Flux Balance Analysis: Alternate optimal solutions

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INTRODUCTION

In this practical, the existence of Alternate Optimal Solutions [2] to a Flux Balance Analysis (FBA) problem is introduced using the E. coli core model[1], with functions in the COBRA Toolbox v3.0 [3].

E. coli core model

A map of the E. coli core model is shown in Figure 1.

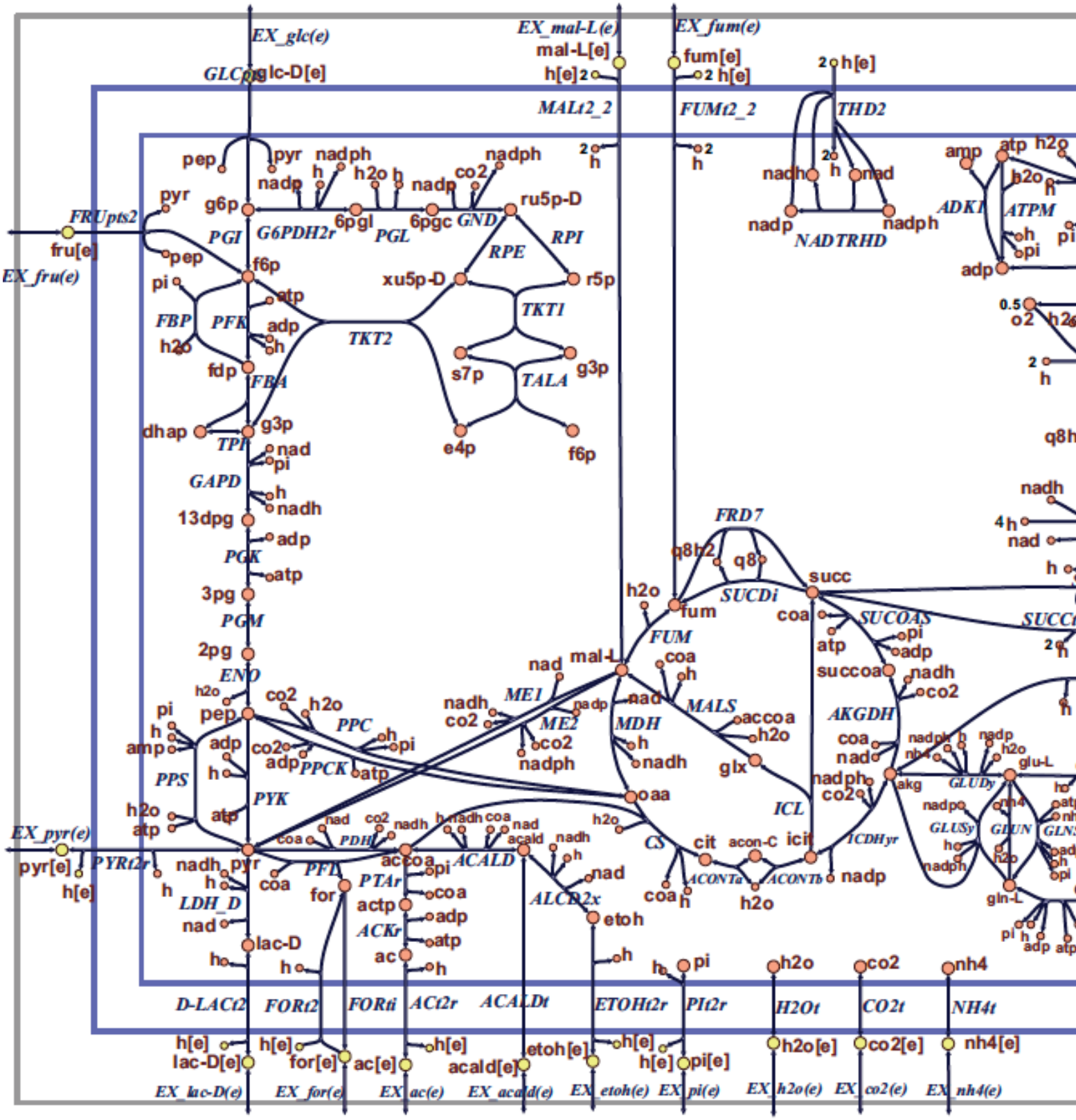


Figure 1 Map of the core E. coli metabolic network. Orange circles represent cytosolic metabolites, yellow circles represent extracellular metabolites, and the blue arrows represent reactions. Reaction name abbreviations are uppercase (blue) and metabolite name abbreviations are lowercase (rust colour). This flux map was drawn using SimPheny and edited for clarity with Adobe Illustrator.

MATERIALS - EQUIPMENT SETUP

Please ensure that all the required dependencies (e.g. , `git` and `curl`) of The COBRA Toolbox have been properly installed by following the installation guide here. Please ensure that the COBRA Toolbox has been initialised (tutorial_initialize.mlx) and verify that the pre-packaged LP and QP solvers are functional (tutorial_verify.mlx).

PROCEDURE

Load E. coli core model

The most direct way to load a model into The COBRA Toolbox is to use the `readCbModel` function. For example, to load a model from a MAT-file, you can simply use the filename (with or without file extension).

```
fileName = 'ecoli_core_model.mat';
if ~exist('modelOri','var')
    modelOri = readCbModel(fileName);
end
%backward compatibility with primer requires relaxation of upper bound on
%ATPM
modelOri = changeRxnBounds(modelOri,'ATPM',1000,'u');
model = modelOri;
```

model

1x1 struct with 28 fields

Field	Value	Size
S	*72x95 sparse do...*	72x95
mets	*72x1 cell*	72x1
b	*72x1 double*	72x1
csense	*72x1 char*	72x1
rxns	*95x1 cell*	95x1
lb	*95x1 double*	95x1
ub	*95x1 double*	95x1
c	*95x1 double*	95x1
osenseStr	'max'	1x3
genes	*137x1 cell*	137x1
rules	*95x1 cell*	95x1
metCharges	*72x1 int32*	72x1
metFormulas	*72x1 cell*	72x1
metNames	*72x1 cell*	72x1
metInChIString	*72x1 cell*	72x1
metKEGGID	*72x1 cell*	72x1
metChEBIID	*72x1 cell*	72x1
metPubChemID	*72x1 cell*	72x1
grRules	*95x1 cell*	95x1
rxnGeneMat	*95x137 sparse d...*	95x137
rxnConfidence...	*95x1 double*	95x1
rxnNames	*95x1 cell*	95x1
rxnNotes	*95x1 cell*	95x1
rxnECNumbers	*95x1 cell*	95x1
rxnReferences	*95x1 cell*	95x1

The meaning of each field in a standard model is defined in the standard COBRA model field definition.

In general, the following fields should always be present:

- **S**, the stoichiometric matrix
- **mets**, the identifiers of the metabolites
- **b**, Accumulation (positive) or depletion (negative) of the corresponding metabolites. 0 Indicates no concentration change.
- **csense**, indicator whether the b vector is a lower bound ('G'), upper bound ('L'), or hard constraint 'E' for the metabolites.
- **rxns**, the identifiers of the reactions
- **lb**, the lower bounds of the reactions
- **ub**, the upper bounds of the reactions
- **c**, the linear objective
- **genes**, the list of genes in your model
- **rules**, the Gene-protein-reaction rules in a computer readable format present in your model.
- **osenseStr**, the objective sense either `'max'` for maximisation or `'min'` for minimisation

Checking the non-trivial constraints on a model

What are the default constraints on the model?

Hint: `printConstraints`

Alternate optimal solutions

The flux distribution calculated by FBA is often not unique. In many cases, it is possible for a biological system to achieve the same objective value by using alternate pathways, so phenotypically different alternate optimal solutions are possible. A method that uses FBA to identify alternate optimal solutions is Flux Variability Analysis (FVA)[13]. This is a method that identifies the maximum and minimum possible fluxes through a particular reaction with the objective value constrained to be close to or equal to its optimal value. Performing FVA on a single reaction using the basic COBRA Toolbox functions is simple. First, use functions changeRxnBounds, changeObjective, and optimizeCbModel to perform FBA as described in the previous examples. Get the optimal objective value (FBAsolution.f), and then set both the lower and upper bounds of the objective reaction to exactly this value. Next, set the reaction of interest as the objective, and use FBA to minimize and maximize this new objective in two separate steps. This will give the minimum and maximum possible fluxes through this reaction while contributing to the optimal objective value.

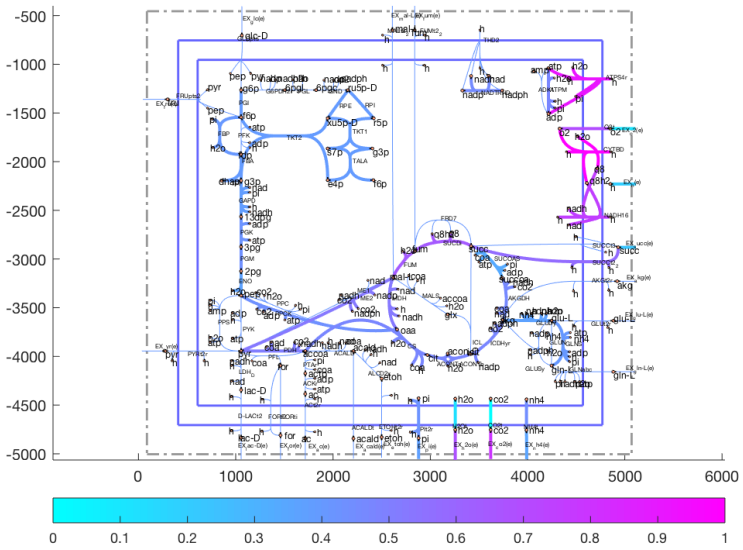
What is the minimum and maximum rate of the malic enzyme reaction (ME1) when the E. coli core model grows at a maximal rate on succinate as a carbon source?

Hint: `changeRxnBounds, printConstraints, optimizeCbModel, changeObjective, solution = optimizeCbModel(model, osenseStr)`

Display a flux map for alternate solutions for maximum aerobic growth on succinate.

```
outputFormatOK = changeCbMapOutput('matlab');
map=readCbMap('ecoli_core_map');
options.zeroFluxWidth = 0.1;
options.rxnDirMultiplier = 10;
```

```
drawFlux(map, model, FBAsolution_ME1_Min.v, options);
```



Systematic evaluation of alternate optima with Flux Variability Analysis

Flux variability analysis minimises and maximises the rate of each reaction in a model to evaluate what range of alternate optima exist for each reaction. The COBRA Toolbox includes a built in function for performing FVA called `fluxVariability`. This function is useful because, by default, it performs FVA on every reaction in a model.

What reactions vary their optimal flux in the set of alternate optimal solutions to maximum growth of E. coli on succinate?

Hint: create a table with varying reactions using the output from `fluxVariability`

Are there any reactions that are not used in one optimal solution but used in another optimal solution?

Hint: study the flux variablity analysis results

What are the computational and biochemical aspects to consider when interpreting these alternate optimal solutions?

Hint: the flux span for some reactions is far larger than for other reactions

In E.coli core, what reactions vary their optimal flux in the set of alternate optimal solutions where PYK (pyruvate kinase) is always at a maximum rate?

Hint: `fluxVariability, drawFlux`

TIMING

1 hrs

ANTICIPATED RESULTS

Understanding that, often, many alternate optimal flux vectors can give rise to the same optimal objective to a flux balance analysis problem.

Acknowledgments

Part of this tutorial was originally written by Jeff Orth and Ines Thiele for the publication "What is flux balance analysis?"

REFERENCES

1. Orth, J.D., Fleming, R.M. & Palsson, B.O. in EcoSal - Escherichia coli and Salmonella Cellular and Molecular Biology. (ed. P.D. Karp) (ASM Press, Washington D.C.; 2009).

2. Mahadevan, R. & Schilling, C.H. The effects of alternate optimal solutions in constraint-based genome-scale metabolic models. Metabolic engineering 5, 264-276 (2003).

3. Laurent Heirendt & Sylvain Arreckx, Thomas Pfau, Sebastian N. Mendoza, Anne Richelle, Almut Heinken, Hulda S. Haraldsdottir, Jacek Wachowiak, Sarah M. Keating, Vanja Vlasov, Stefania Magnusdottir, Chiam Yu Ng, German Preciat, Alise Zagare, Siu H.J. Chan, Maike K. Aurich, Catherine M. Clancy, Jennifer Modamio, John T. Sauls, Alberto Noronha, Aarash Bordbar, Benjamin Cousins, Diana C. El Assal, Luis V. Valcarcel, Inigo Apaolaza, Susan Ghaderi, Masoud Ahookhosh, Marouen Ben Guebila, Andrejs Kostromins, Nicolas Sompairac, Hoai M. Le, Ding Ma, Yuekai Sun, Lin Wang, James T. Yurkovich, Miguel A.P. Oliveira, Phan T. Vuong, Lemmer P. El Assal, Inna Kuperstein, Andrei Zinovyev, H. Scott Hinton, William A. Bryant, Francisco J. Aragon Artacho, Francisco J. Planes, Egils Stalidzans, Alejandro Maass, Santosh Vempala, Michael Hucka, Michael A. Saunders, Costas D. Maranas, Nathan E. Lewis, Thomas Sauter, Bernhard Ø. Palsson, Ines Thiele, Ronan M.T. Fleming, **Creation and analysis of biochemical constraint-based models: the COBRA Toolbox v3.0**, Nature Protocols, volume 14, pages 639–702, 2019 doi.org/10.1038/s41596-018-0098-2.