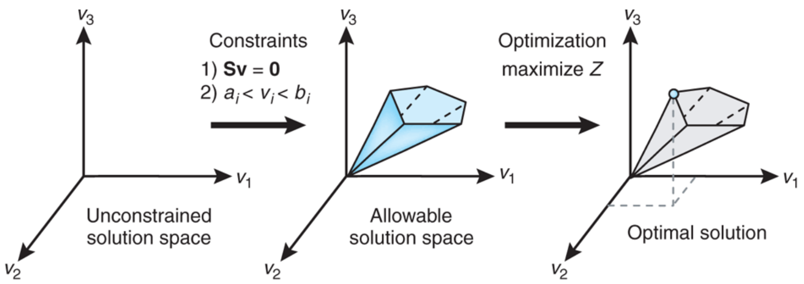


Exercise 3: Flux balance analysis

Iván Domenzain
ivand@chalmers.se

2018-10-05

Learning outcomes

The objectives of this exercise are:

- To get familiar with a MATLAB structure of genome-scale models.
- To realize how undetermined models of metabolism are.
- To be able to find an optimal flux distribution under specific conditions.
- To interpret flux distributions and find trends in metabolism.

Miscellaneous

- Book chapters 18, 19 and 21
- Report deadline: Sunday 21st of October at 23:55
- Requirements:
  - Matlab
  - Any software of your preference for plotting the results

smallModel.mat

- Represents yeast central carbon metabolism (CCM).

- Pathways included:
  - Glycolysis
  - Pentose phosphate pathway
  - TCA cycle
  - Respiration (NADHX and FADHX)
  - Production of byproducts: ethanol, glycerol and acetate.
  - Biomass pseudo-reaction (bioRXN)
  - Non-Growth Associated Maintenance = NGAM (ATPX)

- Exchange reactions (all defined as sinks):
  - Glucose (glcEX), oxygen (o2EX), ethanol (ethEX), acetate (acEX), biomass (bioEX), CO_2 (co2EX), glycerol (glyEX).

Determined/Undetermined problems (problem 1)

$$\textbf{Degrees of Freedom} = \#\textbf{vars} - \#\textbf{equations} \qquad (\textbf{if equations are L.I.})$$

$$\textbf{Degrees of Freedom} = \#\textbf{vars} - \textbf{rank(matrix)} \qquad (\textbf{general case})$$

- Determined problems: Degrees of Freedom = 0
  - Solve the system using the \ command
- Undetermined problems: Degrees of Freedom > 0
  - Define objective function and solve using `linprog()`.

Using `linprog()`

1 = solution found
Anything else = no solution

Solution

[ ]　　[ ]

```
[x,f,flag] = linprog(c,Ai,bi,Ae,be,LB,UB);
```

O.F. value

ALWAYS MINIMIZES!!
But remember:
Min f(x) = - Max -f(x)

smallModel.mat

- Reactions that consume ATP:
  - HXK: GLC_c + ATP_c -> ADP_c + G6P_c
  - PFK: ATP_c + F6P_c -> ADP_c + F16P_c
  - PYC: ATP_c + CO2_c + PYR_c -> ADP_c + OAA_m + PI_c
  - ACS: AC_c + 2 ATP_c + COA_c -> ACCOA_c + 2 ADP_c + 2 PI_c
  - PCK: ATP_c + OAA_m -> ADP_c + CO2_c + PEP_c
  - ATPX: ATP_c -> ADP_c + PI_c (maintenance)
  - bioRXN

- Reactions that produce ATP:
  - PGK: P13G_c + ADP_c <-> P3G_c + ATP_c
  - PYK: ADP_c + PEP_c -> ATP_c + PYR_c
  - LSC1LSC2: ADP_c + PI_c + SUCCOA_m <-> ATP_c + COA_m + SUC_m
  - NADHX: 2.4 ADP_c + 2 NADH_m + O2_c + 2.4 PI_c -> 2.4 ATP_c + 2 NAD_m
  - FADHX : 2.4 ADP_c + 2 FADH2_m + O2_c + 2.4 PI_c -> 2.4 ATP_c + 2 FAD_m

smallModel.mat

- Growth pseudo-reaction:

AKG_m + 6 P3G_c + 24 ACCOA_c + 3 ACCOA_m + 25 G6P_c + 200 ATP_c + 3 E4P_c + 3 R5P_c + GP_c + 16 NAD_c + 6 NAD_m + 90 NADPH_c + 22 NADPH_m + 10 OAA_m + 6 PEP_c + 18 PYR_c ->

200 ADP_c + 10 BIOMASS_c + 24 COA_c + 3 COA_m + 16 NADH_c + 6 NADH_m + 90 NADP_c + 22 NADP_m + 244 PI_c

smallModel.mat

- Growth pseudo-reaction:

AKG_m + 6 P3G_c + 24 ACCOA_c + 3 ACCOA_m + 25 G6P_c + 200 ATP_c + 3 E4P_c + 3 R5P_c + GP_c + 16 NAD_c + 6 NAD_m + 90 NADPH_c + 22 NADPH_m + 10 OAA_m + 6 PEP_c + 18 PYR_c ->

200 ADP_c + 10 BIOMASS_c + 24 COA_c + 3 COA_m + 16 NADH_c + 6 NADH_m + 90 NADP_c + 22 NADP_m + 244 PI_c

Changing Objective Functions (problem 2)

$$\text{P2.1) Max growth} \\ \text{s.t.} \\ Sv = 0 \\ LB \leq v \leq UB \\ \text{gluc uptake} \leq 1$$

$$\text{P2.2) Max acetate prod.} \\ \text{s.t.} \\ Sv = 0 \\ LB \leq v \leq UB \\ \text{gluc uptake} \leq 1$$

$$\text{P2.3) Max ATP maint.} \\ \text{s.t.} \\ Sv = 0 \\ LB \leq v \leq UB \\ \text{gluc uptake} \leq 1$$

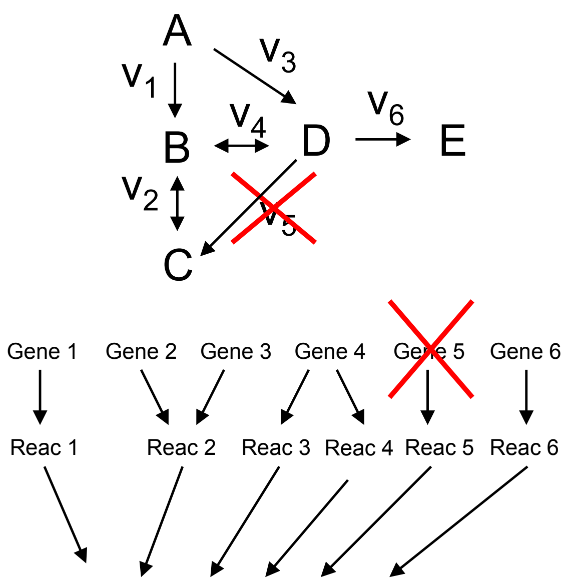
Changing Growth Conditions (problem 3)

- New reactions:
  - ALD2: Acetaldehyde + NAD -> Acetate + NADH (cytosol)
  - Glyoxylate 1: Isocitrate -> Glyoxylate + Succinate (mitochondria)
  - Glyoxylate 2: Glyoxylate + Acetyl CoA -> Malate + CoA (mitochondria)

- Growth conditions: For all, maximize growth rate

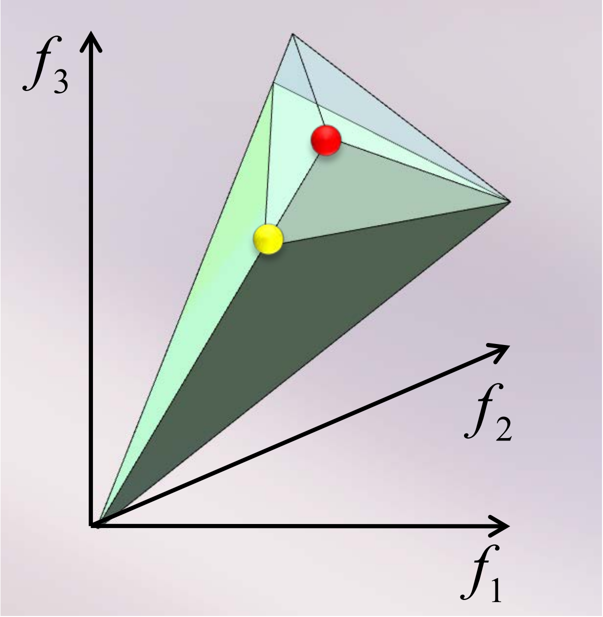
	Aerobic Conditions	Anaerobic Conditions
Carbon Source = Glucose $C_6H_{12}O_6$	$v_{glcEX} \neq 0$ $v_{o2EX} \neq 0$ $v_{ethEX} = 0$	$v_{glcEX} \neq 0$ $v_{o2EX} = 0$ $v_{ethEX} = 0$
Carbon Source = Ethanol C_2H_6O	$v_{glcEX} = 0$ $v_{o2IN} \neq 0$ $v_{ethEX} \neq 0$	$v_{glcEX} = 0$ $v_{o2EX} = 0$ $v_{ethEX} \neq 0$

In Silico gene deletions



$$
\begin{array}{c}
\begin{array}{cccccccc} v_1 & v_2 & v_3 & v_4 & v_5 & v_6 & \ldots & \textit{flux } n \end{array} \\
\left[\begin{array}{cccccccc}
-1 & 0 & -1 & 0 & 0 & 0 & \ldots & 0 \\
1 & -1 & 0 & -1 & 0 & 0 & \ldots & 1 \\
0 & 1 & 0 & 0 & 1 & 0 & \ldots & 0 \\
0 & 0 & 1 & 1 & -1 & -1 & \ldots & -1 \\
0 & 0 & 0 & 0 & 0 & 1 & \ldots & 0 \\
\ldots & \ldots & \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\
0 & 1 & -1 & 0 & 0 & 0 & 0 & 0
\end{array}\right]
\begin{array}{l} A \\ B \\ C \\ D \\ E \\ \ldots \\ \textit{metabolite } m \end{array}
\end{array}
$$

$$0 \leq v_1 \leq +\infty$$
$$-\infty \leq v_2 \leq +\infty$$
$$0 \leq v_3 \leq +\infty$$
$$-\infty \leq v_4 \leq +\infty$$
$$0 \leq v_5 \leq 0$$
$$0 \leq v_6 \leq +\infty$$
$$\ldots$$
$$0 \leq v_n \leq +\infty$$



Example of Bar Plot to Present