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Recursive MILP model for finding all the alternate optima in LP models for metabolic networks

Sangbum Lee, Chan Phalakornkule, Michael M. Domach, Ignacio E. Grossmann *

Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, USA

Abstract

We consider linear programming (LP) models for metabolic networks in which alternate optima often arise, and need to be identified to allow for data interpretation or the effective design of follow-up experiments. A recursive mixed-integer linear programming (MILP) algorithm is proposed for rigorously finding all alternate optima. The carbon trafficking alternatives of an *Escherichia coli* mutant lacking pyruvate kinase are analyzed with the proposed algorithm. The results are discussed in terms of using them as an input to isotopomer mapping matrix calculations in order to design ^{13}C NMR experiments for maximum contrast.

Keywords: Metabolic network; Linear programming; Mixed integer linear programming; Alternate optima

1. Introduction

Metabolic networks consist of an array of numerous, connected reactions that are catalyzed by enzymes. Through the activity of these networks, raw materials can be converted to a myriad of products (e.g. amino acids, vitamins, sterols) with economic value. Modern genetic manipulation tools now allow one to alter the network structure and/or control mechanisms (e.g. feed back loops) in attempt to increase product yield. Such manipulation aimed at steering energetic and material resources toward target products and/or away from undesirable waste products is termed 'metabolic engineering'.

Linear programming (LP) analysis has been proven to be a useful tool for elucidating carbon trafficking patterns in metabolic networks. For example, LP has been used to enumerate potential metabolic flux (reaction rate/mass cell) distributions in adipose tissue (Fell & Small, 1986), and microbial metabolic networks (Majewski & Domach, 1990; Varma & Palsson, 1994; Lee, Goel, Ataai & Domach, 1997). In general, the analysis involves formulating a problem in terms of an objective function, constraints, and flux balance equations. The objective function provides a physiological rationale or desired engineering endpoint, while the constraints account for aggregate requirements (e.g. total energy production for biosynthesis) and the phenomenological impact of the mechanistic details. Linear programming then generates a flux distribution that optimizes the objective function, subject to satisfying flux balance equations and constraints.

Flux distributions obtained from LP analysis can enhance basic understanding metabolism by, for example, indicating where conflicts or trade-offs occur when different objectives are to be considered. From a more pragmatic standpoint, these flux distributions yield upper bounds of product yield calculations. Moreover, optimal flux distributions provide design targets for the metabolic engineer. A related use is the distributions, which can suggest what may occur within the cell when alternate metabolic engineering strategies are used.

Mixed-integer linear programming (MILP) methods have been used to optimize control schemes in metabolic networks with the goal of maximizing the direction of resources to a desired product (Hatzimanikatis, Floudas & Bailey, 1996a,b). To our knowledge, however, less work has been done on automatically enumerating the multiple flux distributions that can satisfy material balances and constraints with the same value of the objective function. The alternate flux distributions will differ by the subset of

* Corresponding author. Tel.: +1-412-2682230; fax: +1-412-2687139.

E-mail address: grossmann@cmu.edu (I.E. Grossmann)

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all the available reaction paths actually used, reaction directionalities, etc. Thus, different candidate 'flowsheets' may exist that reflect alternate induction/repression control options or responses to mutation, and the fluxes can indicate what the feedback control strategies should accomplish. The former is especially important because altering a biological system can change the set of reaction paths being used. Thus, how the 'flowsheet' may change needs to be considered along with the optimization of flux control.

From another stand point, multiple solutions are important because they commonly arise in LP problems that have flexibility and excess capacity with respect to the constraints imposed. Moreover, for metabolic network problems, limited experimental observations are typically available relative to the number of unknowns. Therefore, multiple solutions will reveal the various ways in which the known biochemistry and physiology can account for the limited observations. As illustrated by Phalakornkule, Fry, Zhu, Kopesel, Ataai and Domach (2000), the multiple solutions can be used to scrutinize metabolic regulation hypotheses and design follow-up ^{13}C NMR tracer experiments that are aimed at discriminating between the flux distribution options.

To address the limitation noted above, we have developed a MILP method for finding all alternate optima in LP problems. Apart from having utility for hypothesis generation and data reconciliation, we have begun using this method as a driver for the design of ^{13}C NMR experiments. Such experiments entail inputting a labeled precursor's history (i.e. relative rates through different reaction paths). Having scenarios developed prior to the experiment in tandem with label trafficking models can enable one to choose a labeled precursor compound that will be the most sensitive indicator of how raw materials traffick through the network.

In this study, we first describe a MILP method for finding all alternate optima of a given LP problem. After presenting the general methodology, the MILP-generated carbon trafficking alternatives of an *Escherichia coli* mutant lacking pyruvate kinase activity are presented. This mutation has been suggested as a potential metabolic engineering strategy for minimizing acetate production (Goel, Lee, Domach & Ataai, 1995). We will show that there are multiple flux distribution scenarios that can satisfy the same objective function (consistent with mutation), flux balances, and constraints.

2. Alternate optimal solutions from linear programming

A LP problem can be posed as follows.

$$\min\ Z = c^{\mathrm{T}}x$$

$$\text{s.t.}\quad A^1x = b^1$$

$$A^2x \le b^2$$

$$x^{\mathrm{L}} \le x \le x^{\mathrm{U}};\quad x \in R^n \qquad \text{(P)}$$

Provided the feasible region is non-empty and the optimal solution is bounded, Eq. (P) has a unique optimum objective function value. Furthermore, the optimal value of the vector x corresponds to an extreme point of the polyhedron that corresponds to the feasible region of Eq. (P) (Chvatal, 1983). However, this optimum value of x is not necessarily unique, as there might be multiple solutions to Eq. (P), which have the same objective function value. Our interest is to find all the extreme points in Eq. (P) that have identical objective values. These are commonly known as alternate optima. Alternate optima can also be interpreted as the extreme points that lie at the intersection of the convex polytope given by the linear constraints and the hyperplane of the optimal objective function value. A number of special purpose algorithms have been reported in the literature for finding all the extreme points of the convex polytope of Eq. (P) (e.g. see Swart, 1985; Matheiss & Rubin, 1980). Aside from the fact that these algorithms have exponential complexity, they are not very easy to implement. Therefore, our goal in this study is to develop a recursive MILP method, which has the advantage that it can readily be implemented in a modeling language (e.g. GAMS, AMPL). The basic ideas of the proposed method are as follows.

We first reformulate Eq. (P) in canonical form by introducing slack variables s to the inequalities, and by converting all variables into non-negative variables. This can be accomplished with the introduction of the slack variables s^{L}, s^{U} for the lower bound and upper bound of x, respectively. Here we assume a compact set (i.e. x^{L}, x^{U} are finite). By eliminating x using s^{L} and s^{U}, we obtain the final canonical form for LP.

$$\min\ Z = c^{\mathrm{T}}s^{\mathrm{L}} + c^{\mathrm{T}}x^{\mathrm{L}}$$

$$\text{s.t.}\quad A^1s^{\mathrm{L}} = b^1 - A^1x^{\mathrm{L}}$$

$$A^2s^{\mathrm{L}} + s = b^2 - A^2x^{\mathrm{L}}$$

$$s^{\mathrm{L}} + s^{\mathrm{U}} = x^{\mathrm{U}} - x^{\mathrm{L}}$$

$$s^{\mathrm{L}}, s^{\mathrm{U}}, s \ge 0;\quad s^{\mathrm{L}}, s^{\mathrm{U}} \in R^n;\quad s \in R^m \qquad \text{(PR)}$$

Since the last term $c^{\mathrm{T}}x^{\mathrm{L}}$ in the objective function is constant, we can drop it. Note that in Eq. (PR) all variables are non-negative and all the constraints are equalities. Thus, Eq. (PR) when written in canonical leads to the LP,

$$\min\ Z = \alpha^{\mathrm{T}}z$$

$$\text{s.t.}\quad Bz = q$$

$$z \ge 0 \qquad \text{(CF)}$$

In Eq. (CF), the following relations hold:

$$z=\begin{bmatrix} s^{L} \\ s^{U} \\ s \end{bmatrix}, \quad \alpha=\begin{bmatrix} c \\ 0 \\ 0 \end{bmatrix}, \quad B=\begin{bmatrix} A^{1} & 0 & 0 \\ A^{2} & 0 & I \\ I & I & 0 \end{bmatrix},$$

$$q=\begin{bmatrix} b^{1} & -A^{1}x^{L} \\ b^{2} & -A^{2}x^{L} \\ x^{U} & -x^{L} \end{bmatrix}$$

For the sake of simplicity of the representation, we will explain the proposed algorithm in the context of Eq. (CF).

2.1. Algorithm for finding multiple solutions

In order to find all the alternate optima, the proposed algorithm incorporates Eq. (CF) into a recursive MILP problem that has a set of constraints for changing the basis and identifying a new extreme point corresponding to one of the alternate optima. The search algorithm stops when no other solution with the same objective function can be found in the MILP. As is well known (Chvatal, 1983), non-basic variables in Eq. (CF) have a value of 0 since they are set to the lower bound of z. The basic variables are normally non-zero, but may have the value of 0 when there is degeneracy in Eq. (CF). In order to force the selection of a new basis, we first define the 0-1 variable y_i for each variable z_i that is a non-zero basic variable, $i \in NZ^{K-1}$, at the previous iteration $K-1$. If y_i is set to 1, z_i is selected to become non-basic. If y_i is 0, z_i remains in the basis. Since the change of basis is achieved with the selection of at least one variable y_i, the following constraint holds.

$$\sum_{i \in NZ^{K-1}} y_i \geq 1 \tag{1}$$

Eq. (1) means that at least one of the non-zero basic variables in the $(K-1)$th solution is selected and is set to 0 (non-basic) in the current iteration, K. In order to ensure that all alternate bases are generated, we define the 0-1 variable w_i which is 1 if variable z_i is non-zero at the Kth iteration of the procedure. The two following constraints are imposed on w_i,

$$\sum_{i \in NZ^{k}} w_i \leq |NZ^{k}| - 1, \quad k = 1, 2 \ldots, K-1 \tag{2}$$

$$0 \leq z_i \leq U w_i, \quad i \in I \tag{3}$$

where Eq. (3) forces $w_i = 1$ if $z_i > 0$. U is a valid upper bound for all z_i. Eq. (2) eliminates from consideration at least one of the non-zero variables of the basis that were found at the previous iterations, $k = 1, 2, \ldots, K-1$.

Finally, the variables y_i and w_i are related by the logical condition $y_i \Rightarrow \neg w_i$, meaning that if the ith non-zero variable is selected to be non-basic, then it cannot be non-zero (i.e. it must be 0). Thus, the logical condition can be written as a linear constraint for the current iteration K as,

$$y_i + w_i \leq 1, \quad i \in NZ^{K-1} \tag{4}$$

The proposed algorithm can then be stated as follows:

Step 1. Set iteration counter $K = 1$. Solve Eq. (CF). Define the set NZ^1 and the optimal objective $(Z^1)^*$

Step K, for $K \geq 2$.

(a) Solve the master problem.

$$\min \ Z^K = \alpha^{T} z$$

$$\text{s.t.} \quad Bz = q$$

$$\sum_{i \in NZ^{K-1}} y_i \geq 1$$

$$\sum_{i \in NZ^{k}} w_i \leq |NZ^{k}| - 1, \quad k = 1, 2, \ldots, K-1$$

$$0 \leq z_i \leq U w_i, \quad i \in I$$

$$y_i + w_i \leq 1, \quad i \in NZ^{k-1}$$

$$z \geq 0 \tag{MP}$$

(b) Define set NZ^K and continue until $Z^K > (Z^1)^*$.

The proposed algorithm is guaranteed to find all the alternate optima when there is degeneracy in the LP or not. It should also be noticed that the proposed algorithm may require an exponential number of steps since the number of extreme points is exponential. In practice, however, the number of alternate optima is usually relatively small. The proposed method was implemented in the GAMS modeling system (Brooke, Kendrick, Meeraus & Raman, 1997).

3. Application to metabolic engineering

The algorithm is used to explore a metabolic engineering problem in the bacterium, *E. coli*. When an enzymatic step is eliminated, it has been proposed that the conversion of the raw material, glucose, to the waste product, acetate will cease. The aim of the analysis is to enumerate the different ways in which metabolic rates may respond to the mutation yet still fulfill the constraints that are associated with a functional cell.

The metabolic network of *E. coli* is shown in Fig. 1. In the network, the step that will be subjected to metabolic engineering is represented by r_{18}. For the network, the 30 molar balances and constraints for 33 fluxes (r_i) are as follows.

$$r_1 - r_2 - r_3 - r_{10} = 0 \quad (5a)$$

$$r_{10} - r_{12} - r_{11} + r_7 + r_8 = 0 \quad (5b)$$

$$r_{14} - r_{15} - r_{16} = 0 \quad (5c)$$

$$-r_1 + r_{16} - r_{18} - r_{17} - r_{31} = 0 \quad (5d)$$

$$r_1 + r_{18} - r_{21} - r_{19} - r_{20} + r_{32} = 0 \quad (5e)$$

$$-r_{31} + r_{24} + r_{30} - r_{33} = 0 \quad (5f)$$

$$r_{21} - r_{24} - r_{23} - r_{22} = 0 \quad (5g)$$

$$r_{24} - r_{25} = 0 \quad (5h)$$

$$r_{25} - r_{27} - r_{26} = 0 \quad (5i)$$

$$-r_{29} + r_{27} - r_{28} = 0 \quad (5j)$$

$$r_2 - r_4 - r_5 = 0 \quad (5k)$$

$$-r_7 + r_4 - r_6 = 0 \quad (5l)$$

$$-r_7 - r_8 + r_5 = 0 \quad (5m)$$

$$r_7 - r_8 - r_9 = 0 \quad (5n)$$

$$2r_{12} + r_8 - r_{14} - r_{13} = 0 \quad (5o)$$

$$r_{29} - r_{33} - r_{32} = 0 \quad (5p)$$

The following equations Eqs. (6a), (6b), (6c), (6d), (6e), (6f), (6g), (6h), (6i) and (6j) were derived from biosynthetic loads (units are mmol g^{-1} cell h^{-1}) based on cellular mass composition (Mandelstam, McQuillen & Dawes, 1982; Ingraham, Maaloe & Neidhardt, 1983; Goel, Domach, Hanley, Lee & Ataai, 1996), and specific growth rate equal to 0.4 h^{-1}. Note that these loads must be satisfied by all feasible candidate solutions.

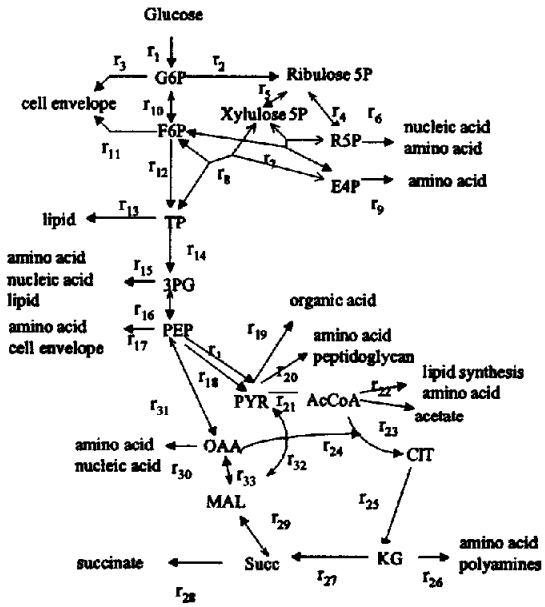

Fig. 1. The metabolic network of *E. coli*. The two-way arrows represent reversible fluxes with the bold heads showing the constrained net directions.

$$2.5r_3 = 0.205 \quad (6a)$$

$$2.5r_{11} = 0.0709 \quad (6b)$$

$$2.5r_{13} = 0.129 \quad (6c)$$

$$2.5r_{15} = 1.493 \quad (6d)$$

$$2.5r_{17} = 0.7191 \quad (6e)$$

$$2.5r_6 = 0.897 \quad (6f)$$

$$2.5r_9 = 0.361 \quad (6g)$$

$$2.5r_{20} = 2.833 \quad (6h)$$

$$2.5r_{22} = 2.928 \quad (6i)$$

$$2.5r_{26} = 1.078 \quad (6j)$$

$$2.5r_{30} = 1.786 \quad (6k)$$

There are also constraints that must be attained through the aggregate activity of different subsets of metabolic reactions. Such constraints are shared loads and provide connectivity between individual rates. These constraints involve NADPH and ATP production. NADPH is the molecule responsible for reducing chemistry and ATP hydrolysis provides the energy for biosynthesis (i.e. free energy of hydrolysis drives otherwise thermodynamically infeasible polymerization). The NADPH and minimum ATP requirements (Goel et al., 1996) are represented by Eqs. (7a), (7b) and (7c). Again, the rate basis is a specific growth rate equal to 0.4 h^{-1}.

$$2r_2 + r_{25} + r_{32} = 7.2 \quad (7a)$$

$$r_{ATP} + r_{12} - 3r_{14} - r_{18} - r_{23} - 3r_{27} - 2r_{21} - 2r_{33} - r_{29} - r_{31} + 2r_{19} = 0 \quad (7b)$$

$$r_{ATP} \geq 13.3 \quad (7c)$$

In the above equation, r_{ATP} denotes the rate of ATP production and Eq. (7b) is based on the assumption that NADH and FADH yield 2 and 1 mol of ATP per mol oxidized, respectively.

4. Results

4.1. Trafficking alternatives with E. coli mutant lacking pyruvate kinase

The minimization of r_{18} was the objective to analyze the flux distributions in an *E. coli* mutant lacking pyruvate kinase. The size of the MILP (MP) is 81 continuous variables, 63 binary variables and 141 constraints, and the algorithm found nine alternate optima requiring a CPU-time of 7.78 s using GAMS/CPLEX 6.5.2. Nine solutions are shown in Table 1. In the model, all the fluxes were constrained to be unidirectional except for r_{10} and r_{33}, which were allowed to be

Table 1
Nine flux[a] distributions found by the MILP method, only selected fluxes[b], carbon and ATP yields (Y_C and Y_{ATP}) are shown

Alternate solutions	Selected fluxes (mmol g^{-1} cell h^{-1})				
	r_1	r_2	r_{10}	r_{18}	r_{19}
1	4.01	2.75	1.18	0	0
2	4.22	3.38	0.76	0	1.49
3	4.22	3.38	0.76	0	0
4	4.94	0.65	4.21	0	0
5	8.21	0.65	7.48	0	0
6	8.78	0.65	8.05	0	11.5
7	8.78	0.65	8.05	0	0
8	8.78	0.65	8.05	0	0.57
9	8.78	0.65	8.05	0	0
	$r23$	$r27$	$r32$	Y_C	Y_{ATP}
1	0	1.27	0	0.69	13.4
2	0	0	0	0.66	22.9
3	1.49	0	0	0.66	16.1
4	0	3.84	1.63	0.56	7.3
5	0	5.47	0	0.34	5.6
6	0	0	5.47	0.32	24.2
7	11.5	0	5.47	0.32	5.4
8	0	5.47	0	0.32	5.2
9	0.57	5.47	0	0.32	5.0

[a] All fluxes are bound between 0 and 20 mmol g^{-1} cell h^{-1}, except r_{10} and r_{33} which are between −20 and 20 mmol g^{-1} cell h^{-1}.

[b] r_1, glucose uptake rate; r_2, glucose-6-phosphate dehydrogenase flux; r_{10}, phosphoglucose isomerase flux; r_{18}, pyruvate kinase flux; r_{19}, lactate dehydrogenase flux; r_{23}, phosphotransacetylase+acetate kinase fluxes; r_{27}, α-ketoglutarate dehydrogenase flux; r_{32}, malic enzyme flux.

reversible (i.e. assigned lower bounds less than 0). These reactions are reported to have free energy changes nearly equal to 0.

Selected fluxes from each scenario, carbon yield (Y_c), and ATP yield (Y_{ATP}), which are calculated from Eqs. (8) and (9), are reported in Table 1. These two yields are a measure of carbon and energy utilization efficiency.

$$Y_c = \frac{500\ \mu}{(72 r_1)} \tag{8}$$

$$Y_{ATP} = \left(\frac{1000}{r_{ATP}}\right)\mu \tag{9}$$

To obtain Eq. (8), cells are assumed to consist of 50% carbon and μ denotes the specific growth rate (assigned as 0.4 h^{-1}).

All scenarios have the same objective function value of 0, but reveal different ways for carbon to traffic through an *E. coli* network lacking pyruvate kinase. For example, alternative 1 has nil acid production and the minimum glucose uptake rate and consequently the maximum carbon yield. Scenarios 2 and 3 suggest that organic acid and acetate formations are possible with higher glucose consumption rate and thus smaller carbon yield (see Fig. 2). Scenarios 1 and 3 have also been previously reported by Lee et al. (1997). They manually relaxed constraints whereas the MILP algorithm automatically found these and other solutions. In scenario 4, some malate is transformed to pyruvate via malic enzyme (see Fig. 3). This scenario thus suggests that malic enzyme may be activated; hence, the algorithm provides a potential input on the scope of subsequent experimental designs.

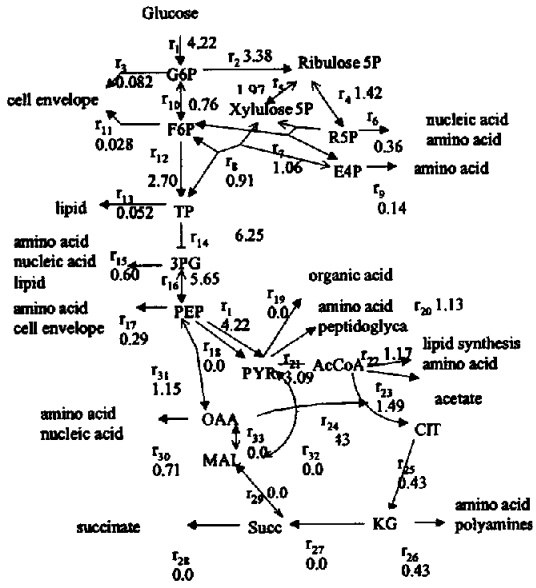

Fig. 2. Two detailed routings of glucose through *E. coli* networks with minimized pyruvate kinase flux (scenario 3 from Table 1).

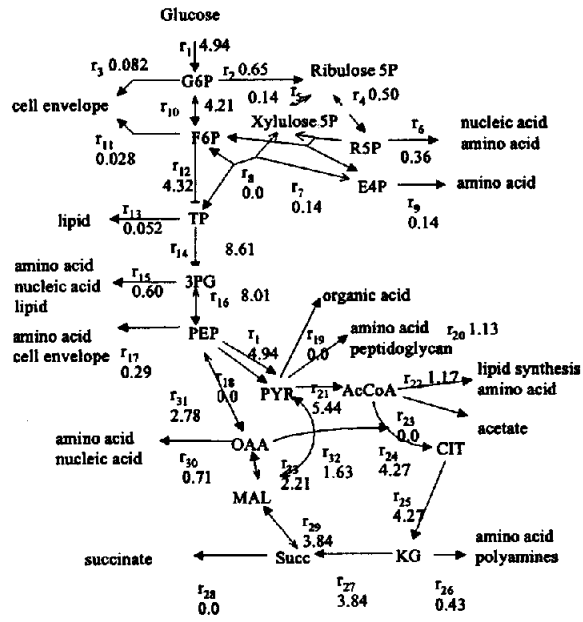

Fig. 3. Two detailed routings of glucose through *E. coli* networks with minimized pyruvate kinase flux (scenario 4 from Table 1).

In scenarios 6 and 7, net fluxes from oxaloacetate to malate (r_{33}, not shown in Table 1), and from malate to pyruvate (r_{32}) are obtained with 0 TCA fluxes (r_{27} and r_{29}). These scenarios thus suggest that the TCA cycle only provide the glutamate precursor, α-ketoglutarate.

Also, note that six scenarios can be grouped into three pairs (2, 3; 6, 7; 8, 9) where for a pair the only difference is whether organic acid or acetate is produced. The scenario with acetate production has a lower ATP yield because one mole of ATP is generated for each mol of acetate produced. The pairs define boundaries to valid solutions where all fluxes other than r_{19} and r_{23} are fixed. All linear combinations of r_{19} and r_{23} that sum to a constant (e.g. for scenarios 2 and 3, $r_{19} + r_{23} = 1.49$) constitute feasible solutions that fall within the boundaries. Thus, the multiple solutions can be reduced to scenario categories with defined bounds.

5. Conclusion

We have developed a search algorithm that solves MILP problem recursively to generate automatically multiple flux distribution alternatives that have the same objective function value and satisfy constraints. This formulation can be used to generate potential flux distribution scenarios that (1) may arise from implementing a metabolic engineering strategy or (2) provide the underlying fluxes that can account for a limited number of experimental observations. Additionally, using the MILP method in combination with ^{13}C NMR spectra simulation allows for the prediction of the NMR spectra associated with a particular flux distribution and labeled precursor compound. These spectra, in turn, can enable the design of ^{13}C NMR experiments. Based on the spectra, the labeled glucose can be chosen such that the resulting spectrum will significantly differ for a subset of flux distribution candidates.

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