Partitioning Enzymatic Reactions with Flux Balance Analysis

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Introduction

Flux Balance Analysis (FBA) is a method used to study the metabolic behavior of a single-cell organism by defining and solving an optimization problem. FBA assumes a cellular objective, usually the maximization of growth, and under certain constraints describing the cell's environment and capabilities of intracellular reactions, solves for intracellular fluxes which achieve optimum growth.

Researchers have used FBA to ascertain properties of the metabolic networks of several organisms, but in doing so, haven't overcome environmental dependencies and solution degeneracy inherent to FBA. This project attempts to discuss these issues and extend definitions to account for them.

An additional area of interest is the relationship between cell growth and environmental resources. We define a minimal environment, which is characterized by minimizing the resources needed for a cell to achieve a certain growth state. When the growth state varies, the minimal environment varies with it. There are certain points at which changing the target growth state causes non-linear changes in the minimal environment. These growth rates identify shifts in metabolic processes.

Mathematically Modelling Metabolic Processes

The standard form linear program (LP) is (in vector notation):

minimize/maximize $\mathbf{c}^T\mathbf{x}$
subject to $\mathbf{Ax = b, x >= 0}$

where $\mathbf{c}^T\mathbf{x}$ is the inner product of vectors **c** and **x**, i.e. $c_1x_1+c_2x_2+c_3x_3+\ldots+c_nx_n$. This allows each member of **x** to have a weight defined by a corresponding member of **c.**

Ax=**b** is a collection of constraints on elements in **x** and can be expanded into a collection of equations of the form $a_{m1}x_1+a_{m2}x_2+\ldots+a_{mn}x_n <= b_m$

Growth is traditionally assumed to be the primary objective of a single-cell organism. FBA is used under the assumption that the metabolic processes are in a steady state, and that the mass of the cell is conserved. Let $[M_i]$ be the concentration of metabolite i and A_{ir} be the stoichiometric coefficient of metabolite i in reaction r. Allowing $\boldsymbol{\nu}_r$ to be the flux of reaction r, we assume

$$\frac{d[M_i]}{dt} = \sum_r A_{ir}\nu_r = 0.$$

This forces all metabolites to balance between what is input to and output from the cell.

The High-Flux Backbone

The High-Flux Backbone (HFB) of a cell is defined as the collection of reactions that both dominate the consumption and production of a metabolite (a metabolite is a precursor or product of a reaction)[1]. These reactions correlate well with established pathways in biology, suggesting that the HFB is a useful as a tool for studying single-cell organisms.

The HFB, however, is dependent on the solution of the LP and the environment[2]. Areas of ambiguity could include the environmental resources available to the cell, but also, the algorithm used by the solver. This is because the solution to the LP is not unique; in fact, the solution space of the linear program is highly dimensional, resulting in an infinite number of ways to reach optimum growth. This calls for an unambiguous and general way to partition cellular reactions.

The High, Intermediate and Low Flux Networks

We have extended the definition of the HFB to the HFN and further define the Intermediate and Low Flux Networks.

The **High Flux Network (HFN)** is the collection of reactions that can be a dominant consumer and producer in an optimal setting.

The **Intermediate Flux Network (IFN)** is the collection of reactions that can be a dominant consumer or producer in an optimal setting, but not both.

The **Low Flux Network (LFN)** is the collection of reactions that are incapable of dominating or consuming a metabolite in an optimal setting.

If a reaction is in one of these sets for at least one but not all environments, then it is in the set dependent on the environment. Otherwise, it is in the set independent of the environment.

By partitioning the metabolic network into these sets, studying relationships between reactions is made easier by identifying reactions that serve similar purposes.

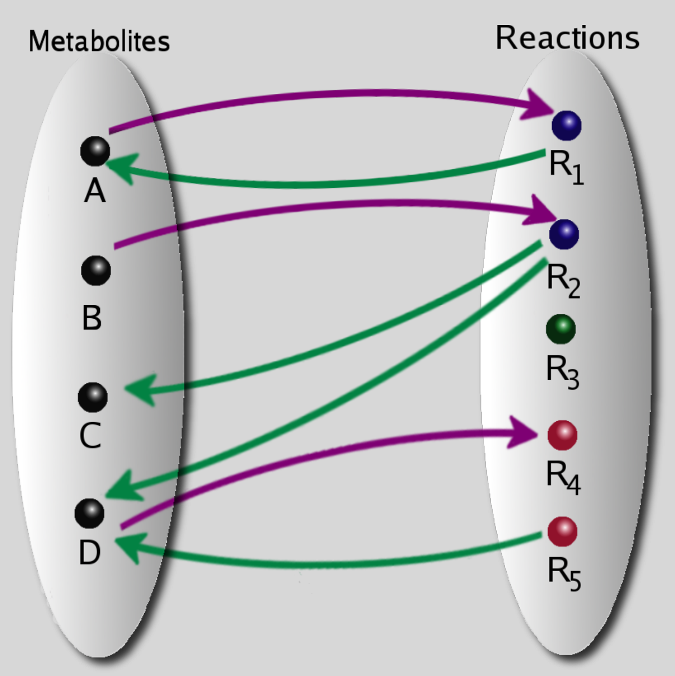

Figure 1. A bipartite graph of metabolite-reaction pairs. Blue reactions are in the HFN, red are in the IFN, and the green reaction is in the LFN.

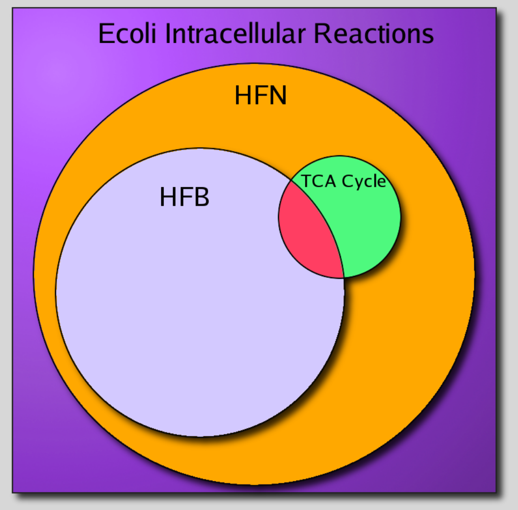

Figure 2. Venn Diagram of the reactions that occur within e.coli. The TCA cycle is partially revealed by the HFB and completely revealed by the HFN.

Minimal Environments

Because the solution fluxes are not unique, the amount of each metabolite required to achieve a certain growth rate is not unique. We have developed an algorithm to minimize each environmental resource to identify a unique set of fluxes for a specified growth rate. The growth rate is fixed at an attainable value and a variable is added which acts as an upper bound to the fluxes of the cellular inputs. This upper bound is minimized until some of the fluxes becomes fixed at this value. We fix these flux at this value, remove them from the set of bounded fluxes, and continue the process to minimize the remaining resources. The result is a unique set of environmental resource fluxes that can be used to attain a particular level of growth.

The minimal environment varies with the target growth rate. This relationship is generally linear on a per-metabolite basis, which indicates that the cost of one unit of growth remains constant regardless of how much growth is attained (within known bounds of how quickly a cell can grow). This relationship is not linear for every metabolite. The rate at which Alanine and Glycine must be imported to e.coli increases at key growth rates, which are important because they indicate that the mechanisms used by the cell to produce growth are changing.

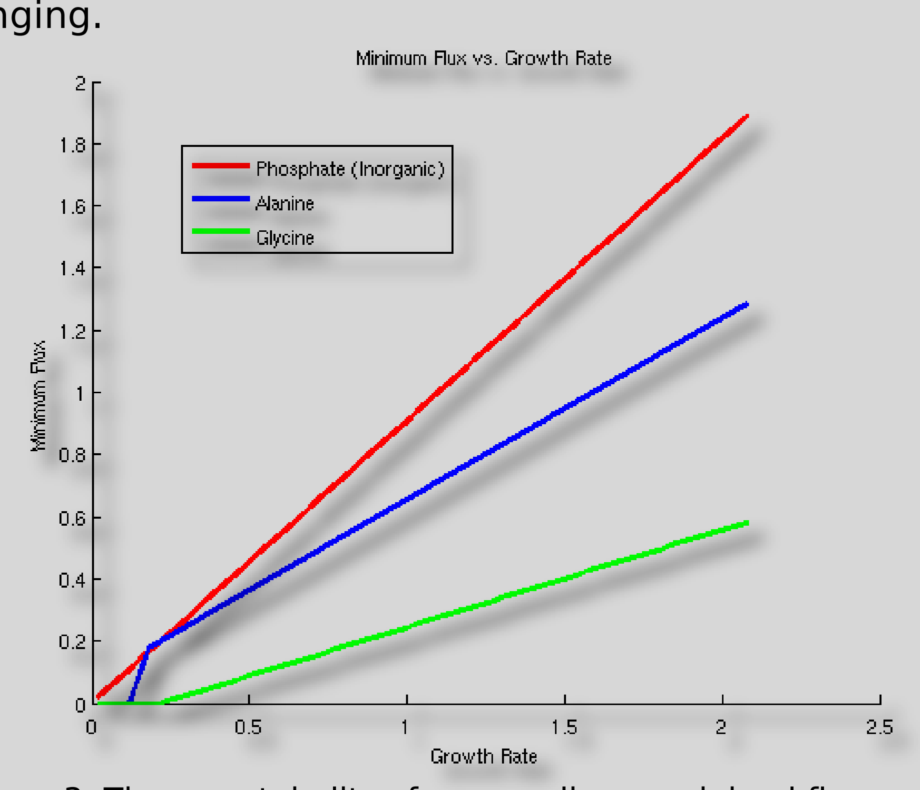

Figure 3. Three metabolites from e.coli on a minimal flux vs. growth rate graph. The relationship is non-linear for Alanine and Glycine.

An interesting result from this algorithm is that groups of metabolites that act as substitutes for one another are highlighted. Flat regions as seen in Figure 4 are likely to occur because single metabolites that provide an important service to the cell are limited by the bounding variable, prompting other metabolites to compensate in such a way that they are all at the maximum flux.

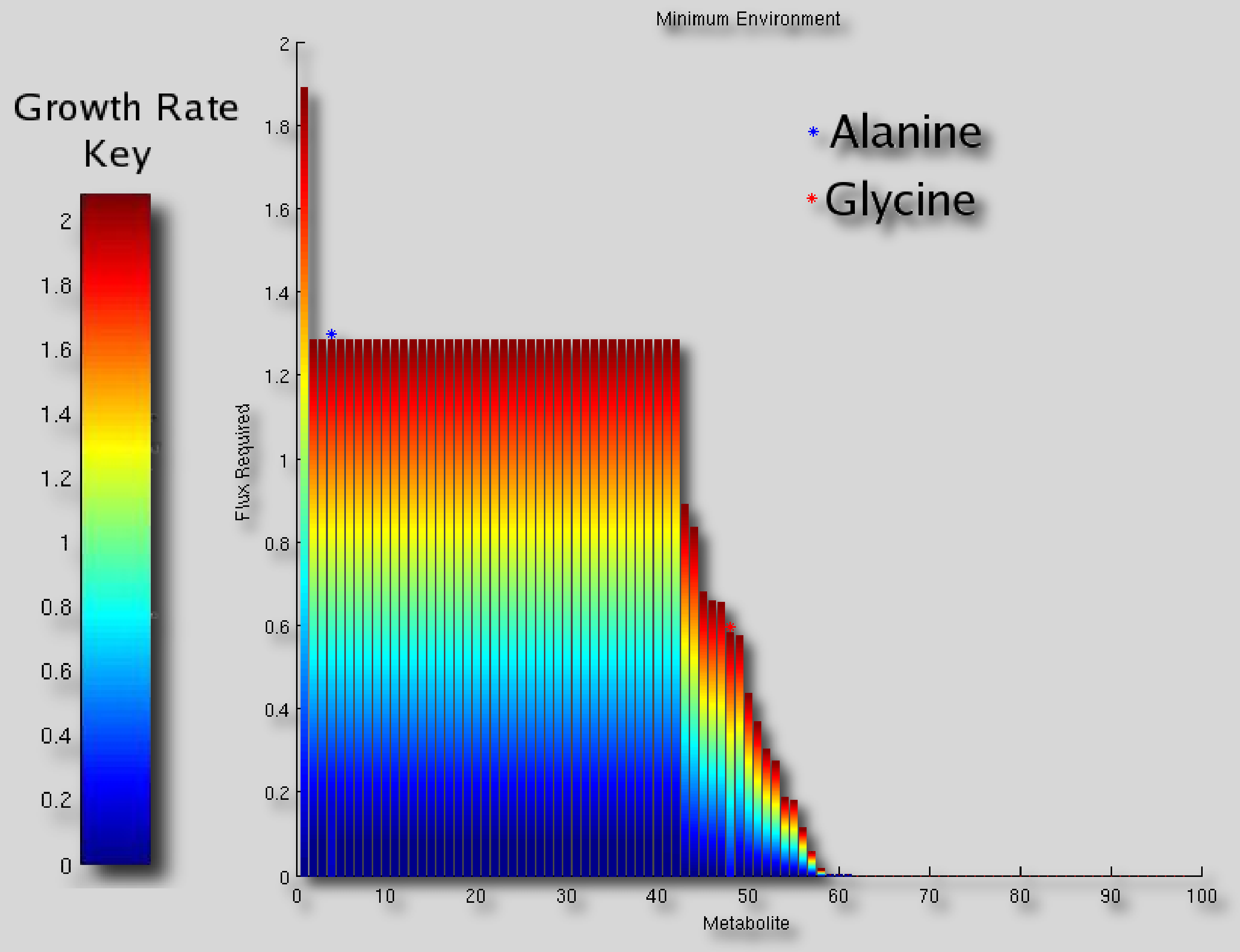

Figure 4. Minimal environment fluxes for e.coli. Red values correspond to high growth rates and blue values correspond to low growth rates. Flat regions indicate metabolites which can be substitutes for one another.

Future Work

Future goals of the project include calculating the dimension of the optimal set in order to determine the extent of degeneracy, to further investigate the relationships between environment and growth, and to precisely define and develop terms used in the FBA literature in order to identify dependencies on algorithms and environments.

References and Acknowledgements

1. Almaas, E., B. Kovacs, T. Vicsek, Z. N. Oltvai, and A.-L. Barabasi, "Global organization of metabolic fluxes in the bacterium Escherichia coli," Nature 427, 839 (2004).
2. Almaas, E., "Optimal flux patterns in cellular metabolic networks," Chaos. Accepted.

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