Colored Petri Net Modeling of the Sucrose Biosynthesis Pathway in Plasmodium falciparum

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***Abstract*—Sucrose is an important macromolecule that is used in organisms including *Plasmodium falciparum* (*P.f.*) to generate glucose which is used for energy production in the glycolysis pathway. In numerous research projects on modelling and analyzing biological pathways, Petri net has been recognized as a promising method for representing biological pathways. A metabolic pathway is an interconnected series of enzymatic reactions that occur within a cell. It consists of consecutive chemical reactions, which transform input compound(s) (substrates) via several intermediate compounds into an output compound (product). This paper focuses on the use of Colored Petri Net to construct an *in-silico* metabolic network that shows the interactions between the metabolites and the reactions in the sucrose biosynthesis pathway of *Plasmodium falciparum (P.f.)* and further analyze the model for its structural and quantitative properties using Petri net theory. Our model gives more insight to the structure of the pathway and helps to improve our understanding of the biological processes within this pathway.**

Keywords-Sucrose, Colored Petri Net, Plasmodium falciparum

I. INTRODUCTION

A model is a representation of the construction and working of a particular system. A model is a similar but simpler version of the system it represents. Modeling is simply the process of creating a model [1]. There are different types of models, qualitative or quantitative, deterministic or stochastic, static or dynamic, continuous or discrete. While a qualitative or structural model e.g. a network graph specifies the interactions among model elements, a quantitative model assigns values to the elements and to their interactions [2].

Colored Petri Nets were first proposed by Jensen [6], it combines Petri nets with capabilities of programming languages to describe data types and operations, this providing a flexible way to create a compact and parameterized model. In colored Petri nets tokens are distinguishable by the "color", rather than having only the "black" one. Colored Petri Nets are colored extension of the basic Petri net. Arc expressions (extension of arc weights from the basic Petri net) specify which tokens can flow over the arcs and guards (Boolean expressions) which can be used to define additional constraints on enabling of transitions. [6]

A colored Petri Net (CPN) (check [7-10] for surveys) is an extension of the ordinary Petri net (PN) that provides a framework for the construction and analysis of distributed and concurrent systems [11]. A CPN model of a system describes the states which the system may be in and the transitions between these states. CPNs have been applied to a vast range of areas, for example in the area of biological systems [5] communication protocols[12,13], audio/video systems[14], operating systems[15,16], hardware design, embedded systems[17], software system designs[18,19] and business process re-engineering [20,21]

II. FORMAL DEFINITION

According to Jensen [6], A colored Petri net is a tuple < P, T, F, P, C, g, f, m0 >, where:

- P is a finite, non-empty set of places.
- T is a finite, non-empty set of transitions.
- F is a finite set of directed arcs, such that $F \subseteq (P \times T) \cup (T \times P)$.
- P is a finite, non-empty set of types, also called color sets.
- C: P →P is a color function that assigns to each place $p \in P$ a color set $C(p) \in P$
- g: T → EXP is a guard function that assigns to each transition $t \in T$ a guard expression that has the Boolean type.
- f : F → EXP is an arc function that assigns to each arc $a \in F$ an arc expression that has a multiset type C(p)MS, where p is the place connected to the arc a, and C(p)MS is the multiset on the color set C(p).
- m0: P → EXP is an initialization function that assigns to each place $p \in P$ an initialization expression that has a multiset type C(p)MS

III. METABOLIC NETWORKS

A metabolic pathway is an interconnected series of enzymatic reactions that occur within a cell. It consists of consecutive chemical reactions, which transform input compound(s) (substrates) via several intermediate compounds into an output compound (product) [3]. It is defined as a subsystem that deals with some specific function, subsystems that generate the core components

for life and energy that is important to synthesize and use them [22]. It can also be defined as a network of chemical reactions catalyzed by one or more enzymes, where some molecules (reactants or substrates) are changed into others (products). The product of a reaction is the substrate for the next reaction.

Reasons for using mathematical models to represent metabolic networks include; organization of disparate information into a coherent, self-consistent whole, to think (and calculate) logically about what components and interactions are important in a complex system, simulation, prediction, and optimization of procedures, experiments and therapies, to disprove hypotheses and to define improved hypotheses and to understand the essential features of a system [23].

Consequently, the task of any metabolic pathway is to modify a principal chemical compound to form another chemical compound which can be used up, passed on to start another pathway or stored up by the cell.

Sucrose is a disaccharide composed of the monosaccharide glucose and fructose with the molecular formula $C_{12}H_{22}O_{11}$. The biosynthesis of sucrose proceeds via a reaction UDP-D-Glucose and fructo 6-phosphate in the presence of an enzyme sucrose-6-phopshate synthase. The energy for this reaction is obtained by the cleavage of Uridine diphosphate (UDP-$C_9H_{11}N_2O_{12}P_2$). Sucrose is formed by plants and cyanobacteria but not by other organisms.

This paper focuses on the use of a modeling tool called Petri net to construct an *in-silico* metabolic network that shows the interactions between the metabolites and the reactions in the sucrose biosynthesis pathway of *Plasmodium falciparum (P.f.)*. Reddy et al., 1993 [4] was the first work to introduce the application of Petri nets to qualitative modelling of biochemical networks. In their paper, simple case condition systems were used for simulation of simple biochemical processes. Since then lot of deeper papers have been published using this method of simulation of metabolic, regulatory, genetic and signal transduction networks.

IV. MATERIALS AND METHODS

The data for the Sucrose Biosynthesis pathway for *P.f.* was gotten from the **BioCyc** database - (www.biocyc.org). BioCyc is a collection of 3530 Pathway/Genome Databases (PGDBs). Sucrose synthesis is performed by first generating the phosphorylated form, sucrose 6^F-phosphate with chemical formula: $C_{12}H_{21}O_{14}P$ (the "F" indicates that the phosphate group is attached to the furanose functional group), then this is followed by dephosphorylation. The first step is catalyzed by sucrose-phosphate synthase (E.C 2.4.1.14), which condenses β-D-fructofuranose 6-phosphate ($C_6H_{11}O_9P$) with UDP-α-D-glucose ($C_{15}H_{22}N_2O_{17}P_2$). The second step is catalyzed by sucrose-phosphate phosphatase (EC 3.1.3.24), which hydrolyzes, sucrose 6^F-phosphate to sucrose ($C_{12}H_{22}O_{11}$). In photosynthetic organisms both precursors originate from photosynthetic-derived carbon, via β-D-fructofuranose 6-phosphate ($C_6H_{11}O_9P$) [29]. The image in figure 2 below is the representation of the Sucrose Biosynthesis pathway for *P.f.* from BioCyc which was transformed into a Colored Petri net model.

The Petri net model was built and analyzed using CPN Tool version 4.0.0. CPN Tool (http://cpntools.org/). CPN Tools is a tool for editing, simulating, and analyzing Colored Petri nets. The tool features incremental syntax checking and code generation, which take place while a net is being constructed. A fast simulator efficiently handles untimed and timed nets. Full and partial state spaces can be generated and analyzed, and a standard state space report contains information, such as boundedness properties and liveness properties. See [30-32] for more details on this tool.

Using a colored Petri net to model a pathway requires us assigning the elements of the colored Petri net to the corresponding metabolic pathway. Places would be equivalent to byproduct of metabolism i.e metabolites, proteins and enzymes. The reactants or substrates represent input places and the products would represent output places. In simple terms an input place has an arc directed outwards from it, while an output place has an arc directed towards it. Transitions represent chemical reactions. The stoichiometric matrix of a pathway is equivalent to the incidence matrix of the petri net and the arc weights can be gotten by the given stoichiometric coefficients. The number of tokens in each place indicates the amount of substance associated with that place, the flux modes and the conservation relations for metabolites correspond to specific properties of PNs. In particular minimal (semi-positive) T-invariants correspond to elementary flux modes of a metabolic pathway, i.e., minimal sets of reactions that can operate at a steady state. Minimal T-invariants form a basis for the set of semi-positive T-invariant (Hilbert basis) which is unique and characteristic of PN [27]. According to Paolo Baldan [28],

TABLE I. shows the relationship between Petri net elements and metabolic pathway elements. For the Colored Petri net model the only change to this table would be the introduction of the colored tokens which are called Colored Sets. The colored sets would also represent metabolites, enzymes, and compounds quantities

An illustration of the colored petri net representation of the well-known chemical reaction $2H_2 + O_2 \rightarrow 2H_2O$ is shown in figure 1 below. The first petri net represent the state before the reaction occurs (i.e. before the transitions fires), while the second represents the state after the reaction has occurred.

TABLE I. RELATIONSHIP BETWEEN PETRINETS AND PATHWAY ELEMENTS

Petri Net Elements	**Pathway Elements**
Places	Metabolites ,enzymes, compounds
Transitions	Reactions, interactions
Input Places	Substrates, reagents
Output places	Reaction Products
Arc Weights	Stoichiometric coefficients
Number of tokens on places	Metabolites, enzymes, compounds quantities
Transition rates	Kinetic laws of reactions

For this Petri net to be constructed an enumerated type color set IN with members H and O had to be defined using the following syntax *closet IN = with H | O;* and two variables of type *closet IN,* in1 and in2 were declared, using this syntax *var in1, in2: IN;* Then a compound type color set OUT was declared using this syntax *closet OUT = product IN * IN;*. This was defined as a product in order to contain hold the different tokens of the various input places (i.e. H and O). The variables in1 and in2 were bound to the various members of the color set IN so that the arc expression for each of the input places contained the condition in which the transition would be enabled to fire. In this example the transition required 2 moles of H^+ and 1 mole of O_2 to be enabled to fire and result in the formation of 2 moles of H_2O.

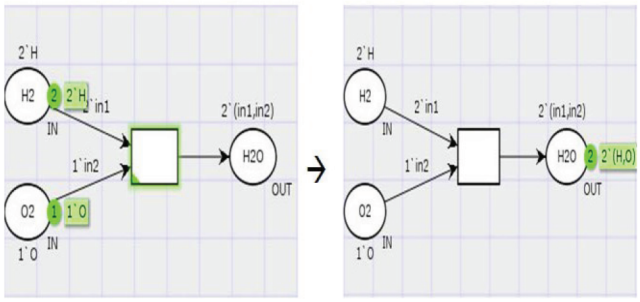

Fig. 1. The pertinent representation of the formation of H_2O

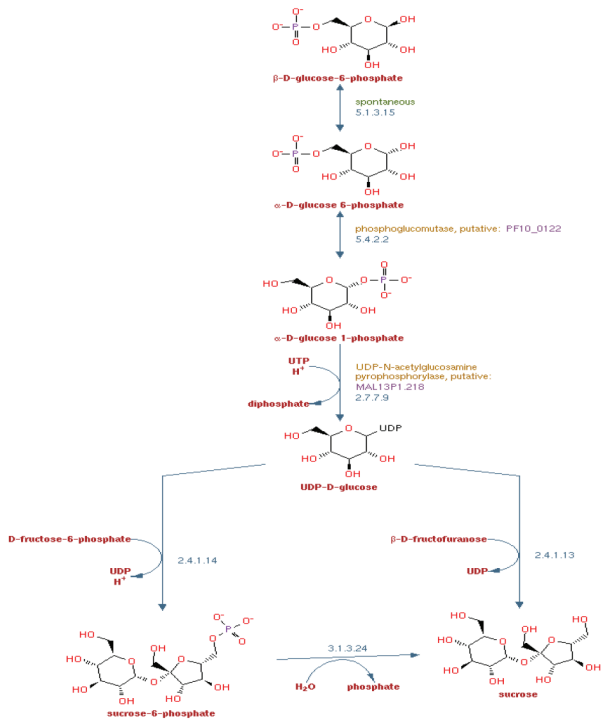

Fig. 2. The Sucrose Biosynthesis Pathway in *Plasmodium falciparum* from Biocyc

V. RESULT AND CONCLUSION

The Constructed Model consists of 6 reactions which have a total of 9 reactants and 11 products. 4 of the reactions are reversible and the other two are irreversible. The reactions were catalyzed by 5 enzymes, one of them (5.1.3.15) being spontaneous (meaning it's not compulsory that enzyme is present for the corresponding reaction to occur). TABLE II. shows the overall reaction layout for the Sucrose biosynthesis pathway in *P.f.*

From the constructed model a corresponding stoichiometric matrix using the stoichiometric coefficients. The substrates are multiplied by -1 and the products by +1. The zero value entries signify that the given metabolite did not participate in the given reaction. This was used in assigning the values of the color sets in the construction of the Colored Petri net model.

TABLE II. OVERALL REACTION LAYOUT OF THE SUCROSE BIOSYNTHESIS PATHWAY

Reaction Number	Reaction Layout	Reversibility	E.C Number
Rx1	β -D-glucose-6-phosphate ⇆ α-D-glucose-6-phosphate	Reversible	5.1.3.15 (spontaneous)
Rx2	α-D-glucose-6-phosphate ⇆ α-D-glucose-1-phosphate	Reversible	5.4.2.2
Rx3	α-D-glucose-1-phosphate + UTP + H ⇆ UDP-D-Glucose + Diphosphate	Reversible	2.7.7.9
Rx4	UDP-D-Glucose+β-D-Fructofuranose ⇆ Sucrose + UDP	Reversible	2.4.1.13
Rx5	UDP-D-Glucose + D-Fructose-6-phosphate →Sucrose-6-Phosphate+UDP+H^+	Irreversible	2.4.1.14
Rx6	Sucrose-6-phosphate + H_2O → Sucrose + Phosphate	Irreversible	3.1.3.24

TABLE III. ABBREVIATIONS OF COMPOUNDS AND THEIR FULL MEANINGS

Abbreviations	Full Meanings
a-D-g6p	α-D-glucose-6-phosphate
b-D-g6p	β -D-glucose-6-phosphate
B-D-FF	β-D-Fructofuranose
Df6p	D-Fructose-6-phosphate
d-p	Diphosphate
Dg1p	α-D-glucose-1-phosphate
s-6-p	Sucrose-6-Phosphate
UDP	Uridine diphosphate
UDP-g	Uridine diphosphate- Glucose
UTP	Uridine triphosphate

	Rx1a	*Rx1b*	*Rx2a*	*Rx2b*	*Rx3a*	*Rx3b*	*Rx4a*	*Rx4b*	*Rx5*	*Rx6*
b-D-g6p	1	-1	0	0	0	0	0	0	0	0
a-D-g6p	-1	1	-1	1	0	0	0	0	0	0
Dglp	0	0	1	-1	-1	1	0	0	0	0
UTP	0	0	0	0	-1	1	0	0	0	0
H+	0	0	0	0	-1	1	0	0	1	0
UDP-G	0	0	0	0	2	−1	−1	1	−1	0
dp	0	0	0	0	1	−1	0	0	0	0
b−DFF	0	0	0	0	0	0	−1	1	0	0
sucrose	0	0	0	0	0	0	1	−1	0	1
UDP	0	0	0	0	0	0	1	−1	1	0
F6P	0	0	0	0	0	0	0	0	−1	0
S6P	0	0	0	0	0	0	0	0	1	−1
H_2O	0	0	0	0	0	0	0	0	0	−1
Phosphate	0	0	0	0	0	0	0	0	0	1

Fig. 3. The Stoichiometric matrix for Sucrose Biosynthesis pathway

Therefore, various Petri Net representations have been successfully used for biological networks such as gene regulation, signal transduction and metabolic systems. Here, we used an extension of the Petri Net model known as the Colored Petri Net which is more compact and readable to build the sucrose biosynthesis pathway and further analyze the model for its structural and quantitative properties using Petri Net theory. Our model gives more insight to the structure of the pathway and helps to improve our understanding of the biological processes within this pathway.

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