Flux Balance Analysis (FBA)

- Flux balance analysis (FBA) determines theoretical capabilities of metabolic networks using only stoichiometry and fundamental physiochemical capacity constraints.
- Metabolic networks reconstructed from annotated genome and literature.
- Capacity constraints include maximum uptake rates of oxygen and substrates such as glucose, acetate, and lactose.
- FBA determines optimal flux distribution for given conditions.
- Cell growth is used as the objective function approximated by production of growth precursors in certain ratios.

FBA Formulation

Constructing a mass balance equation for each species in the network, $X_i$, by examining the fluxes that affect it.

$$\frac{dX_i}{dt} = V_{syn} - V_{deg} - V_{use} \pm V_{trans}$$

where $V_{syn}$ is synthesis rate, $V_{deg}$ is degradation rate, $V_{use}$ is the consumption rate, and $V_{trans}$ is exchange rate with the cell’s environment.

Assuming that these rates are rapid with respect to the rate of cell growth, these rates are assumed to be balanced:

$$0 = V_{syn} - V_{deg} - V_{use} \pm V_{trans}$$
Mass Balances and Flux Constraints

<table>
<thead>
<tr>
<th>Mass Balances</th>
<th>Flux Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>A : ( R_1 - R_2 - R_3 - V_{growth} = 0 )</td>
<td>0 ( \neq 0 )</td>
</tr>
<tr>
<td>B : ( 0 \neq 0 )</td>
<td>( x \neq 0 )</td>
</tr>
<tr>
<td>C : ( R_1 + R_2 = 0 )</td>
<td>( x \neq 0 )</td>
</tr>
<tr>
<td>D : ( R_1 + R_2 - R_3 = 0 )</td>
<td>( x \neq 0 )</td>
</tr>
<tr>
<td>E : ( 0 \neq 0 )</td>
<td>( x \neq 0 )</td>
</tr>
<tr>
<td>F : ( 0 \neq 0 )</td>
<td>( x \neq 0 )</td>
</tr>
<tr>
<td>G : ( 0 \neq 0 )</td>
<td>( x \neq 0 )</td>
</tr>
<tr>
<td>H : ( 0 \neq 0 )</td>
<td>( x \neq 0 )</td>
</tr>
</tbody>
</table>

Objective Function

\[ Z = V_{growth} \]

Flux Balance Equation

\[ \begin{bmatrix} a_1 & a_2 & \cdots & a_n \\ b_1 & b_2 & \cdots & b_n \\ \vdots & \vdots & \ddots & \vdots \\ g_1 & g_2 & \cdots & g_n \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{bmatrix} = \begin{bmatrix} f_1 \\ f_2 \\ \vdots \\ f_n \end{bmatrix} \]

Regulatory Flux Balance Analysis (rFBA)

- Regulatory network derived from the literature.
- Regulatory rules based on both:
  - External (presence/absence of extracellular molecules) and
  - Internal conditions (activity/inactivity of enzymatic flux).
- Regulatory constraints described using Boolean formalism.
- In steady-state, genes evaluated to determine if expressed or repressed.
- Fluxes of repressed gene products are set to zero.

Time-Dependent Cell Behavior

- Metabolic time constants msecs to 10s of secs, regulation is minutes, cell growth is hours to days.
- Simulations consider cell to be in quasi-steady state during short time intervals relative to the environment.
- Time run in small time steps.
- For each time step, regulatory rules evaluated.
- Up-regulated genes result in regulated reactions being unconstrained after a time delay for protein synthesis.
- Down-regulated genes result in regulated reactions being constrained to zero after time delay for protein degradation.
- Next, optimal flux distribution is calculated.
- Calculate cell growth, substrate uptake, by-product secretion.

Central Metabolism in *E. Coli*
**Results**

- The rFBA network accounts for 149 genes.
- The products of which include 16 regulatory proteins and 73 enzymes which catalyze 113 reactions.
- Synthesis of 43 enzymes controlled by transcriptional regulation, 45 reactions controlled by logic statement.
- Used in retrospective analysis of experimental data to determine utility of rFBA model to make accurate predictions.
- Performed both a mutant study as well as dynamic simulation under three environmental conditions.

**Mutant Study**

- To simulate deletion of a metabolic gene, flux through corresponding gene product was set to zero.
- Regulatory gene deletion simulated by setting gene to OFF.
- rFBA model used to determine ability of mutant strains of E. coli to grow on defined media (116 total cases).
- FBA model alone correctly predicted 97 cases (83.6%), 16 cases incorrect while 3 not possible since mutated gene is regulatory.
- rFBA model correct in 106 cases (91.4%).
- Incorrect predictions often due to accumulation of toxic substances, which is not yet predictable by this approach.

**Aerobic Growth on Acetate w/Glucose Reutilization**

**Aerobic Growth on Glucose and Lactose**

**Aneaerobic Growth on Glucose**

**Flux Balance Analysis Summary**

- Most models require extensive kinetic and other environmental information that is difficult to obtain.
- Large-scale constraints-based metabolic models can capture essential behavioral features with relatively few parameters.
- Regulatory constraints improve predictive capacity.
- Regulatory constraints useful in the interpretation of dynamic behaviors.
ODE models assume spatial homogeneity, but time delays due to diffusion or multiple compartments may be important.

Reaction-diffusion models introduced by Turing (1951).

\[ \frac{dx_i}{dt} = f(x) + \delta_i \nabla^2 x_i, 1 \leq i < \rho \]

If number of cells large enough, \( I \) can be continuous:

\[ \frac{dx_i}{dt} = f(x) + \delta_i \frac{\partial^2 x_i}{\partial t^2}, 1 \leq i < \rho \]

Stationary case with extreme long wave-length
Contents of all cells same, no diffusion, as if isolated.

Embryo in spherical blastula stage has spherical symmetry. Systems consist of masses of tissues within which certain substances, morphogens, are reacting chemically and diffusing.

Each morphogen moves from regions of greater to regions of less concentration, at rate proportional to gradient and diffusibility.

Reactions occur at rate proportional to concentrations of reactants, i.e., obey law of mass action.

If there are \( N \) cells and \( M \) morphogens, state of system is \( MN \) numbers giving quantity of morphogens in each cell.

**Turing’s Morphogens**

Goal of this work is a mathematical model of a growing embryo.

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If number of cells large enough, \( I \) can be continuous:

\[ \frac{dx_i}{dt} = f(x) + \delta_i \frac{\partial^2 x_i}{\partial t^2}, 1 \leq i < \rho \]

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**Reactions and Diffusion in a Ring of Cells**

Assume a ring of \( N \) cells with two morphogens, \( X \) and \( Y \).

\[ \frac{dX_r}{dt} = f(X_r, Y_r) + \mu(X_{r+1} - X_r + X_{r-1}) \]

\[ \frac{dY_r}{dt} = g(X_r, Y_r) + \nu(Y_{r+1} - Y_r + Y_{r-1}) \]

where \( r = 1, \ldots, N \).

After much manipulation, solution can be written as:

\[ X_r = h + \sum_{s=1}^{N} (A_s e^{\nu s} + B_s e^{-\nu s}) \exp\left[ \frac{2\pi is}{N} \right] \]

\[ Y_r = k + \sum_{s=1}^{N} (C_s e^{\nu s} + D_s e^{-\nu s}) \exp\left[ \frac{2\pi is}{N} \right] \]

**Examples of Spatial Configurations**

- **Stationary case with extreme long wave-length**
  - Contents of all cells same, no diffusion, as if isolated.
- **Oscillatory case with extreme long wave-length**
  - As if isolated, but departure from equilibrium oscillatory.
- **Stationary waves with extreme short wave-length**
  - If \( N \) even, alternating pattern, but odd varies around ring.
- **Stationary waves with finite wave-length**
  - Most interesting biologically, example coming.
- **Oscillatory case with finite wave-length**
  - Requires 3 morphogens, genuine travelling waves.
- **Oscillatory case with extreme short wave-length**
  - Also requires 3, oscillation 180 degrees out of phase with neighbor.

**Types of Asymptotic Behavior in the Ring**

- **Stationary case with extreme long wave-length**
  - Contents of all cells same, no diffusion, as if isolated.
- **Oscillatory case with extreme long wave-length**
  - As if isolated, but departure from equilibrium oscillatory.
- **Stationary waves with extreme short wave-length**
  - If \( N \) even, alternating pattern, but odd varies around ring.
- **Stationary waves with finite wave-length**
  - Most interesting biologically, example coming.
- **Oscillatory case with finite wave-length**
  - Requires 3 morphogens, genuine travelling waves.
- **Oscillatory case with extreme short wave-length**
  - Also requires 3, oscillation 180 degrees out of phase with neighbor.
A Numerical Example

- Twenty cells in a ring, each with 0.1mm diameter and volume of $10^{-8}$ cm$^3$.
- Two morphogens $X$ ($5 \times 10^{-4}$ cm$^2$s$^{-1}$) and $Y$ ($2.5 \times 10^{-4}$ cm$^2$s$^{-1}$).

\[
Y + X \rightarrow W \quad (\text{slow})
\]
\[
W + A \rightarrow 2Y + B \quad (\text{instantly})
\]
\[
2X \rightarrow W \quad (\text{slow})
\]
\[
A \rightarrow W \quad (\text{slow})
\]
\[
Y \rightarrow B \quad (\text{slow})
\]
\[
Y + C \rightarrow C' \quad (\text{instantly})
\]
\[
C' \rightarrow X + C \quad (\text{slow})
\]

Concentration of $A$ is 1000 s.u. (i.e., $10^{-11}$ mole/cm$^3$).
Concentration of $C$ and $C'$ is $10^{-3}(1 + \gamma)$ s.u. where $\gamma = [-0.5, 0.5]$.

- "quick cooking" - $\gamma$ increases at rate of $2^{-7}$ s.u. from $-\frac{1}{4}$ to $+\frac{1}{4}$, and then allowed to decrease at same rate to 0.
- "slow cooking" - $\gamma$ allowed to increase at rate of $10^{-5}$.

Concentrations of $Y$ in Development of 1st Specimen

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Concentration</th>
<th>Time (min)</th>
<th>Concentration</th>
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<tr>
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<td>60</td>
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<tr>
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</tr>
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</tr>
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<tr>
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</tr>
<tr>
<td>8</td>
<td>0.0000</td>
<td>540</td>
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</table>

Table 1. Some stationary-wave patterns

<table>
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<tr>
<th>Cell number</th>
<th>Concentration</th>
<th>wavenumber</th>
<th>amplitude</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>0</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>1</td>
<td>0.0000</td>
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<td>0.0000</td>
<td>0.0000</td>
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<tr>
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<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>3</td>
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<td>0.0000</td>
<td>0.0000</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
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<tr>
<td>6</td>
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<tr>
<td>8</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Sources
- Web page for ODE solvers.
- Numerical recipes in C.
- Matsuno paper on HPNs
- Gouze paper for PLDEs
- De Jong paper for QDEs
- Covert and Palsson, JBC 2002
- Turing paper for PDEs