IN SILICO ANALYSIS OF STEADY STATE MECHANISMS OF METABOLIC NETWORKS IN COBRA TOOLBOX AND FBA–SIMVIS

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Abstract

Metabolic analysis is one of the research focuses of systems biology. Two aspects of metabolic networks -network topology and stoichiometry - are what current researchers are most interested in, and both studies have revealed significant information. The research of the stoichiometric matrix of metabolic network has generated a series of powerful methodologies such as flux balance analysis (FBA). For FBA different methods execution are used different software like COBRA Toolbox and FBA-SimVis. The aim of this paper is to compare and analyze functionality of these two toolboxes, metabolic network data conformation conditions, and to compare all available FBA methods comparison in calculation possibilities and visual interpretation way. FBA, when analyzing all fluxes using different options, gives results ints metabolic network flow chart, although COBRA Toolbox returns the results in the matrix in number formats. FBA-SimVis for Steady state metabolic network models analysis is provided for a small metabolic network, because making some FBA analysis there are a ten possibilities to change an unlimited count of variables, to choose or change or optimize reactions as variables. COBRA Toolbox for Steady state metabolic network models analysis is provided for greater metabolic networks with hundreds or thousands of reactions. It allows changing an unlimited count manipulating and optimizing reactions fluxes.
Key words: Flux balance analysis, COBRA Toolbox, FBA-SimVis.

Introduction

Systems biology is a rapidly growing science field that is based on building and validating *in silico* models of biological systems. Under different environmental conditions and genetic backgrounds, the mathematical constraint–based modeling approach of metabolism is used to predict an optimal metabolic yield and steady state flux distributions (Varma and Palson, 1994).

A variety of tools have been developed to facilitate simulations and to handle the models like COBRA toolbox *(http://gcrg.ucsd.edu/Downloads/Cobra_Toolbox*), FBA-SimVis (*fbasimvis.ipk-gatersleben.de/installation.html*). Tools called COBRA toolbox (Kauffman et al., 2003; Reed and Palsson, 2003) and FBA-SimVis (Belau et al., 2009) include implementations of many of the commonly used forms of constraint-based analysis such as flux balance analysis (FBA), gene deletions, flux variability analysis, sampling, and batch simulations together with tools to read in and manipulate constraint-based models (Rahmanian et al., 2009*)*.

The methods provided in the COBRA Toolbox and FBA-SimsVis can be used, in principle, on any metabolic network, but the more computationally intensive calculations may require extensive computer processing time. The metabolic network models should be represented in Systems Biology Markup Language (SBML) (Becker et al., 2009). Mathematical constraint–based modeling approach of metabolism in these tools offers a lot of potential steady state solutions of metabolic networks. These solution mechanisms are difficult to analyze and understand without additional data representation. To make different background of scientists better understand and interpret enormous metabolic network data, there is a need for additional metabolism data representation mechanism like much more visualizations, less calculations. The aim of this paper is to compare COBRA toolbox and FBA-SimVis models output types, buil-in metabolic network analyses functions, calculation resources, data representation type, results analysis methods and experimental data input-output possibilities.

Materials and Methods

The experimental data for comparison and analyze opportunities in COBRA and FBA–SimVis Toolboxes has been used steady-state metabolic network of bacteria *Zymomonas mobilis* adaptation for glycerol conversion into bioethanol. The *Zymomonas mobilis* parameter values were conducted in Vitro at the temperature of 30 °C, pH was about 6.5. These in vitro parameters were taken into account making *in Silico* steady state metabolic network models using COBRA and FBA–SimVis Toolbox.

To better understand and interpret enormous metabolic network data, authors use following methods: COBRA Toolbox and FBA-SimVis functionality, and constraint–based model analysis.

COBRA Toolbox and FBA-SimVis functionality (metabolic network model formats, mathematical calculation resources, metabolic network model visualization)

Metabolic network model formats. Although the COBRA Toolbox (http://sbml.org/software/sbmltoolbox/) could (with some modification) handle any reasonable input format like XLS file format for the models, we describe the metabolic network model input as using the Systems Biology Markup Language (SBML) format (Hucka, 2003).

Compared with COBRA Toolbox, FBA-SimVis can use only SBML metabolic network model format (Belau

et al., 2009) integrated in open source software VANTED through import function.

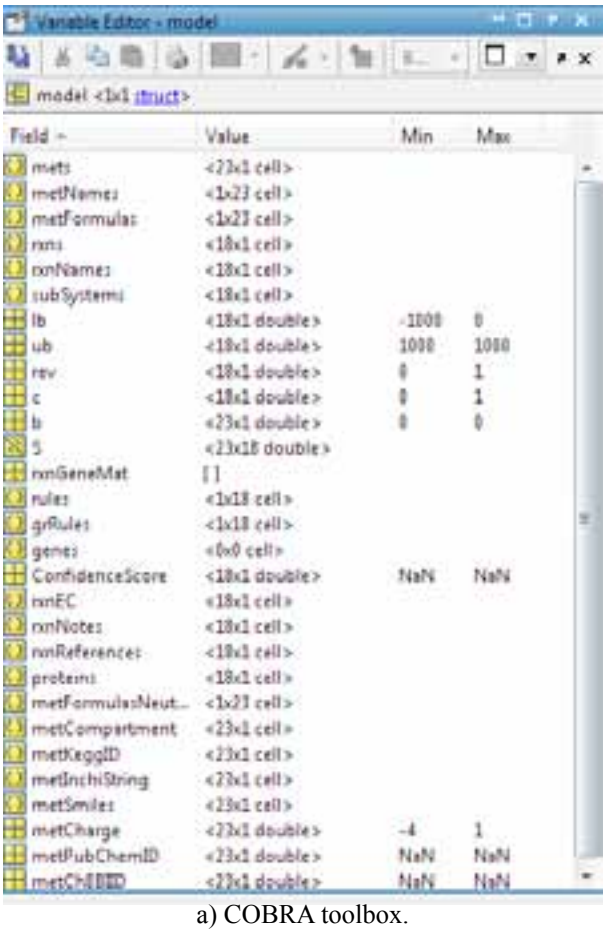
Mathematical calculation resources. COBRA Toolbox Version 6.0 or above of Mathlab (Mathworks Inc.) numerical computation and visualization software (http://www.mathworks.com). COBRA Toolbox uses few different kinds of solvers like:

- lp_solve: (https://sourceforge.net/projects/lpsolve),
- glpk: http:(//www.gnu.org/software/glpk/),
- LINDO (LINDO Systems Inc.) Matlab API: (http://www.lindo.com),
- CPLEX (ILOG Inc.) through the Tomlab (Tomlab Optimization Inc.) optimization environment: (http://tomopt.com/),
- Mosek (MOSEK ApS): (http://www.mosek.com).

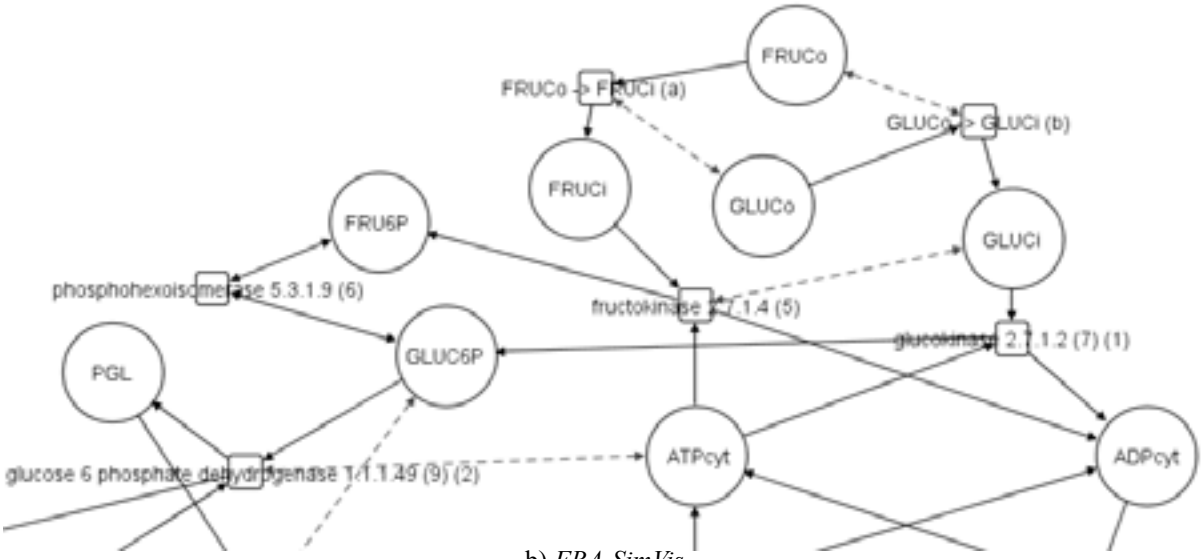
FBA-SimVis is a user-friendly tool providing visual interpretation and analysis of constraint–based metabolic models. FBA-SimVis is implemented in Java-based open source software VANTED as a toolbox. FBA-SimVis uses in Mathlab integrated mathematical approaches for constraint–based model analysis and for user friendly interactive flux visualization way use Java-based VANTED software.

Metabolic network model visualization. COBRA Toolbox metabolic network visualization returns various forms of matrix, which interpretation and data examination for a scientist without specific software resources is a time-consuming process (Fig. 1. a), because all data is displayed as various kinds of matrices in Mathlab simulation environment. Model each data analyses function gained data is represented in own matrix file. This data visualization method for checking data examination and interpretation is based on many number comparing between different matrices without additional usage of comparable software is time consuming process.

FBA–SimVis framework Tool for metabolic network visualization is based on graphical data examination and interpretation. FBA–SimVis tool uses open source software VANTED opportunities, and visualization options for biological networks containing experimental data (Fig. 1. b). To reconstruct a metabolic network model, the user can either create a new model (using the graphical model editor to edit existing or dropping new elements onto the canvas), or import a metabolic network model via SBML from files (by dropping the file onto the canvas) or model databases, and layout the corresponding network map by using the different layout algorithms provided by VANTED (Belau et al., 2009).



a) COBRA toolbox.



b) *FBA-SimVis*.
Figure 1. Metabolic network model visualization.

Metabolic network data conformation. FBA–SimVis and COBRA Toolbox are software for in silico metabolic network reconstruction creation and usage different analysis methods. All metabolites and reactions data must satisfy each specific requirement for software analysis methods. FBA–SimVis and COBRA toolbox has own metabolic network data different requirements and prepared file structure.

COBRA Toolbox uses XLS format file to store additional information into each metabolite and each reaction sheet (Thiele and Palsson, 2010). All this information for metabolites and reactions must be taken from databases and tools on the internet (Klipp et al., 2006) and completed in XLS file without disturbing the file structure. The completed file is loaded with COBRA Toolbox commands in Mathlab, where it is tested for structural or syntactical errors. One of the main conditions is that all reactions must be balanced and all metabolites must have correctly assigned chemical formula. When above motioned steps are reached and metabolic network data are correctly conformed, only then we can make metabolic network model analysis.

FBA–SimVis toolbox integrated in VANTED software uses its possibility to import already made SBML file format metabolic network model or to create a new one with built-in VANTED software possibilities, and then creates several files before FBA functions can be made. From imported SBML model, FBA–SimVis toolbox creates several files:

- reaction file – stores SBML metabolic network reactions information ;
- C-matrix file – stores each metabolite carbon atoms count (if VANTED software cannot find any metabolite with additional name, then this count manually must be tilled in);
- SBML file – stores metabolic network model;
- metabolite file – stores all metabolites information.

Before make metabolic network model analysis can be made, the imported metabolic network model must be conformed to FBA–SimVis standard: all metabolites must be redrawn like circle shape and all reactions – like rectangle shape, because imported metabolic network model is in SBGN standard (Systems Biology Graphical Notation), but FBA–SimVis uses its own standard. As VANTED software has an opportunity to connect to Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/), then all metabolites and reaction abbreviations must be correctly tilled in like abbreviations into KEGG database in order to automatically balance all reactions.

Constraint–based model analysis

Flux balance analysis. Flux balance analysis is based on the optimization of an objective function, which is used as an evaluation criterion to identify an optimal flux distribution among all possible steady state flux distributions that meet the objective (Belau et al., 2009).

Flux balance analysis can be made in Mathlab with COBRA Toolbox, where the models are structures with fields, such as ‘rxns’ (a list of all reaction names), ‘mets’ (a list of metabolite names), and ‘S’ (stoichometric matrix). The function ‘optimizeCBModel’ is used to perform FBA. To change the bounds on reactions, use a function ‘changeRxnBounds’. Flux balance analysis can be performed to optimize one reaction by changing more than one reaction setting for each upper and lower flux flow amplitude limits, but then it must be noticed which of these reactions is the primary balanced reaction. Flux flow amplitude limits can be changed using COBRA Toolbox specific commands or changing them into XLS format file. Also the whole model must be reloaded into the Mathlab environment.

Flux balance analysis in VANTED software using FBA–SimVis toolbox can be performed to optimize only one reaction by changing up to three different reactions setting for each upper and lower flux flow amplitude limits in user friendly graphical interface. Each reaction is chosen from drop list of all metabolic network model reactions.

Knock-out analysis. Knock-out analysis (the deletion of given enzyme or gene) is performed by setting the flux trough a particle reaction to zero and calculating objective function. *In silico* knock-out analysis provides an efficient method to study the essentiality of a reaction in a metabolic network and to gain insight into metabolic changes caused by the deletion (Belau et al., 2009).

Knock-out analysis function in Mathlab environment with COBRA Toolbox is performed to delete one or two enzymes or genes with additional command '*singleGene Deletion(model,method)'*, '*doubleGeneDeletion(model,method)'*.

FBA-SimVis knock–out analysis function can be performed by two different methods.

- Complete – in this scenario we optimize the chosen function by disabling one by one all reactions and seeking from chosen settings the best solution for metabolic network model flux flow. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear.
- Specific – before call knock-out specific function the user must select reaction on canvas which will be knocked out, then call knock–out function from FBA menu list. In this scenario we optimize the chosen function by disabling selected reactions and seeking from chosen settings the best solution for metabolic network model flux flow. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear.

Robustness analysis. Robustness analysis is performed by varying a particular flux over a specified range of values and by recalculating the objective function. As the resulting curve depicts the sensitivity of the objective function to that particular flux, robustness analysis can be used to assess the effect of reducing flux trough particular reaction on a given objective (Belau et al., 2009).

COBRA Toolbox robustness analysis can be made in Mathlab with COBRA Toolbox by calling '*robustness analysis(model,controlRxn,nPoints)'* command, which is used to compute and plot the value of the model objective function as a function of flux values for a reaction of interest (*control Rxn*) as a means to analyze the network robustness with respect to that reaction. A plot with a defined number of points (*nPoints*) can be generated to visually assess how the objective function changes as the flux through the control reaction varies.

FBA–SimVis robustness analysis function can be performed by two different methods.

- Complete – in this scenario we optimize the chosen function by changing one by one all reactions flux flow and seeking from chosen settings the best solution for metabolic network model flux flow. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear;
- Specific – the user calls robustness analysis from FBA menu list. In this scenario we optimize the chosen function flux flow by changing selected reactions flux flow and seeking from chosen settings the best solution. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear and reaction which will be varied.

Flux variability analysis is performed by constraining the objective function to the optimal value and computing the minimal and maximal flux through every reaction in the network (range of fluxes). Flux variability analysis can be used to study the redundancies of the metabolic model under investigation (Belau et al., 2009).

COBRA toolbox determines the minimum and maximum flux value that each reaction in the model can possess while satisfying the steady-state assumption of FBA and the constraints on the system using the function: *'[minFlux,maxFlux]= fluxVariability(model,optPercentage)'*.

FBA-SimVis Simulation results of flux variability analysis are provided by displaying the minimal and maximal flux for each of the network reactions within the respective reaction nodes.

Results and Discussion

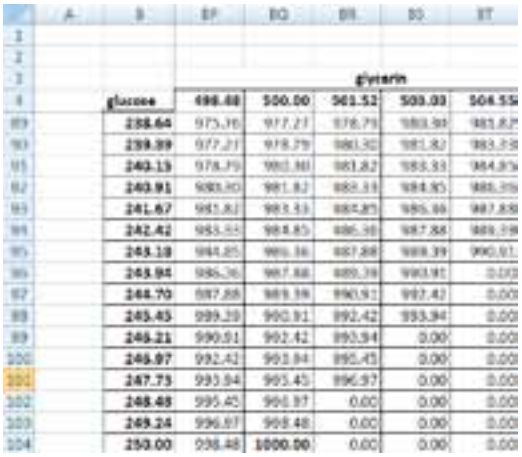
FBA, when analyzing all fluxes using different options, gives results into metabolic network flow chart, although COBRA Toolbox returns the results in the matrix in number formats. This information using COBRA Toolbox on the metabolic network is given into several different matrices. To better analyze these multiple matrices with single software there is need for other software like MS Excel, SPSS, Open Office CALC sheets technology. Each time you need to compare several data from different matrices changing focus from one to another.

FBA flow can be represented in flow chart, but this tool for flow chart creation for researchers is not available for experiments. COBRA Toolbox provides many visualization maps for FBA flow rate, and can show graphical user friendly maps, but free maps versions is difficult to create, because creation tool SimPheny (http://www.gtlifesciences.com/technology/Simpheny.html) is a commercial toolbox for FBA simulation creation. An alternative way to analyze FBA data from COBRA Toolbox is to create built in Mathlab 2D or 3D charts. For example, robustness analysis has been made for *Zymomonas mobilis* bacteria steady state metabolic network adaptation for glycerol conversion into bioethanol. First of all, S matrix has been created and correctly filled, then robustness analysis function has been executed with COBRA toolbox, and afterwards the obtained information can be analyzed numerically through matrices switching between them. A different method is to call 2D or 3D graph commands and analyze these graphs. For example, glucose flux constrain is +250 Unit - mmol gDW^{-1} h^{-1} (mill moles per gram dry cell weight per hour) and maximized Ethanol transport flux (objective). When

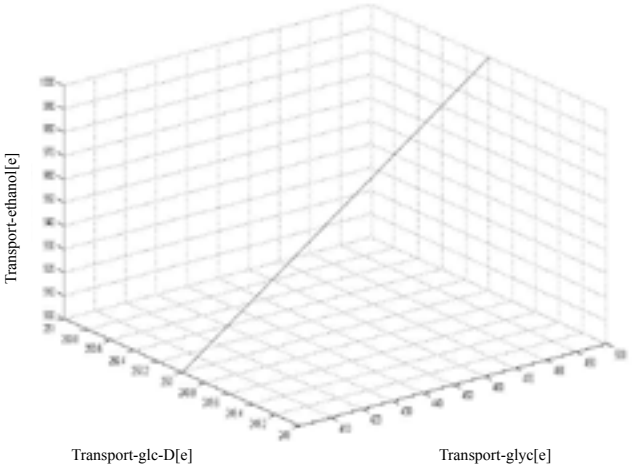
Transport Glycerol flux constrains reaches +500 Unit - mmol gDW^{-1} h^{-1}, then is reached maximal flux of Ethanol in steady state + 1000 Unit - mmol gDW^{-1} h^{-1}. In case when Transport Glycerol flux overruns +500 Unit - mmol gDW^{-1} h^{-1}, then starts inhibition. The results are given in matrix form (Fig. 2. a) and the results can then be plotted as a 3D surface (Fig. 2. b). The chart is easier to analyze and perceive than the numeric value.

FBA-SimVis the visualization of simulation results obtained from robustness analysis is offered in two ways, either displaying the sensitivity curve for each of the network reactions within the respective reaction nodes (Fig. 2. c), or by displaying the flux distribution of a particular enzymatic reaction, which can be varied by slider interactions (Fig. 2. d). While the first option provides a rapid and comparative overview of all reactions included in the network, the second option offers the user the possibility to obtain detailed insights into specific enzyme/objective function dependencies.

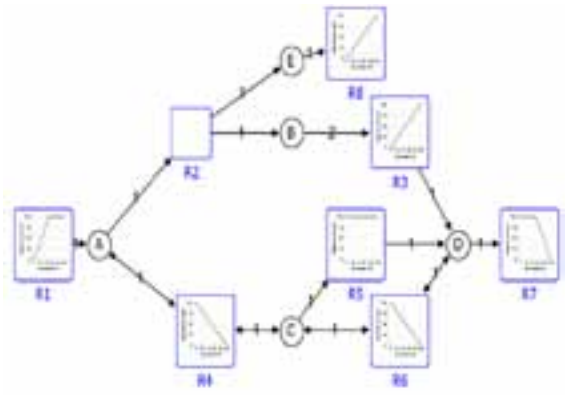
In FBA–SimVis for steady state *Zymomonas mobilis* bacterial metabolic network adaptation for glycerol conversion into bioethanol model was executed robustness analysis function with default constraint, where FBA-SimVis, unlike the COBRA Toolbox, results was represented in user friendly graphical interface as dynamically visually changing thickness of arrows with additional numerical values. FBA–SimVis visually on canvas represents steady state metabolic network each reaction flow rate, direction and numeric rate value, but it offers to vary with only 3 reactions to optimize only one function, unlike the COBRA Toolbox which analyzes all the reaction flows at the same time and optimized can be more than one function. COBRA Toolbox is a powerful FBA calculation tool for metabolic networks, but the FBA-SimVis is a great FBA calculation tool with limited conditions opportunities.



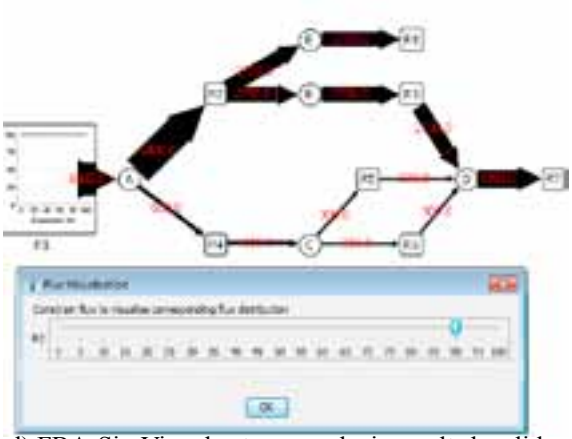
a) COBRA toolbox Glycerol robustness analysis results matrix 100×100.



b) COBRA toolbox Glycerol robustness analysis results chart.



c) FBA-SimVis robustness analysis results.



d) FBA-SimVis robustness analysis results by slider interactions.

Figure 2. Robustness analysis results visualization.

Conclusions

FBA-SimVis for Steady state metabolic network models analysis is provided for a small metabolic network, because making some FBA analysis there are small possibilities to change an unlimited count of variables, to choose or change or optimize reactions as variables.

FBA-SimVis does support SBGN standard to importing SBML files. It would be great advantage to improve FBA-SimVis with a supported SBGN standard automatically change metabolites and reactions shapes to FBA-SimVis eligible shapes. FBA analyses need to extend possibilities to change an unlimited count of variables, to choose change and optimize reactions as variables.

COBRA Toolbox for Steady state metabolic network models analysis is provided for greater metabolic networks with hundreds or thousands of reactions. It allows changing an unlimited count manipulating and optimizing reactions fluxes. To better analyze the obtained data from FBA analysis we can call maps which are made by freeware SimPheny and can get results in user friendly graphical interface in Internet browser. For example, has been free available example for *Escherichia coli* bacteria with full genome scale metabolic network, where, with the help of COBRA toolbox available commands, it is possible to experiment and obtain data but with no full functionality. Our proposal is to release map making software on next nearest COBRA toolbox versions with easy, user friendly graphical interface and functionality between the maps and matrices. Including a freeware map making tool on next releases, COBRA Toolbox would attract much more researchers, researchers' teams in silico analysis of steady state mechanisms of metabolic networks research. COBRA Toolbox is a calculation tool for an enormous amount of data, but FBA - SimVis Tool is at FBA flux visualization at user friendly graphical interface.

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