THE JOURNAL OF COMPUTER SCIENCE AND ITS APPLICATIONS
Vol. 22, No 2 December 2015

# SIMULATION AND ANALYSIS OF PENTOSE PHOSPHATE PATHWAY IN *PLASMODIUM FALCIPARUM* USING COLORED PETRI NETS MODEL

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**ABSTRACT**
*Plasmodium falciparum* is a protozoan parasite and the deadliest of five human malaria species which is responsible for the majority of malaria related deaths in humans. The erythrocytes' stage of *Plasmodium falciparum* depend on Pentose Pathway as an alternative source of energy and it releases electrons used in protecting the *Plasmodium falciparum* from its host. Colored Petri Net has been recognized as one of the important models in modelling and analyzing biological pathways. It is an accurate qualitative and quantitative modelling tool for modeling complex biological systems. In this work, the modeling of the pentose phosphate pathway in *Plasmodium falciparum* is presented using the Petri Net Markup Language (PNML). The Colored Petri Net (CPN) models based on the Petri Net representation and the conservation and kinetic equations were used to examine the dynamic behavior of the metabolic pathway. The usefulness of Petri Nets is demonstrated for the quantitative analysis of the pathway. We obtained data from Biocyc database. The constructed model was viewed through the Colored Petri Net Tool (CPN tool 4.0). Specific drug targets called the essential reactions within the pathway were identified, listed and proposed. These essential reactions would alter the functioning of the pathway which would affect the energy and protection needs of the parasite therefore leading to the death of the parasite in the human red blood cell.

Keywords: *Plasmodium falciparum*, metabolic pathway, Colored Petri Net, Pentose

phosphate pathway

## 1.0 INTRODUCTION

A model is a simplified version of a complex system intended to analyze and solve problems or make predictions on the concerned system. Modeling is the act of creating or developing models for the purpose of understanding and describing it. There are different classifications of models; qualitative and quantitative, deterministic and stochastic, static and dynamic, continuous and discrete. A qualitative (structural model) e.g. a network graph specifies the interactions among model elements; while a quantitative model assigns values to the elements and to their interactions [1]. Mathematical modeling is increasingly used to get insights into the functioning of complex biological networks. Petri Nets (PNs) have recently emerged as a promising tool among the various methods employed for the modeling and analysis of biological systems. One of the great advantages of PNs is that, it comprises the definition of structural and behavioural properties. It allows the formulation of model validation criteria which increases the confidence in the model. PNs support the integration of qualitative as well as quantitative methods by serving as a mathematically unifying description. The combination of the qualitative and quantitative analysesis a significant step toward integrated analysis of biochemical systems. Colored Petri Net (CPN) is an extension of standard PNs where a group of similar components are represented as one component, each of which is defined and distinguished by a color. The concepts of subnetwork abstraction, transition refinement or node fusion, among others, have been explored in Petri Net theory [2]. Computational models such as boolean networks, graph theory, etc. have been used to simulate and model real life systems as well as biological systems to identify the way the systems behave in different situations.

A metabolic network is the complete representation of the set of metabolic and physical processes that determine the physiological and biochemical properties of a cell. It is defined formally as a collection of objects and the interactions that exists among them. The objects correspond to the chemical reactions, enzymes and genes [3]. A metabolic network is a graphical representation of anabolism and catabolism. Metabolism is bridge between genotype and phenotype. It allows for an in-depth insight into the molecular mechanisms of a particular organism. Metabolic pathway is a series of chemical reactions occurring within a cell which involves the modification or change of an initial molecule to form another product. They are complex networks which combine large volume of diverse biological, chemical, and physical data. The resulting product of metabolic pathway can be used immediately or initiate another metabolic pathway or to be stored by the cell. These reactions are catalyzed by enzymes. A fundamental problem is to integrate and interpret biological data to further our understanding in metabolic networks. Mathematical and statistical models provide a possibility of metabolic modeling.

Modeling a metabolic network provides a steady-state description of metabolic

behavior in understanding the genetics and biochemical mechanisms in the networks. Metabolic networks are used to stimulate, predict and optimize procedures, experiments and therapies; to calculate logically what components and interactions are important in a complex network; also to disapprove hypotheses and to define improved hypotheses; to disparate information into a coherent and self-consistent whole and understand the essential features of a system.

The Pentose phosphate pathway (also called the Phosphogluconate pathway and the Hexose monophosphate shunt) is a biochemical pathway parallel to glycolysis that generates NADPH (Nicotinamide adenine dinucleotide phosphate) and Pentoses (5-carbon sugars). The Pentose pathway is an alternative source of energy used during the erythrocytes stage (growth stage) and NADPH used by the erythrocytes, releases electrons to protect it from its host for it to survive in its host. The survival of malaria parasites in human RBCs (red blood cells) depends on the pentose phosphate pathway, both in *Plasmodium falciparum (P.f).* and its human host.

In this study, an *in-silico* metabolic network model of Pentose Phosphate in *P.f* was built using Colored Petri Nets (CPN). The metabolic network model shows the interaction between metabolites and the reactions in the pentose phosphate pathway of *P.f.*

## 2.0 RELATED WORK

Biological data is often faced with challenges of incomplete data, therefore, the development of correct models is a challenge to theoretical biology. Computational methods are needed to model the complex biological processes in order to analyze and understand them. Traditional mathematical models are focused on the construction of kinetic models by solving algebraic equations for steady states and systems of differential equations for time-dependent states [4]. Petri Net theory exhibits a mathematical formalism to model, analyze, and simulate discrete event systems with inherent concurrency [5] and [6]. There are many applications in the field of modeling and control of discrete systems and in the field of concurrent software development [7]. The first application of Petri Nets to modeling of metabolic pathways was published by [8]. In recent years, Petri Net theory has been applied to model metabolic pathways in relation to genetic and cell communication [9], to investigate quantitative properties of biochemical networks [10], and to model stochastic systems using stochastic Petri Nets [11]. A previous study used stochastic Petri Nets in analyzing the stabilizing effect of the protein on the genetic network controlling ColE1 plasmid replication [12]. Petri Nets (PNs) have been suggested to be well suited for modelling metabolic networks by overcoming some challenges faced with ordinary differential equations (ODE) to a very large and complex system which can be an uphill task [13]. Since then, a lot of further conceptual work, technical tool implementations and applications into biological problems have been reported and have demonstrated the usefulness of this concept known today as systems biology. Asides from PNs being intuitively understandable to scientists trained in life sciences, they also have a

strong mathematical foundation and provide the required flexibility with regard to the models' granularity. They have been successfully used in modeling various biological pathways [14, 15]. Sackmann *et al* [16], provide a systemic modeling method of signal transduction pathways in terms of petri Net components. Chaouiya [1], provides an overview of the different types of PNs. They include Coloured Petri Net (CPN), Stochastic Petri Net (SPN), Hybrid Petri Nets (HPN) and Hybrid Function Perti Nets (HFPNs). Hardy and Robillard [17] also discuss the modelling and simulation of molecular biology networks. They identify two categories of goals of Petri Net biological modelling: qualitative and quantitative analysis. Qualitative analysis is the analysis of the different biological properties while quantitative analysis is the simulation system dynamics. For quantitative analysis, a PN representation with sufficient modelling power, kinetic parameters like reaction rates and stoichiometric quantities of reactants are necessary. Heiner *et al*. [18], demonstrate a generalized approach towards modelling and analysis of biological pathways using Petri Nets.

To model and analyse biochemical pathways on a qualitaive level, it is necessary to establish the concept. of elementary modes [19], that is based on the incidence (stochiometric) matrix of the underlying directed graph. Table 1 shows how metabolic pathway elements are represented using PN theory.

## 2.1 Formal definition

Colored Petri Net is defined as a tuple CPN = (S, P, T, A, N, C, G, E, IN) satisfying the following requirements [20]:

1. S is a finite set of non-empty types, called **color sets**.
2. P is a finite set of **places**.
3. T is a finite set of **transitions**.
4. A: is a finite set of arc such that:
$$A \rightarrow P \times T \cup T \times P$$
5. N is a **node** function.
6. C is a **color** function. It is defined from P into S.
7. G is a **guard** function. It is defined from T into
8. E is an **arc expression** function. It is defined from A.
9. IN is an **initialization** function. It is defined from P.

**Table 1: Differences between Petri Net elements and Pathway elements**

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

Colored Petri Net is an extension of ordinary Petri Net for modeling, simulating and analyzing biological networks. It has capabilities of programming languages to describe data types and operations, thus providing a flexible way to create compact and parameterizable models. The major difference between the elements for colored Petri Nets is the introduction of colored tokens called *Color Set* where tokens are distinguished by the "color", rather than having only the "black" one. Also the, arc expressions, an extended version of arc weights, specifies which tokens can flow over the arcs, and guards, that are in Boolean expressions which define additional constraints on

the enabling of the transitions.

The water formation modeling example in figures 1 & 2 will be used to state the differences between the ordinary Petri Net and the CPN. The firing of transition molecules removes two tokens from place *Hydrogen* and one token from place *Oxygen* and adds two tokens in the place *Water*, thus capturing the reaction $2H_2 + O_2 \rightarrow 2H_2O$ in both OPN and CPN.

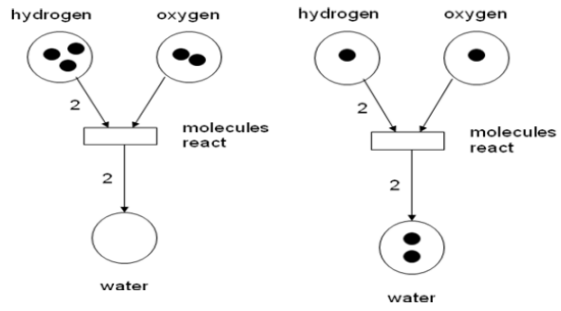


**Figure 1: Marked OPN before (left) and after (right) firing of transition (Water formation).**

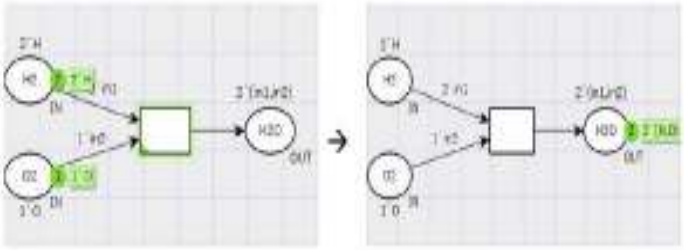

**Figure 2: CPN before (left) and after (right) firing of transition (Water formation).**

Tokens are declared with data types making it easier to know what kind of data the place accepts. transition is always green in a CPN whenever the transition is enabled and can be fired. .

## 3.0 MATERIAL AND METHODS

The implementation was done using Petri Net Markup Language (PNML) for the construction of the model. We obtained data for Pentose phosphate pathway of *P.f* from PlasmoCyc v14.0 from the BioCyc database collection (www.biocyc.org). The pathway map is presented in figure 3.

We then obtained the following for each reaction in the pentose phosphate pathway; Reaction Unique-ID, Common Name of Reaction, Reactants, Reactant Stoichiometry Coefficients, Products, Product Stoichiometry Coefficients, Reversibility, E.C Number, Enzyme, Common Name of Enzyme. With the results of the data obtained, we constructed stoichiometric matrix for the pathway and then built the Petri Net model. The construction of the Petri Net is based on the stoichiometric matrix for the metabolic reactions. For a stoichiometric number matrix; the column represents each reaction and the rows represent each reactant. The reactants on the left (left child) have the stoichiometric numbers with negative signs, while the reactants on the right (right child) have a stoichiometric number with a positive sign [21]. The stoichiometric number matrix, $C = P\ x\ T$, of a place/transition net would be defined as an integer, where the places are listed as rows and the transitions as columns as shown in figure 4.
For example, from figure 3, the first two reactions of the pentose phosphate pathway *P.f* 3D7 according to the data extraction results are:

b-D-Glucose-6-Phosphate + NADP+ ⟶ 6-Phospho-D-Glucono-1,5-Lactone + NADPH + H+6-Phospho-D-Glucono-1,5-Lactone+H20 ⟶ 6-Phospho-D-Gluconate + H+

The stoichiometric matrix is defined as an integer matrix where places are listed as rows and the transitions as columns. The stoichiometric coefficients of the reactants are multiplied by -1 and the

products multiplied by +1. If a compound is not present in the reaction it will be represented as zero. The stoichiometry matrix for the first two reactions would be:

$$\mathbf{C} = \begin{pmatrix} \underline{\textbf{Compounds}} & \text{Rx1} & \text{Rx2} \\ \text{bDG6} & -1 & 0 \\ \text{NADP+} & -1 & 0 \\ \text{6PDG15L} & 1 & -1 \\ \text{NADPH} & 1 & 0 \\ \text{H+} & 1 & 1 \\ \text{H20} & 0 & -1 \\ \text{6PDG} & 0 & 1 \end{pmatrix}$$

The Pentose phosphate pathway (PPP) is both an anabolic (its primary role) and catabolic pathway. It is parallel to the Glycolysis pathway which involves two phases; the oxidative and non-oxidative phase. It is an alternative path for the metabolism of glucose which reduces coenzyme NADPH and produces pentose sugars such as Ribose, the sugar component of the nuclei acid. PPP does not lead to the formation of ATP (Adenosine Triphosphate) but it has 2 major functions which are:

- The generation of NADPH during the oxidative phase for biosynthesis of fatty acids and steroids
- Production of ribose 5-phosphate (R5P) (which takes place during the non-oxidative) phase, used in the synthesis of nucleotides and nucleic acids.

PPP is the only source of NADPH erythrocytes and an alternative route for the glucose breakdown. The biosynthesis of fatty acids biosynthesis is necessary for membranes and DNA. NADPH also acts as an electron acceptor that helps in the defense of *P.f* against its host which sends electrons to try to destroy it. The NADPH in another pathway can donate these electrons to another electron carrier that can supplement energy in burning these electrons in the electron transport chain for energy. Energy and building blocks are the purpose for metabolism in organism - both are equally important. The PPP provide energy via electrons, sugars (which can be passed through Glycolysis), fats (which can be burned for energy). All these process depend on the requirements of the cell at a given time. Therefore, it is shown that the PPP in *P.f* provides energy used by the erythrocytes during its growth stage.

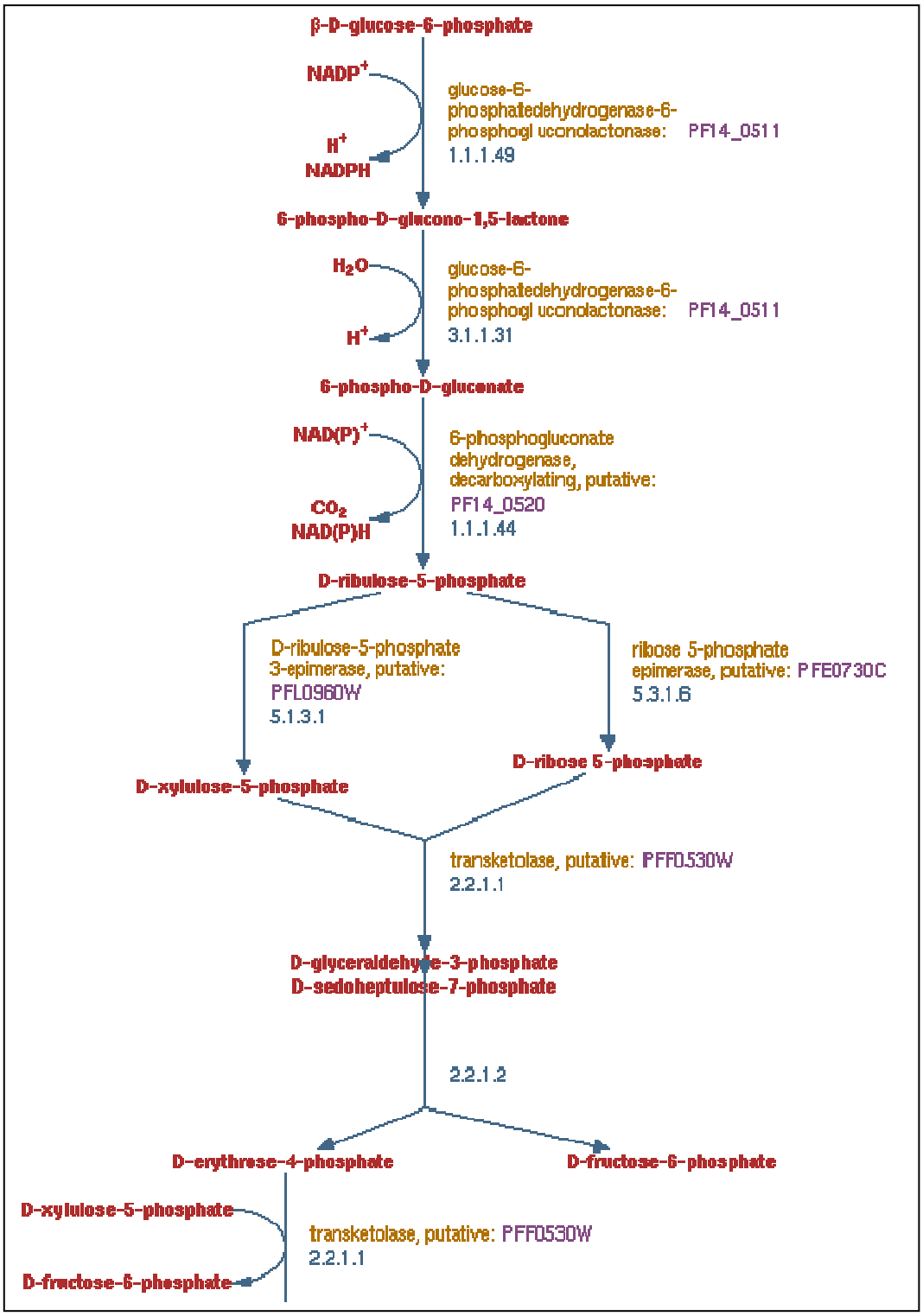


**Figure 3: Pentose Phospahate Pathway for *Plasmodium falciparum* (Biocyc.org)**

| Compounds | Rx1 | Rx2 | Rx3 | Rx4a | Rx4b | Rx5a | Rx5b | Rx6a | Rx6b | Rx7a | Rx7b | Rx8a | Rx8b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BDG6P** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **NADP+** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **6PDG15L** | 6 | -6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **NADPH** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **H2O** | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **6PDG** | 0 | 6 | -6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **H+** | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **NAD(P)+** | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **DRL5P** | 0 | 0 | 1 | -1 | 1 | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **CO2** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **NAD(P)H or NAH** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **DX5P** | 0 | 0 | 0 | 1 | -1 | 0 | 0 | 1 | -1 | 0 | 0 | -1 | 1 |
| **DR5P** | 0 | 0 | 0 | 0 | 0 | -1 | 1 | 1 | -1 | 0 | 0 | 0 | 0 |
| **DS7P** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 1 | -1 | 1 | 0 | 0 |
| **DG3P** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 1 | -1 | 1 | 1 | -1 |
| **DF6P** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | -1 | 1 | -1 |
| **DE4P** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | -1 | -1 | 1 |

**Figure 4: Stoichiometric matrix for the Pentose Phosphate Pathway**

**Table 2: Overall Reaction Layout of the Pentose Phosphate Pathway**

| Reaction Number | E.C Number | Enzyme Gene | Reaction Layout |
|---|---|---|---|
| R x1 | 1.1.1.49 | PF14_0511 | bDG6P + NADP+ → 6PDG15L + NADPH + H+ |
| Rx2 | 3.1.1.31 | PF14_0511 | 6PDG15L + H20 → 6PDG + H+ |
| Rx3 | 1.1.1.44 | PF14_0520 | 6PDG + NAD(P)+ → DRL5P + CO2 + NAH |
| Rx4 | 5.1.3.1 | PFL0960W | DRL5P ⟷ DX5P |
| Rx5 | 5.1.3.6 | PFE0730C | DR5P ⟷ DRL5P |
| Rx6 | 2.2.1.1 | PFF0530W | DS7P + DG3P ⟷ DR5P + DX5P |
| Rx7 | 2.2.1.2 | PFF0530W | DG3P + DS7P ⟷ DF6P + DE4P |
| Rx8 | 2.2.1.1 | PFF0530W | DE4P + DX5P ⟷ DF6P + DG3P |

## 4.0 RESULTS AND DISCUSSION

The CPN Model consists of 8 reactions which have a total of 13 substrates and 13 products. 5 of the reactions are reversible and the other 3 are irreversible. The reactions were catalyzed by 8 enzymes. Table 2 shows the overall reaction layout for the PPP in P.f. The abbreviations of compounds and their corresponding meanings are given in table 3.

From the data extraction, we constructed a stoichiometric matrix (figure 4) using the stoichiometric coefficients. We multiplied by -1 for substrates and +1 for products. The zero entries mean the metabolite did not participate in the given reaction. This was then used to construct the Petri Net model. The CPN model is shown in figure 5 (before it fires) and figure 6 (after it fires).

## MODEL VALIDATION

The aim of model validation is to check the constructed Petri Net for inconsistencies in the given system. It helps in deriving statements on the structural and dynamics properties that reflect the activities of the system in reality.

**Structural Analysis**

The aim of this is to conclude on the structural properties of the CPN model. Structural analysis checks if the net is Ordinary, Homogeneous, Conservative, Pure, Static, Conflict-free and connected or strongly connected in graph theoretical sense. Some of these properties are based on the stoichiometric relations of biochemical networks.

A bounded net is one in which the token in a place is never more than the arc weight. And a net is ordinary if the weight of its entire arc equals 1. The net constructed in this work is a bounded net but it is not an ordinary net because the arc weights (stoichiometric numbers) do not equal one for every reactant. The net is not homogenous due to the fact that all the places in the pathway do not have the same arc weight.

**Table 3: Abbreviations of biological compounds and their full meanings**

| S/N | Abbreviations | Full Meanings |
|---|---|---|
| 1 | bDG6P | b-D-Glucose-6-Phosphate |
| 2 | NADP+ | Nicotinamide Adenine Dinucleotide Phosphate |
| 3 | 6PDG15L | 6-Phospho-D-Glucono-1,5-Lactone |
| 4 | NADPH | Nicotinamide Adenine Dinucleotide Phosphate (Reduced form of NADP+ and the H represent the attached Hydrogen) |
| 5 | H20 | Water |
| 6 | 6PDG | 6-Phospho-D-Gluconate |
| 7 | H+ | Hydrogen |
| 8 | DRL5P | D-Ribulose-5-Phosphate |
| 9 | CO2 | Carbon-Dioxide |
| 10 | DX5P | D-Xylulose-5-Phosphate |
| 11 | DR5P | D-Ribose-5-Phosphate |
| 12 | DS7P | D-Sedoheptulose-7-Phosphate |
| 13 | DG3P | D-Glyceraldehyde-3-Phosphate |
| 14 | DF6P | D-Fructose-6-Phosphate |
| 15 | DE4P | D-Erythrose-4-Phosphate |

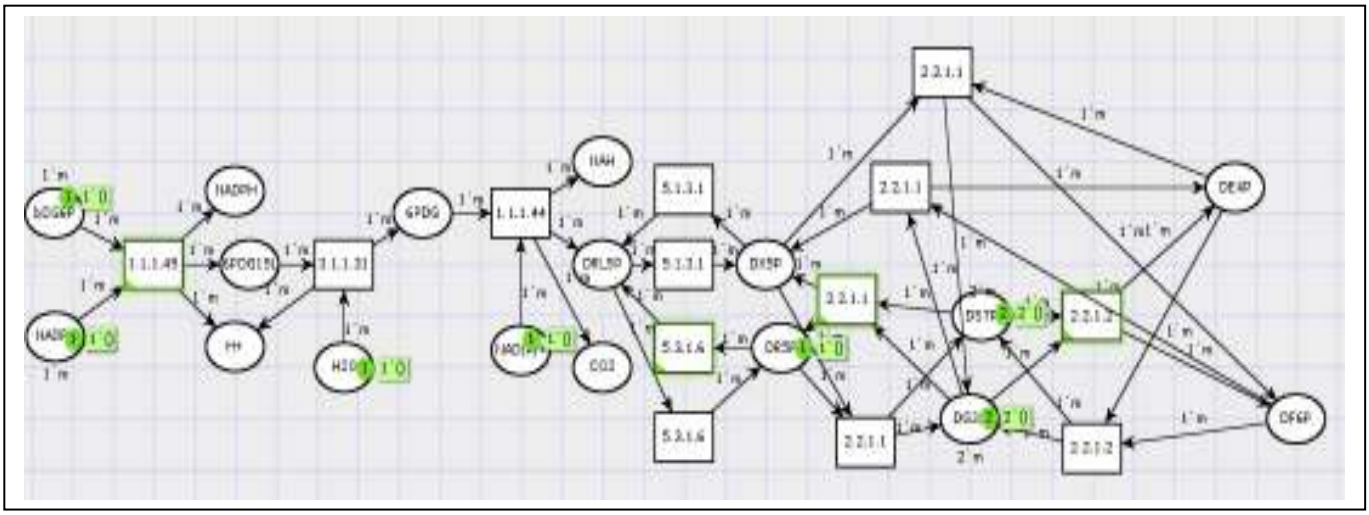

**Figure 5: The CPN model of Pentose phosphate pathway (before firing)**

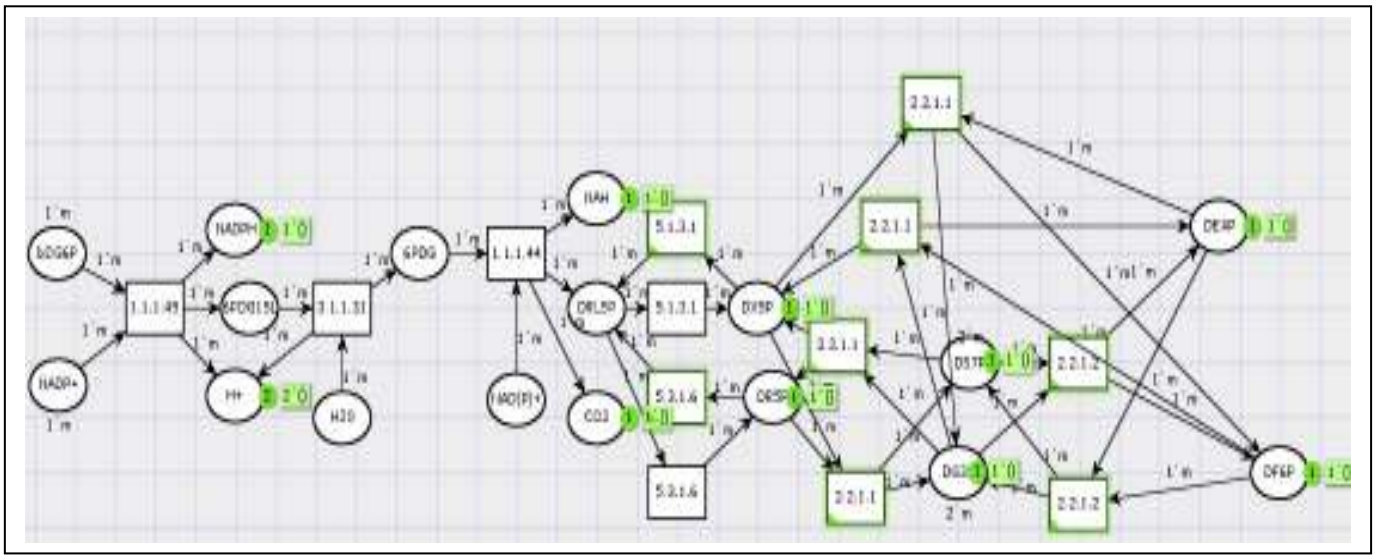

**Figure 6: The CPN model of Pentose phosphate pathway (after firing)**

**Table 4: List of Essential reactions for the Pentose Phosphate**

| S/N | Reaction Name | Enzymes | E.C Number | Reaction Equation |
|---|---|---|---|---|
| 1 | GLU6PDEHYDROG-RXN | Glucose-6-phosphate dehydrogenate-6-phosphogluconolactonase | 1.1.1.49 | b-D-glucose-6-phosphate → 6-phospho-D-glucono-1,5-lactone |
| 2 | 6PGLUCONOLACT-RXN | Glucose-6-phosphatedehydrogenase-6-phosphoGlunconolactonase | 3.1.1.31 | 6-phospho-D-glucono-1,5-lactone → 6-phospho-D-gluconate |
| 3 | Phosphogluconate dehydrogenase (decarboxylating) | 6-phosphogluconate dehydrogenase | 1.1.1.44 | 6-phospho-D-gluconate → D-ribulose-5-phosphate |

A conservative net is one that has its number of tokens constant. Therefore, this net is not conservative because the number of token in each place is not the same. This net is not conservative but it is bounded. A Pure petri-net is one without self-loop where a place is both the input and output place; therefore the constructed net is pure because there is no place that is both a pre-place and a post place. A conflict occurs when the token of one place may be used by two different transitions. A metabolic Petri Net would not be free of static conflicts because different compounds maybe used by several reactions. Due to that fact that this net is a bounded, the reachability graph and coverability graph can be constructed or computed.

**Essential Reactions**

An essential reaction is a reaction that either uniquely consumes a specific metabolite or uniquely produces a specific metabolite. These are important reactions in the metabolic pathway. In this work, the reactions in table 4 were identified as the possible essential reactions (choke points) for the pentose phosphate. These essential reactions are very important because without them, the pathway cannot function. As a result, they are potential drug target. If any of these reactions are inhibited by drug, it is possible that the PPP in P.f will be inactive and will not be able to perform its functions.

## 5.0 CONCLUSION

Malaria is one of the deadliest diseases that kill millions of people across Africa, especially the children. The proposed system is a step to altering the lifecycle of the protozoan *P.f* that causes malaria. The Pentose Phosphate Pathway in the *P.f* is one of the pathways that aid the growth of the parasite during erythrocytes stage. The pathway produces indirect source of energy and helps in immune evasion.

The essential reactions predeicted in the constructed CPN model are catalyzed by enzymes; inhibitors for each of these enzymes can be introduced such the activities of the enzymes can be altered hence causing a deadlock in the pathway.

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