good formalism, which provide parameterized and compact representations of complex biological systems by folding similar components into one and differentiating them using different colors [5].

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While there is a number of reported work on the application of different classes of standard Petri nets to a variety of biological systems, see [6] for a recent review, there are only a few which take advantage of the additional power and ease of modeling offered by colored Petri nets. To our knowledge, the existing applications of colored Petri nets in systems biology can be summarized as follows.

In [7] and [8], they use colored Petri nets is to encode the concentration of species as colored discrete tokens in order to implement continuous simulation in the given net annotation language ML in Design/CPN or CPN tools. While this is a nice exercise in demonstrating the power of colored Petri nets, the burden to implement standard simulation algorithms is left to the modeler. Colored Petri nets have been used for qualitative modeling and analysis in [9] to predict pathological phenotypes based on genetic mutations and in [10] to model signal transduction networks, where, colors encode mutations of molecules or distinguish different molecules. Colors have also been used to discriminate metabolites which follow different T-invariants (elementary flux modes) in [11-13]. Advantages of stochastic colored Petri nets were first demonstrated in [14] using a very simple epidemic model, where color is used to encode the serological state of individuals (*e.g.*, susceptible, infected, removed).

From the review of related work, we can see that existing studies usually resort to Design/CPN [15] or its successor CPN tools [16] to model and analyze biological systems. However neither tool was specifically designed with the requirements of systems biology in mind. Thus they are not suitable in many aspects, *e.g.*, they do not directly support stochastic or continuous modeling, nor the simulative analysis of the models by stochastic or deterministic simulation.

Building upon the lessons learned so far, we extend our software tool Snoopy [17, 18] by specific functionalities and features to support editing, simulating and analyzing of large and complex biological models based on colored qualitative, stochastic, continuous, and hybrid Petri nets [5]. We have given several large case studies, see *e.g.*, [5, 19].

In this paper, we aim to present a colored stochastic Petri net approach for systems biology to modeling complex biological systems. Standard colored stochastic Petri nets are not sufficient for biologists to flexibly build complex models in many aspects. In order to better support the modeling of biological systems, we have to consider features of biological models, *e.g.*, biochemical reactions may obey the law of mass action, and provide support to facilitate biologists to use colored stochastic Petri nets. Therefore, in this paper we present a type of biochemically interpreted colored stochastic Petri nets ($\mathcal{SPN}^{\mathcal{C}}$). We also describe the extensions and analysis techniques of $\mathcal{SPN}^{\mathcal{C}}$, which can strongly support the modeling and analysis of complex biological systems. Besides, we use a well-known biological phenomenon, Ca^{2+} release sites, to illustrate the use of $\mathcal{SPN}^{\mathcal{C}}$.

This paper is organized as follows. In Section 2, we describe the syntax and semantics of stochastic Petri nets. In Section 3, we give a type of biochemically interpreted colored stochastic Petri nets, followed by a discussion of extensions and analysis techniques of $\mathcal{SPN}^{\mathcal{C}}$ to support the modeling of biological systems in Section 4. In Section 5, we give a case study, the modeling of Ca^{2+} release sites, followed by the conclusion in Section 6.

2. Stochastic Petri nets

In this section, we will recall stochastic Petri nets so as to better understand colored stochastic Petri nets that are followed.

Petri nets [20] are weighted, directed, bipartite graphs, consisting of places, transitions and arcs that connect them, see Figure 1 for an example. Places usually represent species or any kind of chemical compounds, *e.g.*, genes, proteins or proteins complexes (here three states of Ca^{2+} channels), while transitions represent any kind of chemical reactions, *e.g.*, association, dissociation, translation or transcription in systems biology. A place may contain an arbitrary (natural) number of tokens, represented as black dots or a natural number, *e.g.*, place *Closed*1 has one token. A distribution of tokens over all places of a Petri net represents a state of it, which is called a marking. The initial marking means the initial state, *e.g.*, the initial marking in Figure 1 is (*Closed*1,*Closed*2,*Open*)=(1,0,0). Each arc gets a weight, also called multiplicity, *e.g.*, all arcs in Figure 1 have a weight 1.

A transition is enabled if each of its preplaces has tokens that are equal to or more than the weight of the arc that starts from the place. If enabled, a transition can be fired. If fired, a transition removes tokens from its preplaces and adds tokens to its postplaces. The number of the removed or added tokens in a place is decided by the weight of the corresponding arc of the place. For example, in Figure 1, transition *Associate*1 is enabled in the initial marking, and if fired, it will remove a token from place *Closed*1 and add a token to place *Closed*2.

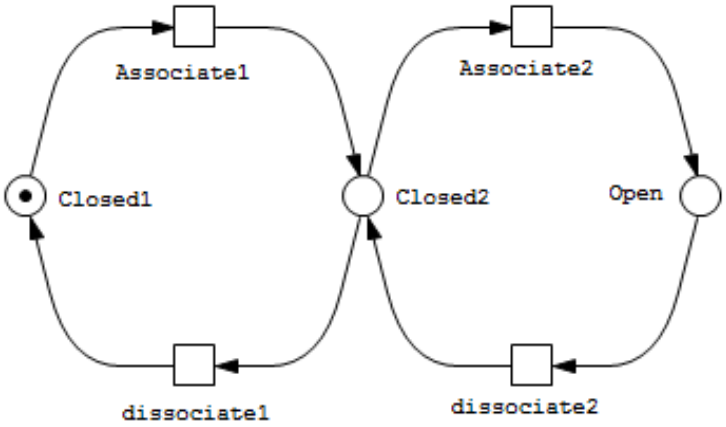

Figure 1. A Petri net for the three-state model of a Ca^{2+} channel [26]

Stochastic Petri nets are an extension of qualitative Petri nets above [4]. Contrary to qualitative Petri nets, a firing delay rate is introduced and associated with each transition t of a stochastic Petri net, which is a random variable X_t, defined by the exponential probability distribution: $F_{X_t}(\tau)=1-e^{-\lambda_t * \tau}, \tau \geq 0$. For example, if we assign a rate to each of the transitions in Figure 1, we can obtain a stochastic Petri net, illustrated in Figure 2, where a marking-dependent rate is labeled over each transition.

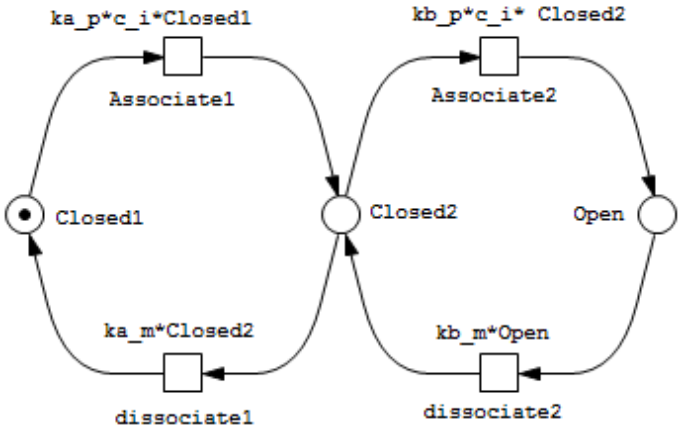

Figure 2. A stochastic Petri net for the three-state model of a Ca^{2+} channel, where the rate function of each transition is labeled over the transition, and *ka_p*, *ka_m*, *kb_p*, *kb_m*, and *c_i* are parameters

The formal definition of a type of biochemically interpreted stochastic Petri nets is as follows [21]. A biochemically interpreted stochastic Petri net is a six-tuple $N=<P,T,F,f,v,m_0>$, where:

- P is a finite, non-empty set of places.
- T is a finite, non-empty set of transitions.
- $F \subseteq (P\times T)\cup(T\times P)$ is a finite set of directed arcs.
- $f:F\rightarrow N_0$ is a function that assigns a non-negative integer to each arc $a\in F$.
- $v:T\rightarrow H$ is a function that assigns a stochastic hazard function $h(t)$ to each transition $t\in T$, whereby $H:=\cup_{t\in T}\{h_t|h_t:N_0^{|{}^\bullet t|}\rightarrow R^+\}$ is the set of all stochastic hazard functions, and $v(t)=h(t)$ for all transitions $t\in T$. ${}^\bullet t$ denotes the preplaces of transition t. R^+ denotes the set of all non-negative real numbers.
- $m_0:P\rightarrow N_0$ is the initial marking.

The stochastic hazard function h_t defines the marking-dependent transition rate $\lambda_t(m)$ for the transition $t\in T$, *i.e.*, $h_t=\lambda_t(m)$. The domain of h_t is restricted to the set of preplaces of t to enforce a close relation between network structure and hazard functions [21]. For example, in Figure 2, the rate function of each transition is marking dependent.

The semantics of a stochastic Petri net is a continuous time Markov chain (CTMC), which has a probability density function whose solution is described by the chemical master equation (CME) [21]. For this, a direct and exact way of computing individual evolution trajectories is to use the Gillespie stochastic simulation algorithm (SSA) [22] or its variants, see *e.g.*, [23]. Besides, in order to accelerate simulation, several approximate methods have been developed in the context of stochastic biochemical kinetics, see *e.g.*, [24].

3. Colored stochastic Petri nets

Let us begin with introducing multisets that will be used in the later definitions of colored stochastic Petri nets.

Multiset. A *multiset* is a set in which there can be several occurrences for the same element. The number of occurrences of an element is called *coefficient* or *multiplicity*. Let S be a finite, non-empty set, a multiset over S is a function $m:S\rightarrow N_0$ that maps each element $s\in S$ onto a non-negative integer $m(s)\in N_0$. It can be denoted by a formal sum: $m=\sum_{s\in S} m(s)`s$. The collection of all the multisets over S is denoted by S_{MS}. The empty multiset over S is denoted by φ_{MS} where the coefficient for each element is zero.

Colored Petri nets. Colored Petri nets (see Figure 3 for an example) consist, as standard Petri nets, of places, transitions and arcs. In addition, a colored Petri net model is characterized by a set of color sets, *e.g.*, a color set *CS* is defined in Figure 3. Each place gets assigned a color set and may contain distinguishable tokens colored with a color of this color set. For example, each place in Figure 3 gets the color set *CS*, and place *Closed*1 has colored tokens 1`*all*(), which means one token for each color in *CS*, *i.e.*, 1`1++1`2. As there can be several tokens of the same color on a given place, the tokens on a place define a multiset over the place's color set. For example, place *Closed*1 now has a multiset of colored tokens, 1`*all*(), instead of an integer number. Each transition has a guard, which is a Boolean expression over defined variables, constants, *etc.* The guard must be evaluated to true for the enabling of the transition. The trivial guard "true" is usually not explicitly given, see the transitions in Figure 3 for such case. Each arc gets assigned an expression and the result type of this expression is a multiset over the color set of the connected place, instead of an integer number. For example, all arcs in Figure 3 have a multiset expression 1`*x* (*x* for short) over *CS*.

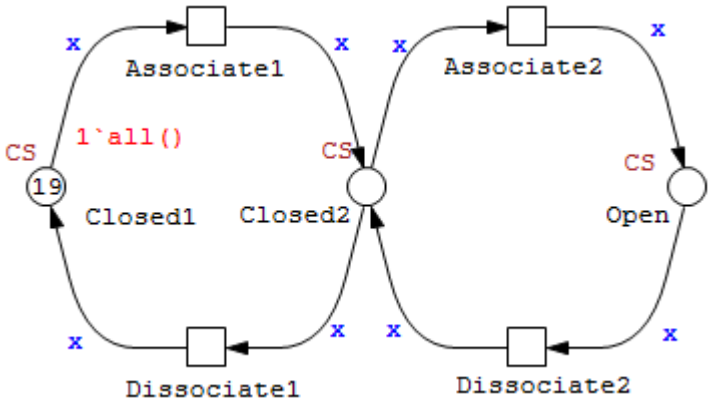

Figure 3. A colored Petri net for the three-state model of a Ca^{2+} channel [26]. Declarations: colorset *CS* = int with 1-2; variable *x*: *CS*. This colored Petri net is obtained by folding two copies of the net in Figure 1

Colored Petri nets can be unfolded to uncolored Petri nets and uncolored Petri nets can be folded to colored Petri nets. Suppose P is a set of colored places of a colored

Petri net, and each place $p \in P$ is associated with a color set $C(p)$. Each color $c \in C(p)$ exactly corresponds to a place instance, *i.e.*, each color will become an uncolored place after unfolding.

Suppose T is a set of colored transitions of a colored Petri net. The variables associated with a transition $t \in T$ are denoted $Var(t)$, which is composed of the variables in the guard of t and in the expressions of arcs connected to t. Before the expressions are evaluated to values, the variables must get assigned values, which is called binding [25]. A binding b of a transition t is a function that maps each variable $v \in Var(t)$ onto a value $b(v)$ that is of the same type as the variable. The set of all bindings for a transition t is denoted $B(t)$. A binding $b \in B(t)$ of a transition t exactly corresponds to a transition instance, denoted by $t(b)$, *i.e.*, it will become an uncolored transition after unfolding. The set of all bindings for a transition t constitutes the set of all the instances of transition t, denoted by $I_T(t)$. The set of all instances of all transitions $t \in T$ is denoted I_T.

In colored Petri nets, there are different types of expressions, *e.g.*, arc expressions, guards, or expressions for defining initial markings. An expression is built up from variables, constants, operation symbols, *etc.* It is not only associated with a particular color set, but also written in terms of a predefined syntax. In the following, we denote by EXP a set of expressions that comply with a predefined syntax.

Colored stochastic Petri nets ($\mathcal{SPN}^C$) are a colored version of stochastic Petri nets. In the following, based on $\mathcal{SPN}$ and colored Petri nets above, we give the formal definition of a type of biochemically interpreted $\mathcal{SPN}^C$.

A biochemically interpreted colored stochastic Petri net is a nine-tuple $N=<P,T,F,\sum,C,g,f,v,m_0>$, where:

- P is a finite, non-empty set of places.
- T is a finite, non-empty set of stochastic transitions with exponentially distributed waiting time.
- F is a finite set of directed arcs. $F \subseteq (P \times T) \cup (T \times P)$.
- $\sum$ is a finite, non-empty set of color sets.
- $C: P \to \sum$ is a color function that assigns to each place $p \in P$ a color set $C(p) \in \sum$.
- $g: T \to EXP$ is a guard function that assigns to each transition $t \in T$ a guard expression of the Boolean type.
- $f: F \to EXP$ is an arc function that assigns to each arc $a \in F$ an arc expression of a multiset type $C(p)_{MS}$, where p is the place connected to the arc a.
- $v: I_T \to H$ is a function that assigns a stochastic hazard function $h(t(b))$ to each transition instance $t(b) \in I_T(t)$ of each transition $t \in T$, whereby $H := \cup_{t(b) \in I_T} \{h_{t(b)} | h_{t(b)}: N_0^{|\bullet t(b)|} \to R^+\}$ is the set of all stochastic hazard functions, and $v(t(b))=h(t(b))$ for all transitions $t \in T$.

- m_0:$P \rightarrow EXP$ is an initialization function that assigns to each place $p \in P$ an initialization expression of a multiset type $C(p)_{MS}$.

Please note, the stochastic hazard function in $\mathcal{SPN}^C$ is defined for each instance of each colored transition. The domain of $h(t(b))$ is also restricted to the set of preplace instances of $t(b)$, denoted by ${}^\bullet t(b)$ with ${}^\bullet t(b):=\{p(c) \in I_P | f(p(c),t(b)) \neq 0\}$.

The semantics of $\mathcal{SPN}^C$ is the same as that of $\mathcal{SPN}$ as $\mathcal{SPN}^C$ can be exactly unfolded to $\mathcal{SPN}$. So all simulation and analysis techniques applicable for $\mathcal{SPN}$ are also applicable for $\mathcal{SPN}^C$.

Here for sake of simplicity, we give a basic definition of $\mathcal{SPN}^C$ and omit their extensions. In the next section, we will in detail discuss some extensions and features that are very helpful for modeling of complex biological systems.

4. Modeling extensions and analysis techniques in $\mathcal{SPN}^C$

In order to better support the modeling of complex biological systems, we include a number of extensions and features in our $\mathcal{SPN}^C$. We also provide a set of analysis techniques for $\mathcal{SPN}^C$. In the following, we will briefly describe them.

4.1. Modeling extensions

Special arcs. We first extend $\mathcal{SPN}^C$ by special arc types such as read arcs (often also called test arcs), inhibitor arcs, equal arcs, reset arcs, and modifier arcs [5], see Figure 4. All these special arcs are only allowed to go from places to transitions. Among them, read, inhibitor, and equal arcs add constraints on the firing of a transition, but the connected places are not affected by the firing.

- A read arc allows to model that some resource is required, but not exclusively consumed upon firing. Hence the same token can be used at the same time by many transitions.
- An inhibitor arc reverses the logic of the enabling condition of a place, *i.e.*, it imposes a precondition that a transition may only fire if the place contains less tokens than the weight of the arc indicates.
- An equal arc imposes the precondition that a transition may only fire if the number of tokens on the place connected by the equal arc is equal to the arc weight.
- A reset arc makes it possible to empty the place connected by this arc once the transition fires; the number of tokens does not matter.
- A modifier arc connects a preplace to a transition, which may modify the transition's firing rate, but does not have an influence on the transition's enabledness.

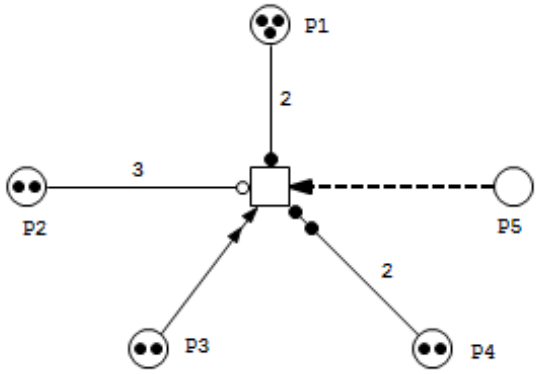

Figure 4. Special arcs of *SPN*^{C} drawn in Snoopy, where the arc terminating with a small hollow circle is the inhibitor arc, the arc with a filled circle is the read/test arc, the arc with double filled circles is the equal arc, the arc with double arrowheads is the reset arc, and the arc with a dashed line is the modifier arc

These special arcs may play an important role in systems biology. For example, inhibitor arcs are especially useful for modeling inhibition functions widely existing in biological systems [27], and read arcs provide a convenient or accurate representation of such chemical interactions as enzymatic reactions, since the enzyme itself is not consumed in the enzyme reaction [28].

Special transitions. We provide furthermore three special transition types, immediate, deterministic and scheduled [5], see Figure 5:

- An immediate transition immediately fires with zero waiting time if it is enabled.
- A deterministic transition fires after a deterministic waiting time, relative to the time point where the transition gets enabled.
- A scheduled transition is scheduled to fire, if any, at single or equidistant, absolute points of the simulation time.

Figure 5. Special transitions of *SPN*^{C} drawn in Snoopy

These special transitions are very helpful in the modeling of biological systems, see *e.g.*, [21] for some applications. Besides, for stochastic transitions, as rate functions are often marking-dependent, popular kinetics like mass action semantics and level semantics [4] are supported by pre-defined function patterns in Snoopy.

4.2. Analysis techniques

Petri net theory offers a rich body of analysis techniques, which can also be used for $\mathcal{SPN}^C$ with the tools, Snoopy and its friends, Charlie [29], Marcie [30] and MC2 tool [31]. In the following, we briefly describe these techniques [5].

Behavioral and structural properties. Petri nets offer a number of behavioral and structural properties. The general behavioral properties include boundedness, liveness, and reversibility. Structural properties can be further classified as elementary graph properties like connectedness, siphons/traps, and place/transition invariants [4]. These properties are usually used as preliminary checks of Petri nets. In order to use these properties to analyze a colored Petri net, we can automatically unfold the colored net to an uncolored Petri net, which is then fed into Charlie to obtain analysis results.

Stochastic animation. Colored Petri nets can be animated in Snoopy, so we can execute a colored Petri net by playing the token game to experience the net behavior. For colored stochastic Petri nets, time-dependent animation is available, which means that an automatic animation corresponds to a stochastic simulation run. Besides, we can choose automatic animation or manually trigger a transition instance from all the enabled transition instances that are automatically computed.

Stochastic simulation. The Gillespie stochastic simulation algorithm (SSA) [22] has been implemented for colored stochastic Petri nets, which is done on automatically unfolded uncolored Petri nets in Snoopy.

Model checking. If the state space is finite and of manageable magnitude, analytical model checking can be used to analyze a Petri net model, otherwise simulative model checking may help to obtain an approximate answer. In order to use analytical model checking for a colored Petri net, we have to first export it to an uncolored Petri net, which is then fed to Marcie to obtain analysis results. But for simulative model checking, we only need simulation traces, by reading which MC2 tool can give analysis results. See [5] for more details.

5. Case study

Calcium is of great interest to cellular signals. Free calcium ions (Ca^{2+}) are usually stored in the endoplasmic or sarcoplasmic reticulum (ER or SR), and mainly mediated by the inositol 1,4,5-trisphosphate (IP3) receptor (IPR) and the ryanodine receptor (RyR), which are also called Ca^{2+} channels [26]. It is an important problem to understand the mechanism of how open Ca^{2+} channels affect other channels in a Ca^{2+}-regulated Ca^{2+} release site in a stochastic manner. Traditional stochastic modeling methods, *e.g.*, stochastic Petri nets or stochastic automata networks, usually produce a very large model or a model that is difficult to understand for a Ca^{2+} release site with many Ca^{2+} channels. Moreover, these models are usually not scalable, *i.e.*, to change the number of Ca^{2+} channels means to change the structure of models. In this section, we will describe how to use colored stochastic Petri nets to facilitate the modeling of Ca^{2+} release sites, which will provide graphical and scalable models that are easy to be built and modified.

We start with a three-state single-channel model with Ca^{2+}-mediated activation that has two closed (Closed1, Closed2) and one open (Open) states, see Figure 6. In Figure 6, $k_i^+ c_\infty$ and k_i^- with $i=a,b$ are transition rates with units of reciprocal time, k_i^+ is

an association rate constant with units of $conc^{-1}\ time^{-1}$, and c_∞ is the fixed background of Ca^{2+} concentration ($[Ca^{2+}]$ for short). This transition diagram can be immediately read as a continuous time Markov chain (CTMC) model.

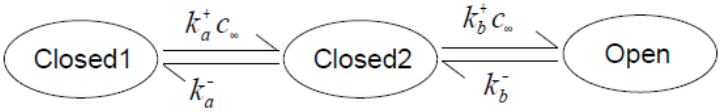

Figure 6. A three-state single-channel model with Ca^{2+}-mediated activation that has two closed (Closed1, Closed2) and one open (Open) states.

Based on Figure 6, we can build a stochastic Petri net model, which is already illustrated in Figure 2. Each state in Figure 6 is converted to a place in Figure 2. Each arc in Figure 6 becomes a transition in Figure 2, and the rate on the arc becomes the rate of the transition.

In a Ca^{2+} release site, Ca^{2+} channels are coupled through buffered diffusion of Ca^{2+}, *i.e.*, the transition probability of a Ca^{2+} channel depend on both the local $[Ca^{2+}]$ and other open channels. This can be written as $f(c)=c_\infty+c_*{*}N_O$ under the mean-field assumption, where c_* is the average $[Ca^{2+}]$ contributed by each open channel, and N_O is the number of open channels. Now, we can give a colored stochastic Petri net model of a Ca^{2+} release site with N channels, illustrated in Figure 7. In this model, an additional place *NumOpen* is added to count the number of open channels, and the coupling effects are reflected by modifier arcs (with dashed lines).

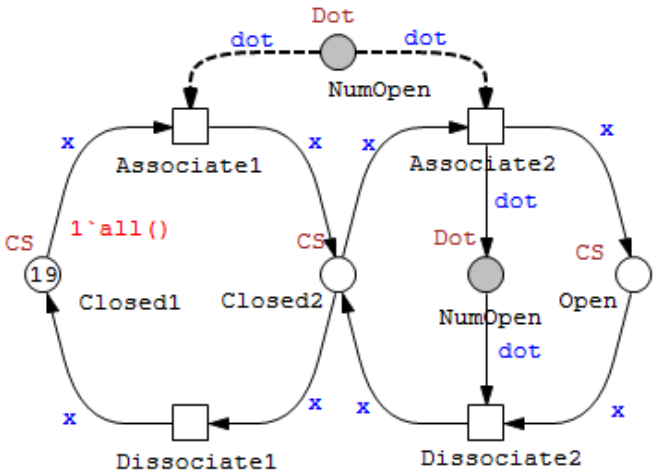

Figure 7. A colored stochastic Petri net model for a Ca^{2+} release site. See Table 1 and Table 2 for declarations and rate functions, respectively

Table 1. Declarations used in Figure 7

Category	Declarations
constant	int: N=19;
colorset	CS = int with 1−N;
colorset	Dot = with *dot*;
variable	x: CS;

Table 2. Rate functions used in Figure 7. $ka\_p=k_a^+$, $kb\_p=k_b^+$, $ka\_m=k_a^-$, **and** $kb\_m=k_b^-$

Category	Declarations
Associate1	$ka\_p*(c\_i+NumOpen*c_s)*Closed1$;
Associate2	$kb\_p*(c\_i+NumOpen*c_s)*Closed2$;
dissociate1	$ka\_m*Closed2$;
dissociate2	kb_m*Open;

Colored Petri nets can be analyzed with a set of techniques, *e.g.*, structural analysis, stochastic animation, stochastic simulation, and model checking. For example, for each channel model, three places, *Closed*1, *Closed*2 and *Open* consist of a place invariant, *i.e.*, there is always a constant token (here 1) in these three places, which shows that at each time step only one state holds for a channel. We also can run stochastic animation to check the model step by step. Besides, if we run stochastic simulation, we can give a simulation plot for the model with 19 channels in Figure 7, illustrated in Figure 8, from which we can see some Ca^{2+} waves.

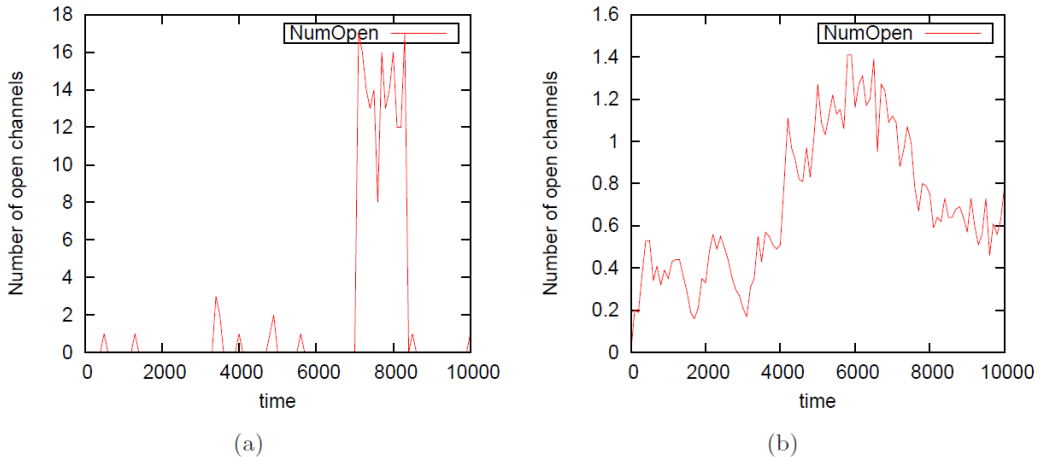

Figure 8. Simulation plots of (a) one simulation run and (b) the average behavior of 100 simulation runs for a Ca^{2+} release site with 19 channels.

Parameters: c_∞=0.05, k_a^+=1.5, k_a^-=50, k_b^+=150, k_b^-=1.5, c^*=0.075

Besides, we can easily increase the size of the model by only changing the number of colors and if possible some parameters. For example, if we set the color set *CS* to 100 colors, we can obtain a model of the Ca^{2+} release site with 100 channels, see Figure 9 for its simulation plots.

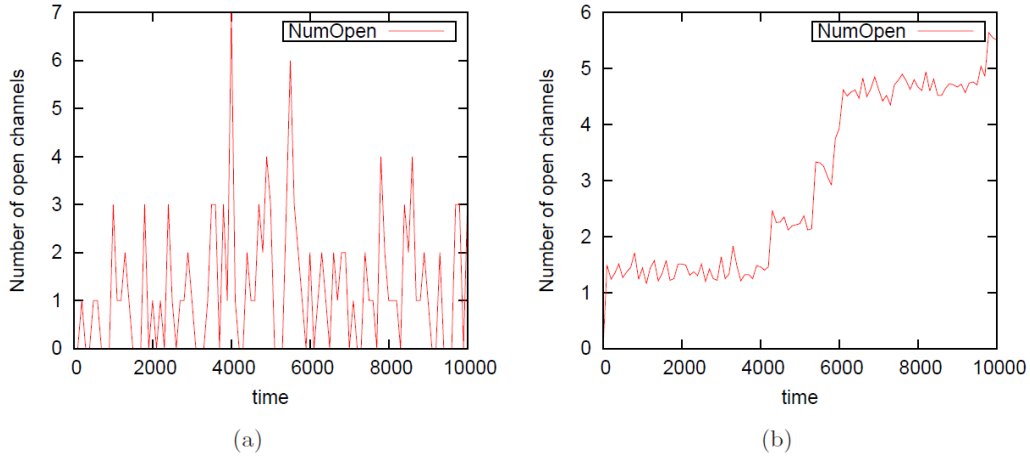

Figure 9. Simulation plots of (a) one simulation run and (b) the average behavior of 100 simulation runs for a Ca^{2+} release site with 100 channels. Parameters: c_∞=0.05, k_a^+=1.5, k_a^-=50, k_b^+=150, k_b^-=1.5, c^*=0.015

6. Conclusions

The stochastic modeling of biological systems is gaining more and more attention when stochastic fluctuations need to be described. On the other hand, the biological systems under investigation are becoming much more complex than before. Traditional methods like stochastic Petri nets do not easily scale and thus become difficult to tackle this problem. Therefore, this paper presented a colored stochastic Petri net approach to modeling and analyzing complex biological systems. This paper described the formal syntax and semantics of a type of biochemically interpreted colored stochastic Petri nets and their extensions. This paper also gave a case study, the modeling of Ca^{2+} release sites, to illustrate the use of colored stochastic Petri nets.

In the next step, we will continue to explore the extensions of colored stochastic Petri nets and their applications in multiscale modeling of systems biology, which is now challenging to biologists.

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