

Regulatory Flux Balance Analysis (rFBA)

Metabolische Netzwerke SS 16

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Iterative algorithm

1. Determine substrate concentration S_c from previous substrate concentration S_{co} and additional supply:

$$S_c = S_{co} + \frac{\text{supply} \cdot \Delta t}{\text{vol}}$$

2. Scale substrate concentration

$$\text{Substr_avail} = \frac{S_c}{X \cdot \Delta t}$$

(X is the cell density)

3. Use FBA to determine actual substrate uptake rate S_u , growth rate μ , and potential by-product secretion.

4. Compute new concentrations

$$\frac{dX}{dt} = \mu X \Rightarrow X = X_0 \cdot e^{\mu \Delta t}$$

$$\frac{\partial S_c}{\partial t} = -S_u \cdot X \Rightarrow S_c = S_{co} + \frac{S_u}{\mu} X_0 (1 - e^{\mu \Delta t})$$

Iterated flux balance analysis

Varma/Palsson 1994

Flux balance model

$$S \cdot v = b$$

(b net metabolic uptake)

Objective

$$\text{minimize } Z = -v_{growth}$$

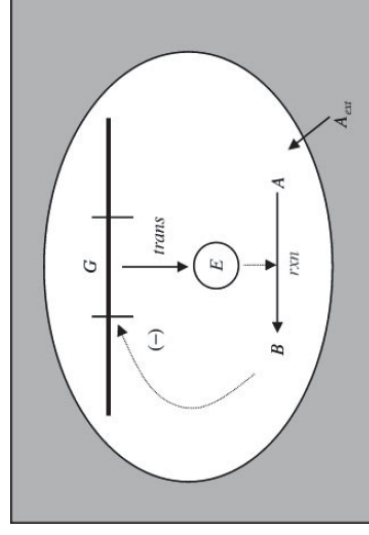
$$\sum_{\text{all } M} d_M \cdot M \xrightarrow{v_{growth}} \text{biomass}$$

Divide experimental time into small time steps Δt .

Specify initial values for **external** concentrations.

Use flux balance model to predict concentrations for the next step (**➡** dynamic profiles).

Regulatory constraints



trans = IF (G) AND NOT (B)

rxn = IF (A) AND (E)

Gene G is transcribed by a process trans to produce an enzyme E.

This enzyme then catalyses a reaction rxn which converts substrate A into product B.

Product B then represses transcription of G, leading to depletion of E.

Regulatory flux balance analysis (rFBA)

Covert/Schilling/Palsson 2001

Refinement of iterative FBA

Divide experimental time into small time steps Δt .

Reactions may happen in a given time interval $[t_1, t_2]$, if corresponding regulatory constraints are satisfied.

If a regulatory constraint for reaction k does not hold in $[t_1, t_2]$, we impose the temporary constraint

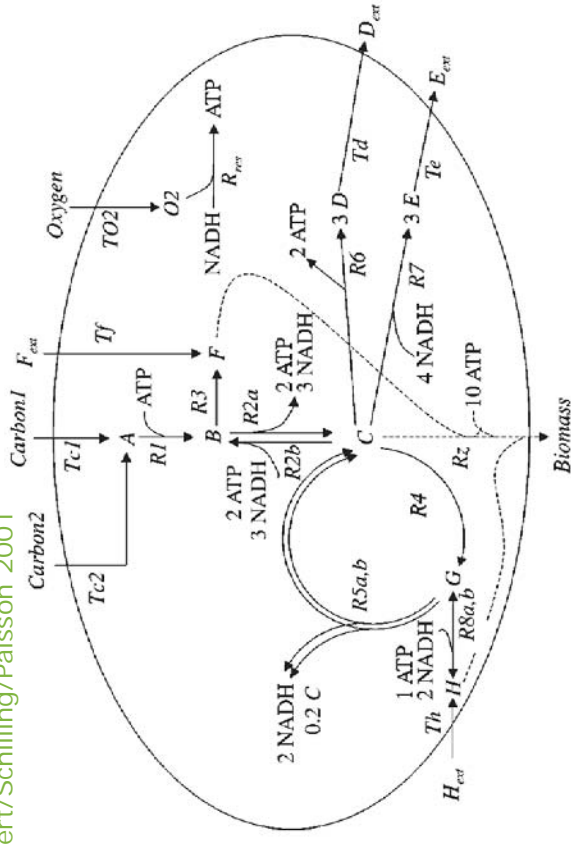
$$v_k(t) = 0 \text{ when } t \in [t_1, t_2].$$

Mathematical model I

| | |
|---|--------|
| <i>Metabolic reactions</i> | |
| -1 A + 1 ATP + 1 B | R1 |
| -1 B + 2 ATP + 2 NADH + 1 C | R2a |
| -1 C - 2 ATP - 2 NADH + 1 B | R2b |
| -1 B + 1 F | R3 |
| -1 C + 1 G | R4 |
| -1 G + 0.8 C + 2 NADH | R5a |
| -1 G + 0.8 C + 2 NADH | R5b |
| -1 C + 2 ATP + 3 D | R6 |
| -1 C - 4 NADH + 3 E | R7 |
| -1 G - 1 ATP - 2 NADH + 1 H | R8a |
| + 1 G + 1 ATP + 2 NADH - 1 H | R8b |
| -1 NADH - 1 O ₂ + 1 ATP | Rres |
| <i>Transport processes</i> | |
| -1 Carbon1 + 1 A | Tc1 |
| -1 Carbon2 + 1 A | Tc2 |
| -1 F _{ext} + 1 F | Tf |
| -1 D + 1 D _{ext} | Td |
| -1 E + 1 E _{ext} | Te |
| -1 H _{ext} + 1 H | Th |
| -1 Oxygen + 1 O ₂ | To2 |
| <i>Maintenance and growth processes</i> | |
| -1 C - 1 F - 1 H - 10 ATP + 1 Biomass | Growth |

Simplified core carbon metabolism

Covert/Schilling/Palsson 2001



Preferential carbon source uptake

Assume Carbon1 to be the preferred carbon source.

Presence of extracellular Carbon1 activates a regulatory protein which inhibits the transcription of the gene which encodes a protein for transport of Carbon2 into the cell, via a transport process Tc2.

RPC1 is the regulatory protein which senses extracellular Carbon1,

tTc2 is the occurrence of a transcription event (which will eventually result in the protein enabling transport process Tc2 and the relaxation of one regulatory constraint, $v_{Tc2} = 0$).

$$\begin{aligned} \text{RPC1} &= \text{IF (Carbon1)} \\ \text{tTc2} &= \text{IF NOT (RPC1)} \end{aligned}$$

Anaerobic growth

The transcription of many enzymes is regulated according to whether or not oxygen is available to the cell

Here, the presence of Oxygen will inactivate regulatory protein RPO2, which inhibits transcription of the genes for Rres and R5a but induces transcription of the gene for R5b.

R5a and R5b are reactions catalyzed by isozymes.

$$RPO2 = \text{IF NOT (Oxygen)}$$

$$tRres = \text{IF NOT (RPO2)}$$

$$tR5a = \text{IF NOT (RPO2)}$$

$$tR5b = \text{IF (RPO2)}.$$

Amino acid biosynthesis pathway repression

The transcription of amino acid biosynthesis genes is often induced by a low intracellular concentration.

Since intracellular concentrations cannot be determined by FBA, use fluxes to approximate the regulation.

Metabolite H represents the amino acid, and can be made by the cell via reaction R8a or transported from the extracellular media through transport process Th.

For the regulatory structure, Th will be used to activate RPh which will repress transcription of the gene encoding R8a.

$$RPh = \text{IF } (v_{Th} > 0),$$

$$tR8a = \text{IF NOT (RPh)}.$$

Maintain concentrations

Transcriptional regulation maintains concentration levels of important metabolites.

The activation or repression of these genes depends on the level of B in the cell.

Use a flux rather than concentration to turn off an enzyme.

Choose R2b as the determining factor; it will activate RPh which in turn will inactivate tR2a and tR7.

$$RPh = \text{IF } (v_{R2b} > 0),$$

$$tR2a = \text{IF NOT (RPh)},$$

$$tR7 = \text{IF NOT (RPh)}.$$

Mathematical model II

| Reaction | Name | Regulation |
|---|--------|---------------------------|
| <i>Metabolic reactions</i> | | |
| -1 A - 1 ATP + 1 B | R1 | |
| -1 B + 2 ATP + 2 NADH + 1 C | R2a | IF NOT(RPh) |
| -1 C - 2 ATP - 2 NADH + 1 B | R2b | |
| -1 B + 1 F | R3 | |
| -1 C + 1 G | R4 | |
| -1 G + 0.8 C + 2 NADH | R5a | IF NOT (RPO2) |
| -1 G + 0.8 C + 2 NADH | R5b | IF RPO2 |
| -1 C + 2 ATP + 3 D | R6 | |
| -1 C - 4 NADH + 3 E | R7 | IF NOT (RPh) |
| -1 G - 1 ATP - 2 NADH + 1 H | R8a | IF NOT (RPh) |
| +1 G + 1 ATP + 2 NADH - 1 H | R8b | IF NOT (RPO2) |
| -1 NADH - 1 O2 + 1 ATP | Rres | |
| <i>Transport processes</i> | | |
| -1 Carbon1 + 1 A | Tc1 | |
| -1 Carbon2 + 1 A | Tc2 | IF NOT(RPh) |
| -1 F _{ext} + 1 F | Tf | |
| -1 D + 1 D _{ext} | Td | |
| -1 E + 1 E _{ext} | Te | |
| -1 H _{ext} + 1 H | Th | |
| -1 Oxygen + 1 O2 | To2 | |
| <i>Maintenance and growth processes</i> | | |
| -1 C - 1 F - 1 H - 10 ATP + 1 Biomass | Growth | |
| <i>Regulatory proteins</i> | | |
| | RPO2 | IF NOT(Oxygen) |
| | RPh | IF Carbon1 |
| | RPh | IF (v _{Rh} > 0) |
| | RPh | IF (v _{R2b} > 0) |

Generating dynamic profiles (rFBA)

Covert/Schilling/Palsson 2001

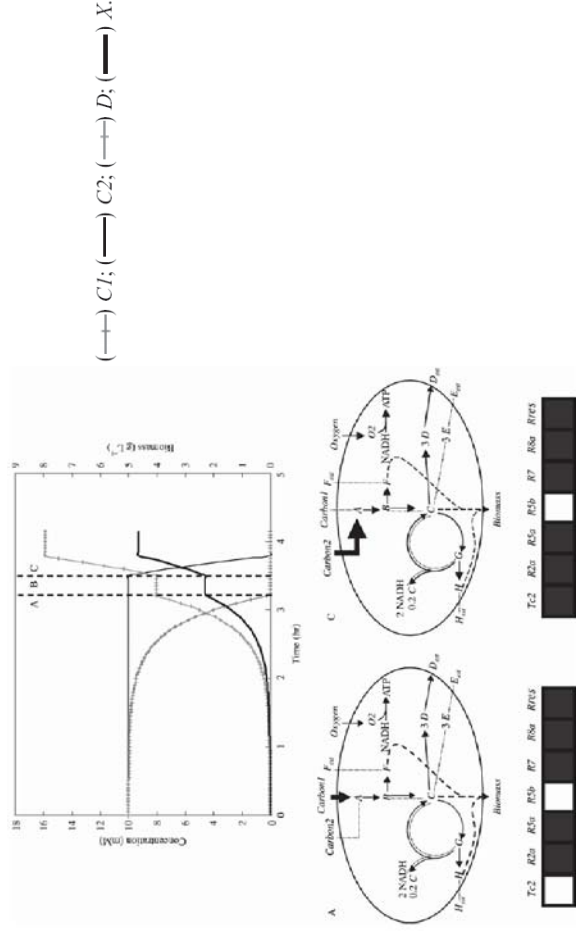
Divide experimental time into small time steps Δt .

At a given time point, use linear programming to identify an optimal metabolic flux distribution (by maximizing the Growth flux).

Using the resulting flux distribution and the conditions of the system in a previous time step, the conditions of the next time step are calculated to obtain biomass as well as extracellular substrate and by-product concentrations.

Not considered: Variability of the optimal flux distribution

1. Catabolite repression

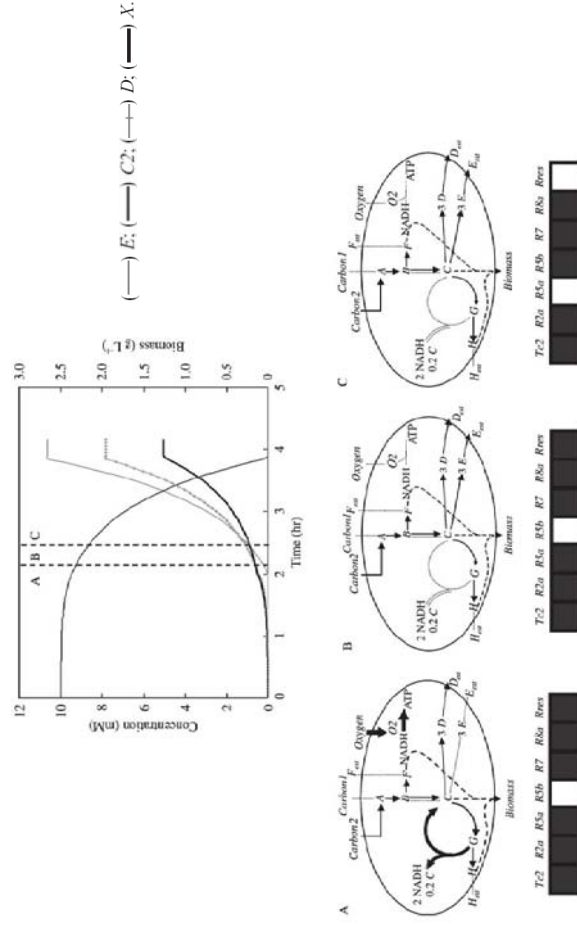


Numerical parameter values

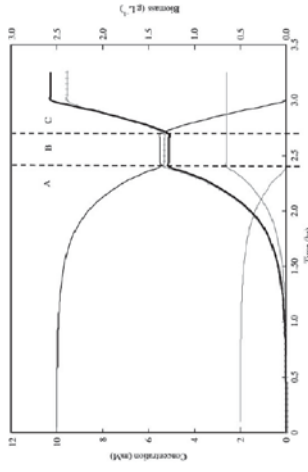
| Parameter | Value |
|---|-------|
| Maximum transport rates ($\text{mmol g-DCW}^{-1} \text{hr}^{-1}$) | |
| Carbon1 | 10.5 |
| Carbon2 | 10.5 |
| D | 12.0 |
| E | 12.0 |
| F | 5.0 |
| H | 5.0 |
| O2 | 15.0 |
| Protein synthesis/decay delay (hr) | 0.25 |

➡ five simulations to illustrate each regulatory element separately and in a complex medium

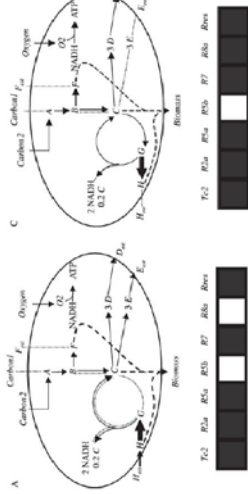
2. Aerobic/anaerobic diauxie



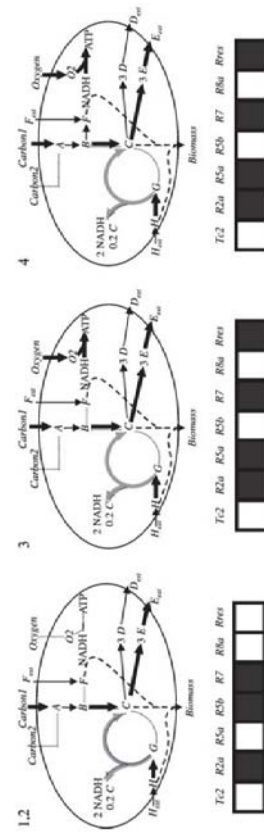
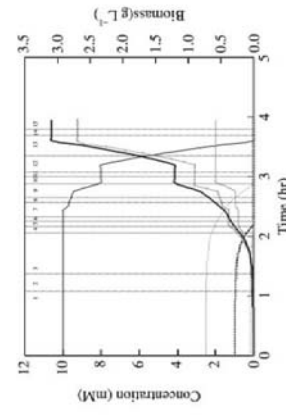
3. Amino acid biosynthesis



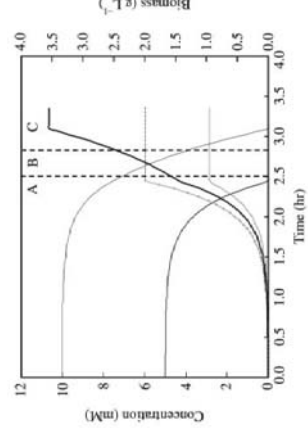
(\longrightarrow) C2; (\dashrightarrow) D; (\dashrightarrow) E; (\dashrightarrow) H; (\dashrightarrow) X.



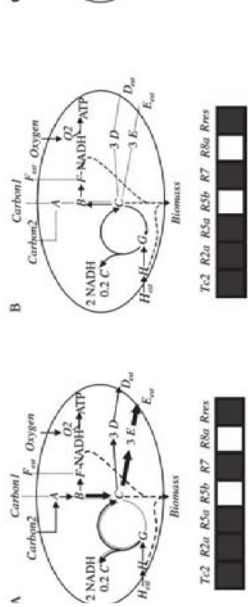
5. Growth on complex media



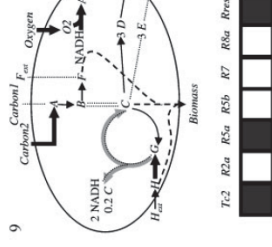
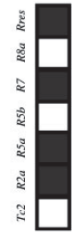
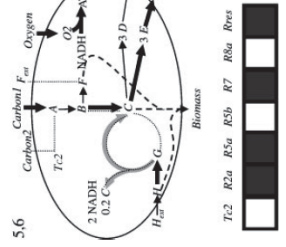
4. Growth on carbon and amino acid



(\longrightarrow) C2; (\dashrightarrow) D;
(\dashrightarrow) E; (\dashrightarrow) H; (\dashrightarrow) X.



5. Growth on complex media (ctd)



Growth on C1, C2 and O2

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 21 | 22 | 23 | 24 |
| 5 | 6 | 7 | 8 | 25 | 26 | 27 | 28 |
| 9 | 10 | 11 | 12 | 29 | 30 | 31 | 32 |
| 13 | 14 | 15 | 16 | 33 | 34 | 35 | 36 |
| 17 | 18 | 19 | 20 | 37 | 38 | 39 | 40 |
| 41 | 42 | 43 | 44 | 61 | 62 | 63 | 64 |
| 45 | 46 | 47 | 48 | 65 | 66 | 67 | 68 |
| 49 | 50 | 51 | 52 | 69 | 70 | 71 | 72 |
| 53 | 54 | 55 | 56 | 73 | 74 | 75 | 76 |
| 57 | 58 | 59 | 60 | 77 | 78 | 79 | 80 |

All possible extreme pathways

Inconsistent regulation
C1, C2 and O2

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 21 | 22 | 23 | 24 |
| 5 | 6 | 7 | 8 | 25 | 26 | 27 | 28 |
| 9 | 10 | 11 | 12 | 29 | 30 | 31 | 32 |
| 13 | 14 | 15 | 16 | 33 | 34 | 35 | 36 |
| 17 | 18 | 19 | 20 | 37 | 38 | 39 | 40 |
| 41 | 42 | 43 | 44 | 61 | 62 | 63 | 64 |
| 45 | 46 | 47 | 48 | 65 | 66 | 67 | 68 |
| 49 | 50 | 51 | 52 | 69 | 70 | 71 | 72 |
| 53 | 54 | 55 | 56 | 73 | 74 | 75 | 76 |
| 57 | 58 | 59 | 60 | 77 | 78 | 79 | 80 |

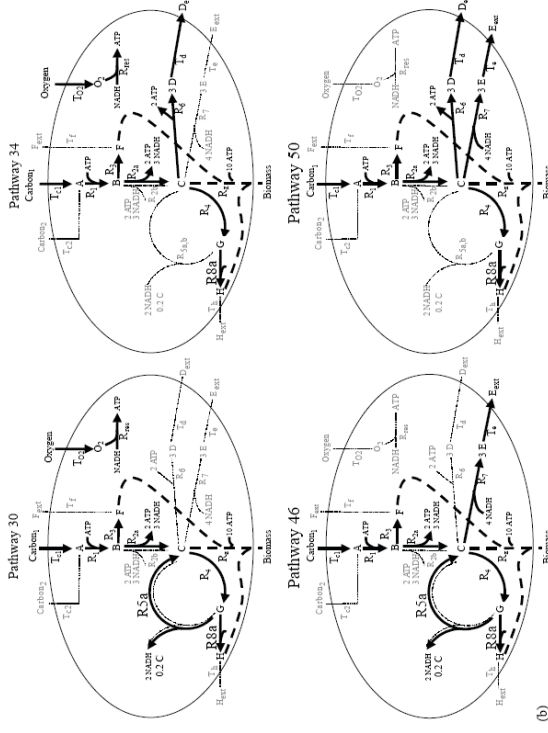
Environment-specificity:
C1, C2 and O2

| | | | | | | | |
|----|----|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 21 | 22 | 23 | 24 |
| 5 | 6 | 7 | 8 | 25 | 26 | 27 | 28 |
| 9 | 10 | 11 | 12 | 29 | 30 | 31 | 32 |
| 13 | 14 | 15 | 16 | 33 | 34 | 35 | 36 |
| 17 | 18 | 19 | 20 | 37 | 38 | 39 | 40 |
| 41 | 42 | 43 | 44 | 61 | 62 | 63 | 64 |
| 45 | 46 | 47 | 48 | 65 | 66 | 67 | 68 |
| 49 | 50 | 51 | 52 | 69 | 70 | 71 | 72 |
| 53 | 54 | 55 | 56 | 73 | 74 | 75 | 76 |
| 57 | 58 | 59 | 60 | 77 | 78 | 79 | 80 |

Environment-specific regulation:
K3b, Tc2

| | |
|----|----|
| 30 | 34 |
| 42 | 46 |
| 50 | 58 |
| 62 | 70 |
| 74 | 78 |

Four remaining extreme pathways



A. Bockmayr, Metabolische Netzwerke, SS 16, 23.6.2016

Growth on C1, C2, F, H and O2

| P# | Group 1 | | Group 2 | |
|----|---------|---------|---------|---------|
| | Biomass | Biomass | Biomass | Biomass |
| 8 | 0.283 | 0.283 | 0.277 | 0.277 |
| 29 | 0.717 | 0.283 | 0.277 | 0.277 |
| 32 | 0.435 | 0.283 | 0.277 | 0.277 |
| 5 | 0.277 | 1.000 | 0.268 | 0.268 |
| 6 | 1.000 | 0.723 | 0.262 | 0.262 |
| 30 | 0.723 | 0.277 | 0.252 | 0.252 |
| 31 | 0.277 | 0.277 | 0.227 | 0.227 |
| 9 | 0.262 | 1.000 | 0.217 | 0.217 |
| 12 | 0.252 | 0.738 | 0.188 | 0.188 |
| 10 | 0.773 | 0.746 | 1.075 | 1.075 |
| 33 | 0.545 | 0.227 | 0.750 | 0.750 |
| 36 | 1.000 | 0.227 | 0.750 | 0.750 |
| 34 | 0.783 | 0.217 | 0.794 | 0.794 |
| 4 | 0.170 | 0.813 | 0.964 | 0.964 |
| 49 | 0.675 | 0.125 | 1.964 | 1.964 |
| 52 | 1.000 | 0.125 | 2.011 | 2.011 |
| 50 | 0.882 | 0.118 | 2.011 | 2.011 |
| 51 | 0.357 | 0.560 | 2.011 | 2.011 |
| 38 | 0.917 | 0.083 | 2.011 | 2.011 |
| 45 | 0.833 | 0.083 | 2.011 | 2.011 |
| 48 | 0.429 | 0.571 | 2.011 | 2.011 |
| 37 | 1.000 | 0.077 | 2.011 | 2.011 |
| 46 | 0.923 | 0.077 | 2.011 | 2.011 |
| 47 | 0.923 | 0.077 | 2.011 | 2.011 |

- 26 extreme pathways, four groups with high similarity
- small degree of variation, once regulatory constraints are taken into account.

A. Bockmayr, Metabolische Netzwerke, SS 16, 23.6.2016

References

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- Regulation of gene expression in flux balance models of metabolism. Covert MW, Schilling CH, Palsson BO. J Theor Biol. 2001 Nov 7;213(1):73-88.
- Transcriptional regulation in constraints-based metabolic models of *Escherichia coli*. Covert MW, Palsson BO. J Biol Chem. 2002 Aug 2;277(31):28058-64.
- Constraints-based models: regulation of gene expression reduces the steady-state solution space. Covert MW, Palsson BO. J Theor Biol. 2003 Apr 7;221(3):309-25.

A. Bockmayr, Metabolische Netzwerke, SS 16, 23.6.2016