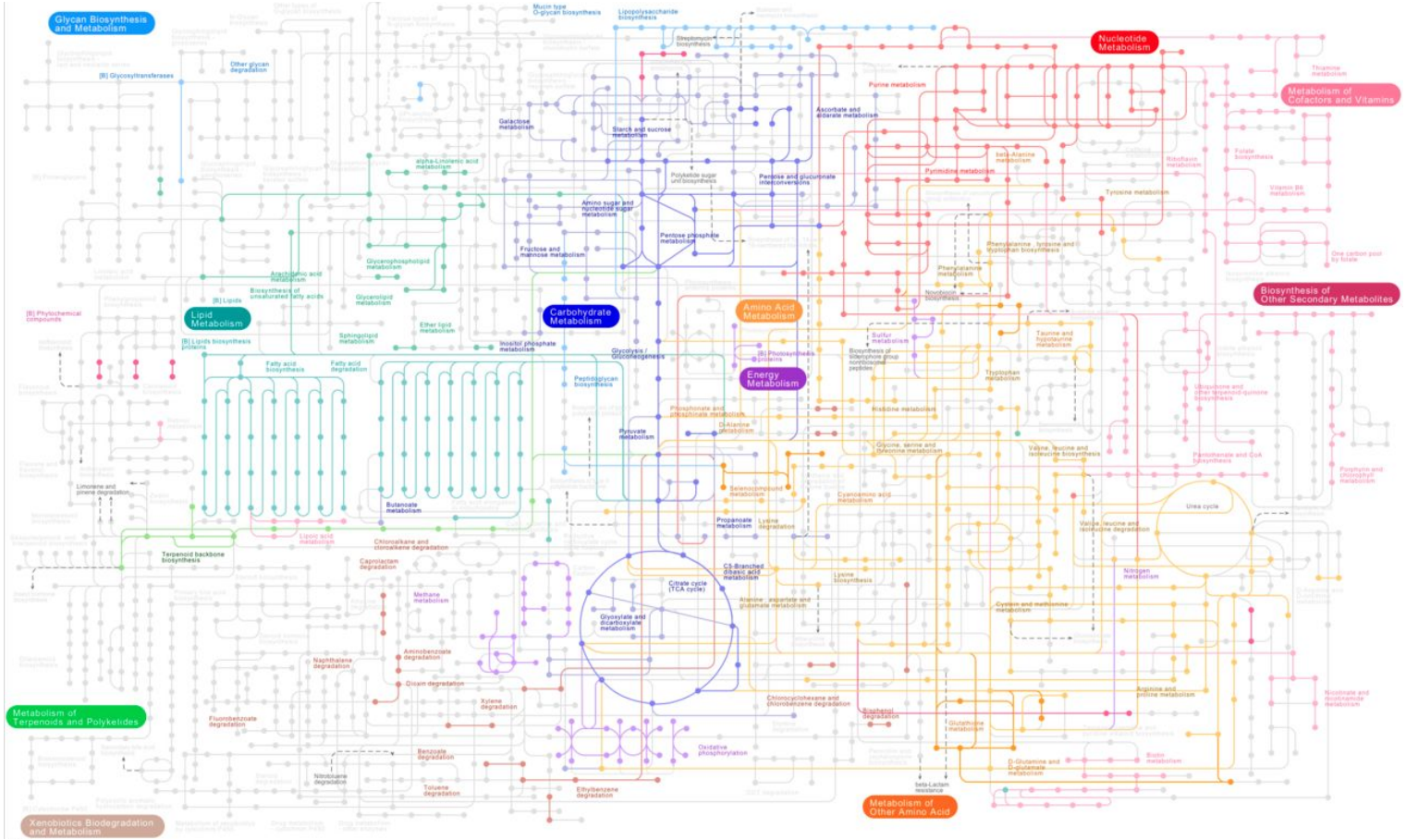
Lecture 13: Metabolomics

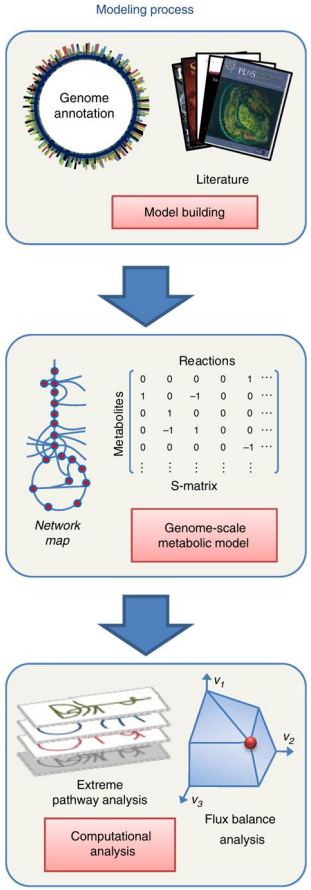
- Genome-scale metabolic models
- Flux balance analysis

Metabolic networks



KEGG

Genome-scale metabolic network reconstruction & model



Genome-scale metabolic network **reconstruction**:

- A collection of biochemical transformation derived from the genome annotation and the literature of a particular organism.
- Formed based on an organism-specific knowledge base.
- A network reconstruction is unique to an organism.

Genome-scale metabolic network **model**:

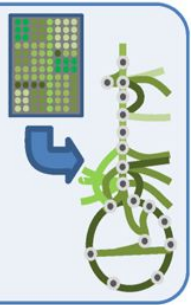
- Derived from a *reconstruction* by converting it into a mathematical form (i.e., an in silico model) and by assessing its phenotypic properties computationally.

Oberhardt (2009) Mol. Sys. biol.
Thiele & Palsson (2010) Nat. Protoc.

Genome-scale metabolic network reconstruction & model

1. Contextualization of HT data

Several studies have overlaid gene microarray data on a metabolic GENRE to determine condition-dependent cell phenotypes. Metabolic GENREs have also been used to interpret metabolomic data, 13C flux data, and to link multiple data types.

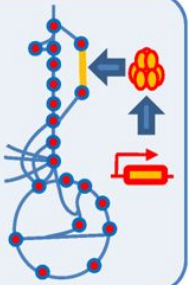


2. Guidance of metabolic engineering

Metabolic GENREs guided efforts to engineer malate and succinate producing strains of *S. cerevisiae* and *M. succiniciproducens*. GENREs have also helped determine ways to increase the respiration rate of *G. sulfurreducens* and scale-up vaccine production against *N. meningitides*.

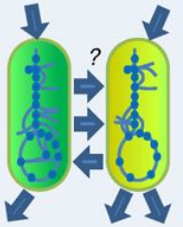
3. Directing hypothesis-driven discovery

A Metabolic GENRE aided in determining pathway usage and discovering a novel citramalate synthase gene in *G. sulfurreducens*. GENREs have also helped study the effects of transposons on downstream genes, and identify transc riptional timing patterns in *S. cerevisiae*.



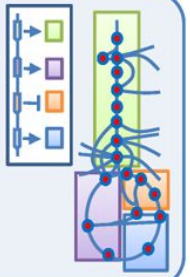
4. Interrogation of multi-species relationships

A dual-species metabolic model was built to study Interactions between the syntrophic bacteria, *D. vulgaris* and *M. maripaludis*. Metabolic models have also been used in comparisons of multiple species, such as an analysis of pathway differences between four halophilic bacteria.



5. Network property discovery

Metabolic GENREs have been used to study metabolite connectivity, and pathway redundancy *in silico*. Pathway-analysis tools have also spawned techniques such as flux coupling analysis, which has helped identify novel drug targets in *M. tuberculosis*.



Oberhardt (2009) Mol. Sys. biol.
Thiele & Palsson (2010) Nat. Protoc.

Constructing a genome-scale metabolic model

Draft construction

Genome databases

Comprehensive Microbial Resource (CMR)	http://cmr.jcvi.org/cgi-bin/CMR/CmrHomePage.cgi
Genomes OnLine Database (GOLD)	http://www.genomesonline.org/
TIGR	http://www.tigr.org/db.shtml
NCBI Entrez Gene	http://www.ncbi.nlm.nih.gov/sites/entrez
SEED database[32]	http://theseed.uchicago.edu/FIG/index.cgi

Biochemical databases

KEGG[41]	http://www.genome.jp/kegg/
BRENDA[42]	http://www.brenda-enzymes.info/
Transport DB[89]	http://www.membranetransport.org/
PubChem[86]	http://pubchem.ncbi.nlm.nih.gov/
Transport Classification Database (TCDB)	http://www.tcdb.org/
pK_a Plugin	http://www.chemaxon.com/product/pka.html
pK_a DB	http://www.acdlabs.com/products/phys_chem_lab/pka/

1. Draft reconstruction

1| Obtain genome annotation.
2| Identify candidate metabolic functions.
3| Obtain candidate metabolic reactions.
4| Assemble draft reconstruction.
5| Collect experimental data.

Organism-specific databases

Ecocyc[43]	http://ecocyc.org/
PyloriGene[37]	http://genolist.pasteur.fr/PyloriGene
Gene Cards	http://www.genecards.org/

Protein localization databases

PSORT[47]	http://www.psort.org/psortb/
PA-SUB[48]	http://www.cs.ualberta.ca/~bioinfo/PA/Sub/

Bio-numbers

CyberCell Database (CCDB)[88]	http://redpoll.pharmacy.ualberta.ca/CCDB/cgi-bin/STAT_NEW.cgi
B10NUMB3R5	http://bionumbers.hms.harvard.edu/

Thiele & Palsson (2010) Nat. Protoc.

Constructing a genome-scale metabolic model

Refinement of draft construction

2. Refinement of reconstruction

6| Determine and verify substrate and cofactor usage.
7| Obtain neutral formula for each metabolite.
8| Determine the charged formula.
9| Calculate reaction stoichiometry.
10| Determine reaction directionality.
11| Add information for gene and reaction localization.
12| Add subsystems information.
13| Verify gene–protein-reaction association.
14| Add metabolite identifier.
15| Determine and add confidence score.
16| Add references and notes.
17| Flag information from other organisms.
18| Repeat Steps 6 to 17 for all genes.
19| Add spontaneous reactions to the reconstruction.
20| Add extracellular and periplasmic transport reactions.
21| Add exchange reactions.
22| Add intracellular transport reactions.
23| Draw metabolic map (optional).
24–32| Determine biomass composition.
33| Add biomass reaction.
34| Add ATP-maintenance reaction (ATPM).
35| Add demand reactions.
36| Add sink reactions.
37| Determine growth medium requirements.

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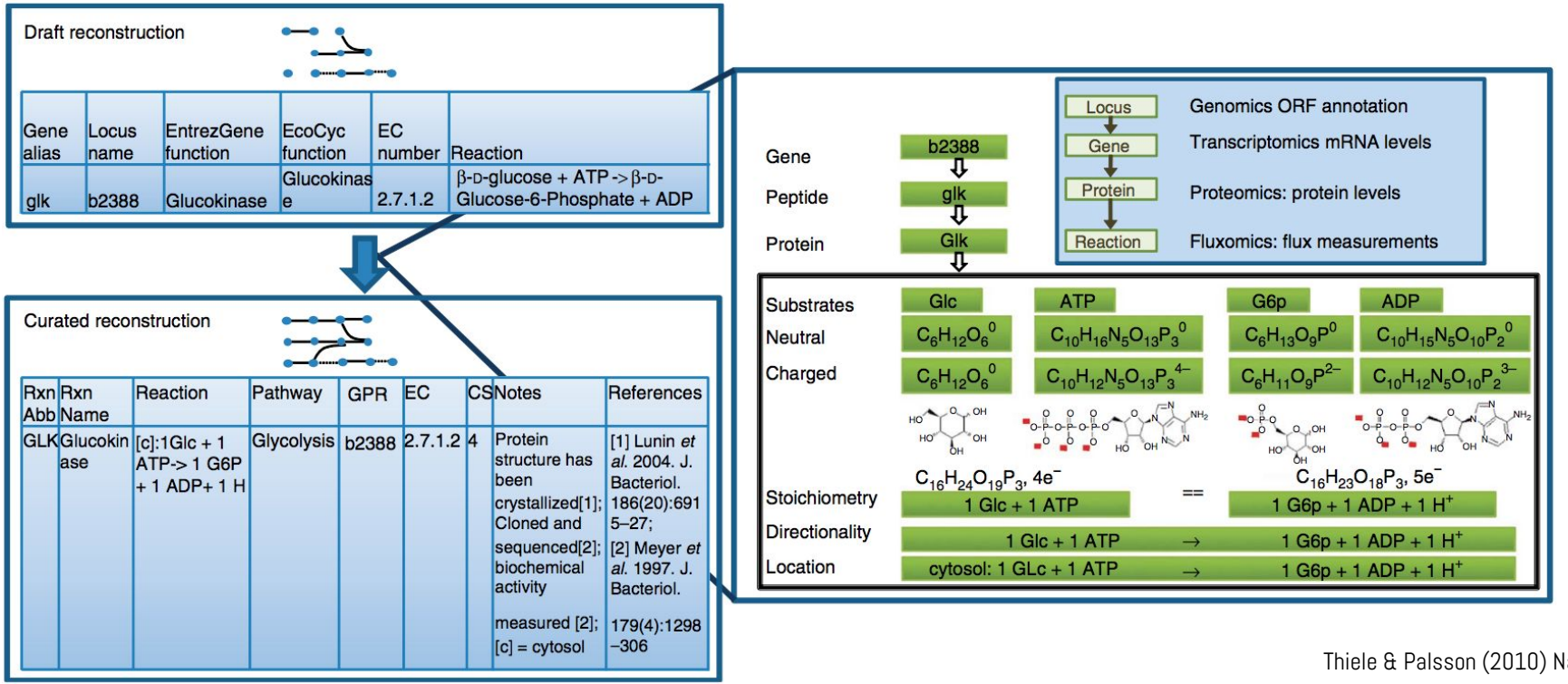
Constructing a genome-scale metabolic model

Refinement of draft construction

Mass & charge balancing; Filling-in H^+ & water; adjusting metabolites to a particular pH



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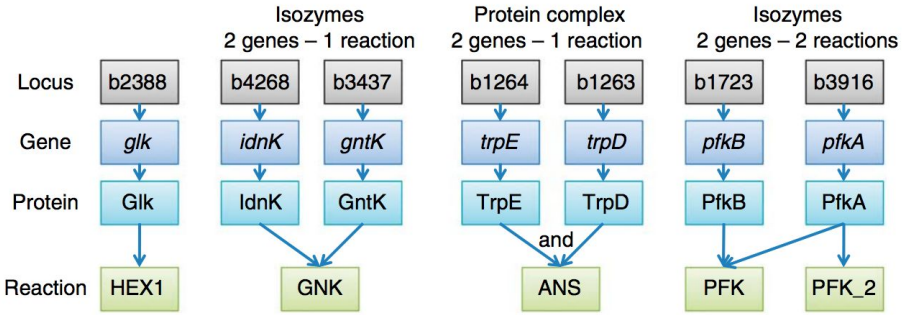
Constructing a genome-scale metabolic model

Refinement of draft construction

Subcellular localization

Compartment	Commonly used symbol[#]	Achaea	Bacteria	Eukaryotic pathogens[a]	Fungi[b]	Photosynthetic eukarya[c]	Baker's yeast	Human
Extracellular space	[e]	■	■		■	■	■	■
Periplasm	[p]		■					
Cytoplasm	[c]	■	■	■	■	■	■	■
Nucleus	[n]			■			■	■
Mitochondrion	[m]			■	■		■	■
Chloroplast	[h]					■		
Lysosome[*]	[l]							
Vacuole	[v]						■	■
Golgi apparatus	[g]						■	■
Endoplasmatic	[r]			■			■	■

Gene–protein-reaction associations



Reaction abbreviation	Reaction name	E. C.number	GPR
HEX1	Hexokinase (D-glucose:ATP)	2.7.1.1	(b2388)
GNK	Gluconokinase	2.7.1.12	(b3437) or (b4268)
ANS	Anthranilate synthase	4.1.3.27	(b1264) and (b1263)
PFK	Phosphofructokinase	2.7.1.11	(b1723) or (b3916)
PFK_2	Phosphofructokinase (2)	2.7.1.11	(b3916)

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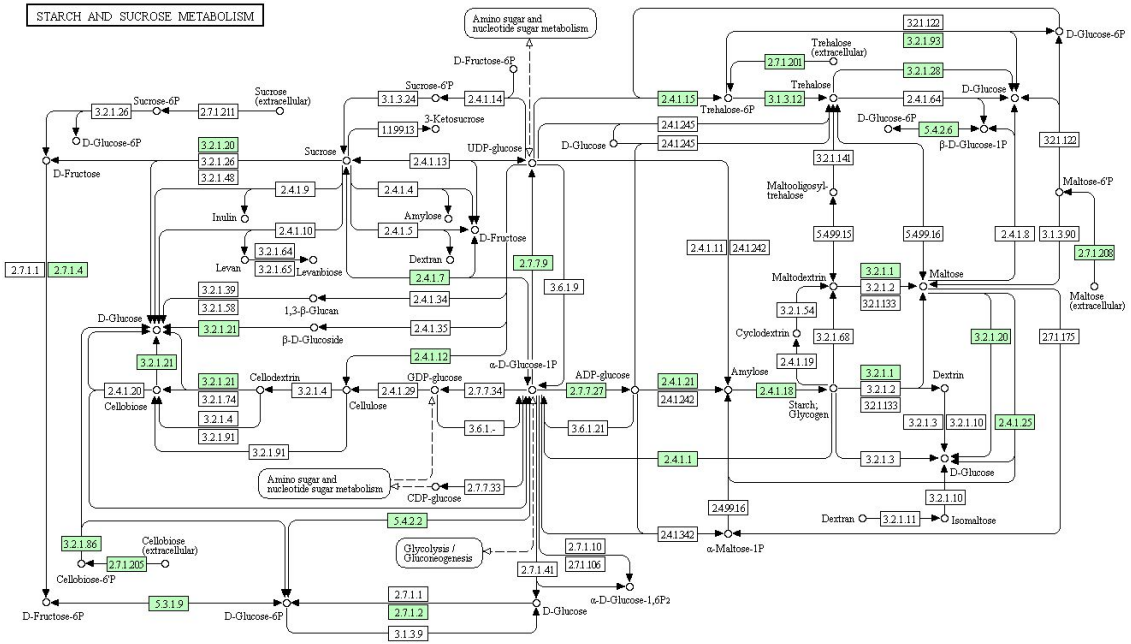
Constructing a genome-scale metabolic model

Refinement of draft construction

Chemical composition of a cell

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

Identification of missing functions



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Constructing a genome-scale metabolic model

Refinement of draft construction

Evidence type	Confidence score	Examples
Biochemical data	4	Direct evidence for gene product function and biochemical reaction: protein purification, biochemical assays, experimentally solved protein structures and comparative gene-expression studies (e.g., Chhabra *et al.*[95])
Genetic data	3	Direct and indirect evidence for gene function: knockout characterization, knock-in characterization and overexpression
Physiological data	2	Indirect evidence for biochemical reactions based on physiological data: secretion products or defined medium components serve as evidence for transport and metabolic reactions
Sequence data	2	Evidence for gene function: genome annotation and SEED annotation[32]
Modeling data	1	No evidence is available, but reaction is required for modeling. The included function is a hypothesis and needs experimental verification. The reaction mechanism may be different from the included reaction(s)
Not evaluated	0	

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Constructing a genome-scale metabolic model

Conversion of reconstruction into a model

3. Conversion of reconstruction into computable format

38| Initialize the COBRA toolbox.
39| Load reconstruction into Matlab.
40| Verify S matrix.
41| Set objective function.
42| Set simulation constraints.

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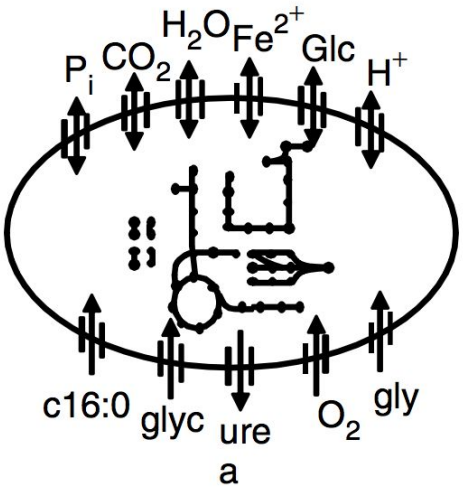
Constructing a genome-scale metabolic model

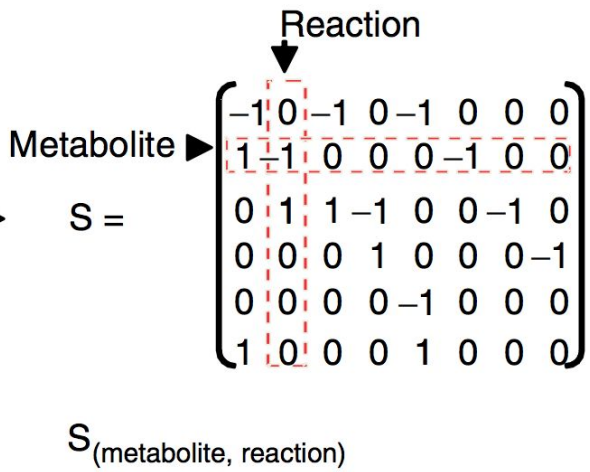
Conversion of reconstruction into a model

Mathematical representation

Metabolic network



Stoichiometric matrix



$S_{(metabolite, reaction)}$

By definition:

- Substrates have negative coefficients (i.e., they are consumed)
- Products have positive coefficients (i.e., they are produced)

v is a vector of reaction fluxes

Conservation of mass: All steady states can be described by **Sv** = 0

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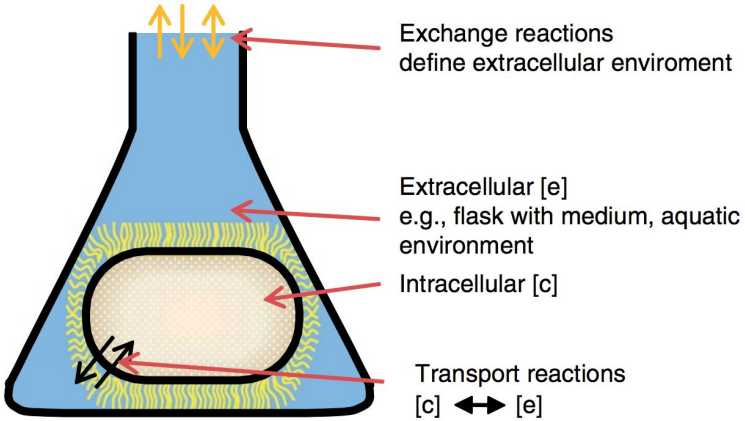
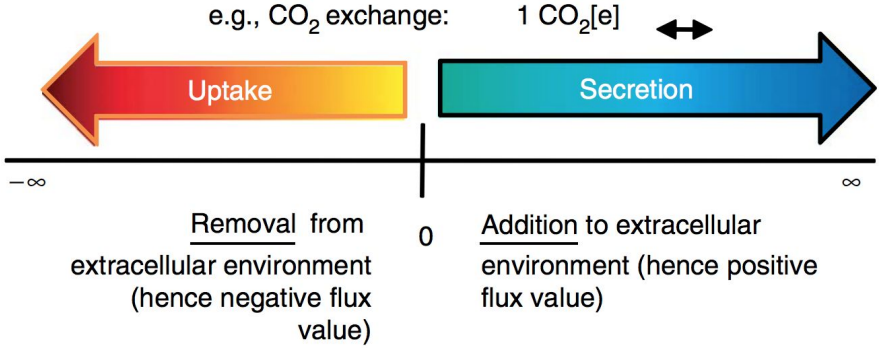
Constructing a genome-scale metabolic model

Conversion of reconstruction into a model

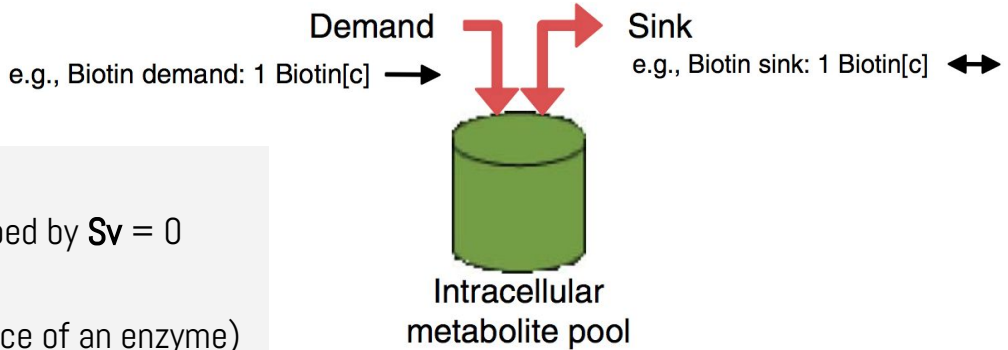
Definition of system boundaries



Exchange reactions



Demand/sink reactions



Constraints:

- Mass conservation: all steady states can be described by **Sv** = 0
- Thermodynamics (reaction directionality)
- Enzyme capacity or regulation (i.e., presence/absence of an enzyme)

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Constructing a genome-scale metabolic model

Network evaluation ≃ Debugging

4. Network evaluation

43–44| Test if network is mass-and charge balanced.
45| Identify metabolic dead-ends.
46–48| Perform gap analysis.
49| Add missing exchange reactions to model.
50| Set exchange constraints for a simulation condition.
51–58| Test for stoichiometrically balanced cycles.
59| Re-compute gap list.
60–65| Test if biomass precursors can be produced in standard medium.
66| Test if biomass precursors can be produced in other growth media.
67–75| Test if the model can produce known secretion products.
76–78| Check for blocked reactions.
79–80| Compute single gene deletion phenotypes.
81–82| Test for known incapabilites of the organism.
83| Compare predicted physiological properties with known properties.
84–87| Test if the model can grow fast enough.
88–94| Test if the model grows too fast.

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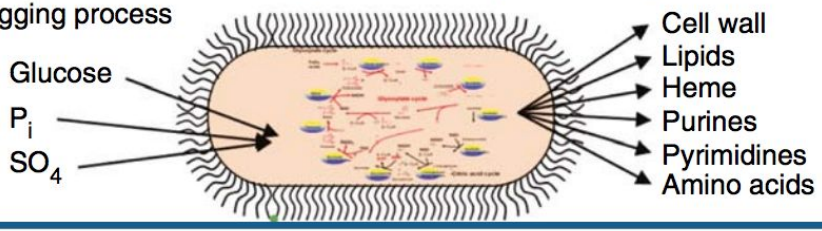
Constructing a genome-scale metabolic model

Network evaluation ≃ Debugging

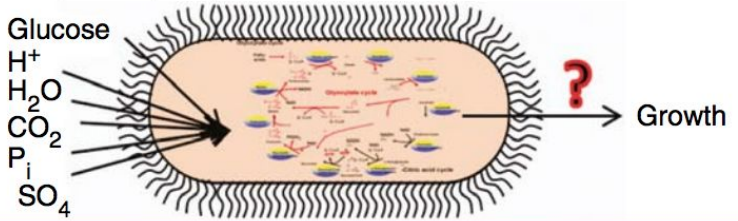
Analysis of biomass precursors synthesis

-Biomass precursors = cellular growth requirements
-Pathways to synthesize precursors must be complete (i.e., functional) for the network to simulate growth
-Testing synthesis of each separate biomass precursor is part of the debugging process



Analysis of growth in minimal medium

-Minimal medium is defined for many organisms and can be found in primary literature
-Contains at least 1 C-, N-, S- and P-source
-Auxotrophs may require the presence of addition metabolites



Test for growth on known carbon sources

-Exchange reactions define medium and environment
-Transport reactions allow network to consume carbon sources
-Biodegradative pathways that are required for carbon utilization

Secretion capability

-Transport and exchange reactions are required in reconstruction to enable secretion
-Secretion may only occur under certain circumstances (e.g., D-lactic acid formation under anoxic conditions)
-Comparison with known secretion pattern of multiple metabolites (e.g., secretion of a certain ratio of CO_2 and acetate)

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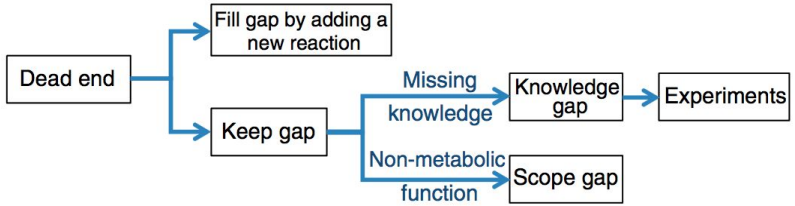
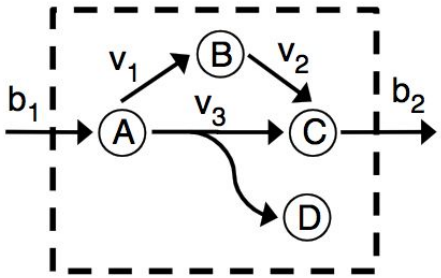
Constructing a genome-scale metabolic model

Network evaluation ≃ Debugging



Identifying gaps

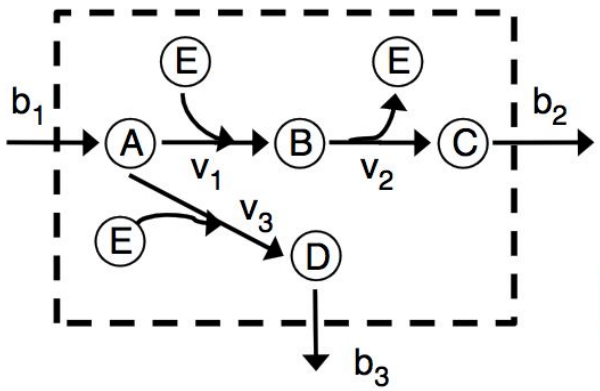
Connectivity based (topology):



	v_1	v_2	v_3	b_1	b_2
A	−1	0	−1	1	0
B	1	−1	0	0	0
C	0	1	1	0	−1
D	0	0	1	0	0

Dead end

Functionality based (computation)



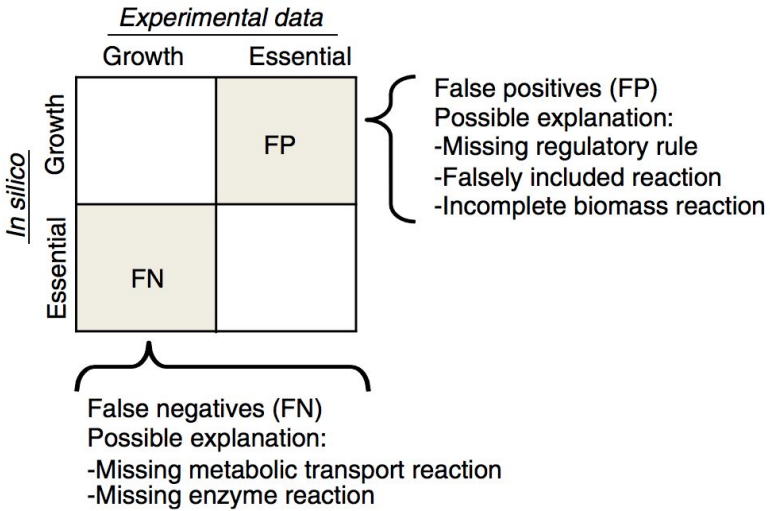
	v_1	v_2	v_3	b_1	b_2	b_3
A	−1	0	−1	1	0	0
B	1	−1	0	0	0	0
C	0	1	0	0	−1	0
D	0	0	1	0	0	−1
E	−1	1	−1	0	0	0

Thiele & Palsson (2010) Nat. Protoc.

Constructing a genome-scale metabolic model

Network evaluation ≃ Debugging

Gene essentiality



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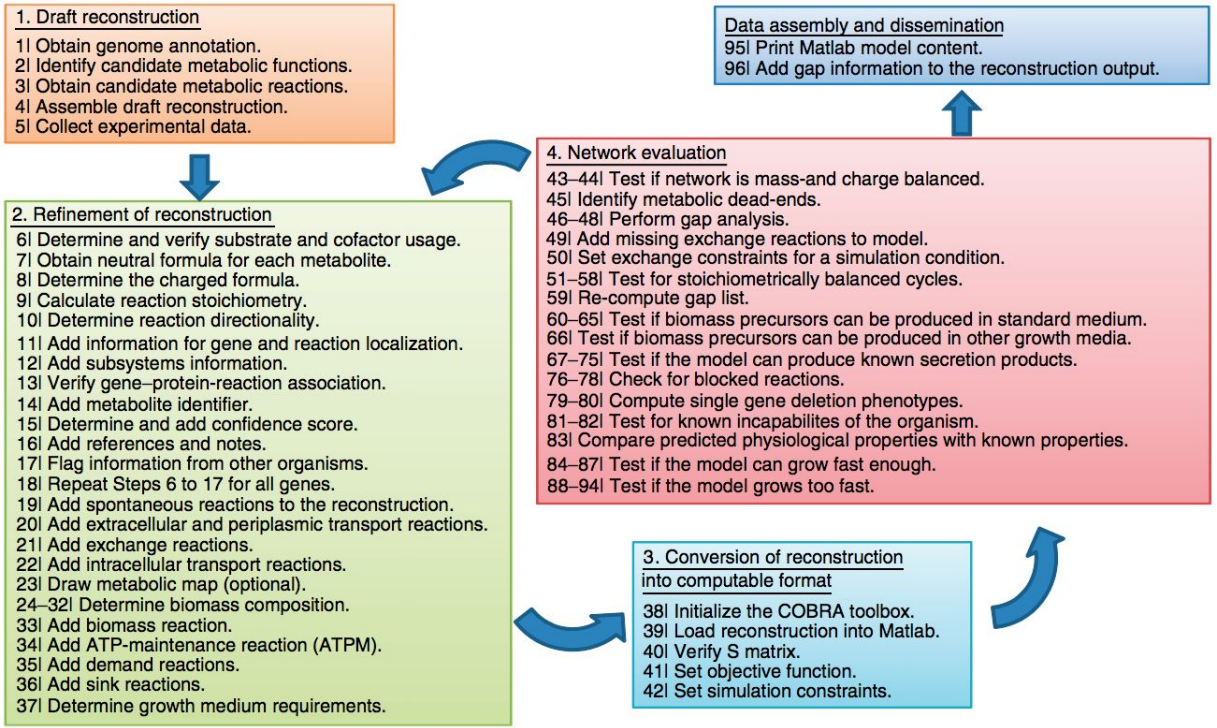
Constructing a genome-scale metabolic model

Procedure to iteratively reconstruct metabolic networks.

- Iterate stages 2–4 are continuously…
- …until model predictions are similar to the phenotypic characteristics of the target organism and/or all experimental data for comparison are exhausted.



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Constructing a genome-scale metabolic model

Organism	Strain	Genes	Version	GR	Mets	Rxns	Comp
Bacillus subtilis		4,225	model_v3	844	988	1,020	2 (c,e)
Escherichia coli	K12 MG1655	4,405	iAF1260	1,260	1,039	2,077	3 (c,e,p)
Helicobacter pylori	26695	1,632	iIT341	341	485	476	2 (c,e)
Pseudomonas putida	KT2440	5,350	iNJ746	746	911	950	3 (c,p,e)
Pseudomonas putida	KT2440	5,350	iJP815	815	886	877	2 (c,e)
Pseudomonas aeruginosa	PA01	5,640	iMO1056	1,056	760	883	2 (c,e)
Mycoplasma genitalium	G-37	521	iPS189	189	274	262	2 (c,e)
Lactobacillus plantarum	WCFS1	3,009		721	531	643	2 (c,e)
Streptomyces coelicolor	A3(2)	8,042		700	500	700	2 (c,e)
Leishmania major	Friedlin	8,370	iAC560	560	1,101	1,112	8 (a,f,y,c,e,m,r,n)
Saccharomyces cerevisiae	Sc288	6,183	iMM904	904	713	1,412	8 (c,e,m,x,n,r,v,g)
Homo sapiens		28,783	Recon 1	1,496	2,766	3,311	8 (c,e,m,x,n,r,v,g)

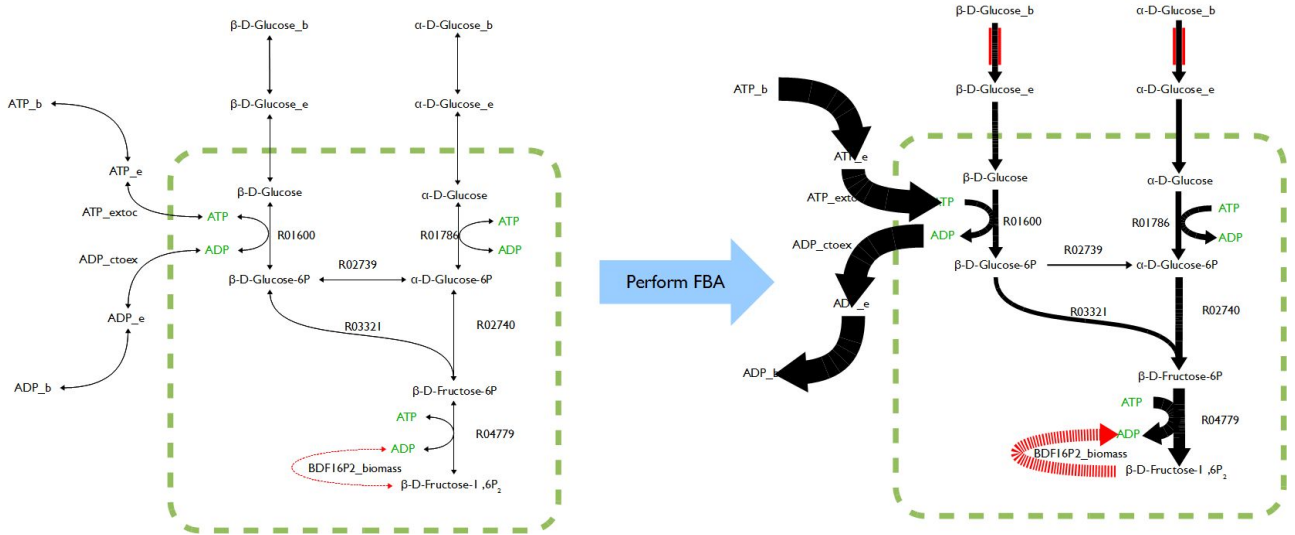
Thiele & Palsson (2010) Nat. Protoc.
http://gcrg.ucsd.edu/InSilicoOrganisms/OtherOrganisms

Flux balance analysis (FBA)

FBA is a formalism that defines the metabolic network as a linear programming optimization problem. The main constraints in FBA are imposed by the steady-state mass conservation of metabolites.

The results of FBA on a metabolic network of the top six reactions of glycolysis.

- The predicted flux through each reaction is proportional to the width of the line.
- **Red springy arrow**: Objective function; **Red bars**: Constraints on α-D-glucose and β-D-glucose import.

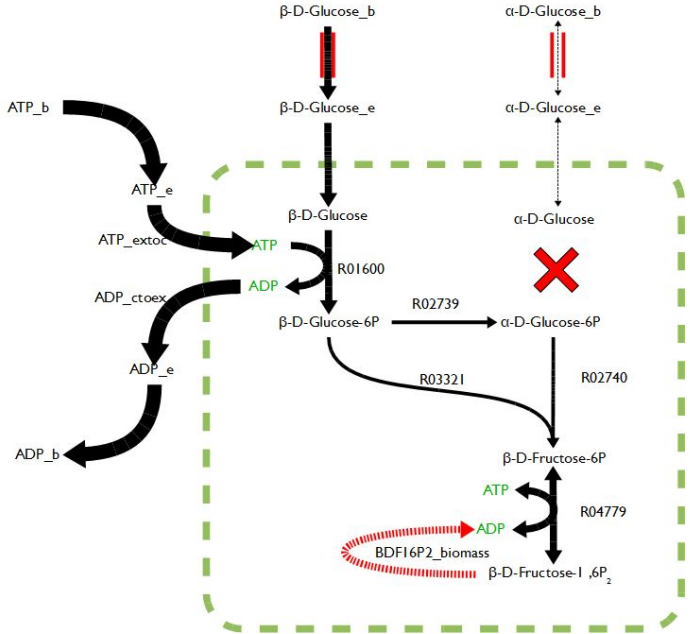


Lee (2006) Brief. Bioinfo.
Wikipedia

Flux balance analysis

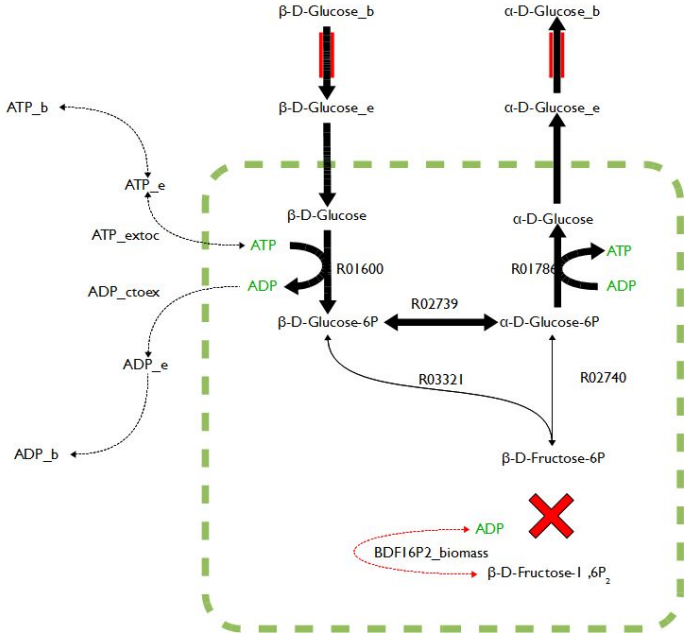
Non-lethal gene deletion in a metabolic network.

- Flux through the objective function is halved but is still present.



Lethal gene deletion in a metabolic network.

- No flux through the objective function → pathway is no longer functional.

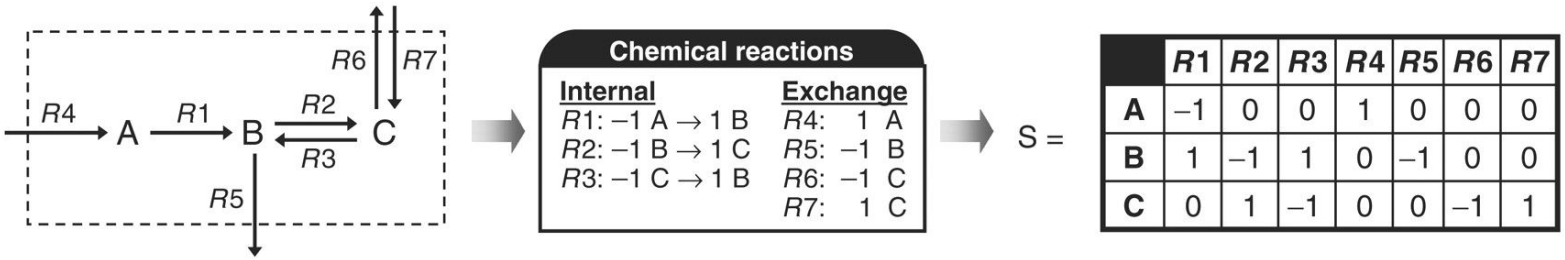


wikipedia

Flux balance analysis

FBA is a formalism that defines the metabolic network as a linear programming optimization problem. The main constraints in FBA are imposed by the steady-state mass conservation of metabolites.

I. Reaction network formalism



	R1	R2	R3	R4	R5	R6	R7
A	−1	0	0	1	0	0	0
B	1	−1	1	0	−1	0	0
C	0	1	−1	0	0	−1	1

Lee (2006) Brief. Bioinfo.

Flux balance analysis

FBA is a formalism that defines the metabolic network as a linear programming optimization problem. The main constraints in FBA are imposed by the steady-state mass conservation of metabolites.

II. FBA formulation

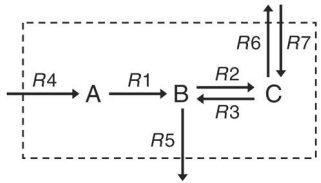
Dynamic mass balance

$$\frac{dC}{dt} = \mathbf{Sv}$$

C : Concentration
t : Time
$\mathbf{S}$: Stoichiometric matrix
$\boldsymbol{v}$: Flux vector

Steady-state assumption

$$\mathbf{Sv} = 0$$



LP formulation

Objective: max $Z = v_5$ $\quad Z = \mathbf{c} \cdot \mathbf{v}$

Constraints:

$$\begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccccccc} R1 & R2 & R3 & R4 & R5 & R6 & R7 \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 & 1 \end{bmatrix} \end{array} \begin{bmatrix} v_1 \\ \vdots \\ v_7 \end{bmatrix} = \mathbf{0}$$

$0 \le v_1, \ldots, v_7 \le 10$

- Principal constraint: mass balance.
- Additional constraints: e.g. reversibility of a reaction, energy requirement for cell maintenance.
- All constraints represent a set of linear equations.
  - No. of equations (one per reactant) << no. of unknown variables (reaction fluxes).
  - An under-determined set of linear equations.
- Therefore, optimize fluxes given cellular objective.

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Flux balance analysis

FBA is a formalism that defines the metabolic network as a linear programming optimization problem. The main constraints in FBA are imposed by the steady-state mass conservation of metabolites.

II. FBA formulation

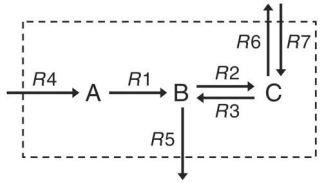
Dynamic mass balance

$$\frac{dC}{dt} = \mathbf{Sv}$$

C : Concentration
t : Time
$\mathbf{S}$: Stoichiometric matrix
$\boldsymbol{v}$: Flux vector

Steady-state assumption

$$\mathbf{Sv} = 0$$



LP formulation

Objective: max $Z = v_5$ $\quad Z = \mathbf{c} \cdot \mathbf{v}$

Constraints:

$$\begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccccccc} R1 & R2 & R3 & R4 & R5 & R6 & R7 \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 & 1 \end{bmatrix} \end{array} \begin{bmatrix} v_1 \\ \vdots \\ v_7 \end{bmatrix} = \mathbf{0}$$

Objective functions: physiologically meaningful objectives OR design objectives for the interrogation or exploitation of a given system.

- Maximizing biomass or cell growth, maximizing ATP production or maximizing the rate of synthesis of a particular product.
- Minimizing ATP production in order to determine conditions of optimal metabolic energy efficiency
- Minimizing nutrient uptake in order to evaluate the conditions under which a cell will perform its metabolic functions while consuming the minimum amount of nutrients.

$0 \le v_1, \ldots, v_7 \le 10$

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Flux balance analysis

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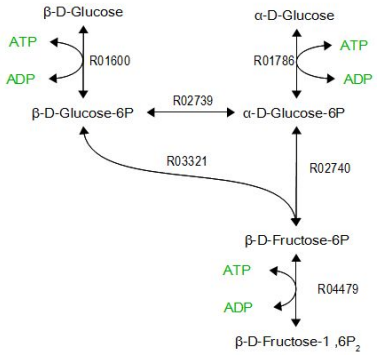
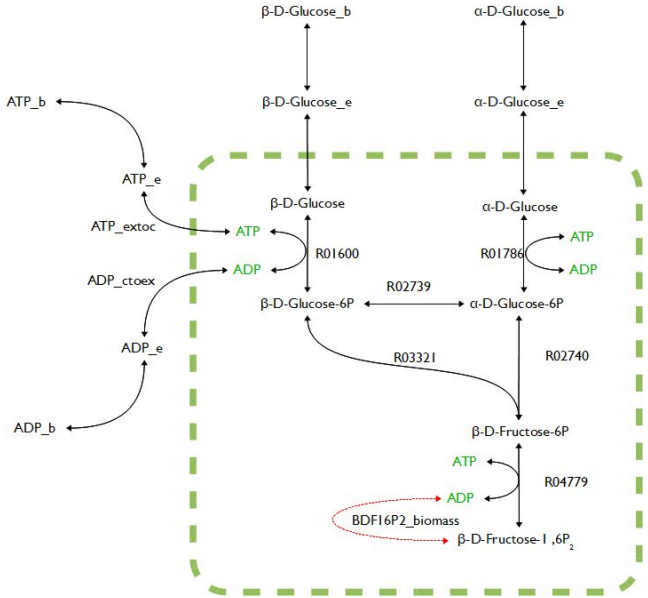
II. FBA formulation

Preparing the first six reactions in glycolysis for FBA:

- Addition of an objective function (red).
- Import & export of nutrients (ATP, ADP, BDG, ADG) across the system boundary (dashed green line).



Prepare for FBA



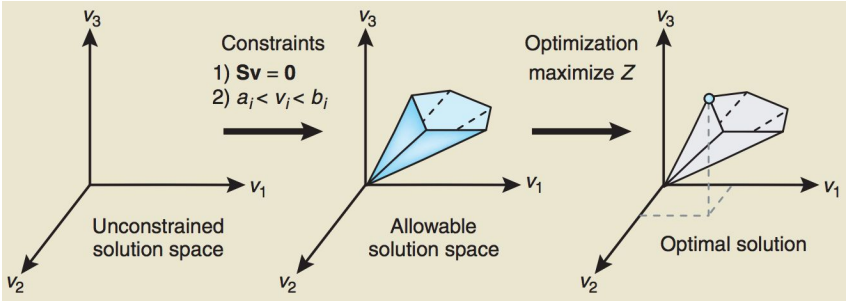
wikipedia

Flux balance analysis

FBA is a formalism that defines the metabolic network as a linear programming optimization problem. The main constraints in FBA are imposed by the steady-state mass conservation of metabolites.

Constraint-based modeling:

- No constraints: flux may lie at any point in solution space.
- Mass balance constraints (imposed by the stoichiometry) and capacity constraints (imposed by the lower and upper bounds (ai & bi): defines allowable solution space.
    - Any flux distribution within this space is allowable
    - Points outside this space are denied
- Optimization of an objective function: A single optimal flux distribution that lies on the edge of the allowable solution space.



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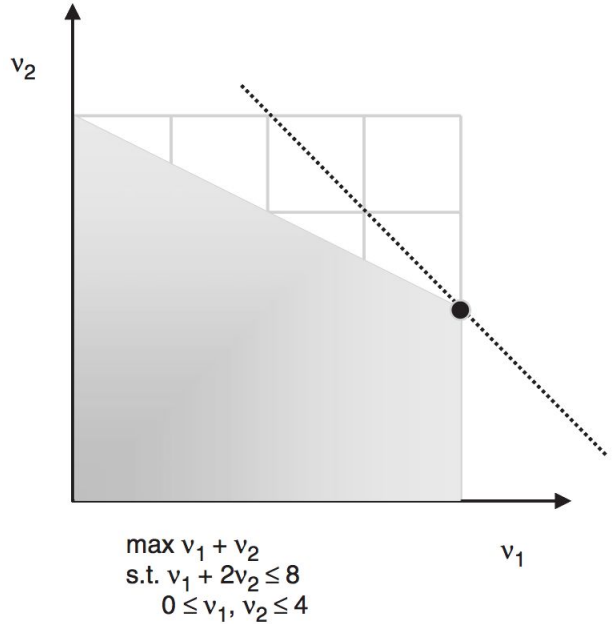
Flux balance analysis

FBA is a formalism that defines the metabolic network as a linear programming optimization problem. The main constraints in FBA are imposed by the steady-state mass conservation of metabolites.

Linear programming

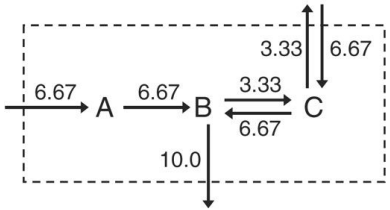
- Fluxes: v1 and v2
- Feasible solution space: shaded area and solid lines, defined by flux capacities, stoichiometric relationships, and design specification (e.g. gene deletions).
- Objective function: dotted line
- Optimal solution: circular dot



III. Hypothetical flux distribution at steady-state

$Z = 10$

$\boldsymbol{v} = [6.67\ 3.33\ 6.67\ 6.6710.0\ 3.33\ 6.67]^T$



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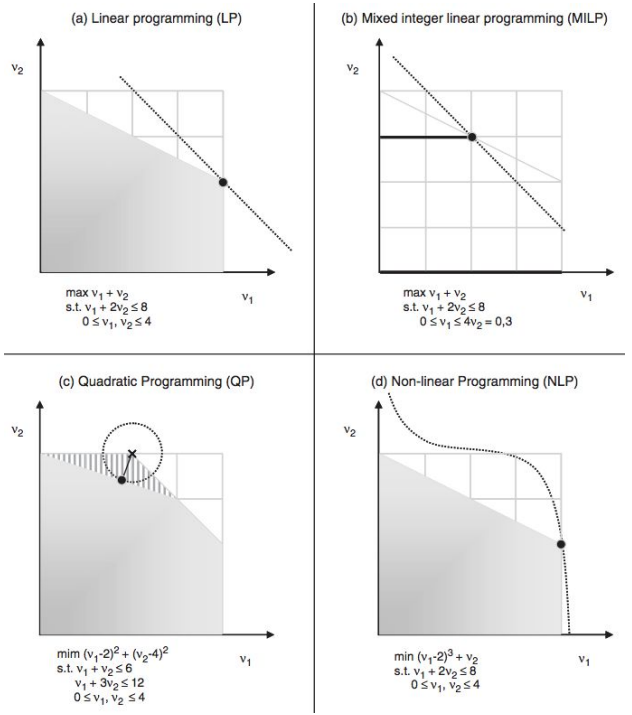

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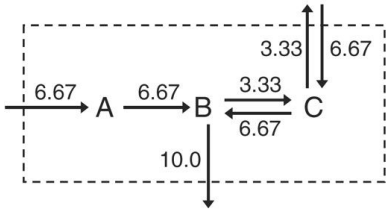

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Flux balance analysis

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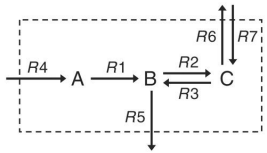
I. Reaction network formalism



Chemical reactions

Internal	Exchange
$R1$: -1 A $\rightarrow 1$ B	$R4$: 1 A
$R2$: -1 B $\rightarrow 1$ C	$R5$: -1 B
$R3$: -1 C $\rightarrow 1$ B	$R6$: -1 C
	$R7$: 1 C

S =

	R1	*R2*	*R3*	*R4*	*R5*	*R6*	*R7*
A	–1	0	0	1	0	0	0
B	1	–1	1	0	–1	0	0
C	0	1	–1	0	0	–1	1

II. FBA formulation

Dynamic mass balance

$$\frac{dC}{dt} = \mathbf{S}\boldsymbol{v}$$

C : Concentration
t : Time
$\mathbf{S}$: Stoichiometric matrix
$\boldsymbol{v}$: Flux vector

Steady-state assumption

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

LP formulation

Objective: $\max Z = v_5$

Constraints:

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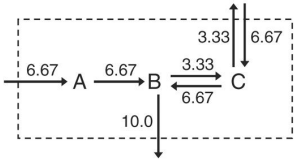
III. Hypothetical flux distribution at steady-state

$Z = 10$

$\boldsymbol{v} = [6.67\ 3.33\ 6.67\ 6.67 10.0\ 3.33\ 6.67]^T$

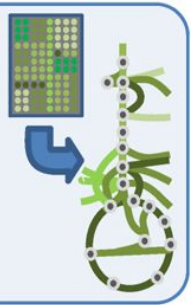


Lee (2006) Brief. Bioinfo.

Genome-scale metabolic network reconstruction & model

1. Contextualization of HT data

Several studies have overlaid gene microarray data on a metabolic GENRE to determine condition-dependent cell phenotypes. Metabolic GENREs have also been used to interpret metabolomic data, 13C flux data, and to link multiple data types.

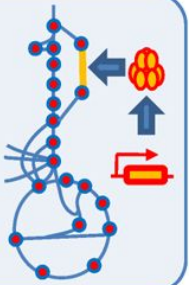


2. Guidance of metabolic engineering

Metabolic GENREs guided efforts to engineer malate and succinate producing strains of *S. cerevisiae* and *M. succiniciproducens*. GENREs have also helped determine ways to increase the respiration rate of *G. sulfurreducens* and scale-up vaccine production against *N. meningitides*.

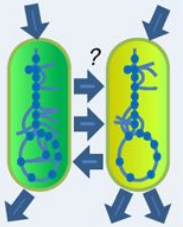
3. Directing hypothesis-driven discovery

A Metabolic GENRE aided in determining pathway usage and discovering a novel citramalate synthase gene in *G. sulfurreducens*. GENREs have also helped study the effects of transposons on downstream genes, and identify transc riptional timing patterns in *S. cerevisiae*.



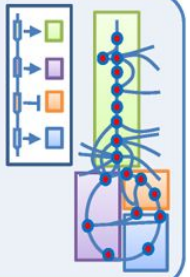
4. Interrogation of multi-species relationships

A dual-species metabolic model was built to study Interactions between the syntrophic bacteria, *D. vulgaris* and *M. maripaludis*. Metabolic models have also been used in comparisons of multiple species, such as an analysis of pathway differences between four halophilic bacteria.



5. Network property discovery

Metabolic GENREs have been used to study metabolite connectivity, and pathway redundancy *in silico*. Pathway-analysis tools have also spawned techniques such as flux coupling analysis, which has helped identify novel drug targets in *M. tuberculosis*.



Oberhardt (2009) Mol. Sys. biol.
Thiele & Palsson (2010) Nat. Protoc.