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.

Mathematically Modelling Metabolic Processes

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

\[
\text{minimize/maximize } c^T x \\
\text{subject to } A x = b, \ x \geq 0
\]

where \( c \) is the inner product of vectors \( c \) and \( x \), i.e. \( \sum c_i x_i \). This allows each member of \( x \) to have a weight defined by a corresponding member of \( c \).

\( A x = b \) is a collection of constraints on elements in \( x \) and can be expanded into a collection of equations of the form \( a_{ir} x_i + a_{jr} x_j + \cdots + a_{mr} x_m = b_j \), which each member of \( x \) to have a definition of \( c \).

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 \( M \) be the mass of the cell is conserved. Let \( \Delta M \) be the concentration of metabolite \( i \) and \( A_i \) be the stoichiometric coefficient of metabolite \( i \) in reaction \( r \). Allowing \( M \) to be the flux of reaction \( r \), we assume

\[
\frac{\Delta M}{dt} = \sum a_{ir} \frac{\Delta x_i}{dt} = -a_{ir} \frac{x_i}{r} = 0
\]

This force all metabolite 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). These reactions correlate well with established pathways in biology, suggesting that the HFB is a useful tool for studying single-cell organisms.

The HFB, however, is dependent on the solution of the LP and the environment. 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 usually high 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 HIN 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.

Growth is attained (within known bounds of how quickly a unit of growth remains constant regardless of how much environmental resource fluxes that can be used to attain a particular level of growth.

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 satisfied. The fluxes become fixed at this value. We fix these fluxes 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.

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


We thank the Howard Hughes Medical Institute for its financial support.

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