Development of Generic System/Synthetic Biology simulation network using Metabolic Flux Balance Analysis

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CERTIFICATION

I hereby declare that the project titled "**Development of Generic System/Synthetic Biology simulation network using Metabolic Flux Balance Analysis"** is submitted as a project work has been carried out by me at Jaypee University of Information Technology, Solan under the guidance of Dr. Sree Krishna Chanumolu**.** Any further extension, continuation or use of this project has to be undertaken with prior express written consent from the Supervisor, Jaypee University of Information Technology, Solan-173234.

I further declare that the project work or any part thereof has not been previously submitted for any degree or diploma in any university.

Signature of Supervisor ……..

Name of Supervisor ……..

Designation ……..

Date ……..

ACKNOWLEDGEMENT

I acknowledge my sincere thanks to **Dr. Sree Krishna Chanumolu**, *Jaypee University of Information Technology, JUIT, Solan* for giving me this great opportunity to have project work under him and throughout this project, enlightening me on various topics and creating a congenial environment for my work. I am sure it would continue to raise research interests in young students like me.

I am grateful to the PhD student Tarun,*at Jaypee University of Information Technology, JUIT,solan*who helped me throughout this project and supported me in this Project.

Signature of the student ……..

Name of Student ……..

Date ……..

ABSTRACT

Renewed interest in metabolic research over the last two decades has inspired an explosion of technological developments for studying metabolism. At the forefront of methodological innovation is an approach referred to as "untargeted" or "discovery" metabolomics. We are referring KEGG data base to get the pathways information as an input which is present in the in XML format. Main aim is to calculate the rate of the reaction which are present in the reaction which is a simulation of the real world reactions. The algorithm platform developed for integration of KEGG data with a method to find the rate of the reaction of all the reaction occurring in the pathways. In this algorithm we are using the Stoichiomatric matrix to find the rate, which can be further use to decide which reaction to be eliminated or which product to be added to get better the results.

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CHAPTER 1

Introduction

1.1 Literature Studied and Code development

1.1.1 Metabolic Networks

The function of cells is based on complex networks of interacting chemical reactions carefully organized in space and time. These biochemical reaction networks produce observable cellular functions. Network re-construction is the process of identifying all the reactions that comprise a network. The reconstruction process for metabolic networks has been developed and implemented for a number of organisms. The main features of metabolic network reconstruction are described in this chapter. We briefly review the key properties of metabolic networks and introduce the hierarchical thinking that goes into the interpretation of complex network functions. A true genome-scale reconstruction of cellular functions necessitates accounting for all cellular networks simultaneously. Such a comprehensive network reconstruction has yet to be established therefore, here, we focus on metabolism and address the reconstruction of transcriptional regulatory and signaling net-works.

1.1.2 Flux balance analysis

Flux balance analysis (FBA) is a mathematical method for simulating metabolism in genome scale reconstructions of metabolic networks. In comparison to traditional methods of modeling, FBA is less intensive in terms of the input data required for constructing the model. Simulations performed using FBA are computationally inexpensive and can calculate steady-state metabolic fluxes for large models (over 2000 reactions) in a few seconds on modern personal computers.

The results of FBA on a prepared metabolic network of the top six reactions of glycolysis. The predicted flux through each reaction is proportional to the width of the line. Objective function in red, constraints on alpha-D-Glucose and beta-D-Glucose import represented as red bars.[citation needed]

FBA finds applications in bioprocess engineering to systematically identify modifications to the

metabolic networks of microbes used in fermentation processes that improve product yields of industrially-important chemicals such as ethanol and succinic acid. It has also been used to identify putative drug targets in cancer and pathogens, rational design of culture media, and more recently host–pathogen interactions have been studied using FBA. The results of FBA can be visualized using flux maps like the image on the right which illustrates the steady-state fluxes carried by reactions in glycolysis. The thickness of the arrows is proportional to the flux through the reaction.

1.1.3 Metabolic Pathways

A metabolic pathway is a series of chemical reactions occurring within a cell. In a pathway, the initial chemical (metabolite) is modified by a sequence of chemical reactions. These reactions are catalyzed by enzymes, where the product of one enzyme acts as the substrate for the next. These enzymes often require dietary minerals, vitamins, and other cofactors to function.

Pathways are required for the maintenance of homeostasis within an organism and the flux of metabolites through a pathway is regulated depending on the needs of the cell and the availability of the substrate. The end product of a pathway may be used immediately, initiate another metabolic pathway or be stored for later use. The metabolism of a cell consists of an elaborate network of interconnected pathways that enable the synthesis and breakdown of molecules (anabolism and catabolism).

1.1.4 Basic Features

Intermediary metabolism can be viewed as a chemical “engine” that converts available raw materials into energy as well as the building blocks needed to produce biological structures, maintain cells, and carry out various cellular functions.

This chemical engine is highly dynamic, obeys the laws of physics and chemistry, and is thus limited by various physicochemical constraints. It also has an elaborate regulatory structure that allows it to respond to a variety of external perturbations. Metabolic imbalance is involved in major human diseases, such as diabetes, obesity, cancer, and heart disease.

Metabolism comprises two types of chemical transformations: catabolic pathways that break

down various substrates into common metabolites and anabolic pathways that collectively synthesis amino acids fatty 'Kids nucleic acids and other needed building blocks. During these processes, an intricate exchange of various chemical groups and reduction oxidation (redox) potentials takes place through a set of carrier molecules.

These carrier molecules and the properties that they transfer thus tie the metabolic network tightly together. Intermediary metabolism can be described at several levels of complexity

1.1.5 Hierarchy in function of metabolic networks

Genome-scale reconstructions of metabolic networks contain hundreds of *metabolites and sometimes over a thousand reactions (see Table 3.6). The functions of such networks are hard for the human mind to comprehend. We thus need mathematical models for the study of their properties and simulation of their function.

We can think of network properties in a hierarchical fashion to simplify the conceptualization of network functions. Such hierarchy can be based on manmade concepts, as discussed later, or can be the result of a nonbiased mathematical analysis of the stoichiometric matrix. In what follows, we briefly describe the traditional view of the hierarchical decomposition of the functions of metabolic networks

Level I:

Cellular inputs and outputs. Overall, intermediary metabolism comprises the enzymatic reactions pertaining to the transformation of substrate molecules into the essential building blocks of macromolecules and other vital products for growth and maintenance.

A coarse-grained description of the overall activity of metabolism thus involves substrates as inputs and biomass and metabolic by-products as outputs. For industrial fermentation processes. a description of cells at this level has sufficed for many purposes.

The description comprises a simple set of coupled mass and energy balances, with various empirically determined yield coefficients that describe partitioning of the consumed substrate. Growth kinetics are given in terms of simple phenomenological models such as the Monod growth model.

Models of this type are useful for a limited set of specific conditions. The yield coefficients are not constants: they change with the physiological state of the cell.

Level 2: Sectors.

A bit finer grained look at intermediary metabolism reveals that it can be divided into two basic sectors. Catabolism carries out the degradation of substrates via a series of con-verging pathways that lead to a set of 11 metabolites of central importance, called the biosynthetic precursors.

Anabolism is a set of diverging pathways that originate from these central metabolites to form monomers or building blocks for macromolecular biosynthesis. Genetically engineered bacteria used for bioprocessing, for instance, can be described at this level of complexity since it is appropriate for assessing host-plasmid interactions.

Level 3: Pathways.

A still finer resolution reveals a situation in which path-ways, and segments thereof, serve a definite role. For instance, catabolism of the major classes of biomolecules follows the same pattern; first substrates are picked up by the cell, hydrolyze if necessary, activated by as cofactor, and then degraded to yield energy and other properties stored on the carrier molecules. At this level of description, the essential features of metabolism begin to depend on basic chemical principles such as stoichiometric structure and kinetic regulation.

Key metabolic pools, such as the energy charge, dominate the description, and key regulatory enzymes influence the motion of these pools and how mass and energy is distributed among them. There is currently much interest in the pathway level characterization of reconstructed biochemical reaction networks.

Level 4: Individual reactions

At the finest level of description one considers all the biochemical transformations that take place in a cell. Available high-throughput data allows us to generate the information needed to describe cells at this resolution.

It is at this level where this book is focused. We can now reconstruct genome-scale stoichiometric matrices of organisms and study them. The dimensions of these matrices are on the order of hundreds of metabolites and sometimes over a thousand chemical reactions reflecting the complexity of a fully functional metabolic network. Biochemical transformations fall into a few major categories. Some examples include transamination, phosphorylation, isomerization, dehydration, and dismutation.

Thus, there are chemical "rules" that dictate what kind of links can exist in metabolic networks. As described later, bio-chemists have devised nomenclature that classifies these types of transformations and an Enzyme Commission (E.C.) number is associated with each enzymatically catalyzed metabolic reaction.

Furthermore, there are thermodynamic restrictions associated with these transformations that dictate the energetic feasibility of a reaction and its equilibrium state. Thus, even though metabolic networks may appear complex, there are underlying physicochemical restrictions on their topological structure and network states.

1.1.6 Anabolic pathway

Anabolism is the set of metabolic pathways that construct molecules from smaller units. These reactions require energy. One way of categorizing metabolic processes, whether at the cellular, organ or organism level is as "anabolic" or as "catabolic", which is the opposite. Anabolism is powered by catabolism, where large molecules are broken down into smaller parts and then used up in respiration. Many anabolic processes are powered by the hydrolysis of adenosine triphosphate (ATP).

Anabolic processes tend toward "building up" organs and tissues. These processes produce growth and differentiation of cells and increase in body size, a process that involves synthesis of complex molecules. Examples of anabolic processes include the growth and mineralization of bone and increases in muscle mass. Endocrinologists have traditionally classified hormones as anabolic or catabolic, depending on which part of metabolism they stimulate. The classic anabolic hormones are the anabolic steroids, which stimulate protein synthesis and muscle

growth, and insulin. The balance between anabolism and catabolism is also regulated by circadian rhythms, with processes such as glucose metabolism fluctuating to match an animal's normal periods of activity throughout the day.

1.1.7Catabolic pathway

Catabolism (from Greek κάτω kato, "downward" and βάλλειν ballein, "to throw") is the set of metabolic pathways that breaks down molecules into smaller units to release energy.[1] Catabolism breaks down large molecules (such as polysaccharides, lipids, nucleic acids and proteins) into smaller units (such as monosaccharides, fatty acids, nucleotides, and amino acids, respectively). As molecules such as polysaccharides, proteins, and nucleic acids comprise long chains of these small monomer units (mono = one + mer = part), the large molecules are called polymers (poly = many).

Cells use the monomers released from breaking down polymers to either construct new polymer molecules, or degrade the monomers further to simple waste products, releasing energy. Cellular wastes include lactic acid, acetic acid, carbon dioxide, ammonia, and urea. The creation of these wastes is usually an oxidation process involving a release of chemical free energy, some of which is lost as heat, but the rest of which is used to drive the synthesis of adenosine triphosphate (ATP). This molecule acts as a way for the cell to transfer the energy released by catabolism to the energy-requiring reactions that make up anabolism. (Catabolism is seen as destructive metabolism and anabolism as constructive metabolism). Catabolism therefore provides the chemical energy necessary for the maintenance and growth of cells. Examples of catabolic processes include glycolysis, the citric acid cycle, the breakdown of muscle protein in order to use amino acids as substrates for gluconeogenesis, the breakdown of fat in adipose tissue to fatty acids, and oxidative deamination of neurotransmitters by monoamine oxidase.

An example of catabolic reaction

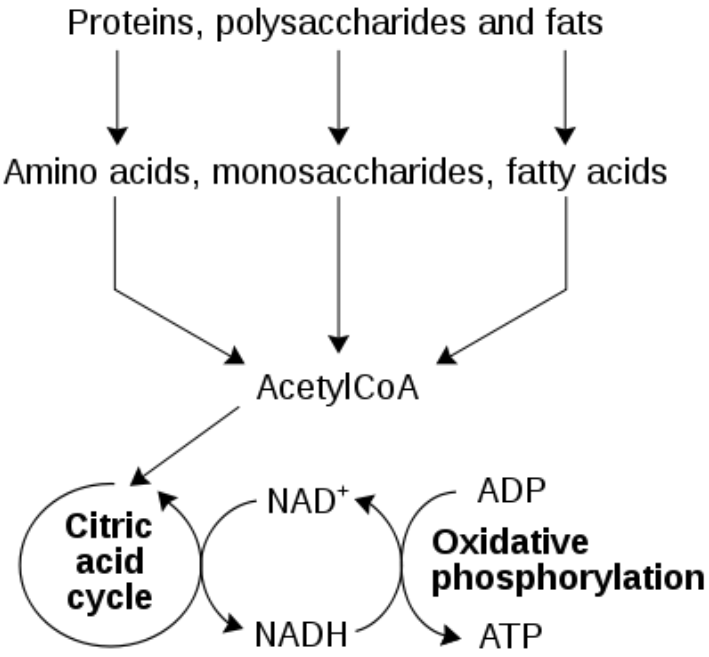


FIG1. Diagrammatic representation of a catabolic reaction

1.1.8 Glycolysis

Glycolysis is the metabolic pathway that converts glucose, into pyruvate. The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide).

Glycolysis is a determined sequence of ten enzyme-catalyzed reactions. The intermediates provide entry points to glycolysis. For example, most monosaccharides, such as fructose and galactose, can be converted to one of these intermediates. The intermediates may also be directly useful. For example, the intermediate dihydroxyacetone phosphate (DHAP) is a source of the glycerol that combines with fatty acids to form fat.

Glycolysis occurs, with variations, in nearly all organisms, both aerobic and anaerobic. The wide occurrence of glycolysis indicates that it is one of the most ancient metabolic pathways. Indeed, the reactions that constitute glycolysis and its parallel pathway, the pentose phosphate pathway, occur metal-catalyzed under conditions of the Archean ocean also in the absence of enzymes. Glycolysis could thus have originated from chemical constraints of the prebiotic world.

Glycolysis occurs in most organisms in the cytosol of the cell. The most common type of glycolysis is the Embden–Meyerhof–Parnas (EMP pathway), which was discovered by Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas. Glycolysis also refers to other pathways, such as the Entner–Doudoroff pathway and various heterofermentative and homofermentative pathways. However, the discussion here will be limited to the Embden–Meyerhof–Parnas pathway.

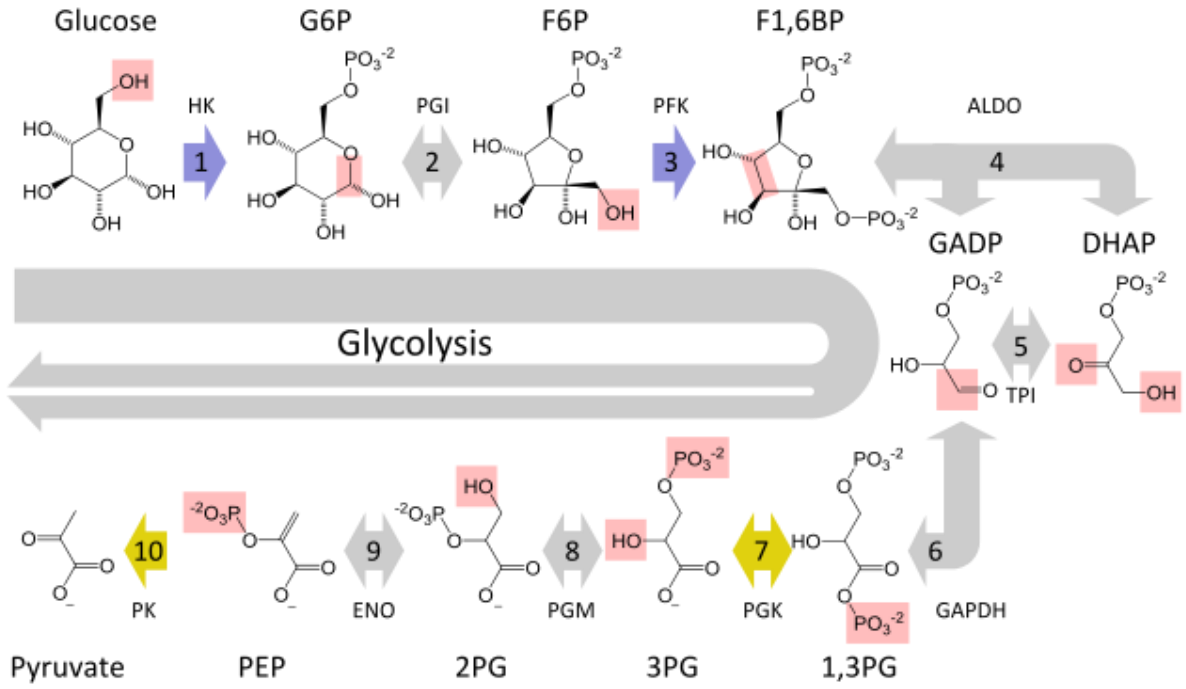


FIG2. Diagrammatic representation of anabolic reaction(glycolysis)

1.1.9Stoichiometric matrix

In complex reactions, stoichiometries are often represented in a more compact form called the stoichiometry matrix. The stoichiometry matrix is denoted by the symbol **N**.

If a reaction network has **n** reactions and **m** participating molecular species then the stoichiometry matrix will have corresponding**m** rows and **n** columns.

For example, consider the system of reactions shown below:

S1 → S2

5 S3 + S2 → 4 S3 + 2 S2

S3 → S4

S4 → S5.

This systems comprises four reactions and five different molecular species. The stoichiometry matrix for this system can be written as:

$$\mathbf{N} = \begin{bmatrix} -1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

Where the rows correspond to S1, S2, S3, S4 and S5, respectively. Note that the process of converting a reaction scheme into a stoichiometry matrix can be a lossy transformation, for example, the stoichiometries in the second reaction simplify when included in the matrix. This means that it is not always possible to recover the original reaction scheme from a stoichiometry matrix.

Often the stoichiometry matrix is combined with the rate vector**v**, and the species vector, **S** to form a compact equation describing the rates of change of the molecular species:

$$\frac{d\mathbf{S}}{dt} = \mathbf{N} \cdot \mathbf{v}.$$

1.2 Languages

1.2.1 R

R is a programming language and software environment for statistical computing and graphics. The R language is widely used among statisticians and data miners for developing statistical software and data analysis.

1.2.2 SBML

The Systems Biology Markup Language (SBML) is a representation format, based on XML, for

communicating and storing computational models of biological processes. It is a free and open standard with widespread software support and a community of users and developers. SBML can represent many different classes of biological phenomena, including metabolic networks, cell signaling pathways, regulatory networks, infectious diseases, and many others. It is the de facto standard for representing computational models in systems biology today.

1.2.3 PHP

PHP is a server-side scripting language designed for web development but also used as a general-purpose programming language. As of January 2013, PHP was installed on more than 240 million websites (39% of those sampled) and 2.1 million web servers. Originally created by Rasmus Lerdorf in 1994, the reference implementation of PHP (powered by the Zend Engine) is now produced by The PHP Group. While PHP originally stood for Personal Home Page, it now stands for PHP: Hypertext Preprocessor, which is a recursive backronym.

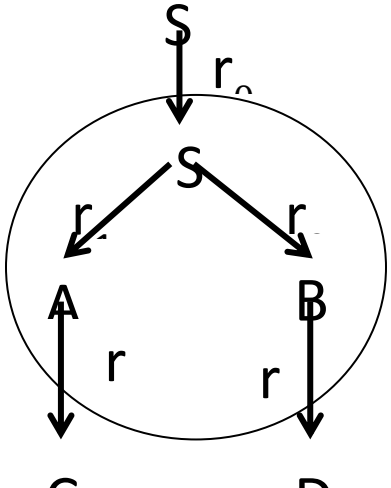
1.3 Linkage of R with SBML

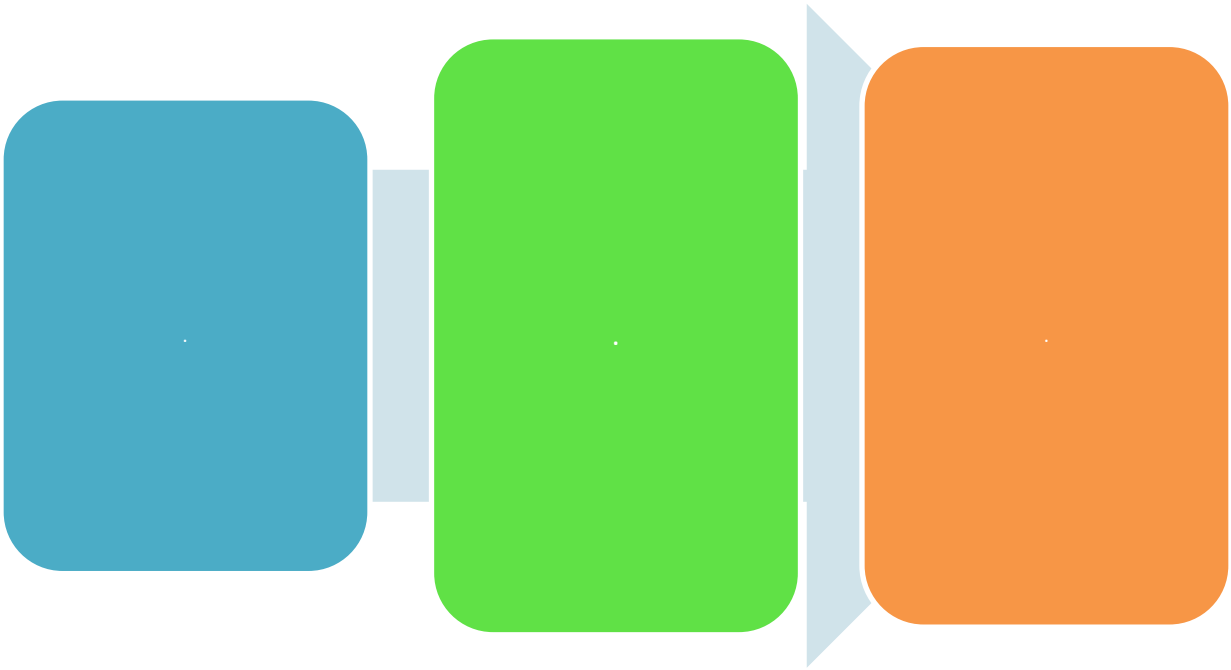
R is a language which is widely used by bioinformaticians all over the world because is it very user friendly easy and full of functions. On the other hand SBML is a language which is specially made for the people working on System biology in which we deal with pathways, cell its functions working etc. There are many ways to use SBML one them is to integrate with R.

To integrate R with SBML we need to download R console, platform on which R runs. So there is a special Library of SBML (LibSBML) should be incorporated with R console version2.15 Because LibSBML is not compatible with newer version of R console.

1.4 Calculation of rate of the reaction.

In case of stoichiometric matric main aim is to find the rate of the reaction in the metabolic pathway this is how we calculate the rate of the reactio





Reaction 0: $S_o \xrightarrow{r0} S$

Reaction 1: $S \xrightarrow{r1} A$

Reaction 2: $S \xrightarrow{r2} B$

Reaction 3: $A \xrightarrow{r3} C$

Reaction 4: $B \xrightarrow{r4} D$

$$\frac{dS_o}{dt} = -r_o$$

$$\frac{dS}{dt} = r_o - r_1 - r_2$$

$$\frac{dA}{dt} = r_1 - r_3$$

$$\frac{dB}{dt} = r_3 - r_4$$

$$\frac{dC}{dt} = r_3$$

$$\frac{dD}{dt} = r_4$$

This method is transformed into Matrix, which is as shown bellow

$$\frac{d}{dt}\begin{bmatrix} S_O \\ S \\ A \\ B \\ C \\ D \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 \\ 0 & 0 & 1 & 0 & -1 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} r_O \\ r_1 \\ r_2 \\ r_3 \\ r_4 \end{bmatrix}$$

FIG3. Stoichiomatric matrix

1.6 The steady-state assumption

The assumption of equilibrium between E and ES is unnecessarily restrictive. Briggs and Haldane showed in 1926 that an equation formally similar to equation may be derived without this assumption. They assumed instead a steady state, i.e. that the

Concentrations of E and ES remained effectively constant over the period of the rate measurement. According to the equilibrium assumption, the rate of formation of ES from E and S equals its rate of dissociation to E and S. The steady state assumption requires only that the rate of formation of ES should equal its rate of breakdown in any direction, including product formation, which need not be slow relative to the back-dissociation to E and S. A useful physical analogy is that of a large jug pouring water at a steady rate into a funnel. At first the rate of inflow greatly exceeds the rate of outflow, but this makes the water level in the funnel (cf. [ES]) rise. The rate of outflow (product formation) therefore increases until it exactly matches the inflow. Thereafter the level of waterin the funnel and the rate of flow remain constant until the jug is emptied. The analogy is not perfect. The rate of formation of ES decreases as the steady state is established, because ES is formed at the expense of E. The jug's pouring rate, by contrast, is independent of the height of the water in the funnel. There is also no counterpart to the

dissociation of ES to E and S: the water does not jump back from the funnel into the jug. This perhaps serves to emphasize the nature of a steady state as opposed to an equilibrium. The steady state is still an assumption, albeit less restrictive than the equilibrium assumption, and its validity has been the subject of recurrent debate. The steady state may, however, sometimes be directly demonstrated, provided that the enzyme-substrate complexes differ appreciably from free enzyme in physical properties that can be conveniently measured on a millisecond timescale. After very rapid mixing of a concentrated solution of enzyme with its substrate(s), e.g. in a 'stopped-flow' apparatus, the build-up and decay of enzyme-substrate complexes is directly observed and recorded. The duration of the steady state, for a fixed enzyme concentration, is a function of the substrate concentration and the turnover capacity of the enzyme. To return to our analogy, if there is not much water in the jug it may all flow through before thelevel in the funnel has a chance to reach its steady state: Likewise if the neck of the funnel is very wide. It seems likely, therefore, that the steady state assumption is justified under the conditions of most experiments in which the enzyme concentration is low (in molar terms) and the substrate concentration is relatively high. Empirically, the assumption has been justified by its success in explaining and predicting kinetic patterns for many enzymes. As we shall see, it is also often possible to make independent checks on the validity of kinetic deductions. Turning now to the mathematical consequences of the steady-state treatment, we have from Fig bellow

$$\text{rate of formation of ES} = k_1 [\text{E}] [\text{S}]$$

$$\text{rate of removal of ES} = k_2 [\text{ES}] + k_3 [\text{ES}] = (k_2 + k_3)[\text{ES}].$$

$$k_1 [\text{E}] [\text{S}] = (k_2 + k_3)[\text{ES}]$$

$$\therefore [\text{E}] = [\text{ES}] \frac{(k_2 + k_3)}{k_1 [\text{S}]}.$$

$$v = \frac{k_3 e}{1 + \dfrac{k_2 + k_3}{k_1 [\text{S}]}} = \frac{k_3 e [\text{S}]}{[\text{S}] + \dfrac{k_2 + k_3}{k_1}}.$$

Since kl , k2 and k3 are all constants, this equation once again may be re-written as equation , but the value of Km, the Michaelis constant, is no longer the same. Whereas the Michaelis—Menten treatment leads to an equation in which K. = k2lki, in the Briggs—Haldane treatment Km = (k2 + k3)/k, Thus effectively the difference between the two treatments lies in the relative values of k2 and k3. These two constants determine the fate of ES. Either it can return whence it came, releasing S at a rate determined by k2, or it can proceed to form product at a rate determined by k3. If k3 is very much smaller than k2, so that the chances of reaction to yield P are small for any given molecule of ES compared to the chances of dissociation back to S, then k2 + k3 will approximate to k2, E and ES will be virtually at equilibrium, and the product-forming step will be, as it were, a small leak out of that equilibrium system. Thus, if K2 k3, the Briggs—Haldane equation reduces to the Michaelis—Menten equation. Equally plausible, however, is the alternative extreme assumption that k3 K2, so that Km becomes k3/k, this exposes the dubious nature of the frequent assertion that Km reflects an enzyme's affinity for its substrate, a low Km representing high affinity and vice versa. This is valid when the Michaelis—Menten assumption holds and Km = since k2/k, is the dissociation constant for ES. Clearly, however, if k3 K2, Km is bound to be much larger than the dissociation constant. The ideal procedure would be to test the Michaelis—Menten assumption by measuring the Km and the dissociation constant for S separately and comparing them. Unfortunately, for a one-substrate enzyme, thiscannot be done, since one cannot mix E and S without a reaction occurring.. In summary, therefore, in the absence of other evidence, K. should be regarded simply as an empirical constant equal to the substrate concentration that gives 11/,,,a,, under defined experimental conditions.

1.7 Pseudo steady state

The assumption of a pseudo-steady state can simplify a wide variety of kinetic problems. The most elementary application of this assumption yields the Michaelis—Menten equation (10a) that is a keystone of theoretical biochemistry. For both of these reasons it is worth carefully working out the conditions under which the pseudo-steady state assumption (7) is expected to be valid. The key concept here is that of 'time scale', the order of magnitude of time that

characterizes the duration of a process or subprocess. For example, what is the time scale of the fast transient process during which the complex concentration changes from its initial value of zero to a pseudo-state condition? Does it take microseconds, milliseconds or seconds? To estimate the duration of this period we can make the approximation S = So in (6c). This transforms (6c) into a linear equation, with the solution

$$C(t) = \bar{C}[1 - \exp(-\mu t)], \qquad \mu \equiv k_{+1}(S_0 + K_m),$$
$$\bar{C} \equiv E_0 S_0/(K_m + S_0).$$

$$t_S \approx \frac{\text{total change in } S \text{ after fast transient}}{\max |dS/dt| \text{ after fast transient}}.$$

The numerator of above equation is approximately S0. Assuming the validity of steady state assumption that denominator is given by above equation.

$S = S_0$. Thus $t_S \approx S_0/[k_{+2}E_0 S_0/(K_m + S_0)]$, i.e.

One necessary criterion for the validity of the pseudo-steady state assump-tion is that the 'fast transient' is indeed brief compared to the time during which the substrate changes appreciably. This criterion is tc <C is or, from above equations.

$$\frac{E_0}{K_m + S_0} \ll \left(1 + \frac{k_{-1}}{k_{+2}}\right)\left(1 + \frac{S_0}{K_m}\right).$$

1.8 S as a Connectivity Matrix

A network can be visually represented as a map. Each node in the map corresponds to a row in a connectivity matrix, and each column corresponds to a link in the map.

1.8.1 The maps of S

The reaction map S represents a map where a compound is a node and the reactions connect (link) the compounds.This map is the reaction map (also called reaction-centered map) and is the standard way of viewing metabolic reactions and pathways in biochemistry textbooks.

1.8.2 The compound map

The negative of the transpose of the stoichiometric matrix, -ST, also represents a map which we will call the compound map (also referred to as the metabolite-centered map). The map that —ST represents has the reactions (now the rows in —ST) as the nodes in the network and the compounds (now the columns of —ST) as the connections, or the links. This representation of a biochemical reaction network is unconventional, but useful in many circumstances.

Examples:

The compound map for glycolysis is shown in Figure bellow. The compound map can be complicated notably by highly connected co-factor molecules.

1.8.3 Biological quantities displayed on maps

It is worth examining the columns (si^v) and rows of S a bit more closely. Let's examine a reaction:

$$x_1 + x_2 \xrightarrow{v_i} x_3 + x_4$$

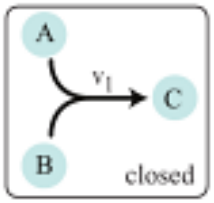
With the corresponding column of S, = (-1, -1, 1, 1) T. This vector is in the column space of S. Moving along this vector is like carrying out this reaction. Note that motion along this vector will conserve the sum x1 + x2 + x3 + x4. Thus, a column in S represents a 'tie' between the compounds participating in a particular reaction. If these compounds participate in other reactions, there will be interactions between the motions along the columns of S. These vectors,

'if', span the column space of S and thus give a conceptually useful basis for the column space of S. As we will see, certain combinations of the column vectors form pathways through the reaction map.

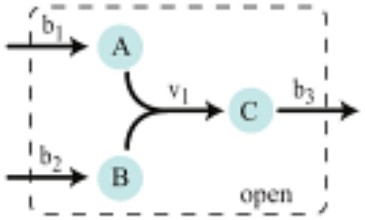
Similar observations apply to the rows of S (or the columns of —ST). A column in—ST will 'tie' together, or connect, all the reactions in which a metabolite participates. These connections, however, do not imply any particular relationship among reac-tions, and therefore are not considered 'hard' connections. As will be further discussed in Chapter 11, metabolite pools form, which are the linear combinations of metabolite concentrations. These pools represent a conservation among metabolites that is mediated by specific reactions, and therefore represents a more meaningful relationship among reactions in which the metabolites participate. Note that the columns of S, in contrast, create a 'hard' connection between the metabolites, as a reaction will simultaneously use and produce the participating compounds. Conversely, the connectivity's created between the reactions are 'soft,' as the reactions in which a compound participates can have varying flux levels that may not have fixed ratios. These ratios are determined by the kinetic properties of the reactions.

Reaction Maps

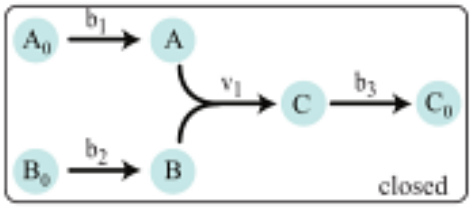
$$S_{int} = \begin{matrix} v_1 \\ \begin{bmatrix} -1 \\ -1 \\ 1 \end{bmatrix} \end{matrix} \begin{matrix} A \\ B \\ C \end{matrix}$$



$$S_{exch} = \begin{matrix} v_1 & b_1 & b_2 & b_3 \\ \begin{bmatrix} -1 & 1 & 0 & 0 \\ -1 & 0 & 1 & 0 \\ 1 & 0 & 0 & -1 \end{bmatrix} \end{matrix} \begin{matrix} A \\ B \\ C \end{matrix}$$



$$S_{tot} = \begin{matrix} v_1 & b_1 & b_2 & b_3 \\ \begin{bmatrix} -1 & 1 & 0 & 0 \\ -1 & 0 & 1 & 0 \\ 1 & 0 & 0 & -1 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \end{matrix} \begin{matrix} A \\ B \\ C \\ A_0 \\ B_0 \\ C_0 \end{matrix}$$



Compound Maps

$$-S_{int}{}^T = \begin{matrix} A & B & C \\ \begin{bmatrix} 1 & 1 & -1 \end{bmatrix} \end{matrix} \begin{matrix} v_1 \end{matrix}$$

$$-S_{exch}{}^T = \begin{matrix} A & B & C \\ \begin{bmatrix} 1 & 1 & -1 \\ -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \end{matrix} \begin{matrix} v_1 \\ b_1 \\ b_2 \\ b_3 \end{matrix}$$

$$-S_{tot}{}^T = \begin{matrix} A & B & C & A_0 & B_0 & C_0 \\ \begin{bmatrix} 1 & 1 & -1 & 0 & 0 & 0 \\ -1 & 0 & 0 & -1 & 0 & 0 \\ 0 & -1 & 0 & 0 & -1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 \end{bmatrix} \end{matrix} \begin{matrix} v_1 \\ b_1 \\ b_2 \\ b_3 \end{matrix}$$

FIG4. Stoichiomatric matrix of 3 reactions.

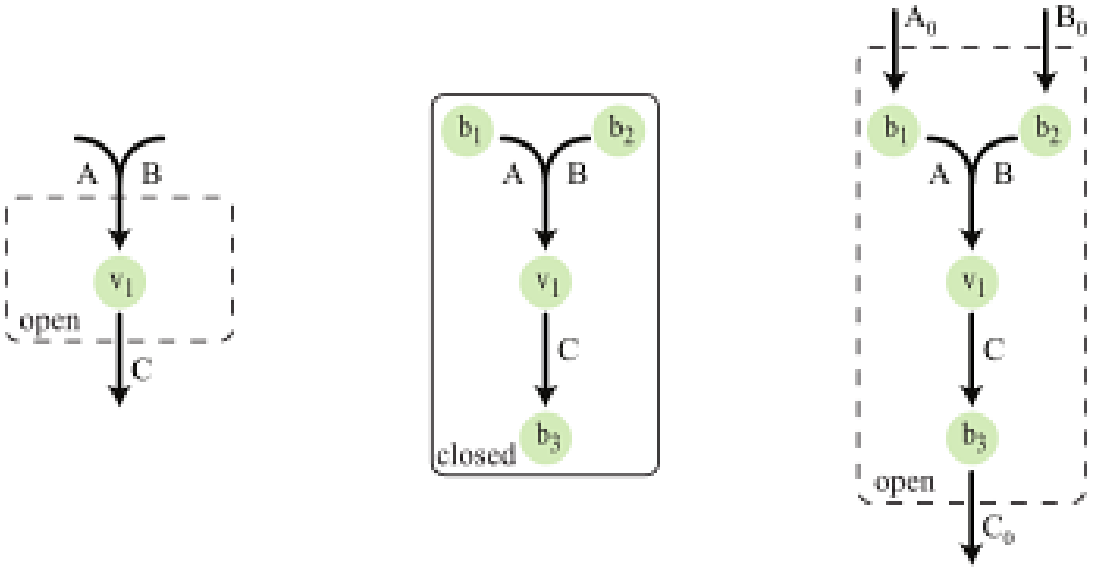


FIG5. Diagram of direction of pathway

1.8.4 The column and left null spaces

The time derivative is in the column space of S (denoted by Col(S)), as can be seen from the expansion of Sv:

dx = at + S2 V2 + • " Snl'n

(6.7)

wher si• are the reaction vectias that form the columns of S. Col(S) is there-fore spanned by the reaction vectors si. The,reactionkaora_ are struciuLal feat1..i..u.si.,s14he network and are fixed. However, the fluxes IT; are scalar quantities and represent the flux through reaction i. The fluxes are vari-ables. We do note that each flux has a maximal value, v < ; VA.max and this limits the size of the time derivatives. Thus, only a portion of the column space is explored, that is, we can cap the size of the column space of S. The vectors in the left null space (1i) of S are orthogonal to the column space, that is, (li si) = 0. The vectors represent a mass conservation.

1.8.5 The row and null spaces

The flux vector can be decomposed into a dynamic component and a steady-state component:

V = Vdvn Vss

The steady state component satisfies Svss =

And v, is thus in the null space of S (see Chapter 9). The dynamic component of the flux vector, vdy,,, is orthogonal to the null space and consequently it is in the row space of S. Each pair of subspaces in the domain and codomain of the dynamic mass balance equation therefore form orthogonal sets to each other, and their dimensions sum up to the dimension of their corresponding vectors, that is, dim(Null(S) + dim(Row(S)) = n and dim(Left null(S)) + dim(Col(S)) = m. These are introductory observations about S and its fundamental sub-spaces. In Chapters 8 through 11, we will study the individual fundamental subspaces in more detail.

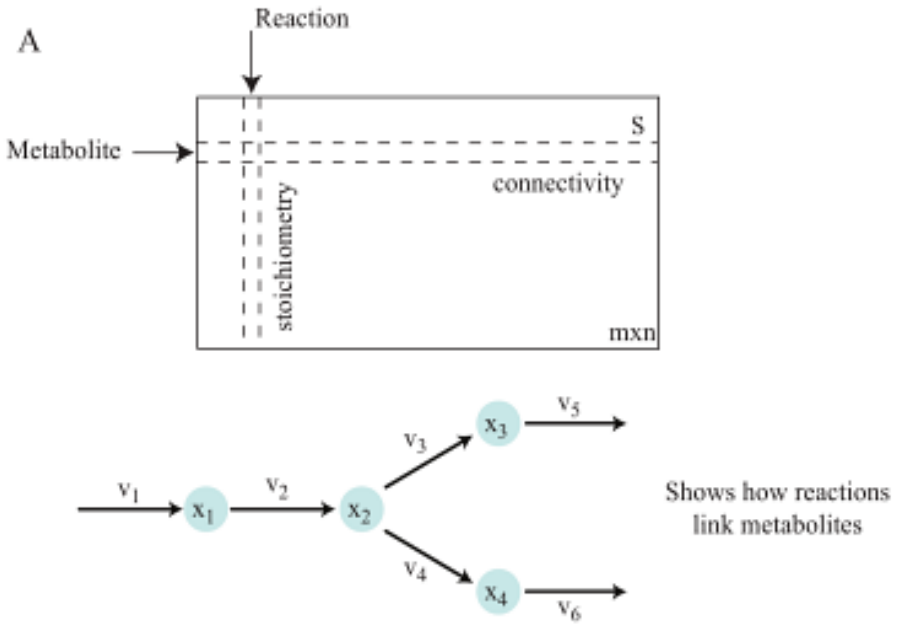


FIG6. Diagram of stoichiomatrix roes and cllumn

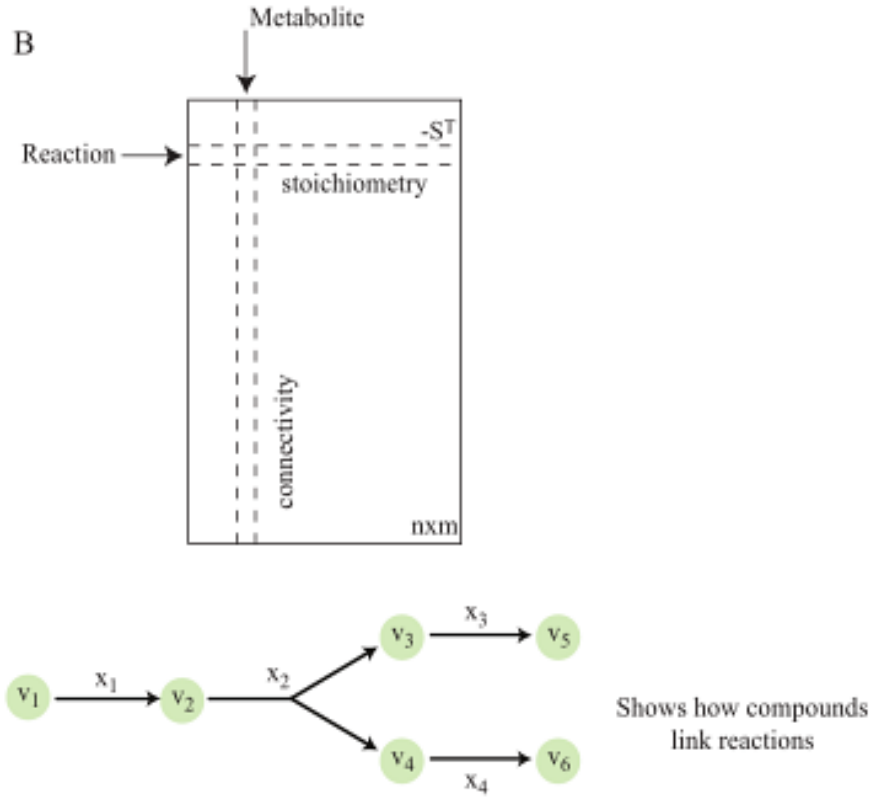


FIG6. Diagram of stoichiomatrix roes and cllumn

CHAPTER 2

Objective

Development of Generic System/Synthetic Biology simulation network using Metabolic Flux Balance Analysis

Now a days, in biological world we are trying to make simulation of every process whether it is a drug action to a target or to refine any protein molecule, we are making tool to solve complex processes through different programs. Metabolic flux balance analysis is one of those complex processes in the biological world. In this project, I aim to find the rate of the reaction of all the metabolic pathway flux in the body, which could be helpful for the wet lab people so that they can find the utilization and production of the compounds.

CHAPTER 3

Methodology

I have gone through KEGG (Kyoto Encyclopedia of Genes and Genomes), because it contain the information about the pathways and other information about metabolic pathways. I have also looked into some other databases also like Biocyc and Metacyc. To see what type of metabolic pathway tools they have.

3.1 Data retrieval

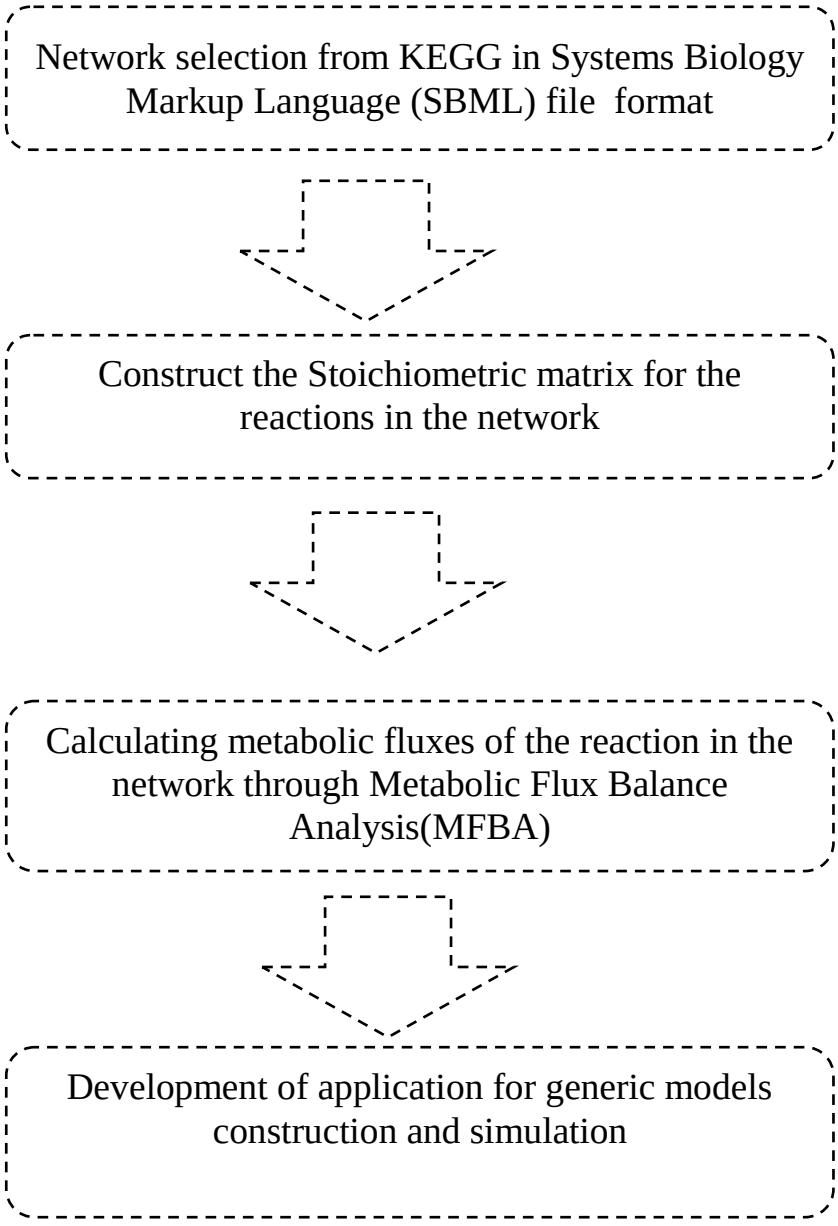
The Glycolytic pathway is most common pathway of all, which is used to produce energy in all organisms. Retrieval of Glycolytic pathway XML file was from KEGG (Kyoto Encyclopedia of Genes and Genomes) subdivision KEGG Pathway. It is one of the best metabolic pathway database in the world.

3.2 Retrieve information

The retrieved reaction ID, substrate ID and product ID were collected from the XML file, which was taken from KEGG Pathways. This information was retrieved form the help of the code which is shown 7^{th} section of this thesis. All the replicating reactions were removed. The information is stored in the Excel file with attributed like Reaction ID, Reaction Type, Substrate ID and Product ID.

3.3 Getting Reaction Rate of the metabolic pathways

To get the reaction rate of all the reactions of the metabolic pathways we construct the Stoichiometric Matrix. Which contain the data that in which reaction which compound is consumed and which compound is produced. After getting all the information about the metabolic pathway, we have to deduce the reaction rate of all the reactions for that we multiply the inverse stoichiometric matrix with the differential of all the reactions.


Network selection from KEGG in Systems Biology Markup Language (SBML) file format
Construct the Stoichiometric matrix for the reactions in the network
Calculating metabolic fluxes of the reaction in the network through Metabolic Flux Balance Analysis(MFBA)
Development of application for generic models construction and simulation

CHAPETR 4

Tools & Techniques

4.1 Tools

a) **KEGG:** KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of databases dealing with genomes, biological pathways, diseases, drugs, and chemical substances. KEGG is utilized for bioinformatics research and education, including data analysis in genomics, metagenomics, metabolomics and other omics studies, modeling and simulation in systems biology, and translational research in drug development.

b) **BioCyc:**The BioCyc database collection is an assortment of organism specific Pathway/ Genome Databases (PGDBs). They provide reference to genome and metabolic pathways of few thousand organisms. As of June 23, 2014, there are 3563 databases within BioCyc. The list of databases can be found here. SRI International, based in Menlo Park, California, maintains the BioCyc database family.

c) **PubMed:**PubMed is a free search engine accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics. The United States National Library of Medicine (NLM) at the National Institutes of Health maintains the database as part of the Entrez system of information retrieval.

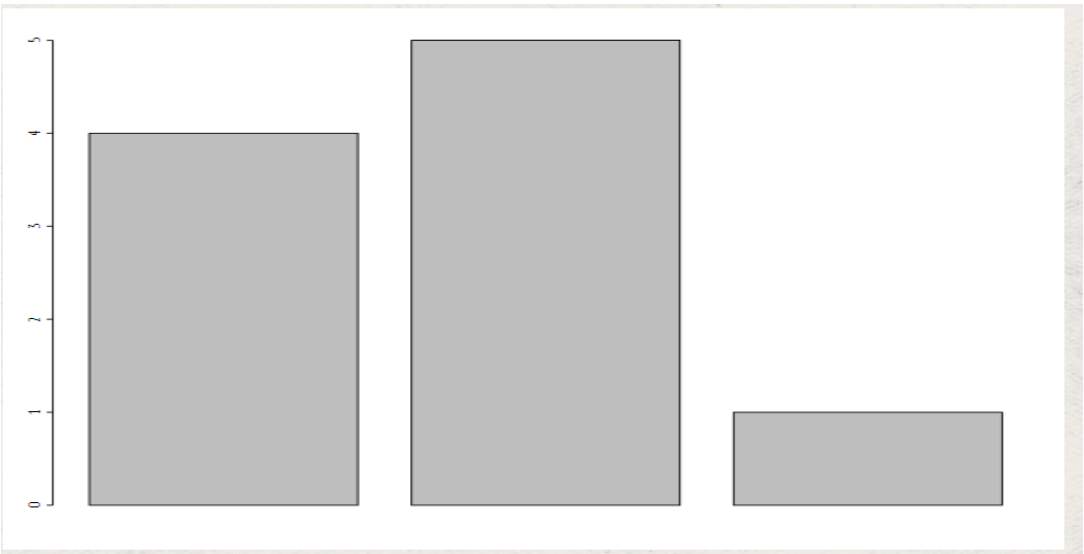
4.2 Techniques

a) **R:** R is a programming language and software environment for statistical computing and graphics. The R language is widely used among statisticians and data miners for developing statistical software and data analysis. Polls, surveys of data miners, and

studies of scholarly literature databases show that R's popularity has increased substantially in recent years.

Example

```
> vesselsSunk <- c(4, 5, 1)
> barplot(vesselsSunk)
```



b) **SBML:** The Systems Biology Markup Language (SBML) is a representation format, based on XML, for communicating and storing computational models of biological processes. It is a free and open standard with widespread software support and a community of users and developers. SBML can represent many different classes of biological phenomena, including metabolic networks, cell signaling pathways, regulatory networks, infectious diseases, and many others. It is the de facto standard for representing

computational models in systems biology today.

c) **LibSBML:** LibSBML is an open-source software library that provides an application programming interface (API) for the SBML (Systems Biology Markup Language) format. The libSBML library can be embedded in a software application or used in a web servlet (such as one that might be served by Apache Tomcat) as part of the application or servlet's implementation of support for reading, writing, and manipulating SBML documents and data streams. The core of libSBML is written in ISO standard C++; the library provides API for many programming languages via interfaces generated with the help of SWIG.

Example for SBML

```
<?xml version="1.0" encoding="UTF-8"?>
<sbml level="3" version="1" xmlns="http://www.sbml.org/sbml/level3/version1/core">
  <model extentUnits="mole" timeUnits="second">
    <listOfUnitDefinitions>
      <unitDefinition id="per_second">
        <listOfUnits>
          <unit kind="second" exponent="-1" scale="0" multiplier="1"/>
        </listOfUnits>
      </unitDefinition>
      <unitDefinition id="litre_per_mole_second">
        <listOfUnits>
          <unit kind="mole"   exponent="-1" scale="0" multiplier="1"/>
          <unit kind="litre"  exponent="1"  scale="0" multiplier="1"/>
          <unit kind="second" exponent="-1" scale="0" multiplier="1"/>
        </listOfUnits>
      </unitDefinition>
    </listOfUnitDefinitions>
    <listOfCompartments>
      <compartment id="comp" size="1e-14" spatialDimensions="3" units="litre" constant="true"/>
    </listOfCompartments>
    <listOfSpecies>
      <species compartment="comp" id="E"  initialAmount="5e-21" boundaryCondition="false"
               hasOnlySubstanceUnits="false" substanceUnits="mole" constant="false"/>
      <species compartment="comp" id="S"  initialAmount="1e-20" boundaryCondition="false"
               hasOnlySubstanceUnits="false" substanceUnits="mole" constant="false"/>
      <species compartment="comp" id="P"  initialAmount="0"     boundaryCondition="false"
               hasOnlySubstanceUnits="false" substanceUnits="mole" constant="false"/>
      <species compartment="comp" id="ES" initialAmount="0"     boundaryCondition="false"
               hasOnlySubstanceUnits="false" substanceUnits="mole" constant="false"/>
    </listOfSpecies>
    <listOfReactions>
      <reaction id="veq" reversible="true" fast="false">
        <listOfReactants>
          <speciesReference species="E"  stoichiometry="1" constant="true"/>
            <speciesReference species="S"  stoichiometry="1" constant="true"/>
        </listOfReactants>
```

The libSBML library is free software released under the terms of the GNU Lesser General Public License as published by the Free Software Foundation; either version 2.1 of the License, or any later version. LibSBML was developed thanks to funding from many agencies, particularly the National Institute of General Medical Sciences (NIGMS, USA) as well as the Defense Advanced Research Projects Agency (DARPA, USA) under the Bio-SPICE program.

d) **PHP:** PHP is a server-side scripting language designed for web development but also used as a general-purpose programming language. While PHP originally stood for Personal Home Page, it now stands for PHP: Hypertext Preprocessor, which is a recursive backronym. PHP code can be simply mixed with HTML code, or it can be used in combination with various templating engines and web frameworks. PHP code is usually processed by a PHP interpreter, which is usually implemented as a web server's native module or a Common Gateway Interface (CGI) executable.

Example

```
<!DOCTYPE html>
<html>
    <head>
        <title>PHP Test</title>
    </head>
    <body>
        <?php echo '<p>Hello World</p>'; ?>
    </body>
</html>
```

CHAPTER 5

Code Used in the Project

5.1 Code to get the Basic Information of the Metabolic Pathways:

```
library(libSBML)
filename = args[1];
d  = readSBML(abc.xml);
errors   = SBMLDocument_getNumErrors(d);
SBMLDocument_printErrors(d);
m = SBMLDocument_getModel(d);
level   = SBase_getLevel  (d);
version = SBase_getVersion(d);
cat("\n");
cat("File: ",abc.xml," (Level ",level,", version ",version,")\n");
if (errors > 0) {
```

```
                    stop("No model present.");

                   }
cat("        ");
cat("  model id: ", ifelse(Model_isSetId(m), Model_getId(m) ,"(empty)"),"\n");
cat( "functionDefinitions: ", Model_getNumFunctionDefinitions(m) ,"\n" );
cat( "    unitDefinitions: ", Model_getNumUnitDefinitions    (m) ,"\n" );
cat( "   compartmentTypes: ", Model_getNumCompartmentTypes   (m) ,"\n" );
cat( "        specieTypes: ", Model_getNumSpeciesTypes       (m) ,"\n" );
cat( "       compartments: ", Model_getNumCompartments       (m) ,"\n" );
cat( "            species: ", Model_getNumSpecies            (m) ,"\n" );
cat( "         parameters: ", Model_getNumParameters         (m) ,"\n" );
cat( " initialAssignments: ", Model_getNumInitialAssignments (m) ,"\n" );
cat( "              rules: ", Model_getNumRules              (m) ,"\n" );
cat( "        constraints: ", Model_getNumConstraints        (m) ,"\n" );
cat( "         reactions: ", Model_getNumReactions          (m) ,"\n" );
cat( "             events: ", Model_getNumEvents            (m) ,"\n" );
cat( "\n" );
q(status=0);
```

5.2 Code to get the information like reaction ID, reaction type, substrate ID and product ID.

```
<?php
$f=fopen("hsa00010.xml","r");
$f2=fopen("result.csv","w");
fwrite($f2,"id,type,substrateid,substratename,productid,productname\n");
while($line=fgets($f))
        {
                if(strstr($line,"<reaction id="))
```

```
                {
                        $data=explode("",$line);
                        $id=$data[1];
                        $type=$data[5];
                        $line=fgets($f);
                        $data=explode("",$line);
                        $substrateid=$data[1];
                        $substratename=$data[3];
                        $line=fgets($f);
                        $data=explode("",$line);
                        $productid=$data[1];
                        $productname=$data[3];

        fwrite($f2,$id.",".$type.",".$substrateid.",".$substratename.",".$productid.",".$product
name."\n");

        }
}
fclose($f);
fclose($f2);
?>
```

File taken as Input in .XML Format is Present in Appendix.

5.3 Code to get the reaction rate of the reactions

```
function invert($A, $debug = FALSE)
{
        /// @todo check rows = columns
```

```
$n = count($A);

// get and append identity matrix
$I = identity_matrix($n);
for ($i = 0; $i < $n; ++ $i) {
	$A[$i] = array_merge($A[$i], $I[$i]);
}

if ($debug) {
	echo "\nStarting matrix: ";
	print_matrix($A);
}

// forward run
for ($j = 0; $j < $n-1; ++ $j) {
	// for all remaining rows (diagonally)
	for ($i = $j+1; $i < $n; ++ $i) {
		// if the value is not already 0
		if ($A[$i][$j] !== 0) {
			// adjust scale to pivot row
			// subtract pivot row from current
			$scalar = $A[$j][$j] / $A[$i][$j];
			for ($jj = $j; $jj < $n*2; ++ $jj) {
				$A[$i][$jj] *= $scalar;
				$A[$i][$jj] -= $A[$j][$jj];
			}
		}
	}
	if ($debug) {
		echo "\nForward iteration $j: ";
		print_matrix($A);
	}
}

// reverse run
for ($j = $n-1; $j > 0; -- $j) {
	for ($i = $j-1; $i >= 0; -- $i) {
		if ($A[$i][$j] !== 0) {
			$scalar = $A[$j][$j] / $A[$i][$j];
			for ($jj = $i; $jj < $n*2; ++ $jj) {
				$A[$i][$jj] *= $scalar;
				$A[$i][$jj] -= $A[$j][$jj];
			}
		}
	}
	if ($debug) {
```

```
                        echo "\nReverse iteration $j: ";
                        print_matrix($A);
                }
        }

        // last run to make all diagonal 1s
        /// @note this can be done in last iteration (i.e. reverse run) too!
        for ($j = 0; $j < $n; ++ $j) {
                if ($A[$j][$j] !== 1) {
                        $scalar = 1 / $A[$j][$j];
                        for ($jj = $j; $jj < $n*2; ++ $jj) {
                                $A[$j][$jj] *= $scalar;
                        }
                }
                if ($debug) {
                        echo "\n1-out iteration $j: ";
                        print_matrix($A);
                }
        }

        // take out the matrix inverse to return
        $Inv = array();
        for ($i = 0; $i < $n; ++ $i) {
                $Inv[$i] = array_slice($A[$i], $n);
        }

        return $Inv;
}
```

5.4 Code to get the rates of the reactions

```
function invert($A, $debug = FALSE)
{
	/// @todo check rows = columns

	$n = count($A);

	// get and append identity matrix
	$I = identity_matrix($n);
	for ($i = 0; $i < $n; ++ $i) {
		$A[$i] = array_merge($A[$i], $I[$i]);
	}

	if ($debug) {
		echo "\nStarting matrix: ";
		print_matrix($A);
	}

	// forward run
	for ($j = 0; $j < $n-1; ++ $j) {
		// for all remaining rows (diagonally)
		for ($i = $j+1; $i < $n; ++ $i) {
			// if the value is not already 0
			if ($A[$i][$j] !== 0) {
				// adjust scale to pivot row
				// subtract pivot row from current
				$scalar = $A[$j][$j] / $A[$i][$j];
				for ($jj = $j; $jj < $n*2; ++ $jj) {
					$A[$i][$jj] *= $scalar;
					$A[$i][$jj] -= $A[$j][$jj];
				}
			}
		}
		if ($debug) {
			echo "\nForward iteration $j: ";
			print_matrix($A);
		}
	}

	// reverse run
	for ($j = $n-1; $j > 0; -- $j) {
		for ($i = $j-1; $i >= 0; -- $i) {
			if ($A[$i][$j] !== 0) {
				$scalar = $A[$j][$j] / $A[$i][$j];
				for ($jj = $i; $jj < $n*2; ++ $jj) {
```

```
                                        $A[$i][$jj] *= $scalar;
                                        $A[$i][$jj] -= $A[$j][$jj];
                                }
                        }
                }
                if ($debug) {
                        echo "\nReverse iteration $j: ";
                        print_matrix($A);
                }
        }

        // last run to make all diagonal 1s
        /// @note this can be done in last iteration (i.e. reverse run) too!
        for ($j = 0; $j < $n; ++ $j) {
                if ($A[$j][$j] !== 1) {
                        $scalar = 1 / $A[$j][$j];
                        for ($jj = $j; $jj < $n*2; ++ $jj) {
                                $A[$j][$jj] *= $scalar;
                        }
                }
                if ($debug) {
                        echo "\n1-out iteration $j: ";
                        print_matrix($A);
                }
        }

        // take out the matrix inverse to return
        $Inv = array();
        for ($i = 0; $i < $n; ++ $i) {
                $Inv[$i] = array_slice($A[$i], $n);
        }

        return $Inv;
}
```

CHAPTER 6

Results

Extracted data

Glycolysis

id	type	substrateid	substratename	productid	productname
37	reversible	101	cpd:C00084	40	cpd:C00033
39	reversible	101	cpd:C00084	40	cpd:C00033
44	reversible	97	cpd:C00469	101	cpd:C00084
45	reversible	97	cpd:C00469	101	cpd:C00084
47	irreversible	98	cpd:C00022	136	cpd:C00068
48	irreversible	99	cpd:C05125	96	cpd:C15972
49	reversible	103	cpd:C15973	100	cpd:C00024
50	reversible	95	cpd:C00186	98	cpd:C00022
52	irreversible	92	cpd:C00074	98	cpd:C00022
54	reversible	85	cpd:C00631	92	cpd:C00074
55	reversible	85	cpd:C00631	93	cpd:C00197
56	reversible	130	cpd:C00118	94	cpd:C00236
57	reversible	130	cpd:C00118	88	cpd:C00111
13	reversible	104	cpd:C05378	130	cpd:C00118
58	irreversible	91	cpd:C05345	104	cpd:C05378
59	irreversible	104	cpd:C05378	91	cpd:C05345
61	reversible	90	cpd:C00668	91	cpd:C05345
62	reversible	84	cpd:C00103	90	cpd:C00668
63	reversible	89	cpd:C01172	91	cpd:C05345
64	irreversible	87	cpd:C00221	89	cpd:C01172
65	irreversible	87	cpd:C00221	89	cpd:C01172
66	reversible	90	cpd:C00668	89	cpd:C01172
68	reversible	86	cpd:C00267	87	cpd:C00221
69	irreversible	86	cpd:C00267	90	cpd:C00668
70	irreversible	86	cpd:C00267	90	cpd:C00668
71	irreversible	90	cpd:C00668	86	cpd:C00267
75	reversible	103	cpd:C15973	96	cpd:C15972
76	reversible	93	cpd:C00197	94	cpd:C00236

112	irreversible	113	cpd:C00036	92	cpd:C00074
128	reversible	125	cpd:C01159	93	cpd:C00197
129	reversible	94	cpd:C00236	125	cpd:C01159
131	irreversible	40	cpd:C00033	100	cpd:C00024
133	irreversible	86	cpd:C00267	90	cpd:C00668
134	reversible	87	cpd:C00221	89	cpd:C01172
140	irreversible	125	cpd:C01159	85	cpd:C00631

CHAPTER 7

Conclusion and future aspects

Chemical reactions vary greatly in the speed at which they occur. Some are essentially instantaneous, while others may take years to reach equilibrium. The Reaction Rate for a given chemical reaction is the measure of the change in concentration of the reactants or the change in concentration of the products per unit time. Calculating the rate of the reaction is a very important entity of the reaction occurring in the metabolic pathways with the help of this we can decide which reactions are helping pathway in more efficient way and as per our convenience we can decide that which reaction are better or they are not helping much in the pathway so that we can direct them manually. Rate of reaction can be use to control the reaction to get the product of interest.

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APPENDIX

The input file for the code is

```
<?xml version="1.0"?>
<!DOCTYPE pathway SYSTEM "http://www.kegg.jp/kegg/xml/KGML_v0.7.1_.dtd">
<!-- Creation date: Aug 7, 2013 15:57:17 +0900 (GMT+09:00) -->
- <pathway title="Prostate cancer" link="http://www.kegg.jp/kegg-bin/show_pathway?hsa05215" image="http://www.kegg.jp/kegg/pathway/hsa/hsa05215.png"
number="05215" org="hsa" name="path:hsa05215">
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa04115" name="path:hsa04115" type="map" id="1">
      <graphics name="p53 signaling pathway" type="roundrectangle" height="34" width="104" y="435" x="1002" bgcolor="#FFFFFF" fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa04060" name="path:hsa04060" type="map" id="2">
      <graphics name="Cytokine-cytokine receptor interaction" type="roundrectangle" height="34" width="104" y="507" x="231" bgcolor="#FFFFFF" fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?map00150" name="path:map00150" type="map" id="3">
      <graphics name="Androgen and estrogen metabolism" type="roundrectangle" height="34" width="126" y="594" x="94" bgcolor="#FFFFFF" fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:6716" name="hsa:6716" type="gene" id="4">
      <graphics name="SRD5A2" type="rectangle" height="17" width="46" y="595" x="209" bgcolor="#BFFFBF" fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:367" name="hsa:367" type="gene" id="5">
      <graphics name="AR, AIS, DHTR, HUMARA, HYSP1, KD, NR3C4, SBMA, SMAX1, TFM" type="rectangle" height="17" width="46" y="706" x="603" bgcolor="#BFFFBF"
        fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:367" name="hsa:367" type="gene" id="6">
      <graphics name="AR, AIS, DHTR, HUMARA, HYSP1, KD, NR3C4, SBMA, SMAX1, TFM" type="rectangle" height="17" width="46" y="689" x="603" bgcolor="#BFFFBF"
        fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:4790+hsa:5970" name="hsa:4790 hsa:5970" type="gene" id="7">
      <graphics name="NFKB1, EBP-1, KBF1, NF-kB1, NF-kappa-B, NF-kappaB, NFKB-p105, NFKB-p50, NFkappaB, p105, p50..." type="rectangle" height="17" width="46"
        y="607" x="700" bgcolor="#BFFFBF" fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:4792" name="hsa:4792" type="gene" id="8">
      <graphics name="NFKBIA, IKBA, MAD-3, NFKBI" type="rectangle" height="17" width="46" y="575" x="700" bgcolor="#BFFFBF" fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1027" name="hsa:1027" type="gene" id="9">
      <graphics name="CDKN1B, CDKN4, KIP1, MEN1B, MEN4, P27KIP1" type="rectangle" height="17" width="46" y="454" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:5728" name="hsa:5728" type="gene" id="10">
      <graphics name="PTEN, 10q23del, BZS, CWS1, DEC, GLM2, MHAM, MMAC1, PTEN1, TEP1" type="rectangle" height="17" width="46" y="419" x="409" bgcolor="#BFFFBF"
        fgcolor="#000000"/>
    </entry>
  - <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:4824" name="hsa:4824" type="gene" id="11">
```

<graphics name="NKX3-1, BAPX2, NKX3, NKX3.1, NKX3A" type="rectangle" height="17" width="46" y="358" x="479" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:354" name="hsa:354" type="gene" id="12">
<graphics name="KLK3, APS, KLK2A1, PSA, hK3" type="rectangle" height="17" width="46" y="680" x="1039" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:596" name="hsa:596" type="gene" id="13">
<graphics name="BCL2, Bcl-2, PPP1R50" type="rectangle" height="17" width="46" y="608" x="1039" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:595" name="hsa:595" type="gene" id="14">
<graphics name="CCND1, BCL1, D11S287E, PRAD1, U21B31" type="rectangle" height="17" width="46" y="545" x="1039" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:51176+hsa:6932+hsa:6934+hsa:83439" name="hsa:51176 hsa:6932 hsa:6934 hsa:83439" type="gene" id="15">
<graphics name="LEF1, LEF-1, TCF10, TCF1ALPHA, TCF7L3..." type="rectangle" height="17" width="46" y="544" x="902" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1387+hsa:2033" name="hsa:1387 hsa:2033" type="gene" id="16">
<graphics name="CREBBP, CBP, KAT3A, RSTS..." type="rectangle" height="17" width="46" y="511" x="854" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?C00951+C00410+C07653+C01227+C00280+C00735+C00762" name="cpd:C00951 cpd:C00410 cpd:C07653 cpd:C01227 cpd:C00280 cpd:C00735 cpd:C00762" type="compound" id="17">
<graphics name="C00951..." type="circle" height="8" width="8" y="703" x="238" bgcolor="#FFFFFF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?C03917" name="cpd:C03917" type="compound" id="18">
<graphics name="C03917" type="circle" height="8" width="8" y="617" x="239" bgcolor="#FFFFFF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?C00535" name="cpd:C00535" type="compound" id="19">
<graphics name="C00535" type="circle" height="8" width="8" y="577" x="239" bgcolor="#FFFFFF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa04110" name="path:hsa04110" type="map" id="20">
<graphics name="Cell cycle" type="roundrectangle" height="34" width="104" y="435" x="872" bgcolor="#FFFFFF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:367" name="hsa:367" type="gene" id="21">
<graphics name="AR, AIS, DHTR, HUMARA, HYSP1, KD, NR3C4, SBMA, SMAX1, TFM" type="rectangle" height="17" width="46" y="697" x="466" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?C16038" name="cpd:C16038" type="compound" id="22">
<graphics name="C16038" type="circle" height="8" width="8" y="242" x="239" bgcolor="#FFFFFF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:4193" name="hsa:4193" type="gene" id="23">
<graphics name="MDM2, ACTFS, HDMX, hdm2" type="rectangle" height="17" width="46" y="485" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1026" name="hsa:1026" type="gene" id="24">
<graphics name="CDKN1A, CAP20, CDKN1, CIP1, MDA-6, P21, SDI1, WAF1, p21CIP1" type="rectangle" height="17" width="46" y="424" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:2308" name="hsa:2308" type="gene" id="25">
<graphics name="FOXO1, FKH1, FKHR, FOXO1A" type="rectangle" height="17" width="46" y="393" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1017" name="hsa:1017" type="gene" id="26">
<graphics name="CDK2, CDKN2, p33(CDK2)" type="rectangle" height="17" width="46" y="314" x="907" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1499" name="hsa:1499" type="gene" id="27">
<graphics name="CTNNB1, CTNNB, MRD19, armadillo" type="rectangle" height="17" width="46" y="544" x="745" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1869+hsa:1870+hsa:1871" name="hsa:1869 hsa:1870 hsa:1871" type="gene" id="28">
<graphics name="E2F1, E2F-1, RBAP1, RBBP3, RBP3..." type="rectangle" height="17" width="46" y="361" x="990" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:3265+hsa:3845+hsa:4893" name="hsa:3265 hsa:3845 hsa:4893" type="gene" id="29">
<graphics name="HRAS, C-BAS/HAS, C-H-RAS, C-HA-RAS1, CTLO, H-RASIDX, HAMSV, HRAS1, RASH1, p21ras..." type="rectangle" height="17" width="46" y="596" x="381" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:7157" name="hsa:7157" type="gene" id="30">
<graphics name="TP53, BCC7, LFS1, P53, TRP53" type="rectangle" height="17" width="46" y="483" x="936" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1956+hsa:2064+hsa:2260+hsa:2263+hsa:3480+hsa:3645+hsa:5156+hsa:5159" name="hsa:1956 hsa:2064 hsa:2260 hsa:2263 hsa:3480 hsa:3645 hsa:5156 hsa:5159" type="gene" id="31">
<graphics name="EGFR, ERBB, ERBB1, HER1, PIG61, mENA..." type="rectangle" height="17" width="46" y="471" x="307" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa04210" name="path:hsa04210" type="map" id="32">
<graphics name="Apoptosis" type="roundrectangle" height="34" width="104" y="311" x="737" bgcolor="#FFFFFF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1147+hsa:3551+hsa:8517" name="hsa:1147 hsa:3551 hsa:8517" type="gene" id="33">
<graphics name="CHUK, IKBKA, IKK-alpha, IKK1, IKKA, NFKBIKA, TCF16..." type="rectangle" height="17" width="46" y="574" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:572" name="hsa:572" type="gene" id="34">
<graphics name="BAD, BBC2, BCL2L8" type="rectangle" height="17" width="46" y="362" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:842" name="hsa:842" type="gene" id="35">
<graphics name="CASP9, APAF-3, APAF3, ICE-LAP6, MCH6, PPP1R56" type="rectangle" height="17" width="46" y="333" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>

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        fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:10488+hsa:1385+hsa:148327+hsa:468+hsa:64764+hsa:84699+hsa:90993+hsa:9586" name="hsa:10488
hsa:1385 hsa:148327 hsa:468 hsa:64764 hsa:84699 hsa:90993 hsa:9586" type="gene" id="36">
    <graphics name="CREB3, LUMAN, LZIP..." type="rectangle" height="17" width="46" y="508" x="744" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:898+hsa:9134" name="hsa:898 hsa:9134" type="gene" id="37">
    <graphics name="CCNE1, CCNE..." type="rectangle" height="17" width="46" y="331" x="907" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1950+hsa:3479+hsa:3630+hsa:5154+hsa:5155+hsa:56034+hsa:7039+hsa:80310" name="hsa:1950
hsa:3479 hsa:3630 hsa:5154 hsa:5155 hsa:56034 hsa:7039 hsa:80310" type="gene" id="38">
    <graphics name="EGF, HOMG4, URG..." type="rectangle" height="17" width="46" y="471" x="230" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:5925" name="hsa:5925" type="gene" id="39">
    <graphics name="RB1, OSRC, PPP1R130, RB, p105-Rb, pRb, pp110" type="rectangle" height="17" width="46" y="324" x="990" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:5170" name="hsa:5170" type="gene" id="40">
    <graphics name="PDPK1, PDK1, PDPK2, PRO0461" type="rectangle" height="17" width="46" y="439" x="470" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:6654+hsa:6655" name="hsa:6654 hsa:6655" type="gene" id="41">
    <graphics name="SOS1, GF1, GGF1, GINGF, HGF, NS4..." type="rectangle" height="17" width="46" y="557" x="381" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:2885" name="hsa:2885" type="gene" id="42">
    <graphics name="GRB2, ASH, EGFRBP-GRB2, Grb3-3, MST084, MSTP084, NCKAP2" type="rectangle" height="17" width="46" y="519" x="381" bgcolor="#BFFFBF"
        fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?C05981" name="cpd:C05981" type="compound" id="43">
    <graphics name="C05981" type="circle" height="8" width="8" y="470" x="434" bgcolor="#FFFFFF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:2932" name="hsa:2932" type="gene" id="44">
    <graphics name="GSK3B" type="rectangle" height="17" width="46" y="525" x="630" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:3320+hsa:3326+hsa:7184" name="hsa:3320 hsa:3326 hsa:7184" type="gene" id="45">
    <graphics name="HSP90AA1, EL52, HSP86, HSP89A, HSP90A, HSP90N, HSPC1, HSPCA, HSPCAL1, HSPCAL4, HSPN, Hsp89, Hsp90, LAP-2, LAP2..." type="rectangle"
        height="17" width="46" y="714" x="466" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
<entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:2475" name="hsa:2475" type="gene" id="46">
    <graphics name="MTOR, FRAP, FRAP1, FRAP2, RAFT1, RAPT1" type="rectangle" height="17" width="46" y="638" x="629" bgcolor="#BFFFBF" fgcolor="#000000"/>
</entry>
```

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- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:1027" name="hsa:1027" type="gene" id="47">
    <graphics name="CDKN1B, CDKN4, KIP1, MEN1B, MEN4, P27KIP1" type="rectangle" height="17" width="46" y="287" x="843" bgcolor="#BFFFBF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa04010" name="path:hsa04010" type="map" id="48">
    <graphics name="MAPK signaling pathway" type="roundrectangle" height="34" width="104" y="540" x="477" bgcolor="#FFFFFF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:10000+hsa:207+hsa:208" name="hsa:10000 hsa:207 hsa:208" type="gene" id="49">
    <graphics name="AKT3, MPPH, PKB-GAMMA, PKBG, PRKBG, RAC-PK-gamma, RAC-gamma, STK-2..." type="rectangle" height="17" width="46" y="471" x="530"
      bgcolor="#BFFFBF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:23533+hsa:5290+hsa:5291+hsa:5293+hsa:5294+hsa:5295+hsa:5296+hsa:8503" name="hsa:23533
  hsa:5290 hsa:5291 hsa:5293 hsa:5294 hsa:5295 hsa:5296 hsa:8503" type="gene" id="50">
    <graphics name="PIK3R5, F730038I15Rik, FOAP-2, P101-PI3K, p101..." type="rectangle" height="17" width="46" y="471" x="381" bgcolor="#BFFFBF"
      fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:2950" name="hsa:2950" type="gene" id="51">
    <graphics name="GSTP1, DFN7, FAEES3, GST3, GSTP, HEL-S-22, PI" type="rectangle" height="17" width="46" y="198" x="688" bgcolor="#BFFFBF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa05215" name="path:hsa05215" type="map" id="52">
    <graphics name="TITLE:Prostate cancer" type="roundrectangle" height="25" width="152" y="58" x="116" bgcolor="#FFFFFF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa04110" name="path:hsa04110" type="map" id="53">
    <graphics name="Cell cycle" type="roundrectangle" height="34" width="104" y="309" x="1081" bgcolor="#FFFFFF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:5594+hsa:5595" name="hsa:5594 hsa:5595" type="gene" id="54">
    <graphics name="MAPK1, ERK, ERK-2, ERK2, ERT1, MAPK2, P42MAPK, PRKM1, PRKM2, p38, p40, p41, p41mapk, p42-MAPK..." type="rectangle" height="17" width="46"
      y="637" x="534" bgcolor="#BFFFBF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:5604+hsa:5605" name="hsa:5604 hsa:5605" type="gene" id="55">
    <graphics name="MAP2K1, CFC3, MAPKK1, MEK1, MKK1, PRKMK1..." type="rectangle" height="17" width="46" y="596" x="533" bgcolor="#BFFFBF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa:369+hsa:5894+hsa:673" name="hsa:369 hsa:5894 hsa:673" type="gene" id="56">
    <graphics name="ARAF, A-RAF, ARAF1, PKS2, RAFA1..." type="rectangle" height="17" width="46" y="596" x="459" bgcolor="#BFFFBF" fgcolor="#000000"/>
  </entry>
- <entry link="http://www.kegg.jp/dbget-bin/www_bget?hsa04151" name="path:hsa04151" type="map" id="57">
    <graphics name="PI3K-Akt signaling pathway" type="roundrectangle" height="34" width="104" y="321" x="479" bgcolor="#FFFFFF" fgcolor="#000000"/>
  </entry>
```

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- <entry name="undefined" type="group" id="58">
    <graphics type="rectangle" height="34" width="46" y="322" x="907" bgcolor="#FFFFFF" fgcolor="#000000"/>
    <component id="26"/>
    <component id="37"/>
  </entry>
- <entry name="undefined" type="group" id="59">
    <graphics type="rectangle" height="34" width="46" y="697" x="603" bgcolor="#FFFFFF" fgcolor="#000000"/>
    <component id="5"/>
    <component id="6"/>
  </entry>
- <entry name="undefined" type="group" id="60">
    <graphics type="rectangle" height="34" width="46" y="705" x="466" bgcolor="#FFFFFF" fgcolor="#000000"/>
    <component id="21"/>
    <component id="45"/>
  </entry>
- <relation type="PPrel" entry2="40" entry1="50">
    <subtype name="compound" value="43"/>
    <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PPrel" entry2="49" entry1="50">
    <subtype name="compound" value="43"/>
    <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PPrel" entry2="31" entry1="38">
    <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PPrel" entry2="50" entry1="31">
    <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PPrel" entry2="42" entry1="31">
    <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PPrel" entry2="41" entry1="42">
    <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PPrel" entry2="29" entry1="41">


- <relation type="PPrel" entry2="25" entry1="49">
    <subtype name="inhibition" value="--|"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="24" entry1="49">
    <subtype name="inhibition" value="--|"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="9" entry1="49">
    <subtype name="inhibition" value="--|"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="44" entry1="49">
    <subtype name="inhibition" value="--|"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="36" entry1="44">
    <subtype name="inhibition" value="--|"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="27" entry1="44">
    <subtype name="inhibition" value="--|"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="8" entry1="33">
    <subtype name="activation" value="-->"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="39" entry1="58">
    <subtype name="activation" value="-->"/>
    <subtype name="phosphorylation" value="+p"/>
  </relation>
- <relation type="PPrel" entry2="59" entry1="54">
    <subtype name="indirect effect" value="..>"/>
  </relation>
- <relation type="PPrel" entry2="39" entry1="28">
    <subtype name="dissociation" value="-+-"/>
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- <relation type="PPrel" entry2="30" entry1="23">
      <subtype name="inhibition" value="--|"/>
  </relation>
- <relation type="PPrel" entry2="16" entry1="36">
      <subtype name="inhibition" value="--|"/>
  </relation>
- <relation type="PPrel" entry2="16" entry1="27">
      <subtype name="inhibition" value="--|"/>
  </relation>
- <relation type="PPrel" entry2="8" entry1="7">
      <subtype name="dissociation" value="-+-"/>
  </relation>
- <relation type="PPrel" entry2="15" entry1="27">
      <subtype name="activation" value="-->"/>
  </relation>
- <relation type="GErel" entry2="14" entry1="15">
      <subtype name="expression" value="-->"/>
  </relation>
- <relation type="GErel" entry2="13" entry1="7">
      <subtype name="expression" value="-->"/>
  </relation>
- <relation type="GErel" entry2="12" entry1="59">
      <subtype name="expression" value="-->"/>
  </relation>
- <relation type="PPrel" entry2="59" entry1="60">
      <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PCrel" entry2="22" entry1="51">
      <subtype name="missing interaction" value="-/-"/>
  </relation>
- <relation type="PPrel" entry2="58" entry1="47">
      <subtype name="missing interaction" value="-/-"/>
  </relation>
- <relation type="PPrel" entry2="30" entry1="16">
      <subtype name="missing interaction" value="-/-"/>
  </relation>
- <relation type="PPrel" entry2="49" entry1="11">
      <subtype name="missing interaction" value="-/-"/>
  </relation>
- <relation type="PCrel" entry2="43" entry1="10">
      <subtype name="missing interaction" value="-/-"/>
  </relation>
- <relation type="PCrel" entry2="60" entry1="17">
      <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PCrel" entry2="60" entry1="19">
      <subtype name="activation" value="-->"/>
  </relation>
- <relation type="PCrel" entry2="60" entry1="18">
      <subtype name="activation" value="-->"/>
  </relation>
'pathway>
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