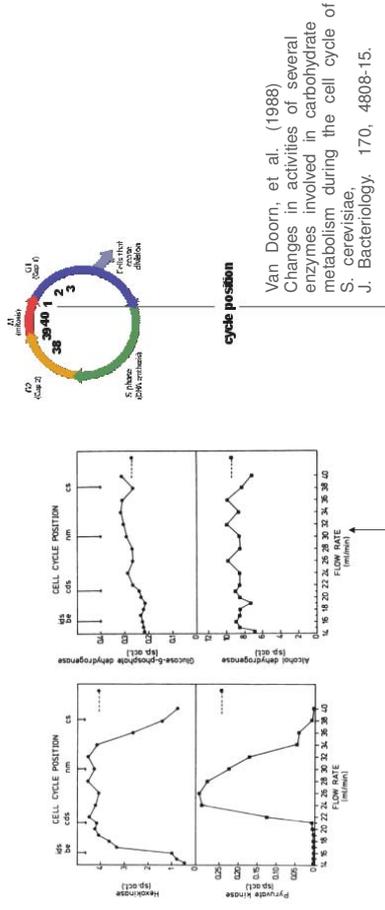




10. Sequential metabolic phases

- ▷ Metabolism adapts to changing environments.
- ▷ Even in constant environments, metabolism exhibits changes in enzyme activity.
- ▷ Significant metabolic alterations during cell cycle.



Van Doorn, et al. (1988)
Changes in activities of several enzymes involved in carbohydrate metabolism during the cell cycle of *S. cerevisiae*,
J. Bacteriology, 170, 4808-15.



When is this possible ?

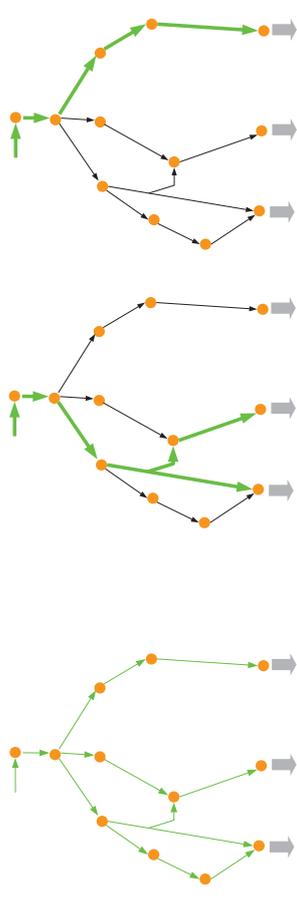
- ▷ Assume metabolic network with internal metabolites $M^\#$ and output metabolites M^* , for which there is a demand Γ .
- ▷ A flux mode v which is executed for some time $\tau \geq 0$ satisfies the demand if $\tau \cdot S^* v \geq \Gamma$.
- ▷ Two flux modes v^1, v^2 which are executed consecutively for time τ_1 resp. τ_2 satisfy the demand if $\tau_1 \cdot S^* v^1 + \tau_2 \cdot S^* v^2 \geq \Gamma$.
- ▷ Can we be more efficient with two flux modes v^1, v^2 than with one flux mode v ?
- ▷ In an FBA context, this is not possible, since we may always choose $v = \frac{\tau_1}{\tau_1 + \tau_2} v^1 + \frac{\tau_2}{\tau_1 + \tau_2} v^2$, which has the same efficiency $\tau = \tau_1 + \tau_2$.



Working hypothesis

Palinkas/Builik/Bockmayr/Holzhtütter 15

Switching between pathways may improve overall efficiency:

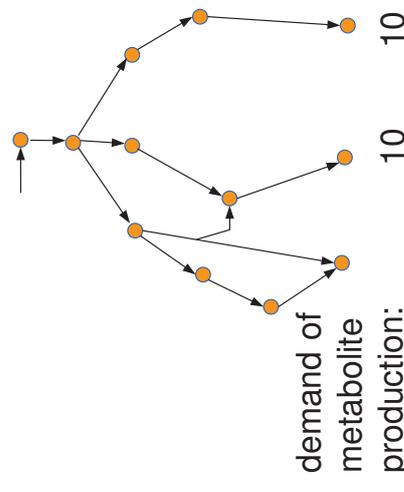


The output is produced by a single flux mode, using all reactions. Two consecutive flux modes produce the output with higher flux rates.



Illustrating the principle

Assume $0 \leq v \leq ub / |supp(v)|$, i.e., flux bound depends on number of active reactions.



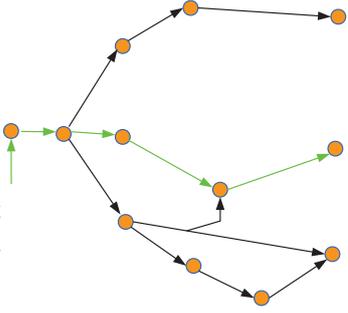


Illustrating the principle

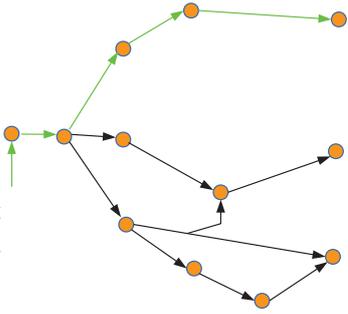
Assume flux bounds $0 \leq v \leq ub/|supp(v)|$, here $ub = 40$.

$$\frac{ub}{|supp(v^1)|} = \frac{40}{5} = 8$$

$$\frac{ub}{|supp(v^2)|} = \frac{40}{5} = 8$$



output: 8



output: 8

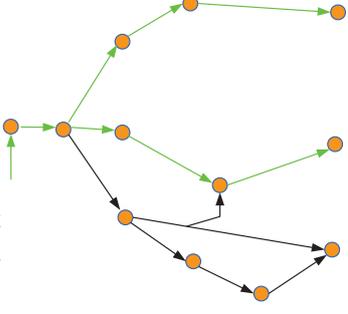
To satisfy demand, need $1.25 \cdot v^1 + 1.25 \cdot v^2$, i.e., $\tau = 2.5$.



Illustrating the principle

Assume flux bounds $0 \leq v \leq ub/|supp(v)|$, here $ub = 40$.

$$\frac{ub}{|supp(v^3)|} = \frac{40}{8} = 5$$



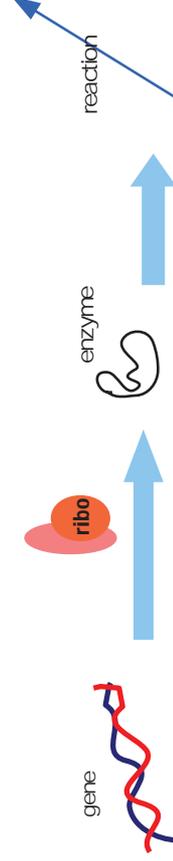
output: 2.5

output: 2.5

To satisfy demand, need $4 \cdot v^3$, i.e., $\tau = 4$.



Modeling approach



gene expression g_j

enzyme amount E_j

reaction flux $v_j \leq k_{c_j} \cdot E_j$

Basic assumption: Total amount of protein in the cell is limited.



Sharing available enzymes

▷ Synthesis and degradation of j -th enzyme:

$$dE_j/dt = g_j k_{s_j} A - k_{d_j} E_j,$$

where A is the total mass of free amino acids.

▷ Steady-state condition:

$$E_j = g_j A \eta_j, \text{ with } \eta_j = k_{s_j}/k_{d_j}.$$

▷ Assume **fixed total mass of amino acids**

$$A_{tot} = A + \sum E_i \gamma_i \left[= A + \sum (g_i A \eta_i) \gamma_i = A(1 + \sum g_i \gamma_i \eta_i) \right],$$

where γ_i is the molecular mass of the i -th enzyme.

▷ Resulting **upper bound**:

$$v_j \leq k_{c_j} \cdot E_j = k_{c_j} \cdot A_{tot} \frac{g_j \eta_j}{1 + \sum_i g_i \gamma_i \eta_i}$$



Optimizing cellular output

- ▷ Internal metabolites $M^\#$, output metabolites M^* , all reactions irreversible.
- ▷ Assume cell has to produce a certain amount Γ of output metabolites M^* .
- ▷ Allow using up to l flux modes v^1, \dots, v^l in l consecutive time intervals of length τ_1, \dots, τ_l .
- ▷ Output in k -th time interval: $\tau_k \cdot S^* \cdot v^k$
- ▷ **Goal:** Minimize the total time $\tau_1 + \dots + \tau_l$ needed to produce Γ .
- ▷ Lower bounds $lb \geq 0$ (minimum activity of certain reactions).



Non-linear mixed 0-1 optimisation problem

$$\min \sum_{k=1}^l \tau_k$$

$$S^\# v^k = 0, \quad \sum_{k=1}^l \tau_k S^* v^k \geq \Gamma,$$

$$lb_j \leq v_j^k \leq v_j^k \leq kc_j \cdot A_{tot} \frac{g_j \eta_j}{1 + \sum_i g_i \eta_i},$$

$$l \in \mathbb{N}, k = 1, \dots, l, j = 1, \dots, n,$$

$$\tau_k \in \mathbb{R}_{\geq 0}, v^k \in \mathbb{R}^n, g^k \in \{0, 1\}^n.$$

↪ can be reformulated as mixed 0-1 quadratic program (MIQP).

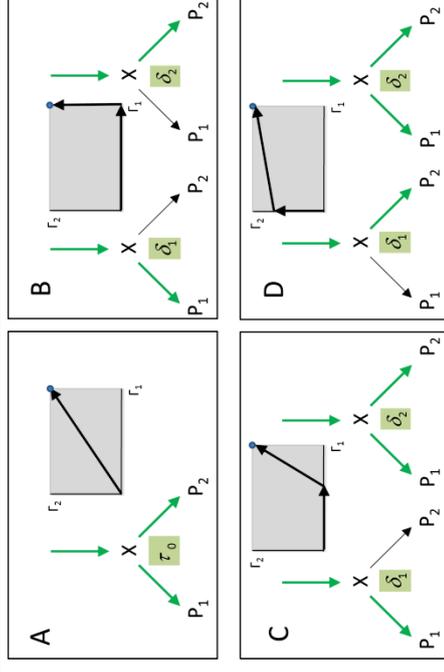


Modeling gene expression levels

- ▷ $g \in [0, 1]^n$: fine tuning of genes (similar to FBA with Molecular Crowding, cf. Beg et al. 2007)
 - ↪ feasible region is convex
 - ↪ no improvement possible through switching
- ▷ $g \in \{0, 1\}^n$: genes switched on or off individually.
- ▷ $g \in \mathcal{U} \subset \{0, 1\}^n$: genes switched on or off in functional groups, biologically more meaningful (e.g. common transcription factors)
 - ↪ may use **MinModes** (Hoffmann et al. 2006)



Toy example

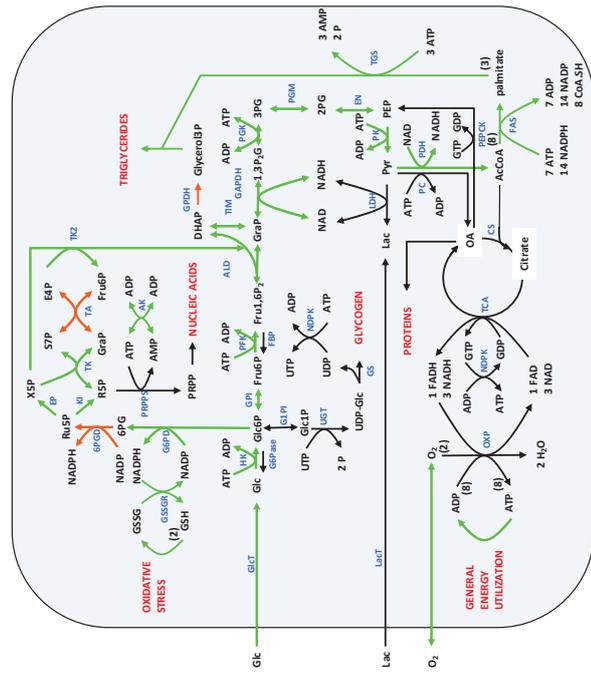
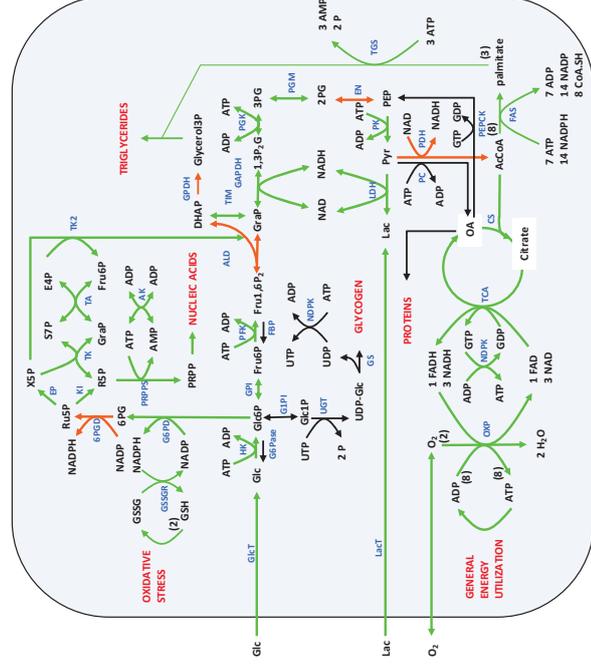
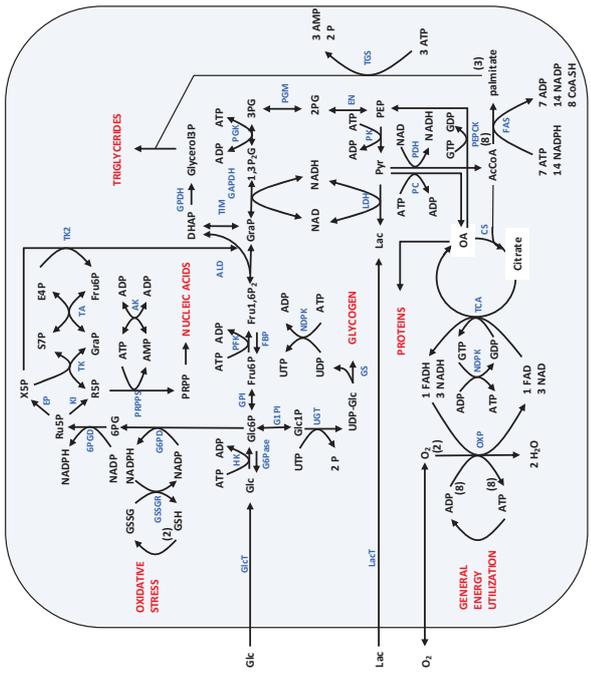


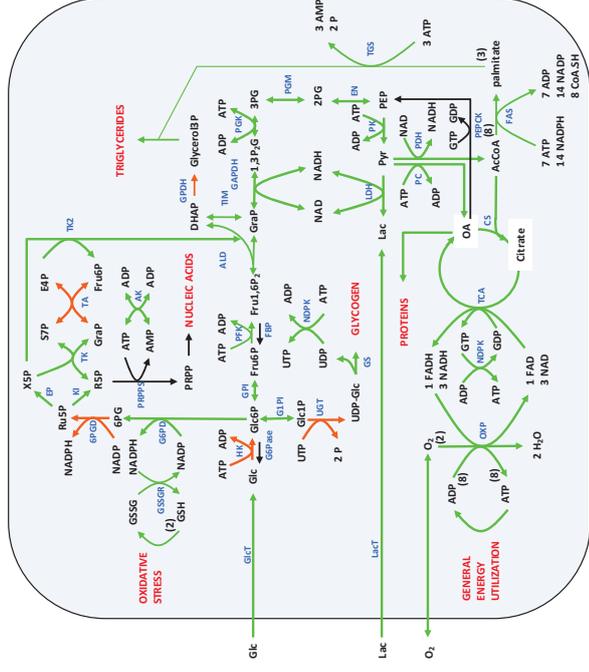
- ▷ Improvement by switching depends on parameter values.
- ▷ Random sampling gives improvement in 83% of the cases.
- ▷ Higher improvement if more protein is spent downstream.



Switching MinModes On and Off

Sub	/	Σ	τ_1	τ_2	τ_3	τ_4	# reaction switches	# active reactions
glc,	1	7.405h	2.302h	3.242h			0	41
lac	2	5.544h	2.429h	0.176h	2.201h		14	29,43
	3	4.805h	2.214h	0.098h	2.362h		26 (8/8/10)	35,41,29
	4	4.792h						29,37,41,35
glc	1	10.700h	0.203h	7.764h			0	41
	2	7.967h	0.203h	5.832h	1.559h		9	38,35
	3	7.631h	0.239h	5.832h	6.029h		11 (8/3)	39,35,32
	4	7.607h	0.071h	1.386h	0.121h		14 (4/3/7)	32,32,35,36
lac	1	7.405h	0.728h	5.214h			0	41
	2	5.942h	2.688h	2.429h	0.728h		7	40,35
	3	5.845h	0.210h	2.688h	2.429h		9 (2/7)	33,35,40
	4	5.764h			0.437h		10 (3/2/5)	36,33,35,38





- ▷ Results depend on the parameter values, but the benefit of switching is persistent.
- ▷ No improvement if feasible region is convex.
- ▷ MinMode solutions ($U \subsetneq \{0, 1\}^n$) biologically more realistic.
- ▷ Number of pathways in an optimal solution can be bound by number of output metabolites ($I \leq |M^*|$).
- ▷ MIQP remains difficult to solve.

