

# MetaFlux in Pathway Tools: Flux Balance Analysis (FBA) (Short Tutorial)

Markus Krummenacker,  
Mario Latendresse,  
Wai Kit Ong

SRI International

November 2021

## Outline

- 1 Introduction to Flux Balance Analysis**
  - What is Flux Balance Analysis (FBA)?
  - Overview of MetaFlux
- 2 Introduction to Development Mode**
  - Single and Multiple Gap-Filling
  - User Input: Fixed and Try Sets, Weights
- 3 Knockouts of Genes and Reactions**
- 4 Dynamic FBA of an Organism Community**

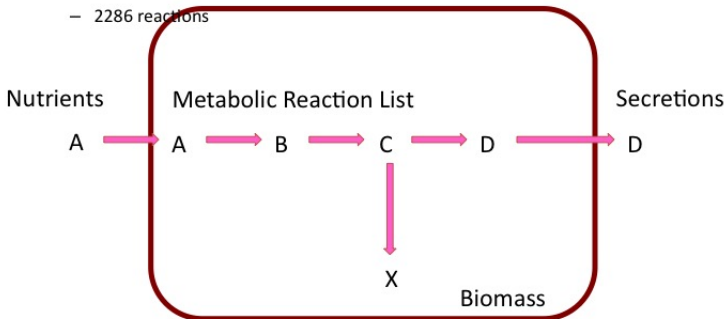
## Metabolic Modeling Applications

# Metabolic Modeling Applications

- Identify gaps in genome annotation
- Predict steady-state reaction fluxes for the metabolic network
- Predict growth rates, nutrient uptake rates
  
- Remove genes/reactions from model to predict knock-out phenotypes
  - EcoCyc model predicts knock-out phenotypes with 95.2% accuracy
- Supply alternative nutrient sets to predict growth phenotypes
  - EcoCyc model predicts growth under 431 growth media with 80.7% accuracy
  
- Metabolic engineering

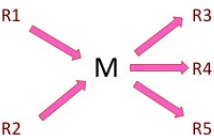
## Flux-Balance Analysis

- Steady state, constraint-based quantitative models of metabolism
- *E. coli* model derived from EcoCyc (*BMC Sys Biol* 2014 8:79):
  - 16 nutrients
  - 108 biomass metabolites
  - 2286 reactions



# Flux Balance Analysis

- Define system of linear equations encoding fluxes on each metabolite M
  - $R1 + R2 = R3 + R4 + R5$
- Boundary reactions:
  - Exchange fluxes for nutrients and secretions
  - Biomass reaction    L-arginine ... + GTP ... + ...  $\rightarrow$  biomass
- Submit to linear optimization package
  - Optimize biomass production
  - Optimize ATP production
  - Optimize production of desired end product

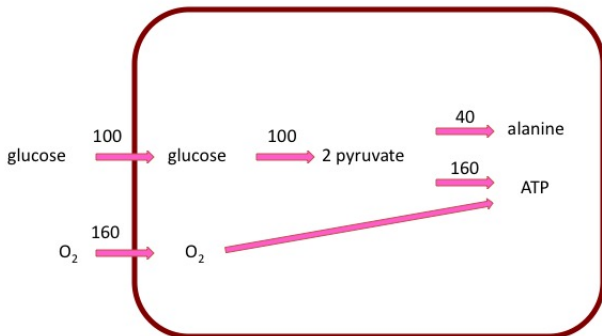


What is Flux Balance Analysis (FBA)?

## Simple Example of Fluxes

### Example

Biomass: ATP:alanine, 4:1



What is Flux Balance Analysis (FBA)?

## What is Flux Balance Analysis (FBA)?

### Computing Fluxes of Reactions for Organism Growth

Given a network of biochemical reactions, nutrients and secretions, assign a flux (a numerical value) to every reaction to produce a set of biomass metabolites for growth. Maximize the biomass. **The system is in a steady state (metabolite concentrations do not vary)**

### Applications

FBA can be used to 1) analyze different growth conditions (e.g., nutrients), 2) effects of gene knockout, 3) do metabolic engineering, and more

What is Flux Balance Analysis (FBA)?

## What is a Flux of a Reaction?

- The flux of a reaction is the rate of substrates consumed and produced, typically in steady state during growth
- Fluxes are typically expressed as mmol per gram dry weight per hour, denoted mmol/gDW/hr
- Solving an FBA model gives the fluxes of all reactions that are needed to create a non-zero flux for **all** biomass metabolites necessary for growth



## MetaFlux: The FBA Tool in Pathway Tools

- 1 The FBA Tool, MetaFlux, was introduced in version 15.0 of Pathway Tools (Feb 2011)
- 2 MetaFlux has three modes: solving, development, and gene knockout
- 3 Solving mode: compute the fluxes of reactions to produce the biomass
- 4 Development mode: trying different biomass, nutrients, secretions, and reactions to create a model
- 5 Gene knockout: deactivating gene(s) from the model and see the effect on growth (testing a model)
- 6 Also, we can simulate a community of organisms in a grid space with multiple interacting organisms (multiple FBA models)

# Assumptions

## MetaFlux Main Assumptions

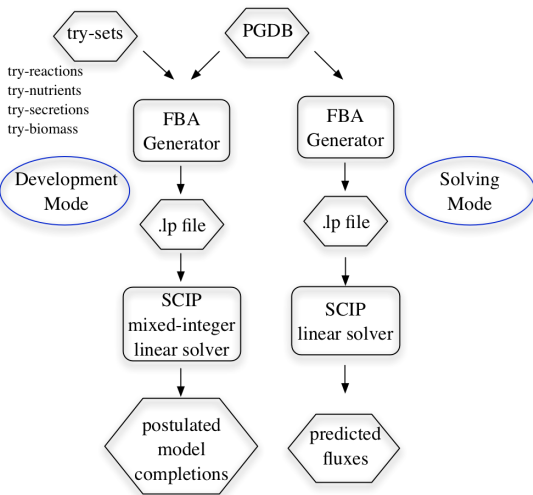
- Regulation (of genes and enzymes) is ignored
- Cofactors (in enzymes) are ignored
- That is, only metabolic reactions are used, and these reactions must specify all relevant metabolites to consider

# MetaFlux Modes of Operation

## MetaFlux Modeling Tool: Modes of Operation

- **Solving mode**
  - Individual organisms, organism communities
  - Steady-state FBA, dynamic FBA
  - Single compartment, 2-D spatial grid with diffusion
  - Cellular-compartment aware
  - Removal of flux loops, inference of biomass reaction
- **Knock-out mode** (single/double gene/reaction knock-outs)
- **Model development mode**
  - Development mode (multiple gap filling)
  - Fast Development mode (reaction gap filling) [Latendresse 2014]
  - Identify dead-end metabolites and blocked reactions

# Creating an FBA Model vs Solving an FBA Model

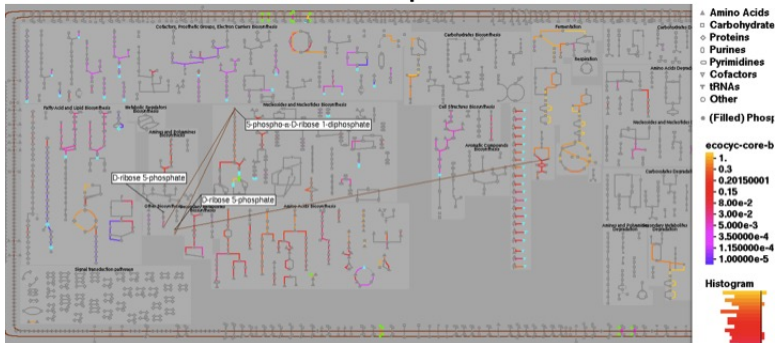


## The Linear Programming (LP) Solver

- Solving a model is done by a Linear Programming (LP) solver
- There are many open source and commercial LP solvers: CPLEX, GLPK, SCIP, Gurobi, and more
- Pathway Tools uses SCIP
- Even with thousands of reactions, typical FBA/LP formulation can be solved in a few seconds
- Solving in development mode could be longer (e.g., 20 seconds) or much longer (e.g., one hour). Unpredictable.
- MetaFlux is available on Mac OS X, Linux and Windows

# Painting Fluxes on Metabolic Map

## Painting *E. coli* Fluxes on Metabolic Map



# Fluxes on Pathway Diagram

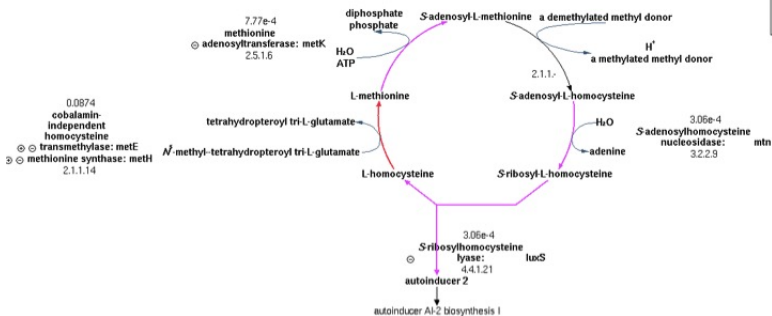
## *E. coli* Fluxes on Pathway Diagram

*Escherichia coli* K-12 substr. MG1655 Pathway: S-adenosyl-L-methionine cycle I 

[More Detail](#)

[Less Detail](#)

[Species Comparison](#)



## MetaFlux Development Mode

- Developing an accurate FBA model usually requires multiple refinements, such as:
- including the addition of new reactions to a PGDB
- altering the directionality of some reactions
- fixing compartments of transport reactions
- modifying the biomass reaction
- adding new nutrients and secretions



## Single and Multiple Gap-Filling

### Typically "Gap-Filling" Means "Completing the Reaction Network"

- Gap-filling suggests to add reactions from a reference database (e.g., MetaCyc) to the FBA model to produce one or more biomass metabolites

### Multiple Gap-Filling on Important Metabolites

Multiple gap-filling applies gap-filling not only on reactions but also on nutrients, secretions, and biomass metabolites. For biomass metabolites, we try to include as many as possible, while still getting growth. For nutrients, we try to use as few as possible while still getting growth.

User Input: Fixed and Try Sets, Weights

## Try-Sets and Weights for Multiple Gap-filling

The user provides four try-sets and weights to control the generation of the model.

- Set of reactions to try to add at a cost: typically all metabolic reactions of MetaCyc
- Sets of nutrients, secretions and biomass metabolites to try to add to the model
- Weights, as integers for gain and cost, for the reactions, nutrients, secretions, and biomass metabolites
- Typically, adding a biomass metabolite is a gain, but adding a reaction or a nutrient is a cost. We have different weights for different type of reactions (e.g., spontaneous, in the taxonomic range, etc.)

## Testing a Model Using Knockouts of Genes

### Knocking Out One Gene

- Knocking out a gene means to deactivate the reactions catalyzed by that gene
- Isozymes are taken into account

### Multiple Knockouts

More than one gene might be knocked out simultaneously, to hit isozymes or alternate pathways

### Batch Knockouts

Typically, MetaFlux is used to run a batch of gene knockouts (e.g., all genes)

## Examples of Knockout FBA Input Files

- Examples of gene knockout run on EcoCyc for
  - 1 A few genes: cysN, cysD, gltX
  - 2 All metabolic genes with summary solution file only (takes about one minute)
  - 3 All metabolic genes with all solution files generated (takes more than one minute)

## Dynamic FBA (dFBA)

- Dynamic FBA (dFBA) goes beyond the steady state of FBA by solving a series of FBAs, which track changing conditions
- Each solved FBA changes the growth environment by keeping track of nutrients used and secretions produced
- dFBA replicates real growth with concentrations of metabolites in the growth environment

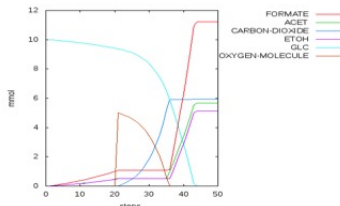
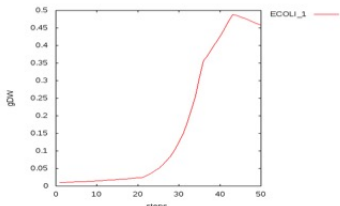
## Dynamic FBA of a Community of Organisms

- A community of organisms can be represented by a set of FBAs exchanging metabolites in a compartment (e.g., extra-cellular)
- The organisms are in a real physical space represented as a grid
- The FBA of each organism is solved and the concentrations of secretions and nutrients are updated in each grid box
- When solving a FBA, the nutrient uptakes are bounded by the concentrations of metabolites

## Dynamic FBA of *E. coli*

### Dynamic FBA Modeling of *E. coli*

- Dynamic FBA modeling of *E. coli* growth under varying nutrient conditions
  - t=1-20: *E. coli* grows anaerobically on 10 mmol glucose
  - t=21-34: O<sub>2</sub> is added to the simulation; *E. coli* grows completely aerobically
  - t=34-35: O<sub>2</sub> availability becomes limiting; acetate forms
  - t=36-44: O<sub>2</sub> is exhausted; anaerobic growth resumes
  - t=45 onwards: glucose is exhausted, cells begin to die

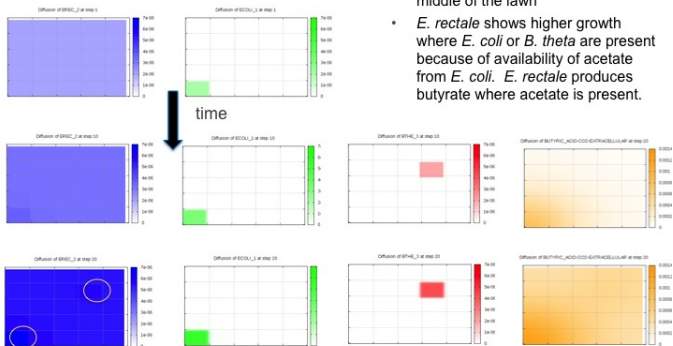


# Dynamic FBA Community of Organisms

## Dynamic Grid Modeling of a Simple Microbial Community

Inspired by Segre's COMETS

- Initially, *E. rectale* is present throughout the grid; *E. coli* is present in southwest corner
- Halfway through simulation, *B. thetaioamicron* is added to the middle of the lawn
- E. rectale* shows higher growth where *E. coli* or *B. theta* are present because of availability of acetate from *E. coli*. *E. rectale* produces butyrate where acetate is present.





## Further Literature on MetaFlux

- 1 Mario Latendresse, Markus Krummenacker, Miles Trupp, and Peter Karp, *Construction and Completion of Flux-Balance Models from Pathway Databases*, Bioinformatics, doi: 10.1093/bioinformatics/btr681, January 2012. Open access
- 2 Mario Latendresse, *Efficiently gap-filling reaction networks*, BMC Bioinformatics, June 28, 2014, 15:225, doi: 10.1186/1471-2105-15-225.
- 3 *Pathway Tools User Guide*