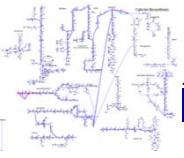


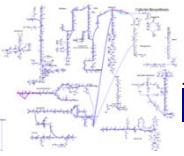
Flux Balance Analysis Overview



Learning Objectives

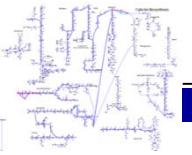
Each student should be able to:

- Explain flux balance analysis (FBA).
- Explain reactions, metabolites, & pathways.
- 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

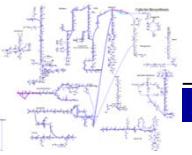
- ➡ • Flux Balance Analysis Overview
- Reactions, Metabolites, & Pathways
- Mathematical Representation of Reactions & Constraints
- Mass Balanced Linear Equations
- Biomass Reaction
- Calculating Fluxes
- Flux Balance Analysis Toolbox



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

a Genome-scale metabolic reconstruction



b Mathematically represent metabolic reactions and constraints



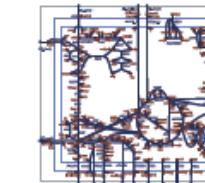
c Mass balance defines a system of linear equations



d Define objective function
($Z = c_1^* v_1 + c_2^* v_2 \dots$)



e Calculate fluxes that maximize Z



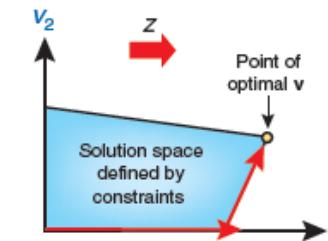
A \leftrightarrow B + C Reaction 1
B + 2C \rightarrow D Reaction 2
...
Reaction n

		Reactions			
		1	2	...	n
Metabolites	A	-1			Biomass
	B	1	-1		Glucose
C	1	-2		Oxygen	
D		1			
...					
m					
					Stoichiometric matrix, S

$* \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_m \end{bmatrix} = 0$

$$\begin{aligned} -v_1 + \dots &= 0 \\ v_1 - v_2 + \dots &= 0 \\ v_1 - 2v_2 + \dots &= 0 \\ v_2 + \dots &= 0 \\ \text{etc.} \end{aligned}$$

To predict growth, $Z = v_{\text{biomass}}$



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



Flux Balance Analysis Overview

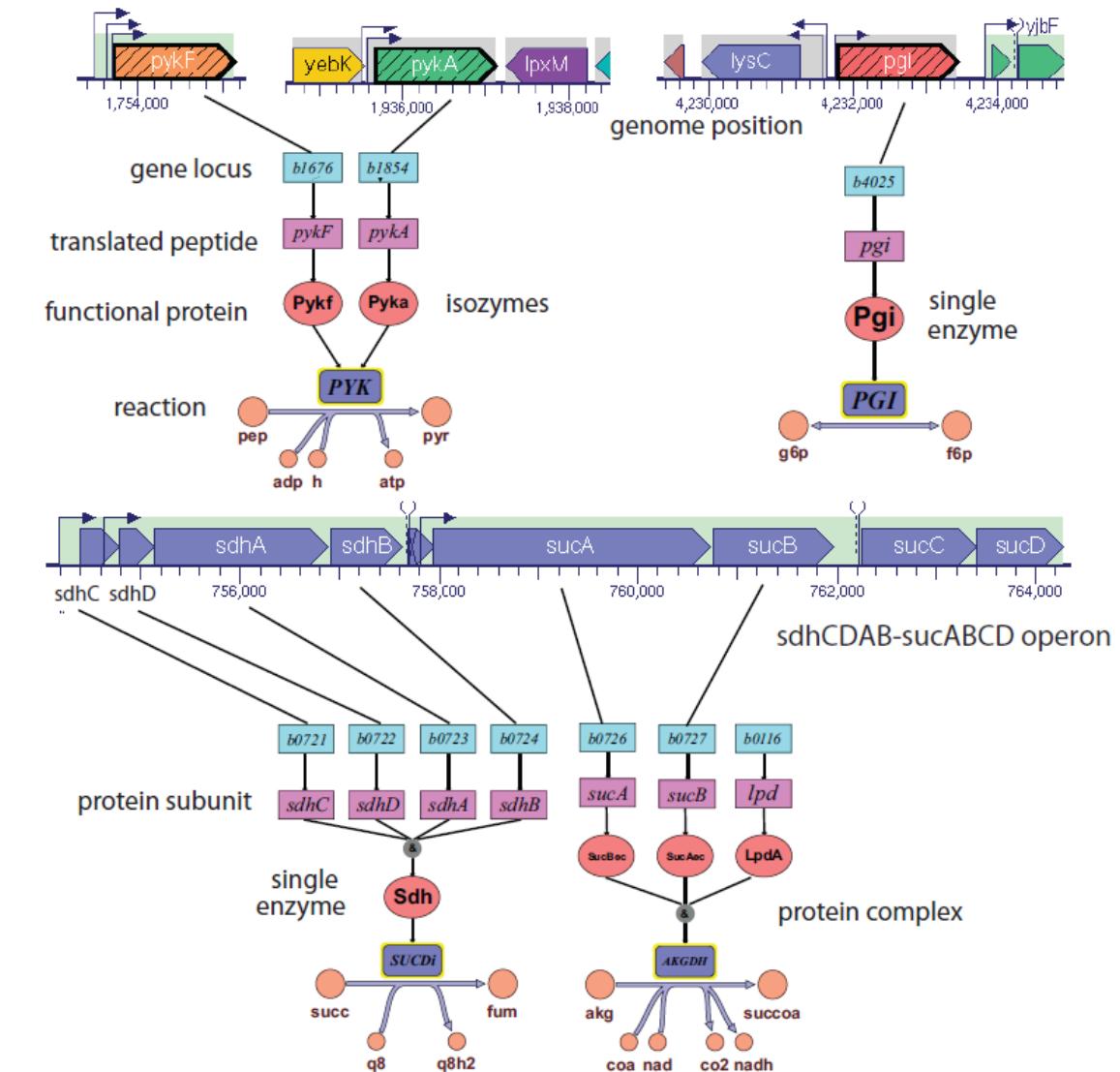
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Identifying Metabolic Reactions and Metabolites (Gene-Protein-Reactions)

Objective:

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

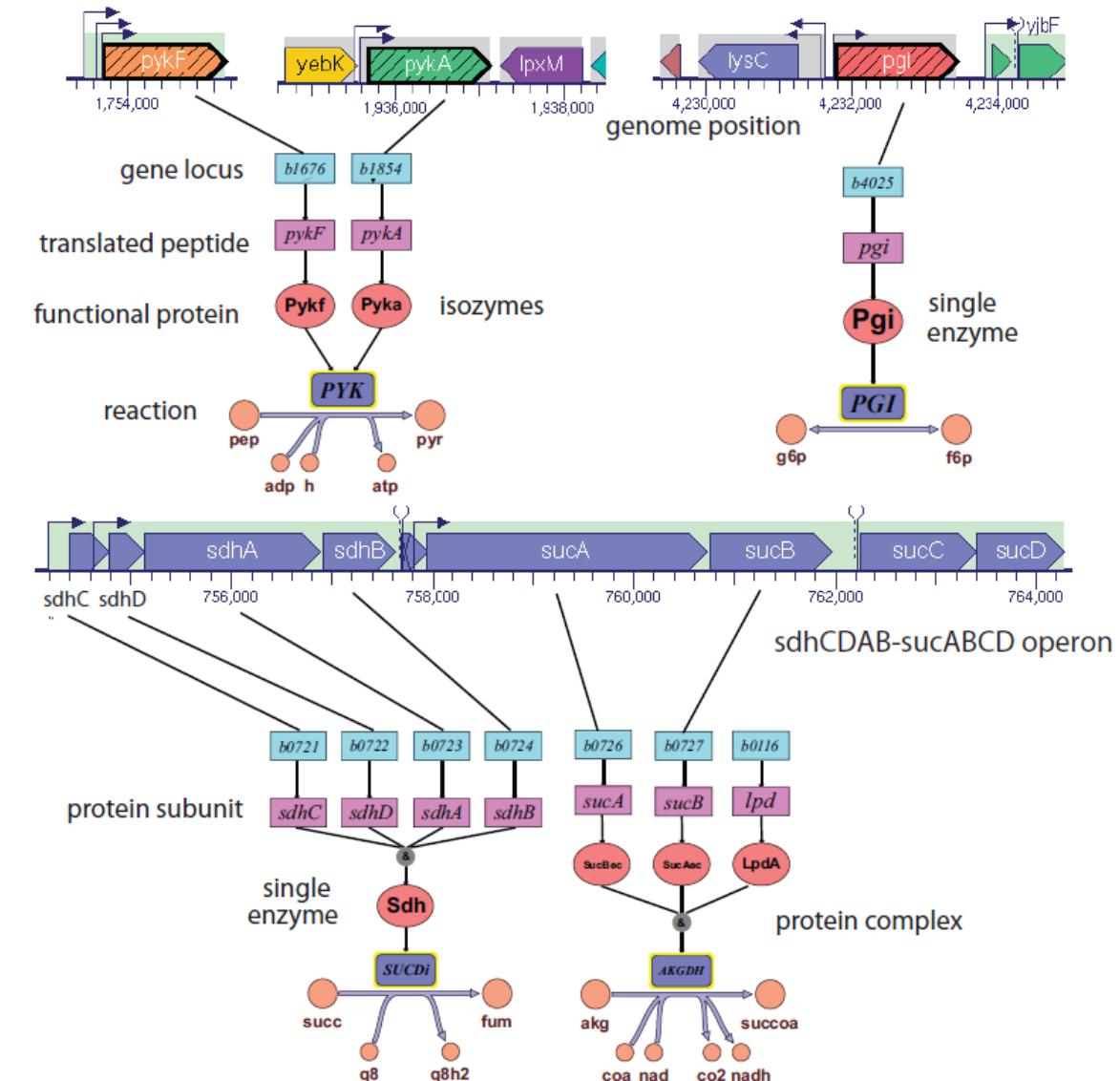




Desired Reaction Information

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





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-Oxoglutarate 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				

ecoli_textbook.xls



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

Gene	b2388	Locus	Genomics ORF annotation	
Peptide	glk	Gene	Transcriptomics mRNA levels	
Protein	Glk	Protein	Proteomics protein levels	
		Reaction	"Fluxomics" flux measurements	
Substrates	glc	atp	g6p	adp
Neutral	C ₆ H ₁₂ O ₆ ⁰	C ₁₀ H ₁₆ N ₅ O ₁₃ P ₃ ⁰	C ₆ H ₁₃ O ₉ P ⁰	C ₁₀ H ₁₅ N ₅ O ₁₀ P ₂ ⁰
Charged	C ₆ H ₁₂ O ₆ ⁰	C ₁₀ H ₁₂ N ₅ O ₁₃ P ₃ ⁻⁴	C ₆ H ₁₁ O ₉ P ²⁻	C ₁₀ H ₁₂ N ₅ O ₁₀ P ₂ ⁻³
Stoichiometry	<chem>C16H24O19P3</chem> , 4e ⁻	$1 \text{ glc} + 1 \text{ atp}$	$\text{C}_{16}\text{H}_{23}\text{O}_{18}\text{P}_3$, 5e ⁻	$1 \text{ g6p} + 1 \text{ adp} + 1 \text{ h}^+$
Directionality	$1 \text{ glc} + 1 \text{ atp}$	\rightarrow	$1 \text{ g6p} + 1 \text{ adp} + 1 \text{ h}^+$	$1 \text{ g6p} + 1 \text{ adp} + 1 \text{ h}^+$
Location	cytosol: $1 \text{ glc} + 1 \text{ atp}$	\rightarrow	$1 \text{ g6p} + 1 \text{ adp} + 1 \text{ h}^+$	$1 \text{ g6p} + 1 \text{ adp} + 1 \text{ h}^+$

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



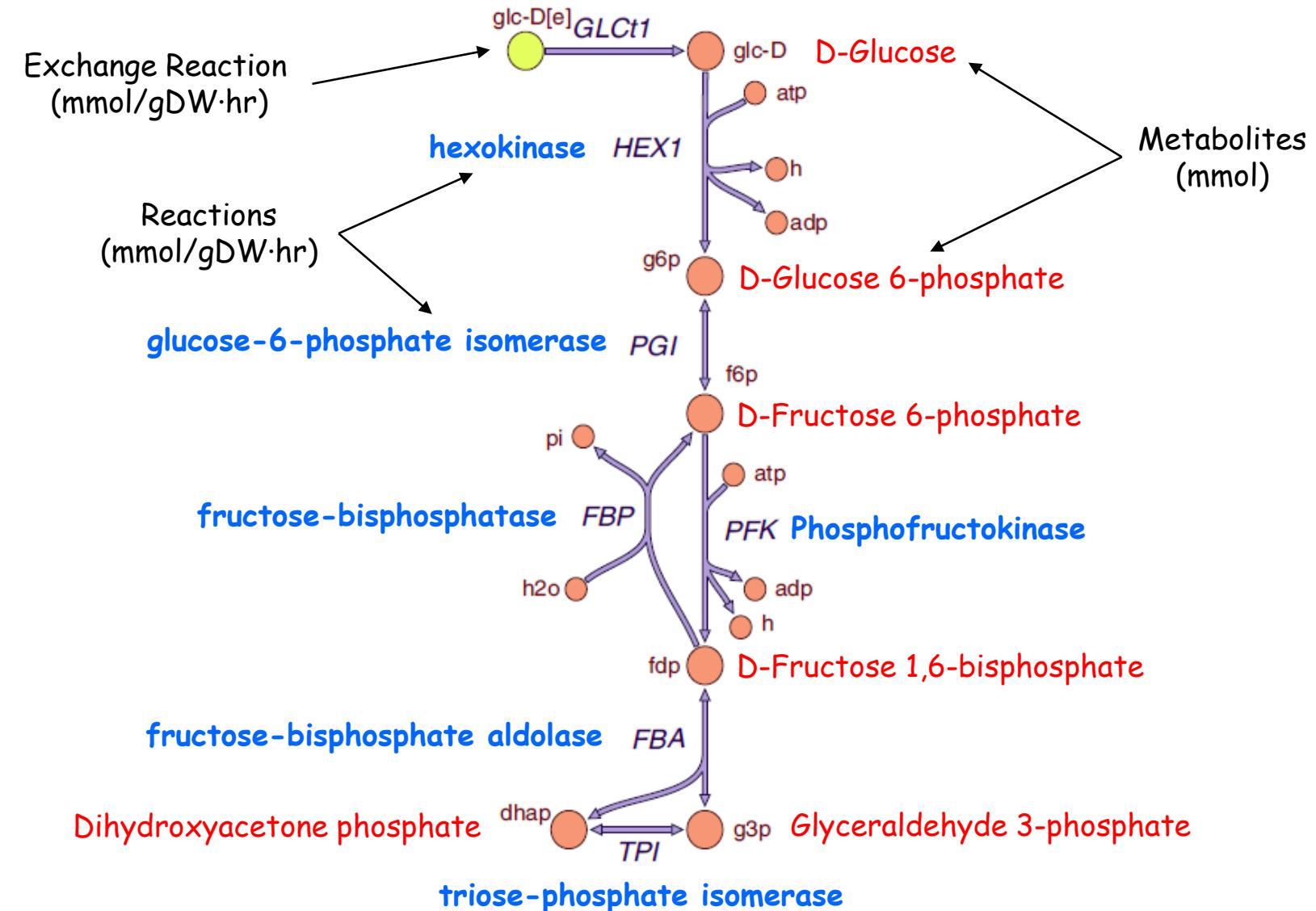
Genome-scale Reconstruction Metabolites

abbreviation	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-tricar	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-fruc	C00354
for	Formate	CH1O2	-1	64-18-6	CH2O2	Methanoic acid/ Formic acid/ Hydrogencarboxylic acid/ aminic	C00058

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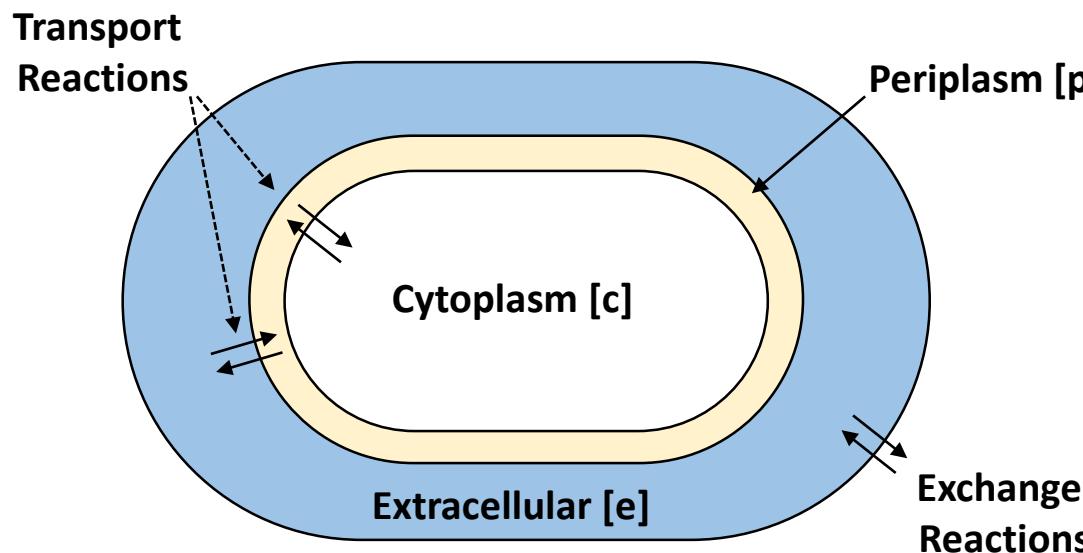
Metabolic Pathway



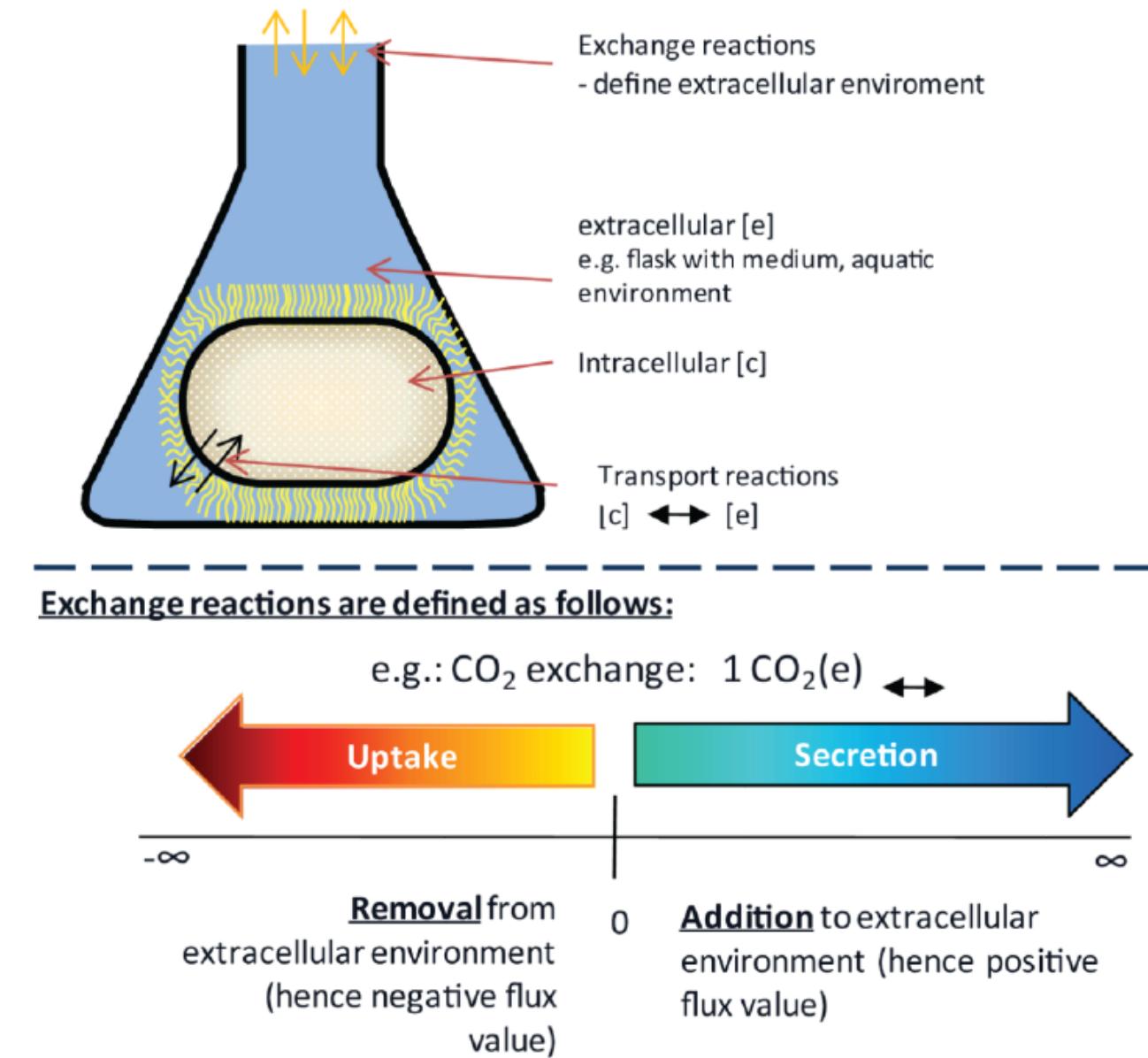
Becker, S. A., et al. (2007). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox." *Nature protocols* 2(3): 727-738.



System Boundaries: Exchange & Transport Reactions



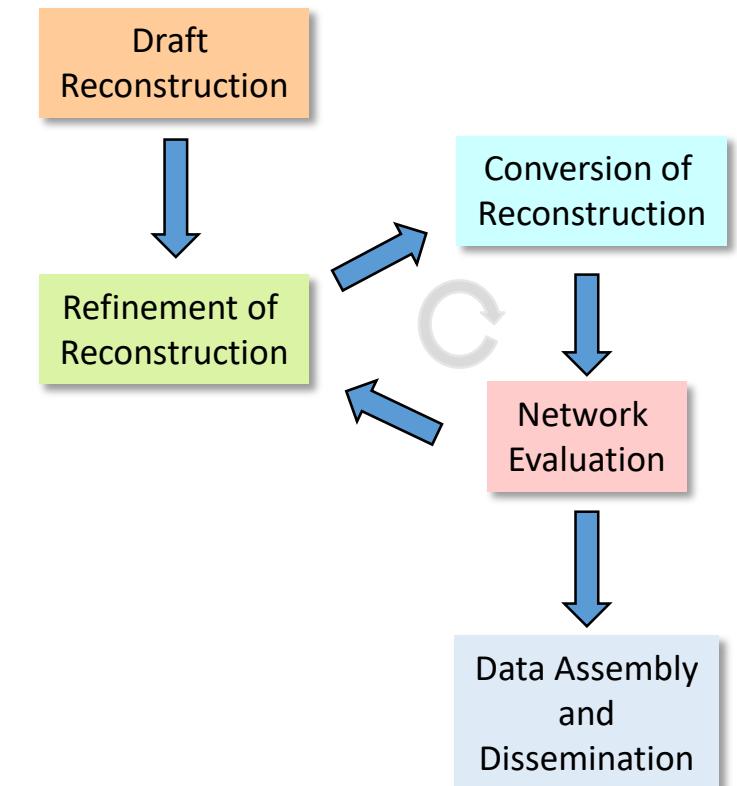
Thiele, I. and B. O. Palsson (2010). "A protocol for generating a high-quality genome-scale metabolic reconstruction." *Nature protocols* 5(1): 93-121.





Genome-scale Metabolic Reconstructions

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

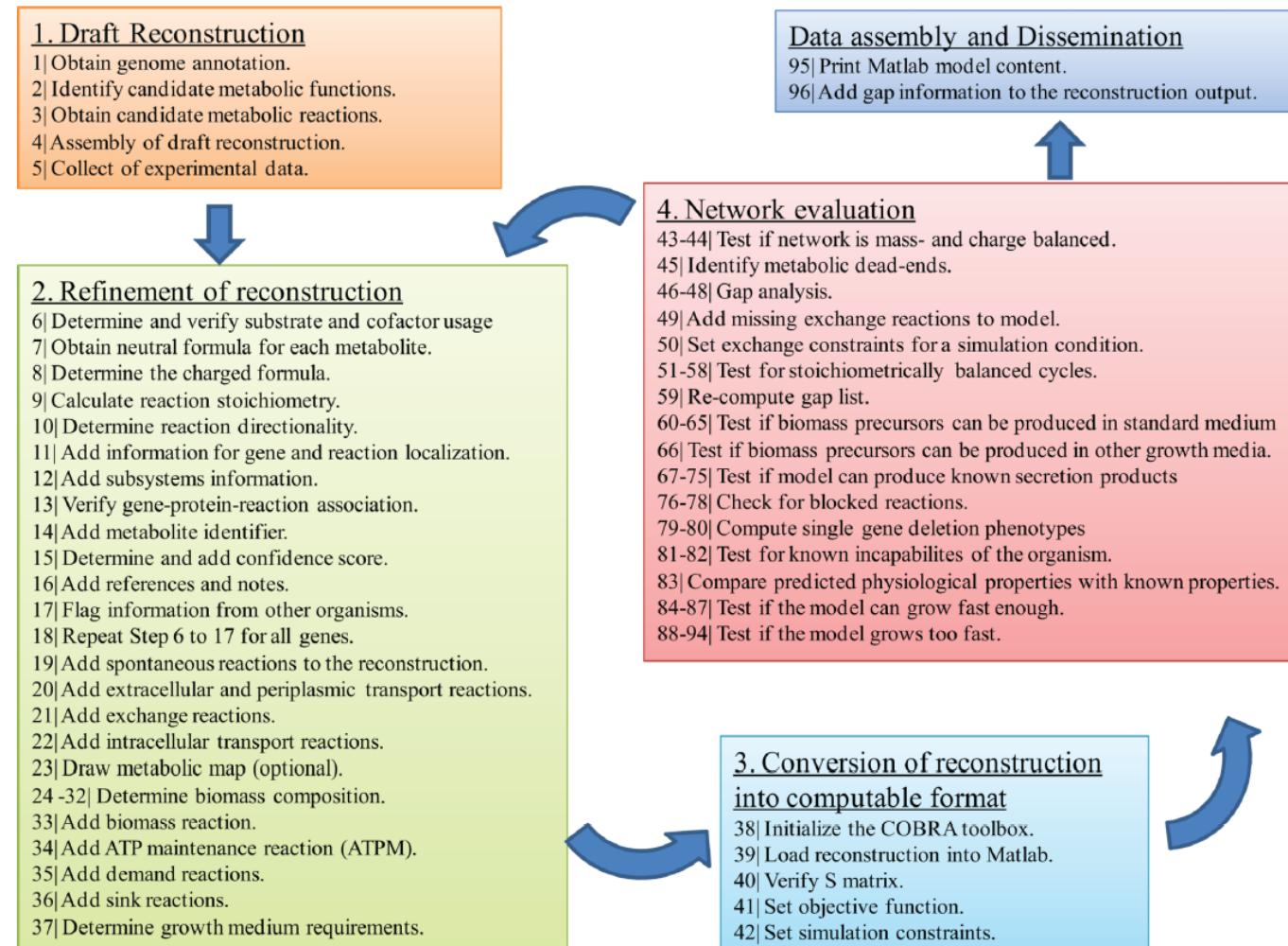


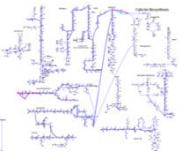
Thiele, I. and B. O. Palsson (2010). "A protocol for generating a high-quality genome-scale metabolic reconstruction." Nature protocols 5(1): 93-121.



Reconstruction Process: 96 Step Protocol

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



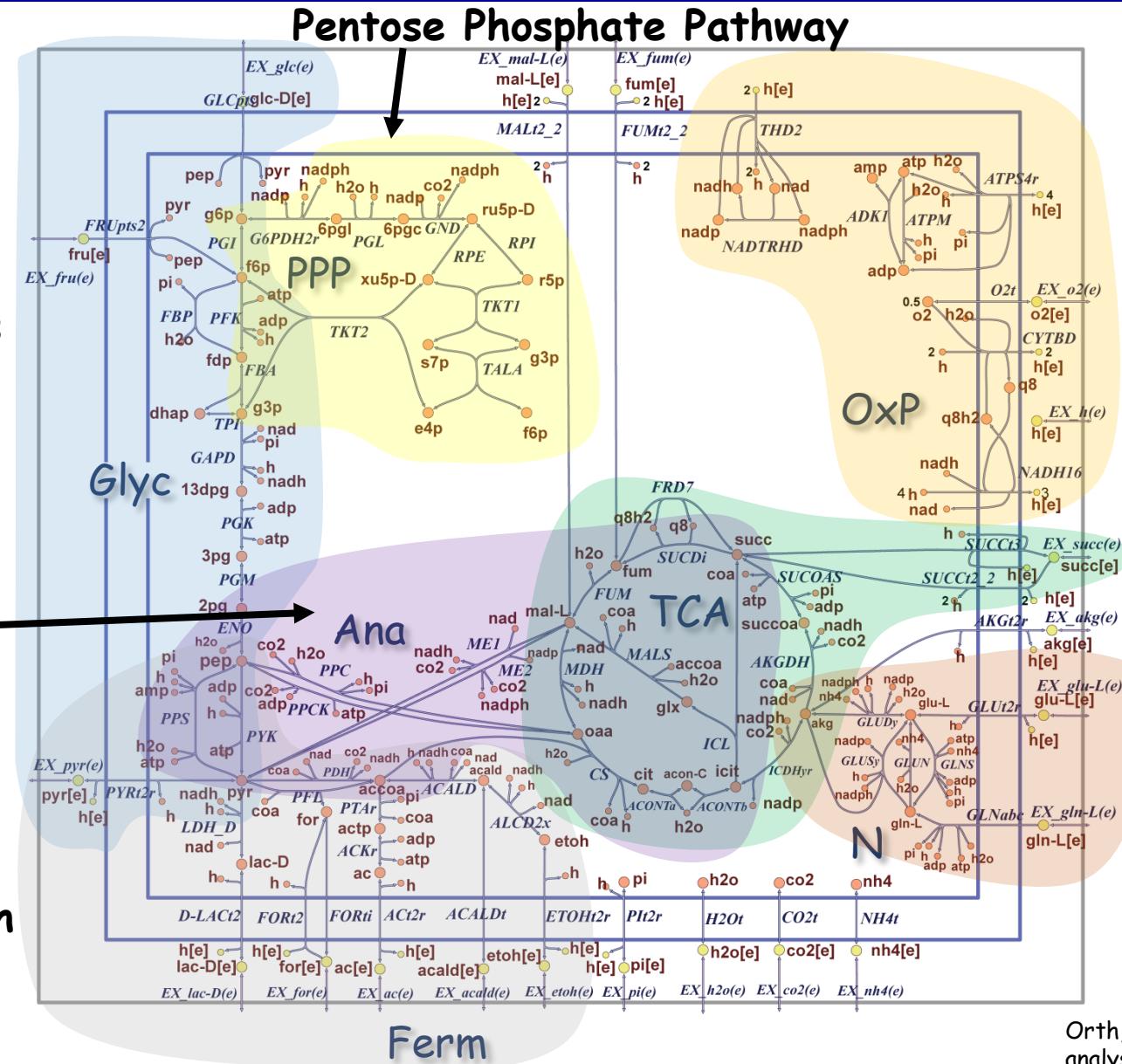


E. coli Core Model

Glycolysis

Glyoxylate Cycle,
Gluconeogenesis, and
Anapleurotic Reactions

Fermentation



Oxidative
Phosphorylation and
Transfer of Reducing
Equivalents

Tricarboxylic Acid
Cycle (TCA)

Nitrogen
Metabolism

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



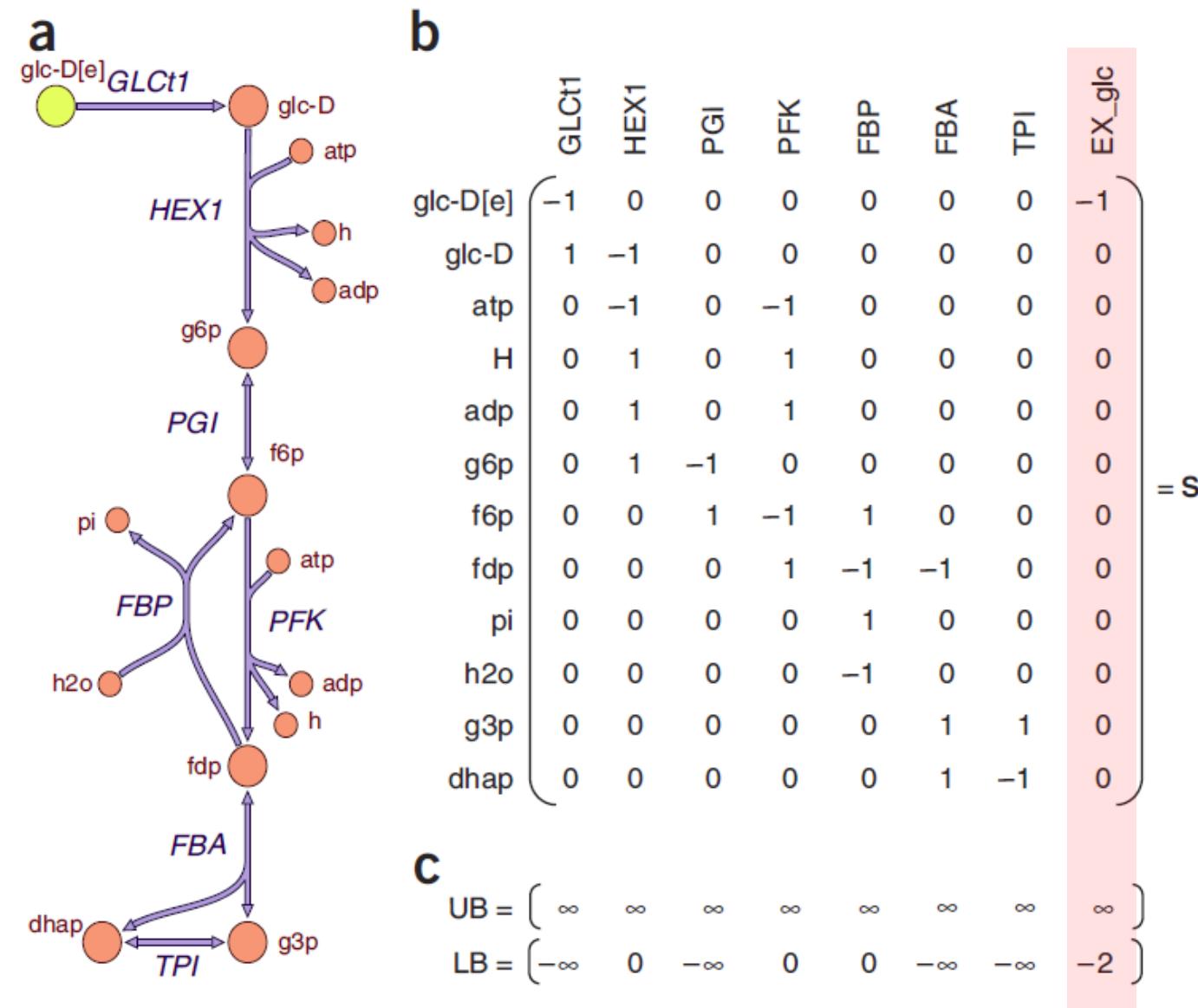
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Creating A Stoichiometric Matrix

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.



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.



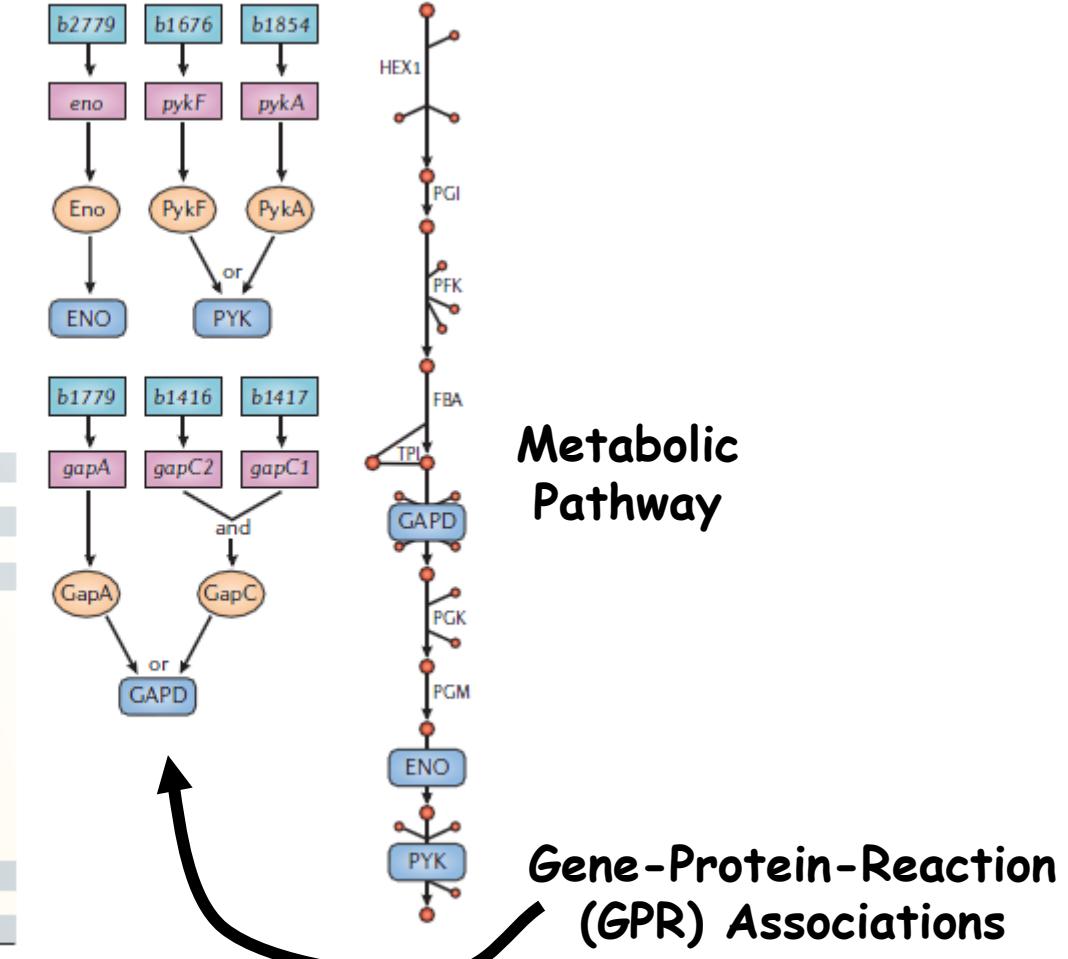
Genome-scale Metabolic Reconstruction

**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 ₂ O + 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 ₂ O	0	0	0	0	0	0	0	0	1	0
PYR	0	0	0	0	0	0	0	0	0	1



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



Flux Balance Analysis Overview

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- Reactions, Metabolites, & Pathways
- Mathematical Representation of Reactions & Constraints
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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 .

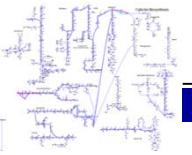
$$\frac{dx}{dt} = S \cdot v$$

- 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.

$$\frac{dx}{dt} = 0 = S \cdot v$$

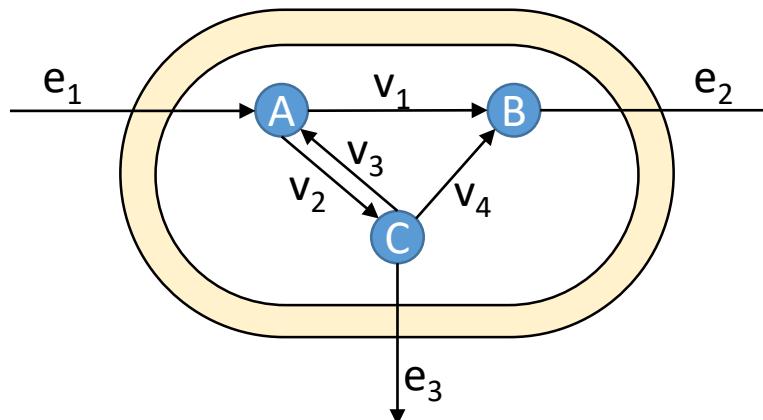
- Since there are normally many more reactions (columns) than metabolites (rows), more unknown variables than equations, then there is no unique solutions (could be a large number of solutions).
- Need to find a way to constrain the solution space!

Metabolites	Reactions							
	-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	0	1	1	0
	0	0	0	0	0	1	-1	0



Dynamic Mass Balance

A simple network



Linear Transformation

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

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

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)

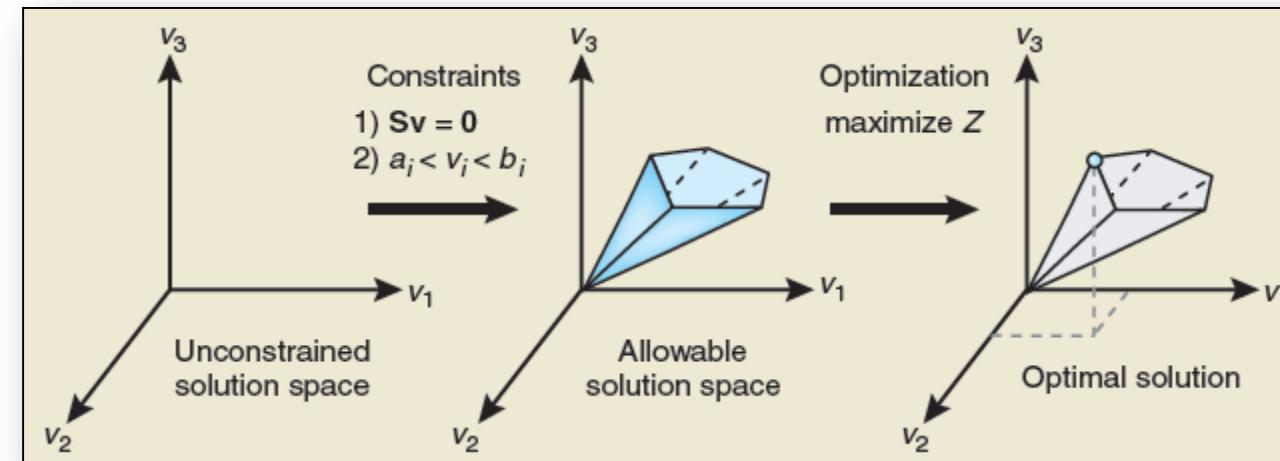
$$0 = \mathbf{S} \cdot \mathbf{v}$$

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

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

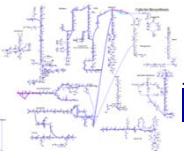


The Conceptual Basis of Constraint-based Modeling



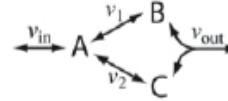
With no constraints, the flux distribution of a biological network may lie at any point in a solution space. When mass balance constraints imposed by the stoichiometric matrix S (label 1) and capacity constraints imposed by the lower and upper bounds (a_i and b_i) (label 2) are applied to a network, 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

Metabolic Network

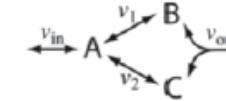


Thermodynamics (reversibility)



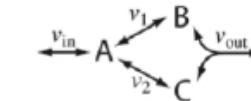
$$v_{out}, v_{in} \geq 0$$

Maximum enzyme capacity



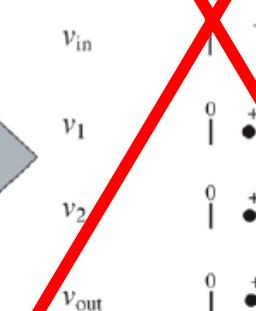
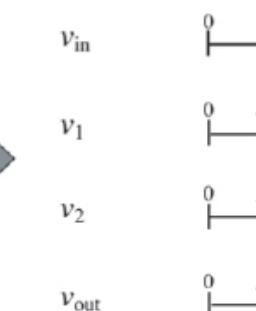
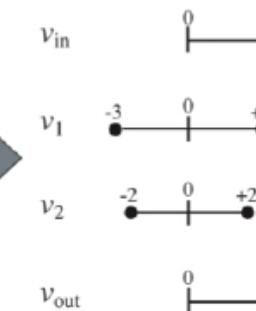
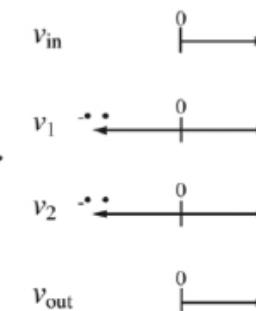
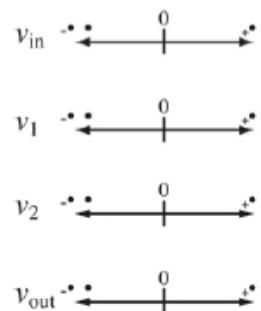
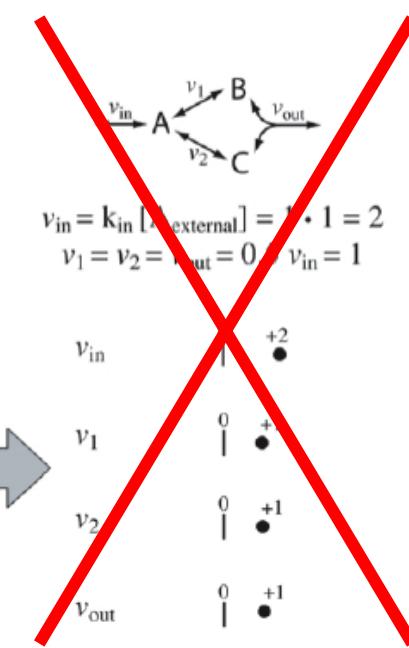
$$|v_1| \leq 3, |v_2| \leq 2$$

Mass balance of metabolites



$$\begin{aligned} v_{in} - v_1 - v_2 &= 0 \\ v_{out} - v_1 &= 0 = v_{out} - v_2 \end{aligned}$$

Kinetics





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

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

$$\alpha_j \leq v_j \leq \beta_j$$

where

\mathbf{x} = concentration vector

\mathbf{v} = flux vector

\mathbf{c} = objective function weights

\mathbf{S} = Stoichiometric matrix

α_j = Lower bound of flux

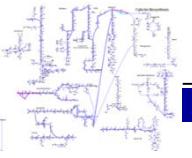
β_j = upper bound of flux

The goal is to create an 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

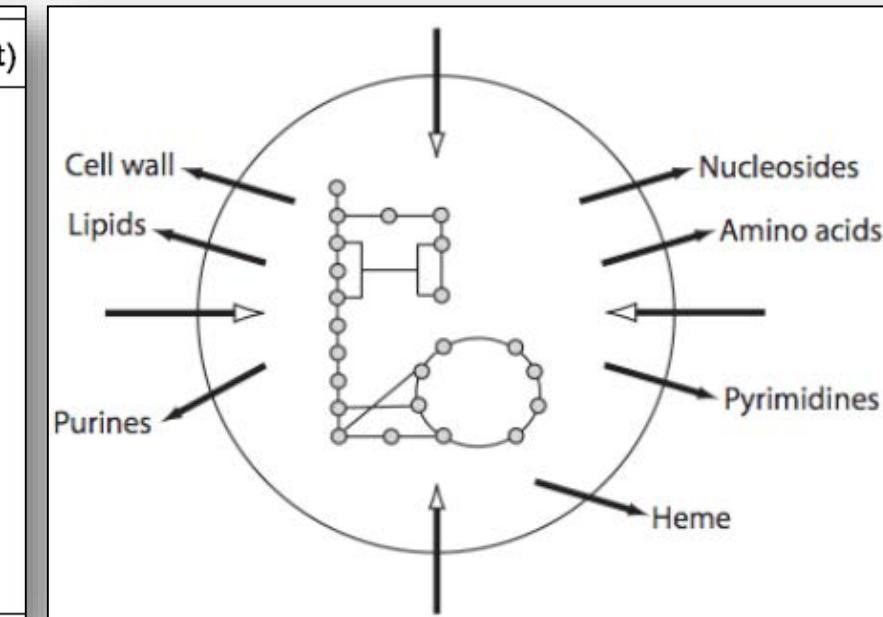
- Flux Balance Analysis Overview
- Reactions, Metabolites, & Pathways
- Mathematical Representation of Reactions & Constraints
- Mass Balanced Linear Equations
- • Biomass Reaction
- Calculating Fluxes
- Flux Balance Analysis Toolbox



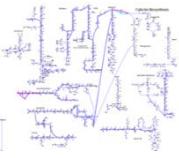
Biomass Precursors

- The biomass reaction accounts for all the fractional contributions from biosynthetic precursors and key cofactors to create 1g of biomass.
- These fractional 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.

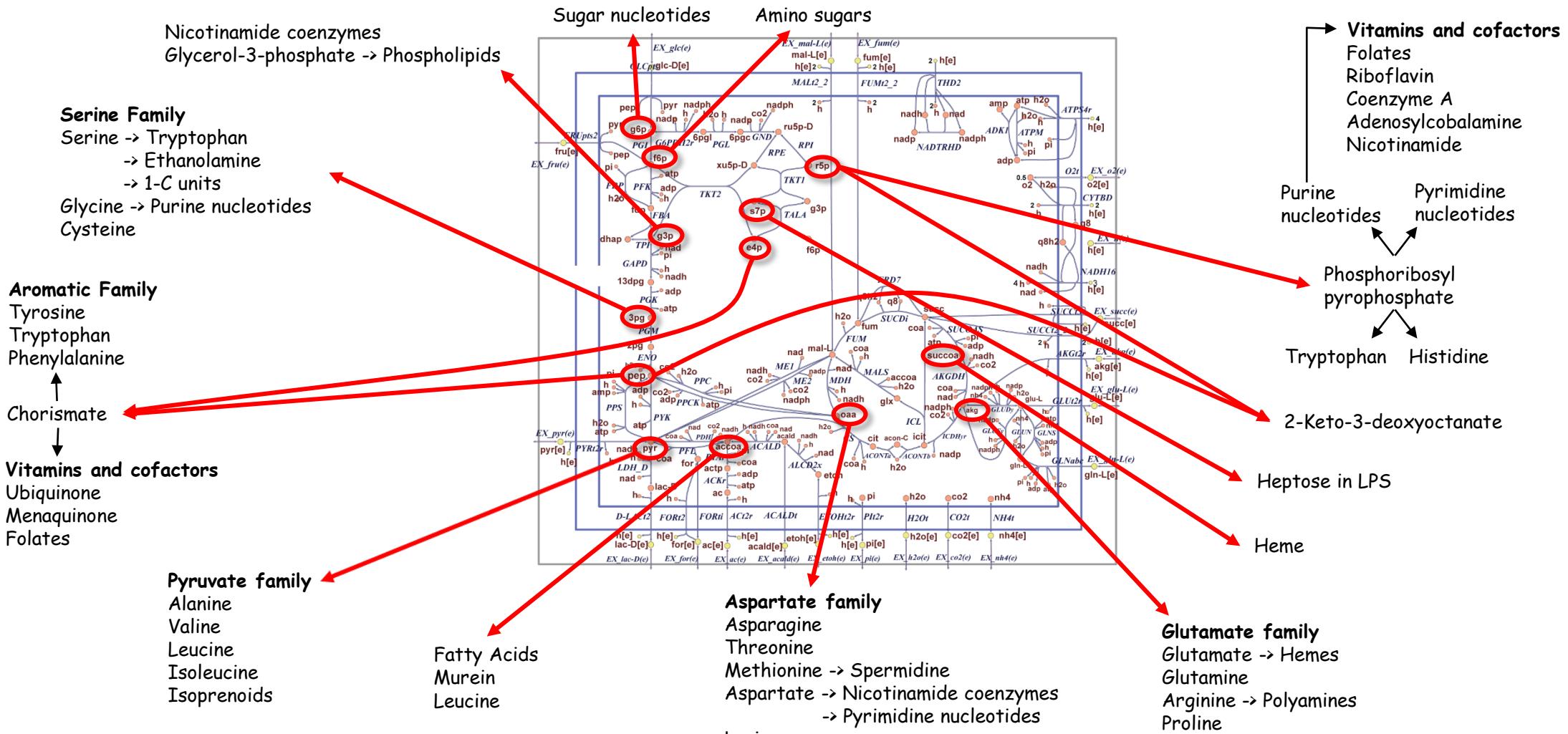
Cellular component	Cellular content % (wt/wt)
Protein	55
RNA	20.5
DNA	3.1
Lipids	9.1
Lipopolsaccharides	3.4
Peptidoglycan	2.5
Glycogen	2.5
Polyamines	0.4
Other	3.5
Total	100.00



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





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 non-growth 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

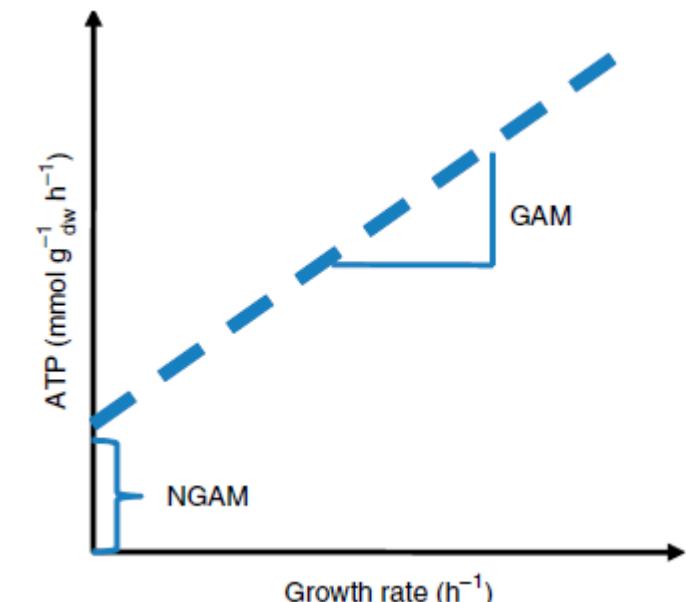


Where x is the number of required phosphate bonds (59.81 in core model). This will be included in the biomass reaction

- The NGAM reaction (ATPM) is given by



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



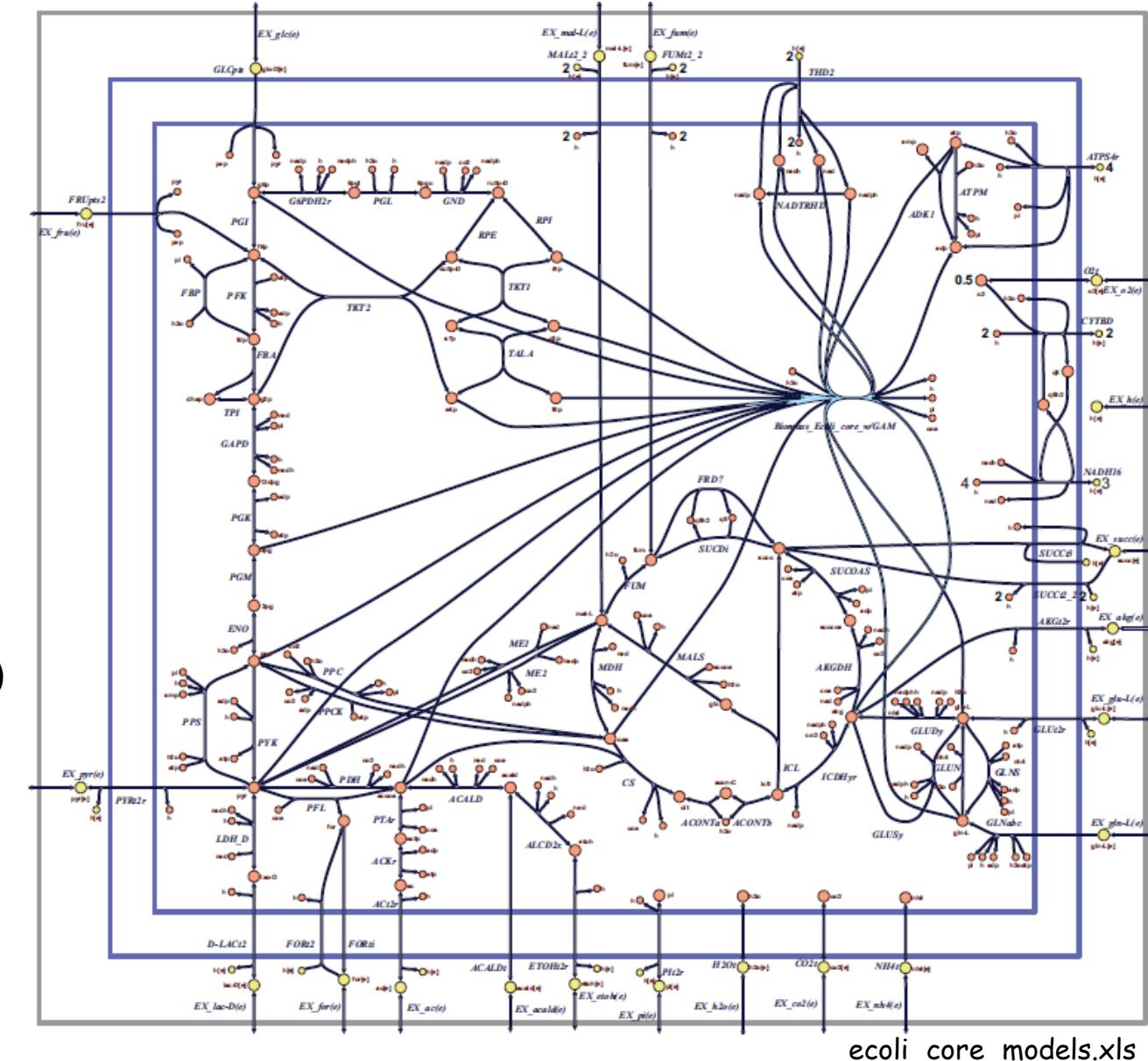
Thiele, I. and B. O. Palsson (2010). "A protocol for generating a high-quality genome-scale metabolic reconstruction." *Nature protocols* 5(1): 93-121.



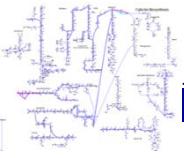
Biomass Reaction For *E.coli* Core Model

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

* Key Cofactors



ecoli_core_models.xls



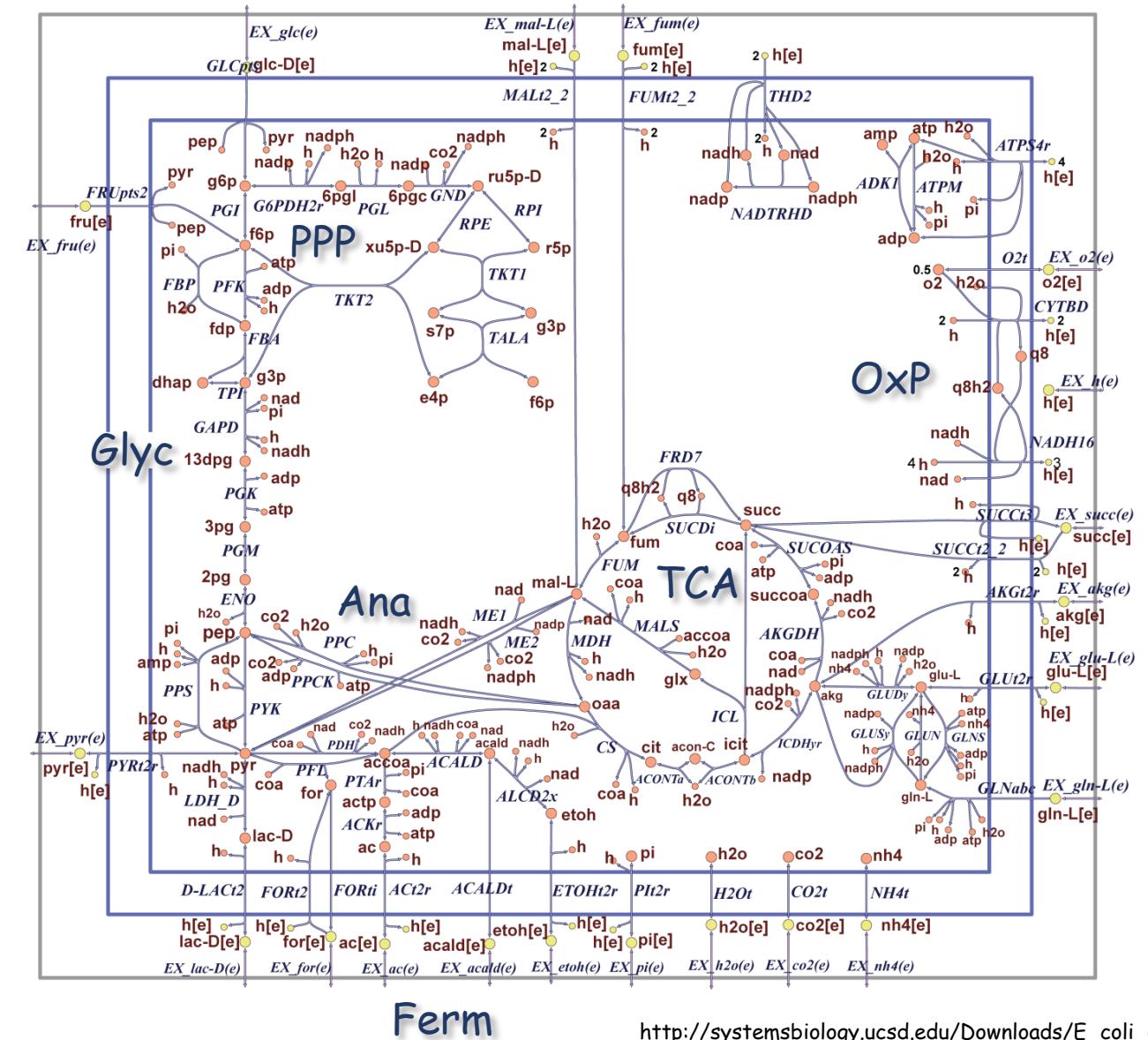
iaf1260 Biomass Objective Function (Ec_biomass_iAF1260_core_59p81M)

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



E.coli Core Model

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



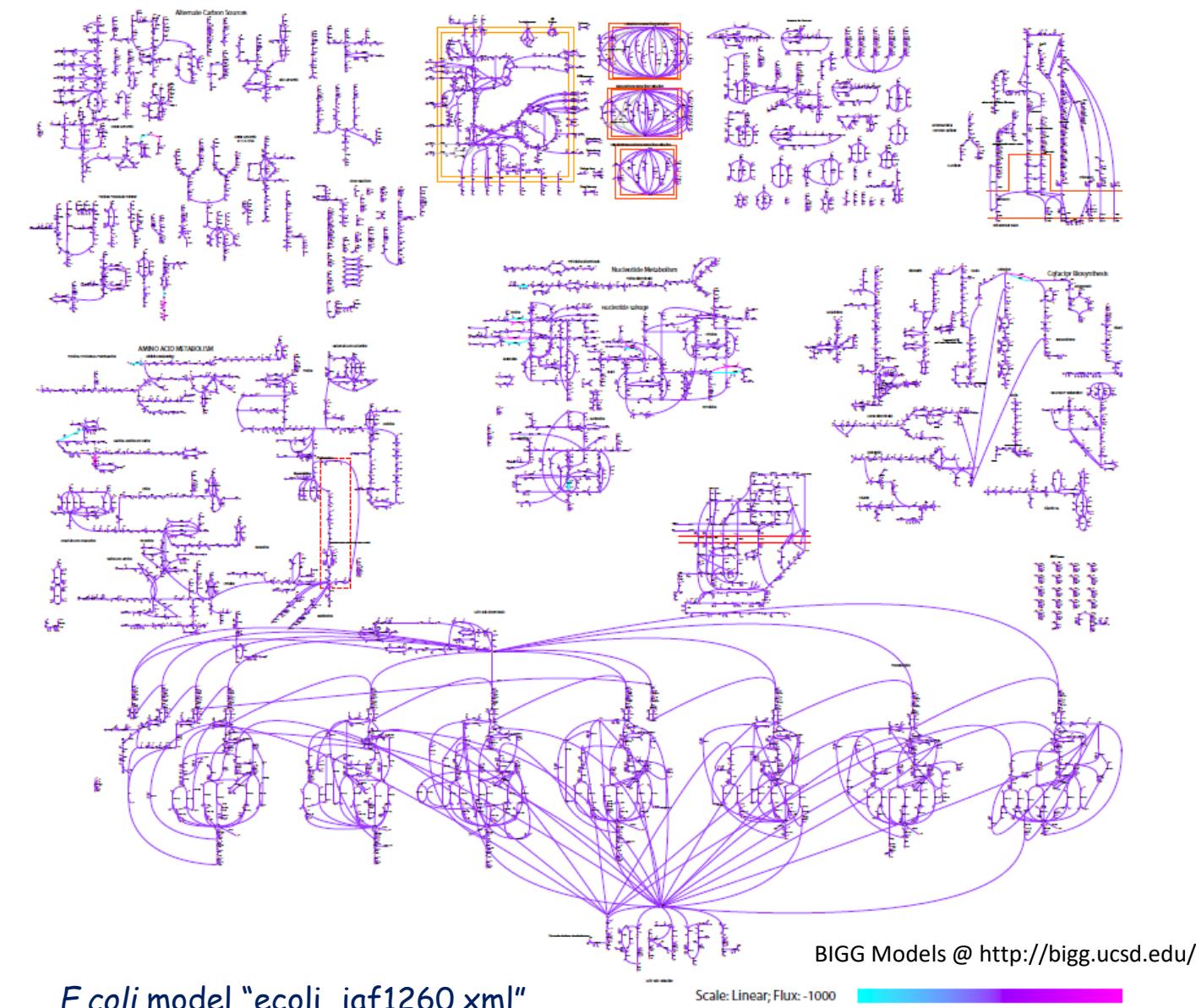
http://systemsbiology.ucsd.edu/Downloads/E_coli_Core



E. coli K-12 MG1655 Genome-Scale Reconstructions

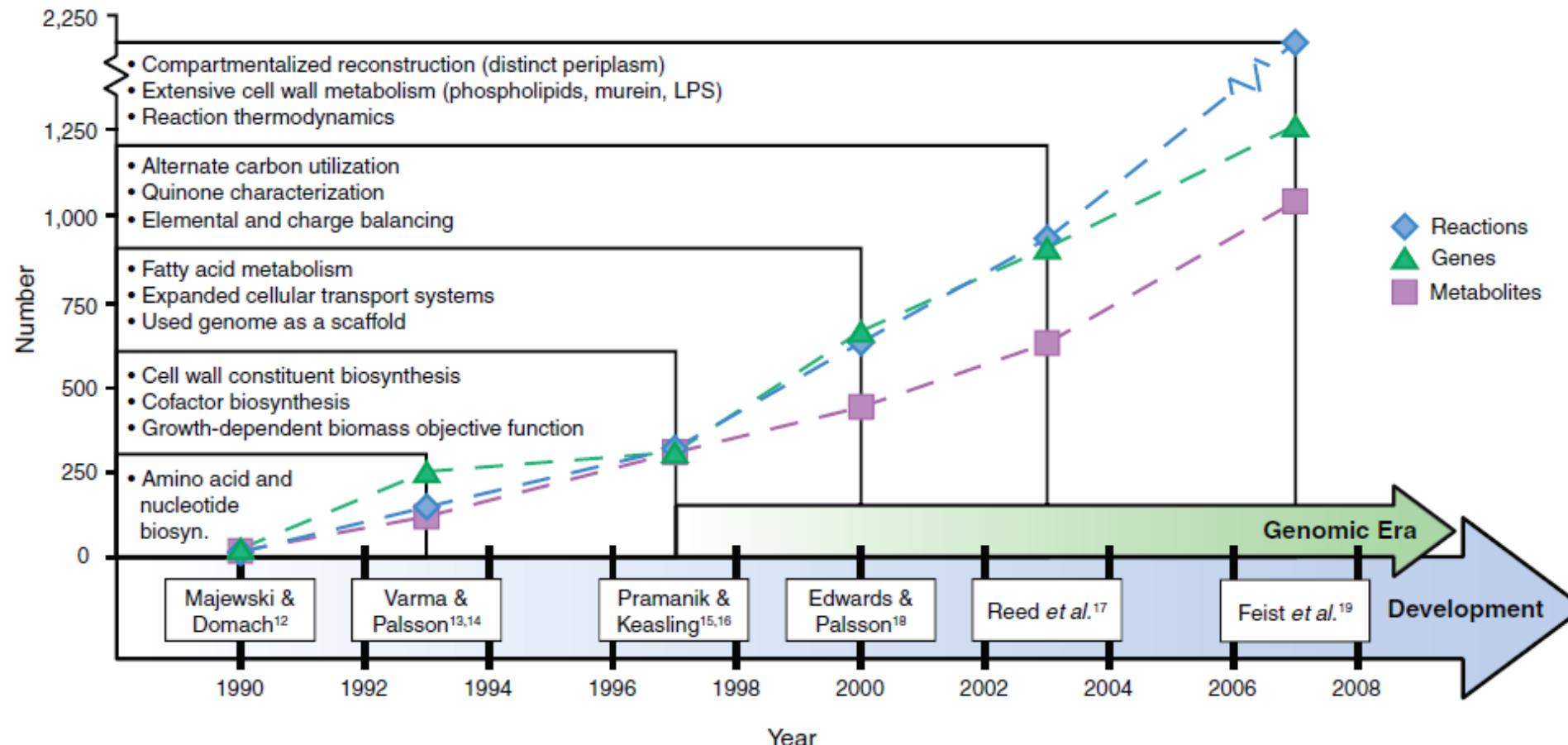
iAF1260 - Feist, A. M., C. S. Henry, et al. (2007).
"A genome-scale metabolic reconstruction for Escherichia coli K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information." Molecular Systems Biology 3: 121.

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.

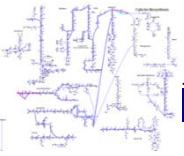




The Iterative Reconstruction and History of the *E. Coli* Metabolic Network



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.



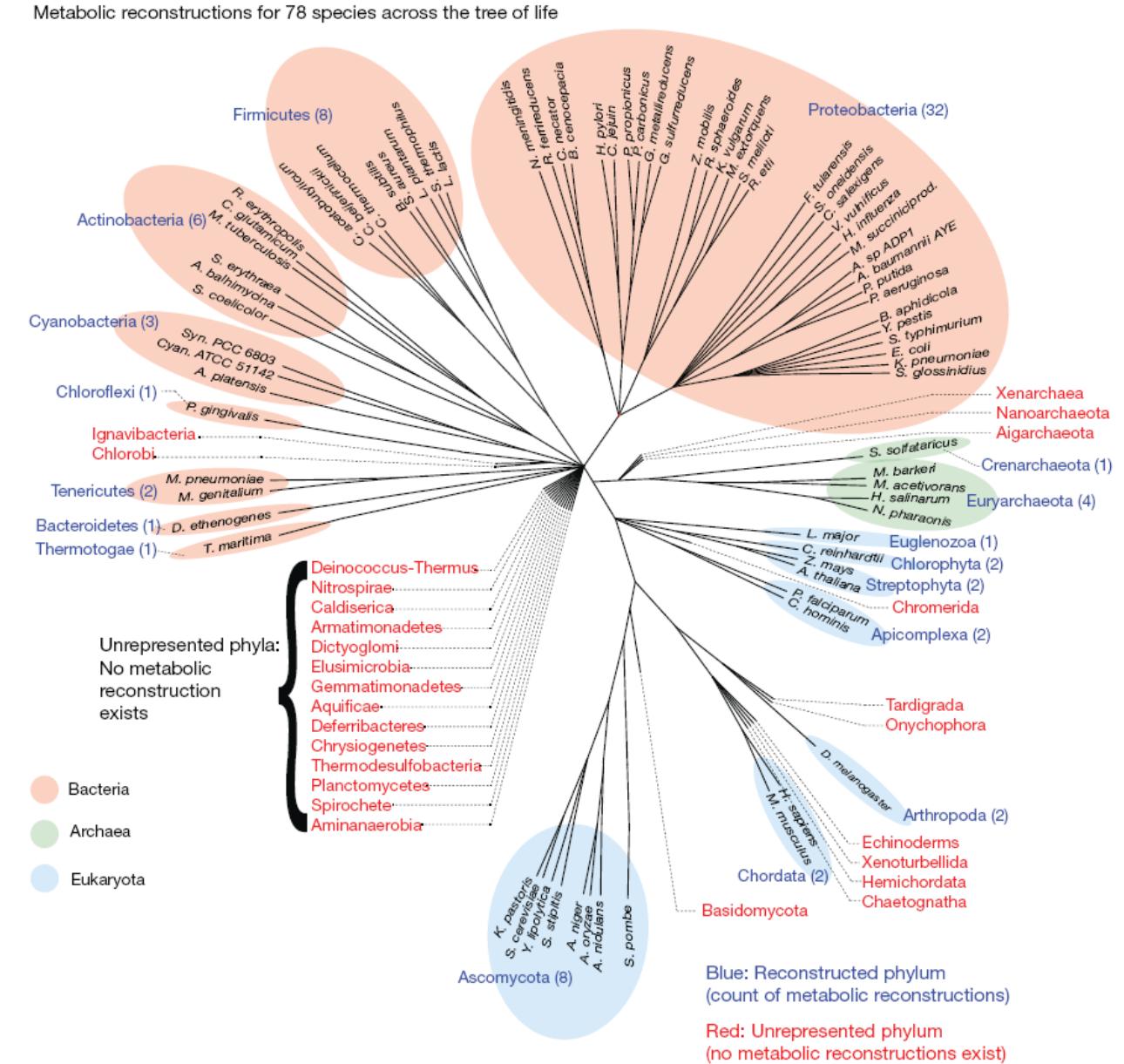
E. coli Genome-scale Reconstructions

- 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

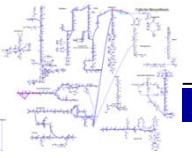
Monk, J. M., P. Charusanti, et al. (2013). Proceedings of the National Academy of Sciences of the United States of America 110(50): 20338-20343.



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

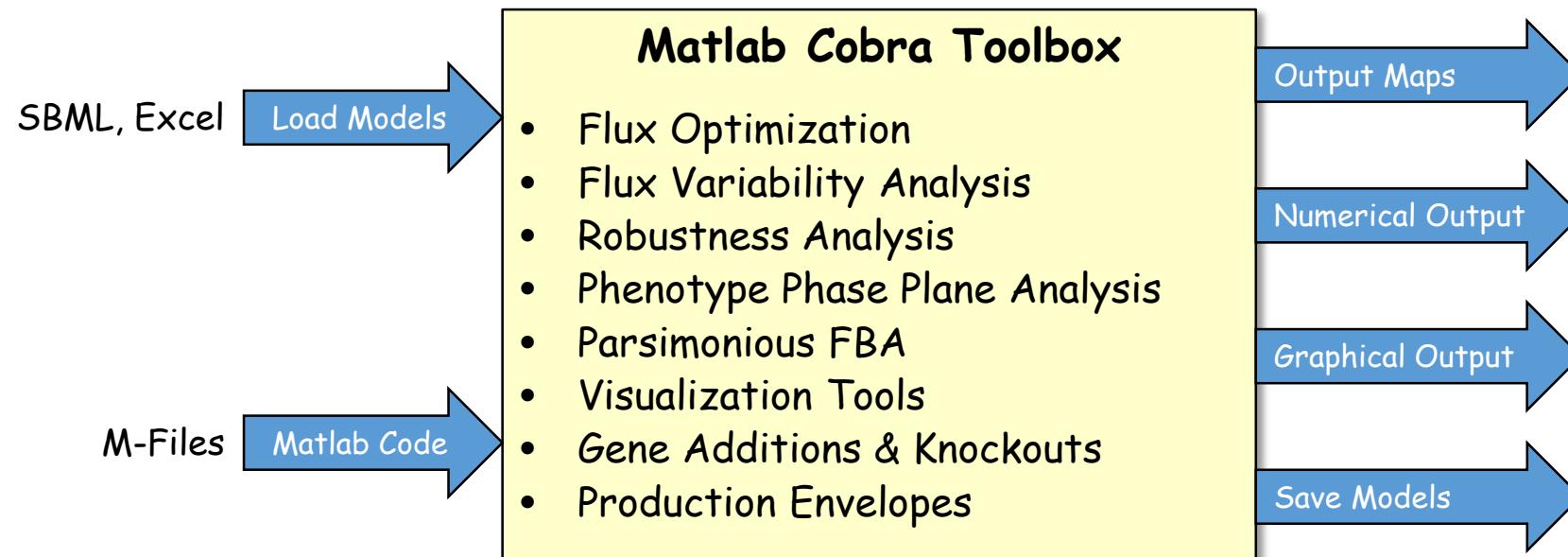


Flux Balance Analysis Overview

- Flux Balance Analysis Overview
- Reactions, Metabolites, & Pathways
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- Mass Balanced Linear Equations
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- • Calculating Fluxes
- Flux Balance Analysis Toolbox



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>



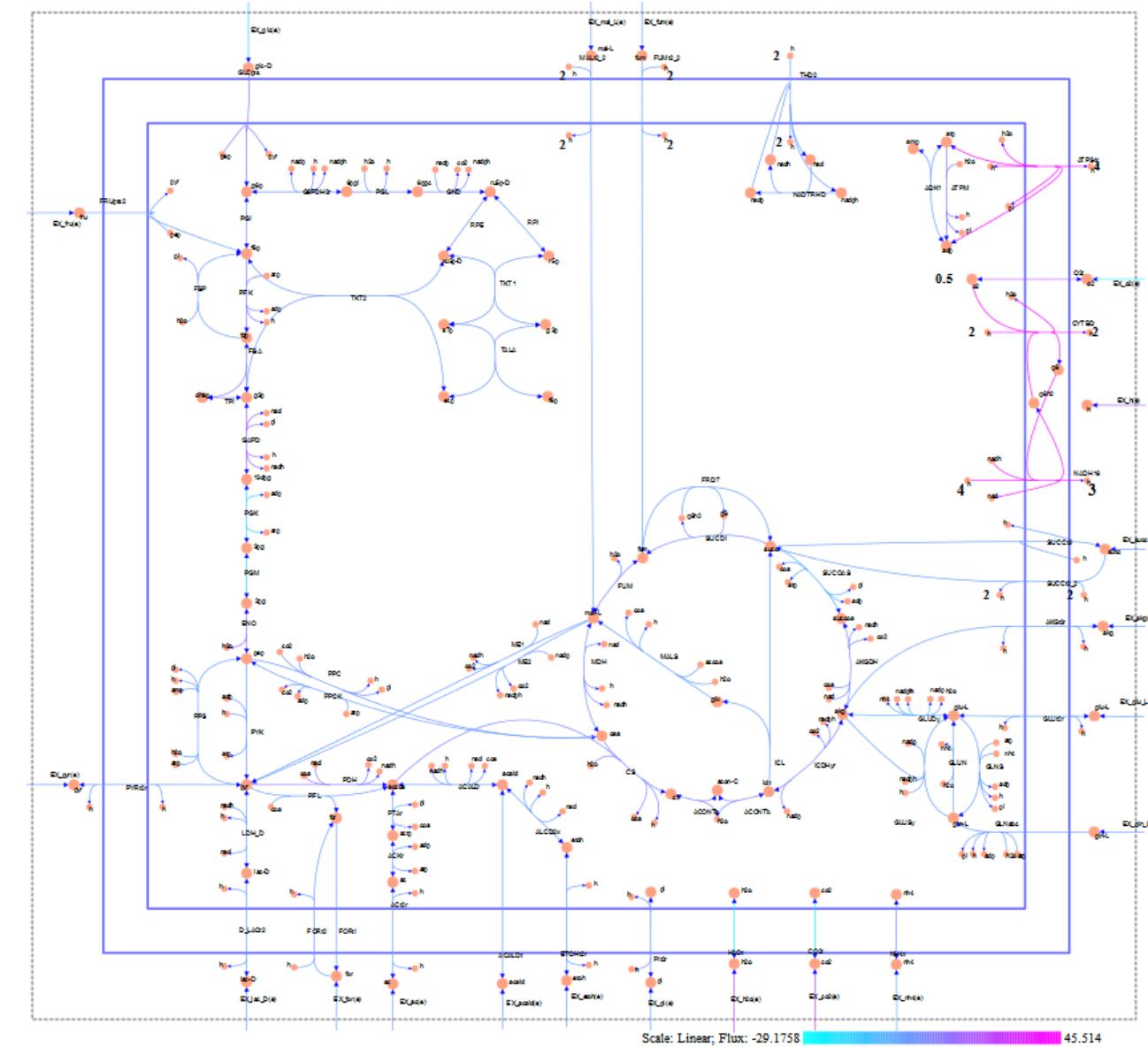
Matlab Interface

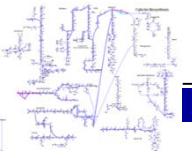
The screenshot shows the MATLAB 7.12.0 (R2011a) interface with the following windows open:

- Variable Editor - solution**: Displays a struct named "solution" with fields: x, f, y, w, stat, origStat, ab, solver, and time. The "x" field is a 95x1 double array ranging from -29.17... to 45.5140.
- Workspace**: Shows variables: ans, map, model, and solution.
- Command History**: Shows the command history for the session, including the execution of optimization scripts.
- Current Folder**: Shows the directory structure for the "Tutorial" folder, containing various files like Succinate.svg, target.svg, and test.ARCH_E.pdf.
- Script Editor**: Displays the script "AerobicGlucoseBioMass.m" which reads a constraint-based model and performs optimization.
- Command Window**: Shows the last few commands entered: `>> solution = optimizeCbModel(model, 'max');` and `f`.



Drawing Flux Values on a Map





Print Flux Values

ACONTa	6.00725	FBA	7.47738	PGK	-16.0235
ACONTb	6.00725	FUM	5.06438	PGL	4.95998
AKGDH	5.06438	G6PDH2r	4.95998	PGM	-14.7161
ATPM	8.39	GAPD	16.0235	PIt2r	3.2149
ATPS4r	45.514	GLCpts	10	PPC	2.50431
Biomass_...	0.873922	GLNS	0.223462	PYK	1.75818
CO2t	-22.8098	GLUDy	-4.54186	RPE	2.67848
CS	6.00725	GND	4.95998	RPI	-2.2815
CYTBD	43.599	H2Ot	-29.1758	SUCDi	5.06438
ENO	14.7161	ICDHyr	6.00725	SUCOAS	-5.06438
EX_co2(e)	22.8098	MDH	5.06438	TALA	1.49698
EX_glc(e)	-10	NADH16	38.5346	TKT1	1.49698
EX_h2o(e)	29.1758	NH4t	4.76532	TKT2	1.1815
EX_h(e)	17.5309	O2t	21.7995	TPI	7.47738
EX_nh4(e)	-4.76532	PDH	9.28253		
EX_o2(e)	-21.7995	PFK	7.47738		
EX_pi(e)	-3.2149	PGI	4.86086		

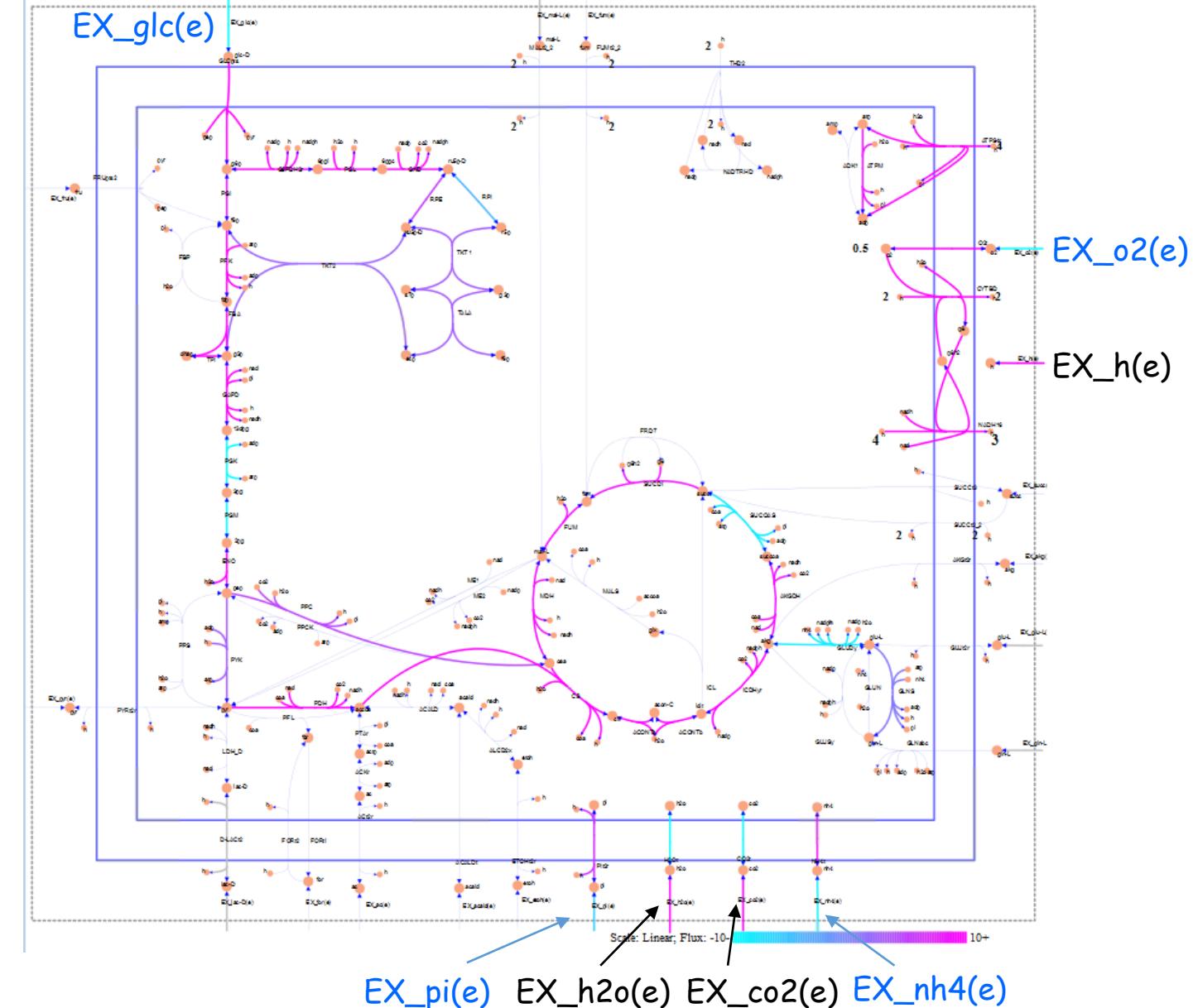
Growth Rate
**Inputs & Outputs
(Exchange Reactions)**



Aerobic Growth on Glucose

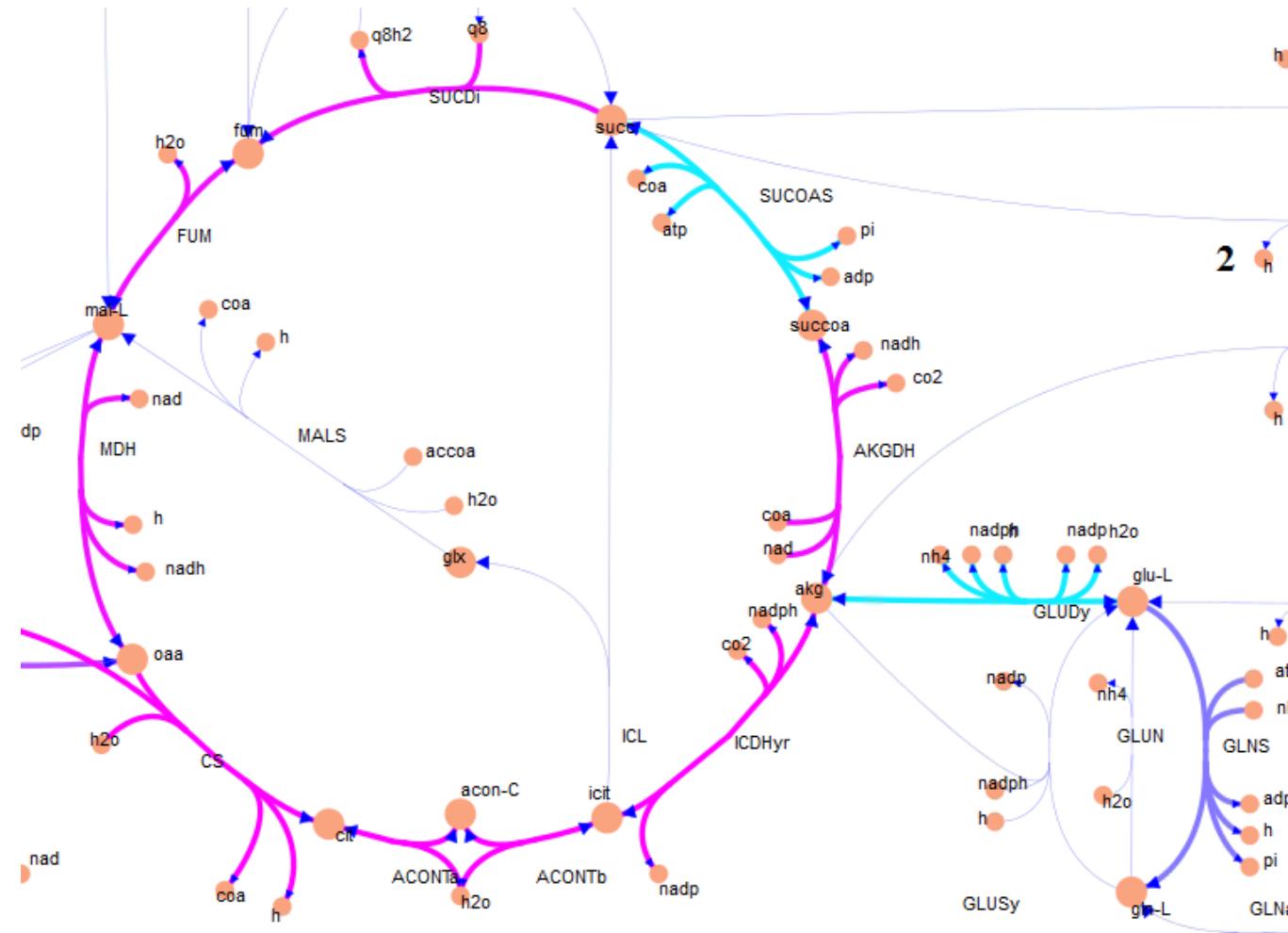
Exchange Reactions

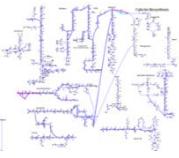
EX_co2(e)	40.6527
EX_glc(e)	-18.5
EX_h2o(e)	52.6943
EX_h(e)	33.1606
EX_nh4(e)	-9.01387
EX_o2(e)	-38.7416
EX_pi(e)	-6.08116





Close-up of TCA Cycle

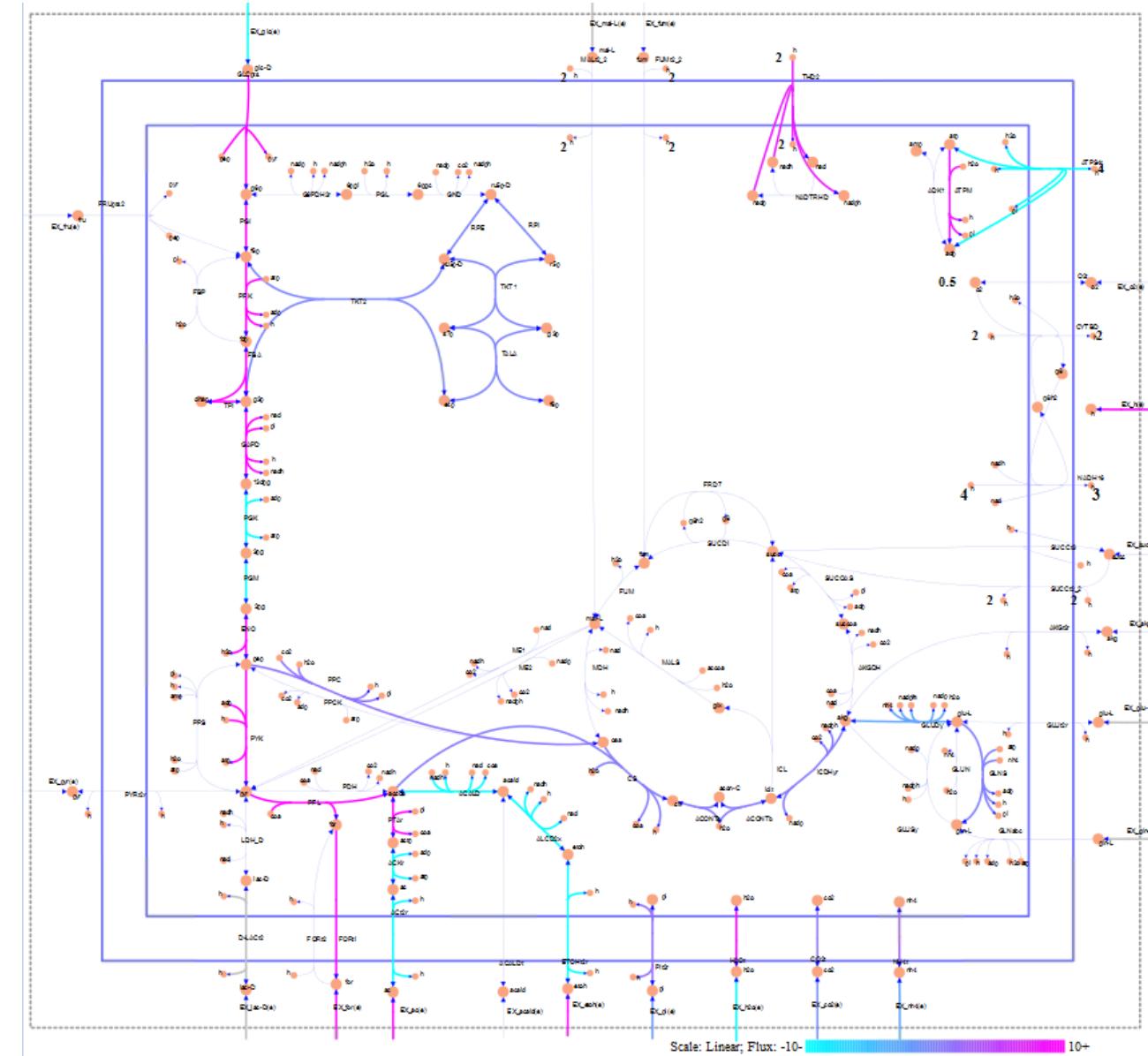




Anaerobic Growth on Glucose

Exchange Reactions

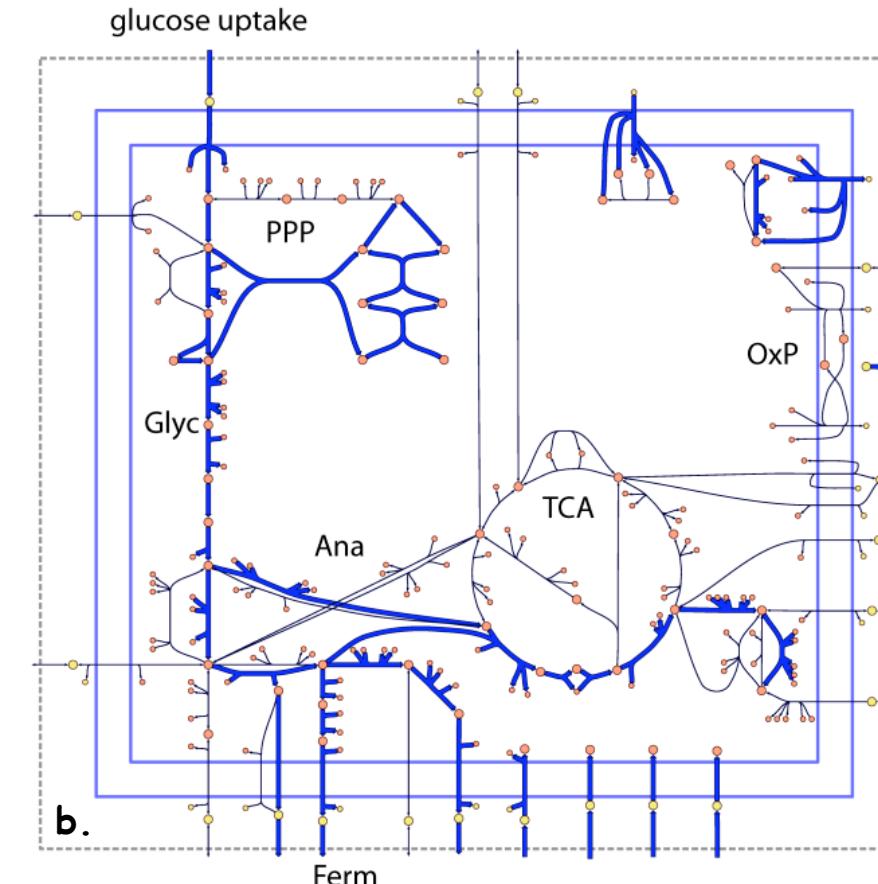
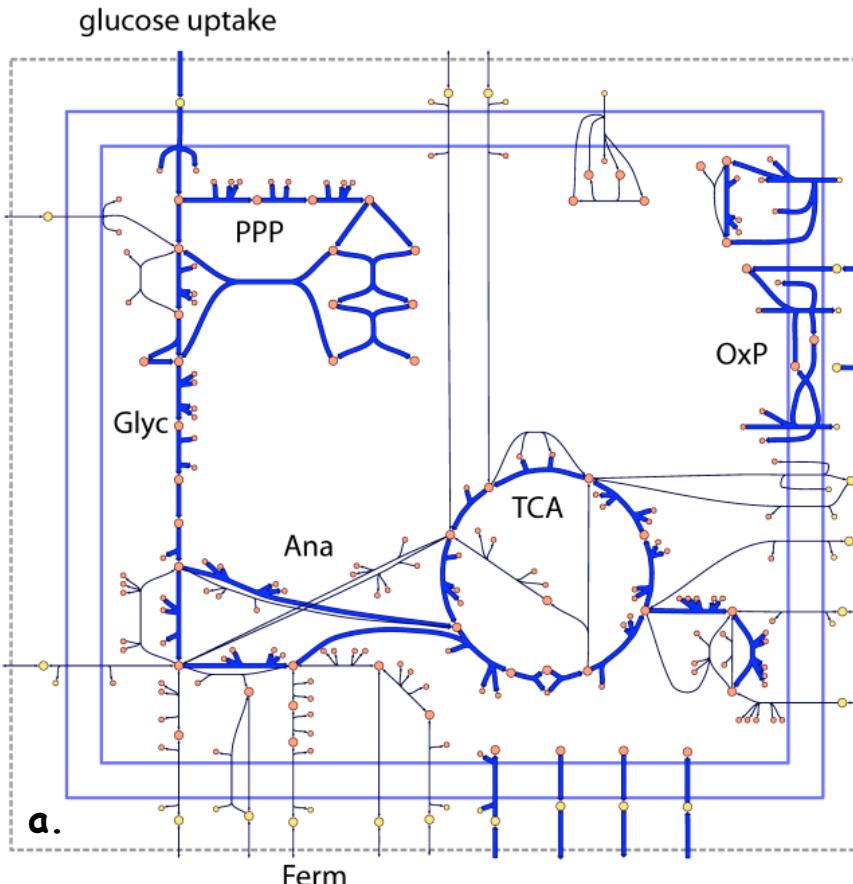
Biomass	0.470565
EX_ac(e)	15.1732
EX_co2(e)	-0.840759
EX_eto(h)	14.6749
EX_for(e)	32.1194
EX_glc(e)	-18.5
EX_h2o(e)	-12.0879
EX_h(e)	56.7321
EX_nh4(e)	-2.5659
EX_pi(e)	-1.73107





Aerobic vs. Anaerobic Growth

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



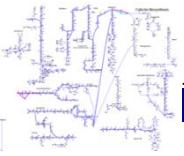


Substrate Maximum Growth Rate

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

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.

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



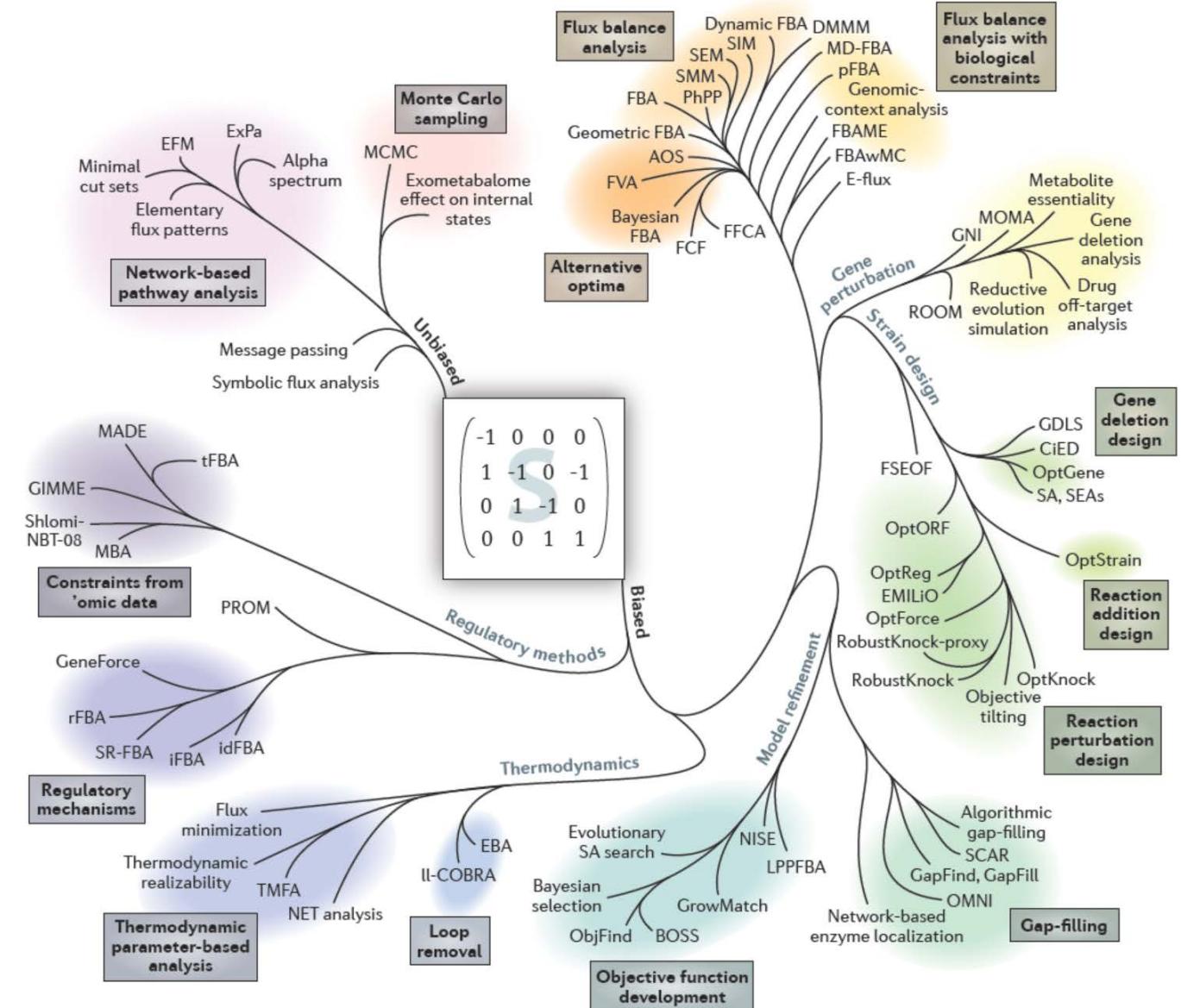
Flux Balance Analysis Overview

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



Methods in Constraint-based Reconstruction and Analysis

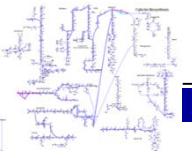
Lewis, N. E., H. Nagarajan, et al. (2012). "Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico methods." *Nature reviews Microbiology* 10(4): 291-305.





Flux Balance Analysis Overview

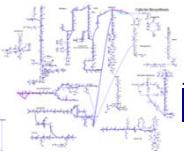
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Learning Objectives

Each student should be able to:

- Explain flux balance analysis (FBA).
- Explain reactions, metabolites, & pathways.
- 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.



Reflective Questions

1. What is flux balance analysis?
2. What does steady state mean in flux balance analysis (FBA)?
3. What is the difference between a reaction, a metabolite, and a pathway?
4. What is a gene-protein-reaction (GPR)?
5. What is a gene locus?
6. What is the difference between a single enzyme, an isozymes, and a protein complex?
7. What is a reaction formula?
8. What is the difference between a metabolite neutral formula and a metabolite charged formula?
9. What is a metabolite compartment?
10. What is an exchange reaction?
11. What are the units of flux?
12. What is the mathematical sign for uptake and secretion?
13. What is the difference between extracellular environment and intracellular space?
14. What are transport reactions?
15. Are the rows of a stoichiometric matrix metabolites or reactions?
16. Why is the product of the stoichiometric matrix and the flux set to zero?
17. What is the purpose of linear programming?
18. What is the purpose on an objective function?
19. What are biomass precursors?
20. What is the purpose of growth associated maintenance (GAM)?
21. What is the purpose of non-growth associated maintenance energy (NGAM)?
22. What is the purpose of the biomass reaction?
23. What are genome-scale metabolic network reconstruction?
24. What phase of growth does FBA assume (lag, exponential, stationary, death)?
25. What are the limits of FBA?
26. What role does the stoichiometric matrix play in FBA?
27. Why are visualization tools needed?