# Minimal Metabolic Behaviors and the Reversible Metabolic Space*

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**Abstract**

We present a new mathematical approach to metabolic pathway analysis, characterizing a metabolic network by minimal metabolic behaviors and the reversible metabolic space. Our method uses an outer description of the steady state flux cone, based on sets of irreversible reactions. This is different from existing approaches, such as elementary flux modes or extreme pathways, which use an inner description, based on sets of generating vectors. The resulting description of the flux cone is much more compact. By focussing on the reversible and irreversible reactions, our approach provides a different view of the network, which may also lead to new biological insights.

## 1 Introduction

Network-based metabolic pathway analysis has received considerable interest in the recent past, see [6] for review. The constraints that have to hold in a metabolic network, which include stoichiometry and thermodynamic irreversibility, have led to the definition of the steady state flux cone, which contains all the possible flux distributions over the network. Two important concepts have been proposed to describe the flux cone in a mathematical and biologically meaningful way: elementary flux modes and extreme metabolic pathways [10, 11, 8, 5, 4].

These two concepts are closely related. Both approaches use an inner description based on generating vectors of the flux cone, and the extreme pathways are a subset of the elementary modes [5]. However, from a mathematical point of view, not all of these generating vectors are needed to

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describe the cone. This observation is important because the computation of these vectors in a high-dimensional space often leads to a combinatorial explosion [6]. Even for small networks, the number of elementary flux modes or extreme pathways is often very large. This makes a complete analysis of the entire network impossible and limits the practical applicability of the approach.

In this paper, we propose a new mathematical approach to metabolic pathway analysis, which uses an outer description of the steady state flux cone, which is based on sets of non-negativity constraints. These can be identified with irreversible reactions, and thus have a direct biochemical interpretation. Our method is different from existing approaches, such as elementary flux modes or extreme pathways, which use an inner description. We characterize a metabolic network by so-called *minimal metabolic behaviors (MMBs)* and the *reversible metabolic space (RMS)*. Like elementary modes or extreme pathways, these are uniquely determined by the network, and yield a complete description of the flux cone. However, this description is much more compact. In addition, by characterizing metabolic pathways through sets of irreversible reactions, our approach provides a different view of the network, and thus may lead to new biological insights.

The organization of this paper is as follows. We start in Sect. 2 with some basic facts about polyhedral cones. In Sect. 3, we recall metabolic pathway analysis, and give a first presentation of our approach. In Sect.4, we formally introduce minimal metabolic behaviors and the reversible metabolic space, and formulate some of their basic properties. Finally, Sect. 5 contains some computational results in order to compare MMBs and the RMS with extreme pathways and elementary flux modes.

## 2 Polyhedral cones

We start with some basic facts about polyhedral cones, see e.g. [9].

A non-empty subset $C \subseteq \mathbb{R}^n$ is called a *(convex) cone* if $\lambda x + \mu y \in C$, whenever $x, y \in C$ and $\lambda, \mu \geq 0$. A cone $C$ is *polyhedral*, if $C = \{x \in \mathbb{R}^n \mid Ax \geq 0\}$, for some real matrix $A \in \mathbb{R}^{m \times n}$. The linear space $\text{lin.space}(C) = \{x \in \mathbb{R}^n \mid Ax = 0\}$ is then called the *lineality space* of $C$.

A cone $C$ is *finitely generated* if there exist $x^1, \dots, x^s \in \mathbb{R}^n$ such that $C = \text{cone}\{x^1, \dots, x^s\} \stackrel{\text{def}}{=} \{\lambda_1 x^1 + \cdots + \lambda_s x^s \mid \lambda_1, \dots, \lambda_s \geq 0\}$. A fundamental theorem of Farkas-Minkowski-Weyl asserts that a convex cone is polyhedral if and only if it is finitely generated. For the rest of this paper, we will consider only polyhedral cones.

An inequality $a^T x \geq 0, a \in \mathbb{R}^n \setminus \{0\}$, is *valid* for $C$ if $C \subseteq \{x \in \mathbb{R}^n \mid a^T x \geq 0\}$. The set $F = C \cap \{x \in \mathbb{R}^n \mid a^T x = 0\}$ is then called a *face* of $C$. The *dimension* of $F$ is defined as the dimension of the linear subspace generated by $F$.

Any non-zero element $r \in C$ is called a *ray* of $C$. We identify two rays $r$ and $r'$, and write $r \cong r'$, if $r = \lambda r'$, for some $\lambda > 0$. A ray $r$ is *extreme* if there do not exist rays $r', r'' \in C, r' \ncong r''$, such that $r = r' + r''$.

**Pointed cones.** A polyhedral cone $C$ is called *pointed* if lin.space$(C) = \{0\}$. Any pointed cone $C$ has a canonical representation

$$C = \text{cone}\{r^1, \ldots, r^s\}, \tag{1}$$

where $r^1, \ldots, r^s$ are the (distinct) extreme rays of $C$. This representation, which is used in the extreme pathway approach, is minimal and unique up to multiplication with positive scalars.

**Non-pointed cones.** If $C$ is not pointed, there is no longer such a unique minimal representation. Let $t$ be the dimension of the lineality space of $C$. Instead of the extreme rays, we consider now the *minimal proper faces* $G^1, \cdots, G^s$ of $C$, which are the faces of $C$ of dimension $t + 1$. Each $G^i$ can be represented by a row vector $a_i^T$ and a submatrix $A_i'$ of $A$, with rank $\begin{pmatrix} A_i' \\ a_i^T \end{pmatrix} = d - t$, such that

$$G^i = \{x \in C \mid a_i^T x \geq 0, A_i' x = 0\}, \tag{2}$$

and lin.space$(C) = \{x \in C \mid a_i^T x = 0, A_i' x = 0\}$.

If we select for each $i = 1, \ldots, s$ a vector $g^i \in G^i \setminus$ lin.space$(C)$, and vectors $b^0, \ldots, b^t \in$ lin.space$(C)$ such that lin.space$(C) = \text{cone}\{b^0, \ldots, b^t\}$, we get

$$C = \text{cone}\{g^1, \ldots, g^s, b^0, \ldots, b^t\}. \tag{3}$$

For additional information, we refer to [9, p.105-106].

Equation (3) generalizes (1), but this representation is no longer unique. We may choose an arbitrary base of lin.space$(C)$, and arbitrary rays $g^i$ in $G^i \setminus$ lin.space$(C)$. As we will see in the following sections, considering the minimal proper faces leads to a new way for describing and analyzing the flux cone associated with a metabolic network.

# 3 Metabolic pathway analysis

From a mathematical point of view, metabolic pathway analysis as considered here can be described as follows [6]. We are interested in the set of all

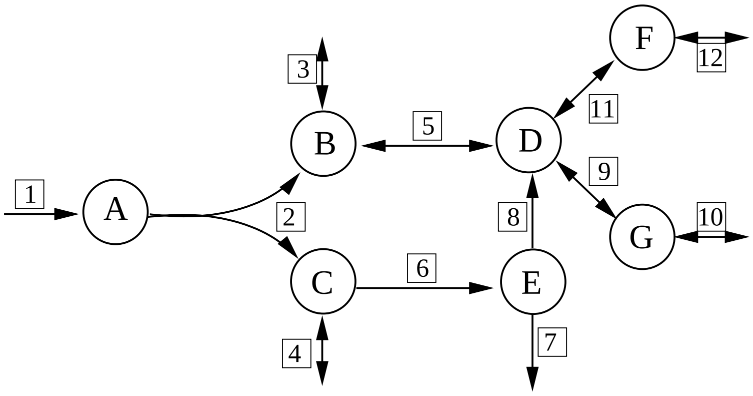


Figure 1: Metabolic network

solutions of the constraint system

$$Sv = 0, v_i \geq 0, \text{ for } i \in \mathit{Irr}. \tag{4}$$

where $S$ is the $m \times n$ stoichiometric matrix of the network, with $m$ metabolites (rows) and $n$ reactions (columns), and $v \in \mathbb{R}^n$ is the *flux vector*. $\mathit{Irr} \subset \{1, \ldots, n\}$ denotes the set of irreversible reactions in the network. The set of all solutions of the constraint system (4), which corresponds to the set of all possible fluxes through the network at steady state, defines a polyhedral cone,

$$C = \{v \in \mathbb{R}^n \mid Sv = 0, \ v_i \geq 0, i \in \mathit{Irr}\} \tag{5}$$

which is called the *flux cone*.

**Example 3.1** Consider the metabolic network depicted in Fig. 1. It consists of seven metabolites $(A, \ldots, G)$, and twelve reactions $(1, \ldots, 12)$. The steady state flux cone is defined by $C = \{v \in \mathbb{R}^{12} \mid Sv = 0, v_i \geq 0$ for all $i \in Irr\}$, with the set of irreversible reactions $\mathit{Irr} = \{1, 2, 6, 7, 8\}$, and the stoichiometric matrix

$$S = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & -1 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \end{pmatrix}.$$

If all reactions are irreversible (and also in some other cases), the flux cone is pointed, and has a minimal and unique set of generators, which correspond to the extreme rays. In general, however, the flux cone is not pointed, and there is no such canonical representation. In the extreme pathways approach, the network is reconfigured and all reversible internal reactions are split into a forward and a backward reaction, which both are constrained to be non-negative [8]. This implies that the flux cone becomes pointed and thus has a minimal and unique (up to multiplication by a positive scalar) set of generators, which correspond to the extreme rays of the (reconfigured) cone. However, the reconfiguration has undesirable consequences. On the one hand, the number of variables and constraints increases, and the constraint system becomes more difficult to solve. On the other hand, a significant number of pathways in the reconfigured cone are extreme for the only reason that the reversible internal reactions have been decomposed into forward and backward reactions. In the initial cone, these extreme pathways are linearly dependent. In addition, the number of extreme pathways (and even more the number of elementary modes) is often very large, even for small networks, which limits the practical applicability of the approach. Computational results illustrating these observations can be found in Sect. 5.

To overcome these problems, we propose a new description of the flux cone. Mathematically speaking, we switch from an inner description of the cone, based on generating flux vectors, to an outer description, based on constraints. In our case, these constraints are inequalities of the form $v_i \geq 0, i \in Irr$, which can be identified with irreversible reactions, and thus have a direct biochemical interpretation. Working on a possibly non-pointed flux cone $C$, we will associate with each minimal proper face a characteristic set of irreversible reactions, which we call a *minimal metabolic behavior (MMB)*. In addition, we include in our description the lineality space lin.space($C$), which will be called the *reversible metabolic space (RMS)*. For a non-pointed cone, this space is different from $\{0\}$. It contains useful biological information, which is no longer handled if we replace a reversible reaction with two irreversible ones. The set of all MMBs together with the RMS yields a complete description of the flux cone. As we will see, it is much more compact than generating sets based on extreme pathways or elementary flux modes. In addition, by focussing on reversible and irreversible reactions, this approach provides a different view of the network, which may also lead to new biological insights.

# 4 Minimal metabolic behaviors

In this section, we formally introduce minimal metabolic behaviors and the reversible metabolic space.

## 4.1 Characterizing minimal proper faces

We start by characterizing the minimal proper faces of the flux cone through irreversible reactions of the network.

**Definition 4.1** Let $G$ be a minimal proper face of the flux cone (5) and let $j \in \mathit{Irr}$ be an irreversible reaction. We say that $G$ is *defined by* $j$ if there exists $I_j \subset \mathit{Irr}$ such that $G = \{v \in C \mid v_j \geq 0,\ v_i = 0,\ \text{for all } i \in I_j\}$, and $\text{lin.space}(C) = \{v \in C \mid v_j = 0,\ v_i = 0,\ \text{for all } i \in I_j\}$.

It follows from the general theory (see Sect. 2) that each minimal proper face $G$ of $C$ is defined by at least one irreversible reaction. However, this reaction need not be unique. In general, there will be several irreversible reactions satisfying the conditions of Def. 4.1. These are further characterized in the next theorem and in Corollary 4.3.

**Theorem 4.2** *Let $G$ be a minimal proper face defined by $j \in \mathit{Irr}$. For an irreversible reaction $k \in \mathit{Irr}$, the following are equivalent:*

- *There exists $g \in G \setminus \text{lin.space}(C)$ with $g_k > 0$.*
- *$G$ is defined by $k$ and there exists $\alpha > 0$ with $v_k = \alpha \cdot v_j$, for all $v \in G$.*

**Proof:** "$\Leftarrow$": Immediate from the definition.

"$\Rightarrow$": Suppose $g \in G \setminus \text{lin.space}(C)$ such that $g_k > 0$. Since $G$ is defined by $j$, we get $g_j > 0$. For all $v \in G \setminus \text{lin.space}(C)$, there exist $\lambda > 0$ and $w \in \text{lin.space}(C)$ such that $v = \lambda \cdot g + w$. It follows that $v_j = \lambda \cdot g_j > 0, v_k = \lambda \cdot g_k > 0$, and therefore $v_k/v_j = g_k/g_j \stackrel{\text{def}}{=} \alpha > 0$, independently from $v$. This shows that $v_k = \alpha \cdot v_j > 0$, for all $v \in G \setminus \text{lin.space}(C)$.

For all $v \in \text{lin.space}(C)$, we have $v_j = v_k = 0$. It follows for all $v \in G$ that $v_k = \alpha \cdot v_j$, and that $v_j \geq 0$ iff $v_k \geq 0$. Choosing $I_k = I_j$, this implies $G = \{v \in C \mid v_k \geq 0,\ v_i = 0,\ \text{for all } i \in I_k\}$, and $\text{lin.space}(C) = \{v \in C \mid v_k = 0,\ v_i = 0,\ \text{for all } i \in I_k\}$, which proves that $G$ is defined by $k$. $\square$

**Corollary 4.3** *Let $G$ be a minimal proper face of $C$ and let $j \in \mathit{Irr}$ be an irreversible reaction. Then the following are equivalent:*

*1. $G$ is defined by $j$.*

*2. $v_j > 0$, for some $v \in G \setminus \mathrm{lin.space}(C)$.*

*3. $v_j > 0$, for all $v \in G \setminus \mathrm{lin.space}(C)$.*

## 4.2 Main definitions

We are now ready to define the key notions of this paper.

**Definition 4.4** Given a minimal proper face $G$ of the flux cone (5), the set

$$D = \{j \in Irr \mid v_j > 0, \text{ for some } v \in G\}$$

of all irreversible reactions defining $G$ is called the *minimal metabolic behavior (MMB)* associated with $G$. The *reversible metabolic space (RMS)* is the lineality space lin.space($C$).

Each minimal proper face $G$ of the flux cone is thus characterized by a set $D$ of irreversible reactions. As the next theorem shows, all pathways $v \in G \setminus \mathrm{lin.space}(C)$ have the following common property: the flux through all irreversible reactions belonging to $D$ is positive, i.e., $v_j > 0$, for $j \in D$, while the flux through all the other irreversible reactions is zero, i.e., $v_j = 0$, for $j \in Irr \setminus D$.

The pathways $v \in \mathrm{lin.space}(C)$ belonging to the reversible metabolic space consist of reversible reactions only, which contribute to the flexibility of the network. This information is no longer present if the network is reconfigured in order to obtain a pointed cone.

**Theorem 4.5** *Let $G$ be a minimal proper face of the cone (5) and $D$ the corresponding MMB. Then*

$$G = \{v \in C \mid v_j \geq 0, \text{ for all } j \in D, \; v_i = 0, \text{ for all } i \in Irr \setminus D\}.$$

**Proof:** Suppose $j \in D$. Then by definition $G = \{v \in C \mid v_j \geq 0, \; v_i = 0, \text{ for } i \in I_j\}$, for some $I_j \subset Irr$. From Corollary 4.3, we see that $I_j \subset Irr \setminus D$. It follows that $\{v \in C \mid v_j \geq 0, \text{ for all } j \in D, \; v_i = 0, \text{ for all } i \in Irr \setminus D\} \subseteq G$. To show the reverse inclusion, suppose $v \in G$. Then $v_j \geq 0$, for all $j \in D$. Suppose $v_i > 0$, for some $i \in Irr \setminus D$. From Corollary 4.3, we would get $i \in D$, which is a contradiction. □

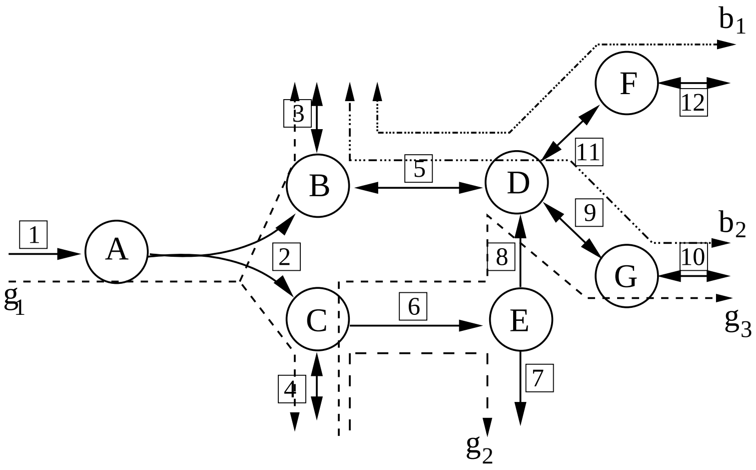


Figure 2: Metabolic network with representative pathways

Note that the set $D$ is uniquely determined by $G$. If $G^1, \ldots, G^s$ are the minimal proper faces of the flux cone $C$, the corresponding sets $D^1, \ldots, D^s$ define the possible minimal metabolic behaviors of $C$, which together with the reversible metabolic space lin.space$(C)$ completely describe $C$.

**Example 4.6** In the metabolic network from Fig. 1, the minimal metabolic behaviors and the corresponding minimal proper faces are as follows:

$$\begin{array}{lcllcl}
D^1 &=& \{1,2\}, & G^1 &=& \{v \in C \mid v_i \geq 0, i \in D^1, v_j = 0, j \in Irr \setminus D^1\}, \\
D^2 &=& \{6,7\}, & G^2 &=& \{v \in C \mid v_i \geq 0, i \in D^2, v_j = 0, j \in Irr \setminus D^2\}, \\
D^3 &=& \{6,8\}, & G^3 &=& \{v \in C \mid v_i \geq 0, i \in D^3, v_j = 0, j \in Irr \setminus D^3\}.
\end{array}$$

Note that the irreversible reaction 6 is participating in the definition of two minimal proper faces, $G^2$ and $G^3$. Fig. 2 shows three pathways

$$\begin{array}{lcl}
g^1 &=& (1,\ \ 1,\ \ 1,\ \ \ \ 1,\ \ 0,\ \ 0,\ \ 0,\ \ 0,\ \ 0,\ \ 0,\ \ 0,\ \ 0) \\
g^2 &=& (0,\ \ 0,\ \ 0,\ \ -1,\ \ 0,\ \ 1,\ \ 1,\ \ 0,\ \ 0,\ \ 0,\ \ 0,\ \ 0) \\
g^3 &=& (0,\ \ 0,\ \ 0,\ \ -1,\ \ 0,\ \ 1,\ \ 0,\ \ 1,\ \ 1,\ \ 1,\ \ 0,\ \ 0)
\end{array}$$

representing, respectively, the minimal proper faces $G^1, G^2$, and $G^3$.

The reversible metabolic space lin.space$(C) = \{v \in C \mid v_i = 0, i \in Irr\}$, has dimension 2. It can be generated by the pathways

$$\begin{array}{lcl}
b^1 &=& (0,\ \ 0-1,\ \ 0,\ \ 1,\ \ 0,\ \ 0,\ \ 0,\ \ 1,\ \ 1,\ \ 0,\ \ 0) \\
b^2 &=& (0,\ \ 0-1,\ \ 0,\ \ 1,\ \ 0,\ \ 0,\ \ 0,\ \ 0,\ \ 0,\ \ 1,\ \ 1)
\end{array}$$

An arbitrary pathway $v \in G^i, i = 1, 2, 3$, can be written as linear combination $v = \alpha g^i + \lambda b^1 + \mu b^2$, with $\alpha \geq 0, \lambda, \mu \in \mathbb{R}$.

The next results shows that, inside a minimal proper face $G$, the fluxes through the irreversible reactions in $D$ are proportional to each other.

**Corollary 4.7** *Let $D$ be the minimal metabolic behavior associated with the minimal proper face $G$. Then for all $j, k \in D$, there exists $\alpha > 0$ such that $v_k = \alpha \cdot v_j$, for all $v \in G$. In particular, $v_j = 0$ implies $v_k = 0$, and $v_j > 0$ implies $v_k > 0$, for all $v \in G$,*

## 4.3 Pseudo-irreversible reactions

We will now distinguish a special class of reversible reactions.

**Definition 4.8** Given the flux cone (5), let $Rev = \{1, \ldots, n\} \setminus Irr$ denote the set of reversible reactions. Then the set

$$Rev^0 = \{i \in Rev \mid v_i = 0, \text{ for all } v \in \text{lin.space}(C)\}$$

is called the set of *pseudo-irreversible reactions*.

The next proposition shows that pseudo-irreversible reactions become irreversible inside minimal proper faces. Within each minimal proper face $G$, any pseudo-irreversible reaction will take a unique direction, which is imposed by the MMB $D$ associated with $G$. This allows one to identify the subspace of the cone in which a reversible reaction can take only one direction.

**Proposition 4.9** *Let $G$ be a minimal proper face of $C$ and let $i \in Rev^0$ be a pseudo-irreversible reaction. Then exactly one of the following three conditions holds:*

1. *$v_i > 0$, for all $v \in G \setminus \text{lin.space}(C)$.*
2. *$v_i = 0$, for all $v \in G \setminus \text{lin.space}(C)$.*
3. *$v_i < 0$, for all $v \in G \setminus \text{lin.space}(C)$.*

**Proof:** Suppose $g \in G \setminus \text{lin.space}(C)$. For any $v \in G \setminus \text{lin.space}(C)$ there exists $\lambda > 0$ and $w \in \text{lin.space}(C)$ such that $v = \lambda \cdot g + w$. It follows that $sign(v_i) = sign(\lambda \cdot g_i) = sign(g_i)$, independently from $v$. □

**Example 4.10** In our example, we have $Rev^0 = \{4\}$, and $Rev \setminus Rev^0 = \{3, 5, 9, 10, 11, 12\}$. In the context of the MMB $D^1$, the pseudo-irreversible reaction 4 becomes positive, i.e., $v_4 > 0$, for all $v \in G^1 \setminus \text{lin.space}(C)$, while it becomes negative in the context of $D^2$ and $D^3$.

### 4.4 Deleting an irreversible reaction from the network

The next proposition shows that the deletion of an irreversible reaction $j$ from the network breaks down all the minimal metabolic behaviors in which $j$ participates. This can be used to determine which irreversible reactions should be deleted from a network in order to stop the production of some metabolites.

**Proposition 4.11** *Given the flux cone $C$ and an irreversible reaction $j \in Irr$, let $C'$ be the flux cone $C' = C \cap \{v \in \mathbb{R}^n \mid v_j = 0\}$. Then the MMBs $D'$ of $C'$ are exactly the MMBs $D$ of $C$ for which $j \notin D$. The reversible metabolic space does not change, i.e.,* $\mathrm{lin.space}(C') = \mathrm{lin.space}(C)$.

**Proof:** Follows from the Strengthened Main Lemma in [3]. □

### 4.5 Computing minimal metabolic behaviors

A simple algorithm to determine the MMBs of a metabolic network is as follows. First compute a set of generators of the flux cone $C$, using some existing software for polyhedral computations such as `cdd` [3]. Second, for each minimal proper face $G$, represented by some generator $g \in G \setminus \mathrm{lin.space}(C)$, identify the set $D$ of irreversible reactions $j \in Irr$, with $v_j > 0$. Another possible approach is to apply the Fourier-Motzkin elimination to eliminate the variables corresponding to the reversible internal reactions. This results in a constraint system that contains only variables corresponding to irreversible reactions.

It follows from the general theory in Sect. 2 that in order to obtain a minimum cardinality set of generators for the flux cone $C$, we have to choose for each minimal proper face $G^k$ a vector $g^k \in G^k \setminus \mathrm{lin.space}(C)$, together with a generating set $\{b^0, \ldots, b^t\}$ of $\mathrm{lin.space}(C)$. If we decompose $g^k = (g^k_{Irr}, g^k_{Rev^0}, g^k_{Rev \setminus Rev^0})$ into components corresponding to irreversible, pseudo-irreversible, and reversible reactions, then the components in $(g^k_{Irr}, g^k_{Rev^0})$ are uniquely determined up to multiplication by positive scalar. There remains some freedom in the choice of the components in $g^k_{Rev \setminus Rev^0}$. Work in the context of the software `cdd` [2] discusses how to obtain generators with a maximum number of zeroes in this part.

## 5 Computational results

In this section, we compare the different approaches on some example networks from the KEGG pathway database[1]. As there is no information about the exchange fluxes of the system, we suppose that there is an unconstrained exchange flux for each metabolite that is not consumed or produced by an internal reaction in the network. The computations of the elementary flux modes, the extreme pathways, the minimal metabolic behaviors and the reversible metabolic space were done using the software `metatool` [7], `cdd` [3], and `lrs` [1]. The results are given in Tables 1 and 2.

Table 1 shows the number of elementary flux modes, extreme pathways, MMBs, and the dimension of the RMS. It shows that the size of our representation, given as the sum of the number of MMBs and $\dim(RMS)$, is typically much smaller than the number of extreme pathways or elementary flux modes. In various examples, the reduction is by several orders of magnitude.

Table 2 describes the distribution of the elementary flux modes and the extreme pathways inside the steady state flux cone. We can see that a very large number of elementary flux modes and extreme pathways lies in the interior of the cone. In addition, the number of elementary flux modes and extreme pathways belonging to the minimal proper faces (MMB) is much larger than the number of MMBs. This shows that many elementary modes/extreme pathways belong to the same minimal proper face, which mathematically can be represented by a single vector. Similarly, the number of elementary modes/extreme pathways belonging to the reversible metabolic space is much larger than its dimension, so that there are many dependencies.

## 6 Conclusion and possible biological implications

Minimal metabolic behaviors and the reversible metabolic space provide a new mathematical description of the possible fluxes through a metabolic network at steady state. Compared to existing approaches, this description is much more compact. Instead of generating a very large number of vectors, it is based on sets of non-negativity constraints (MMBs). These can be identified with irreversible reactions, and thus have a direct biochemical interpretation.

With each MMB, we may associate one or several biological functions

[1] `http://www.genome.ad.jp/kegg/pathway.html`

| Metabolic Network | Network size | | | Number of | | Number of MMBs | dim RMS |
|---|---|---|---|---|---|---|---|
| | Number of metabolites | Reactions | | EFMs | EPs | | |
| | | irrev. | rev. | | | | |
| **Glycolysis / Gluconeogenesis** | 32 | 18 | 29 | 19464 | 1745 | 17 | 13 |
| **Citrate cycle (TCA cycle)** | 22 | 4 | 25 | 3870 | 1588 | 5 | 12 |
| **Pentose phosphate pathway** | 34 | 19 | 24 | 5155 | 1630 | 20 | 8 |
| **Pentose and glucuronate interconversions** | 50 | 13 | 46 | 2258 | 231 | 8 | 23 |
| **Fructose and mannose metabolism** | 46 | 37 | 31 | 2411 | 2102 | 30 | 6 |
| **Galactose metabolism** | 41 | 22 | 28 | 623 | 524 | 14 | 9 |
| **Starch and sucrose metabolism** | 47 | 35 | 30 | 2097 | 1718 | 31 | 5 |
| **Pyruvate metabolism** | 28 | 40 | 29 | 47708 | 27390 | 38 | 16 |
| **Glyoxylate and dicarboxylate metabolism** | 50 | 34 | 28 | 18785 | 1987 | 32 | 11 |
| **Propanoate metabolism** | 34 | 20 | 29 | 877 | 233 | 18 | 13 |
| **Butanoate metabolism** | 40 | 23 | 30 | 2138 | 541 | 19 | 11 |
| **Nitrogen metabolism** | 41 | 53 | 14 | 601 | 612 | 45 | 9 |
| **Sulfur metabolism** | 18 | 26 | 4 | 321 | 326 | 29 | 1 |

Table 1: Metabolic networks, with corresponding elementary flux modes (EFM), extreme pathways (EP), minimal metabolic behaviors (MMB), and the dimension of the reversible metabolic space (dim(RMS)). Contrary to the calculation of EFMs, the calculation of the EPs required a reconfiguration of the network. Except for the two-cycle extreme pathways, the set of EPs is always a (proper or non-proper) subset of the EFMs.

| **Metabolic Network** | **Characteristic pathways in** | | | | | | **Two-cycle EPs** | **Number of MMBs** | **dim RMS** |
|---|---|---|---|---|---|---|---|---|---|
| | **MMBs** | | **RMS** | | **Interior** | | | | |
| | **EFMs** | **EPs** | **EFMs** | **EPs** | **EFMs** | **EPs** | | | |
| **Glycolysis / Gluconeogenesis** | 1226 | 529 | 48 | 46 | 18190 | 1095 | 29 | 17 | 13 |
| **Citrate cycle (TCA cycle)** | 1608 | 568 | 502 | 258 | 1760 | 480 | 24 | 5 | 12 |
| **Pentose phosphate pathway** | 489 | 340 | 25 | 25 | 4641 | 1217 | 23 | 20 | 8 |
| **Pentose and glucuronate interconversions** | 1076 | 32 | 642 | 76 | 540 | 3 | 44 | 8 | 23 |
| **Fructose and mannose metabolism** | 154 | 148 | 14 | 14 | 2243 | 1895 | 31 | 30 | 6 |
| **Galactose metabolism** | 212 | 152 | 45 | 45 | 366 | 254 | 28 | 14 | 9 |
| **Starch and sucrose metabolism** | 108 | 107 | 8 | 8 | 1981 | 1565 | 30 | 31 | 5 |
| **Pyruvate metabolism** | 2016 | 1776 | 146 | 146 | 45546 | 25293 | 29 | 38 | 16 |
| **Glyoxylate and dicarboxylate metabolism** | 869 | 124 | 112 | 18 | 17804 | 1801 | 26 | 32 | 11 |
| **Propanoate metabolism** | 449 | 93 | 133 | 32 | 295 | 50 | 26 | 18 | 13 |
| **Butanoate metabolism** | 357 | 244 | 35 | 34 | 1746 | 201 | 28 | 19 | 11 |
| **Nitrogen metabolism** | 183 | 171 | 22 | 22 | 396 | 384 | 13 | 45 | 9 |
| **Sulfur metabolism** | 44 | 44 | 1 | 1 | 276 | 276 | 4 | 29 | 1 |

Table 2: The distribution of the elementary flux modes (EFMs) and the extreme pathways (EPs) in the three parts of the steady state flux cone: the minimal proper faces (MMB), the lineality space (RMS), and the interior of the cone. Each pair of opposite extreme pathways is considered as one reversible pathway belonging to the RMS. The two-cycle extreme pathways made from a forward and a backward reaction are indicated separately.

corresponding to the transformation of certain metabolites in the direction of the irreversible reactions that define the MMB. The same biological function can be realized by several MMBs. This reflects the redundancy and robustness present in biological systems. The reversible metabolic space, which consists of reversible reactions only, can be used by the biological system to balance the ratio between certain metabolites. Some irreversible reactions turn out to participate in the definition of several MMBs. The number of MMBs in which some irreversible reaction participates is an indicator of its importance. Its deletion will break down these MMBs and thus change the global behavior of the system. The deletion of reversible reactions may affect the ratio between certain metabolites, but will not necessarily change the overall behavior of the network.

Since the flux cone is defined by both stoichiometric and thermodynamic irreversibility constraints, the reversibility of biochemical reactions is a meaningful biological property, which should be dealt with explicitly. Our description involving both MMBs and the RMS highlights the importance of the reversible reactions for the analysis of a network.

Finally, MMBs suggest a modular approach to the study of metabolic networks. Each MMB (i.e, the pathways belonging to the corresponding minimal proper face) could be seen as a module that can cooperate with other modules through the reversible reactions. The overall metabolic network could then be understood as a combination of these different modules.

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