Metabolism in states of maximal enzyme efficiency

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Abstract

Enzyme-efficient states are metabolic states that realize a given flux objective at a minimal enzyme cost. Enzyme-efficient states can be hypothesized to be used under growth rate optimization regimes, as biomass production rate per enzyme can be converted to into cell growth rate. In models without further constraints, enzyme-efficient states are Elementary Flux Modes (EFMs). This allows for a algorithm to find enzyme-efficient states: Enumerate the EFMs, calculate the minimal enzyme cost per EFM, and choose the one with the lowest enzyme investment. This algorithm allows for finding of enzyme efficient states for larger models then can be optimized by 'brute force', but still need to be small enough to enumerate the EFMs. Such optimization has lead to the insights on the effect of changing external nutrient conditions: As growth conditions are changing, the optimal flux profile either changes continuously (and metabolite and enzyme concentrations change continuously as well) when the same EFM remains optimal, or fluxes change discontinuously together with metabolite and enzyme concentrations when a different EFM becomes optimal.

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Chapter overview

- Enzyme-efficient states are metabolic states that realize a given flux objective at a minimal enzyme cost.
- In models without further constraints, enzyme-efficient states are Elementary Flux Modes (EFMs).
- Elementary Flux Modes can be used to find enzyme-efficient states in networks that would be too large to optimize metabolic states "by brute force".
- Biomass per enzyme efficiency can be converted to into cell growth rate.
- As growth conditions are changing, the flux profile either changes continuously (and metabolite and enzyme concentrations as well) or fluxes change discontinuously together with metabolite and enzyme concentrations.

7.1. Introduction

In a simple economic picture of cells, we assume that cells adjust their metabolic state in each environment to obtain a maximal fitness advantage. This may be impossible in reality, but it remains an interesting question what this best metabolic state would look like, according to our knowledge of cells. So what is the best metabolic state overall (comprising metabolic fluxes, metabolite concentrations and enzyme levels)? What pathways should a cell use, which enzymes should be induced or repressed, and how should this change in a new environment? To answer this, we need to remember that all metabolic variables (fluxes, metabolite levels, enzyme levels, and enzyme efficiencies) depend on each other. Physically, fluxes depend on metabolite concentrations through kinetics and enzyme regulation (e.g. competitive inhibition) and metabolites are produced and consumed by the fluxes until a steady state is reached. Hence, if we think in terms of cellular economics (treating enzymes as control variables), then all metabolic variables must be optimized together.

In the previous chapters we saw some ways to predict optimal metabolic fluxes, metabolite concentrations and enzyme levels separately: in Flux Balance Analysis (FBA, Chapter 5 in [1]), we optimized fluxes (while ignoring kinetics or assuming constant enzyme efficiencies); in Enzyme Costs Minimization (Chapter 6 in [1]), in contrast, fluxes were fixed and given and concentrations (and enzyme efficiencies) were optimized. Each of these methods is based on a strong assumption: FBA requires measured flux ranges and/or apparent catalytic rates and assumes enzyme saturation effects can be neglected, while enzyme cost minimization requires a given flux distribution. But what if we dont know any of the variables in advance? How can we predict all of them from first principles?

Before thinking about this, let us briefly step back: what do we actually mean by an "optimal state"? What quantity should be maximized in metabolism? There could be very different aims (e.g. production in biotechnology, vs offspring and survival in a wild-type cell). However, in both cases an important aim is cell growth – or at least, avoiding strong growth deficits. Below we will see that cell growth depends, to a good approximation, on biomass/enzyme efficiency, that is, biomass production per total enzyme invested. Hence, whenever fast growth is important, cells should maximize this efficiency.

Thus, we will consider the following optimality problem: maximize biomass/enzyme efficiency, defined as the production flux per invested enzyme with respect to all metabolic variables (metabolites, enzymes and fluxes) and under all constraints (steady state, enzyme kinetics, etc). Solutions to this problem are considered optimal states.

7.2. The enzyme-efficient metabolic states are elementary flux modes

The optimization problem in this chapter is to reach a maximal flux objective at a minimal enzyme investment. The biological interpretation is that this would lead to the highest growth rate, because it optimizes the ratio between gains (fluxes) and costs (enzymes). When we solve this optimization problem with mathematical tools, it is convenient to either find the minimal enzyme investment for a certain flux, or the maximum flux for a fixed enzyme investment. Although one could think of different biological explanations for those two ways to state the optimization problem, mathematically they are equivalent. For the outline of the proof that optimal states are elementary flux modes, it is convenient to fix the objective flux to an arbitrary value (we choose 1) and then minimize the enzyme investment. This leads to the following optimization problem:

$$
\begin{aligned}
&\underset{\mathbf{v},\mathbf{e},\mathbf{c}}{\text{minimize}} && \sum_{i=1}^{r} h_i e_i && && (7.1)\\
&\text{subject to:} && \mathbf{N}\cdot\mathbf{v} = \mathbf{0} && \text{steady state}\\
& && \forall i : v_i = e_i f_i(\mathbf{c}) && \text{enzyme kinetics}\\
& && \mathbf{e},\mathbf{c} \geq 0 && \text{positive concentrations}\\
& && v_r = 1 && \text{fixed objective flux}\\
& && \mathbf{c} \leq c_{\max} && \text{metabolite bounds}
\end{aligned}
$$

where $\forall i$ means for all reactions i, r is the number of reactions (with the last the objective), h_i are the weights, and bold face items denote vectors. This optimization problem states that by adjusting the fluxes (v), metabolite concentrations (c) and enzyme concentrations (e), the cost (sum of the costs ($h_i e_i$) for every reaction) is minimized, while keeping the objective flux constant. The weights (h_i) can be thought of as the size or production costs of the enzymes (measured in e.g. molecular weight or gene length), which might be different for different enzymes. We need certain constraints to be met for this solution: (i) the metabolic network needs to be in steady state to avoid built-up of intermediates, (ii) enzyme kinetics: the flux of each reaction (v_i) has to be equal to the enzyme concentration (e_i) times a metabolite dependent term ($f_i(\mathbf{c})$), (iii) all enzyme metabolite concentrations have to be positive, and (iv) the objective flux is equal to 1. Optionally, we can add bounds on the metabolite levels, which is mostly necessary with irreversible kinetics. Reversible kinetics usually lead to bounded metabolite levels, because very high concentrations of products will inhibit the reaction forming the products.

In this section we will explain why the optimal state is reached at an Elementary Flux Mode (EFM). One important starting point is that, as we have seen before in Chapter 4 in [1], convex optimization problems with only positivity or equality constraints (no other inequalities) lead to an optimal solution at an extreme points of the feasible solution space, and those extreme points are elementary flux modes. However, the optimization problem (7.1) is not convex, mainly due to the hyperbolic dependency of reaction rates on the metabolite concentrations ($f_i(\mathbf{c})$ is usually not linear).

There are several ways to prove that the solution of this optimization problem is an EFM, of which some are outlined in the papers by Wortel et al. [2] and Müller et al. [3]. Here we will outline a proof by assuming a solution that is an EFM and showing that this leads to a contradiction. Assume we have some optimal state that is not an EFM. Any optimal solution is associated with a set of fluxes, and enzyme and metabolite concentrations. Now we set the metabolite concentrations to the concentrations of the assumed optimal state. Then all metabolite dependent terms ($f_i(\mathbf{c})$) become constants and we return to a convex problem. As explained in Chapter 9 in [1] and Figure 7.1, the optimum of this problem (now in terms of enzyme

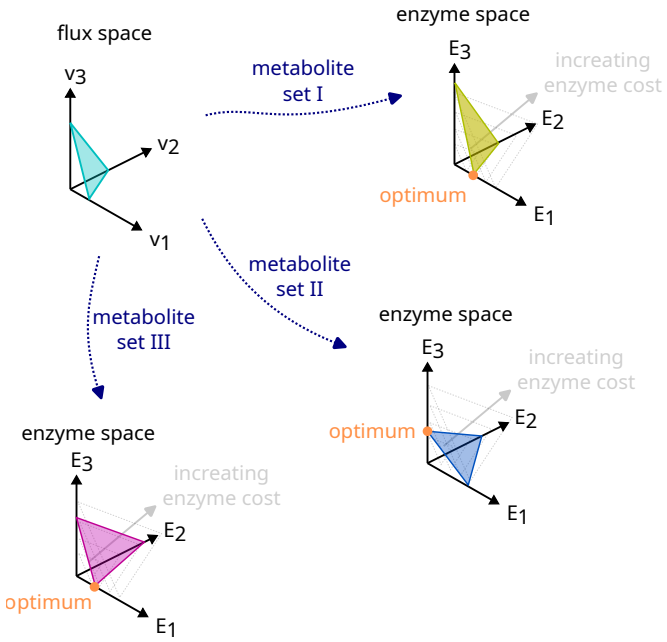

Figure 7.1: Translation from flux to enzyme space retains EFMs as extreme rays. The top left panel shows the feasible flux space with the steady state constraints, all fluxes postive (using splitting of fluxes, as explained in the text, if necessary) and a fixed objective flux. The extreme points here are points where one flux becomes 0 and are elementary flux modes (see Chapter 5 in [1]). Here we show that when we have assumed metabolite concentrations, such as when we keep them at an optimal solution, we get a linear transformation and the extreme rays are maintained. Different metabolite levels, for example solutions to different environmental conditions, can lead to different transformations and therefore different optima (miminal total enzyme), but those are always located at an EFM.

concentrations and fluxes) is a flux distribution that is an EFM. But this contradicts our initial assumption that the optimal state from which we took the set of enzyme concentrations was not an EFM. The proof by contradiction shows that the optimal state must be an EFM.

7.3. Illustration with an example network

To illustrate the proof, we study a simple network that we have seen previously in Chapter 5 in [1] (Figure 7.2), in which we will now include enzyme kinetics. We will use the factorized rate law as in Chapter 6 in [1], but then generalized for n_s substrates and n_p products (also compare Eq. (3.15 in [1]) in Chapter 3 in [1]):

$$v = e \cdot k_{\text{cat}}^{+} \cdot \frac{\prod_{n_s}^{j=1} s_j/K_{\text{S},j}}{1 + \prod_{n_p}^{k=1} p_k/K_{\text{P},k} + \prod_{n_s}^{j=1} s_j/K_{\text{S},j}} \cdot \left(1 - e^{\Delta G_r'/RT}\right) \tag{7.2}$$

See Box **??** for all detailed rate laws of the example networks. We can simplify this equation by combining the forward catalytic constant, the thermodynamic efficiency factor, the saturation efficiency factor, and the regulation efficiency factor (if that exists) in a function $f(\mathbf{c})$, which only depends on the metabolites, and

not on the enzyme concentrations. We will below write f for $f(\mathbf{c})$.

$$v_i = e_i \cdot f_i \tag{7.3}$$

Now we take $v_{\mathrm{BM}} = 1$ and optimize all fluxes, enzymes concentrations and metabolite concentrations to minimize the enzyme costs, while satisfying the constraints posed in Equations (7.1), for different levels of external glucose and standard levels of the other external metabolites. We see that for different external glucose concentrations, we get different optimal fluxes, enzyme levels and metabolite levels (Table 7.1).

We notice that the total enzyme needed for the biomass flux of one decreases with the increasing glucose levels, as we expect. Also, the optimal level of internal glucose increases with increasing external glucose. Moreover, the fluxes of the solutions do follow an EFM (see Figure 7.2b). We can now reformulate the problem for only the flux and enzyme levels, while keeping the metabolite levels as they are in the table. With the metabolite levels in the first row of the table, we can linearly relate the enzyme and flux levels (with the factors f_i), and thereby the extreme rays of the enzyme and flux space will be equal and EFMs, as pointed out above (see also Chapter 5 in [1], Figure 7.1). Optimization in this space will lead to the optimal flux distributions following an EFM. If the fluxes in the table would not follow an EFM, this will lead to an inconsistency; if there is an optimum, this should still be the outcome if we keep some variables in the optimum constant and optimize over the rest of the variables.

We point out two important aspects, using the network (Figure 7.2) as an example. First, it is convenient to split reversible reactions, such that fluxes are always positive. In this case that means that the reversible reaction from P to P_1 is split into the forward reaction v_2 and the reverse reaction v_4, which both can have only positive flux. This splitting makes sure that EFMs are the extreme rays of the flux space (see Chapter 5 in [1]). This splitting is purely a mathematical conveniency, we still assume this to be one reaction in the biological sense, and therefore the kinetic equations of both the forward and the backward reaction will be exactly the same. Only, depending on in which direction the flux goes, either one of the reactions will be positive. Any solution with both reactions positive is infeasible, but minimizing enzyme levels will never lead to such a solution, therefore we do not need to set additional constrains. Second, the feasibility of EFMs can depend on external concentrations. In this network the biomass reaction (v_{BM}) is the objective flux and there are three EFMs leading to the production of biomass: EFM1 consisting of v_0, v_1, v_2 and v_{BM}, EFM2 consisting of v_0, v_1, v_3 and v_{BM} and EFM3 consisting of v_4, v_3 and v_{BM}. However, if P_1 is absent in the environment, the uptake flux v_4 will always be 0 and therefore EFM3 will not be feasible.

7.4. Computation of the optimal state

We can now use the result that states of maximal enzyme efficiency are reached at an elementary flux mode to calculate these states. We can follow the following steps to calculate these states:

1. Enumerate the elementary flux modes

$[\mathrm{G}_{\mathrm{ex}}]$	E_{tot}	v_0	v_1	v_2	v_3	v_4	v_{BM}	e_0	e_1	e_2	e_3	e_4	e_{BM}	[G]	[P]	[ATP]	[ADP]
0.01	156.2	5	5	0	9	0	1	54.4	4.4	0	94.4	0	2.9	0.08	15.14	0.05	20.09
0.1	91.3	50	50	99	0	0	1	61.3	11.3	14.2	0	0	4.4	0.13	4.55	0.11	20.09
1	36.2	50	50	99	0	0	1	13.0	8.0	12.5	0	0	2.7	0.60	7.65	0.11	20.09

Table 7.1: Outcomes of the optimization of the example network with standard kinetics, parameter values and external concentrations (see Box **??**) for varying levels of $[\mathrm{G}_{\mathrm{ex}}]$.

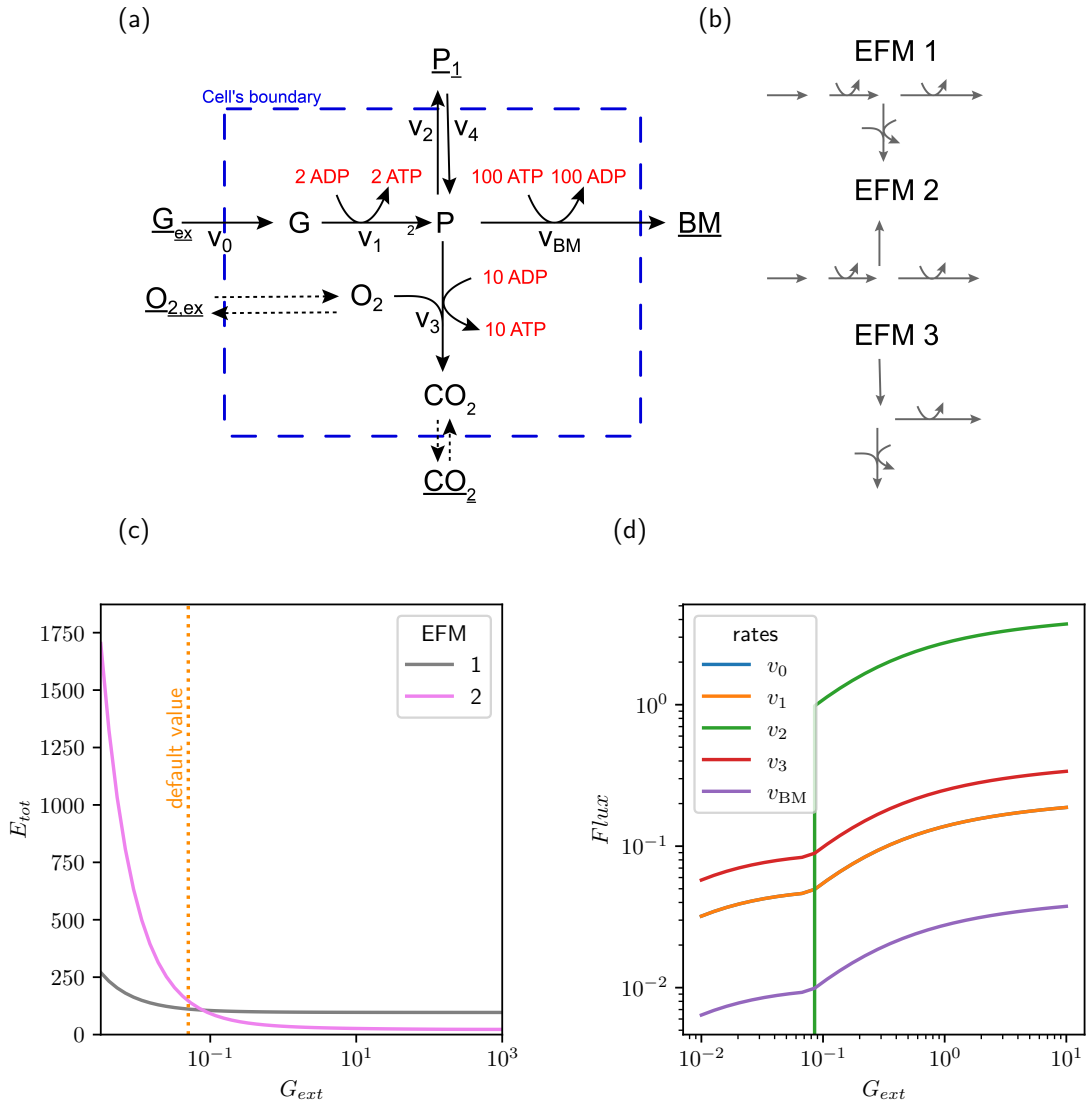

Figure 7.2: States of maximal efficiency in an example model. (a) Example network from Chapter 5 in [1] with added stoichiometry. (b) Three elementary flux modes of this network. (c) Calculated enzyme investment needed for a biomass flux of 1. There is a single concentration of G_{ex} for which the optimal EFM switches from EFM2 to EFM1. (d) Specific fluxes (flux divided by total enzyme) associated with the optimal EFM for different levels of G_{ex}. The rates show a discontinuity when the optimital EFM switches form EFM1 to EFM2.

2. Calculate the minimal enzyme for each EFM scaled to an objective flux of 1
3. Compare the EFMs and select the one with minimal enzyme demands

Step 2 is a convex optimization problem as we have seen in Chapter 6 in [1]. Step 1 is possible for relatively large networks, although usually not for genome scale metabolic networks. The method of these three steps is called Enzyme Flux Cost Minimization, because it is similar to Enzyme Cost Minimization, but while that is focussed on fixes fluxes, Enzyme Flux Cost Minimization simultaneously finds the optimal fluxes. In this section we will show the method on the example network of Figure 7.2.

First, we describe the network with the toichiometric matrix ($\mathbf{N}$) and the concentration vector ($\mathbf{c}$):

$$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 2 & -1 & -1 & 1 & -1 \\ 0 & 2 & 0 & 10 & 0 & -100 \\ 0 & -2 & 0 & -10 & 0 & 100 \end{pmatrix}, \qquad \mathbf{c} \equiv \begin{pmatrix} [\mathrm{G}] \\ [\mathrm{P}] \\ [\mathrm{ATP}] \\ [\mathrm{ADP}] \end{pmatrix} \tag{7.4}$$

And with the stoichiometric matrix we can describe the steady state constraints:

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{c} = \mathbf{N}\ \mathbf{v} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 2 & -1 & -1 & 1 & -1 \\ 0 & 2 & 0 & 10 & 0 & -100 \\ 0 & -2 & 0 & -10 & 0 & 100 \end{pmatrix} \begin{pmatrix} v_0 \\ v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_{\mathrm{BM}} \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} \tag{7.5}$$

Now we find the EFMs (for example with EFMtool [4]). It can easily be checked that the following EFMs are in the nullspace of the stoichiometric matrix:

$$\mathrm{EFM1} = \begin{pmatrix} 5 \\ 5 \\ 0 \\ 9 \\ 0 \\ 1 \end{pmatrix}, \quad \mathrm{EFM2} = \begin{pmatrix} 50 \\ 50 \\ 99 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \quad \mathrm{EFM3} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 10 \\ 11 \\ 1 \end{pmatrix} \tag{7.6}$$

The next step is to perform the convex optimization over the metabolite levels for all three EFMs. Therefore we convert the enzyme levels to ratio of the flux and the function $f(\mathbf{c})$, using Equation 7.3. By taking the sum of those we make a function for the total enzyme as a function of fluxes, metabolite concentrations and parameters. We use the standard parameters (Box **??**) and for each EFM input the fluxes. We are then left with a convex optimization over the metabolite levels, an Enzyme Cost Minimization problem as in Chapter 6 in [1]. For $[\mathrm{G_{ex}}] = 0.05$ we obtain a total enzyme of 111.1 for EFM1 and of 146.3 for EFM2. That means that for this conditions we will conclude that EFM1 is optimal, and we obtain the metabolite concentrations from the optimization of $[\mathrm{G}] = 0.08$, $[\mathrm{P}] = 3.93$, $[\mathrm{ATP}] = 0.11$ and $[\mathrm{ADP}] = 20.09$. We can next use the rate equations to calculate the enzyme levels from the fluxes and metabolite levels, using the values for the parameters and external concentrations.

We can repeat this procedure for different levels of external concentrations and see that the optimal EFM can change depending on the external concentration (Figure 7.2c). When the optimum shifts to using a different EFM, there is a discontinuity in the fluxes at the external metabolite concentration (Figure 7.2d). Many cells show shifts in metabolic strategies depending on the external conditions and with Enzyme Flux Cost Minimization those shift could be explained.

In conclusion, we now found the metabolic state of maximum enzyme efficiency. Even though in our calculation we obtained the enzyme concentrations last, it is by enzyme concentrations that the cell controls the system. If the cells produces enzymes in the concentrations we calculated and reaches a steady state, this

Box 7.A : Kinetics of the example network

The detailed kinetic equations for the example model (Figure 7.2) using the factorized rate law (see Equation (7.2) and Chapters 3 in [1] and 6 in [1]) are:

$$
\begin{aligned}
v_0 &= e_0 \cdot k^+_{\text{cat},0} \cdot \frac{[\text{G}_\text{ex}]/K_{\text{G}_\text{ex}}}{1 + [\text{G}]/K_\text{G} + [\text{G}_\text{ex}]/K_{\text{G}_\text{ex}}} \cdot \left(1 - e^{\Delta G'_{r,0}/RT}\right) \\
v_1 &= e_1 \cdot k^+_{\text{cat},1} \cdot \frac{([\text{G}]/K_\text{G})([\text{ADP}]/K_\text{ADP})}{1 + ([\text{P}]/K_\text{P})([\text{P}]/K_\text{P})([\text{ATP}]/K_\text{ATP}) + ([\text{G}]/K_\text{G})([\text{ADP}]/K_\text{ADP})} \cdot \left(1 - e^{\Delta G'_{r,1}/RT}\right) \\
v_2 &= e_2 \cdot k^+_{\text{cat},2} \cdot \frac{[\text{P}]/K_\text{P}}{1 + [\text{P}_1]/K_{\text{P}_1} + [\text{P}]/K_p} \cdot \left(1 - e^{\Delta G'_{r,2}/RT}\right) \\
v_3 &= e_3 \cdot k^+_{\text{cat},3} \cdot \frac{([\text{P}]/K_\text{P})([\text{ADP}]/K_\text{ADP})([\text{O}_2]/K_{\text{O}_2})}{1 + ([\text{CO}_2]/K_{\text{CO}_2})([\text{ATP}]/K_\text{ATP}) + ([\text{P}]/K_\text{P})([\text{ADP}]/K_\text{ADP})([\text{O}_2]/K_{\text{O}_2})} \cdot \left(1 - e^{\Delta G'_{r,3}/RT}\right) \\
v_4 &= e_4 \cdot k^+_{\text{cat},4} \cdot \frac{[\text{P}_1]/K_{\text{P}_1}}{1 + [\text{P}_1]/K_{\text{P}_1} + [\text{P}]/K_\text{P}} \cdot \left(1 - e^{\Delta G'_{r,4}/RT}\right) \\
v_\text{BM} &= e_\text{BM} \cdot k^+_{\text{cat,BM}} \cdot \frac{([\text{P}]/K_\text{P})([\text{ATP}]/K_\text{ATP})}{1 + ([\text{BM}]/K_\text{BM})([\text{ADP}]/K_\text{ADP}) + ([\text{P}]/K_\text{P})(\text{ATP}/K_\text{ATP})} \cdot \left(1 - e^{\Delta G'_{r,0}/RT}\right)
\end{aligned}
\tag{7.7}
$$

Note that P is a product twice in v_1, as v_1 produces 2P. Note that v_2 and v_4 have a very similar rate equation, but in the different direction, and both should only be used in the positive direction. The standard set of parameters we used for the toy model is all $k_{\text{cat},i} = 10$ except $k_{\text{cat},3} = 0.1$, all $\Delta G'_{r,i} = -1000$, $R = 8.3$, $T = 293$ and all $K_\text{M} = 1$. For the external metabolites $[\text{P}_1] = 1$, $[\text{G}_\text{ex}] = 0.05$, $[\text{O}_2] = 0.1$, $[\text{BM}] = 1$ and $[\text{CO}_2] = 10$ unless mentioned otherwise.

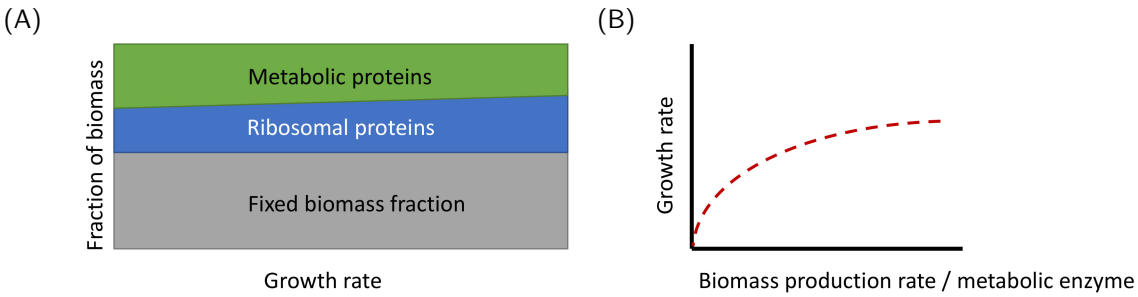

Figure 7.3: Translation of enzyme-specific biomass rate to growth rate. (A) Both from experimental data and a cell-optimization point of view, the ribosomal fraction of the proteome increases with the growth rate, while the metabolic fraction decreases. (B) This leads to a hyperbolic dependency of the growth rate on the biomass production rate.

state will realize the fluxes and metabolite levels that lead to our optimal state.

7.5. Translating enzyme efficiency into cell growth rate

In the section above, we learned how to optimize metabolic states for a maximal overall enzyme efficiency. Why is this quantity relevant? One reason is that overall enzyme efficiency, according to some simple reasoning, determines the cell growth rates. If microbes compete by growing fast, their fitness is largely determined by their momentary growth rate in their respective environment. In such environments, the biomass/enzyme efficiency will be under selection, which makes it one of the important objective functions in this book. If higher enzyme efficiency means higher growth rate, and if we have a conversion formula for this, we can put growth rate instead of overall enzyme efficiency on the axes of our plots.

Enzyme-efficient metabolic states allow us to compute specific biomass production rates, i.e. the rate of biomass production per metabolic enzyme invested. If biomass consisted only of enzymes, the ratio "enzyme

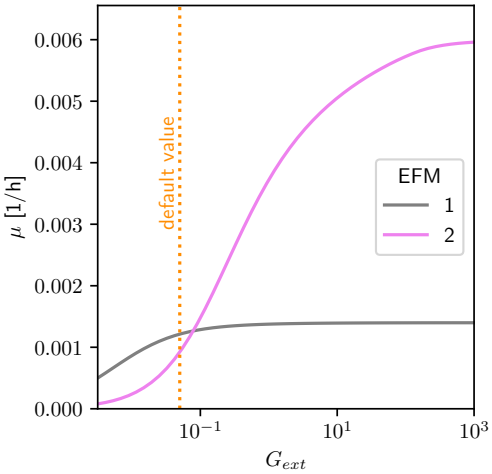

Figure 7.4: Optimal growth rates of the two EFMs for different levels of the external metabolite $\mathrm{G_{ex}}$ (using Equation 7.8)

production rate / enzyme concentration" would give us directly the growth rate. However, biomass does not only consist of metabolic enzymes, but includes ribosomal enzymes, RNA, DNA, lipids, and other compounds. Therefore we need a formula for converting biomass/enzyme efficiency into cellular growth rate.

Mathematically, a cell's growth rate is given by $\mu = v_{\mathrm{BM}}/c_{\mathrm{BM}}$, where v_{BM} is the biomass production rate (biomass produced per cell volume and time) and c_{BM} is the biomass amount per cell volume. If a cell consisted only of metabolic enzymes (more precisely, of the enzymes described in our model) the biomass/enzyme efficiency $K_{\mathrm{BM}} = v_{\mathrm{BM}}/h_{\mathrm{enz}}$ would directly describe the cellular growth rate. Since that is not the case, we need to convert between h_{enz} and c_{BM}. The metabolic protein fraction decreases with the growth rate, leading to a hyperbolic dependency of the growth rate on the biomass production rate (Figure 7.3). We may use the empirical approximation $h_{\mathrm{enz}}/c_{\mathrm{BM}} = f_{\mathrm{prot}}(a - b\ \mu)$, where $f_{\mathrm{prot}} = 0.5$ is the fraction of protein mass within the cell dry mass and the parameters $a = 0.27$ and $b = 0.2\,\mathrm{h}$ were fitted to describe the metabolic enzyme fraction in proteomics data, assuming a linear dependence on growth rate [5]. This yields the conversion formula (see also [6]):

$$\mu = \frac{a\ f_{\mathrm{prot}}\ v_{\mathrm{BM}}}{h_{\mathrm{enz}} + b\ f_{\mathrm{prot}}\ v_{\mathrm{BM}}}\,. \tag{7.8}$$

This formula has been used to convert the minimal enzyme cost per biomass flux for different external concentrations in the toy model (Figure 7.2c) to the maximal growth for each EFM (Figure 7.4).

7.6. Concluding remarks

In this chapter we considered the metabolic network of a cell - and enzyme levels, metabolite concentrations, and fluxes as the state variables - and studied its maximally efficient states. Finding such states can be difficult because fluxes, metabolite concentrations, and enzyme levels are tightly coupled: metabolite concentrations determine enzyme efficiencies, enzyme efficiencies determine optimal enzyme levels, and enzyme levels determine fluxes and metabolite concentrations, which in turn determine enzyme efficiencies. To find an optimal state, all variables need to be optimized at the same time, which is a non-linear optimality problem with (possibly) many local optima. In small toy models, solutions can be found numerically, but for large

detailed models, the computational effort becomes enormous. Instead of simplifying the problem (as in the previous chapters) we here used the insight that (in models without extra flux bounds) the optimal solutions must be EFMs. Biologically, it is enzyme levels rather than fluxes that are regulated directly (by transcriptional regulation), while metabolite concentrations and fluxes respond dynamically. But from a functional point of view, we may see this differently: we may first think of a task (a flux to be realized) and then ask how this flux can be performed optimally.

Recommended readings

- M.T. Wortel, H. Peters, J. Hulshof, B. Teusink, and F.J. Bruggeman. Metabolic states with maximal specific rate carry flux through an elementary flux mode. FEBS Journal, 281(6):15471555, 2014.
- S. Müller, G. Regensburger, and R. Steuer. Enzyme allocation problems in kinetic metabolic networks: Optimal solutions are elementary flux modes. Journal of Theoretical Biology, 347:182190, 2014.

Problems

Problem 7.1 Consider the model in Figure 7.2. What would be the qualitative effect of a change in oxygen concentration on the enzyme cost of the three EFMs and on the choice of the optimal strategy?

Bibliography

[1] The Economic Cell Collective, editor. *Economic Principles in Cell Biology*. Free online book, 2023. doi: 10.5281/zenodo.8156386.

[2] M.T. Wortel, H. Peters, J. Hulshof, B. Teusink, and F.J. Bruggeman. Metabolic states with maximal specific rate carry flux through an elementary flux mode. *FEBS Journal*, 281(6):1547–1555, 2014.

[3] S. Müller, G. Regensburger, and R. Steuer. Enzyme allocation problems in kinetic metabolic networks: Optimal solutions are elementary flux modes. *Journal of Theoretical Biology*, 347:182–190, 2014.

[4] Marco Terzer and Jörg Stelling. Large-scale computation of elementary flux modes with bit pattern trees. *Bioinformatics*, 24(19):2229–2235, 08 2008. ISSN 1367-4803. doi: 10.1093/bioinformatics/btn401.

[5] Matthew Scott, Carl W Gunderson, Eduard M Mateescu, Zhongge Zhang, and Terence Hwa. Interdependence of cell growth and gene expression: origins and consequences. *Science*, 330(6007):1099–1102, 2010. doi: 10.1126/science.1192588.

[6] Meike T Wortel, Elad Noor, Michael Ferris, Frank J Bruggeman, and Wolfram Liebermeister. Metabolic enzyme cost explains variable trade-offs between microbial growth rate and yield. *PLoS Comput. Biol.*, 14(2):e1006010, February 2018. doi: 10.1371/journal.pcbi.1006010.