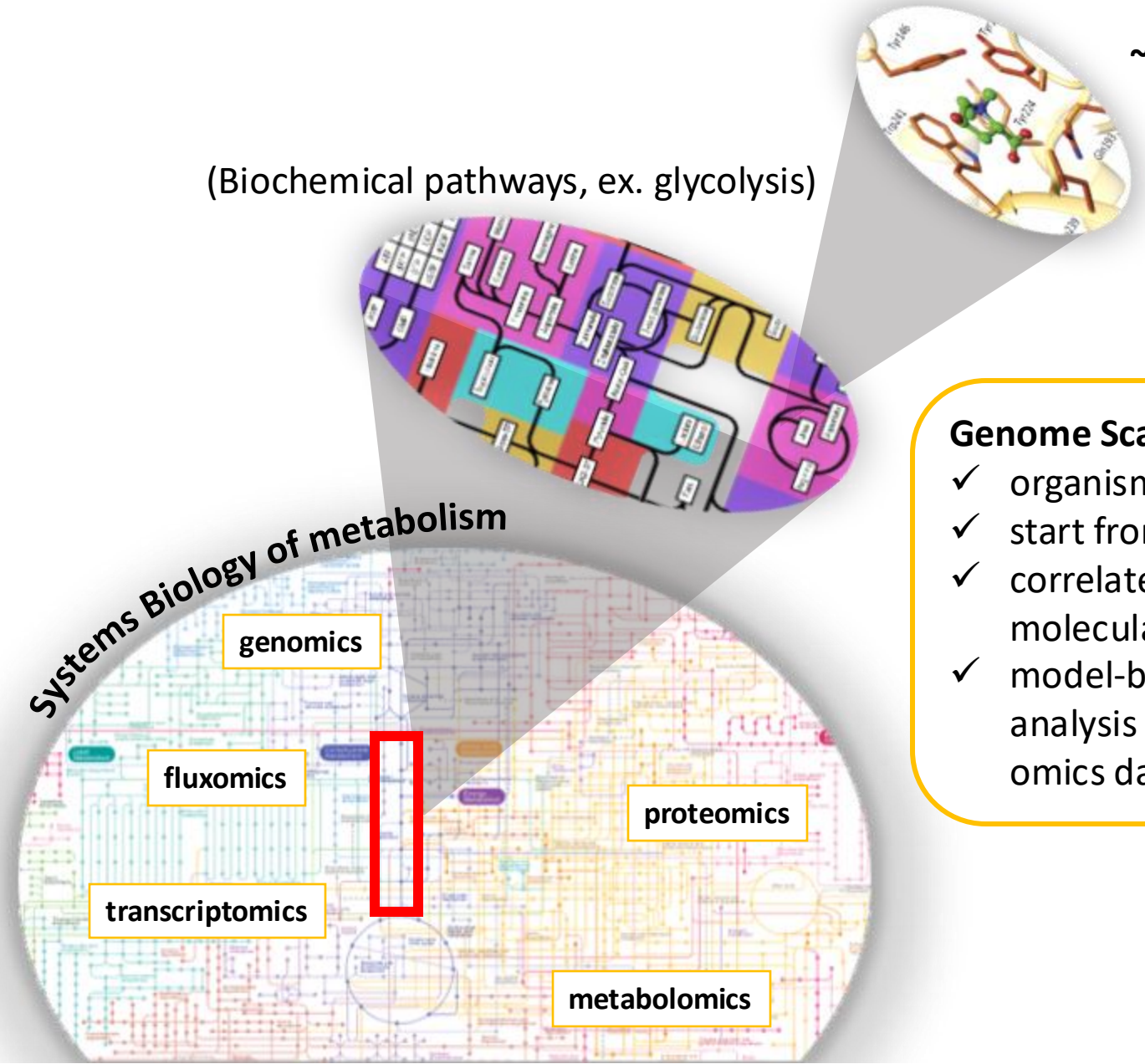
System components



Genome Scale Metabolic Modeling

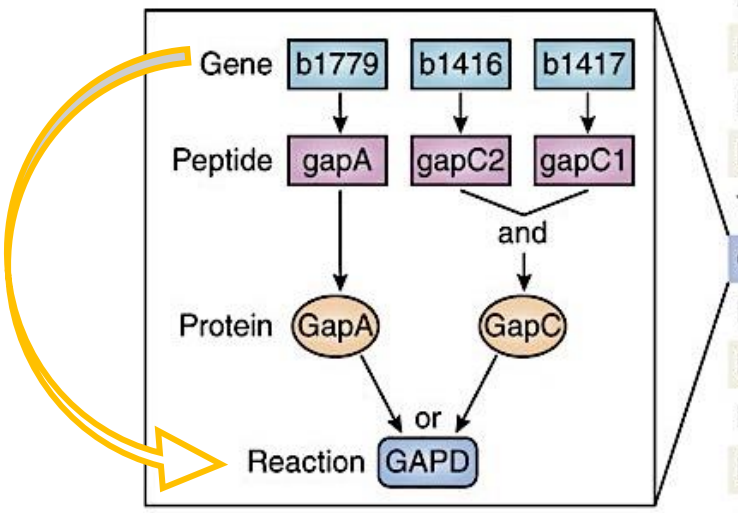
- ✓ organism-specific models
- ✓ start from sequenced genome
- ✓ correlate genome with molecular physiology
- ✓ model-based integration and analysis of large- and multi-scale omics data

Genome Scale Metabolic Models

✓ Systems Analysis of Metabolism

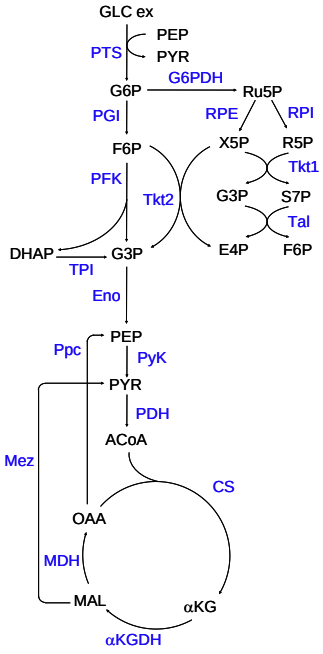
Sequenced and Annotated Genome

Functional Annotation



Metabolic Reactions -> Glycolysis pathway

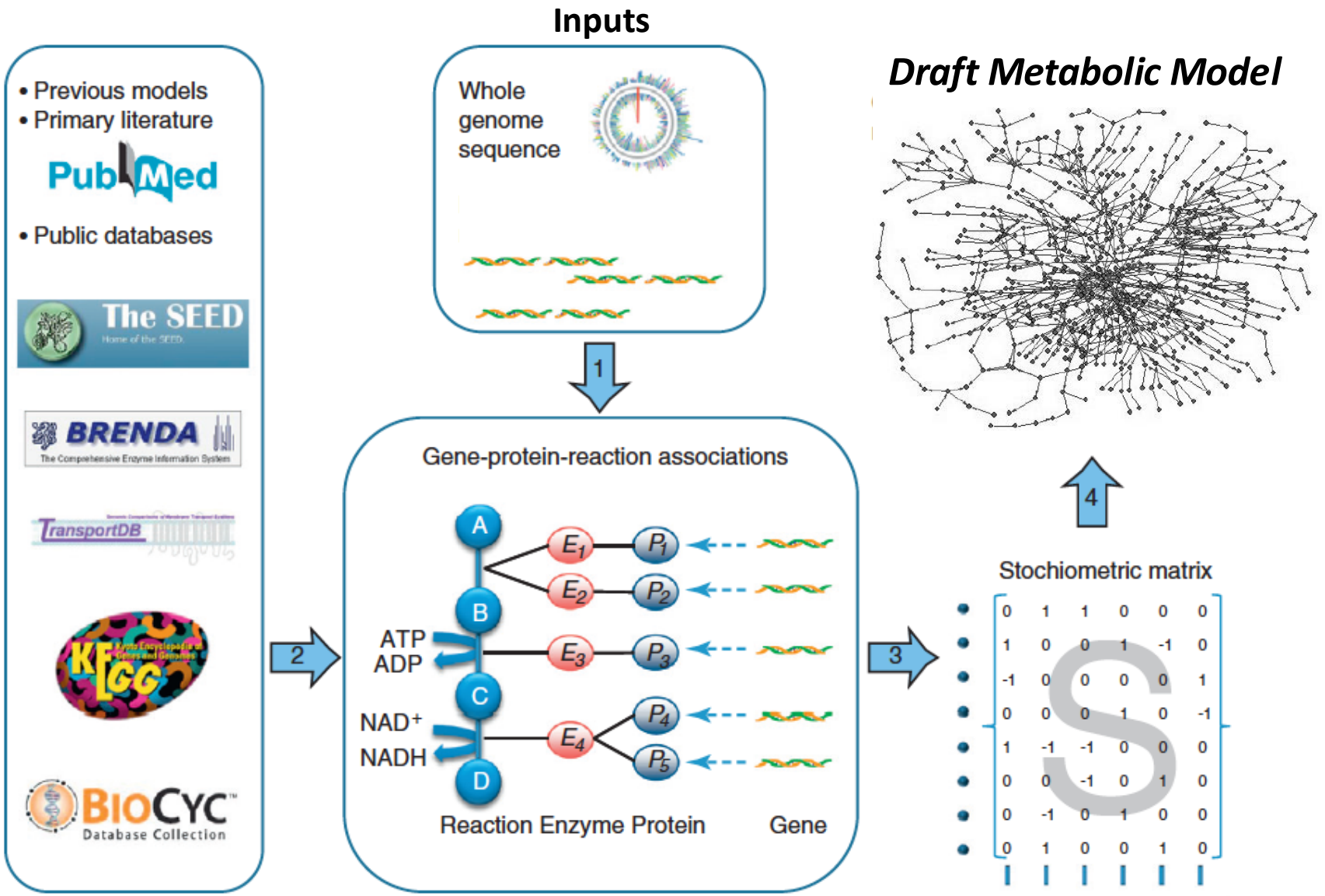
Abbreviation	Glycolytic reactions
HEX1	[c]GLC + ATP → G6P + ADP+ H
PGI	[c]G6P ↔ F6P
PFK	[c]ATP + F6P → ADP + FDP + H
FBA	[c]FDP ↔ DHAP + G3P
TPI	[c]DHAP ↔ G3P
GAPD	[c]G3P + NAD + PI ↔ 13DPG + H + NADH
PGK	[c]13DPG + ADP ↔ 3PG + ATP
PGM	[c]3PG ↔ 2PG
ENO	[c]2PG ↔ H_2O + PEP
PYK	[c]ADP + H + PEP → ATP + PYR



1000s of reactions for a single species

- Computers can keep this information "in mind" and analyze it in various ways.
- How to digitalize this information and show the interactions between metabolites and reactions?
- ***Interaction Matrices*** summarize any kind of interaction between elements of a system.

Genome Scale Model Reconstruction



Agren et al., 2013, The RAVEN Toolbox and Its Use for Generating a Genome-scale Metabolic Model for Penicillium chrysogenum. PLoS Comput Biol

THE RAVEN TOOLBOX

Genome Scale Model Reconstruction

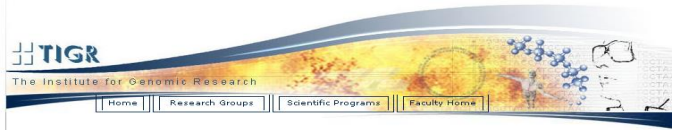
Image adopted from Agren et al., 2013, PLoS Comput Biol 9(3): e1002980

Genome Online Databases

Genome Databases

NCBI Entrez Gene

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=gene



http://pathema.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi

GOLD

http://genomesonline.org/index2.htm

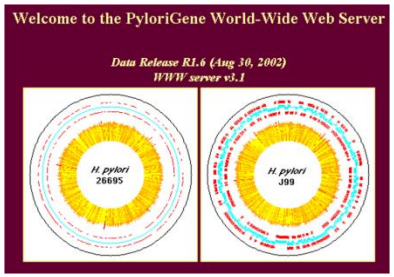
Vega

http://vega.sanger.ac.uk/index.html

TIGR CMR Comprehensive Microbial Resource

http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi

Organism-specific databases



H. pylori: http://genolist.pasteur.fr/PyloriGene/

EcoCyc™ Encyclopedia of *Escherichia coli* K-12 Genes and Metabolism

http://ecocyc.org/

SGD *Saccharomyces* Genome Database

http://www.yeastgenome.org/

Genome Scale Model Reconstruction

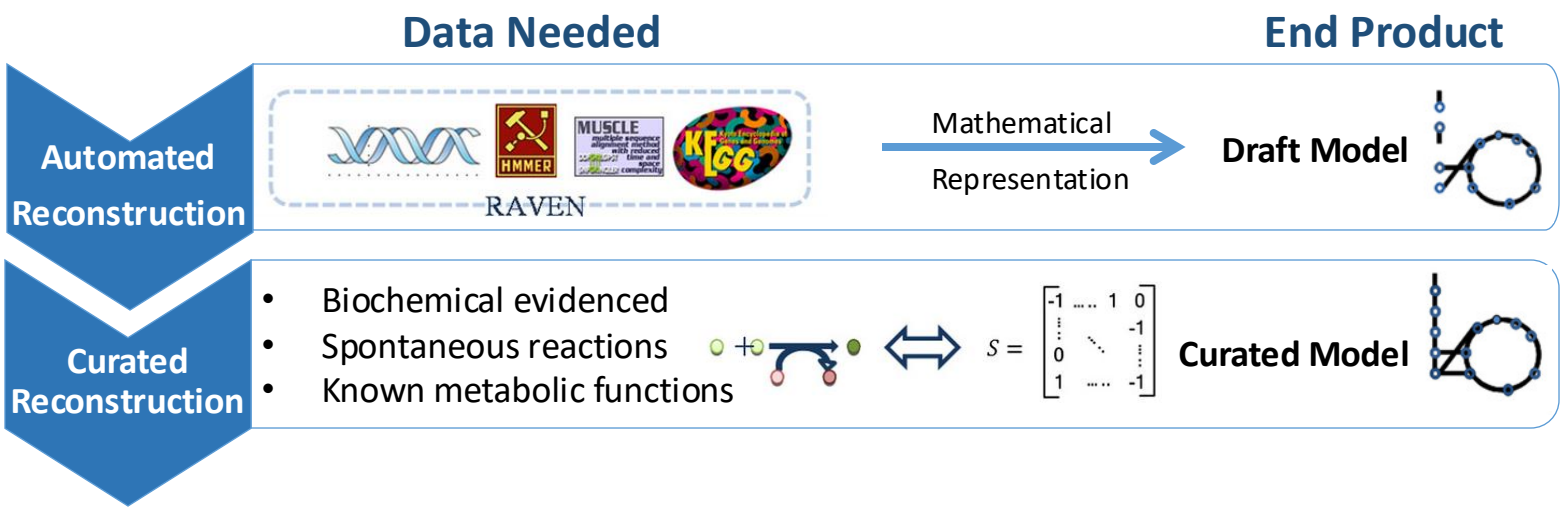


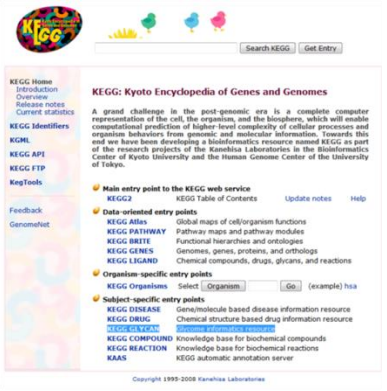
Image adopted from Agren et al., 2013, PLoS Comput Biol 9(3): e1002980

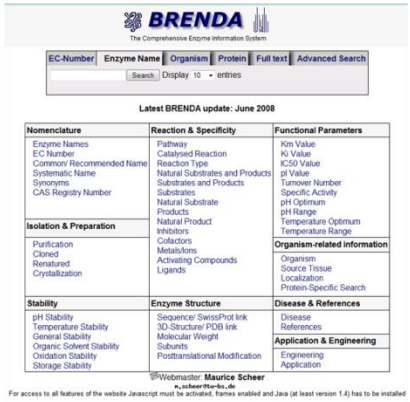
Biochemical databases

- **Enzyme databases:**
  - KEGG:

    http://www.genome.jp/kegg/
  - BRENDA:

    http://www.brenda-enzymes.info/





  - Both databases are great resources for biochemical reactions, but there information are organism-unspecific!

- **Transport database:**
  - Transport DB:

    http://www.membranetransport.org/
  - Transport Classification Database

http://www.tcdb.org/

Genome Scale Model Reconstruction

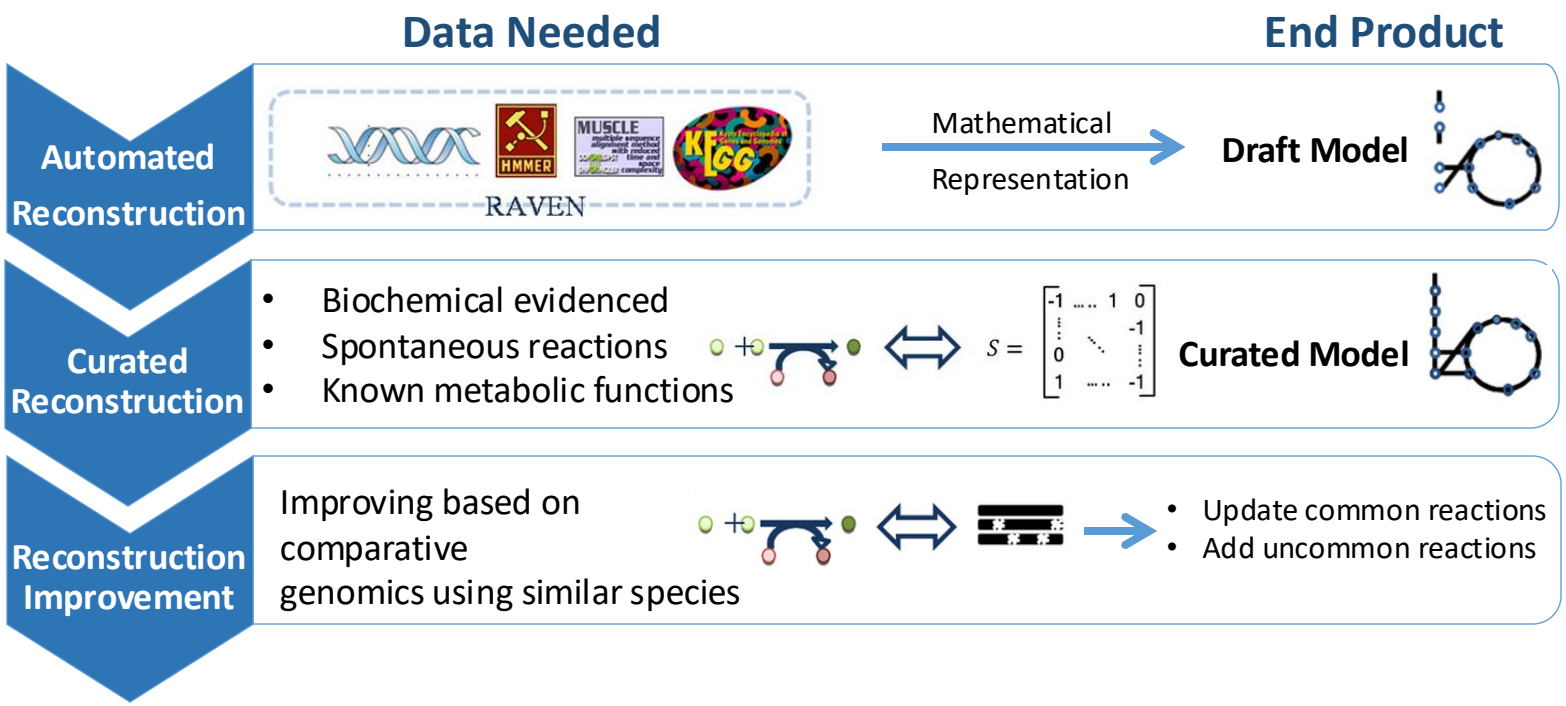


Image adopted from Agren et al., 2013, PLoS Comput Biol 9(3): e1002980

Genome Scale Model Reconstruction

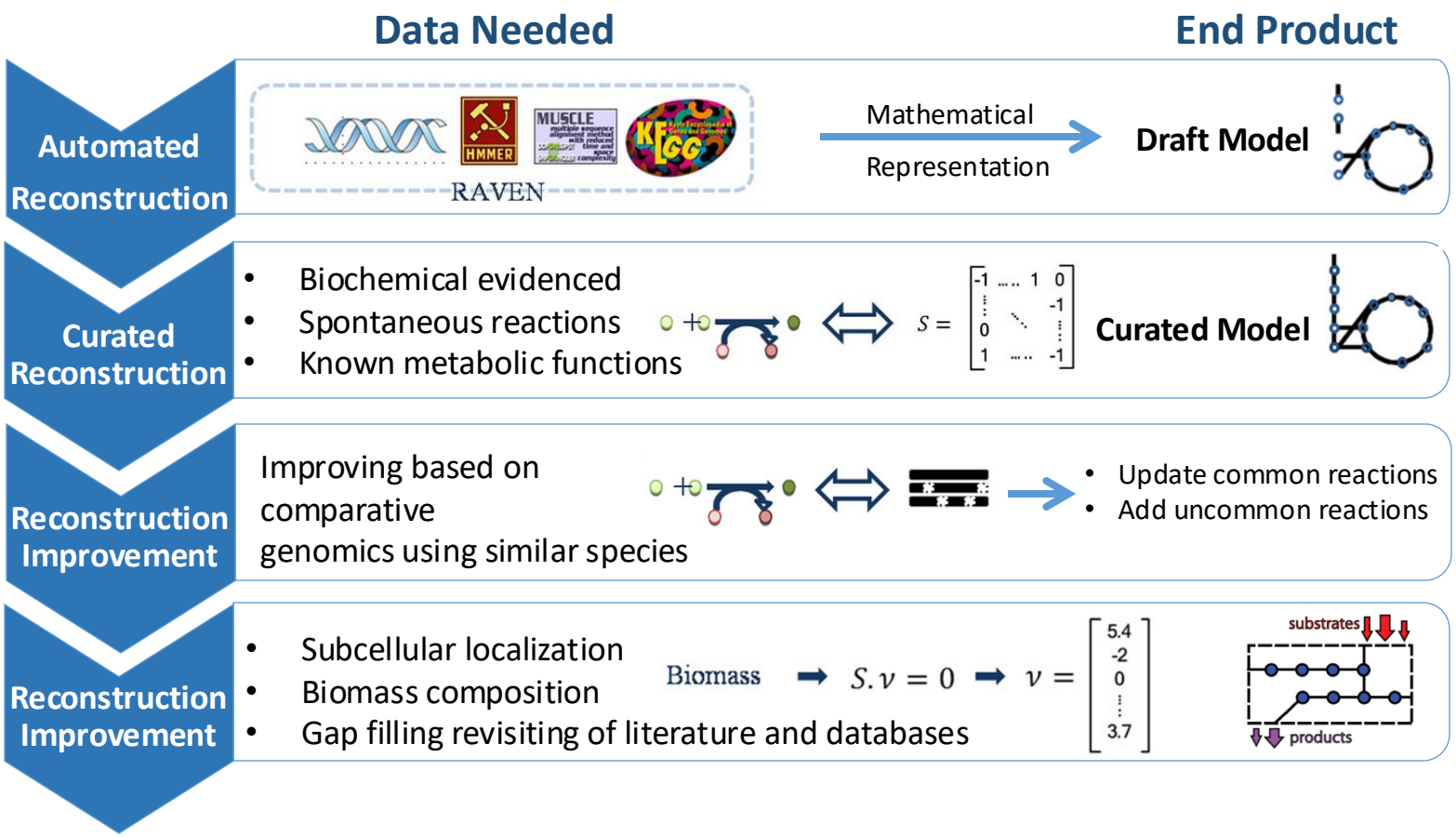
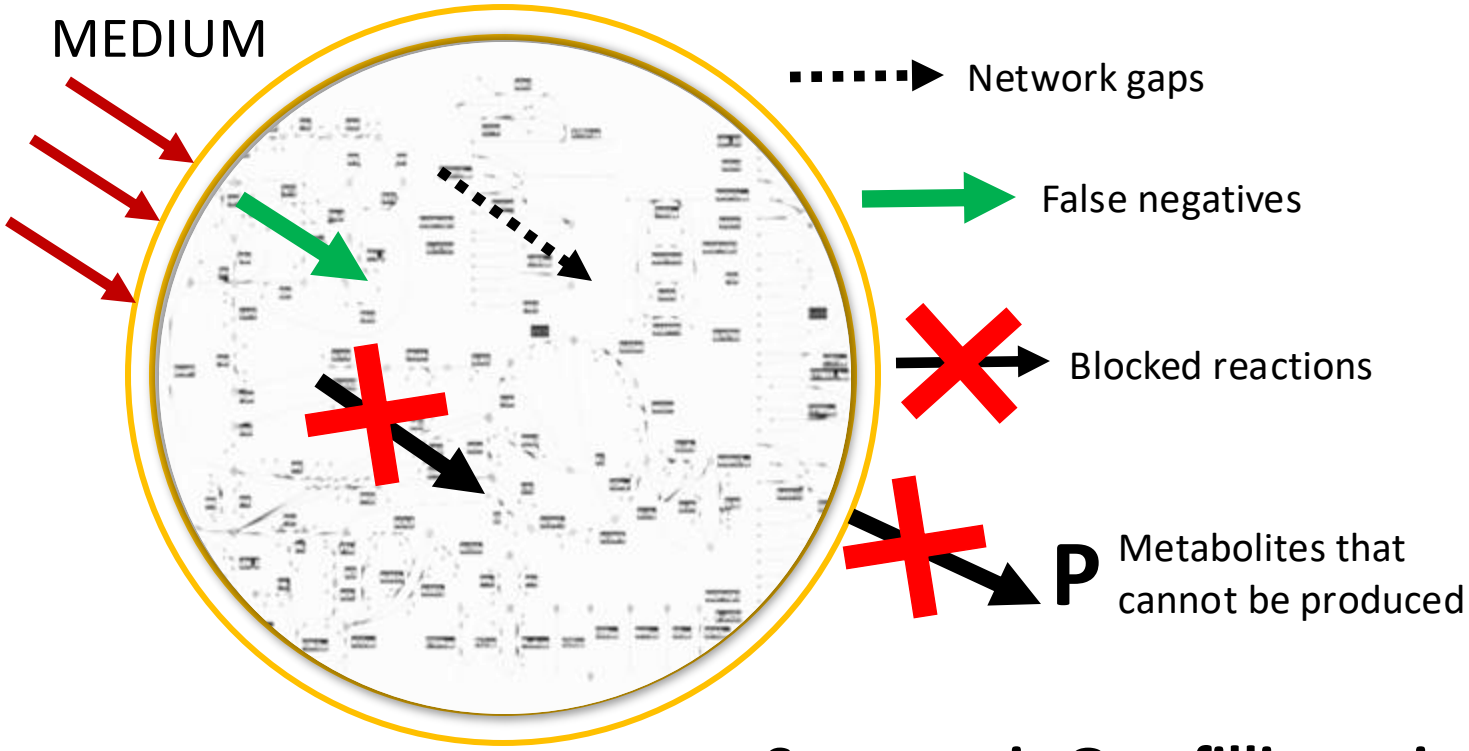


Image adopted from Agren et al., 2013, PLoS Comput Biol 9(3): e1002980

Knowledge Gaps in metabolic networks



Systematic Gap-filling using:

- ✓ Other annotation platforms
- ✓ *Close organism*
- ✓ KEGG database
- ✓ ATLAS of Biochemistry

Genome Scale Model Reconstruction

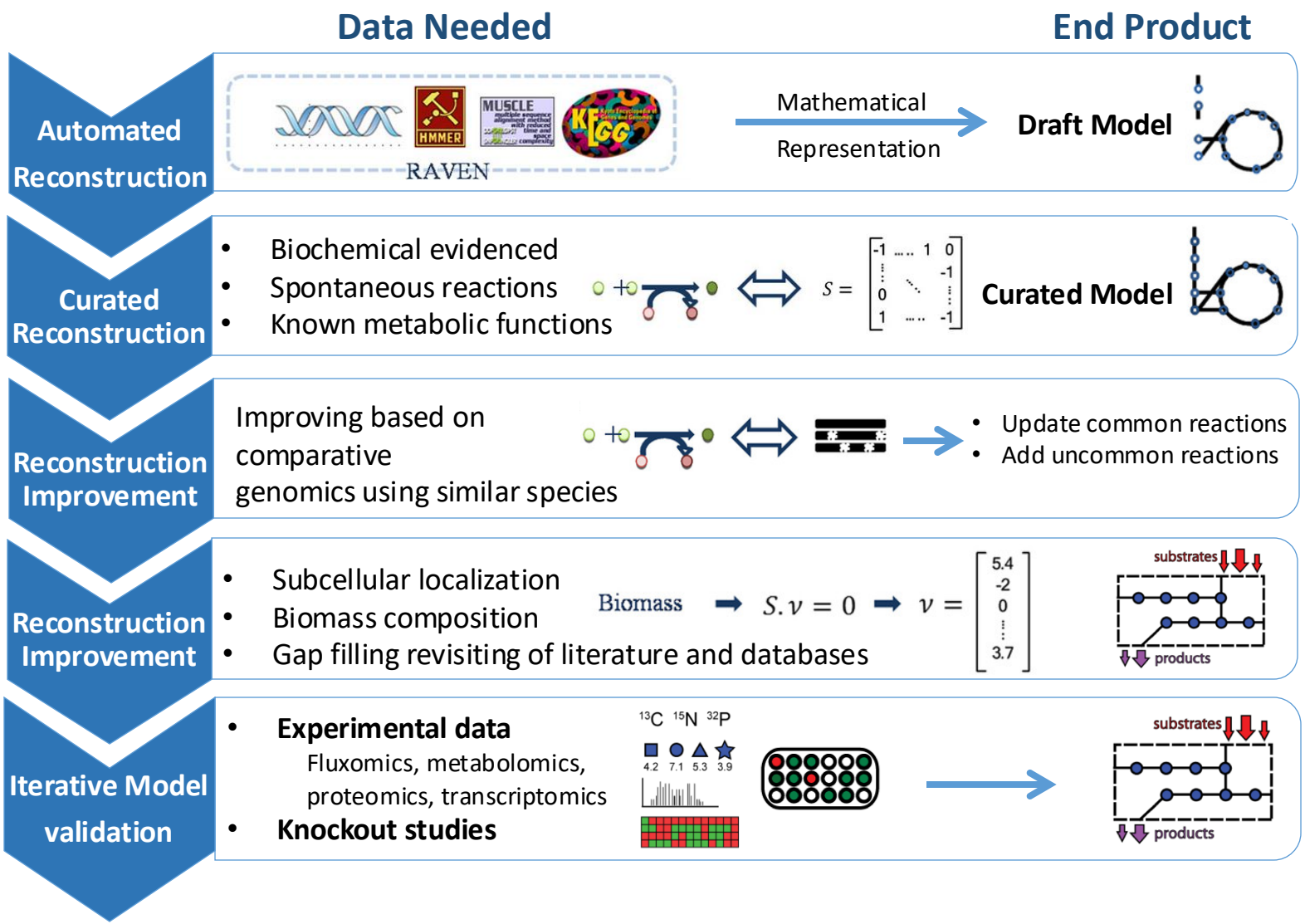


Image adopted from Agren et al., 2013, PLoS Comput Biol 9(3): e1002980

Genome Scale Metabolic Models

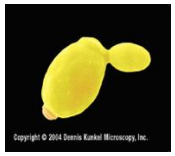
Genome Scale Models are driven from **sequenced genome**

- They started with the reconstruction of metabolic network of microbes
- Now they exist for several mice strains and human

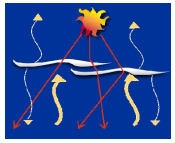
Industrially relevant organisms

E. coli
- 2712 Reactions
- 1516 Genes

S. cerevisiae
- 1402 Reactions
- 910 Genes



M. barkeri
- 619 Reactions
- 692 Genes



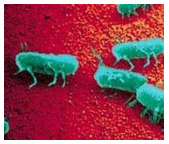
G. sulfurreducens
- 608 Reactions
- 588 Genes

B. subtilis
- 1020 Reactions
- 844 Genes

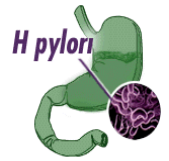
Pathogens

S. aureus
- 640 Reactions
- 619 Genes

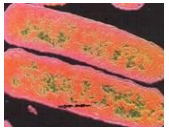
S. typhimurium
- 2545 Reactions
- 1271 Genes



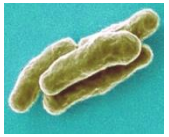
H. pylori
- 558 Reactions
- 341 Genes



H. influenzae
- 472 Reactions
- 376 Genes



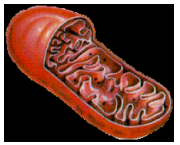
M. tuberculosis
- 939 Reactions
- 661 Genes



Mammalian cells

H. sapiens
- 10600 Reactions
- 2248 Genes

Human Mitochondria
- 218 Reactions

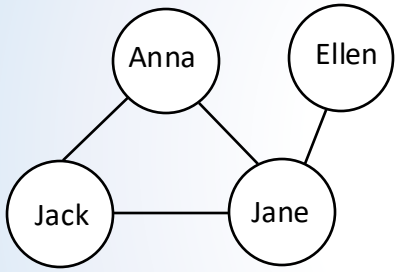


Red blood cell
- 39 Reactions

Interaction Matrices

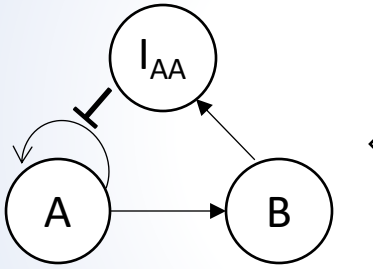
Represents the **relationship** between all the **elements** (constituent) of a system.

Example 1: social networks



	Anna	Ellen	Jack	Jane
Anna	0	0	1	1
Ellen	0	0	0	1
Jack	1	0	0	1
Jane	1	1	1	0

Example 2: gene Regulatory Network



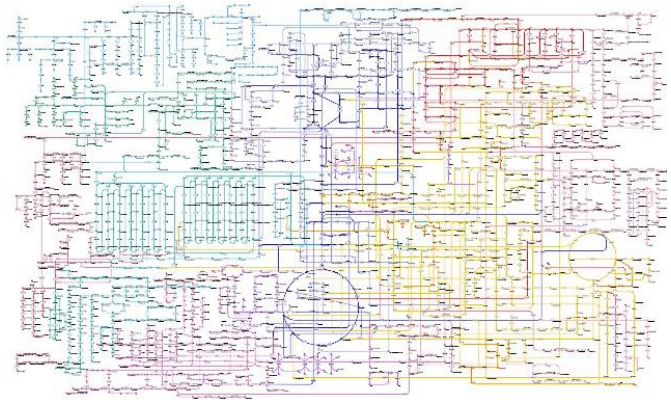
	A	B	I_{AA}
A	1	1	0
B	0	0	1
I_{AA}	-1	0	0

Stoichiometric Matrix

Represents the relationship between *all the* ***metabolites*** in *all the* ***reactions*** in a metabolic network

Metabolic Reactions

Abbreviation	Glycolytic reactions
HEX1	[c]GLC + ATP → G6P + ADP+ H
PGI	[c]G6P ↔ F6P
PFK	[c]ATP + F6P → ADP + FDP + H
FBA	[c]FDP ↔ DHAP + G3P
TPI	[c]DHAP ↔ G3P
GAPD	[c]G3P + NAD + PI ↔ 13DPG + H + NADH
PGK	[c]13DPG + ADP ↔ 3PG + ATP
PGM	[c]3PG ↔ 2PG
ENO	[c]2PG ↔ H_2O + PEP
PYK	[c]ADP + H + PEP → ATP + PYR



Reactions →

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

Metabolites ↑

Reactions

Metabolites

$$\begin{bmatrix} 1 & 0 & \cdots & 1 \\ 0 & -1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 1 & 0 & \cdots & -1 \end{bmatrix}$$

Closing Remarks

Genome-scale metabolic model is a platform that

- agrees with experimentally observed data
- allows testing hypotheses and answer metabolically relevant questions
- allows generating new hypothesis for experimental validation

RECONSTRUCTION OF METABOLIC NETWORKS & FLUX BALANCE ANALYSIS

Principles and Applications of Systems Biology

EPFL

September 2024

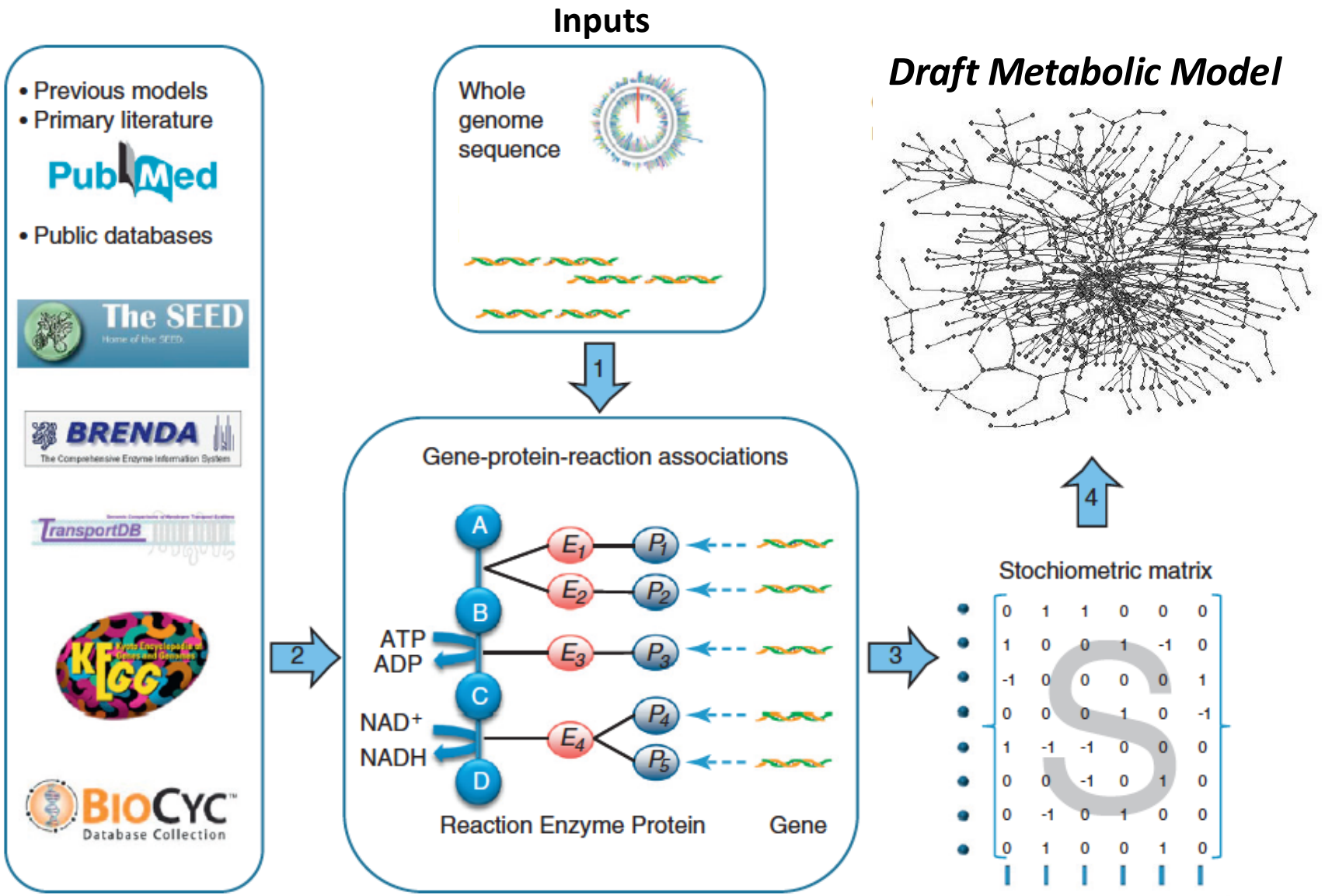
Vassily Hatzimanikatis

LECTURE OBJECTIVES

- GENERAL CONCEPTS OF MASS BALANCES IN METABOLIC NETWORKS
- METABOLIC NETWORK RECONSTRUCTION:
  - GENERAL WORKFLOW
  - BASIC CONCEPTS
- INTRODUCTION TO FLUX BALANCE ANALYSIS
  - LINEAR PROGRAMMING & OPTIMIZATION IN METABOLIC NETWORKS

- ***WHAT IS THE OPTIMAL GROWTH OF BACTERIUM?***

Genome Scale Model Reconstruction



Agren et al., 2013, The RAVEN Toolbox and Its Use for Generating a Genome-scale Metabolic Model for Penicillium chrysogenum. PLoS Comput Biol

THE RAVEN TOOLBOX

Stoichiometric Matrix

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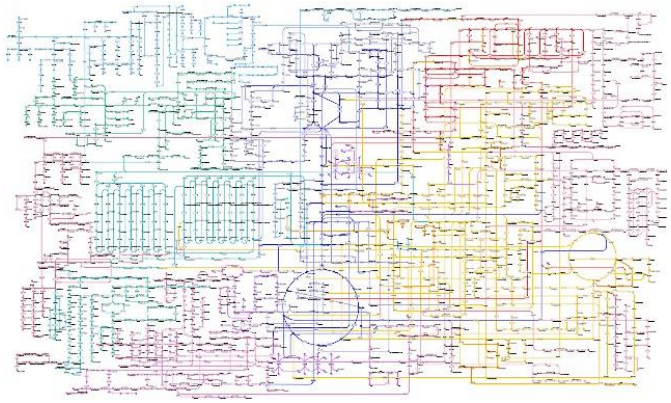


Reactions →

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

Metabolites ↑

Reactions

Metabolites

$$\begin{bmatrix} 1 & 0 & \cdots & 1 \\ 0 & -1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 1 & 0 & \cdots & -1 \end{bmatrix}$$

Mass Balances

Metabolic Reactions

$R1: Substrate \rightarrow A$

$R2: A \rightarrow B$

$R3: A \rightarrow C$

$R4: B \rightarrow product1$

$R5: C \rightarrow product2$

S matrix

Reactions

Metabolites	R1	R2	R3	R4	R5
Substrate	-1	0	0	0	0
A	1	-1	-1	0	0
B	0	1	0	-1	0
C	0	0	1	0	-1
Product 1	0	0	0	1	0
Product 2	0	0	0	0	1

How much **substrates ($d_{susbstrtae}/dt$)** for how much **products (d_{P1}/dt)**?

Fluxes

Metabolic Reactions

$R1: Substrate \rightarrow A$

$R2: A \rightarrow B$

$R3: A \rightarrow C$

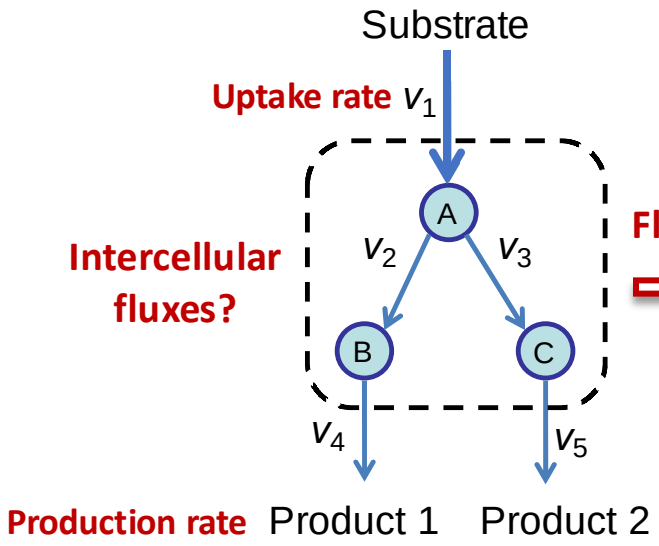
$R4: B \rightarrow product1$

$R5: C \rightarrow product2$

S matrix

Metabolites \ Reactions	R1	R2	R3	R4	R5
Substrate	-1	0	0	0	0
A	1	-1	-1	0	0
B	0	1	0	-1	0
C	0	0	1	0	-1
Product1	0	0	0	1	0
Product2	0	0	0	0	1

How much **substrates ($d_{susbstrtae}/dt$)** for how much **products (d_{P1}/dt)**?



Flux values ?

✓ v_1 $R1: Substrate \rightarrow A$

? v_2 $R2: A \rightarrow B$

? v_3 $R3: A \rightarrow C$

✓ v_4 $R4: B \rightarrow product1$

✓ v_5 $R5: C \rightarrow product2$

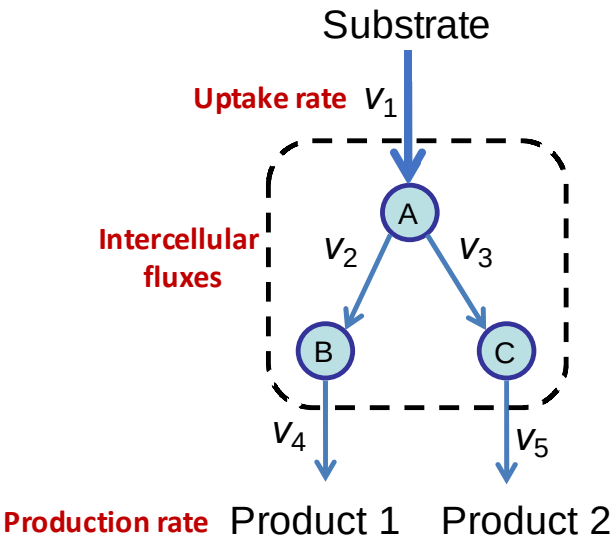
Why calculating the intercellular fluxes?

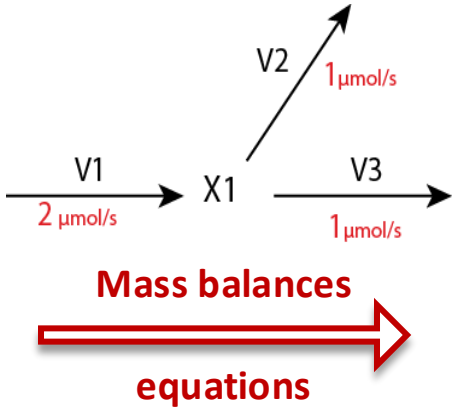
How to calculate the intercellular fluxes?

Metabolic Fluxes Analysis

quantitatively analyze metabolic pathways (calculating/estimating all the missing fluxes).

- ✓ Simulation of the effect of environmental or genetic changes
- ✓ Identification of important/critical/bottleneck reactions or pathways
- ✓ Understanding/controlling the pathway branching points





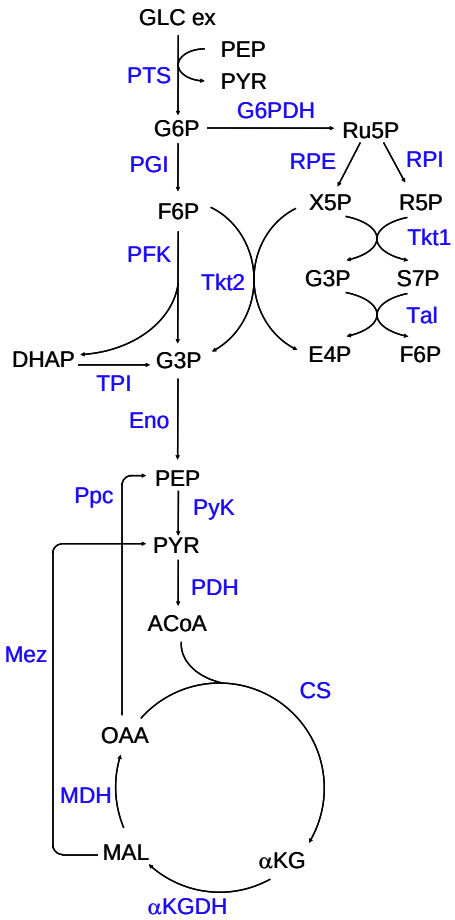
$$\frac{d[A]}{dt} = v_1 - v_2 - v_3$$

$$\frac{d[B]}{dt} = v_2 - v_4$$

$$\frac{d[C]}{dt} = v_3 - v_5$$

Central carbon metabolism of *E. coli*

"Fluxes in many biological networks cannot be uniquely determined"



Measurable flux

$$
\begin{array}{c}
\\
G6P \\
F6P \\
DHAP \\
G3P \\
PEP \\
PYR \\
Ru5P \\
X5P \\
R5P \\
S7P \\
E4P \\
ACoA \\
\alpha KG \\
MAL \\
OAA
\end{array}
\begin{bmatrix}
1 & -1 & & & & -1 & & & & & & & & & & \\
 & 1 & -1 & & & & & & & & 1 & 1 & & & & \\
 & & 1 & -1 & & & & & & & & & & & & \\
 & & 1 & 1 & -1 & & & & 1 & -1 & 1 & & & & & \\
-1 & & & & 1 & -1 & & & & & & & & & -1 & \\
1 & & & & & 1 & & & & & & -1 & & & & 1 \\
 & & & & & & 1 & -1 & -1 & & & & & & & \\
 & & & & & & & 1 & & -1 & & -1 & & & & \\
 & & & & & & & & 1 & -1 & & & & & & \\
 & & & & & & & & & 1 & -1 & & & & & \\
 & & & & & & & & & & 1 & -1 & & & & \\
 & & & & & & & & & & & & 1 & -1 & & \\
 & & & & & & & & & & & & & 1 & -1 & -1 \\
 & & & & & & & & & & & & -1 & & 1 & 1
\end{bmatrix}
\cdot
\begin{bmatrix}
v_{PTS} \\
v_{PGI} \\
v_{PFK} \\
v_{TPI} \\
v_{Eno} \\
v_{Pyk} \\
v_{G6PDH} \\
v_{RPE} \\
v_{RPI} \\
v_{TK1} \\
v_{Tal} \\
v_{TK2} \\
v_{PDH} \\
v_{CS} \\
v_{\alpha KGDH} \\
v_{MDH} \\
v_{Ppc} \\
v_{Mez}
\end{bmatrix}
= 0
$$

$$\frac{d[F6P]}{dt} = v_{PGI} - v_{PFK} + v_{Tal} + v_{TK2} = 0$$

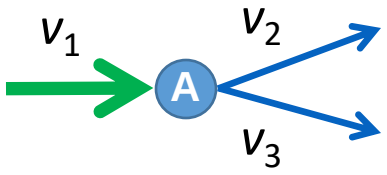
⋮

18 mass balance equations

(Quasi-)Steady state assumption

- Metabolism does not change with respect to time
  - All intermediates do not accumulate
  - The sum of influxes equals the sum of effluxes

$$v_1 = v_2 + v_3$$



Under the ***steady state assumption:***

$$\frac{d[A]}{dt} = v_1 - v_2 - v_3$$
$$\frac{d[B]}{dt} = v_2 - v_4$$
$$\frac{d[C]}{dt} = v_3 - v_5$$

$$\Rightarrow \quad \frac{d[A]}{dt} = \frac{d[B]}{dt} = \frac{d[C]}{dt} = 0 \quad \Rightarrow$$

$$v_1 - v_2 - v_3 = 0$$
$$v_2 - v_4 = 0$$
$$v_3 - v_5 = 0$$

(Quasi-)Steady state assumption

The mass balance equations can be described as follows:

$$\frac{d[A]}{dt} = v_1 - v_2 - v_3 = 0$$

$$\frac{d[B]}{dt} = v_2 - v_4 = 0$$

$$\frac{d[C]}{dt} = v_3 - v_5 = 0$$

matrix multiplication

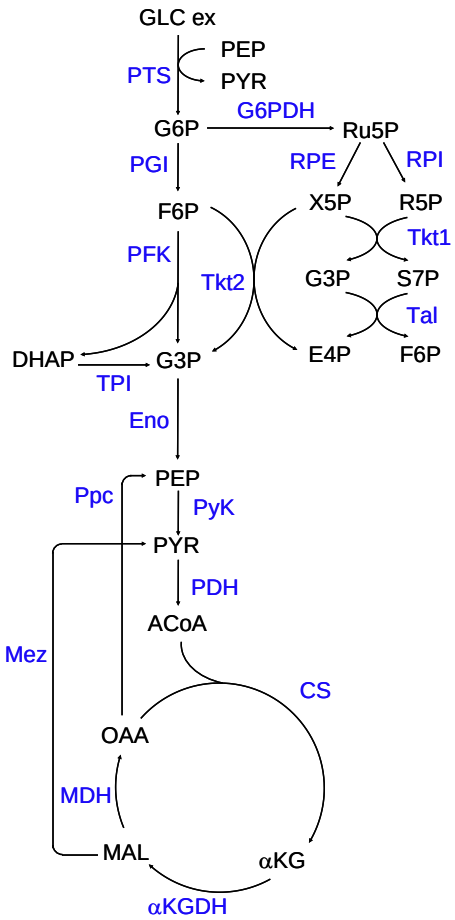
Reactions

Metabolites	R1	R2	R3	R4	R5
Substrat	-1	0	0	0	0
A	1	-1	-1	0	0
B	0	1	0	-1	0
C	0	0	1	0	-1
Product 1	0	0	0	1	0
Product 2	0	0	0	0	1

$$\begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix} = 0 \rightarrow Sv = 0$$

Central carbon metabolism of *E. coli*

"Fluxes in many biological networks cannot be uniquely determined"



Measurable flux

$$
\begin{array}{c}
\\
G6P \\
F6P \\
DHAP \\
G3P \\
PEP \\
PYR \\
Ru5P \\
X5P \\
R5P \\
S7P \\
E4P \\
ACoA \\
\alpha KG \\
MAL \\
OAA
\end{array}
\begin{bmatrix}
1 & -1 & & & & -1 & & & & & & & & & & \\
 & 1 & -1 & & & & & & & & 1 & 1 & & & & \\
 & & 1 & -1 & & & & & & & & & & & & \\
 & & 1 & 1 & -1 & & & & 1 & -1 & 1 & & & & & \\
-1 & & & & 1 & -1 & & & & & & & & & -1 & \\
1 & & & & & 1 & & & & & & -1 & & & & 1 \\
 & & & & & & 1 & -1 & -1 & & & & & & & \\
 & & & & & & & 1 & & -1 & & -1 & & & & \\
 & & & & & & & & 1 & -1 & & & & & & \\
 & & & & & & & & & 1 & -1 & & & & & \\
 & & & & & & & & & & 1 & -1 & & & & \\
 & & & & & & & & & & & & 1 & -1 & & \\
 & & & & & & & & & & & & & 1 & -1 & -1 \\
 & & & & & & & & & & & & -1 & & 1 & 1
\end{bmatrix}
\cdot
\begin{bmatrix}
v_{PTS} \\
v_{PGI} \\
v_{PFK} \\
v_{TPI} \\
v_{Eno} \\
v_{Pyk} \\
v_{G6PDH} \\
v_{RPE} \\
v_{RPI} \\
v_{TK1} \\
v_{Tal} \\
v_{TK2} \\
v_{PDH} \\
v_{CS} \\
v_{\alpha KGDH} \\
v_{MDH} \\
v_{Ppc} \\
v_{Mez}
\end{bmatrix}
= 0
$$

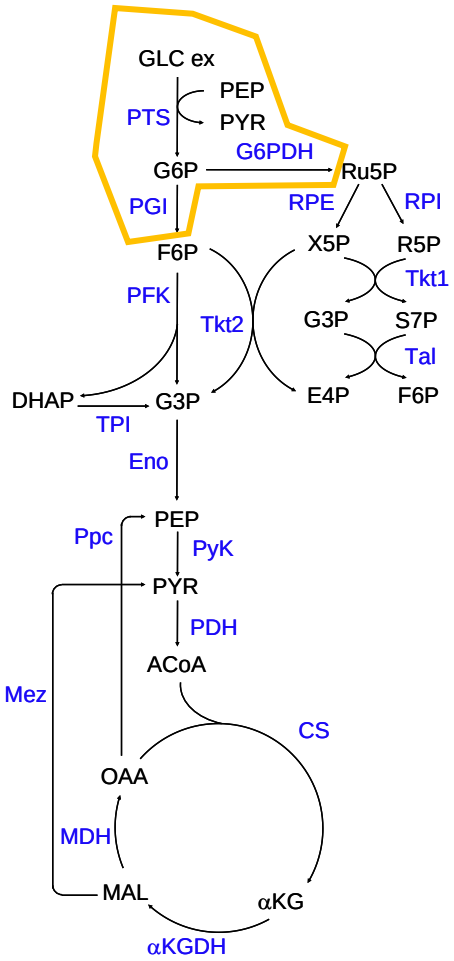
$$\frac{d[F6P]}{dt} = v_{PGI} - v_{PFK} + v_{Tal} + v_{TK2} = 0$$

⋮

18 mass balance equations

Underdetermined Systems

"there are fewer equations than unknowns"



$$V_{PTS} = V_{PGI} + V_{G6PDH}$$

$$10 = V_{PGI} + V_{G6PDH}$$

2 UNKNOWN
1 EQUATION

10 & 0
1 & 9
9 & 1
2 & 8
3 & 7
4 & 6
6 & 4
5 & 5
-100 & 110
1200 & -1190
.
.
.

space of hundreds of feasible solutions

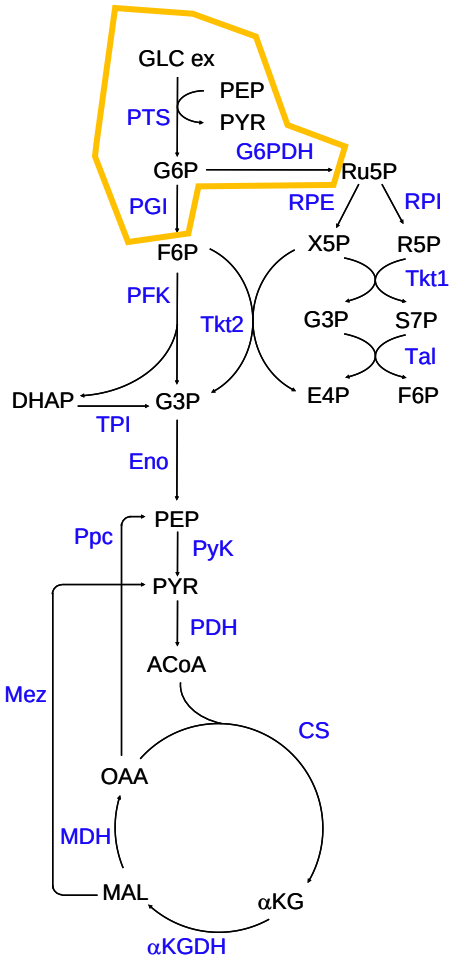
Constraint-based Modeling

Achieving a certain **objective**
in a system
that is shaped by the **defined constraints.**

*There are many different ways from Lausanne to Zurich, but they will all result in reaching Zurich (**objective**) and if I say I should definitely pass through Fribourg & Bern (**defined constraints**) some results will be eliminated.*

Undetermined Systems

"there are fewer equations than unknowns"



$$V_{PTS} = V_{PGI} + V_{G6PDH}$$

$$10 = V_{PGI} + V_{G6PDH}$$

2 UNKNOWN- 1 EQUATION

10 & 0
1 & 9
9 & 1
2 & 8
3 & 7
-20 & 30
6 & 4
5 & 5
-100 & 110
1200 & -1190
.
.
.

Trascriptomics

Thermodynamics

Metabolomics

Fluxomics

space of hundreds of feasible solutions

Flux Balance Analysis

from math to growth

Back to metabolic models ….

Equality constrains:

Stoichiometry:

$$Sv = 0$$

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

Inequality constraints:

Which substrates are available?

Which of them are less abundant?

What can be secreted?

Constraints of metabolism

Equality constrains:

Stoichiometry:

$$Sv = 0$$

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

Inequality constraints:

Uptakes:

$$-10 \leq v_{EX\_glu\_e} \leq 0$$

$$-20 \leq v_{EX\_O2\_e} \leq 0$$

Secretions:

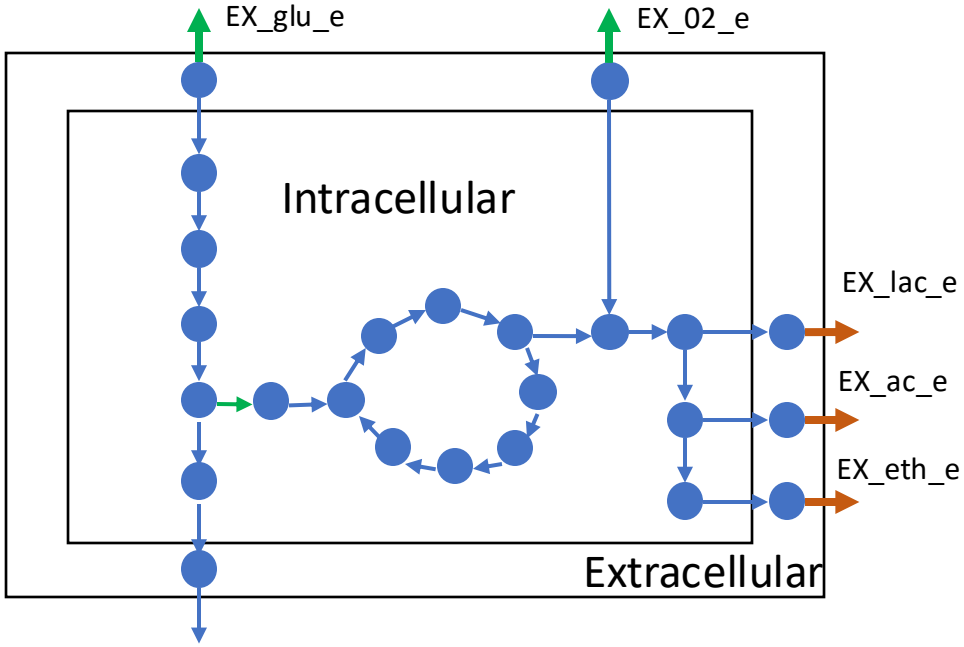
$$0 \leq v_{EX\_lac\_e} \leq 1000$$

$$0 \leq v_{EX\_ac\_e} \leq 1000$$

$$0 \leq v_{EX\_eth\_e} \leq 1000$$

Internal fluxes:

$$10 \leq v_{PYK} \leq 20$$



Constraints of metabolism

Equality constrains:

Stoichiometry:

$$Sv = 0$$

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

Inequality constraints:

Uptakes:

$$-10 \leq v_{EX\_glu\_e} \leq 0$$

$$-20 \leq v_{EX\_O2\_e} \leq 0$$

Secretions:

$$0 \leq v_{EX\_lac\_e} \leq 1000$$

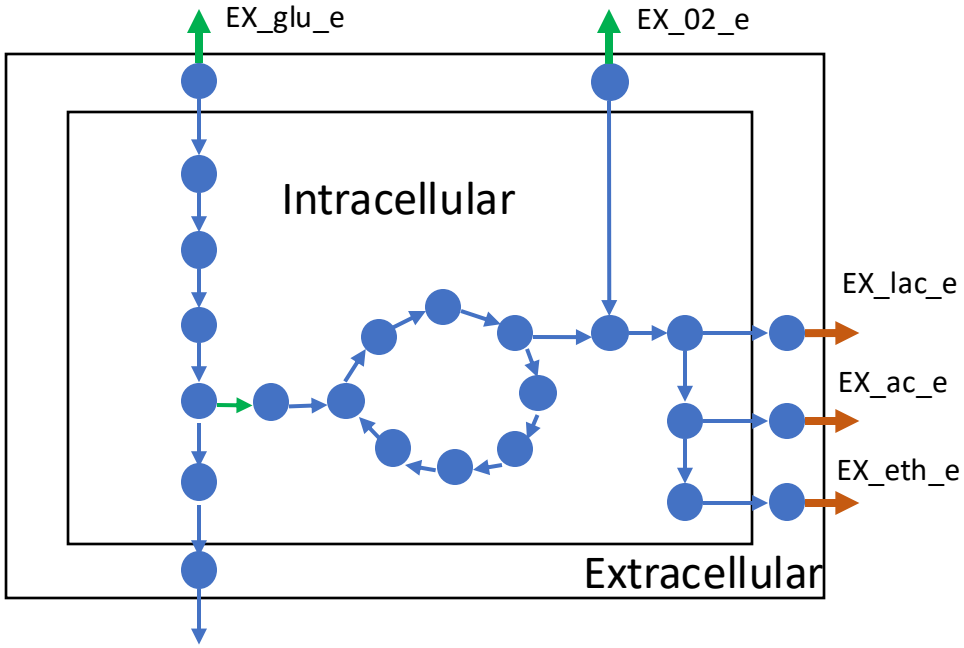
$$0 \leq v_{EX\_ac\_e} \leq 1000$$

$$0 \leq v_{EX\_eth\_e} \leq 1000$$

Internal fluxes:

$$10 \leq v_{PYK} \leq 20$$

If available from data….



Does metabolism have an "*Objective*" ?

Stoichiometry:

$$Sv = 0$$

Uptakes:

$$-10 \leq v_{EX\_glu\_e} \leq 0$$

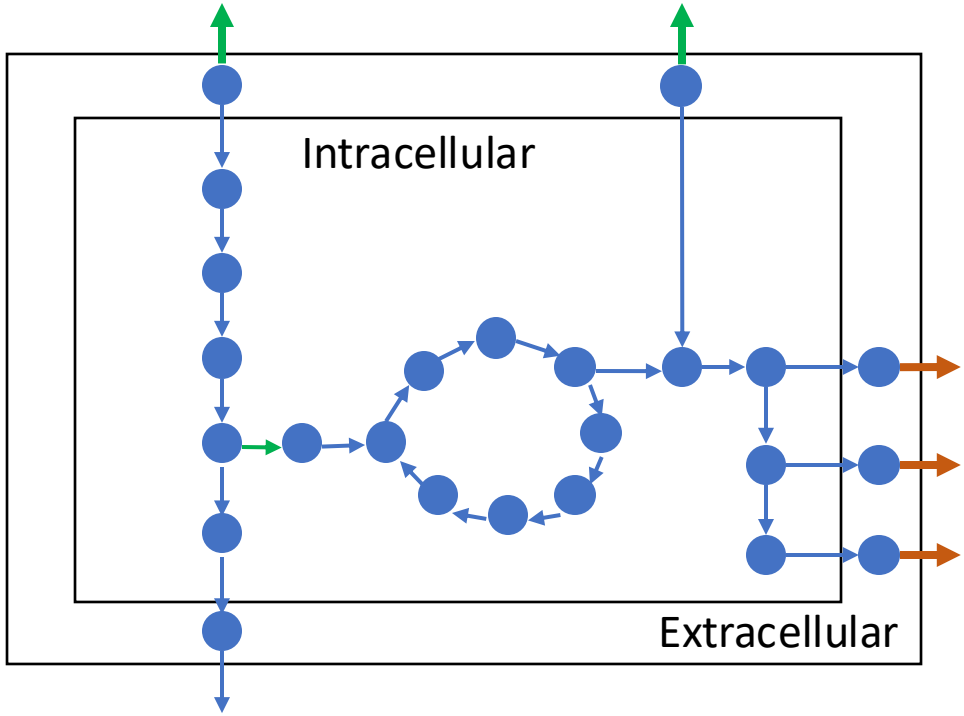
$$-20 \leq v_{EX\_O2\_e} \leq 0$$

Secretions:

$$0 \leq v_{EX\_lac\_e} \leq 1000$$

$$0 \leq v_{EX\_ac\_e} \leq 1000$$

$$0 \leq v_{EX\_eth\_e} \leq 1000$$



What is the objective function?

Does metabolism have an "*Objective*" ?

Stoichiometry:

$$Sv = 0$$

Uptakes:

$$-10 \leq v_{EX\_glu\_e} \leq 0$$

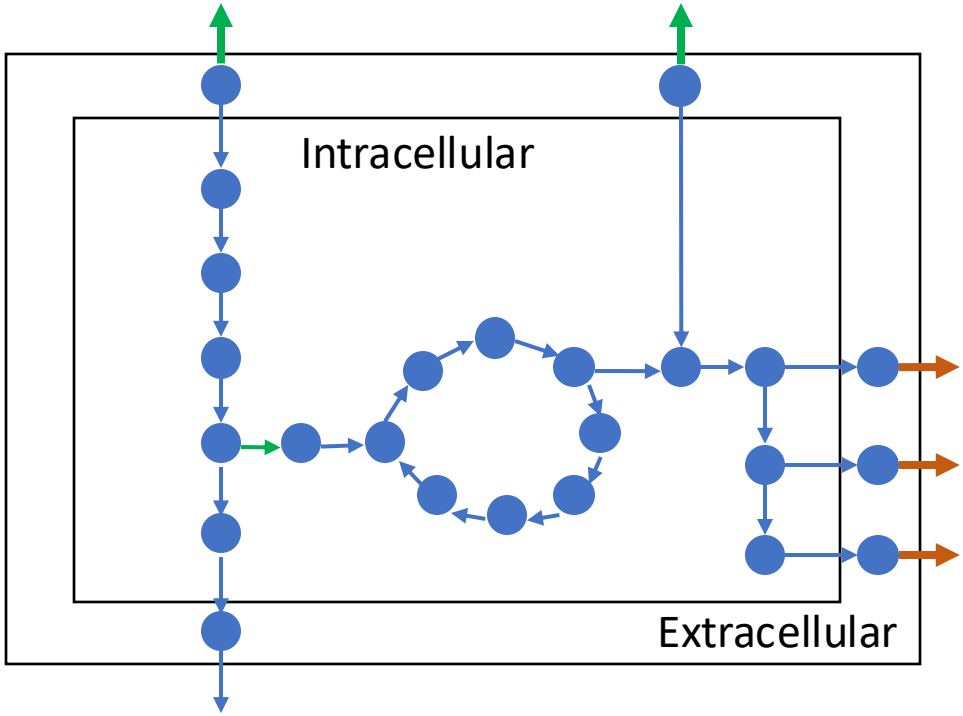
$$-20 \leq v_{EX\_O2\_e} \leq 0$$

Secretions:

$$0 \leq v_{EX\_lac\_e} \leq 1000$$

$$0 \leq v_{EX\_ac\_e} \leq 1000$$

$$0 \leq v_{EX\_eth\_e} \leq 1000$$



What is the objective function?

Evolutionary objective: Utilize **minimal** necessary **resources** for **maximum "growth"**

Does metabolism have an "*Objective*" ?

What is growth?

Does metabolism have an "*Objective*" ?

What is growth?

… doubling time

… maximum theoretical growth rate given a maximum theoretical uptake rate

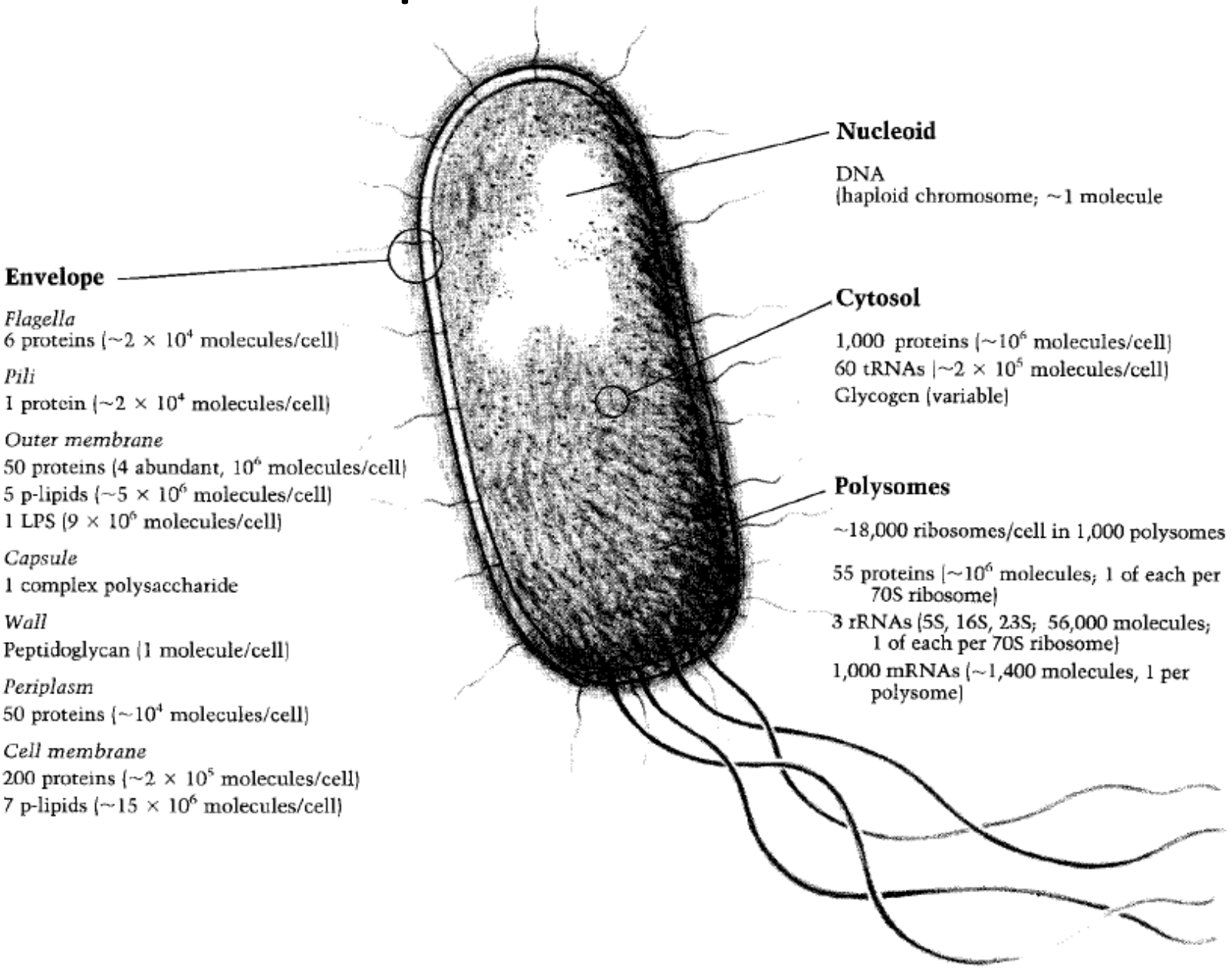
… specific growth rate, yield

Phase diagram … slope of log growth

Is maximization of growth an objective of the cell?
(Darwin…)

How to define growth in the model?

Biomass composition



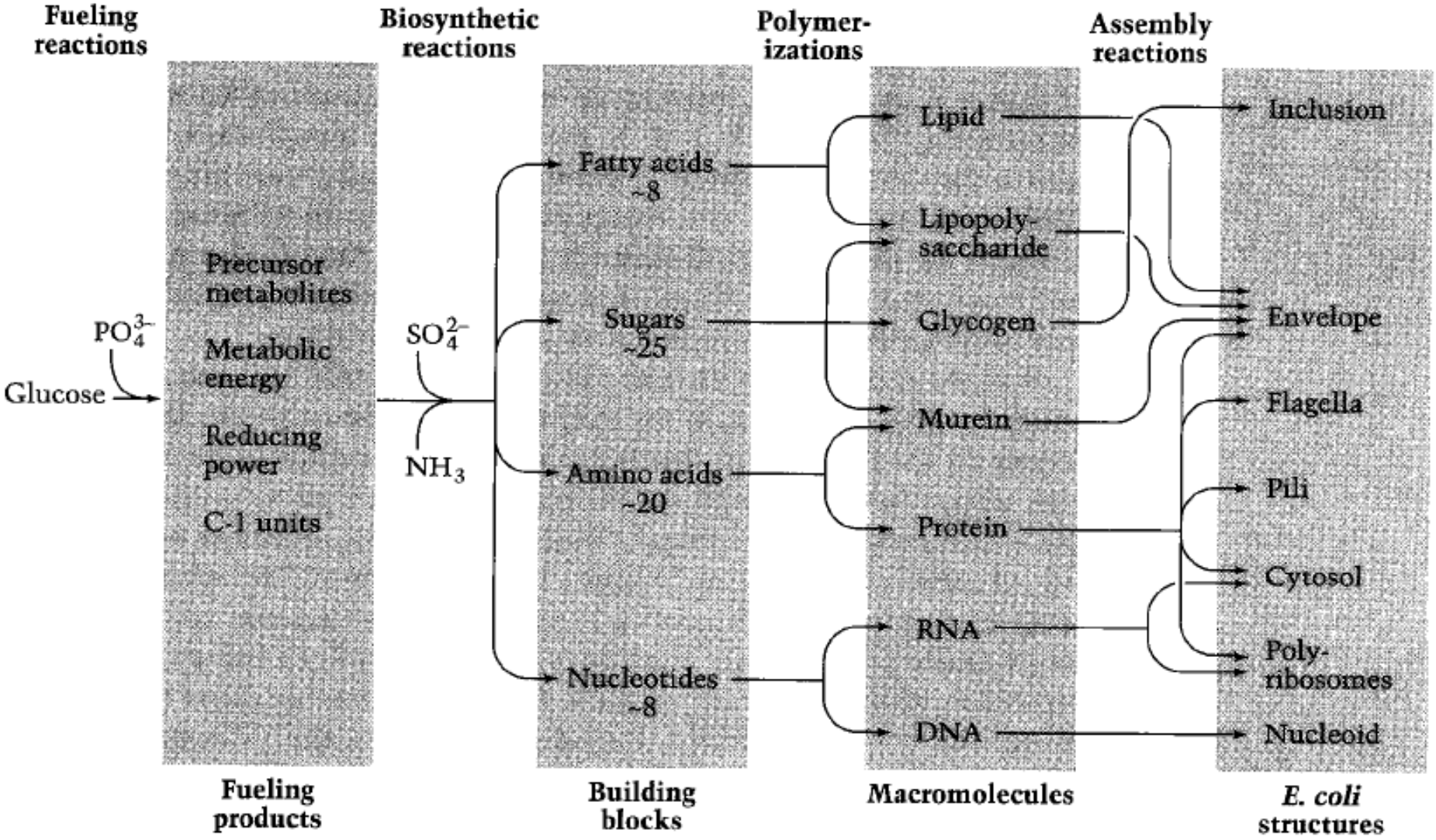


Figure 1

Overview of metabolism leading to the chemical synthesis from glucose of a chemoheterotroph like *E. coli*.

Table 3. Costs of biosynthesis of cellular components from the precursor metabolites

Cellular component	Energy cost[a] (μmole ~P/g cells)	Reducing power cost (μmole NADPH/g cells)
Protein	7,287	11,523
RNA	6,540	427
DNA	1,090	200
Lipid	2,578	5,270
LPS	470	564
Murein	248	193
Glycogen	154	0
1-Carbon	0	48
Polyamines	118	0
Total	18,485	18,225

[a]Each nucleoside triphosphate is assumed to be made by consecutive reactions with ATP that consume 3 ~P per NTP produced. Formation of sugar–nucleotide derivatives are assumed to occur by direct reaction with the appropriate nucleoside triphosphate.

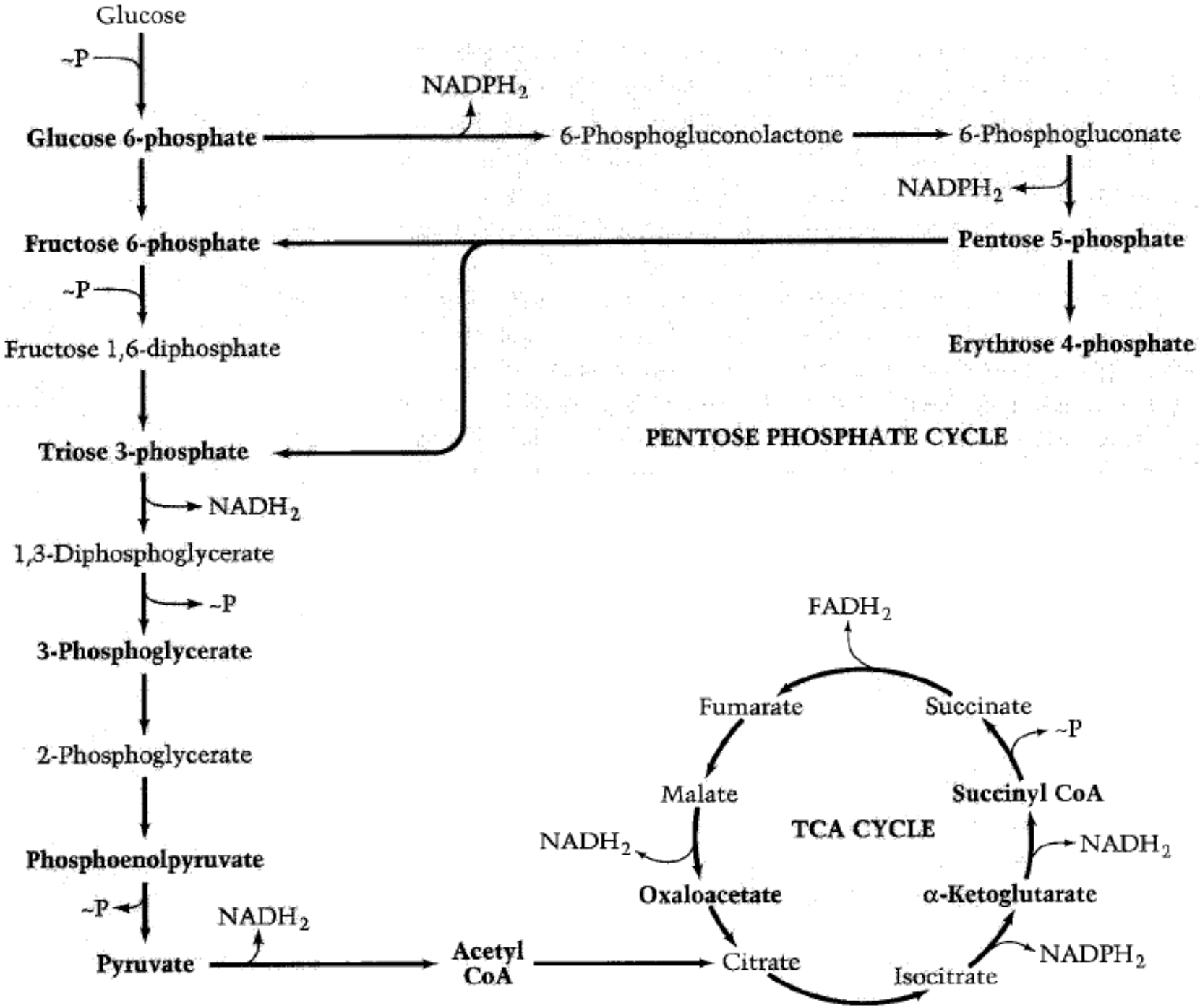
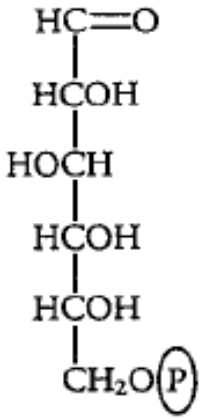
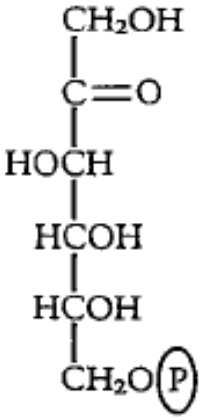

Glucose
~P
Glucose 6-phosphate
NADPH2
6-Phosphogluconolactone
6-Phosphogluconate
NADPH2
Fructose 6-phosphate
Pentose 5-phosphate
~P
Fructose 1,6-diphosphate
Erythrose 4-phosphate
Triose 3-phosphate
PENTOSE PHOSPHATE CYCLE
NADH2
1,3-Diphosphoglycerate
~P
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
~P
NADH2
Pyruvate
Acetyl CoA
EMP PATHWAY
FADH2
Fumarate
Succinate
~P
Malate
Succinyl CoA
NADH2
TCA CYCLE
NADH2
Oxaloacetate
α-Ketoglutarate
Citrate
NADPH2
Isocitrate

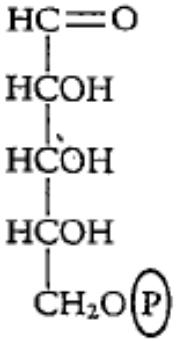
Figure 4



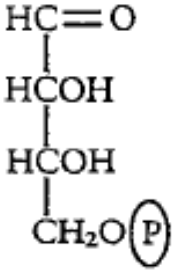
Glucose 6-phosphate



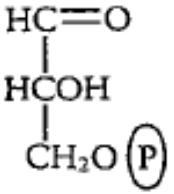
Fructose 6-phosphate



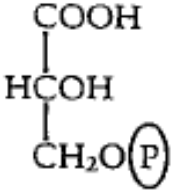
Ribose 5-phosphate



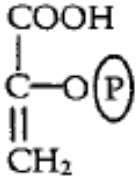
Erythrose 4-phosphate



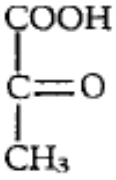
Triose phosphate



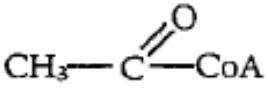
3-Phosphoglycerate



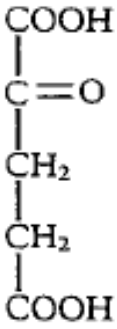
Phosphoenolpyruvate



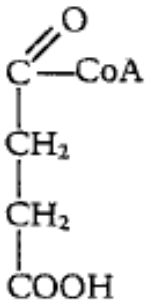
Pyruvate



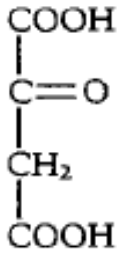
Acetyl CoA



α-Ketoglutarate



Succinyl CoA



Oxaloacetate

Figure 1

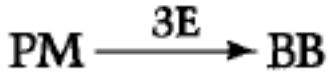
Structures of the 12 precursor metabolites.

Biosynthetic pathways

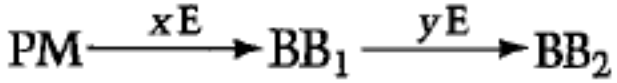
Biosynthetic pathways differ markedly in complexity—some are linear, others branch or are interconnected. A simple pathway, consisting of three sequential enzymatic reactions, might be represented as

$$\underset{(PM)}{\text{Precursor metabolite}} \xrightarrow[(E_1)]{\text{Enzyme 1}} \text{A} \xrightarrow[(E_2)]{\text{Enzyme 2}} \text{B} \xrightarrow[(E_3)]{\text{Enzyme 3}} \underset{(BB)}{\text{Building block}}$$

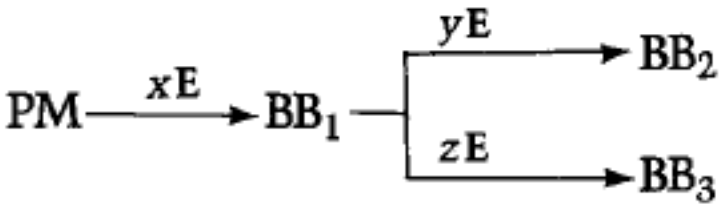
where A and B are intermediate products in the pathway. Recognizing



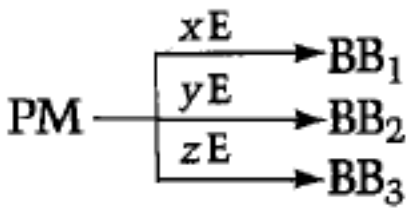
Some pathways produce a building block that, in turn, is converted by a second pathway into another building block:



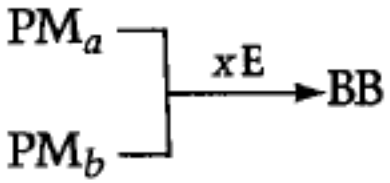
where x and y represent the number of enzymes in the two pathways. In some cases the pathway is branched:



Most of the 12 precursor metabolites actually serve as the starting point for several pathways:



Finally, in many cases more than one precursor molecule is involved in the biosynthesis of a building block:



Branching and interlocking of this sort are common among biosynthetic pathways. Building blocks that are produced from a common precursor are called a FAMILY. The ASPARTATE FAMILY, for example, consists of seven amino acids (asparagine, aspartate, diaminopimelate, isoleucine, lysine, methionine, and threonine) that are synthesized from the common precursor metabolite oxaloacetate (Figure 2).

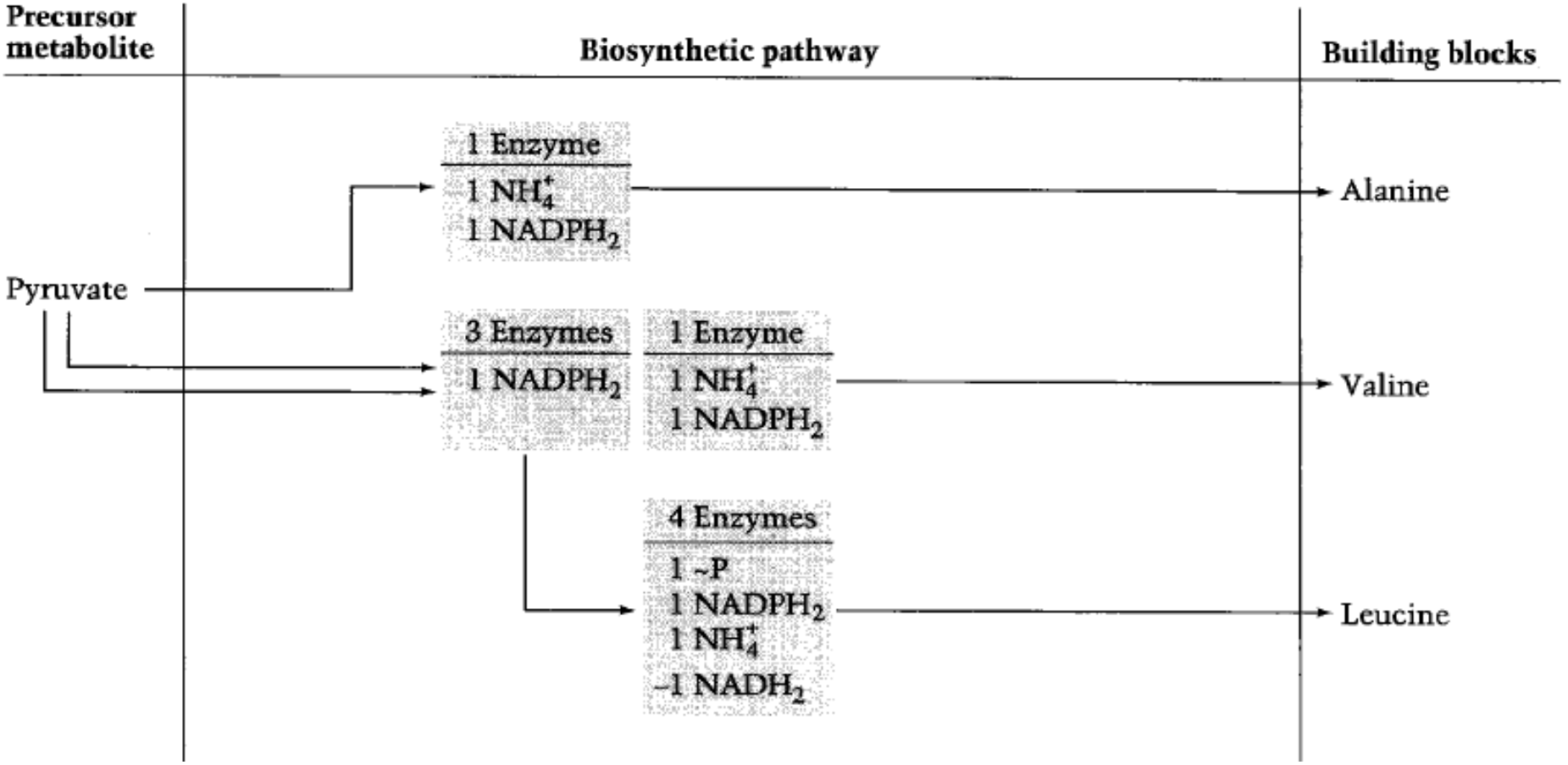
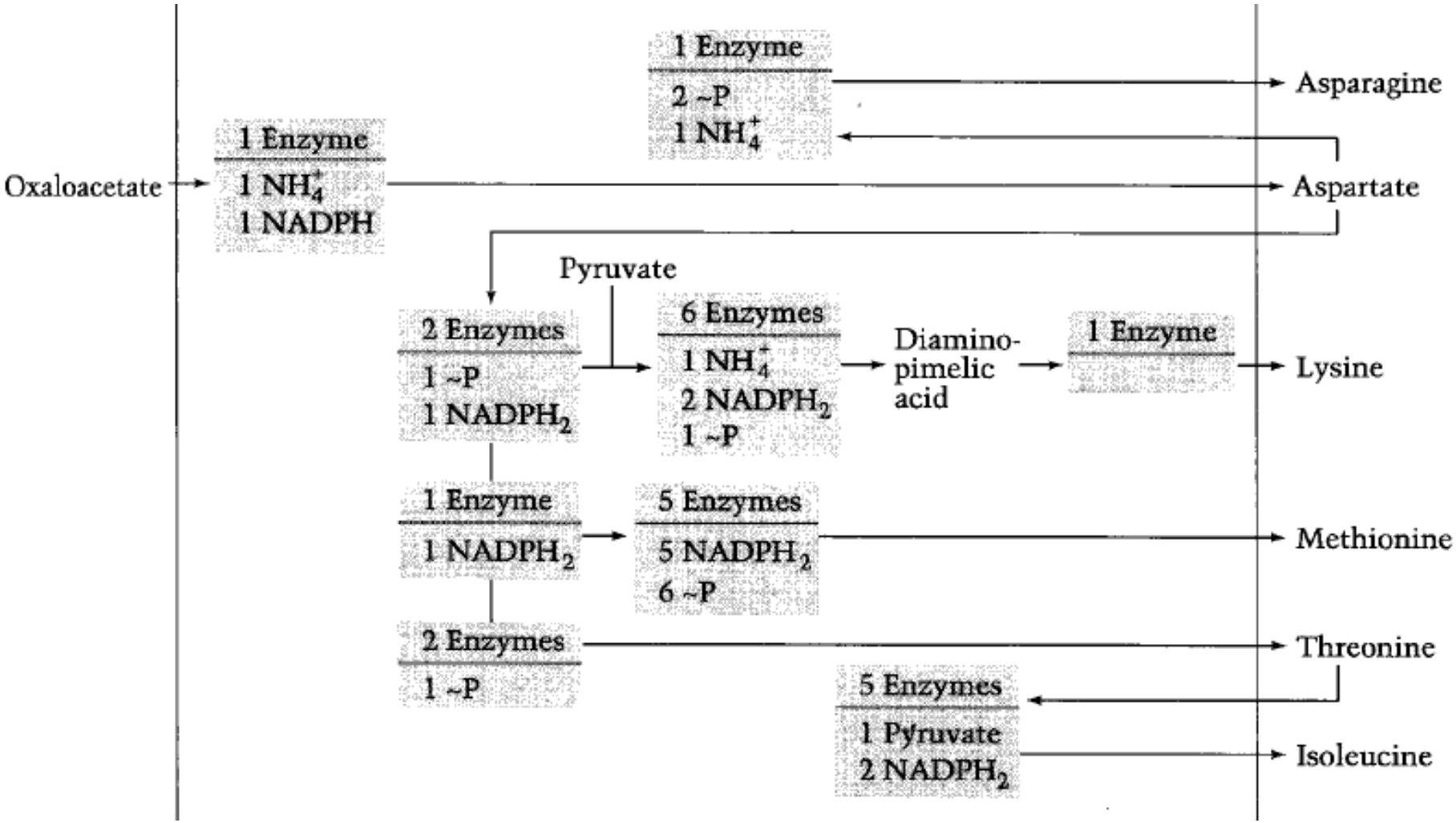
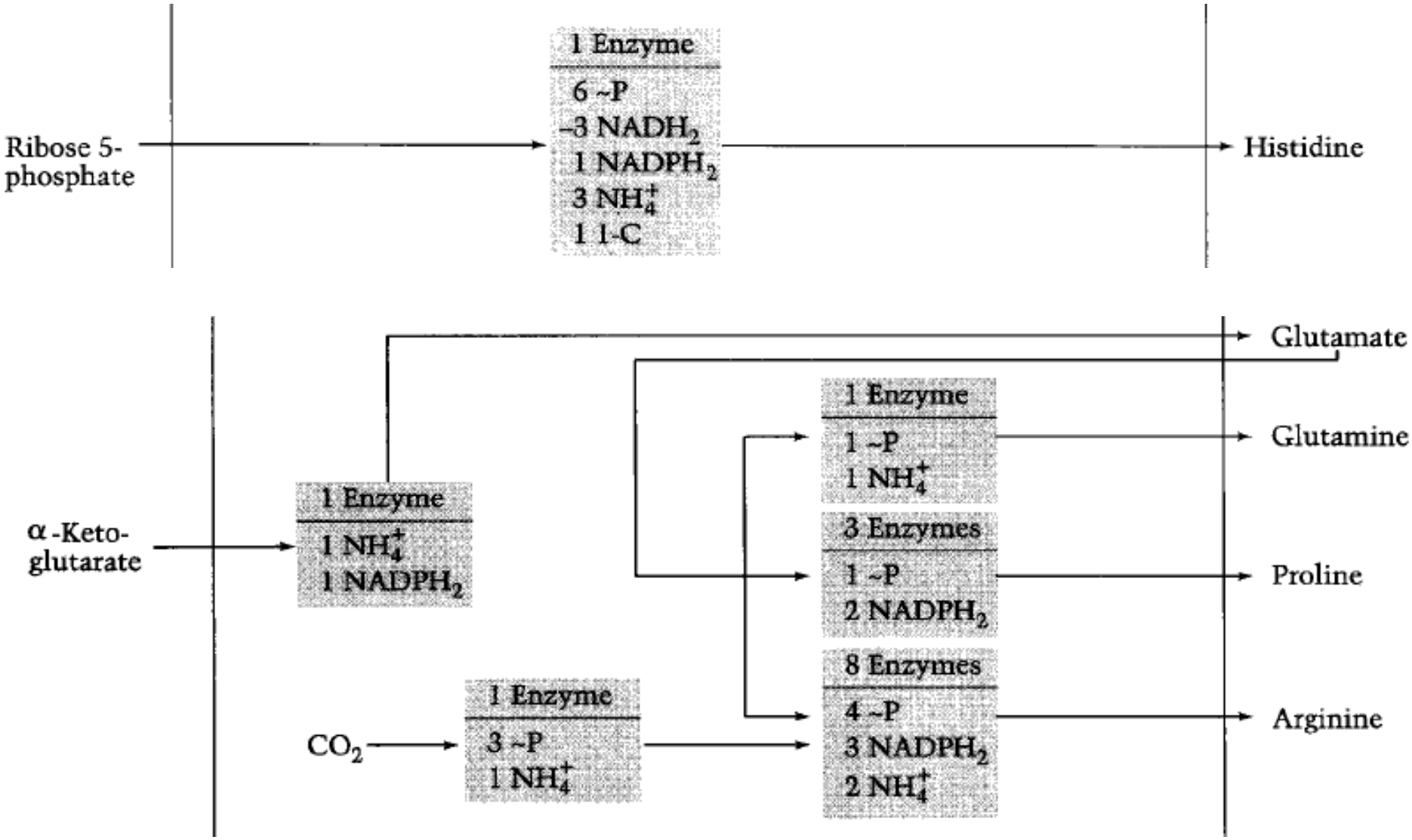
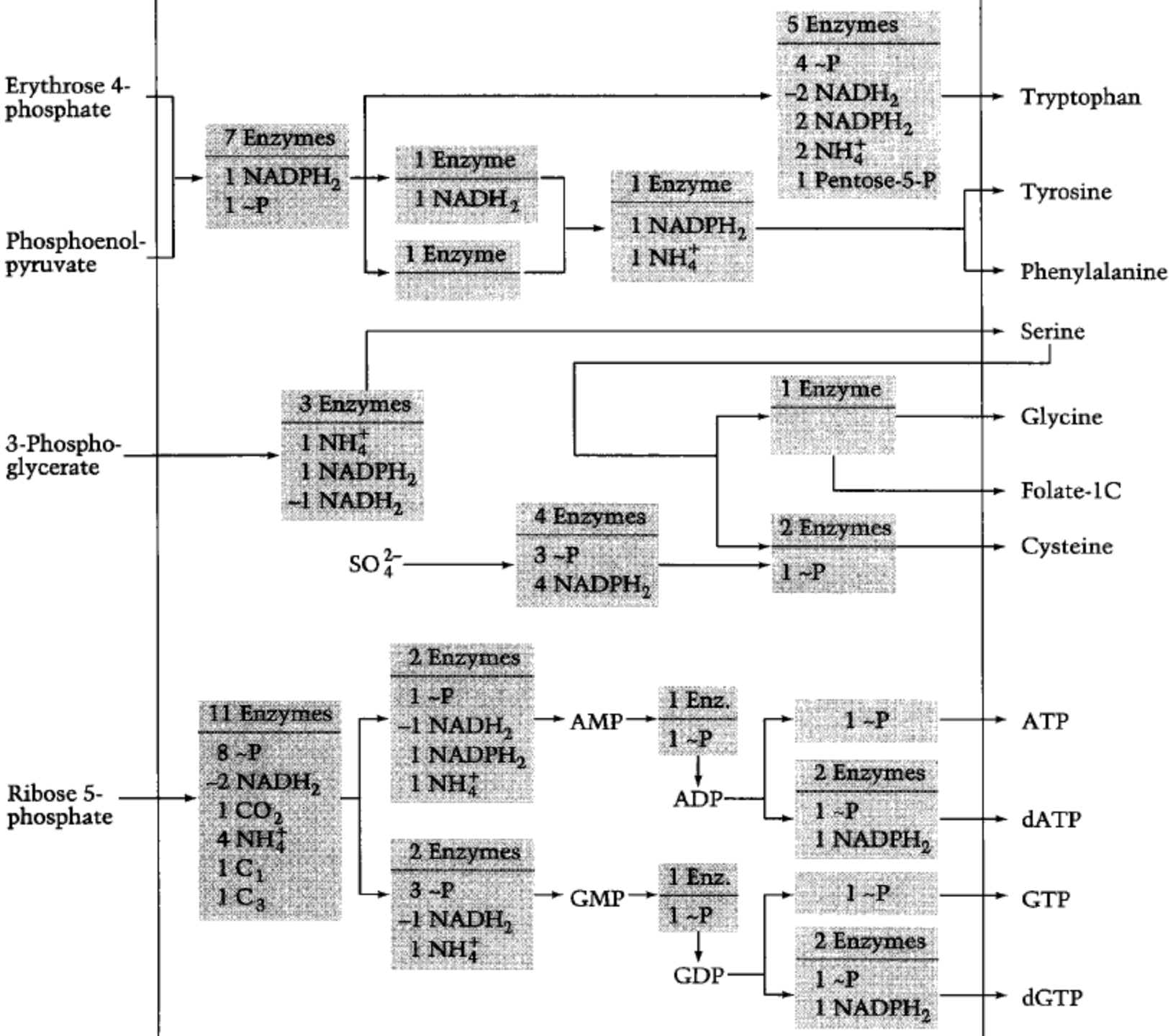

Precursor metabolite
Biosynthetic pathway
Building blocks
1 Enzyme
1 NH_4^+
1 $NADPH_2$
Alanine
Pyruvate
3 Enzymes
1 $NADPH_2$
1 Enzyme
1 NH_4^+
1 $NADPH_2$
Valine
4 Enzymes
1 ~P
1 $NADPH_2$
1 NH_4^+
–1 $NADH_2$
Leucine

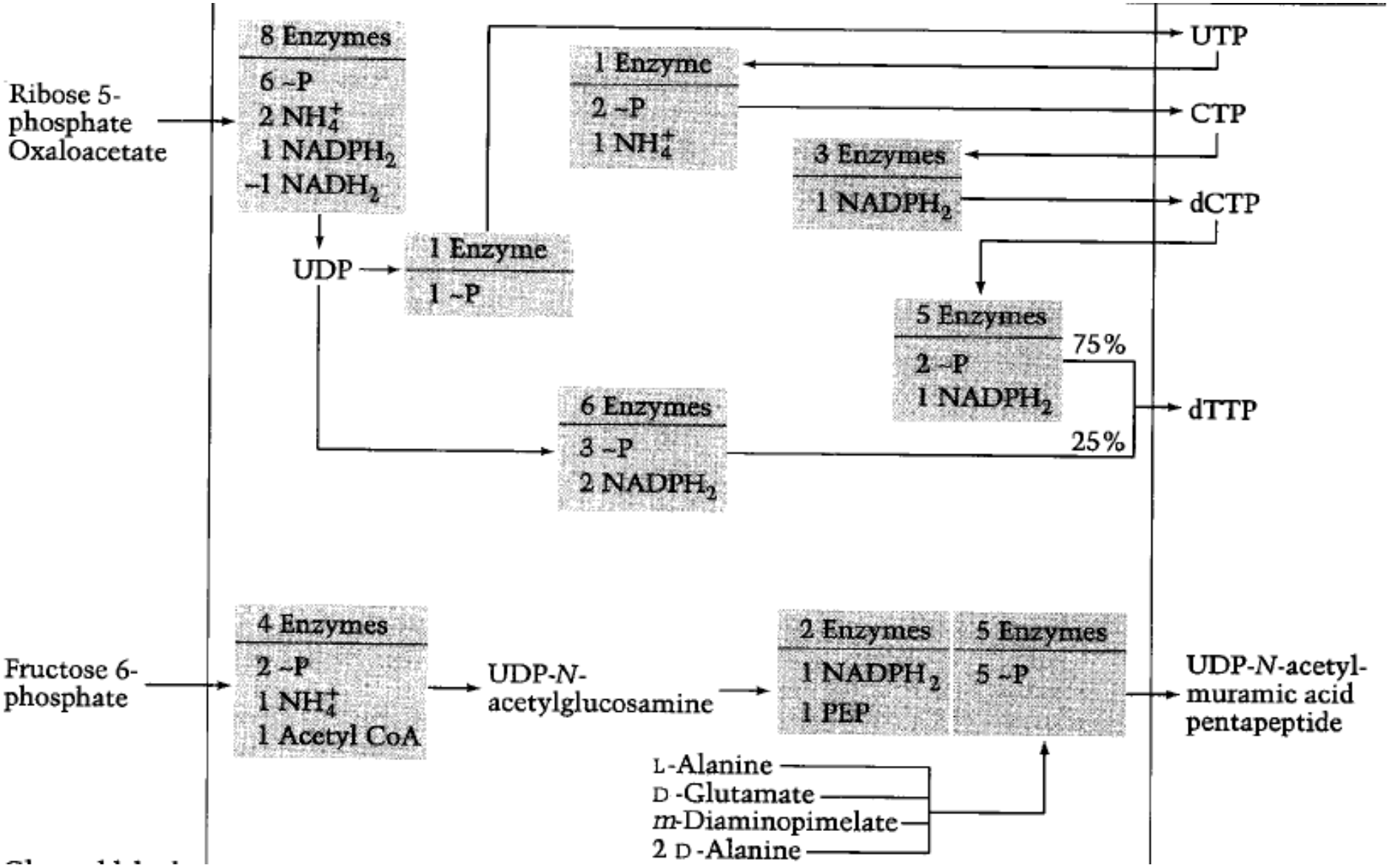
Table 1. Building blocks needed to produce 1 g of *E. coli* protoplasm

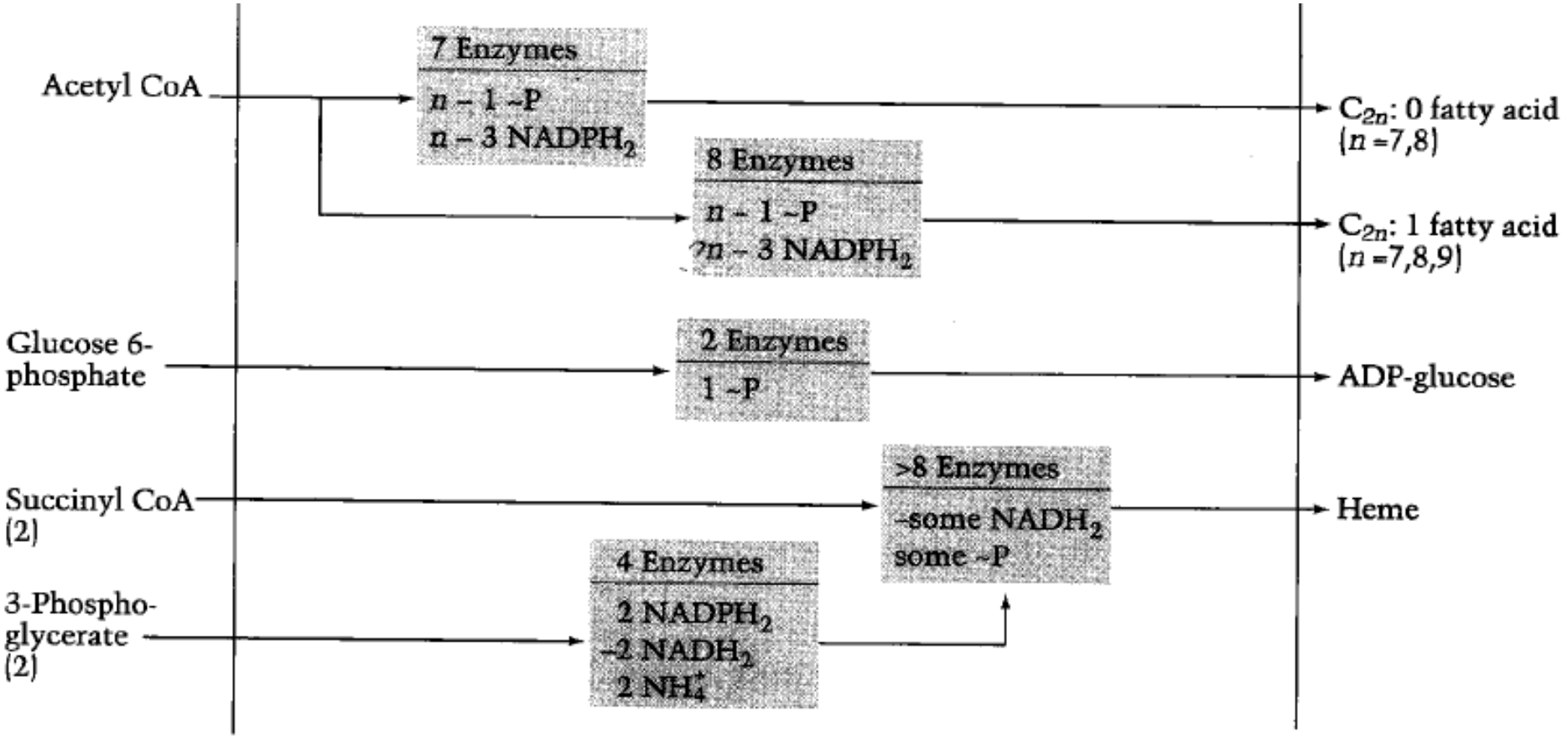
Building block	Amount present in *E. coli* B/r (μmol/g dried cells)	Cost of making 1 μmol of each of these building blocks (μmol/μmol)						
		Metabolites[a]	ATP	NADH	NADPH	1-C	NH_4^+	S
Protein amino acids								
Alanine	488	1 pyr	0	0	1	0	1	0
Arginine	281	1 αkg	7	−1	4	0	4	0
Asparagine	229	1 oaa	3	0	1	0	2	0
Aspartate	229	1 oaa	0	0	1	0	1	0
Cysteine	87	1 pga	4	−1	5	0	1	1
Glutamate	250	1 αkg	0	0	1	0	1	0
Glutamine	250	1 αkg	1	0	1	0	2	0
Glycine	582	1 pga	0	−1	1	−1	1	0
Histidine	90	1 penP	6	−3	1	1	3	0
Isoleucine	276	1 oaa, 1 pyr	2	0	5	0	1	0
Leucine	428	2 pyr, 1 acCoA	0	−1	2	0	1	0
Lysine	326	1 oaa, 1 pyr	2	0	4	0	2	0
Methionine	146	1 oaa	7	0	8	1	1	1
Phenylalanine	176	1 eryP, 2 pep	1	0	2	0	1	0
Proline	210	1 αkg	1	0	3	0	1	0
Serine	205	1 pga	0	−1	1	0	1	0
Threonine	241	1 oaa	2	0	3	0	1	0
Tryptophan	54	1 penP, 1 eryP, 1 pep	5	−2	3	0	2	0
Tyrosine	131	1 eryP, 2 pep	1	−1	2	0	1	0
Valine	402	2 pyr	0	0	2	0	1	0


1 Enzyme
2 ~P
1 NH_4^+
Asparagine
Oxaloacetate
1 Enzyme
1 NH_4^+
1 NADPH
Aspartate
Pyruvate
2 Enzymes
1 ~P
1 $NADPH_2$
6 Enzymes
1 NH_4^+
2 $NADPH_2$
1 ~P
Diamino-
pimelic
acid
1 Enzyme
Lysine
1 Enzyme
1 $NADPH_2$
5 Enzymes
5 $NADPH_2$
6 ~P
Methionine
2 Enzymes
1 ~P
Threonine
5 Enzymes
1 Pyruvate
2 $NADPH_2$
Isoleucine


Ribose 5-phosphate
1 Enzyme
6 ~P
−3 NADH2
1 NADPH2
3 NH4+
1 1-C
Histidine
α-Keto-glutarate
1 Enzyme
1 NH4+
1 NADPH2
Glutamate
1 Enzyme
1 ~P
1 NH4+
Glutamine
3 Enzymes
1 ~P
2 NADPH2
Proline
CO2
1 Enzyme
3 ~P
1 NH4+
8 Enzymes
4 ~P
3 NADPH2
2 NH4+
Arginine


Erythrose 4-phosphate
Phosphoenol-pyruvate
7 Enzymes
1 NADPH2
1 ~P
1 Enzyme
1 NADH2
1 Enzyme
1 Enzyme
1 NADPH2
1 NH4+
5 Enzymes
4 ~P
–2 NADH2
2 NADPH2
2 NH4+
1 Pentose-5-P
Tryptophan
Tyrosine
Phenylalanine
Serine
3-Phospho-glycerate
3 Enzymes
1 NH4+
1 NADPH2
–1 NADH2
1 Enzyme
Glycine
Folate-1C
SO4 2–
4 Enzymes
3 ~P
4 NADPH2
2 Enzymes
1 ~P
Cysteine
Ribose 5-phosphate
11 Enzymes
8 ~P
–2 NADH2
1 CO2
4 NH4+
1 C1
1 C3
2 Enzymes
1 ~P
–1 NADH2
1 NADPH2
1 NH4+
AMP
1 Enz.
1 ~P
ADP
1 ~P
ATP
2 Enzymes
1 ~P
1 NADPH2
dATP
2 Enzymes
3 ~P
–1 NADH2
1 NH4+
GMP
1 Enz.
1 ~P
GDP
1 ~P
GTP
2 Enzymes
1 ~P
1 NADPH2
dGTP

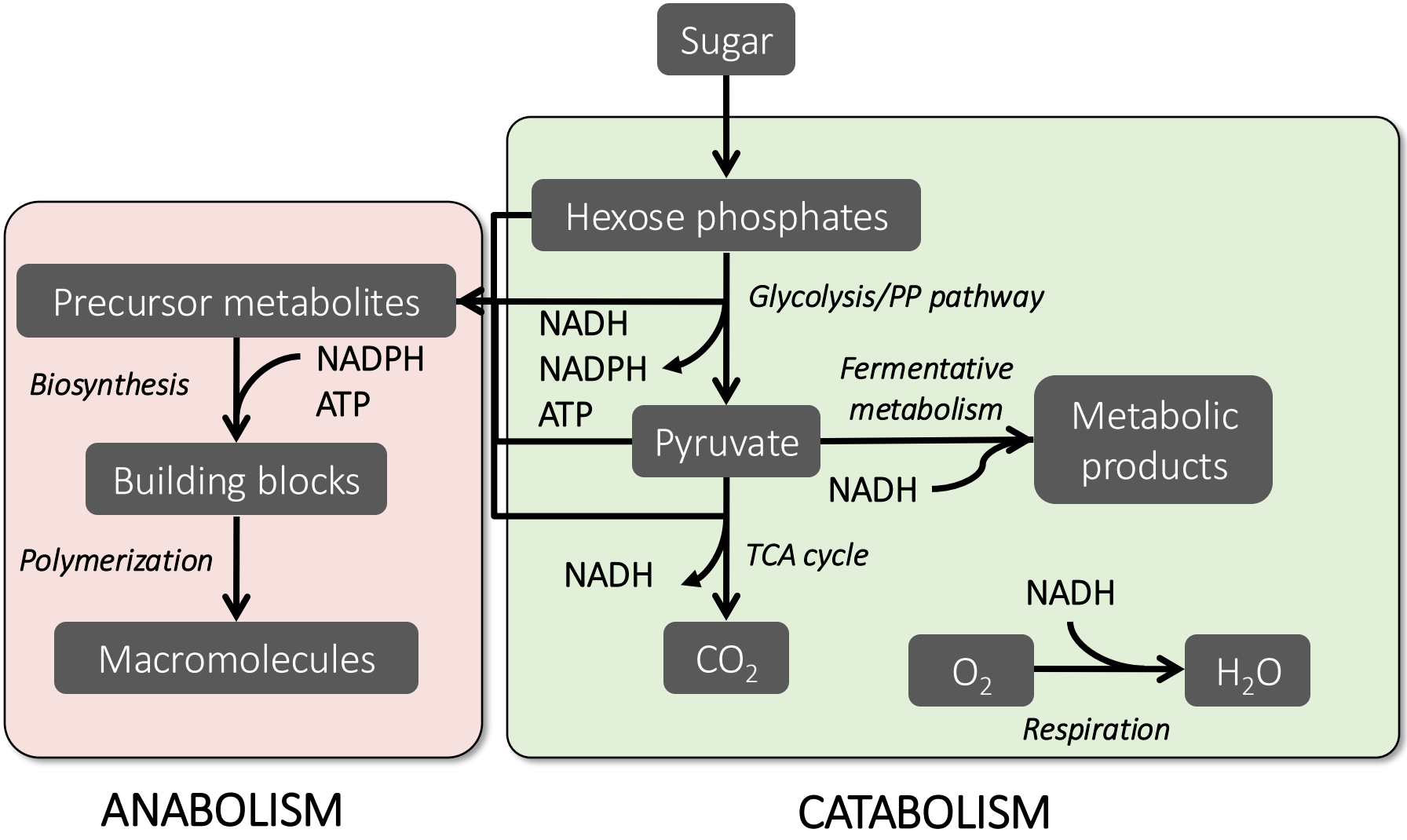

Ribose 5-phosphate
Oxaloacetate
8 Enzymes
6 ~P
2 NH_4^+
1 $NADPH_2$
−1 $NADH_2$
UDP
1 Enzyme
1 ~P
1 Enzyme
2 ~P
1 NH_4^+
UTP
CTP
3 Enzymes
1 $NADPH_2$
dCTP
5 Enzymes
2 ~P
1 $NADPH_2$
75%
25%
dTTP
6 Enzymes
3 ~P
2 $NADPH_2$
Fructose 6-phosphate
4 Enzymes
2 ~P
1 NH_4^+
1 Acetyl CoA
UDP-*N*-acetylglucosamine
2 Enzymes
1 $NADPH_2$
1 PEP
5 Enzymes
5 ~P
UDP-*N*-acetyl-muramic acid pentapeptide
L-Alanine
D-Glutamate
m-Diaminopimelate
2 D-Alanine


Acetyl CoA
7 Enzymes
n – 1 ~P
n – 3 NADPH2
C2n: 0 fatty acid
(n =7,8)
8 Enzymes
n – 1 ~P
2n – 3 NADPH2
C2n: 1 fatty acid
(n =7,8,9)
Glucose 6-phosphate
2 Enzymes
1 ~P
ADP-glucose
Succinyl CoA (2)
>8 Enzymes
~some NADH2
some ~P
Heme
3-Phospho-glycerate (2)
4 Enzymes
2 NADPH2
–2 NADH2
2 NH4+

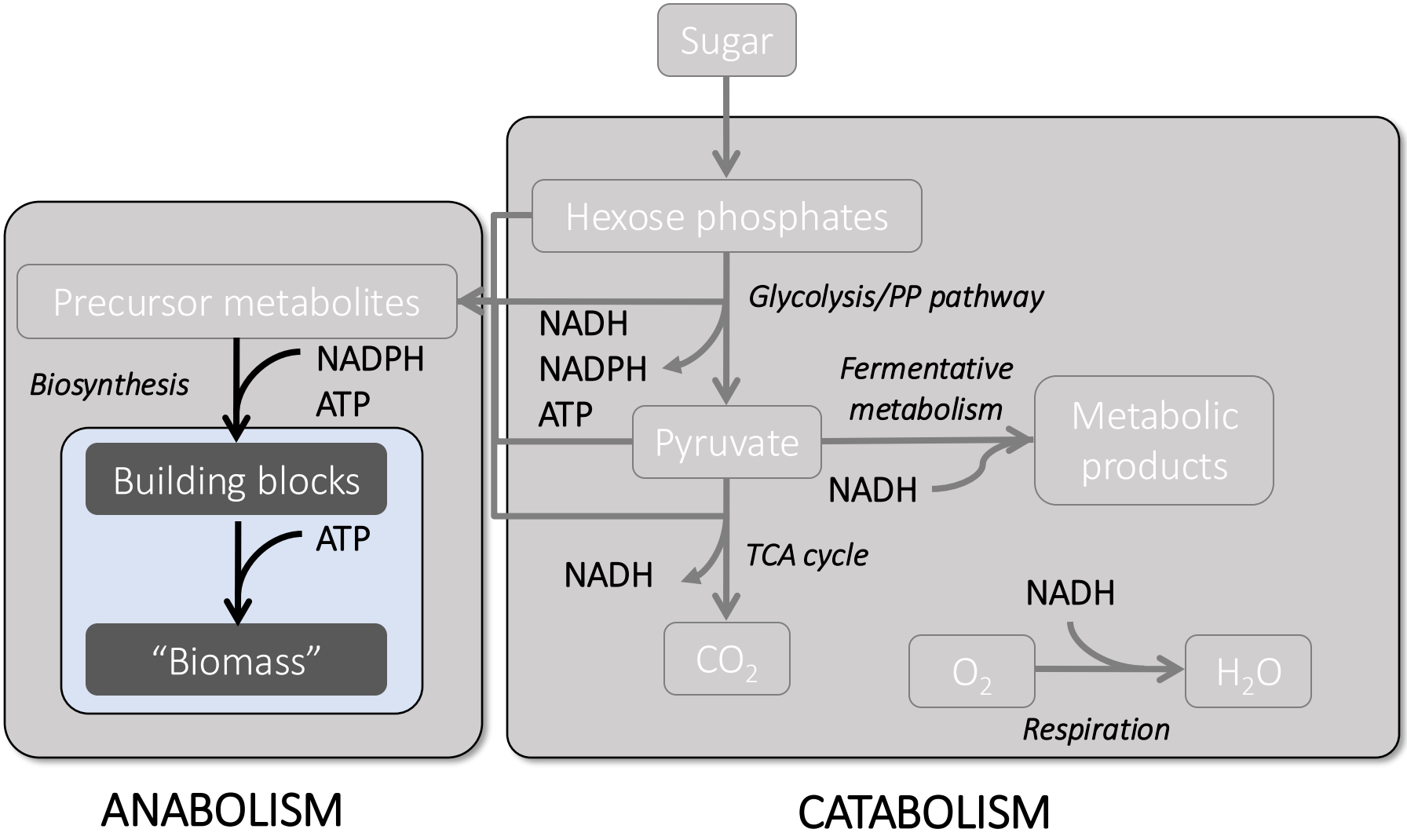
RNA nucleotides								
ATP	165	1 penP, 1 pga	11	−3	1	1	5	0
GTP	203	1 penP, 1 pga	13	−3	0	1	5	0
CTP	126	1 penP, 1 oaa	9	0	1	0	3	0
UTP	136	1 penP, 1 oaa	7	0	1	0	2	0
DNA nucleotides								
dATP	24.7	1 penP, 1 pga	11	−3	2	1	5	0
dGTP	25.4	1 penP, 1 pga	13	−3	1	1	5	0
dCTP	25.4	1 penP, 1 oaa	9	0	2	0	3	0
dTTP	24.7	1 penP, 1 oaa	10.5	0	3	1	2	0
Lipid components								
Glycerol phosphate	129	1 triosP	0	0	1	0	0	0
Serine	129	1 pga	0	−1	1	0	1	0
$C_{16:0}$ fatty acid (43%)		8 acCoA	7	0	14	0	0	0
$C_{16:1}$ fatty acid (33%)		8 acCoA	7	0	13	0	0	0
$C_{18:1}$ fatty acid (24%)		9 acCoA	8	0	15	0	0	0
Average fatty acid	258	8.2 acCoA	7.2	0	14	0	0	0

Building block	Amount present in *E. coli* B/r (μmol/g dried cells)	Cost of making 1 μmol of each of these building blocks (μmol/μmol)						
		Metabolites[a]	ATP	NADH	NADPH	1-C	NH_4^+	S
LPS components								
UDP-glucose	15.7	1 gluP	1	0	0	0	0	0
(CDP) ethanolamine	23.5	1 pga	3	−1	1	0	1	0
OH-myristic acid	23.5	7 acCoA	6	0	11	0	0	0
$C_{14:0}$ fatty acid	23.5	7 acCoA	6	0	12	0	0	0
(CMP) KDO	23.5	1 penP, 1 pep	2	0	0	0	0	0
(NDP) heptose	23.5	1.5 gluP	1	0	−4	0	0	0
(TDP) glucosamine	15.7	1 fruP	2	0	0	0	1	0
Peptidoglycan monomers								
UDP-*N*-acetylglucosamine	27.6	1 fruP, 1 acCoA	3	0	0	0	1	0
UDP-*N*-acetylmuramic acid	27.6	1 fruP, 1 pep, 1 acCoA	4	0	1	0	1	0
Alanine	55.2	1 pyr	0	0	1	0	1	0
Diaminopimelate	27.6	1 oaa, 1 pyr	2	0	3	0	2	0
Glutamate	27.6	1 αkg	0	0	1	0	1	0
Glycogen monomers								
Glucose	154	1 gluP	1	0	0	0	0	0
1-Carbon requirement								
Serine	48.5	1 pga	0	−1	1	0	0	0
Polyamines								
Ornithine equivalents	59.3	1 αkg	2	0	3	0	2	0

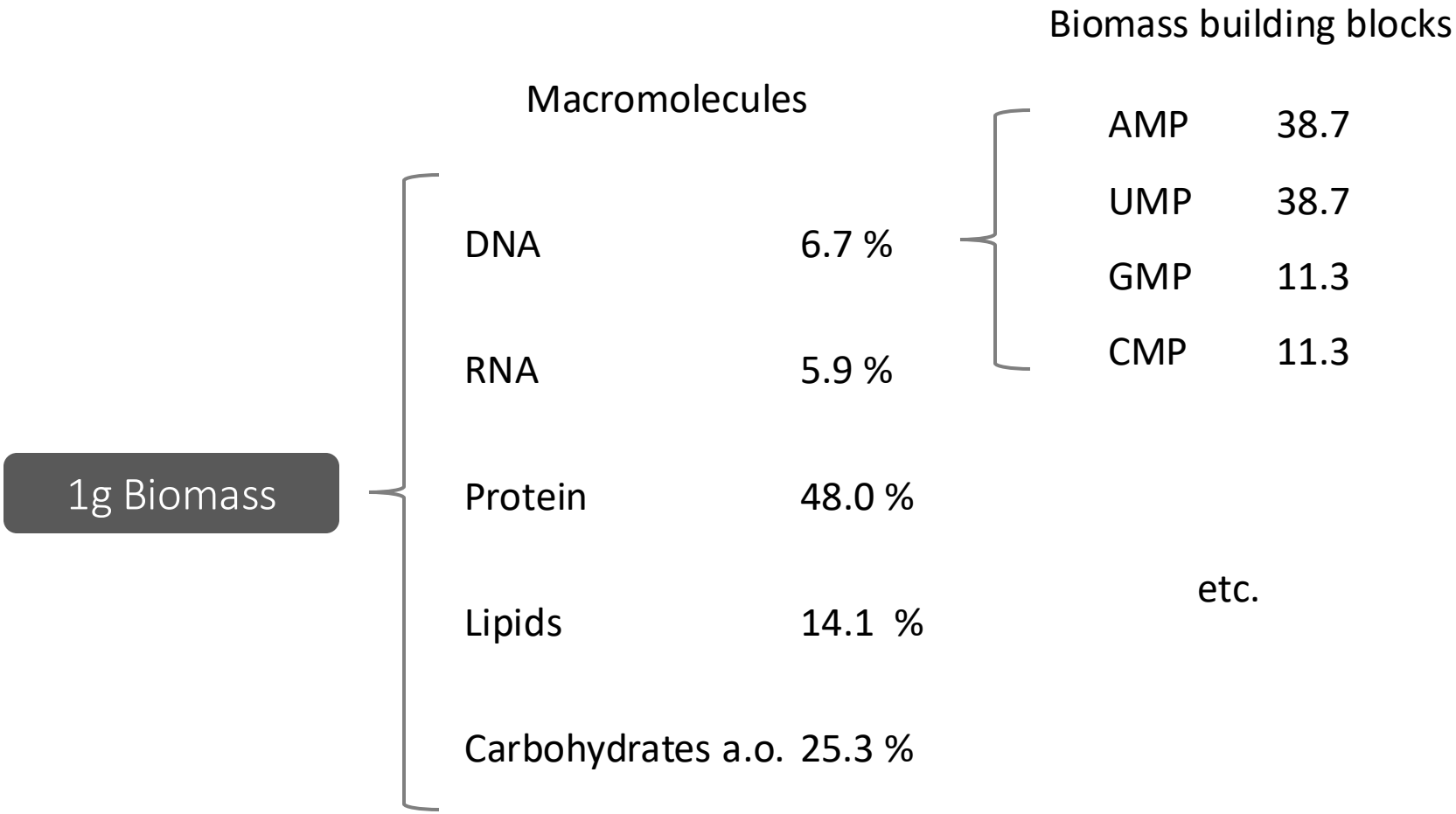
The biomass reactions



The biomass reactions

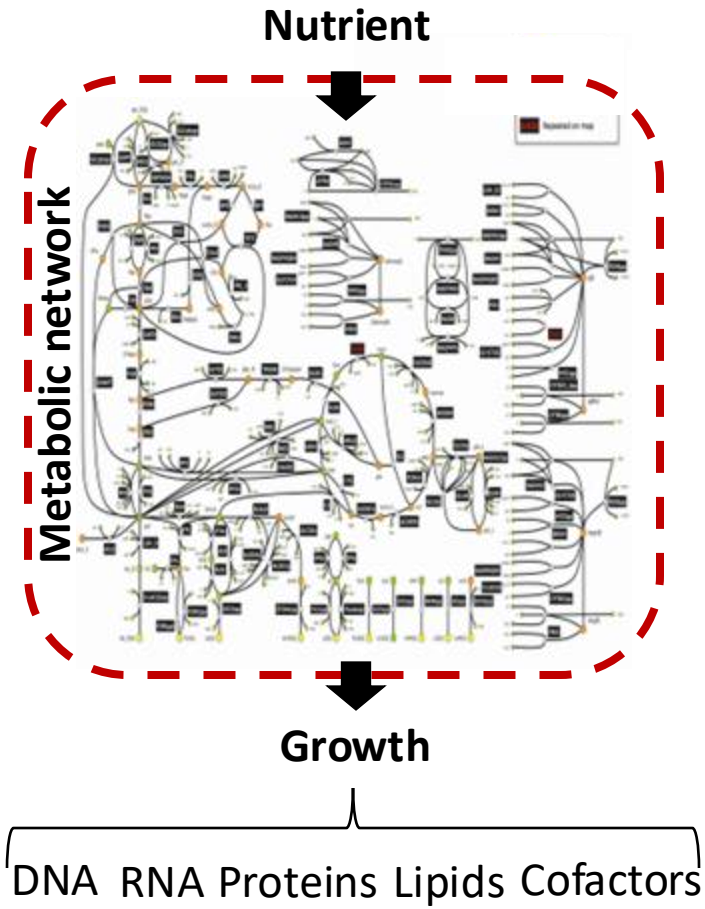


The biomass reactions



Data for toxoplasma :Thomas Forth PhD thesis, Sept 2012

The biomass reactions



- Organism-specific models
- Start from sequenced genome
- Correlate genome with molecular physiology

THE RAVEN TOOLBOX

Malaria Parasite Metabolic Pathways

Defining the growth problem

Equality constrains:

$$Sv = 0$$

Inequality constrains:

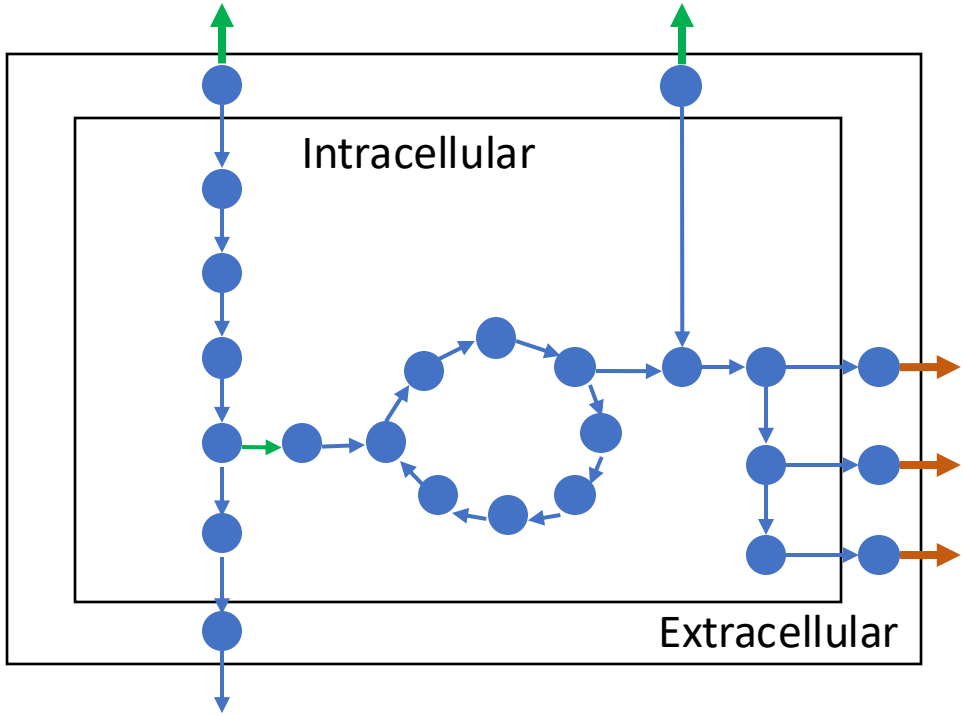
$$-10 \leq v_{EX\_glu\_e} \leq 0$$

$$-20 \leq v_{EX\_O2\_e} \leq 0$$

$$0 \leq v_{EX\_lac\_e} \leq 1000$$

$$0 \leq v_{EX\_ac\_e} \leq 1000$$

$$0 \leq v_{EX\_eth\_e} \leq 1000$$



Objective function:

max(**biomass reaction**):

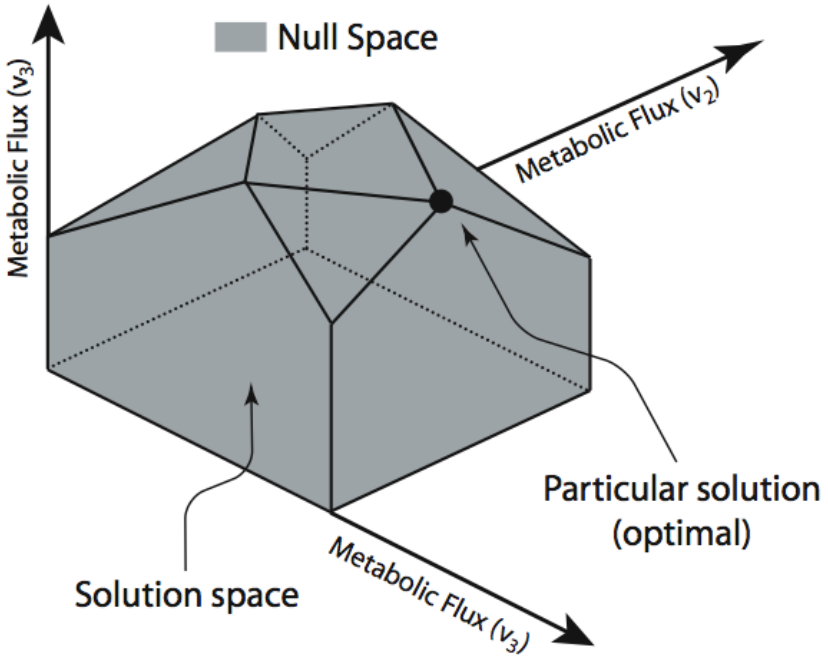
Linear programing

1. Forming the Solution Space

$$\mathbf{Sv} = 0 \quad , \qquad 0 \leq v_i \leq v_{i,m} \qquad \text{internal reactions}$$
$$min \leq b_i \leq max \qquad \text{inputs \& outputs}$$

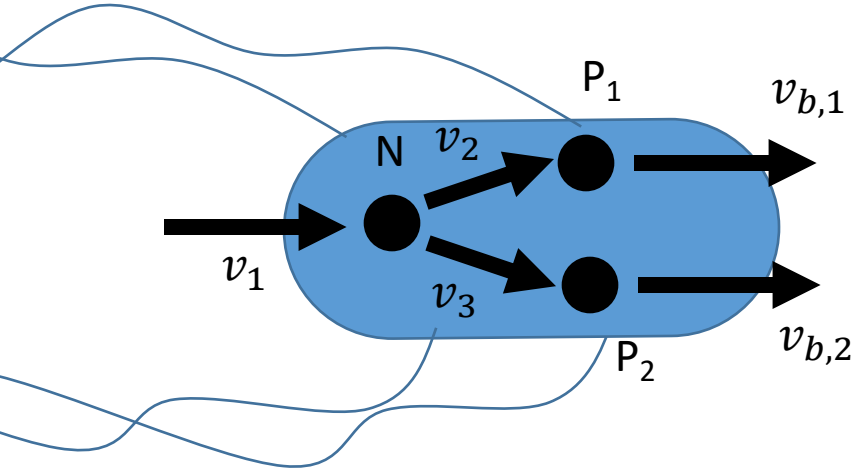
2. Objective Function

$$Z = \sum_{i=1}^{n} c_i v_i$$



Originally from **economics** to **optimize** production processes subject to **linear constraints**.

How Does LP work: 2D example



Biomass building block b_1 and b_2

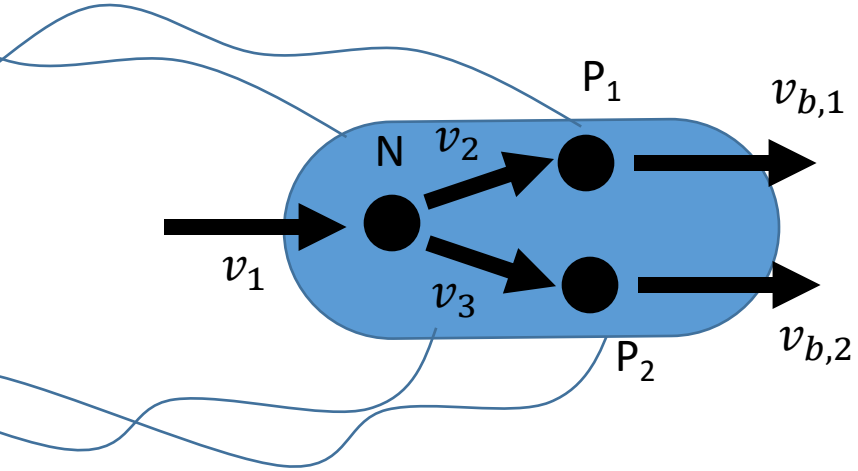
Boundary conditions: $v_1 = 10$

Mass balances:

$$v_1 - v_2 - v_3 = 0$$
$$v_2 - v_{b,1} = 0$$
$$v_3 - v_{b,2} = 0$$

Biomass function: $v_{b,1} + 2v_{b,2} = v_{bio}$

How Does LP work: 2D example



Boundary conditions:

Mass balances:

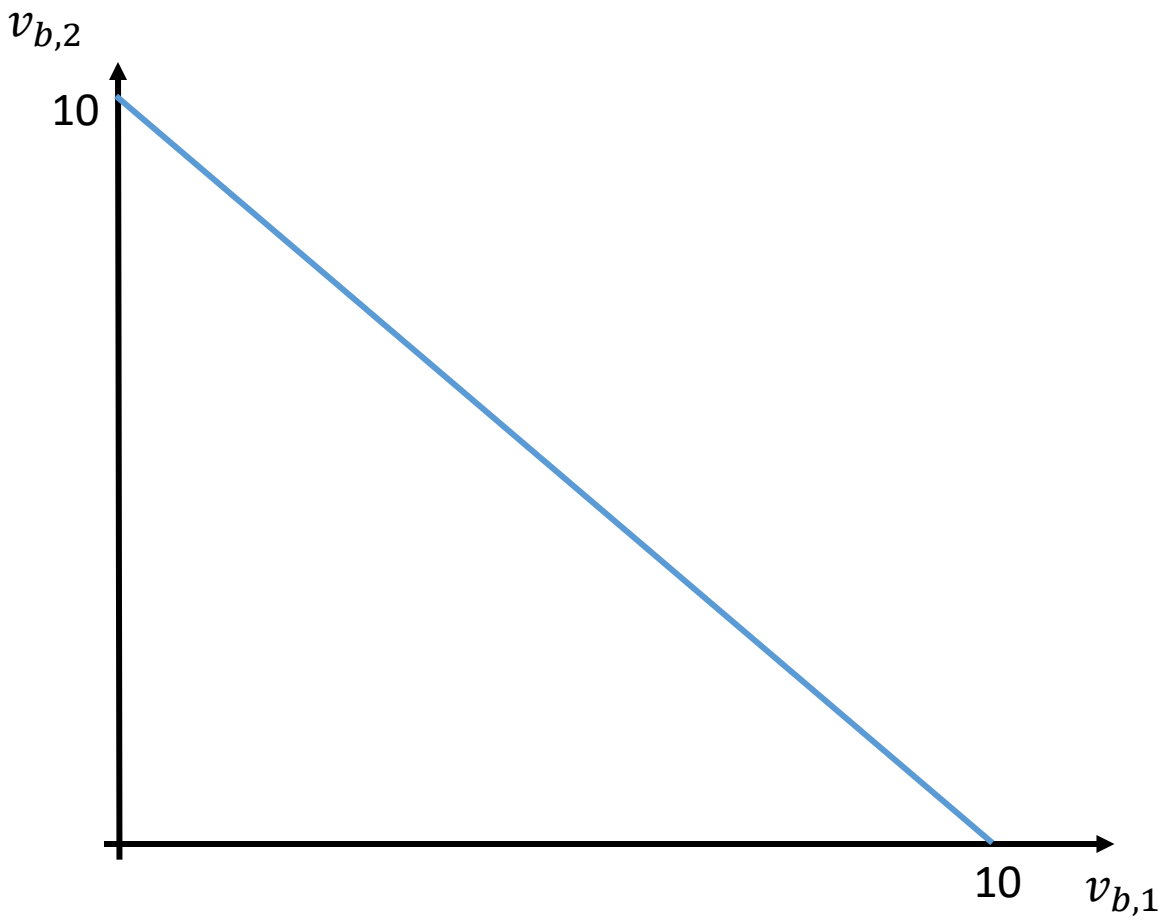
$$\left.\begin{aligned} v_1 &= 10 \\ v_1 - v_2 - v_3 &= 0 \\ v_2 - v_{b,1} &= 0 \\ v_3 - v_{b,2} &= 0 \end{aligned}\right\} \quad 10 - v_{b,1} - v_{b,2} = 0$$

Biomass function: $v_{b,1} + 2v_{b,2} = v_{bio}$

How Does LP work: 2D example

Equality constraints:

$$v_{b,1} = 10 - v_{b,2}$$



How Does LP work: 2D example

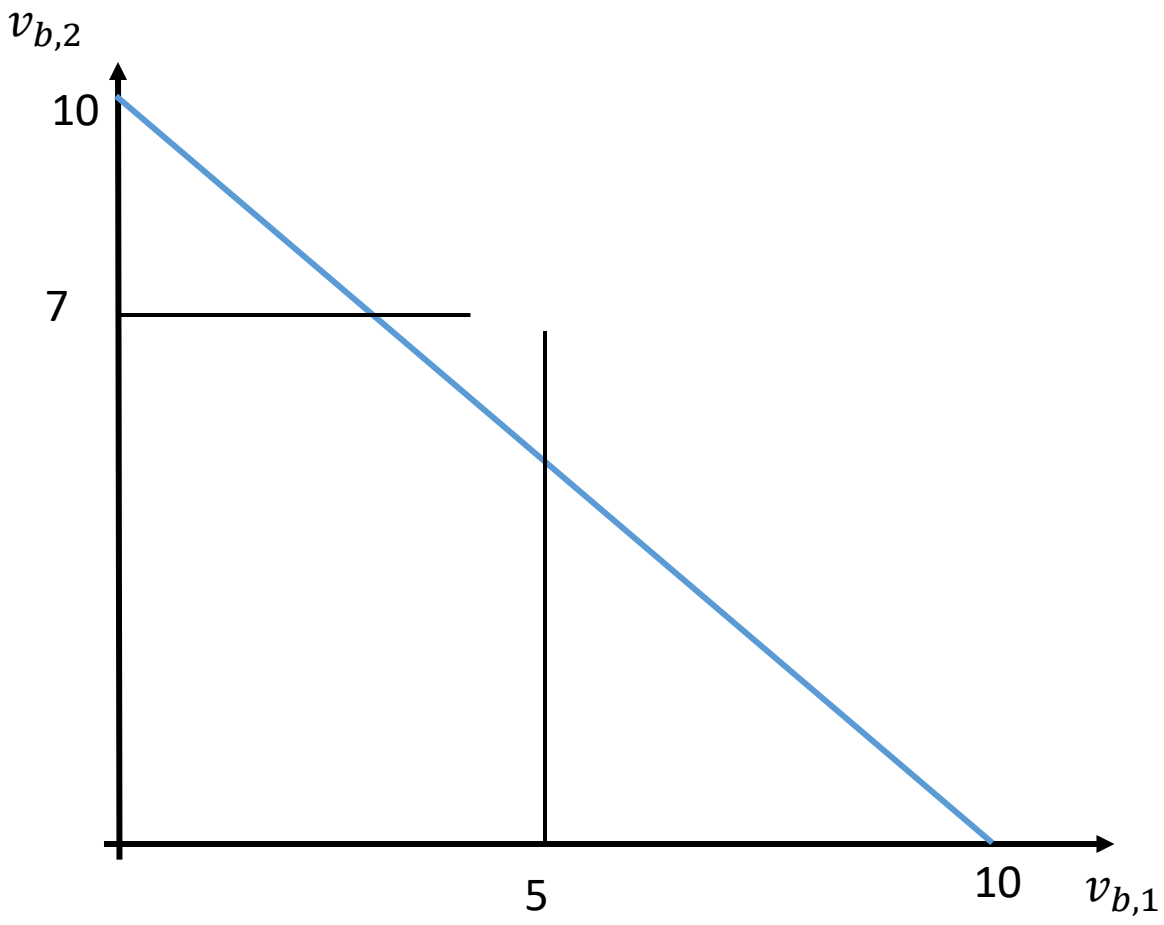
Equality constraints:

$$v_{b,1} = 10 - v_{b,2}$$

Inequality constraints:

$$0 \leq v_{b,2} \leq 7$$

$$0 \leq v_{b,1} \leq 5$$



How Does LP work: 2D example

Equality constraints:

$$v_{b,1} = 10 - v_{b,2}$$

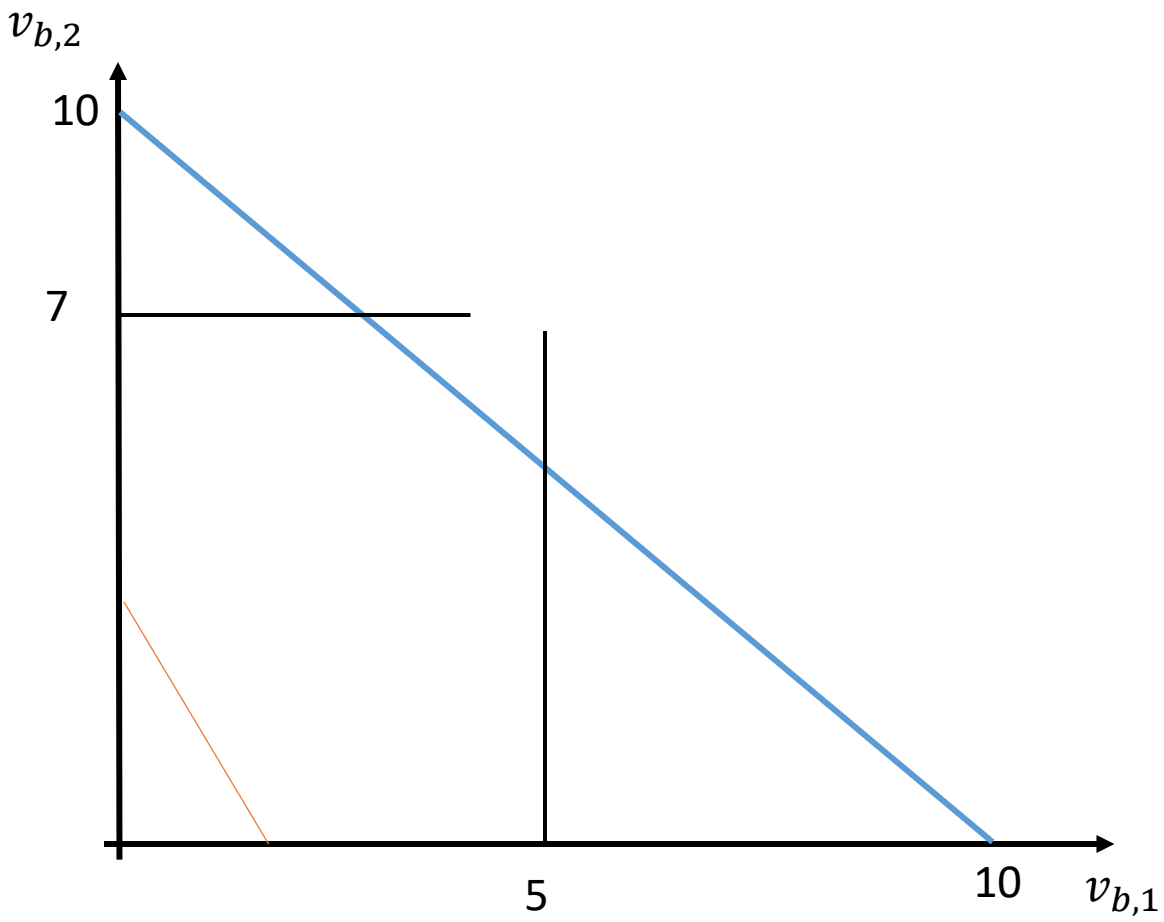
Inequality constraints:

$$0 \leq v_{b,2} \leq 7$$

$$0 \leq v_{b,1} \leq 5$$

Objective function:

$$v_{b,1} + 2v_{b,2} = v_{bio}$$



How Does LP work: 2D example

Equality constraints:

$$v_{b,1} = 10 - v_{b,2}$$

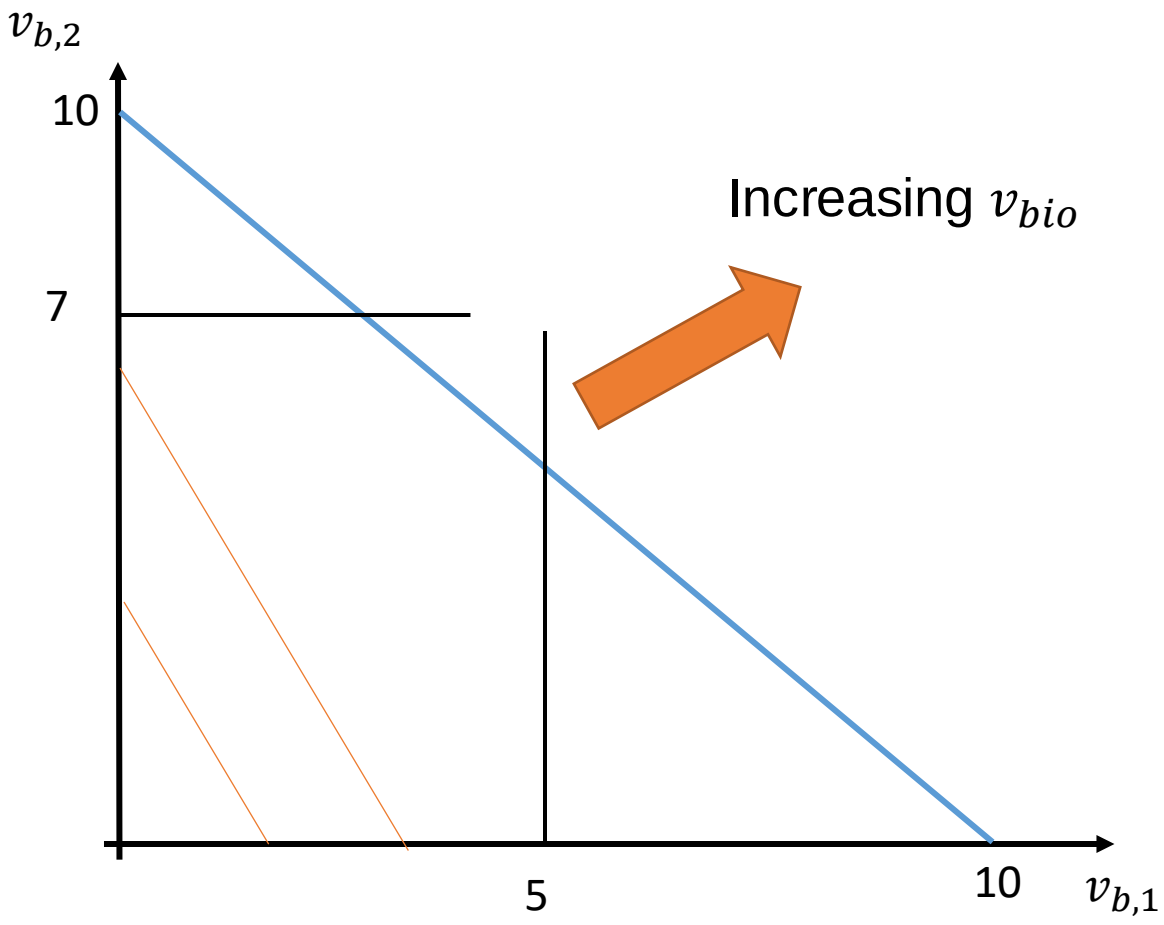
Inequality constraints:

$$0 \leq v_{b,2} \leq 7$$

$$0 \leq v_{b,1} \leq 5$$

Objective function:

$$v_{b,1} + 2v_{b,2} = v_{bio}$$



How Does LP work: 2D example

Equality constraints:

$$v_{b,1} = 10 - v_{b,2}$$

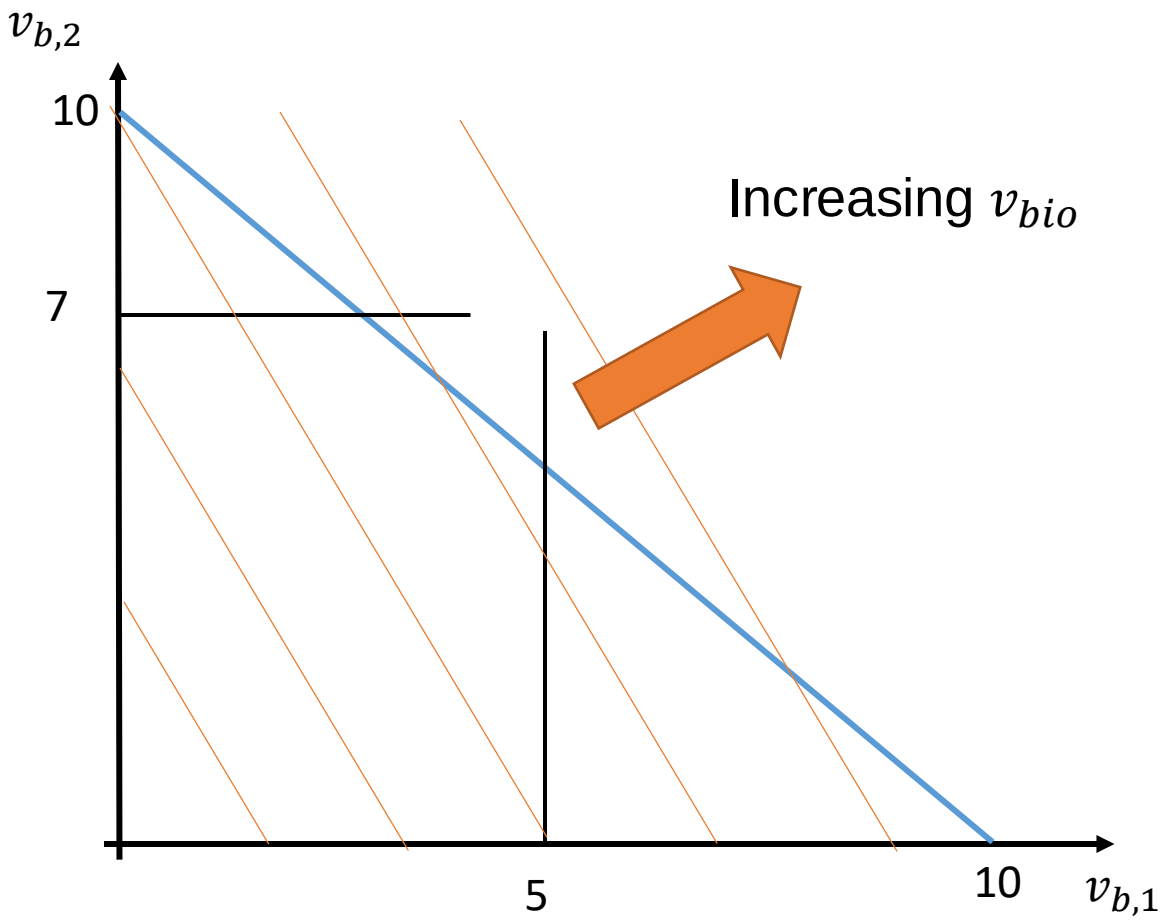
Inequality constraints:

$$0 \leq v_{b,2} \leq 7$$

$$0 \leq v_{b,1} \leq 5$$

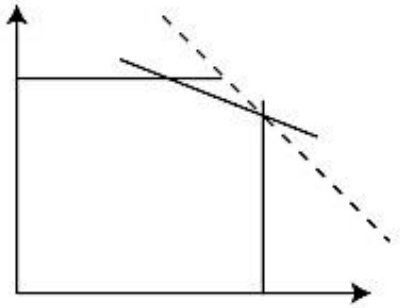
Objective function:

$$v_{b,1} + 2v_{b,2} = v_{bio}$$



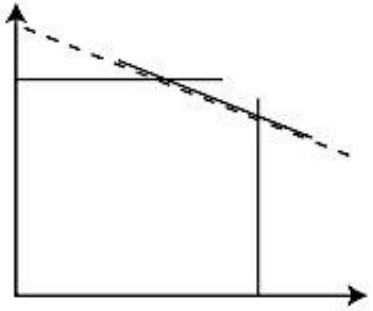
Types of Feasible Solutions found by LP

Unique solution



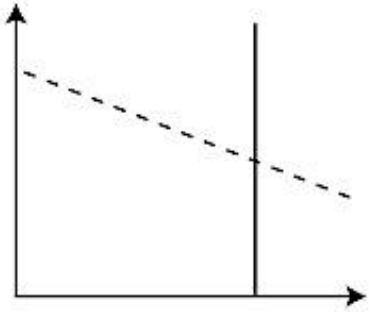
Optimal solution in a corner

Degenerate solution



Optimal solution along an edge

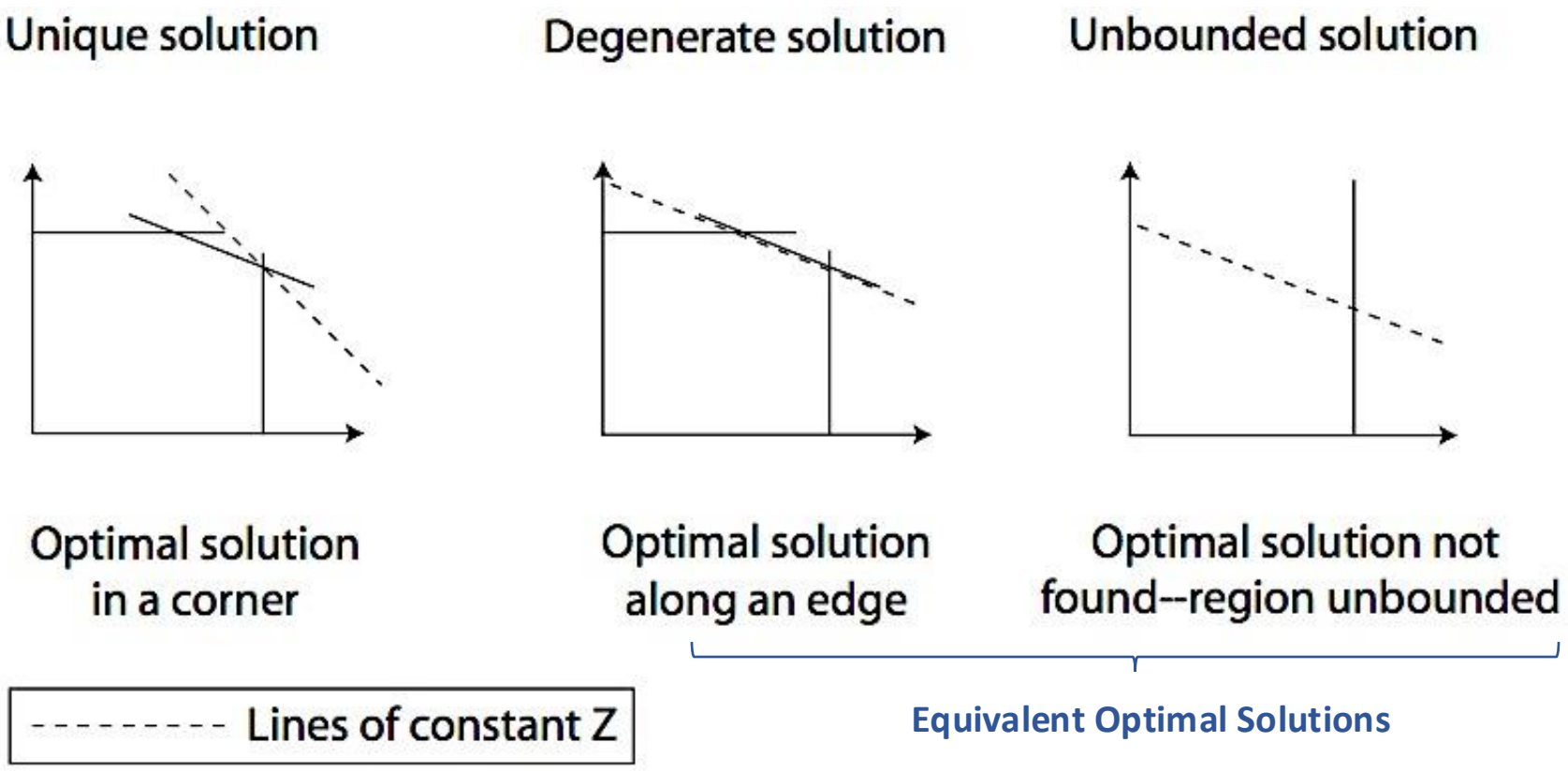
Unbounded solution



Optimal solution not found--region unbounded

--------- Lines of constant Z

Types of Feasible Solutions found by LP



Equivalent optimal solutions occur frequently in genome-scale networks.
Since, Genome-scale networks are typically able to achieve the same overall functional network state in many different ways.

Flux Balance Analysis

Flux balance analysis (FBA) applies defined **constraints**
on an **objective function**
and find the optimum solutions (flux profiles)

Table II. Questions that can be addressed using flux-balance analysis.

Question	Objective	Reference
What are the biochemical production capabilities?	Maximize metabolite product	Varma, Boesch, & Palsson, 1993
What is the maximal growth rate and biomass yield?	Maximize growth rate	Varma & Palsson, 1993; Varma & Palsson, 1994b
How efficiently can metabolism channel metabolites through the network?	Minimize the Euclidean norm	Bonarius et al., 1996
How energetically efficient can metabolism operate?	Minimize ATP production or minimize nutrient uptake	Majewski & Domach, 1990; Savinell & Palsson, 1992; Fell & Small, 1986
What is the tradeoff between biomass production and metabolite overproduction?	Maximize biomass production for a given metabolite production	Varma et al., 1993

For a given growth condition (e.g. known input nutrients), considering:

- metabolic system operates in a **quasi-steady state**.
- certain **constraints** on system (flux limitations, stoichiometric and reversibility constraints).
- an “objective” that is expected to be maximized (e.g. **biomass** production).

FBA ***predicts*** reaction fluxes and essential enzymes
under a given growth condition

Table from: Edwards, J. S., Palsson, B. O., How will bioinformatics influence metabolic engineering? *Biotechnol Bioeng* 1998

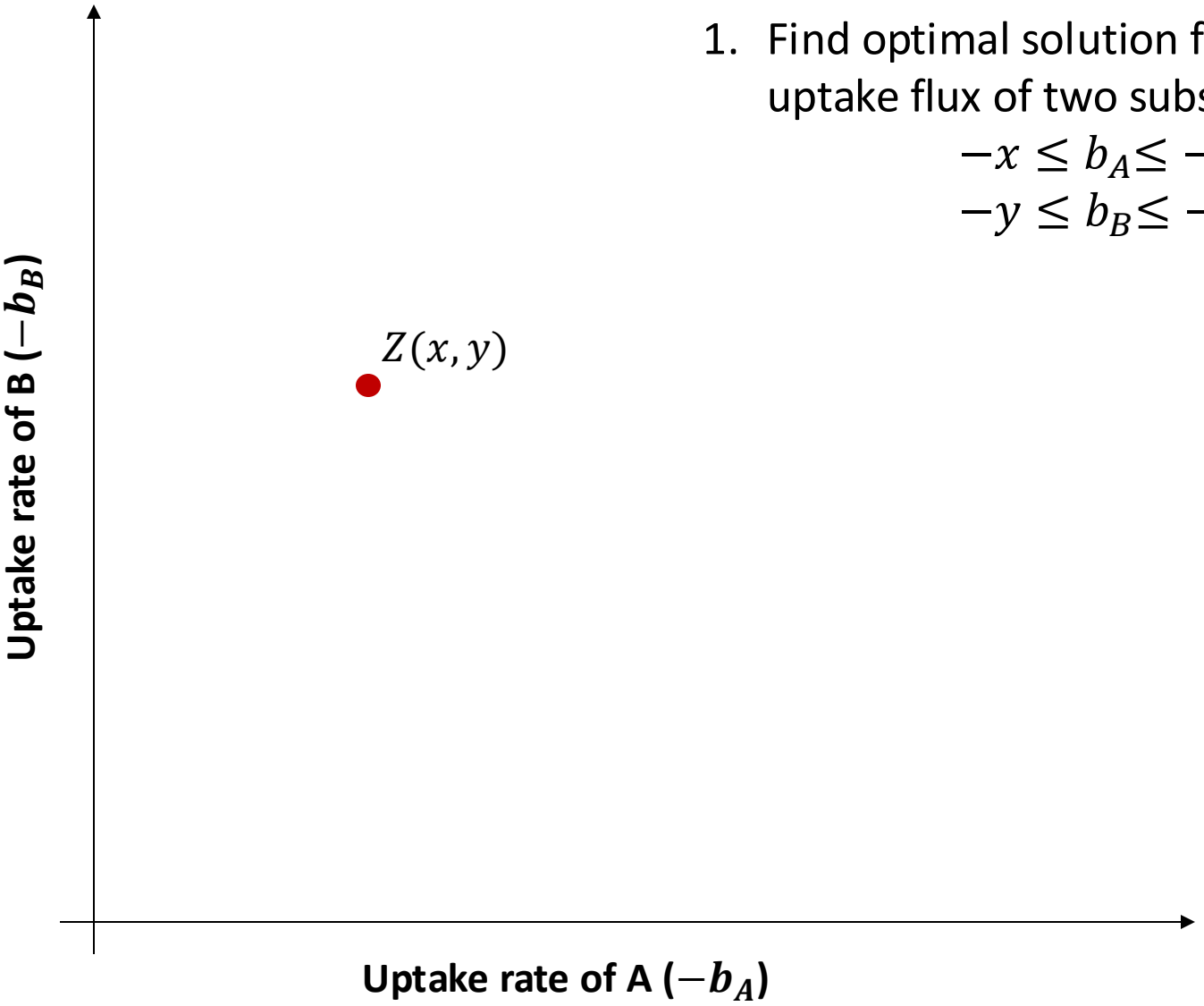
Other biological objectives

Table III Objective functions implemented in constraint-based FBA

Objective function[a]	Mathematical definition	Explanation	Rationale	Reference
Max biomass[b]	$\max \frac{v_{biomass}}{v_{glucose}}$	Maximization of biomass yield	Evolution drives selection for maximal biomass yield ($Y_{X/S}$)	(van Gulik and Heijnen, 1995; Edwards and Palsson, 2000b; Price *et al*, 2004)
Max ATP	$\max \frac{v_{ATP}}{v_{glucose}}$	Maximization of ATP yield	Evolution drives maximal energetic efficiency ($Y_{ATP/S}$)	(van Gulik and Heijnen, 1995; Ramakrishna *et al*, 2001)
Min$\sum v^{2c}$	$\min \sum_{i=1}^{n} v_i^2$	Minimization of the overall intracellular flux	Postulates maximal enzymatic efficiency for cellular growth (analogous to minimization of the Euclidean norm)	(Bonarius *et al*, 1996; Blank *et al*, 2005a)
Max ATP per flux unit[c]	$\max \frac{v_{ATP}}{\sum_{i=1}^{n} v_i^2}$	Maximization of ATP yield per flux unit	Cells operate to maximize ATP yield while minimizing enzyme usage	(Dauner and Sauer, 2001)
Max biomass per flux unit[c]	$\max \frac{v_{biomass}}{\sum_{i=1}^{n} v_i^2}$	Maximization of biomass yield per flux unit	Cells operate to maximize biomass yield while minimizing enzyme usage	
Min glucose	$\min \frac{v_{glucose}}{v_{biomass}}$	Minimization of glucose consumption	Evolution drives selection for most efficient usage of substrate	(Oliveira *et al*, 2005)
Min reaction steps[c]	$\min \sum_{i=1}^{n} y_i^2, y_i \in \{0, 1\}$	Minimization of reaction steps	Cells minimizes number of reaction steps to produce biomass	(Melendez-Hevia and Isidoro, 1985)
Max ATP per reaction step[c]	$\min \frac{v_{ATP}}{\sum_{i=1}^{n} y_i^2}, y_i \in \{0, 1\}$	Maximization of ATP yield per reaction step	Cells operate to maximize ATP yield per reaction step	
Min redox potential[d,e]	$\min \frac{\sum_{n} v_{NADH}}{v_{glucose}}$	Minimization of redox potential[f]	Cells decrease number of oxidizing reactions thus conserving their energy or using their energy in the most efficient way possible	(Knorr *et al*, 2007)
Min ATP production[d,e]	$\min \frac{\sum_{n} v_{ATP}}{v_{glucose}}$	Minimization of ATP producing fluxes[g]	Cells grow while using the minimal amount of energy, thus conserving energy	(Knorr *et al*, 2007)
Max ATP production[d,e]	$\max \frac{\sum_{n} v_{ATP}}{v_{glucose}}$	Maximization of ATP producing fluxes[h]	Cells produce as much ATP as possible	(Heinrich *et al*, 1997; Ebenhoh and Heinrich, 2001; Knorr *et al*, 2007)

[a]Both maximization of biomass objectives (absolute and per flux unit) require no *a priori* assumptions. For all other objectives the specific growth rate was set to the experimentally determined value under each condition.
[b]Often also referred to as optimization of growth rate (Price *et al*, 2004).
[c]n refers to the overall number of reactions in the network, that is 98 in the present case.
[d]Reaction name is that specified in Supplementary Table I; '_R' refers to the reverse reaction.
[e]All reversible reactions in Supplementary Table I were converted to two irreversible reactions resulting in a final stoichiometric model of 60 metabolites and 151 reactions.
[f]Reactions: *gapA, aceE/F, maeA, sucAB, mdh, udhA, fdhF, fdoGHI, fdnGHI, ldhA, adhE_R, mhpF_R, adhP_R, adhC_R, maeB, zwf, gnd, icd, pntAB, frdABCD, sdhAB, dld, sdhABCD_R.*
[g]Reactions: *pgk, pykA, pykF, sucCD, atpA-H, ackA, ackB, tdcD, purT.*
[h]Reactions: *pgk, pykA, pykF, sucCD, atpA-H, ackA, ackB, tdcD, purT.*

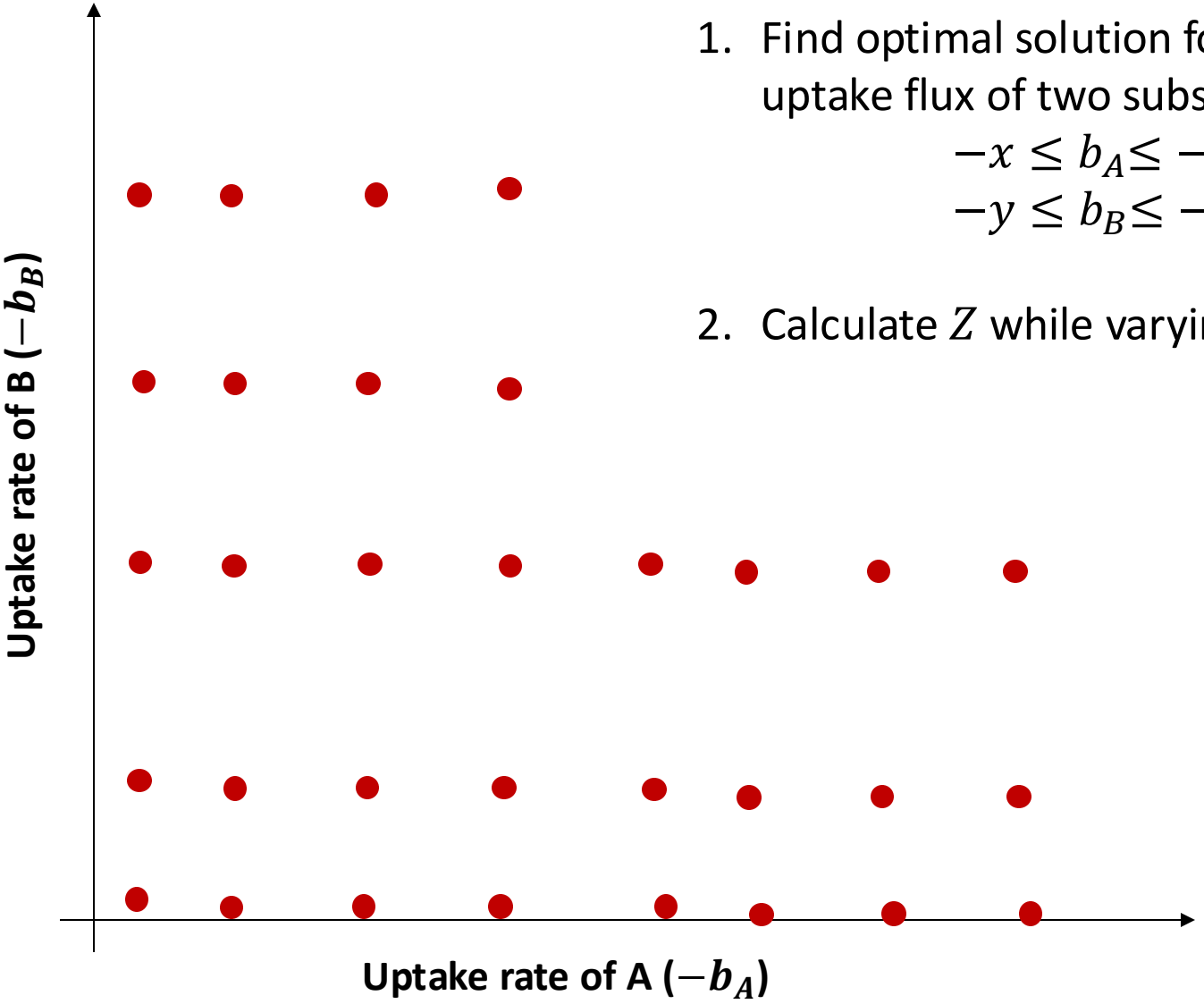
Phenotypic phase planes



1. Find optimal solution for a fixed uptake flux of two substrates (b_A and b_B)

$$-x \leq b_A \leq -x$$
$$-y \leq b_B \leq -y$$

Phenotypic phase planes

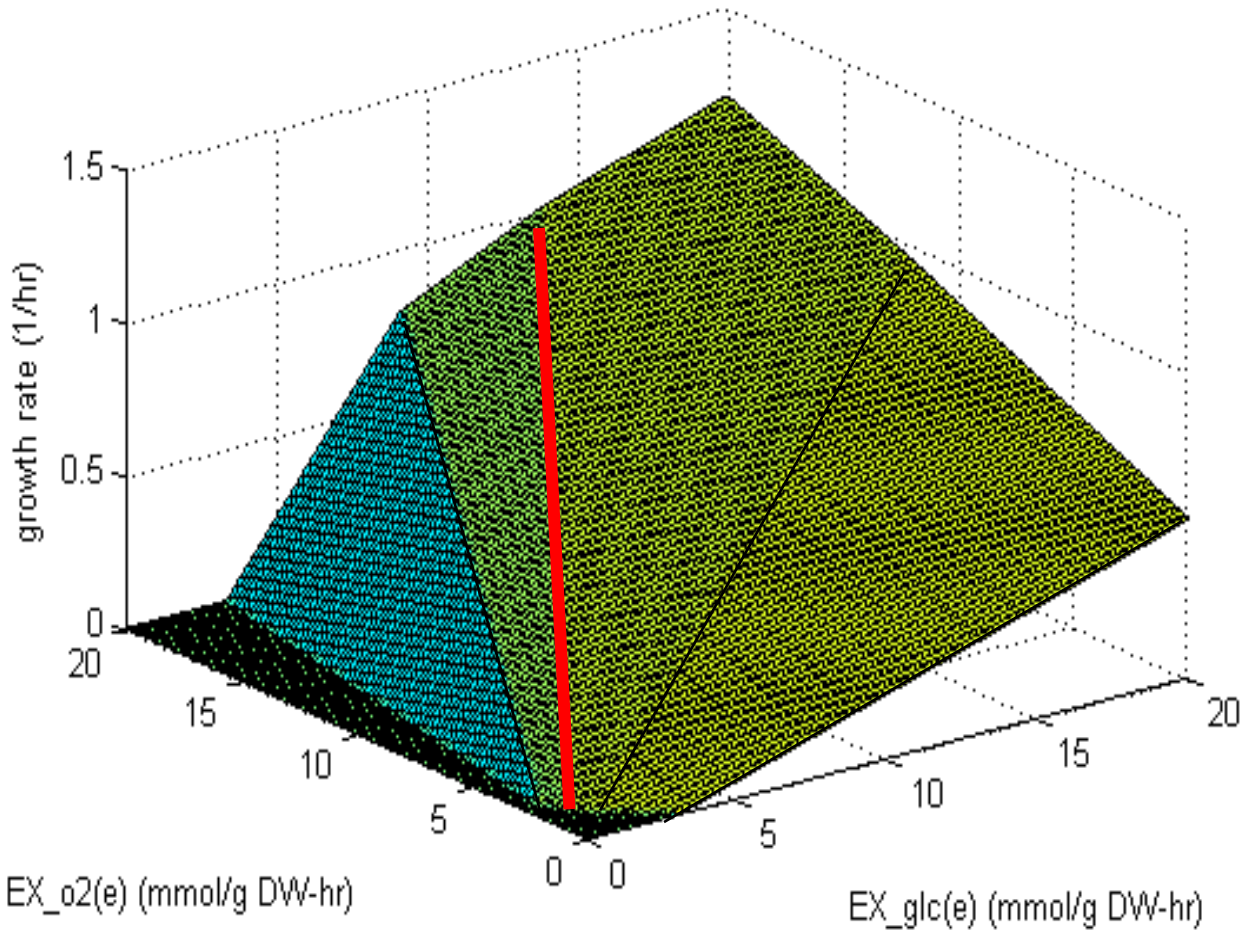


1. Find optimal solution for a fixed uptake flux of two substrates (b_A and b_B)

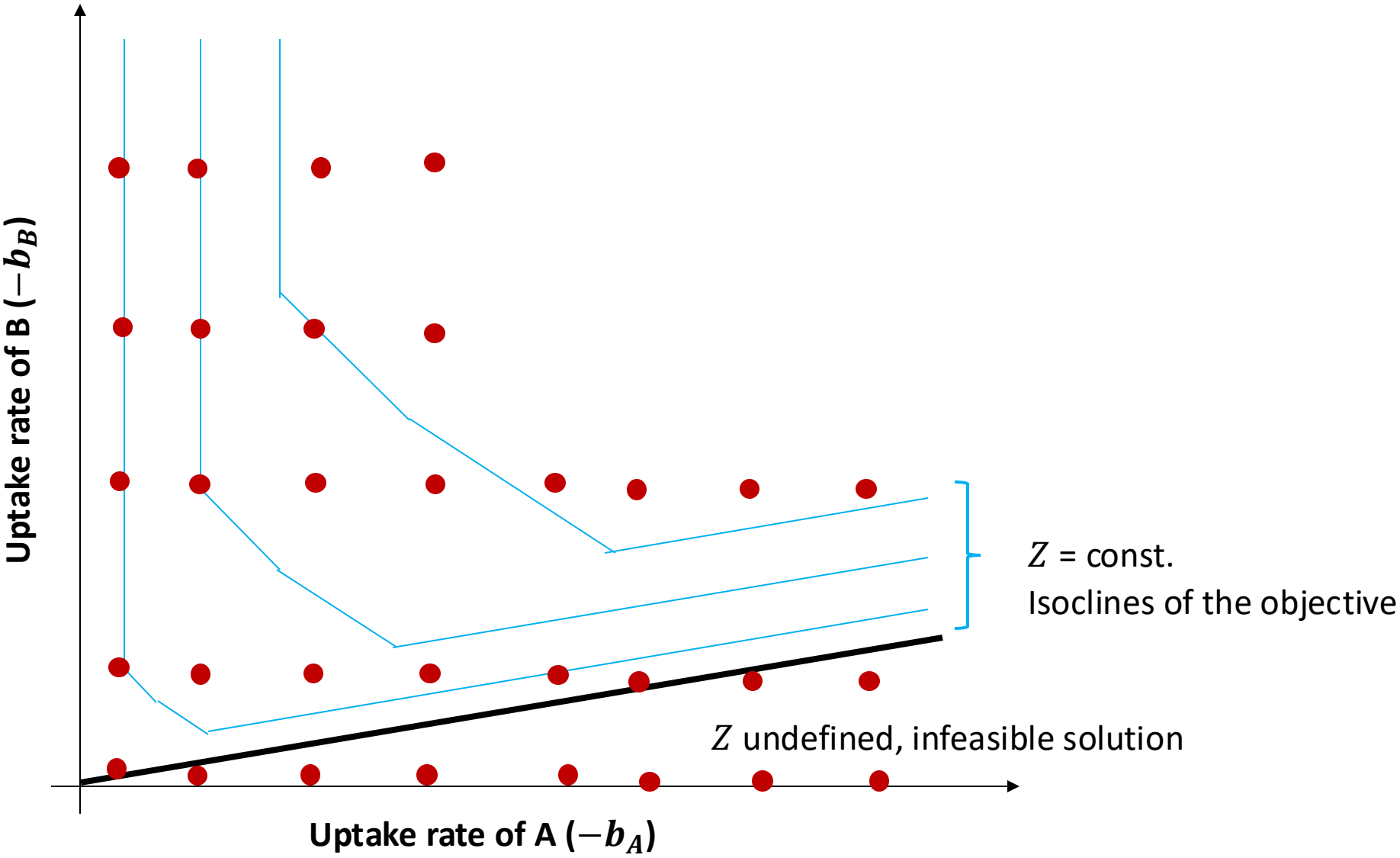
$$-x \leq b_A \leq -x$$
$$-y \leq b_B \leq -y$$

2. Calculate Z while varying x and y

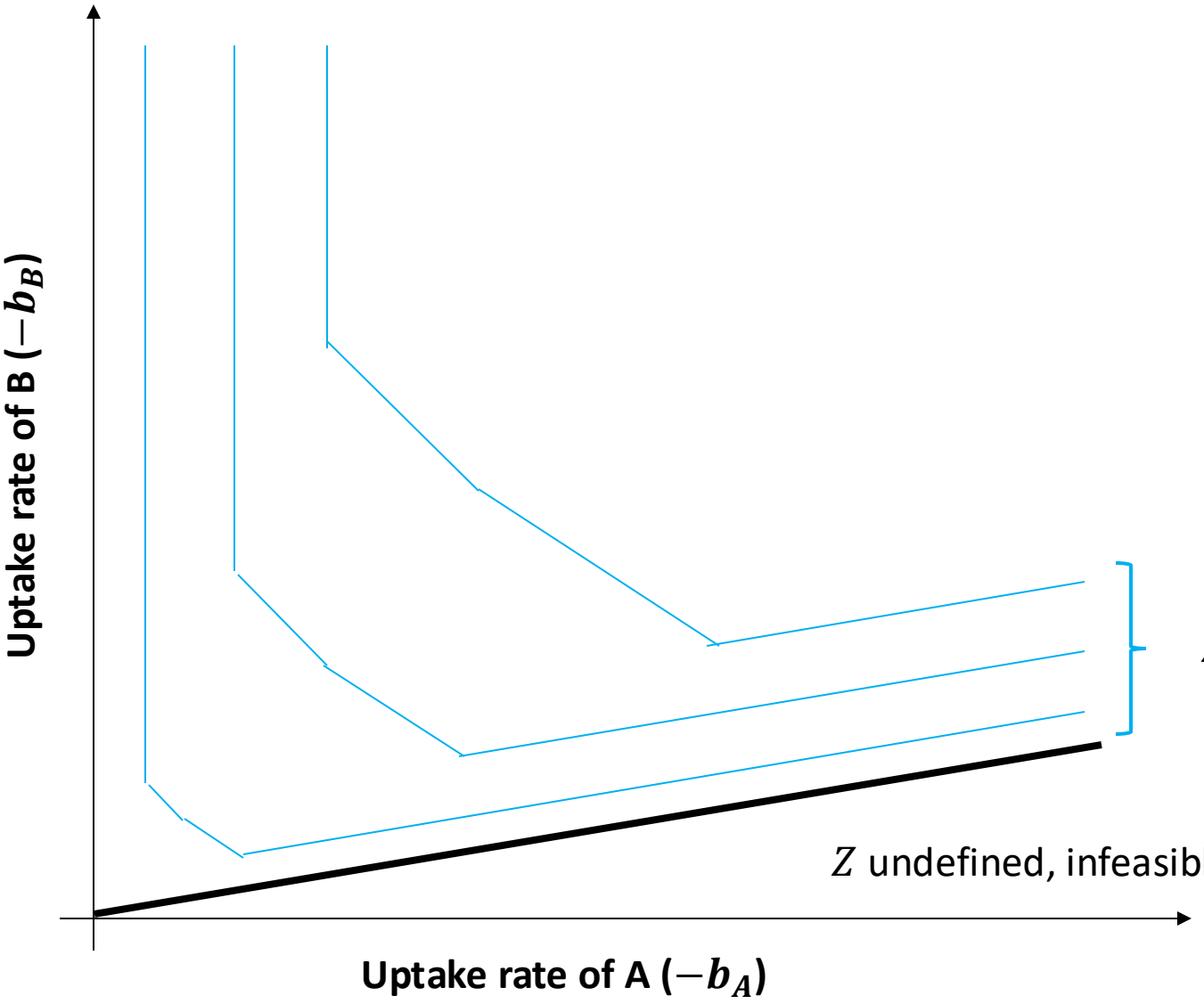
Phenotypic phase planes



Phenotypic phase planes



Phenotypic phase planes



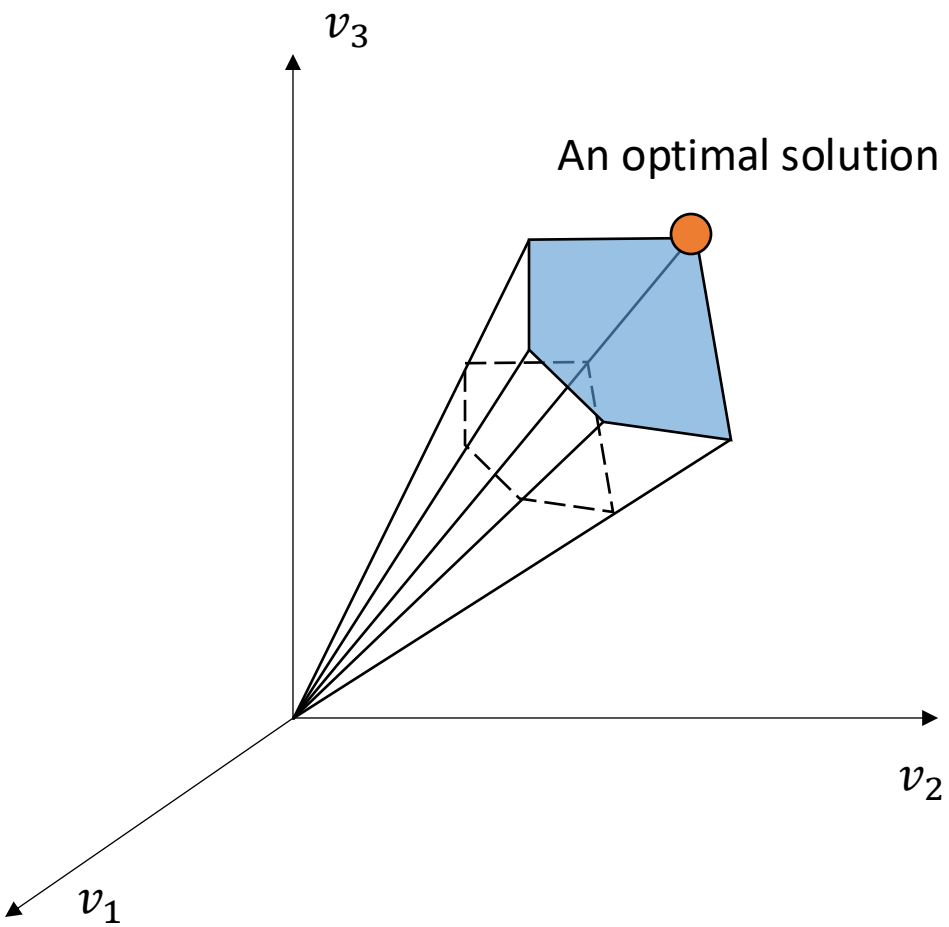
Flux Variability Analysis

Flux Variability Analysis

1) Perform an **FBA** with a given objective e.g:

$$S\vec{v} = \vec{0} \text{ with } v_{j,lb} \leq v_j \leq v_{j,ub}$$

$$b_{max} = max(biomass)$$



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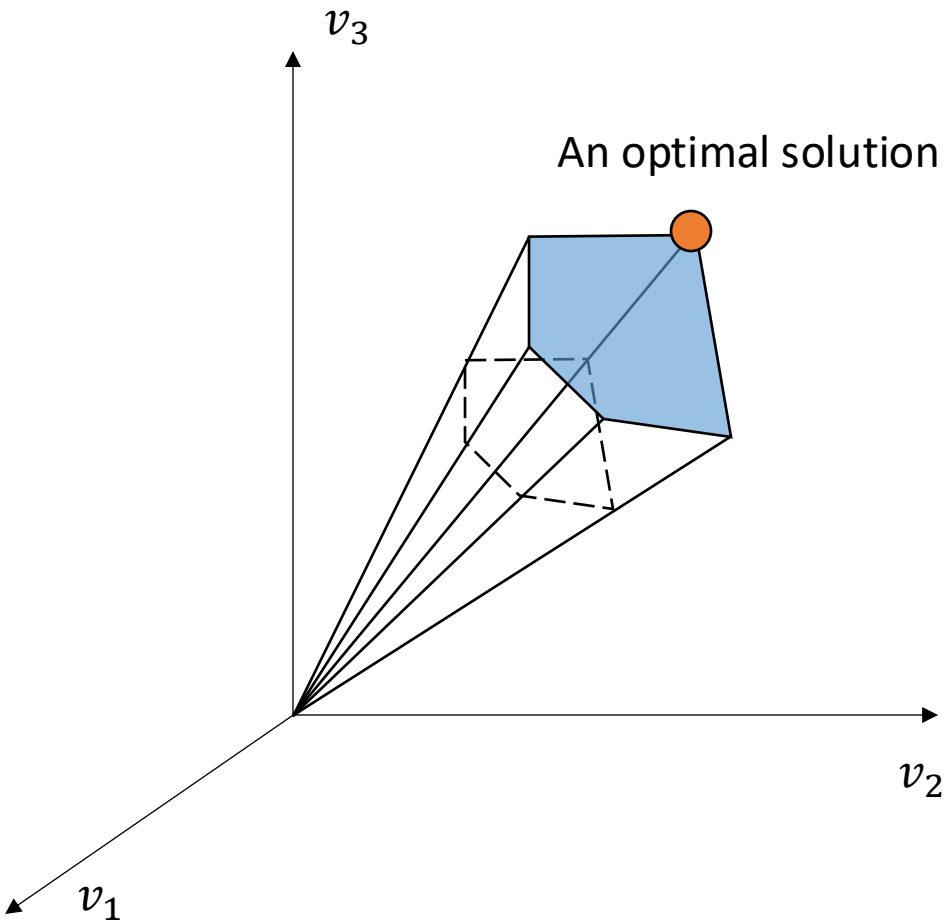
2) **Constraint** the **objective** e.g:

$$b_{max} \leq biomass$$



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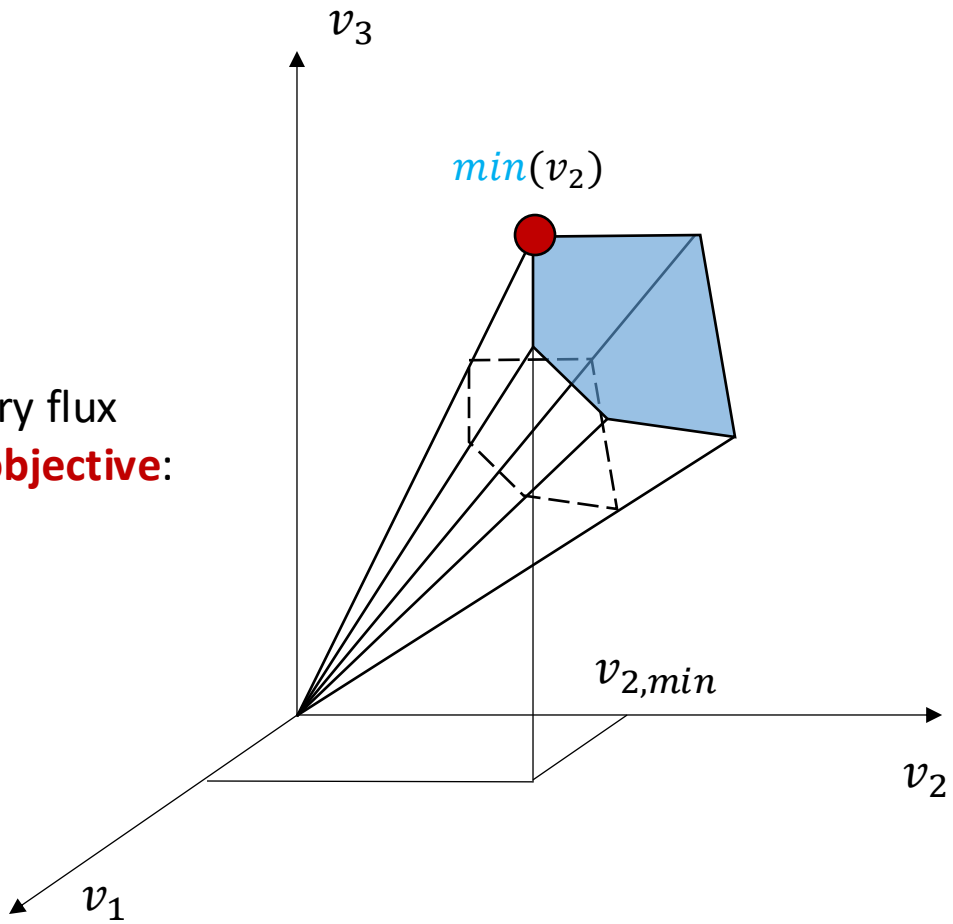
3) Find **minimum** and **maximum** of every flux given the **constraint** of the **optimal objective**:

```
for v_i in v⃗:
    Sv⃗ = 0⃗ with:
        v_j,lb ≤ v_j ≤ v_j,ub and
        v_max ≤ biomass
    v_i,min = min(v_i)
    v_i,max = max(v_i)
end
```



Flux Variability Analysis

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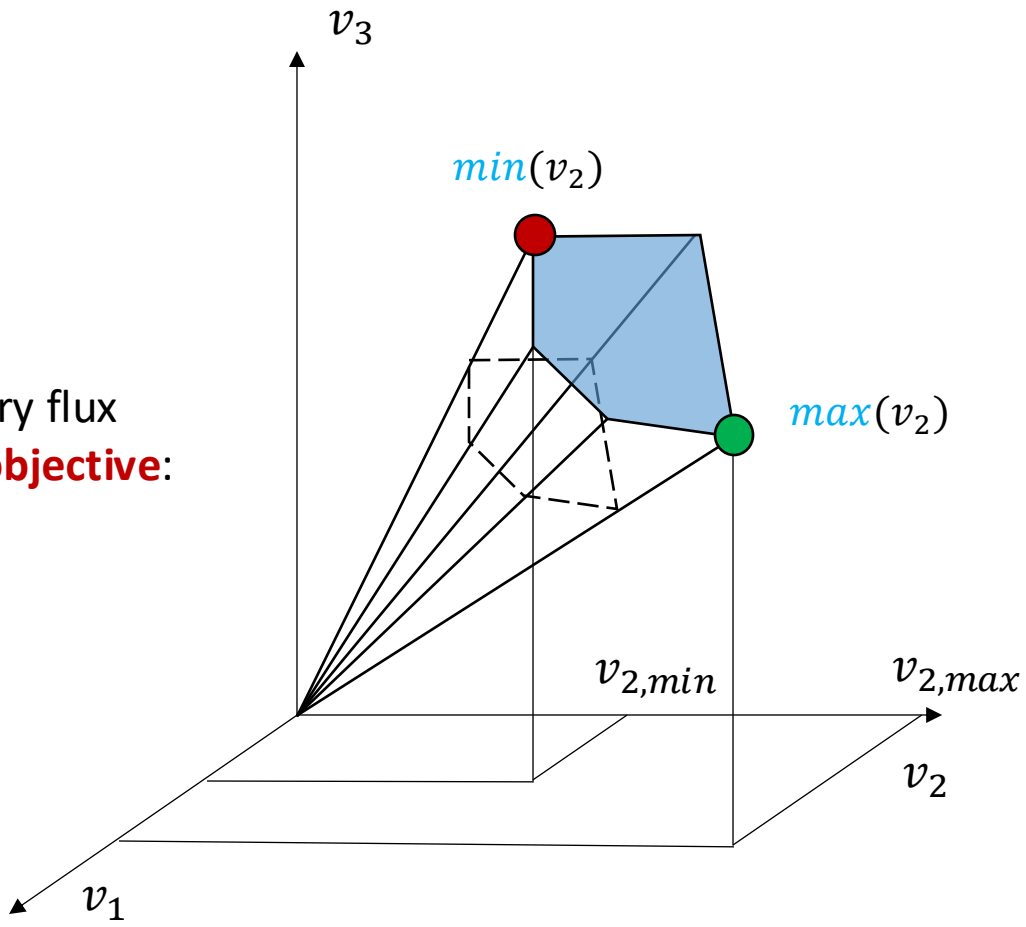
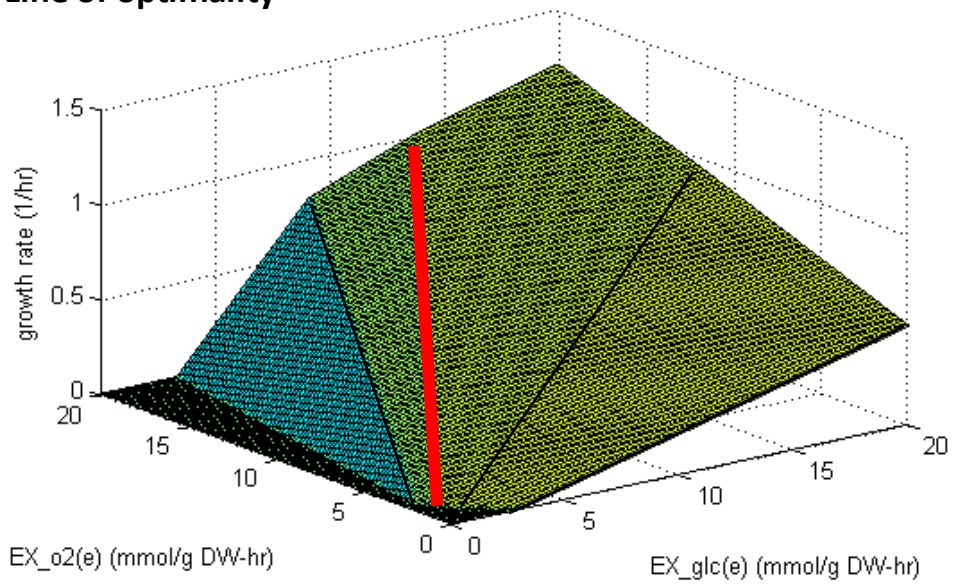
    v_i,max = max(v_i)
end
```



Phenotypic phase planes

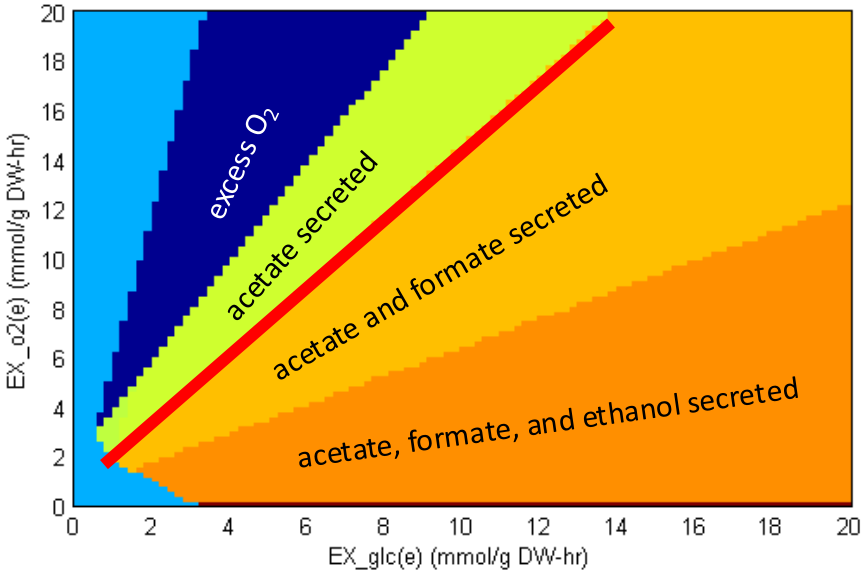
Line of optimality



Line of optimality:
Maximal biomass yield with respect to a carbon source (no oxygen limitation!)

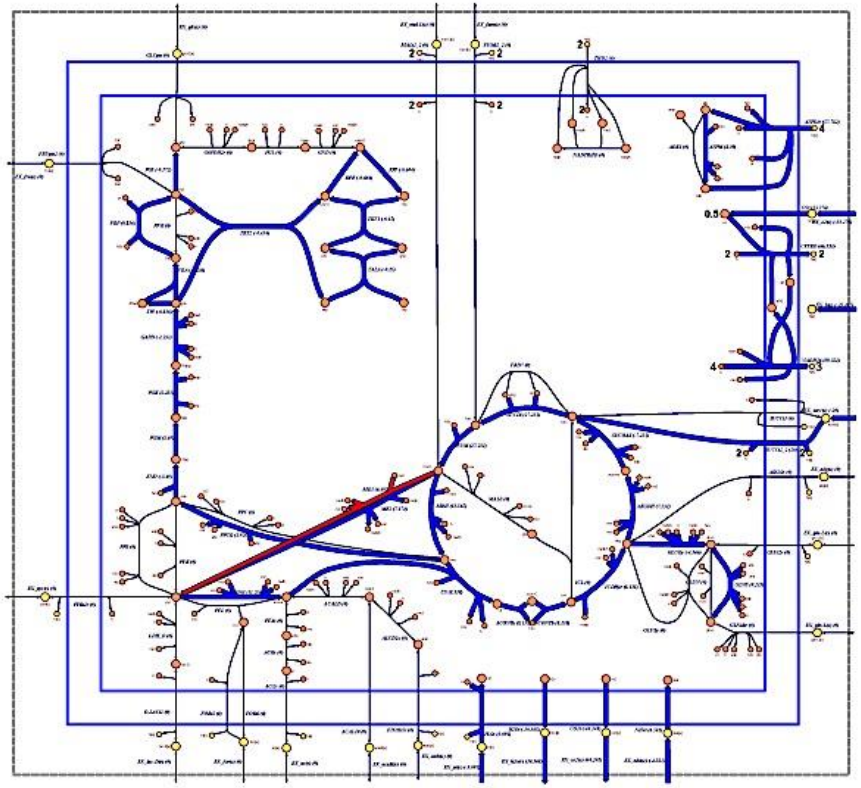
Line of optimality

Line of optimality

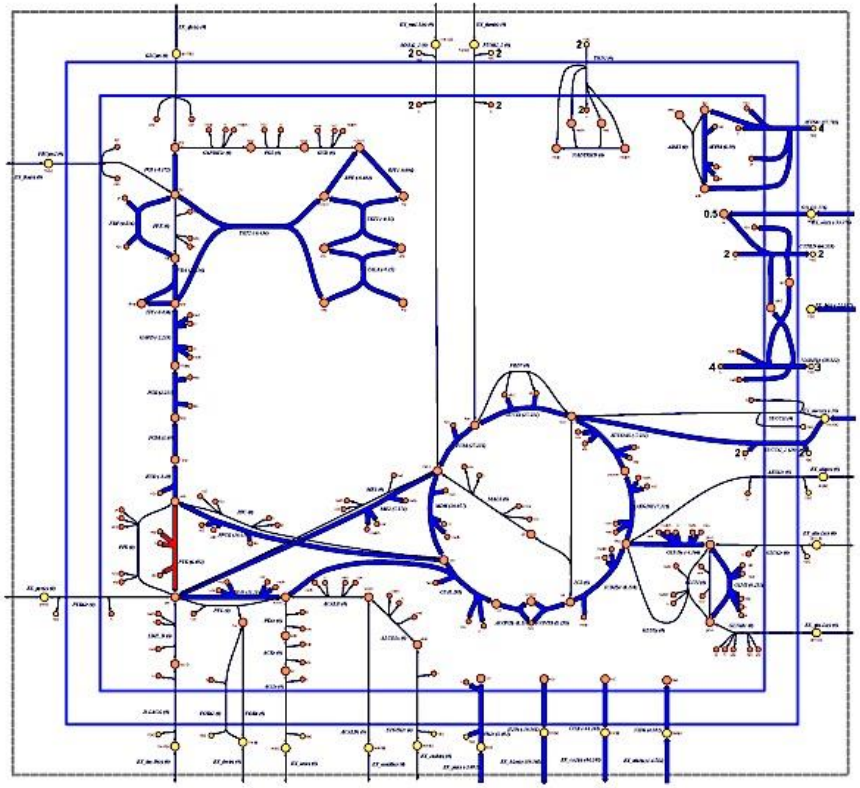


Alternative flux profiles

a



b



Reaction	Minimum Flux (mmol $gDW^{-1} hr^{-1}$)	Maximum Flux (mmol $gDW^{-1} hr^{-1}$)
FRD7	0	972.77
MDH	13.56	20.06
ME1	0	6.49
ME2	7.17	13.67
NADTRHD	0	6.49
PPCK	3.93	10.42
PYK	0	6.49
SUCDi	27.23	1000

Two alternate solutions for maximum aerobic growth on succinate.
a) Reaction ME1 is used to convert L-malate to pyruvate

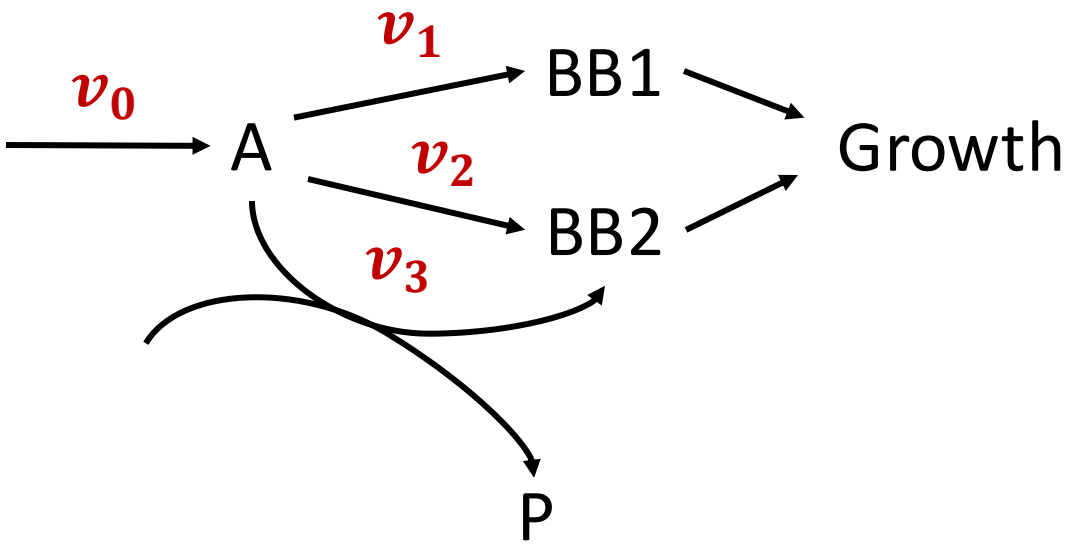
b)The reaction PYK is used to perform this function.

The two alternative reactions are highlighted in red.

Alternative flux profiles

Where do they come from?

$$v_0 = 3 \frac{\text{mmol}}{gDW\ h}$$

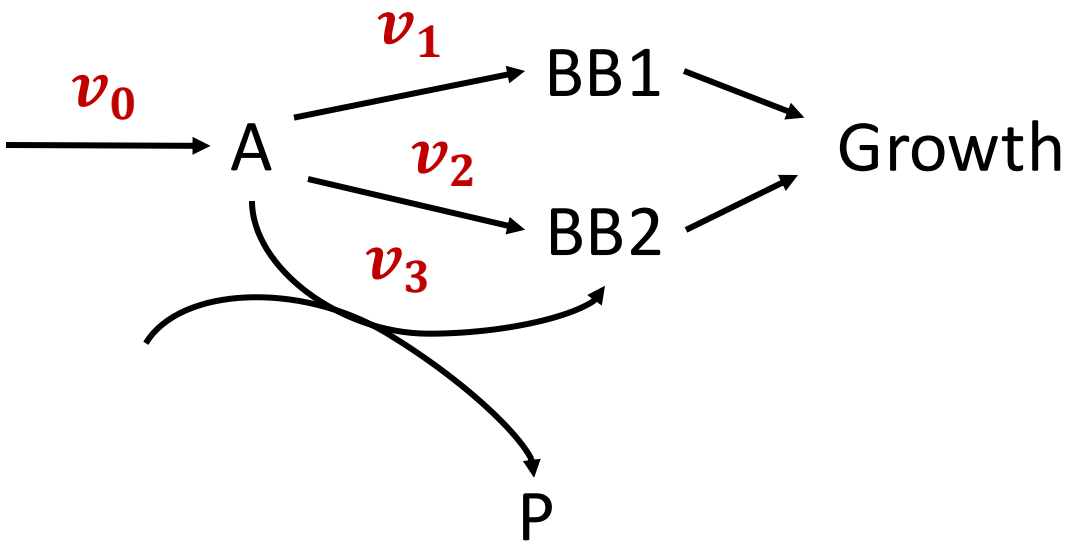


Flux Variability		
Reaction	Min Flux	Max Flux
Growth		

Alternative flux profiles

Where do they come from?

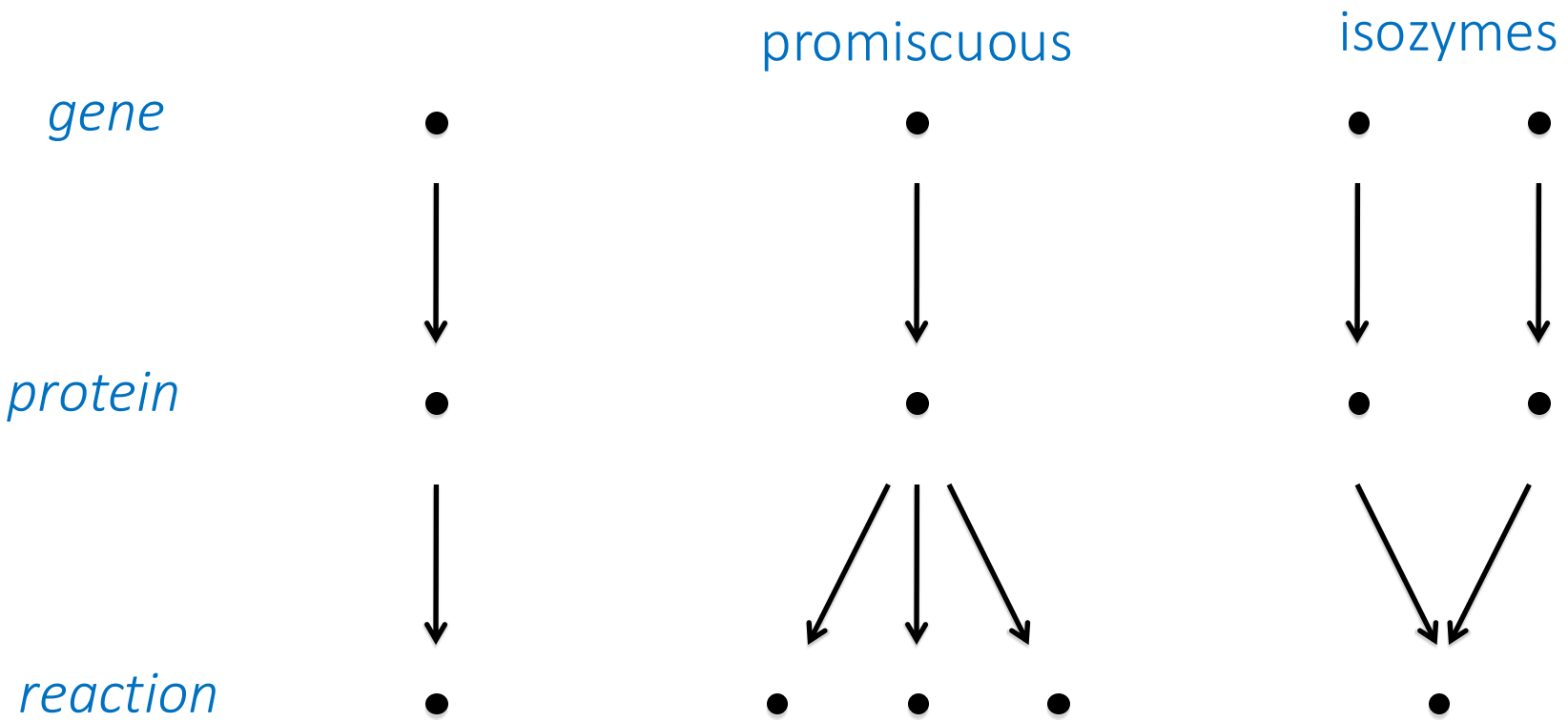
$$v_0 = 3 \frac{\text{mmol}}{gDW\ h}$$



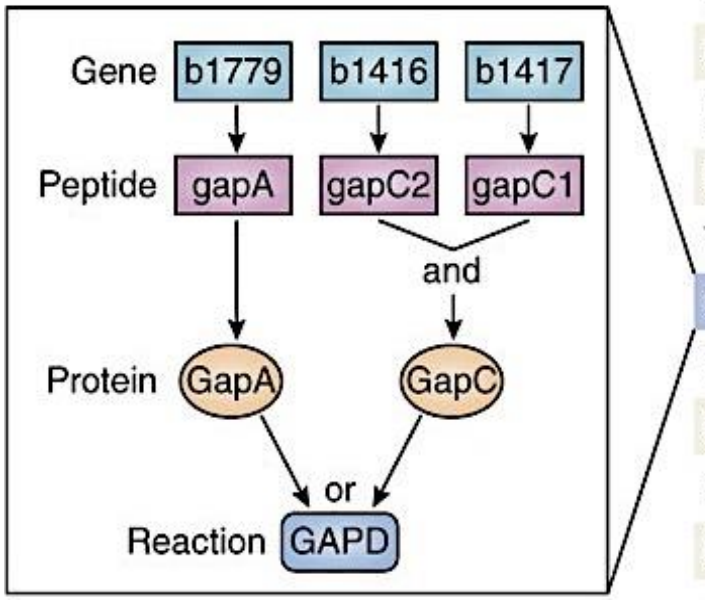
Flux Variability		
Reaction	Min Flux	Max Flux
	1.5	1.5
	0	1.5
	0	1.5
Growth	1.5	1.5

Gene Essentiality

From genes to function



From genes to function

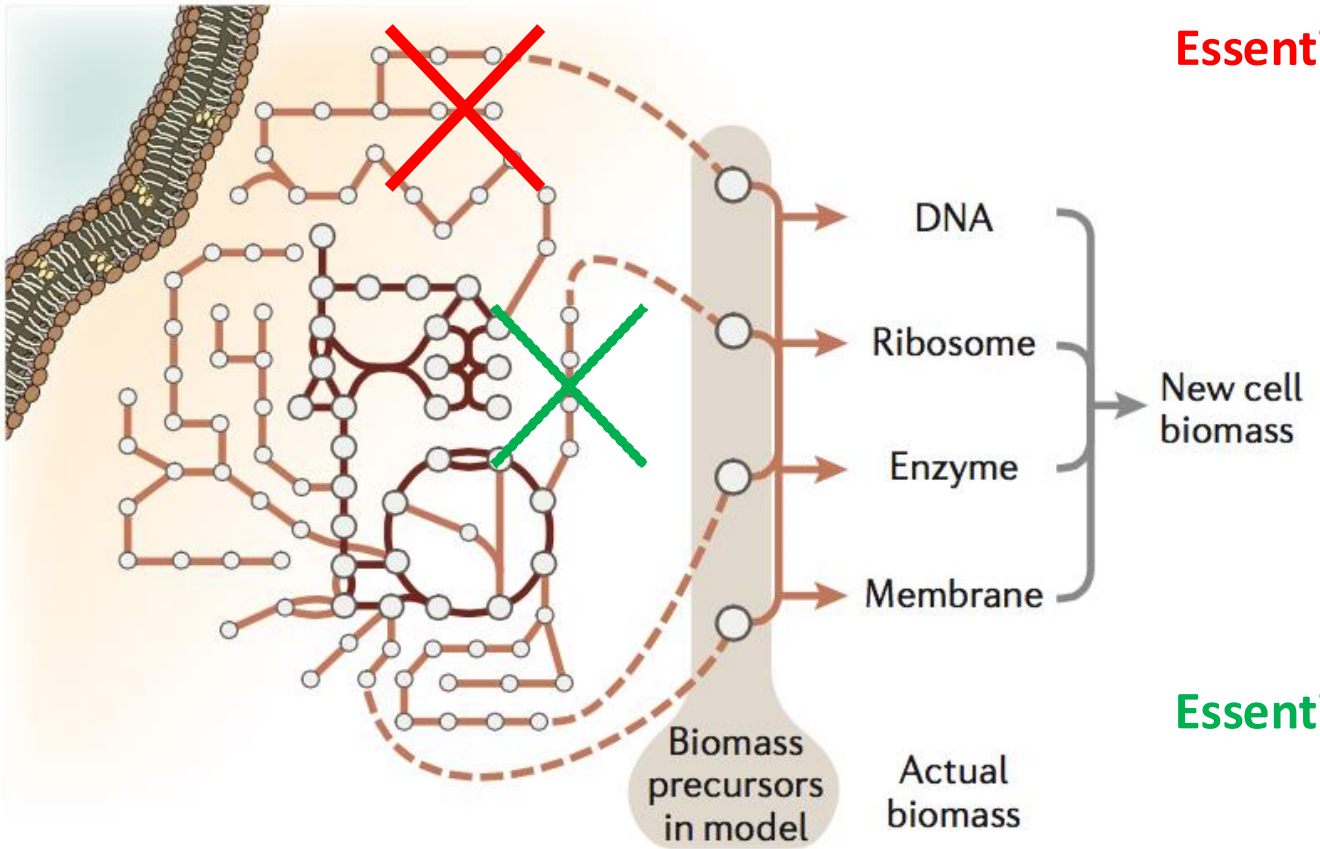


Abbreviation	Glycolytic reactions
HEX1	[c]GLC + ATP → G6P + ADP+ H
PGI	[c]G6P ↔ F6P
PFK	[c]ATP + F6P → ADP + FDP + H
FBA	[c]FDP ↔ DHAP + G3P
TPI	[c]DHAP ↔ G3P
GAPD	[c]G3P + NAD + PI ↔ 13DPG + H + NADH
PGK	[c]13DPG + ADP ↔ 3PG + ATP
PGM	[c]3PG ↔ 2PG
ENO	[c]2PG ↔ H_2O + PEP
PYK	[c]ADP + H + PEP → ATP + PYR

Knockout Studies -> Gene Essentiality

What makes a gene essential ?

- Biomass precursors required for growth,…



Essential for growth?

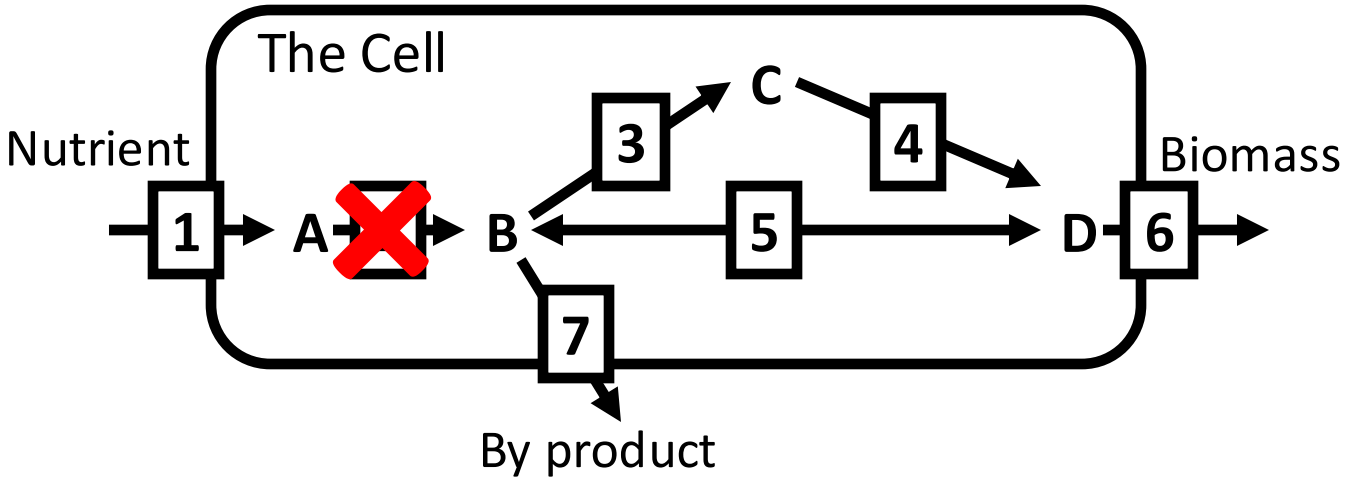
NO!

Essential for growth?

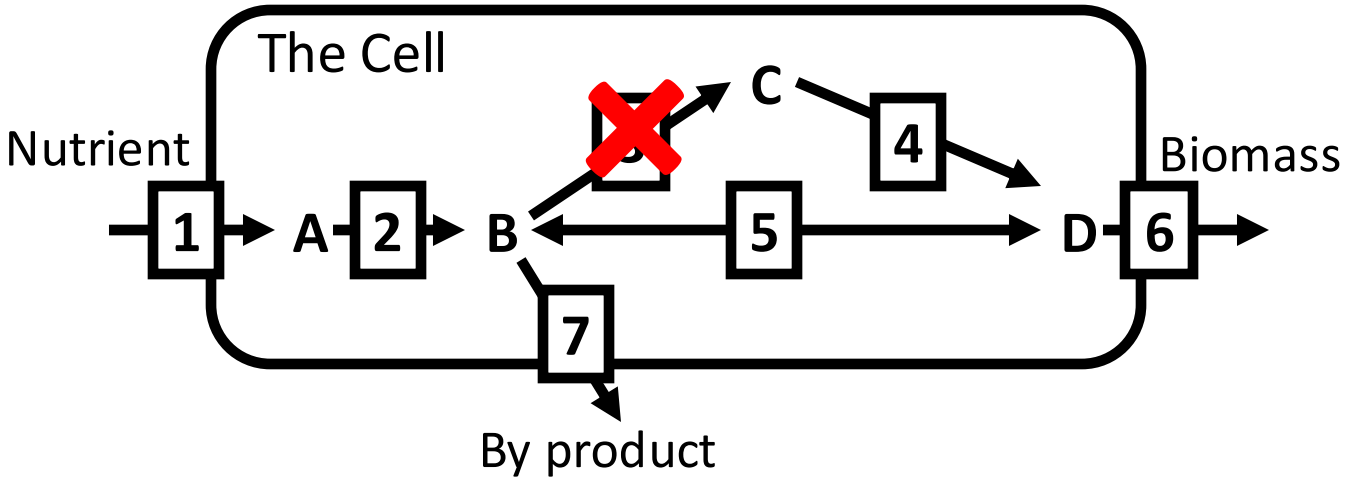
YES!

Lewis, N. E., Nagarajan, H., Palsson, B. O., Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico methods. *Nature reviews. Microbiology* 2012

Example

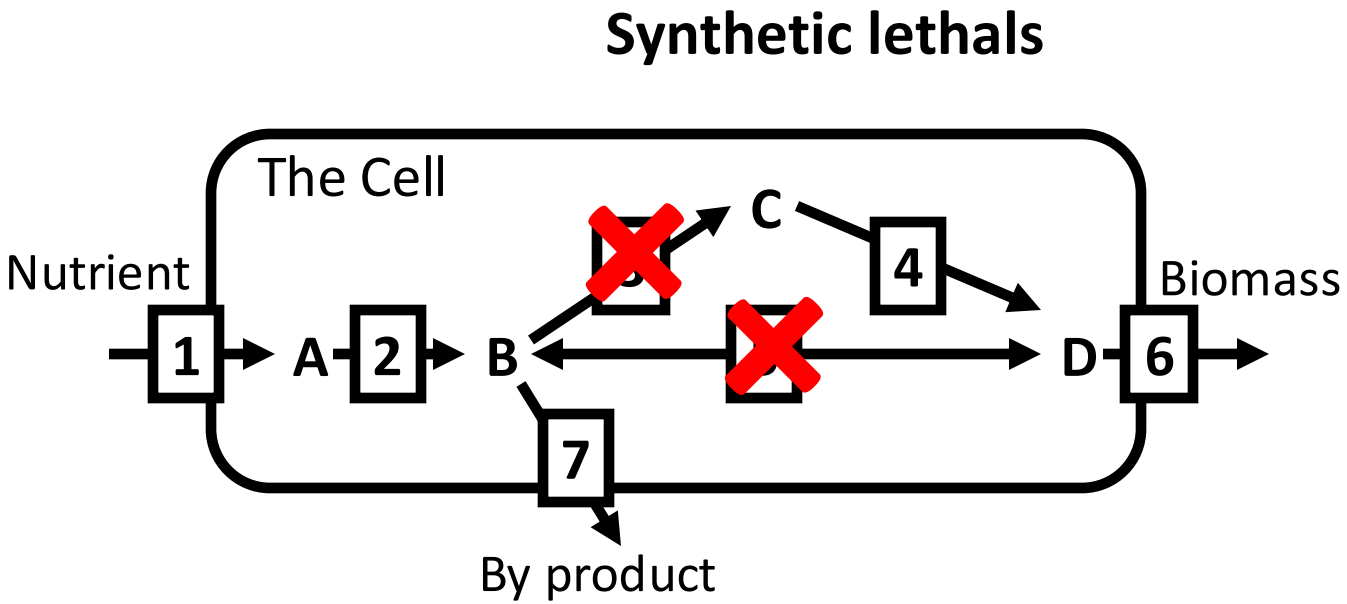


Example



- **Localization:** gene 3 is localized in the cytosol
- **Function:** gene 3 encodes for enzyme 3 that catalyzes reaction from B to C
- **Lethal effect ?:** No the presence of gene 5 enables the production of molecule D that is required for growth

Example



- **Localization:** genes 3 & 5 are localized in the cytosol
- **Function:** both encode for enzymes/pathways that enable production of D
- **Lethal effect:** The knockout of genes 3 & 5 impedes the production of molecule D that is required for growth