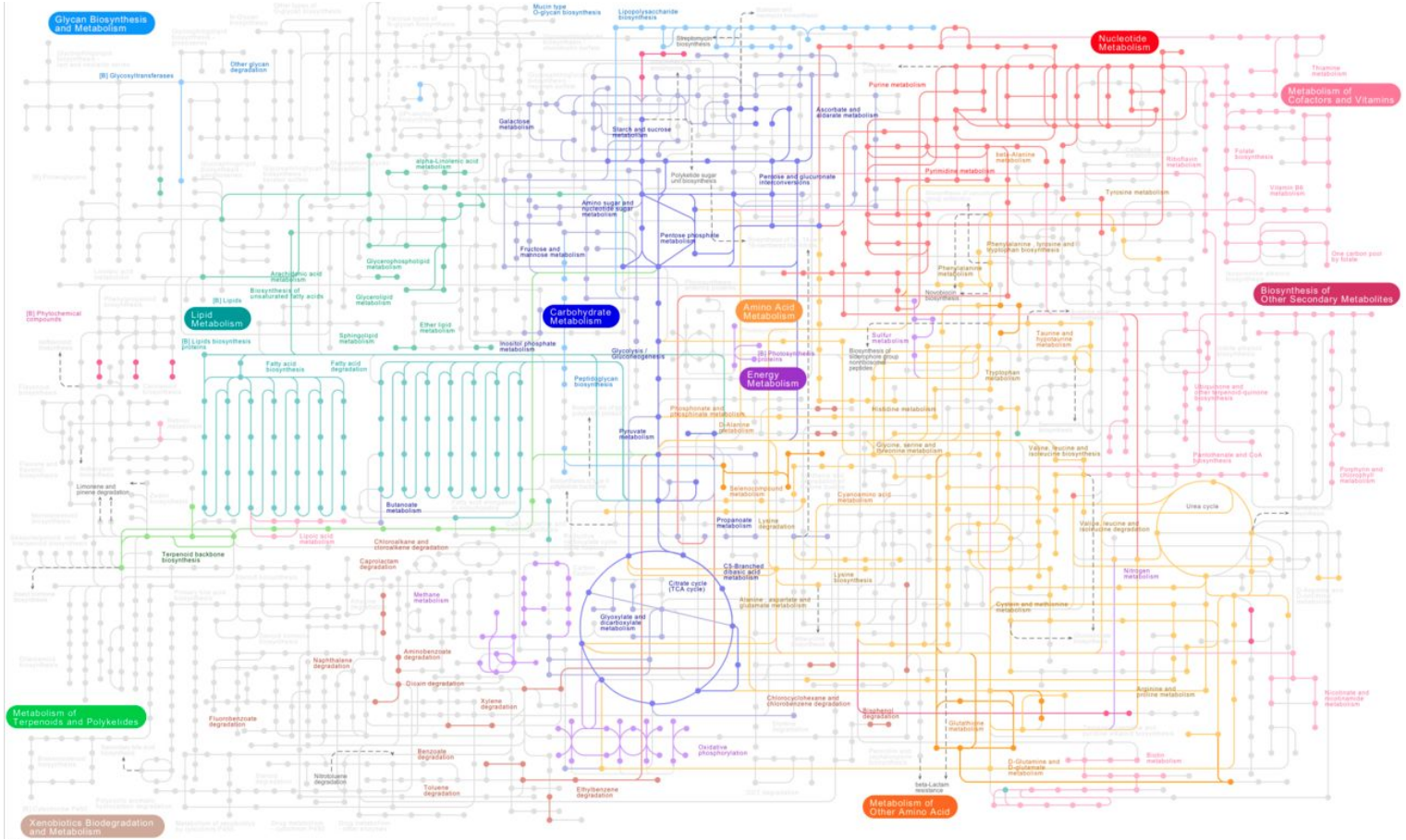
# Lecture 24: Modeling metabolic pathways

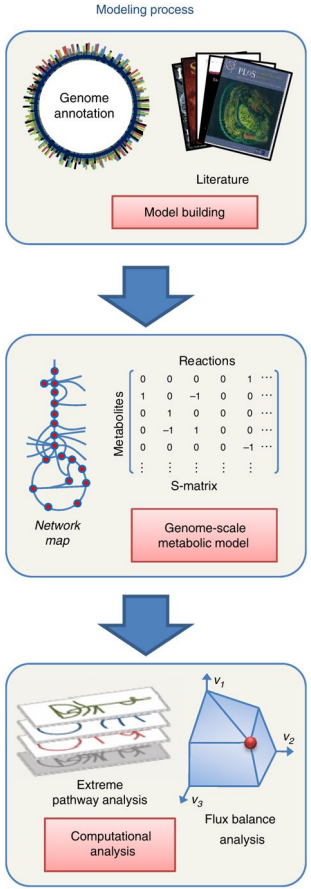
- Genome-scale metabolic models
- Flux balance analysis

# 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**:

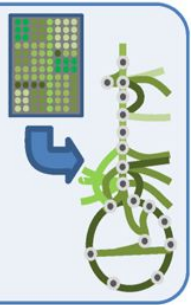
- Derived from a *reconstruction* by converting it into a mathematical form (i.e., an in silico model) and by assessing its phenotypic properties computationally.

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.

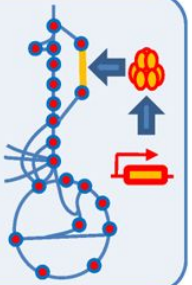


**2. Guidance of metabolic engineering**

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*.

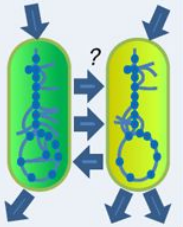
**3. Directing hypothesis-driven discovery**

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*.



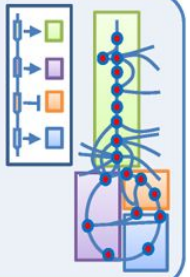
**4. Interrogation of multi-species relationships**

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/ |
| p$K_a$ Plugin | http://www.chemaxon.com/product/pka.html |
| p$K_a$ 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

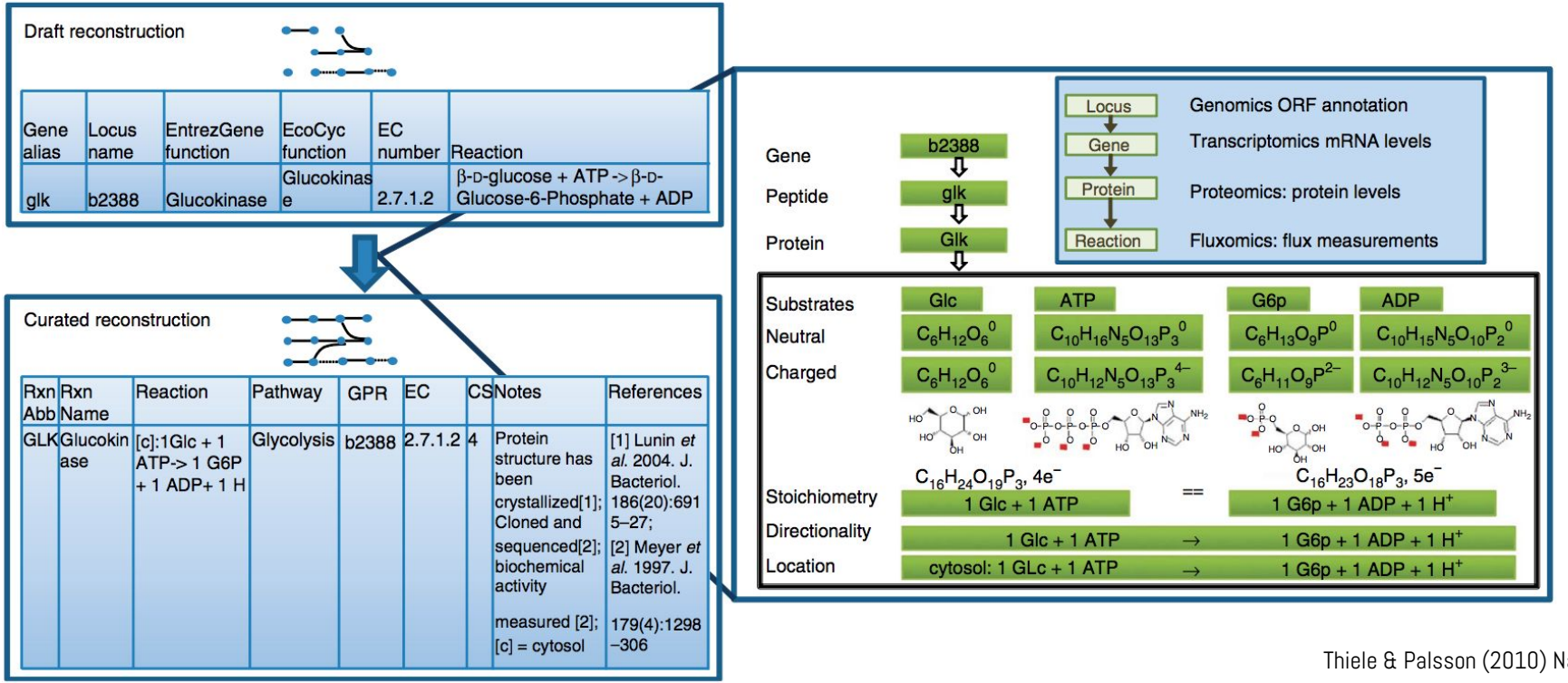
6| Determine and verify substrate and cofactor usage.
7| Obtain neutral formula for each metabolite.
8| Determine the charged formula.
9| Calculate reaction stoichiometry.
10| Determine reaction directionality.
11| Add information for gene and reaction localization.
12| Add subsystems information.
13| 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.

Thiele & Palsson (2010) Nat. Protoc.

# Constructing a genome-scale metabolic model

## Refinement of draft construction

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




Thiele & Palsson (2010) Nat. Protoc.

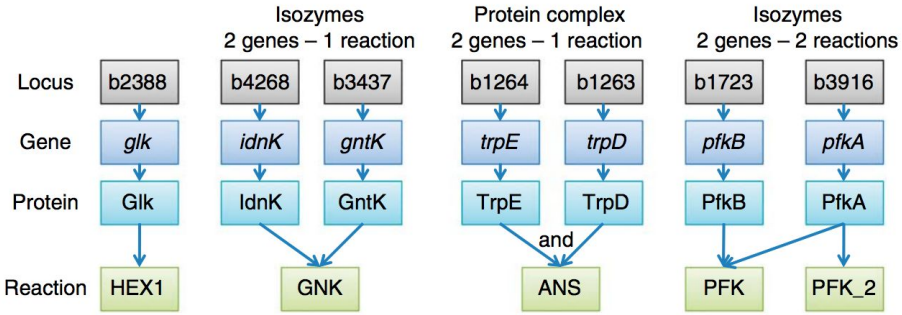
# Constructing a genome-scale metabolic model

## 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) |

Thiele & Palsson (2010) Nat. Protoc.

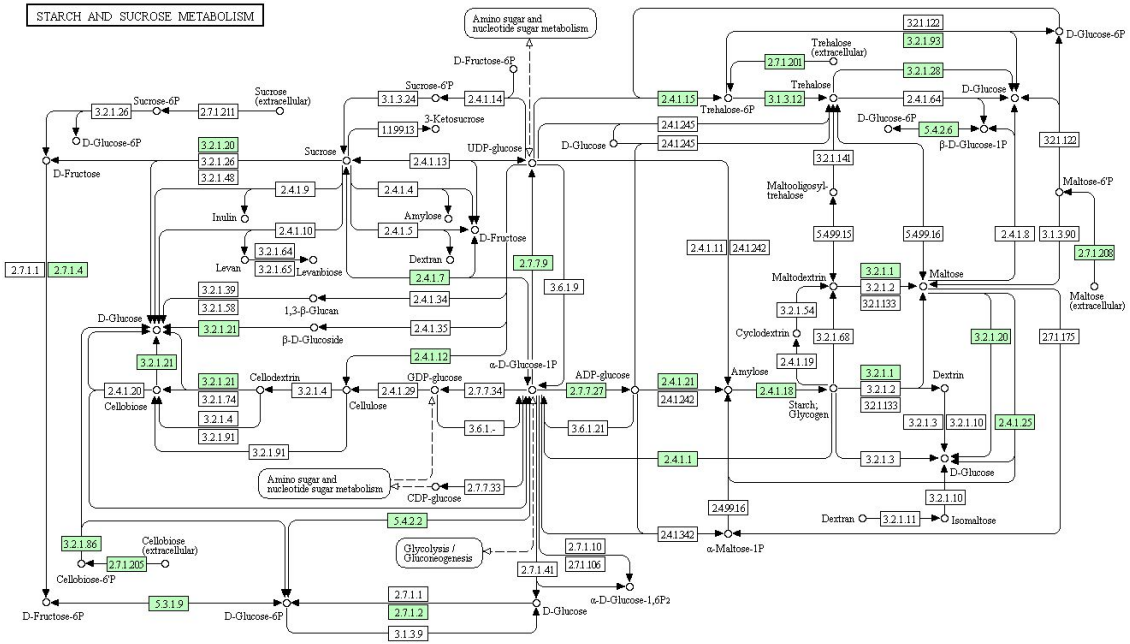
# Constructing a genome-scale metabolic model

## 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




Thiele & Palsson (2010) Nat. Protoc.

# 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 | |

Thiele & Palsson (2010) Nat. Protoc.

# 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.

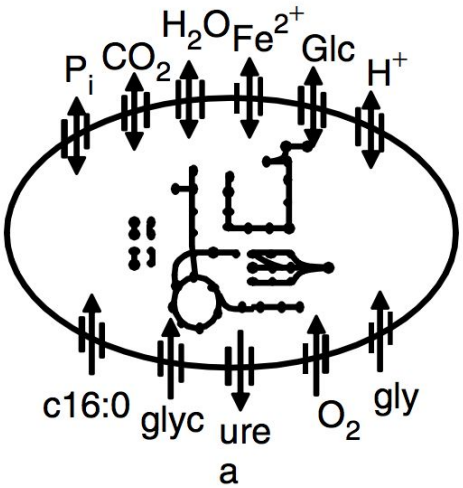
Thiele & Palsson (2010) Nat. Protoc.

# Constructing a genome-scale metabolic model

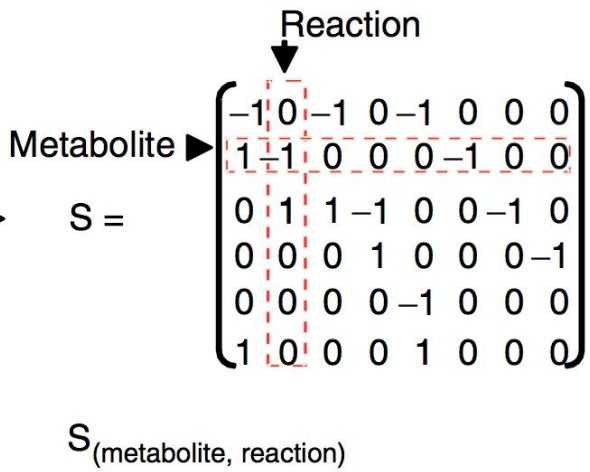
## Conversion of reconstruction into a model

Mathematical representation

*Metabolic network*




*Stoichiometric matrix*




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

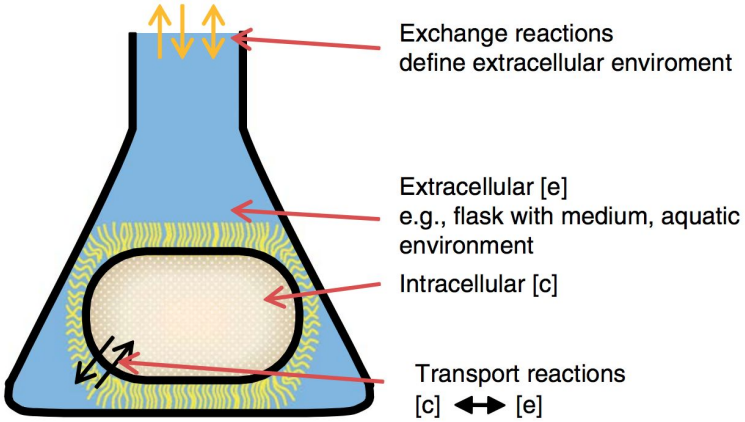
Conservation of mass: All steady states can be described by **Sv** = 0

Thiele & Palsson (2010) Nat. Protoc.

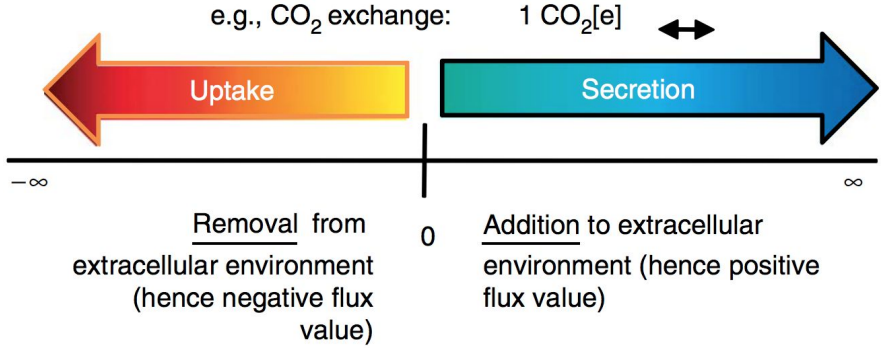
# Constructing a genome-scale metabolic model

## Conversion of reconstruction into a model

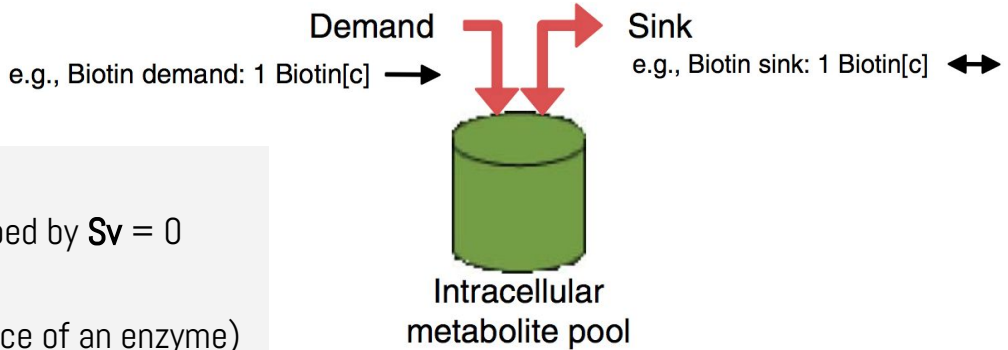
### Definition of system boundaries




### Exchange reactions




### Demand/sink reactions




Constraints:

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

Thiele & Palsson (2010) Nat. Protoc.

# 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.

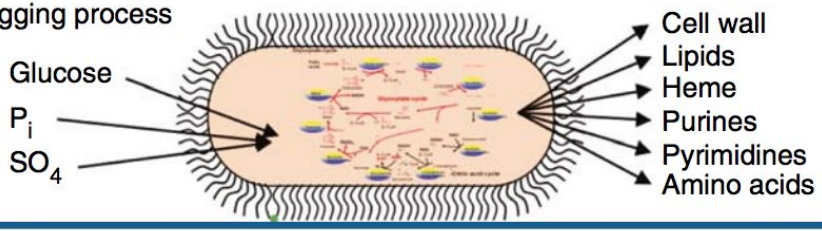
Thiele & Palsson (2010) Nat. Protoc.

# Constructing a genome-scale metabolic model

Network evaluation ≃ Debugging

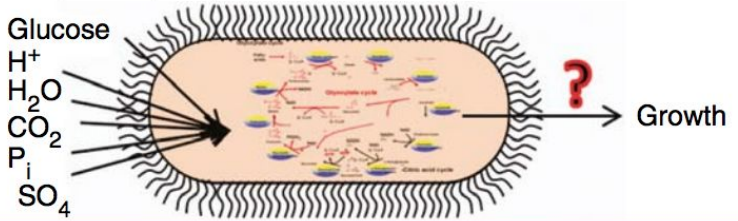
## Analysis of biomass precursors synthesis

-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

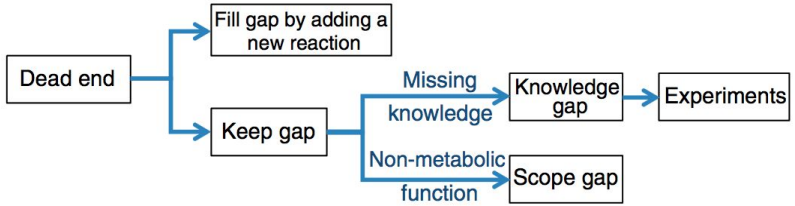
## Secretion capability

-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)

Thiele & Palsson (2010) Nat. Protoc.

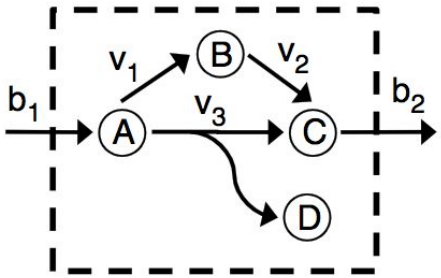
# Constructing a genome-scale metabolic model

Network evaluation ≃ Debugging




Identifying gaps

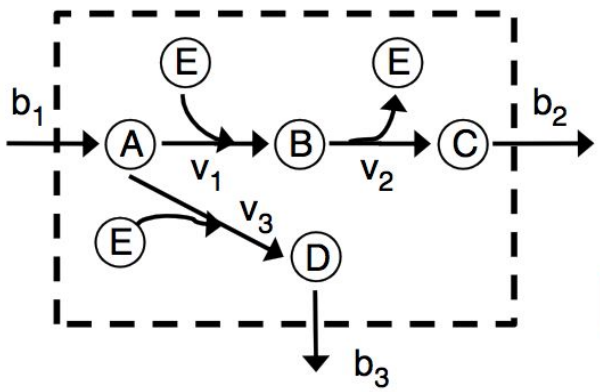
Connectivity based (topology):




| | $v_1$ | $v_2$ | $v_3$ | $b_1$ | $b_2$ |
|---|---|---|---|---|---|
| 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)



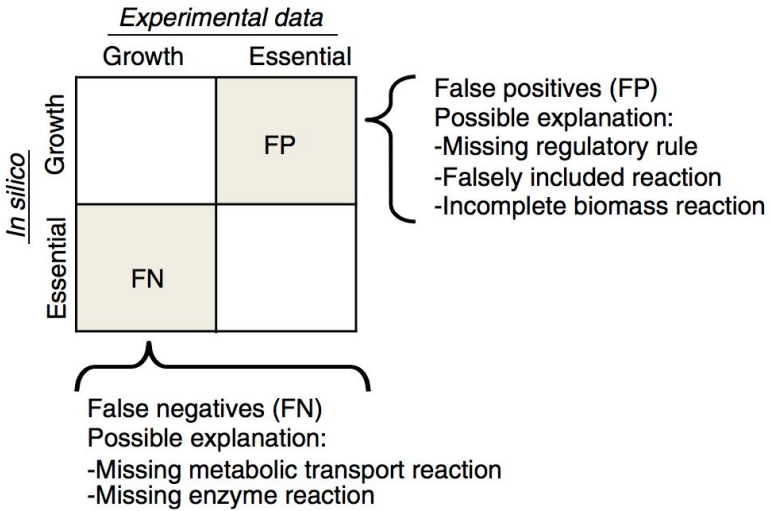

| | $v_1$ | $v_2$ | $v_3$ | $b_1$ | $b_2$ | $b_3$ |
|---|---|---|---|---|---|---|
| A | −1 | 0 | −1 | 1 | 0 | 0 |
| B | 1 | −1 | 0 | 0 | 0 | 0 |
| C | 0 | 1 | 0 | 0 | −1 | 0 |
| D | 0 | 0 | 1 | 0 | 0 | −1 |
| E | −1 | 1 | −1 | 0 | 0 | 0 |

Thiele & Palsson (2010) Nat. Protoc.

# Constructing a genome-scale metabolic model

Network evaluation ≃ Debugging

Gene essentiality

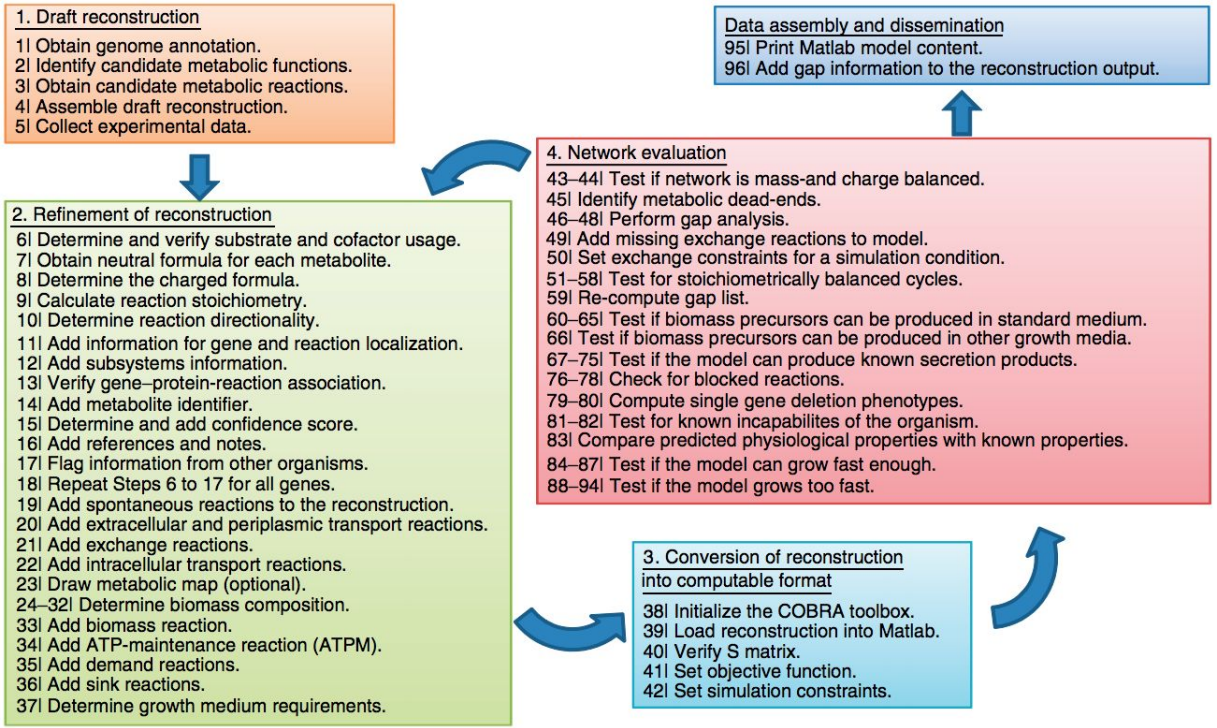



Thiele & Palsson (2010) Nat. Protoc.

# 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.




Thiele & Palsson (2010) Nat. Protoc.

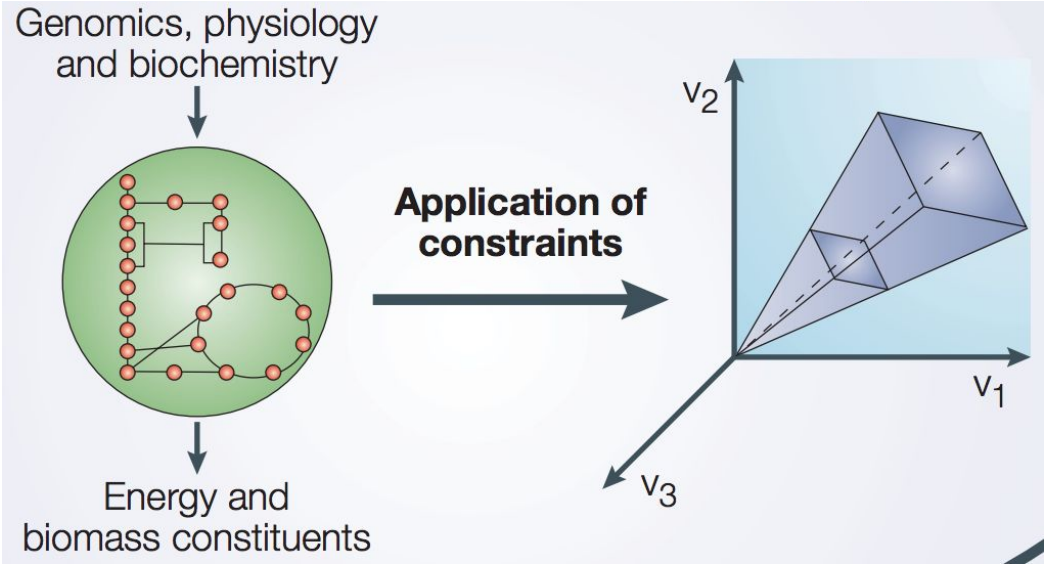
# Constructing a genome-scale metabolic model

| 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

# Constructing a genome-scale metabolic model

**Network construction** **Allowable solution space**




Price (2004) Nat. Rev. Micro.

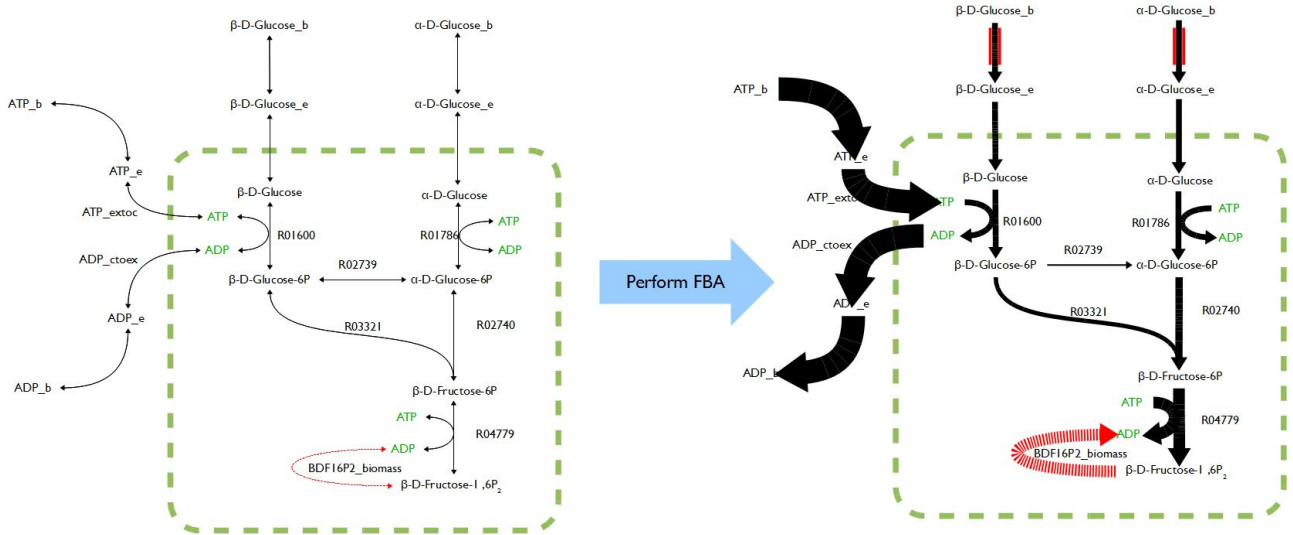
# Flux balance analysis (FBA)

FBA: metabolic network → linear programming optimization problem.

The main constraints in FBA: 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.




Wikipedia

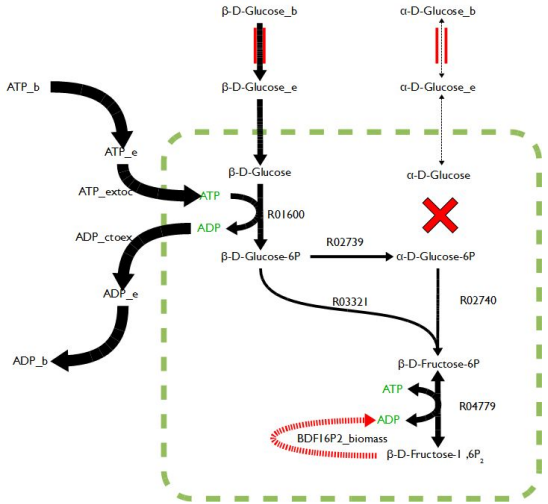
# Flux balance analysis (FBA)

FBA: metabolic network → linear programming optimization problem.

The main constraints in FBA: steady-state mass conservation of metabolites.

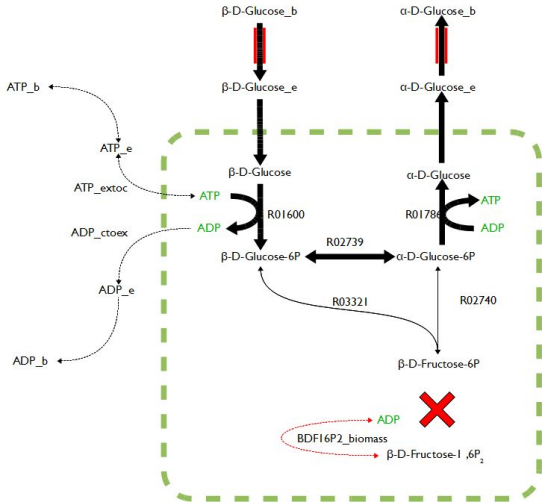
*Non-lethal gene deletion* in a metabolic network.

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




*Lethal gene deletion* in a metabolic network.

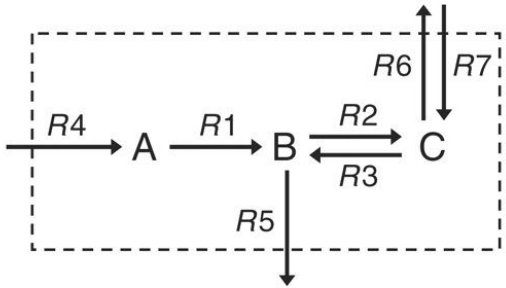
- No flux through the objective function → pathway is no longer functional.




Wikipedia

# Flux balance analysis

**I. Reaction network formalism**




**Chemical reactions**

| Internal | Exchange |
|---|---|
| *R*1: –1 A → 1 B | *R*4: 1 A |
| *R*2: –1 B → 1 C | *R*5: –1 B |
| *R*3: –1 C → 1 B | *R*6: –1 C |
| | *R*7: 1 C |

S =

| | *R*1 | *R*2 | *R*3 | *R*4 | *R*5 | *R*6 | *R*7 |
|---|---|---|---|---|---|---|---|
| **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

## II. FBA formulation

Dynamic mass balance

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

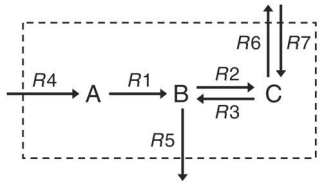
$C$ : Concentration
$t$ : Time
$\mathbf{S}$ : Stoichiometric matrix
$\boldsymbol{v}$ : Flux vector

Steady-state assumption

$$\mathbf{Sv} = \mathbf{0}$$

LP formulation

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




Constraints:

$$\begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccccccc} R1 & R2 & R3 & R4 & R5 & R6 & R7 \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 & 1 \end{bmatrix} \end{array} \begin{bmatrix} v_1 \\ \vdots \\ v_7 \end{bmatrix} = \mathbf{0} \qquad 0 \le v_1, \ldots, v_7 \le 10$$

Lee (2006) Brief. Bioinfo.

# Flux balance analysis: Objective function & Constraints

- **Objective function**:
  - Physiologically-meaningful or design-based objective for the interrogation or exploitation of a given system.
- Examples:
  - Maximizing…
    - biomass or cell growth
    - maximizing ATP production
    - maximizing the rate of synthesis of a particular product
  - Minimizing…
    - ATP production
    - nutrient uptake (both to determine conditions of optimal metabolic energy efficiency)

Lee (2006) Brief. Bioinfo.

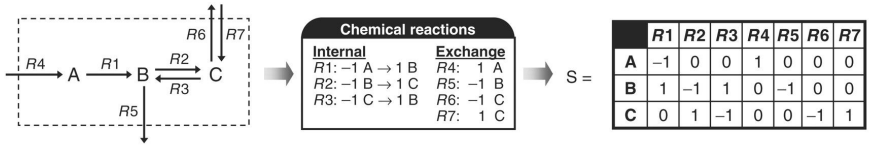
# Flux balance analysis: Objective function & Constraints

- **Principal constraint**: mass balance.
- **Additional constraints**:
  - physico-chemical constraints
  - spatial or topological constraints
  - condition dependent environmental constraints
  - regulatory constraints
- All constraints together 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.

Lee (2006) Brief. Bioinfo.

# Flux balance analysis

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 |

## II. FBA formulation

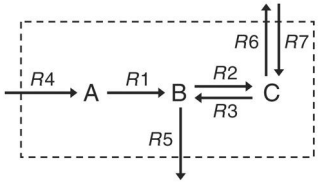
### Dynamic mass balance

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

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

### Steady-state assumption

$$\mathbf{Sv} = \mathbf{0}$$




### LP formulation

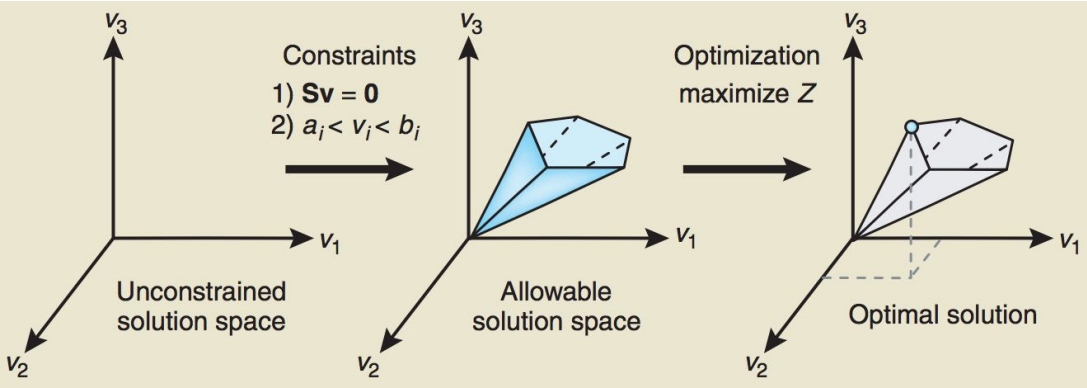
Objective: max $Z = \mathbf{c} \cdot \mathbf{v}$

Constraints:

$$\begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccccccc} R1 & R2 & R3 & R4 & R5 & R6 & R7 \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 & 1 \end{bmatrix} \end{array} \begin{bmatrix} v_1 \\ \vdots \\ v_7 \end{bmatrix} = \mathbf{0} \qquad 0 \le v_1, \ldots, v_7 \le 10$$

Lee (2006) Brief. Bioinfo.

# Flux balance analysis




## Constraint-based modeling

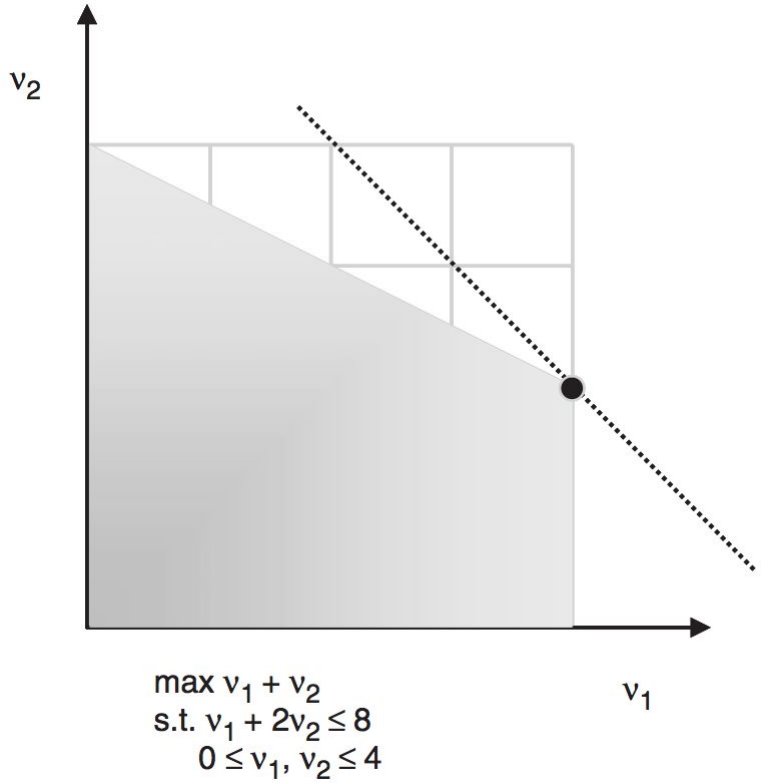
1. No constraints: flux may lie at any point in solution space.
2. Mass balance constraints (imposed by the stoichiometry) and capacity constraints (imposed by the lower and upper bounds: $a_i$ & $b_i$): defines allowable solution space.
    a. Any flux distribution within this space is allowable; Points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

Orth (2010) Nat. Biotech.

# Flux balance analysis

**Linear programming**

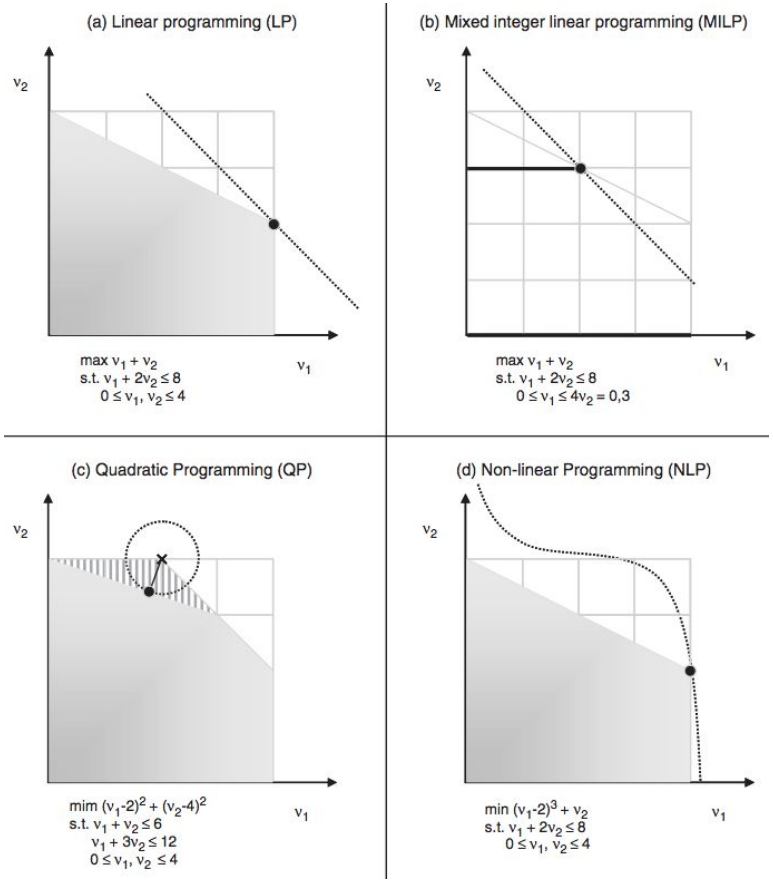
- Feasible solution space:
  - shaded area and solid lines
  - defined by:
    - flux capacities,
    - stoichiometric relationships, and
    - design specification (e.g. gene deletions).
- Objective function: dotted line
- Optimal solution: circular dot




Lee (2006) Brief. Bioinfo.

# Flux balance analysis

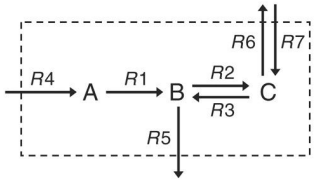
- Mixed Integer LP (MILP):
  - Integer variables are involved in a linear programming problem (e.g. binary variable formulation for gene deletion).
- Quadratic programming (QP):
  - Quadratic objective function subject to linear constraints.
  - This technique is generally used for finding the closest point to a specified point.
- Nonlinear programming (NLP):
  - Nonlinear objectives or constraints.
  - Generally difficult to solve for global optimal solution because of its non-convexity.




Lee (2006) Brief. Bioinfo.

# Flux balance analysis

**I. Reaction network formalism**




**Chemical reactions**

| Internal | Exchange |
|---|---|
| *R*1: –1 A → 1 B | *R*4: 1 A |
| *R*2: –1 B → 1 C | *R*5: –1 B |
| *R*3: –1 C → 1 B | *R*6: –1 C |
| | *R*7: 1 C |

S =

| | *R*1 | *R*2 | *R*3 | *R*4 | *R*5 | *R*6 | *R*7 |
|---|---|---|---|---|---|---|---|
| **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

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

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

Steady-state assumption

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

LP formulation

Objective: max $Z = v_5$

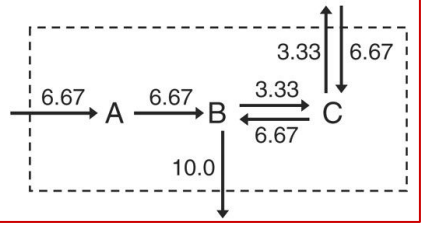
Constraints:

$$\begin{array}{c} \\ A \\ B \\ C \end{array}\begin{array}{c} \begin{array}{ccccccc} R1 & R2 & R3 & R4 & R5 & R6 & R7 \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 & 1 \end{bmatrix} \end{array} \begin{bmatrix} v_1 \\ \vdots \\ v_7 \end{bmatrix} = \mathbf{0} \qquad 0 \le v_1,\ldots,v_7 \le 10$$

**III. Hypothetical flux distribution at steady-state**

$Z = 10$

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

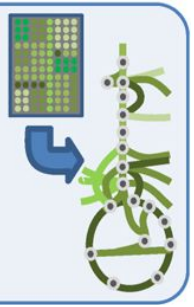



Lee (2006) Brief. Bioinfo.

# 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.

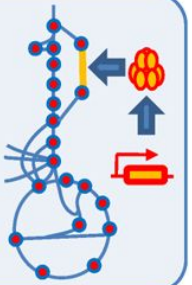


**2. Guidance of metabolic engineering**

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*.

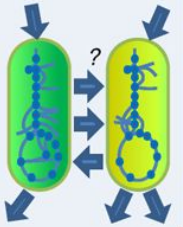
**3. Directing hypothesis-driven discovery**

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*.



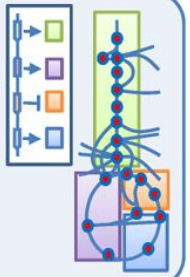
**4. Interrogation of multi-species relationships**

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.