

# Modeling of the Glycolysis Pathway in *Plasmodium falciparum* using Petri Nets



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**ABSTRACT:** Malaria is one of the deadly diseases, which affects a large number of the world's population. The *Plasmodium falciparum* parasite during erythrocyte stages produces its energy mainly through anaerobic glycolysis, with pyruvate being converted into lactate. The glycolysis metabolism in *P. falciparum* is one of the important metabolic pathways of the parasite because the parasite is entirely dependent on it for energy. Also, several glycolytic enzymes have been proposed as drug targets. Petri nets (PNs) have been recognized as one of the important models for representing biological pathways. In this work, we built a qualitative PN model for the glycolysis pathway in *P. falciparum* and analyzed the model for its structural and quantitative properties using PN theory. From Plasmocyc files, a total of 11 reactions were extracted; 6 of these were reversible and 5 were irreversible. These reactions were catalyzed by a total number of 13 enzymes. We extracted some of the essential reactions in the pathway using PN model, which are the possible drug targets without which the pathway cannot function. This model also helps to improve the understanding of the biological processes within this pathway.

**KEYWORDS:** glycolysis, petri nets, *plasmodium falciparum*, Plasmocyc

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## Introduction

Models have been defined as a simplified description of a physical system, which is meant to represent the important aspects of the system in a simple form.<sup>1</sup> The act of creating these models with the purpose of describing it, understanding it, or deriving some hidden properties is known as modeling. Several classifications of models exist, which include qualitative or structural models and quantitative models; deterministic and stochastic models; and discrete and continuous models, and others. Although a qualitative or structural model, eg, a network graph, specifies the interactions among model elements, a quantitative model assigns values to the elements and to their interactions.<sup>2</sup>

Computational modeling of biological networks, such as metabolic pathways, protein–protein interaction networks, neural network, food webs, gene regulatory networks, and signaling networks, has received much attention in the past decade. Likewise, mathematical modeling has been used increasingly to get insights into the functioning of complex biology networks. It is of utmost importance to understand and to interpret the complex interactions between biological systems and also certain behavioral patterns in organisms, cells, or a given metabolism; these interactions are too intrinsic to comprehend intuitively. For example, the human brain is estimated to be built of about a hundred billion neural

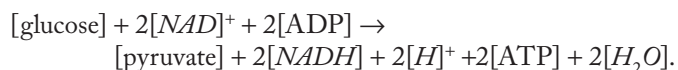
components with hundreds of trillions interconnections,<sup>3</sup> the mere description of this requires computational techniques. There is almost no real understanding of the design principles that govern intact biological systems.

A metabolic network is simply a graphical representation of metabolism. Formally, it is defined as a collection of objects and the interactions that exists among them. The objects correspond to chemical compounds, biochemical reactions, enzymes, and genes.<sup>4</sup> A metabolic network consists of the chemical reactions of metabolism as well as the regulatory interactions that guide these reactions. It is important to develop an understanding of how the cell meets its metabolic objectives through the analysis of metabolic networks, so that the goal of creating, destroying, or even enhancing the production capabilities of an organism can be easily achieved.<sup>5</sup>

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).<sup>6</sup> Consequently, the task of any metabolic pathway is to modify a principal chemical compound to form another chemical compound that can be used up, passed on to start another pathway, or stored up by the cell. The vast occurrence of the glycolysis pathway indicates that it is one of the most ancient known metabolic pathways.<sup>7</sup> It converts glucose



$C_6H_{12}O_6$  into pyruvate  $CH_3COCOO^- + H^+$ . The free energy that is released during glycolysis is in the form of two high-energy compounds: adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). The overall reaction for glycolysis is represented as follows:



According to the World Health Organization's (WHO) annual malaria report, an estimated 207 million cases (uncertainty interval, 135–287 million) and 627,000 malaria deaths (uncertainty interval, 473,000–789,000) have occurred in 2012 in Africa.<sup>8</sup> It is well established that *Plasmodium falciparum*, the causative agent of the most serious form of malaria has its erythrocytes' stages rely mainly on glycolysis for their energy supply.<sup>9</sup> It has also been known for some time that *P. falciparum*-parasitized erythrocytes increase their utilization of glucose as much as 100 times the rate of uninfected host red cells.<sup>10</sup>

## Related Works

Petri nets (PNs) have been suggested to be well suited for modeling metabolic networks by overcoming some limitations such as applying ordinary differential equations (ODEs) to a very large and complex system, which can be an uphill task.<sup>11</sup> 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 that is known today as systems biology. Apart 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.<sup>12</sup> Hence, PN technology appears to be a very promising approach to modeling various forms of biological systems.<sup>13</sup> Sackmann et al.<sup>14</sup> provided a systemic modeling method of signal transduction pathways in terms of PN components. Chaouiya<sup>2</sup> provided an overview of the different types of biological networks. This includes colored PN, stochastic PN, hybrid PNs, and hybrid function PNs. Hardy and Robillard<sup>15</sup> also discussed the modeling and simulation of molecular biology networks. They identified two categories of the goals of PN biological modeling: qualitative and quantitative analyses. Qualitative analysis is the analysis of different biological properties, while quantitative analysis is the simulation of system dynamics. For quantitative analysis, a PN representation with sufficient modeling power should be kinetic parameters, in which reaction rates and stoichiometric quantities of reactants are necessary. Heiner et al.<sup>16</sup> demonstrated a generalized approach toward modeling and analysis of biological pathways using PNs.

Therefore, to model and analyze biochemical pathways on a qualitative level, we need to establish the concept of elementary modes,<sup>17</sup> which are based on the incidence (stoichiometric) matrix of the underlying directed graph. Our

modeling approach is based on the use of the modeling tool called PNs to construct an in silico metabolic network that shows the interactions between the metabolites and the reactions in the glycolysis pathway of *P. falciparum*.

## Materials and Methods

We obtained data for the glycolysis pathway of *P. falciparum* from Plasmocyc v14.0 from the BioCyc database collection ([www.biocyc.org](http://www.biocyc.org)). BioCyc is a collection of 3530 pathway/genome databases. Three files from Plasmocyc were used, namely, *pathways.dat*, *reactions.dat*, and *enzrxns.dat*, and these are available in Supplementary File 1 (a pathway map for the glycolysis pathway in *P. falciparum* can also be found at [www.biocyc.org/PLASMO/NEW-IMAGE?type=PATHWAY&object=GLYCOLYSIS](http://www.biocyc.org/PLASMO/NEW-IMAGE?type=PATHWAY&object=GLYCOLYSIS)).

A data extraction program was written in Java to extract data from these files. The following details were extracted for each reaction in the glycolysis pathway: reaction unique-ID, common name of the reaction, reactants, reactant stoichiometry coefficients, products, product stoichiometry coefficients, reversibility, E.C. number, enzyme, and the common name of the enzyme. With the results of the data extraction, we got a stoichiometric matrix for the pathway and then built the PN model. The construction of the PN is based on the stoichiometric matrix for the metabolic reactions. For a stoichiometric number matrix, the column represents each reaction and the row represents 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.<sup>18</sup> The stoichiometric number matrix,  $C = P \times T$ , of a place/transition net would be defined as an integer, where the places are listed as rows and the transitions as columns. The PN model was built using PIPE2 version 4.2.1.<sup>19</sup> Platform Independent Petri Net Editor (PIPE) is an open-source platform-independent tool, used in creating and analyzing PNs. It also offers a full suite of analysis modules to check the behavioral properties and produce performance statistics and some less common features such as PN comparison and classification.

## Definitions and Notation

**Petri nets.** PNs were first proposed by Carl Adam Petri in 1962. They can be used for describing and modeling dynamic systems that can be characterized as concurrent, synchronous, distributed, parallel, nondeterministic, and/or stochastic systems. A PN is a directed weighted bipartite graph that consists of two types of nodes: places and transitions represented by circles and boxes, respectively. There can only be arcs from places to transitions as well as from transitions to places. The arc weights are positive integers and the absence of a weight implies weight units. A marking is a vector that represents an assignment of a nonnegative number of tokens (denoted by dots) in all places in a given PN. In a PN model of a dynamic system, conditions are represented by places and events by transitions.

As a mathematical tool, PNs provide a uniform environment for the formal analysis, design, and modeling of discrete event systems. The simplest form of a PN is a bipartite digraph, which is a graph with two nodes, namely, input and output. The main idea with PNs is to represent the states of subsystems separately. Then, the distributed activities of a system can be represented very effectively.<sup>20</sup> A formal description of PNs can be seen in Refs. 21 and 22. PN is a promising tool for describing and studying information-processing systems, which are characterized as being concurrent, asynchronous, distributed, parallel, nondeterministic, and/or stochastic. PNs are constructed from four basic elements, as follows.

**Places.** It is represented by a circle/oval. It shows a system's state. It is either input or output, and every PN must have an input and an output place. Places might be marked by an integer number of tokens. The overall state of a system of  $n$  places is represented by a vector of size  $n$ .

**Transitions.** It is represented by a rectangular bar. It is used to change a system's state, it receives tokens from the input place(s) and distributes it to the available output place(s).

**Arcs.** It is represented by a directed arrow, and it is either an inward arc (arrow directed from a place to a transition) or an outward arc (arrow directed from a transition to a place). The arcs may be labeled with an integer representing the weight, if unlabeled it is assumed that the weight = 1.

**Tokens.** It is represented by a dot or a shaded circle; it is usually inside of a place. A token is what usually transferred (this is what we move from one place to another).

**Formal definition.** PN is defined as a 5-tuple  $\alpha = (P, T, E, W, M_0)$ , where  $P = \{p_1, p_2, \dots, p_n\}$  represents a set of places,  $T = \{t_1, t_2, \dots, t_n\}$  represents a set of transitions,  $E$  defines flow relations in terms of arcs,  $W: E \Rightarrow (1, 2, 3, \dots)$  is an arc weight function, and  $M_0: P \Rightarrow (0, 1, 2, \dots)$  is the initial marking and the sets  $P$  and  $T$  are disjoint sets.

A *preplace* of transition  $t$  is a place that is adjacent to  $t$ . The set of *preplaces* of  $t$  is denoted by  $Pre(t)$ . Mathematically,

$$Pre(t) = \{p \mid (p, t) \in E\}.$$

Similarly, a *postplace* of a transition  $t$  is a place adjacent from  $t$  and the set of *postplaces* of  $t$  is denoted by  $post(t)$ . Mathematically,

$$Post(t) = \{p \mid (t, p) \in E\}.$$

The *pretransition* and *posttransition* concepts are defined similarly.

$$Pre(p) = \{t \mid (t, p) \in E\}$$

$$\text{and } post(p) = \{t \mid (p, t) \in E\}.$$

A set of rules defined below control the behavior of a PN model for simulating a dynamic system.

1. Suppose  $w(p, t)$  defines the weight of an arc between  $p$  and  $t$ . Then, transition  $t$  is enabled if each  $p \in pre(t)$  has at least  $w(p, t)$  tokens.
2. If an event takes place, the corresponding enabled transition will fire, otherwise it will not.
3. Let  $|p|$  denote the number of tokens in place  $p$ . Let  $w(t, p)$  define the weight of an arc between  $t$  and  $p$ . After a transition  $t$  has been fired, the tokens will be updated as follows:

$$\forall p \in pre(t), |p| = |p| - w(p, t)$$

$$\forall p \in post(t), |p| = |p| + w(p, t)$$

From the biological point of view, the tokens residing in places indicate whether the corresponding chemical species is present, ie, its concentration is above a certain concentration level (threshold) in the cell. This presence enables the chemical reactions modeled by the place's posttransitions to take place.

A current distribution of the tokens over all places, given as  $m \in \mathbb{N}_0^n$ , describes a certain state of the system and is called a *marking* of the net. Therefore, the initial marking  $m_0$  of a net describes the state of the system before any transition has fired.

The incidence matrix  $C$  of a given PN is a  $P \times T$  matrix (where  $P$  denotes the number of places and  $T$  is the number of transitions). Every matrix entry  $c_{ij}$  gives the token change on the place  $p_i$  by firing the transition  $t_j$ . A  $t$ -invariant is defined as a nonzero vector  $x \in \mathbb{N}_0^T$ . Therefore, we get all minimal semipositive  $t$ -invariants by solving the following integer linear programming problem, which holds for this equation

$$C \cdot x = 0 \tag{1}$$

where  $C = (PxT)$  is the incidence matrix and  $x \neq 0, \forall x \geq 0$ ,  $x$  is the transition vector.

A  $t$ -invariant represents a multiset of transitions, which have altogether a zero effect on the marking, ie, if all of them have fired the required number of times, a given marking is reproduced. A  $t$ -invariant is called *realizable*, if a marking is reachable, such that all transitions of the  $t$ -invariant are able to fire in a suitable partial order.

Similarly, for all minimal semipositive  $p$ -invariants, which is defined as a nonzero vector,  $\beta \in \mathbb{N}_0^n$  holds for this equation

$$\beta \cdot C = 0 \tag{2}$$

where  $C = (PxT)$  is the incidence matrix and  $\beta \neq 0, \forall \beta \geq 0$ ,  $\beta$  is the place vector.

A  $p$ -invariant characterizes a token conservation rule for a set of places, over which the weighted sum of token is constantly independent of any firing, ie, for a  $p$ -invariant  $\beta$  and any markings  $m_i, m_j \in \mathbb{N}_0^n$ , which are reachable from  $m_0$  by the firing of transitions, it holds:

$$\beta \cdot m_i = \beta \cdot m_j \quad (3)$$

The nodes corresponding to the nonzero entries of an invariant  $x$  are called the support of  $x$ , written as  $supp(x)$ . From Equations (1) and (2), it is observed that a sum of  $t$ -invariants ( $p$ -invariants) gives again a  $t$ -invariant ( $p$ -invariant). An invariant  $x$  is called minimal, if its support does not contain the support of any other invariant  $z$ , ie,

$$\text{Invariant } z: supp(z) \subseteq supp(x), \quad (4)$$

and the greatest common divisor of all nonzero entries of  $x$  is one. A net is covered by  $t$ -invariants ( $p$ -invariants), if every transition (place) participates in a  $t$ -invariant ( $p$ -invariant). A  $t$ -invariant ( $p$ -invariant) defines a connected subnet, consisting of its support, the support's pre- and postplaces (pre- and posttransitions), and all arcs in between.

**Metabolic networks.** Reasons for using mathematical models to represent metabolic networks include the following: 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, to define improved hypotheses, and to understand the essential features of a system.<sup>23</sup> Other models apart from PNs that have been proposed to model biological systems include the following: ODEs, process calculi, Boolean networks, Bayesian networks, bipartite graphs, stochastic equations, and Markov chains.<sup>24–27</sup> The applications of these techniques have given rise to a new branch of study called system biology or in silico biology.<sup>28</sup>

To give a PN representation of a metabolic pathway, places represent the by-products of metabolism, ie, metabolites, proteins, and enzymes; transitions represent chemical reactions; input places represent reactants or substrates; and output places represent reaction products. The stoichiometric matrix of a pathway is equivalent to the incidence matrix of the PN, and the arc weights can be gotten by the given stoichiometric coefficients. The number of tokens in each place indicate 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 (semipositive)  $t$ -invariants correspond to elementary flux modes of a metabolic pathway, ie, minimal sets of reactions that can operate at a steady state. Minimal  $t$ -invariants form a basis for the set of semipositive  $t$ -invariant (Hilbert basis), which is unique and characteristic of PN.<sup>29</sup> Table 1 shows

**Table 1.** Relationship between PN elements and pathway elements.<sup>29</sup>

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

the relationship between metabolic pathway elements and PN elements.

An illustration of the PN representation of the well-known chemical reaction  $2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$  is shown in Figure 2. The first PN represents the state before the reaction occurs (ie, before the transitions fire), while the second represents the state after the reaction has occurred.

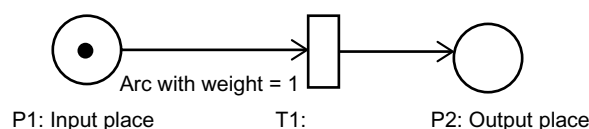
## Results and Discussion

From our data extraction from the Plasmocyc files, a total of 11 reactions were extracted. A total of six of these reactions were reversible and five were irreversible. These reactions were catalyzed by a total of 13 enzymes. The results of the data extraction processes are summarized in Table 2. The abbreviations of compounds and their corresponding meanings are given in Table 3, while Table 4 summarizes the gene identification (Gene ID) and the corresponding enzyme name.

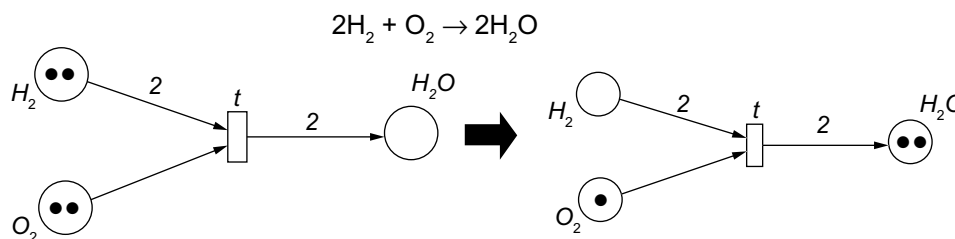
From the data extraction, we constructed a stoichiometric matrix (Fig. 3) using the stoichiometric coefficients. We multiplied by  $-1$  for substrates and  $+1$  for products. The zero entries mean that the metabolite did not participate in the given reaction. This was then used to construct the PN model (Figs. 4 and 5). The model is available in PNML format in Supplementary File 2.

Figures 4 and 5 show the construction of the glycolysis pathways before and after firing. In Figure 5, the following transitions were fired: 4.1.2.13(r), 5.3.1.1, 1.2.1.12(r), 2.7.2.3(r), 5.4.2.1(r), 4.2.1.11(r), 2.7.1.40, and 2.7.9.2.

**Model validation.** The aim of model validation is to check the constructed PN for inconsistencies in the given system, hence deriving statements on the structural and



**Figure 1.** A simple PN.



**Figure 2.** A PN representation of a chemical reaction.<sup>22</sup> © 1989 IEEE. Reprinted with permission from Murata, T. Petrinets: properties, analysis and applications. Proceedings of the IEEE; 1989;77(4):541–80.

**Table 2.** Overall reaction layout of the glycolysis pathway.

REACTION NUMBER	REACTION NAME	REACTION LAYOUT
R × 1	F16BDEPHOS-RXN	F16DP + H <sub>2</sub> O → F6P + Pi
R × 2	PEPSYNTH-RXN	Pyruvate + ATP + H <sub>2</sub> O → 3Proton + Pi + PEP + AMP
R × 3	PGLUCISOM-RXN	G6P ⇌ F6P
R × 4	6PFRUCTPHOS-RXN	ATP + F6P → 2Proton + ADP + F16DP
R × 5	F16ALDOLASE-RXN	F16DP ⇌ GAP + DHAP
R × 6	TRIOSEPISEMERIZATION-RXN	DHAP → GAP
R × 7	GAPOXNPHOSPHN-RXN	GAP + Pi + NAD ⇌ Proton + DPG + NADH
R × 8	PHOSGLYPHOS-RXN	G3P ATP ⇌ Proton + DPG + ADP
R × 9	3PGAREARR-RXN	PROTON + G3P ⇌ 2PG
R × 10	2PGADEHYDRAT-RXN	2PG ⇌ Proton + PEP + H <sub>2</sub> O
R × 11	PEPDEPHOS-RXN	2Proton + ADP + PEP → ATP + Pyruvate

**Table 3.** Abbreviations of compounds and their full meanings.

ABBREVIATIONS	FULL MEANINGS
1. ADP	Adenosine Diphosphate
2. AMP	Adenosine Monophosphate
3. ATP	Adenosine Triphosphate
4. DHAP	Dihydroxy-Acetone-Phosphate
5. F16DP	Fructose1–6, Diphosphate
6. F6P	Fructose 6 Phosphate
7. G3P	Glucose 3 Phosphate
8. G6P	Glucose 6 Phosphate
9. GAP	Glyceraldehyde 3-Phosphate
10. NAD	Nicotinamide Adenine Dinucleotide
11. PEP	Phospho Enol Pyruvate
12. PG	Prostaglandin

dynamic properties that reflect the activities of the system in reality.<sup>30</sup>

The PN model of the glycolysis pathway after firing shows one token each in the final output places (ATP-3, pyruvate), verifying that the model constructed conforms to the results from the data analysis since the stoichiometric coefficients of both ATP-3 and pyruvate are 1. The constructed PN model consists of 32 places and 17 transitions. The reversible reactions are modeled as forward and backward reactions.

**Structural analysis.** This is to discover certain structures that allow conclusions on dynamic properties. It involves the calculation of elementary properties. Structural analysis checks if the net is ordinary, homogenous, conservative, pure, static, conflict free, and connected or strongly connected in graph-theoretical sense. The unbounded net is not ordinary because the arc weights (stoichiometric numbers) in the reaction equation are not equal to one. The net is not homogenous because not all the places in the pathway have the arcs starting there having the same weight. This property usually holds for signal-transduction pathways,<sup>14</sup> and the pathway being modeled in this work is a metabolic pathway.

The construction of the network is not conservative because not all the transitions have the sum of the input arc weights equal to the sum of the output arc weights. Therefore, all reactions do not work in a token-preserving way. It is not

**Table 4.** Gene ID and the corresponding enzyme name.

	GENE ID	ENZYME NAME
1.	PF14_0341	Glucose-6-phosphate isomerase
2.	PF11_0294	Phosphofructokinase, putative
3.	PF10755C	6-phosphofructokinase, putative
4.	PF14_0425	Fructose-biphosphate aldolase
5.	PF14_0378	Triose phosphate isomerase
6.	PFC0831W	Triosephosphate isomerase, putative
7.	PF14_0598	Glyceraldehyde3phosphate hydrogenase
8.	PFI1105W	Phosphoglycerate kinase
9.	PF11_0208	Phosphoglycerate mutase, putative
10.	PFD0660W	Phosphoglycerate mutase, putative
11.	PF10_0155	Enolase
12.	PF10_0363	Pyruvate kinase putative
13.	PFF1300W	Pyruvate kinase putative



	rx1	rx2	rx3a	rx3b	rx4	rx5a	rx5b	rx6	rx7a	rx7b	rx8a	rx8b	rx9a	rx9b	rx10a	rx10b	rx11
<i>F6P</i>	1	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pi</i>	1	1	0	0	0	0	0	0	-1	1	0	0	0	0	0	0	0
<i>F16DP</i>	-1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>water</i>	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0
<i>Proton</i>	0	3	0	0	2	0	0	0	1	-1	1	-1	1	-1	1	-1	2
<i>PEP</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	1
<i>AMP</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pyruvate</i>	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>ATP</i>	0	-1	0	0	-1	0	0	0	0	0	-1	1	0	0	0	0	1
<i>GLC6P</i>	0	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>ADP</i>	0	0	0	0	1	0	0	0	0	0	1	-1	0	0	0	0	-1
<i>DAP</i>	0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0
<i>GAP</i>	0	0	0	0	0	1	-1	1	-1	1	0	0	0	0	0	0	0
<i>DPG</i>	0	0	0	0	0	0	0	0	1	-1	1	-1	0	0	0	0	0
<i>NADH</i>	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0
<i>NAD</i>	0	0	0	0	0	0	0	0	-1	1	0	0	0	0	0	0	0
<i>2PG</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	-1	1	0
<i>G3P</i>	0	0	0	0	0	0	0	0	0	0	-1	1	-1	1	0	0	0

Figure 3. Stoichiometric matrix for glycolysis pathway.

static-conflict free because there are transitions sharing the same input places.

A metabolic PN 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.

Figures 6 and 7 are the PN constructions of the inhibited glycolysis pathways before and after firing. From Figure 7, the following transitions were fired: 5.3.1.9(r), 2.7.1.11, 3.1.3.11, and 5.3.1.9.

The purpose of inhibiting certain reactions is to show that they are essential reactions and are absolutely required for the pathway to function. To simulate reaction inhibition,

an additional place was introduced to the corresponding transition, representing the enzyme responsible for the reaction. The place was then connected to the transition with an inhibitor arc, resulting in the transition not firing; hence the final product of the pathway cannot be produced. The essential reactions in the pathway are listed in Table 5.

**Invariant analysis.** The net contains, but is not covered by, the following *p*-invariants.

Invariant A – ATP, AMP, and ADP (these are the metabolites containing adenosine residues).

Invariant B – 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

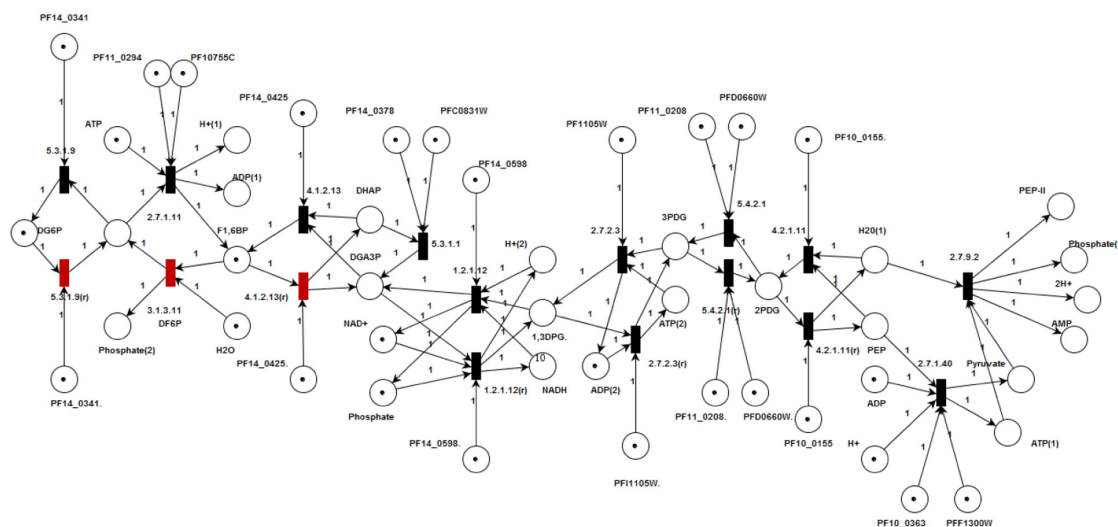
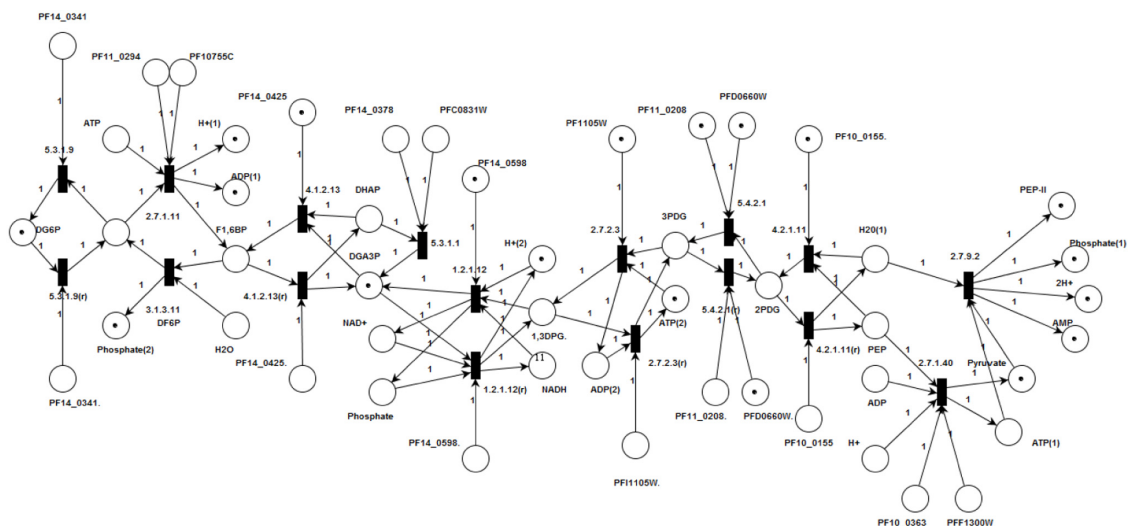


Figure 4. PN construction of the glycolysis pathway (before firing).



**Figure 5.** PN construction of the glycolysis pathway (after firing).

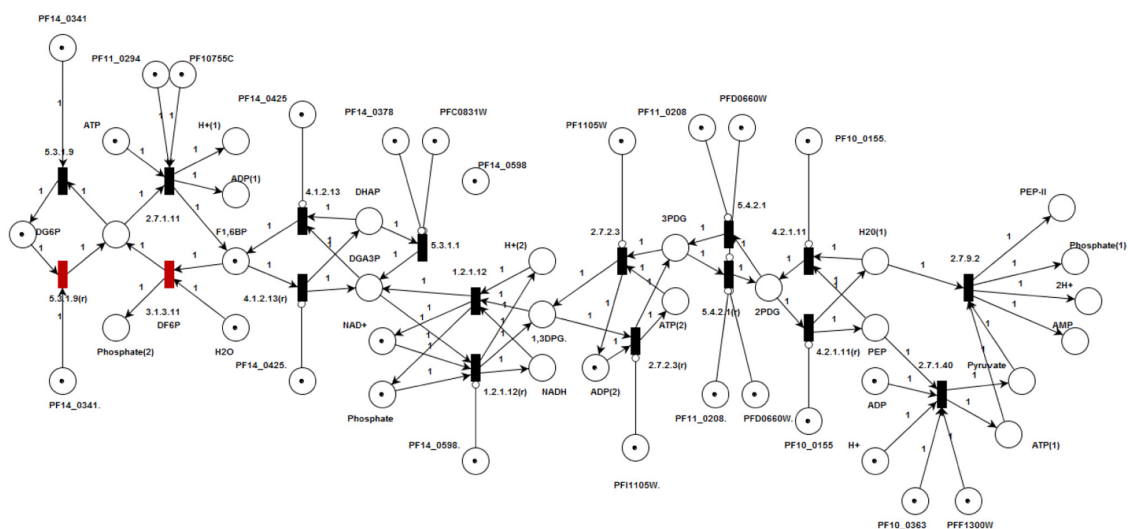
of the phosphorylated metabolite remains unchanged. If no phosphate is taken up or secreted by a cell, the sum of phosphate groups in all metabolites, including inorganic phosphate, would also remain unchanged.

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

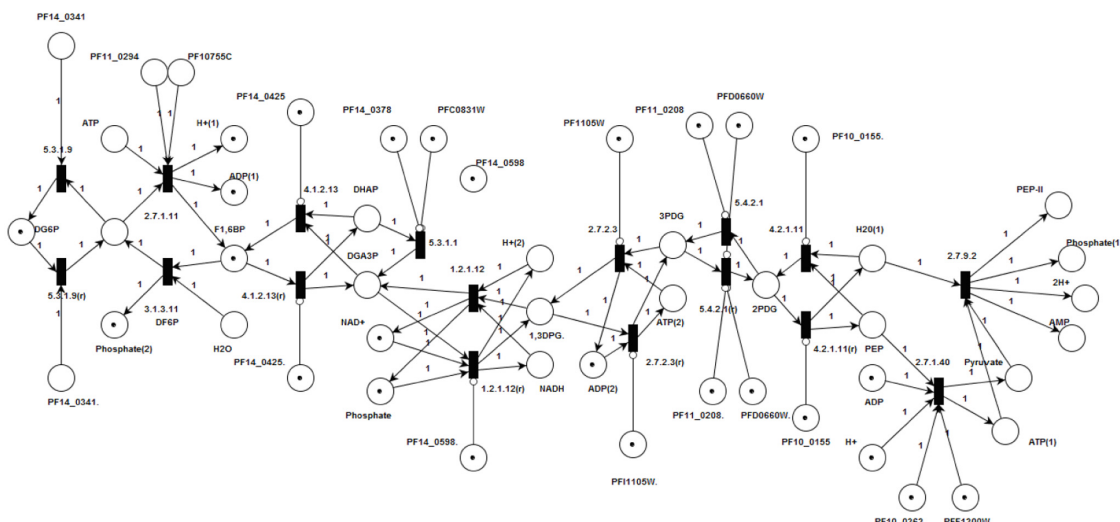
1. Fructose 6-phosphate;
2. Fructose-1,6-diphosphate;
3. Glucose 6-phosphate;
4. Phosphoenolpyruvate;
5. Dihydroxylacetone phosphate;
6. Glyceraldehyde 3-phosphate;
7. Phosphopyruvate;
8. Adenosine triphosphate (ATP);
9. Adenosine diphosphate (ADP); and
10. Pi.

## Discussion

This model of glycolytic pathway in *P. falciparum* has highlighted some key enzymes that can serve as drug targets for antimalarial drug development (Table 5). *P. falciparum* lacks a functional tricarboxylic acid (TCA) cycle; hence, both the asexual erythrocyte stages and gametocytes are dependent primarily on glucose uptake and glycolysis for ATP synthesis and survival. As a result, glucose utilization by infected red blood cells (RBCs) are over 75-fold higher, compared with uninfected RBCs.<sup>31</sup> This upregulation of glycolytic pathway has been reported to be accompanied by concomitant increase in the activities of key glycolytic enzymes.<sup>32</sup> Therefore, our model reaffirms that the ATP-dependent phosphorylation of glucose by hexokinase, being the first step that traps glucose, is very critical to ATP generation in *P. falciparum*.<sup>33</sup> This makes hexokinase, the enzyme that catalyzes this reaction, a very good candidate for drug development.



**Figure 6.** PN construction of the inhibited glycolysis pathway (before firing).



**Figure 7.** PN construction of the inhibited glycolysis pathway (after firing).

Another enzyme of importance is *Plasmodium's* fructose-1,6-phosphate aldolase, which also plays a critical role in the hepatocyte invasion by sporozoites as well as erythrocyte invasion by merozoites. Therefore, this enzyme is essential, not just for the survival of the parasite but for its multiplication in the host liver and erythrocytes.<sup>34,35</sup> Identification of this enzyme as one of the essential enzymes by PN modeling in this study is noteworthy.

*Plasmodium's* triosephosphate isomerase is another essential enzyme identified by this model, and this enzyme plays an important role not just in glycolysis but also in hexose monophosphate shunt and fatty acid biosynthesis.<sup>32</sup> Blocking this enzyme will not just deprive the parasite of needed energy but also of fatty acid needed to maintain its membrane integrity.<sup>36</sup>

Although there is a dearth of information on *Plasmodium's* glyceraldehyde-3-phosphate dehydrogenase, this enzyme is known to bind DNA and RNA and also involves in membrane

vesicle trafficking, tRNA transport, and DNA replication and repair.<sup>37</sup> These are very essential processes required for *Plasmodium's* survival in the erythrocytes and inhibiting this enzyme will not just deprive the parasite of energy but alter the equilibrium of these other processes.

The uniqueness in the structure and function of *Plasmodium's* phosphoglycerate kinase and characterization of phosphoglycerate mutase has established that this enzyme is involved in the phosphate metabolism or regulatory functions in parasite life cycle.<sup>38</sup> Pyruvate kinase actively expressed during the intraerythrocytic stages of *P. falciparum* growth suggests the involvement of this enzyme during infection. A previous study has identified it as an important enzyme for drug development.<sup>39</sup> This is consistent with the findings of this study, as our PN model identified pyruvate kinase as one of the important enzymes.

### Conclusion

Various forms of PN representation have been successfully used in the analysis of many biological networks especially for gene regulation, signal transduction, and metabolic systems. This study was done to show the use of PNs as a tool to model, quantitatively and qualitatively, the glycolysis metabolic pathway. The results characterize the net structure and give insights into the complex net behavior of the pathway.

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### Author Contributions

Conceived the concepts and the case study of this work: JO, II. Designed and analyzed the work: JO, II, IO. Made some valuable contributions in the application of PN techniques: II, IO. Defined the topic and gave substantial contributions

**Table 5.** List of important reactions.

E.C NUMBER	GENE ID	ENZYME NAME
4.1.2.13	PF14_0425	Fructose-biphosphate aldolase
5.3.1.1	PF14_0378	Triose phosphate isomerase
	PFC0831W	Triosephosphate isomerase, putative
1.2.1.12	PF14_0598	Glyceraldehyde3phosphate hydrogenase
2.7.2.3	PFI1105W	Phosphoglycerate kinase
5.4.2.1	PF11_0208	Phosphoglycerate mutase, putative
	PFD0660W	Phosphoglycerate mutase, putative
4.2.1.11	PF10_0155	enolase
2.7.1.40	PF10_0363	Pyruvate kinase putative
	PFF1300W	Pyruvate kinase putative
2.7.9.2	Nil	Nil





to the design of the research project: JO, II. Performed the analysis of the biological interpretation: SR. All the authors conceived the article preparation and read and approved the final article.

## Supplementary Materials

**Supplementary File 1.** Data for the glycolysis pathway of *P. falciparum* from PlasmoCyc v14.0 from the BioCyc database collection ([www.biocyc.org](http://www.biocyc.org)), comprising three files, pathways.dat, reactions.dat and enzrxns.dat.

**Supplementary File 2.** Choked glycolysis model in PNML format.

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