Resource allocation in metabolic networks: kinetic optimization and approximations by FBA (Supplementary material)

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1 Definitions

1.1 Kinetic metabolic networks

We consider a kinetic metabolic network with n metabolites and r reactions. In particular, a stoichiometric matrix $N \in \mathbb{R}^{n\times r}$, metabolite concentrations $x \in \mathbb{R}^n_{\geq}$, enzyme concentrations $c \in \mathbb{R}^r_{\geq}$, and reaction rates

$$v_i = c_i\, \kappa_i(x,p), \quad i = 1,\dots,r.$$

The kinetic functions $\kappa_i(x,p)$ of reactions $i = 1,\dots,r$ depend on metabolite concentrations x and parameters p. The vector p includes k_{cat}, K_{m}, and K_{equ} values as well as external metabolite concentrations.

1.2 Dynamics

Using the component-wise product $\circ$, we write $v = c \circ \kappa(x,p)$ and obtain the dynamical system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = Nv = N(c \circ \kappa(x,p)).$$

1.3 Enzyme constraint

In the following, we maximize the steady-state rate of a target reaction under a biophysical enzyme constraint,

$$\sum_{i=1}^{r} w_i\, c_i \leq W$$

with $w \in \mathbb{R}^r_{>}$ and $W > 0$. In the sum over all enzymatic reactions, the weights w_i are the resources needed per unit enzyme concentration, and W is the available amount of the resource.

2 Example

2.1 Metabolic network

We consider a minimal metabolic network for the study of fermentation vs. aerobic respiration:

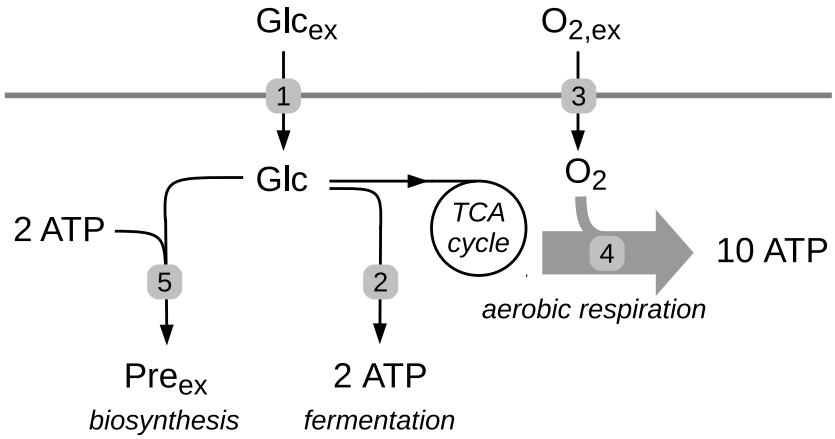

Figure 1: A minimal metabolic network: two exchange reactions (1,3), two intracellular conversions (2,4), and the formation of a precursor molecule (5). Reprinted from [1].

The network involves three internal metabolites (glucose Glc, oxygen O_2, and ATP) and five reactions:

$$\begin{aligned}
1: &\quad \mathrm{Glc_{ex}} &&\rightleftharpoons \mathrm{Glc}\\
2: &\quad \mathrm{Glc} &&\rightleftharpoons 2\,\mathrm{ATP}\\
3: &\quad \mathrm{O_{2,ex}} &&\rightleftharpoons \mathrm{O_2}\\
4: &\quad \mathrm{Glc} + \mathrm{O_2} &&\rightleftharpoons 10\,\mathrm{ATP}\\
5: &\quad \mathrm{Glc} + 2\,\mathrm{ATP} &&\rightarrow \mathrm{Pre_{ex}}
\end{aligned}$$

2.2 Dynamics

The dynamical system amounts to:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = N(c \circ \kappa(x,p)) = \begin{pmatrix} +1 & -1 & 0 & -1 & -1 \\ 0 & 0 & +1 & -1 & 0 \\ 0 & +2 & 0 & +10 & -2 \end{pmatrix} \begin{pmatrix} c_1 \cdot \kappa_1(x,p) \\ c_2 \cdot \kappa_2(x,p) \\ c_3 \cdot \kappa_3(x,p) \\ c_4 \cdot \kappa_4(x,p) \\ c_5 \cdot \kappa_5(x,p) \end{pmatrix}$$

with

$$\begin{aligned} x &= ([\mathrm{Glc}], [\mathrm{O_2}], [\mathrm{ATP}])^T \\ p &= ([\mathrm{Glc_{ex}}], [\mathrm{O_{2,ex}}], \ldots)^T \end{aligned}$$

2.3 Kinetics

In contrast to [1], we assume Michaelis-Menten kinetics:

$$\kappa_1 = \frac{[\mathrm{Glc_{ex}}] - [\mathrm{Glc}]}{10 + [\mathrm{Glc_{ex}}] + [\mathrm{Glc}]}$$
$$\kappa_2 = \frac{[\mathrm{Glc}] - [\mathrm{ATP}]/10}{10 + [\mathrm{Glc}] + [\mathrm{ATP}]}$$
$$\kappa_3 = \frac{[\mathrm{O_{2,ex}}] - [\mathrm{O_2}]}{1 + [\mathrm{O_{2,ex}}] + [\mathrm{O_2}]}$$
$$\kappa_4 = \frac{[\mathrm{Glc}][\mathrm{O_2}] - [\mathrm{ATP}]/10}{(10 + [\mathrm{Glc}])(1 + [\mathrm{O_2}]) + [\mathrm{ATP}]}$$
$$\kappa_5 = \frac{[\mathrm{Glc}][\mathrm{ATP}]}{(10 + [\mathrm{Glc}])(10 + [\mathrm{ATP}])}$$

k_{cat} values (for the forward directions) are set to 1, whereas K_{m} and K_{equ} values are either 1 or 10.

2.4 Enzyme allocation

We consider the following enzyme allocation problem:

"For given external metabolite concentrations,
maximize the rate of biosynthesis by varying metabolite and enzyme concentrations
at steady state
for limited enzyme."

More formally, we solve a non-linear optimization problem (given a certain input).

Input:
stoichiometric matrix N, kinetics κ incl. parameters $p = ([\mathrm{Glc_{ex}}], [\mathrm{O_{2,ex}}], \ldots)^T$;
target reaction 5;
weights $w \in \mathbb{R}^5_>$ and resource $W > 0$ for enzyme constraint;

Kinetic optimization:

$$\max_{x,c} v_5, \tag{1a}$$

where

$$v = c \circ \kappa(x, p), \tag{1b}$$
$$x \in \mathbb{R}^3_{\geq}, \; c \in \mathbb{R}^5_{\geq}, \tag{1c}$$

subject to

$$Nv = 0, \tag{1d}$$
$$\textstyle\sum_{i=1}^{5} w_i \, c_i \leq W. \tag{1e}$$

Theorem: Optimal solutions are EFMs. [1, 3]

In fact, the network has 2 EFMs with non-zero rate of biosynthesis (normalized to 1): $e^1 = (2, 1, 0, 0, 1)^T$ corresponding to pure fermentation (low yield) and $e^2 = \left(\frac{6}{5}, 0, \frac{1}{5}, \frac{1}{5}, 1\right)^T$ corresponding to pure respiration (high yield).

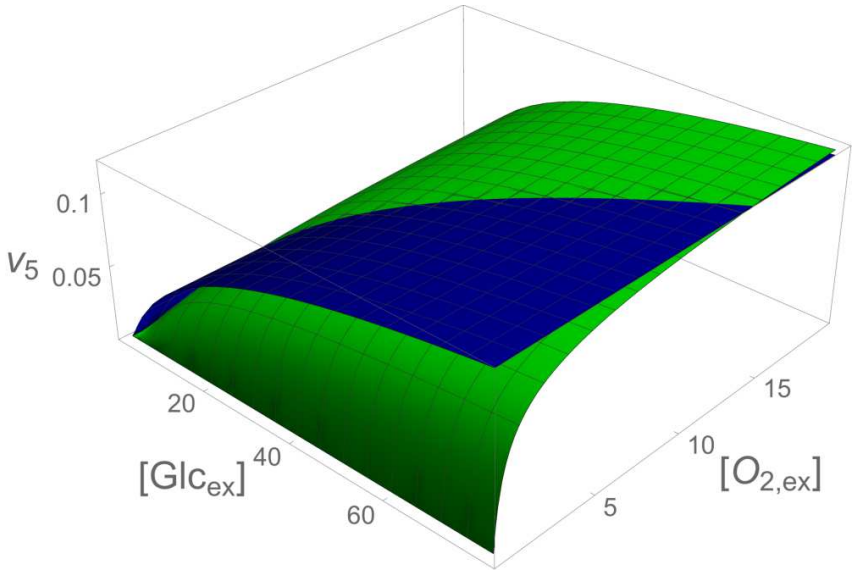

Figure 2: Maximal rate of biosynthesis v_5 as a function of external metabolite concentrations $[Glc_{ex}]$ and $[O_{2,ex}]$ for EFM e^1 (pure fermentation) and EFM e^2 (pure respiration).

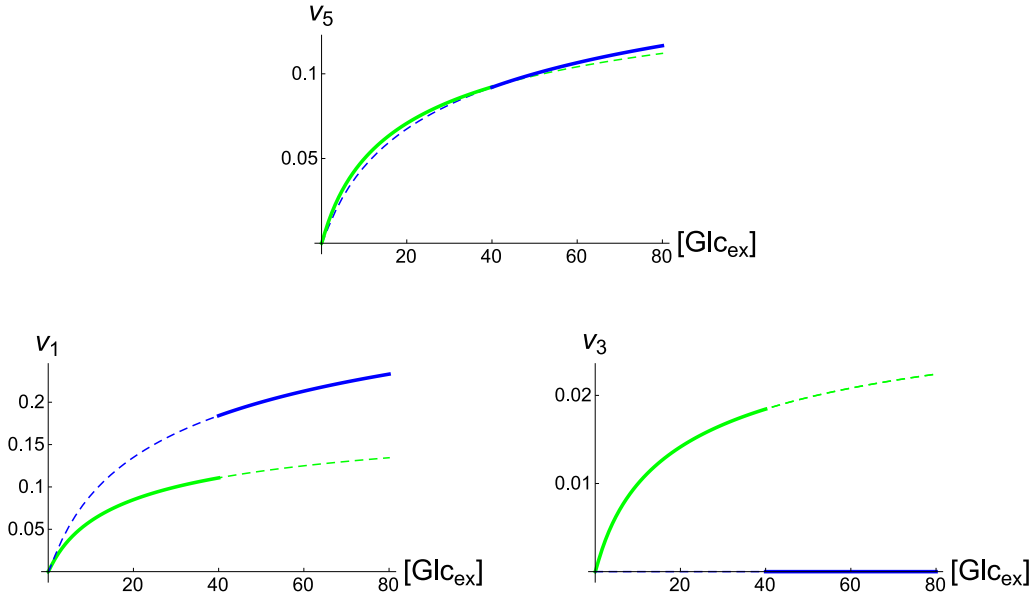

Figure 3: Optimal fluxes as functions of external glucose concentration $[Glc_{ex}]$ (at fixed external oxygen concentration $[O_{2,ex}] = 10$) for EFM e^1 (pure fermentation) and EFM e^2 (pure respiration).

For increasing external glucose, the optimal solution switches from pure respiration (high yield) to pure fermentation (low yield). As a consequence, our theoretical result applied to the minimal network explains the occurrence of low-yield pathways such as the Crabtree and Warburg effects.

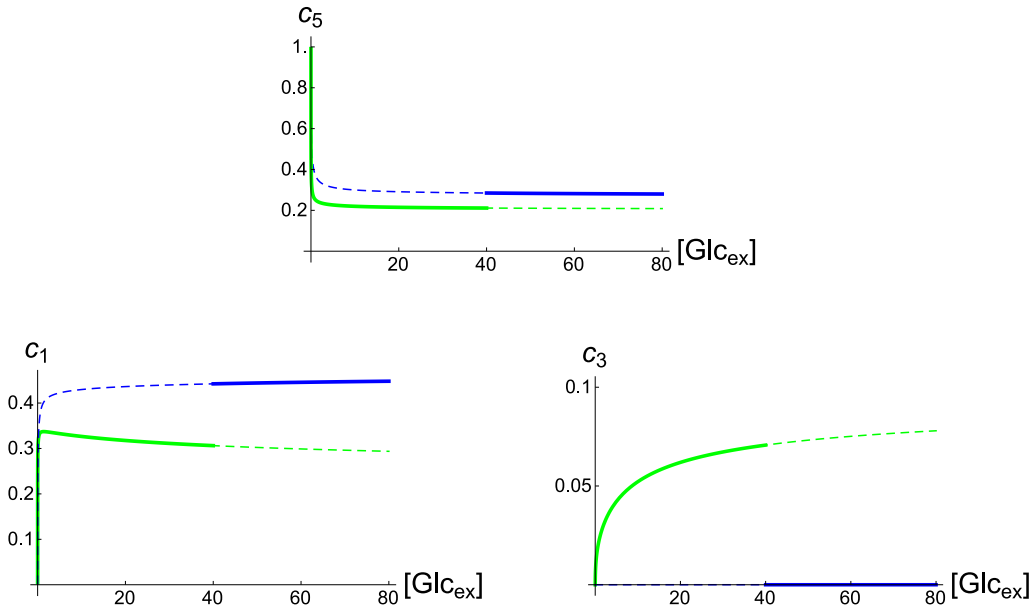

Figure 4: Optimal enzyme concentrations as functions of external glucose concentration [Glc$_{ex}$] (at fixed external oxygen concentration [O$_{2,ex}$] = 10) for EFM e^1 (pure fermentation) and EFM e^2 (pure respiration).

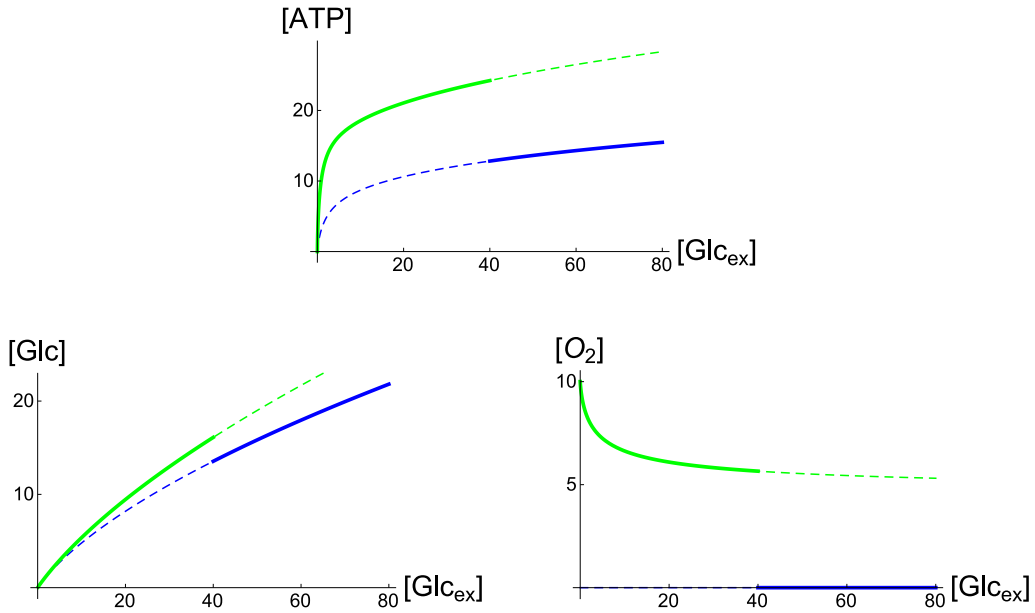

Figure 5: Optimal metabolite concentrations as functions of external glucose concentration [Glc$_{ex}$] (at fixed external oxygen concentration [O$_{2,ex}$] = 10) for EFM e^1 (pure fermentation) and EFM e^2 (pure respiration).

3 Theory

3.1 Kinetic model with enzyme constraint

Input:
stoichiometric matrix N, kinetics κ incl. parameters p;
target reaction i^*;
weights $w \in \mathbb{R}^r_>$ and resource $W > 0$ for enzyme constraint;
region $X \subseteq \mathbb{R}^n_\geq$ for metabolite constraint;

Kinetic optimization:

$$\max_{x,c} v_{i^*}, \tag{2a}$$

where

$$v = c \circ \kappa(x, p), \tag{2b}$$
$$x \in X,\, c \in \mathbb{R}^r_\geq, \tag{2c}$$

subject to

$$Nv = 0, \tag{2d}$$
$$\textstyle\sum_i w_i\, c_i \leq W. \tag{2e}$$

Theorem: For arbitrary kinetics (incl. arbitrary allosteric regulation), optimal solutions are EFMs. [1, 3]

In particular, the Theorem predicts metabolic switches (between EFMs) and explains the occurrence of low-yield pathways (see Example).

3.2 Approximation: Purely stoichiometric model with flux constraint

Enzyme concentrations c (and parameters p) are not considered explicitly. (Logarithms of) metabolite concentrations x may appear in optional thermodynamic feasibility constraints.

A flux constraint can be motivated by the biophysical enzyme constraint, using k_{cat} as an upper bound for κ:

$$v_i = c_i\, \kappa_i(x, p) \leq c_i\, k_{\text{cat},i}$$

implies

$$\tilde{w}_i\, v_i = \frac{w_i}{k_{\text{cat},i}}\, v_i \leq w_i\, c_i$$

with

$$\tilde{w}_i = \frac{w_i}{k_{\text{cat},i}}.$$

Now,

$$\sum_i w_i\, c_i \leq W$$

implies

$$\sum_i \tilde{w}_i\, v_i \leq W.$$

However, the flux constraint is weaker than the enzyme constraint.

(Technical detail: all reversible reactions are split into forward- and backward-reactions to consider different k_{cat} values. Due to the flux constraint, only one direction carries flux, in the end.)

Input:
stoichiometric matrix N;
target reaction i^*;
scaled weights $\tilde{w} \in \mathbb{R}^r_>$ and resource $W > 0$ for flux constraint;
upper bounds $V \in \mathbb{R}^r_\geq$ for fluxes;

FBA:

$$\max_v v_{i^*} \tag{3a}$$

subject to

$$Nv = 0,\ v \geq 0, \tag{3b}$$
$$\textstyle\sum_i \tilde{w}_i\, v_i \leq W, \tag{3c}$$
$$v \leq V, \tag{3d}$$

and optional

$$\text{thermodynamic feasibility constraints.} \tag{3e}$$

3.3 Exchange reactions

In a kinetic model, we can vary external metabolite concentrations involved in the kinetics of exchange reactions and solve the resulting non-linear optimization problems. In the corresponding stoichiometric model, we can approximate the variation of these parameters by the variation of

1. upper bounds for exchange fluxes (standard FBA), or
2. saturation values for exchange reactions (satFBA).

For this purpose, we divide the set of reactions R into the sets of exchange reactions R_{ex} and internal reactions R_{in}:

$$R = R_{\text{ex}} \cup R_{\text{in}}$$

3.3.1 Upper bounds for exchange fluxes

Input:
stoichiometric matrix N;
target reaction i^*;
scaled weights $\tilde{w} \in \mathbb{R}^r_>$ and resource $W > 0$ for flux constraint;
(variable) upper bounds $V_i \geq 0$, $i \in R_{\text{ex}}$, for exchange fluxes;

Standard FBA:

$$\max_v v_{i^*} \tag{4a}$$

subject to

$$Nv = 0,\ v \geq 0, \tag{4b}$$
$$\textstyle\sum_i \tilde{w}_i\, v_i \leq W, \tag{4c}$$
$$v_i \leq V_i \text{ for } i \in R_{\text{ex}}. \tag{4d}$$

Result: In general, optimal solutions are combinations of EFMs, not single EFMs.

As a consequence, standard FBA does not predict switches between EFMs, but continuous transitions.

3.3.2 Saturation values for exchange reactions

In general, reactions are not saturated. In particular, for $i \in R_{\text{ex}}$, we have

$$v_i = c_i\, \kappa_i(x,p) \leq c_i\, k_{\text{cat,i}}.$$

We write

$$w_i\, c_i = \frac{w_i}{\kappa_i}\, v_i = \frac{\frac{w_i}{k_{\text{cat,i}}}}{\frac{\kappa_i}{k_{\text{cat,i}}}}\, v_i = \frac{\tilde{w}_i}{\phi_i}\, v_i$$

with

$$\phi_i = \frac{\kappa_i}{k_{\text{cat,i}}} \leq 1.$$

Now,

$$\textstyle\sum_i w_i\, c_i \leq W$$

implies

$$\sum_{i \in R_{\text{ex}}} \frac{\tilde{w}_i}{\phi_i}\, v_i + \sum_{i \in R_{\text{in}}} \tilde{w}_i\, v_i \leq W.$$

Cf. [2] for an informal argument. Using the concept of saturation values, we introduce a new variant of FBA called satFBA.

Input:
stoichiometric matrix N;
target reaction i^*;
scaled weights $\tilde{w} \in \mathbb{R}^r_>$ and resource $W > 0$ for flux constraint;
(variable) saturation values $\phi_i \leq 1$, $i \in R_{\text{ex}}$, for exchange reactions;

satFBA:

$$\max_v\ v_{i^*} \tag{5a}$$

subject to

$$Nv = 0,\ v \geq 0, \tag{5b}$$

$$\textstyle\sum_{i \in R_{\text{ex}}} \frac{\tilde{w}_i}{\phi_i}\, v_i + \sum_{i \in R_{\text{in}}} \tilde{w}_i\, v_i \leq W. \tag{5c}$$

Result: Optimal solutions are EFMs.

In other words, satFBA predicts a qualitatively correct result.

4 Example (revisited)

4.1 Kinetic model with enzyme constraint

In a kinetic model, enzyme concentrations c, metabolite concentrations x, and parameters p determine fluxes $v = c \circ \kappa(x, p)$. In the corresponding stoichiometric model, fluxes v are basic objects. Hence, we need to express results obtained by kinetic optimization in terms of fluxes, without using external metabolite concentrations.

In the example, $[\mathrm{Glc_{ex}}]$ appears in figures "v_5 vs. $[\mathrm{Glc_{ex}}]$" and "v_1 vs. $[\mathrm{Glc_{ex}}]$". For comparison with FBA, we show the figure "v_5 vs. v_1".

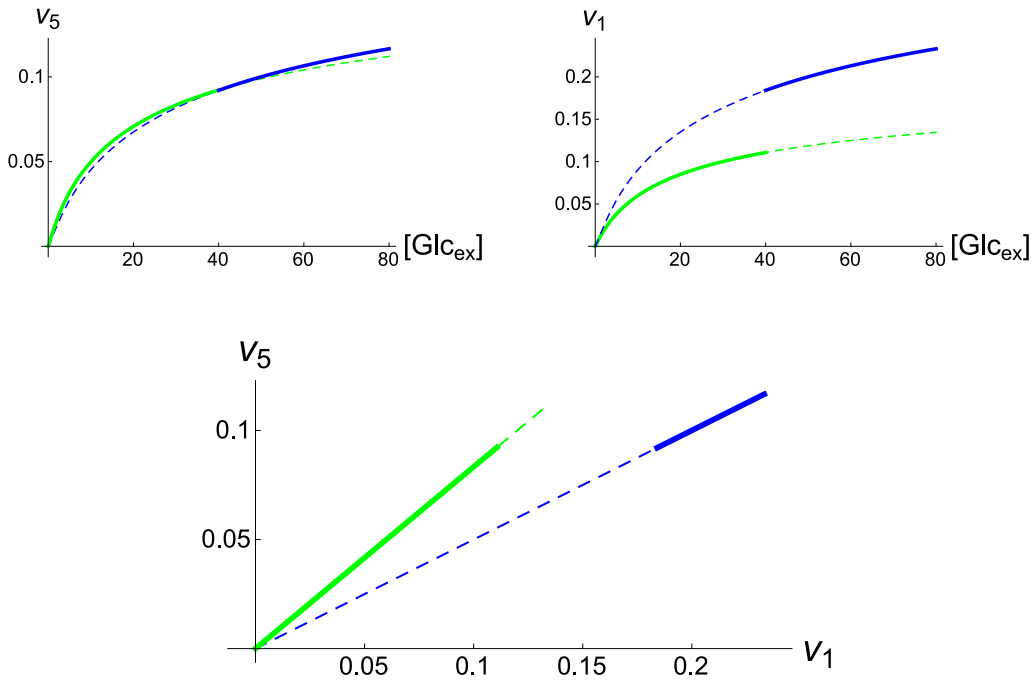

Figure 6: Optimal fluxes v_5 vs. v_1 (rate of biosynthesis vs. rate of glucose import). Optimal fluxes correspond to either EFM e^1 (pure fermentation) or EFM e^2 (pure respiration), but are not combinations of EFMs. (Results obtained by kinetic optimization, displayed for comparison with FBA.)

4.2 Approximation: Purely stoichiometric model with flux constraint

4.2.1 Upper bounds for exchange fluxes

Input:
stoichiometric matrix N;
target reaction 5;
scaled weights $\tilde{w} \in \mathbb{R}^5_>$ and resource $W > 0$ for flux constraint;
(variable) upper bounds $V_1, V_3 \geq 0$ for exchange fluxes;

Standard FBA:

$$\max_v \; v_5 \tag{6a}$$

subject to

$$Nv = 0,\; v \geq 0, \tag{6b}$$
$$\textstyle\sum_{i=1}^{5} \tilde{w}_i\, v_i \leq W, \tag{6c}$$
$$v_1 \leq V_1,\; v_3 \leq V_3. \tag{6d}$$

We assume $\tilde{w}_1 = \tilde{w}_2 = \tilde{w}_3 = \tilde{w}_5 = 1$ and either $\tilde{w}_4 = 1$ or 10. In other words, glucose import, fermentation, oxygen import, and biosynthesis have equal costs, whereas respiration has either equal or higher costs. In both cases, we vary V_1 (at fixed $V_3 = \infty$) and determine the optimal fluxes v_5 and v_1.

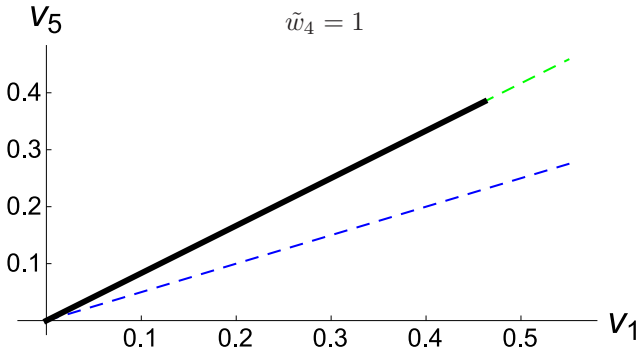

Figure 7: Optimal fluxes v_5 vs. v_1 (rate of biosynthesis vs. rate of glucose import). Results obtained by FBA. The costs of respiration $\tilde{w}_4 = 1$ equal the costs of other processes. Optimal fluxes correspond to EFM e^2 (pure respiration), but not to EFM e^1 (pure fermentation).

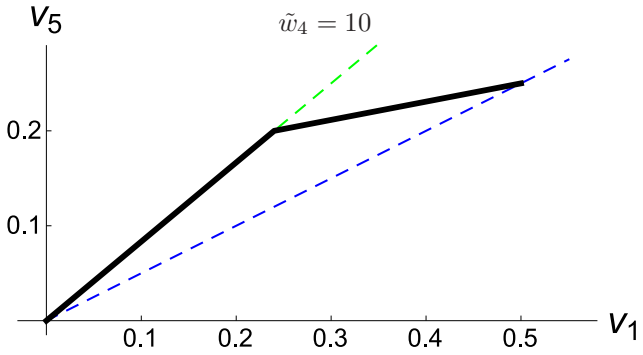

Figure 8: Optimal fluxes v_5 vs. v_1 (rate of biosynthesis vs. rate of glucose import). Results obtained by FBA. The costs of respiration $\tilde{w}_4 = 10$ are higher than the costs of other processes. Optimal fluxes correspond to either EFM e^2 (pure respiration) or combinations of EFM e^2 and EFM e^1 (pure fermentation).

Compare the results of kinetic optimization (Figure 6) and its approximation by satFBA (Figure 8). Standard FBA does not predict a switch between EFMs, but a continuous transition.

4.2.2 Saturation values for exchange reactions

Input:
stoichiometric matrix N;
target reaction 5;
scaled weights $\tilde{w} \in \mathbb{R}^5_>$ and resource $W > 0$ for flux constraint;
(variable) saturation values $\phi_1, \phi_3 \leq 1$ for exchange reactions;

satFBA:

$$\max_v \; v_5 \tag{7a}$$

subject to

$$Nv = 0,\; v \geq 0, \tag{7b}$$

$$\tfrac{\tilde{w}_1}{\phi_1}\, v_1 + \tfrac{\tilde{w}_3}{\phi_3}\, v_3 + \textstyle\sum_{i=2,4,5} \tilde{w}_i\, v_i \leq W. \tag{7c}$$

We assume $\tilde{w}_1 = \tilde{w}_2 = \tilde{w}_3 = \tilde{w}_5 = 1$ and $\tilde{w}_4 = 10$, that is, higher costs for respiration than for other processes. We vary ϕ_1 (at fixed $\phi_3 = 0.90$) and determine the optimal fluxes v_5 and v_1.

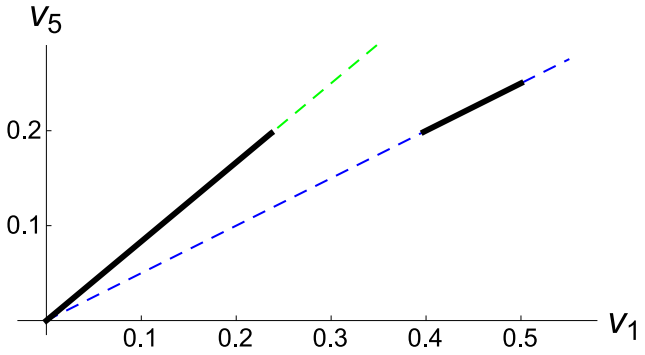

Figure 9: Optimal fluxes v_5 vs. v_1 (rate of biosynthesis vs. rate of glucose import). Results obtained by satFBA. Optimal fluxes correspond to either EFM e^1 (pure fermentation) or EFM e^2 (pure respiration).

Compare the results of kinetic optimization (Figure 6) and its approximation by satFBA (Figure 9). Although satFBA does not use kinetic information, it predicts a qualitatively correct result.

Finally, we vary both ϕ_1 and ϕ_3.

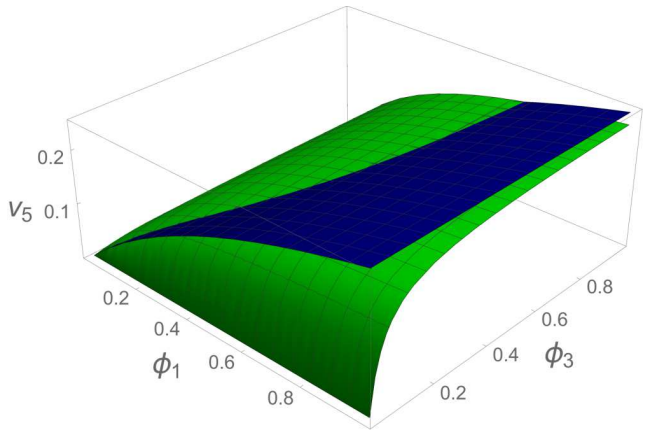

Figure 10: Optimal flux v_5 (rate of biosynthesis) as a function of saturation values ϕ_1 and ϕ_3 (for glucose and oxygen import). Results obtained by satFBA.

Compare the results of kinetic optimization (Figure 2) and its approximation by satFBA (Figure 10).

References

[1] Stefan Müller, Georg Regensburger, and Ralf Steuer. Enzyme allocation problems in kinetic metabolic networks: Optimal solutions are elementary flux modes. *J. Theor. Biol.*, 347:182–190, 2014.

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[3] Meike T. Wortel, Han Peters, Josephus Hulshof, Bas Teusink, and Frank J. Bruggeman. Metabolic states with maximal specific rate carry flux through an elementary flux mode. *FEBS J.*, 281(6):1547–1555, 2014.