3. Flux balance analysis (FBA)

- Assume cellular behavior is determined by a certain biological objective.
- Determine a corresponding “best” flux distribution.
- Use mathematical optimization to predict phenotype.
- Simplest case: Linear programming (LP)

\[
\max \{ c^T x \mid Ax \leq b, x \in \mathbb{R}^n \}
\]

- Flux balance problem (FBA)

\[
\max \{ c^T v \mid Sv = 0, l \leq v \leq u \} \quad \text{(FBA)}
\]

Example

To predict growth, \( Z = v_{\text{biomass}} \)

\[
\begin{bmatrix}
A & B + C \\
B + 2C & D
\end{bmatrix}
\]

Mathematically represent metabolic reactions and constraints

Genome-scale metabolic reconstruction

Mass balance defines a system of linear equations

Define objective function \((Z = c_1^* v_1 + c_2^* v_2 + \ldots)\)

Calculate fluxes that maximize \(Z\)

Solution space defined by constraints

E. coli metabolism

- Genome-scale reconstruction (iJO1366)
- 1336 metabolites, 2251 reactions
- Objective function: biomass
- Glucose and oxygen uptake reactions
- Aerobic and anaerobic growth
- Software: e.g. COBRA Toolbox 2.0

4. Flux variability analysis (FVA)

- Optimal solutions to FBA problems need not be unique.
- Enumerating all optimal solutions is computationally expensive.
- Alternative: Flux variability analysis (FVA)

\[
z_{opt} = \max \{ z = c^T v \mid Sv = 0, l \leq v \leq u \} \quad \text{(FBA)}
\]

For all \( j = 1, \ldots, n \):

\[
\max \{ \pm v_j \mid Sv = 0, l \leq v \leq u, c^T v = z_{opt} \} \quad \text{(FVA)}
\]

Thermodynamic constraints

- Flux through internal cycles is unbounded \(\Rightarrow\) thermodynamically infeasible
- Include additional (non-linear) thermodynamic constraints (Beard/Qian 02)
- Fast thermodynamic FVA (tFVA) (Müller/Bockmayr 12)
- Application: Modules in the optimal flux space (Müller/Bockmayr 13)
5. Flux coupling analysis (FCA)

**Burgard et al. 04**

- \( C = \{ v \mid Sv = 0, v_k \geq 0, k \in \text{IRR} \} \) 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 \( j \), \( i \to j \), if for all \( v \in C, v_i = 0 \)
    implies \( v_j = 0 \).
  - \( i \) and \( j \) are partially coupled, \( i \leftrightarrow j \), if for all \( v \in C, v_i = 0 \)
    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 \( v \in C, v_j = \lambda v_i \).
  - \( i \sim \lambda j \) implies \( i \leftrightarrow j \), which is equivalent to \( i \to j \) and \( j \to i \).

**Example**

\[
\begin{array}{cccc}
1 & 2 & 3 & 4 \\
2 & 3 & 0 & 1,2,3,5,6 \\
3 & 0 & 2 & 5 \\
4 & 0 & 1,2,3,5,6 & 6 \\
5 & 0 & 6 & 5 \\
6 & 0 & 5 & 2 \\
\end{array}
\]

**LP-based flux coupling analysis**

- Reaction \( i \) is blocked iff
  \[
  \max\{\pm v_i \mid Sv = 0, v_k \geq 0, k \in \text{IRR} \} = 0
  \]
- Two unblocked reactions \( i \) and \( j \) are directionally coupled, i.e., \( i \to j \) iff
  \[
  \max\{\pm v_j \mid Sv = 0, v_k \geq 0, k \in \text{IRR}, v_i = 0 \} = 0
  \]
- \( O(n^2) \) linear programming problems

**Fast Flux Coupling Calculation F2C2**

Larhlimi/David/Selbig/Bockmayr 12

<table>
<thead>
<tr>
<th>Network</th>
<th>FFCA</th>
<th>F2C2</th>
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<tbody>
<tr>
<td></td>
<td>ILPs</td>
<td>time</td>
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<td>M. barkeri, iAF692</td>
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<td>59m40s</td>
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