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BioMet Toolbox: genome-wide analysis of metabolism

Marija Cvijovic[1], Roberto Olivares-Hernández[1], Rasmus Agren[1], Niklas Dahr[1], Wanwipa Vongsangnak[1], Intawat Nookaew[1], Kiran Raosaheb Patil[2] and Jens Nielsen[1,*]

[1]Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden and [2]Center for Microbial Biotechnology, Technical University of Denmark, Lyngby, Denmark

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ABSTRACT

The rapid progress of molecular biology tools for directed genetic modifications, accurate quantitative experimental approaches, high-throughput measurements, together with development of genome sequencing has made the foundation for a new area of metabolic engineering that is driven by metabolic models. Systematic analysis of biological processes by means of modelling and simulations has made the identification of metabolic networks and prediction of metabolic capabilities under different conditions possible. For facilitating such systemic analysis, we have developed the BioMet Toolbox, a web-based resource for stoichiometric analysis and for integration of transcriptome and interactome data, thereby exploiting the capabilities of genome-scale metabolic models. The BioMet Toolbox provides an effective user-friendly way to perform linear programming simulations towards maximized or minimized growth rates, substrate uptake rates and metabolic production rates by detecting relevant fluxes, simulate single and double gene deletions or detect metabolites around which major transcriptional changes are concentrated. These tools can be used for high-throughput *in silico* screening and allows fully standardized simulations. Model files for various model organisms (fungi and bacteria) are included. Overall, the BioMet Toolbox serves as a valuable resource for exploring the capabilities of these metabolic networks. BioMet Toolbox is freely available at www.sysbio.se/BioMet/.

INTRODUCTION

The rapid expansion of systems biology has led to the development of a vast number of mathematical models and integrative strategies. Use of computational modelling has emerged as a powerful descriptive and predictive tool that allows the study of complex systems to investigate biological phenomena and represents the core of systems biology. The role of mathematical modelling and data integration is to generate testable hypothesis, design experiments and enrich the information content of experimental data.

One of the major applications of systems biology is within the field of metabolic engineering, which refers to directed genetic modification of cell factories with the goal to improve their phenotype for industrial application. The use of metabolic engineering for exploiting microorganisms in industrial biotechnology is not a novel concept. Fermentation processes have been applied for production of antibiotics, e.g. penicillin by *Penicillium chrysogenum*, amino acids, e.g. lysine by *Corynebacterium glutamicum*, organic acids, e.g. citric acid by *Aspergillus niger*, and enzymes, e.g. α-amylase by *Aspergillus oryzae*. *Escherichia coli* has been used for production of many different recombinant proteins (like human growth hormone) and the yeast *Saccharomyces cerevisiae* is used for bioethanol production, production of a range of pharmaceutical proteins, chemicals, bulk chemicals and nutraceuticals. The development of computational tools for *omics* data integration and genome-scale metabolic models (GSMMs) of cell factories enables the analysis of the effects of different media and specific mutations on growth and the operation of the metabolic network, moving biology from a phenomenological to a predictive science. One of the most accepted methods for providing general information on how the metabolic network is

*To whom correspondence should be addressed. Tel: +46 31 772 3804; Fax: +46 31 772 3801; Email: nielsenj@chalmers.se

The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors.

operating at different growth conditions is metabolic flux analysis. Metabolic fluxes can either be estimated through the use of flux balance analysis (1) or through the use of ^{13}C-labelled substrate feeding followed by analysis of the labelling patterns in intracellular metabolites (2). Thus, flux analysis today represents a standard technique not only for rapid phenotypic characterization of metabolically engineered strains, but also as an important aid in designing metabolic engineering strategies. Although flux analysis contributes strongly to the understanding of metabolic networks it is well known that transcriptional regulatory programs strongly control the flux distribution. Therefore, it is important to identify regulatory patterns in the interactions within the metabolic network and this can be achieved by combining the topology of the underlying network with *omics* data (3,4).

Here, we introduce the BioMet Toolbox, a web-based resource for analysis and integration of transcriptome and interactome data, and thereby exploiting the capabilities of metabolic networks described in genome-scale models. The BioMet Toolbox also includes GSMMs of various cell factories used both in industrial biotechnology and in fundamental research.

FEATURES

The BioMet Toolbox consists of: (i) a suite of applications ('Tools') developed for studying metabolism and high-throughput analysis, and (ii) a collection of GSMMs ('Models') of different organisms (Figure 1).

Tools

The core of the Tools section consists of a client applet that acts as a graphical interface to the server with the following analyses methods: (i) calculation of all internal mass balance fluxes, reduced costs and shadow prices for the assessment of *in silico* metabolic model predictive capabilities (BioOpt); (ii) identification of key biological features (metabolites, transcription factors, protein–protein interactions and GO association) around which transcriptional changes are significant (Reporter Features, 5); (iii) identification of significantly correlated metabolic sub-networks after direct or indirect perturbations of the metabolism (Reporter Subnetwork, 4).

Each analysis has a choice of sub-options that become available on its selection together with extensive examples and help menu.

The BioMetToolbox website is written in HTML and JavaScript. The backend is based on PHP5 and tools are written in MATLAB and C++.

BioOpt: flux distribution calculations using flux balance analysis

BioOpt focuses on flux balance analysis (FBA), using linear programming as the mathematical support. Flux distribution calculations using FBA is a widely used method for analysis of the capabilities of a metabolic network. Given a set of constraints, such as maximal uptake rates of nutrients, BioOpt returns the set of metabolic fluxes that maximizes a specified objective function (usually the growth rate of the organism). FBA of genome-scale networks provide an excellent platform for evaluation of gene essentiality as well as the more general study of metabolic perturbations following gene knock-outs. This has been used to identify drug targets (6) and to suggest possible metabolic engineering strategies to optimize by-product formation in microbial fermentations (7). BioOpt implements several analyses to deal with this type of problem, including an exhaustive combinatorial search for combinations of gene deletions and a mixed integer linear programming application to identify the best set of gene deletions given a target objective value. Other analyses include overexpression of fluxes and basic sensitivity analysis.

Required input is a genome-scale model in BioOpt format [a converter from SBML to BioOpt is provided in the BioMet toolbox and hence it is possible to use custom models available in SBML format in addition to those provided in the library of models (see 'Models' section)]. The input is a text file that contains the reactions in the model together with the constraints and the objective function needed to perform a simulation. For a detailed description of the syntax, consult BioOpt format instruction at the website www.sysbio.se/BioMet. The output is dependent on the type of analysis, but generally includes the optimal solution to the linear programming problem generated with the model, shadow prices for metabolites, and the internal fluxes and reduced costs associated to each reaction. The shadow prices give information on how the availability of metabolites will affect the objective function and the reduced costs give information about how beneficial/detrimental it is to have each reaction carry an additional unit flux. Taken together, this information can give valuable insight into the functionality of the metabolic system.

Reporter Features: identification of transcriptional regulatory circuits in metabolic networks

Reporter Features is a hypothesis-driven algorithm that integrates transcriptome/proteome/metabolome data with the topology of bimolecular networks. Reporter features algorithm exploits the connectivity structure of bio-molecular interaction networks for data integration. For example, the metabolic network can be treated as a bipartite undirected graph, where the nodes are the metabolites and the enzymes composing each reaction, while the edges represent the association between the metabolites and enzymes due to the corresponding reactions. All enzyme nodes are scored based on the *P*-value for significance of change in the expression level of the corresponding gene (across different conditions/mutants). Each metabolite in the graph is then statistically assessed for collective transcriptional response in the neighbouring enzyme nodes. Metabolites with significant scores represent metabolic hot spots with significant degree of transcriptional regulation around them. The algorithm can also be applied to different biological networks to identify corresponding reporter features (such as transcription factors, protein–protein interactions, GO association, protein complexes and *ad hoc* interactions of

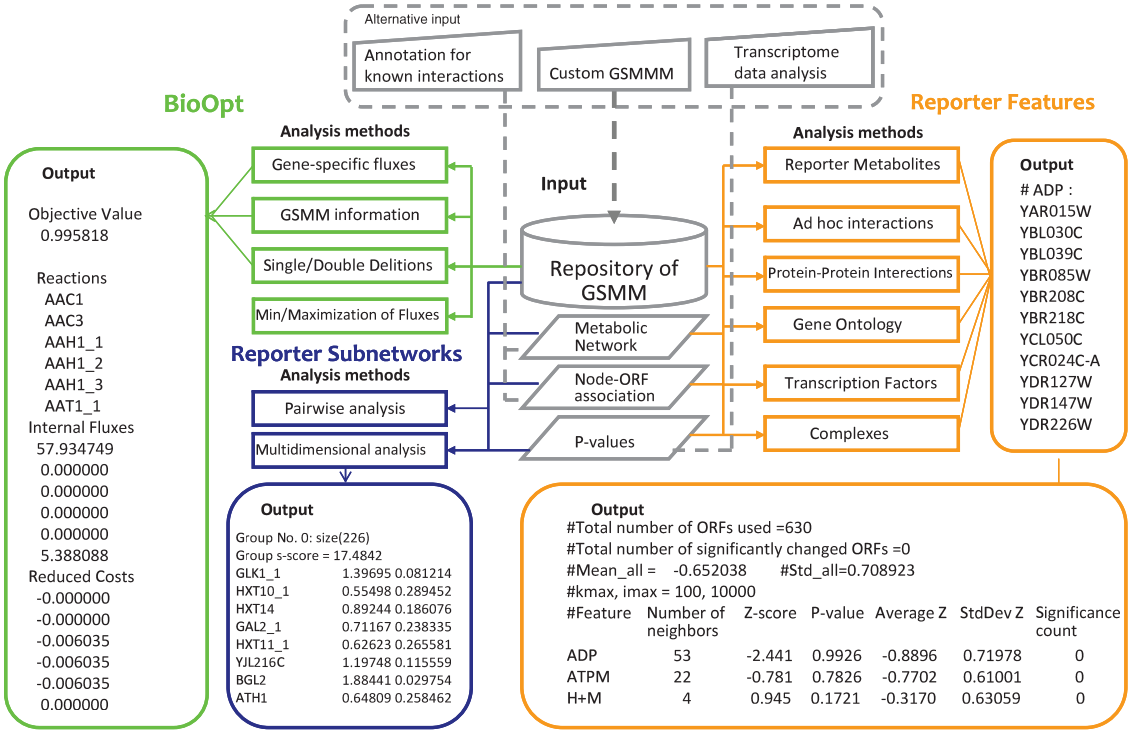

Figure 1. System flow of the BioMet Toolbox. Central panel (gray color) represents required input files [GSMM from our repository, Metabolic Network file with interaction, Association files: Node-ORF and file with *P*-values (more detailed descriptions of input files can be found in the text)]. Alternative input files (dashed gray color) are allowed (Annotation for know interaction can replace Metabolic Network file with interaction and Node-ORF Association files; Transcriptome data analysis instead of file with *P*-values, and custom GSMM instead of models provided in the repository). Three applications with available sub-options (color-coded as corresponding application) are represented in rectangular boxes (Analysis methods) and example of the results (output files) in oval boxes: Reporter Features (orange), Reporter Subnetworks (blue) and BioOpt (green).

interest) around which transcriptional changes are collectively significant. For example, if the algorithm is applied to the regulatory network that represents interactions between transcription factors and the regulated genes, the result will mark the reporter transcription factors—indicating significant change in the corresponding TF activities (5).

The use of the Reporter Features tool requires three input files: (i) a data file with *P*-values from a significance of change test (e.g. Student's *t*-test for a pair-wise comparison) for each ORF represented in the interaction network. In case of a multidimensional data, the algorithm can take the expression levels for each condition (Pearson correlation coefficient is then used as a scoring metric); (ii) an interaction network file containing feature name, together with type of interaction and node name. The format is the same as the SIF format, commonly used in other platforms as Cytoscape (8). For reporter metabolite analysis, a genome-scale model in BioOpt format can directly be used as an input, the computational algorithm internally transforms the BioOpt annotation into required interaction map; (iii) Node-ORF relation file containing node names used in the interaction file and the corresponding ORF names as in the *P*-values data file (i). The output contains a list with the feature (e.g. metabolites or transcription factors) and a *z*-score pertaining to the null hypothesis that the observed collective transcriptional response around the feature is by chance. For instance, if the input file is the interaction network constructed from the GSMM, the output will show a list of metabolites with their *z*-scores, the metabolites with significant scores are defined as reporter metabolites. For ranking the transcription factors that execute the control on the metabolic network the Reporter Transcription Factor analysis option is available. As a supplementary analysis, the user can perform GO enrichment analysis using Reporter Gene Ontology that has been demonstrated to be as powerful as other methods created for gene set enrichment analysis (9).

Reporter Subnetwork: identification of significantly responsive/correlated metabolic subnetworks

The aim of Reporter Subnetwork tool is to identify significantly responsive/correlated metabolic subnetworks after direct or indirect perturbations of the metabolism (4,10). Interaction networks are constructed based on the metabolic genes. Specifically, metabolic enzymes are represented as an undirected graph by using the topological information from the GSMM. In such undirected graph, two metabolic enzymes are connected if they share any common metabolite in the corresponding reactions.

Similar to Reporter Features, Reporter Subnetwork requires three input files. The first input file required is the file containing, either *P*-values (for a pair-wise comparison), or, multiple columns for expression under different condition (for multidimensional data). The second required input is Node-ORF association file and the last

required file is a genome-scale model in BioOpt format. The output consists of two text files, with the same format to those files used in reporter features: the first file contains the gene groups forming the highly responsive/correlated Subnetworks and the corresponding significance score. The second file consists of results from an additional run of the algorithm and reports the high-scoring Subnetwork identified within the first Subnetwork.

The tools described above represent valuable resources for exploring the capabilities of the metabolic networks. The user can perform analysis on the models from our database or upload their custom GSMM.

Comparison to other software

To the best of our knowledge, the only other web-based application offering similar type of analysis as BioMet Toolbox is CycSim (11). CycSim has more advantages in terms of pathways visualization; while on the other hand, BioMet offers the option to analyse custom GSMMs and provides tools for transcriptome analysis and *omics* data integration.

In terms of constrain-based modelling, similar type of analysis as BioOpt can be performed using COBRA TOOLBOX (12), albeit only using MATLAB® environment. BioOpt offers the advantage of being available as a web-based platform, as well as in a standalone version running on the Windows environment. Another strength of BioMet relies on the fact that it also contains transcriptome integrative algorithms using the same format of GSMM. BioMet standalone version allows for formatting of the output so as to be compatible with other software, in particular METATOOL (13). METATOOL can be used to calculate the null space matrix, elementary flux modes and other structural properties of the metabolic network. The standalone version also includes a direct interface to METATOOL.

Another tool available in public domain, FluxAnalyzer (14) is limited to small networks and it is focused on metabolic network simulation and topology analysis; the algorithms used in this tool are not suitable for GSMM.

The major advantage of BioMet Toolbox is that it combines flux balance analysis, transcriptome analysis and *omics* data integration in a single package with user-friendly interface which makes it available for broader audience.

In Table 1, we compare some of the features among the aforementioned tools.

The site also serves as a repository of tools for other types of analysis including C13 and ClusterLustre, with the potential to cover many more tools in the future. Details on these methods are provided on the site.

Models

In the bottom-up systems biology approach, mathematical models have proven to be useful for analysis of high-throughput data (15), as the complexity and integrative nature of biological systems makes it difficult to extract information on molecular processes from such data.

In particular, genome-scale models are used as scaffolds for analysis of *omics* data or for hypothesis-driven analysis of the data with the objective to understand global responses to nutrients and diseases.

Hereby, these GSMMs may be used to predict a theoretical landscape of genetic perturbations that can maximize product and biomass formation under preferred conditions, e.g. during growth on different carbon sources.

We have previously manually reconstructed GSMMs for several important cell factories: *S. cerevisiae* (16–18), *S. coelicolor* (19), *L. lactis* (20), *A. niger* (21), *A. nidulans* (22), *A. oryzae* (23) and *C. glutamicum* (24) (Table 2).

All models included in the BioMet Toolbox are available in three formats: SBML, BioOpt and Excel. For the custom models in SBML format, uploaded by the user, we provide a converter to BioOpt format (SBML2BioOpt).

Following efforts of the systems biology community for model standardization, we compiled all models into SBML format (25). We encourage the community to submit their own GSMMs to be included in the repository; such that the BioMet Toolbox can expand to cover many more models in the future.

CASE STUDY

Aerobic chemostat fermentations on either glucose or ethanol (26) were selected for demonstration of the

Table 1. Comparison of key features among BioMet, COBRA, FluxAnalyzer and CycSim

	BioMet	COBRA	FluxAnalyzer	CycSim
Web-based	Yes	No	No	Yes
Stand Alone version (Platform)	Yes (MS-DOS prompt)	No (MATLAB)	No (MATLAB)	No
Flux Analysis	Yes	Yes	Yes	Yes
Metabolic Flux Analysis	No	No	Yes	No
Elementary Flux Mode	Yes[a]	No	Yes	No
Extreme Pathways	No	No	Yes	No
Pathways Visualization	No	No	Yes	Yes
SBML	Yes	Yes	No	Yes
Transcriptome Analysis and Integration	Reporter Analysis[b] Clustering	Reporter Metabolites	No	No

[a]Elementary Flux Mode Analysis is available in Stand Alone version.
[b]The Reporter Analysis in BioMet toolbox is more comprehensive (covers Reporter Features, Metabolites and Subnetworks Analysis) then the one available in COBRA.

Table 2. Overview of available GSMMs in the BioMet Toolbox

Organism	Genome sequence		Model statistics		
	Size (kb)	ORFs	Reactions	Metabolites	ORFs
Lactococcus lactis	2365	2310	621	509	358
Coryne glutamicum	3282	3002	446	411	446
Streptomyces coelicolor	8667	7825	769	500	769
Saccharomyces cerevisiae	12 069	6294	1149	646	750
Aspergillus niger	35 900	14 165	1190	1045	871
Aspergillus nidulans	30 100	9451	1213	732	666
Aspergilus oryzae	37 200	13 120	1053	1073	1314

applications of the BioMet toolbox. The fermentation characteristics were predicted using the BioOpt application via the web interface. This was performed by first downloading the provided example file for the yeast GSMM (iIN800). The upper bound of the uptake rate (GLCxtI for glucose and ETHxtI for ethanol) was specified to be within the experimentally measured values. Each model file was then submitted to BioOpt for maximization of biomass production. A comparison between measured production/consumption rates and those obtained from simulations is shown in Table 3. The model captures several important differences between the two conditions, such as the lower biomass yield and higher respiratory activity typically seen with growth on ethanol.

The same model file was then submitted to the Reporter Features algorithm together with the *P*-values for differential expression under the two conditions. The top 10 ranking metabolites out of 54 significant hits ($P < 0.05$) are shown in Table 4.

Drastic metabolic changes are associated with the change from growth on sugars to growth on C2-compounds. During growth on glucose, all building blocks for biomass can be derived from glycolysis, TCA cycle and the pentose phosphate pathway. For growth on ethanol, gluconeogenesis and the glyoxylate shunt are needed for the production of some of these precursors. Energy metabolism is shifted towards increased respiration since energy cannot be harvested from glycolysis. The output from Reporter Features analysis accurately captures several of these differences: α-D-glucose[c] and β-D-fructofuranose 6-phosphate[c] are involved in glycolysis and α-D-glucose[e] corresponds to the uptake of glucose. Carnitine[c] and *O*-acetylcarnitine[c] participates in the carnitine shuttle of acetyl to produce mitochondrial acetyl-CoA, which is utilized to a much larger degree in the ethanol case. 6-Phospho-D-gluconate[c] is an important intermediate in the pentose phosphate pathway. α-D-mannose[c] and D-fructose[e] are reported because several proteins, such as hexokinases and sugar transporters, acting on glucose can also act on these metabolites.

This small example shows how FBA can be used to get quantitative estimations of growth characteristics that are in good agreement with what is seen experimentally, and how Reporter Features can be used to identify metabolic hot spots around which transcriptional changes occur.

Table 3. Comparison of measured and simulated fluxes for growth on two different carbon sources (all values are in mmol/gDW/h)

	Glucose	Glucose sim	EtOH	EtOH sim
Glucose consumption	1.15	1.15	–	–
O_2 consumption	2.74	2.88	6.87	7.20
CO_2 production	2.85	2.90	3.26	3.41
EtOH production	–	–	3.78	3.78
Biomass production	0.10	0.11	0.10	0.12

Table 4. The 10 most significant reporter metabolites from Reporter Features

Reporter Metabolite
Beta-D-fructofuranose 6-phosphate[c]
Carnitine[c]
Alpha-D-glucose[e]
Alpha-D-glucose[c]
O-acetylcarnitine[c]
6-Phospho-D-gluconate[c]
Fumarate[m]
Alpha-D-mannose[c]
2-Oxoglutarate[c]
D-fructose[e]

The characters in brackets correspond to the sub cellular localization ([c], cytosol; [m], mitochondria; [e], extracellular).

SUMMARY

The BioMet Toolbox combines a variety of algorithms for genome-wide exploration of metabolism. It allows users to make important predictions using GSMMs and elucidate unexplored properties of biological networks with the means of computational tools. There are a number of advantages for using BioMet Toolbox: (i) it is web-based, platform-independent toolbox for analysis of transcriptome data and GSMMs; (ii) it is not organism-specific; (iii) it is suitable for both, inexperienced and advanced users. The Toolbox accepts genome-scale models in easy to read and write text format (knowledge about compiling models in SBML format is not prerequisite for using BioMet Toolbox), and the more advanced users can analyse their own models written in SBML format.

The BioMet Toolbox is built in a flexible and easily extendible platform to allow incorporation of more tools and genome-scale models in the future.

AVAILABILITY

The BioMet Toolbox will be continuously maintained and updated. The web server is freely available at www.sysbio.se/BioMet/.

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