*In-silico*Validation of the Essentiality of Reactions in *Plasmodium Falciparum* Metabolic Network

Jelili Oyelade, Efosa Uwoghiren,Itunuoluwa Isewon, Femi Aromolaran & Olufunke Oladipupo

***Abstract*—*Plasmodium falciparum* are instrumental in causing malaria and have developed complex life cycles, thus, it becomes very possible for the malaria parasite to take advantage of the uniqueness of its pathways to design therapeutic strategies. Despite the colossal efforts put in to fight malaria the disease still affects up to over 200 million people every year amongst which close to half a million dies. The treatment of the disease, could be done successfully if the essential enzymes of this parasite is precisely targeted. Nevertheless, the development of the parasite to resisting existing drugs now makes it a core responsibility to discover novel drugs. In this study, existing essential reactions from different literature are considered and evaluated to determine reactions that are common in all literature and evaluated to determine their essentiality level. This study evaluates essential reactions that has been predicted in literature computationally and validates its essentiality based on the reconstructed metabolic network and identifies 10 essential reactions that are common to all existing literature of which all this reaction were validated to be essential by our method. This study has established a simple novel *in-silico* method that validates predicted essential reactions in a metabolic network which makes validation of predicted anti-malarial drug target cheaper, easier and faster. This study *in-silico* model serves as a valuable tool for validation of *Plasmodium falciparum* metabolic states under various perturbations.**

***Index Terms*— drug targets, essential reactions, malaria, metabolic network, plasmodium falciparum**

I. Introduction

A. The Overview of Plasmodium falciparum

NOVELanti-malaria cures are in immediate need to combat the drug-resistant malaria parasite [1], [2]. The metabolism of *Plasmodium falciparum (P. f.)* in cells that are infected would be quite a potential source of targets for novel drugs but it's rather complex. *In-silico* methods can handle and take care of this complexity and gives room for integrative analyses of the cell metabolism [3]. The dominance of malaria in resistance to all identified anti-malarial drugs in current circulation has given rise to the increase of anti-malarial drug discovery research [4]–[6]. *Plasmodium falciparum* are instrumental in causing malaria and have developed complex life cycles [7], [8]. Malaria, which is a disease that threatens life, is initiated by 5 different species of the *Plasmodium* genre: *P. f., Plasmodium ovale, Plasmodium malariae*and *Plasmodium vivax Plasmodium knowlesi*, of which the dangerous of them all are *P. f.* and *Plasmodium vivax*[4], [6].The feminine mosquito of the *Anopheles* genre is responsible for all the transference of malaria from one patient to another [1], [9], [10]. Despite the colossal efforts put in to fight malaria the disease still affects up to over 200 million people every year amongst which close to half a million dies [2], [4], [9]–[11]. The comprehensive *Plasmodium falciparum* lifecycle comprises of 3 important developmental stages: the mosquito stage, the liver stage, and the blood stage [12]. The malaria parasite metabolic pathways are in a number of ways different from that of a human being because of the uniqueness in the malaria parasite life-cycle, thus it becomes very possible for the malaria parasite to take advantage of the uniqueness of its pathways to design therapeutic strategies [13]–[15] which helps the parasite to resisting existing drugs and makes it a core responsibility to discover new drugs [1], [2]. The treatment of the parasite could be done effectively if the essential enzymes of this parasite is specifically targeted. Metabolic pathways are chains of connected enzymatic reactions that takes place inside a cell [16], [17], a metabolic pathway help form a different chemical compound by modifying principal chemical which is then passed on to start an alternative pathway or used up or kept by the cell [18]. The representation of a metabolic pathway is generally a graphical network of chemical reactions [18]. It is essential to identify the components of a metabolic pathway which are (reactants, enzymes, products and reactions) and their relations in order to absolutely describe a metabolic pathway. The stoichiometry represents the quantifiable relations amid reactants and products in a balanced chemical reaction. Combinations of information from different sources such as, genomics, network analysis and simulation, biochemistry, etc. are necessary in the study of a metabolic pathway [19].

B. Metabolic Network

More than one metabolic pathway which consists of a chain of reactions that contribute in the synthesis or degradation of the same metabolite makes up a metabolic network. Diverse data sources guides the genome-scale reconstruction of metabolic networks [2]. [20], regarded metabolic networks as a flow of substance from side to side of biochemical intermediates that are converted into each other [20]. A metabolic network is simply a graphical representation of metabolism [21]. Metabolic networks are characterized by a flow of substance through biochemical intermediates that are interconverted into each other [20]. Metabolic Networks are useful tools for deepening our understanding of metabolism and the role of genes through

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the evaluation of gene essentiality [22].

C. Essentiality of a Reaction in a Metabolic Network

Identifying essential reactions in a network allows the identification of potential drug targets [23], [24]. Essential reactions are widely recognized as ideal drug target candidates since their deletion can lead to compromising the integrity of the network [24], [25]. Essential reactions are those reactions of an organism that are thought to be critical for its survival. These essential reactions are very important because without them, the network cannot function [26]. The prediction of experimental essential genes have need for substantial time and resources, even for organisms that are well-studied, and they are not at all times practical [2], [24], [27], while the prediction of computational essential reactions are faster and quite less expensive and they have the capability to decrease the search space for new targets for drugs in a metabolic network [2], [24].

D. Methods to detect Essential reactions

Thermodynamics-based Flux Analysis (TFA)

It's a variant of metabolic flux analysis presented with the capacity of producing thermodynamically feasible flux and metabolite movement profiles on a genome scale [28], [29]. TMFA includes the utilization of an arrangement of straight thermodynamic constraints notwithstanding the mass balance limitations ordinarily utilized as a part of MFA [3], [29]. TMFA is used to analyse the thermodynamically possible extents for the free energy of fluxes and the Gibbs, of the actions and reactions the metabolites in the metabolic genome-scale network [3], [28].

Flux balance analysis (FBA)

It's a computational method to gain insight into the metabolic behaviour and capabilities of a cell. [2], [9]. It is a widely used and well-established method to assess the essentiality of genes for an organism. However, one of the puzzling failures of FBA techniques has been precisely the lack of even moderate correlation between predicted gene dispensability and evolutionary rate [30], [31]. FBA suffers from incomplete annotation of the proteins in a genome [32]. FBA suffers greatly in defining biologically relevant objective function [32]. FBA requires the information about the stoichiometry of the reaction pathway [9].

Extreme pathways (ExPas)

ExPas are metabolic pathways that are defined mathematically calculated from a metabolic network that has been reconstructed [33]. ExPas of a particular metabolic network are set of vectors that are irreducible and describes the root of the null-space of the network's stoichiometric matrix [34]–[36].

Metabolic Flux Analysis (MFA)

By using metabolic flux analysis [37], [38], any change made in the metabolic pathway fluxes are measured that is the outcome from genetic or environmental interventions. Information like this gives more insights into how the metabolic pathways are being regulated and could likely suggest novel targets for added metabolic engineering of the strains [39]. One major advantage of Metabolic flux analysis is that it can be easily applied making it easily accessible to lots of researchers, since it entails only simple linear algebra and depend on moderately robust measurements of extracellular metabolites [39]. A major setback of Metabolic flux analysis for a lot of biological systems is however, that the amount of constraint is often not sufficient to observe all essential intracellular metabolic pathways [39], [40].

Load point and Choke point Analysis

Choke points can be defined as the enzymes that exclusively produces or/and consumes a particular metabolite and they play an essential role in a specific reaction. The choke points are ordered by the amount or number of k-shortest paths that passes through them and the load point on it. When a choke point in an organism is absent, the organism can rarely survive [41], [42]. It is proposed and suggested that 'load points' has the capabilities to accompaniment other already existing technique of metabolic network analysis [43]. It is also proposed that choke point enzymes are very essential in the organism and are therefore possible targets for drugs [44]. Choke point analysis helps in examining the reliability between assumptions about the regulation of the biochemical pathway and the way it's organized and of its interdependencies with other processes and experimental data [41].

E. Reaction Deletion / Perturbation studies

Perturbation in a pathway occurs by interrupting the signal flow in a network which gives insight into both their structure and their downstream targets. First, with the interruption at a certain node in the pathway, the signal cannot be transmitted further. Second, each node in the pathway may have its own (direct or indirect) contribution to the perturbation effects, such as reaction expression changes [45].

In this study, existing essential reactions from different literature are considered and evaluated to determine reactions that are common in all literature and evaluated to determine their essentiality level. This study determines the essentiality of the different reactions from existing studies and validate their essentiality. Therefore a list of indispensable reactions in the *Plasmodium falciparum* metabolic network was identified and proposed as potential drug target for *Plasmodium falciparum.*

II. Materials and Method

A. Reconstruction of the metabolic network

In this study the resource that was considered for the reconstruction of the metabolic network, is the genome-scale metabolic dataset of the 3D7 strain of *Plasmodium falciparum* which was extracted from the BIOCYC flat file database version 19.5 [46] because of its comprehensiveness and robustness of which the dataset contains 894 metabolic reactions and these reactions were catalyzed by a total number of 710 enzymes. The BIOCYC identifiers were chosen for this study as the generally accepted means of identification. In this study currency metabolites of the 24 currency metabolites outlined by [47] were removed from the reconstructed genome-scale metabolic dataset.

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B. *The Algorithm*

In the network a reaction is knocked out to determine the dependent reaction on the knocked out reaction. The procedure for determining which reaction is connected to the knocked out is outlined in the following steps below:

1. A reaction in the network is selected
2. The selected reaction is being knocked out
3. For every selected reaction that's knocked out, it also knocks out every reaction in the network that its reactant is the product of the selected reaction except the reactant of the reaction to be knocked out by the selected knocked out reaction is a product of any other reaction in the network
4. For every selected reaction that's knocked out, it also knocks out every reaction in the network that its product is the reactant of the selected reaction except the product of the reaction to be knocked out by the selected knocked out reaction is a reactant of any other reaction in the network
5. Step 3 and 4 is repeated until all knocked out reaction for selected knocked out reaction is gotten

All above steps are performed for all reaction in the network enabling this study to predict the essentiality of all reaction in the network and exporting them in hierarchical order. In this study reconstructed network, we determined the essentiality of every reaction in the network and validated the essentiality of previous proposed reactions in literature. The essentiality of all reactions are saved in a .txt file for easy access. The formula for determining the essentiality of every reaction in the network is outlined in the following steps below:

$$E(r) = \frac{\sum k(ri)}{\sum N(ri)}\%$$

C. *Existing essential reactions in literature*

A detailed list of essential reactions in *Plasmodium falciparum* that have already been predicted in several literature using computation methods were considered.

III. Results

Computationally predicted Essential reactions from 5 different literatures were considered for this study, this literatures were compared and 10 reactions that were common to over 80% of all literature considered in this study were identified and validated to be essential by our method. We evaluated all 10 reactions by gold standard consisting of reactions that are targets of already accepted and tested drugs and they were all confirmed to be essential thereby confirming our method as a valuable method for the validation of drug computationally predicted drug target. The list of the 10 reactions are given in TableI, respectively and figure 4 & 5 shows the essentiality of the reactions in the metabolic network used in this study.

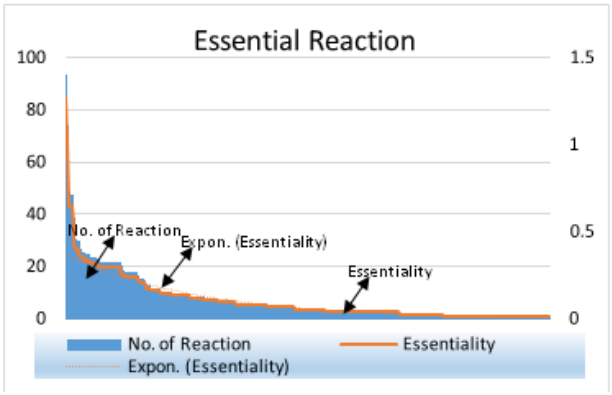

Fig. 1. Graph to show No. of reactions considered and essentiality of reactions.

Table 1: RESULTS USING OUR METHOD TO VALIDATE EXISTING POTENTIAL DRUG TARGET PREDICTED COMPUTATIONALLY

S/N	Reaction	EC Number	Yeh[44]	Fatumo[48]	Huthmacher[2]	Plata [1]	Bazzani[49]	This Study
1	DIHYDROFOLATEREDUCT-RXN	1.5.1.3	Yes	Yes	Yes	Yes	Yes	Yes
2	THYMIDYLATESYN-RXN	2.1.1.45	Yes	Yes	Yes	Yes	Yes	Yes
3	H2PTEROATESYNTH-RXN	2.5.1.15	Yes	Yes	Yes	Yes	No	Yes
4	IMP-DEHYDROG-RXN	1.1.1.205	Yes	Yes	Yes	No	Yes	Yes
5	SUPEROX-DISMUT-RXN	1.15.1.1	Yes	Yes	Yes	No	Yes	Yes
6	ADENOSYLHOMOCYSTEINASE-RXN	3.3.11	Yes	Yes	Yes	No	Yes	Yes
7	OROTPDECARB-RXN	4.1.1.23	Yes	Yes	Yes	Yes	Yes	Yes
8	SAMDECARB-RXN	4.1.1.50	Yes	Yes	Yes	Yes	Yes	Yes
9	PORPHOBILSYNTH-RXN	4.2.1.24	Yes	Yes	Yes	Yes	Yes	Yes
10	GLUTCYSLIG-RXN	6.3.2.2	Yes	Yes	Yes	No	Yes	Yes

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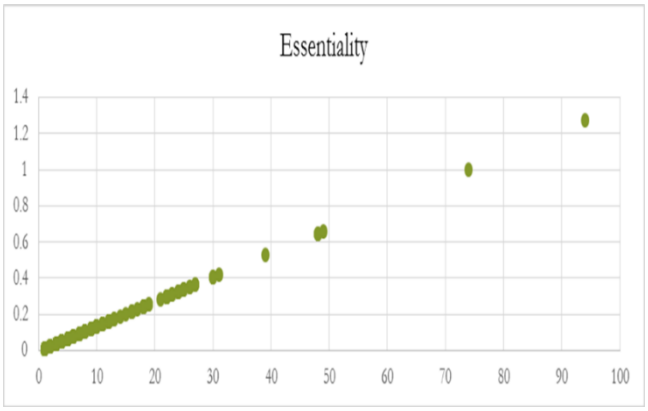

Fig. 2. Graph to represent essentiality of reactions

IV. Discussion

This study evaluates essential reactions that has been predicted in literature computationally and validates its essentiality based on the reconstructed metabolic network and identifies 10 essential reactions that are common to all existing literature of which all this reaction were validated to be essential by our method. This study has established a simple novel *in-silico* method that validates predicted essential reactions in a metabolic network which makes validation of predicted anti-malarial drug target cheaper, easier and faster. This study *in-silico* model serves as a valuable tool for validation of *Plasmodium falciparum* metabolic states under various perturbations. This study contribution is important because the genome-scale model can be used to investigate and predict genetic perturbations from a network level perspective.

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References

[1] G. Plata, T. Hsiao, K. L. Olszewski, M. Llinás, and D. Vitkup, "Reconstruction and flux- balance analysis of the Plasmodium falciparum metabolic network," *Mol. Syst. Biol.*, vol. 6, no. 1, p. 408, 2010.

[2] C. Huthmacher, A. Hoppe, S. Bulik, and H.-G. Holzhütter, "Antimalarial drug targets in Plasmodium falciparum predicted by stage-specific metabolic network analysis," *BMC Syst. Biol.*, vol. 4, no. 1, p. 1, 2010.

[3] A. Chiappino-Pepe, S. Tymoshenko, M. Ataman, D. Soldati-Favre, V. Hatzimanikatis, and A. Schaffer, "Bioenergetics-based modeling of Plasmodium falciparum metabolism reveals its essential genes, nutritional requirements, and thermodynamic bottlenecks," *PLOS Comput. Biol.*, vol. 13, no. 3, p. e1005397, Mar. 2017.

[4] C. Ekenna, S. Fatumo, and E. Adebiyi, "In-silico evaluation of malaria drug targets," *Int. J.*, 2010.

[5] A. Golldack, B. Henke, B. Bergmann, and M. Wiechert, "Substrate-analogous inhibitors exert antimalarial action by targeting the Plasmodium lactate transporter PfFNT at nanomolar scale," *PLoS*, 2017.

[6] K. Sahan, C. Pell, and F. Smithuis, "Community engagement and the social context of targeted malaria treatment: a qualitative study in Kayin (Karen) State, Myanmar," *Malaria*, 2017.

[7] K. Modrzynska *et al.*, "A Knockout Screen of ApiAP2 Genes Reveals Networks of Interacting Transcriptional Regulators Controlling the Plasmodium Life Cycle," *Cell Host Microbe*, vol. 21, no. 1, pp. 11–22, 2017.

[8] K. Koussis, E. Goulielmaki, and A. Chalari, "Targeted Deletion of a Plasmodium Site-2 Protease Impairs Life Cycle Progression in the Mammalian Host," *PLoS One*, 2017.

[9] N. Dholakia, P. Dhandhukia, and N. Roy, "Screening of potential targets in Plasmodium falciparum using stage-specific metabolic network analysis," *Mol. Divers.*, 2015.

[10] J. Teahton, "In vitro and in vivo anti-plasmodial activities of senna occidentalis roots extracts against plasmodium falciparum and plasmodium berghei," 2016.

[11] "WHO | World Malaria Report 2016," *WHO*, 2016.

[12] Z. Bozdech, M. Llinás, B. L. Pulliam, E. D. Wong, J. Zhu, and J. L. DeRisi, "The Transcriptome of the Intraerythrocytic Developmental Cycle of Plasmodium falciparum," *PLoS Biol.*, vol. 1, no. 1, p. e5, Aug. 2003.

[13] K. Nakatani, H. Ishikawa, S. Aono, and Y. Mizutani, "Identification of Essential Histidine Residues Involved in Heme Binding and Hemozoin Formation in Heme Detoxification Protein from Plasmodium falciparum," *Sci. Rep.*, vol. 4, no. 1, p. 6137, May 2015.

[14] E. Hempelmann, "Hemozoin Biocrystallization in Plasmodium falciparum and the antimalarial activity of crystallization inhibitors," *Parasitol. Res.*, vol. 100, no. 4, pp. 671–676, Jan. 2007.

[15] N. Lang-Unnasch and A. D. Murphy, "METABOLIC CHANGES OF THE MALARIA PARASITE DURING THE TRANSITION FROM THE HUMAN TO THE MOSQUITO HOST," *Annu. Rev. Microbiol.*, vol. 52, no. 1, pp. 561–590, Oct. 1998.

[16] M. Klapa and G. Stephanopoulos, "Metabolic flux analysis," *Bioreact. Eng.*, 2000.

[17] L. Popova-Zeugmann and M. Heiner, "Time Petri nets for modelling and analysis of biochemical networks," *Fundamenta*, 2005.

[18] A. Romano and T. Conway, "Evolution of carbohydrate metabolic pathways," *Res. Microbiol.*, 1996.

[19] P. Baldan, N. Cocco, A. Marin, and M. Simeoni, "Petri nets for modelling metabolic pathways: a survey," *Nat. Comput.*, 2010.

[20] W. Marwan, A. Wagler, and R. Weismantel, "Petri nets as a framework for the reconstruction and analysis of signal transduction pathways and regulatory networks," *Nat. Comput.*, vol. 10, no. 2, pp. 639–654, Jun. 2011.

[21] V. Lacroix, L. Cottret, and P. Thébault, "An introduction to metabolic networks and their structural analysis," *IEEE/ACM Trans.*, 2008.

[22] H. Song, R. McClure, H. Bernstein, and C. Overall, "Integrated in silico analyses of regulatory and metabolic networks of Synechococcus sp. PCC 7002 reveal relationships between gene centrality and," *Life*, 2015.

[23] K. Plaimas, R. Eils, and R. König, "Identifying essential genes in bacterial metabolic networks with machine learning methods," *BMC Syst. Biol.*, vol. 4, no. 1, p. 56, 2010.

[24] C. Qin, Y. Sun, and Y. Dong, "A New Method for Identifying Essential Proteins Based on Network Topology Properties and Protein Complexes," *PLoS One*, vol. 11, no. 8, p. e0161042, Aug. 2016.

[25] M. JING, "Metabolic Network Based Gene Essentiality Analysis," 2012.

[26] O. Oyelade, I. Isewon, S. Mba-Kalu, and S. Rotimi, "SIMULATION AND ANALYSIS OF PENTOSE PHOSPHATE PATHWAY IN PLASMODIUM FALCIPARUM USING COLORED PETRI NETS MODEL," *J. Comput. Sci. ITS Appl.*, vol. 22, no. 5, 2015.

[27] X. Zhang, M. Acencio, and N. Lemke, "Predicting essential genes and proteins based on machine learning and network topological features: a comprehensive review," *Front. Physiol.*, 2016.

[28] C. S. Henry, L. J. Broadbelt, and V. Hatzimanikatis, "Thermodynamics-Based Metabolic Flux Analysis," *Biophys. J.*, vol. 92, no. 5, pp. 1792–1805, Mar. 2007.

[29] S. Garg, L. Yang, and R. Mahadevan, "Thermodynamic analysis of regulation in metabolic networks using constraint-based modeling," *BMC Res. Notes*, vol. 3, no. 1, p. 125, 2010.

[30] B. Papp, C. Pal, and L. Hurst, "Metabolic network analysis of the causes and evolution of enzyme dispensability in yeast," *Nature*, 2004.

[31] C. Jacobs, L. Lambourne, Y. Xia, and D. Segrè, "Upon Accounting for the Impact of Isoenzyme Loss, Gene Deletion Costs Anticorrelate with Their Evolutionary Rates," *PLoS One*, 2017.

[32] K. Raman and N. Chandra, "Flux balance analysis of biological systems: applications and challenges," *Brief. Bioinform.*, 2009.

[33] N. Price, J. Papin, and B. Palsson, "Determination of redundancy and systems properties of the metabolic network of Helicobacter pylori using genome-scale extreme pathway analysis," *Genome Res.*, 2002.

[34] M. Yeung, I. Thiele, and B. Palsson, "Estimation of the number of extreme pathways for metabolic networks," *BMC*, 2007.

[35] C. H. Schilling, S. Schuster, B. O. Palsson, and R. Heinrich, "Metabolic Pathway Analysis: Basic Concepts and Scientific Applications in the Post-genomic Era," *Biotechnol. Prog.*, vol. 15, no. 3, pp. 296–303, Jun. 1999.

[36] N. Price, J. Reed, J. Papin, and S. Wiback, "Network-based analysis

of metabolic regulation in the human red blood cell," *J. Theor.*, 2003.
[37] G. Stephanopoulos, A. Aristidou, and J. Nielsen, *Metabolic engineering: principles and methodologies*. 1998.
[38] J. Heijnen, W. Van Gulik, and H. Shimizu, "Metabolic flux control analysis of branch points: an improved approach to obtain flux control coefficients from large perturbation data," *Metabolic*, 2004.
[39] M. R. Antoniewicz, "Methods and advances in metabolic flux analysis: a mini-review," *J. Ind. Microbiol. Biotechnol.*, vol. 42, no. 3, pp. 317–325, Mar. 2015.
[40] L.-E. Quek, S. Dietmair, J. O. Krömer, and L. K. Nielsen, "Metabolic flux analysis in mammalian cell culture," *Metab. Eng.*, vol. 12, no. 2, pp. 161–171, 2010.
[41] S. Mehta and S. Tagore, "Functional module analysis in metabolomics: chokes," *Adv. Comput. Res.* , vol. 1, no. 1, pp. 1–4, 2009.
[42] T. Kim, H. Kim, and S. Lee, "Metabolite-centric approaches for the discovery of antibacterials using genome-scale metabolic networks," *Metab. Eng.*, pp. 105–111, 2010.
[43] S. Rahman and D. Schomburg, "Observing local and global properties of metabolic pathways:'load points' and 'choke points' in the metabolic networks," *Bioinformatics*, vol. 22, no. 14, pp. 1767–1774, 2006.
[44] I. Yeh, T. Hanekamp, S. Tsoka, P. D. Karp, and R. B. Altman, "Computational analysis of Plasmodium falciparum metabolism: organizing genomic information to facilitate drug discovery," *Genome Res.*, vol. 14, no. 5, pp. 917–924, 2004.
[45] E. Szczurek and N. Beerenwinkel, "Linear effects models of signaling pathways from combinatorial perturbation data," *Bioinformatics*, 2016.
[46] R. Caspi *et al.*, "The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases," *Nucleic Acids Res.*, vol. 44, no. D1, pp. D471–D480, Jan. 2016.
[47] T. Kim *et al.*, "Patterns of metabolite changes identified from large-scale gene perturbations in Arabidopsis using a genome-scale metabolic network.," *Plant Physiol.*, vol. 167, no. 4, pp. 1685–98, Apr. 2015.
[48] S. Fatumo *et al.*, "Estimating novel potential drug targets of Plasmodium falciparum by analysing the metabolic network of knock-out strains in silico," *Infect. Genet. Evol.*, vol. 9, no. 3, pp. 351–358, 2009.
[49] S. Bazzani, A. Hoppe, and H.-G. Holz, "Network-based assessment of the selectivity of metabolic drug targets in Plasmodium falciparum with respect to human liver metabolism," *BMC Syst. Biol.*, vol. 6, 2012.

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