Colored Petri Net: Its application to Sucrose Biosynthesis Pathway in *Plasmodium falciparum*

Jelili Oyelade[1, 2], Itunuoluwa Isewon[1, 2], Ifeoluwa Okunoren[1]

[1]Dept. of Computer and Information Science
Covenant University, Ota, Ogun State, Nigeria.
[2]Covenant University Bioinformatics Research (CUBRe), Ota, Nigeria

ola.oyelade@covenantuniversity.edu.ng
itunu.isewon@covenantuniversity.edu.ng
ifeoluwababa@gmail.com

Abstract: Sucrose plays major role as macromolecule used in organisms including *Plasmodium falciparum* (P.f.) to generate glucose for energy production in the glycolysis pathway. A metabolic pathway is a series of chemical reactions, which goes through various intermediate compounds to transform input compounds into a product. In this work, we modelled a metabolic pathway in *Plasmodium falciparum* using Colored Petri Net Markup Language (CPNML). The model was used to examine the dynamic behavior of the sucrose biosynthesis pathway which shows the interactions between the metabolites and the reactions in the sucrose biosynthesis pathway of *Plasmodium falciparum*. We further analyzed 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*

Introduction

A model is a representation of the build-up and working process 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 (Anu, 1997). 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 indicates the relationship among model elements, a quantitative model attaches weights to those relationships. (Riesig, 1982).

Petri Net (PN) was suggested by (Reddy *et al*., 1993) for modeling metabolic networks to overcome some limitations like applying ordinary differential equations (ODE) to a very large complex system which can be a very tediuos task. Lots of further conceptual works, technical tool implementations and applications into biological problems have been reported since then and have

demonstrated the importance and usefulness of this concept referred today as systems biology.

Colored Petri Nets (CPN) was first proposed by (Jensen, 1981). They combine PN alongside the advantages of computer programming to narrate data type and operations, which gives an easier approach to produce a parameterized model. Colors are used to distinguish the tokens within a colored Petri Net. An arc expression (extension of arc weights from the basic PN) indicates the flow of the tokens either above the arcs or guards (Boolean expressions) which can be used to define additional constraints on enabling of transitions (Jensen *et al*. 2007).

CPN is an extension of an ordinary PN which provides a framework for the construction and analysis of distributed and concurrent systems (Kristensen *et al*., 1998). Its representation in a system shows the status of the system and the possible changes between the states involved. CPNs have been applied to a vast range of areas, such as biological systems (Liu & Heiner, 2012) communication protocols (Huber & Pinci, 1991, Floreani *et al*., 1996), audio/video systems (Christensen & Jørgensen, 1997), operating systems (Cherkasova *et al*., 1993a & Cherkasova *et al*., 1993b), hardware design, embedded systems (Rasmussen & Singh, 1996), software system designs (McLendon & Vidale, 1992, Scheschonk & Timpe, 1994) and business process re-engineering (Pinci & Shapiro, 1991 & Mortensen & Pinci, 1994).

Definition1: Colored Petri Net (CPN) can be formally defined as a 9-tuple (Jensen *et a*l. 2007):

$CPN = (Pl, TR, Ar, \sum, VR, Cl, Gd, E, I)$, where:

Pl denotes a bounded set places

TR denotes a bounded set transitions $TR \ni Pl \cap TR = \phi$

$Ar \subseteq PlXTR \cup TRXPl$ denotes directed arcs

$\sum$ denotes bounded non-empty color sets

VR is a bounded typed variables $\ni$ Type$[VR] \in \sum$ $\forall$ variables $v \in VR$.

$Cl : Pl \to \sum$ denotes colored set function attaching color set to each place.

$Gd : TR \to PR_v$ denotes a guard function attaching guard to each transition

$tr \ni Type[Gd(tr)] = bool$.

$E : Ar \to PR_v$ denotes arc expression function attaching arc expression to each arc $ar \ni Type[E(ar)] = Cl(p_l)$, where p_l denoted the place connected to the arc ar.

$I : Pl \to PR_\theta$ denotes initialization function connecting initialization expression to each place

$p_l \ni Type[I(p_l)] = C(p_l)$.

Definition2: A Colored Petri Net module contains 4-tuples Jensen *et al*. 2007):

$CPN_m = (CPN, T_s, P_{pt}, PT)$, where

CPN is a non-hierachical colored petri Net defined in definition 1 above.

$T_s \subseteq TR$ denoted substitution transitions

$P_{pt} \subseteq Pl$ denoted port places

$P_T : Pl \rightarrow \{I_{IN}, O_{OUT}; I/O\}$ denoted port type function connecting port type to each port places.

Metabolic Networks

A Metabolic pathway is a series of connected enzymatic reactions that occur within a cell. It consists of consecutive chemical reactions, which changes input compound(s) (substrates) into an output compound (product) with the aid of enzymes (Reddy *et al*., 1993). It is defined as a subsystem that deals with some specific function, generate the most essential components that are vital for the synthesis, usage for life and energy (Baldan *et al*., 2011). It can also be defined as a combination of reactions that are chemical in nature that is combined with enzymes in which some of the reactants are converted to other products. These products can in-turn serve as the substrate for the next reaction.

There are various advantages in the use of mathematical representations in the modeling of metabolic networks such as: organization of disparate information into coherent and self-consistent whole, to think logically about the essential components and interactions in a complex system, simulation, prediction, and optimization of procedures, experiments and therapies, in order to validate and to define improved hypotheses and to understand the important features of a given system (van Riel, 2006).

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 consists of monosaccharide glucose and fructose with the molecular formula $C_{12}H_{22}O_{11}$. The sucrose biosynthesis is obtained through UDP-D-Glucose and fructo-6-phosphate reaction in the presence of an enzyme called sucrose-6-phopshate synthase. This reaction obtained energy from the cleavage of Uridine diphosphate (UDP-$C_9H_{11}N_2O_{12}P_2$).

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 were the first to introduce the use of Petri Nets to qualitative modeling of biochemical Networks. In their work, 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 nnetworks.

Materials and Methods

The data for the Sucrose Biosynthesis pathway for *P.f.* was obtained from the **BioCyc** database - (www.biocyc.org) which consists 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 ($C_6H_{11}O_9P$) (Hasunuma et al., 2010). The image in figure 2 below is the representation of the Sucrose Biosynthesis pathway for *P.f.* from BioCyc, which was then converted by building a Colored Petri Net model.

The Petri Net model was built and analyzed using CPN Tool version 4.0.0. (http://cpntools.org/). Colored Petri Net tool helps to gain better understanding of a colored Petri Net by providing room for making changes, implementing a prototype and carrying out analysis. For more details on this tool see (Ratzer *et al*, 2003, Jensen *et al*. 2007, Westergaard & Kristensen, 2009).

The use of colored Petri Net model for pathway modelling requires to appoint the elements of the colored Petri Net to the corresponding metabolic pathway. Places (P_l) would be equivalent to byproduct of metabolism (i.e Metabolites (M_b), Enzymes (E_z) and Compound(C_p)). The reactants or substrates represent input places (I_p) and the products(R_p) represent output places (O_p). In simple terms, an arc is directed outwards from an input place, while an arc is directed inwards into an output place. Transitions (T_R) represent chemical reactions (such as Reaction (R_t) and Interaction

(Int). The pathway's stoichiometric matrix is equivalent to the incidence matrix of the petri Net and the arc weights are obtained by the given stoichiometric coefficients. In each place, the number of tokens suggests the amount of substance integrated with that place, the flux's modes and the conservation relations of metabolites correlate with the specific properties of PNs. For example, the minimal (semi-positive) T-invariants correlate with the flux modes of a metabolic pathway, this form a basis for the set of semi-positive T-invariant (Hilbert basis) which is unique and characteristic of PN (Hofestadt, 1994).

According to (Baldan *et al*., 2013), Table 1 shows the relationship between Petri Net elements and metabolic pathway elements. For the Colored Petri Net model, the only change to this table is the inclusion of the colored tokens refered to as Colored Sets (metabolites, enzymes, and compounds quantities). Figure 1 is the colored Petri Net representation of the well-known chemical reaction $2H_2 + O_2 \rightarrow 2H_2O$. The first Petri Net represents the state before the reaction occurs (i.e. before the transition fires), while the second represents the state after the reaction has occurred.

Table 1. Relationship Between Petri Nets and Pathway Elements

PN Elements	**Biological Pathway Elements**
Places(P_l)	Metabolism(M_b, E_z, C_p)
Transitions	Chemical reaction(R_t, Int)
Input Places	Substrates, reagents
Output places	Products(R_p)
Incident matrix (Arc Weights)	Stoichiometric coefficients
Number of tokens on places	Metabolism(M_b, E_z, C_p)
Transition rates	Kinetic 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 in 1 and 2 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.

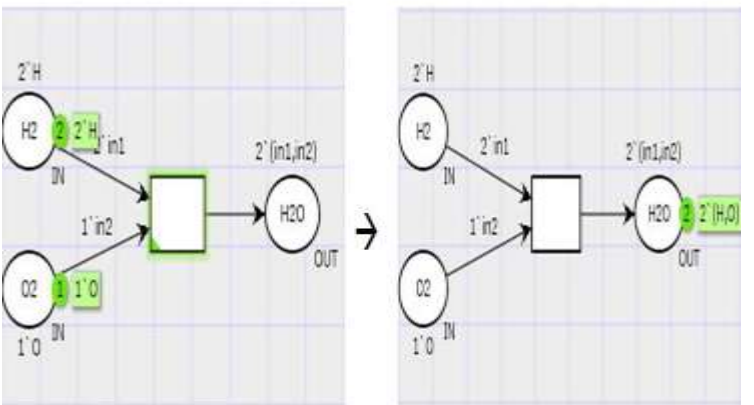

Figure 1. The Petri Net Representation of the Formation OF H_2O

Table 2. Overall Reaction Layout of the Sucrose Biosynthesis Pathway

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-	Irreversible	2.4.1.14

	Phosphate+UDP $+H^+$		
Rx6	Sucrose-6-phosphate + H_2O → Sucrose + Phosphate	Irreversible	3.1.3.24

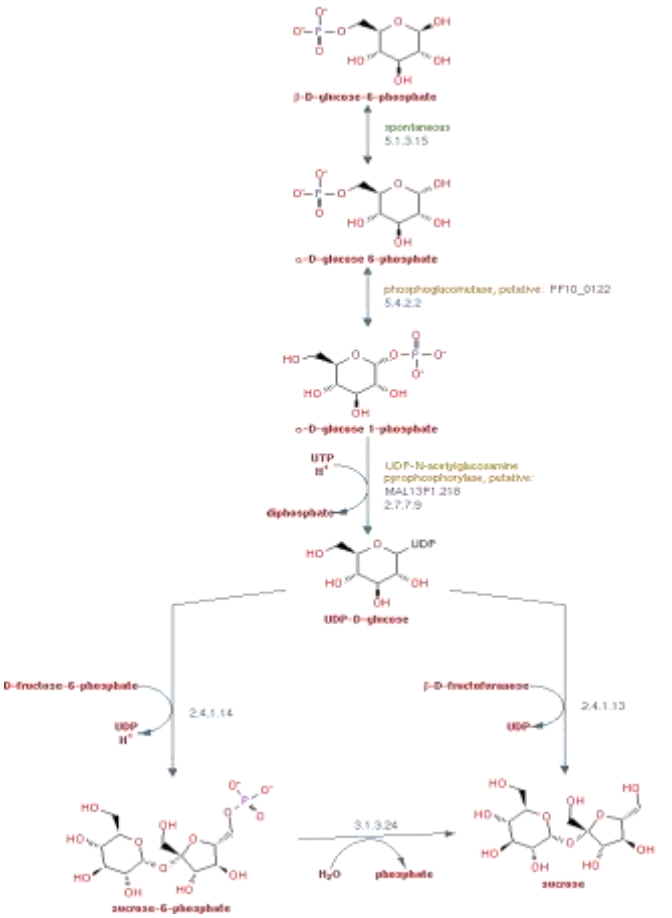

Figure 2. The Sucrose Biosynthesis Pathway in *Plasmodium Falciparum* from BioCyc

Results And Discussion

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 2 shows the overall reaction layout for the Sucrose biosynthesis pathway in *P.f.*

From the reaction equations, a corresponding stoichiometric matrix (shown in figure 3) was built using the stoichiometric coefficients. The substrates are multiplied by -1 and the products by +1. The zero value entries signify 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. The CPN model is shown in figure 4 (before it fires) and figure 5 (after it fires).

Table 3. Abbreviations of Compounds and their full meaning

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

	*Rx*1*a*	*Rx*1*b*	*Rx*2*a*	*Rx*2*b*	*Rx*3*a*	*Rx*3*b*	*Rx*4*a*	*Rx*4*b*	*Rx*5	*Rx*6
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
Dg1p	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
*F*6*P*	0	0	0	0	0	0	0	0	−1	0
*S*6*P*	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

Figure 3. The Stoichiometric Matrix for Sucrose Biosynthesis Pathway

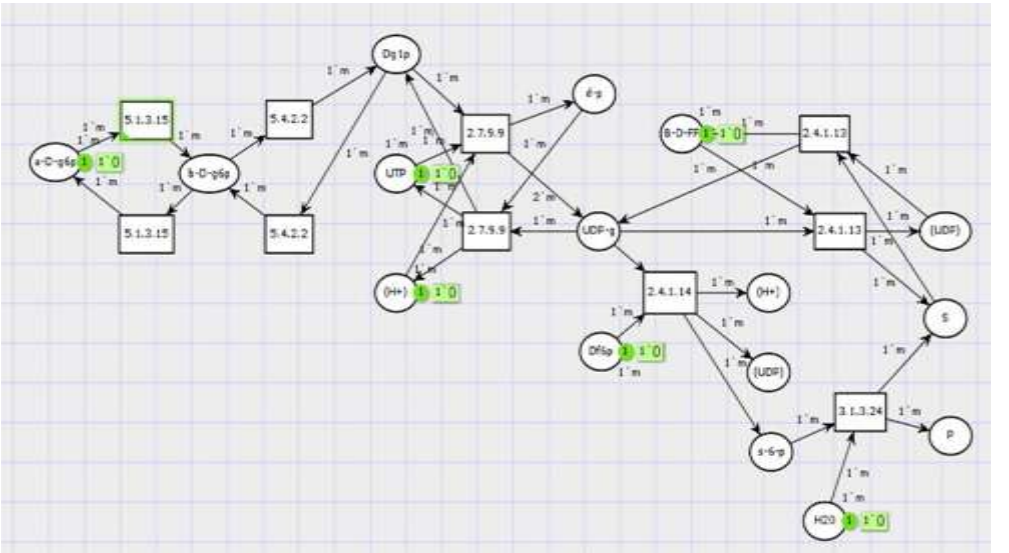

Figure 4: Petri Net Construction of the Sucrose Biosynthesis Pathway (before Firing)

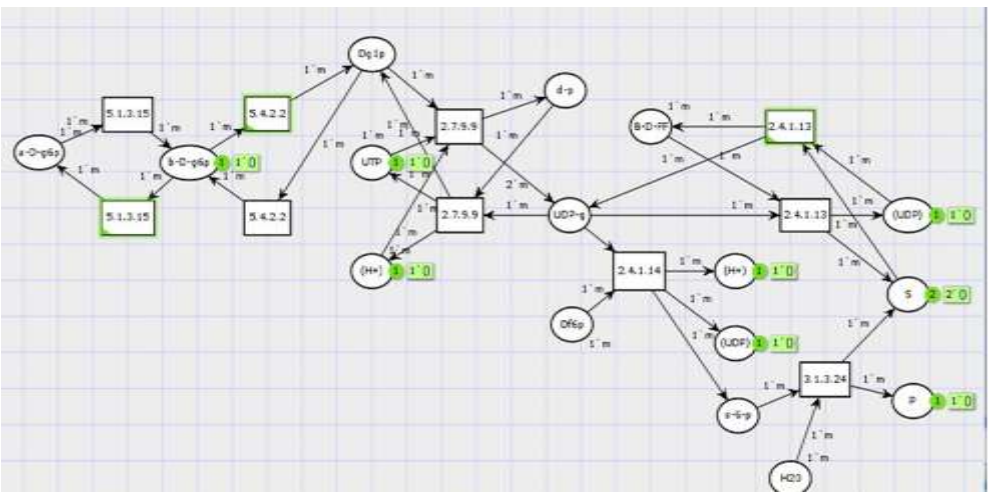

Figure 5: Petri Net Construction of the Sucrose Biosynthesis Pathway (after Firing)

Model Validation & Analysis

The aim of model validation of a constructed model is to check for inconsistencies in that given system, hence deriving statements on the structural and dynamic properties that reflect the activities of that system in real life (Koch et al, 2005). The CPN model of the sucrose biosynthesis pathway in P.f. after firing shows one token of UDP (Uridine diphosphate), one token of Phosphate and two tokens of Sucrose. The two tokens of Sucrose produced is because there are two distinguishing "routes" in which Sucrose is produced from UDP-D-Glucose (UDP being an activator).

The first being the hydrolysis of Sucrose-6-phoshpate to form sucrose and one mole of Phosphate and the second is the condensation of β-D-fructofuranose with D-Glucose to form another mole of sucrose and a mole of UDP.

This verifies that the constructed model conforms to the reaction layout. The constructed model consists of 6 reactions which have a total of 9 reactants and 11 products. Four of the reactions are reversible and the other two are irreversible, and the reversible reactions are modeled as forward and backward reactions. 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).

Structural Analysis

The aim of structural analysis is to discover underlying structures that allows conclusions on dynamic properties. It involves the calculation of elementary properties. Structural analysis checks if the constructed net is ordinary, homogenous, conservative, pure, static, conflict free & connected or strongly connected in the graph theoretical sense. The net constructed is unbounded and it is not ordinary because the arc weights are not all equal to one. The net is also not homogenous (the pathway modeled in a metabolic pathway). It is also not conservative because not all transitions have the sum of the input arc weights equal to the sum of the output arc weights.

For example: Reaction 1: 1 mole of α-D-glucose-1-phosphate and 1 mole of UTP and 1 mole of H^+would result in one mole UDP-D-Glucose and one mole of Diphosphate. For this example we have a total of 2 moles of reactants and 3 moles of products.

Since the net is not conservative then it's not bounded. The net is not pure because there's no transition in which the pre-place is also a post-place (except in reversible reactions). This net is not static conflict free because there are transitions sharing the same input places. A metabolic petri net would not be free of static conflicts because compounds may be used by several reactions. Since the model is not a bounded model, we cannot compute the reachability graph. Also the cover ability graph which is a subset of the reachability graph cannot be computed because there is a huge amount of possible states.

Invariant Analysis

The net contains but is not covered by the following P invariants;

Invariant B – These are the set of all the compounds that provide a phosphate group either directly or indirectly. If the phosphate group is transferred from one compound to another the sum of the phosphorylated metabolite remains unchanged. If no phosphate is consumed or secreted by a cell, the sum of phosphate groups in all metabolites, including inorganic

phosphate would also remain unchanged.

The following compounds are the compounds that contain a phosphate group.

UTP (Uridine triphosphate), UDP (Uridine diphosphate) and Phosphate.

5. Conclusion

Various Petri Net representations have been successfully used for biological networks such as gene regulation, signal transduction and metabolic systems. In this work, we used Colored Petri Net model 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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