Flux coupling relations (reminder)

Burgard et al. 04

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

Flux coupling and EFMs

Marashi/Bockmayr 11

Proposition

Let $\mathcal{N}$ 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 $\nu \in C$, $v_i = 0$ 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 $\mathcal{N}$ with set of elementary modes $E$. Then:

- $i \rightarrow 0 j$ iff for all $e \in E$, $e_i = 0$ implies $e_j = 0$.
- $i \leftrightarrow 0 j$ iff for all $e \in E$, $e_i = 0$ is equivalent to $e_j = 0$.
- $i \sim \lambda j$ 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 $i \rightarrow 0 j$ nor $j \rightarrow 0 i$.
- Equivalently, there exist EFMs $e, e' \in E$ such that
  - $e_i = 0$, $e_j \neq 0$ and $e'_i \neq 0$, $e'_j = 0$.
- 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 $\Rightarrow$ targeted search

Problem statement

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

Output:
- Set $E$ of EFMs in $\mathcal{N}$, $|E| = k$
- $\text{supp}(e) \supseteq \{r_1, \ldots, r_t\}$, 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 $\mathcal{N}^i = (\text{Met}, \text{Reac}^i)$ and EFMs $e^i$ of $\mathcal{N}$ such that for all $i \in \{1, \ldots, k\}$:

- The target reactions $r_1, \ldots, r_t$ belong to $\mathcal{N}^i$.
- $e^i$ is an EFM in $\mathcal{N}^i$ involving $r_1$.
- $r_2, \ldots, r_t$ 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

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

$\Rightarrow$ use Simplex method to compute a non-zero vertex (basic feasible solution)
One target reaction, \( k \) EFMs

- \( k \)-shortest EFMs
- Sequence of mixed-integer linear optimisation problems

\[
\text{MILP1}(E) : \begin{aligned}
\min \sum_{r \in \text{Reac}} a_r \\
\text{s.t.} \quad S_v &= 0, \\
\nu_1 &\geq 1, \\
a_r &\leq \nu_r \leq M a_r, \quad \forall r \in \text{Reac}, \\
\nu_r &\geq 0, \quad \forall r \in \text{Reac}, \\
a_r &\in \{0, 1\}, \quad \forall r \in \text{Reac}, \\
\sum_{r \in \text{supp}(e)} a_r &\leq |\text{supp}(e)| - 1, \quad \forall e \in E.
\end{aligned}
\]

Generic approach: one target reaction

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

```
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 \( \mathcal{N}^i \).
4. Find a basic feasible solution \( e' \) of LP(\( \mathcal{N}^i \)).
5. Let \( E := E \cup \{e'\} \) 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

<table>
<thead>
<tr>
<th>Method</th>
<th>NoR</th>
<th>LI</th>
<th>AHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortest EFMs</td>
<td>54</td>
<td>25-26</td>
<td>12.79</td>
</tr>
<tr>
<td>Algorithm 1</td>
<td>272</td>
<td>25-57</td>
<td>26.082</td>
</tr>
</tbody>
</table>

- 20 EFMs, \( S. cerevisiae \) iND750

<table>
<thead>
<tr>
<th>Method</th>
<th>Integer variables</th>
<th>Continuous variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Length</td>
<td>Time</td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>M = 10 Shortest</td>
<td>6-10</td>
<td>1719s</td>
</tr>
<tr>
<td>M = 10 Shortest</td>
<td>6-15</td>
<td>16s</td>
</tr>
<tr>
<td>M = 100 Shortest</td>
<td>6-10</td>
<td>8158s</td>
</tr>
<tr>
<td>M = 100 Shortest</td>
<td>6-21</td>
<td>21s</td>
</tr>
<tr>
<td>M = 1000 Shortest</td>
<td>6-10</td>
<td>14362s</td>
</tr>
<tr>
<td>M = 1000 Shortest</td>
<td>6-31</td>
<td>16s</td>
</tr>
</tbody>
</table>

Two target reactions

First attempt

\[
\text{(MILP2)} : \begin{aligned}
\min \sum_{r \in \text{Reac}} a_r \\
\text{s.t.} \quad S_v &= 0, \\
\nu_1 &\geq 1, \\
\nu_2 &\geq 1, \\
a_r &\leq \nu_r \leq M a_r, \quad \forall r \in \text{Reac}, \\
\nu_r &\geq 0, \quad \forall r \in \text{Reac}, \\
a_r &\in \{0, 1\}, \quad \forall r \in \text{Reac}.
\end{aligned}
\]
Two target reactions: Using FCA

**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

$$u^T A = 0, \quad u^T b = -1, \quad u \geq 0$$

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

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Two target reactions: Extended MILP

**MILP3**

$$\text{min} \quad 0$$

subject to

$$Sv = 0,$$

$$v_{r_1} \geq 1,$$

$$v_{r_2} \geq 1,$$

$$a_r \leq v_r \leq M_0 a_r, \quad \forall r \in \text{Reac},$$

$$S^T \mathbf{y} + u^r x \geq M_1 (a - 1 - u^r),$$

$$-x \geq 1, \quad \text{DirC}(r_1, r_2),$$

$$\sum_{r \in \text{supp}(e)} a_r \leq |\text{supp}(e)| - 1, \quad \forall e \in E,$$

$$v_r \geq 0, \quad \forall r \in \text{Reac},$$

$$a_r \in \{0, 1\}, \quad \forall r \in \text{Reac},$$

$$x, y_m \in \mathbb{R}, \quad \forall m \in \text{Met}.$$  

($u^r$ denotes the $r$-unit vector)

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Generic approach: two target reactions

**Algorithm 2**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.</td>
<td>Initialize $i := 1, E := \emptyset$.</td>
</tr>
<tr>
<td>1.</td>
<td>Try to find a feasible solution $(v', a')$ of MILP3($E$).</td>
</tr>
<tr>
<td>2.</td>
<td>If MILP3($E$) is infeasible, then STOP.</td>
</tr>
<tr>
<td>3.</td>
<td>From $(v', a')$ derive subnetwork $\mathcal{N}^i$.</td>
</tr>
<tr>
<td>4.</td>
<td>Find a basic feasible solution $\mathbf{e}'$ of LP($\mathcal{N}^i$).</td>
</tr>
<tr>
<td>5.</td>
<td>Let $E := E \cup {e'}$ and $i := i + 1$.</td>
</tr>
<tr>
<td>6.</td>
<td>If $i &gt; k$ then STOP.</td>
</tr>
<tr>
<td>7.</td>
<td>Go to Step 1.</td>
</tr>
</tbody>
</table>
General case

- For \( t \) target reactions, \( t > 2 \), it is enough to add directional coupling constraints \( \text{DirC}(r_1, r_2), \text{DirC}(r_1, r_3), \ldots, \text{DirC}(r_1, r_t) \).

- 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

<table>
<thead>
<tr>
<th>Network</th>
<th># Reac.</th>
<th># Pairs</th>
<th>EFMs found</th>
<th>Mean length</th>
<th>No EFM exists</th>
<th>No answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>90</td>
<td>8010</td>
<td>7691</td>
<td>24.36</td>
<td>176</td>
<td>143</td>
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<tr>
<td>H. pylori</td>
<td>269</td>
<td>72092</td>
<td>66749</td>
<td>46.57</td>
<td>1862</td>
<td>3481</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Network</th>
<th># Reac.</th>
<th># Pairs</th>
<th>EFMs found with MILP3</th>
<th>EFMs found with MILP2</th>
<th>False pos with MILP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli central</td>
<td>90</td>
<td>8010</td>
<td>7691</td>
<td>5212</td>
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<td>H. pylori</td>
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<td>9213</td>
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</tbody>
</table>

⇝ MILP2 in general not sufficient.