MCS^2: Minimal coordinated supports for fast enumeration of minimal cut sets in metabolic networks

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Abstract

Constraint-based modeling helps researchers to understand metabolic networks. Minimal Cut Sets (MCSs) are minimal knock-out sets that block a target reaction in metabolic networks. Most approaches for finding the MCSs for a target reaction in constrained-based models require the computation of the set of elementary flux modes (EFMs) either as an intermediate step or as a byproduct of the calculation. Recently, Ballerstein et al. [BvKKH11] proposed a method of computing the MCSs directly. We propose an alternate method to compute the MCSs directly, based on linear programming duality. We prove the correctness of our new approach, extending the last author's doctoral work [Chi10]. The key idea is to find the EFMs of a fully reversible network with stoichiometric matrix equal to the transposed nullspace matrix of the original network's stoichiometric matrix. We implement our method and show that it succeeds in calculating the set of MCSs in many models where other approaches are not able to finish within a reasonable amount of time. Thus, in addition to its theoretical novelty, our approach provides a practical advantage over existing methods.

1 Introduction

Constraint-based modeling of metabolic networks has been a major subfield of systems biology thanks to its ability to identify key qualitative characteristics of networks for analyzing and extracting useful information [PRP04] A metabolic network is a collection of chemical reactions which comprise the metabolic activities (i.e. the biochemical transformation of molecules into other molecules for the purpose of maintenance and growth) of a specific organism. One important application of metabolic network analysis is to find interventions that can block a reaction of interest, typically referred to as the *target reaction*, with applications in drug target identification [HFR$^+$14] and metabolic engineering [MvKK15]. When this is achieved by disabling one or more other reactions, the disabled reactions are called a *cut set*. A cut set is called "minimal" if no proper subset of it can disable the target reaction. The concept of minimal cut sets (MCS) was introduced by Klamt and Gilles [KG04] and its applications are examined in detail in [Kla06].

At the moment, the main approach used for enumerating the MCSs for a target reaction is to compute the elementary flux modes containing the target and then use a dualization procedure to produce the MCSs [GDVL17]. Here, *flux modes* are possible distributions of fluxes through

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the reactions, and those can be modelled as hyperedges on the vertex set of possible reactions. *Elementary flux modes* (EFMs) are flux modes which are support-minimal, and it is known that any flux mode can be written as a non-negative linear combination of EFMs. Given the full set of elementary flux modes, minimal cut sets can be obtained through the dualization of the hypergraph they define [KG04, HKS08]. Two approaches to do this are Berge's algorithm [Ber84] and Fredman and Khachiyan's dualization procedure [FK96]. However, both suffer from poor worst-case complexity and produce mixed results in practice. A comparatively new approach [BvKKH11] produces MCSs without EFMs. It works by generating a dual network and computing the EFMs of that network that contain the target reaction. We call this the *dual network method*. In this paper, we develop a new method which finds the minimal cut sets from the row space of the stoichiometric matrix, based on a generalization of some theoretical results by the last author [Chi10].

We implement the method, and find it to be effective on most instances. We compare it to three alternate methods for enumerating all the MCSs for a target set. The first two methods are to compute the EFMs, and then dualize with Berge's algorithm, or an optimized implementation of Fredman-Khachiyan dualization, respectively. For Berge's algorithm, we used the implementation in CellNetAnalayzer [KSRG07], containing the enhancements described in [HKS08, EMG08]. For Fredman-Khachiyan dualization, we used the recent implementation of [SSC18].

The *dual network method* [BvKKH11] first creates a dual network based on the given stoichiometric matrix and given target reactions. Then it proceeds to compute the EFMs of that dual network, mimicking the calculations that produce the EFMs in the original network. Following some post-processing, these EFMs are reduced to give the required MCSs; because the MCSs correspond to only part of the vectors produced, this postprocessing includes purging the result of supersets. Like our method, the dual network method reports all the MCSs without first producing the EFMs or requiring them as an input. The authors of [BvKKH11] did not implement their method, so we did so ourselves, including all the enhancements mentioned in their supplementary material. Most of these enhancements have improved the performance, for instance by reducing the size of intermediate results.

However, for the majority of the models we investigate, we find that our approach is more efficient in terms of both running time and memory use. On the negative side, we show that our approach does not allow the enumeration of all MCSs through a given target reaction in incremental polynomial time, something that therefore remains a major open problem in the field. We conclude that the row space-based approach is a promising approach for the computation of structural elements of metabolic networks such as MCSs, and expect it to be a beneficial addition to the analysis tools available for metabolic network models.

1.1 Formal definitions

Definition 1 (Stoichiometric matrix). The *stoichiometric matrix* S of a metabolic network is the $m \times n$ stoichiometric matrix encoding in its columns the coefficients of consumed and produced metabolites in each chemical reaction. Here m is the number of metabolites and n is the number of reactions. As a result, each row represents a metabolite, showing how much is being consumed or produced by each reaction.

Definition 2 (Steady state). The assumption of *steady state* means that internal quantities of metabolites in the network stay the same over time. It follows that if v is the vector of fluxes through each of the reactions in the network, $Sv = 0$.

Definition 3 (Reaction reversibility). Thermodynamics forces some reactions to have fluxes in one direction while others have the possibility of both converting their reactants to products and vice versa. We call these *irreversible* and *reversible* reactions respectively, and denote the set of all irreversible reactions with $\mathcal{I}$.

Definition 4 (Stoichiometric matrix reconfiguration). Let S be a stoichiometric matrix with irreversible reactions $\mathcal{I}$. We can *reconfigure* the stoichiometric matrix to $[S' = S| - S_{\cdot\mathcal{I}^C}]$ add opposite directions of reversible reactions to give an equivalent system where all reactions are irreversible. We denote by $\mathcal{I}^C$ the set of indices of reversible reactions.

Definition 5 (Nullspace matrix). Let S be a matrix over $\mathbb{Q}$ (where $\mathbb{Q}$ is the set of rational numbers). A *nullspace matrix* of S is a matrix whose rows form a basis of the nullspace of S over $\mathbb{Q}$.

Definition 6 (Nullspace network). The nullspace network of stoichiometric matrix S is a fully reversible metabolic network whose stoichiometric matrix is nullspace of S.

Definition 7 (Positive and negative support). Let v be a vector. The *positive support* of v is the set of positions i where flux v_i is positive: $\mathcal{R}_+(v) := \{i|v_i > 0\}$. The *negative support* of v is the set of positions i where flux v_i is negative: $\mathcal{R}_-(v) := \{i|v_i > 0\}$. Their union is the *support* of v: $\mathcal{R}(v) := \mathcal{R}_+(v) \cup \mathcal{R}_-(v)$.

Definition 8 (Coordinated support). Let v be a vector and let A be a set of positions. The *A-coordinated support* of v is the union of the negative support on positions A and the support everywhere else: $\mathcal{R}_A(v) := (\mathcal{R}_-(v) \cap A) \cup (\mathcal{R}(v) \cap A^C)$.

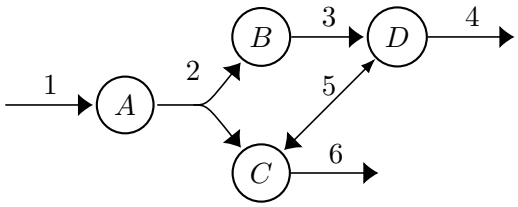
Definition 9 (Elementary flux mode (EFM)). Let $\mathcal{M}$ be a metabolic network with stoichiometric matrix S and set of irreversible reactions $\mathcal{I}$. A vector v is a *flux mode* if $Sv = 0$ and $v_\mathcal{I} \geq 0$. It is an *elementary flux mode* if its support is minimal among all flux modes: $Sw = 0, w_\mathcal{I} \geq 0, \mathcal{R}(w) \subsetneq \mathcal{R}(v) \implies w = 0$ [SFD00, GK04].

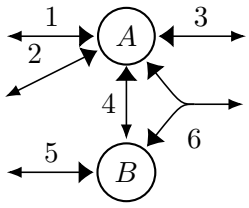
Definition 10 (Minimal cut set (MCS)). Let $\mathcal{M}$ be a metabolic network with stoichiometric matrix S and set of irreversible reactions $\mathcal{I}$. Let t be a reaction. A set C is a *cut set* for t if $Sv = 0, v_\mathcal{I} \geq 0, v_C = 0 \implies v_t = 0$. It is a **minimal cut set** if it is inclusion-minimal: $D \subsetneq C \implies \exists\ v$ s.t. $Sv = 0,\ v_\mathcal{I} \geq 0, v_D = 0, v_t \neq 0$[KG04].

Definition 11 (Canonical form of a network). Let $\mathcal{M}$ be a metabolic network with stoichiometric matrix S and set of irreversible reactions $\mathcal{I}$. We say that S is in *canonical form* if it satisfies:

1. No blocked reactions: for every reaction i, there exists a flux vector v with $v_i = 1$;
2. Proper directedness: for every reaction $i \in \mathcal{I}^C$, there exists a flux vector w with $w_i = -1$;
3. No enzyme subsets: no reaction pair $i \neq j$ satisfies $v_i = \kappa v_j$ with $\kappa \in \mathbb{R}$ for all flux vectors v;
4. No redundant constraints: S has full row rank.

A metabolic network can be reduced to an equivalent one in canonical form (a.k.a. compressed form) in polynomial time [Chi10].





Stoichiometric matrix $= \begin{bmatrix} 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & -1 \\ 0 & 0 & 1 & -1 & -1 & 0 \end{bmatrix}$

Reversibility vector = [No No No No Yes No]

The nullspace network's stoichiometric matrix$= \begin{bmatrix} 1 & 1 & 1 & 1 & 0 & 1 \\ 0 & 0 & 0 & -1 & 1 & 1 \end{bmatrix}$

Reversibility vector = [Yes Yes Yes Yes Yes Yes]

Cut sets for reaction 1 $\left\{ \begin{array}{ccc} \{4,6\} & \xleftarrow{\mathcal{I}\text{-Coordinated support}} & [1,0,0,\frac{-1}{2},0,\frac{-1}{2}] \\ \{3\} & \xleftarrow{\mathcal{I}\text{-Coordinated support}} & [1,1,-2,0,0,0] \\ \{5,6\} & \xleftarrow{\mathcal{I}\text{-Coordinated support}} & [1,0,0,0,1,-1] \end{array} \right\}$ EFMs in nullspace network

Figure 1: Example of a metabolic network and an associated row-space network.

2 Row-space method

Let S_i be a row of the stoichiometric matrix S. Then S_{ir} represents the amount of metabolite i consumed or produced by reaction r (in these cases, $S_{ir} < 0$ and $S_{ir} > 0$, respectively). Assume that reaction r produces metabolite i if it has a positive flux. Then, in a steady state where no reaction consuming metabolite i is active, reaction r must be inactive in the forward direction. If reaction r happens to be reversible, it must be consuming metabolite i, and its flux must be negative. This shows that reaction r is blocked in the forward direction if we disable every reaction that can consume metabolite i, i.e. every irreversible reaction with a negative value in row i and every reversible reaction with a non-zero value in row i. The set of such reactions is then is a cut set for the forward direction of reaction r. Every row gives us some, not necessarily minimal, cut set in this manner.

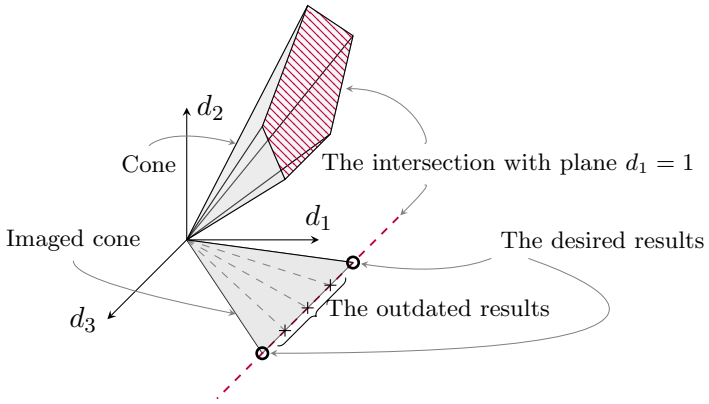
In fact, we can apply the same reasoning to linear combinations of the metabolites. Consider a new *virtual* metabolite x, which represents a linear combination of rows S_i and S_j corresponding to metabolites i and j respectively, say for example $v_x = 2S_i - S_j$. Since virtual metabolite x has the balance properties, it should not be produced or consumed in any steady state; in other words, since the fluxes of each metabolite are balanced, so are the fluxes of their linear combinations. If we pick a reaction with positive value in v_x, it produces a virtual metabolite x when it has a positive flux. Similarly, it will be blocked if we cut irreversible reactions with negative values in v_x and reversible reactions with non-zero values in v_x. Thus, we can obtain cut sets from the vector v_x, which is a member of rowspace of S, as we did with i and j.

A proposal for finding cut sets via the row space of the stoichiometric matrix was introduced in the Ph.D. thesis of Chindelevitch [Chi10]. The described intuition shows why a vector in the row space can generate cut sets. However, the lemma presented in [Chi10] only works for the fully irreversible or fully reversible networks. We generalize here to networks with both irreversible reactions and reversible reactions.

In our row space method, we build a new network based on the transposed null space matrix of the original matrix with full reversibility. EFMs in this new matrix represent cut sets in the

$$\left[\begin{array}{c|c} S & -S \\ \hline I & I \end{array}\right]$$

(a) This $(m + n) \times 2n$ matrix is the nullspace of reconfigured nullspace of stoichiometry matrix S. The double description method will start its process on this space and it will find extreme rays with length $2n$. However, what we need are the extreme rays of this space while ignoring non-related dimensions. The dimensions that are not counted in coordinated support.



(b) All extreme rays of the projected cone are an image of an extreme ray in original cone, while some extreme rays of the original cone do not project to extreme rays. It's also possible that two or more extreme rays project into one. Our desired results lay on the plane where the value in target position is one.

Figure 2: Minimal supports correspond to extreme rays of the cone while minimal coordinated supports are extreme rays of the projected cone in a lower dimension space. Our desired results are the intersection of plane $t = 1$ with the projected cone's extreme rays, where t is the position of our target reaction in the nullspace matrix of the reconfigured nullspace matrix (Figure 2a).

original network. The new network has the same number of reactions as the original one, but in most cases, it has a lot fewer metabolites.

Lemma 1 (MCSs for an irreversible reaction). *Let $\mathcal{M}$ be a metabolic network with stoichiometric matrix S and irreversible reactions $\mathcal{I}$ and reversible reactions Rev. Let $t \in \mathcal{I}$ be an irreversible target reaction. Then C is a cut set for t if and only if there exists a vector $u \in Row(S)$ such that $u_t = 1$ and $\mathcal{R}_{\mathcal{I}}(u) \subseteq C$.*

Proof. This lemma is an extension of Lemma 3 of [Chi10]. We observe that C being a cut set for irreversible reaction t is equivalent to:

$$S_{-C}v = 0 \text{ and } v_i \geq 0 \; \forall \; i \in \mathcal{I} - C \implies v_t = 0 \tag{1}$$

Based on Farkas' Lemma, we only need to find a constraint that implies $v_t \leq 0$. Thus, there exists a y such that:

$$y^T S_{-C} = e_t + \sum \alpha_i e_i \; \forall \; i \in \mathcal{I} - \{C \cup t\} \tag{2}$$

Here α_i are non-negative and e_i is a vector with 1 in ith value and zero everywhere else. Thus we have:

$$y^T S = u \tag{3}$$

Which u has a non-negative value $u_i \; \forall i \in \mathcal{I} - \{C \cup i\}$ and zero value $u_j \; \forall j \in Rev - C$ and $u_t = 1$, i.e. $\mathcal{R}_-(u) \cap \mathcal{I} \subseteq C \cap \mathcal{I}$ and $\mathcal{R}(u) \cap Rev \subseteq C \cap Rev$. For the other direction of proof, check if u in equality (3) has value 1 in u_t. Then pick indices i in $\mathcal{I}$ which $u_i < 0$ and indices i in Rev which $u_i \neq 0$ to get a cut set. Equality (2) holds and so does (1). □

Lemma 2 (MCSs for one direction of a reversible reaction). *Let $\mathcal{M}$ be a metabolic network with stoichiometric matrix S and irreversible reactions $\mathcal{I}$. Let t be a reversible target reaction. Then C is a cut set for forward direction of t if and only if there exists a vector $u \in Row(S)$ such that $u_t = 1$ and $\mathcal{R}_{\mathcal{I}}(u) \subseteq C$. Furthermore, C is a cut set for backward direction of t if and only if there exists a vector $u \in Row(S)$ such that $u_t = -1$ and $\mathcal{R}_{\mathcal{I}}(u) \subseteq C$.*

Proof. If we assume t is irreversible for a moment, the first part is already proved in the previous lemma. For the second part, replace t with $-t$ in S and create S'. Reaction t in S' is blocked in forward direction if and only if t in S is blocked in backward direction. And every vector in $Row(S)$ has a corresponding vector in $Row(S')$ with negated value in index t. □

With these lemmas, Algorithm 1 below can be used to find the minimal cut sets for a set of target reactions $T = \{t_1, t_2, ..., t_k\}$ in an arbitrary metabolic network $\mathcal{M}$ with stoichiometric matrix S and set of irreversible reactions $\mathcal{I}$, where T has separate elements for the opposite directions of a reversible reaction.

Algorithm 1 MCS enumeration via rowspace method

Input: Stoichiometric matrix S, set of Irreversible reaction $\mathcal{I}$, and Target set $T = \{t_1, t_2, \ldots, t_k\}$
Output: Minimal cut sets of target reactions T.

1: **function** $MCS_$ENUMERATION$(S, \mathcal{I}, T)$
2: Reduce S to its canonical form.
3: Compute the nullspace matrix N of S.
4: Compute all elementary flux modes $\mathcal{F}$ of N.
5: **for all** $1 \leq i \leq k$ **do** Compute $\mathcal{F}_i$, the set of all elements of $\mathcal{F}$ involving target t_i.
6: **for all** $1 \leq i \leq k$ **do** Let $\mathcal{C}_i$ be the set of minimal $\mathcal{I}$-coordinated supports of the elements of $\mathcal{F}_i$.
7: Let $\mathcal{C} = \{x = x_1 \cup x_2 \cup ... \cup x_k | x_i \in \mathcal{C}_i \; \forall \; i\}$.
8: Let $\mathcal{C}'$ be the result of pruning $\mathcal{C}$ to remove any supersets.
9: Return $\mathcal{C}'$.

Flux modes finders such as FluxModeCalculator reconfigure the network to apply the double description method. That is, they work with $N' = [N \mid -N]$ where N is the nullspace network of S. Figure 2a shows the nullspace matrix of N', which is the starting point of double description method. At the very beginning of applying the double description method, the matrix is converted by elementary row operations into the suggested shape described in [Wag04] which contains an identity matrix of size $m + n$. At the end of the double description method, we will have extreme rays describing a cone in $2n$-dimensional space [TS08]. The extreme rays are non-zero members of our cone with minimal support. On the other hand, coordinated support does not count non-zero values in some dimensions, namely, those that correspond to positive value of irreversible reactions. If we ignore these dimensions, we project the cone into a lower-dimensional subspace. While the image of a pointed cone remains a pointed cone, extreme rays of the new cone are the one in our feasible space with minimal support in the remaining dimensions. Figure 2b shows why all minimal coordinated supports are among the minimal supports, while there may be some redundant or unwanted results among them as well.

Theorem 1 (Correctness of the method). *Algorithm 1 returns precisely the set of minimal cut sets of the network $\mathcal{M}$ for reaction t.*

Proof. We prove the inclusion in both directions. First, let $C \in \mathcal{C}'$ be one of the sets returned by the method above. Then C is a cut set for t in the reconfigured network, by lemma 1 and by construction. Indeed, $\mathcal{F}$ contains flux modes of N involving t, which are precisely the vectors in the row space of S involving t, and $\mathcal{C}$ (and *a fortiori* its subset $\mathcal{C}'$) contains the $\mathcal{I}$-coordinated supports of these vectors.

Now, let C be a minimal cut set for t in $\mathcal{M}$. We will show that $C \in \mathcal{C}'$. By lemma 1, there exists a vector $u \in Row(S)$ such that $u_t = 1$ and $C = \mathcal{R}_{\mathcal{I}}(u)$. Since $u \in Row(S) \iff u \in Null(N)$, u is a conical combination of the elementary flux modes of N. Note that the results of Müller and Regensburger [MR16] imply that since the space to which u belongs is linear (i.e. it does not need to satisfy any non-negativity constraints), this conical combination can be chosen to be **conformal**, meaning that there are no cancellations involved in any component. Let such a conformal conical combination be given by

$$u = \alpha_1 f_1 + \cdots + \alpha_k f_k, \alpha_i > 0 \; \forall \; 1 \leq i \leq k. \tag{4}$$

Since all the coefficients are strictly positive in (4), we deduce that

$$\mathcal{R}_{\mathcal{I}}(u) = \mathcal{R}_{\mathcal{I}}(f_1) \cup \cdots \cup \mathcal{R}_{\mathcal{I}}(f_k).$$

Indeed, each $j \in \mathcal{R}_{\mathcal{I}}(u) \cap \mathcal{I}$ must have a negative component in at least one of the f_i, as otherwise the j-th component of the right-hand side of (4) will be non-negative, which gives the $\subseteq$ direction, and the fact that the combination is conformal gives the $\supseteq$ direction, as otherwise there would be a cancellation.

In particular, we deduce that $\mathcal{R}_{\mathcal{I}}(f_i) \subseteq \mathcal{R}_{\mathcal{I}}(u)$ for each $1 \leq i \leq k$. In this case, the minimality of C implies that either $\mathcal{R}_{\mathcal{I}}(f_i) = \mathcal{R}_{\mathcal{I}}(u)$ or f_i has a 0 in position t, for each $1 \leq i \leq k$. But since u has a 1 in position t, there must be at least one f_i in the first category, so that $\mathcal{R}_{\mathcal{I}}(f_i) = \mathcal{R}_{\mathcal{I}}(u) = C$ and therefore, $C \in \mathcal{C}$. Once again, by the minimality of C we conclude that $C \in \mathcal{C}'$ since it cannot be a superset of the $\mathcal{I}$-coordinated support of another vector in $\mathcal{C}$. This concludes the proof. □

2.1 Limitations

Our method is limited to blocking one direction of a given reaction. However, in practice, blocking one direction of a given reaction is the typical objective [LB07]. To block multiple reactions it is possible to compute the MCSs of every target reaction, take unions of all possible combinations of them, and then remove the supersets. However, this is not efficient.

A more critical issue is the possibility of generating a large number of non-minimal cut sets before the post-processing. The following Lemma, proved in Appendix A, shows this type of blow-up can occur:

Lemma 3 (Large number of supersets in the final step). *For every integer $k \geq 2$ there exists a network containing $k+2$ metabolites, $3k+3$ reactions and $2^{k-1}+1$ elementary vectors for the target reaction $t = 1$ that map to the exact same minimal cut set. This network is in canonical form in the sense of [Chi10] and elementally balanced in the sense of [ZSBC18].*

2.2 Advantages

An advantage of the new approach is that we find the MCSs directly. We do not need to find the EFMs before or during the calculation. Also, we do not need to reconfigure or alter the stochiometric matrix: every step is applied directly to the given stoichiometric matrix. Compression or reduction may be done in preprocessing before going through the main procedure, but these are only for reducing running time and space and can be skipped.

The preprocessing step in which we compute the EFMs of the transposed nullspace is independent of the target reaction. This means that we can calculate these EFMs once and use them for any given target reaction to block.

The most important advantage of the row space method is that the null space where we work will be small if the stoichiometric matrix is nearly full-rank. In that situation, which is easy to identify, our method usually performs well enough to beat other methods.

3 Implementation details

Except where noted, the implementations we discuss are in MATLAB. Each method that we consider requires an extreme ray computation, with the underlying cone varying. We used FluxModeCalculator's EFM generator [vKWvD15] for this purpose. Note that the optimized Berge algorithm implemented by CellNetAnalyzer [KSRG07] uses the older EFM finder of CellNetAnalyzer by default. However, we observed that it is a slower implementation of an identical calculation, so we rewrote this part to use FluxModeCalculator to make a fair comparison. The row space method and the dual method both need to removing redundant (super)sets from the obtained extreme rays. We use an implementation in Java whose time complexity is $O(N^2)$ for a collection of N sets. The stoichiometric matrices are compressed by the MongooseGUI3 [CTRB14] beforehand, which converts them to a canonical form.

Since the nullspace is needed for the row space method, we calculate the nullspace basis matrix using MongooseGUI3. Since finding the MCSs in every method takes several seconds to several minutes and the computation time of the nullspace basis matrix was less than a second in every case, we ignored its computation time. The reduced matrix given by Mongoose, the dual matrix, and the nullspace basis matrix get further compressed by FluxModeCalculator before processing. For the Berge algorithm we used CellNetAnalyzer. We also used an existing implementation of the improved modified Fredman-Khachiyan (MFK) algorithm [SSC18]. However, we implemented the dual method from scratch using MATLAB and the source code of FluxModeCalculator. All the enhancements mentioned in the supplementary material of the dual paper [BvKKH11] were implemented as well.

Our implementation is freely available at `https://github.com/RezaMash/MCS` under the GNU 3.0 license.

4 Results

Table 1: Result of running the methods on the *glucose* network with 106 reactions and 89 metabolites (the first reaction is the target)

	Optimized Berge	Improved MFK	Dual with Enhancements	Row-Space
extreme ray computation	69.9	69.9	>18000	410.2
secondary process time	1.9	1544.7	-	519.9
total time	71.8	1614.6	>18000	930.1

All times are in seconds.

Table 2: Result of running the methods on the *hepatic polyamine and sulfur aminoacid network* [RPMSJM12] with 73 reactions and 53 metabolites (the first reaction is the target)

	Optimized Berge	Improved MFK	Dual with Enhancements	Row-Space
extreme ray computation	270.2	270.2	1191.9	79.8
secondary process time	>18000	>18000	591.3	157.4
total time	>18000	>18000	1783.2	237.2

All times are in seconds.

We ran the methods on some models from BioModels database [LDR+10]. There were a few models on which our method either wasn't able to finish in the given time (5 hours) or took much longer to report the MCSs, while the optimized Berge was able to finish in time and beat our method (See table 4 for an example). This is due to the large set of supersets generated in that example by our method. However, the row-space method always performs better than the dual approach, even with its enhancements included. In addition, as can be seen for the *hepatic polyamine and sulfur aminoacid combined* model [RPMSJM12], the Berge and MFK methods could not finish in five hours, but the rowspace method generated results in four minutes, and the dual method in half an hour. The first task of every method is an extreme ray computation, which for Berge and MFK is the well-known EFM computation. Berge and MFK then proceed to generate the MCSs through dualization, while the secondary process of the dual approach and our row-space approach is removing the redundant cut sets. In the first two provided examples, the target is the forward direction of the first reaction. Table 3 shows the computation task for calculating the MCSs for all possible target reactions. In the *kinetic model of yeast metabolic network*, described in [SLS+13], our method's advantage is clear - it was able to finish computing the MCSs for all reactions in only 13.6 seconds. Note that the dimensions stated in the tables are the ones before any compression is applied.

5 Conclusion and future works

One limitation to our method is that can not find the MCSs of set of a reactions directly unless we find MCSs for each direction individually and prune supersets from the union of these MCSs.

An alternate strategy for computing EFMs is via mixed integer linear programming (MILP) particularly when only a few good vectors are required, rather than a full enumeration [DFPR+09, RPdF+13, RPT+14]. We describe some preliminary progress in this direction in Appendix B.

Table 3: Result of running the methods on the *kinetic model of yeast* network [SLS+13] with 285 reactions and 295 metabolites (all reactions used as targets)

	Optimized berge	Improved MFK	Dual with Enhancements	Row-Space
extreme ray computation	86.0	86.0	>18000	53.0
secondary process time	>18000*	>18000	-	13.6
total time	>18000*	>18000	>18000	66.6

All times are in seconds.

*: Berge wasn't able to finish calculating MCSs for all the reactions but it computed MCSs for first five reactions before running out of time. The FK and Dual methods weren't able to finish the computation of MCSs for reaction 1.

Table 4: Result of running the methods on *Fernandez2006 ModelB* [FSGLN06] with 152 reactions and 75 metabolites (the first reaction is the target)

	Optimized berge	Improved MFK	Dual with Enhancements	Row-Space
extreme ray computation	99.5	99.5	>18000	>18000
secondary process time	2.1	1445.1	-	-
total time	101.6	1544.6	>18000	>18000

All times are in seconds.

This is an example where our method and the Dual method weren't able to finish in time while the Berge and MFK methods report all 194689 MCSs of the compressed network's first reaction fairly fast.

Another idea is to alter the double description method to find rays with minimal coordinated support instead of minimal support, e.g. by ignoring some of dimensions of the reconfigured network. Here it is important to be careful about zero-cycle flux modes which are flux modes that can have fluxes in both direction of split reversible reaction. These are not valid flux modes, but they appear in the output of Double Description method and they can cause omission of some rays which contain them in their support.

As we mentioned, there are models for which our method surpass other methods while there are other models where the best performance is by the Berge algorithm. The challenge is to find out what traits of these models are different and how we can decide to chose what method to run on the model.

In general, our method is new and still in need of refinement. Possible additional sources of improvement include identifying and removing unwanted super-sets in the middle of double description method and applying optimization on removing super sets.

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A Proof of Lemma 3

Proof. We construct the network as follows. There are two special metabolites, denoted M_I (initial) and M_F (final), and k intermediate metabolites, denoted M_i for $1 \leq i \leq k$. For each metabolite, we have an export reaction and an import reaction, with the export reactions for each intermediate metabolite coupled with an import of the final metabolite. Lastly, each intermediate metabolite except the first one can be transformed into the first one, M_1, which itself can also be transformed into the initial metabolite M_I. All reactions in the network are irreversible and all the stoichiometric coefficients are ± 1.

We order these reactions as follows (for simplicity of argument):

$$\begin{aligned}
R_1 &: \emptyset \to M_I && \\
R_2 &: M_I \to \emptyset && \\
R_3 &: M_1 \to M_I && \\
R_{3+i} &: M_{i+1} \to M_1 && 1 \leq i \leq k-1 \\
R_{k+2+i} &: \emptyset \to M_i && 1 \leq i \leq k \\
R_{2k+2+i} &: M_i \to M_F && 1 \leq i \leq k \\
R_{3k+3} &: M_F \to \emptyset. &&
\end{aligned}$$

The stoichiometric matrix then looks as follows (shown here for $k = 2$):

$$S = \begin{pmatrix} + & - & + & & & & & & \\ & & - & + & + & & - & & \\ & & & - & & + & & - & \\ & & & & & & + & + & - \end{pmatrix}$$

Here, a $+$ represents a 1 and a $-$ represents a -1. We now proceed to show each part of the desired statement:

- The network is elementally balanced because every reaction that is not pure import or pure export is an exchange of one metabolite for another in a 1-1 ratio, so we can consider each metabolite as containing exactly 1 atom.

- The network is in canonical form because every reaction can be active and no pair of reactions is constrained to have proportional fluxes; this is evidenced by the following flux modes:

$$\begin{aligned} &R_1 + R_2 && \\ &R_2 + R_3 + R_{k+3} && \\ &R_1 + 2R_2 + R_3 + R_{k+3} && \\ &R_2 + R_3 + R_{3+i} + R_{k+3+i} && 1 \leq i \leq k-1 \\ &R_{k+2+i} + R_{2k+2+i} + R_{3k+3} && 1 \leq i \leq k \end{aligned}$$

  This set of fluxes includes every reaction at least twice, and in at least two of these the sets of other active reactions are disjoint. The only exceptions are R_1 and R_3, which need R_2 to be active in order to occur, but the first and third flux modes (respectively second and third flux modes) show that their fluxes are not proportional to that of R_2 or to each other; and and the reactions R_{3+i} and R_{2k+2+i} for $1 \leq i \leq k-1$, both of which need R_{k+3+i} to be active in order to occur, but not in a fixed ratio, as evidenced by taking linear combinations of the last two sets of flux vectors.

- Furthermore, the stoichiometric matrix has full row rank, i.e. no metabolite generates a redundant constraint, because every metabolite except M_F has a pure import reaction, while M_F has a pure export reaction.

- There is a unique MCS for target reaction R_1, namely, R_2. This is because R_2 is the only reaction consuming M_I (recall that all reactions are irreversible). The first row of S, $u := e_1 - e_2 + e_3$ produces this MCS via its negative support.

- Lastly, there are 2^{k-1} additional vectors in the rowspace of S that produce supersets of this MCS via their negative support. The first one is obtained from u by adding the second row of S, to replace it with:

$$v_\emptyset := e_1 - e_2 + e_4 + e_5 + \cdots + e_{k+2} + e_{k+3} - e_{2k+3},$$

  and then picking any subset P of the set of $k-1$ entries $E := \{4, 5, \ldots, k+2\}$ to form a new vector v_P, as follows.

Let $3+j \in P$ be an element of the chosen subset, where $1 \leq j \leq k-1$. We will replace e_{3+j} with $e_{k+3+j} - e_{2k+3+j}$ via the addition of the $(j+2)$-nd row of S (corresponding to the intermediate metabolite M_{j+1}) to the starting vector. Indeed, this row contains three non-zero entries: a -1 from reaction R_{3+j} (which cancels out the 1 in position e_{3+j}), as well as another 1 from reaction R_{k+3+j} and another -1 from reaction R_{2k+3+j}.

We do this addition independently for each element of P to get v_P (if $P = \emptyset$ we get $v_\emptyset$). It is easy to check that all the v_P are elementary and that v_P has support:

$$\{1, 2, 2k+3\} \cup \{3+j | 3+j \notin P\} \cup \{3+k+j | 3+j \in P\} \cup \{3+2k+j | 3+j \in P\},$$

and no proper subset of this support can produce a non-trivial vector in the rowspace of S, as otherwise it would be possible to add a linear combination (possibly with negative coefficients) of the rows of S to v_P without adding any new elements to its support, which is impossible by construction. Furthermore, the negative support of v_P is:

$$\{2, 2k+3\} \cup \{3+2k+j | 3+j \in P\},$$

which is a strict superset of the negative support $\{2\}$ of u. This completes the proof.

□

B Mixed Integer Linear Programming

An alternate strategy for computing EFMs is via mixed integer linear programming (MILP), particularly when only a few good vectors are required, rather than a full enumeration [DFPR$^+$09, RPdF$^+$13, RPT$^+$14]. Recall that EFMs are minimum support vectors in the nullspace. Our method for finding MCSs similarly looks for vectors with minimal coordinated support in the rowspace, so a similar approach may be effective.

Lemma 4 (MCSs of a target set of reactions in a fully irreversible metabolic network). *Let S be a stoichiometric matrix of a fully irreversible metabolic network $\mathcal{M}$. Let T be a set of target reactions which may contain more than one reaction. C is a cut set for all reactions in T if and only if there exist a vector $u \in Row(S)$ such that $T \subseteq \mathcal{R}_+(u)$ and $\mathcal{R}_-(u) = C$.*

Proof. We need to show that every cut set for the set of target reactions appears as a vector in the row space with the described constraints, and every vector in the row space with those constraints represents a cut set.
Let C be a cut set for all reactions in T. Therefore, C is a cut set for each reaction in $T = \{t_1, t_2, ..., t_k\}$ individually. Based on Lemma 1, there exist vectors $u_1, u_2, ..., u_k \in Row(S)$ such that $t_i \in \mathcal{R}_+(u_i)$ and $\mathcal{R}_-(u_i) = C$ for $1 \leq i \leq k$. In other words, for all vectors u_i $(1 \leq i \leq k)$ the only negative elements are the ones with indices belonging to C, and all other elements are non-negative, with a strictly positive value in the one with index t_i in the vector u_i, for $1 \leq i \leq k$. If we define the vector $u := u_1 + u_2 + ... + u_k$, then $\mathcal{R}_-(u) = C$ and $T \subseteq \mathcal{R}_+(u)$, and u is clearly in $Row(S)$.
Now, let u be a vector in $Row(S)$ such that $T \subseteq \mathcal{R}_+(u)$ and $\mathcal{R}_-(u) = C$. Then $t_i \in \mathcal{R}_+(u)$ for all $1 \leq i \leq k$. Based on Lemma 1, $C = \mathcal{R}_-(u)$ is a cut set for the reaction t_i, for each $1 \leq i \leq k$. Therefore, C is a cut set for all the reactions in T, completing the proof. □

Based on this Lemma we are able to find minimal cut sets for every set of target reactions without the restriction of only blocking one direction of a reaction. Since reversible reactions split into two reactions after reconfiguration, we can block a reversible reaction in one direction or in both directions.

Let S' be the $m \times n'$ reconfigured matrix of $m \times n$ stoichiometric matrix S with irreversible reactions $\mathcal{I}$. Since all the values in the stoichiometric matrix are proportions of consumed and produced metabolites, we can scale each row of S to have only integer entries without changing its structural properties.

We now describe how to encode the problem of finding the smallest MCS for a target set T as a mixed-integer linear program (MILP). Let $v \in \mathbb{Z}^{n'}$ be a vector in the row space of matrix S corresponding to the smallest MCS for target reaction set $T = \{t_1, t_2, ..., t_k\}$. Then there exists a vector $y \in \mathbb{Z}^m$ s.t $y^T S = v$. If we define $r^+, r^- \in \{0,1\}^n$ as the positive and negative supports of v, respectively, we force to v_i to be negative if r_i^- is one, and force it to be positive if r_i^+ is one; similarly, we force v_i to be non-negative when r_i^- is zero and force it to be non-positive when r_i^+ is zero, by adding the following constraints:

$$v_i + W r_i^- \leq W - 1 \qquad -v_i + W r_i^+ \leq W - 1 \qquad \forall 1 \leq i \leq n'; \tag{5}$$

$$v_i \leq W r_i^+ \qquad -v_i \leq W r_i^- \qquad \forall 1 \leq i \leq n', \tag{6}$$

where W is a large constant number. There must also be positive values in the target positions:

$$r_i^+ = 1 \qquad \forall i \in T \tag{7}$$

This constraint also implies r_i^- for any target position i is zero. Indeed, both r_i^+ and r_i^- can not be one at the same time, as otherwise, v_i would be a negative integer and a positive integer at the same time. These constraints also ensure that $v = 0$ is not in our feasible space.

To find the smallest minimal cut set, the objective function is as follows:

$$\text{minimize} \sum_{i=1}^{n'} r_i^-, \tag{8}$$

since the cut set is the negative support of v, i.e r^-.

Suppose that we have found the smallest MCS $\mathcal{C} \subsetneq \{1, 2, ..., n'\}$. To find the next smallest MCS we need to remove $\mathcal{C}$ and all its super-sets from our feasible space. The following constraint excludes $\mathcal{C}$ and all its super-sets:

$$\sum_{i \in \mathcal{C}} r_i^- \leq |\mathcal{C}| - 1 \tag{9}$$

We can keep excluding newly found MCSs and thus enumerate them by size. As we stated above, in most scenarios we only wish to block an irreversible reaction or one direction of a reversible reaction. In those cases, we can avoid re-configuring the network to have a smaller stoichiometric matrix. Let t be the only target reaction. Instead of the constraints (7), we only need one constraint $r_t^+ = 1$ if we want to block it in forward direction, and we need the constraint $r_t^- = 1$ if we need to block it in the backward direction. The objective function (8) and constraints (9) can be updated as follows to reflect the coordinated support instead of the negative support:

$$\text{minimize}\Big(\sum_{\substack{i=1 \\ i \neq t}}^{n} r_i^- + \sum_{\substack{i \in \mathcal{I} \\ i \neq t}} r_i^+\Big) \qquad \sum_{i \in \mathcal{C}} r_i^- + \sum_{i \in \mathcal{C} \cap \mathcal{I}} r_i^+ \leq |\mathcal{C}| - 1$$

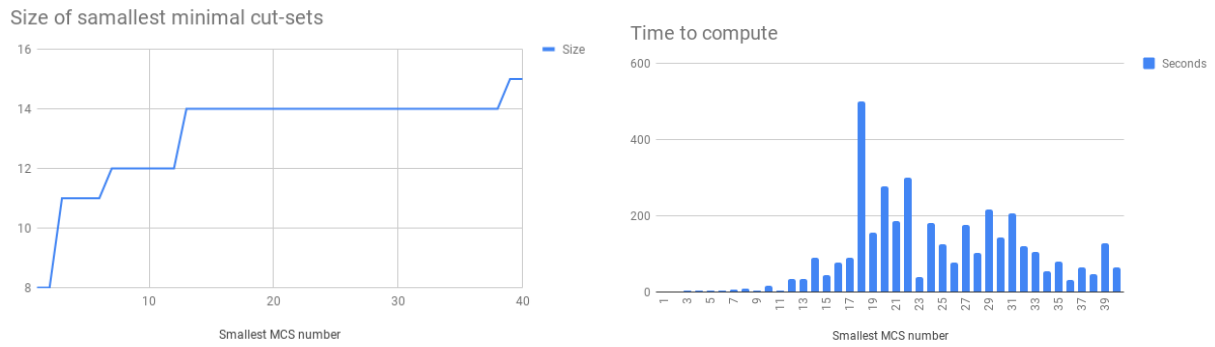


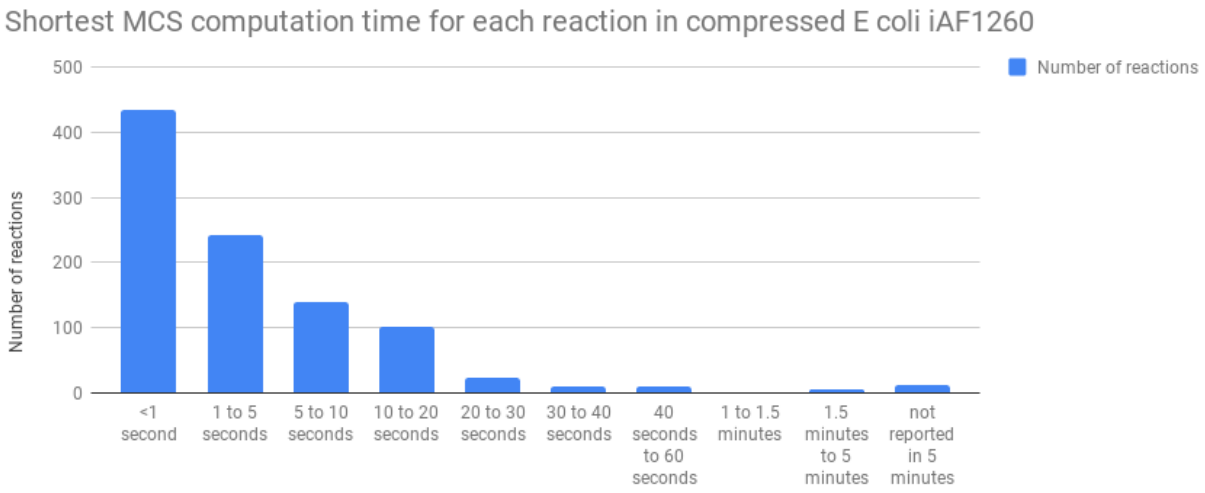
Figure 3: Result of computing the smallest MCSs for reaction 10 (the first reaction which has at least 100 MCSs) of the Li2012 Calcium-mediated synaptic plasticity model [LSLN12]

B.1 Implementation

We used CPLEX [IBM] to solve the MILPs. The implementation was done in Java and has been implemented for both single target reactions without re-configuration, and for multiple target reactions with network reconfiguration. Since the stoichiometric matrices needed to contain only integers, we used the `integralize` function of MONGOOSE [CTRB14] to multiply each row by the smallest possible integer that makes all the values integer (which is, of course, the least common multiple of the denominators of the entries). We also tested the results of our MILP in small networks against other implementations to make sure the results are consistent. The implementation of all the methods and the MILP version of our method are publicly available. In some cases they require the use of non-public modules, such as CellNetAnalyzer [KSRG07] and CPLEX, which are available for academic use.

B.2 Results

We ran the MILP version on larger networks. With this implementation we were now able to find partial MCSs for any target reaction. However, these partial MCSs are adjustable to our needs, and we always find the smallest MCSs in order. We were able to compute 100 MCSs of reaction 10 (the first reaction with at least 100 MCSs) in the Li2012 Model [LSLN12], which has 578 reactions after compression. The results are shown in Figure 3. The smallest MCS has eight reactions, and this goes up to 16 for the 100-th smallest MCS. Integer linear programming has been used for finding a subset of MCSs [SGMR17]. There have also been approaches using Boolean duality to find a subset of the MCSs [vKK14, VMRR16, HKS08]. As these approaches state, not all the EFMs in the dual space result in valid MCSs, but by adding the proper constraints one can remove the redundant results from the ILP's feasible space. To get a sense of how our approach perform compared to these earlier ones, we also ran our method on *E.coli iAF1260* to compare with [HKS08], which showed a superior performance relative to other implementations that enumerate a subset of the MCSs with MILP. We were not able to find the MCSs for 13 of the reactions in the reduced network, which maps to 30 reactions in the original, uncompressed network. Compared to [HKS08], where they missed 209 reactions, this is a notable improvement. The results are shown in Figure 4.



Computation time	Number of reactions
1 second	435
1 to 5 seconds	241
5 to 10 seconds	140
10 to 20 seconds	102
20 to 30 seconds	23
30 to 40 seconds	11
40 seconds to 1 minute	9
1 to 1.5 minutes	2
1.5 minutes to 5 minutes	5
not reported	13

Figure 4: Result of running our MILP on *E coli iAF1260* with 2382 reactions (981 reactions after reduction)