# 8. Geometry of the flux cone

- Convex cone

$$C \subseteq \mathbb{R}^n, \text{ with } \lambda x + \mu y \in C \text{ whenever } x, y \in C \text{ and } \lambda, \mu \geq 0.$$

- Polyhedral cone — Outer

$$C = \{x \in \mathbb{R}^n \mid Ax \geq 0\}, \text{ for some } A \in \mathbb{R}^{m \times n}.$$

- Finitely generated cone — Inner

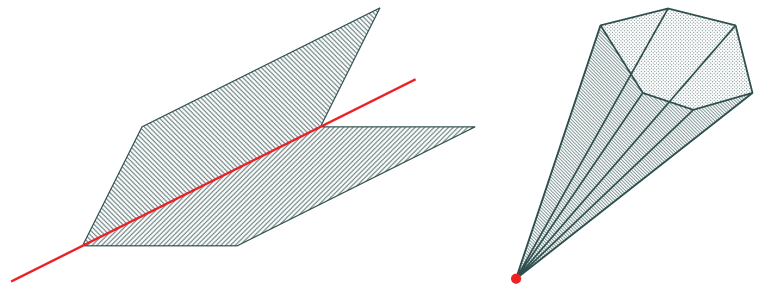
$$C = \operatorname{cone}\{g^1, \dots, g^s\} = \{\lambda_1 g^1 + \cdots + \lambda_s g^s \mid \lambda_1, \dots, \lambda_s \geq 0\},$$

  for some $g^1, \dots, g^s \in \mathbb{R}^n$.
- Theorem (Farkas-Minkowski-Weyl)
  A convex cone is polyhedral iff it is finitely generated.



# Pointed vs. non-pointed cones

- Polyhedral cone $C = \{x \in \mathbb{R}^n \mid Ax \geq 0\}$
- Lineality space $\operatorname{linsp}(C) = \{x \in \mathbb{R}^n \mid Ax = 0\}$
- $C$ is pointed if $\operatorname{linsp}(C) = \{0\}$.



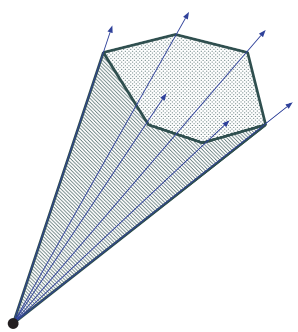


# Pointed cones

- $C$ pointed polyhedral cone
- $r \in C$ is an extreme ray if there exist no linearly independent $r', r'' \in C$ such that $r = r' + r''$ (identify $r^1, r^2 \in C$ if $r^1 = \lambda r^2$, for some $\lambda > 0$).
- Theorem. If $C$ is pointed, then

$$\begin{aligned} C &= \operatorname{cone}\{r^1, \dots, r^s\} \\ &= \{\lambda_1 r^1 + \cdots + \lambda_s r^s \mid \lambda_1, \dots, \lambda_s \geq 0\}, \end{aligned}$$

  where $r^1, \dots, r^s$ are the extreme rays of $C$.
- This representation is minimal and unique up to multiplication with positive scalars.

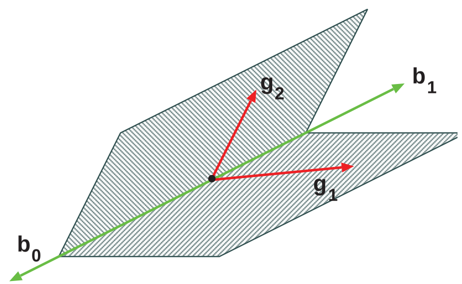




# Non-pointed cones

- $C$ polyhedral cone, $t = \dim(\operatorname{linsp}(C)) \geq 0$
- Faces $G$ of dimension $t + 1$ are called minimal proper faces.
- Theorem. If $g^i \in G^i \setminus \operatorname{linsp}(C)$, for $i = 1, \dots, s$, and $\operatorname{linsp}(C) = \operatorname{cone}\{b^0, \dots, b^t\}$, then

$$C = \operatorname{cone}\{g^1, \dots, g^s, b^0, \dots, b^t\}$$



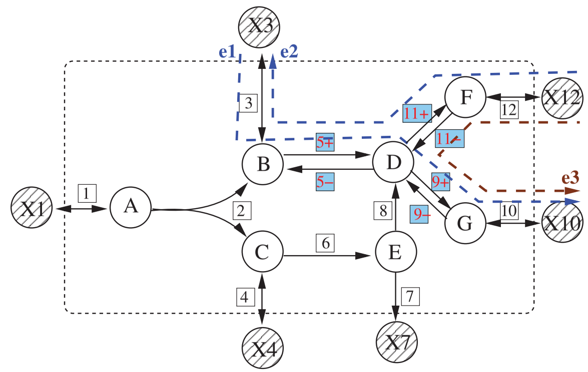

- For non-pointed cones, this representation is still minimal, but not unique.

# Inner descriptions of the flux cone

- Idea: Make the cone pointed in case it is not.
- Split reversible reactions into two irreversible reactions
  $\rightsquigarrow$ pointed cone in a higher-dimensional space
  $\rightsquigarrow$ compute the extreme rays
- Split all reversible reactions $\rightsquigarrow$ extremal currents (Clarke 80)
- Split all internal reversible reactions $\rightsquigarrow$ extreme pathways (Palsson et al., 2000)
- Alternatively, consider all flux vectors with minimum support in the original cone $\rightsquigarrow$ elementary flux modes (Schuster et al. 94)



# Example




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



# Example (ctd)

- $e_r^1, e_r^2, e_r^3$ are extreme pathways in the reconfigured space, whereas in the original space $e^3 = e^1 + e^2$.
- The reason is that the reversible reaction 5 is split in the two irreversible reactions $5^+$ and $5^-$.
- The number of elementary flux modes is 18, while the number of extreme pathways (after reconfiguration) is 14.



# Mathematical results

Theorem

- Extreme pathways correspond to a subset of the elementary modes (up to 2-cycles).
- Elementary modes are equivalent to extremal currents (up to 2-cycles).

Corollary

Elementary modes and extreme pathways can be computed by algorithms that enumerate the extreme rays of a pointed cone.

Software: `efmtool` (`http://www.csb.ethz.ch/tools/efmtool`)

## Inner descriptions: Overview

| Inner description | Split react. | Reconfigured flux cone $C'$ | | | Properties | |
|---|---|---|---|---|---|---|
| | | Dim. | # Constraints | $linsp(C')$ | Unique | Minim. |
| Extr. pathways | $Rev_{int}$ | $n+\|Rev_{int}\|$ | $m+\|Irr\|+2\|Rev_{int}\|$ | $\{0\}$ | $\surd$ | yes |
| Extr. currents | $Rev$ | $n+\|Rev\|$ | $m+\|Irr\|+2\|Rev\|$ | $\{0\}$ | $\surd$ | yes |
| Elem. modes | $\emptyset$ | $n$ | $m+\|Irr\|$ | $linsp(C)$ | $\surd$ | no |



## Computational results

Larhlimi/Bockmayr'09

| Metabolic network | Met | Irr | Rev | EFM | EP | $s$ | $t$ |
|---|---|---|---|---|---|---|---|
| Glycolysis / Gluconeogenesis | 32 | 18 | 29 | 19464 | 1745 | 16 | 13 |
| Citrate cycle (TCA cycle) | 22 | 4 | 25 | 3870 | 1588 | 4 | 12 |
| Pentose phosphate pathway | 34 | 19 | 24 | 5155 | 1630 | 19 | 8 |
| Pentose and glucuronate | 50 | 13 | 46 | 2258 | 231 | 7 | 23 |
| Fructose and mannose | 46 | 37 | 31 | 2411 | 2102 | 30 | 6 |
| Galactose | 41 | 22 | 28 | 623 | 524 | 13 | 9 |
| Starch and sucrose | 47 | 35 | 30 | 2097 | 1718 | 30 | 5 |
| Pyruvate | 28 | 40 | 29 | 47708 | 27390 | 37 | 16 |
| Propanoate | 34 | 20 | 29 | 877 | 233 | 17 | 13 |
| Butanoate | 40 | 23 | 30 | 2138 | 541 | 18 | 11 |
| Nitrogen | 41 | 53 | 14 | 601 | 612 | 44 | 9 |
| Sulfur | 18 | 26 | 4 | 321 | 326 | 28 | 1 |

($s$ is the number of minimal proper faces and $t$ the dimension of the lineality space)



## 9. Minimal metabolic behaviors (MMBs)

Larhlimi/Bockmayr 09

- Consider outer descriptions of the flux cone $C$.
- Characterize $C$ directly by its minimal proper faces and the lineality space.
- The lineality space $\mathrm{linsp}(C) = \{v \in C \mid v_i = 0, \text{ for all } i \in Irr\}$ has a natural description by equality constraints
  $\rightsquigarrow$ reversible metabolic space.
- Each minimal proper face can be characterized by a unique set of inequality constraints (= irreversible reactions)
  $\rightsquigarrow$ minimal metabolic behaviors (MMBs).
- The resulting outer description is both minimal and unique.



## Metabolic behaviors

- A metabolic behavior is a set of irreversible reactions $D \subseteq Irr, D \neq \emptyset$, such that there exists a flux vector $v \in C$ with

$$D = \{i \in Irr \mid v_i \neq 0\}.$$

- A metabolic behavior $D$ is minimal, if there is no metabolic behavior $D' \subsetneq D$ strictly contained in $D$.

# Elementary modes vs. MMBs

- An elementary flux mode is a vector $v \in C$ with a maximum number of zero components, i.e.,

$$\{i \in \{1,\dots,n\} \mid v_i \neq 0\}$$

is a minimal set of active reactions.

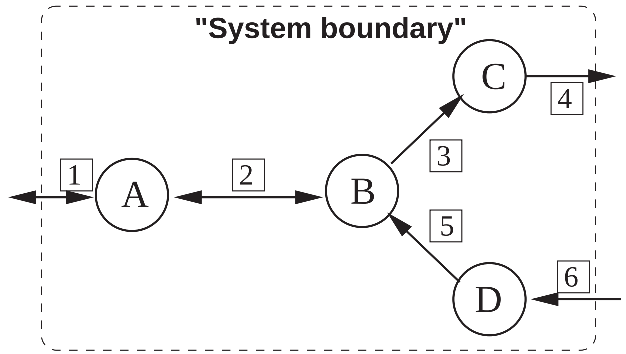
- Similarly, a minimal metabolic behavior

$$\{i \in Irr \mid v_i > 0\} = \{i \in Irr \mid v_i \neq 0\},$$

for some $v \in C$, is a minimal set of active irreversible reactions.



# Example




$e^1 = (1,1,1,1,0,0)$, $e^2 = (-1,-1,0,0,1,1)$, $e^3 = (0,0,1,1,1,1)$

$D^1 = \{3,4\}$ $D^2 = \{5,6\}$



# Characteristic set of a minimal face

- Lemma Given a minimal proper face $G$ of $C$ and an irreversible reaction $j \in Irr$, the following are equivalent:
  - $v_j > 0$, for some $v \in G \setminus \mathrm{linsp}(C)$.
  - $v_j > 0$, for all $v \in G \setminus \mathrm{linsp}(C)$.
- Call

$$D = D_G = \{j \in Irr \mid v_j > 0, \text{ for all } v \in G \setminus \mathrm{linsp}(C)\}$$

the characteristic set of $G$.

- It follows

$$G = \{v \in C \mid v_j > 0, j \in D, \ v_i = 0, i \in Irr \setminus D\} \ \cup \ \mathrm{linsp}(C).$$



# MMBs and minimal faces

Theorem

Let $C = \{v \in \mathbb{R}^n \mid Sv = 0, \ v_i \geq 0, i \in Irr\}$ be the flux cone of a metabolic network.
For any set of irreversible reactions $D \subseteq Irr$, the following are equivalent:

- $D$ is a minimal metabolic behavior.
- There exists a minimal proper face $G$ of $C$ whose characteristic set is $D$.

# MIP to enumerate shortest MMBs/GFMs

Rezola et al. 11

$\min W \cdot \sum_{j \in Irr} z_j + \sum_{j \notin Irr} z_j$

$$Sv = 0, v_{Irr} \geq 0,$$
$$-Mz_j \leq v_j \leq Mz_j, \quad \text{for } j = 1, \ldots, n,$$
$$\textstyle\sum_{j \in Irr} z_j \geq 1,$$
$$v \in \mathbb{R}^n, z \in \{0,1\}^n$$

Forbidding the $k$-th solution $(v^k, z^k)$: Let $D^k = \{j \in Irr \mid z_j^k = 1\}$.

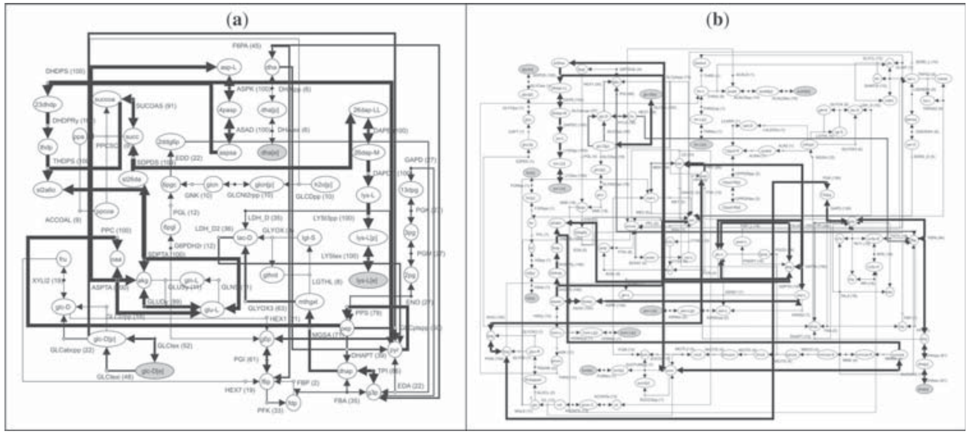
$$\sum_{j \in D^k} z_j \leq |D^k| - 1, \text{ for } k = 1, \ldots, K-1.$$

$v^k$ is called the $k$-th generating flux mode (GFM)



# EFMs vs. GFMs in *E. coli*

Rezola et al. 11




(thickness of arrows proportional to the number of times a reaction appears in the 100 shortest EFMs / GFMs)



# EFMs vs. GFMs in *E. coli* (2)

| | NoR | LI | OvR | AHD | No EFMs | No EFMs(R) |
|---|---|---|---|---|---|---|
| 100 shortest EFMs | 54 | 25–26 | 90.74% (49/54) | 12.79 | 1665<br>Lys: 1636 | 5398<br>Lys: 3960 |
| 100 shortest GFMs | 132 | 25–37 | 37.12% (49/132) | 16.21 | 354238<br>Lys: 25007 | $5 \times 10^5$ – – $5 \times 10^{10}$<br>Lys: $*$ |

NoR, number of reactions; LI, length interval; OvR, overlapping percentage of reactions of the 100 shortest EFMs (GFMs) in the shortest GFMs (EFMs); AHD, average hamming distance; No EFMs, number of EFMs emerging from the network formed by the 100 shortest EFMs (GFMs); No EFMs (R), number of EFMs emerging from the network formed by the 100 shortest EFMs (GFMs) and the reversible metabolic space (RMS); Lys, L-lysine.



# Novel pathway for lysine production