FLUX BALANCE ANALYSIS OVERVIEW

Learning Objectives

Each student should be able to:

- Explain flux balance analysis (FBA).
- Explain the stoichiometric reactions and metabolites.
- Explain mass balanced linear equations.
- Explain the biomass reaction.
- Explain how to create a stoichiometric matrix from reactions and metabolites.
- Explain gene-protein-reaction associations.
- Explain the constraint-based modeling.

Flux Balance Analysis Overview

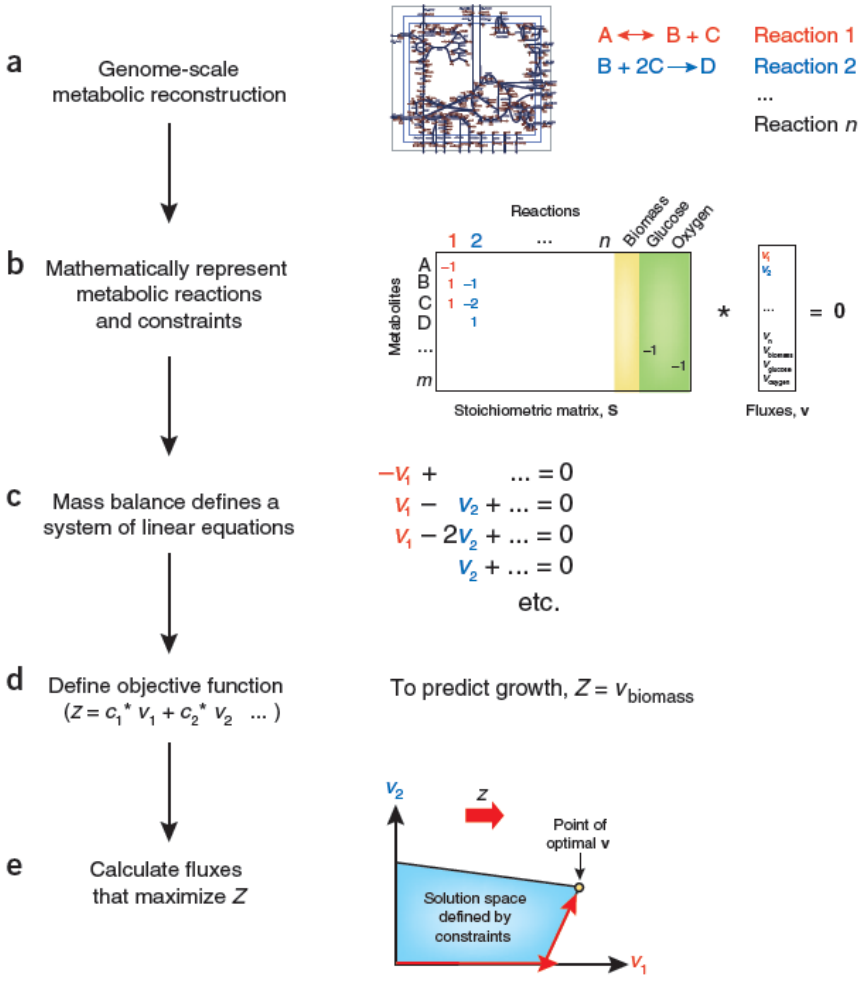
- Flux Balance Analysis Overview
- Stoichiometric Reactions & Metabolites
- Mathematical Representation of Reactions & Constraints
- Mass Balanced Linear Equations
- Biomass Reaction
- Calculating Fluxes
- Flux Balance Analysis Toolbox

FLUX BALANCE ANALYSIS

Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Nature biotechnology 28(3): 245-248.

- Through the use of genome-scale metabolic network reconstructions, Flux Balance Analysis (FBA) can be used to calculate the flow of metabolites through a metabolic network. This capability makes it possible to predict the growth rate of an organism and/or the rate of production of a given metabolite.
- FBA has limitations! It does not use kinetic parameters, thus it cannot predict metabolite concentrations. It is also only capable of determining fluxes at steady state. Typically, FBA does not account for regulatory effects such as activation of enzymes by protein kinases or regulation of gene expression. Therefore, its predictions may not always be accurate.

Formulation of Flux Balance Analysis



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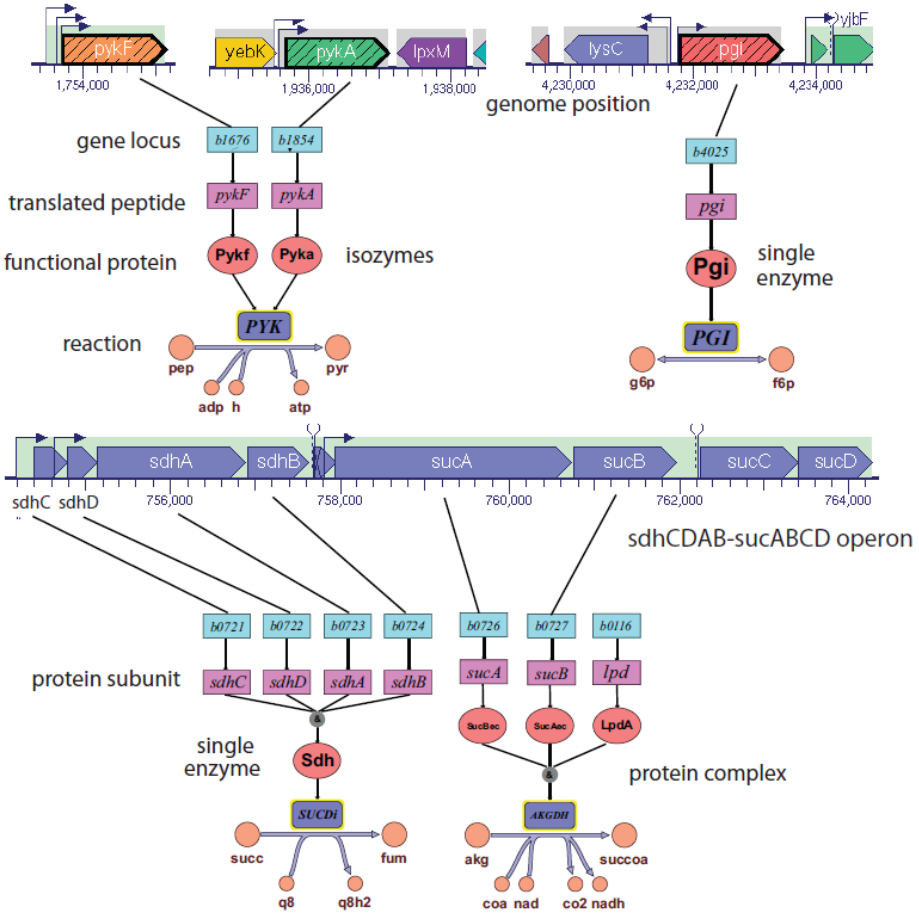
Identifying Metabolic Reactions and Metabolites

(Gene-Protein-Reactions)



Objective:

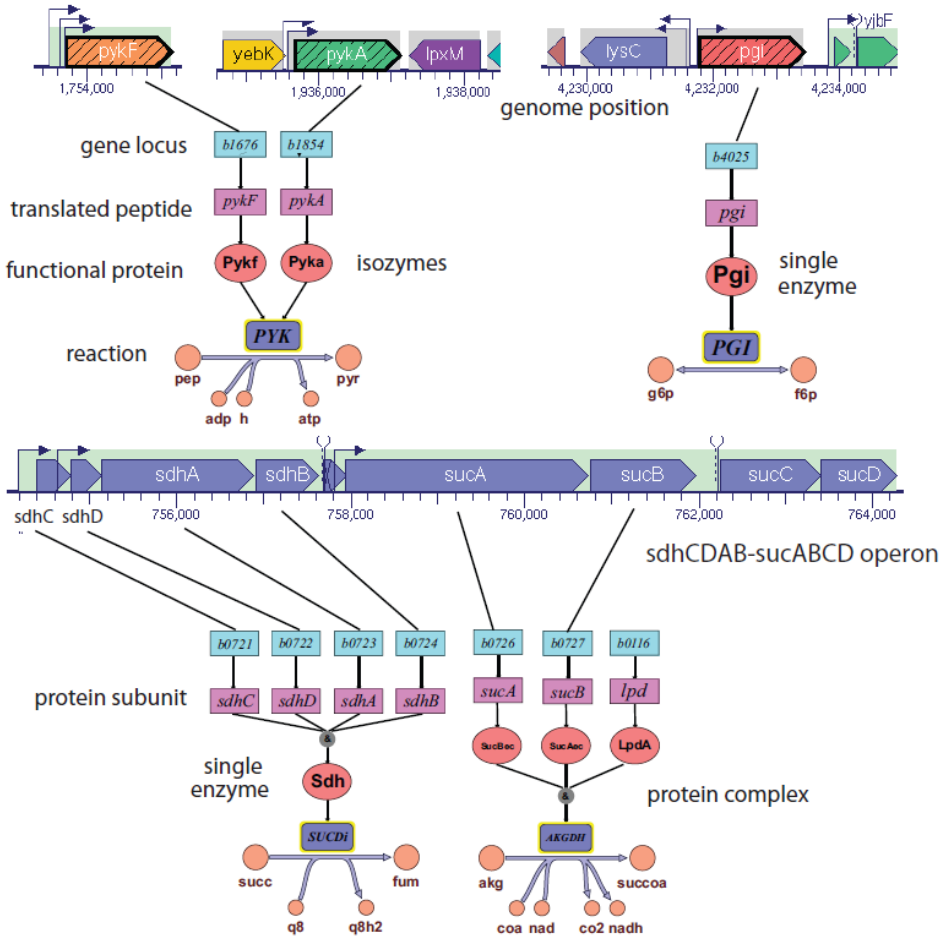
Create A biochemically, genetically and genomically (BiGG) structured knowledge base.

Reconstruction and Use of Microbial Metabolic Networks: the Core Escherichia coli Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)

Desired Reaction Information

1. Reaction Name*
2. Reaction Description*
3. Reaction Formula*
4. Gene-reaction Association*
5. Genes (Gene Locus) *
6. Proteins
7. Cellular Subsystem * (e.g. Glycolysis)
8. Reaction Direction*
9. Flux Lower Bound*
10. Flux Upper Bound*
11. Confidence Score (1-5)
12. EC Number
13. Notes
14. References

* Required



Reconstruction and Use of Microbial Metabolic Networks: the Core Escherichia coli Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)

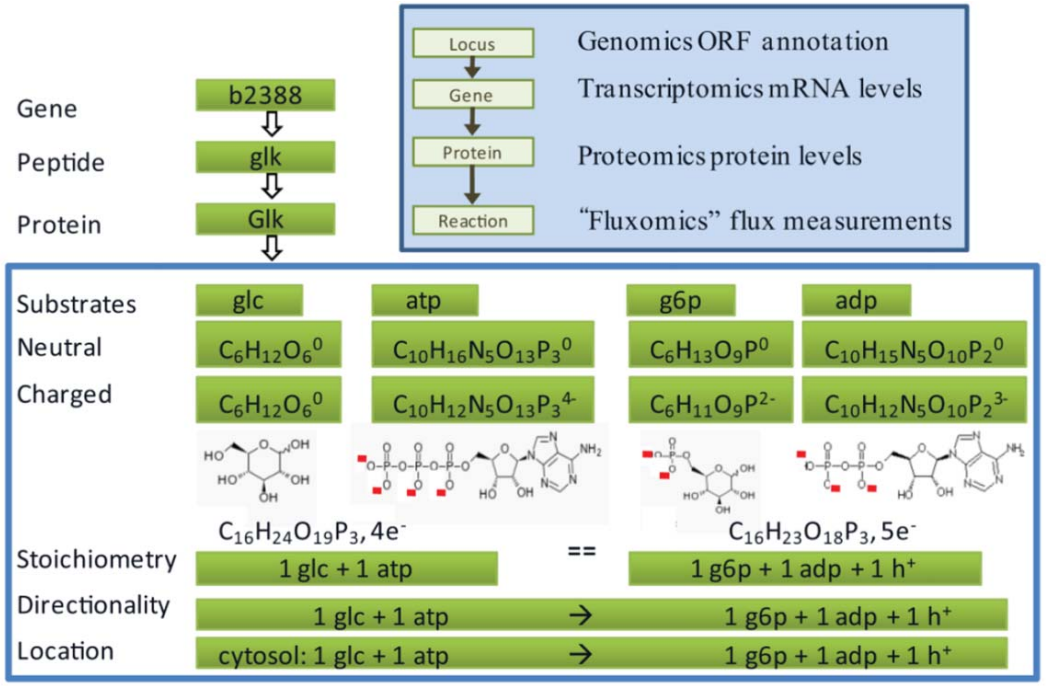
Genome-scale Reconstruction Reactions

abbreviation	officialName	equation	subSystem	proteinClass	delta G (pH 7.2)	Keq	v
ACALD	acetaldehyde dehydrogenase (acetylating)	[c] : acald + coa + nad <==> accoa + h + nadh	Pyruvate Metabolism	1.2.1.10	-4.4	1.66E+03	
ACALDt	acetaldehyde reversible transport	acald[e] <==> acald[c]	Transport, Extracellular		0.0	1.00E+00	
ACKr	acetate kinase	[c] : ac + atp <==> actp + adp	Pyruvate Metabolism	2.7.2.1	4.3	7.1204E-04	
ACONTa	aconitase (half-reaction A, Citrate hydro-lyase)	[c] : cit <==> acon-C + h2o	Citric Acid Cycle	4.2.1.3	1.5	7.98E-02	
ACONTb	aconitase (half-reaction B, Isocitrate hydro-lyase)	[c] : acon-C + h2o <==> icit	Citric Acid Cycle	4.2.1.3	-0.2	1.40E+00	
ACt2r	acetate reversible transport via proton symport	ac[e] + h[e] <==> ac[c] + h[c]	Transport, Extracellular		0.0	1.0000E+00	
ADK1	adenylate kinase	[c] : amp + atp <==> (2) adp	Oxidative Phosphorylation	2.7.4.3	-0.1	1.1836E+00	
AKGDH	2-Oxogluterate dehydrogenase	[c] : akg + coa + nad --> co2 + nadh + succoa	Citric Acid Cycle		-8.3	1.1896E+06	
AKGt2r	2-oxoglutarate reversible transport via symport	akg[e] + h[e] <==> akg[c] + h[c]	Transport, Extracellular		0.0	1.0000E+00	
ALCD2x	alcohol dehydrogenase (ethanol)	[c] : etoh + nad <==> acald + h + nadh	Pyruvate Metabolism	1.1.1.1	6.0	4.06E-05	
ATPM	ATP maintenance requirement	[c] : atp + h2o --> adp + h + pi	Oxidative Phosphorylation		-6.6	6.7770E+04	
ATPS4r	ATP synthase (four protons for one ATP)	adp[c] + (4) h[e] + pi[c] <==> atp[c] + (3) h[c] + h2o[c]	Oxidative Phosphorylation	3.6.3.14	6.6	1.4756E-05	
Biomass_Ecoli_	Biomass Objective Function with GAM	[c] : (1.496) 3pg + (3.7478) accoa + (59.8100) atp + (0.3610) e4p + (0.0709) f6p + (0.1290) g3p + (0.2050) g6p + (0.2557) gln-L + (4.9414) glu-L -					
CO2t	CO2 transporter via diffusion	co2[e] <==> co2[c]	Transport, Extracellular		0.0	1.0000E+00	
CS	citrate synthase	[c] : accoa + h2o + oaa --> cit + coa + h	Citric Acid Cycle		-8.6	1.9724E+06	
CYTBD	cytochrome oxidase bd (ubiquinol-8: 2 protons)	(2) h[c] + (0.5) o2[c] + q8h2[c] --> (2) h[e] + h2o[c] + q8	Oxidative Phosphorylation		-37.2	1.6962E+27	
D_LACt2	D-lactate transport via proton symport	h[e] + lac-D[e] <==> h[c] + lac-D[c]	Transport, Extracellular		0.0	1.0000E+00	
ENO	enolase	[c] : 2pg <==> h2o + pep	Glycolysis/Gluconeogenesis	4.2.1.11	-0.9	4.5580E+00	
ETOHt2r	ethanol reversible transport via proton symport	etoh[e] + h[e] <==> etoh[c] + h[c]	Transport, Extracellular		0.0	1.0000E+00	
EX_ac(e)	Acetate exchange	[e] : ac <==>	Exchange				
EX_acald(e)	Acetaldehyde exchange	[e] : acald <==>	Exchange				
EX_akg(e)	2-Oxoglutarate exchange	[e] : akg <==>	Exchange				
EX_co2(e)	CO2 exchange	[e] : co2 <==>	Exchange				
EX_etoh(e)	Ethanol exchange	[e] : etoh <==>	Exchange				
EX_for(e)	Formate exchange	[e] : for <==>	Exchange				
EX_fru(e)	D-Fructose exchange	[e] : fru <==>	Exchange				
EX_fum(e)	Fumarate exchange	[e] : fum <==>	Exchange				
EX_glc(e)	D-Glucose exchange	[e] : glc-D <==>	Exchange				
EX_gln_L(e)	L-Glutamine exchange	[e] : gln-L <==>	Exchange				
EX_glu_L(e)	L-Glutamate exchange	[e] : glu-L <==>	Exchange				

Desired Metabolite Information

1. Metabolite Name*
2. Metabolite Description*
3. Metabolite Neutral Formula
4. Metabolite Charged Formula*
5. Metabolite Charge*
6. Metabolite Compartment*
7. Metabolite KEGGID
8. Metabolite PubChemID
9. Metabolite CheBI ID
10. Metabolite Inchi String
11. Metabolite Smile

* Required



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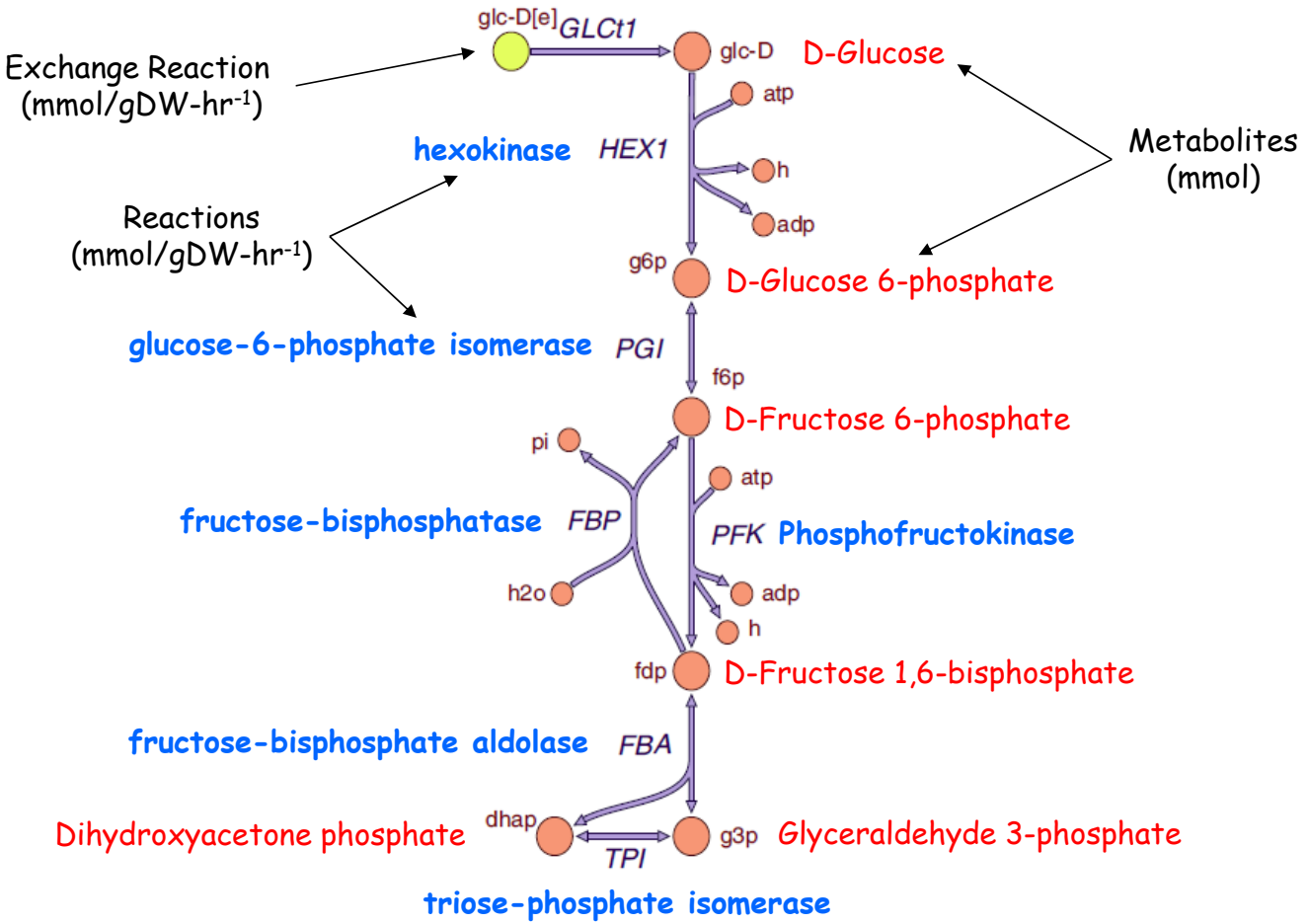
Genome-scale Reconstruction Metabolites

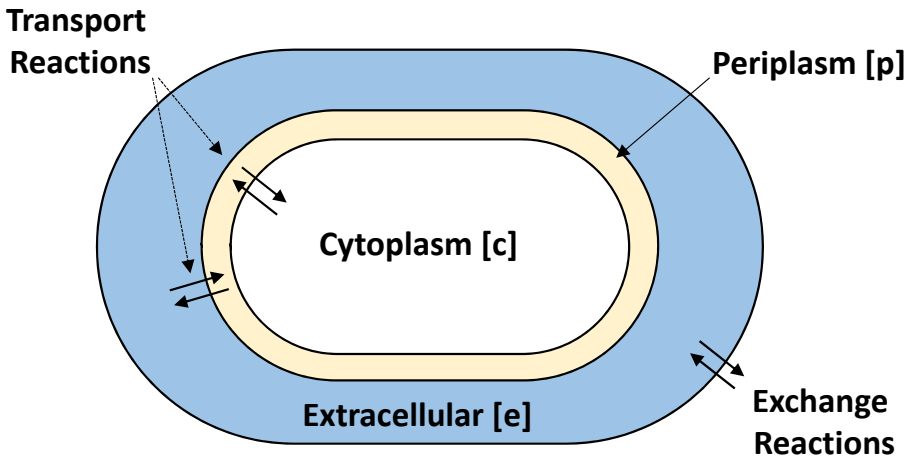
abbreviatio	officialName	formula	charge	casNumber	formulaNeutral	CompoundNames	KeggID
13dpg	3-Phospho-D-glyceroyl phosphate	C3H4O10P2	-4	38168-82-0	C3H8O10P2	1,3-bis-phosphoglycerate/ 3-Phospho-D-glyceroyl phosphate/	C00236
2pg	D-Glycerate 2-phosphate	C3H4O7P	-3	None	C3H7O7P	2-phosphoglyceric acid/ 2-Phospho-D-glycerate	C00631
3pg	3-Phospho-D-glycerate	C3H4O7P	-3	None	C3H7O7P	D-Glycerate 3-phosphate/ 3-Phosphoglycerate/ 3-phosphogly	C00197
6pgc	6-Phospho-D-gluconate	C6H10O10P	-3	None	C6H13O10P	6-phosphogluconic acid/ D-gluconate 6-phosphate	C00345
6pgl	6-phospho-D-glucono-1,5-lactone	C6H9O9P	-2	None	C6H11O9P	D-Glucono-1,5-lactone 6-phosphate	C01236
ac	Acetate	C2H3O2	-1	71-50-1	C2H4O2	vinegar/ Ethylic acid/ Vinegar acid/ Methanecarboxylic acid/ A	C00033
ac[e]	Acetate (extracellular)	C2H3O2	-1	71-50-1	C2H4O2	vinegar/ Ethylic acid/ Vinegar acid/ Methanecarboxylic acid/ A	C00033
acald	Acetaldehyde	C2H4O	0	75-07-0		Ethanal/ Aldehyde C(2)/ acetylaldehyde/ Aceteldehyde/ Acet	C00084
acald[e]	Acetaldehyde (extracellular)	C2H4O	0	75-07-0		Ethanal/ Aldehyde C(2)/ acetylaldehyde/ Aceteldehyde/ Acet	C00084
accoa	Acetyl-CoA	C23H34N7O17P3	-4	72-89-9	C23H38N7O17P3S	Acetyl coenzyme A	C00024
acon-C	cis-Aconitate	C6H3O6	-3	585-84-2	C6H6O6	cis-1,2,3-Propenetricarboxylic acid/ (Z)-1-Propene-1,2,3-tricarl	C00417
actp	Acetyl phosphate	C2H3O5P	-2	19926-71-7	C2H5O5P		C00227
adp	ADP	C10H12N5O10P2	-3	58-64-0	C10H15N5O10P2	Adenosine 5'-diphosphate	C00008
akg	2-Oxoglutarate	C5H4O5	-2	328-50-7	C5H6O5	Oxoglutaric acid/ 2-Ketoglutaric acid/ alpha-Ketoglutarate/ alp	C00026
akg[e]	2-Oxoglutarate (extracellular)	C5H4O5	-2	328-50-7	C5H6O5	Oxoglutaric acid/ 2-Ketoglutaric acid/ alpha-Ketoglutarate/ alp	C00026
amp	AMP	C10H12N5O7P	-2	61-19-8	C10H14N5O7P	Adenosine 5'-monophosphate/ Adenylic acid/ Adenylate/ 5'-A	C00020
atp	ATP	C10H12N5O13P3	-4	56-65-5	C10H16N5O13P3	Adenosine 5'-triphosphate/ 5'-adenylate triphosphate	C00002
cit	Citrate	C6H5O7	-3	77-92-9	C6H8O7	Citric acid/ 2-Hydroxytricarballylic acid/ 2/ 2-Hydroxy-1/ 3-pro	C00158
co2	CO2	CO2	0	124-38-9		Carbonic anhydride/ Carbon dioxide	C00011
co2[e]	CO2 (extracellular)	CO2	0	124-38-9		Carbonic anhydride/ Carbon dioxide	C00011
coa	Coenzyme A	C21H32N7O16P3	-4	85-61-0	C21H36N7O16P3S	CoA/ CoA-SH/ CoASH	C00010
dhap	Dihydroxyacetone phosphate	C3H5O6P	-2	57-04-5	C3H7O6P	Glycerone phosphate	C00111
e4p	D-Erythrose 4-phosphate	C4H7O7P	-2	585-18-2	C4H9O7P	4-phospho D-erythrose	C00279
etoh	Ethanol	C2H6O	0	64-17-5		Ethyl alcohol/ Methylcarbinol	C00469
etoh[e]	Ethanol (extracellular)	C2H6O	0	64-17-5		Ethyl alcohol/ Methylcarbinol	C00469
f6p	D-Fructose 6-phosphate	C6H11O9P	-2	643-13-0	C6H13O9P	D-Fructose 6-phosphoric acid/ Neuberg ester/ beta-D-Fructos	C00085
fdp	D-Fructose 1,6-bisphosphate	C6H10O12P2	-4	488-69-7	C6H14O12P2	fructose-1,6-bisphosphate/ fructose diphosphate/ beta-D-fru	C00354
for	Formate	CH1O2	-1	64-18-6	CH2O2	Methanoic acid/ Formic acid/ Hydrogencarboxylic acid/ aminic	C00058
for[e]	Formate (extracellular)	CH1O2	-1	64-18-6	CH2O2	Methanoic acid/ Formic acid/ Hydrogencarboxylic acid/ aminic	C00058
fru[e]	D-Fructose (extracellular)	C6H12O6	0	57-48-7		Levulose/ Fruit sugar/ D-Arabino-hexulose	C00095

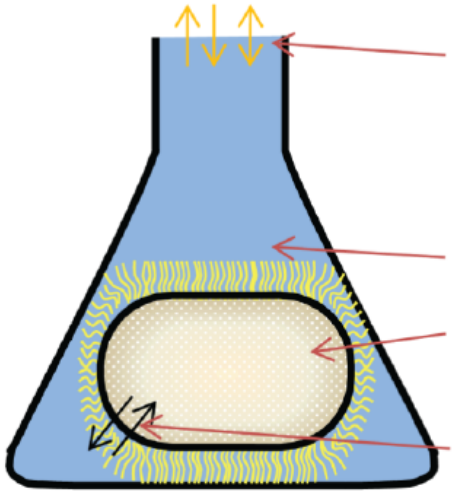
Metabolic Pathway



Becker, S. A., et al. (2007). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox." Nature protocols **2(3): 727-738.**

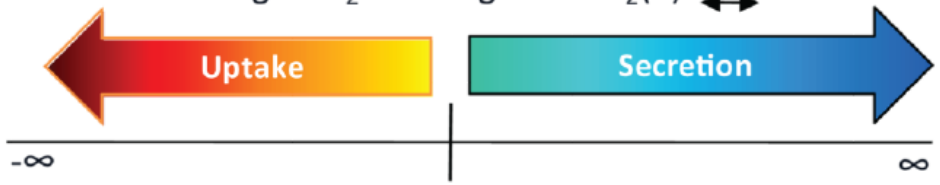
System Boundaries:
Exchange & Transport Reactions





Exchange reactions are defined as follows:

e.g.: CO_2 exchange: $1\ CO_2(e)$ ↔



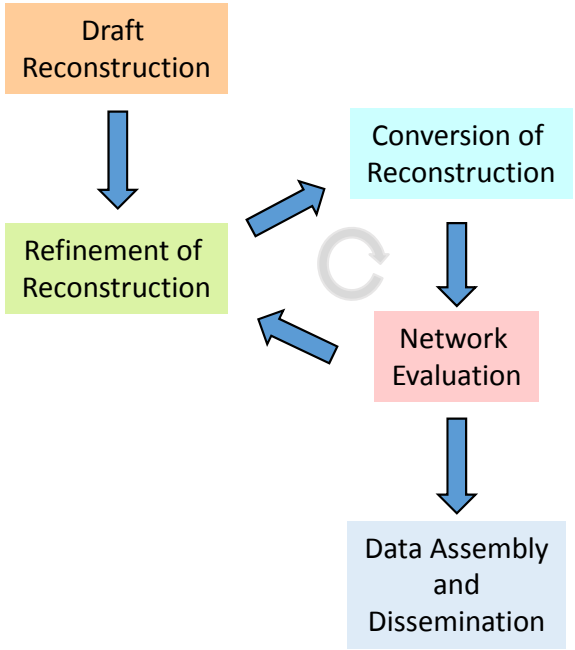
Removal from extracellular environment (hence negative flux value)

Addition to extracellular environment (hence positive flux value)

Thiele, I. and B. O. Palsson (2010). "A protocol for generating a high-quality genome-scale metabolic reconstruction." Nature protocols 5(1): 93-121.

GENOME-SCALE METABOLIC RECONSTRUCTIONS

- Overview
- Draft Reconstruction
- Refinement of Reconstruction
- Conversion of Reconstruction into Computable Format
- Network Evaluation
- Data Assembly and Dissemination



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Reconstruction Process: 96 Step Protocol

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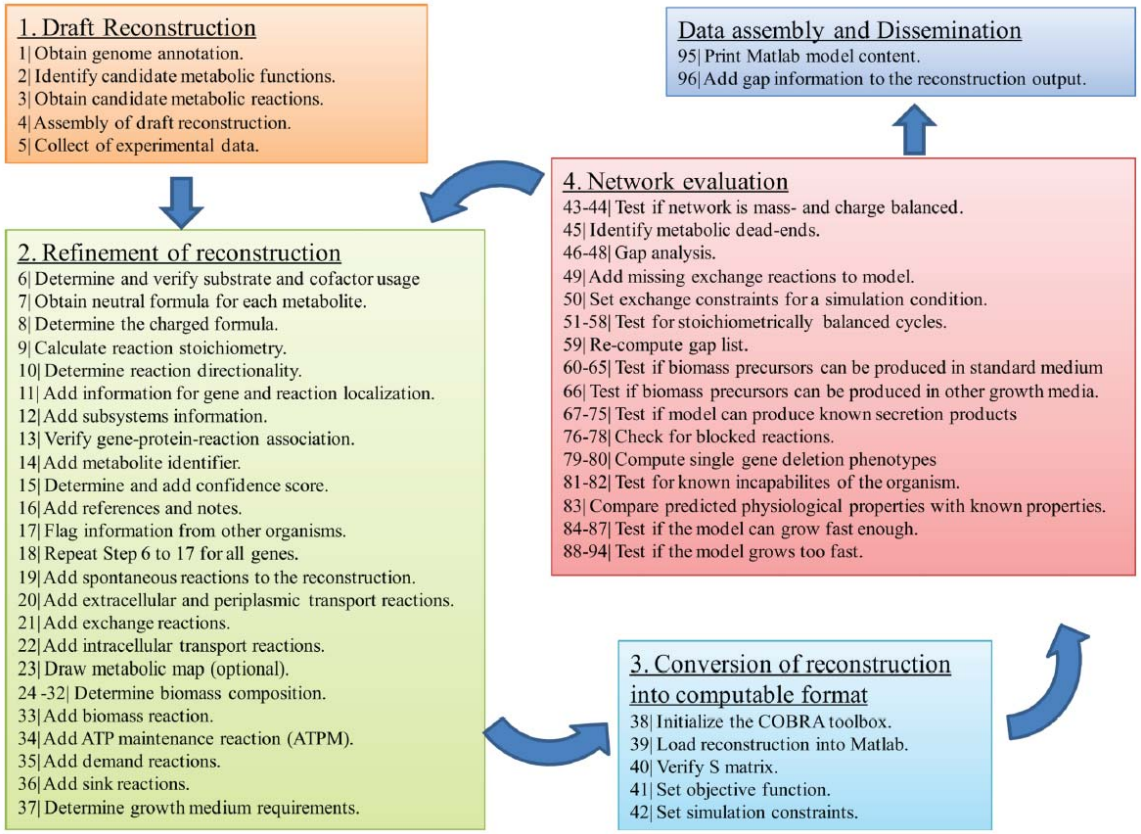
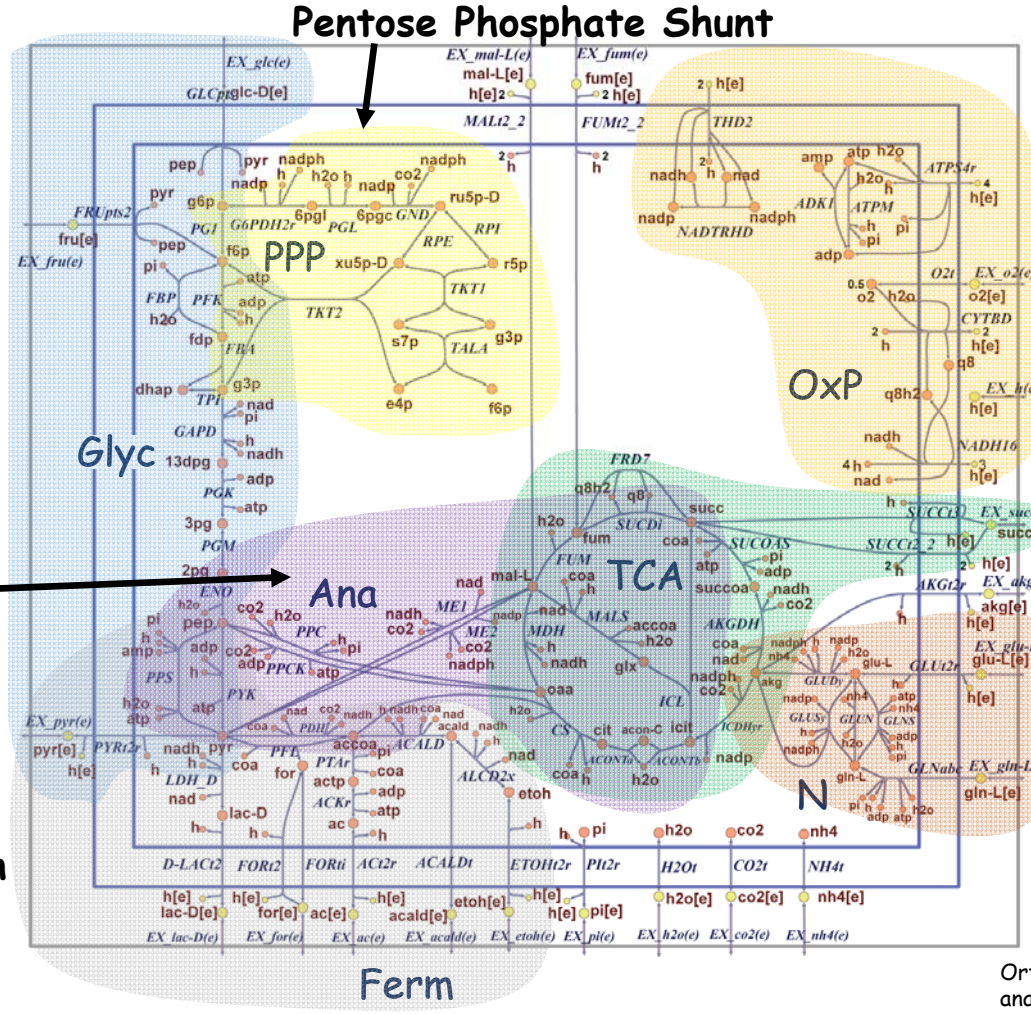


E.coli Core Model



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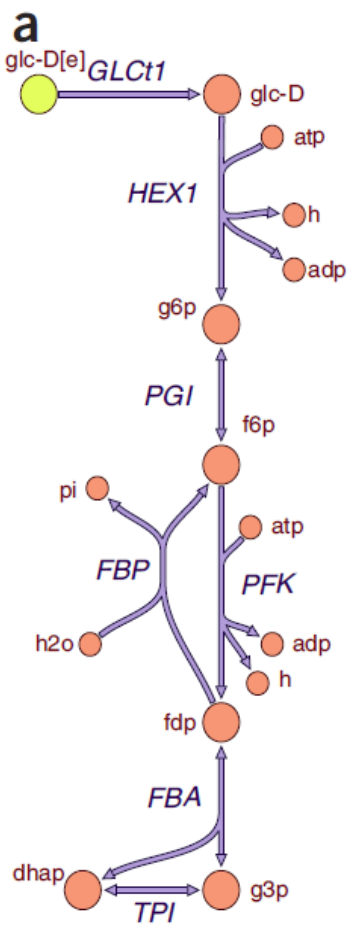
Creating A Stoichiometric Matrix

The stoichiometric matrix, S, is the centerpiece of a mathematical representation of genome-scale metabolic networks. This matrix represents each reaction as a column and each metabolite as a row, where each numerical element is the corresponding stoichiometric coefficient.



b

	GLCt1	HEX1	PGI	PFK	FBP	FBA	TPI	EX_glc
glc-D[e]	−1	0	0	0	0	0	0	−1
glc-D	1	−1	0	0	0	0	0	0
atp	0	−1	0	−1	0	0	0	0
H	0	1	0	1	0	0	0	0
adp	0	1	0	1	0	0	0	0
g6p	0	1	−1	0	0	0	0	0
f6p	0	0	1	−1	1	0	0	0
fdp	0	0	0	1	−1	−1	0	0
pi	0	0	0	0	1	0	0	0
h2o	0	0	0	0	−1	0	0	0
g3p	0	0	0	0	0	1	1	0
dhap	0	0	0	0	0	1	−1	0

= S

c

UB =	∞	∞	∞	∞	∞	∞	∞	∞
LB =	−∞	0	−∞	0	0	−∞	−∞	−2

Becker, S. A., A. M. Feist, et al. (2007). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox." Nature protocols **2(3): 727-738.**

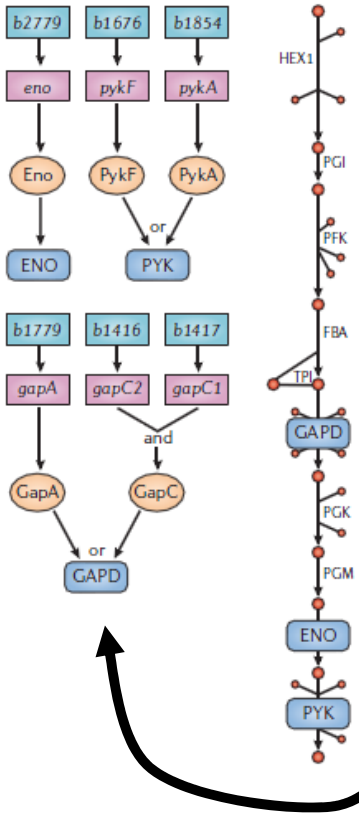
Genome-scale Metabolic Reconstruction

BIGG Database

Abbreviation	Glycolytic reactions	Genes
HEX1	[c]GLC + ATP → G6P + ADP + H	*glk*
PGI	[c]G6P ↔ F6P	*pgi*
PFK	[c]ATP + F6P → ADP + FDP + H	*pfkA, pfkB*
FBA	[c]FDP ↔ DHAP + G3P	*fbaA, fbaB*
TPI	[c]DHAP ↔ G3P	*tpiA*
GAPD	[c]G3P + NAD + PI ↔ 13DPG + H + NADH	*gapA, gapC1, gapC2*
PGK	[c]13DPG + ADP ↔ 3PG + ATP	*pgk*
PGM	[c]3PG ↔ 2PG	*gpmA, gpmB*
ENO	[c]2PG ↔ H_2O + PEP	*eno*
PYK	[c]ADP + H + PEP → ATP + PYR	*pykA, pykF*

Stoichiometric Matrix

	HEX1	PGI	PFK	FBA	TPI	GAPD	PGK	PGM	ENO	PYK
ATP	−1	0	−1	0	0	0	1	0	0	1
GLC	−1	0	0	0	0	0	0	0	0	0
ADP	1	0	1	0	0	0	−1	0	0	−1
G6P	1	−1	0	0	0	0	0	0	0	0
H	1	0	1	0	0	1	0	0	0	−1
F6P	0	1	−1	0	0	0	0	0	0	0
FDP	0	0	1	−1	0	0	0	0	0	0
DHAP	0	0	0	1	−1	0	0	0	0	0
G3P	0	0	0	1	1	−1	0	0	0	0
NAD	0	0	0	0	0	−1	0	0	0	0
PI	0	0	0	0	0	−1	0	0	0	0
13DPG	0	0	0	0	0	1	−1	0	0	0
NADH	0	0	0	0	0	1	0	0	0	0
3PG	0	0	0	0	0	0	1	−1	0	0
2PG	0	0	0	0	0	0	0	1	−1	0
PEP	0	0	0	0	0	0	0	0	1	−1
H_2O	0	0	0	0	0	0	0	0	1	0
PYR	0	0	0	0	0	0	0	0	0	1



Metabolic Pathway

Gene-Protein-Reaction (GPR) Associations

Reed, J. L., I. Famili, et al. (2006). "Towards multidimensional genome annotation." Nature reviews. Genetics 7(2): 130-141.

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How can we use the Stoichiometric Matrix?

- The stoichiometric matrix, **S**, is a linear transformation of the flux vector, **v** to a vector of time derivatives of the concentration vector, **x**.

$$\frac{d\mathbf{x}}{dt} = \mathbf{S} \cdot \mathbf{v}$$

- The concentration vector, **x**, represents the concentration of each of the metabolites.
- If we assume that a cell will be in a particular phenotype for a time much larger than the changing time of metabolites then we can also assume that the concentration pools for the metabolites will be non-changing thus setting $dx/dt = 0$. This is the steady state assumption of flux balance analysis.

$$\frac{d\mathbf{x}}{dt} = 0 = \mathbf{S} \cdot \mathbf{v}$$

- Since there are normally many more reactions (columns) than metabolites (rows), more unknown variables than equations, then there is no unique solutions (could be a large number of solutions).
- Need to find a way to constrain the solution space!

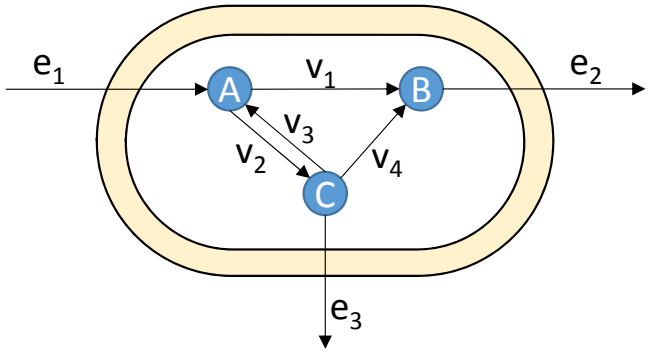
Reactions

Metabolites

$$\begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 \end{pmatrix} = \mathbf{S}$$

Dynamic Mass Balance

A simple network



Linear Transformation

$$\frac{d\mathbf{x}}{dt} = \mathbf{S}\cdot\mathbf{v}$$

$$\begin{bmatrix} \frac{dA}{dt} \\ \frac{dB}{dt} \\ \frac{dC}{dt} \end{bmatrix} = \begin{bmatrix} -1 & -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ e_1 \\ e_2 \\ e_3 \end{bmatrix}$$

Stoichiometric Matrix

Linear Differential Equations

$$\frac{dA}{dt} = -v_1 - v_2 + v_3 + e_1$$

$$\frac{dB}{dt} = v_1 + v_4 - e_2$$

$$\frac{dA}{dt} = v_2 - v_3 - v_4 - e_3$$

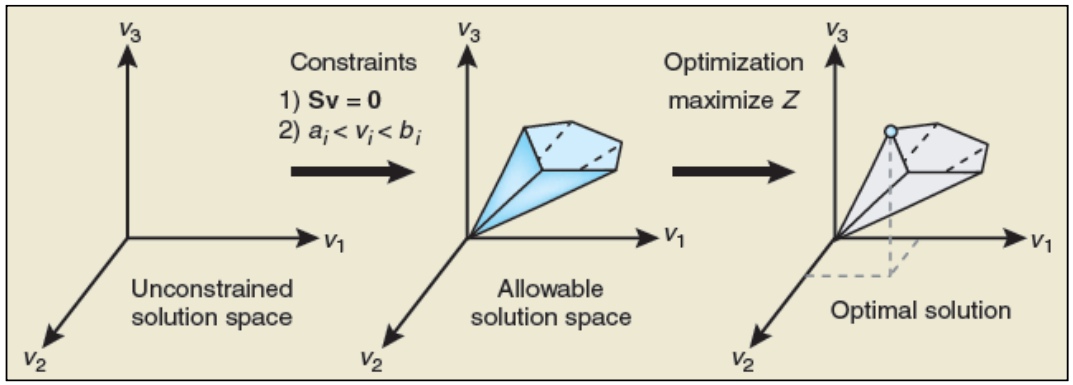
Dynamic Mass Balance (Steady State)

$$0 = \mathbf{S}\cdot\mathbf{v}$$

$$\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix} = \begin{bmatrix} -1 & -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ e_1 \\ e_2 \\ e_3 \end{bmatrix}$$

Note: More unknown variables than equations, thus no unique solutions! Need constraints!

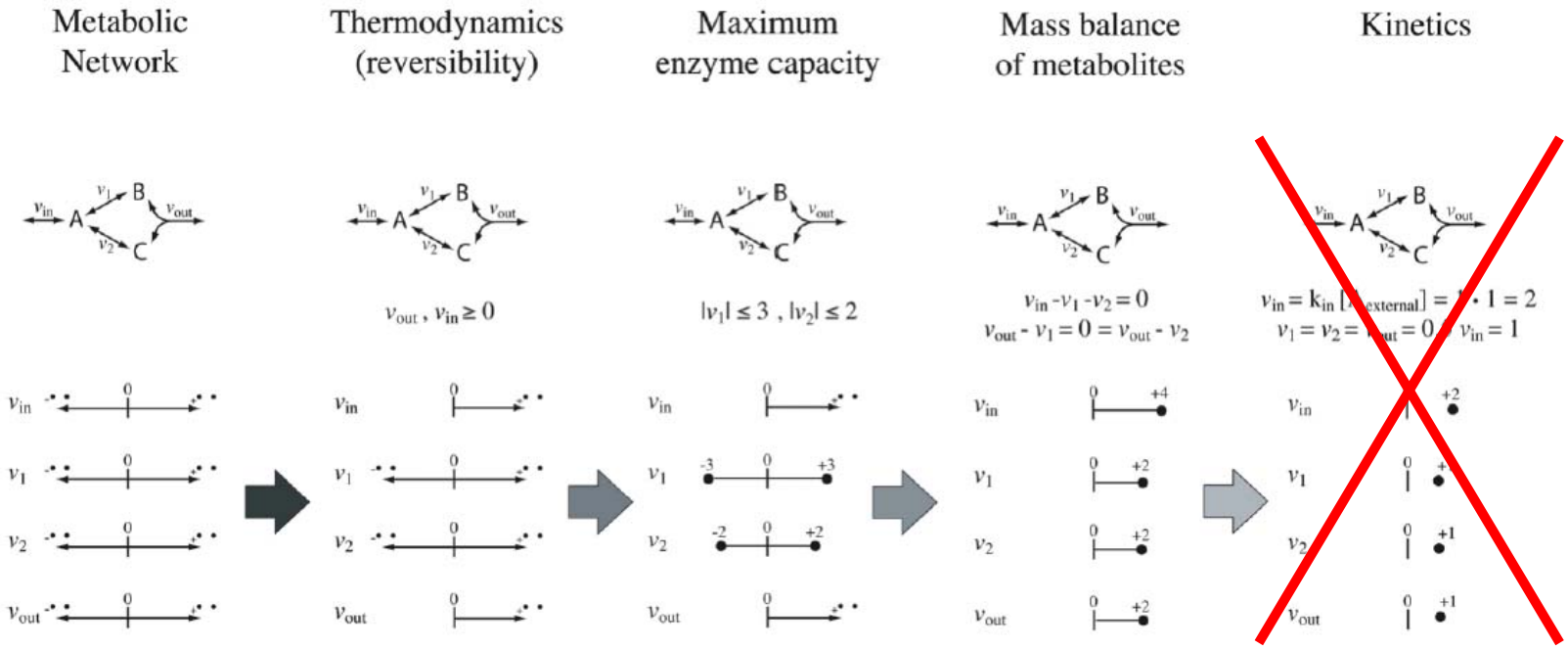
The Conceptual Basis of Constraint-based Modeling



With no constraints, the flux distribution of a biological network may lie at any point in a solution space. When mass balance constraints imposed by the stoichiometric matrix S (label 1) and capacity constraints imposed by the lower and upper bounds (a_i and b_i) (label 2) are applied to a network, it defines an allowable solution space. The network may acquire any flux distribution within this space, but points outside this space are denied by the constraints. Through optimization of an objective function using linear programming, FBA can identify a single optimal flux distribution that lies on the edge of the allowable solution space.

Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Nature biotechnology 28(3): 245-248.

Role of Constraints



REI601M,Introduction to Systems Biology, Dr. Innes Thiele,2012, https://systemsbiology.hi.is/wiki/REI601M

FLUX OPTIMIZATION

(Linear Programming or Linear Optimization Problem)

Maximize the objective function

$$Z = \sum_i c_i v_i = \mathbf{c} \cdot \mathbf{v}$$

with the following constraints

$$\frac{d\mathbf{x}}{dt} = \mathbf{S} \cdot \mathbf{v} = \mathbf{0}$$

$$\alpha_j \le v_j \le \beta_j$$

where

- $\mathbf{x}$ = concentration vector
- $\mathbf{v}$ = flux vector
- $\mathbf{c}$ = objective function weights
- $\mathbf{S}$ = Stoichiometric matrix
- α_j = Lower bound of flux
- β_j = upper bound of flux

The goal is to create and objective function that is biologically meaningful. These could include;

1. Cellular growth (maximization)
2. Particular metabolite engineering (maximization)
3. Energy consumption (minimization)

For the case of cellular growth as the objective function (Biomass Function)

1. "It has been shown that under rich growth conditions (i.e. no lack of phosphate and nitrogen), *E. coli* grows in a stoichiometrically optimal manner." (Schilling 2001, Edwards 1994)
2. "It is reasonable to hypothesize that unicellular organisms have evolved toward maximal growth performance." (Segre, 2002.)

Flux Balance Analysis Overview

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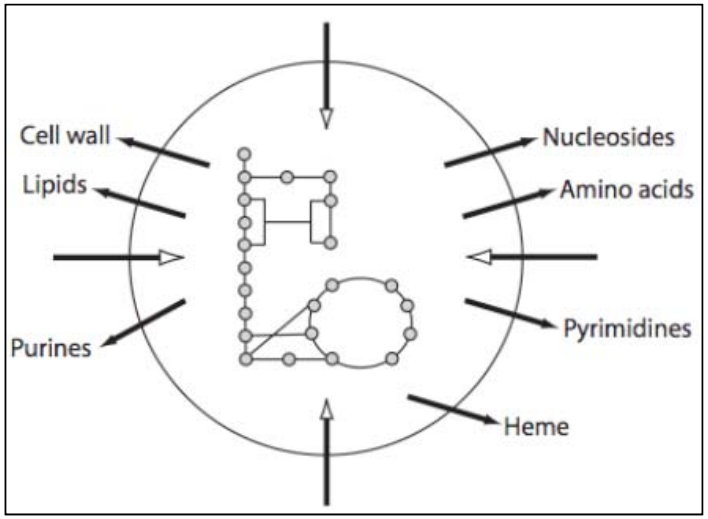
Biomass Precursors

- The biomass reaction accounts for all the fractional contributions from biosynthetic precursors and key cofactors to create 1g of biomass.
- These factional contributions need to be determined experimentally for cells growing in **log phase**.
- It may not be possible to obtain a detailed biomass composition for the target organism. In this case, one can estimate the relative fraction of each precursor from existing databases.

Cellular component	Cellular content %(wt/wt)
Protein	55
RNA	20.5
DNA	3.1
Lipids	9.1
Lipopolysaccharides	3.4
Peptidoglycan	2.5
Glycogen	2.5
Polyamines	0.4
Other	3.5
Total	100.00



Thiele, I. and B. O. Palsson (2010). "A protocol for generating a high-quality genome-scale metabolic reconstruction." Nature protocols 5(1): 93-121.

E.coli Precursor Metabolites

Nicotinamide coenzymes
Glycerol-3-phosphate -> Phospholipids

Sugar nucleotides

Amino sugars

Serine Family
Serine -> Tryptophan
-> Ethanolamine
-> 1-C units
Glycine -> Purine nucleotides
Cysteine

Aromatic Family
Tyrosine
Tryptophan
Phenylalanine

Chorismate

Vitamins and cofactors
Ubiquinone
Menaquinone
Folates

Pyruvate family
Alanine
Valine
Leucine
Isoleucine
Isoprenoids

Fatty Acids
Murein
Leucine

Aspartate family
Asparagine
Threonine
Methionine -> Spermidine
Aspartate -> Nicotinamide coenzymes
-> Pyrimidine nucleotides
Lysine

Glutamate family
Glutamate -> Hemes
Glutamine
Arginine -> Polyamines
Proline

Vitamins and cofactors
Folates
Riboflavin
Coenzyme A
Adenosylcobalamine
Nicotinamide

Purine nucleotides
Pyrimidine nucleotides

Phosphoribosyl pyrophosphate

Tryptophan
Histidine

2-Keto-3-deoxyoctanate

Heptose in LPS

Heme

Maintenance Energy Requirements

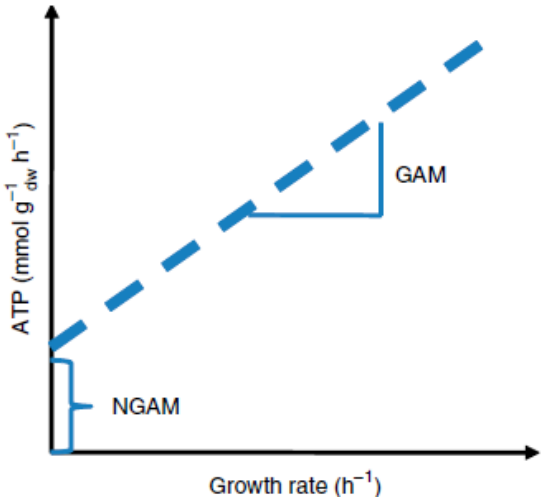
- To simulate growth, the energy required to maintain the cell growth must be accounted for.
- Two forms of energy are required; growth associated maintenance (GAM) energy and nongrowth associated maintenance (NGAM) energy (e.g. turgor pressure).
- GAM reaction accounts for the energy (ATP) necessary to replicate a cell. It is represented in the model by

  $$x\ ATP + x\ H_2O \rightarrow x\ ADP + x\ P_i + x\ H^+$$

  Where x is the number of required phosphate bonds (59.81 in core model). This will be included in the biomass reaction
- The NGAM reaction (ATPM) is given by

  $$1\ ATP + 1H_2O \rightarrow 1\ ADP + 1\ P_i + 1\ H^+$$

  where the flux through this reaction is constrained by experimental data to 8.39 mmol $g_{DW}^{-1}h^{-1}$

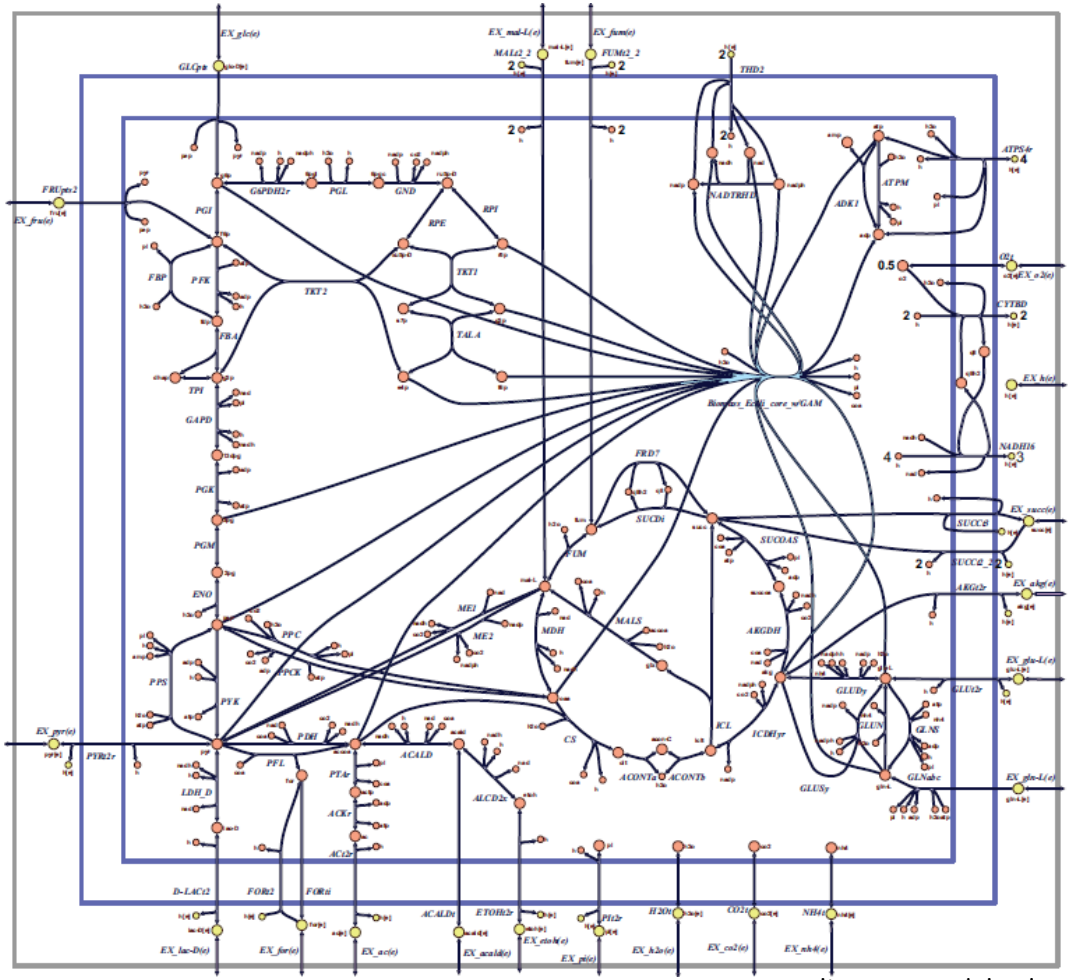


Thiele, I. and B. O. Palsson (2010). "A protocol for generating a high-quality genome-scale metabolic reconstruction." Nature protocols 5(1): 93-121.

Biomass Reaction For *E.coli* Core Model

(1.496) 3pg + (3.7478) accoa + (59.8100) atp + (0.3610) e4p + (0.0709) f6p + (0.1290) g3p + (0.2050) g6p + (0.2557) gln-L + (4.9414) glu-L + (59.8100) h2o + (3.5470) nad + (13.0279) nadph + (1.7867) oaa + (0.5191) pep + (2.8328) pyr + (0.8977) r5p --> (59.8100) adp + (4.1182) akg + (3.7478) coa + (59.8100) h + (3.5470) nadh + (13.0279) nadp + (59.8100) pi

* Key Cofactors



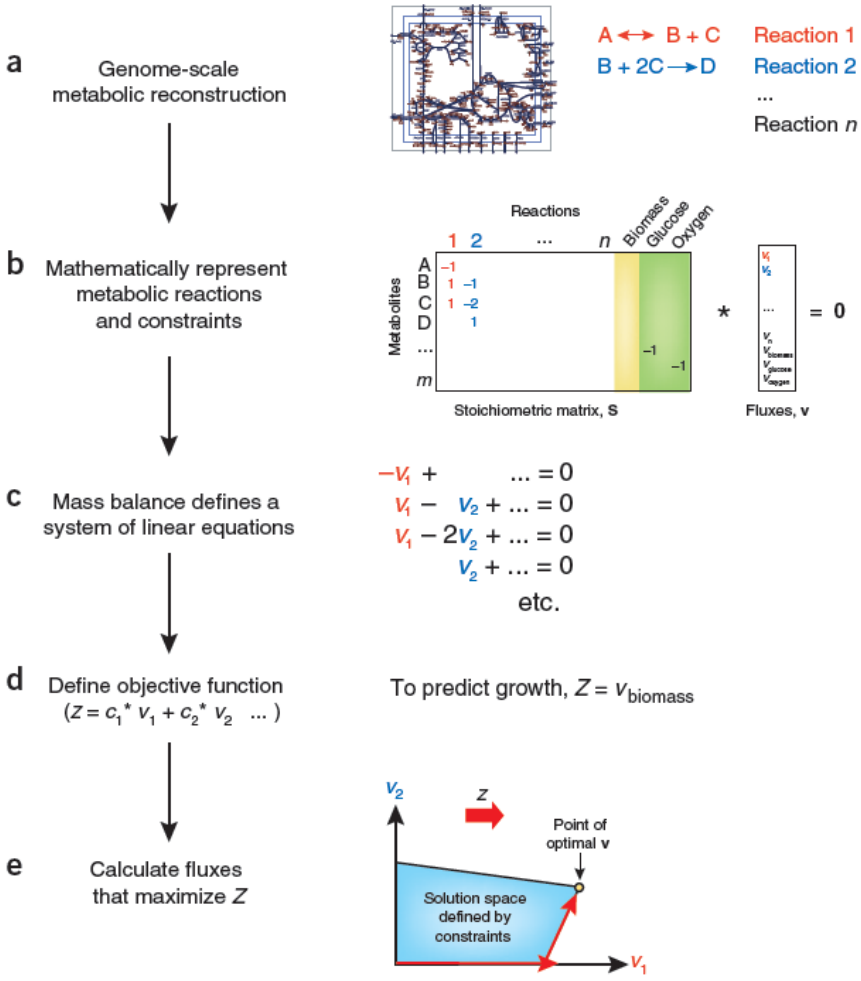
ecoli_core_models.xls

iaf1260 BIOMASS OBJECTIVE FUNCTION

(Ec_biomass_iAF1260_core_59p81M)

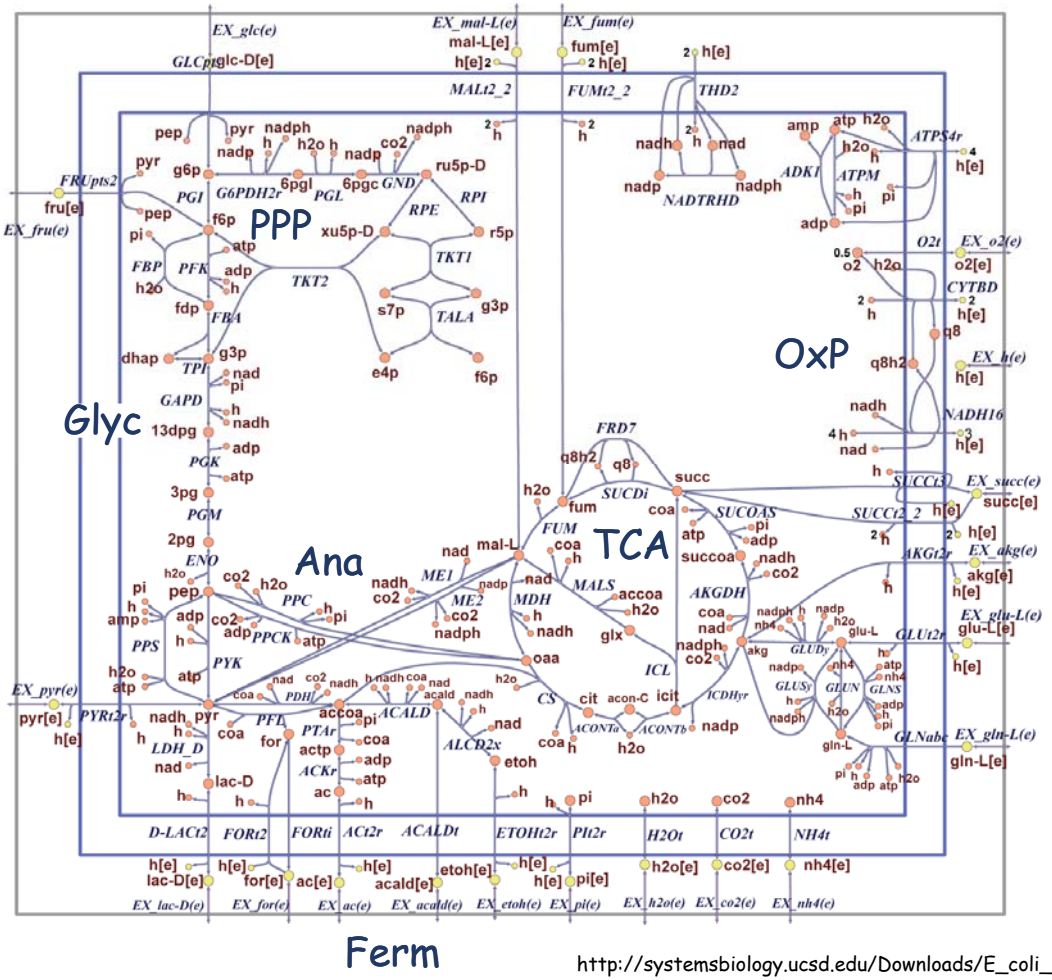
Z = 0.000223 10fthf[c] + 0.000223 2ohph[c] + 0.5137 ala-L[c] + 0.000223 amet[c] + 0.2958 arg-L[c] + 0.2411 asn-L[c] + 0.2411 asp-L[c] + 59.984 atp[c] + 0.004737 ca2[c] + 0.004737 cl[c] + 0.000576 coa[c] + 0.003158 cobalt2[c] + 0.1335 ctp[c] + 0.003158 cu2[c] + 0.09158 cys-L[c] + 0.02617 datp[c] + 0.02702 dctp[c] + 0.02702 dgtp[c] + 0.02617 dttp[c] + 0.000223 fad[c] + 0.007106 fe2[c] + 0.007106 fe3[c] + 0.2632 gln-L[c] + 0.2632 glu-L[c] + 0.6126 gly[c] + 0.2151 gtp[c] + 54.462 h2o[c] + 0.09474 his-L[c] + 0.2905 ile-L[c] + 0.1776 k[c] + 0.01945 kdo2lipid4[e] + 0.4505 leu-L[c] + 0.3432 lys-L[c] + 0.1537 met-L[c] + 0.007895 mg2[c] + 0.000223 mlthf[c] + 0.003158 mn2[c] + 0.003158 mobd[c] + 0.01389 murein5px4p[p] + 0.001831 nad[c] + 0.000447 nadp[c] + 0.011843 nh4[c] + 0.02233 pe160[c] + 0.04148 pe160[p] + 0.02632 pe161[c] + 0.04889 pe161[p] + 0.1759 phe-L[c] + 0.000223 pheme[c] + 0.2211 pro-L[c] + 0.000223 pydx5p[c] + 0.000223 ribflv[c] + 0.2158 ser-L[c] + 0.000223 sheme[c] + 0.003948 so4[c] + 0.000223 thf[c] + 0.000223 thmpp[c] + 0.2537 thr-L[c] + 0.05684 trp-L[c] + 0.1379 tyr-L[c] + 5.5e-005 udcpdp[c] + 0.1441 utp[c] + 0.4232 val-L[c] + 0.003158 zn2[c] -> 59.81 adp[c] + 59.81 h[c] + 59.806 pi[c] + 0.7739 ppi[c]

Formulation of Flux Balance Analysis



Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Nature biotechnology 28(3): 245-248.

E.coli Core Model



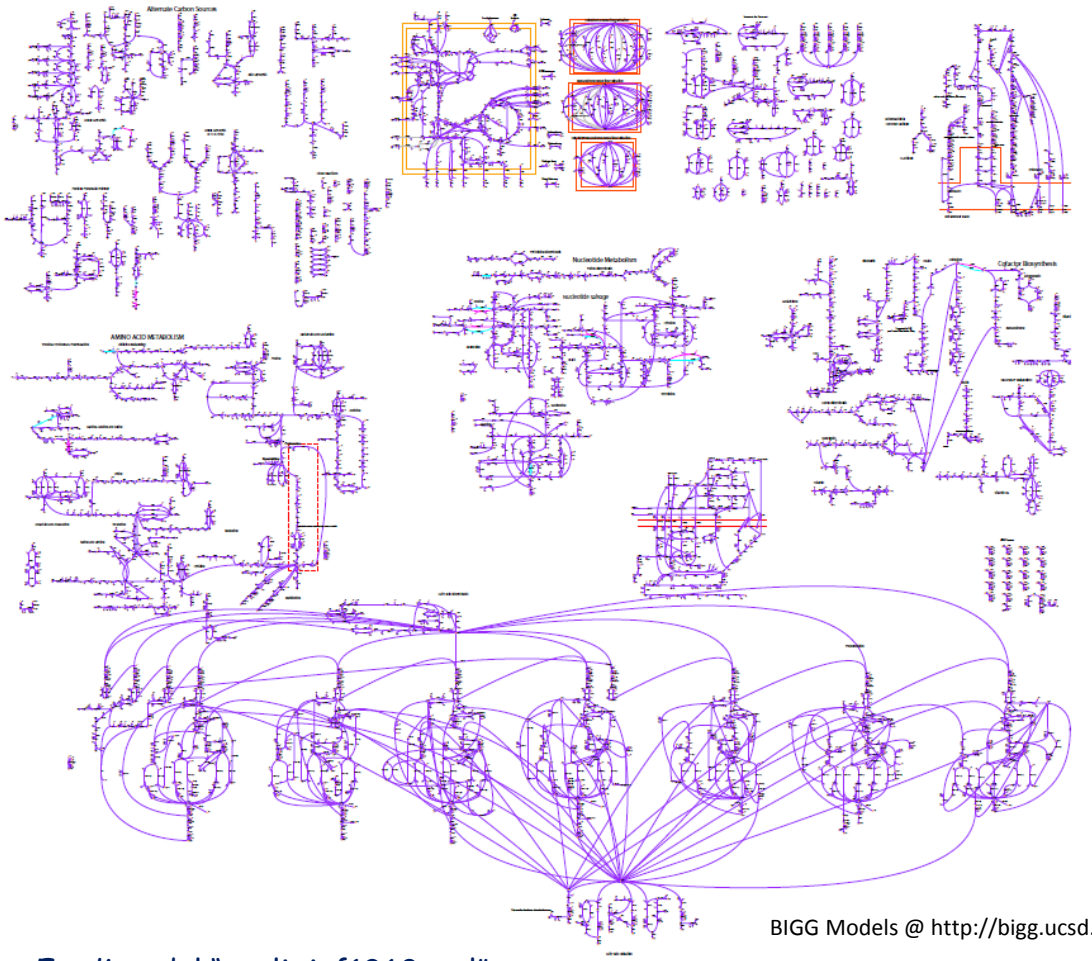
Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Nature biotechnology **28(3): 245-248.**

http://systemsbiology.ucsd.edu/Downloads/E_coli_Core

E.coli K-12 MG1655 Genome-Scale Reconstructions

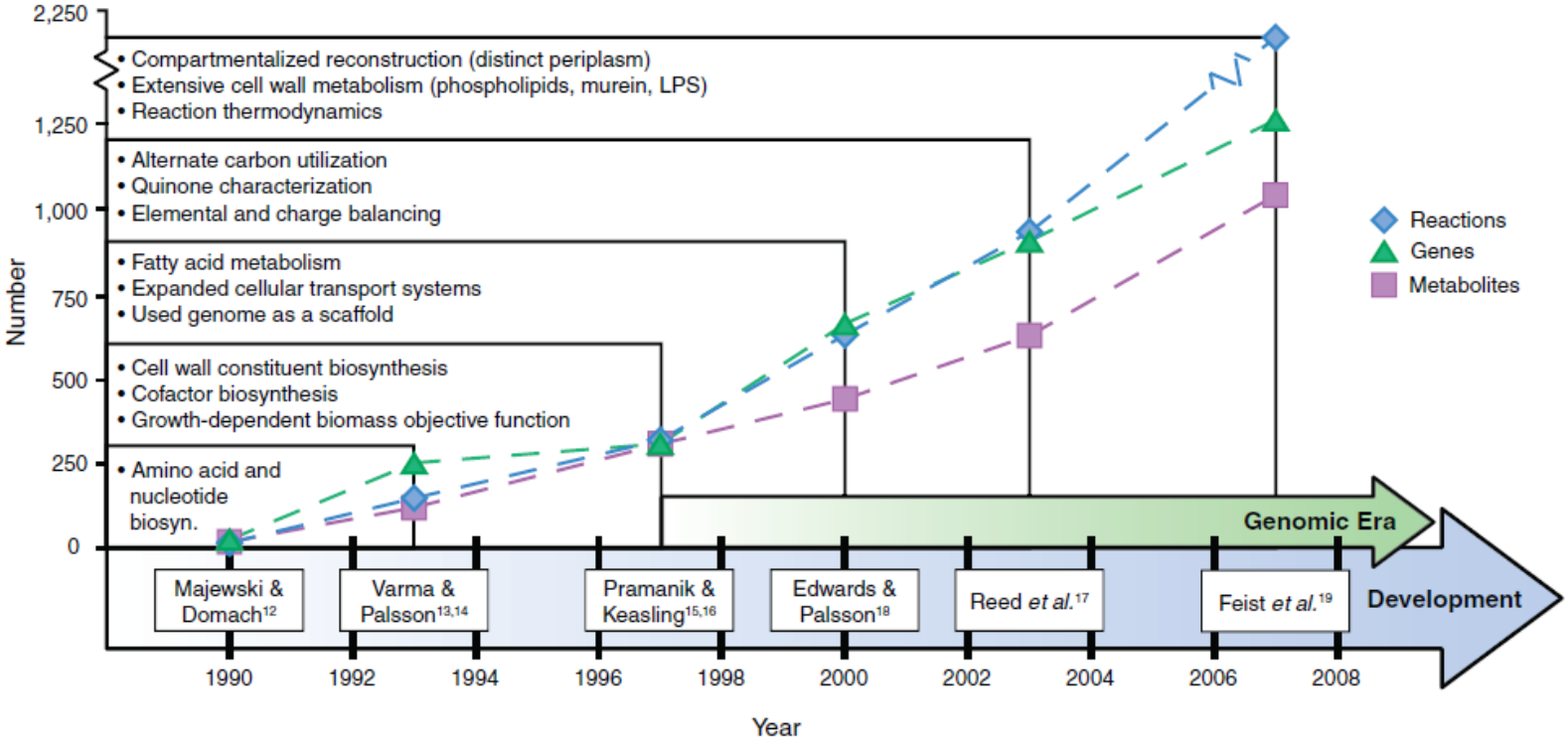
iAF1260 – 6.Feist, A. M., C. S. Henry, et al. (2007). "A genome-scale metabolic reconstruction for Escherichia coli K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information." Molecular Systems Biology 3: 121.

iJO1366 - Orth, J. D. and B. O. Palsson (2012). "Gap-filling analysis of the iJO1366 Escherichia coli metabolic network reconstruction for discovery of metabolic functions." BMC systems biology 6(1): 30.



E.coli model "ecoli_iaf1260.xml"

The Iterative Reconstruction and History of the *E. Coli* Metabolic Network



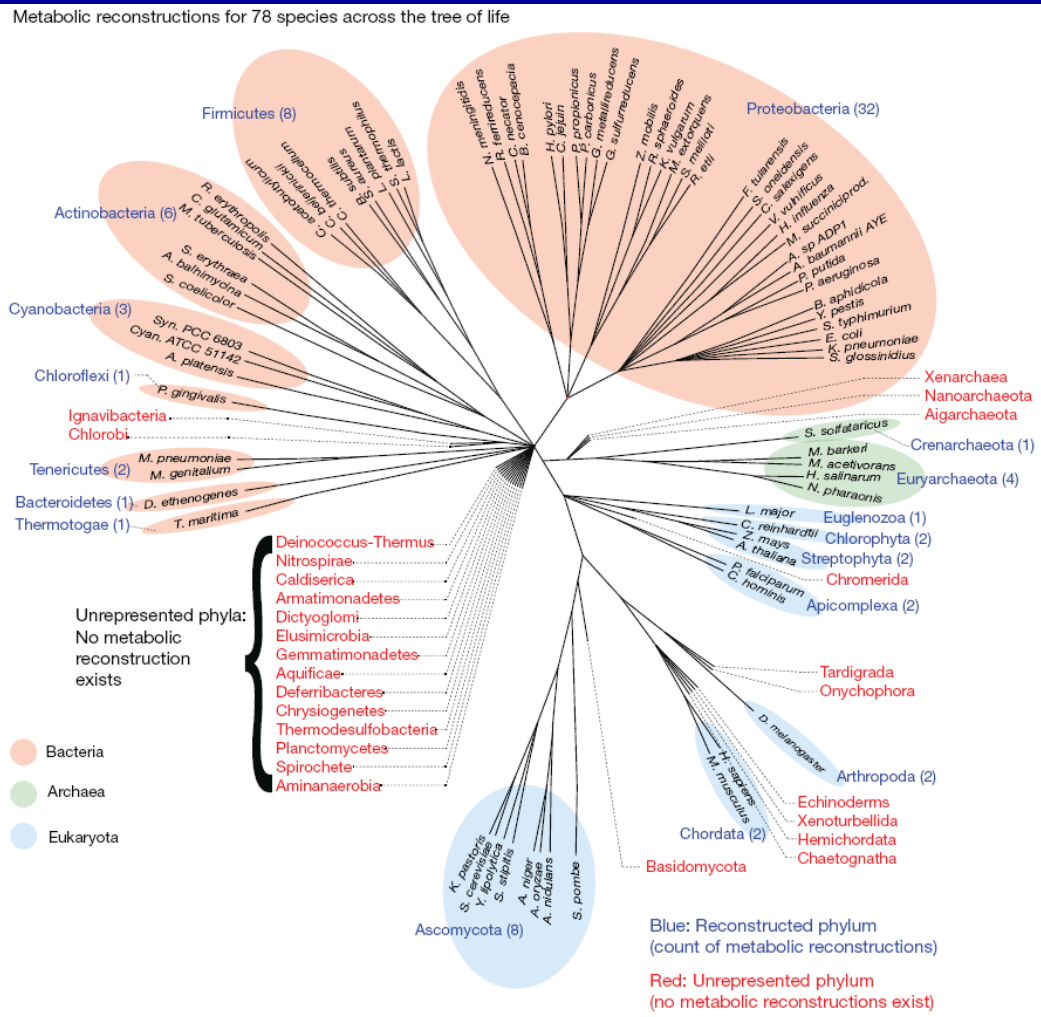
Feist, A. M. and B. O. Palsson (2008). "The growing scope of applications of genome-scale metabolic reconstructions using Escherichia coli." Nature biotechnology 26(6): 659-667..

E.coli Genome-scale Reconstructions

- Escherichia coli 042
- Escherichia coli 536
- Escherichia coli 55989
- Escherichia coli ABU 83972
- Escherichia coli APEC O1
- Escherichia coli ATCC 8739
- Escherichia coli B str. REL606
- Escherichia coli BL21(DE3) AM946981
- Escherichia coli BL21(DE3) BL21-Gold(DE3)pLysS AG
- Escherichia coli BL21(DE3) CP001509
- Escherichia coli BW2952
- Escherichia coli CFT073
- Escherichia coli DH1
- Escherichia coli DH1 ME8569
- Escherichia coli E24377A
- Escherichia coli ED1a
- Escherichia coli ETEC H10407
- Escherichia coli HS
- Escherichia coli IAI1
- Escherichia coli IAI39
- Escherichia coli IHE3034
- Escherichia coli KO11FL
- Escherichia coli LF82
- Escherichia coli NA114
- Escherichia coli O103:H2 str. 12009
- Escherichia coli O111:H- str. 11128
- Escherichia coli O127:H6 str. E2348/69
- Escherichia coli O157:H7 EDL933
- Escherichia coli O157:H7 str. EC4115
- Escherichia coli O157:H7 str. Sakai
- Escherichia coli O157:H7 str. TW14359
- Escherichia coli O26:H11 str. 11368
- Escherichia coli O55:H7 str. CB9615
- Escherichia coli O83:H1 str. NRG 857C
- Escherichia coli S88
- Escherichia coli SE11
- Escherichia coli SE15
- Escherichia coli SMS-3-5
- Escherichia coli str. K-12 substr. DH10B
- Escherichia coli str. K-12 substr. MG1655
- Escherichia coli str. K-12 substr. W3110
- Escherichia coli UM146
- Escherichia coli UMN026
- Escherichia coli UMNK88
- Escherichia coli UTI89
- Escherichia coli W
- Escherichia coli W CP002185
- Escherichia coli K-12 MG1655

Monk, J. M., P. Charusanti, et al. (2013). Proceedings of the National Academy of Sciences of the United States of America 110(50): 20338-20343.

Phylogenetic Coverage of Genome-scale Network Reconstructions



Monk, J., J. Nogales, et al. (2014). "Optimizing genome-scale network reconstructions." Nature biotechnology 32(5): 447-452

Flux Balance Analysis Overview

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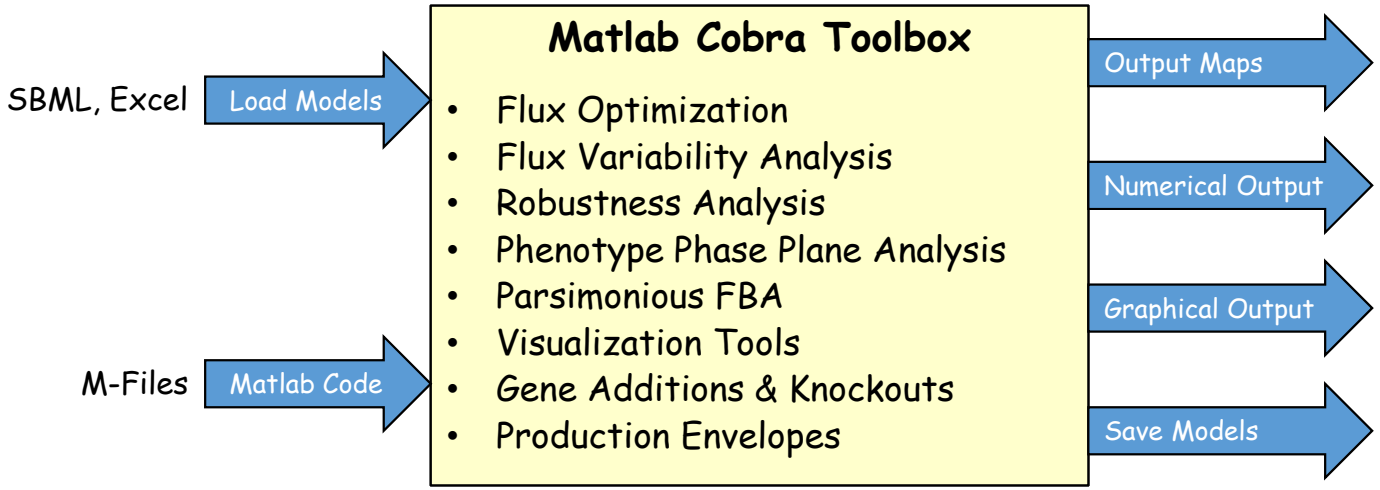
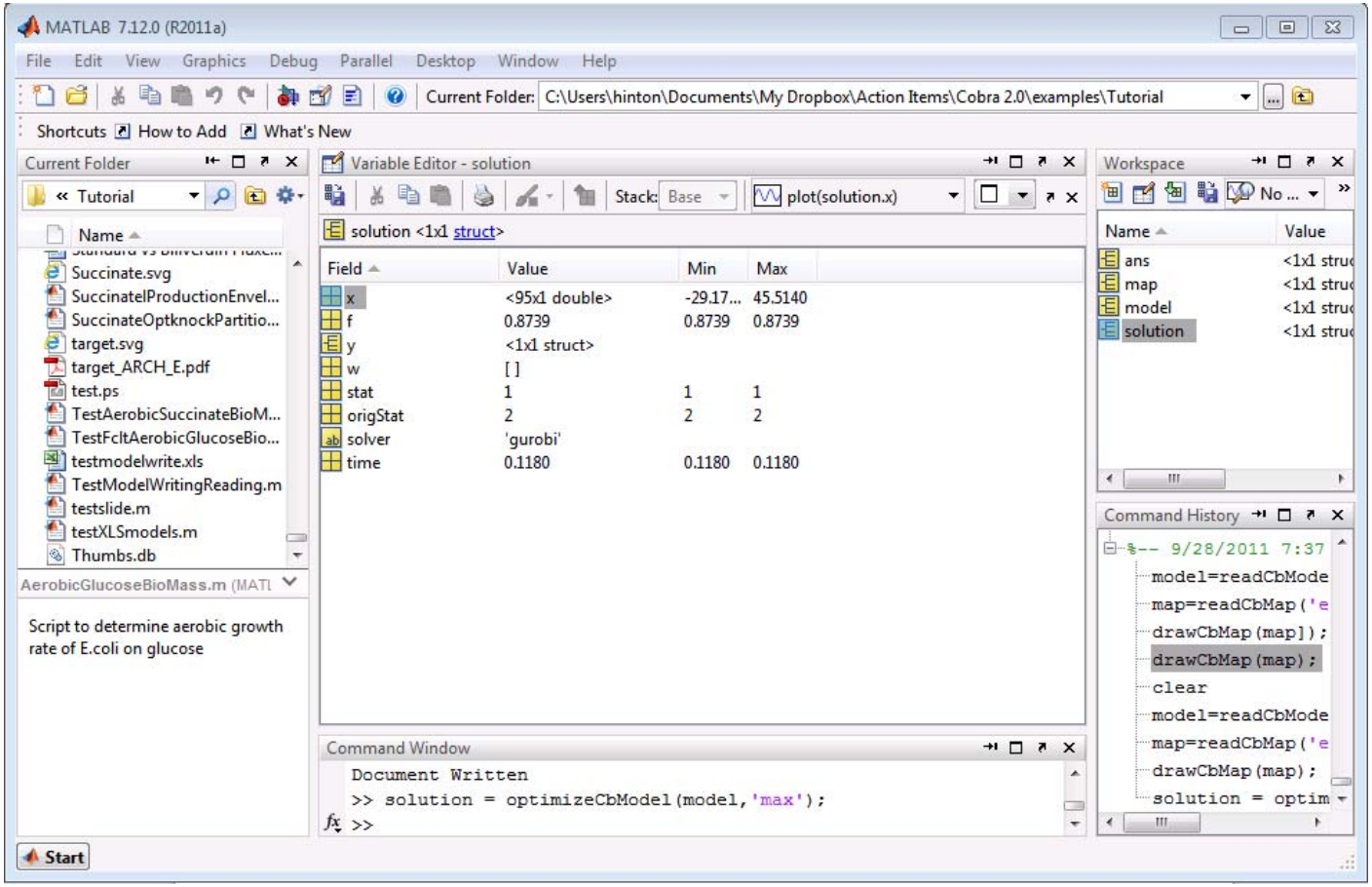
COBRA TOOLBOX



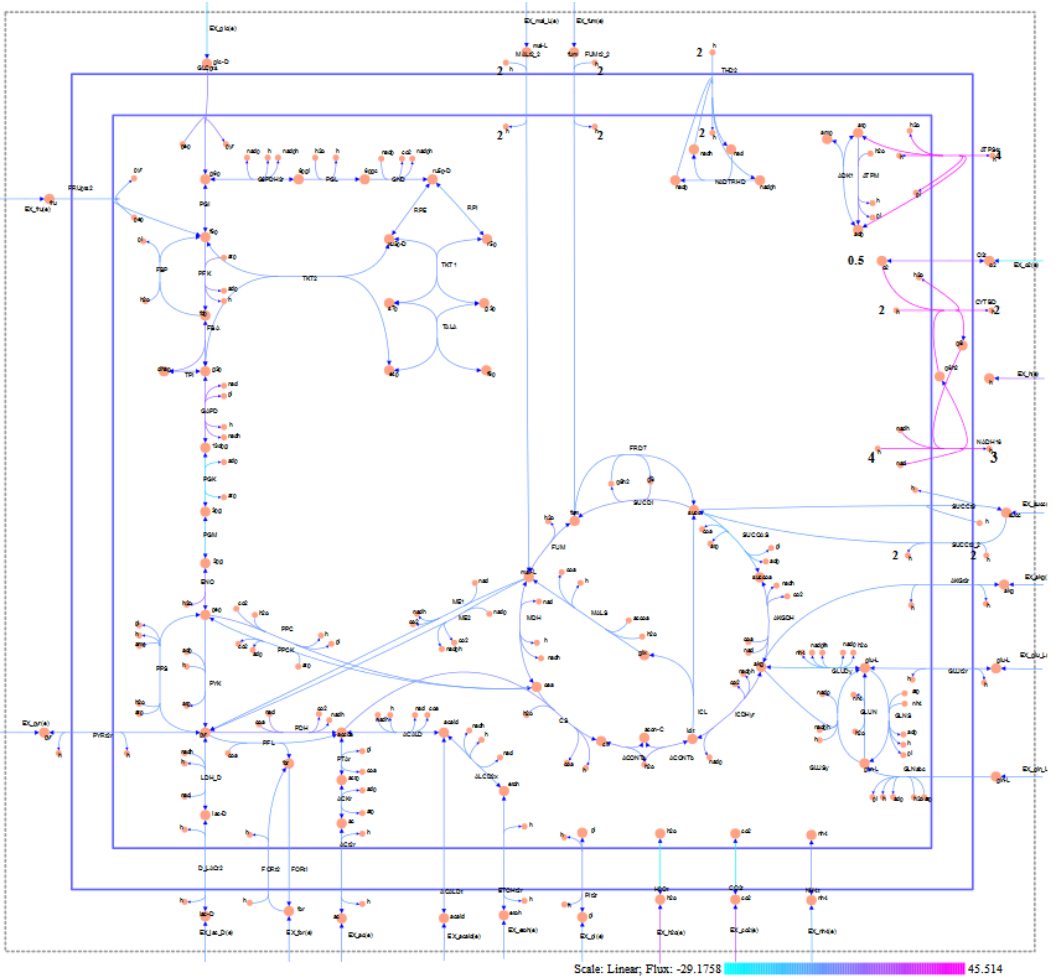
Links for installing COBRA toolbox for MATLAB

- http://www.nature.com/protocolexchange/protocols/2097#/introduction
- http://benheavner.com/systemsbio/index.php?title=Installing_COBRA_toolbox_for_MATLAB
- http://opencobra.sourceforge.net/openCOBRA/Install.html

Matlab Interface



DRAWING FLUX VALUES ONTO A MAP



Print Flux Values

ACONTa	6.00725
ACONTb	6.00725
AKGDH	5.06438
ATPM	8.39
ATPS4r	45.514
Biomass_...	0.873922
CO2t	-22.8098
CS	6.00725
CYTBD	43.599
ENO	14.7161
EX_co2(e)	22.8098
EX_glc(e)	-10
EX_h2o(e)	29.1758
EX_h(e)	17.5309
EX_nh4(e)	-4.76532
EX_o2(e)	-21.7995
EX_pi(e)	-3.2149

Growth Rate

Inputs & Outputs (Exchange Reactions)

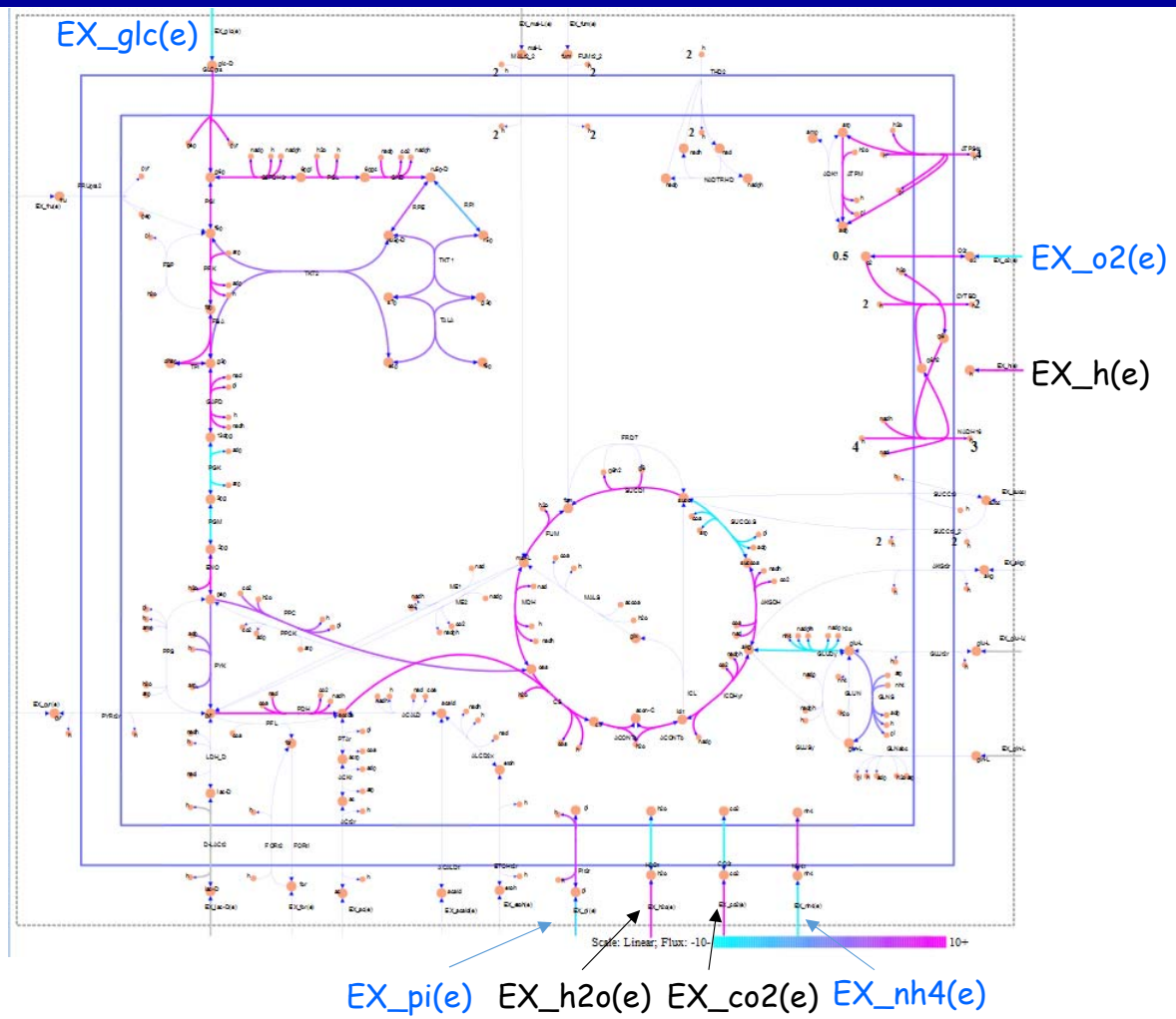
FBA	7.47738
FUM	5.06438
G6PDH2r	4.95998
GAPD	16.0235
GLCpts	10
GLNS	0.223462
GLUDy	-4.54186
GND	4.95998
H2Ot	-29.1758
ICDHyr	6.00725
MDH	5.06438
NADH16	38.5346
NH4t	4.76532
O2t	21.7995
PDH	9.28253
PFK	7.47738
PGI	4.86086

PGK	-16.0235
PGL	4.95998
PGM	-14.7161
PIt2r	3.2149
PPC	2.50431
PYK	1.75818
RPE	2.67848
RPI	-2.2815
SUCDi	5.06438
SUCOAS	-5.06438
TALA	1.49698
TKT1	1.49698
TKT2	1.1815
TPI	7.47738

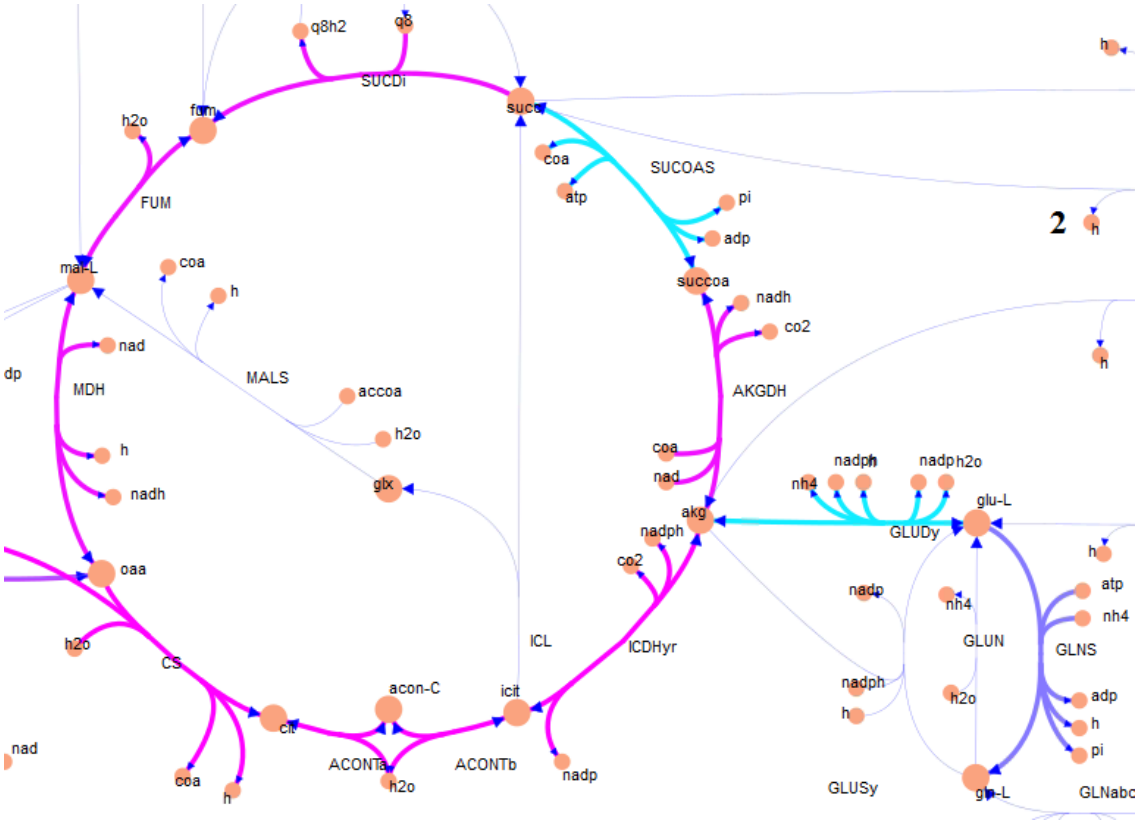
Aerobic Growth on Glucose

Exchange Reactions

EX_co2(e)	40.6527
EX_glc(e)	-18.5
EX_h2o(e)	52.6943
EX_h(e)	33.1606
EX_nh4(e)	-9.01387
EX_o2(e)	-38.7416
EX_pi(e)	-6.08116



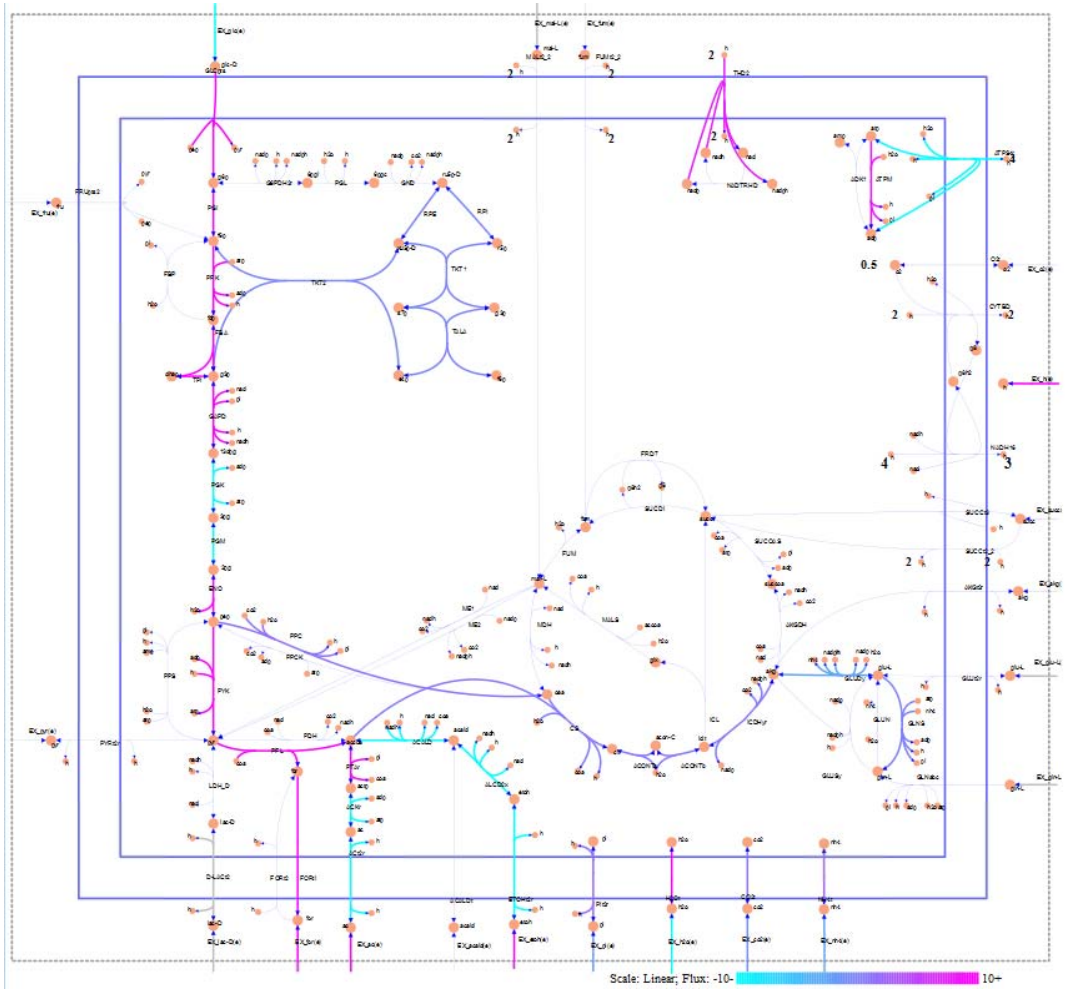
Close-up of TCA Cycle



Anaerobic Growth on Glucose

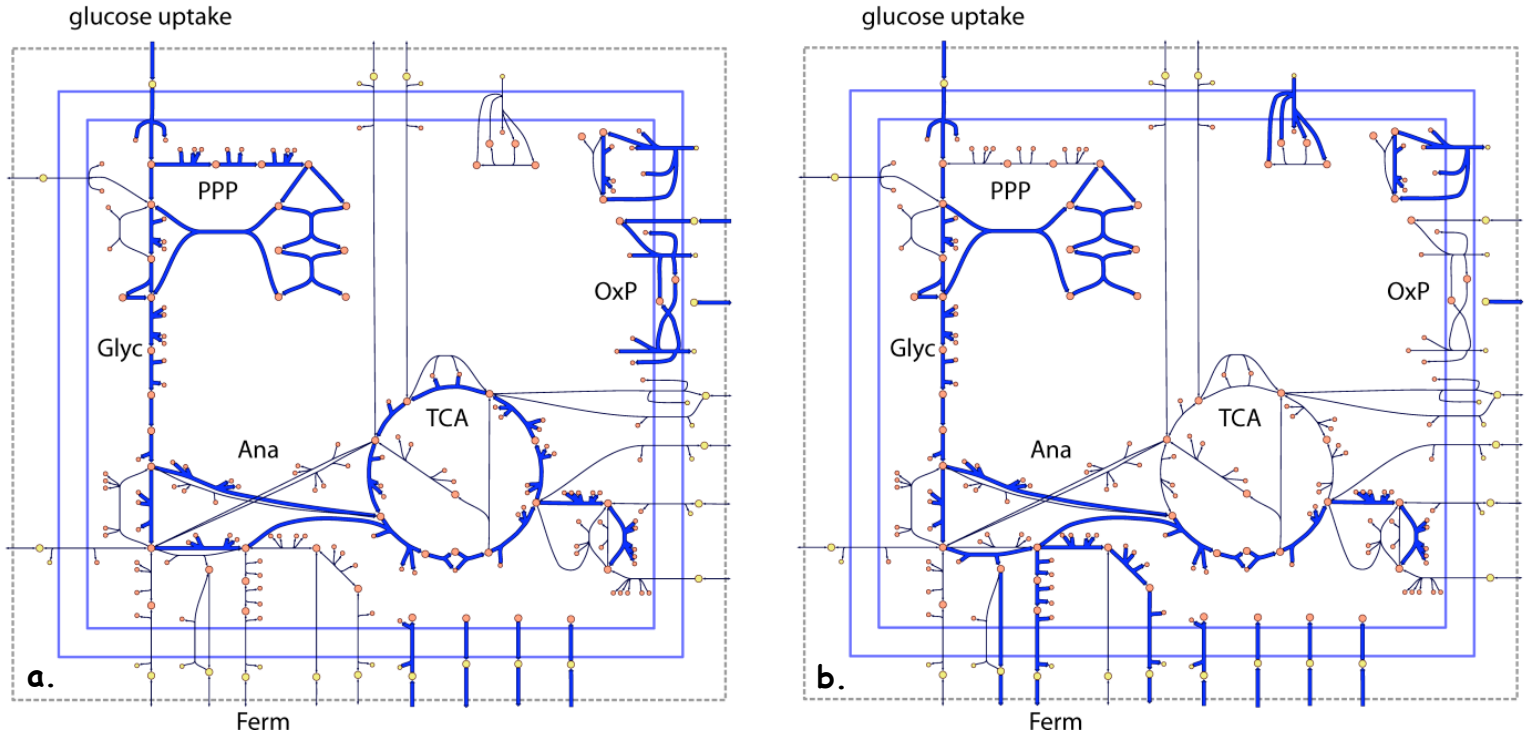
Exchange Reactions

Biomass	0.470565
EX_ac(e)	15.1732
EX_co2(e)	-0.840759
EX_etoh(e)	14.6749
EX_for(e)	32.1194
EX_glc(e)	-18.5
EX_h2o(e)	-12.0879
EX_h(e)	56.7321
EX_nh4(e)	-2.5659
EX_pi(e)	-1.73107



AEROBIC vs. ANAEROBIC GROWTH

Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Nature biotechnology 28(3): 245-248.



Aerobic Growth **Anaerobic Growth**

Substrate Maximum Growth Rate

The core *E. coli* model contains exchange reactions for 13 different organic compounds, each of which can be used as the sole carbon source under aerobic or anaerobic conditions.

Substrate	Aerobic (hr^{-1})	Anaerobic (hr^{-1})
acetate	0.3893	0
acetaldehyde	0.6073	0
2-oxoglutarate	1.0982	0
ethanol	0.6996	0
D-fructose	1.7906	0.5163
fumarate	0.7865	0
D-glucose	1.7906	0.5163
L-glutamine	1.1636	0
L-glutamate	1.2425	0
D-lactate	0.7403	0
L-malate	0.7865	0
pyruvate	0.6221	0.0655
succinate	0.8401	0

("What is flux balance analysis? - Supplementary tutorial")

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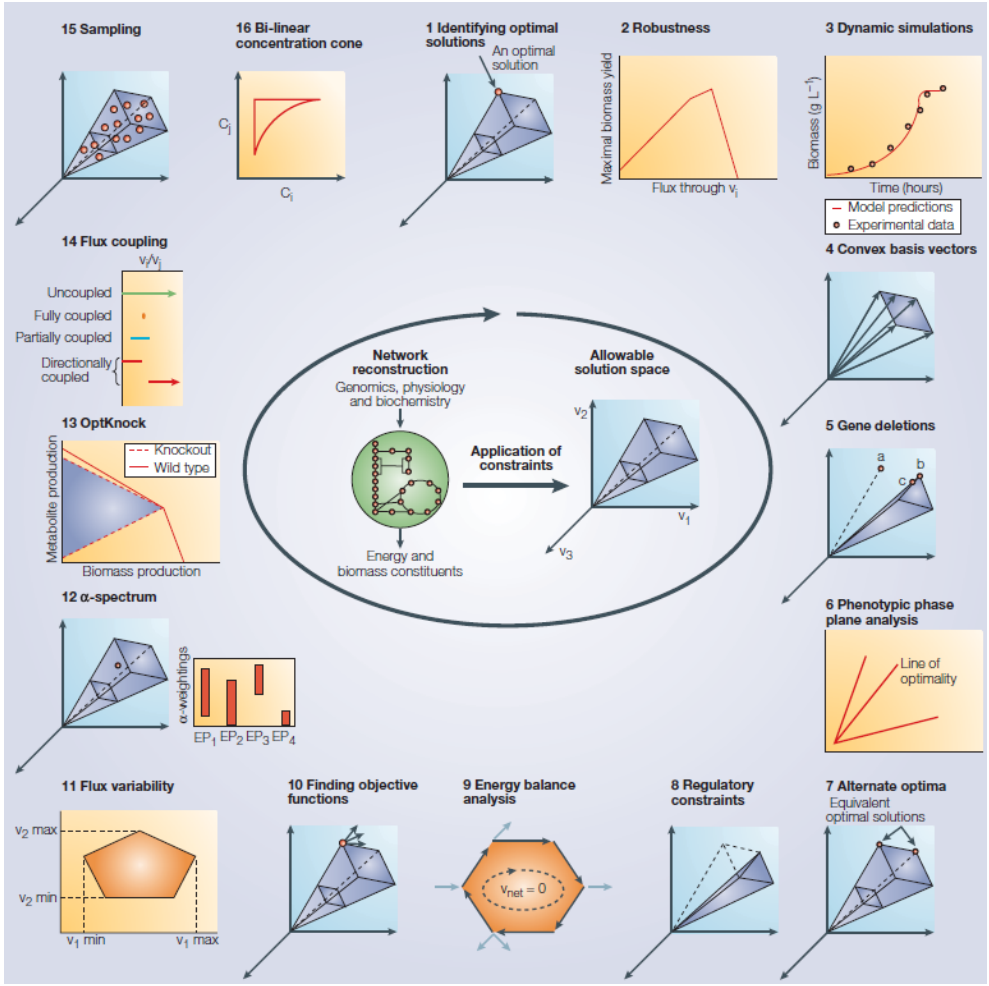
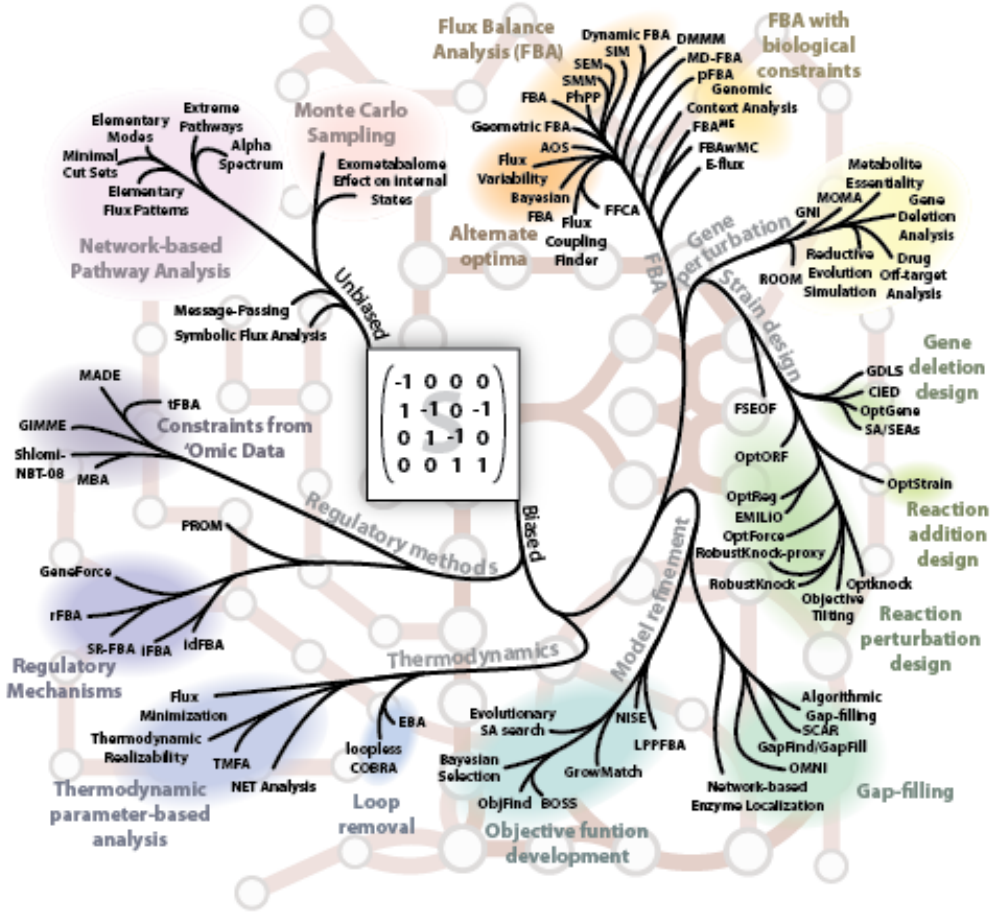
A Growing Toolbox for Constraint-based Analysis



Price, N. D., J. L. Reed, et al. (2004). "Genome-scale models of microbial cells: evaluating the consequences of constraints." Nature reviews. Microbiology 2(11): 886-897.

Methods in Constraint-based Reconstruction and Analysis



http://sourceforge.net/apps/mediawiki/opencobra/index.php?title=Main_Page

Flux Balance Analysis Overview

- Flux Balance Analysis Overview
- Stoichiometric Reactions & Metabolites
- Mathematical Representation of Reactions & Constraints
- Mass Balanced Linear Equations
- Biomass Reaction
- Calculating Fluxes
- Flux Balance Analysis Toolbox