8. 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.,

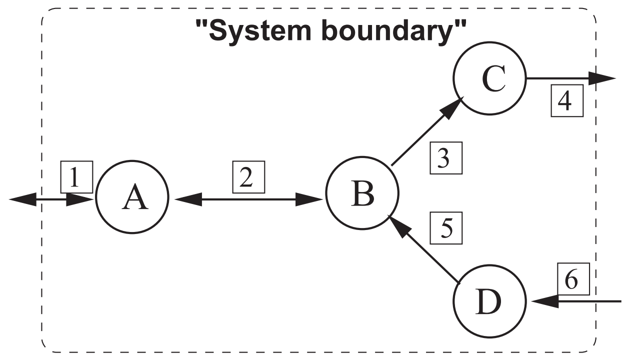
$$\{i \in \{1, \ldots, n\} \mid v_i \neq 0\}$$

  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

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

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

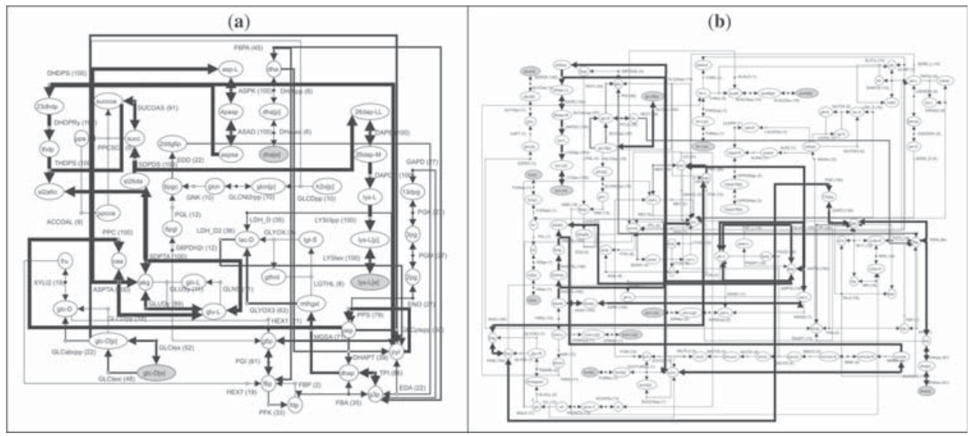
Forbidding the k-th solution (v^k, z^k): Let $D^k = \{j \in Irr \mid z_j^k = 1\}$.

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

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

EFMs vs. GFMs in *E. coli*

Rezola et al. 11



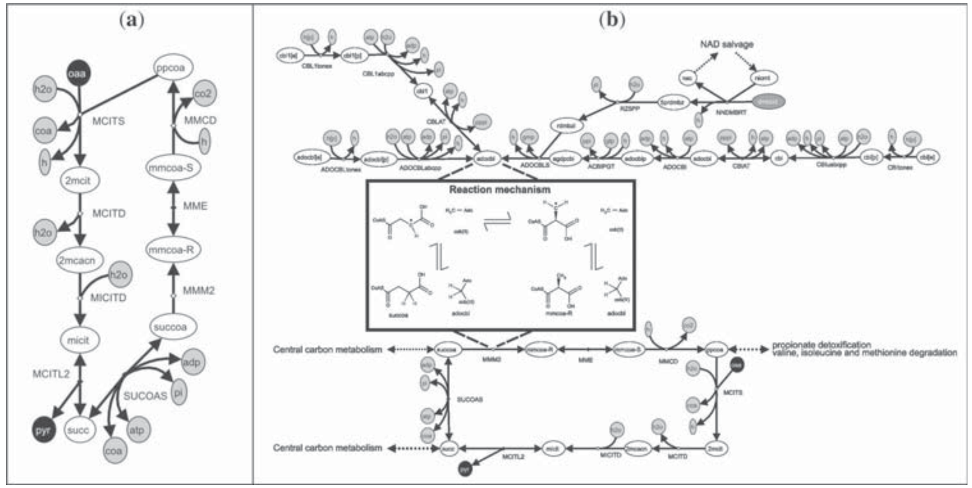
(thickness of arrows proportional to the number of times a reaction appears in the 100 shortest EFMs / GFMs)

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 Lys: 1636	5398 Lys: 3960
100 shortest GFMs	132	25–37	37.12% (49/132)	16.21	354238 Lys: 25007	5×10^5 – -5×10^{10} 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



Pseudo-irreversibility and minimal faces

- A reversible reaction i is called fully reversible if there exists a flux vector $v \in C$ such that $v_i \neq 0$ and $v_j = 0$ for all $j \in Irr$.
- Otherwise, reaction i is called pseudo-irreversible.

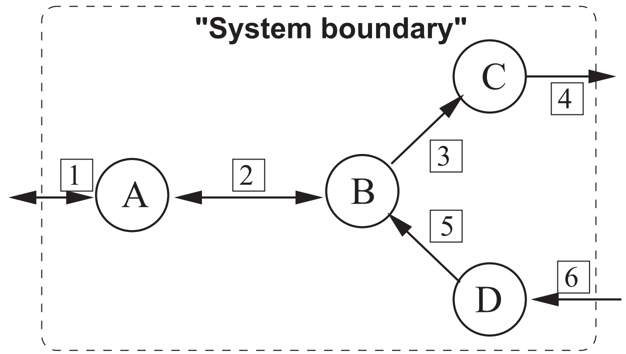
Lemma

Given a minimal proper face G of C and a pseudo-irreversible reaction $i \in Prev$, exactly one of the following conditions holds:

- $v_i > 0$, for all $v \in G \setminus \mathrm{linsp}(C)$.
- $v_i = 0$, for all $v \in G \setminus \mathrm{linsp}(C)$.
- $v_i < 0$, for all $v \in G \setminus \mathrm{linsp}(C)$.

In other words, pseudo-irreversible reactions become irreversible (or zero) within each minimal proper face.

Example



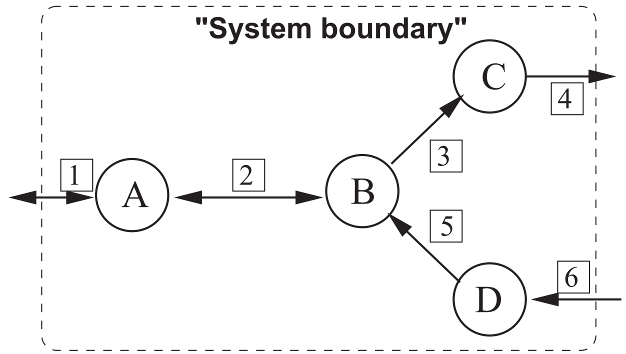
Reactions 1 and 2 are pseudo-irreversible:

- $v_1, v_2 > 0$, for all $v \in G^1 = \{v \in C \mid v_3, v_4 > 0, v_5, v_6 = 0\}$.
- $v_1, v_2 < 0$, for all $v \in G^2 = \{v \in C \mid v_3, v_4 = 0, v_5, v_6 > 0\}$.

Reaction classification

- Irreversible reactions j
  For all minimal proper faces G, either $v_j > 0$, for all $v \in G \setminus \mathrm{linsp}(C)$, or $v_j = 0$, for all $v \in G$.
  Furthermore, $v_j = 0$, for all $v \in \mathrm{linsp}(C)$.
- Pseudo-irreversible reactions j
  Inside each minimal proper face, the flux v_j through j has a unique sign $(+, -,$ or $0)$.
  For all $v \in \mathrm{linsp}(C)$, we have again $v_j = 0$.
- Fully reversible reactions j
  There exists $v \in \mathrm{linsp}(C)$ such that $v_j \neq 0$.
  Therefore, in each minimal proper face G there exist $v^p, v^n, v^0 \in G \setminus \mathrm{linsp}(C)$ with $v_j^p > 0$, $v_j^n < 0$ and $v_j^0 = 0$.

Sign patterns of MMBs



$$D^1 \equiv (+, +, +, +, 0, 0)$$
$$D^2 \equiv (-, -, 0, 0, +, +)$$

Splitting reversible reactions

Larhlimi/Bockmayr 08

- What happens to the steady-state flux cone

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

  if we split some reversible reaction $j \in Rev$?
- Reconfigured cone

$$C' = \{(v, v_{n+1}) \in \mathbb{R}^{n+1} \mid S'v' = 0, v_i \geq 0, i \in Irr, v_j \geq 0, v_{n+1} \geq 0\}$$

- $\mathrm{linsp}(C') = \{(v, v_{n+1}) \in \mathbb{R}^{n+1} \mid v \in \mathrm{linsp}(C), v_j = 0, v_{n+1} = 0\}$
- What is the relationship between the minimal proper faces of C and C'?

Case 1: Reaction j is fully reversible

- $\dim(\mathrm{linsp}(C')) = \dim(\mathrm{linsp}(C)) - 1$
- C' has two minimal proper faces related to the splitting

$$G^j = \{(v, v_{n+1}) \in C' \mid v_i = 0, i \in Irr, \ v_j \geq 0, v_{n+1} = 0\},$$
$$G^{n+1} = \{(v, v_{n+1}) \in C' \mid v_i = 0, i \in Irr, \ v_j = 0, v_{n+1} \geq 0\}$$

- All other minimal proper faces of C' are in a 1-1 correspondence with the minimal proper faces of C.
- Define $size(C) = s + t$, where s is the number of minimal proper faces of C and $t = \dim(\mathrm{linsp}(C))$.
- It follows: $size(C') = size(C) + 1$

Case 2: Reaction j is pseudo-irreversible

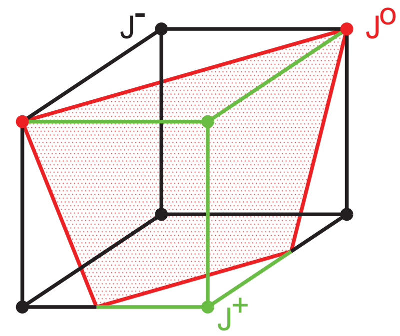
- $\dim(\operatorname{linsp}(C')) = \dim(\operatorname{linsp}(C))$
- To determine the minimal proper faces, we have to look at adjacent minimal proper faces of C.
- Let $J = \{G^1, \ldots, G^s\}$ be the set of minimal proper faces of C

$$\begin{aligned} J^0 &= \{G \in J \mid v_j = 0 \text{ for all } v \in G\}, \\ J^+ &= \{G \in J \mid v_j > 0 \text{ for all } v \in G \setminus \operatorname{linsp}(C)\}, \\ J^- &= \{G \in J \mid v_j < 0 \text{ for all } v \in G \setminus \operatorname{linsp}(C)\}. \end{aligned}$$

- Adjacent minimal proper faces

$$\Phi = \{(G^k, G^l) \in J^+ \times J^- \mid D^i \not\subseteq D^k \cup D^l \text{ for all } i \in \{1, \ldots, s\} \setminus \{k, l\}\}$$

Illustration



- Each pair $(G^k, G^l) \in \Phi$ defines a minimal proper face of C'

$$\zeta(G^k, G^l) = \{v \in C \mid v_j = 0,\ v_i = 0, \text{ for all } i \in Irr \setminus (D^k \cup D^l)\}.$$

- $Adj = \{\zeta(G^k, G^l) \mid (G^k, G^l) \in \Phi\}$

Minimal proper faces of C', j pseudo-irrev.

- Minimal proper face

$$G^c = \{(v, v_{n+1}) \in C' \mid v_i = 0, i \in Irr,\ v_j \geq 0, v_{n+1} \geq 0\}$$

- All other minimal proper faces G' of C' are of the form

$$\begin{aligned} G' &= \{(v, 0) \in \mathbb{R}^{n+1} \mid v \in G\}, \text{ for } G \in J^0 \cup J^+ \cup Adj, \text{ and} \\ G' &= \{v' \in C' \mid v'_i = 0, \text{ for all } i \in (Irr \cup \{j\}) \setminus D^k\}, \text{ for } G^k \in J^-. \end{aligned}$$

- $size(C') = 1 + size(C) + |Adj|$

From outer to inner descriptions

- Split a set of reversible reactions $SR = \{j_1, \ldots, j_p\}$, e.g.
  - $SR = Rev \rightsquigarrow$ extreme currents, or
  - $SR = Rev_{int} \rightsquigarrow$ extreme pathways.
- Need $p \geq t = \dim(\operatorname{linsp}(C))$ to obtain a pointed cone
  $\rightsquigarrow$ split t fully reversible and $p - t$ pseudo-irreversible reactions
  $\rightsquigarrow$ significant increase of the description size.
- Fully reversible reactions may become pseudo-irreversible after splitting.

Computational results

Metabolic network	Network size				Outer descript.		Inner descript.		
	Met	Irr	Rev Int	Rev Ext	RMS	MMB	EM	EP	EC
Chloropl. stroma	19	9	12	3	0	11	15	27	30
Red blood cell	38	18	17	15	1	48	3557	127	3590
S. cerevesiae	48	30	17	0	0	657	8726	8743	8743
E. coli	90	83	27	1	0	3560	507632	?	?
Purple bacteria	77	61	24	3	2	12	393524	?	?