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IDENTIFYING MULTIPLE DRUG TARGETS FOR HEME DETOXIFICATION BY USING PLASMODIUM METABOLISM MODEL IN RED BLOOD CELL

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A Thesis Submitted in Partial Fulfillment of the Requirements
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การกำจัดสารพิษเป็นกระบวนการหนึ่งที่จำเป็นของสิ่งมีชีวิตเพื่อที่จะนำสารพิษออกจากเซลล์ พลาสโมเดียมฟาลซิพารัมซึ่งเป็นปรสิตที่ก่อให้เกิดโรคมาลาเรียในวงจรชีวิตทีอาศัยอยู่ในเม็ดเลือดแดง จะทำการย่อยสลายฮีโมโกลบินแล้วทำการสร้างฮีโมโซอินเพื่อลดฮีมอิสระซึ่งมีความเป็นพิษต่อตัวปรสิตเอง ดังนั้น กระบวนการสร้างฮีโมโซอินในตัวปรสิตซึ่งจำเป็นต่อการรอดชีวิตจึงกลายเป็นเป้าหมายในการพัฒนายารักษามาลาเรีย ซึ่งในปัจจุบัน การประมวลผลด้วยคอมพิวเตอร์เป็นเครื่องมือสำคัญที่ช่วยลดเวลาและค่าใช้จ่ายในกระบวนการพัฒนายา งานชิ้นนี้ ทำการสร้างแบบจำลองเมตาโบลิกแบบข้อจำกัดขึ้นใหม่จากแบบจำลองของพลาสโมเดียมฟาซิพาลัมและเม็ดเลือดแดงของมนุษย์ เพื่อที่จะทำการระบุเป้าหมายของยา เราได้ใช้การวิเคราะห์ฟลักซ์สมดุลกับแบบจำลองนี้ในสองเป้าหมาย หนึ่งคือสภาวะก่อโรคในมนุษย์ อีกหนึ่งคือสภาวะที่ได้รับการรักษา ปฏิกิริยาเคมีในแบบจำลองที่มีการเปลี่ยนค่าฟลักซ์เป็นศูนย์ จะถูกตั้งสมมติฐานว่าเป้าหมายขั้นต้นซึ่งได้รับผลจากการรักษาด้วยยา จำนวนของเป้าหมายขั้นต้นนี้ถูกลดจำนวนลง ด้วยการทดสอบทุกๆ การผสมกันของเป้าหมายเพื่อหากลุ่มของเป้าหมายที่มีศักยภาพที่ดีที่สุด ในท้ายที่สุด เราได้ฮีมลิเกสเป็นหนึ่งเป้าหมายที่มีศักยภาพมากที่สุด ร่วมกับไนไตรทรีดักเทส และ ไอโนซิโทลไตรฟอสเฟสซินเทส เป็นเป้าหมายของยาที่เหมาะสม โดยสรุป วิธีการนี้ ได้แสดงให้เห็นหนทางหนึ่งที่มีประสิทธิผลในการระบุเป้าหมายของยา ซึ่งมีประโยชน์ในกระบวนการพัฒนายา

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SUTHAT PHAIPHINIT: IDENTIFYING MULTIPLE DRUG TARGETS FOR HEME DETOXIFICATION BY USING PLASMODIUM METABOLISM MODEL IN RED BLOOD CELL. ADVISOR: KITIPORN PLAIMAS, Ph.D., CO-ADVISOR: PROF. CHIDCHANOK LURSINSAP, Ph.D., 61 pp.

Detoxification is an essential process in a living organism for removing toxic substances from the cell. In red blood cell cycle, Plasmodium falciparum, the most devastating malaria parasite, digests hemoglobin from blood and forms hemozoin to eliminate free heme which is toxic to the parasite. Therefore, the hemozoin formation is essential to the survival of the parasite and become an attractive target for developing drugs. Nowadays, computational approaches have been a great tool to reduce time and cost in drug development process. In this work, we reconstructed constraint-based metabolic model of P. falciparum and human red blood cell. To identify drug target we apply flux balance analysis to this model in two objectives. One is for pathological stage in human, another one is for medication stage. The reactions which its flux value are change to zero in medication stage are assumed to be preliminary potential targets due to inhibited by drug treatment. The number of preliminary potential targets were reduced by test all combination to search the best set of potential drug targets to inhibit heme detoxification in P. falciparum. Finally, we obtained heme ligase is one potential drug targets and combination with nitrite reductase[NAD(P)H] and inositol-3-phosphate synthase for optimal drug targets. In conclusion, this method shown an effective way to identify drug targets which is useful in drug development process.

Department:	Mathematics and Computer Science	Student's Signature	.....
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Chapter 1
Introduction

Background

Malaria has been widespread in tropical zone and is caused by Plasmodium parasite, especially the species: *Plasmodium falciparum* and *Plasmodium vivax*. In the past, we used quinine from cinchona for treatment. Later, the synthesis of antimalarial drug was developed through literature and experiment both *in vitro* and *in vivo*. Recently, the drug development deploys computer approach (*in silico*) to reduce cost and time.

Malaria parasite's life cycle exists both in mosquito and human. In mosquito, a parasite lives in salivary glands but it does not cause any harm to the host. When a mosquito bites human, parasites are transported to the liver, called the hepatic stage. After that they release to circulatory system, called the erythrocytic stage. During the erythrocytic stage, the parasite evades red blood cells to consume some essential metabolites; especially, hemoglobin for its reproduction. The parasites digest hemoglobin and release high quantities of free heme which is toxic to the cells. Thus, the parasites detoxicate the free heme by converting it into hemozoin, so-called the malaria pigment. Therefore, the hemozoin formation is an attractive target for developing drugs because it is essential for the parasite survival. Several antimalarial drugs, such as chloroquine and mefloquine [1], are found to kill malaria parasites by inhibiting hemozoin production.

However, according to the WHO report [1], there is still an urgent need for the design of a new generation of malaria drugs targeting multiple essential proteins [1, 2]. By mimicking this detoxification process of the parasite, we employed a large-scale metabolic model of Plasmodium falciparum, including the hemozoin formation, and proposed the promising combinations of drug targets whose inhibition may give the same effect and solve drug resistance problem in the malaria treatment by targeting the heme detoxification.

Literatures review

A way to find drug targets is based on metabolic network which is able to be analyzed by linear programming called Flux Balance Analysis. The flux balance analysis method has been introduced and shown an example to analyze quantity of flux distribution at steady state of both the normal strain and the modified strain in the network model [3]. Smallbone and Simeonidis [4] analyzed the flux balance analysis method in geometry perspective and visualized it. An a modification method of flux balance analysis, OptKnock [5], used programming technique namely bi-level programming to identify genes as targets of the objective strain. The first level (inner problem) optimized cellular objectives and the second level (outer problem) optimized bioengineering objective. OptCom [6] is another modification method of flux balance analysis for microbial communities. This method models each species as inner problem of bi-level programming and makes inter-flow in a community into the community constraint. The outer problem optimizes the objective of the community. The combined method of OptKnock and Minimization of Metabolic Adjustment (MOMA) was proposed in 2013 [7]. Other approaches of flux balance analysis have been continuously introduced in the later years. To avoid kinetic parameters but still keeps physical constraint in the model, Energy Balance Analysis (EBA) [8] has been proposed energy constraint from the laws of thermodynamics in the simulation which contrasts the flux optimization. Combining Energy balance analysis with Flux balance analysis [9] results in non-linear model and have been solved by a sequential quadratic programming algorithm. Another thermodynamic-based model, metabolic flux analysis (TMFA) [10] added linear thermodynamic constraints to flux constraints in the model. A statistical approach was also proposed in Bayesian flux balance analysis [11] in an example two-compartment model. The Bayes estimator and likelihood function are used to estimate fluxes in the system. The heuristics algorithm was also applied to optimize the gene knockout to obtain the desired phenotypes. Evolution programming demonstrated the capability to identify genes for improvement the yeast's fermentation [12]. A well-known evolution algorithm, Simulated Annealing (SA) was used and compared with Evolutionary Algorithms (EAs)

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in [13]. Flux Balance Analysis gave flux distribution at steady state which flux did not change in time, but the flux rate in continuous time was also computed and shown in [14-16]. Some computational methods were based on optimal control theory [17-20]. The original flux balance analysis has one objective but in some engineering situation that wants more objectives than one such as maximizing a metabolic flux and minimizing the number of knockout genes, it has been solved by adopted multi-objectives with flux balance based method [21-24].

To design a drug, a selected drug target [25] is one thing needed to be considered. In recent years, several computational approaches help to find the targets for the drug design and development. A multi-target drug is then being proposed as it can provide more effective in the treatment than a single-target drug [26], Later, the network-based approach was suggested to be used in the drug target identification and the drug design [27, 28]. Many computational methods were proposed, such as machine learning based method [29], minimization between healthy state and disease state [30-32] or metabolic network based methods [33, 34].

Based on graph techniques, choke-point analysis was used to find essential enzymes in *Plasmodium falciparum* metabolism model [35]. These enzymes have been proposed as potential drug targets to be inhibited when treating malaria in human. Later, this method was improved by Fatumo et al. [36] to reduce the number of potential drug targets by a new method to identify essential reactions in metabolic network. Ludin et al. [37] clustered *P. falciparum* proteome to predict essential proteins and then provided the druggability score for each of that proteins. By specific stage life-cycle, the stage of parasite was combined with optimization method. Huthmacher et al. [38] was proposed potential drug targets specific on blood-stage in the parasite life cycle.

This thesis proposed a novel *Plasmodium falciparum* metabolism model integrated with human red blood cell model and heme detoxification pathway. The new metabolism model was analyzed by flux balance analysis method, and also used to simulate the optimal number of drug targets which were found by heuristic optimization. Statistical methods were used to measure the performance of the

model. Based on the integration, the workflow analysis in identifying potential drug targets were developed and the set of potential drug targets in malaria was proposed.

Outlines

Chapter 2 provides details about methods in this thesis as shown below:

- Workflow in this thesis
- Mathematics of metabolic model and flux balance analysis
- Genetic Algorithm
- Performance measurement
- Pathway enrichment test

Chapter 3 shows output from the workflow described in chapter 2, the characteristics of the integrated model, and list of potential drug targets compared to those of the other literature finding. Finally, this thesis proposes an optimal set of drug targets in associating with those potential targets. Chapter 4 concludes the result of this thesis, limitation and the future work.

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Chapter 2
Methods

2.1 Workflow

To find new antimalarial drug targets by *in silico*, we applied an existing metabolic network of Plasmodium to develop a metabolic network which can represent the malaria parasite when living in human red blood cell. The network was used for exploring the metabolic processes in various conditions; especially, during the treatment condition by using a drug. By simulating a treated and an untreated condition of the metabolic network, we then identified a list of potential targets and further analyzed these targets for multiple targeting.

The main works of this task, thus, consists of three main steps as shown in Figure 2.1 and as the following.

Step 1 Create multi-cellular metabolic model

Plasmodium falciparum is a parasite living in human's liver and red blood cell, and anopheles (malaria mosquito). The life cycle is modeled by combining constraint-based metabolism model of *Plasmodium falciparum* [39] and human's red blood cell [40]. All exchanged metabolites of parasite with external environment are focused.

Step 2 Compare flux distribution between two states

To find potential reactions which could make *Plasmodium falciparum* die from severed toxicity in cell, the flux distribution of multi-cellular metabolic model is separated into two states. The first state is normal condition of the living parasite; this state the parasite can detoxify toxin caused by hemoglobin degradation after consuming it from the red blood cell. The second state, which has toxin from hemoglobin degradation, is mimicked an abnormal condition of the parasite. The difference of flux distribution between two states was assumed to be the effect from a drug which disturbed the parasite in the normal state.

Step 3 Find the optimal drug target

The reactions in the parasite which were disturbed from a drug in an abnormal state have potential to be drug targets. Especially the flux rates of reactions were changed to be zero. This step reduced the number of potential drug targets by heuristic optimization and tests the response of potential drug targets by Minimization of Metabolic Adjustment method to find targets which give high toxic flux rate.

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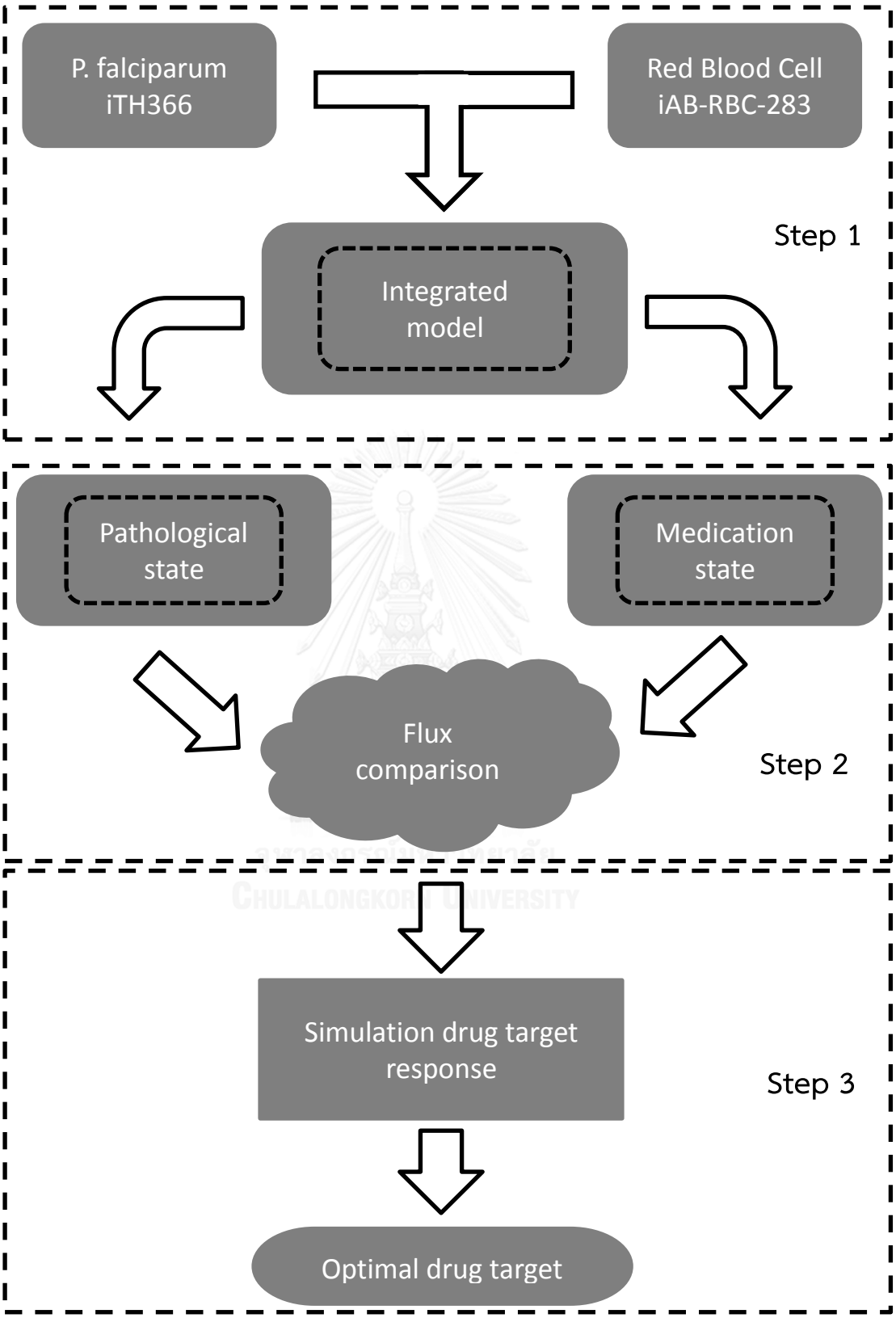

Figure 2.1 Workflow.

2.2 Constraint-based metabolic model

There are many kinds of models that describe any natural behavior. For chemical reactions in metabolism, one kind of the models to represent relations between metabolites is a dynamical model. For example, below is a set of four chemical reactions (R_1, R_2, R_3, and R_4) with their reaction equations of consuming or producing 7 substances (A, B, C, D, E, F and G).

R1: A + B -> C + D

R2: 2D -> E + G

R3: C + E -> F

R4: F + G -> 2H

From the above simple chemical reactions, by using conversion ratio of substance in reaction and defines decreasing (reactant) as minus, increasing (product) as plus, they can be modeled as follows

$$
\begin{aligned}
\frac{d[A]}{dt} &= -1v_1 \\
\frac{d[B]}{dt} &= -1v_1 \\
\frac{d[C]}{dt} &= +1v_1 \quad -1v_3 \\
\frac{d[D]}{dt} &= +1v_1 \quad -2v_2 \\
\frac{d[E]}{dt} &= \quad +1v_2 \quad -1v_3 \\
\frac{d[F]}{dt} &= \quad +1v_3 \quad -1v_4 \\
\frac{d[G]}{dt} &= \quad +1v_2 \quad -1v_4 \\
\frac{d[H]}{dt} &= \quad +2v_4
\end{aligned}
\qquad (1)
$$

where v_i is the flux rate of reaction i.

Each row indicates the change ratio of each substance for all reactions. For example, C is a product in reaction R1 and a reactant in reaction R3. Therefore, the coefficient of C is +1 for R1 and -1 for R3. Each column indicates the change ratio of all substances associated with each reaction. For example, reaction R2 converts D to E and G by the ratio at 2:1. Therefore, the coefficient of D is -2, E and G both are +1. The flux rate is the conversion rate of reactants to products in the reaction which is denoted by v.

This system can be written in the following matrix form.

$$\begin{bmatrix} \frac{d[A]}{dt} \\ \frac{d[B]}{dt} \\ \frac{d[C]}{dt} \\ \frac{d[D]}{dt} \\ \frac{d[E]}{dt} \\ \frac{d[F]}{dt} \\ \frac{d[G]}{dt} \\ \frac{d[H]}{dt} \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 \\ +1 & 0 & -1 & 0 \\ +1 & -2 & 0 & 0 \\ 0 & +1 & -1 & 0 \\ 0 & 0 & +1 & -1 \\ 0 & +1 & 0 & -1 \\ 0 & 0 & 0 & +2 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix} \quad (2)$$

$$X = S \cdot v$$

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$$X = \begin{bmatrix} \frac{d[A]}{dt} \\ \frac{d[B]}{dt} \\ \frac{d[C]}{dt} \\ \frac{d[D]}{dt} \\ \frac{d[E]}{dt} \\ \frac{d[F]}{dt} \\ \frac{d[G]}{dt} \\ \frac{d[H]}{dt} \end{bmatrix}, S = \begin{bmatrix} -1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 \\ +1 & 0 & -1 & 0 \\ +1 & -2 & 0 & 0 \\ 0 & +1 & -1 & 0 \\ 0 & 0 & +1 & -1 \\ 0 & +1 & 0 & -1 \\ 0 & 0 & 0 & +2 \end{bmatrix}, v = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix}$$

where X is the rate of change of metabolite vector.

S is called stoichiometric matrix.

v is a flux vector.

The experiment showed that each reaction in the system has a limited value (lower bound and upper bound) so the model will be constrained by their limit in the following form:

$$\begin{aligned} lb_1 \leq v_1 \leq ub_1 \\ lb_2 \leq v_2 \leq ub_2 \\ lb_3 \leq v_3 \leq ub_3 \\ lb_4 \leq v_4 \leq ub_4 \end{aligned} \tag{3}$$

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where lb is a lower bound constraint of flux rate.

ub is a upper bound constraint of flux rate.

2.3 Flux balance analysis

Most models of the metabolic system in a constraint-based approach cannot be directly solved because of non-square matrix properties. Furthermore, the concentration of each metabolite at steady state is more interesting than a certain time. When computing concentration of metabolite at steady value, we assume the differential concentration of each metabolite is zero (no concentration changes any time). To solve system of linear equations with a certain objective, linear

programming called flux balance analysis (FBA) [3] was proposed. The objective function is defined by a reaction representing a main activity (do/produce something) in the system.

$$\begin{aligned} max \quad & c^T \cdot v \\ subject\ to \quad & S \cdot v = 0 \\ & lb_i \leq v_i \leq ub_i \end{aligned} \tag{4}$$

c is a coefficient vector.

v is a flux vector.

S is a stoichiometric matrix.

lb is a lower bound constraint vector of flux rate.

ub is a upper bound constraint vector of flux rate.

After solving the system equation (4), a solution vector represents a flux of reactions at the steady state. [3, 41]

In our work, FBA was used to find the flux distributions of the metabolic networks under the pathological state and the treatment state. These two metabolic networks derived from our integrated model iPFRBC-713 by having different objective functions. The objective in the pathological state is to maximize the production rate of biomass constitutions according to [39], including Na+/K+ exchanging ATPase which plays an important role of homeostasis in red blood cell [42, 43]. In treatment state, the model represents the metabolic network when the parasite stays inside an infected red blood cell during the drug treatment, which usually inhibits the detoxification process of the parasite (Figure 2.2). This means the drug causes the toxicity of free heme (Fe(III)-protoporphyrin IX [Fe3+PPIX]) in *P. falciparum*. Thus, during the treatment state the toxic flux of heme production rate should be forced to occur by adding it in the objective function. This is to ensure that the toxic flux is not zero under this state and to analyze which reactions or enzymes should be blocked during the treatment. After that, the flux distributions of both models were compared to get a preliminary list of candidate targets by the criteria that the

reactions with zero fluxes in the treatment state but non-zero fluxes in the pathological state could be potential targets in inhibiting heme detoxification.

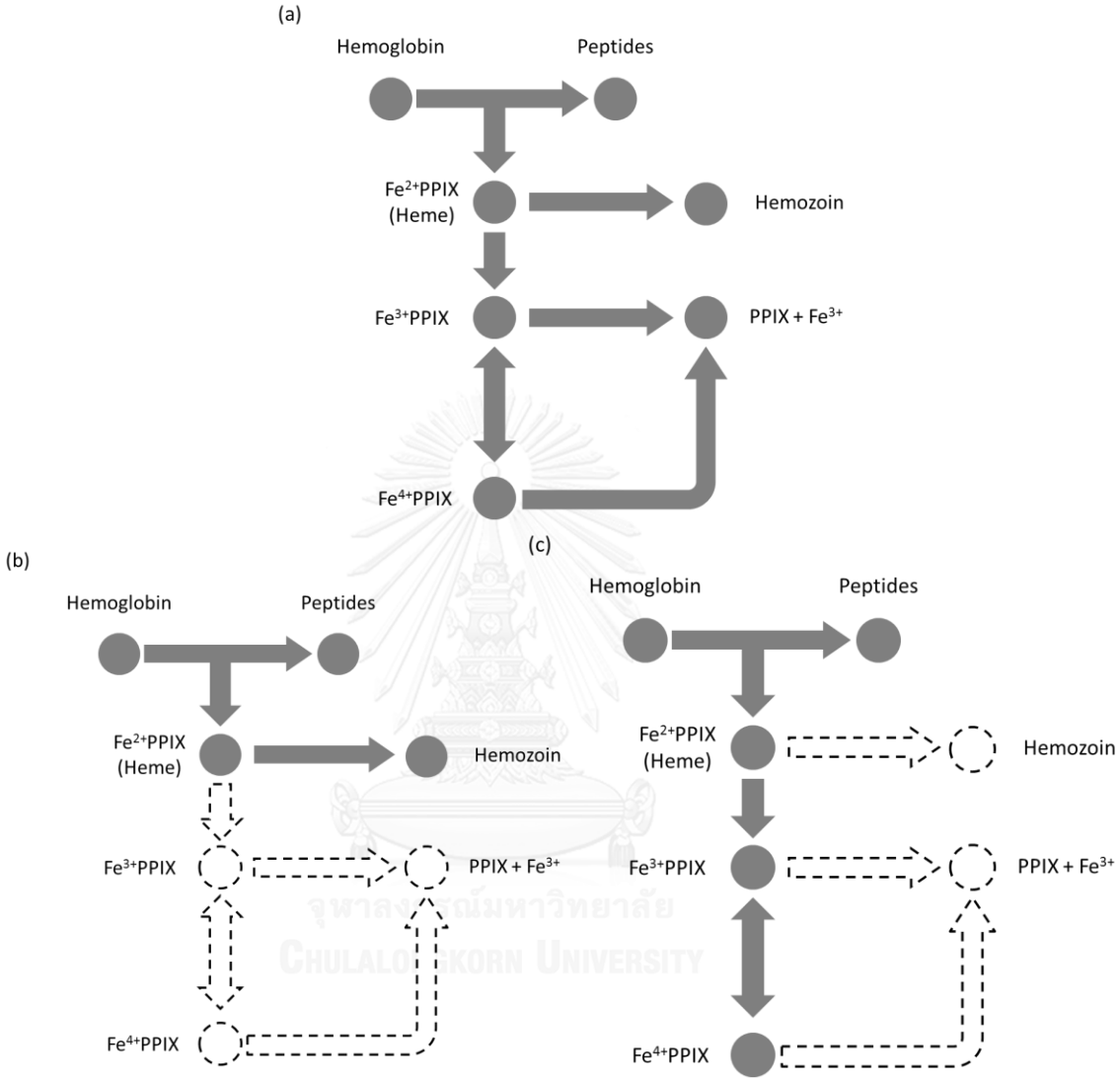

Figure 2.2 Detoxification pathway. (a) Hemoglobin degradation pathway. (b) Pathological stage simulation where heme is converted to the nontoxic metabolite (hemozoin). (c) Ideal treatment stage where heme is changed to cause toxic metabolites and the detoxification pathway or hemozion formation are inhibited.

2.4 Flux variability analysis

To find the range of flux rate of each reaction in constrain-based model, the maximum and minimum values of flux at steady state will be calculated. Both

values are obtained by solving linear programming in equation (5). This is called flux variability or flux span analysis [3, 41]. If both maximum and minimum values of flux are zero, it means that the reaction is not possible to occur in flux balance analysis. The better the model is the higher the number of the reactions with non-zero flux range. Therefore, flux span analysis was used to examine the connectivity of flux flows within our integrated model.

$$\begin{aligned} &max/min && v_i \\ &subject\ to && S \cdot v = 0 \\ & && lb_i \leq v_i \leq ub_i \\ & && c^T \cdot v \geq \alpha Z \end{aligned} \tag{5}$$

where v_i is each reaction in the model, c is a vector indicating the objective reactions α is a parameter ($0 \leq \alpha \leq 1$) controlling the result approaching the optimal solution Z which retrieves from solving flux balance analysis.

2.5 Minimization of Metabolic Adjustment

At steady state, the flux rates in the system are computed by flux balance analysis when disturbing the system by knocking out some reactions. The system is adjusted to a new steady state. *Minimization of metabolic adjustment* (MOMA) was proposed to estimate new flux rate at the new steady state [44]. This method based on quadratic programming technique whose objective is minimized by the overall different fluxes in the system as shown in equation (6)

$$\sqrt{\sum_i (v_i - x_i)^2} \tag{6}$$

where v is wild-type flux and x is new optimal flux of reaction i. In standard QP form, equation (6) can be written as

$$\frac{1}{2} z^T Q z + L z \tag{7}$$

where z is $v - x$ constrained by the steady state and reaction rate limit.

$$\textit{subject to} \qquad S \cdot v = 0 \tag{8}$$
$$lb_i \leq v_i \leq ub_i$$
$$v_j = 0$$

where v_j is a knockout reaction.

In our case, the wild-type network is the integrated model under the pathological state, the disruption is the knockout reaction strategy by forcing the flux rate of a knocked out reaction to be zero ($v_j = 0$). This method follows the assumption that all fluxes in the metabolic processes after the disruption differ from the original fluxes (wild-type network) as small as possible. Like the parasite, Plasmodium in our case, if it lacks of some reactions or enzymes are blocked by drug, it will try to recover all important processes in the metabolic network as much as possible. Therefore, the method was used to perform the knockout strategy to simulate the process during the drug treatment.

2.6 Genetic algorithm

This algorithm is used to optimize the objective function based on the evolution process in nature. It represents a solution vector by a chromosome and encodes a solution element to gene. It consists of three operations on chromosome: selection, crossover, and mutation.

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Selection will select some individual chromosomes to be the parent for a next state. The simple selection algorithm used a roulette wheel to assign selection probability depending on chromosome's fitness value defined by

$$p_i = \frac{f_i}{\sum_i f_i} \tag{9}$$

where f_i is the fitness value of each chromosome.

The selected chromosome will replace its predecessor. The best chromosome is retained in the next generation regardless its probability.

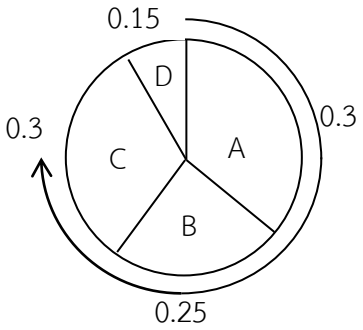

Figure 2.3 An example of roulette wheel selection.

Figure 2.3 shows the fitness value of each chromosome: Chromosome A is 0.3, Chromosome B is 0.25, Chromosome C is 0.3 and Chromosome D is 0.15. The summation of all fitness value is 1.0. The pseudo-code of roulette wheel selection is shown below:

Algorithm RouletteWheelSelection:

```
For 1 to number of newChromosomes:
    r = Random(0,1)
    p = 0
    While p < r and Chromosome in population:
        p +=  Chromosome.fitness
    endloop
    newChromosomes.add(Chromosome)
endloop
return newChromosomes
end
```

From the above algorithm, each chromosome in a new generation is selected by its fitness value which makes the summation of the fitness value (p) more than a random value (r).

Crossover combines parent chromosome to produce child chromosome. Each new child chromosome has some information from both parents. In case of one-point crossover (Figure 2.4), it swaps the information between chromosomes at a specific point. The two-point crossover (Figure 2.5) produces child which has more combination information.

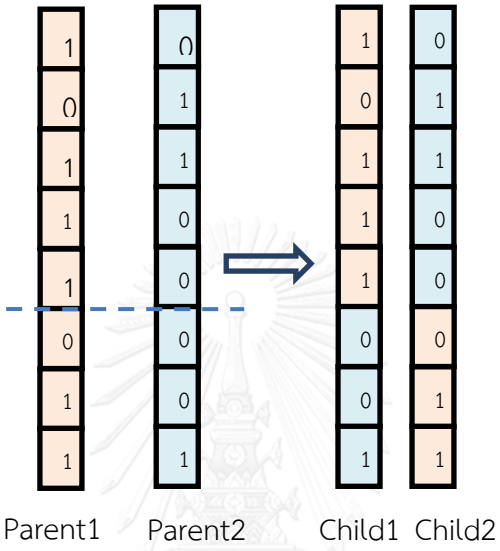

Figure 2.4 One-point crossover. The genes from Parent1 are split into two parts. The first part is in Child1 and the second part is in Child2. The genes from Parent2 are also split into two parts. The first part is in Child2 and the second part is in Child1.

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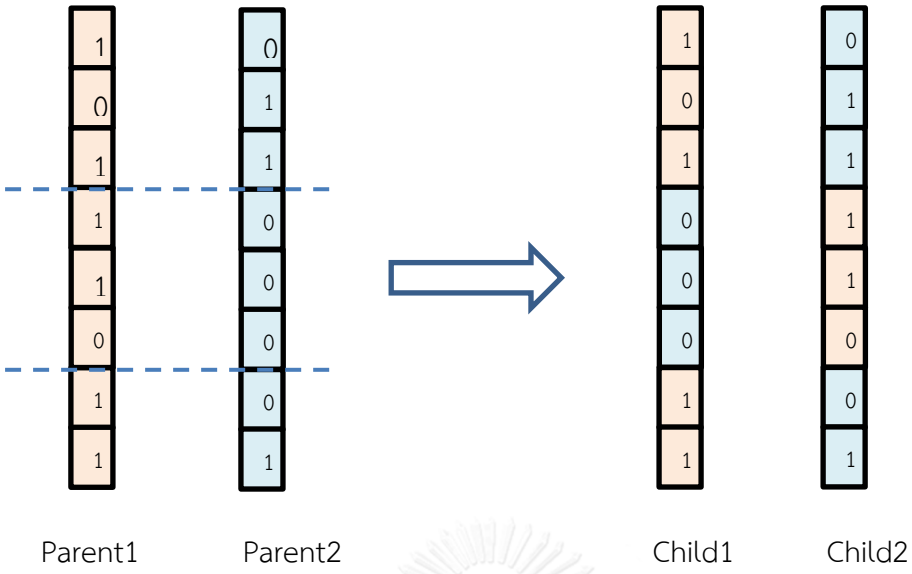

Figure 2.5 Two-point crossover. Child1 consists of the genes from Parent1 two parts and one part from Parent2. Child2 consists of the genes from the Parent1 one and two parts from Parent2.

Mutation randomly changes gene in a selected chromosome and assigns a new value.

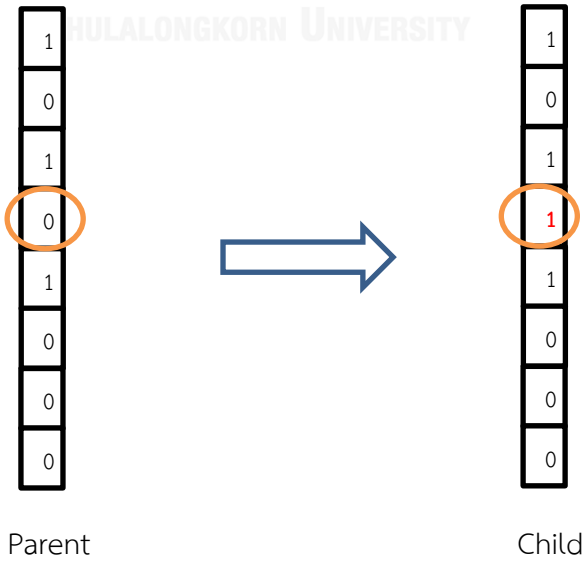

Figure 2.6 Mutation. The 4th gene is mutated from 0 to 1.

This genetic algorithm used a knockout reaction vector as a chromosome and the toxic flux obtained from MOMA as a value for the fitness function. Each chromosome contained the activity status of all reactions in the pathological model. After getting small changes between the wild type and the knockout, the toxic flux was observed and measured as a fitness function of the genetic algorithm to find the best smallest set of knockouts yielding high toxicity. During the process of the genetic algorithm, each generation chromosomes giving the highest toxic flux were selected to be the parents for the next generation. This approach guarantees that a new set of knockout reaction vectors, representing an offspring in the next generation, does not give toxic flux less than the parent generation. This work repeated 50 generations for each iteration and used Gaussian distribution with scale of 1 and shrink of 0.1 as parameters to determine mutation position and has crossover 80% in each generation. Thus, the genetic algorithm was conducted by setting a chromosome as a vector of knockout reactions and using toxic flux calculated from MOMA as the fitness function. Each knockout reaction vector (chromosome) was used to mark which reactions are active (allow to have some flux rate) or inactive (force to be zero flux) in the pathological model (Figure 2.7).

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0	1	1	0	1	0
j=1	j=2	j=3	j=4	j=5	j=6

Figure 2.7 Example of a chromosome which represents knockout reactions in MOMA. From this chromosome the constraint of v_1, v_4 and v_6 are 0 and constraint of v_2, v_3 and v_5 are their upper bound and lower bound.

All the programs were done under MATLAB environment. FBA and MOMA can be found in the COBRA toolbox [41] and genetic algorithm was implemented under Global Optimization toolbox (www.mathworks.com/products/global-optimization/). Gurobi was used to solve the linear programming problems (www.gurobi.com).

2.7 Pathway enrichment test

There are many genes which are detected during drug development may be potential targets. Genes in the model are separated into two types (detected and non-detected) and two groups (same pathway and different pathway). These data were put into contingency table then uses Fisher's exact test to find the significance of detected genes. Fisher's exact test is a statistical method used to determine association between categorical data commonly use with small samples. Fisher's exact test is calculated by equation (10).

Table 2.1 An example of 2 x 2 contingency table.

	Group 1	Group 2	Sum in row
Type 1	A	B	A+B
Type 2	C	D	C+D
Sum in column	A+C	B+D	A+B+C+D = n

$$p = \frac{\binom{A+C}{A}\binom{B+D}{B}}{\binom{n}{A+B}} \tag{10}$$

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2.8 Performance measures

There are four keys to measure performance in our model.

		Truth	
		Positive	**Negative**
Prediction	**Positive**	True Positive (TP)	False Positive (FP)
	Negative	False Negative (FN)	True Negative (TN)

Accuracy measures proportion of truth prediction values among all prediction and known values, defined by

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \tag{11}$$

Precision measures the proportion of true prediction values among all truth values, defined by

$$Precision = \frac{TP}{TP + FP} \tag{12}$$

Sensitivity measures the proportion of the correction prediction in true value, defined by

$$Sensitivity = \frac{TP}{TP + FN} \tag{13}$$

Specificity measures the proportion of the correction prediction in false value, defined by

$$Specificity = \frac{TN}{TN + FP} \tag{14}$$

These four values perform on outputs and reference data in classification by the model. Accuracy value tells that the model can classify the truth value and the falsity value. If the model accuracy is 1, the model perfectly classifies inputs. Precision value considers the true prediction value in classification from the truth reference. Sensitivity tells how much the model can classify truth value from positive prediction and Specificity tells how much the model can classify falsity value from negative prediction.

Chapter 3
Result

3.1 Metabolic models of Plasmodium in red blood cell

We constructed a large-scale metabolic model of the parasite in the infected red blood cell by integrating both the existing blood-stage specific metabolic model of *Plasmodium falciparum* strain iTH366 [39] and the metabolic model of human's red blood cell iAB-RBC-283 [40]. All exchange metabolites were assumed to exist in the red blood cell [45-47] with the same constrains in iTH366. In addition, the detoxification pathway [48, 49] from the hemoglobin degradation during the parasite's growth in the red blood cell was included into the model. Finally, the integrated model, iPF-RBC-713, was a metabolic network consisting of 1206 metabolites, 1479 metabolic reactions, the total of 713 metabolic genes responsible for 962 reactions (Table 3.1) and contained both objective flux rates, biomass from iTH366 and Na+/K+ exchange from iAB-RBC-283. Our integrated model was verified by performing the flux balance analysis and then we compared the objective's flux rate of *Plasmodium falciparum* and the red blood cell to those of the original metabolic network model. The same objective's flux rates were found and represented valid reactions in both models. The analysis of flux spans over the integrated model showed that the model covers with non-zero flux spans of 77% while the original parasite model covered with non-zero flux spans only 66%. It means that our integrated metabolic model of Plasmodium in the red blood cell was also sufficient in describing the metabolic processes of the malaria parasite in the red blood cell.

Table 3.1 Characteristics of the integrated Plasmodium and red blood cell metabolic network.

	P. falciparum iTH366	Human's red blood cell iAB-RBC-283	Our Integrated model iPF-RBC-713
Genes	367	346	713
Metabolites	915	342	1206
Reactions	1001	469	1479
Exchange reactions between these two species			111
Transport reactions between apicoplast, mitochondria and cytoplasm in P. falciparum			51
Non zero flux span			77%

By applying the two-state flux balanced analysis [30] for Plasmodium, the integrated model was used as a template to create a pathological-state metabolic model and a treatment-state metabolic model. Both derived some reactions from the original with different definition of objective fluxes for FBA (Table 3.2). The objective fluxes of the pathological-state model was to ensure that the parasite can stay in red blood cell while those of the treatment-state model were to ensure the non-zero toxic flux as mentioned in section 2.3.

Table 3.2 The objective flux rate of each model.

Model	iTH366	iAB-RBC-283	iPF-RBC-713	
			Pathological state	Treatment state
Objective flux rate (max)	$v_{biomass}$	$v_{Na/K}$	$v_{biomass} + v_{Na/K}$	$v_{biomass} + v_{Na/K} + v_{Fe3Heme}$

3.2. Identification of drug target candidates

After getting the metabolic models for the pathological state and the treatment state, the FBAs of these two were performed and the flux distributions for all reactions in both models were compared. The flux distributions of all reactions under the pathological state represented the mass-flow pathways commonly used by the parasite to stay alive in the red blood cell while those under the treatment state

represented the possible pathways which were used after the drug action. After excluding source or sink reactions, unknown-associated gene reactions, and exchanging reactions, the remaining reactions having non-zero fluxes in the pathological state but being changed to zero fluxes in the treatment state were predicted as potential drug targets in the treatment. According to this criterion, twenty-three genes were predicted as potential drug targets and listed together with literatures in Table 3.3. Notice that fourteen of them were found as potential targets in the other studies. Thus, the precision of this identification was 61% (14/23). The number of our detected drug targets and other literatures were calculated in percentage and shown in Table 3.4. Ludin et al. [37] detected potential drug targets by clustering essential proteins in the malaria parasite and then provided the druggability score by comparing with known targets in the database. Fatumo et al. [36] analyzed metabolic network topology of *Plasmodium falciparum* to identify essential enzymes then knockout them to investigate potential of drug targets. Huthmacher et al. [38] assembled metabolic model of *Plasmodium falciparum* integrated with gene expression data and find essential reactions by flux balance analysis approach. The enzymes catalyzed by the detected reactions were proposed as potential drug targets. Plata et al. [39] reconstructed metabolic network of *Plasmodium falciparum* at blood-stage life cycle and then used flux balance analysis to identify essential genes. The original parasite model (iTH366) was proposed the most drug targets compared with other.

Table 3.3 Candidate target reactions.

No.	Gene	Enzyme	E.C. Number	Pathway	References
1	MAL13P1_324	aldo-keto reductase	1.1.1.21	Pyruvate metabolism	-
2	PF13_0242	isocitrate dehydrogenase (NADP), mitochondrial precursor (IDH)	1.1.1.42	Citrate cycle	[50]
3	PF11_0256	pyruvate dehydrogenase E1 alpha subunit (pdhA)	1.2.4.1	Pyruvate metabolism	[51]
4	PF14_0441	pyruvate dehydrogenase E1 beta subunit (pdhB)	1.2.4.1	Pyruvate metabolism	[51]
5	PF11_0436	coproporphyrinogen III oxidase (CPO)	1.3.3.3	Porphyrin metabolism	-

No.	Gene	Enzyme	E.C. Number	Pathway	References
6	PF13_0353	NADH-cytochrome b5 reductase	1.7.1.3	Nitrogen metabolism	[52]
7	PF07_0085	ferrodoxin reductase-like protein	1.7.1.4	Nitrogen metabolism	[53]
8	PF08_0066	lipoamide dehydrogenase (aLipDH)	1.8.1.4	Pyruvate metabolism	-
9	PFL1550w	lipoamide dehydrogenase (mLipDH)	1.8.1.4	Pyruvate metabolism	-
10	PF14_0192	glutathione reductase (GR)	1.8.1.7	Glutathione metabolism	[54]
11	PFL0595c	glutathione peroxidase	1.11.1.9	Glutathione metabolism	-
12	PF10_0407	dihydrolipoamide acyltransferase component E2 (DLAT)	2.3.1.12	Pyruvate metabolism	-
13	PFC0170c	dihydrolipoamide acyltransferase, putative	2.3.1.168	Valine, leucine and isoleucine degradation	-
14	PF10_0218	citrate synthase, mitochondrial precursor	2.3.3.1	Citrate cycle	-
15	PFE0660c	purine nucleoside phosphorylase (PNP)	2.4.2.1	Purine metabolism	[55, 56]
16	PF13_0229	aconitate hydratase	4.2.1.3	Citrate cycle	[57]
17	MAL13P1.326	ferrochelatase	4.99.1.1	Porphyrin metabolism	[58]
18	PF14_0446	Heme ligase	4.99.1.8	Hemoglobin degradation	[59, 60]
19	PFE0585c	myo-inositol 1-phosphate synthase	5.5.1.4	Inositol Phosphate Metabolism	-
20	PFB0210c	hexose transporter (HT)	-	-	[61]
21	PF13_0252	nucleoside transporter 1 (NT1)	-	-	[62]
22	MAL8P1.32	nucleoside transporter 2 (NT2)	-	-	[62]
23	PF14_0662	nucleoside transporter 3, putative (NT3)	-	-	[62]

Table 3.4 Percentage of number of drug targets in Table A.1.

	Ludin et al. [37]	Huthmacher et el. [38]	Fatumo et el. [36]	Plata et el. [39]	Our Flux comparison
%	34.09	39.77	13.64	46.59	20.45

Focusing on these 14 targets, we found some interesting results as follows. Isocitrate dehydrogenase (NADP), mitochondrial precursor (IDH) (Gene PF13_0242, EC 1.1.1.42) plays an important role in energy supply for the parasites and was studied and reported that it involved in mitochondrial redox control rather than energy metabolism of Plasmodium [50]. Inhibitors of pyruvate dehydrogenase E1 alpha subunit pdhA and pdhB (Gene PF13_0242 and PF11_0256, EC 1.2.4.1) were designed and proposed [51]. NADH-cytochrome b5 reductase (Gene PF13_0353, EC 1.7.1.3) was found malfunction during Plasmodium infection [52]. Ferrodoxin reductase-like protein (Gene PF07_0085, EC 1.7.1.4) Nitrogen metabolism was purposed as a drug target against Apicomplexan human parasite [53]. Inhibitors of glutathione reductase (Gene PF14_0192, EC 1.8.1.7) were designed and their kinetic effect was studied [54]. Several inhibitors for purine nucleoside phosphorylase (Gene PFE0660c, EC 2.4.2.1) were reported for Plasmodium and other parasites [55, 56]. Aconitate hydratase (Gene PF13_0229, EC 4.2.1.3) was found related to an iron regulatory-like protein [57]. Ferrochelatase (Gene MAL13P1.326, EC 4.99.1.1) in porphyrin metabolism and heme ligase (Gene PF14_0446, EC 4.99.1.8) in hemoglobin degradation were found to be related to heme production and its inhibitors were reported in [58-60]. Hexose transporter (HT) (Gene PFB-210c) and nucleoside transporter (Gene PF13_0252, MAL8P1.32 and PF14_0662) were reported as potential targets for malaria treatment [61, 62].

The remaining nine targets in Table 3.3 were purposed to be further investigated as novel potential targets which distribute in several pathways. Detected enzymes in pyruvate metabolism and citrate cycle for energy supply are aldo–keto reductase (Gene MAL13P1_324, EC 1.1.1.21), lipoamide dehydrogenase (Gene PF08_0066 (aLipDH), PFL1550w(mLipDH), EC 1.8.1.4), dihydrolipoamide acyltransferase, putative (Gene PFC0170c, EC 2.3.1.12), and citrate synthase, mitochondrial precursor (Gene PF10_0218, EC 2.3.3.1). Interestingly, we found that coproporphyrinogen III oxidase (CPO) (Gene PF11_0436, EC 1.3.3.3) in porphyrinmetabolismwhich is an enzyme in human that its deficiency results in a reduced production of heme [63, 64]. Glutathione peroxidase (Gene PFL0595c, EC 1.11.1.9) is in glutathione metabolism.

Finally, myo-inositol 1-phosphate synthase (Gene PFE0585c, EC 5.5.1.4) is in inositol phosphate metabolism. Table 3.5 shows the results of pathway enrichment test (Fisher's exact test) with our proposed targets in Kegg database (www.kegg.org) [65]. Most of the genes detected as potential targets were found in pyruvate metabolism (p-value = 0.00024) playing the important role to convert pyruvate to acetyl-CoA, and citrate cycle (p-value= 0.0051) which is a process of energy production.

Table 3.5 Pathway Enrichment test of proposed drug targets.

Pathway ID	Pathway name	# of genes in the pathway	# genes detected as potential targets	p-value
pfa00620	Pyruvate metabolism	17	6	0.00024
pfa00020	Citrate cycle	20	5	0.00510
pfa00910	Nitrogen metabolism	7	2	0.06514
pfa00860	Porphyrin metabolism	11	2	0.14640
pfa00480	Glutathione metabolism	12	2	0.16910
pfa00562	Inositol phosphate metabolism	8	1	0.40720
pfa00230	Purine metabolism	30	1	0.86810

Note that our results cannot directly be compared to those of the other methods because our technique specified drug targets whose absence in the treatment state resulted in high heme toxicity. In another word, our established technique focused on reactions that should be blocked during the drug treatment and might reflect to the high production of the toxicity for the parasite in red blood cell. It is different from the other methods became mostly proposed potential drug targets in terms of survival by measuring the amount of biomass [38, 39], or in terms of the assumption

that blocking a suitable target that matched with some criteria the parasite might die [36, 37, 66, 67]. Neither of them focuses on a specific heme detoxification.

When we measured performance of targets detection from the original *P. falciparum* model and our new development model, the results in Table 3.6 showed that no significant difference between classification performances by single-gene knockout. We selected reactions which made the flux rate of the biomass to zero as potential targets. Accuracy of our integrated model was a little bit lower than those of the original model because new exchange flux rates with red blood cell gave alternative ways to produce such a compound in the metabolism of *Plasmodium falciparum*. However, in this work the only parasite metabolites were investigated. Notice that, the model could yield better in precision.

Table 3.6 Comparing performance between original and new model by using single-gene knockout of normal condition (Pathological state).

	iTH366	iPF-RBC-713
Accuracy	0.1220	0.1031
Precision	0.2131	0.2212
Sensitivity	0.1781	0.1540
Specificity	0.8876	0.9058

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Moreover, our method aims to propose a new set of multiple targets to effectively inhibit the heme detoxification or produce the toxicity to the parasite (discussed in the next section). However, it is still interesting to see the overlaps or the differences of our finding and the other technique. Therefore, the result of this comparison was provided in (Table A.1). This table contained the information of current drug targets found by the other methods including our 18 enzymes with EC numbers out of 23 candidates and known targets used in DrugBank database [68]. As expected, heme ligase was found in DrugBank and our method, but not found by the other methods.

3.3 Promising target combinations to kill the parasite

Our aim is to find an optimal set of multiple targets that might yield high heme production rate. According to the flux comparison, it implied that if we block all reactions associated with 23 candidate targets in Table 3.3, the treatment metabolic model give high heme toxicity. Notice that it is not necessary to block all of these 23 candidates simultaneously. It should be a small set of reactions that might give the similar level of the toxicity. All possible combinations of reactions from the result of flux comparison in Table B.1 should be tested by the mutant simulation to find an optimal set and to get rid of unnecessary reactions that might not involve with the toxic. The process of filtering unnecessary reactions while keeping high toxic flux with smallest number of knockout reactions, leads to a multi-objectives optimization problem. The heuristic optimization algorithm [13] was used to find the knockout reactions which make the model giving the highest toxic flux regardless of biomass. The smallest set of multiple targets was obtained for further drug designs and development. The candidate target reactions were found by finding plasmodium's reactions which have zero flux distribution in remedy state. The first candidate reactions are assumed to be knocked out due to antimalarial drug (Table 3.3).

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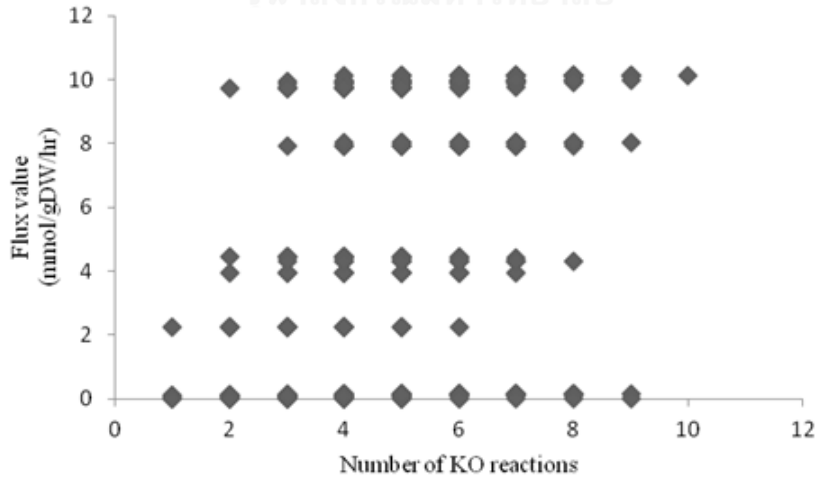

Figure 3.1 Heme toxicity production rates against the number of multiple knockouts (KO).

Figure 3.1 shows all toxic flux production rates against the number of multiple knockouts. From our integrated model, most of single knockouts gave very low toxic flux rate and some did not give any toxic flux rate. Obviously, individual knockout heme ligase yielded the highest toxic flux rate at 2.2479 mmol/gDW/hr. However, the toxic flux rate could be improved by knocking out more enzymes together with blocking the heme ligase. The best double knockouts which gave higher toxic flux rate than the single heme ligase knockout were the inhibition of heme ligase and adenosine transporter. These knockouts gave the value of toxic flux approximately five times higher than that of the single heme ligase knockout. In the same manger, increasing the number of more targets to be blocked gave an improvement of toxic flux rate. Most of triple knockouts showed a little higher toxic flux rate than those of the double knockouts. The toxic flux rate slightly increased and then stayed stable around 10 mmol/gDW/hr until the number of knockouts reached to 5 targets. The best combination of each knockout is summarized in Table 3.7.

Therefore, the smallest set of multiple targets was five corresponding to five targets: heme ligase, adenosine transporter, myo-inositol 1-phosphate synthase, ferrodoxim reductase-like protein, and guanine transporter. Reasonably, these five targets could be blocked simultaneously to yield the highest toxic. Table 3.7 shows the five knocked out reactions which gave the highest toxic flux rate by specific number of knocked out reactions. Obviously, the main reaction to be inhibited in all combinations was heme ligase or heme polymerization which converts Fe(II)-PPIX (or heme) to hemozoin [69, 70].

There are several known drugs to block the heme polymerization such as chloroquine, quinine, mefloquine, halofantrine and sitamaquine (www.drugbank.ca [68]). They all are antimalarial drugs whose mechanism action is to inhibit heme polymerase that allows accumulation of toxic heme in the parasites. Chloroquine is the most widely used to treat all types of malaria. Quinine and mefloquine are used to treat the disease caused by chloroquine resistant Plasmodium falciparum [68]. We might first block the heme ligase by one of those established drugs and find other drugs to block the remaining four targets. Notice that double knockouts when blocking only heme ligase and adenosine transporter gave a good toxic flux

comparable to the knockouts of these five targets. Adenosine transport and its inhibitor have been studied in Plasmodium falciparum-infected and uninfected human erythrocytes recently [71]. Their results clearly showed that purine transporters from host are the major route of purine into the parasitized red blood cell and their inhibitors should be considered in the development of anti-malarials targeted to purine transport. Therefore, it is highly possible to do an experimental test of these two knockouts. After that, finding out and designing of inhibitors or drugs are still required to end the goal of our multiple targets. Myo-inositol 1-phosphate synthase is one of the enzymes in the myo-inositol group to synthesize phospholipid from red blood cell. Recently, a study showed that Plasmodium falciparum asexual stages are critically dependent on de novo myo-inositol biosynthesis [72]. No any inhibitor has been studied for ferrodoxim reductase-like protein. This enzyme is putative and related to oxidation-reduction process (http://www.genedb.org/gene/PF07_0085). Like the adenosine transporter, guanine transporter is one of purine to be transported into the parasite. Since the parasite cannot synthesize the purine de novo, it depends on purine salvage from the host for survival. Thus, this transporter is essential. Its inhibitors were studied from time to time [71, 73].

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Table 3.7 Optimal knockout reactions.

Number of knockout reactions	E.C. number	Target
1	4.99.1.8	Heme ligase
2	4.99.1.8	Heme ligase
	-	Adenosine transporter
3	4.99.1.8	Heme ligase
	-	Adenosine transporter
	5.5.1.4/3.1.3.25	inositol-3-phosphate synthase/ inositol-phosphate phosphatase
4	4.99.1.8	Heme ligase

Number of knockout reactions	E.C. number	Target
	-	Adenosine transporter
	5.5.1.4/3.1.3.25	inositol-3-phosphate synthase/ inositol-phosphate phosphatase
	1.7.1.4	nitrite reductase [NAD(P)H]
5	4.99.1.8	Heme ligase
	-	Adenosine transporter
	5.5.1.4/3.1.3.25	inositol-3-phosphate synthase/ inositol-phosphate phosphatase
	1.7.1.4	nitrite reductase [NAD(P)H]
	-	Guanine transporter

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Chapter 4
Conclusion

Multiple targets identification specifically for heme detoxification in the malaria parasite was proposed in this work by using various computational approaches based on the analysis of flux flows in the metabolic networks.

Metabolic model of *P. falciparum* in red blood cell

The model consisted of two sub-models; *P. falciparum* and human's red blood cell which connected together by modified exchange reactions of the parasite model (*P. falciparum*) to make host model (red blood cell) was sink and source of external flux. The new model also included new pathway which represented detoxification process after hemoglobin digestion. The non-zero flux span of the new model was higher than the original parasite model.

Identification of drug targets

The main idea of this method imitated how the antimalarial drug especially quinine killed the parasite in red blood cell. The combined model was analyzed by flux balance analysis in two states, pathological and treatment state. The change of flux rate of each reaction was detected from non-zero value in pathological state to zero value in treatment state. There were 23 reactions detected and assumed to be candidates for drug targets. The model's performance was evaluated by comparing with the list of the other known targets and also with the literature search. In biological view of view, the pathway enrichment test was also performed to clarify the proposed targets were mostly from essential biological processes. The result from the first method did not differ from the original parasite model while the second method showed that detected enzymes in pyruvate and citrate pathway are more significant than the remaining pathways.

Promising combination drug targets

The number of candidate drug targets is too many for development a real antimalarial drug. These targets were reduced by drug response test. Genetic

Algorithm helped to select targets and their combination for simulation when these targets are inhibited. As for inhibition simulation, MOMA were used to calculate toxic flux in the *P. falciparum* model. The combination of five targets from simulation which gave higher toxic flux than single target or other combinations were promised to be new antimalarial drug target.

Future work

The accuracy of flux balance analysis in parasite state and clinical state can be improved by variant flux balance analysis methods which include high-throughput gene expression data to such as GIMME [74], iMAT [75], MADE [76].

Limitation

In this work, we need a model which has two independent conditions for flux balance analysis. If there are too many reactions after comparing flux, it will consume time to select the best combination of drug targets. The method used to simulate drug response also is limited to base on flux analysis.

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REFERENCES

1. WHO, *Global Report on Antimalarial drug efficacy and drug resistance: 2000-2010.* 2011.
2. WHO, *GUIDE LINES FOR THE TREATMENT OF MALARIA.* 2010.
3. Orth, J.D., I. Thiele, and B.Ø. Palsson, *What is flux balance analysis?* Nature biotechnology, 2010. **28**: p. 245-248.
4. Smallbone, K. and E. Simeonidis, *Flux balance analysis: a geometric perspective.* Journal of theoretical biology, 2009. **258**: p. 311-315.
5. Burgard, A.P., P. Pharkya, and C.D. Maranas, *Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization.* Biotechnology and bioengineering, 2003. **84**: p. 647-657.
6. Zomorrodi, A.R. and C.D. Maranas, *OptCom : A Multi-Level Optimization Framework for the Metabolic Modeling and Analysis of Microbial Communities.* 2012. **8**.
7. Ren, S., B. Zeng, and X. Qian, *Adaptive bi-level programming for optimal gene knockouts for targeted overproduction under phenotypic constraints.* BMC bioinformatics, 2013. **14 Suppl 2**: p. S17.
8. Beard, D.a., S.-d. Liang, and H. Qian, *Energy balance for analysis of complex metabolic networks.* Biophysical journal, 2002. **83**: p. 79-86.
9. Heuett, W.J., *Combining flux and energy balance analysis to model large-scale biochemical networks.* Journal of Bioinformatics and Computational Biology, 2006. **4**: p. 1227-1243.
10. Henry, C.S., L.J. Broadbelt, and V. Hatzimanikatis, *Thermodynamics-based metabolic flux analysis.* Biophysical journal, 2007. **92**: p. 1792-1805.
11. Heino, J., et al., *Bayesian flux balance analysis applied to a skeletal muscle metabolic model.* Journal of theoretical biology, 2007. **248**: p. 91-110.
12. Patil, K.R., et al., *Evolutionary programming as a platform for in silico metabolic engineering.* BMC bioinformatics, 2005. **6**: p. 308.

13. Rocha, M., et al., *Natural computation meta-heuristics for the in silico optimization of microbial strains.* BMC bioinformatics, 2008. **9**: p. 499.
14. Mahadevan, R., J.S. Edwards, and F.J. Doyle, *Dynamic flux balance analysis of diauxic growth in Escherichia coli.* Biophysical journal, 2002. **83**: p. 1331-1340.
15. Lequeux, G., et al., *Dynamic metabolic flux analysis demonstrated on cultures where the limiting substrate is changed from carbon to nitrogen and vice versa.* Journal of biomedicine & biotechnology, 2010. **2010**.
16. Zhou, Q., D. Wang, and M. Xiong, *Dynamic flux balance analysis of metabolic networks using the penalty function methods.* 2007 IEEE International Conference on Systems, Man and Cybernetics, 2007: p. 3594-3599.
17. Uygun, K., H.W.T. Matthew, and Y. Huang, *DFBA-LQR: An Optimal Control Approach to Flux Balance Analysis.* Industrial & Engineering Chemistry Research, 2006. **45**: p. 8554-8564.
18. Uygun, K. and Y. Huang. *A dynamic cybernetic approach: Optimal control for predicting regulatory metabolism actions*. in *Proceedings of the AIChE Annual National Meeting, San Franciso, CA*. 2003. Citeseer.
19. van Riel, N.a., M.L. Giuseppin, and C.T. Verrips, *Dynamic optimal control of homeostasis: an integrative system approach for modeling of the central nitrogen metabolism in Saccharomyces cerevisiae.* Metabolic engineering, 2000. **2**: p. 49-68.
20. Oyarz, D.A., *A control-theoretic approach to dynamic optimization of metabolic networks.* 2010.
21. Agrath, D.E.N., et al., *Integrated Energy and Flux Balance Based Multiobjective Framework for Large-Scale Metabolic Networks.* 2007. **35**: p. 863-885.
22. Nagrath, D., et al., *Soft constraints-based multiobjective framework for flux balance analysis.* 2010. **12**: p. 429-445.
23. Wu, W.-H., F.-S. Wang, and M.-S. Chang, *Multi-objective optimization of enzyme manipulations in metabolic networks considering resilience effects.* BMC systems biology, 2011. **5**: p. 145.
24. Costanza, J., et al., *Multi-objective Optimisation, Sensitivity and Robustness Analysis in FBA Modelling.* 2012: p. 127-147.

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25. Imming, P., C. Sinning, and A. Meyer, *Drugs, their targets and the nature and number of drug targets.* Nature, 2007. **5**: p. 821-835.
26. Zimmermann, G.R., J. Lehár, and C.T. Keith, *Multi-target therapeutics: when the whole is greater than the sum of the parts.* Drug discovery today, 2007. **12**: p. 34-42.
27. Csermely, P., V. Agoston, and S. Pongor, *The efficiency of multi-target drugs: the network approach might help drug design.* Trends in pharmacological sciences, 2005. **26**: p. 178-182.
28. Korcsmáros, T., et al., *How to design multi-target drugs : Target search options in cellular networks.* 2007: p. 1-10.
29. Ding, H., et al., *Similarity-based machine learning methods for predicting drug-target interactions: a brief review.* Briefings in bioinformatics, 2013.
30. Li, Z., R.-S. Wang, and X.-S. Zhang, *Two-stage flux balance analysis of metabolic networks for drug target identification.* BMC systems biology, 2011. **5 Suppl 1**: p. S11.
31. Li, Z. and R.-s. Wang, *Drug Target Identification Based on Flux Balance Analysis of Metabolic Networks.* 2010: p. 331-338.
32. Vera, J., et al., *Detection of potential enzyme targets by metabolic modelling and optimization: application to a simple enzymopathy.* Bioinformatics (Oxford, England), 2007. **23**: p. 2281-2289.
33. Bazzani, S., A. Hoppe, and H.-G. Holzhütter, *Network-based assessment of the selectivity of metabolic drug targets in Plasmodium falciparum with respect to human liver metabolism.* BMC systems biology, 2012. **6**: p. 118.
34. van Laarhoven, T. and E. Marchiori, *Predicting Drug-Target Interactions for New Drug Compounds Using a Weighted Nearest Neighbor Profile.* PloS one, 2013. **8**: p. e66952.
35. Yeh, I., et al., *Computational analysis of Plasmodium falciparum metabolism: organizing genomic information to facilitate drug discovery.* Genome research, 2004. **14**: p. 917-924.
36. Fatumo, S., et al., *Estimating novel potential drug targets of Plasmodium falciparum by analysing the metabolic network of knock-out strains in silico.*

Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases, 2009. **9**: p. 351-358.

37. Ludin, P., et al., *In silico prediction of antimalarial drug target candidates.* Int J Parasitol Drugs Drug Resist, 2012. **2**: p. 191-199.
38. Huthmacher, C., et al., *Antimalarial drug targets in Plasmodium falciparum predicted by stage-specific metabolic network analysis.* BMC Systems Biology, 2010. **4**.
39. Plata, G., et al., *Reconstruction and flux-balance analysis of the Plasmodium falciparum metabolic network.* Molecular systems biology, 2010. **6**: p. 408.
40. Bordbar, A., N. Jamshidi, and B.O. Palsson, *iAB-RBC-283: A proteomically derived knowledge-base of erythrocyte metabolism that can be used to simulate its physiological and patho-physiological states.* BMC systems biology, 2011. **5**: p. 110.
41. Schellenberger, J., et al., *Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0.* Nat Protoc, 2011. **6**(9): p. 1290-1307.
42. Mauritz, J.M.A., et al., *The Homeostasis of Plasmodium falciparum-Infected Red Blood Cells.* PLoS computational biology, 2009. **5**: p. 11-14.
43. Wiback, S.J. and B.O. Palsson, *Extreme pathway analysis of human red blood cell metabolism.* Biophysical journal, 2002. **83**: p. 808-818.
44. Segre, D., D. Vitkup, and G.M. Church, *Analysis of optimality in natural and perturbed metabolic networks.* PNAS, 2002. **99**.
45. Bordbar, A., et al., *A multi-tissue type genome-scale metabolic network for analysis of whole-body systems physiology.* BMC Systems Biology, 2011. **5**: p. 180.
46. Lewis, N.E., et al., *Large-scale in silico modeling of metabolic interactions between cell types in the human brain.* Nature biotechnology, 2010. **28**: p. 1279-1285.
47. Stolyar, S., et al., *Metabolic modeling of a mutualistic microbial community.* Molecular systems biology, 2007. **3**.

48. Kumar, S., et al., *Antimalarial drugs inhibiting hemozoin (beta-hematin) formation: a mechanistic update.* Life sciences, 2007. **80**: p. 813-828.

49. Tekwani, B.L. and L.a. Walker, *Targeting the hemozoin synthesis pathway for new antimalarial drug discovery: technologies for in vitro beta-hematin formation assay.* Combinatorial chemistry & high throughput screening, 2005. **8**: p. 63-79.

50. Wrenger, C. and S. Muller, *Isocitrate dehydrogenase of Plasmodium falciparum. Energy metabolism or redox control?* European Journal of Biochemistry, 2003. **270**: p. 1775-1783.

51. Granchi, C., et al., *Inhibitors of lactate dehydrogenase isoforms and their therapeutic potentials.* Curr Med Chem, 2010. **17**: p. 672-697.

52. Saxena, N., et al., *Effect of Plasmodium yoelii nigeriensis infection and chloroquine on the hepatic mixed function oxidase system of mice.* Mol Biochem Parasitol, 1987. **24**: p. 283-287.

53. Seeber, F., A. Aliverti, and G. Zanetti, *The Plant-Type Ferredoxin-NADP+ Reductase/Ferredoxin Redox System as a Possible Drug Target Against Apicomplexan Human Parasites.* Current Pharmaceutical Design. **11**: p. 3159-3172.

54. Lüönd, R., et al., *Inhibitors of glutathione reductase as potential antimalarial drugs. Kinetic cooperativity and effect of dimethyl sulphoxide on inhibition kinetics.* J Enzyme Inhib., 1998. **13**: p. 327-345.

55. Chaudhary, K., et al., *Toxoplasma gondii purine nucleoside phosphorylase biochemical characterization, inhibitor profiles, and comparison with the Plasmodium falciparum ortholog.* The Journal of biological chemistry, 2006. **281**: p. 25652-25658.

56. Hazleton, K.Z., et al., *Acyclic Immucillin Phosphonates: Second generation inhibitors of Plasmodium falciparum hypoxanthine-guanine-xanthine phosphoribosyltransferase.* Chem Biol., 2013. **19**: p. 721-730.

57. Hodges, M., et al., *An iron regulatory-like protein expressed in Plasmodium falciparum displays aconitase activity.* Molecular and biochemical parasitology, 2005. **143**: p. 29-38.

58. Nagaraj, V.A., et al., *Malaria parasite-synthesized heme is essential in the mosquito and liver stages and complements host heme in the blood stages of infection.* PLoS pathogens, 2013. **9**: p. e1003522.

59. Martiney, J.a., a. Cerami, and a.F. Slater, *Inhibition of hemozoin formation in Plasmodium falciparum trophozoite extracts by heme analogs: possible implication in the resistance to malaria conferred by the beta-thalassemia trait.* Molecular medicine (Cambridge, Mass.), 1996. **2**: p. 236-246.

60. Ziegler, J., R. Linck, and D. Wright, *Heme Aggregation inhibitors: antimalarial drugs targeting an essential biomineralization process.* Curr Med Chem, 2001. **8**: p. 171-189.

61. Joet, T., et al., *Validation of the hexose transporter of Plasmodium falciparum as a novel drug target.* Proceedings of the National Academy of Sciences of the United States of America, 2003. **100**: p. 7476-7479.

62. Baldwin, S., et al., *Nucleoside transport as a potential target for chemotherapy in malaria.* Current Pharmaceutical Design, 2007. **13**: p. 569-580.

63. Kohno, H., et al., *Coproporphyrinogen Oxidase.* The Journal Of Biological Chemistry, 1993. **268**: p. 21359-21363.

64. Lamoril, J., et al., *A molecular defect in coproporphyrinogen oxidase gene causing harderoporphyria, a variant form of hereditary coproporphyria.* Human Molecular Genetics 1995. **4** p. 275-278.

65. Tanabe, M. and M. Kanehisa, *Using the KEGG database resource.* Curr Protoc Bioinformatics, 2012. **Chapter 1**: p. Unit1 12.

66. Alam, A., et al., *Novel antimalarial drug targets: hope for new antimalarial drugs.* Expert Rev Clin Pharmacol, 2009. **2**(5): p. 469-489.

67. Rout, S., et al., *In silico comparative genomics analysis of Plasmodium falciparum for the identification of putative essential genes and therapeutic candidates.* J Microbiol Methods, 2014. **109**: p. 1-8.

68. Law, V., et al., *DrugBank 4.0: shedding new light on drug metabolism.* Nucleic Acids Res, 2013. **42**(Database issue): p. D1091-1097.

69. Jani, D., et al., *HDP-a novel heme detoxification protein from the malaria parasite.* PLoS pathogens, 2008. **4**: p. e1000053.

70. Vinayak, S., et al., *Limited genetic variation in the Plasmodium falciparum heme detoxification protein (HDP).* Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases, 2009. **9**: p. 286-289.

71. Quashie, N.B., L.C. Ranford-Cartwright, and H.P. de Koning, *Uptake of purines in Plasmodium falciparum-infected human erythrocytes is mostly mediated by the human equilibrative nucleoside transporter and the human facilitative nucleobase transporter.* Malar J, 2010. **9**: p. 36.

72. Macrae, J.I., et al., *Plasmodium falciparum is dependent on de novo myo-inositol biosynthesis for assembly of GPI glycolipids and infectivity.* Mol Microbiol, 2014. **91**(4): p. 762-776.

73. El Bissati, K., et al., *Genetic evidence for the essential role of PfNT1 in the transport and utilization of xanthine, guanine, guanosine and adenine by Plasmodium falciparum.* Mol Biochem Parasitol, 2008. **161**(2): p. 130-139.

74. Becker, S.a. and B.O. Palsson, *Context-specific metabolic networks are consistent with experiments.* PLoS computational biology, 2008. **4**: p. e1000082.

75. Zur, H., E. Ruppin, and T. Shlomi, *iMAT: an integrative metabolic analysis tool.* Bioinformatics (Oxford, England), 2010. **26**: p. 3140-3142.

76. Jensen, P.a. and J.a. Papin, *Functional integration of a metabolic network model and expression data without arbitrary thresholding.* Bioinformatics (Oxford, England), 2011. **27**: p. 541-547.

77. Razakantoanina V, Nguyen Kim PP, and J. G, *Antimalarial activity of new gossypol derivatives.* Parasitol Res, 2000. **86**: p. 665-668.

78. Webster, H.K. and J.M. Whaun, *Antimalarial properties of bredinin. Prediction based on identification of differences in human host-parasite purine metabolism.* Journal of Clinical Investigation, 1982. **70**(2): p. 461-469.

79. Cassera, M.B., et al., *Effect of fosmidomycin on metabolic and transcript profiles of the methylerythritol phosphate pathway in Plasmodium falciparum.* Memórias do Instituto Oswaldo Cruz, 2007. **102**: p. 377-384.

80. Jomaa, H., et al., *Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs.* Science, 1999. **285**(5433): p. 1573-1576.

81. Lau, K.S., A.J.L. Cooper, and D.T. Chuang, *Inhibition of the bovine branched-chain 2-oxo acid dehydrogenase complex and its kinase by arylidenepyruvates.* Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 1990. **1038**(3): p. 360-366.

82. Vaughan, A.M., et al., *Type II fatty acid synthesis is essential only for malaria parasite late liver stage development.* Cellular Microbiology, 2009. **11**(3): p. 506-520.

83. Yu, M., et al., *The Fatty Acid Biosynthesis Enzyme FabI Plays a Key Role In the Development of Liver Stage Malarial Parasites.* Cell host & microbe, 2008. **4**(6): p. 567-578.

84. Heath, R.J. and C.O. Rock, *Enoyl-acyl carrier protein reductase (fabI) plays a determinant role in completing cycles of fatty acid elongation in Escherichia coli.* J Biol Chem, 1995. **270**(44): p. 26538-26542.

85. Surolia, N. and A. Surolia, *Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase of Plasmodium falciparum.* Nat Med, 2001. **7**(2): p. 167-173.

86. Spalding, M.D. and S.T. Prigge, *Malaria pulls a FASt one.* Cell Host Microbe, 2008. **4**(6): p. 509-511.

87. McRobert, L. and G.A. McConkey, *RNA interference (RNAi) inhibits growth of Plasmodium falciparum.* Molecular and Biochemical Parasitology, 2002. **119**(2): p. 273-278.

88. Baldwin, J., et al., *High-throughput screening for potent and selective inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase.* J Biol Chem, 2005. **280**(23): p. 21847-21853.

89. Boa, A.N., et al., *Synthesis of brequinar analogue inhibitors of malaria parasite dihydroorotate dehydrogenase.* Bioorg Med Chem, 2005. **13**(6): p. 1945-1967.

90. Heikkilä, T., et al., *The first de novo designed inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase.* Bioorg Med Chem Lett, 2006. **16**: p. 88-92.

91. Krungkrai, J., et al., *Mitochondrial NADH Dehydrogenase from Plasmodium falciparum and Plasmodium berghei.* Experimental Parasitology, 2002. **100**(1): p. 54-61.

92. Ramya, T.N.C., et al., *Inhibitors of Nonhousekeeping Functions of the Apicoplast Defy Delayed Death in Plasmodium falciparum.* Antimicrobial Agents and Chemotherapy, 2007. **51**(1): p. 307-316.

93. Suraveratum, N., et al., *Purification and characterization of Plasmodium falciparum succinate dehydrogenase.* Molecular and Biochemical Parasitology, 2000. **105**(2): p. 215-222.

94. Jiang, L., et al., *Potent and Selective Activity of a Combination of Thymidine and 1843U89, a Folate-Based Thymidylate Synthase Inhibitor, against Plasmodium falciparum.* Antimicrobial Agents and Chemotherapy, 2000. **44**(4): p. 1047-1050.

95. Bras, J.L. and R. Durand, *The mechanisms of resistance to antimalarial drugs in Plasmodium falciparum.* Fundamental & Clinical Pharmacology, 2003. **17**(2): p. 147–153.

96. Fidock, D.A., T. Nomura, and T.E. Wellems, *Cycloguanil and Its Parent Compound Proguanil Demonstrate Distinct Activities against Plasmodium falciparumMalaria Parasites Transformed with Human Dihydrofolate Reductase.* Molecular Pharmacology, 1998. **54**(6): p. 1140-1147.

97. Dar, O., M.S. Khan, and I. Adagu, *The potential use of methotrexate in the treatment of falciparum malaria: in vitro assays against sensitive and multidrug-resistant falciparum strains.* Jpn J Infect Dis, 2008. **61**(3): p. 210-211.

98. Zhang, K. and P.K. Rathod, *Divergent Regulation of Dihydrofolate Reductase Between Malaria Parasite and Human Host.* Science (New York, N.Y.), 2002. **296**(5567): p. 545–547.

99. Biot, C., et al., *5-Substituted Tetrazoles as Bioisosteres of Carboxylic Acids. Bioisosterism and Mechanistic Studies on Glutathione Reductase Inhibitors as Antimalarials.* Journal of Medicinal Chemistry, 2004. **47**(24): p. 5972-5983.

100. Zhang, Y.A., E. Hempelmann, and R.H. Schirmer, *Glutathione reductase inhibitors as potential antimalarial drugs. Effects of nitrosoureas on Plasmodium falciparum in vitro.* Biochem Pharmacol, 1988. **37**(5): p. 855-860.

101. Krnajski, Z., et al., *Thioredoxin Reductase Is Essential for the Survival ofPlasmodium falciparum Erythrocytic Stages.* Journal of Biological Chemistry, 2002. **277**(29): p. 25970-25975.

102. Lüersen, K., R.D. Walter, and S. Müller, *Plasmodium falciparum-infected red blood cells depend on a functional glutathione de novo synthesis attributable to an enhanced loss of glutathione.* Biochem J, 2000. **346**(Pt 2): p. 545-552.

103. Fieser, L.F. and E. Berliner, *Naphthoquinone antimalarials; general survey.* J Am Chem Soc, 1948. **70**(10): p. 3151-3155.

104. Yano, K., et al., *2-Cys Peroxiredoxin TPx-1 is involved in gametocyte development in Plasmodium berghei.* Molecular and Biochemical Parasitology, 2006. **148**(1): p. 44-51.

105. Yano, K., et al., *Disruption of the Plasmodium berghei 2-Cys peroxiredoxin TPx-1 gene hinders the sporozoite development in the vector mosquito.* Molecular and Biochemical Parasitology, 2008. **159**(2): p. 142-145.

106. Soulère, L., et al., *Screening of Plasmodium falciparum iron superoxide dismutase inhibitors and accuracy of the SOD-assays.* Bioorg Med Chem, 2003. **11**(23): p. 4941-4944.

107. Chakrabarti, D., S.M. Schuster, and R. Chakrabarti, *Cloning and characterization of subunit genes of ribonucleotide reductase, a cell-cycle-regulated enzyme, from Plasmodium falciparum.* Proceedings of the National

Academy of Sciences of the United States of America, 1993. **90**(24): p. 12020-12024.

108. Barker, R.H., et al., *Inhibition of Plasmodium falciparum malaria using antisense oligodeoxynucleotides.* Proc Natl Acad Sci USA, 1996. **93**: p. 514-518.

109. Lytton, S.D., et al., *Mode of action of iron (III) chelators as antimalarials: II. Evidence for differential effects on parasite iron-dependent nucleic acid synthesis.* Blood, 1994. **84**(3): p. 910-915.

110. Nduati, E., et al., *2,4-diaminopteridine-based compounds as precursors for de novo synthesis of antifolates: a novel class of antimalarials.* Antimicrob Agents Chemother, 2005. **49**(9): p. 3652-3657.

111. Massimine, K.M., et al., *Eosin B as a novel antimalarial agent for drug-resistant Plasmodium falciparum.* Antimicrob Agents Chemother, 2006. **50**(9): p. 3132-3141.

112. Baron, R.A., et al., *Time-dependent inhibition of isoprenylcysteine carboxyl methyltransferase by indole-based small molecules.* Biochemistry, 2007. **46**(2): p. 554-560.

113. Winter-Vann, A.M., et al., *A small-molecule inhibitor of isoprenylcysteine carboxyl methyltransferase with antitumor activity in cancer cells.* Proc Natl Acad Sci USA, 2005. **102**(12): p. 4336-4341.

114. Pessi, G., G. Kociubinski, and C.B. Mamoun, *A pathway for phosphatidylcholine biosynthesis in Plasmodium falciparum involving phosphoethanolamine methylation.* Proceedings of the National Academy of Sciences of the United States of America, 2004. **101**(16): p. 6206-6211.

115. Witola, W.H., et al., *Disruption of the Plasmodium falciparum PfPMT Gene Results in a Complete Loss of Phosphatidylcholine Biosynthesis via the Serine-Decarboxylase-Phosphoethanolamine-Methyltransferase Pathway and Severe Growth and Survival Defects.* The Journal of Biological Chemistry, 2008. **283**(41): p. 27636-27643.

116. Banerjee, A.K., N. Arora, and U.S.N. Murty, *Aspartate carbamoyltransferase of Plasmodium falciparum as a potential drug target for designing anti-malarial*

chemotherapeutic agents. Medicinal Chemistry Research, 2011. **21**(9): p. 2480-2493.

117. Ginsburg, H., *Progress in in silico functional genomics: the malaria Metabolic Pathways database.* Trends in Parasitology, 2006. **22**(6): p. 238-240.

118. Gerold, P. and R.T. Schwarz, *Biosynthesis of glycosphingolipids de-novo by the human malaria parasite Plasmodium falciparum.* Mol Biochem Parasitol, 2001. **112**: p. 29-37.

119. Surolia, N. and G. Padmanaban, *de novo biosynthesis of heme offers a new chemotherapeutic target in the human malarial parasite.* Biochem Biophys Res Commun, 1992. **187**(2): p. 744-750.

120. Varadharajan, S., et al., *Involvement of delta-aminolaevulinate synthase encoded by the parasite gene in de novo haem synthesis by Plasmodium falciparum.* Biochem J, 2002. **367**(Pt 2): p. 321-327.

121. Waller, R.F., et al., *A type II pathway for fatty acid biosynthesis presents drug targets in Plasmodium falciparum.* Antimicrob Agents Chemother, 2003. **47**: p. 297-301.

122. Prigge, S.T., et al., *The initiating steps of a type II fatty acid synthase in Plasmodium falciparum are catalyzed by pfACP, pfMCAT, and pfKASIII.* Biochemistry, 2003. **42**(4): p. 1160-1169.

123. Bhanot, P., et al., *A Surface Phospholipase Is Involved in the Migration of Plasmodium Sporozoites through Cells.* Journal of Biological Chemistry, 2005. **280**(8): p. 6752-6760.

124. Kicska, G.A., et al., *Purine-less Death in Plasmodium falciparumInduced by Immucillin-H, a Transition State Analogue of Purine Nucleoside Phosphorylase.* Journal of Biological Chemistry, 2002. **277**(5): p. 3226-3231.

125. Dawson, P.A., et al., *Inhibition of Plasmodium falciparum hypoxanthine-guanine phosphoribosyltransferase mRNA by antisense oligodeoxynucleotide sequence.* Molecular and Biochemical Parasitology, 1993. **60**(1): p. 153-156.

126. Deng, X., et al., *Structural Plasticity of Malaria Dihydroorotate Dehydrogenase Allows Selective Binding of Diverse Chemical Scaffolds.* The Journal of Biological Chemistry, 2009. **284**(39): p. 26999-27009.

127. Sarma, P.S. and R.S. Kumar, *Abdominal pain in a patient with falciparum malaria.* Postgrad Med J, 1998. **74**(873): p. 425-427.

128. Li, C.M., et al., *Transition-state analogs as inhibitors of human and malarial hypoxanthine-guanine phosphoribosyltransferases.* Nat Struct Biol, 1999. **6**(6): p. 582-587.

129. Mukkamala, D., et al., *Bisphosphonate Inhibition of a Plasmodium Farnesyl Diphosphate Synthase and a General Method for Predicting Cell-Based Activity from Enzyme Data.* Journal of medicinal chemistry, 2008. **51**(24): p. 7827-7833.

130. Zhang, Y. and S.R. Meshnick, *Inhibition of Plasmodium falciparum dihydropteroate synthetase and growth in vitro by sulfa drugs.* Antimicrobial Agents and Chemotherapy, 1991. **35**(2): p. 267-271.

131. McCullough, J.L. and T.H. Maren, *Dihydropteroate synthetase from Plasmodium berghei: isolation, properties, and inhibition by dapsone and sulfadiazine.* Mol Pharmacol, 1974. **10**: p. 140-145.

132. Triglia, T., et al., *Mutations in dihydropteroate synthase are responsible for sulfone and sulfonamide resistance in Plasmodium falciparum.* Proc Natl Acad Sci USA, 1997. **94**(25): p. 13944-13949.

133. Mycek, M.J., R.A. Harvey, and P.C. Champe, *Pharmacology*. 2000.

134. Haider, N., et al., *The spermidine synthase of the malaria parasite Plasmodium falciparum: Molecular and biochemical characterisation of the polyamine synthesis enzyme.* Molecular and Biochemical Parasitology, 2005. **142**(2): p. 224-236.

135. Fritz-Wolf, K., et al., *X-ray structure of glutathione S-transferase from the malarial parasite Plasmodium falciparum.* Proc Natl Acad Sci USA, 2003. **100**(24): p. 13821-13826.

136. Harwaldt, P., S. Rahlfs, and K. Becker, *Glutathione S-transferase of the malarial parasite Plasmodium falciparum: characterization of a potential drug target.* Biol Chem, 2002. **383**(5): p. 821-830.

137. Liebau, E., et al., *The glutathione S-transferase from Plasmodium falciparum.* Mol Biochem Parasitol, 2002. **124**(1-2): p. 85-90.

138. Perbandt, M., et al., *Native and inhibited structure of a Mu class-related glutathione S-transferase from Plasmodium falciparum.* J Biol Chem, 2004. **279**(2): p. 1336-1342.

139. Roberts, F., et al., *Evidence for the shikimate pathway in apicomplexan parasites.* Nature, 1998. **393**(6687): p. 801-805.

140. Chakrabarti, D., et al., *Protein farnesyltransferase and protein prenylation in Plasmodium falciparum.* J Biol Chem, 2002. **277**(44): p. 42066-42073.

141. Schnick, C., et al., *Structure and non-essential function of glycerol kinase in Plasmodium falciparum blood stages.* Molecular Microbiology, 2009. **71**(2): p. 533-545.

142. Choubey, V., et al., *Inhibition of Plasmodium falciparum choline kinase by hexadecyltrimethylammonium bromide: a possible antimalarial mechanism.* Antimicrob Agents Chemother, 2007. **51**(2): p. 696-706.

143. Ancelin, M.L. and H.J. Vial, *Quaternary ammonium compounds efficiently inhibit Plasmodium falciparum growth in vitro by impairment of choline transport.* Antimicrobial Agents and Chemotherapy, 1986. **29**(5): p. 814-820.

144. Spry, C., et al., *A Class of Pantothenic Acid Analogs Inhibits Plasmodium falciparum Pantothenate Kinase and Represses the Proliferation of Malaria Parasites.* Antimicrobial Agents and Chemotherapy, 2005. **49**(11): p. 4649-4657.

145. Ogwan'g, R., et al., *Use of pharmacological agents to implicate a role for phosphoinositide hydrolysis products in malaria gamete formation.* Biochemical Pharmacology, 1993. **46**(9): p. 1601-1606.

146. Hanada, K., et al., *Plasmodium falciparum Phospholipase C Hydrolyzing Sphingomyelin and Lysocholinephospholipids Is a Possible Target for Malaria Chemotherapy.* The Journal of Experimental Medicine, 2002. **195**(1): p. 23-34.

147. Yuasa, K., et al., *PfPDE1, a novel cGMP-specific phosphodiesterase from the human malaria parasite Plasmodium falciparum.* Biochemical Journal, 2005. **392**(Pt 1): p. 221-229.

Chulalongkorn University

148. Dessens, J.T., et al., *Knockout of the Rodent Malaria Parasite Chitinase PbCHT1 Reduces Infectivity to Mosquitoes.* Infection and Immunity, 2001. **69**(6): p. 4041-4047.

149. Messika, E., et al., *Effect of sinefungin on macromolecular biosynthesis and cell cycle of Plasmodium falciparum.* Trop Med Parasitol, 1990. **41**(3): p. 273-278.

150. Kitade, Y., et al., *Synthesis of S-adenosyl-L-homocysteine hydrolase inhibitors and their biological activities.* Nucleic Acids Symp Ser, 1999. **42**: p. 25-26.

151. Shuto, S., et al., *New neplanocin analogues. 12. Alternative synthesis and antimalarial effect of (6'R)-6'-C-methylneplanocin A, a potent AdoHcy hydrolase inhibitor.* J Med Chem, 2002. **45**(3): p. 748-751.

152. Bujnicki, J.M., et al., *Structure, evolution, and inhibitor interaction of S-adenosyl-L-homocysteine hydrolase from Plasmodium falciparum.* Proteins, 2003. **52**(4): p. 624-632.

153. Nankya-Kitaka, F.M., et al., *Plasmodium chabaudi chabaudi and P. falciparum: inhibition of aminopeptidase and parasite growth by bestatin and nitrobestatin.* Parasitology Research, 1998. **84**(7): p. 552-558.

154. Klemba, M., I. Gluzman, and D.E. Goldberg, *A Plasmodium falciparum dipeptidyl aminopeptidase I participates in vacuolar hemoglobin degradation.* J Biol Chem, 2004. **279**(41): p. 43000-43007.

155. Nöteberg, D., et al., *Design and Synthesis of Plasmepsin I and Plasmepsin II Inhibitors with Activity in Plasmodium falciparum-Infected Cultured Human Erythrocytes.* Journal of Medicinal Chemistry, 2003. **46**(5): p. 734-746.

156. Romeo, S., et al., *Plasmepsin II inhibition and antiplasmodial activity of Primaquine–Statine `double-drugs'.* Bioorganic & Medicinal Chemistry Letters, 2004. **14**(11): p. 2931-2934.

157. Smith, T.K., et al., *Substrate specificity of the Plasmodium falciparum glycosylphosphatidylinositol biosynthetic pathway and inhibition by species-specific suicide substrates.* Biochemistry, 2002. **41**(41): p. 12395-12406.

158. Seymour, K.K., et al., *dCTP levels are maintained in Plasmodium falciparum subjected to pyrimidine deficiency or excess.* Ann Trop Med Parasitol, 1997. **91**(6): p. 603-609.

159. Krungkrai, J., S.R. Krungkrai, and K. Phakanont, *Antimalarial activity of orotate analogs that inhibit dihydroorotase and dihydroorotate dehydrogenase.* Biochem Pharmacol, 1992. **43**(6): p. 1295-1301.

160. Olszewski, K.L., et al., *Host-parasite Interactions Revealed by Plasmodium falciparum Metabolomics.* Cell host & microbe, 2009. **5**(2): p. 191-199.

161. Ho, M.-C., et al., *Structural and metabolic specificity of methylthiocoformycin for malarial adenosine deaminases.* Biochemistry, 2009. **48**(40): p. 9618-9626.

162. Gero, A.M., et al., *New malaria chemotherapy developed by utilization of a unique parasite transport system.* Curr Pharm Des, 2003. **9**(11): p. 867-877.

163. Tyler, P.C., et al., *Synthesis of 5'-methylthio coformycins: specific inhibitors for malarial adenosine deaminase.* J Am Chem Soc, 2007. **129**(21): p. 6872-6879.

164. Guranowski, A., et al., *Adenosine-5'-O-phosphorylated and adenosine-5'-O-phosphorothioylated polyols as strong inhibitors of (symmetrical) and (asymmetrical) dinucleoside tetraphosphatases.* Biochemical Journal, 2003. **373**(Pt 2): p. 635-640.

165. Nguyen, C., et al., *Deoxyuridine Triphosphate Nucleotidohydrolase as a Potential Antiparasitic Drug Target.* Journal of Medicinal Chemistry, 2005. **48**(19): p. 5942-5954.

166. Das Gupta, R., et al., *3-Aminooxy-1-Aminopropane and Derivatives Have an Antiproliferative Effect on Cultured Plasmodium falciparum by Decreasing Intracellular Polyamine Concentrations.* Antimicrobial Agents and Chemotherapy, 2005. **49**(7): p. 2857-2864.

167. Müller, I.B., et al., *Assessing the polyamine metabolism of Plasmodium falciparum as chemotherapeutic target.* Molecular and Biochemical Parasitology, 2008. **160**(1): p. 1-7.

168. Ramya, T.N.C., N. Surolia, and A. Surolia, *Polyamine synthesis and salvage pathways in the malaria parasite Plasmodium falciparum.* Biochemical and Biophysical Research Communications, 2006. **348**(2): p. 579-584.

169. Assaraf, Y.G., et al., *Polyamine levels and the activity of their biosynthetic enzymes in human erythrocytes infected with the malarial parasite, Plasmodium falciparum.* Biochem J, 1984. **222**(3): p. 815-819.

170. Bitonti, A.J., et al., *Bis(benzyl)polyamine analogs inhibit the growth of chloroquine-resistant humanmalaria parasites (Plasmodium falciparum) in vitro and in combination with alpha-difluoromethylornithine cure murine malaria.* Proc Natl Acad Sci USA, 1989. **86**(2): p. 651-655.

171. Berger, B.J., *Antimalarial activities of aminooxy compounds.* Antimicrob Agents Chemother, 2000. **44**(9): p. 2540-2542.

172. Scott, H.V., A.M. Gero, and W.J. O'Sullivan, *In vitro inhibition of Plasmodium falciparum by pyrazofurin, an inhibitor of pyrimidine biosynthesis de novo.* Mol Biochem Parasitol, 1986. **18**: p. 3-15.

173. Seymour, K.K., et al., *Cytotoxic effects of inhibitors of de novo pyrimidine biosynthesis upon Plasmodium falciparum.* Biochemistry, 1994. **33**(17): p. 5268-5274.

174. Krungkrai, S.R., et al., *A novel enzyme complex of orotate phosphoribosyltransferase and orotidine 5'-monophosphate decarboxylase in human malaria parasite Plasmodium falciparum: physical association, kinetics, and inhibition characterization.* Biochemistry, 2005. **44**(5): p. 1643-1652.

175. Bello, A.M., et al., *Structure-activity relationships of C6-uridine derivatives targeting plasmodia orotidine monophosphate decarboxylase.* J Med Chem, 2008. **51**(3): p. 439-448.

176. Meza-Avina, M.E., et al., *Inhibition of orotidine 5'-monophosphate decarboxylase and its therapeutic potential.* Mini Rev Med Chem, 2008. **8**(3): p. 239-247.

177. Wright, P.S., et al., *Irreversible inhibition of S-adenosylmethionine decarboxylase in Plasmodium falciparum-infected erythrocytes: growth inhibition in vitro.* Biochem Pharmacol, 1991. **41**(11): p. 1713-1718.

Chulalongkorn University

178. Wanidworanun, C., R.L. Nagel, and H.L. Shear, *Antisense oligonucleotides targeting malarial aldolase inhibit the asexual erythrocytic stages of Plasmodium falciparum.* Mol Biochem Parasitol, 1999. **102**: p. 91-101.

179. Jewett, T.J. and L.D. Sibley, *Aldolase forms a bridge between cell surface adhesins and the actin cytoskeleton in apicomplexan parasites.* Mol Cell, 2003. **11**(4): p. 885-894.

180. Krungkrai, J., S.R. Krungkrai, and C.T. Supuran, *Carbonic anhydrase inhibitors: Inhibition of Plasmodium falciparum carbonic anhydrase with aromatic/heterocyclic sulfonamides—in vitro and in vivo studies.* Bioorganic & Medicinal Chemistry Letters, 2008. **18**(20): p. 5466-5471.

181. Reungprapavut, S., S.R. Krungkrai, and J. Krungkrai, *Plasmodium falciparum carbonic anhydrase is a possible target for malaria chemotherapy.* J Enzyme Inhib Med Chem, 2004. **19**(3): p. 249-256.

182. Bonday, Z.Q., et al., *Import of host delta- aminolevulinate dehydratase into the malarial parasite: identification of a new drug target.* Nat Med, 2000. **6**(8): p. 898-903.

183. Sharma, S.K., et al., *Identification, characterization, and inhibition of Plasmodium falciparum beta-hydroxyacyl-acyl carrier protein dehydratase (FabZ).* J Biol Chem, 2003. **278**(46): p. 45661-45671.

184. Bulusu, V., et al., *Elucidation of the substrate specificity, kinetic and catalytic mechanism of adenylosuccinate lyase from Plasmodium falciparum.* Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 2009. **1794**(4): p. 642-654.

185. Thornalley, P.J., M. Strath, and R.J.M. Wilson, *Antimalarial activity in vitro of the glyoxalase I inhibitor diester, S-p-bromobenzylglutathione diethyl ester.* Biochemical Pharmacology, 1994. **47**(2): p. 418-420.

186. Ono, T., et al., *Adenylyl cyclase alpha and cAMP signaling mediate Plasmodium sporozoite apical regulated exocytosis and hepatocyte infection.* PLoS Pathog, 2008. **4**.

187. Hirai, M., et al., *PbGC β Is Essential for Plasmodium Ookinete Motility to Invade Midgut Cell and for Successful Completion of Parasite Life Cycle in Mosquitoes.* Journal of Biochemistry, 2006. **140**(5): p. 747-757.

188. Crane, C.M., et al., *Fluorescent inhibitors for IspF, an enzyme in the non-mevalonate pathway for isoprenoid biosynthesis and a potential target for antimalarial therapy.* Angew Chem Int Ed Engl, 2006. **45**(7): p. 1069-1074.

189. Bodley, A.L., J.N. Cumming, and T.A. Shapiro, *Effects of camptothecin, a topoisomerase I inhibitor, on Plasmodium falciparum.* Biochem Pharmacol, 1998. **55**(5): p. 709-711.

190. Gamage, S.A., et al., *Synthesis and in vitro evaluation of 9-anilino-3,6-diaminoacridines active against a multidrug-resistant strain of the malaria parasite Plasmodium falciparum.* J Med Chem, 1994. **37**(10): p. 1486-1494.

191. Noonpakdee, W., et al., *Inhibition of Plasmodium falciparum proliferation in vitro by antisense oligodeoxynucleotides against malarial topoisomerase II.* Biochem Biophys Res Commun, 2003. **302**(4): p. 659-664.

192. Chavalitshewinkoon-Petmitr, P., et al., *Gametocytocidal activity of pyronaridine and DNA topoisomerase II inhibitors against multidrug-resistant Plasmodium falciparum in vitro.* Parasitol Int, 2000. **48**(4): p. 275-280.

193. Ruan, B., et al., *A unique hydrophobic cluster near the active site contributes to differences in borrelidin inhibition among threonyl-tRNA synthetases.* J Biol Chem, 2005. **280**: p. 571-577.

194. Corvaisier, S., V. Bordeau, and B. Felden, *Inhibition of transfer messenger RNA aminoacylation and trans-translation by aminoglycoside antibiotics.* J Biol Chem, 2003. **278**(17): p. 14788-14797.

195. Vega-Rodríguez, J., et al., *The Glutathione Biosynthetic Pathway of Plasmodium Is Essential for Mosquito Transmission.* PLoS Pathogens, 2009. **5**(2): p. e1000302.

196. Platel, D.F., F. Mangou, and J. Tribouley-Duret, *Role of glutathione in the detoxification of ferripro-toporphyrin IX in chloroquine resistant Plasmodium berghei.* Mol Biochem Parasitol, 1999. **98**(2): p. 215-223.

197. Meierjohann, S., R.D. Walter, and S. Müller, *Regulation of intracellular glutathione levels in erythrocytes infected with chloroquine-sensitive and chloroquine-resistant Plasmodium falciparum.* Biochem J, 2002. **368**(Pt 3): p. 761-768.

198. Webster, H.K., et al., *Synthesis of adenosine nucleotides from hypoxanthine by human malaria parasites (Plasmodium Falciparum) in continuous erythrocyte culture: inhibition by hadacidin but not alanosine.* Biochemical Pharmacology, 1984. **33**(9): p. 1555-1557.

199. Eaazhisai, K., et al., *Crystalstructure of fully ligated adenylosuccinate synthetase from Plasmodium falciparum.* J MolBiol, 2004. **335**(5): p. 1251-1264.

200. McConkey, G.A., *Plasmodium falciparum: Isolation and Characterisation of a Gene Encoding Protozoan GMP Synthase.* Experimental Parasitology, 2000. **94**(1): p. 23-32.

201. Flores, M.V.C., et al., *Inhibition of Plasmodium falciparum Proliferationin Vitro by Ribozymes.* Journal of Biological Chemistry, 1997. **272**(27): p. 16940-16945.

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APPENDIX

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Table A.1 List of potential drug targets colleting from literatures.

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
1.1.1.21	aldo-keto reductase*						y
1.1.1.27	L-lactate dehydrogenase[77]				y		
1.1.1.42	isocitrate dehydrogenase (NADP), mitochondrial precursor (IDH) [50]						y
1.1.1.205	IMP dehydrogenase [78]						
1.1.1.267	4-hydroxy-3-methylbut-2-enyl_diphosphate_reductase[79, 80]		Y		y		
1.2.4.1	pyruvate dehydrogenase E1 alpha subunit (pdhA, pdhB) [51]						y
1.2.4.4	3-Methyl-2-oxobutanoate dehydrogenase (lipoamide)[81]			y			

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
1.3.1.9	enoyl-ACP-reductase[82-86]		y		y		
1.3.3.1	Dihydroorotate oxidase[87-91]		y		y		
1.3.3.3	coproporphyrinogen III oxidase (CPO)*						y
1.3.3.4	protoporphyrinogen oxidase[92]				y		
1.3.5.2	Dihydroorotate dehydrogenase (quinone), mitochondrial					y	
1.3.99.1	Succinate dehydrogenase [93]						
1.5.1.3	Dihydrofolate reductase[94-98]	y			y	y	
1.6.5.3	NADH dehydrogenase (ubiquinone) [91]						
1.7.1.3	NADH-cytochrome b5 reductase[52]						y

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
1.7.1.4	ferrodoxin reductase-like protein[53]						y
1.8.1.4	lipoamide dehydrogenase*						y
1.8.1.7	glutathione reductase[54, 99, 100]		y				y
1.8.1.9	thioredoxin reductase[101, 102]		y		y		
1.10.2.2	cytochrome c reductase[103]		y				
1.11.1.9	glutathione peroxidase*						y
1.11.1.15	Thioredoxin peroxidase[104, 105]				y		
1.15.1.1	superoxide dismutase[106]		y				
1.17.4.1	Ribonucleoside-diphosphate reductase[107-109]		y		y		

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
2.1.1.45	Thymidylate synthase[94, 110]		y		y	y	
2.1.1.64	3-Demethylubiquinone-9,3-Omethyltransferase[111]		y				
2.1.1.100	Protein-S-isoprenylcysteine-Omethyltransferase[112, 113]		y				
2.1.1.103	Phosphoethanolamine methyltransferase [114, 115]						
2.1.3.2	Aspartate carbamoyltransferase [116]						
2.3.1.12	dihydrolipoamide acyltransferase component E2 (DLAT)*						y
2.3.1.15	Glycerol-3-phosphate-O-acyltransferase [117]						
2.3.1.24	sphingosine-N-acyltransferase[118]		y				
2.3.1.37	delta-aminolevulinate synthase[119, 120]		y				

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
2.3.1.41	3-Oxoacyl-[acyl-carrier protein] synthase[82, 121, 122]		y		y		
2.3.1.43	Phosphatidylcholine-sterol acyltransferase[123]				y		
2.3.1.50	serine-palmitoyl transferase[118]		y				
2.3.1.168	dihydrolipoamide acyltransferase, putative*						y
2.3.3.1	citrate synthase, mitochondrial precursor*						y
2.4.2.1	Purine-nucleoside phosphorylase 56, 124]				y		y
2.4.2.8	Hypoxanthine phosphoribosyltransferase[125-128]				y		
2.5.1.1	Dimethylallyltranstransferase[129]				y		
2.5.1.10	geranyltranstransferase[129]				y		

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
2.5.1.15	Dihydropteroate synthase[130-133]	y			y	y	
2.5.1.16	Spermidine synthase[134]			y	y		
2.5.1.18	Glutathione transferase[135-138]		y	y		y	
2.5.1.19	3-Phosphoshikimate 1-carboxyvinyltransferase[139]			y	y		
2.5.1.21	Farnesyl-diphosphate farnesyltransferase[140]			y			
2.5.1.58	Farnesyl-diphosphate Farnesyltransferase[140]		y				
2.7.1.30	Glycerol kinase[141]				y		
2.7.1.32	Choline kinase[142, 143]			y			
2.7.1.33	pantothenate kinase[144]				y		

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Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
2.7.8.3	Ceramidecholinephosphotransferase[118]		y				
3.1.3.25	inositol-phosphate phosphatase						y
3.1.3.56	Inositol-1,4,5-trisphosphate 5-phosphatase[145]			y			
3.1.4.12	Sphingomyelin phosphodiesterase[146]				y		
3.1.4.17	3',5'-Cyclic-nucleotide phosphodiesterase[147]			y			
3.2.1.14	Chitinase[148]				y		
3.3.1.1	S-adenosyl-l-homocysteinehydrolase[149-152]		y				
3.4.11.1	leucine aminopeptidase [153]						
3.4.14.1	dipeptidyl aminopeptidase 1 [154]						

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
3.4.23.38	plasmepsins (aspartic acid proteases) [155, 156]						
3.4.23.39	plasmepsins (aspartic acid proteases) [155, 156]						
3.5.1.89	N-acetyl glucosaminylphosphatidylinositol deacetylase[157]		y				
3.5.2.3	Dihydroorotase[158, 159]		y				
3.5.3.1	Arginase[160]				y		
3.5.4.4	Adenosine deaminase[161-163]				y		
3.6.1.17	Bis(5'-nucleosyl)-tetraphosphatase (asymmetrical) [164]						
3.6.1.23	Deoxyuridine 5'-triphosphate nucleotidohydrolase[165]				y		
4.1.1.17	Ornithine decarboxylase[166-171]				y		

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
4.1.1.23	Orotidine-5'-phosphate decarboxylase[+][172-176]		y	y	y		
4.1.1.50	Adenosylmethionine decarboxylase[166-168, 177]				y		
4.1.2.13	Fructose-bisphosphate aldolase[27, 178, 179]				y		
4.2.1.1	carbonic anhydrase[180, 181]		y		y		
4.2.1.3	aconitate hydratase						y
4.2.1.11	phosphopyruvate hydratase	y					
4.2.1.24	Delta-aminolevulinic acid dehydratase/porphobilinogen synthase[92, 182]		y		y		
4.2.1.58-61	3-hydroxyacyl-ACP dehydratase[82, 183]		y		y		

Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
4.2.3.5	Chorismate synthase[87]				y		
4.3.2.2	adenylosuccinate lyase[184]				y		
4.4.1.5	Lactoylglutathione lyase[185]		y	y	y		
4.6.1.1	adenylate cyclase[186]				y		
4.6.1.2	Guanylate cylcase[187]				y		
4.6.1.12	2-C-methyl-D-erythritol-2,4-Cyclodiphosphate-synthase[188]		y				
4.99.1.1	Ferrochelatase[58]						y
4.99.1.8	Heme ligase[59, 60]					y	y

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Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
5.5.1.4	myo-inositol 1-phosphate synthase*						y
5.99.1.2	topoisomerase I[189]		y				
5.99.1.3	topoisomerase II[190-192]		y				
6.1.1.3	Threonine-tRNA ligase[193]		y	y			
6.1.1.7	Alanine-tRNA ligase[194]		y				
6.3.2.2	Gamma-glutamylcysteine synthetase[102, 195-197]		y	y	y		
6.3.4.4	Adenylosuccinate synthetase[198, 199]		y		y		
6.3.5.2	GMP synthetase [200]						
6.3.5.5	Carbamoyl-phosphate synthase (glutamine-hydrolysing)[201]		y		y		

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Table A.1 List of potential drug targets colleting from literatures. (cont.)

EC	Reaction	Ludin et al.	Hutchmacher et al.	Fatumo et al.	Plata et al.	DrugBank	Flux Comparison
6.4.1.2	Acetyl-CoA carboxylase[121]		y				

* No evidence, proposed by flux comparison method.

+ Leiden Malaria Research Group, unpublished data

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Appendix B

The Table B.1 lists all reactions which are the results from flux comparison without considering gene associated reactions, sink or source reactions, exchange reactions.

Table B.1 Reactions as the result from flux comparison

nitrate reductase (NADPH)	nitrite reductase [NAD(P)H]
aldehyde reductase	coproporphyrinogen oxidase
Ferrochelatase	inositol-phosphate phosphatase
inositol-3-phosphate synthase	purine-nucleoside phosphorylase
aconitate hydratase (ACONTam_mt)	aconitate hydratase (ACONTbm_mt)
citrate (Si)-synthase	isocitrate dehydrogenase (NADP+)
glutathione-disulfide reductase	glutathione peroxidase
PDH ap (pyruvate dehydrogenase)	EX_glc(e) (D_Glucose external)*
EX_sbt_D(e) (D_Sorbitol external)*	GLCt1r (D_Glucose transport)
H2O2t (Hydrogen peroxide exchange)	OXAHCOtex (xalate- Bicarbonate exchange)
SBT_Dt (D_Sorbitol exchange)	SO4OXAtex2 (Sulfate-xalate exchange)
NADtap (Nicotinamide_adenine_dinucleotide transport AP)	ACCOAtap (Acetyl_CoA transport AP)
NADHtap (NADH transport AP)	ADNt (Adenosine exchange)
GUAt (Guanine exchange)	COAtmt (Coenzyme_A transport MT)
H2O2tmt (Hydrogen peroxide transport MT)	NH4tmt (Ammonium transport MT)
PPPG9tmt (Protoporphyrinogen_IX transport MT)	ACCOAtm (Acetyl_CoA transport MT)
HEMOZOIN (Hemozoin storing)*	HEMECRYS (heme polymerization)

*****This reaction is excluded from multiple drug target optimization.

To show drug response simulation results, we defined some symbols to represent reactions as follow:

nitrite reductase [NAD(P)H] := A

coproporphyrinogen oxidase := B

ferrochelatase := C

inositol-phosphate phosphatase := D

inositol-3-phosphate synthase := E

xalate- Bicarbonate exchange := F

Adenosine exchange := G

Guanine exchange := H

Protoporphyrinogen IX transport MT := I

heme polymerization := J

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Table B.2 Show single target's drug response

Combination	Flux rate
A	0.013292
B	0
C	0
D	0.096409
E	0.096409
F	0
G	0.065605
H	0.000112
I	0
J	2.247908

Table B.3 Show double targets' drug response

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
G + J	9.73445	A + G	0.094609	E + H	0.013292
A + J	4.437808	G + H	0.070385	E + I	0.013292
D + J	3.939342	F + G	0.065605	E + J	0.013292
E + J	3.939342	G + I	0.065605	F + G	0.013292
H + J	2.25484	B + G	0.065605	F + H	0.013292
I + J	2.247908	C + G	0.065605	F + I	0.013292
F + J	2.247908	A + I	0.013292	F + J	0.013292
C + J	2.247908	A + C	0.013292	G + H	0.013292
B + J	2.247908	A + F	0.013292	G + I	0.013292
A + D	0.105945	A + B	0.013292	G + J	0.013292
A + E	0.105945	C + E	0.013292	H + I	0.013292
D + G	0.105176	C + F	0.013292	H + J	0.013292
E + G	0.105176	C + G	0.013292	I + J	0.013292
D + H	0.097003	C + H	0.013292	A + H	0.013072
E + H	0.097003	C + I	0.013292	C + H	0.000112
C + D	0.096409	C + J	0.013292	F + H	0.000112
C + E	0.096409	D + E	0.013292	H + I	0.000112
D + E	0.096409	D + F	0.013292	B + H	0.000111
D + I	0.096409	D + G	0.013292	B + C	0
E + I	0.096409	D + H	0.013292	B + F	0
D + F	0.096409	D + I	0.013292	B + I	0
E + F	0.096409	D + J	0.013292	C + F	0
B + D	0.096409	E + F	0.013292	C + I	0
B + E	0.096409	E + G	0.013292	F + I	0

Table B.4 Show triple targets' drug response

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
D + G + J	9.932973	A + D + H	0.108564	C + E + F	0.096409
E + G + J	9.932973	A + E + H	0.108564	C + E + I	0.096409
A + G + J	9.876417	A + B + D	0.105945	D + E + F	0.096409
G + H + J	9.756865	A + B + E	0.105945	D + E + I	0.096409
B + G + J	9.73445	A + C + D	0.105945	D + F + I	0.096409
C + G + J	9.73445	A + C + E	0.105945	E + F + I	0.096409
F + G + J	9.73445	A + D + E	0.105945	A + B + G	0.094609
G + I + J	9.73445	A + D + F	0.105945	A + C + G	0.094609
A + D + J	7.926124	A + D + I	0.105945	A + F + G	0.094609
A + E + J	7.926124	A + E + F	0.105945	A + G + I	0.094609
A + C + J	4.437808	A + E + I	0.105945	B + G + H	0.070385
A + F + J	4.437808	B + D + G	0.105176	C + G + H	0.070385
A + I + J	4.437808	B + E + G	0.105176	F + G + H	0.070385
A + B + J	4.437745	C + D + G	0.105176	G + H + I	0.070385
A + H + J	4.403181	C + E + G	0.105176	B + C + G	0.065605
D + H + J	4.313564	D + E + G	0.105176	B + F + G	0.065605
E + H + J	4.313564	D + F + G	0.105176	B + G + I	0.065605
B + D + J	3.939342	D + G + I	0.105176	C + F + G	0.065605
B + E + J	3.939342	E + F + G	0.105176	C + G + I	0.065605
D + E + J	3.939342	E + G + I	0.105176	F + G + I	0.065605
D + F + J	3.939342	A + G + H	0.09865	A + B + C	0.013292
D + I + J	3.939342	B + D + H	0.097003	A + B + F	0.013292
E + F + J	3.939342	B + E + H	0.097003	A + B + I	0.013292
E + I + J	3.939342	C + D + H	0.097003	A + C + F	0.013292
C + D + J	3.939322	C + E + H	0.097003	A + C + I	0.013292
C + E + J	3.939322	D + E + H	0.097003	A + F + I	0.013292
C + H + J	2.25484	D + F + H	0.097003	A + B + H	0.013072
F + H + J	2.25484	D + H + I	0.097003	A + C + H	0.013072
H + I + J	2.25484	E + F + H	0.097003	A + F + H	0.013072
B + H + J	2.254813	E + H + I	0.097003	A + H + I	0.013072
B + C + J	2.247908	B + C + D	0.096409	C + F + H	0.000112
B + F + J	2.247908	B + C + E	0.096409	C + H + I	0.000112
B + I + J	2.247908	B + D + E	0.096409	F + H + I	0.000112
C + F + J	2.247908	B + D + F	0.096409	B + C + H	0.000111
C + I + J	2.247908	B + D + I	0.096409	B + F + H	0.000111
F + I + J	2.247908	B + E + F	0.096409	B + H + I	0.000111
A + D + G	0.141034	B + E + I	0.096409	B + C + F	0
A + E + G	0.141034	C + D + E	0.096409	B + C + I	0
D + G + H	0.112646	C + D + F	0.096409	B + F + I	0
E + G + H	0.112646	C + D + I	0.096409	C + F + I	0

Table B.5 Show 4 targets' drug response

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
A + D + G + J	10.112	C + D + F + J	3.939322	C + E + G + I	0.105176
A + E + G + J	10.112	C + D + I + J	3.939322	D + E + F + G	0.105176
D + G + H + J	9.968775	C + E + F + J	3.939322	D + E + G + I	0.105176
E + G + H + J	9.968775	C + E + I + J	3.939322	D + F + G + I	0.105176
B + D + G + J	9.932973	C + F + H + J	2.25484	E + F + G + I	0.105176
B + E + G + J	9.932973	C + H + I + J	2.25484	A + B + G + H	0.09865
C + D + G + J	9.932973	F + H + I + J	2.25484	A + C + G + H	0.09865
C + E + G + J	9.932973	B + C + H + J	2.254813	A + F + G + H	0.09865
D + E + G + J	9.932973	B + F + H + J	2.254813	A + G + H + I	0.09865
D + F + G + J	9.932973	B + H + I + J	2.254813	B + C + D + H	0.097003
D + G + I + J	9.932973	B + C + F + J	2.247908	B + C + E + H	0.097003
E + F + G + J	9.932973	B + C + I + J	2.247908	B + D + E + H	0.097003
E + G + I + J	9.932973	B + F + I + J	2.247908	B + D + F + H	0.097003
A + G + H + J	9.898187	C + F + I + J	2.247908	B + D + H + I	0.097003
A + C + G + J	9.876418	A + D + G + H	0.14599	B + E + F + H	0.097003
A + B + G + J	9.876417	A + E + G + H	0.14599	B + E + H + I	0.097003
A + F + G + J	9.876417	A + B + D + G	0.141034	C + D + E + H	0.097003
A + G + I + J	9.876417	A + B + E + G	0.141034	C + D + F + H	0.097003
B + G + H + J	9.756865	A + C + D + G	0.141034	C + D + H + I	0.097003
C + G + H + J	9.756865	A + C + E + G	0.141034	C + E + F + H	0.097003
F + G + H + J	9.756865	A + D + E + G	0.141034	C + E + H + I	0.097003
G + H + I + J	9.756865	A + D + F + G	0.141034	D + E + F + H	0.097003
B + C + G + J	9.73445	A + D + G + I	0.141034	D + E + H + I	0.097003
B + F + G + J	9.73445	A + E + F + G	0.141034	D + F + H + I	0.097003
B + G + I + J	9.73445	A + E + G + I	0.141034	E + F + H + I	0.097003
C + F + G + J	9.73445	B + D + G + H	0.112646	B + C + D + E	0.096409
C + G + I + J	9.73445	B + E + G + H	0.112646	B + C + D + F	0.096409
F + G + I + J	9.73445	C + D + G + H	0.112646	B + C + D + I	0.096409
A + D + H + J	8.020111	C + E + G + H	0.112646	B + C + E + F	0.096409
A + E + H + J	8.020111	D + E + G + H	0.112646	B + C + E + I	0.096409
A + B + D + J	7.926124	D + F + G + H	0.112646	B + D + E + F	0.096409
A + B + E + J	7.926124	D + G + H + I	0.112646	B + D + E + I	0.096409
A + C + D + J	7.926124	E + F + G + H	0.112646	B + D + F + I	0.096409
A + C + E + J	7.926124	E + G + H + I	0.112646	B + E + F + I	0.096409
A + D + E + J	7.926124	A + B + D + H	0.108564	C + D + E + F	0.096409
A + D + F + J	7.926124	A + B + E + H	0.108564	C + D + E + I	0.096409
A + D + I + J	7.926124	A + C + D + H	0.108564	C + D + F + I	0.096409
A + E + F + J	7.926124	A + C + E + H	0.108564	C + E + F + I	0.096409

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
A + E + I + J	7.926124	A + D + E + H	0.108564	D + E + F + I	0.096409
A + C + F + J	4.437808	A + D + F + H	0.108564	A + B + C + G	0.094609
A + C + I + J	4.437808	A + D + H + I	0.108564	A + B + F + G	0.094609
A + F + I + J	4.437808	A + E + F + H	0.108564	A + B + G + I	0.094609
A + B + C + J	4.437745	A + E + H + I	0.108564	A + C + F + G	0.094609
A + B + F + J	4.437745	A + B + C + D	0.105945	A + C + G + I	0.094609
A + B + I + J	4.437745	A + B + C + E	0.105945	A + F + G + I	0.094609
A + C + H + J	4.403181	A + B + D + E	0.105945	B + C + G + H	0.070385
A + F + H + J	4.403181	A + B + D + F	0.105945	B + F + G + H	0.070385
A + H + I + J	4.403181	A + B + D + I	0.105945	B + G + H + I	0.070385
A + B + H + J	4.403116	A + B + E + F	0.105945	C + F + G + H	0.070385
B + D + H + J	4.313564	A + B + E + I	0.105945	C + G + H + I	0.070385
B + E + H + J	4.313564	A + C + D + E	0.105945	F + G + H + I	0.070385
C + D + H + J	4.313564	A + C + D + F	0.105945	B + C + F + G	0.065605
C + E + H + J	4.313564	A + C + D + I	0.105945	B + C + G + I	0.065605
D + E + H + J	4.313564	A + C + E + F	0.105945	B + F + G + I	0.065605
D + F + H + J	4.313564	A + C + E + I	0.105945	C + F + G + I	0.065605
D + H + I + J	4.313564	A + D + E + F	0.105945	A + B + C + F	0.013292
E + F + H + J	4.313564	A + D + E + I	0.105945	A + B + C + I	0.013292
E + H + I + J	4.313564	A + D + F + I	0.105945	A + B + F + I	0.013292
B + D + E + J	3.939342	A + E + F + I	0.105945	A + C + F + I	0.013292
B + D + F + J	3.939342	B + C + D + G	0.105176	A + B + C + H	0.013072
B + D + I + J	3.939342	B + C + E + G	0.105176	A + B + F + H	0.013072
B + E + F + J	3.939342	B + D + E + G	0.105176	A + B + H + I	0.013072
B + E + I + J	3.939342	B + D + F + G	0.105176	A + C + F + H	0.013072
D + E + F + J	3.939342	B + D + G + I	0.105176	A + C + H + I	0.013072
D + E + I + J	3.939342	B + E + F + G	0.105176	A + F + H + I	0.013072
D + F + I + J	3.939342	B + E + G + I	0.105176	C + F + H + I	0.000112
E + F + I + J	3.939342	C + D + E + G	0.105176	B + C + F + H	0.000111
B + C + D + J	3.939322	C + D + F + G	0.105176	B + C + H + I	0.000111
B + C + E + J	3.939322	C + D + G + I	0.105176	B + F + H + I	0.000111
C + D + E + J	3.939322	C + E + F + G	0.105176	B + C + F + I	0

Table B.6 Show 5 targets' drug response

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
A + D + G + H + J	10.13531	A + B + F + I + J	4.437745	A + B + C + E + H	0.108564
A + E + G + H + J	10.13531	A + C + F + H + J	4.403181	A + B + D + E + H	0.108564
A + B + D + G + J	10.112	A + C + H + I + J	4.403181	A + B + D + F + H	0.108564
A + B + E + G + J	10.112	A + F + H + I + J	4.403181	A + B + D + H + I	0.108564
A + C + D + G + J	10.112	A + B + C + H + J	4.403116	A + B + E + F + H	0.108564

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
A + C + E + G + J	10.112	A + B + F + H + J	4.403116	A + B + E + H + I	0.108564
A + D + E + G + J	10.112	A + B + H + I + J	4.403116	A + C + D + E + H	0.108564
A + D + F + G + J	10.112	B + C + D + H + J	4.313564	A + C + D + F + H	0.108564
A + D + G + I + J	10.112	B + C + E + H + J	4.313564	A + C + D + H + I	0.108564
A + E + F + G + J	10.112	B + D + E + H + J	4.313564	A + C + E + F + H	0.108564
A + E + G + I + J	10.112	B + D + F + H + J	4.313564	A + C + E + H + I	0.108564
B + D + G + H + J	9.968775	B + D + H + I + J	4.313564	A + D + E + F + H	0.108564
B + E + G + H + J	9.968775	B + E + F + H + J	4.313564	A + D + E + H + I	0.108564
C + D + G + H + J	9.968775	B + E + H + I + J	4.313564	A + D + F + H + I	0.108564
C + E + G + H + J	9.968775	C + D + E + H + J	4.313564	A + E + F + H + I	0.108564
D + E + G + H + J	9.968775	C + D + F + H + J	4.313564	A + B + C + D + E	0.105945
D + F + G + H + J	9.968775	C + D + H + I + J	4.313564	A + B + C + D + F	0.105945
D + G + H + I + J	9.968775	C + E + F + H + J	4.313564	A + B + C + D + I	0.105945
E + F + G + H + J	9.968775	C + E + H + I + J	4.313564	A + B + C + E + F	0.105945
E + G + H + I + J	9.968775	D + E + F + H + J	4.313564	A + B + C + E + I	0.105945
B + C + D + G + J	9.932973	D + E + H + I + J	4.313564	A + B + D + E + F	0.105945
B + C + E + G + J	9.932973	D + F + H + I + J	4.313564	A + B + D + E + I	0.105945
B + D + E + G + J	9.932973	E + F + H + I + J	4.313564	A + B + D + F + I	0.105945
B + D + F + G + J	9.932973	B + D + E + F + J	3.939342	A + B + E + F + I	0.105945
B + D + G + I + J	9.932973	B + D + E + I + J	3.939342	A + C + D + E + F	0.105945
B + E + F + G + J	9.932973	B + D + F + I + J	3.939342	A + C + D + E + I	0.105945
B + E + G + I + J	9.932973	B + E + F + I + J	3.939342	A + C + D + F + I	0.105945
C + D + E + G + J	9.932973	D + E + F + I + J	3.939342	A + C + E + F + I	0.105945
C + D + F + G + J	9.932973	B + C + D + E + J	3.939322	A + D + E + F + I	0.105945
C + D + G + I + J	9.932973	B + C + D + F + J	3.939322	B + C + D + E + G	0.105176
C + E + F + G + J	9.932973	B + C + D + I + J	3.939322	B + C + D + F + G	0.105176
C + E + G + I + J	9.932973	B + C + E + F + J	3.939322	B + C + D + G + I	0.105176
D + E + F + G + J	9.932973	B + C + E + I + J	3.939322	B + C + E + F + G	0.105176
D + E + G + I + J	9.932973	C + D + E + F + J	3.939322	B + C + E + G + I	0.105176
D + F + G + I + J	9.932973	C + D + E + I + J	3.939322	B + D + E + F + G	0.105176
E + F + G + I + J	9.932973	C + D + F + I + J	3.939322	B + D + E + G + I	0.105176
A + B + G + H + J	9.898187	C + E + F + I + J	3.939322	B + D + F + G + I	0.105176
A + C + G + H + J	9.898187	C + F + H + I + J	2.25484	B + E + F + G + I	0.105176
A + F + G + H + J	9.898187	B + C + F + H + J	2.254813	C + D + E + F + G	0.105176
A + G + H + I + J	9.898187	B + C + H + I + J	2.254813	C + D + E + G + I	0.105176
A + B + C + G + J	9.876418	B + F + H + I + J	2.254813	C + D + F + G + I	0.105176
A + C + F + G + J	9.876418	B + C + F + I + J	2.247908	C + E + F + G + I	0.105176
A + C + G + I + J	9.876418	A + B + D + G + H	0.14599	D + E + F + G + I	0.105176
A + B + F + G + J	9.876417	A + B + E + G + H	0.14599	A + B + C + G + H	0.09865
A + B + G + I + J	9.876417	A + C + D + G + H	0.14599	A + B + F + G + H	0.09865
A + F + G + I + J	9.876417	A + C + E + G + H	0.14599	A + B + G + H + I	0.09865

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
B + C + G + H + J	9.756865	A + D + E + G + H	0.14599	A + C + F + G + H	0.09865
B + F + G + H + J	9.756865	A + D + F + G + H	0.14599	A + C + G + H + I	0.09865
B + G + H + I + J	9.756865	A + D + G + H + I	0.14599	A + F + G + H + I	0.09865
C + F + G + H + J	9.756865	A + E + F + G + H	0.14599	B + C + D + E + H	0.097003
C + G + H + I + J	9.756865	A + E + G + H + I	0.14599	B + C + D + F + H	0.097003
F + G + H + I + J	9.756865	A + B + C + D + G	0.141034	B + C + D + H + I	0.097003
B + C + F + G + J	9.73445	A + B + C + E + G	0.141034	B + C + E + F + H	0.097003
B + C + G + I + J	9.73445	A + B + D + E + G	0.141034	B + C + E + H + I	0.097003
B + F + G + I + J	9.73445	A + B + D + F + G	0.141034	B + D + E + F + H	0.097003
C + F + G + I + J	9.73445	A + B + D + G + I	0.141034	B + D + E + H + I	0.097003
A + B + D + H + J	8.020111	A + B + E + F + G	0.141034	B + D + F + H + I	0.097003
A + B + E + H + J	8.020111	A + B + E + G + I	0.141034	B + E + F + H + I	0.097003
A + C + D + H + J	8.020111	A + C + D + E + G	0.141034	C + D + E + F + H	0.097003
A + C + E + H + J	8.020111	A + C + D + F + G	0.141034	C + D + E + H + I	0.097003
A + D + E + H + J	8.020111	A + C + D + G + I	0.141034	C + D + F + H + I	0.097003
A + D + F + H + J	8.020111	A + C + E + F + G	0.141034	C + E + F + H + I	0.097003
A + D + H + I + J	8.020111	A + C + E + G + I	0.141034	D + E + F + H + I	0.097003
A + E + F + H + J	8.020111	A + D + E + F + G	0.141034	B + C + D + E + F	0.096409
A + E + H + I + J	8.020111	A + D + E + G + I	0.141034	B + C + D + E + I	0.096409
A + B + C + D + J	7.926124	A + D + F + G + I	0.141034	B + C + D + F + I	0.096409
A + B + C + E + J	7.926124	A + E + F + G + I	0.141034	B + C + E + F + I	0.096409
A + B + D + E + J	7.926124	B + C + D + G + H	0.112646	B + D + E + F + I	0.096409
A + B + D + F + J	7.926124	B + C + E + G + H	0.112646	C + D + E + F + I	0.096409
A + B + D + I + J	7.926124	B + D + E + G + H	0.112646	A + B + C + F + G	0.094609
A + B + E + F + J	7.926124	B + D + F + G + H	0.112646	A + B + C + G + I	0.094609
A + B + E + I + J	7.926124	B + D + G + H + I	0.112646	A + B + F + G + I	0.094609
A + C + D + E + J	7.926124	B + E + F + G + H	0.112646	A + C + F + G + I	0.094609
A + C + D + F + J	7.926124	B + E + G + H + I	0.112646	B + C + F + G + H	0.070385
A + C + D + I + J	7.926124	C + D + E + G + H	0.112646	B + C + G + H + I	0.070385
A + C + E + F + J	7.926124	C + D + F + G + H	0.112646	B + F + G + H + I	0.070385
A + C + E + I + J	7.926124	C + D + G + H + I	0.112646	C + F + G + H + I	0.070385
A + D + E + F + J	7.926124	C + E + F + G + H	0.112646	B + C + F + G + I	0.065605
A + D + E + I + J	7.926124	C + E + G + H + I	0.112646	A + B + C + F + I	0.013292
A + D + F + I + J	7.926124	D + E + F + G + H	0.112646	A + B + C + F + H	0.013072
A + E + F + I + J	7.926124	D + E + G + H + I	0.112646	A + B + C + H + I	0.013072
A + C + F + I + J	4.437808	D + F + G + H + I	0.112646	A + B + F + H + I	0.013072
A + B + C + F + J	4.437745	E + F + G + H + I	0.112646	A + C + F + H + I	0.013072
A + B + C + I + J	4.437745	A + B + C + D + H	0.108564	B + C + F + H + I	0.000111

Table B.7 Show 6 targets' drug response

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
A + B + D + G + H + J	10.13531	A + B + C + D + H + J	8.020111	A + D + F + G + H + I	0.14599
A + B + E + G + H + J	10.13531	A + B + C + E + H + J	8.020111	A + E + F + G + H + I	0.14599
A + C + D + G + H + J	10.13531	A + B + D + E + H + J	8.020111	A + B + C + D + E + G	0.141034
A + C + E + G + H + J	10.13531	A + B + D + F + H + J	8.020111	A + B + C + D + F + G	0.141034
A + D + E + G + H + J	10.13531	A + B + D + H + I + J	8.020111	A + B + C + D + G + I	0.141034
A + D + F + G + H + J	10.13531	A + B + E + F + H + J	8.020111	A + B + C + E + F + G	0.141034
A + D + G + H + I + J	10.13531	A + B + E + H + I + J	8.020111	A + B + C + E + G + I	0.141034
A + E + F + G + H + J	10.13531	A + C + D + E + H + J	8.020111	A + B + D + E + F + G	0.141034
A + E + G + H + I + J	10.13531	A + C + D + F + H + J	8.020111	A + B + D + E + G + I	0.141034
A + B + C + D + G + J	10.112	A + C + D + H + I + J	8.020111	A + B + D + F + G + I	0.141034
A + B + C + E + G + J	10.112	A + C + E + F + H + J	8.020111	A + B + E + F + G + I	0.141034
A + B + D + E + G + J	10.112	A + C + E + H + I + J	8.020111	A + C + D + E + F + G	0.141034
A + B + D + F + G + J	10.112	A + D + E + F + H + J	8.020111	A + C + D + E + G + I	0.141034
A + B + D + G + I + J	10.112	A + D + E + H + I + J	8.020111	A + C + D + F + G + I	0.141034
A + B + E + F + G + J	10.112	A + D + F + H + I + J	8.020111	A + C + E + F + G + I	0.141034
A + B + E + G + I + J	10.112	A + E + F + H + I + J	8.020111	A + D + E + F + G + I	0.141034
A + C + D + E + G + J	10.112	A + B + C + D + E + J	7.926124	B + C + D + E + G + H	0.112646
A + C + D + F + G + J	10.112	A + B + C + D + F + J	7.926124	B + C + D + F + G + H	0.112646
A + C + D + G + I + J	10.112	A + B + C + D + I + J	7.926124	B + C + D + G + H + I	0.112646
A + C + E + F + G + J	10.112	A + B + C + E + F + J	7.926124	B + C + E + F + G + H	0.112646
A + C + E + G + I + J	10.112	A + B + C + E + I + J	7.926124	B + C + E + G + H + I	0.112646
A + D + E + F + G + J	10.112	A + B + D + E + F + J	7.926124	B + D + E + F + G + H	0.112646
A + D + E + G + I + J	10.112	A + B + D + E + I + J	7.926124	B + D + E + G + H + I	0.112646
A + D + F + G + I + J	10.112	A + B + D + F + I + J	7.926124	B + D + F + G + H + I	0.112646
A + E + F + G + I + J	10.112	A + B + E + F + I + J	7.926124	B + E + F + G + H + I	0.112646
B + C + D + G + H + J	9.968775	A + C + D + E + F + J	7.926124	C + D + E + F + G + H	0.112646
B + C + E + G + H + J	9.968775	A + C + D + E + I + J	7.926124	C + D + E + G + H + I	0.112646
B + D + E + G + H + J	9.968775	A + C + D + F + I + J	7.926124	C + D + F + G + H + I	0.112646
B + D + F + G + H + J	9.968775	A + C + E + F + I + J	7.926124	C + E + F + G + H + I	0.112646
B + D + G + H + I + J	9.968775	A + D + E + F + I + J	7.926124	D + E + F + G + H + I	0.112646
B + E + F + G + H + J	9.968775	A + B + C + F + I + J	4.437745	A + B + C + D + E + H	0.108564
B + E + G + H + I + J	9.968775	A + C + F + H + I + J	4.403181	A + B + C + D + F + H	0.108564
C + D + E + G + H + J	9.968775	A + B + C + F + H + J	4.403116	A + B + C + D + H + I	0.108564
C + D + F + G + H + J	9.968775	A + B + C + H + I + J	4.403116	A + B + C + E + F + H	0.108564
C + D + G + H + I + J	9.968775	A + B + F + H + I + J	4.403116	A + B + C + E + H + I	0.108564
C + E + F + G + H + J	9.968775	B + C + D + E + H + J	4.313564	A + B + D + E + F + H	0.108564
C + E + G + H + I + J	9.968775	B + C + D + F + H + J	4.313564	A + B + D + E + H + I	0.108564
D + E + F + G + H + J	9.968775	B + C + D + H + I + J	4.313564	A + B + D + F + H + I	0.108564
D + E + G + H + I + J	9.968775	B + C + E + F + H + J	4.313564	A + B + E + F + H + I	0.108564
D + F + G + H + I + J	9.968775	B + C + E + H + I + J	4.313564	A + C + D + E + F + H	0.108564

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
E + F + G + H + I + J	9.968775	B + D + E + F + H + J	4.313564	A + C + D + E + H + I	0.108564
B + C + D + E + G + J	9.932973	B + D + E + H + I + J	4.313564	A + C + D + F + H + I	0.108564
B + C + D + F + G + J	9.932973	B + D + F + H + I + J	4.313564	A + C + E + F + H + I	0.108564
B + C + D + G + I + J	9.932973	B + E + F + H + I + J	4.313564	A + D + E + F + H + I	0.108564
B + C + E + F + G + J	9.932973	C + D + E + F + H + J	4.313564	A + B + C + D + E + F	0.105945
B + C + E + G + I + J	9.932973	C + D + E + H + I + J	4.313564	A + B + C + D + E + I	0.105945
B + D + E + F + G + J	9.932973	C + D + F + H + I + J	4.313564	A + B + C + D + F + I	0.105945
B + D + E + G + I + J	9.932973	C + E + F + H + I + J	4.313564	A + B + C + E + F + I	0.105945
B + D + F + G + I + J	9.932973	D + E + F + H + I + J	4.313564	A + B + D + E + F + I	0.105945
B + E + F + G + I + J	9.932973	B + D + E + F + I + J	3.939342	A + C + D + E + F + I	0.105945
C + D + E + F + G + J	9.932973	B + C + D + E + F + J	3.939322	B + C + D + E + F + G	0.105176
C + D + E + G + I + J	9.932973	B + C + D + E + I + J	3.939322	B + C + D + E + G + I	0.105176
C + D + F + G + I + J	9.932973	B + C + D + F + I + J	3.939322	B + C + D + F + G + I	0.105176
C + E + F + G + I + J	9.932973	B + C + E + F + I + J	3.939322	B + C + E + F + G + I	0.105176
D + E + F + G + I + J	9.932973	C + D + E + F + I + J	3.939322	B + D + E + F + G + I	0.105176
A + B + C + G + H + J	9.898187	B + C + F + H + I + J	2.254813	C + D + E + F + G + I	0.105176
A + B + F + G + H + J	9.898187	A + B + C + D + G + H	0.14599	A + B + C + F + G + H	0.09865
A + B + G + H + I + J	9.898187	A + B + C + E + G + H	0.14599	A + B + C + G + H + I	0.09865
A + C + F + G + H + J	9.898187	A + B + D + E + G + H	0.14599	A + B + F + G + H + I	0.09865
A + C + G + H + I + J	9.898187	A + B + D + F + G + H	0.14599	A + C + F + G + H + I	0.09865
A + F + G + H + I + J	9.898187	A + B + D + G + H + I	0.14599	B + C + D + E + F + H	0.097003
A + B + C + F + G + J	9.876418	A + B + E + F + G + H	0.14599	B + C + D + E + H + I	0.097003
A + B + C + G + I + J	9.876418	A + B + E + G + H + I	0.14599	B + C + D + F + H + I	0.097003
A + C + F + G + I + J	9.876418	A + C + D + E + G + H	0.14599	B + C + E + F + H + I	0.097003
A + B + F + G + I + J	9.876417	A + C + D + F + G + H	0.14599	B + D + E + F + H + I	0.097003
B + C + F + G + H + J	9.756865	A + C + D + G + H + I	0.14599	C + D + E + F + H + I	0.097003
B + C + G + H + I + J	9.756865	A + C + E + F + G + H	0.14599	B + C + D + E + F + I	0.096409
B + F + G + H + I + J	9.756865	A + C + E + G + H + I	0.14599	A + B + C + F + G + I	0.094609
C + F + G + H + I + J	9.756865	A + D + E + F + G + H	0.14599	B + C + F + G + H + I	0.070385
B + C + F + G + I + J	9.73445	A + D + E + G + H + I	0.14599	A + B + C + F + H + I	0.013072

Table B.8 Show 7 targets' drug response

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
A + B + C + D + G + H + J	10.13531	C + D + E + G + H + I + J	9.968775	B + C + E + F + H + I + J	4.313564
A + B + C + E + G + H + J	10.13531	C + D + F + G + H + I + J	9.968775	B + D + E + F + H + I + J	4.313564
A + B + D + E + G + H + J	10.13531	C + E + F + G + H + I + J	9.968775	C + D + E + F + H + I + J	4.313564
A + B + D + F + G + H + J	10.13531	D + E + F + G + H + I + J	9.968775	B + C + D + E + F + I + J	3.939322
A + B + D + G + H + I + J	10.13531	B + C + D + E + F + G + J	9.932973	A + B + C + D + E + G + H	0.14599
A + B + E + F + G + H + J	10.13531	B + C + D + E + G + I + J	9.932973	A + B + C + D + F + G + H	0.14599
A + B + E + G + H + I + J	10.13531	B + C + D + F + G + I + J	9.932973	A + B + C + D + G + H + I	0.14599

Combination	Flux rate	Combination	Flux rate	Combination	Flux rate
A + C + D + E + G + H + J	10.13531	B + C + E + F + G + I + J	9.932973	A + B + C + E + F + G + H	0.14599
A + C + D + F + G + H + J	10.13531	B + D + E + F + G + I + J	9.932973	A + B + C + E + G + H + I	0.14599
A + C + D + G + H + I + J	10.13531	C + D + E + F + G + I + J	9.932973	A + B + D + E + F + G + H	0.14599
A + C + E + F + G + H + J	10.13531	A + B + C + F + G + H + J	9.898187	A + B + D + E + G + H + I	0.14599
A + C + E + G + H + I + J	10.13531	A + B + C + G + H + I + J	9.898187	A + B + D + F + G + H + I	0.14599
A + D + E + F + G + H + J	10.13531	A + B + F + G + H + I + J	9.898187	A + B + E + F + G + H + I	0.14599
A + D + E + G + H + I + J	10.13531	A + C + F + G + H + I + J	9.898187	A + C + D + E + F + G + H	0.14599
A + D + F + G + H + I + J	10.13531	A + B + C + F + G + I + J	9.876418	A + C + D + E + G + H + I	0.14599
A + E + F + G + H + I + J	10.13531	B + C + F + G + H + I + J	9.756865	A + C + D + F + G + H + I	0.14599
A + B + C + D + E + G + J	10.112	A + B + C + D + E + H + J	8.020111	A + C + E + F + G + H + I	0.14599
A + B + C + D + F + G + J	10.112	A + B + C + D + F + H + J	8.020111	A + D + E + F + G + H + I	0.14599
A + B + C + D + G + I + J	10.112	A + B + C + D + H + I + J	8.020111	A + B + C + D + E + F + G	0.141034
A + B + C + E + F + G + J	10.112	A + B + C + E + F + H + J	8.020111	A + B + C + D + E + G + I	0.141034
A + B + C + E + G + I + J	10.112	A + B + C + E + H + I + J	8.020111	A + B + C + D + F + G + I	0.141034
A + B + D + E + F + G + J	10.112	A + B + D + E + F + H + J	8.020111	A + B + C + E + F + G + I	0.141034
A + B + D + E + G + I + J	10.112	A + B + D + E + H + I + J	8.020111	A + B + D + E + F + G + I	0.141034
A + B + D + F + G + I + J	10.112	A + B + D + F + H + I + J	8.020111	A + C + D + E + F + G + I	0.141034
A + B + E + F + G + I + J	10.112	A + B + E + F + H + I + J	8.020111	B + C + D + E + F + G + H	0.112646
A + C + D + E + F + G + J	10.112	A + C + D + E + F + H + J	8.020111	B + C + D + E + G + H + I	0.112646
A + C + D + E + G + I + J	10.112	A + C + D + E + H + I + J	8.020111	B + C + D + F + G + H + I	0.112646
A + C + D + F + G + I + J	10.112	A + C + D + F + H + I + J	8.020111	B + C + E + F + G + H + I	0.112646
A + C + E + F + G + I + J	10.112	A + C + E + F + H + I + J	8.020111	B + D + E + F + G + H + I	0.112646
A + D + E + F + G + I + J	10.112	A + D + E + F + H + I + J	8.020111	C + D + E + F + G + H + I	0.112646
B + C + D + E + G + H + J	9.968775	A + B + C + D + E + F + J	7.926124	A + B + C + D + E + F + H	0.108564
B + C + D + F + G + H + J	9.968775	A + B + C + D + E + I + J	7.926124	A + B + C + D + E + H + I	0.108564
B + C + D + G + H + I + J	9.968775	A + B + C + D + F + I + J	7.926124	A + B + C + D + F + H + I	0.108564
B + C + E + F + G + H + J	9.968775	A + B + C + E + F + I + J	7.926124	A + B + C + E + F + H + I	0.108564
B + C + E + G + H + I + J	9.968775	A + B + D + E + F + I + J	7.926124	A + B + D + E + F + H + I	0.108564
B + D + E + F + G + H + J	9.968775	A + C + D + E + F + I + J	7.926124	A + C + D + E + F + H + I	0.108564
B + D + E + G + H + I + J	9.968775	A + B + C + F + H + I + J	4.403116	A + B + C + D + E + F + I	0.105945
B + D + F + G + H + I + J	9.968775	B + C + D + E + F + H + J	4.313564	B + C + D + E + F + G + I	0.105176
B + E + F + G + H + I + J	9.968775	B + C + D + E + H + I + J	4.313564	A + B + C + F + G + H + I	0.09865
C + D + E + F + G + H + J	9.968775	B + C + D + F + H + I + J	4.313564	B + C + D + E + F + H + I	0.097003

Table B.9 Show 8 targets' drug response

Combination	Flux rate	Combination	Flux rate
A + B + C + D + E + G + H + J	10.13531	B + C + E + F + G + H + I + J	9.968775
A + B + C + D + F + G + H + J	10.13531	B + D + E + F + G + H + I + J	9.968775
A + B + C + D + G + H + I + J	10.13531	C + D + E + F + G + H + I + J	9.968775
A + B + C + E + F + G + H + J	10.13531	B + C + D + E + F + G + I + J	9.932973

Combination	Flux rate	Combination	Flux rate
A + B + C + E + G + H + I + J	10.13531	A + B + C + F + G + H + I + J	9.898187
A + B + D + E + F + G + H + J	10.13531	A + B + C + D + E + F + H + J	8.020111
A + B + D + E + G + H + I + J	10.13531	A + B + C + D + E + H + I + J	8.020111
A + B + D + F + G + H + I + J	10.13531	A + B + C + D + F + H + I + J	8.020111
A + B + E + F + G + H + I + J	10.13531	A + B + C + E + F + H + I + J	8.020111
A + C + D + E + F + G + H + J	10.13531	A + B + D + E + F + H + I + J	8.020111
A + C + D + E + G + H + I + J	10.13531	A + C + D + E + F + H + I + J	8.020111
A + C + D + F + G + H + I + J	10.13531	A + B + C + D + E + F + I + J	7.926124
A + C + E + F + G + H + I + J	10.13531	B + C + D + E + F + H + I + J	4.313564
A + D + E + F + G + H + I + J	10.13531	A + B + C + D + E + F + G + H	0.14599
A + B + C + D + E + F + G + J	10.112	A + B + C + D + E + G + H + I	0.14599
A + B + C + D + E + G + I + J	10.112	A + B + C + D + F + G + H + I	0.14599
A + B + C + D + F + G + I + J	10.112	A + B + C + E + F + G + H + I	0.14599
A + B + C + E + F + G + I + J	10.112	A + B + D + E + F + G + H + I	0.14599
A + B + D + E + F + G + I + J	10.112	A + C + D + E + F + G + H + I	0.14599
A + C + D + E + F + G + I + J	10.112	A + B + C + D + E + F + G + I	0.141034
B + C + D + E + F + G + H + J	9.968775	B + C + D + E + F + G + H + I	0.112646
B + C + D + E + G + H + I + J	9.968775	A + B + C + D + E + F + H + I	0.108564
B + C + D + F + G + H + I + J	9.968775		

Table B.10 Show 9 targets' drug response

Combination	Flux rate
A + B + C + D + E + F + G + H + I	0.14599
A + B + C + D + E + F + G + H + J	10.13531
A + B + C + D + E + F + G + I + J	10.112
A + B + C + D + E + F + H + I + J	8.020111
A + B + C + D + E + G + H + I + J	10.13531
A + B + C + D + F + G + H + I + J	10.13531
A + B + C + E + F + G + H + I + J	10.13531
A + B + D + E + F + G + H + I + J	10.13531
A + C + D + E + F + G + H + I + J	10.13531
B + C + D + E + F + G + H + I + J	9.968775

Table B.11 Show 10 targets' drug response

Combination	Flux rate
A + B + C + D + E + F + G + H + I + J	10.13530622

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