Flux coupling relations (reminder)

Burgard et al. 04

- $C = \{v \mid Sv = 0,\ v_k \geq 0, k \in Irr\}$ flux cone
- A reaction i is blocked if $v_i = 0$, for all $v \in C$.
- Let i and j be two unblocked reactions.
  - i is directionally coupled to j, $i \overset{=0}{\rightarrow} j$, if for all $v \in C$, $v_i = 0$ implies $v_j = 0$.
  - i and j are partially coupled, $i \overset{=0}{\leftrightarrow} j$, if for all $v \in C$, $v_i = 0$ is equivalent to $v_j = 0$.
  - i and j are fully coupled, $i \backsim^{\lambda} j$, if there exists $\lambda \in \mathbb{R} \setminus \{0\}$ such that for all $v \in C, v_j = \lambda v_i$.

Flux coupling and EFMs

Marashi/Bockmayr 11

Proposition
Let $\mathscr{N}$ be a metabolic network with flux cone C and set of elementary modes E.

For any two reactions i and j, the following are equivalent:

(i) For all $v \in C$, $v_i = 0$ implies $v_j = 0$.
(ii) For all $e \in E$, $e_i = 0$ implies $e_j = 0$.

Flux coupling and EFMs (ctd)

Corollary
Let i, j be two non-blocked reactions in a metabolic network $\mathscr{N}$ with set of elementary modes E. Then:

- $i \overset{=0}{\rightarrow} j$ iff for all $e \in E$, $e_i = 0$ implies $e_j = 0$.
- $i \overset{=0}{\leftrightarrow} j$ iff for all $e \in E$, $e_i = 0$ is equivalent to $e_j = 0$.
- $i \backsim^{\lambda} j$ iff there exists $\lambda \neq 0$ such that for all $e \in E$, $e_j = \lambda \cdot e_i$.

Flux uncoupling

- Two reactions i, j are uncoupled if neither $i \overset{=0}{\rightarrow} j$ nor $j \overset{=0}{\rightarrow} i$.
- Equivalently, there exist EFMs $e, e' \in E$ such that

$$e_i = 0,\ e_j \neq 0 \quad \text{and} \quad e'_i \neq 0,\ e'_j = 0.$$

- Two uncoupled reactions i, j are called mutually exclusive if there is no EFM $e \in E$ with

$$e_i \neq 0,\ \ e_j \neq 0.$$

(i and j never occur together in the same EFM).

7. EFMs for given target reactions

Motivation: Huge number of EFMs in genome-scale metabolic network reconstructions ⇝ targeted search

Problem statement

Input:
- ▷ Metabolic network $\mathcal{N} = (Met, Reac, S)$ (assume $Irr = Reac$)
- ▷ Set of t target reactions $\{r_1, \ldots, r_t\} \subseteq Reac$,
- ▷ Natural number $k \geq 1$.

Output:
- ▷ Set E of EFMs in $\mathcal{N}$, $|E| = k$,
- ▷ $supp(e) \supseteq \{r_1, \ldots, r_t\}$, for all $e \in E$.

Complexity

Acuña et al. 09 and 10

Theorem
1. Computing an EFM containing one given target reaction ($t = 1$) can be done in polynomial time.
2. Deciding whether there exists an EFM containing $t \geq 2$ target reactions is NP-complete (even for $t = 2$).

Generic solution approach

David/Bockmayr 13

Iteratively, compute a sequence

$$\mathcal{N}^1, e^1, \ \mathcal{N}^2, e^2, \ \ldots, \ \mathcal{N}^k, e^k,$$

of subnetworks $\mathcal{N}^i = (Met, Reac^i)$ and EFMs e^i of $\mathcal{N}$ such that for all $i \in \{1, \ldots, k\}$:

- ▷ The target reactions r_1, ..., r_t belong to $\mathcal{N}^i$.
- ▷ e^i is an EFM in $\mathcal{N}^i$ involving r_1.
- ▷ r_2, ..., r_t are directionally coupled to r_1 in $\mathcal{N}^i$, i.e., $v_{r_1} \neq 0$ implies $v_{r_2} \neq 0, \ldots, v_{r_t} \neq 0$, for all $v \in C^i$.
- ▷ None of $e^1, \ldots, e^{i-1}$ is a flux mode in $\mathcal{N}^i$.

One target reaction, one EFM

Acuña et al. 09

Linear optimisation problem

$$\begin{array}{llcll} \mathrm{LP}(\mathcal{N}): \min & 0 & & & \\ \text{s.t.} & Sv & = & 0, & \\ & v_{r_1} & \geq & 1, & \\ & v_r & \geq & 0, & \forall r \in Reac. \end{array}$$

⇝ use Simplex method to compute a non-zero vertex (basic feasible solution)

One target reaction, k EFMs

de Figueiredo et al. 09

- k-shortest EFMs
- Sequence of mixed-integer linear optimisation problems

$$\begin{aligned} \text{MILP1}(E): \min \ & \textstyle\sum_{r \in Reac} a_r \\ \text{s.t.} \ & Sv = 0, \\ & v_{r_1} \geq 1, \\ & a_r \leq v_r \leq M a_r, && \forall r \in Reac, \\ & v_r \geq 0, && \forall r \in Reac, \\ & a_r \in \{0,1\}, && \forall r \in Reac, \\ & \textstyle\sum_{r \in supp(e)} a_r \leq |supp(e)| - 1, && \forall e \in E. \end{aligned}$$

Generic approach: one target reaction

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- Compute feasible solution of (MILP1) $\rightsquigarrow$ subnetwork $\mathcal{N}^i$
- Solve LP($\mathcal{N}^i$) to obtain EFM e^i

Step	Action (**Algorithm 1**)
0.	Initialize $i := 1, E := \emptyset$.
1.	Try to find a feasible solution (v', a') of MILP1(E).
2.	If MILP1(E) is infeasible, then STOP.
3.	Otherwise, use (v', a') to derive subnetwork N^i.
4.	Find a basic feasible solution e^i of LP(N^i).
5.	Let $E := E \cup \{e^i\}$ and $i := i + 1$.
6.	If $i > k$ then STOP.
7.	Go to Step 1.

Computational results: one target reaction

- 100 EFMs, *E. coli* iAF1260

Method	NoR	LI	AHD
Shortest EFMs	54	25-26	12.79
Algorithm 1	272	25-57	26.082

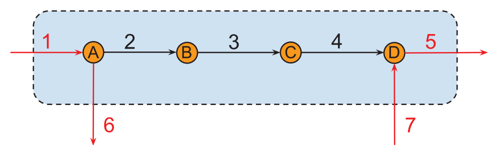
- 20 EFMs, *S. cerevisiae* iND750

		Integer variables		Continuous variables	
	Method	Length	Time	Length	Time
M = 10	Shortest	6-10	1719s	6-10	2074s
	Algo 1	6-15	16s	6-23	15s
M = 100	Shortest	6-10	8158s	6-10	3421s
	Algo 1	6-21	21s	6-31	18s
M = 1000	Shortest	6-10	14362s	6-10	7780s
	Algo 1	6-31	16s	6-50	29s

Two target reactions

First attempt

$$\begin{aligned} \text{(MILP2)}: \min \ & \textstyle\sum_{r \in Reac} a_r \\ \text{s.t.} \ & Sv = 0, \\ & v_{r_1} \geq 1, \\ & v_{r_2} \geq 1, \\ & a_r \leq v_r \leq M a_r, && \forall r \in Reac, \\ & v_r \geq 0, && \forall r \in Reac, \\ & a_r \in \{0,1\}, && \forall r \in Reac. \end{aligned}$$



Two target reactions: Using FCA

David/Bockmayr 13

Proposition

- An optimal solution v^* of (MILP2) is either an EFM or the sum of two EFMs.
- In the subnetwork $\mathcal{N}^*$ defined by (v^*, a^*), the reactions r_1 and r_2 are either fully coupled or mutually exclusive.

Consequences

- Refine (MILP2) by excluding the second case.
- Require that r_1 is directionally coupled to r_2 (or vice versa).
- Use Farkas' Lemma to express this condition by a set of linear constraints in the dual space.

Farkas' Lemma

Theorem

Let $A \in \mathbb{R}^{m\times n}$ and $b \in \mathbb{R}^n$.

The system of linear inequalities

$$Ax \leq b$$

has no solution $x \in \mathbb{R}^n$ if and only if the system

$$u^T A = 0,\ u^T b = -1,\ u \geq 0 \quad \Leftrightarrow \quad \begin{pmatrix} A^T \\ b^T \end{pmatrix} u = \begin{pmatrix} 0 \\ -1 \end{pmatrix},\ u \geq 0$$

has a solution $u \in \mathbb{R}^m$.

Two target reactions: Extended MILP

$$\begin{array}{rrcll}
\text{MILP3}(E):\min & 0 & & & \\
\text{s.t.} & Sv & = & 0 & \\
 & v_{r_1} & \geq & 1, & \\
 & v_{r_2} & \geq & 1, & \\
a_r \leq & v_r & \leq & M_0\, a_r, & \forall r \in Reac, \\
 & S^T y + u^{r_1} x & \geq & M_1\,(a - \mathbf{1} - u^{r_2}), & \\
 & -x & \geq & 1, & \text{DirC}(r_1, r_2) \\
 & \sum_{r\in supp(e)} a_r & \leq & |supp(e)| - 1, & \forall e \in E, \\
 & v_r & \geq & 0, & \forall r \in Reac, \\
 & a_r & \in & \{0,1\}, & \forall r \in Reac, \\
 & x, y_m & \in & \mathbb{R}, & \forall m \in Met.
\end{array}$$

(u^r denotes the r-unit vector)

Generic approach: two target reactions

Step	Action **Algorithm 2**
0.	Initialize $i := 1, E := \emptyset$.
1.	Try to find a feasible solution (v', a') of MILP3(E).
2.	If MILP3(E) is infeasible, then STOP.
3.	From (v', a') derive subnetwork $\mathcal{N}^i$.
4.	Find a basic feasible solution e^i of LP($\mathcal{N}^i$).
5.	Let $E := E \cup \{e^i\}$ and $i := i+1$.
6.	If $i > k$ then STOP.
7.	Go to Step 1.

General case

- For t target reactions, $t > 2$, it is enough to add directional coupling constraints DirC(r_1, r_2), DirC(r_1, r_3), ..., DirC(r_1, r_t).
- Prototype software available:
  `https://sourceforge.net/projects/caefm`

EFMs for all reaction pairs

- Compute EFMs for every pair of reactions
- Time out of 60 secs per pair

Network	# Reac.	# Pairs	EFMs found	Mean length	No EFM exists	No answer
E. coli central	90	8010	7691	24.36	176	143
H. pylori	269	72092	66749	46.57	1862	3481

Shortest flux modes vs. EFMs

- Two target reactions
- Computing shortest flux modes with MILP2 vs. computing EFMs with MILP3
- Time out of 60 secs per pair

Network	# Reac.	# Pairs	EFMs found with MILP3	EFMs found with MILP2	False pos with MILP2
E. coli central	90	8010	7691	5212	2686
H. pylori	269	72092	66749	206	9213

⇝ MILP2 in general not sufficient.