Flux Balance Analysis of Melanogenesis Pathway

Prashana Balaji V., Anvita Gupta Malhotra, Khushhali Menaria

Abstract*— A computational model could serve as a conventional engineering approach to uncover the biochemistry of the metabolic pathways. These would dynamically mimic the pathways in-silico. Flux Balance Analysis (FBA) is one such method wherein characterization of growth yields, bio-energy production, environmental conditions and robustness under knock out & knock down can be studied. We have built a comprehensive dynamic platform of integrated network for melanogenesis pathway containing 6 major reactions. Wherein detailed stoichiometric matrix of the pathway reactions is constructed followed by defining constrains and objective function. Subsequently, these are optimized using linear programming to give us resultant fluxes. Using this model, vulnerability of the enzymes in these pathways are studied; essentiality of participating enzymes are established and varied computational gene knock-out experiments which can decipher effect of inhibition on metabolic circuit are performed. Results of the simulations were in corroboration with published results and predictions were validated. However, this platform can enables us to make elaborate prediction in the known modeled domain and later with amalgamation of more modelled pathways into this network; a comprehensive virtual cell can be constructed.

***Index Terms*— Melanogenesis, Flux Balance Analysis (FBA), Pheomelanin, Eumelanin, Systems Biology.**

I. INTRODUCTION

There is a paradigm shift in the field of bioinformatics; it is not just genomics and sequence analysis anymore. It has grown beyond it in the field of network biology, protein structure modelling, docking and molecular dynamics [1]. On the other hand in the department of experimental sciences, over several years reductionism in biochemistry, molecular biology and cellular biology, has dominated this research arena. It has provided a wealth of knowledge about individual cellular components and their functions [2]. Despite its enormous success, it is clear that the biological systems are greater than sum of their parts. Therefore, the main challenge today lies in integration of knowledge from both bioinformatics & experimental research to systematically catalogue all molecules within a living cell [3]. This would aid in understanding the dynamics of the complex intercellular web of interactions that contribute to the structure and function of a living cell.

Manuscript Received February 09, 2011.

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Systems Biology is the bird eye view of the biological networks. This is a holistic approach for modeling and analysis of metabolic, regulatory and signaling pathways. This will lead to a better understanding of cellular behavior. In the current scenario, there are several methods to study the dynamics of the pathways, one of the most common one being the mechanistic modeling. This implies the generation of biochemical models based on the enzymatic kinetic parameters and substrate concentration. Fetching this kinetic data is difficult due to its scare availability. Since experiments performed to discover them have their own limitations [4]. As a result, there are few number and size of systems in different species that can be studied through this approach. A viable alternative to this approach is constrain based modeling via FBA [5]. Fluxes play a key role in describing the functionality of the pathways and studying response to perturbation. Moreover, this protocol enforces cellular limitation in terms of physicochemical, growth, topological, environmental and gene regulation constrains to model the system.

Flux balance analysis is a widely used approach for studying biochemical networks, in particular the genome-scale metabolic network reconstructions. FBA calculates the flow of metabolites through this metabolic network, thereby making it possible to predict the growth rate of an organism or the rate of production of a biotechnologically important metabolite. The first step in FBA is to mathematically represent metabolic reactions. The core feature of this representation is tabulation, in the form of a numerical matrix, of the stoichiometric coefficients of each reaction. The matrix of stoichiometries imposes flux (that is, mass balance) constraints on the system, ensuring that the total amount of any compound being produced must be equal to the total amount being consumed at steady state. Every reaction can also be given upper and lower bounds, which define the maximum and minimum allowable fluxes of the reactions. These balances and bounds define the space of allowable flux distributions of a system—that is, the rates at which every metabolite is consumed or produced by each reaction. The next step in FBA is to define a phenotype in the form of a biological objective that is relevant to the problem being studied. Biomass production is mathematically represented by adding an artificial 'biomass reaction'— that is, an extra column of coefficients in the matrix of stoichiometries—that consumes precursor metabolites at stoichiometries that simulate biomass production. The biomass reaction is based on experimental measurements of biomass components. This reaction is scaled so that the flux through it is equal to the exponential growth rate (μ) of the organism[5]. Flux

Balance Analysis (FBA), is a constraint based approach which is widely used for understanding the interaction and functioning of the biochemical pathways. This constraint based modeling has been successful in metabolic and signaling regulations [6]. It involves computing the metabolic fluxes under steady state by optimizing the objective function under a set of physiochemical, topological or environmental constraints implied on the system. This flux distribution is then used to interpret the metabolic capabilities of the system.

The mathematical representation of the metabolic reactions and objective function, defines a system of linear equations. In flux balance analysis, these equations are solved using linear programming [7]. Many computational linear programming algorithms exist, and they can very quickly identify optimal solutions to large systems of equations.

In the present study an attempt has been taken to exploit this method for unrevealing the intricacies of melanogenesis pathway. Melanogenesis is a process of melanin production by melanocyte. These are special skin cells located in the basal layer of the epidermis. In humans, melanin is the primary determinant of skin color. The most common are reddish color pheomelanin and brownish eumelanin. Both pheomelanin and eumelanin are found in human skin and hair, but eumelanin is the most abundant melanin in humans, as well as the form most likely to be deficient in albinism. The major role of melanins is to protect skin from the harmful effects of UV rays and to prevent skin cancer. Besides this, both eumelanin and pheomelanin play an important protective role within melanocytes and keratinocytes due to their ability to bind cations, anions, drugs, and chemicals.

II. PROBLEM STATEMENT

A large number of experiments are performed but till date the researchers are unable to produce significant data to model entire system. Therefore the methods like FBA can be important tool to move forward and predict the abnormalities in any metabolic path, in our case this is melanogenesis. Melanogenesis pathway is not much explored for in-silico modeling due to limited kinetic data. Moreover, hyper or hypo melanin production leads to disorders in skin pigmentation like vitilogo etc. Hence building model for this network will give useful insights about the disease. Herein the model generated using FBA was used for the prediction of the rate limiting step of the cascade. Furthermore, several in-silico gene knockout experiments are also performed, which would aid in identification of target protein.

III. MODEL DESCRIPTION

A. Construction of static pathway

Melanogenesis pathway consist of two major branches i.e., one for the synthesis of Eumelanin and another for Pheomelanin production [8] [Figure 1]. Eumelanin is a highly heterogeneous polymer consisting of DHI (DiHydroxyIndole) and DHICA ((DiHydroxyIndole Carboxylic Acid) units in reduced or oxidized states. Pheomelanin consists mainly of sulfur-containing benzothiazine derivatives. Melanins are synthesized from tyrosine exogenously supplied by blood. However tyrosine is produced from phenyl-alanine via different path. Tyrosines are oxidized by tyrosinase and metabolised into DOPAs and then into DOPA quinones which are automatically oxidized into indole compounds. Indole compounds connect to each other to produce eumelanins.

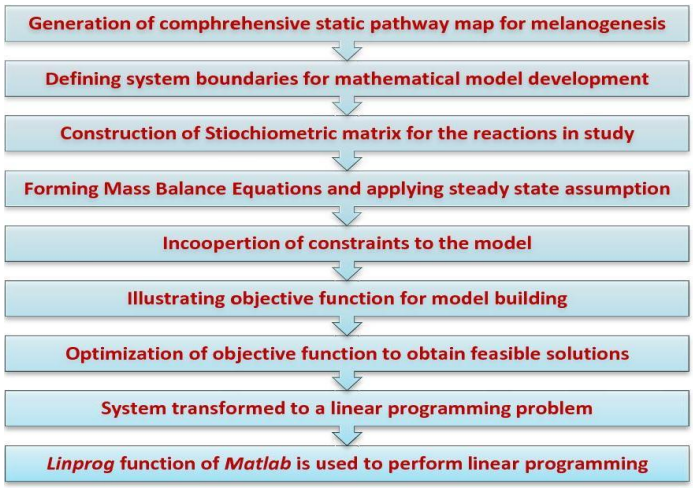
The pheomelanin synthesis pathway involves sulfur compounds, the amino acid cystein or glutathion that liberates cysteins through the action of a glutamyl-transpeptidase. In presence of cysteins, DOPAquinones connect with cysteins to form 5-S-cysteinyl DOPA and 2-S-cysteinylDOPA which give benzothiazin intermediates that polymerise to produce pheomelanins.

The primary step of FBA is the building a comprehensive pathway in study. These are done by collating and integrate existing information from available pathway resources like KEGG [9], and BIOCYC [10], literature survey and protein interaction data. The melanin synthesis network considered can be referred in Figure 1. Furthermore enzymes missing from the network after initial construction can be identified subsequently by additional experimentation as a part of iterative model refinement process.

B. Model Development

FBA, which uses linear optimization to determine the steady-state reaction flux distribution in a metabolic network by maximizing an objective function is the soul in model development for the primary study. Essentially our model involves four steps [11]: (i) system definition, (ii) obtaining reaction stoichiometries, (iii) defining biologically relevant objective function and addition of other biochemical constraints and (iv) optimization [Figure 2]. This flux distribution is then used to interpret the metabolic capabilities of the system.

Figure 2: Work Flow for FBA model construction used to explore melanogenesis pathway.



C. Formulation of Flux Balance Analysis

1. Reconstruction of the biochemical networks: [system definition]

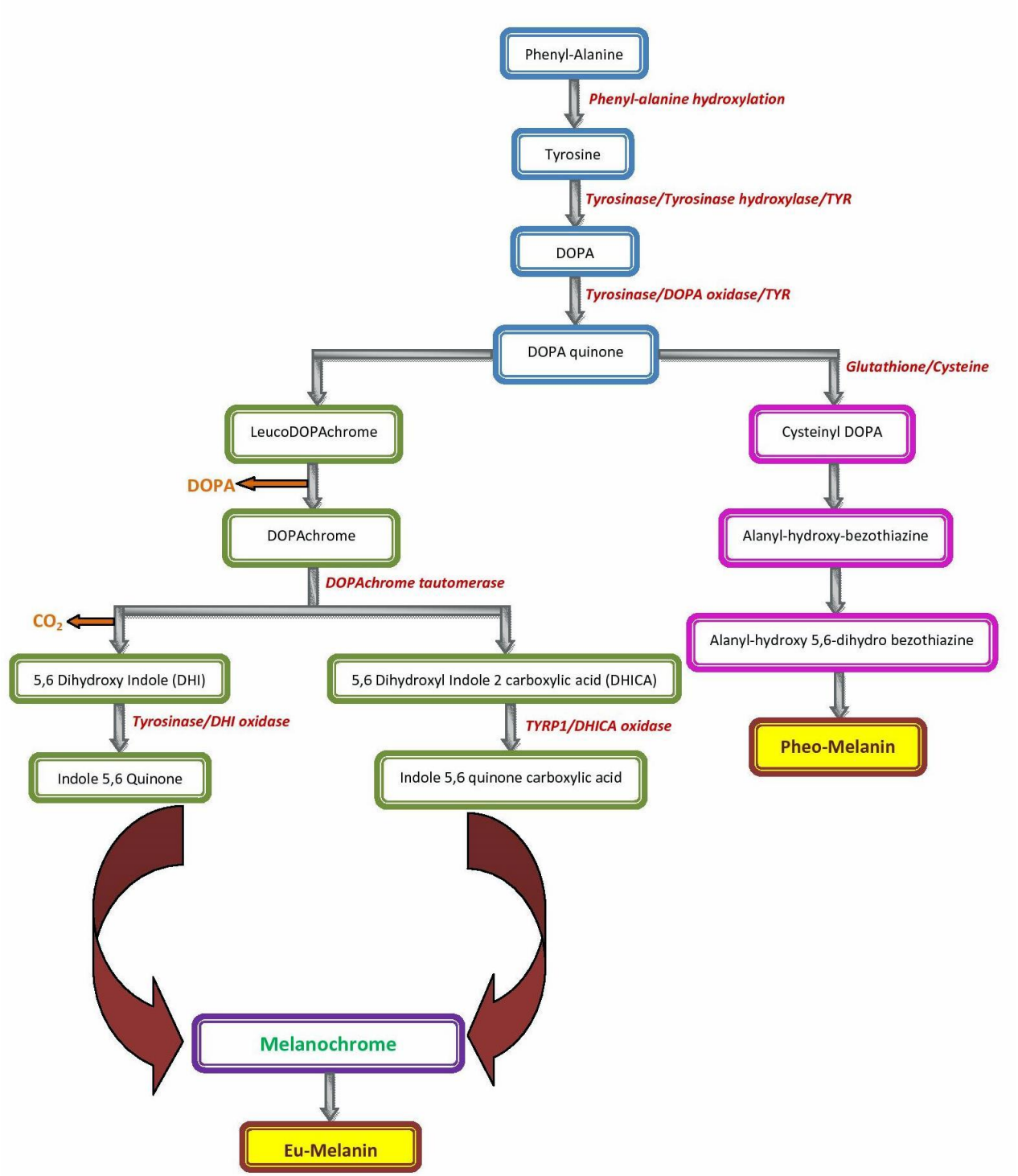


Figure-1: Schematic diagram of Melanogenesis pathway.

To add fluxes into the static path it is necessary to reconstruct the system (Melanogenesis). It would result in the creation of dynamic model of the system in study. This is the defined system on which the entire analysis will be later carried out.

Here we have built a dynamic map of melanogenesis pathway [Figure 3]. All the reactions and regulations of the system are studied in detail and a network is generated. This network is used for further analysis and calculations.

Herein we have defined our fluxes in two different terms, internal fluxes and external fluxes. The internal fluxes are the flux flowing within a defined system of study and the external fluxes are the ones coming from outside into the system or leaving the system [Table 1].

Table 1: List of internal and external fluxes.

External Fluxes	Internal Fluxes
b1, b2, b3 & b4	v1, v2, v3, v4, v5, v6, v7, v8, v9, v10 &v11

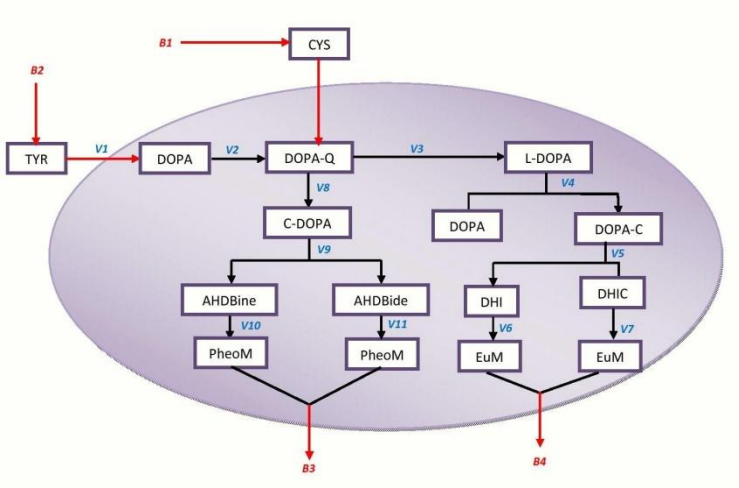


Figure 3: Flux Flow of Melanogenesis pathway.

2. Construction of Stoichiometric matrix:

The metabolic network constructed is later transformed into a stoichiometric matrix 'S' [Table 2]. The matrix describes the relationship between the metabolites and the products. This S is an m×n matrix of stoichiometric coefficients corresponding to the various reactions under the pathway in study. The rows in S are the compounds or metabolites (m) and the columns assimilate the chemical reactions or fluxes within the metabolic network (n). The elements of the matrix are the associated stoichiometric coefficients. The negative element in the matrix signifies the consumption of the compound and the positive means the production of the same [12].

The stoichiometric matrix of the melanogenesis pathway is mentioned in the table [Table 2].

Table 2: Matrix 'S' representing stoichiometric coefficients of the corresponding metabolites

METABOLITES	REACTION FLUXES														
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	B1	B2	B3	B4
A	-1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
B	1	-2	0	1	0	0	0	0	0	0	0	0	0	0	0
C	0	2	-1	-1	0	0	0	-2	0	0	0	0	0	0	0
D	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0
G	0	0	0	0	1	0	-1	0	0	0	0	0	0	0	0
J	0	0	0	0	0	1	1	0	0	0	0	0	0	-2	0
K	0	0	0	0	0	0	0	-1	0	0	0	1	0	0	0
L	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0
M	0	0	0	0	0	0	0	0	1	0	-1	0	0	0	0
N	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0
Q	0	0	0	0	0	0	0	0	0	1	1	0	0	0	-2

3. Mass Balance:

The steady state behavior of the network can be explained in terms of mass balance equations. These equations describe the change in concentration of the metabolite over time with respect to the flux through the reactions. The rate of change in concentration of the metabolites is the difference of the rate at which the compound is produced and consumed. It is observed that the flux entering the reaction is equal to the flux exiting the reaction thus, obeying the law of conservation of the mass i.e., there is no loss of mass during the process [13]. The mass balance table for the various steps of the melanogenesis pathway also represents the same behavior [Table 2].

4. Steady State Assumption:

The metabolism has extremely fast transients which results in steady state of the system within seconds. Changes that occur as an outcome of gene regulation are noticeable only after minutes to hours. This assumption is known as quasi steady state approximation [14].

The relationship between the concentration of the metabolites and stoichiometric matrix is

$$\begin{pmatrix} \frac{dA}{dt} \\ . \\ . \\ . \\ \frac{dQ}{dt} \end{pmatrix} = S.v$$

Wherein S is the stoichiometric matrix and v is the quantity of flux within the metabolic network.

At steady state the concentration of the metabolites are constant i.e., change in the amount of compound over time for all the reactions within the system becomes zero [Table 3].

$$\begin{pmatrix} \frac{dA}{dt} \\ . \\ . \\ . \\ \frac{dQ}{dt} \end{pmatrix} = S.v = 0$$

Therefore the required flux distribution belongs to the null space 'S'. Since there are many more reactions than the metabolites (n>m) the system is underdetermine (with n-m degrees of freedom). Thus it requires imposition of additional constant to obtain meaningful solution for steady state flux distribution (15).

5. System Constraints:

Once the system is been defined in terms of the mass balance equation, the constraints can be imposed on to the model to reduce its solution space. This steady state is the primary constraint for FBA. Additionally, physico-chemical constraints were conveniently in cooperated in the system. These were like concentration limit of the metabolites, gene regulation, reversibility of the reactions, energy requirement for cell maintenance, etc.

Table – 2 Mass and flux balance equations of the participating reactions

$$\frac{dA}{dt} = b_2 - v_1 \qquad \frac{dA}{dx} = +1 - 1 = 0$$
$$\frac{dB}{dt} = v_1 - v_2 + v_4 \qquad \frac{dB}{dt} = 1 - 2 + 1 = 0$$
$$\frac{dC}{dt} = v_2 - v_3 - v_8 \qquad \frac{dC}{dt} = 2 - 1 - 1 = 0$$
$$\frac{dD}{dt} = v_3 - v_4 \qquad \frac{dD}{dt} = 1 - 1 = 0$$
$$\frac{dE}{dt} = v_4 - v_5 \qquad \frac{dE}{dt} = 1 - 1 = 0$$
$$\frac{dF}{dt} = v_5 - v_6 \qquad \frac{dF}{dt} = 1 - 1 = 0$$
$$\frac{dG}{dt} = v_6 - v_7 \qquad \frac{dG}{dt} = 1 - 1 = 0$$
$$\frac{dJ}{dt} = v_6 + v_7 - b_3 \qquad \frac{dJ}{dt} = 1 + 1 - 2 = 0$$
$$\frac{dK}{dt} = b_1 - v_8 \qquad \frac{dK}{dt} = 1 - 1 = 0$$
$$\frac{dL}{dt} = v_8 - v_9 \qquad \frac{dL}{dt} = 1 - 1 = 0$$
$$\frac{dM}{dt} = v_9 - v_{11} \qquad \frac{dM}{dt} = 1 - 1 = 0$$
$$\frac{dN}{dt} = v_9 - v_{10} \qquad \frac{dN}{dt} = 1 - 1 = 0$$
$$\frac{dQ}{dt} = v_{10} + v_{11} - b_4 \qquad \frac{dQ}{dt} = 1 + 1 - 2 = 0$$

The most common technique to impose constraint is by defining upper and lower bound for the fluxes [16].

$$0 < v_i < \infty$$

$$-\infty < b_i < +\infty$$

This signifies that all the internal reactions will have fluxes directed in positive direction and external fluxes can be in either direction. However, a finite upper bound can be imposed, based on knowledge of cellular, thermodynamic and actual measurements [15]

6. Model Optimization:

The constraints can also be rewritten as a set of linear equations and the network is already represented in terms of mass balance equations. Here the number of equations are far less than number of known variables (reaction fluxes). Consequently, this set of linear equations are under determined [17]. Thus FBA always involves in optimizing the fluxes so that a particular cellular function can be achieved.

Objective function used in FBA is also of the linear form[18]

$$Z = c \cdot v$$

Where Z is the objective function and c denotes the vector that defines the coefficients or weights for each of the fluxes in v and v is the set of fluxes in the defined system.

Here the optimization strategy attempts to find a solution v that optimizes Z, remaining in the bounded solution space defined by the set of physicochemical constraints.

Since the objective function is a linear equation, FBA becomes a linear programming problem. This can be easily implemented computationally for large systems as well [19].

The objective functions can be of various categories, from physiologically essential to functions that are required for interrogation or exploitation of the given system. The commonly perused objective includes maximization of biomass, ATP production or particular product concentration. The ATP production, may lead to the predictions which will demonstrate the minimal energy requirement for the growth and survival of the organism. Similarly for the metabolites, minimal concentration required for the existence can be determined. However the cell growth objective function (i.e., maximization of biomass) yields insilico predictions that are in accordance to the experimental observation and hence is highly preferred [15].

It is well known that the measurement of fluxes is a tedious job. Although it is possible to solve for a flux distribution by assuming that the underdetermine system is optimized with respect to a objective thus this system is transformed into an optimization problem and furthermore if the objective function is linear in nature it becomes a linear programming query.

7. Linear Programming:

Linear programming is used to calculate solutions for linear equation subjected to constraints. The objective is to optimize a function that is under constraint of a number of inequality functions. The most common procedure to do so is the simplex method [20]. This is the primary method to convert the linear programming problem into a system of linear equations. Here for our system we have a set of mass balance constraints (along with the other linear constraint) in an optimization phase for a given objective function. This is to be solved to obtain the steady state flux distribution.

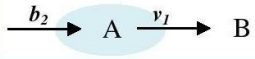
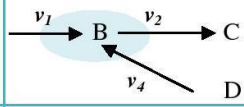
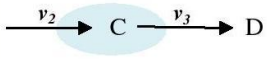
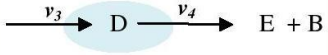
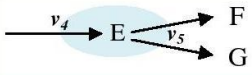
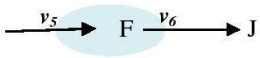
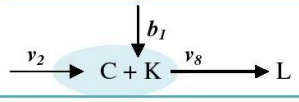
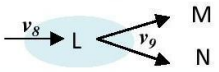
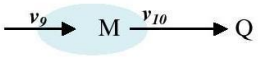
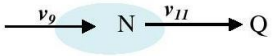
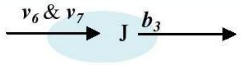
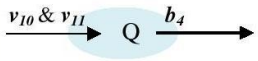
There are a variety of solvers available like COBRA [21], LINDO, CPLEX, GLFK etc but for our analysis “linprog” [22] function packaged with Matlab [23] was used. Operativity of the linprog function is defined as:

[x,fval] = linprog (f,A,b,Aeq,beq,lb,ub)

This defines a set of lower and upper bounds on the design variables, x, so that the solution is always in the range lb <= x <= ub. Set Aeq=[] and beq=[] if no equalities exist.

The codes were written in MATLAB program and the results were observed in graph format. This study was extended to knockout analysis and the results were interpreted and discusses based on the experimentally validated literature.

Table 3: Reactions used to model the melanogenesis pathway by FBA

Equations	Flux Balance
Tyr ⟶ DOPA	
DOPA ⟶ DOPA-Quinone Leuco-DOPA-Quinone ⟶ DOPA	
DOPA-Quinone ⟶ Leuco-DOPA-Quinone	
Leuco-DOPA-Quinone ⟶ DOPA-Chrome + DOPA	
DOPA-Chrome ⟶ DHI + DHICA	
DHI ⟶ Eumelanin	
DHICA ⟶ Eumelanin	
DOPA-Quinone + Cys ⟶ Cysteinyl-DOPA	
Cysteinyl-DOPA ⟶ AHDBine + AHDBide	
AHDBine ⟶ Pheomelanin	
AHDBide ⟶ Pheomelanin	
Eumelanin ⟶ Out of system boundries	
Pheomelanin ⟶ Out of system boundries	

IV. RESULTS AND DISCUSSION

The result of flux analysis reveals the theoretical solutions for the dynamic modeling of melanogenesis pathway. The FBA helps to identify the key rate limiting steps of the pathway [24]. Moreover, this along-with the insilico gene knockout analysis will aid in drug target identification and validation.

A. Theoretical solution:

For the first time in program the flux for all the reactions in melanin synthesis were considered and the graphs obtained shows the steady state behavior with three high variation regions. This is mainly because random number generation for external fluxes is a part of optimization protocol. The first one is in between v1 – v3, since these are the start of the path and the initial concentration is determine by random number generation so high variation is seen. An average of the values can be considered for all practical purposes. The second region is transition from v7 to v8, it is because v7 is eumelanin production and v8 is the onset of pheomelanin pathway, so depending on which path is initiated the flux will be directed accordingly. However, in our study we have considered activation of both the paths at different time so these variations are observed. The last patch of variation lies in region of

external fluxes i.e, v12-v15, since the influx and

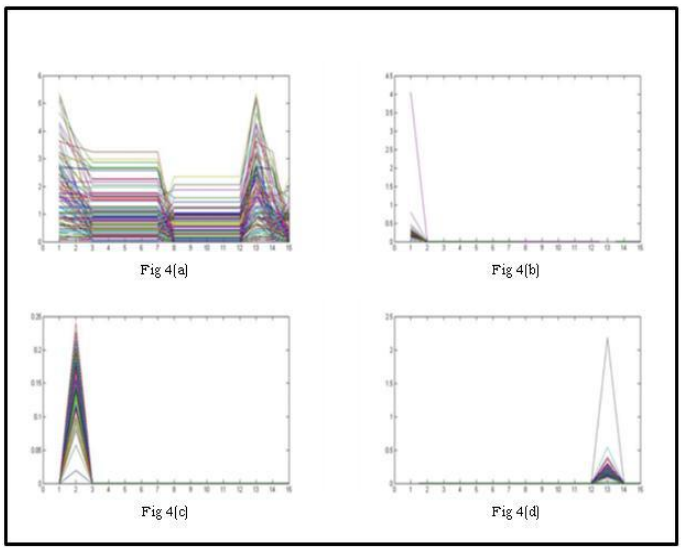


Figure 4: (a) Steady state flux distribution of the system, Flux distribution on (b) v1 inhibition (c) v2 inhibition (d) b2 inhibition

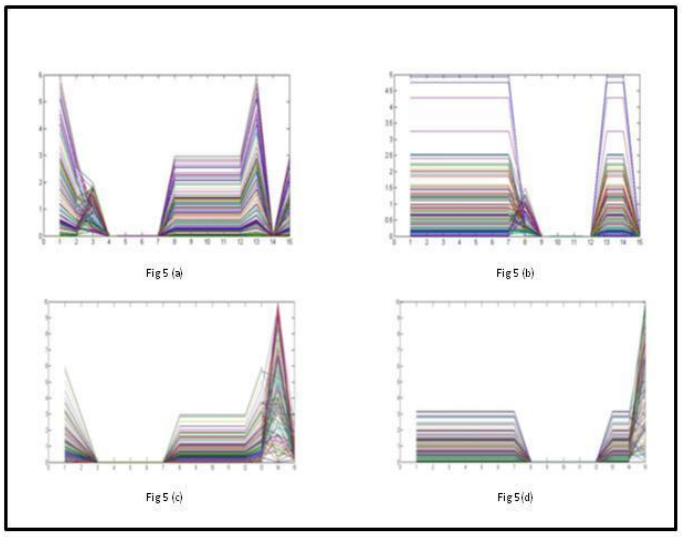


Figure 5: Flux distribution on (a) v3 inhibition, (b) v8 inhibition (c) b3 inhibition (d) b4 inhibition

outflux from the system is not known, hence the program assigns it values within the constraint limit and these values are different each time so variation is observed.

B. Analysis of perturbation for knock-out studies:

The most common perturbation studied using FBA is the deletion of one or more genes from the system. Gene knock-outs in our analysis are performed by constraining flux into the reaction that is catalyzed by the enzyme produced by that gene to zero. Similarly, the effect of inhibitor on the protein can be studied by constraining the upper bound of the fluxes to any defined fraction of the normal flux corresponding to the extents of inhibition.

On the similar grounds knock out analysis of this network is performed. To mimic knock out condition, flux through the metabolite or gene product was blocked as a result of which that gene activity was prohibited. All the fluxes of the system were inhibited one by one and the resultant profiles are studied.

To begin knockout experiments we suppressed v1 first and observed the complete blockage of the melanogenesis. The same results were observed with v2 and b2. These observations are due to the fact that the synthesis path for eumelanin and pheomelanin shares the common entry point till formation of DOPA quinone [Figure 4]. The DOPA quinone is the third product of the network and is also a junction from where the separation of synthesis path of the melanins occurs. Moreover, any inhibition in the first two reactions of the pathway will lead to complete blockage of the network. Hence, suppression of v1, v2, b2 fluxes resulted in entire pathway inhibition [Figure 4 b,c,d].

Further the knockout analysis was performed for v3-v7 and v14 (b3). This results in the inhibition of eumelanin production. Whereas the knockout of v8-v11 along with v12 (b1) and v15 (b4) stopped the pheomelanin synthesis [Figure 5]. This is because the fluxes v3 to v7 and v14 (b3) belongs to Eumelanin synthesis pathway and fluxes ranging from v8 to v11 alongwith v12 (b1) and v15 (b4) are a part of pheomelanin synthesis cascade. Therefore, inhibition of these fluxes will lead to the prevention of the flux from the respective network.

C. Bifurcation of melanogenesis into eumelanin and pheomelanin synthesis cascade:

It is noticeable [Figure 4, 5] that both Eumelanin and pheomelanin paths are independent and can co-occur, it means that on inhibition of fluxes from one path did not affect the other. For example blocking of pheomelanin synthesis cascade will disturb or interfere with production of Eumelanin and vice-versa.

D. Detecting Rate-controlling step of the pathway:

These knock-out studies gives us information about the vulnerability of the enzymes involved and rate limiting step of the network. The results in our study were explored to find the rate limiting step of the path [Figure 2]. It revealed that the enzyme involved in first two steps seems to be the rate limiting because when they are knocked out, melanin synthesis was completely stopped. Since no other alternate route is available to complete the synthesis. Moreover literature also suggests the same. Hence, tyrosinase enzyme is the rate limiting enzyme, inhibition of which results in complete inhibition of the melanogenesis pathway [25].

V. FUTURE PROSPECTS:

Flux Balance Analysis gives a general idea of the metabolic capabilities of an organism in study. In-silico models that are generated via this approach can be extensively used in drug discovery path, detecting essentiality of a gene and in providing necessary information to the biologist that will aid in designing experiments which will lead to conclusive discovery. The gene knock out studies performed on the generated models can be extensively used to predict lethality of the gene deletion [26] and also helps in evaluation of gene essentiality.

To anticipate indispensable gene pair, double knockout studies can be performed. However, by constraining gene expression at different levels can provide knowledge about the vulnerability of the protein. All these are vital informations, which are required for suitable target identification [27]. Thereby fitness of the suggested inhibitor can be learned.
Furthermore, growth of microbes and gene essentiality under different media & other conditions can be monitored.
FBA can help in identification of core metabolic reactions and also in optimization of bioprocess in microbes based industries [28]. Analysis of these networks assists in finding gene function and also in augmentation of already annotated genes [29]. Besides this, it can also predict novel regulatory mechanism which can assist in refinement of existing model. Based on the information from these models genetically engineered organism can be generated which will produced desired metabolite in high concentrations. Discrepancies results in model refinement so by integrating experimental data and modeling results in an iterative manner can lead to robust model and yield valuable insights about biology of the system modeled [30].

VI. CONCLUSION:

Identification of an adept drug target is a requisite of rational drug design process. Enzymes, in this regard scores over other macromolecular classes since they provide a great deal of information about essentiality, vulnerability and insights about its % inhibition required for cell cidality. All the above stated features can be studied with FBA.
Within melanosomes, at least three key-enzymes, named tyrosinase, Tyrosinase-related protein 1 (Tyrp1), and Tyrosinase-related protein 2 (Tyrp2/Dct) are absolutely required for the synthesis of different types of melanin. The tyrosinase gene family members are the main enzymes and regulatory proteins of melanogenesis (Tsukamoto et al, 1992). Tyrosinase is responsible for the critical initial rate limiting steps of melanogenesis and hence is the most suitable target to study. This is an experimentally validated outcome [ref].
FBA doesn't generate precise solution but it gives a solution space which would contain all the possible solution to the network is present [31]. This range of solution will generate new ideas, hypothesis and direction for the investigation by the biologist. This is an essential part of the systems based experimental-computational paradigm also known as wiechert's algorithm [32]. This would lead to synergistic outcomes. One of the key advantages of FBA over classical kinetic modeling is that it enables prediction of network phenotypes given far less knowledge of enzyme kinetic parameters. However, integration of all forms of modeling approaches like kinetic, topological, constraint based etc will be helpful in deriving meaningful conclusions for biological challenges.

ACKNOWLEDGMENT

This project is supported by the M.P. Council for Science and Technology (MPCST), India, under Endt No. 6087/CST/BTAC/2011. The authors gratefully acknowledge Maulana Azad National Institute of Technology, Bhopal (INDIA) for its facilities and support.

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