Mini Review

Computational Methods for Modification of Metabolic Networks

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ABSTRACT

In metabolic engineering, modification of metabolic networks is an important biotechnology and a challenging computational task. In the metabolic network modification, we should modify metabolic networks by newly adding enzymes or/and knocking-out genes to maximize the biomass production with minimum side-effect. In this mini-review, we briefly review constraint-based formalizations for Minimum Reaction Cut (MRC) problem where the minimum set of reactions is deleted so that the target compound becomes non-producible from the view point of the flux balance analysis (FBA), elementary mode (EM), and Boolean models. Minimum Reaction Insertion (MRI) problem where the minimum set of reactions is added so that the target compound newly becomes producible is also explained with a similar formalization approach. The relation between the accuracy of the models and the risk of overfitting is also discussed.

Contents

1. Introduction

A metabolic network represents relations between chemical reactions and compounds in a cell of organisms. Although much knowledge about metabolic networks is available in public databases and references, we often have to modify metabolic networks in various situations. For example, in metabolic engineering, we should modify metabolic networks by newly adding enzymes or/and knocking-out genes to maximize the biomass production with minimum side-effect. The former and latter correspond to adding and deleting chemical reactions, respectively, in a metabolic network. For another example, when we reconstruct a genome-scale metabolic network from a newly determined DNA sequence, the reconstructed metabolic network may need some modification to be consistent with the existing knowledge. Thus, in metabolic network modification, we often add or/and delete reactions so that specified constraints are satisfied.

Although there may exist various modification problems, we focus on the following two major problems in this mini-review: (i) *Minimum Reaction Cut* (MRC) problem: delete the minimum set of reactions so that the target compound becomes non-producible, and (ii) *Minimum Reaction Insertion* (MRI) problem: add the minimum set of reactions so that the target compound newly becomes producible. It should be noted that, for most cases, a target compound can be replaced by a set of target compounds in a straight-forward manner. In order to solve

these problems, three mathematical models have been utilized: flux balance analysis (FBA) model, elementary mode (EM) model, and Boolean model. In this mini-review, we explain these three models in the context of MRC, and briefly review MRI.

Before explaining details of each model, we briefly explain MRC. Suppose that a metabolic network of Fig. 1 is given. Rectangles and circles represent reactions and compounds, respectively. $\{c_1,...,c_{13}\}$ is a set of compounds, and $\{r_1,...,r_5\}$ is a set of reactions. For example, reaction r_1 has the substrates (reactants) $\{c_1, c_2\}$, and products $\{c_6, c_7\}$. If either indegree (the number of input nodes) or outdegree (the number of output nodes) of a compound node is 0, it is called an *external node*. $\{c_1, c_2, c_3, c_4, c_5, c_6, c_9, c_{10}, c_{13}\}$ is a set of external nodes in Fig. 1, and the external nodes consist of *source nodes* and *sink nodes*. Compound nodes with indegree 0 are called source nodes and are assumed to be supplied by the external environment. $\{c_1, c_2, c_3, c_4, c_5\}$ are source nodes. On the other hand, compound nodes with outdegree 0 are called sink nodes. $\{c_6, c_9, c_{10}, c_{13}\}$ are sink nodes. *Target nodes* are chosen from sink nodes. In Fig. 1, $\{c_9\}$ is chosen as a target node.

For example, in MRC, the solution in the Boolean model is deleting $\{r_2, r_3\}$ because c_9 is produced only from r_2 or r_2. However, in the EM and FBA models, if there is a chemical reaction "$A + B \rightarrow C + D$", C and D should also exist for the reaction to take place, in addition to A and B, because steady states are assumed (see latter sections for details of EM and FBA models). Therefore, deletion of any single reaction is the solution of MRC in the FBA and EM models since all reactions must take place at a time if some reaction takes place.

2. Flux Balance Analysis-based Method

Flux balance analysis (FBA) is a constraint-based mathematical framework using the stoichiometry of a given metabolic network. In many cases, FBA is used to optimize a biologically relevant objective function to identify optimal flux distributions [19,32]. In FBA, the state of the whole metabolic network is represented by fluxes for all reactions, and the sum of incoming fluxes must be equal to the sum of outgoing fluxes for each compound, where fluxes may be weighted according to the stoichiometry coefficients.

For MRC and MRI in the FBA model, in addition to the objective function in the standard FBA, the number of added or deleted reactions should also be taken into account. Furthermore, we may need to consider two objectives: cellular objective and bioengineering objective.

In order to identify gene knockout strategies for microbial strain optimization under such a complex situation, a bilevel programming framework was introduced in [2] in which there are outer and inner optimization problems as shown in Fig. 2. The outer problem optimizes the bioengineering objective, whereas the inner problem optimizes the cellular objective.

Here, we consider MRC under the bilevel programming framework. Let v_{target} denote the flux of the reaction that produces the target compound. Our purpose is to find the minimum number of reactions
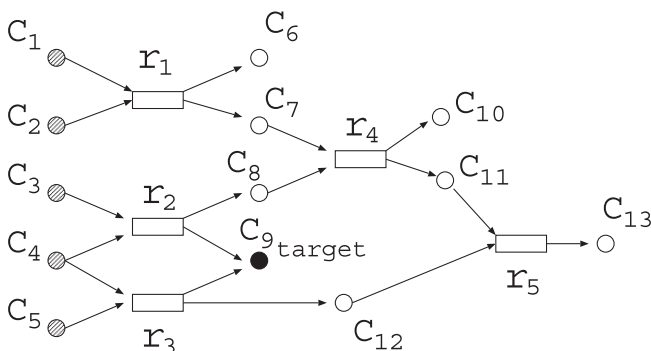

Fig. 1. An example of a metabolic network. Rectangles and circles represent chemical reactions and compounds, respectively.

Fig. 2. A framework of the bilevel programming [2].

deletion of which always makes $v_{target} = 0$. Then, MRC in the FBA model can be formalized as follows by starting with $K = 0$, and increment K by 1 until $v_{target} = 0$ is obtained, where K is the upper limit of the number of deleted reactions.

$$\underset{s_j}{Maximize} \quad -v_{target}$$

subject to

$$\underset{v_j}{Maximize} \quad v_{target}$$

subject to

$$\sum_j S_{ij} \cdot v_j = 0, \forall i \in I,$$
$$LB_j \cdot s_j \le v_j \le UB_j \cdot s_j, \forall j \in J,$$
$$s_j \in \{0, 1\}, \forall j \in J,$$
$$\sum_{j \in J} (1 - s_j) \le K,$$

where s_j is a 0–1 variable, s_{ij} is a stoichiometry matrix for the ith compound and jth reaction, v_j ($j = 1,..., n$) is a flux vector, I is a set of compounds, J is a set of reactions, and LB_j and UB_j are the lower and upper bounds of v_j ($j = 1,..., n$), respectively. s_j represents whether jth reaction is knocked-out, where $s_j = 0$ indicates that jth reaction is knocked-out since v_j is forced to be 0.

In the above, we used the same function (but with different signs) as the objective functions in outer and inner optimization problems. However, there are various versions of the problem setting based on objective functions for the inner problem and the outer problem.

For example, the minimization of metabolic adjustment method (MOMA) minimizes the difference between the wild and the knocked-out flows [25]. In the flux variability analysis (FVA), both the maximum and minimum values of the objective function are calculated, and the range of them is accounted for [26]. OptKnock maximizes the bioengineering objective in the outer problem, and the cellular objective in the inner problem [2], where the upper bound of the number of removed reactions is given as in the above. On the other hand, RobustKnock maximizes the minimal possible rate of the bioengineering objective in the outer max–min problem, while the cellular objective is maximized in the inner min–max structure [30].

3. Elementary Mode-based Method

An elementary mode (EM) represents a feasible and balanced (steady-state) flux distribution of the network [24,23]. It must be minimal with respect to utilized reactions (enzymes). Suppose that a metabolic network of Fig. 3 is given, where reaction nodes are omitted. $\{A_{ex}, B_{ex}, C_{ex}, D_{ex}\}$ is a set of external compounds. In this network, there are 5 EMs, which are shown in Table 1. Although all values in Table 1
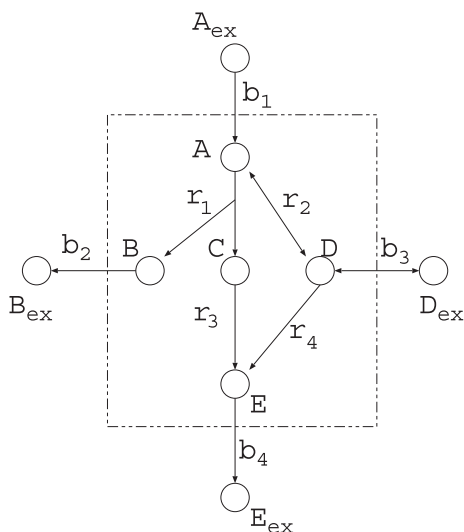
Fig. 3. An example of a metabolic network, where reaction nodes are omitted. Elementary modes (EMs) of this network is shown in Table 1.

are either 0 or 1, any real number is allowed according to the coefficients of chemical reaction formula.

The most standard version of MRC in the EM model is formalized as a minimal cut set (MCS) problem by [7]. MCS is a minimal set of reactions in the network whose inactivation leads to a failure in certain network functions. EMs and MCSs can be calculated by their developed software tools called FluxAnalyzer and CellNetAnalyzer [9,8]. Because MCS can induce side effects disabling desired functionalities, constraint MCSs (cMCSs) have been proposed, which generalize MCSs and allow for the additional definition of a set of desired modes [3]. In cMCSs, a minimum number must be preserved for the desired modes.

Once EMs are given, MRC in the EM model (MCS) can be formalized by Integer Linear Programming (ILP) [17]. Suppose that the objective is to suppress production of E_{ex} in the metabolic network of Fig. 3 by knocking-out the minimum number of reactions. To this end, it is enough to inactivate EMs including b_4, which can be represented as:

Maximize $r_1 + r_2 + r_3 + r_4$

subject to

$r_1 \wedge r_3 = 0,$
$r_4 = 0,$
$r_1 \wedge r_2 \wedge r_3 = 0,$
$r_2 \wedge r_4 = 0,$

where all variables are binary, and $x \wedge y$ denotes the logical "AND" of x and y (i.e., $x \wedge y = 1$ if and only if $x = 1$ and $y = 1$). The Boolean

Table 1
5 elementary modes (EMs) of a metabolic network of Fig. 3 when every coefficient of chemical reaction formula is assumed to be 1.

	r_1	r_2	r_3	r_4	b_1	b_2	b_3	b_4
EM1	1	0	1	0	1	1	0	1
EM2	0	0	0	1	0	0	1	1
EM3	1	−1	1	0	0	1	1	1
EM4	0	1	0	0	1	0	−1	0
EM5	0	1	0	1	1	0	0	1

constraints in the above are converted into the following linear inequalities.

$r_1 + r_3 \leq 1,$
$r_4 = 0,$
$r_1 + r_2 + r_3 \leq 2,$
$r_2 + r_4 \leq 1.$

4. Boolean Model-based Method

In the Boolean model, all reaction and compound nodes are assigned either 0 or 1. If 1 is assigned, it means that the compound is producible, or the reaction can take place. On the other hand, if 0 is assigned, it means that the compound is not producible or the reaction cannot take place.

Moreover, reaction and compound nodes are represented by logical "AND" and "OR" functions, respectively. For example, in Fig. 1, r_1 represents a chemical reaction "$c_1 + c_2 \rightarrow c_6 + c_7$". In the Boolean model, for r_1 to take place, both c_1 and c_2 are necessary. Therefore, the condition of r_1 is represented by $r_1 = c_1 \wedge c_2$. Similarly, c_9 is producible if either r_2 or r_3 takes place. Therefore, the condition of c_9 is represented by $c_9 = r_2 \vee r_3$.

For MRC in the Boolean model, the Boolean reaction cut (BRC) problem has one of the most standard problem settings. In BRC, the number of deleted reactions is minimized to make the target compounds non-producible, and an ILP-based method for solving it was developed in [29].

Another standard problem setting is to minimize the side effect instead of the number of deleted reactions. The Optimal enzyme drug target identification algorithm based on metabolic networks (OPMET) was developed in [28]. OPMET identifies the optimal enzyme combination whose inhibition achieves the required effect of eliminating a given target set of compounds, while incurring minimal side-effects.

As MRC in the FBA model can be formalized by the bilevel programming with the inner and outer problems, MRC in the Boolean model also has such two layers of problems. Because each set of deleted reactions can have multiple 0/1 assignments which satisfies all Boolean constrains, some objective function should be optimized in the inner problem even in the Boolean model. This is necessary especially for properly accounting for the effect of directed cycles in metabolic networks. For this purpose, [29] introduced the notion of maximal valid assignment (MaxVA), where MaxVA is a 0/1 assignment that is maximal with respect to the number of 1s, when a set of deleted reaction is given.

In the above problem settings, the main desired effects and side non-desired effects are considered in a single metabolic network. A reasonable extension is to consider them in multiple networks. [17] developed an ILP-based method for the minimum knockout for multiple metabolic network problem (MKMN). In MKMN, when a set of source compounds and a set of target compounds are given, we must find the minimum set of reactions whose knockout ensures that the target compounds are not producible in N_1, but are producible in N_2.

In ILP, every constraint must be represented by linear equations or inequalities. Boolean constraints can be transformed into linear equations or inequalities as follows.

LP1 [29]: Since the Boolean "AND" relation $y = x_1 \wedge x_2 \wedge \cdots \wedge x_k$ can be converted into $(y \vee \overline{x_1} \vee \overline{x_2} \vee \cdots \vee \overline{x_k}) \wedge (\overline{y} \vee x_1) \wedge (\overline{y} \vee x_2) \wedge \cdots \wedge (\overline{y} \vee x_k) = 1$, it can be represented by the following linear inequalities:

$y + (1-x_1) + (1-x_2) + \cdots + (1-x_k) \geq 1,$
$\qquad (1-y) + x_1 \geq 1,$
$\qquad (1-y) + x_2 \geq 1,$
$\qquad \cdots$
$\qquad (1-y) + x_k \geq 1,$

where all variables are binary.

Similarly, as the Boolean "OR" relation $y = x_1 \vee x_2 \vee \cdots \vee x_k$ can be converted into $(\overline{y} \vee x_1 \vee x_2 \vee \cdots \vee x_k) \wedge (y \vee \overline{x_1}) \wedge (y \vee \overline{x_2}) \wedge \cdots \wedge (y \vee \overline{x_k}) = 1$, it can be represented by the following linear inequalities:

$$(1-y) + x_1 + x_2 + \cdots + x_k \geq 1,$$
$$y + (1-x_1) \geq 1,$$
$$y + (1-x_2) \geq 1,$$
$$\cdots$$
$$y + (1-x_k) \geq 1,$$

where all variables are binary.

LP2 [1]: Another type of linear function representation of Boolean functions is as follows: The Boolean "AND" can be represented by the following linear inequalities:

$$ky \leq x_1 + x_2 + \ldots + x_k,$$
$$y \geq x_1 + \ldots + x_k - (k-1),$$

where all variables are binary.

Similarly, the Boolean "OR" can be represented by the following linear inequalities:

$$ky \geq x_1 + x_2 + \ldots + x_k,$$
$$y \leq x_1 + \ldots + x_k,$$

where all variables are binary.

To calculate the MaxVA, using the notion of time is convenient in the ILP formalization [29]. However, in a naive algorithm, the number of variables is $O((m + n)^2)$, where m and n are the numbers of compounds and reactions, respectively. Because, the computational time for solving ILP is often exponential for the number of variables, the naive method cannot be applied for large scale networks.

To handle this problem, [29] developed an feedback vertex set (FVS)-based method. An FVS is a set of nodes whose removal makes a network acyclic. They formalized the MRC in the Boolean model by ILP in which the number of variables is $O(f(m + n + f))$, where f is the size of the FVS.

The problems formalized by ILP can also be solved by SAT-based methods since both are NP-complete problems, where SAT denotes the Boolean satisfiability problem. MRC may also be represented as an abduction problem if it is formalized by a logic programming-based method. Meta-level abduction is a method of abducting missing rules to account for observations. [5] showed that meta-level abduction can consistently produce both positive and negative causal relations. [31] developed an inductive logic programming approach to estimate possible reaction states. Their method finds hypotheses that logically explains the causal relations. Because the reaction states correspond to which reactions are active, the problem setting may correspond to MCS in the Boolean model.

5. Minimum Reaction Insertion

MRC problems are for finding reaction deletion strategies to satisfy given constrains. Different from MRC, the minimum reaction insertion (MRI) problems are for finding reaction addition strategies to satisfy constraints.

In the given network of MRI, reactions are classified into the currently available part and the currently non-available part. We call the former and latter a host network and a reference network, respectively. In Fig. 4, only $\{r_1\}$ belongs to the host network, whereas none of $\{r_2, r_3, r_4, r_5\}$ belongs to the host network. Suppose that $\{c_1, c_2, c_3, c_4, c_5\}$ is a set of source nodes, $\{c_6, c_9, c_{10}, c_{13}\}$ is a set of sink nodes, and c_{10} is a target node. In the Boolean model, adding $\{r_2, r_4\}$ is the solution, whereas adding $\{r_2, r_3, r_4, r_5\}$ is the solution in the FBA model.

When a metabolic network is newly reconstructed from a DNA sequence, a host network is often constructed according to the ortholog information of DNAs. However, this network often has gaps, and some
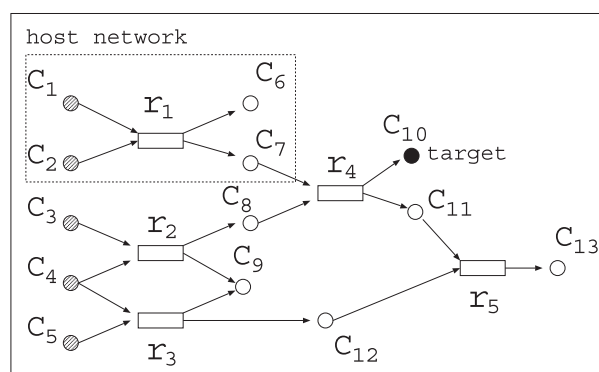

Fig. 4. An example of a metabolic network for minimum reaction insertion (MRI) problem. The area of the dotted line is a host network and initially available. We should add the minimum set of reactions so that the target compound becomes producible.

of necessary compounds are not producible in this initial model. In such a case, gaps are often found and filled by FBA-based simulations. GapFind identifies non-producible metabolites based on the initial FBA model, and GapFill fills these gaps by the minimum set of additional reactions [10]. GapFill utilizes a customized multi-organism database that restores the connectivity of these metabolites to the parent network.

[16] developed a software tool, minRect, for solving MRI in the Boolean model. They call the parent network and multi-organism database as the host network and reference network, respectively. In the inner problem of MRI in the Boolean model, the notion of the minimal valid assignment is employed in order to account for the effect of directed cycles instead of the maximal valid assignment, since MRC and MRI are complementary problems.

Cell growth rate and gene essentiality are also utilized for the modification of metabolic networks. GrowMatch identifies the gaps based on the inconsistencies of cell growth rates between the simulation results on the model and the biological experiment results [11].

6. Discussion

6.1. Comparison Among the FBA, EM, and Boolean-based Models

In this article, we have briefly reviewed studies on MRC and MRI problems in the FBA, EM and Boolean-based models. The FBA and EM models can be classified into flow-based models. The flow-based models can realize more detailed simulation, because they account for the chemical reaction coefficients, the upper and lower bounds of each flux, and the steady state. Therefore, if the purpose of the research is to construct an exact model which meets the data obtained in biological experiments, the flow-based models are better than the Boolean model.

However, at the same time, this high performance of the flow models includes the risk of the overfitting. Because knowledge and data about metabolic networks are not yet perfect, an exact model may be useful only for the data used to reconstruct the model. Although the Boolean model is less detailed than the flow-based models, it is considered to be more robust from this point of view.

For example, in MRC of Fig. 1, deleting r_5 is one of the optimal solution of the flow models. However, if there is an unknown reaction r_6 whose substrates are c_8 and c_{12} and products are c_{10} and c_{13}, then c_9 is still producible even if r_5 is deleted. This shows that the flow-based models are less robust for the lack of information in the downstream of a flux. On the other hand, the optimal solution of the Boolean model is to delete $\{r_2, r_3\}$. Even if there is the hidden reaction r_6, deleting $\{r_2, r_3\}$ is still the optimal solution in the Boolean model. Thus, the Boolean model is more robust for the lack of information in the

downstream of a flux, and there is a tradeoff between the accuracy of the model and the risk of the overfitting.

In the flow models, if free variables are introduced or inequalities for the stoichiometric constraint are allowed for internal nodes, the flow model may become more robust for the incorrectness of the distant nodes. However, in such a case, there may be a risk that constraints are not appropriately propagated.

The main difference between the FBA model and EM model is as follows. The basic version of the FBA model requires an objective function, and is formalized as a linear program. On the other hand, in the EM model, all EMs are often enumerated and then are utilized in some optimization problem. These suggest that the FBA model needs an objective function on some target flow(s), whereas the EM model (and also the Boolean model) does not. Therefore, it is suggested that the FBA model is suitable if the objective function is well-defined, otherwise the EM model and/or the Boolean model might be more suitable.

The basic version of the FBA model is efficiently solvable since the linear programming problem is solvable in polynomial time. However, most optimization problems including MCS are NP-hard. For the EM-based model, since the number of EMs may be exponential to the size of the network, the EM-based methods are not feasible for many optimization problems with large-scale networks. The EM-based methods are useful for analyzing details of the flow-based models. Although the MRC of the Boolean model is also NP-hard, improvements of ILP solvers such as CPLEX lead to the speedup for solving the Boolean MRC.

Here, we briefly discuss the scalability of each method based on several reports on computational experiments using real metabolic network data. Related to the FBA-based MRI, the gap-filler method was applied to completion of the metabolic network in the EcoCyc database (version 15.5), which contained 1888 reactions [12]. For the FBA-based MRC, a more complex version was implemented by SimOptStrain, which simultaneously adds and deletes reactions. Although the number of deleted reactions is limited to 10, SimOptStrain was successfully applied to iAF1260, which includes 2077 reactions and 1039 unique metabolites [6]. For the Boolean-based MRC, a more complex version, MKMN (Multiple Knockout for Multiple Networks), was solved by a fast approximation algorithm for the network with 609 reactions and 622 compounds [17]. The Boolean-based MRI was solved for the network with 150 reactions and 93 compounds [16], where an approximation algorithm may be applicable for larger networks. For the EM-based methods, enumerating all EMs was succeeded for the network with 328 nodes, but failed with 1881 nodes [17]. These results suggest that the FBA-based methods are the most scalable, the Boolean-based methods are modestly scalable, and the EM-based methods are less scalable.

6.2. Implementation and Application

Many of the constraint-based methods are realized in a software package called COBRA Toolbox, which works on MATLAB [22]. CPLEX is a software tool for solving many types of constraint programming problems including linear programming, mixed integer programming, quadratic programming, and quadratically constrained programming [4].

Although we separately discussed MRC and MRI in this article, adding and deleting reactions at the same time is a reasonable approach in the metabolic network modification. OptReg identifies reaction activations or inhibitions to suggest up-regulation or down-regulation of genes to overproduce biochemicals [21]. OptStrain firstly adds the minimum set of reactions to maximize the theoretical maximum production, and then maximizes the biochemical production by the bilevel programming where the number of deleted reactions is limited [20]. Different from OptStrain, SimOptStrain simultaneously adds and deletes reactions to maximize the biochemical production and the cellular growth in the outer and inner problems, respectively [6]. Applying

existing ideas for MRC and MRI to this problem may be a promising future direction.

The FBA-based methods are often used in the process of reconstructing genome-scale metabolic networks, and industrial metabolic engineering. For example, iJO1366 is an FBA-based genome-scale metabolic network of *Escherichia coli*, and can be used for the simulation of cell growth rate for gene knockouts [18]. iNL403 is a core metabolic network of human brains, and used for the simulation of Alzheimer's disease [15]. In these models, the initial models are constructed from genome-sequences and literatures, and then the modification is often conducted to satisfy the experimental data and/or the connectivity of the network. Efficient production of biofuel using microorganisms with metabolic engineering is another important application [13].

For the Boolean model, measures of the impact of knockouts were studied for finding novel drug targets and/or crucial genes for diseases. For example, [14] studied the effect of deletion of each enzyme in the metabolic network of a Boolean model, and [27] considered almost the same problem from the viewpoint of the Boolean aspect of the flux balance mode. Formalizing an optimization problem as MRC with such measures may be useful for finding a gene set as a drug target and/or factors of diseases.

References

[1] Akutsu T, Yang Z, Hayashida M, Tamura T. Integer programming-based approach to attractor detection and control of Boolean networks. IEICE Trans Inf Syst 2012; 95(12):2960–70.
[2] Burgard AP, Pharkya P, Maranas CD. Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. Biotechnol Bioeng 2003;84(6):647–57.
[3] Hädicke O, Klamt S. Computing complex metabolic intervention strategies using constrained minimal cut sets. Metab Eng 2011;13(2):204–13.
[4] IBM. IBM ILOG CPLEX Optimizer. http://www-01.ibm.com/software/integration/optimization/cplex-optimize r/; 2010.
[5] Inoue K, Doncescu A, Nabeshima H. Completing causal networks by meta-level abduction. Mach Learn 2013;91(2):239–77.
[6] Kim J, Reed JL, Maravelias CT. Large-scale bi-level strain design approaches and mixed-integer programming solution techniques. PLoS ONE 2011;6(9) e24162.
[7] Klamt S, Gilles ED. Minimal cut sets in biochemical reaction networks. Bioinformatics 2004;20(2):226–34.
[8] Klamt S, Saez-Rodriguez J, Gilles ED. Structural and functional analysis of cellular networks with cellnetanalyzer. BMC Syst Biol 2007;1(1):2.
[9] Klamt S, Stelling J, Ginkel M, Gilles ED. Fluxanalyzer: exploring structure, pathways, and flux distributions in metabolic networks on interactive flux maps. Bioinformatics 2003;19(2):261–9.
[10] Kumar VS, Dasika MS, Maranas CD. Optimization based automated curation of metabolic reconstructions. BMC bioinformatics 2007;8(1):212.
[11] Kumar VS, Maranas CD. Growmatch: an automated method for reconciling in silico/in vivo growth predictions. PLoS Comput Biol 2009;5(3) [e1000308].
[12] Latendresse M, Krummenacker M, Trupp M, Karp PD. Construction and completion of flux balance models from pathway databases. Bioinformatics 2012;28(3):388–96.
[13] Lee SK, Chou H, Ham TS, Lee TS, Keasling JD. Metabolic engineering of microorganisms for biofuels production: from bugs to synthetic biology to fuels. Curr Opin Biotechnol 2008;19(6):556–63.
[14] Lemke N, Herédia F, Barcellos CK, Dos Reis AN, Mombach JC. Essentiality and damage in metabolic networks. Bioinformatics 2004;20(1):115–9.
[15] Lewis NE, Schramm G, Bordbar A, Schellenberger J, Andersen MP, Cheng JK, et al. Large-scale in silico modeling of metabolic interactions between cell types in the human brain. Nat Biotechnol 2010;28(12):1279–85.
[16] Lu W, Tamura T, Song J, Akutsu T. Integer programming-based method for designing synthetic metabolic networks by minimum reaction insertion in a Boolean model. PLoS ONE 2014;9(3) [e92637].
[17] Lu W, Tamura T, Song J, Akutsu T. Computing smallest intervention strategies for multiple metabolic networks in a Boolean model. J Comput Biol 2015;22(2):85–110.
[18] Orth JD, Conrad TM, Na J, Lerman JA, Nam H, Feist AM, et al. A comprehensive genome-scale reconstruction of escherichia coli metabolism. Mol Syst Biol 2011; 7(1).
[19] Orth JD, Thiele I, Palsson BØ. What is flux balance analysis? Nat Biotechnol 2010; 28(3):245–8.

[20] Pharkya P, Burgard AP, Maranas CD. Optstrain: a computational framework for redesign of microbial production systems. Genome Res 2004;14(11):2367–76.

[21] Pharkya P, Maranas CD. An optimization framework for identifying reaction activation/inhibition or elimination candidates for overproduction in microbial systems. Metab Eng 2006;8(1):1–13.

[22] Schellenberger J, Que R, Fleming RM, Thiele I, Orth JD, Feist AM, et al. Quantitative prediction of cellular metabolism with constraint-based models: the cobra toolbox v2. 0. Nat Protoc 2011;6(9):1290–307.

[23] Schuster S, Fell DA, Dandekar T. A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks. Nat Biotechnol 2000;18(3):326–32.

[24] Schuster S, Hilgetag C. On elementary flux modes in biochemical reaction systems at steady state. J Biol Syst 1994;2(02):165–82.

[25] Segre D, Vitkup D, Church GM. Analysis of optimality in natural and perturbed metabolic networks. Proc Natl Acad Sci 2002;99(23):15112–7.

[26] Shlomi T, Cabili MN, Ruppin E. Predicting metabolic biomarkers of human inborn errors of metabolism. Mol Syst Biol 2009;5:263.

[27] Smart AG, Amaral LAN, Ottino JM. Cascading failure and robustness in metabolic networks. Proc Natl Acad Sci 2008;105(36):13223–8 [URL http://www.pnas.org/content/105/36/13223.abstract].

[28] Sridhar P, Song B, Kahveci T, Ranka S. Mining metabolic networks for optimal drug targets. Pac Symp Biocomput 2008;13:291–302.

[29] Tamura T, Takemoto K, Akutsu T. Finding minimum reaction cuts of metabolic networks under a Boolean model using integer programming and feedback vertex sets. Int J Knowl Discov Bioinformatics (IJKDB) 2010;1(1):14–31.

[30] Tepper N, Shlomi T. Predicting metabolic engineering knockout strategies for chemical production: accounting for competing pathways. Bioinformatics 2010;26(4):536–43.

[31] Yamamoto Y, Inoue K, Doncescu A. Integrating abduction and induction in biological inference using cf-induction. Elements Comput Syst Biol 2010:213–34.

[32] Zhao Y, Tamura T, Akutsu T, Vert J-P. Flux balance impact degree: a new definition of impact degree to properly treat reversible reactions in metabolic networks. Bioinformatics 2013;29(17):2178–85.