

Resource allocation in metabolic networks: kinetic optimization and approximations by FBA

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Abstract

Based on recent theoretical results on optimal flux distributions in kinetic metabolic networks, we explore the congruences and differences between solutions of kinetic optimization problems and results obtained by constraint-based methods. We demonstrate that, for a certain resource allocation problem, kinetic optimization and standard flux balance analysis (FBA) give rise to qualitatively different results. Furthermore, we introduce a variant of FBA, called satFBA, whose predictions are in qualitative agreement with kinetic optimization.

Introduction

The construction and analysis of genome-scale metabolic networks are undoubtedly true success stories of systems biology. Based on the increasing number of fully sequenced genomes, large-scale metabolic reconstructions have enabled us to establish a computational link between a given genome and properties of the resulting metabolic phenotype [1,2]. As a prerequisite for the application to large-scale networks, flux balance analysis (FBA) and related constraint-based methods do not require extensive kinetic information on individual enzymatic reactions. Rather, these methods build upon constraints for the feasible biochemical flux space, induced by the principles of mass and charge conservation in biochemical reactions.

Considering only stoichiometric constraints however, the fluxes in cellular metabolism are still highly under-determined. The predictive power of constraint-based methods derives from the assumption that the activities of biochemical reactions are organized according to certain evolutionary plausible optimality principles. In the terminology of FBA, an objective function is maximized or minimized. It is the assumption of optimality in metabolic network operation that enables specific, albeit not necessarily unique, flux predictions for given constraints [3–5].

Although highly successful, the limits of FBA and related methods are increasingly recognized [5–7]. The existence of seemingly sub-optimal flux solutions, specifically low-yield flux phenotypes, is well known, for example, aerobic fermentation by microorganisms and many cancer cells. An increasing number of constraint-based methods have been proposed to explain such observed transitions to low-yield pathways. The respective extensions of standard FBA

typically incorporate additional cellular capacity constraints, for example, induced by limited cytosolic space (molecular crowding), limited membrane space, finite availability of micro- and macro-nutrients or limited energy expenditure for amino-acid synthesis [8–12].

Although additional constraints can often be justified based on biophysical principles, the success of such extensions in reproducing overflow metabolism and low-yield pathways gives rise to a computational conundrum: To what extent can constraint-based methods truly substitute for kinetic optimization? To what extent are they sufficient to identify the underlying causes of observed metabolic transitions? In order to answer these questions, we must go beyond constraint-based methods and consider the non-linear optimization problem arising from a kinetic metabolic network, taking into account the system of differential equations that governs its dynamics.

Currently, kinetic optimization problems for large-scale metabolic networks are not practicable. In addition to being computationally hard, the solution requires detailed and quantitative knowledge of the underlying rate equations and their parameters, information that is not yet available. Nonetheless, recent theoretical findings about the optimal state of kinetic metabolic networks allow, for the first time, to approach these questions from a fundamental perspective. Two independent publications [13,14] showed that any metabolic network that maximizes the rate of a particular reaction given a limited enzymatic resource operates in an elementary flux mode (EFM). However, the specific EFM that exhibits optimal rate depends on kinetic parameters, such as external metabolite concentrations, in particular, its identity is not determined by its yield.

In this contribution, we explore the differences and congruences between kinetic and purely stoichiometric models of metabolic networks, in particular, between kinetic optimization and FBA.

Key words: elementary flux modes, enzyme kinetics, metabolic optimization, non-linear optimization, satFBA.

Abbreviations: EFM, elementary flux mode; FBA, flux balance analysis.

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The enzyme allocation problem

A kinetic metabolic network with n metabolites and r reactions gives rise to the dynamical system

$$\frac{dx}{dt} = N v(x; c, p),$$

where $N \in \mathbb{R}^{n \times r}$ denotes the stoichiometric matrix and $v \in \mathbb{R}^r$ the vector of reaction rates as a function of metabolite concentrations $x \in \mathbb{R}^n$, enzyme concentrations $c \in \mathbb{R}^r$ and parameters p . (Clearly, the concentrations fulfill the natural nonnegativity constraints $x \geq 0$ and $c \geq 0$.)

More specifically, the rate v_i of reaction i is a product of the corresponding enzyme concentration c_i and the kinetic function $\kappa_i(x, p)$ which may depend nonlinearly on metabolite concentrations and parameters,

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

Using the component-wise vector-product \circ , we write $v = c \circ \kappa(x, p)$.

In addition to stoichiometric constraints, we consider a cellular capacity constraint, in particular, a limited enzymatic resource,

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

In the sum over all enzymatic reactions, the weights w_i are the amounts of the resource needed per unit enzyme concentration and W is the available amount. The weights might relate to cytosolic space, membrane space, nutrient or energy expenditure.

Under this enzyme constraint, we maximize the steady-state rate v_{i^*} of a particular reaction i^* by varying metabolite and enzyme concentrations, i.e., we solve an enzyme allocation problem using kinetic optimization. For reasons of comparison, we approximate the kinetic model (with the enzyme constraint) by a purely stoichiometric model (with a corresponding flux constraint) and directly vary fluxes, i.e., we solve the problem by FBA. We define the two problems in Table 1.

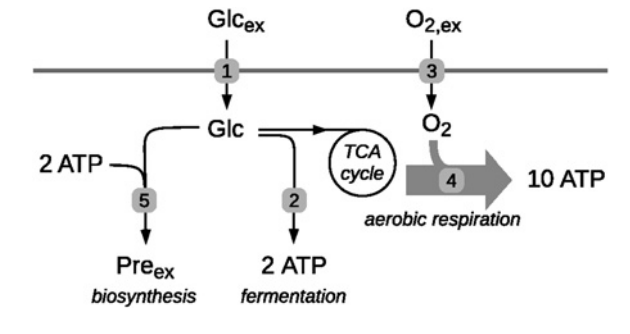
The approximation of the kinetic by a purely stoichiometric model is described in detail in the sub-section '3.2 Approximation: purely stoichiometric model with flux constraint' of the Supplementary Material. In particular, reversible reactions are split into forward and backward directions (and hence $v \geq 0$), weights are scaled ($\tilde{w}_i = w_i/k_{\text{cat},i}$) and fluxes have upper bounds ($v \leq V$).

The properties of the two approaches are compared in Table 1. Kinetic optimization is non-linear, it contains the biophysical enzyme constraint and thermodynamic feasibility is guaranteed by the kinetics. In contrast, FBA is linear, the corresponding flux constraint is weaker than the original enzyme constraint and thermodynamic feasibility can be incorporated as an optional constraint.

Most importantly, the results obtained for the two approaches are qualitatively different. As shown previously,

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

Reproduced from [13]: Müller, S., Regensburger, G. and Steuer, R. (2014) Enzyme allocation problems in kinetic metabolic networks: Optimal solutions are EFMs. *J. Theor. Biol.* **347**, 182–190.



optimal solutions of kinetic optimization are EFMs [13,14], whereas optimal solutions of FBA are combinations of EFMs, in general.

Kinetic optimization and FBA

We illustrate the comparison of kinetic optimization and FBA by an example. We consider a minimal metabolic network including fermentation, aerobic respiration and synthesis of a biomass precursor from glucose (Figure 1).

The network contains three internal metabolites (glucose, oxygen, ATP) and five reactions (glucose import, fermentation, oxygen import, aerobic respiration, biosynthesis) for which we assume Michaelis–Menten kinetics. In particular, the rates of the import reactions depend on the external glucose and oxygen concentrations. The network and the kinetics are described in detail in the sub-sections '2.1 Metabolic network' and '2.3 Kinetics' of the Supplementary Material.

We note that the network can operate in two EFMs with non-zero rate of biosynthesis (normalized to 1),

$$e^1 = (2, 1, 0, 0, 1)^T \quad \text{and} \quad e^2 = (6/5, 0, 1/5, 1/5, 1)^T$$

EFM e^1 corresponds to 'pure fermentation' since reactions 3 and 4 have zero flux, whereas EFM e^2 corresponds to 'pure respiration' since reaction 2 has zero flux. Further, e^1 has low yield, since two glucose are needed to produce one precursor, whereas e^2 has high yield, since only 6/5 glucose are needed.

Kinetic optimization

As discussed in the introduction and formalized in the previous section, we consider a cellular capacity constraint, in particular, a limited enzymatic resource. Under this constraint, we maximize the steady-state rate of biosynthesis by varying metabolite and enzyme concentrations. In other

Table 1 | The enzyme allocation problem for kinetic and purely stoichiometric metabolic networks: definitions, properties and results

Enzyme allocation problem	
Kinetic optimization	FBA
$\max_{x,c} v_j^*$ <p>subject to</p> $N v = 0,$ $\sum_i w_i c_i \leq W,$ <p>where</p> $v = c \circ \kappa(x, \rho),$ $x \geq 0, c \geq 0.$	$\max_v v_j^*$ <p>subject to</p> $N v = 0, v \geq 0,$ $\sum_i \tilde{w}_i v_i \leq W,$ $v \leq V,$ <p>and</p> <p>(thermodynamic feasibility).</p>
Non-linear in metabolite concentrations x , linear in enzyme concentrations c	Linear in fluxes v
Biophysical enzyme constraint	Flux constraint
Thermodynamic feasibility guaranteed by kinetics	Optional thermodynamic feasibility constraint
Theorem: for arbitrary kinetics, optimal solutions are EFMs.	In general, optimal solutions are combinations of EFMs.

words, we solve an enzyme allocation problem using kinetic optimization.

In fact, we are interested in the maximal rate of biosynthesis v_5 for different external metabolite concentrations $[\text{Glc}_{\text{ex}}]$ and $[\text{O}_{2,\text{ex}}]$. In particular, since optimal solutions are necessarily EFMs [13,14], we are interested in which EFM is optimal and where a transition between EFMs occurs. Hence, we solve the resulting non-linear optimization problems for EFM e^1 (pure fermentation) and EFM e^2 (pure respiration) individually and display the maximal rates in Figure 2.

Indeed, for high external glucose and low external oxygen concentrations, pure fermentation is optimal (i.e., achieves maximal rate under the enzyme constraint), whereas for low glucose and high oxygen pure respiration is optimal.

In Figure 3 (top), we show the optimal rates of biosynthesis and glucose import as functions of the external glucose concentration (at fixed external oxygen concentration). Clearly, there is a transition from pure respiration to pure fermentation which is continuous in the rate of biosynthesis, but discontinuous in the rate of glucose import. In fact, the transition is discontinuous in all other rates and all enzyme and metabolite concentrations (for details, see sub-section ‘2.4 Enzyme allocation’ of the Supplementary Material).

For comparison with FBA, we need to express results obtained by kinetic optimization in terms of fluxes, without using external metabolite concentrations. In Figure 3 (bottom), we show the optimal fluxes of biosynthesis compared with glucose import. In this representation, EFMs are the extreme rays of the projected flux cone and optimal solutions lie on these rays, but not in the interior of the cone.

Approximations by FBA

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).

In the example of the minimal metabolic network, instead of varying the external metabolite concentrations $[\text{Glc}_{\text{ex}}]$ and $[\text{O}_{2,\text{ex}}]$, we can either vary the upper bounds V_1 and V_3 for the corresponding exchange fluxes or the saturation values $\varphi_1 = \kappa_1/k_{\text{cat},1}$ and $\varphi_3 = \kappa_3/k_{\text{cat},3}$ for the corresponding exchange reactions.

The approximations of kinetic optimization by standard FBA and satFBA are described in detail in the sub-section ‘3.3 Exchange reactions’ of the Supplementary Material. In both approximations, we consider a linear optimization problem involving the flux constraint $\sum_i \tilde{w}_i v_i \leq W$ arising from the enzyme constraint.

In standard FBA, the constraints $v_i \leq V_i$ for exchange fluxes lead to optimal solutions that are combinations of EFMs, in general. As a consequence, standard FBA does not predict switches between EFMs, but continuous transitions.

In satFBA, exchange reactions contribute terms $(\tilde{w}_i/\varphi_i)v_i$ to the flux constraint arising from the enzyme constraint. In other words, the saturation values $\varphi_i = \kappa_i/k_{\text{cat},i}$ modulate the weights \tilde{w}_i for exchange fluxes, cf. [7] for an informal

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, blue) and EFM e^2 (pure respiration, green)

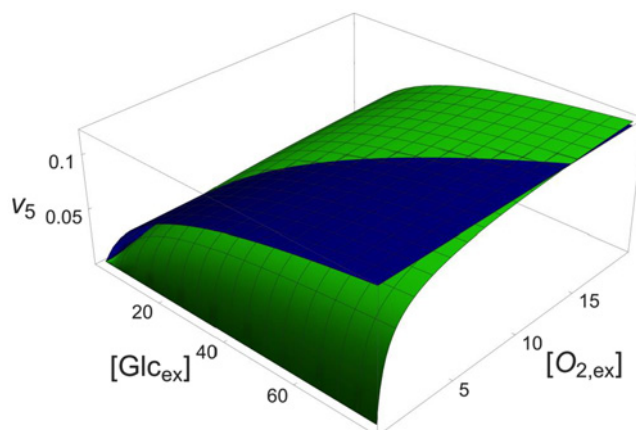
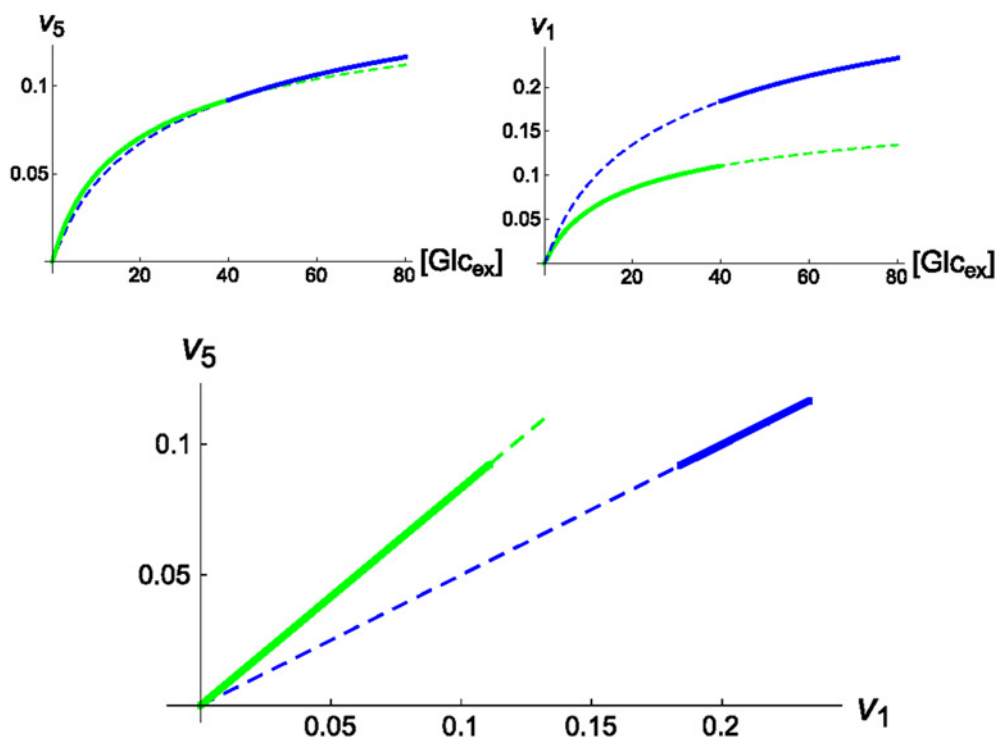
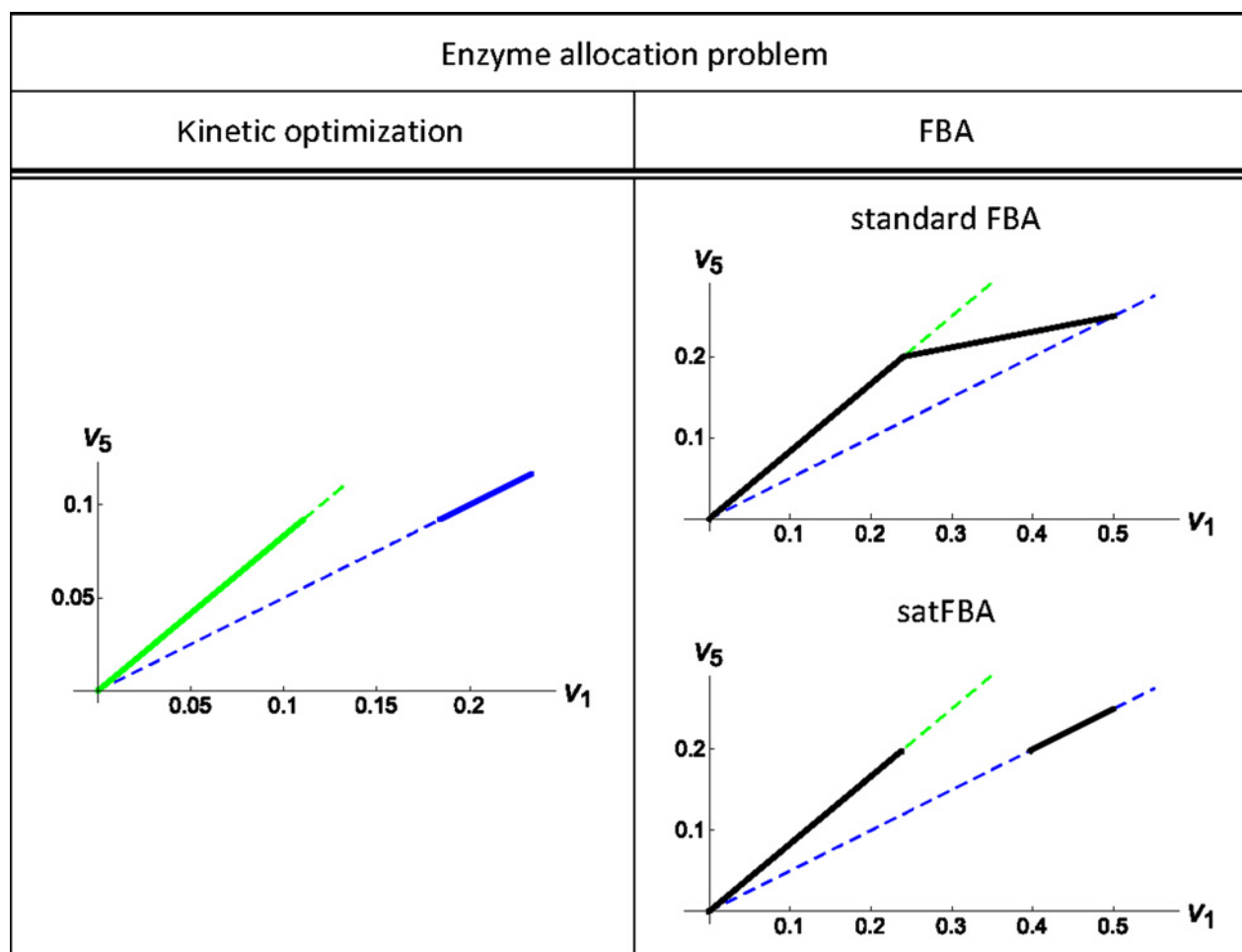


Figure 3 | Optimal rates of biosynthesis v_5 and glucose import v_1 as functions of external glucose concentration $[Glc_{ex}]$ (at fixed external oxygen concentration $[O_{2,ex}] = 10$) for EFM e^1 (pure fermentation, blue) and EFM e^2 (pure respiration, green) [top]. Optimal fluxes v_5 compared with v_1 as a result of kinetic optimization [bottom].



argument. Clearly, low saturation values lead to high weights. Due to the absence of constraints for individual exchange fluxes, optimal solutions of the resulting optimization problems are EFMs, as in the case of kinetic optimization. As a consequence, satFBA predicts switches between EFMs.

In Table 2, we compare optimal solutions obtained by kinetic optimization, standard FBA and satFBA for the example of the minimal metabolic network. Whereas kinetic optimization predicts a switch between aerobic respiration and fermentation, its approximation by standard FBA predicts a continuous transition. The result of satFBA

Table 2 | The enzyme allocation problem for the minimal network. Optimal solutions obtained by kinetic optimization and two variants of FBA

agrees with the result of kinetic optimization, at least qualitatively.

Conclusions

We have reviewed the modelling of resource allocation in metabolic networks given a cellular capacity constraint. This scenario can be caused by limited cytosolic space, limited membrane space, finite availability of micro- and macro-nutrients or limited energy expenditure for amino-acid synthesis. In each of these instances, the resulting enzyme constraint involves a weighted sum of enzyme concentrations, where the weights are the resources needed per enzyme.

Strictly speaking, enzyme constraints can only be formulated in kinetic models of metabolic networks, where metabolite and enzyme concentrations determine fluxes. Such enzyme constraints however have motivated the formulation of corresponding flux constraints in large-scale, purely stoichiometric models, most notably to explain the occurrence of low-yield pathways [7,11,12,15]. In fact, every

kinetic model with an enzyme constraint can be approximated by a purely stoichiometric model with a corresponding flux constraint. Analogously, kinetic optimization can be approximated by FBA. However, the results of the two approaches are qualitatively different. Optimal solutions of kinetic optimization are EFMs [13,14], whereas optimal solutions of FBA are combinations of EFMs, in general.

We have illustrated the comparison of kinetic optimization and FBA by an example. We considered a minimal metabolic network including fermentation and aerobic respiration and maximized the rate of biosynthesis of a precursor from glucose. Whereas kinetic optimization predicts an abrupt switch between aerobic respiration (high-yield) and fermentation (low-yield), its approximation by standard FBA predicts a continuous transition. To address this discrepancy, we have introduced a new variant of FBA, called satFBA, which does not use upper bounds for individual fluxes, but instead considers saturation values for exchange reactions to mimic the effect of varying external metabolite concentrations. The result of satFBA qualitatively agrees with the result of kinetic optimization.

We note that experimentally both continuous and discontinuous transitions are observed. Whereas metabolic switches indicate an exclusive choice between alternative metabolic states, such as in catabolite repression, different metabolic strategies can also operate simultaneously, such as fermentation and residual respiration in cancer cells [16]. The theoretical results in the studies by Müller et al. [13] and Wortel et al. [14] suggest that such a co-occurrence of metabolic strategies is either caused by additional constraints or an instance of sub-optimal adaptation.

We claim that kinetic models of metabolic networks will lead to a better understanding of resource allocation. Using enzyme kinetics and assuming that metabolic activity is organized according to optimality principles, we can identify the relevant constraints and objective functions. However, at the moment, kinetic optimization for large-scale metabolic networks is not practicable and approximations by FBA are being used. Whereas constraint-based methods can never substitute for non-linear optimization from a quantitative perspective, the predictions of satFBA and kinetic optimization are in qualitative agreement.

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