Lætitia Laura Diemer

Pathway Analysis of Metabolic Networks

Bachelor Thesis
Supervisor: Dr. Sander C. Hille

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Introduction

The full metabolic network of an organism is the collection of all chemical reactions which may occur in and between the cells of the organism. Sometimes, a subset of reactions is also called a metabolic network (e.g. glycolysis). Due to the mapping of the genome of many organisms, there are now many metabolic networks to be studied.
Metabolic pathway analysis consists of describing the complete set of possible pathways in a metabolic network. This set is a convex cone which may be described (generated) by a finite set of vectors (pathways). In the literature, different sets of generating vectors exist, the two most frequently used are the extreme pathways and the elementary modes.
Chapter 1 covers the description of the general model used in metabolic network analysis. In Chapter 2 we describe the different sets of generating vectors and theirs properties. Motivation is given for choosing elementary modes over extreme pathways as a set of generating vectors. In Chapter 3 we describe the algorithm for calculating elementary modes and in Chapter 4 a method for calculating elementary modes through subdivision of the network is given.
The main goal of the thesis was to try to find a better method to compute the extreme pathways of a large metabolic network by subdividing the network, as was done in [7]. Naturally, this was changed to computing elementary modes as well. The goals of the thesis gradually changed as I became more immersed in the subject matter. Initially, the idea was to expand the concept of an extreme pathway, by allowing reversible reactions. The set of extreme pathways is unique. Extreme pathways are said to be conically independent (I discovered later on that this is not always the case), so we tried to expand the definition using this property. This turned out to be a dead end, as a generating set of conically independent vectors is not always unique. By setting a new goal, namely studying the different sets of generating vectors and their differences, it became clear that the elementary modes were much more useful in a system without restrictions on the reversible reactions.

Chapter 1

Fundamental model and mathematical concepts

In this chapter we present the fundamental mathematical description of a (general) chemical reaction network and various graphical representations. Moreover, we introduce concepts from convex analysis needed to understand the pathway analysis of the network, which we will cover in the following chapters.

A metabolic network $\mathcal{N}$ is a list of possible chemical reactions between metabolites in the organism that is being studied. The information may be obtained from the genome through reaction network reconstruction which is a scientific research field in itself. We may consider only part of the full metabolic network of an organism. In a later chapter, we will discuss how to subdivide networks. The reactions are always considered to take place within at least one compartment, which may also contain other compartments. This compartment may only be put in place theoretically or may be an actual compartment within the cell, e.g. the mitochondrion.

The *exchange reactions* describe which molecules are transported to and from the compartment (between the interior of the compartment and the exterior, the environment.) From the point of view of the interior compartment an exchange reaction is a constant flux in and/or out of the compartment. In a exchange reaction the chemical structure of the molecules not changed, it merely changed location. This may be executed through a physical process (diffusion through a membrane) or with the help of a transporter molecule. Due to the exchange reactions, the system has inputs and outputs, it is called an *open system*. Reactions that are not exchange reactions are called internal reactions.

In a biological metabolic network almost all reactions are catalysed by a specific enzyme. This is a molecule which is needed to start the reaction without itself undergoing chemical change. In our model we assume that these enzymes are always present and we consider two reactions that are

catalysed by a different enzyme or take place in different compartments to be different reactions.In this way the effects of gene-knock-outs or the inhibition of access of enzymes to a compartment can be readily interpreted.
Note that all chemical reactions are reversible in theory but due to the conditions inside the compartment one0 direction may be very unlikely, in which case it is considered to be irreversible.

1.1 Description of the model

Using the information available we will make

- a network representation, which shows how the reactions are connected to each other, and
- a stoichiometric matrix S, which describes the relative quantities of the reactants and products in each reaction.

Various types of network representations exist. The neatest from a mathematical perspective is a bipartite graph with (weighted) arrows between the set of reactions and the set of metabolites, but more often than not (as this is easier to draw) the network representation shows the reactions as arrows (which may be forked and bidirectional) between metabolites. See Figure 1.1 for an example. Let m be the number of metabolites and n the number of reactions in the system.

Collection of metabolites: $M(\mathcal{N}) = \{M_1, ..., M_m\}$
Collection of reactions: $R(\mathcal{N}) = \{R_1, ..., R_n\}$

The metabolic network can be represented by a directed 'graph', with m vertices and n arrows.
The entries of the *stoichiometric matrix* are defined as follows:

$$s_{ij} = \begin{cases} x & \text{if } x \text{ molecules of metabolite } M_i \text{ are produced by reaction } R_j \\ -x & \text{if } x \text{ molecules of metabolite } M_i \text{ are used in reaction } R_j \\ 0 & \text{otherwise} \end{cases}$$

(Note that even though the network representation is technically not a graph, the stoichiometric matrix may be interpreted as the 'weighted' incidence matrix of the network.)
We keep track of the bidirectional reactions in the system (the reactions that are reversible). To display these in the stoichiometric matrix, a positive direction is chosen for each reaction.

$\text{Irr}(\mathcal{N}) = \{i : R_i \text{ irreversible}\}$, $\text{Rev}(\mathcal{N}) = \{i : R_i \text{ reversible}\}$

The behaviour of the metabolic network is described by the following differential equation:

$$\frac{dx(t)}{dt} = S \cdot v(t)$$

where $x(t)$ is the total number vector of size m (the numbers of each metabolite in the system at time t) and $v(t)$ is the total reaction flux vector of size n (the number of occurrences of each reaction in the system at time t). The vector $v(t)$ will depend on $x(t)$, possibly time t and a vector of parameters p. The precise functional dependence of v on $x(t)$ and t depends on the specific chemical reactions involved such as detailed enzymatic kinetics.
To study the system we would like to find paths for which the system is in steady state, i.e. the number of each metabolite does not change over time ($\frac{dx(t)}{dt} = 0$). We would therefore like to find vectors $v \in \mathbb{R}^n$ such that $Sv = 0$. These vectors are called *steady state flux distributions*.

The solution space Γ of this equation is called the *steady state flux cone*.

$$\Gamma = \{v \in \mathbb{R}^n | Sv = 0 \text{ and } v_i \geq 0 \text{ for } i \in \text{Irr}(\mathcal{N})\} \tag{1.1}$$

Vectors belonging to the steady state flux cone are called *flux vectors*.

The purpose of metabolic pathway analysis is to provide a description of the set of steady state flux distributions v, through 'elementary' or fundamental flux distributions, which can be interpreted as biochemical pathways.
The strength of pathway analysis is, that predictions on essential pathways in the network, network yield at steady state, etc can be made without knowledge of these detailed kinetics.

Example (A simplified reaction scheme for glycolysis)

Internal reactions and exchange reactions

R_1	$\text{Glucose} + 2 \times \text{ATP} \longrightarrow 2 \times \text{ADP} + \text{Fru1,6BP}$
R_2	$\text{Fru1,6BP} \longrightarrow 2 \times \text{GAP}$
R_3	$\text{Fru1,6BP} \longleftarrow 2 \times \text{GAP}$
R_4	$2 \times \text{GAP} + 2 \times \text{NAD}^+ \longrightarrow 2 \times \text{NADH} + 2 \times \text{1,3BPGA}$
R_5	$2 \times \text{1,3BPGA} + 2 \times \text{ADP} \longrightarrow 2 \times \text{ATP} + 2 \times \text{3PGA}$
ER_1	$\text{Glucose} \longleftrightarrow \text{Glucose}$
ER_2	$2 \times \text{3PGA} \longleftrightarrow 2 \times \text{3PGA}$

Metabolites

M_1	Glucose
M_2	Fructose 1,6BP
M_3	GAP
M_4	1,3BPGA
M_5	3PGA
M_6	ATP
M_7	ADP
M_8	NAD+
M_9	NADH

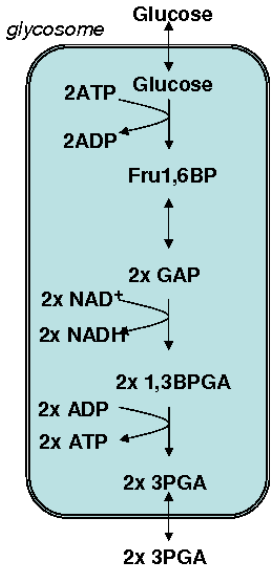


Figure 1.1: Part of glycolysis in *T. brucei*, in the glycosome [replace with glycolysis in general]

Below the columns represent reactions, numbered as R_1 through R_5, ER_1 and ER_2. The rows represent the metabolites numbered as M_1 through M_9.

$$S = \left[\begin{array}{ccccc|cc} -1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 2 & -2 & -2 & 0 & 0 & 0 \\ 0 & 0 & 0 & 2 & -2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2 & 0 & 2 \\ -2 & 0 & 0 & 0 & 2 & 0 & 0 \\ 2 & 0 & 0 & 0 & -2 & 0 & 0 \\ 0 & 0 & 0 & -2 & 0 & 0 & 0 \\ 0 & 0 & 0 & 2 & 0 & 0 & 0 \end{array}\right]$$

1.2 Convex analysis

What is covered in this section mostly comes from [5].

The *closed line segment* that connects two points v and w in $\mathbb{R}^n$ is

$$[v, w] := \{x \in \mathbb{R}^n | x = \lambda v + (1 - \lambda)w \text{ and } 0 \leq \lambda \leq 1\}$$

An *interior point* of the closed line segment is any point of the segment not equal to the endpoints v or w.

A *ray* is a half-line emanating from the origin:

$$\{\lambda y \mid \lambda \geq 0, y \in \mathbb{R}^n\},$$

We say that the ray is *generated* by y.

Let S be a subset of $\mathbb{R}^n$, then ray(S) is the union of all rays generated by non-zero vectors $y \in S$.

A *direction* of a half-line $L_1 = \{x + \lambda y, \lambda \geq 0\}$ is an equivalence class on the set of all closed half-lines under the equivalence relation: "L_1 is equivalent to L_2 when L_1 is a translate of L_2".
The direction of $\{x + \lambda y, \lambda \geq 0\}$ is also called the direction of y. Two vectors have the same direction if and only if they are scalar multiples of each other.

Definition 1.1. Let C be a subset of $\mathbb{R}^n$,
then C is *convex* if

$$v, w \in C \implies \lambda v + (1 - \lambda)w \in C, \quad \text{for all } \ 0 \leq \lambda \leq 1$$

In other words, C is convex if every line segment connecting two points of C is contained in C.
It should be noted that various definitions of 'cone' are used in mathematical literature. We will use the following:

Definition 1.2. C is a *cone* if

$$v \in C \text{ and } \lambda \geq 0 \implies \lambda v \in C$$

That is, a cone is a union of rays. In another often used definition a cone does not necessarily contain the origin, in which case λ is strictly positive. Cones that are convex, called *convex cones*, will play an important role later on and the following results should be viewed with these subsets of $\mathbb{R}^n$ in mind. Often cones are considered to be convex, as part of the definition.
Let $x \cdot y$ denote the standard scalar product in $\mathbb{R}^n$.

Definition 1.3. For any non-zero $b \in \mathbb{R}^n$ and any $\beta \in \mathbb{R}$, the set $\{x \in \mathbb{R}^n | x \cdot b \geq \beta\}$ is called closed *half spaces.*

Definition 1.4. A *polyhedral convex set* is an intersection of a finite collection of closed half-spaces.

This means that a polyhedral set can be expressed as the set of solutions to a system of weak linear inequalities: $\{x \in \mathbb{R}^n |\ x \cdot a_i \geq \alpha_i, i = 1, \ldots, m\}$. Since an equation $x \cdot a = \alpha$ can be written as two inequalities $x \cdot a \geq \alpha$ and $x \cdot a \leq \alpha$, any mixed system of linear equations and weak linear inequalities defines a polyhedral convex set.
Remark. A polyhedral convex set is a cone when it is the set of solutions to a system of homogeneous weak linear inequalities: $\{x \in \mathbb{R}^n |\ x \cdot a_i \geq 0\}$.

Definition 1.5. The *convex hull* of a subset S is the intersection of all convex sets containing S. It is the smallest convex set containing S. It is denoted by $\text{conv}(S)$.

Definition 1.6. Let S be a non-empty subset of R^n, and let K be the set of all non-negative linear combinations of S. Then K is the smallest convex cone that includes S. K is known as the convex cone *generated by* S and is denoted by cone(S).

The convex cone generated by S is the smallest convex set containing all rays generated by vectors of S:
$\text{cone}(S) = \text{conv}(\text{ray}(S))$

The convex hull of a set of points S_0 and directions S_1:
$S = S_0 \cup S_1, \text{conv}(S) = \text{conv}(S_0 + \text{ray}(S_1)) = \text{conv}(S_0) + \text{cone}(S_1)$.

Definition 1.7. A *finitely generated* convex cone is the convex hull of the origin and finitely many directions.

A *face* of a convex set C is a convex subset C' of C such that every closed line segment in C with an interior point in C' has both endpoints in C'.

An *extreme ray* of a convex cone is a face that is a ray. The direction of an extreme ray is called an *extreme direction.* It corresponds to a vector y which is unique up to positive scalar multiplication.

Theorem 1.1. *([5], Theorem 19.1) The following are equivalent:*

1. *C is polyhedral,*
2. *C is closed and has finitely many faces,*
3. *C is finitely generated.*

Definition 1.8. The *orthogonal complement* of L:

$$L^{\perp} = \{x \in R^n | x \cdot y = 0 \text{ for all } y \in L\}$$

Definition 1.9. If C is a non-empty convex set containing the origin, the set $(-C) \cap C$ is called the *lineality space* of C. It consists of vectors y such that, for every $x \in C$, the line through x in the direction of y is contained in C. The directions of the vectors y in the lineality space are called directions in which C is linear. The lineality space is the same as the set of vectors y such that $C + y = C$. The lineality space is the largest vector space contained in the convex cone C.

Definition 1.10. A convex cone C is called *pointed* if it contains no lines, i.e. there is no vector $x \in C$ s.t. $-x \in C$.

A pointed cone has a trivial lineality space : $L = C \cap (-C) = \{0\}$. If the convex cone C is *non-pointed* then its lineality space is non-trivial.
Moreover, C has a 'unique' decomposition $C = C_0 + L$ and $C_0 = C \cap L^{\perp}$ is a pointed cone. Note that this decomposition does depend on the choice of scalar product. We may also choose the cone C_0 in a different way.
Each $v \in C$ has a unique representation $v = x + y$ with $x \in C_0$ and $y \in L$. In fact, if $v = x + y$ and there exist vectors $x' \neq x \in C_0$ and $y' \neq y \in L$ such that $x = x' + y'$ then $x - x' = y' - y$ and $x - x' \in C_0$, $y' - y \in L$. $L \cap C_0 = \{0\}$ implies $x = x', y = y'$.

Theorem 1.2. *([5], Theorem 18.5) Let C be a closed pointed convex set and let E be the set of all extreme points and extreme directions of C. Then $C = conv(E)$.*

The set E is *minimal* in the sense that if E' is any set of points and directions such that $C = \text{conv}(E')$, then $S \subset S'$.
Let $ext(C)$ denote the set of extreme points and directions of a set C.

Proposition 1.1. *If C is pointed, then there exists a hyperplane V that contains the origin such that $C \cap V = \{0\}$. Furthermore, an affine hyperplane V' exists such that $V' = v_0 + V$ and $ext(C \cap V')$ is a set of representatives for the extreme rays of C.*

1.3 The steady state flux cone

The theory covered in the previous section applies to the steady state flux cone, due to the following.

Proposition 1.2. *The steady state flux cone is a polyhedral convex cone.*

Proof. The steady state flux cone 1.1 is given by a finite set of weak homogeneous linear inequalities. □

This means, according to Theorem 1, that it can be finitely generated by a set of directions. If the cone is pointed, it can be generated by the unique set of extreme directions (Theorem 2).

If the cone is non-pointed, a generating set of vectors can be found by finding the lineality space of Γ and choosing a pointed convex cone C_0 such that $\Gamma = C_0 + L$. For example $C_0 = C \cap L^{\perp}$. The basis vectors of L and the extreme directions of C_0 are a set of generating vectors. Due to the basis vectors for L being chosen arbitrarily the generating set of vectors is not unique in this case.

Definition 1.11. A *flux vector* or *flux mode* is a vector in the steady state flux cone. Two flux vectors x, x' are equivalent when $x' = \lambda x$, $\lambda \geq 0$

1.4 The augmented reaction network

Recall that reactions in a chemical network $\mathcal{N}$ may be reversible or irreversible. The augmented network $\mathcal{N}'$ consists of the network associated with the set of reactions where each reversible reaction is split into two irreversible reactions. It has an augmented stoichiometric matrix S' and a steady state flux cone Γ'.
Each $i \in \text{Rev}(\mathcal{N})$ corresponds to a pair $\{(i,-1),(i,+1)\}$

Let $v \in \mathbb{R}^r$ be a vector of Γ and vector $v' \in \mathbb{R}_+^{Irr \cup Rev \times \{+1,-1\}}$ a vector of $\in \Gamma'$.

Map from Γ' to Γ, which will refer to as map A:

$$\begin{cases} v_i = v'_i & \text{if } i \in \text{Irr} \\ v_i = v'_{(i,+1)} - v'_{(i,-1)} & \text{if } i \in \text{Rev} \end{cases}$$

Then a map from Γ to Γ' may be defined as follows:

$$\begin{cases} v'_i = v_i & \text{if } i \in \text{Irr} \\ v'_{(i,+1)} = v_i \text{ and } v'_{(i,-1)} = 0 & \text{if } i \in \text{Rev and } v_i \geq 0 \\ v'_{(i,+1)} = 0 \text{ and } v'_{(i,-1)} = -v_i & \text{if } i \in \text{Rev and } v_i \leq 0 \end{cases}$$

We will refer to this as map B.

Note that this map is not reversible. Two different vectors in Γ' may have the same corresponding vector in Γ. Biochemically they are the same as

they produce the same net result.

Chapter 2

Types of generating sets

The steady state flux cone Γ of a chemical reaction network yields all possible steady state flux distributions. The purpose of pathway analysis is to provide a concise description of the steady state flux cone that is useful in the analysis of the functioning of these networks. A concise description is furnished by a set of generating vectors for the cone.
We have seen in Chapter 1 that there exists a unique set of conically independent generating vectors, the extreme rays, when the cone is pointed. It turns out that these vectors also have an important biological property. If the cone is non-pointed, choosing a unique set of generating vectors which is biologically relevant is less obvious.
Below, we will describe different sets of generating vectors which are used in the literature and describe their properties and differences.

In the first models of metabolic networks a direction was chosen for each reaction (based on which direction was more likely), making all reactions irreversible. In this case the steady state flux cone is always pointed and a minimal generating set of vectors is equal to the set of extreme rays/directions. If we allow reversible reactions, there exist

- extreme currents, these are the extreme rays in the augmented network,
- elementary modes, these are the non-decomposable flux vectors,
- and extreme pathways, which were developed at the same time as elementary modes.

2.1 Description

Definition 2.1. The *null-component index set*: $\nu(v) := \{i|v_i = 0\}$
and its complement the *support* of v: $\text{supp}(v) := \{i|v_i \neq 0\}$.

2.1.1 Extreme currents

Extreme currents are obtained in the augmented network $\mathcal{N}'$ from Chapter 1. They are the extreme directions (extreme rays) of the cone Γ'.

Definition 2.2. A set of generating vectors $p_1, ..., p_k$ is called *conically independent* if there are no $\lambda_1, \ldots, \lambda_k \geq 0$, not all zero, such that

$$\lambda_1 p_1 + \cdots + \lambda_k p_k = 0$$

In the context of metabolic network analysis conical independence is often called *systemic* independence.

Theorem 2.1. *If the steady state flux cone Γ is pointed, the following are equivalent for a collection of flux vectors $P = \{p_1, \cdots, p_k\}$:*

1. *P is a collection of representatives for the extreme rays,*
2. *P is a conically independent set of generating vectors.*

Proof. ($\Longrightarrow$) Let $C = \Gamma \cap V$ (V is an affine hyperplane as in Prop 1.1), then C is convex and we may assume without loss of generality that $p_1, ..., p_k$ are the extreme points of C. Assume there exist $\lambda_1, ..., \lambda_k \geq 0$, not all zero such that $\sum_j \lambda_j p_j = 0$. Then $p_i = \sum_j \lambda'_j p_j$ and $\bar{\lambda} := \sum_j \lambda'_j > 0$. Then $\frac{1}{\bar{\lambda}} p_i = \sum_j \frac{\lambda_j}{\bar{\lambda}} p_j \in C$ and $\sum_j \frac{\lambda_j}{\bar{\lambda}} = 1$
Since $p_i \in C$ and $0 \notin C$, we find that $\bar{\lambda} = 1$. Because p_i is extreme, $\lambda'_i = 0$ for $j \neq i$ and $\lambda'_i = 1$. Thus $\lambda_j = 0$ for all j, which is a contradiction.

($\Longleftarrow$) Let V' be a hyperplane as in Proposition 1.1. Then each p_i in P can be scaled to a vector $\tilde{p}_i$ contained in $V' \cap \Gamma$. $\tilde{P}$ is still a conically independent set.
Claim: $\tilde{P} = \{\tilde{p}_i, ..., \tilde{p}_k\} = \text{ext}(\Gamma \cap V')$
($\subset$) Then, since $\tilde{p}_j \in \Gamma \cap V'$, $\tilde{p}_i = \sum_j \lambda_j e_j$, with $e_j \in \text{ext}(\Gamma \cap V')$ and $\lambda_i \geq 0$, $\sum \lambda_j = 1$ (Theorem 1.2)
Every $e_j \in \Gamma$, so $e_j = \sum_k \mu_{j,k} \tilde{p_k}$, $\mu_{j,k} \geq 0$ because the $\tilde{p_k}$ are generating vectors. So, $\tilde{p}_i = \sum_j \tilde{\lambda}_j \tilde{p}_j$, $\tilde{\lambda}_j = \sum_l \lambda_l \mu_{l,j}$ ($\tilde{\lambda}_i \neq 0$).
Because the $\tilde{p}_j$ are conically independent, $\tilde{\lambda}_j = 0$ for $j \neq i$. Thus

$$\tilde{\lambda}_i = \sum_l \lambda_l \mu_{l,i} \neq 0 \tag{2.1}$$

$$\tilde{\lambda}_j \sum_l \lambda_l \mu_{l,j} = 0 \text{ for } j \neq i \tag{2.2}$$

From (2.1) we conclude that for some j', both $\lambda_{j'} \neq 0$, and $\mu_{j',i} = 0$. Looking at (2.2), the term with $l = j'$ should vanish. So $\mu_{j',j} = 0$ for $j \neq i$. So $e_{j'} = \sum_k \mu_{j',k} \tilde{p_k} = \mu_{j,i} \tilde{p}_i$. That is $\tilde{p}_i = \mu_{j,i}^{-1} e'_j$. So $\tilde{p}_i$ is an extreme point.

($\supset$) Let $e^* \in \text{ext}(\Gamma \cap V')$, but $e^* \neq \tilde{p_i}$ for all i. Then $e^* = \sum_i \lambda_i \tilde{p_i}, \lambda_i \geq 0$ with not all $\lambda_i = 0$.
Because $\tilde{p_i} \in \text{ext}(\Gamma \cap V')$ e^* must be a combination of the $\tilde{p_i}$. This implies that e^* is not extreme. This is a contradiction.

□

Theorem 2.2. *(Prop 3.2.2 from [2]) Let C be a pointed convex cone in the positive orthant, and $p_1, ..., p_k$ a set of generating vectors, then the following statements are equivalent:*

1. *the vectors p_i are conically independent,*
2. *for all i, i' $i \neq i'$ $\nu(p_i) \not\subset \nu(p_{i'})$*

Proof. See [2]. □

2.1.2 Elementary modes

The concept of an elementary mode was first described in [8] as a set of generating vectors which could be used for the more general case in which some reactions are reversible.

Definition 2.3. A *flux mode* m is a set

$$m = \{x \in \Gamma | x = \lambda v, \lambda > 0, v' \neq 0\}$$

v' is a representative of m.

Definition 2.4. A flux mode is called *reversible* if the set $m' = \{-x | x \in m\}$ is a flux mode as well.

Definition 2.5. $v \in \mathbb{R}^n$ is *simple* when there exists no non-zero $x \neq v \in \Gamma, x \neq v$, such that $\nu(v) \subsetneq \nu(x)$ (or $\text{supp}(x) \subsetneq \text{supp}(v)$)

This means that v involves a minimal number of reactions. If a reaction is omitted it is no longer functioning as a steady state, i.e. $Sv \neq 0$.

Definition 2.6. $v \in \mathbb{R}^n$ is *non-decomposable* (or *indecomposable*) when there exist no non-zero $x, y \in \Gamma$ defining different flux modes such that

$$v = \lambda_1 x + \lambda_2 y, \qquad \lambda_1, \lambda_2 > 0$$

and $\nu(v) \subset \nu(x)$, $\nu(v) \subset \nu(y)$.

Theorem 2.3. *The two properties above are equivalent.*

Proof. Non-decomposable $\implies$ simple: See Lemma 2 in[10].
Simple $\implies$ non-decomposable: If v is decomposable then it can be decomposed into two modes which involve more zero components, therefore v is not simple. □

Definition 2.7. An *elementary mode* (EM) is a flux mode m with a representative v such that v is non-decomposable (or simple).

Theorem 2.4. *(from [10]) Each flux vector v can be written as a positive linear combination of elementary modes* without cancellations*, that is there exist elementary modes e_i such that*

$$v = \sum \lambda_i e_i, \lambda_i \geq 0, \tag{2.3}$$

and

$$\nu(v) \subset \nu(e_i) \text{ for all } i \tag{2.4}$$

.

Proof. Let v be a flux vector which doesn't represent an elementary mode , then v is decomposable, it can be written as:

$$v = \lambda_1 x + \lambda_2 y, \quad \lambda_1, \lambda_2 > 0 \text{ with } \nu(v) \subset \nu(x) \text{ and } \nu(v) \subset \nu(y).$$

If x and y are elementary modes then equation 2.3 holds. Otherwise x, y or both can be further decomposed, and so forth. Since the number of zero components strictly increases at each decomposition, eventually we end up with a set of vectors that cannot be split further. This implies that they are elementary modes which satisfy 2.4.

□

This means that each admissible flux distribution can be written as a superposition of elementary modes.
Remark: In [5] (Section 22) we can find the definition an *elementary vector* which is defined through simplicity. It is not unlikely that the idea of an elementary mode sprang from this concept.

2.1.3 Crucial relationship between ECs and EMs

Extreme currents and elementary modes are closely related. This relationship will be the driver of our subdivision method presented in Chapter 4. As before let $\mathcal{N}'$ denote the augmented network of $\mathcal{N}$ where each reversible reaction of $\mathcal{N}$ is replaced by a pair of irreversible reactions. (Γ' and S' are the corresponding steady state flux cone and stoichiometric matrix).

Theorem 2.5. *The set of EMs of Γ' is exactly equal to the union of the sets*

a) *the EMs of Γ augmented to Γ'*

b) *a 2-cycle of a reversible reaction.*

Proof. (from [1])
$\Rightarrow$ Let e' be a flux vector in Γ' originating from a) or b). Then $S'e' = 0$ and $e' \geq 0$.
If b) , e' is EM of Γ' because a single forward or backward reaction can only be at steady state if it involves only external metabolites (e' is simple).
If a), if e' is not EM of Γ' then $\exists x'$ s.t. $x' \geq 0$, $S'x' = 0$ and $\nu(e') \subset \nu(x')$.
For $i \in \text{Rev}(\mathcal{N})$ either $e'_{(i,+1)} = 0$ or $e'_{(i,-1)} = 0$, so this also holds for x'.
Apply map B to e' and x'. Now $\nu(e) \subset \nu(x)$, so e is not an EM, which is a contradiction.
$\Leftarrow$ Assume there is an e' that is an EM of Γ' such that a) nor b) holds.
If e' contains a reversible pair for which both directions are non-zero, then the 2-cycle for that pair is a subvector of e', so e' is not an EM.
So, for each $i \in \text{Rev}$ either $e'_{(i,+1)} = 0$ of $e'_{(i,-1)} = 0$ $(\star)$.
e isn't an EM of Γ (by assumption), so $\exists x \neq 0 \in \Gamma$ s.t. $\nu(e) \subset \nu(x)$. Map e back onto Γ', this is equal to e' because of $(\star)$.
Then we have $\nu(e') \subset \nu(x')$. This contradicts the assumption. □

Theorem 2.6. *If the network only has irreversible reactions, then the set of extreme currents and elementary modes coincide.*

Proof. Let p be an EC, and suppose P is decomposable. Then there exist v_1, $v_2 \in \Gamma$, $v_i \neq 0$ defining different flux modes, and $\lambda_i > 0$, such that

$$p = \lambda_1 v_1 + \lambda_2 v_2$$

and $\nu(p) \subset \nu(v_i)$, for $i = 1, 2$.
Since the set of ECs $\{p_1, ..., p_k\}$ generate Γ, for each i there exists $\mu_j^{(i)} \geq 0$ such that

$$v_i = \sum_j \mu_j^{(i)} p_j$$

Hence

$$p = \sum_j (\lambda_1 \mu_j^{(1)} + \lambda_2 \mu_j^{(2)}) p_j$$

$p = p_{j_0}$ for some $j_0 \in \{1, ..., k\}$
By conical independence it follows that (Theorem 2.1)

$$\lambda_1 \mu_j^{(1)} + \lambda_2 \mu_j^{(2)} = 0 \text{ for } j \neq 0, (*)$$
$$\lambda_1 \mu_{j_0}^{(1)} + \lambda_2 \mu_{j_0}^{(2)} = 1.$$

Because $\lambda_i > 0$, $(*)$ implies that $\mu_j^{(i)} = 0$ for all $j \neq j_0$. Consequently, $v_i = \mu_{j_0}^{(i)} p_{j_0}$ and $v_1 = \lambda v_2$ for some $\lambda > 0$. Hence v_1 and v_2 define the same flux mode, a contradiction.
Let $\{q_1, ..., q_m\}$ be the set of EMs. These generate Γ. So we need to show that this set is conically independent (Theorem 2.1). Suppose they are not conically independent. According to Theorem 2.2 (part 2) there exist $i, i', i \neq i'$ such that$\nu(q_i) \subset \nu(q_i')$. As in the proof of Proposition 3.2.2 (in [2]) there exists a $\mu > 0$ such that $v^* := q_i - \mu q_i'$ is a non-zero steady state flux distribution. This yield $q_i = v^* + \mu q_i'$. So q_i is decomposable which is a contradiction.

□

Thus we can compute EMs by means of computing ECs, which can be done using algorithms for computing extreme rays. These will be discussed in Chapter 3.

2.1.4 Extreme pathways

Extreme pathways (EPs) were invented by Pallsson (see [3]).
Like the ECs, the extreme pathways are calculated in an augmented vector space. This time only the internal reactions $\Gamma \to \Gamma''$.
let S'' be the $m \times n'$ stoichiometric matrix of the altered metabolic system. $m = q + e'$ is the number of metabolites, where q is the number of metabolites not involved in a bidirectional exchange reaction.
$n' = d + e + e'$, where d is the number of internal reactions, e is the number of unidirectional exchange reactions and e' the number of bidirectional exchange reactions.

$$S'' = \begin{array}{c} \\ q \\ e' \end{array} \overbrace{\phantom{S^0_{\text{int}}}}^{d} \overbrace{}^{e} \overbrace{}^{e'} \begin{bmatrix} S^0_{\text{int}} & B^0 & 0 \\ S^u_{\text{int}} & B^u & I \end{bmatrix}$$

N.B.: The lower right sub matrix is equal to the identity matrix if and only if each metabolite in the system has at most one unconstrained exchange flux.
Up until now, we have used EPs because they are a unique conically independent generating set of vectors for the steady state flux cone. However, this is only true when all the internal reactions are irreversible.

2.2 Discussion

For a more detailed comparison of the different sets of generating vectors, see [4].
There are a few properties that are important when choosing which set of generating vectors to use.

1. The set is unique.
2. The set is equal to the set of all non-decomposable flux modes.
3. The set is conically independent.

In general, 2 $\implies$ 1.
If there are only irreversible reactions, the steady state flux cone is pointed. However, the reverse does not hold.
To study the differences between the different sets of generating vectors, we need to look at the different types of networks that can occur.
We can distinguish seven different networks:

1. all reactions are irreversible (the steady state flux cone is pointed),
2. internal reactions are irreversible, there are reversible exchange reactions,
   a) the steady state flux cone is pointed,
   b) the steady state flux cone is non-pointed,
3. there are reversible internal reactions, all exchange reactions are irreversible,
   a) pointed,
   b) non-pointed,
4. there are reversible internal reactions and exchange reactions,
   a) pointed,
   b) non-pointed.

In system 1, EMs = EPs.

Theorem 2.7. *In system 2a, an EP is non-decomposable.*

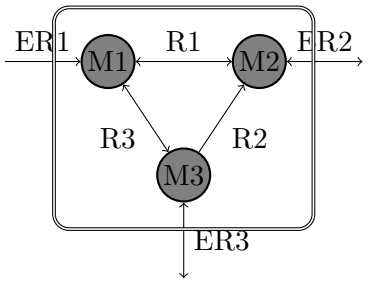
The following is a short description of the algorithm for computing extreme pathways (see [2]):

Prop 2.2 proves that properties 2. and 3. are equivalent when there are no bidirectional reactions.

Using this property, a set of conically independent (and non-decomposable) vectors are calculated for the unidirectional reactions of the system in step 1 of the the algorithm.
The extended vectors for the steady state flux cone computed in step 3 are still conically independent because we have used the fact that each metabolite has at most one bidirectional exchange reaction. (See note in chapter 1)
The EPs do not remain conically independent when they are translated back to the original system (with bidirectional internal reactions).

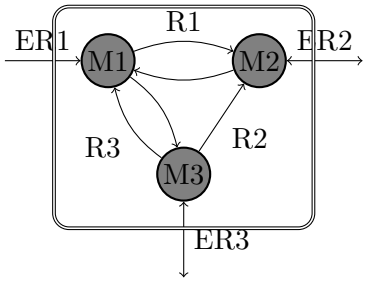
2.3 Example calculations

Example 2.1. From [4], example 5 (reversible vector)



$$S = \left[\begin{array}{ccc|ccc} 1 & 0 & 1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 & 1 \end{array}\right]$$

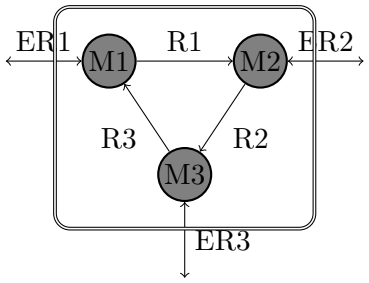
To compute the EPs, we first split the internal reversible reactions. We then get the following graph:



The stoichiometric matrix of the augmented space:

$$S = \left[\begin{array}{ccccc|ccc} -1 & 1 & 0 & 1 & -1 & 1 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 1 \end{array}\right]$$

Example 2.2. In the following graph all the metabolites are involved in bidirectional exchange reactions.



$$S = \left[\begin{array}{ccc|ccc} -1 & 0 & 1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 & 1 & 0 \\ 0 & 1 & -1 & 0 & 0 & 1 \end{array}\right]$$

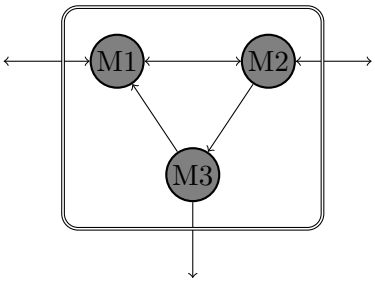
$$T^{(0)} = \left[\begin{array}{cccccc||ccc} 1 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & -1 \\ \hline 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 \end{array}\right]$$

2.3.1 Problems with EPs

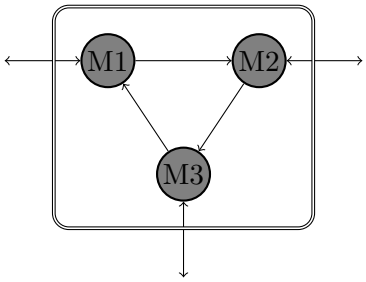
The following is a list of questions that arose when studying the algorithm for computing extreme pathways.

- The EPs are equal to the extreme rays when the system contains no reversible internal reactions.

  For a system that contains reversible reactions the EPs can be calculated when we split these into two irreversible reactions. However when we translate the EPs back to the original system they are no longer conically independent. (Give example). Furthermore, this isn't very realistic as one internal bidirectional reaction is not equivalent to two unidirectional reactions, these can be catalysed by different enzymes.

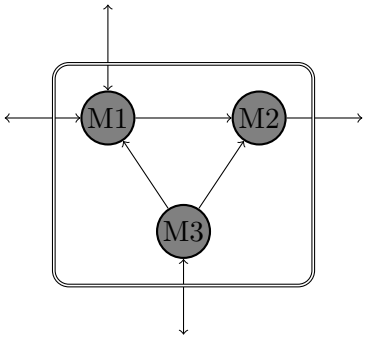


- How to compute EPs when all metabolites are involved in a bidirectional exchange reaction?



- EPs cannot be calculated when a metabolite has more than one unconstrained exchange flux.

  Again, this isn't realistic: when there are multiple compartments for example, there will often be exchanges from one metabolite to the different compartments.



These problems sparked the idea to expand the definition of extreme pathways, but this idea was abandoned altogether in favour of using elementary modes.

2.3.2 Why EMs are useful

Due to the no cancellation rule (theorem 2.4) many questions may be answered.

Q: Which reactions are essential to produce a metabolite Y?
A: Those that occur in all elementary modes producing Y.

Q: Is there a path connecting a reactant X with the product Y?
A: Only if there is an elementary mode connecting them.

Q: Which are the capabilities of the network if a reaction R can no longer function?
A: The elementary modes not containing R describe the steady state flux cone.

Q: What is the highest possible yield to produce Y from X?
A: The highest possible yield is given by the elementary mode which produces Y from X with the highest yield.

Chapter 3

Computing ECs and EMs

In the previous chapter we have described elementary modes and their properties and have shown that they are essentially equal to the set of extreme currents of the augmented network in which all reversible reactions have been split into two irreversible reactions(cf. theorem 2.5).
Both sets can be computed using a simple algorithm as we will demonstrate in this chapter. It turns out that it is much easier to compute the extreme currents, therefore it makes sense to compute the extreme currents and translate them back to the original steady state flux cone to obtain the elementary modes.

To compute a set of generating vectors, we need to

I. Determine a set of generating vectors for the (augmented) steady state flux cone,

II. Check for conical independence (ECs) or check for non-decomposability (EMs).

These steps may be executed simultaneously, but it is useful to look at step I. individually, to see how a set of generating vectors is computed in general, regardless of its properties.
Note. The steps for computing a set of EPs (which can be found in [2]) are slightly different. A set of generating vectors is found for the positive part of the augmented steady state flux cone (the irreversible reactions). These are checked for conical independence and the vectors are then expanded to add the fluxes for the reversible exchange reactions. This is only possible because it is assumed that each metabolite is involved in at most one reversible exchange reaction (see the stoichiometric matrix in Section 2.1.4). This assumption is quite restrictive if one considers 'real-life' compartmentalised networks.

3.1 Computing a generating set

In [2] it is shown in proposition 3.2.1 how a generating set of vectors can be obtained for a positive cone. The proposition may be generalized for a cone of the form

$$C_A = \{x \in \mathbb{R} \mid x_i \geq 0; i = 1, ..., h; h \leq n; Ax = 0\}$$

for some $m \times n$ matrix A, i.e. a cone whose vectors may contain negative entries. This proposition may then be used for steady state flux cones of metabolic networks with reversible reactions.
We say that C_A is generated by a set of non-zero vectors $p_1, ..., p_k$ and a set of basis vectors $b_1, ..., b_l$ when, for $x \in C_A$:

$$x = \sum_{i=1}^{k} \lambda_i p_i + \sum_{i=1}^{l} \mu_i b_i, \qquad \lambda_i \geq 0, \mu_i \in \mathbb{R}, \forall i.$$

Note that the set $b_1, ..., b_l$ does not exist when $L_A = C_A \cap (-C_A) = \{0\}$.

Theorem 3.1. *Let A be a $m \times n$ matrix such that C_A is non-empty. Then there exist non-zero vectors $p_1, ..., p_k$ and (when $L_A \neq \{0\}$) basis vectors $b_1, ..., b_l$ that generate C_A.*

Proof. Let $a_1, ..., a_m$ be the m row vectors of the matrix A. Define inductively,

$$C_A^0 := \{x \in \mathbb{R} \mid x_i \geq 0; i = 1, ..., h; h \leq n; \}, \quad C_A^j = \{x \in C_A^{j-1} \mid a_j x = 0\}$$

For C_A^0 the generating sets consist of the standard basis vectors: $p_i = e_i$ for $i = 1, ..., h$ and $b_i = e_{h+i}$ for $i = h+1, ..., n$. We shall now construct generating sets of vectors for C_A^{j+1} out of the generating sets $p_1^{(j)}, ..., p_{k_j}^{(j)}$ and $b_1^{(j)}, ..., b_{l_j}^{(j)}$ of C_A^j.

1. Rescale the vectors $p_1^{(j)}, ..., p_{k_j}^{(j)}$ and $b_1^{(j)}, ..., b_{l_j}^{(j)}$ s.t.:

   $a_{j+1} \cdot \hat{p}_i^{(j)} \in \{0, +1, -1\}, \forall i$

   $a_{j+1} \cdot \hat{b}_i^{(j)} \in \{0, 1\}, \forall i$

2. Renumber the vectors $\hat{p}_1^{(j)}, ..., \hat{p}_{k_j}^{(j)}$ and the vectors $\hat{b}_1^{(j)}, ..., \hat{b}_{l_j}^{(j)}$ (From now on we will drop the (j) superscript).

   Rename the vectors s.t.:

   $a_{j+1} \cdot \hat{p}_i^{+} = +1$, $i = 1, ..., k_j^{+}$,

   $a_{j+1} \cdot \hat{p}_i^{-} = -1$, $i = 1, ..., k_j^{-}$,

$a_{j+1} \cdot \hat{p}_i^0 = 0,\ i = 1, ..., k_j^0,$

$a_{j+1} \cdot \hat{b}_i = 1,\ i = 1, ..., l_j,$

$a_{j+1} \cdot \hat{b}_i^0 = 0,\ i = 1, ..., l_j^0.$

Where $k_j^+ + k_j^- + k_j^0 = k_j$, $l_j + l_j^0 = l_j$.

3. *Claim*: the set of vectors
   $P^{j+1} = \{p_i^- + p_{i'}^+ | i = 1, ..., k_j^-; i' = 1, ..., k_j^+\} \cup \{p_i^- + b_{i'}\} \cup \{p_i^+ - b_{i'}\} \cup \{b_i - b_{i'}\} \cup \{p_i^0\} \cup \{b_i^0\}$
   is generating for C_A^{j+1}.

(Proof of claim) By construction $P^{j+1} \subseteq C_A^{j+1}$, thus the cone generated by the vectors in P^{j+1} is contained in C_A^{j+1}. It remains to prove that these two are equal. To that end, pick $x \in C_A^{j+1}$. Then $x \in C_A^j$. The vectors $\hat{p}_1^{(j)}, ..., \hat{p}_{k_j}^{(j)}$ and $\hat{b}_1^{(j)}, ..., \hat{b}_{l_j}^{(j)}$ are generating for C_A^j, hence there exist $\lambda_i^+, \lambda_i^-, \lambda_i^0 \geq 0$ and $\mu_i, \mu_i^0 \in \mathbb{R}$ s.t.:

$$x = \sum_{i=1}^{k_j^-} \lambda_i^- \hat{p}_i^- + \sum_{i=1}^{k_j^+} \lambda_i^+ \hat{p}_i^+ + \sum_{i=1}^{l_j} \mu_i \hat{b}_i + \sum_{i=1}^{k_j^0} \lambda_i^0 \hat{p}_i^0 + \sum_{i=1}^{l_j^0} \mu_i^0 \hat{b}_i^0$$

We need to write x in terms of the vectors in P^{j+1}. Let $x = X + Y$, where X is the part of x that is written in terms of the vectors in P^{j+1}. We start with $X = \sum_{i=1}^{k_j^0} \lambda_i^0 \hat{p}_i^0 + \sum_{i=1}^{l_j^0} \mu_i^0 \hat{b}_i^0$. Y can be a combination of:

1. p^-'s and b's,
2. p^+'s and b's,
3. only b's,
4. p^-'s, p^+'s and b's.

(only p's: see proof of 3.2.1) We will use that:

$$a_{j+1} \cdot x = 0 \implies \sum_{i=1}^{l_j} \mu_i + \sum_{i=1}^{k_j^+} \lambda_i^+ - \sum_{i=1}^{k_j^-} \lambda_i^- = 0$$

1.

$$
\begin{aligned}
Y &= \sum_{i=1}^{k_j^-} \lambda_i^- \hat{p}_i^- + \sum_{i=1}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^-} \lambda_i^- \left(\hat{p}_i^- + \hat{b}_1 \right) - \sum_{i=1}^{k_j^-} \lambda_i^- \hat{b}_1 + \mu_1 \hat{b}_1 + \sum_{i=2}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^-} \lambda_i^- \left(\hat{p}_i^- + \hat{b}_1 \right) + \left(\mu_1 - \sum_{i=1}^{k_j^-} \lambda_i^- \right) \hat{b}_1 + \sum_{i=2}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^-} \lambda_i^- \left(\hat{p}_i^- + \hat{b}_1 \right) + \left(- \sum_{i=2}^{l_j} \mu_i \right) \hat{b}_1 + \sum_{i=2}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^-} \lambda_i^- \left(\hat{p}_i^- + \hat{b}_1 \right) - \sum_{i=2}^{l_j} \mu_i \hat{b}_1 + \sum_{i=2}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^-} \lambda_i^- \left(\hat{p}_i^- + \hat{b}_1 \right) + \sum_{i=2}^{l_j} \mu_i \left(\hat{b}_i - \hat{b}_1 \right)
\end{aligned}
$$

2.

$$
\begin{aligned}
Y &= \sum_{i=1}^{k_j^+} \lambda_i^+ \hat{p}_i^+ + \sum_{i=1}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^+} \lambda_i^+ \left(\hat{p}_i^+ - \hat{b}_1 \right) + \sum_{i=1}^{k_j^+} \lambda_i^+ \hat{b}_1 + \mu_1 \hat{b}_1 + \sum_{i=2}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^+} \lambda_i^+ \left(\hat{p}_i^+ - \hat{b}_1 \right) + \left(\mu_1 + \sum_{i=1}^{k_j^+} \lambda_i^+ \right) \hat{b}_1 + \sum_{i=2}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^+} \lambda_i^+ \left(\hat{p}_i^+ - \hat{b}_1 \right) + \left(- \sum_{i=2}^{l_j} \mu_i \right) \hat{b}_1 + \sum_{i=2}^{l_j} \mu_i \hat{b}_i \\
&= \sum_{i=1}^{k_j^+} \lambda_i^+ \left(\hat{p}_i^+ - \hat{b}_1 \right) + \sum_{i=2}^{l_j} \mu_i \left(\hat{b}_i - \hat{b}_1 \right)
\end{aligned}
$$

3.

$$\begin{aligned} Y &= \sum_{i=1}^{l_j} \mu_i \hat{b}_i \\ &= \sum_{i=1}^{l_j-1} \left(\sum_{i'=1}^{i} \mu_{i'} \right) \left(\hat{b}_i - \hat{b}_{i+1} \right) + \left(\sum_{i=1}^{l_j} \mu_i \right) \hat{b}_{l_j} \end{aligned}$$

$a_{j+1} \cdot x = 0$ so $\sum_{i=1}^{l_j} \mu_i = 0$

4.

$$Y = \sum_{i=1}^{k_j^+} \lambda_i^+ \hat{p}_i^+ \sum_{i=1}^{k_j^-} \lambda_i^- \hat{p}_i^- + \sum_{i=1}^{l_j} \mu_i \hat{b}_i$$

If Y is a combination of p^-'s, p^+'s and b's, many possibilities arise and the proof needs to be written recursively. The proof is a short algorithm which keeps track of the new variables which arise when when we combine generating vectors. Recall that X is the part of x that is written in terms of the vectors of P^{j+1}. In the algorithm we keep removing conbinations of vectors from Y and adding them to X, until Y is empty.

We keep a list of all p^+'s (variable i) and p^-'s (variable j) and their respective variables (Λ_i^+ and Λ_j^-) and try to pair as many up as we can (step (c)). When we choose a pair, we first need to compare their variables (step (b)), so that when rewriting (see (c)i and (c)ii) we do not end up with a negative variable. Whatever remains when rewriting is added back to the list. We keep doing this until no more pairs can be made, which means that we are left with a sum of p^+'s and b's, p^-'s and b's or just b's. In each case we may refer back to steps 1, 2 and 3 of the proof, respectively, to rewrite Y (step (e)).

($i++$ means that the variable i is ... with 1.)

(a) Start with $i = 1$, $j = 1$, $\Lambda_1^+ = \lambda_1^+$ and $\Lambda_1^- = \lambda_1^-$.

(b) Check if $\Lambda_i^+ > \Lambda_j^-$ (i), $\Lambda_i^+ < \Lambda_j^-$ (ii), or $\Lambda_i^+ = \Lambda_j^-$ (iii).

(c) Rewrite $\Lambda_i^+ p_i^+ + \Lambda_j^- p_j^-$ to

i. $\Lambda_j^- \left(p_i^+ + p_j^- \right) + \left(\Lambda_i^+ - \Lambda_j^- \right) p_i^+$. Now $X = X + \Lambda_j^- \left(p_i^+ + p_j^- \right)$
If p_{j+1}^- exists, go to (d), otherwise go to (e).

ii. $\Lambda_i^+ \left(p_i^+ + p_j^- \right) + \left(\Lambda_j^- - \Lambda_i^+ \right) p_j^-$. Now $X = X + \Lambda_i^+ \left(p_i^+ + p_j^- \right)$
If p_{i+1}^+ exists, go to (d), otherwise go to (e).

iii. $\Lambda_i^+ \left(p_i^+ + p_j^-\right)$. Now $X = X + \Lambda_i^+ \left(p_i^+ + p_j^-\right)$ and Y hasn't changed. If p_{j+1}^- and p_{i+1}^+ exist, go to (d), otherwise go to (e).

(d) Set

i. $\Lambda_i^+ = \left(\Lambda_i^+ - \Lambda_j^-\right)$, j++. Go to (b).

ii. $\Lambda_j^- = \left(\Lambda_j^- - \Lambda_i^+\right)$, i++. Go to (b).

iii. i++, j++. Go to (b).

(e) i. Go to 2.

ii. Go to 1.

iii. If $\begin{cases} p_{j+1}^- \text{ exists}, & \text{go to 1.} \\ p_{i+1}^+ \text{ exists}, & \text{go to 2.} \\ \text{otherwise}, & \text{go to 3.} \end{cases}$

□

3.2 Computing extreme currents

We will use a *tableau* (see [8]) to keep track of the sets of generating vectors and the values of $a_i \cdot p_{j'}^{(j)}$. A tableau $T^{(j)}$ is made up of two matrices:

- $P^{(j)}$ which is made up of the generating vectors $p_i^{(j)}$ and $b_i^{(j)}$ written as row vectors (with respect to the standard basis $e_1, ..., e_n$), and
- $\Pi^{(j)}$ which is equal to $P^{(j)} \cdot S^T$. Each column of $\Pi^{(j)}$ corresponds to a metabolite.

$$T^{(j)} = \left[\begin{array}{c||c} P^{(j)} & \Pi^{(j)} \end{array}\right]$$

In the starting tableau $T^{(0)}$, $P^{(0)}$ is equal to the identity matrix and $\Pi^{(0)}$ is then equal to S^T.
Let s_i be a row vector of S.

At each step (new tableau $T^{(j)}$) we will 'balance' a metabolite M_j.
To obtain the next tableau:

1. rescale the rows i so that the values of $s_j \cdot p_i^{(j)} \in \{0, -1, +1\}$,
2. copy the the rows that have 0 in the j-th column of $\Pi^{(j)}$
3. with the remaining rows, form possible (positive) combinations such that the entries in the j-th row add up to 0.

When adding up rows to compute extreme currents, there is only one rule to consider. This makes it much faster and easier to compute ECs than to compute EMs.
We only need to check whether $\nu(p_i) \subset \nu(p_{i'})$ for some i, i' $i \neq i'$. If this is the case, remove p_i.
We may perform this check at each step of the process (each tableau) or at the end. Recall theorem 2.2.

3.3 Computing elementary modes

The algorithm for computing elementary modes is given in [10], and [6]. Using the same principle as in the algorithm shown above, more care is required to determine which rows are added together in step 3. Rows may also be subtracted if they correspond to a reversible reaction.
When forming combinations of rows, the following conditions (step II) are to be met:

1. When adding a positive multiple of a unidirectional row to a multiple of a bidirectional row, the sum of these is added to the unidirectional rows in the next tableau.

2. a pair of rows i and k from the j-th tableau is combined only if: $\nu(r_i^{(j)}) \cap \nu(r_k^{(j)}) \not\subset \nu(r_l^{(j+1)})$ for some row l in the $j+1$-th tableau.

3. before starting a new tableau check if $\nu(r_l^{(j+1)}) \subset \nu(r_i^{(j)}) \cap \nu(r_k^{(j)})$, for all rows j previously added to the tableau. If this is the case, delete the l-th row.

The second condition ensures that each new row added to the tableau contains a set of zeros not yet generated. The third condition deletes any rows (including those that were added in step 2) of which the null-set is contained in the null-set of a newly added row. This check may also be done at the end of the algorithm.

Using Theorem 2.5 we may compute the elementary modes by first computing the extreme currents in the augmented network and 'translating' them back to the original network (removing all 2-cycles).

$$\begin{array}{lcl} \text{(GVs)} & \Gamma' \xrightarrow{\text{Step II'}} \Gamma' & \text{(ECs)} \\ & \qquad\quad \Big\downarrow \text{A} & \\ \text{(GVs)} & \Gamma \xrightarrow{\text{Step II}} \Gamma & \text{(EMs)} \end{array}$$

(GVs=generating set of vectors)

This construction leads to the conditions in Step II' (for Γ') translating to a set of conditions in Step II for the original steady state flux cone Γ. It is not yet clear how the three conditions given above follow from the simple condition for the extreme currents, but this is not of importance for the method of subdivision we will use in Chapter 4.

Chapter 4

Subdivision of the system

Schilling and Palsson [7] present an approach to computing extreme pathways (and possibly elementary modes) for large metabolic networks through subdivision of the network into subnetworks. They do so by splitting the set of reactions of the network into pairwise disjoint subsets and putting each of these into virtual compartments. The compartments are connected to each other through metabolites which they call common metabolites, as they are contained in more than one compartment. Furthermore, additional exchange reactions are added to these common metabolites. The process of calculating the extreme pathways of the subnetworks seems overly complicated (it contains eight steps) and the brief explanation of the algorithm as provided in [7] did not give sufficient confidence in its proper functioning. In fact, the risen confusion started this bachelor project with the objective to understand this subdivision algorithm or devise a new one, with proper mathematical underpinning.
We have chosen to subdivide the system by using a different approach: subdividing the network *by splitting the set of metabolites*, instead of the reactions, into pairwise disjoint sets. This links closer to physical reality: metabolites reside in one compartment only, while (exchange) reactions furnish a means of transition. The choice of subdivision may be based on either mathematical or biological reasoning. To compute the ECs, we use the method of Section 3.2.
We have not looked at the efficiency of our method, merely tried to find a method that 'works' and for which we can mathematically prove it works. The problem of efficiency may be explored further, see for example the [9].

4.1 Subdivision of a network

Let $\mathcal{N}$ be a network of metabolic reactions, and let $\mathrm{M}(\mathcal{N})$ and $\mathrm{R}(\mathcal{N})$ be the associated sets of metabolites and reactions. A subdivision of the network $\mathcal{N}$ consists of making a partition on $\mathrm{M}(\mathcal{N})$ into s subsets $\mathcal{M}_1, \ldots, \mathcal{M}_s$.

We think of the metabolites in $\mathcal{M}_i$ as lying in a (virtual) compartment C_i $(i = 1, ..., s)$. Let $\mathcal{N}_i$ be the list of of all reactions that involve at least one or more of the metabolites $\mathcal{M}_i$ as substrate (reactant) or product and let $\mathcal{R}_i \subset R(\mathcal{N})$ be the set of labels for the reactions in $\mathcal{N}_i$. All reactions in $\mathcal{N}_i$ that involve a metabolite not in $\mathcal{M}_i$ are considered as exchange reactions for the compartment C_i. The network $\mathcal{N}_i$ defines a stoichiometric matrix S_i for each compartment C_i. For each network $\mathcal{N}_i$ one can compute the ECs using the associated augmented network $\mathcal{N}_i'$.
We define:

- a block-diagonal stoichiometric matrix S^* for the subdivided network $\mathcal{N}^*$ in which all the stoichiometric matrices S_i of the subsystems $\mathcal{N}_i$ are contained,
- a compartmentalised flux vector $v^* = (v^{(1)}| \dots |v^{(s)})^T$,
- a compatibility matrix Q (for $\mathcal{N}^*$) which describes how the exchange reactions between the subsystems fit together (incoming flux = - outgoing flux).

The steady state flux cone for $\mathcal{N}^*$ comprises vectors v^* such that $S^*v^* = 0$ and $Qv^* = 0$ ($v_i^* \geq 0$ for $i \in \mathrm{Irr}(\mathcal{N}^*)$).A vector that satisfies $Qv^* = 0$ can be reduced to a vector in Γ' by identifying corresponding exchange reactions and removing duplicates.

4.2 Subdivision algorithm

The algorithm together with arguments for proof of its functioning is given below.

1. Compute the ECs for each compartment $\mathcal{N}_i$ $(i = 1, ..., s)$, by using the algorithm described in Section 3.2, or if the EMs are already known translate these to get ECs, through theorem 2.5.

   Embedding these ECs for the $\mathcal{N}_i$ into the steady state flux cone for $\mathcal{N}^*$ yields elements v^* in Γ^*: $S^*v^* = 0$. Because of the block-diagonal structure of S^*, the vectors thus obtained generate C_{S^*}. Further constraints are imposed by Q.

2. Combine generating vectors for C_{S^*} such that $Qv^* = 0$ is satisfied (using the standard algorithm from section 3.1).

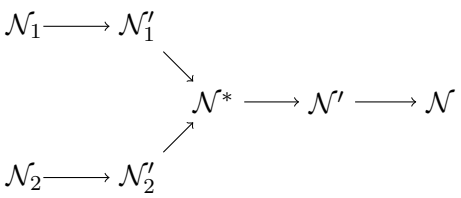
   (All ECs which do not contain exchange reactions between subsystems are also ECs for $\mathcal{N}^*$.)

3. Check for conical independence in $\mathcal{N}^*$, using theorem 2.2. This yields the ECs of $\mathcal{N}^*$.

The conditions imposed by Q ensure that exchange reactions between subsystems can be identified yielding a single reaction and vectors in $\mathcal{N}'$.

4. Restrict the resulting vectors to the flux space of $\mathcal{N}$. This yields the EMs of $\mathcal{N}$, according to theorem 2.5.

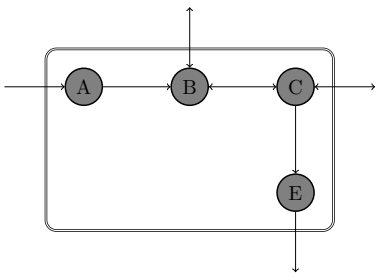
Diagram showing the case for s=2:



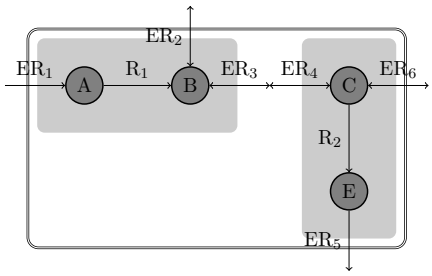
4.3 Example subdivision of a system

We will subdivide a simple metabolic network with four metabolites and seven reactions into two subnetworks.

Network $\mathcal{N}$:



Subdivided network $\mathcal{N}^*$, into $\mathcal{N}_1$ and $\mathcal{N}_2$:



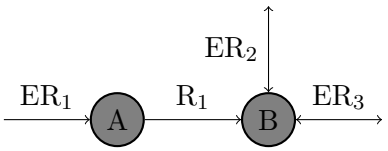
The compatibility matrix:
(Note that the signs in the compatibility matrix depend on the chosen positive directions for the reversible reactions.) $v_3 = -v_4 \implies v_3 + v_4 = 0$

$$Q = \begin{array}{c} \begin{matrix} \text{R1} & \text{ER1} & \text{ER2} & \text{ER3} & \text{R2} & \text{ER4} & \text{ER5} & \text{ER6} \end{matrix} \\ \left[\begin{array}{cccc|cccc} 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \end{array}\right] \end{array}$$

In the augmented network $\mathcal{N}'$ this corresponds to the following compatibility matrix:

$$Q' = \begin{array}{c} \begin{matrix} \text{R}_1 & \text{ER}_1 & \text{ER}_2 & \text{ER}'_2 & \text{ER}_3 & \text{ER}'_3 & \text{R}_2 & \text{ER}_4 & \text{ER}'_4 & \text{ER}_5 & \text{ER}_6 & \text{ER}'_6 \end{matrix} \\ \left[\begin{array}{cccccc|cccccc} 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 \end{array}\right] \end{array}$$

Network $\mathcal{N}_1$:



$$S_1 = \begin{array}{c} \begin{matrix} \text{R}_1 & \text{ER}_1 & \text{ER}_2 & \text{ER}_3 \end{matrix} \\ \begin{bmatrix} -1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 1 \end{bmatrix} \end{array}$$

$$S'_1 = \begin{array}{c} \begin{matrix} \text{R}_1 & \text{ER}_1 & \text{ER}_2 & \text{ER}'_2 & \text{ER}_3 & \text{ER}'_3 \end{matrix} \\ \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & -1 & 1 & -1 \end{bmatrix} \end{array}$$

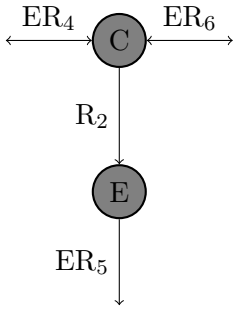
We get the following elementary modes:

$$p_1 = \begin{pmatrix} 1 \\ 1 \\ -1 \\ 0 \end{pmatrix}, b_1 = \begin{pmatrix} 0 \\ 0 \\ -1 \\ 1 \end{pmatrix}$$

These correspond to the following extreme currents in the augmented network $\mathcal{N}'_1$, together with the 2-cycles:

$$p_1 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix}, b_1 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \end{pmatrix}, b'_1 = \begin{pmatrix} 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 1 \end{pmatrix}, c_1 = \begin{pmatrix} 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \end{pmatrix}, c'_1 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \end{pmatrix}$$

Network $\mathcal{N}_2$:



$$S_2 = \begin{array}{c} \begin{matrix} \mathrm{R}_2 & \mathrm{ER}_5 & \mathrm{ER}_4 & \mathrm{ER}_6 \end{matrix} \\ \begin{bmatrix} -1 & 0 & 1 & 1 \\ 1 & -1 & 0 & 0 \end{bmatrix} \end{array}$$

$$S_2' = \begin{array}{c} \begin{matrix} \mathrm{R}_2 & \mathrm{ER}_5 & \mathrm{ER}_4 & \mathrm{ER}_4' & \mathrm{ER}_6 & \mathrm{ER}_6' \end{matrix} \\ \begin{bmatrix} -1 & 0 & 1 & -1 & 1 & -1 \\ 1 & -1 & 0 & 0 & 0 & 0 \end{bmatrix} \end{array}$$

Elementary modes:

$$p_2 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 1 \end{pmatrix}, \; b_2 = \begin{pmatrix} 0 \\ 1 \\ -1 \\ 0 \end{pmatrix}$$

$$p_2 = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 1 \\ 0 \end{pmatrix}, \; b_2 = \begin{pmatrix} 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix}, \; b_2' = \begin{pmatrix} 0 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \; c_2' = \begin{pmatrix} 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \end{pmatrix}, \; c_2' = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \end{pmatrix},$$

Next, we will combine these vectors to get the elementary modes for the network $\mathcal{N}'$, with stoichiometric matrix S^* and compatibility matrix Q'.

$$S^* = \begin{bmatrix} S_1 & \mathbf{0} \\ \mathbf{0} & S_2 \end{bmatrix}$$

Vector p_1 and the 2-cycles are elementary modes for the whole network since they do not involve common reactions.

$$A = \begin{bmatrix} S \\ Q \end{bmatrix}$$

[7] [10] [5] [6]

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