Chapter 9

Optimal cell behavior in time

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Chapter highlights

- Microorganisms live in continually changing environments, which require them to develop adaptation strategies.
- These strategies have been profitably studied under the assumption that microorganisms have evolved to optimize one or several aspects of their adaptive response.
- The mathematical formalization of this assumption leads to dynamic optimization problems that can be solved by means of techniques from optimal control theory.
- The chapter discusses three example problems: dynamic optimization of enzyme expression in metabolic pathways, dynamic optimization of coarse-grained models of cellular growth, and dynamic flux balance analysis.
- The results obtained for these problems illustrate the interest of studying adaptation strategies from the perspective of dynamic optimization, and the strengths and weaknesses of this approach.

9.1 Introduction

The study of microorganisms in the laboratory has often focused on the creation of stable conditions enabling balanced, reproducible growth of the population. Such conditions are almost never found in nature. Microorganisms live in continually changing environments in which nutrients are only intermittently available and in which the cells are submitted to a variety of other temporally varying stresses (acidity, heat and cold, drought, ...). In order to survive in these conditions, microorganisms have developed a range of molecular mechanisms to detect changes in the environment, or signals announcing such changes, and to adapt their functioning accordingly.

A well-studied example of the dynamic response of bacteria to changes in their environment is the phenomenon of diauxic growth, discovered by Jacques Monod ([1] (see also Chapter 7). When *Escherichia coli* is grown in a medium containing a mixture of two carbon sources, *e.g.*, glucose and lactose, the cells generally first deplete the carbon source supporting the highest growth rate (glucose) before starting to assimilate the other carbon source (lactose). A variety of mechanisms are involved in this switch from a preferred to a secondary carbon source, including the release of the repression of enzymes necessary for lactose utilization, the release of the inhibition of lactose transporters, and the global regulation of a large number of other genes [2, 3].

In many situations, the precise functioning of the molecular mechanisms regulating the adaptation of microbial physiology

to changes in the environment is not or only qualitatively understood. This precludes their inclusion in quantitative models that accurately predict the dynamic response of the cell in a variety of conditions. The lack of mechanistic, quantitative information can be bypassed by making appropriate assumptions about the regulatory systems, in particular that the latter have evolved under the selection pressure of the environment to optimize the response to external perturbations. More precisely, it is assumed that microorganisms have developed mechanisms that allocate limiting resources (proteins, fluxes, ...) to cellular processes so as to maximize or minimize some objective function, or combination of objective functions, over the time-interval of environment changes.

The use of an optimality assumption to make up for missing or incomplete information was already exploited with success in Chapter 5 of this book. The difference with those approaches is that here we are interested in cases where the optimality criterion is defined over an interval of time rather than at steady state, and thus we need to consider dynamic instead of static optimization. Moreover, some methods take into account that cells may vary the allocation of limiting resources to cellular processes over the time interval in which the environmental changes occur, instead of only considering a constant response in a stable environment. This generalization of the problem enormously increases its complexity. It may also lead to nontrivial dynamical effects that are not found in the case of static optimization, such as the accumulation of resource buffers to anticipate future changes in the environment [4].

The classical argument motivating the optimality assumption in the case of microorganisms is that mutants of genes coding for enzymes in central metabolism often have a lower growth rate than the wild-type strain, where growth rate is interpreted as indicating fitness [5]. This argument, however, derives from observations of balanced growth in a stable environment. Is there any evidence that, in the case of changing environments, microorganisms have evolved to perform dynamic optimization? Some circumstantial evidence is provided by the observed capacity of microorganisms to anticipate changes in their environment. For example, when moving along the digestive tract, *E. coli* cells are exposed first to lactose and then to maltose, thus requiring the ability to switch from growth on lactose to growth on maltose (reminiscent of diauxic growth in the laboratory) [6]. Interestingly, reporter gene studies found that the enzymes required for maltose assimilation are expressed at a much higher level in the presence than in the absence of lactose, in otherwise identical conditions [7]. This suggests a specific effect of the presence of lactose on the expression of maltose enzymes, preparing the cells for the expected future availability of maltose. This and other examples of anticipatory behavior are not conclusive in themselves, but they suggest that dynamic optimization is a plausible working hypothesis that may be useful in practice.

The aim of this chapter is to show how microbial physiology can be studied by means of dynamic optimization, by combining a specific objective function, or combination of objective functions, with models of different scope and granularity, while taking into account a number of biophysical and biochemical constraints. We first provide a general definition of dynamic optimization problems in the mathematical framework of optimal control. We then instantiate this general definition for three types of biological problems, each giving rise to a specific class of models. In particular, we discuss (i) dynamic optimization of enzyme expression in metabolic pathways, (ii) dynamic optimization of resource allocation in coarse-grained models of cellular growth, and (iii) dynamic flux balance analysis (dFBA) of metabolic networks. Across the different examples, the scope of the models varies from metabolic pathways (i) to metabolic networks (iii) to the entire cell (ii). The increase in scope is sometimes traded against a lower granularity of the description of cellular process (ii). Some of the models provide a kinetic description of the rates of the individual reactions (i and ii), whereas other models only provide constraints on the reaction rates (iii). In every case, different objective functions are tried, for example the minimization of the time to produce a given compound or the maximization of the amount of biomass produced.

For each of the biological problems and corresponding models considered, we give the precise definition of the modeling formalism and the optimization problem, a small example as an illustration, a discussion of the solution of the problem, and a brief description of more realistic applications and the insights they have given into the functioning of cellular networks. The chapter does not give a detailed explanation of the mathematical methods that are used for solving different classes of optimal control problems, because this would require knowledge of specialized mathematical concepts with which the average reader of the book may not be familiar. Moreover, these methods have been the subject of

dedicated textbooks [8, 9]. Rather, we focus on the definition of the dynamic optimization problems and the interpretation of the solutions returned by available numerical solvers of optimal control problems.

9.2 Mathematical formalization of dynamic optimization problems

The models of cellular processes considered in this chapter have the form of systems of ordinary differential equations (ODEs) 3. Dynamic optimization problems for such systems take the form of so-called optimal control problems, which have their roots in physics and engineering [8, 9].

Let $\mathbf{x}(t)$ be the (time-varying) state of the dynamical system, typically concentrations of (intracellular or extracellular) metabolites or proteins, and let $\mathbf{f}(\cdot)$ describe the (linear or nonlinear) dynamics of the state. $\mathbf{u}(t)$ denotes the (time-varying) control variables, *e.g.*, fluxes allocated to specific reactions or protein fractions allocated to specific enzymes. The time-points 0 and $T > 0$ indicate the bounds of the interval over which the behavior of the system is optimized, with respect to an objective function J. The behavior of the system, given the control exerted by $\mathbf{u}(t)$, is subject to constraints $\mathbf{c_1}(\cdot)$ and $\mathbf{c_2}(\cdot)$ on the admissible control inputs at specific time-points t or over the entire time-interval $[t_0, t_e]$, respectively. The constraints express physical limitations, such as the intracellular density of molecular constituents 2, or biochemical limitations, such as the maximum protein synthesis rate. Combining the above elements, we obtain the following definition of dynamic optimization problems:

$$\max_{u \in U} J(\mathbf{x}(t), \mathbf{u}(t), 0, T), \tag{9.1}$$

such that

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}(t), \mathbf{u}(t)), \quad \mathbf{x}(0) = \mathbf{x_0}, \tag{9.2}$$

$$0 \geq \mathbf{c_1}(\mathbf{x}(t), \mathbf{u}(t)), \tag{9.3}$$

$$0 \geq \mathbf{c_2}(\mathbf{x}(0), \mathbf{x}(T)). \tag{9.4}$$

In summary, the problem consists in finding controls that, given the dynamics of the system, maximize the objective function and satisfy the constraints [10].

The above definition makes no specific assumptions about the dynamics of the system under consideration. Given that we deal with biochemical reaction systems, the dynamics can be refined to

$$\frac{d\mathbf{x}}{dt} = \mathbf{N}\,\mathbf{v}(\mathbf{x}(t), \mathbf{u}(t)) - \mu(t)\,\mathbf{x}(t), \quad \mathbf{x}(0) = \mathbf{x_0}, \tag{9.5}$$

where $\mathbf{N}$ represents the stoichiometry matrix and μ is the (time-varying) growth rate. The principles of describing the structure of biochemical reactions systems by means of a stoichiometry matrix were described in Chapter 3 above.

The problem definition assumes that there is only a single objective function to be optimized. This may not be appropriate, since microorganisms seem to optimize several criteria in parallel, for example growth rate and survival under stress [11]. In many situations, it is therefore more appropriate to generalize the above problem to the case where $\mathbf{J}(\ldots)$ represents a vector of n objective functions $\mathbf{J} = [J_1, \ldots, J_n]$. Thus generalized, the problem does not usually have a single solution, but rather an infinite set of solutions located on a so-called Pareto surface [12]. Solutions on the Pareto surface have the property that every alternative solution improving the performance with respect to some objective necessarily degrades the performance with respect to at least one of the other objectives. In the problems to be developed in the sections below, we consider both optimality in the case of a single objective and Pareto optimality in the case of several objectives.

Many methods for solving optimal control problems (9.1)-(9.4) exist. While some optimal control problems can be solved analytically, most of the problems considered in the examples below require numerical approximations to be solved. All examples developed in the sections below have been solved by means of freely available solvers.

9.3 Dynamic optimization of enzyme expression in metabolic pathways

A number of experimental works suggest that metabolic regulation encodes temporal patterns in enzyme expression that may be beneficial for cell physiology [13, 14]. Since the timing of gene expression can directly control resource expenditure, several authors have attempted to rationalize such patterns as solutions of optimal control problems defined as in (9.1)-(9.4). The general idea is to optimize the temporal evolution of enzyme concentrations using objective functions that are representative of cellular goals. This provides a rationale to reverse-engineer optimality principles that underlie the expression patterns observed in experiments. In this section, we briefly describe results obtained for unbranched metabolic pathways, the basic building blocks of the metabolic networks of the cell.

Dynamic optimization of enzymatic concentrations was first considered by Klipp and co-workers [15]. The problem under study was the minimal-time activation of an unbranched network from an "off" state, where only the precursor is present, to a state where all substrate has been converted into product. To this end, the authors considered an unbranched pathway with n enzymes and $(n+1)$ metabolites:

$$\begin{aligned} \frac{\mathrm{d}x_0}{\mathrm{d}t} &= -k_1 e_1 x_0, \\ \frac{\mathrm{d}x_i}{\mathrm{d}t} &= k_i e_i x_{i-1} - k_{i+1} e_{i+1} x_i, \\ \frac{\mathrm{d}x_n}{\mathrm{d}t} &= k_n e_n x_{n-1}, \end{aligned} \tag{9.6}$$

with a given initial condition $x_0(0) \neq 0$ and $x_i(0) = 0$ for $i = 1, 2, \ldots, n$, and where all enzymatic reactions are assumed to follow mass-action kinetics with rate constant k_i. To model the "off" state prior to pathway activation, the initial conditions can be set to $x_0(0) = s$, where s is the concentration of precursor at $t = 0$, and $x_i(0) = e_i(0) = 0$ for all $i = 1, \ldots n$. The goal was to determine a vector of optimal enzyme concentrations $\mathbf{e}(t)$ that solve the following problem:

$$\mathbf{e}^\star(t) = \arg\min_{\mathbf{e}\in U} \frac{1}{s} \int_0^\infty (s - x_n(t))\, \mathrm{d}t, \tag{9.7}$$

subject to the dynamic model in (9.6) and constraint set U as in (9.1) defined by a limited overall enzyme abundance over the optimization horizon:

$$\sum_{i=1}^{n} e_i(t) = e_{\mathrm{tot}}, \tag{9.8}$$

where e_{tot} is a constant amount of total enzyme concentration. The objective function in (9.7) is called the *transition time* of the pathway and quantifies the time needed to convert all precursor into product. Note that the optimization problem (9.6)-(9.8) falls within the general class of problems defined by (9.1)-(9.4).

Numerical solutions of the optimization problem reveal that the enzyme profiles have a temporal sequence that matches the order in which the enzymes appear in the pathway. Crucially, such pattern resembles the "just-in-time" strategies widely studies in operations research [16], whereby costly resources are deployed only when needed in a production line. In the context of cellular metabolism, such a strategy implies that minimal time activation tends to express biosynthetic enzymes only when their substrates have been built up to sufficiently high concentrations, and thus avoid wasteful protein expression.

The first experimental demonstration of the just-in-time principle was presented by Zaslaver and colleagues [13]. This work employed luminescent and fluorescent reporters to measure the temporal adaptation of *Escherichia coli* upon withdrawal of amino acids from the growth media. Clear just-in-time patterns of enzyme expression were found in the serine, methionine and arginine biosynthetic pathways. To better understand such patterns, the authors studied a model

for an unbranched pathway with three enzymatic steps and Michaelis-Menten kinetics:

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = k_{\mathrm{cat},i}\, e_i \frac{x_{i-1}}{x_{i-1} + K_{\mathrm{M},i}} - k_{\mathrm{cat},i+1}\, e_{i+1} \frac{x_i}{x_i + K_{\mathrm{M},i}} - \mu\, x_i, \quad i = 1, \ldots, 3, \tag{9.9}$$

with given initial conditions $x_1(0) \neq 0$, $x_2(0) = x_3(0) = 0$, and where $(k_{\mathrm{cat},i},\, K_{\mathrm{M},i})$ are the enzyme turnover rate and Michaelis-Menten constants of each enzyme, respectively. The precursor concentration x_0 is assumed to be constant. The model also includes a dilution term that accounts for dilution by cell growth at rate μ. In contrast to previous works, this model also includes an explicit description of enzyme expression:

$$\frac{\mathrm{d}e_i}{\mathrm{d}t} = \frac{\beta_i}{1 + r/\kappa_i} - \mu\, e_i, \quad i = 1, \ldots, 3, \tag{9.10}$$

where the first term is a lumped model of enzyme expression controlled by a time-varying (active) repressor concentration $r(t)$, with maximal expression rate β_i, and κ_i being the concentration of (active) repressor required for half-maximal expression. Moreover, since bacterial amino acid pathways are often subject to end-product feedback, the model assumed that the repressor gets activated by the pathway product:

$$r(t) = r_{\mathrm{T}}(t) \frac{x_3(t)}{K_{\mathrm{r}} + x_3(t)}, \tag{9.11}$$

where $r_{\mathrm{T}}(t)$ denotes the total (active and inactive) repressor concentration. The model also included negative autoregulation of the repressor itself:

$$\frac{\mathrm{d}r_{\mathrm{T}}}{\mathrm{d}t} = \frac{\beta_0}{1 + r/\kappa_0} - \mu\, r_{\mathrm{T}}, \tag{9.12}$$

where β_0 and κ_0 define the strength of autoregulation similarly as in the lumped model for enzyme expression in (9.10).

The authors constructed an optimization problem so as to study the relation between optimality, and the strength of the regulatory parameters $\mathbf{k} = (k_1, k_2, k_3)$ and $\boldsymbol{\beta} = (\beta_1, \beta_2, \beta_3)$. To this end, they defined the optimization problem

$$\min_{\mathbf{k},\boldsymbol{\beta}} a \cdot \sum_{i=1}^{3} \underbrace{\int_0^T \frac{\beta_0}{1 + r(t)/\kappa_0} \mathrm{d}t}_{\text{total amount of repressor}} + \underbrace{\int_0^T |F - F_{\mathrm{goal}}|\, \mathrm{d}t}_{\text{deviation from steady state}}, \tag{9.13}$$

where a is a scalar weight accounting for the protein costs, T is the optimization horizon, and F is the rate of product synthesis:

$$F = k_{\mathrm{cat},3}\, e_3 \frac{x_2}{x_2 + K_{\mathrm{M},3}}. \tag{9.14}$$

In problem (9.13), the constant F_{goal} is a prescribed production flux that the pathway should achieve at steady state. Minimization of the objective in (9.13) accounts for the activation of the pathway from an "off" state until it reaches a prescribed flux F_{goal}. This formulation differs from the previous example [15] in two important ways. First, it accounts for cellular resources in the objective function itself. The first term of the objective quantifies the total amount of repressor produced through the optimization horizon, and thus relates to the amount of cellular resources required to activate the pathway. Second, the decision variables are the regulatory parameters, not the temporal profiles of the molecular species. Therefore, strictly speaking, this is not an optimal control problem but rather a static optimization problem subject to dynamic constraints encapsulated by the pathway ODE model. Through numerical solutions for different values of the protein cost weight a and optimization horizon T, the authors determined conditions under which the optimal solutions showed two features of the just-in-time property, namely:

$$\tau_1 < \tau_2 < \tau_3, \quad \max_t e_1 > \max_t e_2 > \max_t e_3, \tag{9.15}$$

where τ_i is the response time, i.e. the time to reach 50% of maximal concentration, and $\max_t e_i$ is the peak concentration of each enzyme. This theoretical model was designed to mimic the architecture of gene regulation in such pathways, whereby the end product commonly represses the expression of upstream enzymes, and thus gave both experimental and computational evidence that just-in-time patterns may be the result of optimality principles underlying the regulation of metabolic pathways.

Further experimental evidence of temporal patterns in enzyme expression have been found in other pathways [17] and organisms [14], and number of subsequent works have explored their optimality in more detail; we refer the reader to the review in [18] for a detailed discusson on such approaches. Oyarzún and colleagues [19], in particular, gave the first mathematical proof that just-in-time dynamics are a general property in models of unbranched metabolic pathways. Using a cost-benefit objective function that balances the speed of response against the cost of expressing pathway enzymes, they showed that the just-in-time patterns emerge in pathways of arbitrary length and with minimal assumptions on the enzyme kinetics. Specifically, they considered a general model for an unbranched pathway with $n+1$ reactions:

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = g_{i-1}(x_{i-1})\, e_{i-1} - g_i(x_i)\, e_i, \quad i = 1, \ldots, n, \tag{9.16}$$

with initial conditions $x_i(0) = 0$ for $i = 1, 2, \ldots n$, and the precursor x_0 assumed to be at a constant concentration. The functions g_i represent a general kinetic turnover rate satisfying the following conditions:

$$\begin{aligned} g_i(0) &= 0, \\ \frac{\partial g_i(x_i)}{\partial x_i} &> 0. \end{aligned} \tag{9.17}$$

The above assumptions are generally satisfied by most enzyme kinetic functions, as catalytic rates are typically a monotonic function of the substrate concentration. In particular, the assumptions in (9.17) are met by common kinetics such as mass-action, Michaelis-Menten and Hill equations. The optimization problem considered in [19] corresponds to a free final-time optimal control problem:

$$\mathbf{e}^\star(t) = \arg\min_{\mathbf{e}\in U} \int_0^T (1 + \boldsymbol{\alpha}'\, \mathbf{e}(t))\, \mathrm{d}t, \tag{9.18}$$

where $\mathbf{e}(t)$ is the vector of enzyme concentration, $\boldsymbol{\alpha}$ is an $(n+1)-$dimensional vector of tuneable weights, T is a free optimization horizon, and U is a constraint set as in (9.1). The first term in the objective function (9.18) accounts for the total time taken to activate the pathway from the "off" state up to a steady state flux, while the second term weighs the cost of pathway activation. To account for limited availability of cellular resources, the authors also included a temporal constraint on the enzyme concentrations:

$$\sum_{i=0}^{n} e_i(t) \leq e_{\mathsf{tot}}, \tag{9.19}$$

which is a relaxation of the constraint originally employed by Klipp *et al* in (9.8), as well as a terminal constraint of the form:

$$e_i(t) = \frac{F_{\mathsf{goal}}}{g_i(x_i(T))}, \quad \text{for } t \geq T, \tag{9.20}$$

where F_{goal} is a (constant) target pathway flux, similar as in (9.13). The terminal constraint ensures that the pathway reaches a steady state at the final time T. Using Pontryagin's Minimum Principle [9], the authors showed that the optimal enzyme concentrations follow a bang-bang temporal profile that matches the order in the which they act on the pathway. This result was shown to be independent of the weight α, the number of enzymatic steps, and valid for a wide range of enzyme kinetics satisfying the assumptions in (9.17), thus extending the original finding in [15] to a larger class of pathways. Figure 9.1 shows a numerical example of the optimal activation pattern obtained for an unbranched metabolic pathway of length three.

A

$$\boldsymbol{x}_0 \longrightarrow \boldsymbol{x}_1 \longrightarrow \boldsymbol{x}_2 \longrightarrow \boldsymbol{x}_3$$

B

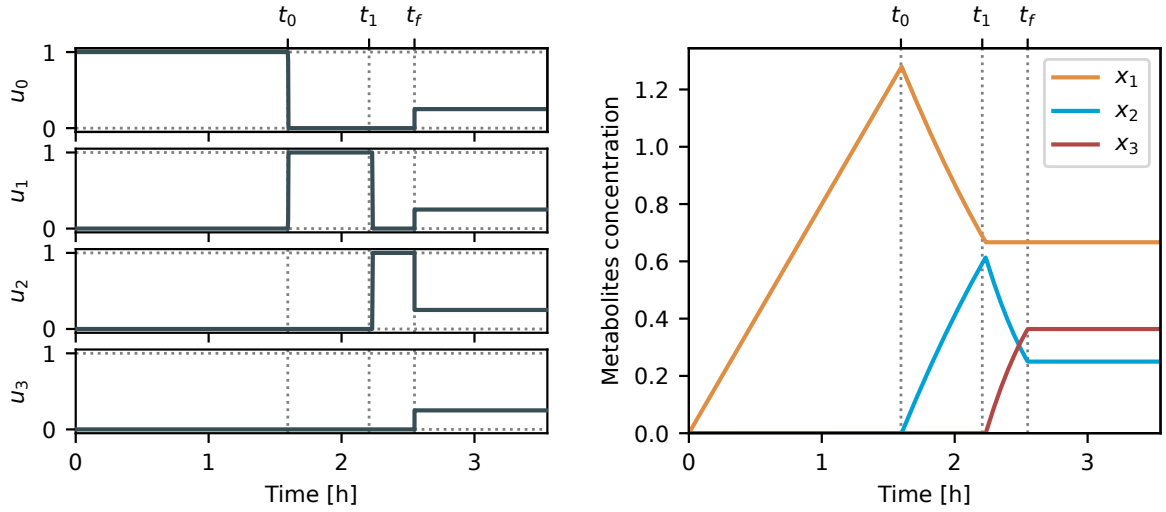

Figure 9.1: Example of optimal enzyme expression in an unbranched metabolic pathway. **A.** A simple scheme of the metabolic pathway. **B.** Time evolution of the optimal enzyme expression u_i and metabolite concentration x_i. For the simulations, the functions g_i are Michaelis-Menten with constants $k = (1, 2, 4, 3)$ s^{-1}, $K = 1$ mM, $V = 0.2$ mM s^{-1} and $x_0 = 5$ mM. Enzymatic weights are set to $\alpha_i = 1$ mM^{-1} s and maximum enzyme availability $E_{\text{tot}} = 1$ mM. Resulting activation times are $t_0 = 1.59$ s, $t_1 = 2.2$ s and $t_f = 2.55$ s.

In this section we have reviewed some optimal control approaches for the optimization of unbranched metabolic pathways. While differing in their formulations and solution strategies, these approaches provide substantial computational evidence that some temporal patterns observed in metabolic dynamics can be understood as the solution of an optimal control problem. In the next section we focus on approaches that go beyond individual pathways and include additional components and processes of the cellular machinery.

9.4 Dynamic optimization of resource allocation in coarse-grained models of cellular growth

In the previous section, we considered models that were essentially limited to metabolic pathways. The optimization problems were formulated in terms of the allocation of enzymes to the different reactions in the pathway. In this section, we generalize the perspective by increasing the scope of the models from metabolism to protein synthesis and growth. The optimization problems concern the allocation of resources to the synthesis of enzymes catalyzing different metabolic reactions, but also to the synthesis of ribosomes in charge of the production of proteins. Growth is explicitly defined in terms of the increase of protein mass, and leads to growth dilution of all cellular components. The models are very similar to those considered in Chapter 7, but the optimization problems are dynamic rather than static. That is, instead of searching an allocation of cellular resources to the synthesis of different classes of proteins that is optimal at steady state, during balanced growth, we are interested in finding a time-varying resource allocation strategy optimizing an objective defined over an interval of time, *e.g.*, during a transition between two states of balanced growth.

We consider the class of models with dynamics given by Eq. 9.5, where the input **u** is interpreted as the (time-varying) resource allocation strategy. Among the cellular components **x**, we distinguish between metabolites and proteins, with concentrations **c** and **p**, respectively. Accordingly, the concentration vector can be written as $\mathbf{x} = [\mathbf{c}, \mathbf{p}]'$. We also

distinguish between enzymatic reactions and protein synthesis reactions. While the former have metabolites as substrates and products, the latter convert metabolites (amino acids) into proteins. An enzymatic reaction i has the following reaction rate function:

$$v_i(t) = k_i\, p_j(t)\, h_i(c), \tag{9.21}$$

where k_i is a catalytic constant, p_j the concentration of protein j, and h_i a function describing enzyme saturation. Enzyme saturation is determined by the substrates, products, and activators/inhibitors of the reaction. Typical rate functions v_i follow mass-action kinetics or (ir)reversible Michaelis-Menten kinetics. The synthesis of protein i is associated with the reaction-rate function

$$v_i(t) = u_i(t)\, v_R(t), \tag{9.22}$$

where v_R is the total protein synthesis rate defined by

$$v_R(t) = k_R\, p_R(t)\, h_R(c(t)), \tag{9.23}$$

with k_R the maximum protein synthesis rate, p_R the concentration of ribosomes, and h_R a function describing the saturation of ribosomes by their substrate, that is, amino acids (or more precisely, tRNAs charged with amino acids). The function u_i in Eq. 9.22 is a time-varying resource allocation function, describing the fraction of the total protein synthesis rate allocated to the synthesis of protein i. The fractions are non-negative and sum to 1, that is, for every time t,

$$\sum_i u_i(t) = 1, \quad \text{and} \quad u_i(t) \geq 0, \text{ for all } i. \tag{9.24}$$

In most models, the biomass of a growing cell population is equated with the mass of proteins, the most abundant cellular component. (Chapter 2). Under the further assumption that the biomass density is constant, it follows that the total protein concentration p_{tot} must be constant, where

$$p_{tot} = \sum_i p_i(t), \tag{9.25}$$

with the index i running over all proteins. Moreover, the growth rate reduces to the relative (or specific) increase of the protein mass, which leads to

$$\mu(t) = \frac{v_R(t)}{p_{tot}} = \frac{k_R\, p_R(t)\, h_R(c(t))}{p_{tot}}. \tag{9.26}$$

The above model couples metabolism, protein synthesis, and growth in a single formalism, in the spirit of the small resource allocation models discussed in Chapter 7.

Figure 9.2 gives an example of a resource allocation model, describing a simple self-replicatory microbial system [20, 21] inspired by the model of Scott *et al.* [22]. The model divides the proteome into three categories: ribosomes, enzymes, and housekeeping proteins, with concentrations p_Q, p_R, and p_M, respectively. In addition to the three categories of protein, we add a metabolite representing the precursors for protein synthesis, with concentration c. The precursors are produced from nutrients in the environment at a rate v_M, a macroreaction catalyzed by the enzymes. Protein synthesis occurs at a rate v_R, catalyzed by the ribosomes. The resource allocation functions u_Q, u_R, and u_M determine the fraction of the protein synthesis rate assigned to each of the three protein categories, where u_Q is assumed to be a constant, growth-rate-independent fraction. The rate equations for the metabolic and protein synthesis reactions follow irreversible Michaelis-Menten kinetics, where the substrate concentration in the medium is assumed saturating.

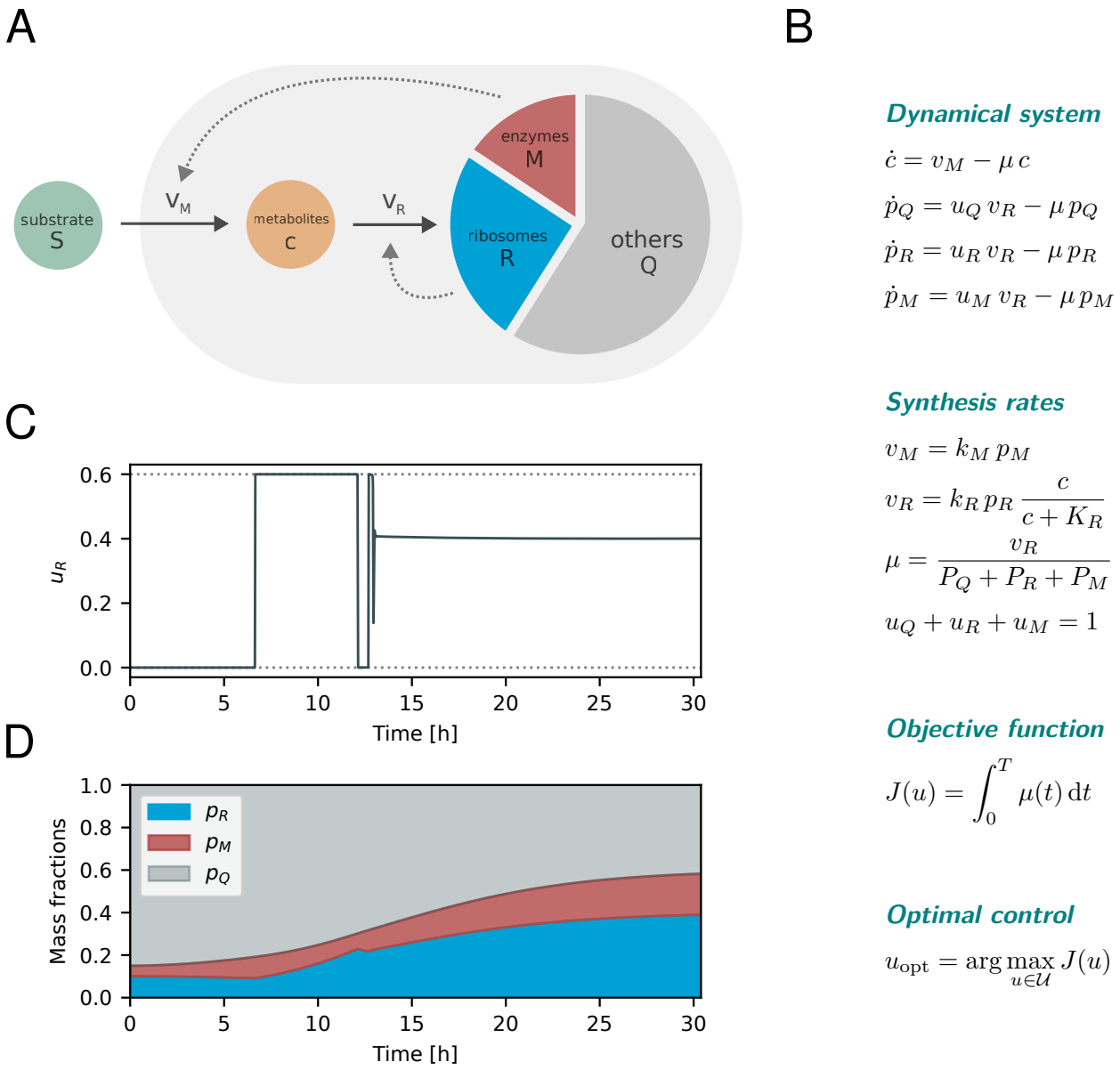

Figure 9.2: Example of optimal resource allocation strategy in a coarse-grained model of microbial growth. **A.** Representation of simple self-replicator model of microbial growth. **B.** Model and optimization problem for the self-replicator shown in panel A, as discussed in the text. **C.** Optimal dynamic resource allocation strategy, in terms of the fraction of resources attributed to ribosome synthesis (u_R). **D.** Time-varying protein mass fractions corresponding to the optimal solution shown in panel C. The parameter values used for the simulation are $k_M = 0.5$, $k_R = 1$, $K_R = 0.5$ and $u_Q = 0.6$.

The resource allocation functions in the model are not explicitly specified by regulatory mechanisms, but assumed to follow a dynamic pattern optimizing an objective criterion. In many cases, the objective criterion is based on the hypothesis that microorganisms have evolved to maximize growth, that is, the accumulation of biomass. While this hypothesis can be criticized on theoretical and empirical reasons, it is a reasonable choice in well-mixed environments and provides an interesting baseline in other environments. (Chapter **??**). In the model framework considered here, this gives rise to the following objective function:

$$\max_{u \in U} J(x(t), u(t), 0, T) = \int_0^T k_R\, p_R(t)\, h_R(c(t))\, dt, \tag{9.27}$$

where like in the general case of Eq. 9.1, U denotes the set of admissible profiles for the resource allocation functions u. Note that the maximization of growth over the time-interval $[0, T]$ amounts to taking the integral of the instantaneous

growth rate over that time-interval, defined by Eq. 9.26. This objective does not generally reduce to maximizing the instantaneous growth rate at every time-point of this interval.

The question can be asked, for the microbial self-replicator in Figure 9.2, how the cells redistribute their resources over the different protein categories after a change in environment, in particular a shift of the cells from a poor to a rich carbon source. In the case of *E. coli*, for example, such as shift might involve a change from minimal medium with acetate to minimal medium with glucose. Given that *E. coli* grows faster on glucose than on acetate, and that a higher growth rate requires an increased proportion of resources to be allocated to ribosomes according to the growth law (Chapter 9), one expects u_R to increase after the shift. Since u_Q is assumed constant, and the resource allocation functions must sum to 1 at every time-point, this overall increase of u_R must be balanced by a decrease of u_M. These expectations concern resource allocation before the shift (balanced growth on acetate) and a long time after the shift (balanced growth on glucose), but the growth law provides no information on the pattern of adaptation immediately after the shift.

In order to investigate the optimal adaptation pattern of u_R immediately after the growth transition, we solve the dynamic optimization problem specified in Figure 9.2. For the simple example considered here, the optimal solution can be characterized analytically [23, 20, 21]. This is not possible for more complicated examples, however, which require the optimal solution to be constructed numerically, using one of the tools discussed in Appendix []. Figure 9.2C-D show a typical solution for parameter values estimated from experimental data [21]. Starting from a low value of u_R during balanced growth on acetate, the optimal resource allocation scheme consists of a sequence of switches between $u_R = 1$ (maximal ribosome synthesis) and $u_R = 0$ (no ribosome synthesis), until an intermediate value of u_R for balanced growth on glucose is attained. The value of u_R during balanced growth on glucose is higher than that for balanced growth on acetate, as expected from the growth law.

The sequence of on-off switches followed by the intermediate steady-state value called a bang-bang-singular solution in optimal control theory [**?**]. The solution reflects a dynamic trade-off between the two different functions contributing to growth: metabolism and protein synthesis. When, due to growth dilution, the ribosome concentration falls to a level that is limiting for maximal protein synthesis, the synthesis of ribosomal proteins is switched on ($u_R = 1$), leading to an increase of the ribosome concentration. Switching on the synthesis of ribosomal proteins causes the synthesis of metabolic enzymes to be switched off. When, due to growth dilution, the concentration of metabolic enzymes next falls to a level that the precursors produced by the latter become limiting, the synthesis of metabolic enzymes is switched on ($u_R = 0$) to replenish the precursor pool, and so on.

Optimal solutions with a similar bang-bang pattern were already encountered in the previous section. They also occur in a model with a more detailed description of different precursor (amino acid) synthesis pathways under the objective under the minimal time of adaptation after a shift from a medium supplemented with amino acid to a medium lacking amino acids [24]. In a different type of problem, the development of intestinal crypts, the minimal time to mature crypts was found to depend on the on-off control of the proliferation of stem and non-stem cells [25]. There is no convincing experimental evidence that the adaptation of ribosomal synthesis after a nutrient upshift from a poor to a carbon source follows a bang-bang singular pattern. The interpretation of proteomics data after a nutrient upshift in *E. coli* shows that the simple upregulation of ribosomal resource allocation to the steady-state value for growth on the rich nutrient captures the ribosomal protein expression data well [26].

This example serves to emphasize that, while the optimality assumption may lead to thought-provoking predictions, these need to be confronted with experimental data. In case the optimal solutions do not agree with the data, several revisions of the problem could be considered. While growth optimization was chosen as the objective criterion in the example of Figure 9.2, there is evidence that during balanced growth, microorganisms find a trade-off between maximizing growth rate in a given environment and minimizing necessary adjustments to other environments [11]. The problem could therefore be generalized to a multi-criteria optimization problem. An example is the analysis of a model similar to that considered here under the objectives of biomass maximization and minimal adaptation time after a nutrient shift [27]. The formulation of the optimization problem in Figure 9.2 does not put any constraints on valid optimal resource allocation strategies, except that the individual functions u_i components need to sum to 1 (Eq. 9.24). Bearing in mind that the regulatory mechanisms underlying a resource allocation come with a cost, and need to respect certain

physical constraints, the predicted resource allocation strategy may not be feasible. When such constraints are taken into account, the optimal solution may no longer be bang-bang singular, but resemble the observed adaptation pattern [28, 21].

In summary, the dynamical optimization approach for studying microbial growth presented here provides a way to test the consequences of hypothesized objective functions in combination with simple resource allocation models. The predictions can be confronted with experimental data, but for the appropriate choice of objective functions, can also inform the redesign of microbial strains for metabolic engineering purposes (Box []).

9.5 Dynamic flux balance analysis (dFBA) of metabolic networks

Dynamic Flux Balance Analysis (dFBA) is an extension of Flux Balance Analysis (FBA) as described in Chapter 5, that can simulate the interactions between the metabolism of an organism and its dynamic environment. In contrast to the constant, steady-state flux solutions that are generated by classical FBA, dFBA yields flux solutions that may dynamically depend on concentrations of extracellular metabolites, such as sugars or other carbon sources, dissolved oxygen, or secreted waste metabolites. Applying these fluxes to the concentration balance of extracellular metabolites also permits to capture dynamic changes in these concentrations due to the cells' metabolic activity, and track the resulting overall biomass growth. It is noted that a basic assumption of dFBA is that organisms rapidly reach intracellular steady state in response to extracellular perturbations, and on the long run no metabolite can accumulate or deplete.

In general, a dFBA model comprises three main parts as demonstrated in Figure 9.3: the dynamic equations, in the form of differential equations, for biomass and extracellular metabolites, constraints on the fluxes as in the FBA model, and an optimization objective that determines how to choose the optimal fluxes.

We first consider the dynamic equations used for dFBA.

The biomass dynamics are given by

$$\dot{X} = \mu X, \tag{9.28}$$

where X denotes the biomass concentration, typically measured as dry mass in $\mathrm{g/L}$, and μ denotes the growth rate, typically measured in $1/\mathrm{h}$. In principle, this equation follows the equations for balanced growth. However, instead of using simple models, like a Monod equation for the growth rate, the growth rate is taken from the value of the biomass reaction in an FBA model (check in Chapter 5!).

Denoting the concentrations of the extracellular metabolites that are modelled dynamically as the vector c, the dynamics for these metabolites can be formulated as the differential equation

$$\dot{c} = S_{exch} v X. \tag{9.29}$$

Here, v is the flux vector for the complete metabolic network, including uptake and production reactions for exchange metabolites, and S_{exch} is the stoichiometric matrix that links these reaction fluxes to the metabolite concentrations which are balanced dynamically. Multiplication with the biomass X is necessary, since the flux values in the FBA model are determined relative to biomass, whereas the concentrations c of the dynamic metabolites are relative to the system volume. The equations are not yet closed, because the fluxes v (including the growth rate μ as one element of the flux vector) still need to be determined by optimization.

As constraints, two types of constraints are used in dFBA models. A flux balance constraint as in steady state FBA models is applied to the concentrations of all metabolites that are not dynamically balanced in (9.29), e.g., intracellular metabolites. This steady state constraint is given by

$$S_{int} v = 0, \tag{9.30}$$

where S_{int} is the stoichiometric matrix that links reaction fluxes in the vector v to the steady state metabolites. Further,

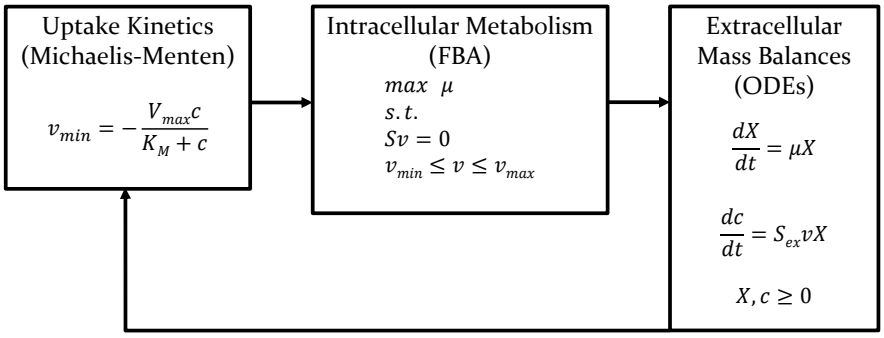

Figure 9.3: Schematic representation of dFBA. As in FBA, the intracellular environment in dFBA is represented by a linear programming (LP) optimization problem that describes the metabolism of the microorganism based on its genome-scale metabolic model (GSMM). FBA assumes that all intracellular metabolite concentrations remain constant while the cells optimally distribute their metabolic fluxes to maximize their growth rate and hence, an LP can calculate the growth rate, as well as the intracellular and exchange fluxes of the GSMM. The calculated growth rate and exchange fluxes can be used to update the extracellular environment. The extracellular environment in dFBA is represented by ordinary differential equations (ODEs) that describe the mass balance equations for biomass and metabolites found outside of the cell. Moreover, the intracellular GSMM and the extracellular mass balance equations can be linked through kinetic rules for substrate uptake, like the Michaelis-Menten equations, that can raise concentration-dependent constraints for exchange fluxes and predict growth rate dependencies on substrate concentrations.

upper and lower bounds need to be put on the individual reaction fluxes. In contrast to classical FBA, where these bounds are constant, in dFBA flux bounds can depend on concentrations of metabolites in the vector c. This is mostly applied to uptake reactions for nutrients, and often as Michelis-Menten kinetics. For example, if c_i is the concentration of a sugar substrate, and v_i is the uptake reaction for this substrate (conventionally negative in FBA models), bounds of the form

$$-\frac{V_{i,max}c_i}{K_M + c_i} \leq v_i \leq 0 \tag{9.31}$$

would be used, where $V_{i,max}$ and K_M are the common parameters of the Michealis-Menten kinetics (Chapter 3 [MET]).

In recent years, dFBA is increasingly applied for the simulation of dynamic biological systems, especially due to the promising use of GSMMs for interpreting cell physiology and evolution, as well as for guiding metabolic engineering and bioprocess design and optimization [29, 30, 31]. The dFBA applications based on GSMMs include the bacteria *Escherichia coli* [32, 33, 34, 35, 36, 37, 38, 39] and *Lactococcus lactis* [40], as well as the yeast species *Saccharomyces cerevisiae* [41, 42, 43, 44, 45, 33, 36, 46, 47, 39] and *Scheffersomyces (Pichia) stipites* [36]. However, the majority of dFBA applications use small-scale metabolic models, most of which include less than 100 reactions. Such applications include models of bacteria, like *Escherichia coli* [48, 49, 50, 36, 51, 52, 53, 54], *Corynebacterium glutamicum* [55, 35, 56, 38] and *Bordetella pertussis* [57], models of yeast for wine fermentation [58] and *Saccharomyces cerevisiae* [59, 47, 60, 61], but also plant and animal models, such as a model for the photosynthetic metabolism of C3 plants [62], a four-tissue (leaf, root, seed, and stem) model of the core metabolism of *Arabidopsis thaliana* [63], a model for fatty acid metabolism and lipid accumulation in rat hepatocytes [64], and a model for energy metabolism in myocardial cells [65].

It is noted that most of the dFBA applications for microorganisms simulate microbial fermentations under batch or fed-batch conditions. Since dFBA can be used for the analysis, control and optimization of biochemical processes,

many dFBA applications focus on either dynamic metabolic engineering or optimal control of bioreactors, or both simultaneously. Dynamic metabolic engineering studies can predict the effect of strain gene insertion and deletion on the dynamic behavior and productivity of a bioprocess [41, 32, 42, 43], while optimal control of batch or fed-batch operation of bioreactors is important for the production of desired chemicals [41, 61]. Finally, dFBA has also been expanded for the study of microbial communities, where each microorganism is represented by an LP that is solved independently [66, 67, 68]. Co-culture simulations with dFBA can predict possible consortia compositions, as well as metabolic engineering approaches to improve the productivity of the consortia, but they are out of the scope of this chapter.

Coming to the mathematical formulation of dFBA models, dFBA is an optimization problem coupled with a system of ordinary differential equations, that can be solved with the help of various mathematical and numerical techniques. Even though dFBA was first introduced in 1994 [48], it was not formalized until 2002 [49]. The existing formalized solution approaches that are going to be discussed here involve the static optimization approach (SOA), the dynamic optimization approach (DOA), and the direct approach (DA). More recently, reformulation approaches and surrogate models for the optimization problem have also been proposed in order to ease the computational complexity of dFBA simulations. This complexity arises from several characteristics of dFBA. More specifically, the solution of dFBA problems faces challenges in terms of:

1. problem size and scalability: As the size of the metabolic network increases, the computational cost increases. For this reason, simulations that involve large genome-scale metabolic models or multispecies microbial communities are limited.
2. stiffness: The stiff behavior of dFBA has been observed in many cases, such as the simulation of the diauxic growth in *E. coli* [49].
3. nonlinearity: The presence of nonlinear constraints or objective functions can significantly increase the computational cost.
4. feasibility: The intracellular optimization problem can become infeasible and lead to failure of the integration of the extracellular ODEs.
5. differentiability: The optimal value of the intracellular optimization problem may not be continuously differentiable, which poses an obstacle when dFBA is used for optimal control or parameter estimation.
6. non-unique solutions: The solution of the intracellular optimization problem is usually not unique which can make fluxes unrealistically "jump" between different optimal solutions.

Static Optimization Approach (SOA) divides the total time horizon of the dFBA simulation into several smaller time intervals. The optimization problem is solved to obtain the flux distribution at the beginning of each time interval, and then the ODEs are integrated over the time interval with this fixed flux distribution. The dynamics calculated from this time step are used to constrain the optimization problem solved at the beginning of the next time interval, and the process is repeated until the end of the simulation time is reached. SOA can be implemented easily with the use of an Euler scheme for integrating the system and a suitable existing LP solver for solving the FBA at each time step. SOA is also implemented in the constraint-based reconstruction and analysis (COBRA) toolbox for MATLAB [69] which can perform dFBA simulations. Since its implementation is relatively simple, SOA has been widely used in studies for the diauxic [49, 51, 70], aerobic and fermentative [48, 71, 72, 32] growth of *E. coli*, for *S. cerevisiae* fermentations [59, 43, 44, 45], as well as for the growth of other bacterial [40] and plant organisms [63]. Many of these applications include larger-scale or genome-scale metabolic networks, due to the scalability of SOA. However, the main drawback is that SOA is inefficient and can become computationally expensive because it has to solve the optimization problem at each time step. This can be challenging for most dFBA problems which are stiff and require small time steps to ensure accuracy, convergence, and stability of the solution.

Dynamic Optimization Approach (DOA) follows closely the general dynamic optimization framework described in Section 9.2: an objective function that depends on the dynamic states of the system over the complete time horizon of interest is formulated, and the dynamics (9.28)–(9.29) and algebraic constraints (9.30)–(9.31) are added as optimization constraints. In other words, DOA discretizes the total time horizon of the dFBA simulation, and then transforms the dynamic optimization problem into a non-linear programming (NLP) problem, which is solved once by simultaneously optimizing over the entire time of the simulation. In this way, DOA obtains the time profiles of fluxes and metabolite concentrations in the system, and allows the formulation of a dynamic objective function, which could provide useful information about the design of genetically modified metabolic networks or the maximization of bioprocess productivity. Because of this characteristic, DOA is often used in dynamic metabolic engineering, parameter estimation and optimal control applications. For example, DOA has been used for simulating the diauxic growth of *E. coli* [49, 73, 74], as well as the growth of engineered *E. coli* strains on glucose [75, 76], and the growth of various eukaryotic organisms, such as *S. cerevisiae* [60, 77], plant [62] and animal [64, 65] cells under genetic and environmental perturbations. On the downside, even though the optimization problem does not need to be repeatedly solved like in SOA, the single NLP of DOA can become easily intractable, as its dimension increases with the fineness of time discretization. Additionally, DOA has been mainly limited to small-scale metabolic networks, since it cannot be easily applied to genome-scale metabolic networks due to the large number of variables and constraints that are introduced in the NLP as the size of the network increases.

Direct Approach (DA) has been formulated more recently than SOA and DOA, and directly includes the LP solver for the FBA in the right-hand side evaluator function of the ODEs. In this way, it can take advantage of existing ODE integrators with adaptive step size and error control that can reduce the number of integration steps and provide better solution accuracy compared to SOA. DA has been implemented in the ORCA toolbox [78], which complements the constraint-based reconstruction and analysis (COBRA) toolbox for MATLAB [69]. Furthermore, DA has been used for studying the diauxic [79], aerobic and anaerobic[50, 80] growth of wild type and engineered *E. coli* strains, the aerobic growth of *Corynebacterium glutamicum* on glucose and xylose in biorefinery simulations [56], as well as the aerobic and anaerobic growth of wild type and engineered *S. cerevisiae* strains [41, 42, 44]. Some of these applications involve dynamic metabolic engineering for product maximization, and many of them include genome-scale metabolic networks, since DA is relatively easily scalable like SOA.

However, DA requires the LP to be resolved at least once, every time the right-hand side of the ODEs is evaluated [81]. This can make DA computationally demanding, especially for larger metabolic networks. Another major challenge is that when evaluating the right-hand side of the ODEs close to the boundary of feasibility, the LP can become infeasible and make the dFBA simulation fail. The LP can become infeasible either because it is really infeasible and the simulation should be terminated, or because the ODE integrator becomes unable to evaluate the right-hand side of the ODEs and the simulation is discontinued, or erroneous death phase messages are being displayed. The latter can happen as dFBA simulations involve discrete events that correspond to switches in the active set of the LP solution. More specifically, different bases for the optimal solution of the LP can emerge at each time step. Moreover, at the points of change of the active set, the dFBA model is not differentiable, since the optimal value of the LP as a function of the right-hand side of the constraints is not continuously differentiable. This is a problem because the first and second derivatives of the model must be computed when dFBA is used for optimal control or parameter estimation applications. Finally, another drawback emerges due to the primal multiplicity of the LP. As it is well-known, FBA is formulated as an underdetermined problem and therefore, the LP does not have a unique solution [82]. Non-unique optimal reaction fluxes can lead different ODE integrators to different results.

Recommended readings

Exercises

Mathematical details 9.A: dFBAlab

In order to address some of the computational challenges of dFBA, Höffner, Harwood, and Barton proposed a simulator for dFBA, which was initially coded in FORTRAN [83], but gained popularity when implemented in MATLAB with the name Dynamic Flux Balance Analysis laboratory (DFBAlab) [68], and more recently in Python [84]. It is noted that the DFBAlab is compatible with the COBRA toolbox [69]. Based on this dFBA simulator, it is not necessary to resolve the LP each time the right-hand side of the ODEs is evaluated and consequently, the solution process becomes faster. This is possible because the FBA solution at an initial time can be used to compute future optimal solutions by detecting changes in the active set [38] or by computing the optimal basis [85] of the FBA. Unfortunately, such formulations need to continuously monitor the active set of the LP, which increases with the size of the metabolic network, or need to choose a basis for the optimal solution that is most likely to remain optimal as the simulation proceeds [85]. The latter is challenging since the optimal basis can be non-unique even for a unique optimal solution. Nevertheless, DFBAlab manages to reduce the number of times that the LP is resolved, and also avoids obtaining infeasible LPs and numerical failure by using the LP feasibility problem and the Karush-Kuhn-Tucker (KKT) optimality conditions of the FBA problem (see below) [33, 35, 38]. In addition, the differentiability problem could be solved with the help of non-smooth analysis which provides optimality conditions in terms of sub-gradients or generalized gradients, for convex and non-convex functions respectively [34]. Furthermore, to tackle the issue of primal multiplicity of the FBA problem, DFBAlab performs lexicographic optimization [33, 34, 35, 38].

Mathematical details 9.B: Lexicographic Optimization

Lexicographic or hierarchical optimization involves the solution of a series of LPs with auxiliary objectives ranked in priority order. The use of auxiliary objectives reduces the feasible space and leads to a unique optimal solution, while the auxiliary objectives can have specific biological meanings and can be selected based on prior knowledge about the organism [86]. Some of the most used auxiliary objectives are based on the assumption that evolution leads to the exclusion of inefficient pathways so that cells can biosynthesize the smaller possible number of enzymes. Examples of such auxiliary objectives include the minimization of enzyme cost [87], the minimization of the total reaction flux [88], and the minimization of the number of active reactions [89]. However, it has been shown that such objectives may not be suitable for some engineered cells [57]. In general, it is not trivial to find a series of auxiliary objectives that are consistent with experimental data, assure uniqueness and preserve continuity of the optimal solution. In some cases, even when all auxiliary objectives have been used, hierarchical optimization cannot ensure the uniqueness of the dFBA solution. Apart from the use of auxiliary objectives, auxiliary rules or auxiliary parameters have also been proposed to address the primal multiplicity of FBA. For example, geometric methods have been proposed to identify a unique distribution of reaction fluxes for FBA [90], even though there is no biological evidence to justify such methods.

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