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Software applications for flux balance analysis

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Abstract

Flux balance analysis (FBA) is a widely used computational method for characterizing and engineering intrinsic cellular metabolism. The increasing number of its successful applications and growing popularity are possibly attributable to the availability of specific software tools for FBA. Each tool has its unique features and limitations with respect to operational environment, user-interface and supported analysis algorithms. Presented herein is an in-depth evaluation of currently available FBA applications, focusing mainly on usability, functionality, graphical representation and inter-operability. Overall, most of the applications are able to perform basic features of model creation and FBA simulation. COBRA toolbox, OptFlux and FASIMU are versatile to support advanced *in silico* algorithms to identify environmental and genetic targets for strain design. SurreyFBA, WEbcoli, Acorn, FAME, GEMSiRV and MetaFluxNet are the distinct tools which provide the user friendly interfaces in model handling. In terms of software architecture, FBA-SimVis and OptFlux have the flexible environments as they enable the plug-in/add-on feature to aid prospective functional extensions. Notably, an increasing trend towards the implementation of more tailored e-services such as central model repository and assistance to collaborative efforts was observed among the web-based applications with the help of advanced web-technologies. Furthermore, most recent applications such as the Model SEED, FAME, MetaFlux and MicrobesFlux have even included several routines to facilitate the reconstruction of genome-scale metabolic models. Finally, a brief discussion on the future directions of FBA applications was made for the benefit of potential tool developers.

Keywords: *systems biology; flux balance analysis (FBA); FBA tools; genome-scale model reconstruction; web applications*

INTRODUCTION

In the past decade, flux balance analysis (FBA) has been one of the most widely employed computational techniques for systems-level analysis of living organisms [1, 2]. It has been successfully applied to a multitude of species for modelling their cellular metabolisms, and therefore, enabled a variety of applications such as metabolic engineering for the over-production of biochemicals [3–5], identification of anti-microbial drug-targets [6–8] and the elucidation of cell–cell interactions [9–12]. FBA has been increasingly recognized due to its simplicity and extensibility: it requires only the information on metabolic reactions stoichiometry and mass balances around the metabolites under pseudo-steady state assumption [13]; additional biological

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constraints can also be easily incorporated into the model formulation [14]. Once the model is well defined, the metabolic fluxes can be quantified by resorting to linear programming (LP) where a plausible cellular objective is optimized, satisfying various balance and capacity constraints. Detailed information on the theory and formulation of FBA model can be found elsewhere [1, 2, 13, 14].

Although the theoretical formulation for FBA has been well established, it may not be easy for researchers to implement it without the familiarity in computational coding and basic programming skills since a large number of metabolites and reactions should be properly handled to quantify the metabolic fluxes. Particularly, recent genome-scale models for a variety of species involve >1000 reactions and metabolites [15]. So, it is often cumbersome and error-prone to manually define stoichiometrically balanced model equation and constraints, and manipulate them for subsequent optimization using LP solver. Clearly, this has motivated systems biologists, bioinformaticians and bioengineers to develop specialized FBA software packages. Initially, such applications were designed to perform only FBA on the given network models. They simply re-formulate the network specifications in certain formats (e.g. text files and spreadsheets) into their LP equivalents for subsequent execution of the solvers. However, with the increasing advancements in software technologies and algorithm development, newer tools have improved/added some of the features, e.g. enhanced graphical user-interfaces to view and manipulate network models, implemented algorithms for more advanced analyses such as strain design methods, and recently, adopted platform-independent web-technologies.

Remarkably, since 2000, there has been a steady increase in the number of FBA software and currently there are already >20 applications available (Figure 1). Thus, it is now timely to evaluate the capabilities and characteristics of FBA applications, as such identifying desirable features and bottlenecks for further improvements. In this regard, providing a comprehensive review on their strengths and weaknesses would be extremely useful to potential users in selecting the suitable tool for their project requirements as well as to developers for improving the FBA tools. Toward this end, we surveyed all currently available FBA tools under academic free license and compared them in various features such as operating platforms, ease of use, model creation facilities, additionally supported flux analysis techniques, visualization of network models and model exchange capabilities. Based on the comparison, we discuss some of the notable limitations in current software and provide the perspectives on future FBA tool development.

EVALUATION OF SOFTWARE APPLICATIONS

The surveyed applications herein can be grouped into any one of three classes, i.e.: (i) stand-alone, (ii) toolbox-based library and (iii) web-based, on the basis of their platform and software dependencies (Table 1). Stand-alone applications are independently installed onto the users' computers and executed locally. Toolbox-based applications, on the other hand, are not self-contained software, but add-on libraries installed in the general-purpose computation or network visualization tools such as MATLAB (http://www.mathworks.com/products/matlab/), Mathematica (http://www.wolfram.com/mathematica/) or VANTED [16], leveraging upon their existing capabilities, e.g. matrix computations, data manipulation and/or visualization for FBA. The third class is web-based applications which are accessible online regardless of the users' platform, only requiring a web-browser with moderately fast internet connection to build models and conduct FBA simulations. Considering the differences among the classes, we evaluated the FBA software applications using a set of test models including a genome-scale metabolic model of *Escherichia coli* [17] (Supplementary Note S1), in terms of five distinct characteristics: platform and software requirements, user friendliness nature, model reconstruction and analysis, visualization and model exchange formats (Figure 2). The resultant comparisons of their unique and common features are summarized in Tables 2–4.

Usability

Usability can be defined as a measure of how easy it is to use the FBA software for building and analysing metabolic models. Availability of intuitive user interface (UI), comprehensive user documentation and other user friendly attributes can enhance this characteristic of the software considerably.

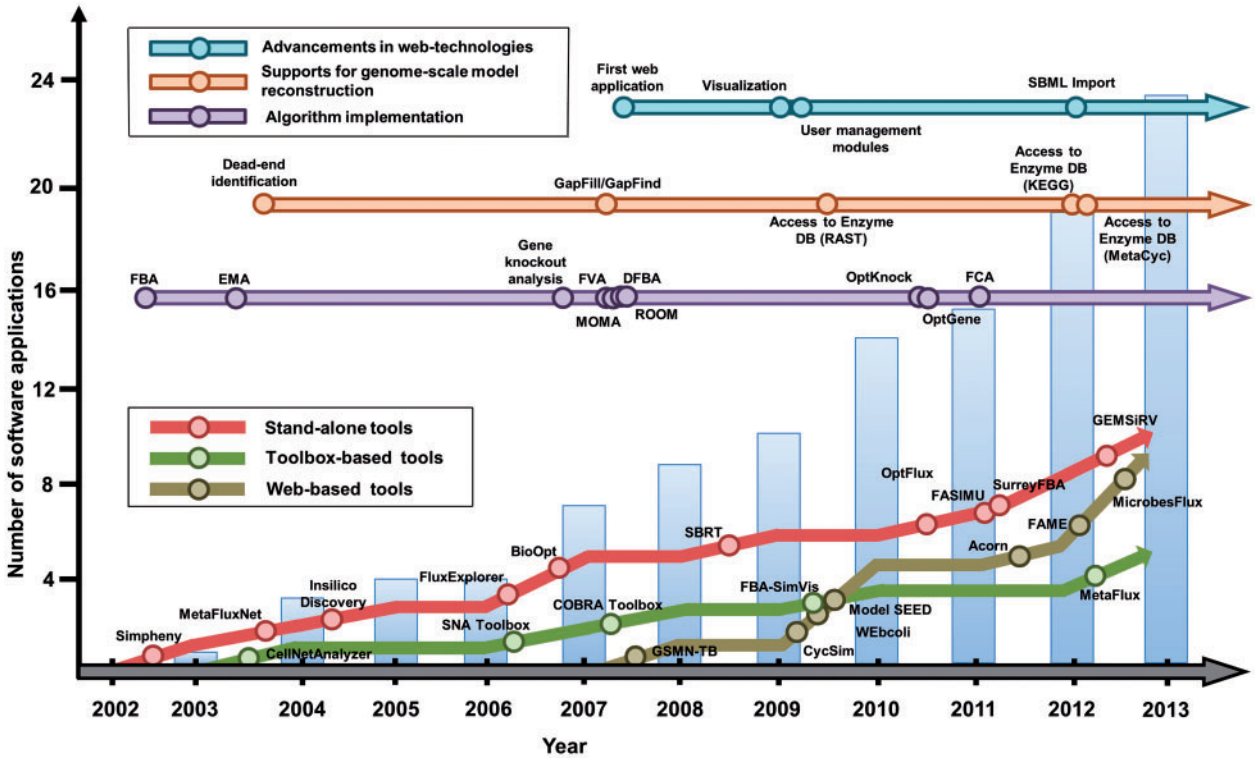

Figure 1: Timeline and development of FBA software. Timeline showing the launch of FBA software tools and the milestones of key features implemented in them. Software tools are classified into any one of the three categories: stand-alone, web-based and toolbox-based. Milestones are grouped into three classes: advancements in web-technologies, supports for genome-scale model reconstruction and algorithm implementation. The vertical bars at the background denote the number of available software at the end of each year (*Note:* If the software has an corresponding published journal article or patent associated with them, then the date of publication is considered as the launch date of the software).

Table 1: List of software applications evaluated in this study and their general characteristics

Type of the software application	Name	Tested version	URL	References
Stand-alone	OptFlux	2.1	http://www.optflux.org/	[18]
	SBRT	2.0.0	http://www.bioc.uzh.ch/wagner/software/SBRT/	[19]
	MetaFluxNet	1.8	http://metafluxnet.kaist.ac.kr/	[20, 21]
	BioOpt	–	http://129.16.106.142/tools.php?c=bioopt	[22]
	SurreyFBA	–	http://sysbio3.fhms.surrey.ac.uk/	[23]
	FASIMU	2.3.1	http://www.bioinformatics.org/fasimu/downloads/	[24]
	GEMSiRV	–	http://sb.nhri.org.tw/GEMSiRV/en/	[25]
Toolbox-based	CellNetAnalyzer/FluxAnalyzer	9.5	http://www.mpi-magdeburg.mpg.de/projects/cna/cna.html	[26, 27]
	COBRA Toolbox	2.0	http://opencobra.sourceforge.net/	[28, 29]
	SNA Toolbox	1.0	http://bioinformatics.org/project/?group_id=546	[30]
	FBA-SimVis	–	http://fbasimvis.ipk-gatersleben.de/	[31]
	MetaFlux	–	http://www.biocyc.org/download.shtml	[32]
Web-based	CycSim	1.0.0	http://www.genoscope.cns.fr/cycsim/	[33]
	WEbcoli	1.5	http://webcoli.org/	[34]
	GSMN-TB	–	http://sysbio3.fhms.surrey.ac.uk/cgi-bin/fba/fbapy	[35]
	Acorn	–	http://sysbio3.fhms.surrey.ac.uk:8080/acorn/homepage.jsf	[36]
	Model SEED	1.0	http://seed-viewer.theseed.org/seedviewer.cgi?page=ModelView	[37]
	FAME	–	http://f-a-m-e.org/	[38]
	MicrobesFlux	–	http://tanglab.engineering.wustl.edu/static/MicrobesFlux.html	[39]

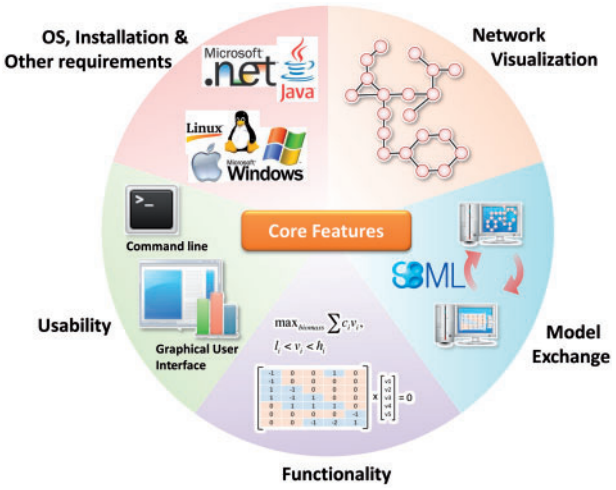

Figure 2: Core features of FBA software. The five core features of any FBA software include the following: (i) OS, installation and other requirements, (ii) usability, (iii) functionality, (iv) model exchange and (v) network visualization.

User interface

Invariably, users need some forms of interface to interact with the FBA application. In this aspect, the models can be defined through the following: (i) script/console, (ii) forms or tables and/or (iii) drag-and-drop operations on a graphical canvas. Script-based applications, e.g. BioOpt, SBRT, COBRA toolbox, FASIMU, MetaFlux and GSMN, require users to specify models in flat files that list down all the molecules, reactions and/or their stoichiometric matrices. Ensuring model correctness could be often a tedious task in this approach since it is easy to commit typographical mistakes, especially for large network models. On the other hand, form-based software applications, including MetaFluxNet, OptFlux, SurreyFBA with JyMet GUI, CellNetAnalyzer, WEbcoli and CycSim, alleviate this issue by providing more error detection features as the user can input the various elements of the model using interactive screens which contain specified forms or tables. The last type of interface, i.e. the diagrammatic UI is more sophisticated, allowing users to create network models by visually dragging and dropping nodes (metabolites or compounds) and edges (reactions or interactions) onto a canvas. This is, indeed, an intuitive approach for model creation: users can quickly glance at the network topology and infer the correctness of the model. However, one severe problem with the interface is the proper layout of large-size network diagrams as it becomes very random and cluttered. In addition, the visualization of metabolic networks with hundreds to thousands of nodes remains a big challenge. Currently, among all surveyed applications, FBA-SimVis is the distinct tool to provide the drag-and-drop UI. Apart from model specification, UI is also critical in FBA simulations and the subsequent analysis of results. In this regard, script-based applications execute FBA upon a command line call and generates some specific file formats (e.g. text files and spreadsheets) containing the quantified metabolic flux values while form-based applications provide specific buttons/tabs within the software environment for FBA execution and employ grids to display the results. Detailed evaluation on the user-friendliness nature of UIs is provided in Supplementary Note S2.

User documentation and miscellaneous features

User manuals and documentations are available in the form of online help or separate documents detailing the implementation and execution methods of the FBA software. The availability of tutorials and case studies with relevant screenshots can also help the user acclimatize to the software much faster.

We evaluated the quality of user documentations provided in all applications according to three core criteria: completeness, understandability and availability of case studies/tutorials (Table 2). The user manuals in most of the applications contain comprehensive information about five major components: (i) installation and software requirements, (ii) steps to create or edit models, (iii) FBA simulation procedures, (iv) network visualization methods and (v) export/import of model and analysis results. Interestingly, along with the typical user manuals, Acorn, FBA-SimVis, MicrobesFlux and WEbcoli also include the multi-media as a part of their documentation by providing video demos for tutorials. Obviously, the presence of such materials could reduce the user's learning curve through more interactive communication than normal text documents. However, despite these intuitive contents, none of the tools provides additional information such as troubleshooting steps for commonly encountered errors or exceptions. Furthermore, the help documents in SBRT, SurreyFBA and CycSim are relatively poor with little/no screenshots and illustrations; the documentation of SNA Toolbox contains many technical terms related to Mathematica without proper explanation.

Table 2: Dependency and usability features of FBA applications

Software applications	Required software environment			Type of UI provided	User documentation rating
	Platforms supported	Additional software requirements	Optimization solver used		
OptFlux	Windows and Linux	Java JRE 1.6.x	GLPK	Wizard/Script	E
SBRT	Windows, Linux and Mac OS X	≥Java JRE 1.5.x	GLPK	Script	P
MetaFluxNet	Windows	≥.NET framework 1.1, ≥Java JRE 1.4.2	Qsopt, LPSOLVE	Wizard	E
BioOpt	Windows	NA	GLPK	Script	G
SurreyFBA	Windows, Linux and Mac OS X	Java JRE 1.6.x (4)	GLPK	Script/Wizard[a]	P
FASIMU[b]	Linux and Mac OS X	Optimization solver	GLPK, CPLEX, LINDO, LPSOLVE	Script	F
GEMSiRV	Windows, Linux and Mac OS X	≥Java JRE 1.7.x	GLPK	Wizard	E
CellNetAnalyzer[b]	Windows, Linux and Mac OS X	≥MATLAB 7.1, Optimization solver, SBML Toolbox 3.0.0	GLPK, Optimization Toolbox (MATLAB)[c]	Wizard/Script	G
COBRA Toolbox[b]	Windows and Mac OS X	≥MATLAB 6.5, Optimization solver, ≥libSBML 4.0, ≥SBML Toolbox 3.0.0	GLPK, TOMLAB.CPLEX[d], LINDO, Gurobi[d]	Script	F
SNA Toolbox	Linux	≥Mathematica 5	–	Script	P
FBA-SimVis	Windows	≥VANTED 1.8, Java JRE 1.6.x	GLPK	Pictorial	E
MetaFlux	Linux and Mac OS X	Pathway Tools	SCIP	Wizard/Script	F
CycSim	–	Web Browser: ≥Firefox 2.0[e]		Wizard	F
WEbcoli	–	Web Browser: ≥IE 6.0 or ≥Firefox 2.0[e]	ILOG.CPLEX	Pictorial/Wizard/Script	E
GSMN-TB	–	Web Browser	GLPK	Script	P
Model SEED	–	Web Browser: ≥Firefox 2.0[e]	ILOG.CPLEX	Wizard	G
Acorn	–	Web Browser, Java JRE 1.6.x	GLPK	Wizard	G
FAME	–	Web Browser: Google Chrome, Firefox[e]	GLPK/ILOG.CPLEX	Wizard	E
MicrobesFlux	–	Web Browser	IPOPT	Wizard	G

[a]Form-based interface is available through a separate program called 'JyMet'. It is a GUI designed to access all installed routines of SurreyFBA. [b]Requires LP solver installation and configuration separately by the user. [c]Optimization toolbox of MATLAB can be used for some functionality instead of GLPK; however developers recommend use of GLPK. [d]COBRA toolbox recommends TOMLAB CPLEX and Gurobi as fast and accurate solvers. [e]Recommended Web Browsers for accessing the application. User documentation rating: E—Excellent, G—Good, F—Fair and P—Poor.

Table 3: Functionalities supported by FBA applications

Software applications	Support for model reconstruction		FBA and other phenotype prediction analyses									Strain design algorithms			Elementary mode analysis
			FBA	Gene knockout analysis			Integration of 'omics' data		DFBA	FCA	FVA				
	Pathway import from DBs	Assistance in gap filling		FBA	MOMA	ROOM	GIMME	iMAT				OptKnock	OptGene	GDLS	
OptFlux			√	√	√	√					√	√	√		√
SBRT			√	√							√				√
MetaFluxNet		√	√	√											
BioOpt			√	√[a]							√				
SurreyFBA			√	√[a,b]							√				√
FASIMU		√	√	√	√	√	√	√		√	√				
GEMSiRV	√[c,d,e]	√	√	√[f]							√				
CellNetAnalyzer			√	√							√				√
COBRA Toolbox		√	√	√[a,g]	√		√	√	√		√	√	√	√	
SNA Toolbox			√												√
FBA-SimVis	√[c,h]		√	√							√				
MetaFlux	√[d]	√	√	√[a,f]											
CycSim			√	√											√
Webcoli			√	√											
GSMN-TB			√	√[g]							√				
Model SEED	√[i,j]	√	√												
Acorn			√	√[g]							√				
FAME	√[d]		√	√							√				
MicrobesFlux	√[d]		√						√						

[a]Can perform in automated format, i.e. run simulations by knocking out each reaction/gene at a single instance in continuous format. [b]Both gene essentiality and reaction essentiality is available separately. [c]Connects with KEGG. [d]Connects withMetaCyc. [e]Contains even a reference DB of certain existing genome-scale models to support AUTOGRAPH method. [f]Can compare the predictions with available experimental datasets in PGDBs. [g]Based on the gene-reaction association; therefore, requires a mention of gene-reaction association. [h]When tested for the import of reactions from KEGG, failed due to the new licensing policy of KEGG, i.e. no free data download through FTP. [i]Automatically curates for network gaps and other inconsistencies. [j]Connects with RAST.

Therefore, we believe that most tools require substantial improvement in the user documentation. More detailed evaluation result can be found in Supplementary Note S2.

In addition to UI and help documents, availability of certain miscellaneous features can also enhance the usability of software applications. For example, the tooltips/succinct descriptions on the buttons/tabs inside a software environment are currently available in Acorn, GEMSiRV, MetaFluxNet and OptFlux. Similarly, Acorn, GEMSiRV, MetaFluxNet, OptFlux and SurreyFBA support the option to store, navigate, sort and/or filter the model information and analysis results.

Functionality

Functionality of an FBA software application can be defined as its capability to support various functional *in silico* analyses for cellular phenotype prediction and strain design under perturbed environmental and/or genetic conditions. We also consider the advanced features for model reconstruction and evaluation as one of the relevant attributes in this facet.

Analytical algorithms for phenotype prediction and strain design

Over the years, several FBA-based analytical algorithms have been developed to incorporate additional constraints, analyse the network flexibility, predict the cellular phenotype under perturbed conditions and to postulate strain design strategies [15, 40]. We evaluated the capabilities of the applications supporting such algorithms by performing various *in silico* analyses using *E. coli* genome-scale model [17] as summarized in Table 3. Details on evaluation methods and results can be found in Supplementary Note S3. Currently, only COBRA toolbox, FASIMU and OptFlux offer various functional analysis for predicting the phenotype of genetically perturbed strains using minimization of metabolic adjustment (MOMA) [41] and regulatory on/off minimization of metabolic flux (ROOM) [42]. COBRA toolbox and OptFlux also provide some strain design algorithms, such as OptKnock [43] and OptGene [44]. Interestingly, COBRA toolbox and FASIMU can incorporate the available 'omics' data sets such as gene expression data for analysing environment- and tissue-specific metabolisms using GIMME [45] and iMAT [46, 47] algorithms, respectively.

Supports for genome-scale model reconstruction and evaluation

The genome-scale model reconstruction is initiated by collecting metabolic reactions of any specific organism from various biochemical databases (DBs) such as KEGG [48], MetaCyc [49] and BRENDA [50] based on their genome annotation [51]. It is followed by assembling of the reactions, resulting in the draft network model which can be further improved by manual curation via dead-end identification, reaction balance checking and gap filling. Currently, some of the applications have such facilities in automating the data collection from different DBs (Table 3). FBA-SimVis, FAME and MicrobesFlux can import organism-specific pathway information from KEGG while MetaFlux and Model SEED can obtain relevant reaction data from MetaCyc and RAST server [52], respectively. As an alternative, genome-scale models can even be drafted from the existing models, based on sequence homology between the genes of target and query organisms. Currently, GEMSiRV supports this approach to reconstruct metabolic models, connecting to the model repository from BiGG [53].

Apart from the support to automate the reconstruction of draft genome-scale models, software applications may also assist users in evaluating the networks connectivity and their gaps (Table 3). Currently, MetaFluxNet, MetaFlux, COBRA toolbox, GEMSiRV and FASIMU helps the user identify the dead-end/blocked metabolites in the model. Similarly, COBRA toolbox and FASIMU also allows the user to check the ability of any particular metabolite for its synthesis or degradation. Interestingly, MetaFlux, Model SEED and COBRA toolbox implement the MILP-based analytical algorithms—GapFind and/or GapFill [54] for gap filling process.

Network visualization

Metabolic networks can be intuitively viewed as graphs where the metabolites and their interactions are represented by nodes and edges linking them, respectively. Most of applications provide basic static images to visualize the networks. However, laying out large-scale networks, e.g. genome-scale models and magnifying any particular pathway are often cumbersome in such static maps because of their intractable nature. Therefore, dynamic network visualization features, supported by FBA-SimVis, remedy this issue by offering sophisticated graphical

Table 4: Network visualization and model-exchange supports

Software applications	Network visualization		Model-exchange			
			SBML		Other formats	
	Visualization type	Flux visualization	Import	Export	Import	Export
OptFlux	S[a]	√	√	√	Text files, MetaTool	Text files
SBRT	–		√		Text files	
MetaFluxNet	S	√	√	√	MFAML	MFAML, GAMS, LINDO, LP SOLVE, CPLEX
BioOpt	–		√[b]		Text Files	
SurreyFBA	S[c]	√	√		Text Files	
FASIMU	–	√[N]	√	√	Text files, FA format[d]	MetaTool, Expa
GEMSiRV	S[a]	√	√	√	Microsoft Excel	Microsoft Excel
CellNetAnalyzer	S[a]	√	√	√	Text files, MetaTool	Text files, MetaTool
COBRA Toolbox	S[a]	√	√	√	Microsoft Excel, SimPheny,	Text files, Microsoft Excel
SNA Toolbox	–	–			Text files	Text Files
FBA-SimVis	D	√	√	√	GML, KGML, GraphML, XGMML, Text files	GML, KGML, GraphML, XGMML, Text files
MetaFlux	S	√		√	Text files	Text files
CycSim	S	√		√		Text files
WEbcoli	D	√		√		Text files
GSMN-TB	–	–		√		Text files
Model SEED	–	√[N]		√		Microsoft Excel, LP format
Acorn	D[e]	√	√			Text files
FAME	S	√	√	√		
MicrobesFlux	–	–		√		

[a]Requires a user supplied 'map' file to execute generate network topology; GEMSiRV can use even KGML/CellDesigner formats whereas OptFlux can use CellDesigner format. [b]Through another program/separate utility (available online at http://129.16.106.142/toolbox.php). [c]Available only while accessing through its GUI: 'JyMet'. [d]FluxAnalyzer format: a format that can be exported from CellNetAnalyzer. [e]Implemented through separate program which runs on desktop PC locally. S, static image; D, dynamic interface; [N], through specialized network visualization software such as Cytoscape.

interfaces where users can re-align the nodes and edges, adjust their sizes and interestingly allow users to visualize sub-networks of certain pathways from a large model for a biologically intuitive network analysis.

In addition to the basic network visualization feature, some of the tools also offer advanced facilities such as incorporation of resultant flux values onto the network map either by simply overlaying the values itself or by providing additional visual cues via variations in the thickness of edges based on flux values (Table 4). Interestingly, some tools accommodate this function by exploiting specific plug-ins under network visualization environments such as Cytoscape [55] and BiNA [56]. For example, FASIMU can export compatible model formats to Cytoscape and BiNA, which can further link the resulting fluxes using its corresponding plug-ins FluxViz [57] and faBiNA [56], respectively. Similarly, Model SEED generates models which can be visualized under Cytoscape environment, followed by flux mapping using its plug-in, CytoSEED [58]. Remarkably, among all applications, FBA-SimVis is a unique tool in flux visualization as it enables the user to even perform flux perturbation analysis through the network diagram. Thus, the network map of the perturbed state can be instantaneously visualized while manipulating the flux of a certain reaction. It should be highlighted that except a few features such as automatic re-alignment of networks based on layout algorithms and filtering option based on sub-networks available in FBA-SimVis, none of the tools can handle large-scale models. In this regard, availability of options to visualize interactive multiple sub-networks of a large-size model with appropriate links among them will improve the intuitive understanding of overall functional interactions along with their organization. Detailed information on network visualization evaluation procedure and results could be found in Supplementary Note S4.

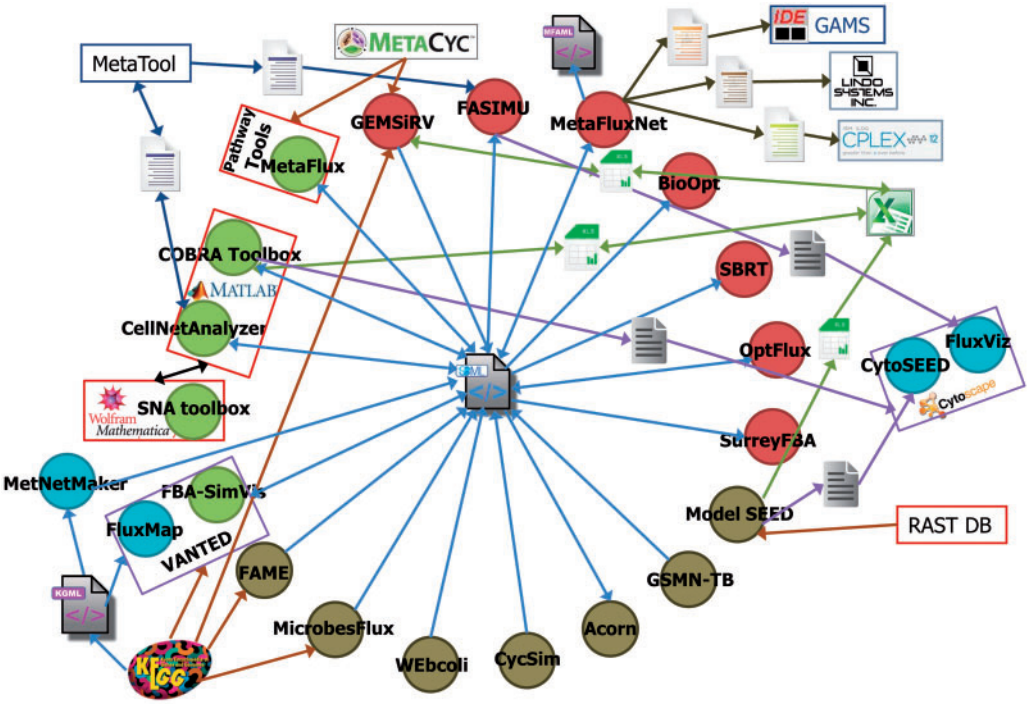

Figure 3: Model exchange capabilities of FBA applications. Brown-, green- and tan-coloured nodes represent the stand-alone, web-based and toolbox-based applications. Nodes with aquatic blue colour represent the software which cannot implement FBA but can help converting model format from one to another. The nodes inside rectangular represents the toolbox-based (red-coloured rectangles) or plug-in based (purple-coloured rectangles) software where the boxes denote base software and nodes inside them represent the additional libraries.

Model exchange

The model exchange via standardized formats across different FBA applications allows us to fully utilize all the unique features supported in each application. Systems biology markup language (SBML) [59, 60] is one of the most widely accepted standards for model representation. Other formats include the metabolic flux analysis markup language (MFAML) [61] and cell markup language [62]. The model exchange feature in various formats is summarized in Table 4 and Figure 3.

SBML compatibility

Most of the stand-alone and toolbox-based applications support the import and/or export of SBML files although BioOpt, FASIMU and SBRT require additional step by the translator to convert them into compatible formats (Figure 3). However, none of the web-based applications, except FAME, have SBML import function as they can conduct *in silico* analysis only on the existing pre-defined models. We tested the SBML compatibility for all the applications using our test models (Supplementary Note S1) by validating generated SBML files through the online SBML validator (http://sbml.org/validator/) and the ability of all software applications to recognize the SBML exported by other software (i.e. the readiness of the application to perform FBA upon import of SBML). Here, we wish to report some of the compatibility issues which should be properly addressed for ensuring model consistency. The results revealed that SBML generated by COBRA toolbox and OptFlux are readily inter-transferable between them. However, certain FBA specific information such as reaction bounds and objective function are missing in the SBML created by all other applications. Detailed discussion on the SBML issues can be found in Supplementary Note S5.

Compatibility in other formats

For the exchange of FBA models among different applications, other than SBML, a similar XML-based standard, MFAML, was specifically designed with relevant XML tags for reaction bounds and objective functions [61]. Unfortunately, it is not widely adopted and currently, MetaFluxNet is the only tool that supports this format. We also analysed the ability of software applications to create

programming scripts/computer codes that represent the models in other general computational or optimization software (Table 4). In this aspect, MetaFluxNet is the most versatile software as it can generate various scripts which are compatible with MATLAB, GAMS (http://www.gams.com/), LP SOLVE (http://lpsolve.sourceforge.net/), IBM ILOG CPLEX (http://www.ibm.com/software/integration/optimization/cplex-optimizer/) and LINDO (http://www.lindo.com) for the given FBA problem. Availability of such options certainly provides more flexibility in implementing complex algorithms which are currently not supported by any of the software applications surveyed. Similarly, OptFlux and FASIMU can generate MetaTool [63] format, thus enabling elementary mode analysis.

DISCUSSION

Genome-scale model reconstruction

As one of important features, the current applications can provide functional environment to accelerate the reconstruction of genome-scale metabolic models which is otherwise too laborious, requiring substantial time and manual efforts [15, 51]. Thus, it is highly required to implement specific functions for (semi-)automatically gathering information from various online enzyme DBs and subsequently refining them with the help of network evaluation algorithms such as GapFill/GapFind [54], GrowMatch [64] and GeneForce [65]. In this regard, as highlighted before, most recent applications, FAME, GEMSiRV, MetaFlux, MicrobesFlux and Model SEED, have already incorporated certain features in facilitating the reconstruction process by importing reaction data from KEGG, MetaCyc or RAST and improving the network connectivity using GapFill/GapFind. Nonetheless, we suggest that the quality of such draft models can be further enhanced by additional model curation steps such as elemental and charge balance check in reactions, verification of reaction directionality from more specific enzymatic DBs, e.g. BRENDA and UniProt [66], assignment of reactions to appropriate cellular compartments based on the protein subcellular localization prediction software, e.g. PSORT [67] and MultiLoc [68] and inclusion of transport reactions from TCDB [69]. Moreover, software applications can also consider linking them with biological model storage DB such as BiGG [53], Model SEED [37], BioMet toolbox [22] and BioModels [70] since this support will not only allow users to directly perform simulations on existing models but can also help them reconstruct new genome-scale models using the AUTOGRAPH method [71]. It should be noticed that the data from multiple-sources may often conflict with each other or even miss several information from one another. These issues can be appropriately resolved by implementing methods for data integration and standardization. In such an attempt, a new knowledgebase, MetRxn, has been, recently, developed by Kumar *et al*. [72], integrating the data from 4 biochemical DBs and 44 genome-scale metabolic models available from literature. Therefore, utilization of this knowledgebase for integrating the data collected from different data sources would be an interesting option for model reconstruction.

FBA-based functional algorithms

Another critical functionality feature along with model reconstruction is to support various analytical algorithms for phenotype prediction and strain design. Although COBRA toolbox, FASIMU and OptFlux have already incorporated basic algorithms for mutant phenotype analysis such as MOMA and ROOM, a multitude of other FBA-based *in silico* techniques can be considered to enhance the functionality and broaden the analytical capability. For example, incorporation of regulatory constraints into the FBA framework via Boolean logic representation, e.g. rFBA [73] and iFBA [74] or by integrating the transcriptomic and/or proteomic data sets using various algorithms such as GIMME, iMAT, E-Flux [75], INIT [76], MBA [77] and MADE [78], offers a wide variety of applications. They include environment-specific phenotype prediction [45, 74, 78], development of tissue-specific sub-network models in higher organisms [76, 77, 79], identification of drug-targets [75, 80] and the analysis of host-pathogen interactions [81]. In terms of strain design, OptKnock, OptGene, GDLS, OptReg [82], OptORF [83], RobustKnock [84] and OptForce [85] could help to devise novel strain engineering strategies by identifying the gene/reaction targets to be manipulated for the improved productivity. It should be highlighted that such advanced algorithms are mostly formulated as MILP problem [86], whereby the combinatorial explosion as a result of the huge metabolic network models may lead to inefficient solving of the optimization problem based on default solver settings [87]. In this regard, it is desirable to have flexible options in selecting the

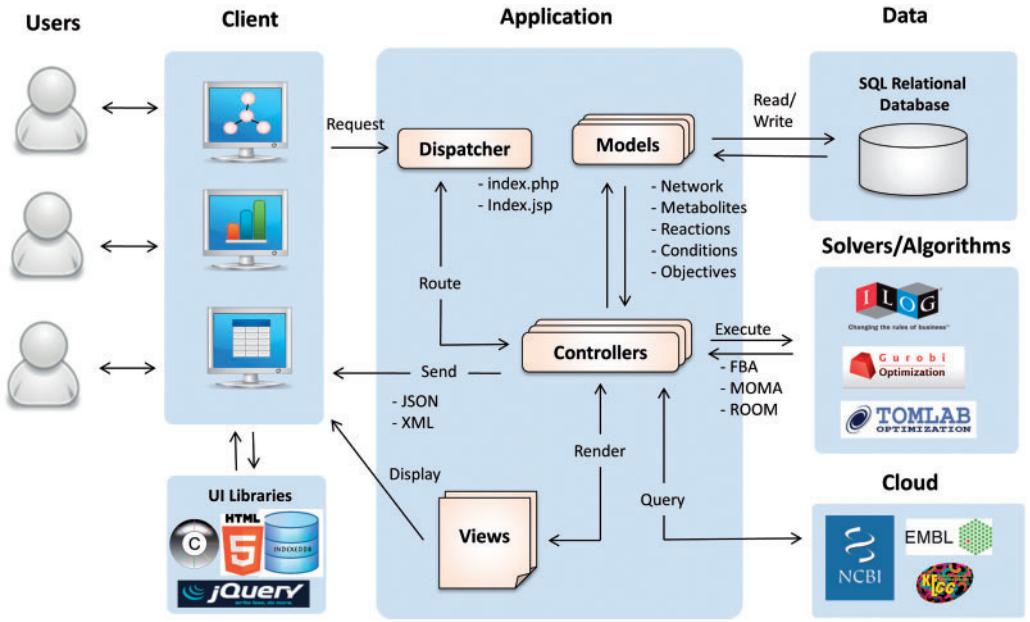

Figure 4: Typical architecture of web application. A typical three-tier architecture of web applications that incorporates the Model-View-Controller pattern for application design.

solver algorithms and specifying the corresponding parameters.

Flexible and extensible software design

As stated earlier, it is highly necessary to standardize model exchange format across different applications in order to utilize various interesting features which are unique in each tool. However, the widely accepted SBML standard currently may not capture some of the key information for FBA, thus initiating community discussion and proposal to include additional attributes for better representing FBA models (http://sbml.org/Community/Wiki/SBML_Level_3_Proposals/Flux_Constraints_Proposal).

Alternatively, we can consider flexible and extensible software environment capable of basic model processing and construction, where such interesting functions can be additionally implemented via plug-ins/add-ons as successfully adopted by Cytoscape, VANTED and CellDesigner [88]. Interestingly, Microsoft Excel (http://office.microsoft.com/en-us/excel/), the commonly used spreadsheet package for storing metabolic models, has all the necessary components to create and analyse FBA models including well-designed form-based interface, plotting and drawing facilities, in-built optimization solvers and scripting language for automating tasks (i.e. MACROS). It should be noticed that Excel-based FBA application may face technical challenges in handling large-size models due to the limitation in its in-built optimization solver. However, this issue can be appropriately resolved by using relevant software technologies such as OpenSolver (http://opensolver.org), an open-source optimization solver for Excel that runs on advanced COIN-OR CBC optimization engine (https://projects.coin-or.org/Cbc) and SolverStudio (http://solverstudio.org), a software framework that can integrate Excel with other open sources as well as commercial solvers, e.g. GLPK (http://www.gnu.org/software/glpk/), COIN CLP (http://www.coin-or.org/Clp/), CPLEX and GUROBI (http://www.gurobi.com/).

Web-based applications: future outlook

Figure 1 clearly indicates the increasing popularity of web-based FBA applications. Such an augmented interest towards web applications is mainly attributable to their multiple advantages over the stand-alone or toolbox-based applications: it provides not only distributed computing for implementing complex analytical frameworks but a medium for collaborative research where the combined efforts of scientific community could be facilitated. Importantly, rich UIs and enhanced interoperability can be manifested by resorting to recent web-technologies, e.g. Ajax, service-oriented architecture and Semantic Web, adopting three-tier MVC

(Model-View-Controller) architecture as depicted in Figure 4. Additionally, the use of Javascript libraries such as Cytoscape web and ExtJS can also improve interactivity, mimicking a desktop application within a web browser. For example, Discovery Studio® provides a thin-client local front-end for users to interact with their models while performing expensive operations at the backend server. However, despite the recent technology improvements, web applications still suffer a major disadvantage in the form of the necessity of being online always. Therefore, future web applications should possibly allow us to perform at least few functions such as model construction offline. Implementation of this facility is very much plausible with the help of offline application cache (appcache) or cache manifest from the emerging HTML5 framework and Indexed DB; the information can be temporarily cached in the client browser through their cookies while updating the transaction information from client-to-server and vice versa upon availability of the network connection. Finally, web-based FBA tools could even consider developing specialized applications (Apps) for smartphones so that the users could access their models and conduct FBA via portable devices.

CONCLUSIONS

In this review, we presented a comprehensive evaluation of all existing FBA software applications based on five major criteria: installation and operational requirements, usability, functionality, network visualization and model-exchange. All the surveyed tools sufficiently performed the basic FBA simulations while each of them has its unique features which may suit specific needs and preferences of the user. Interactive UIs for model building, analysis and basic network visualization are provided in MetaFluxNet, GEMSiRV and Surrey FBA, which are thus suitable for beginners. Expert users may prefer the applications such as OptFlux, FASIMU, CellNetAnalyzer and COBRA toolbox due to their capabilities of implementing advanced *in silico* algorithms including MOMA, ROOM, OptKnock and OptGene. For reconstructing new genome-scale metabolic models, users can utilize several supporting features available in FAME, MetaFlux, MicrobesFlux and Model SEED. Finally, FBA-SimVis provides the attractive diagrammatic UIs to perform FBA. Overall, the development of FBA applications has significantly matured in the past decade with the increasing software technological advancements. Particularly, web-based applications have garnered much interest in the recent years due to their overwhelming advantages than the stand-alone and toolbox-based applications. In conclusion, all the FBA tools need to improve several-folds such that they can perform the functionalities in a user-friendly manner, at the same time being amenable to future extensions.

SUPPLEMENTARY DATA

Supplementary data are available online at http://bib.oxfordjournals.org/.

Key Points

- Flux balance analysis (FBA) is commonly used *in silico* technique for analysing cellular metabolisms with wide applications.
- The availability of various FBA tools classified into three types, stand-alone, toolbox-based and web-based applications, allows users to leverage their analytical capabilities for better understanding and engineering of metabolic systems.
- All the current FBA tools were surveyed and evaluated based on five key criteria: operational requirements, usability, functionality, network visualization and model-exchange. We highlighted their unique supporting features such as advanced analytical algorithms, genome-scale modelling and dynamic visualization, and discussed software architecture in terms of flexibility and extensibility.
- Recent advancements in web-technologies resulted in the increasing trend of web-based FBA tools.

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