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Computational Investigation of Consistency and Performance of the Biochemical Network of the Malaria Parasite, *Plasmodium falciparum*

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Abstract. Malaria has been a problem in the public health sector and Sub-saharan African. The most prevalent symptoms of this disease is caused by *Plasmodium falciparum,* a blood borne pathogen, there has also been a disclosure of resistance to anti malaria drugs in *Pf*. An intimate process of acquiring insight to an organism's metabolism is to analyze her network topology deploying computational techniques.

In this research, the Flux Balance Analysis (FBA) of the metabolism of malaria parasite, *Plasmodium falciparum* that has been converted to a System Biology Markup Language (SBML) format in another work (Segun et al. 2014) was used to predict the metabolic activities and to investigate the consistency of the multi-compartment biochemical metabolic network of the parasite using FASIMU software.

With a projected output in view, a flux-balance computation was first deployed on an energy model and redox metabolism of the human red blood cells to learn the internal structure of FASIMU, this was a simpler model compared to *Plasmodium falciparum* model. The results of the analysis generated a file that consists of the flux values, reaction identifiers, equilibrium constants adopted and the concentration values. It was also discovered that transporters conveyed metabolites among the various cellular compartments of the organism. Further results of the flux balance analysis of the compartmented *Plasmodium falciparum* metabolic network generated a comprehensive list of target metabolites indispensable to the growth of the organism, which have been confirmed by recent literature. It is evident that the results generated from this research represent a significant step towards discovering drug targets.

Keywords: *Plasmodium falciparum* · *FASIMU* · Computational investigation · Biochemical network · Malaria parasite

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1 Introduction

The name given to Malaria was invented in Italy and it is interpreted to mean "bad air was the root cause of the disease" (WHO 2004). Malaria is not a new disease caused by infection with protozoan parasites which belongs to the genus *Plasmodium* transmitted by the female *Anopheles* specie mosquitoes (Francis 2010). Its a severe mosquito-borne disease and a potentially life threatening illness conveyed to humans by the bite of the female *Anopheles* mosquito (NaTHNaC 2010), "*Anopheles* has over 420 different species of which about 50 are medically significant vectors of malaria" (Stürchler 2008). *Anopheles* biting patterns and the parasite/disease transmitted differ depending on world region and species, but malaria parasite, *Plasmodium falciparum Pf.* transmission occurs mostly between dusk and dawn. *Anopheles gambiae*, the main malaria transmitting mosquito in West Africa, is identified to be very active indoors once its midnight (NaTHNaC 2010). A single-celled parasite from this genus *Plasmodium* is what causes malaria, this specie of *plasmodium* has not less than 100 diverse species in existences and is capable of transmitting malaria to other mammalian host, humans inclusive (NIH 2010).

Often symptoms of malaria include headache, malaise, weakness, cough, muscle pain, and diarrhea. Malaria caused by *Plasmodium falciparum* can aggravate to become a serious and dangerous illness if not diagnosed and treated on time, leading to cerebral malaria, kidney failure and severe anemia, which can eventually end in coma and death. (NaTHNaC 2010). It is described as the major human parasitic disease, and still a core source of morbidity, and death, especially in the third world countries. WHO in 2015 reported that malaria makes up for about 438 000 deaths, and 90% of this deaths are from Africa despite the 60% global decline, this is against the reports that death rates may keep increasing yearly" (Krogstad 1996). People who are mostly at risk are those with no or little protective immunity against the disease (WHO 2008). In areas such as Africa, south of the Sahara where there is high transmission, the most susceptible groups are young children, who have not yet built immunity to malaria, pregnant women, whose immunity is affected by pregnancy, particularly during the first two trimesters and pregnancies, and finally travellers coming from areas with little malaria spread. (WHO E Science 2010).

A metabolic network can simply be represented as a list of reactions. Metabolic networks depict every known possible path that connects cellular compounds to one another by the use of biochemical transformations (Chalancon et al. 2013). Understanding the functions of a metabolic network and a good grasp of its topological properties is very essential. By constructing a metabolic network of *P. falciparum* based mainly on data extracted from different biological database, and integrating it sufficiently with supporting experimental data, it might produce a model that will replicate the inner workings of the organism (Ginsburg 2006). Poolman grouped the techniques for analysing metabolic networks into two: structural and kinetic analysis (Poolman et al. 2004).

Jeffrey (2010) defined Flux Balance Analysis as a structural modelling mathematical method for analysing the flow of metabolites within a metabolic network. Flux-balance analysis of the whole organism metabolic networks of *P. falciparum* has been used to predict essential reactions and thus drug targets (Huthmacher et al. 2010).

2 Methodology

FASIMU is a command line adapted software for flux distribution calculation was deployed to implement this system, it is an extremely flexible computation environment for various flux-balance analysis, it calculates flux distributions using varieties of the most popular FBA algorithms (Baird 2013; Biomed Central 2013), and uses the optimization capabilities of free Linear Programming (LP) solve and GNU Linear Programming Kit (GLPK) and commercial solvers CPLEX, Linear, INteractive, and Discrete Optimizer (LINDO) (Andreas et al. 2011). "The results can be visualized in Cytoscape or BiNA using newly developed plugins" (www.sbml.org). Motivation to use FASIMU includes an opportunity to be incorporated on some level of the operating system, it distinctly out-stands other similar tools with the idea of a summarizing description file for flux-balance simulations (Biomed Central 2013). The extracted metabolic network from various databases was compiled in a semi-automated manner using System Biology Markup Language (SBML) format (Adebiyi et al. 2014). Moreso, it has a well defined protocol for network analysis and allows Incorporation of effective commercial and free solvers (www.Biomedcentral.com). It permits simple application of new algorithmic solutions (Andreas 2011) and faster.

Structural modelling was deployed in analysing the extracted metabolic network, which has been compiled and converted to SBML format (Adebiyi et al. 2014); this network was extracted and tested for consistency. A compartmentalized metabolic network was needed for this work to be able to create specific location or cellular addresses for which processes should occur, to impound metabolites from participating in undesirable reactions, to establish physical boundaries for biological processes that enable the cell to carry out different metabolic activities at the same time and finally, to generate a specific micro-environment to temporally regulate a biological process (Andreas 2007).

FBA was used to calculate the flow of metabolites through the metabolic network and it also investigated the metabolic capability of the cellular system. Justifiably, structural modelling was preferred because despite that kinetic modelling can offer a more accurate description of an organism's metabolism than structural but at the same time it is substantially more difficult because is a larger scope for errors when constructing kinetic models and finding errors can take a long time (Hayton and Su 2004). Literature shows that it is currently not suitable for malaria research. Kinetic modelling of large-scale metabolic networks is extremely difficult which is traceable to the scarcity of particular enzyme rate data (Afonso 2006).

Furthermore, large problems can become computationally expensive and the resulting systems are frequently unstable and give results that are difficult to trust. There is an easy and effective substitute for analysis of metabolic capabilities of cellular systems which is flux balance analysis (Constraint-based reconstruction and analysis) (Klann et al. 2011). There is insufficiency of Kinetic data obtainable for the simulation of networks, constraining the number and size of systems in various species that can be analyzed via this approach (Raman and Chandra 2009).

To deploy FBA, the following processes were carried out step wisely,

I. The Metabolic reactions were illustrated as a stoichiometric matrix (S), with size m * n. Each row of the stoichiometric matrix denotes one distinct compound (aimed at a system with m compounds) and each column denotes one reaction (n reactions).
II. The values in each of the columns are the stoichiometric coefficients of the metabolites partaking in a reaction. With every metabolite produced, there is a positive (+) coefficient.
III. A phenotype was described in a biological objective function structure that is applicable to the network being considered. Mathematically, an 'objective function' is to quantitatively state the amount each reaction put in to the phenotype. The mathematical illustrations of the metabolic reactions together with the objective describe a system of linear equations. In flux balance analysis, linear programming is applied to mathematically solve these linear equations (Orth 2010).
FBA seeks to maximize or minimize an objective function $Z = c^{T}v$, which could be several linear combination of fluxes. c = vector of weights representing the amount each reaction v (for example, biomass reaction when simulating maximum growth) put in to the objective function (www.ncbi.nlm.nih.gov). Through the optimization of objective functions, FBA found a single optimal flux distribution that lies on the edge of the allowable solution space (Dal'Molin et al. 2010).

3 Implementation and Results

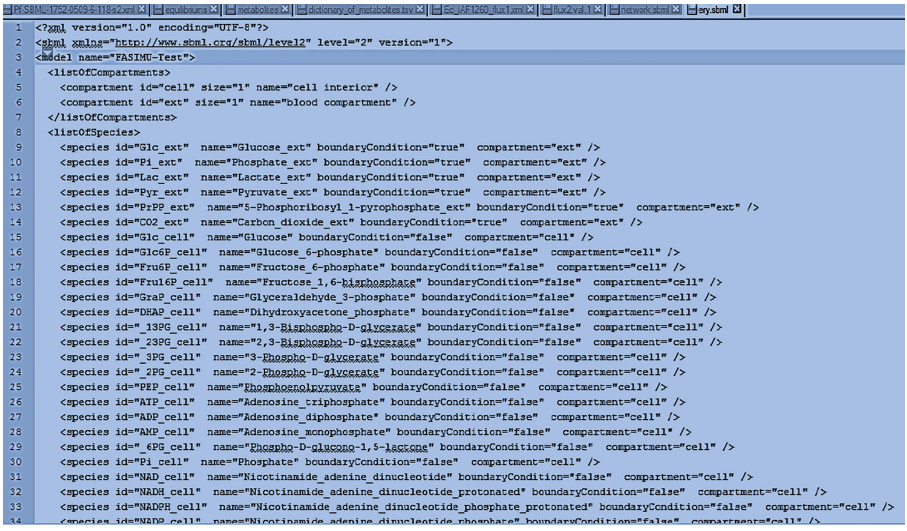
3.1 To Run the Consistency Check of the Metabolic Network

The network was checked accordingly for consistency and capability to generate important metabolites by modifying the subsequent set of biochemical reactions and transport processes to realize a consistent network; this was justified by flux balance simulations to achieve metabolic processes. The first step was completed by substituting highly defined metabolites by more typical metabolites, using a physically compiled list of substitutions. However, if the same metabolite appears with uncommon IDs in reactions, one of the IDs is selected to substitute alternative IDs. Reactions having generic terms were either eliminated or substituted by more definite reactions. Lastly, reaction duplets that previously existed in the initial data or were produced by the consistency procedure were deleted (Sabrina et al. 2007).

The consistency check process made sure that reactions with neither generic stoichiometry (the quantitative relationship between reactants and products) nor reaction duplets existed. To connect related reactions, equivalent metabolites were verified to have the same level of specificity.

3.2 Fasimu Functions Demonstrated on a Small Human Erythrocyte Model

In this test, flux-balance computation was demonstrated on a model of the energy and redox metabolism of human red blood cells (Schuster and Holzhutter 1995), thereby learning the internal structure of a FASIMU session and applying several of the implemented algorithms available. This was a simpler model compared to the *E.coli* and *Plasmodium falciparum* simulation (Fig. 1).



```
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<model name="FASIMU-Test">
  <listOfCompartments>
    <compartment id="cell" size="1" name="cell interior" />
    <compartment id="ext" size="1" name="blood compartment" />
  </listOfCompartments>
  <listOfSpecies>
    <species id="Glc_ext"  name="Glucose_ext" boundaryCondition="true"  compartment="ext" />
    <species id="Pi_ext"  name="Phosphate_ext" boundaryCondition="true"  compartment="ext" />
    <species id="Lac_ext"  name="Lactate_ext" boundaryCondition="true"  compartment="ext" />
    <species id="Pyr_ext"  name="Pyruvate_ext" boundaryCondition="true"  compartment="ext" />
    <species id="PrPP_ext"  name="5-Phosphoribosyl_1-pyrophosphate_ext" boundaryCondition="true"  compartment="ext" />
    <species id="CO2_ext"  name="Carbon_dioxide_ext" boundaryCondition="true"  compartment="ext" />
    <species id="Glc_cell"  name="Glucose" boundaryCondition="false"  compartment="cell" />
    <species id="Glc6P_cell"  name="Glucose_6-phosphate" boundaryCondition="false"  compartment="cell" />
    <species id="Fru6P_cell"  name="Fructose_6-phosphate" boundaryCondition="false"  compartment="cell" />
    <species id="Fru16P_cell"  name="Fructose_1,6-bisphosphate" boundaryCondition="false"  compartment="cell" />
    <species id="GraP_cell"  name="Glyceraldehyde_3-phosphate" boundaryCondition="false"  compartment="cell" />
    <species id="DHAP_cell"  name="Dihydroxyacetone_phosphate" boundaryCondition="false"  compartment="cell" />
    <species id="_13PG_cell"  name="1,3-Bisphospho-D-glycerate" boundaryCondition="false"  compartment="cell" />
    <species id="_23PG_cell"  name="2,3-Bisphospho-D-glycerate" boundaryCondition="false"  compartment="cell" />
    <species id="_3PG_cell"  name="3-Phospho-D-glycerate" boundaryCondition="false"  compartment="cell" />
    <species id="_2PG_cell"  name="2-Phospho-D-glycerate" boundaryCondition="false"  compartment="cell" />
    <species id="PEP_cell"  name="Phosphoenolpyruvate" boundaryCondition="false"  compartment="cell" />
    <species id="ATP_cell"  name="Adenosine_triphosphate" boundaryCondition="false"  compartment="cell" />
    <species id="ADP_cell"  name="Adenosine_diphosphate" boundaryCondition="false"  compartment="cell" />
    <species id="AMP_cell"  name="Adenosine_monophosphate" boundaryCondition="false"  compartment="cell" />
    <species id="_6PG_cell"  name="Phospho-D-glucono-1,5-lactone" boundaryCondition="false"  compartment="cell" />
    <species id="Pi_cell"  name="Phosphate" boundaryCondition="false"  compartment="cell" />
    <species id="NAD_cell"  name="Nicotinamide_adenine_dinucleotide" boundaryCondition="false"  compartment="cell" />
    <species id="NADH_cell"  name="Nicotinamide_adenine_dinucleotide_protonated" boundaryCondition="false"  compartment="cell" />
    <species id="NADPH_cell"  name="Nicotinamide_adenine_dinucleotide_phosphate_protonated" boundaryCondition="false"  compartment="cell" />
```

Fig. 1. SBML file for the Human Erythrocyte

Several files have been generated from the SBML file which was used for the simulations. The SBML file is not regarded any more.

This file shows all flux distributions of a computation series. The flux distributions are separated by lines with many # characters followed by the name of the simulation. In the next line there is a description of the system boundaries of this particular simulation. The next line gives a short comment whether a solution is computed with optional comments from the solver. Additionally warnings are shown here. Then the solution is given in a tab-separated format. The first column provides the name of the reaction (www.alecjacobson.com). The second column provides the flux value (www.alecjacobson.com). Note that zero fluxes are not recorded in the solution. Next column gives the equilibrium constant just for information, then the reaction written with metabolite identifiers follows. FASIMU can also be ordered to write them with metabolite names given in the file metabolites: most conveniently this can be done in the initial start-up of

For computations where the thermodynamic feasibility is checked a section follows given hypothetical concentrations which are compatible with the flux distribution.

The fourth column holds possible reaction names given in the file reaction-names. For computations where the thermodynamic feasibility is checked a section follows given hypothetical concentrations which are compatible with the flux distribution (Scuster et al. 1999; Schuster et al. 2000).

3.3 The Output of the Evaluation.txt

See Fig. 2.

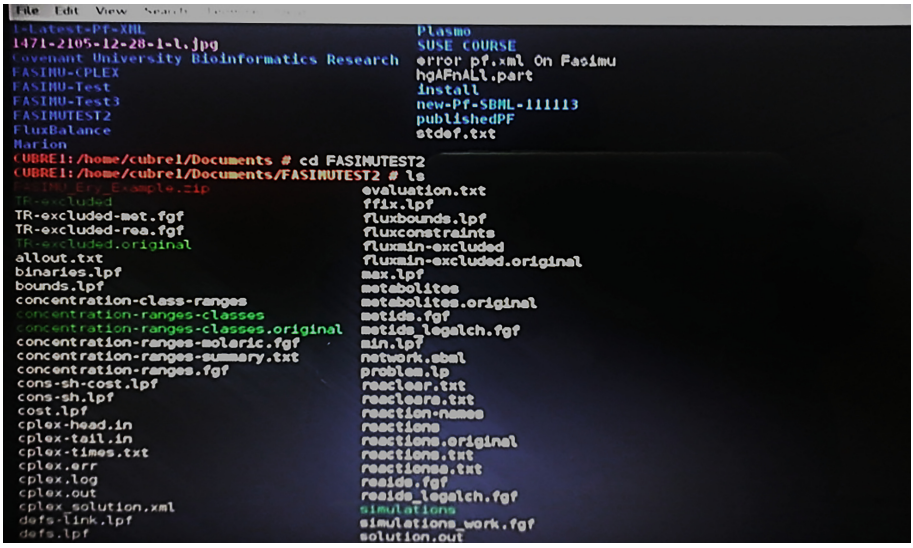


Fig. 2. Screenshot of the Output of the demonstration FASIMU Functions on Human Erythrocyte Model

3.4 Validating and Analyzing the Network

Constraints were defined to make sure essential biomass precursors such as phospholipids are generated, which are required for the parasite reproduction (Huthmacher et al. 2010). In this case;

i. Mass balance constraints: metabolite production and consumption rates are equal
i. Boundary constraints: that limited nutrient uptake/excretion
ii. Internal constraints: that limited the flux through reactions within the organism, transport reactions were introduced only where it is needed.
iii. Thermodynamically reaction constraints: irreversibility of reactions

More constraints were added to compel the network to generate a set of target metabolites essential for the growth of the organism. Constraints were also included to reduce the amount of active reactions whose survival is not sustained by genome annotations. This was important as data obtain from the BioCyc database include reactions exclusive of any associated gene and are present in *P.falciparium.*

Some of the constraints involved were Constraints −Glc_ext … −8 <= Glc_ext <= 8 interpreted as the glucose exchange flux between (−8,8), not (−8,0). Constraints stddef … %O2_ext is interpreted as Oxygen uptake allowed in the file stddef.txt. FASIMU equilibrium constants were used as weights for the backward fluxes in the flux minimization and to implement the thermodynamic feasibility constraint. The Gibb's free energies was not given directly, so we computed the equilibrium constants with this formula

$$K_{eq} = e^{-\frac{\Delta G_r^0}{RT}} \tag{1}$$

Where
R - the universal gas constant
T - is the absolute temperature (FASIMU model has a fixed temperature)
eq - *Equilibrium Constant*
Gr - Gibb's free energies

Solution space is more restricted as a result of Constraints gotten from thermodynamics and cellular environment conditions. More constraints were added to compel the network to generate a set of target metabolites essential for the growth of the organism.

3.5 Defining Objective Functions for the Analysis

Mathematically, an 'objective function' is used to quantitatively define how much each reaction contributes to the phenotype. In flux balance analysis, these linear equations are solved using linear programming. FBA maximize or minimize the objective function.

The Objective function defined in FBA analysis of the *plasmodium falciparum*

i. *Minimize ATP production*: This objective is stated to determine conditions of optimal metabolic energy efficiency.
ii. *Minimize nutrient uptake*: is used to determine the conditions under which the cell will perform its metabolic functions while consuming the minimum amount of available nutrients.
iii. *Maximize metabolite production*: to determine the biochemical production capabilities of *plasmodium falciparum.*

- FASIMU allowed flexibility in the description of objectives and constraints; so sequence of execution was calculated, varying the objectives and constraints of the automatically generated results. More results are available at the appendices section. The ensuing set of biochemical reactions and transport processes was modified to achieve a consistent network verified by flux balance simulations to implement metabolic processes. The metabolic capabilities of the cellular system of the parasite was investigate, this allowed us to evaluate unknown fluxes in metabolic networks according to the cellular constraints. The simulation was successful; the essential metabolites that must be exchange over the system boundary was successfully extracted and these set of target metabolites was established from literature to be indispensable to the growth of the organism, *Plasmodium falciparum.* Hence the network is valid.
- **less allout.txt** shows the list of the essential metabolites that must be exchanged over the system boundary of the parasite.

The allout.txt output is available at the Appendix IX section of the project (Fig. 3).

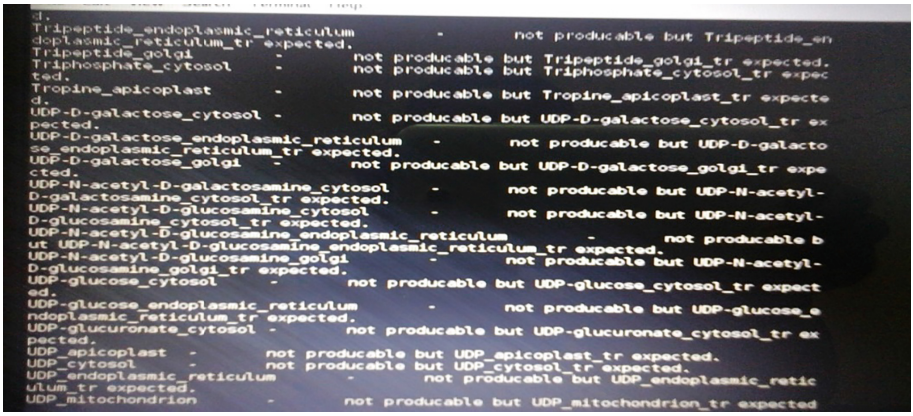
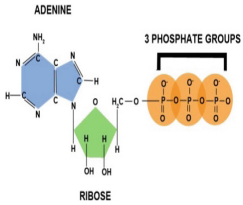
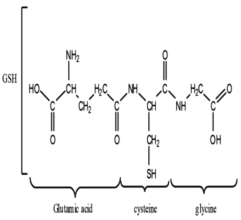
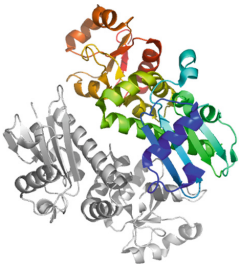


Fig. 3. Screenshot of the Output of the demonstration FASIMU *Pf*

The results provided us with the list of some of the essential metabolites that should be exchanged over the system boundary of a valid *Plasmodium falciparum* network. Table 1 below.

Table 1. Results obtained from the analysis of the *Pf* network, listing the essential metabolites/compound names, structure, compartments where they belong and their known function.

Compound	Compound-Name	Compound Structure	Compartment	Functions
ATP_regeneration	Adenosine Triphosphate		Cytosol	ATP plays a central role in energy exchanges in *plasmodiumfalciparu,* serves as the main donor of free energy. They are present in the glycolytic pathway. (Hutmatcher et al., 2010)
GSH	Glutathione		Cytosol	*Plasmodium falciparum* exhibits an intense glutathione metabolism. Glutathione plays a role not only in antioxidative defence and in maintaining the reducing environment of the cytosol. (Becker et al., 2003)
Hsp90 protein	Heat shock protein 90		Cytosol, Mitochondrion	The Hsp90 protein parasite Plasmodium falciparum is critical for the survival; it is proven that the anti-Hsp90 drug geldanamycin is toxic to P. falciparum growth. (Lelièvre et al., 2010)

4 Conclusion

This work presents an FBA analysis of the compartmented metabolic network of *Plasmodium falciparum* System Biology Mark-up Language (SBML) format and FASIMU tools; this provided us with information on transporters that transferred metabolites between the different cellular compartments of the organism. This serves as input or a promising starting point for further drug targets and development and target discovery. It is a functional description of the network in terms of essential metabolites that must be exchanged over the system boundary of *Plasmodium falciparum* network. The result of the compartmented metabolic network in SBML format validated by flux

balance approach generated a set of target metabolites established from literature to be essential to the growth of this organism and this presents a promising point (input) for further drug target and development.

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