

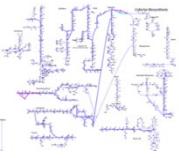
E.coli Core Model: Metabolic Core



LEARNING OBJECTIVES

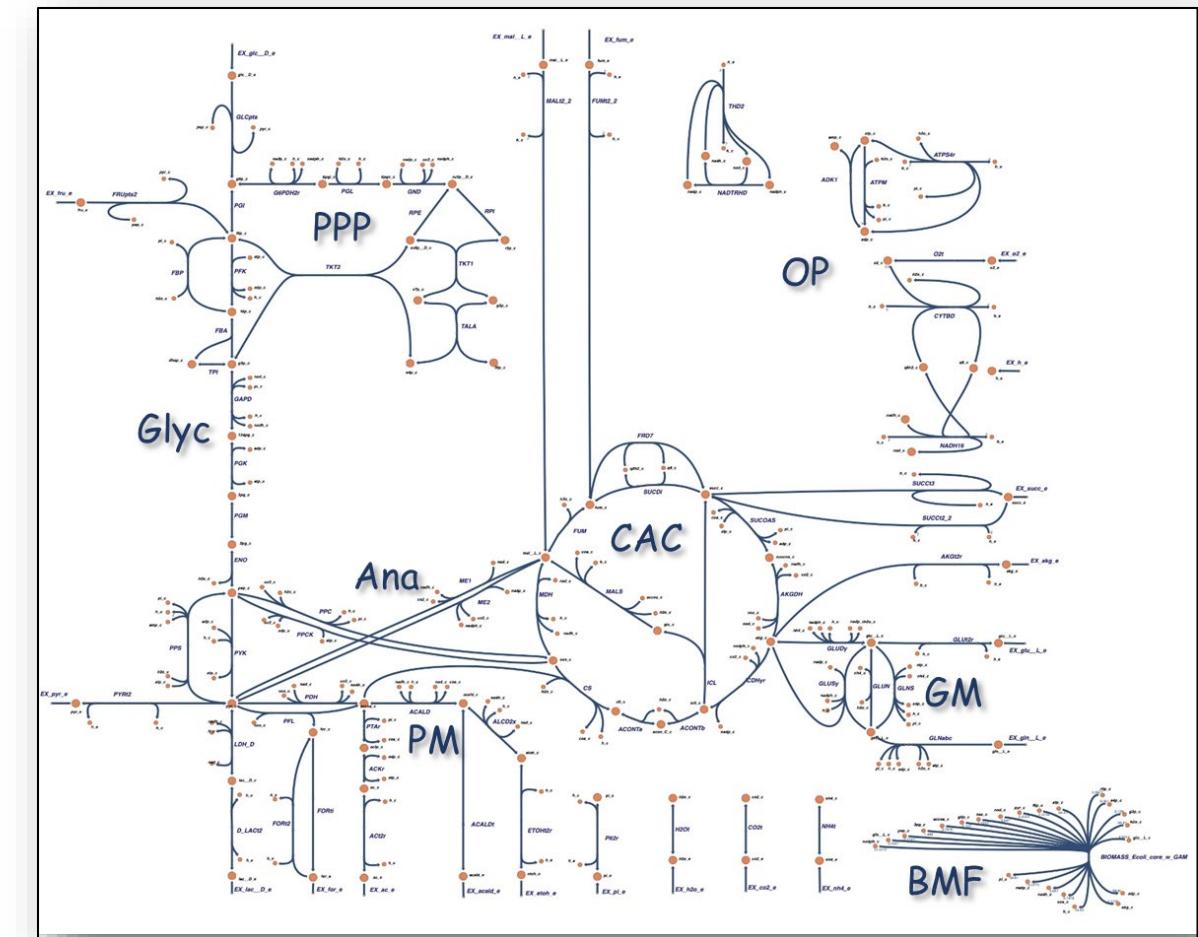
Each student should be able to:

- Describe the glycolysis pathway in the core model.
- Describe the TCA cycle in the core model.
- Explain gluconeogenesis.
- Describe the pentose phosphate pathway in the core model.
- Describe the glyoxylate cycle and anapleurotic pathways in the core model.
- Describe the oxidative phosphorylation and electron transport chain pathways in the core model.
- Describe the fermentation pathways in the core model.
- Describe the nitrogen metabolism pathways in the core model.



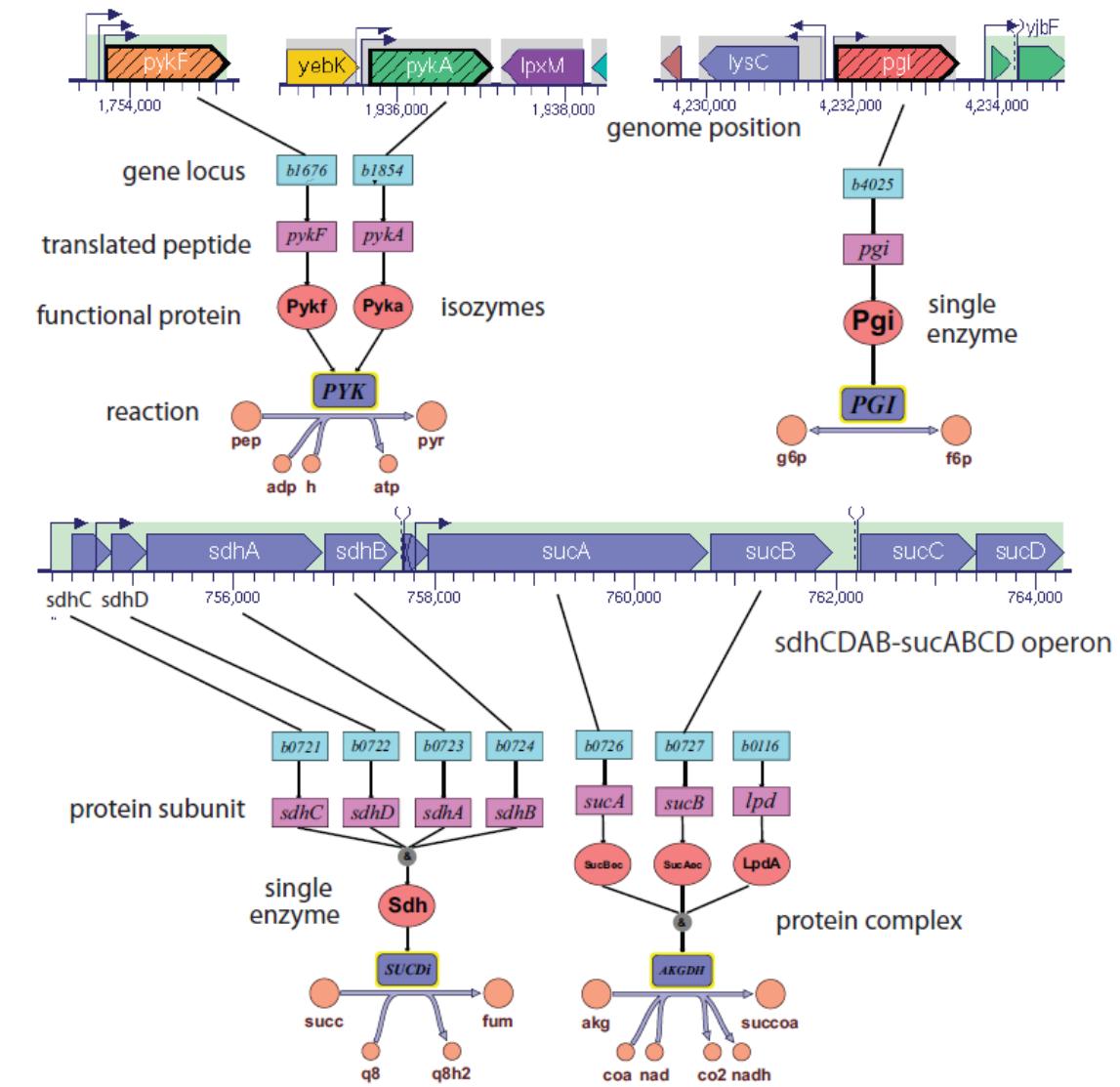
E.coli Core Model Subsystems

- Component Parts of the *E. coli* Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
- Citric Acid Cycle
- Anaplerotic Reactions
- Oxidative Phosphorylation
- Pyruvate Metabolism
- Glutamate Metabolism
- Biomass and Maintenance Functions
- Inorganic Ion Transport and Metabolism
- Transport, Extracellular
- Extracellular Exchange





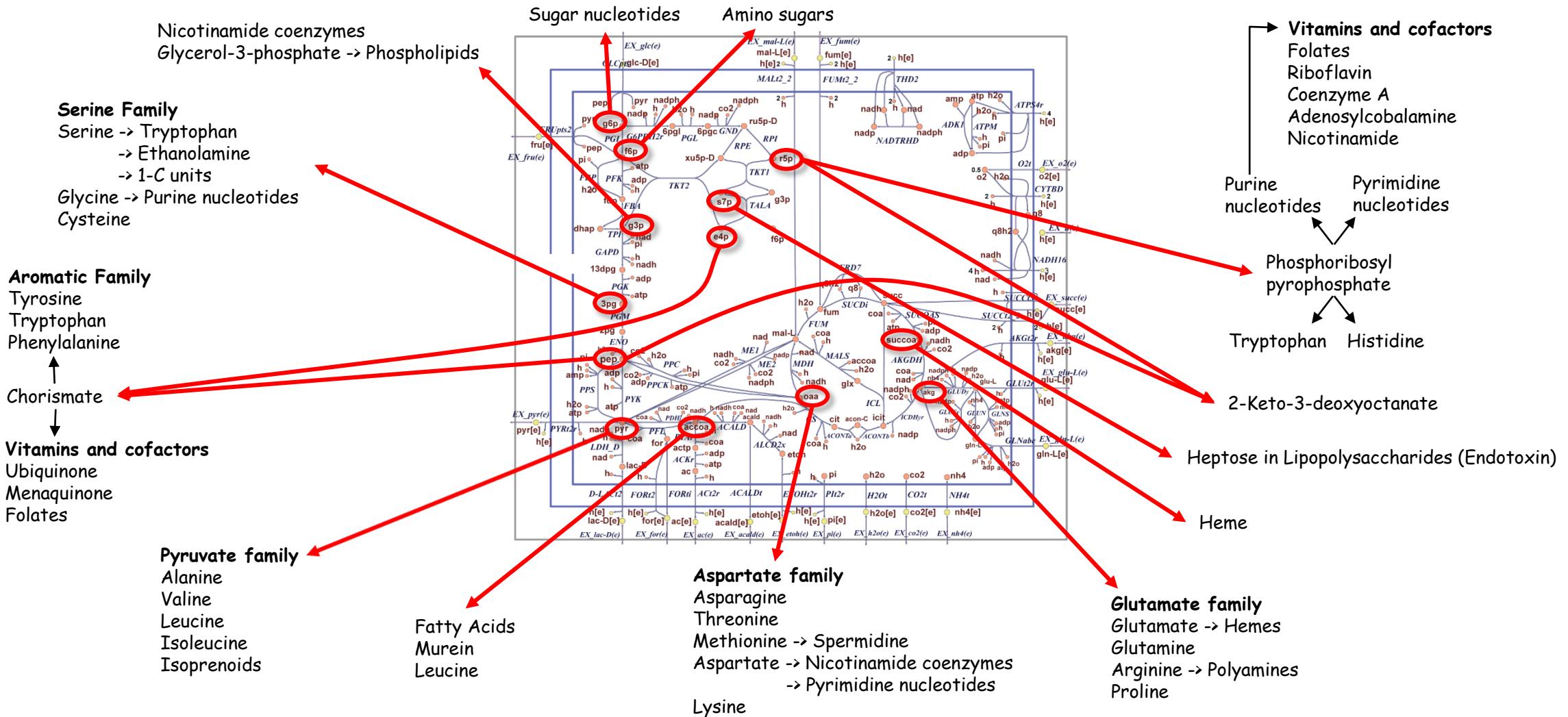
Component Parts of the *E. coli* Core Model

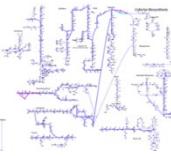


Reconstruction and Use of Microbial Metabolic Networks: the Core Escherichia coli Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)



E.coli Precursor Metabolites





E.coli Core Model Subsystems

Glyc - Glycolysis/Gluconeogenesis

PPP - Pentose Phosphate Pathway

CAC - Citric Acid Cycle

ANA - Anaplerotic Reactions

OP - Oxidative Phosphorylation

PM - Pyruvate Metabolism

GM - Glutamate Metabolism

BM - Biomass and Maintenance Functions

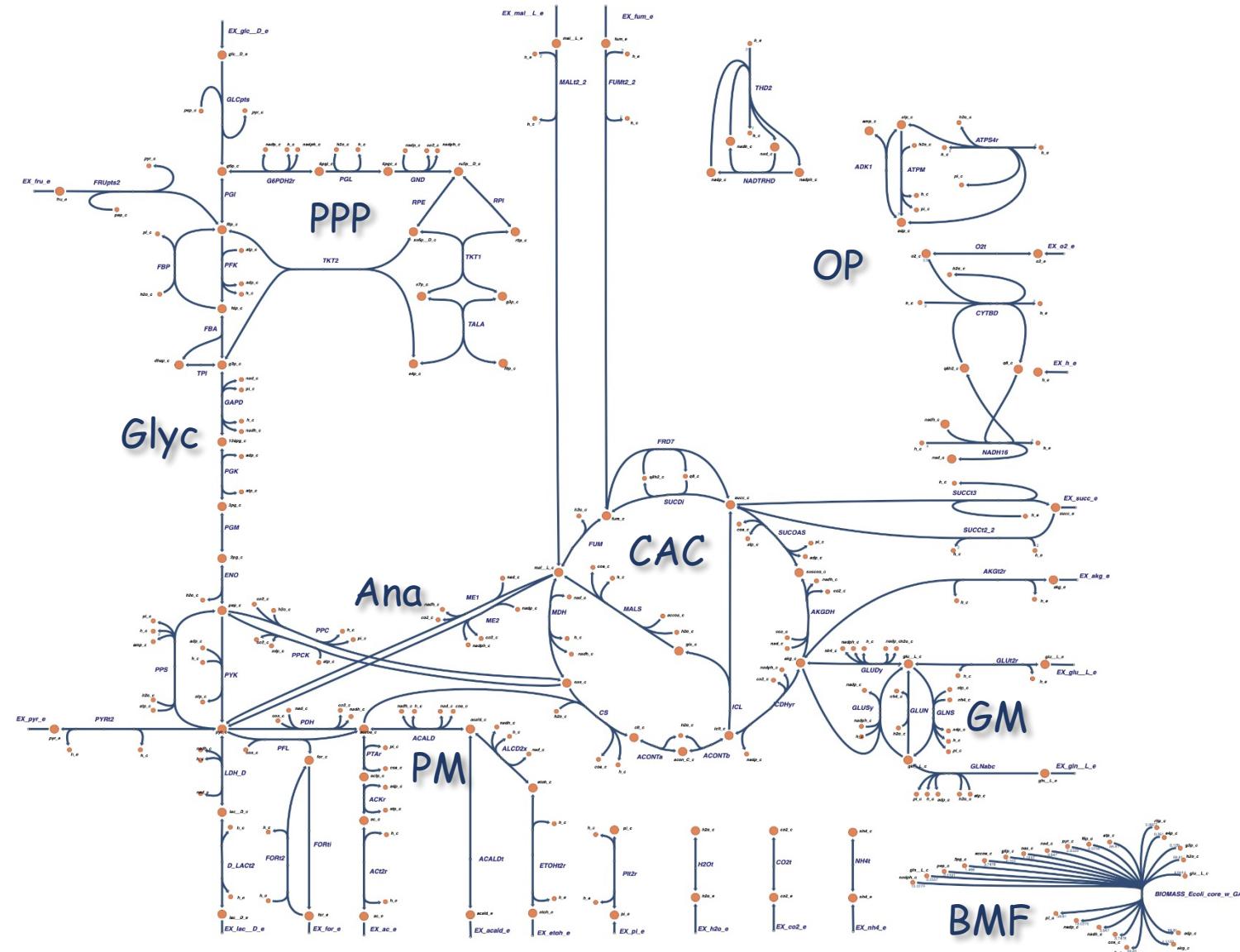
Not shown on Map

TE - Transport, Extracellular

IITM - Inorganic Ion Transport and Metabolism

EE - Extracellular Exchange

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





E.coli Core Model Subsystems

Ecoli_core_model_subsystems.ipynb

Set the model environment

```
In [1]: import cobra.test
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import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
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pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory
```

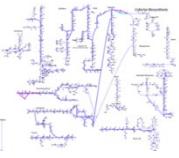
Set parameter Username

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Introduction

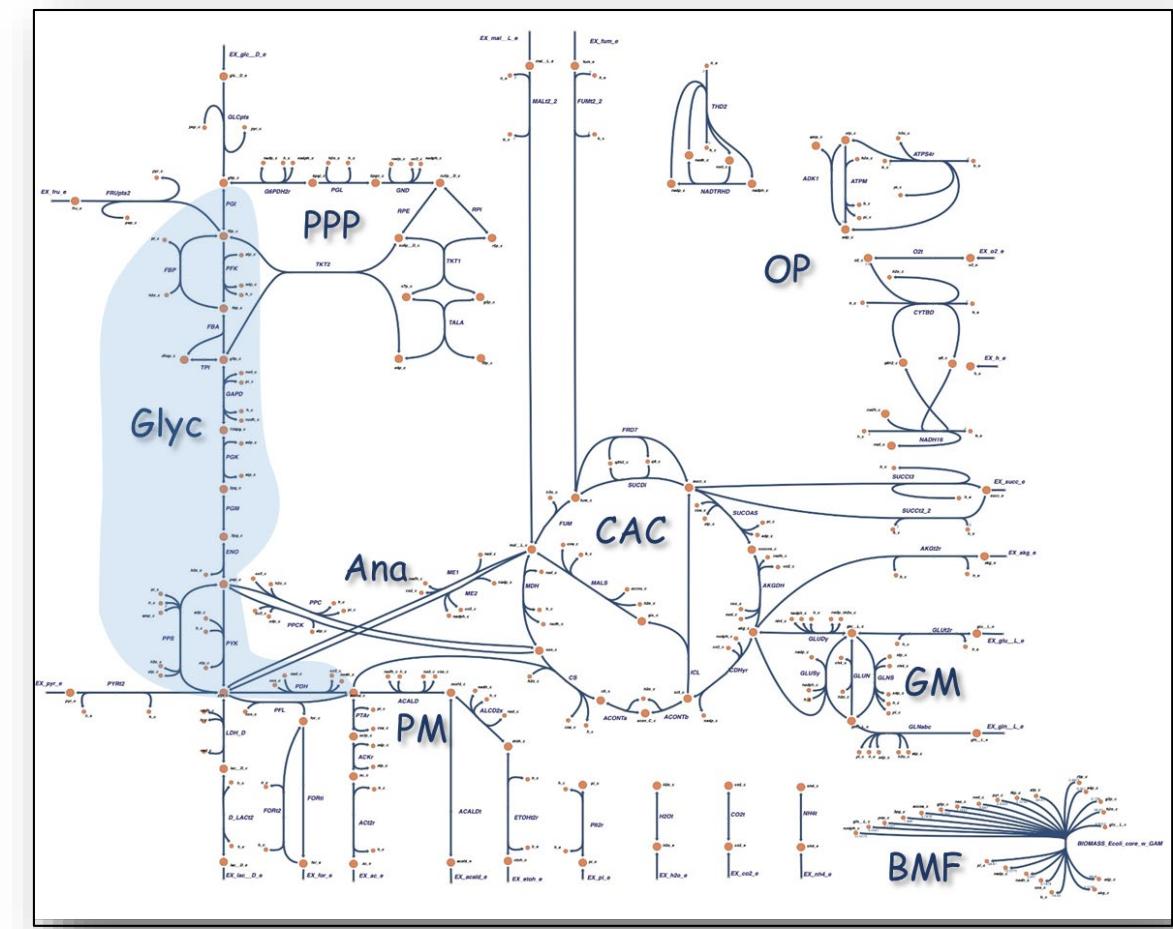
This tutorial will explore the operation of the *E.coli* core model by looking at each of the model subsystems. The different subsystems can be found to be.

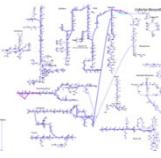
```
In [2]: reactionSubsystem = [r.subsystem for r in model.reactions]
df2 = pd.DataFrame(reactionSubsystem)
df3 = df2.drop_duplicates()
df3
```



E.coli Core Model Subsystems

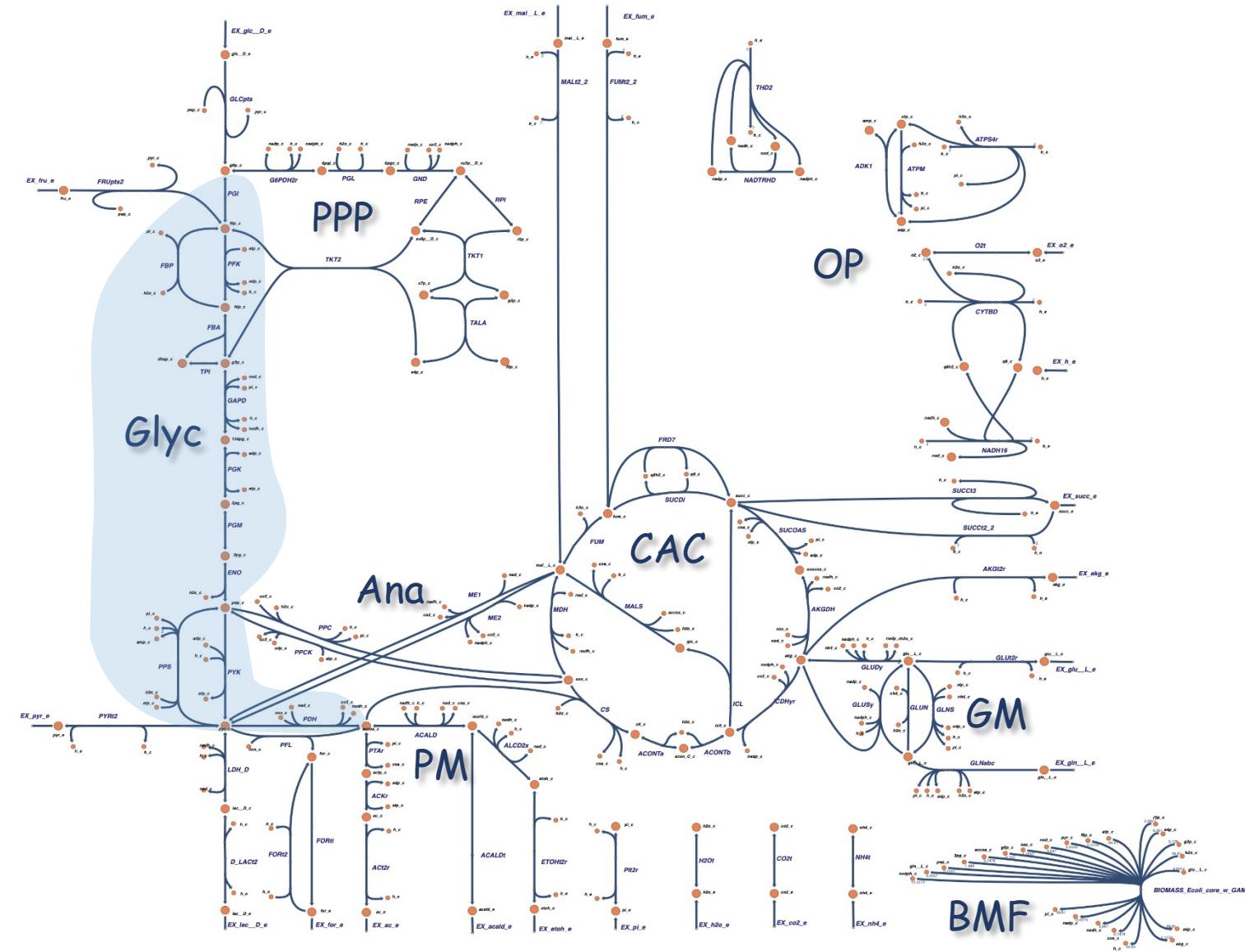
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- Pyruvate Metabolism
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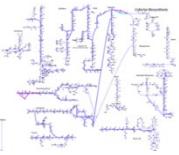


Glycolysis/Gluconeogenesis Subsystem

E.coli Core Model

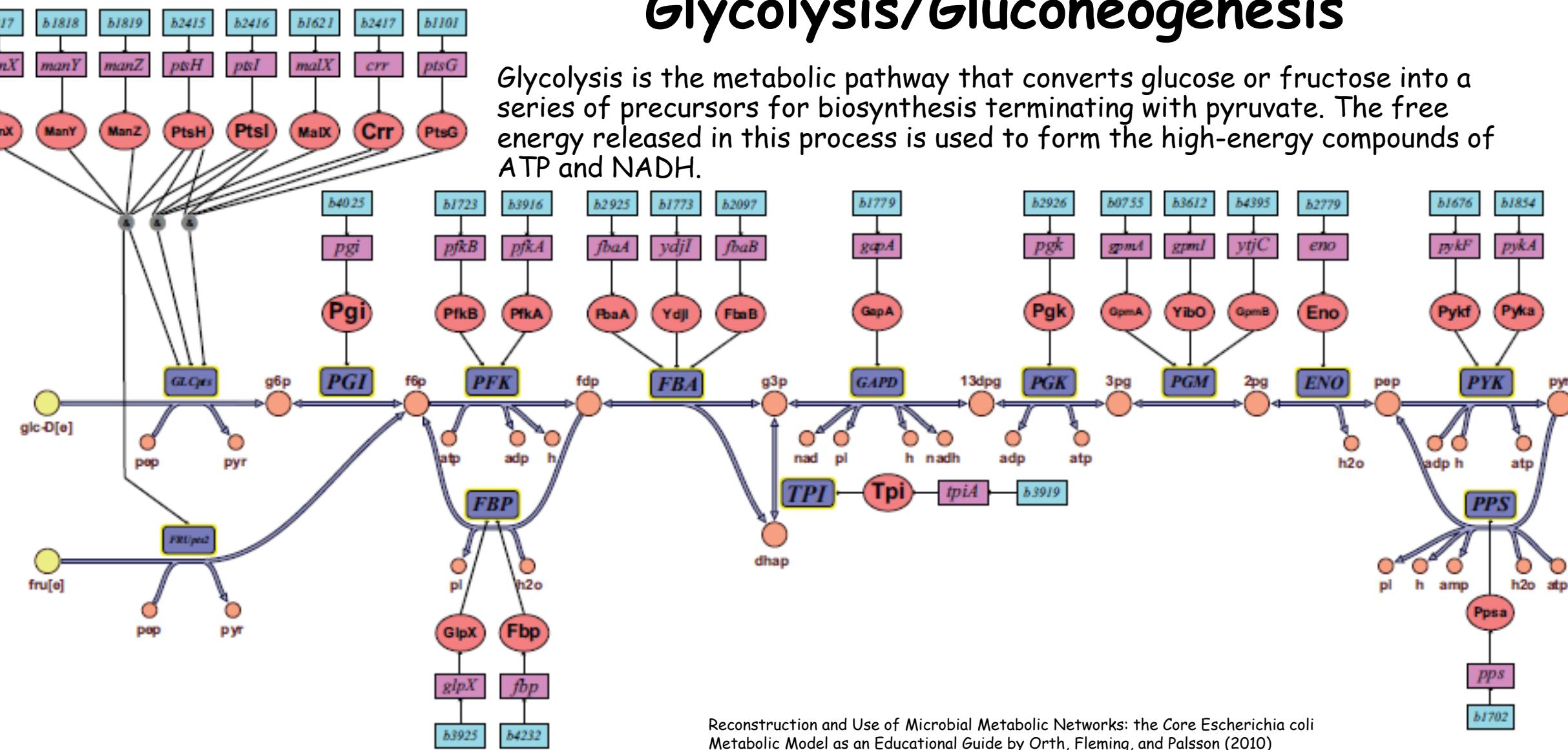


Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" *Nature biotechnology* 28(3): 245-248.

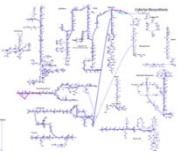


Glycolysis/Gluconeogenesis

Glycolysis is the metabolic pathway that converts glucose or fructose into a series of precursors for biosynthesis terminating with pyruvate. The free energy released in this process is used to form the high-energy compounds of ATP and NADH.

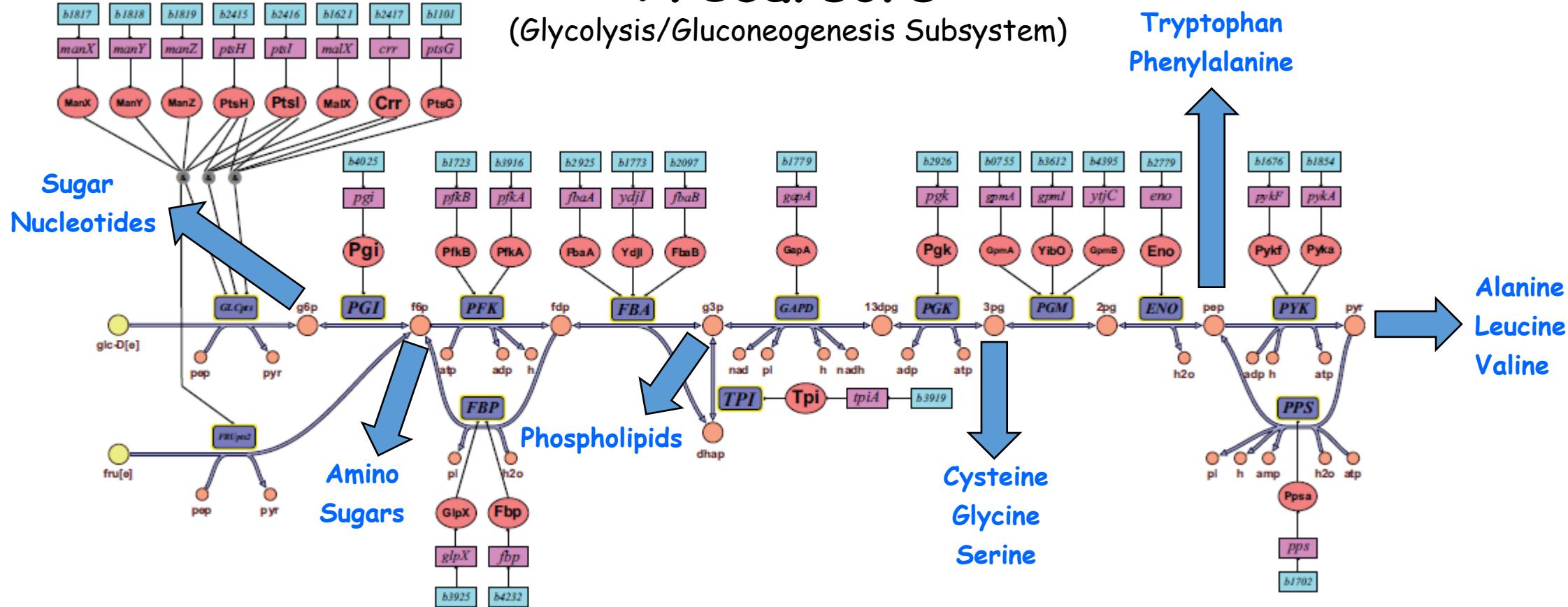


Reconstruction and Use of Microbial Metabolic Networks: the Core *Escherichia coli* Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)



Biosynthetic Precursors

(Glycolysis/Gluconeogenesis Subsystem)

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



Glycolysis/Gluconeogenesis Subsystem Reactions

Reaction ID	Reaction Name	Reaction Formula
PFK	Phosphofructokinase	atp_c + f6p_c --> adp_c + fdp_c + h_c
PGI	Glucose-6-phosphate isomerase	g6p_c <=> f6p_c
PGK	Phosphoglycerate kinase	3pg_c + atp_c <=> 13dpg_c + adp_c
PGM	Phosphoglycerate mutase	2pg_c <=> 3pg_c
PPS	Phosphoenolpyruvate synthase	atp_c + h2o_c + pyr_c --> amp_c + 2.0 h_c + pep_c + pi_c
PYK	Pyruvate kinase	adp_c + h_c + pep_c --> atp_c + pyr_c
ENO	Enolase	2pg_c <=> h2o_c + pep_c
TPI	Triose-phosphate isomerase	dhap_c <=> g3p_c
FBA	Fructose-bisphosphate aldolase	fdp_c <=> dhap_c + g3p_c
FBP	Fructose-bisphosphatase	fdp_c + h2o_c --> f6p_c + pi_c
GAPD	Glyceraldehyde-3-phosphate dehydrogenase	g3p_c + nad_c + pi_c <=> 13dpg_c + h_c + nadh_c
PDH	Pyruvate dehydrogenase	coa_c + nad_c + pyr_c --> accoa_c + co2_c + nadh_c



Constraint-based Metabolic Reconstructions & Analysis

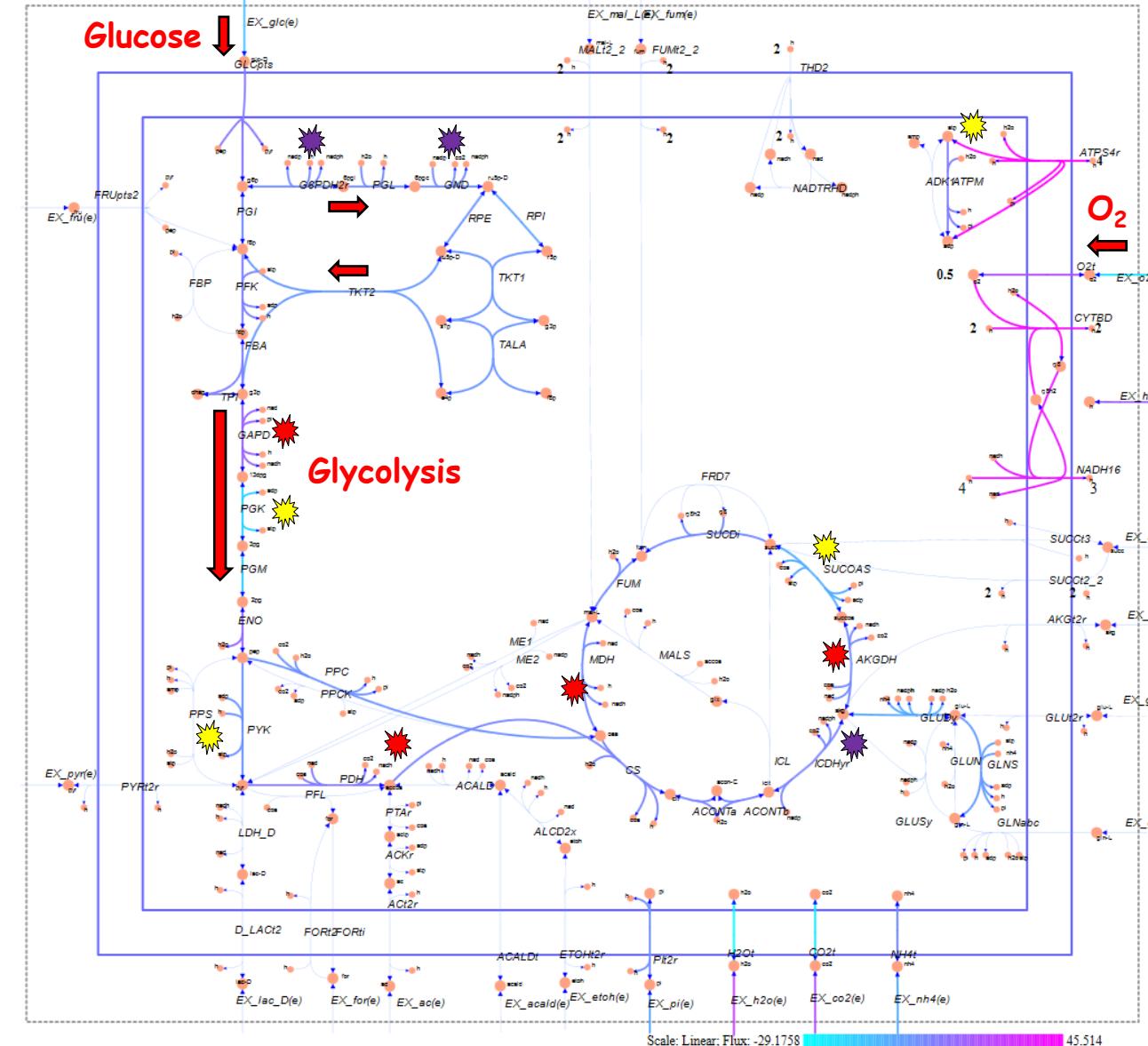
2022 H. Scott Hinton

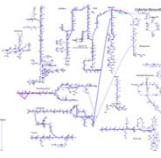
13

Aerobic Conditions
Carbon Source: Glucose

ATP =
NADPH =
NADH =

AerobicGlucoseBioMass.ipynb



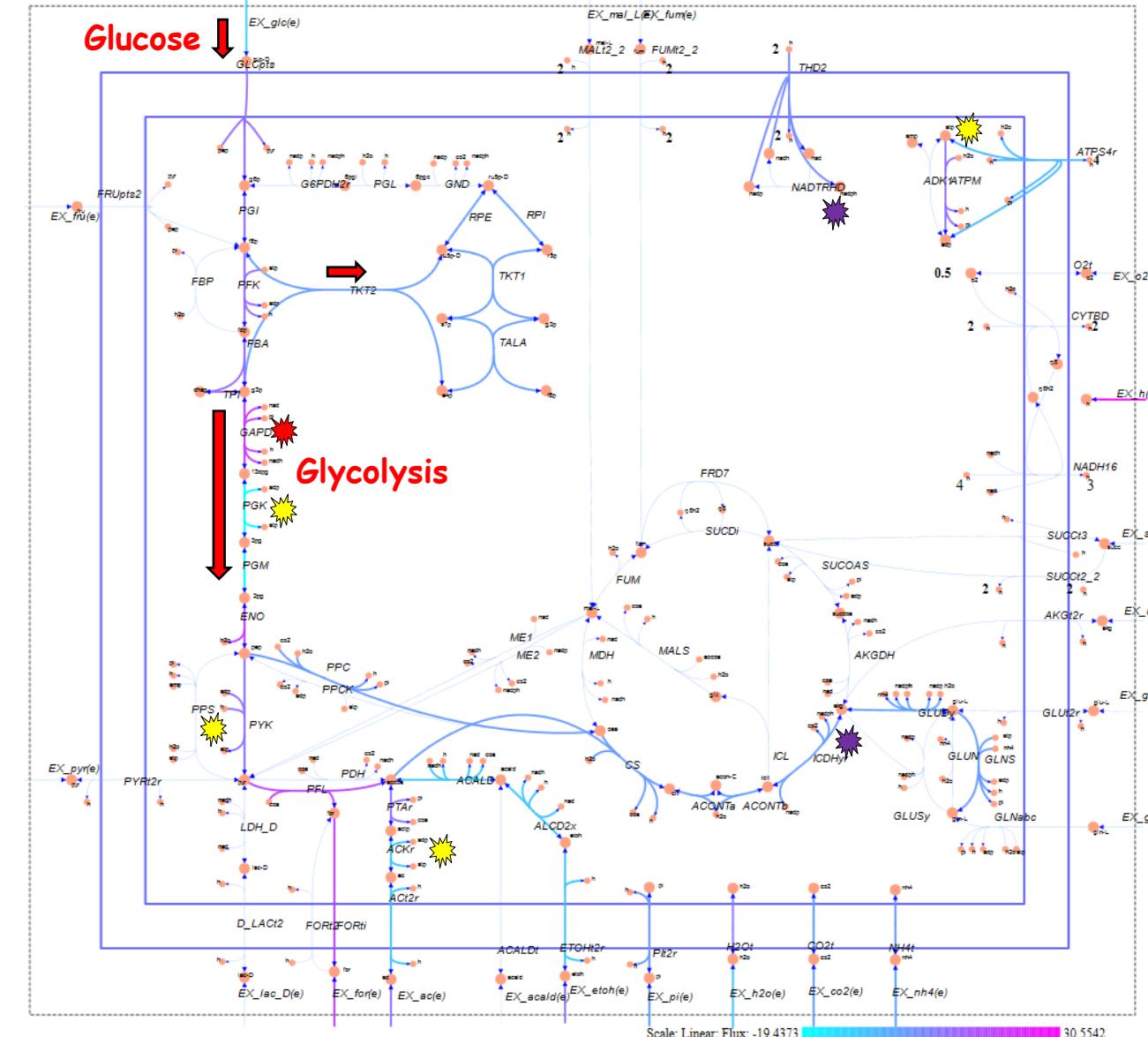


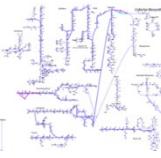
Anaerobic Conditions
Carbon Source: Glucose

ATP =

NADPH =

NADH =





**Aerobic Conditions
Carbon Source: Fructose**

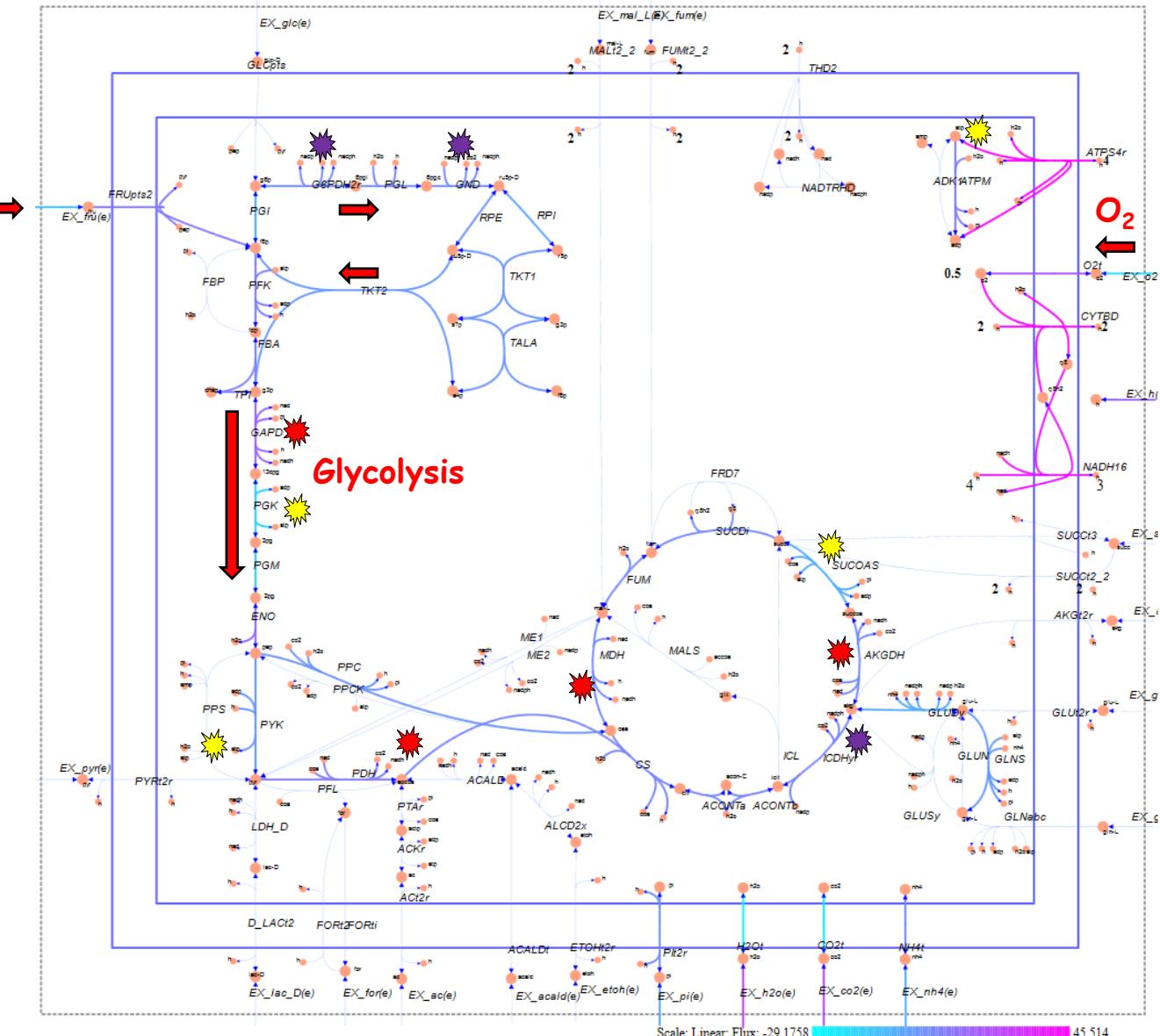
Fructose →

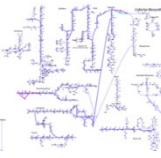
Glycolysis

ATP =

NADPH =

NADH =





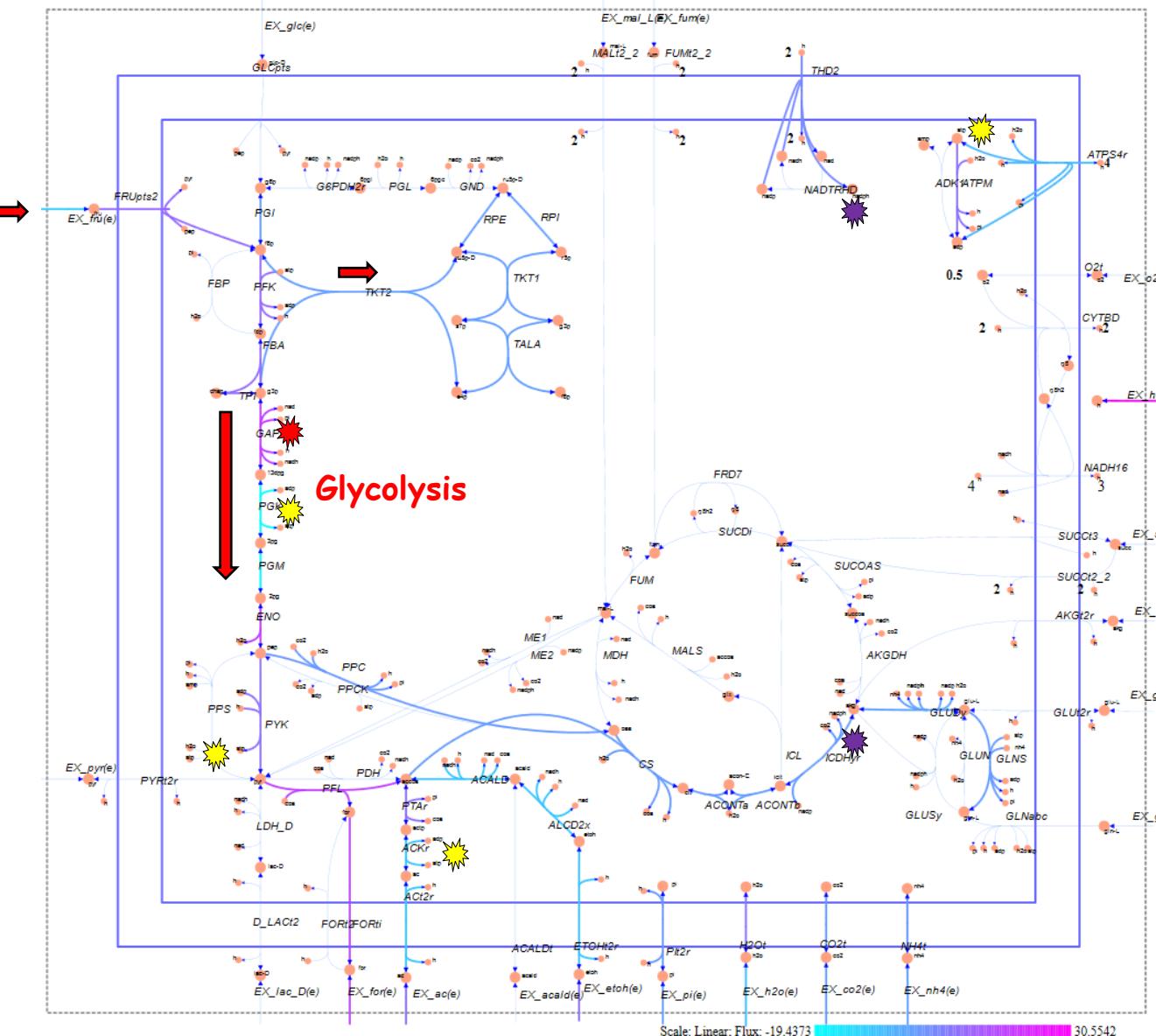
Fructose →

Anaerobic Conditions
Carbon Source: Fructose

ATP =

NADPH =

NADH =





Glycolysis/Gluconeogenesis Subsystem

Glycolysis_subsystem.ipynb

Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
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Set parameter Username
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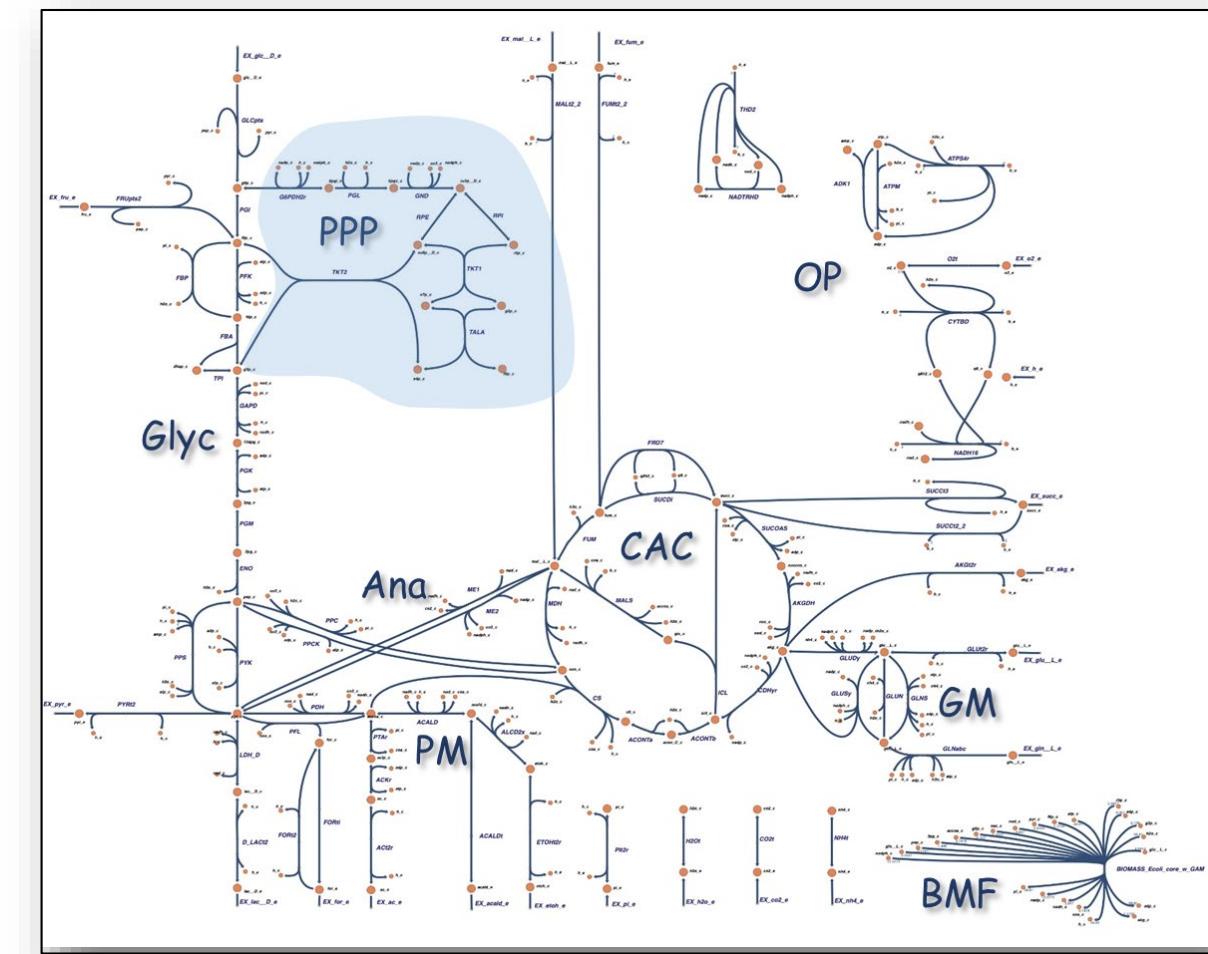
Glycolysis

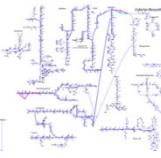
Glycolysis is the metabolic pathway in the *E.coli* core model that converts glucose and fructose into pyruvate. The free energy released in this process is used to form the high-energy compounds of atp_c]and nadh_c. The location of the glycolysis pathway on the *E.coli* core map is highlighted in the figure below.



E.coli Core Model Subsystems

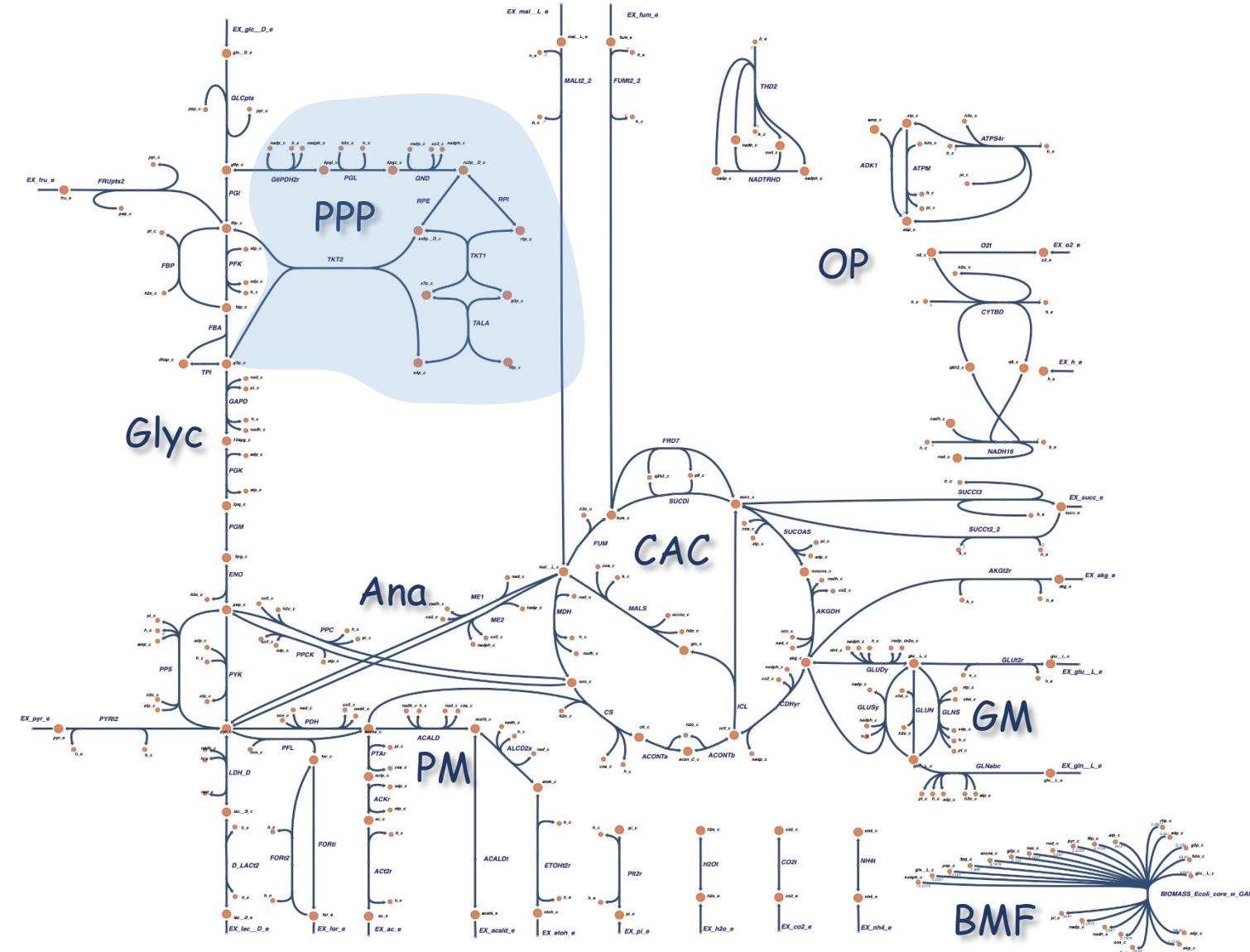
- Component Parts of the *E. coli* Core Model
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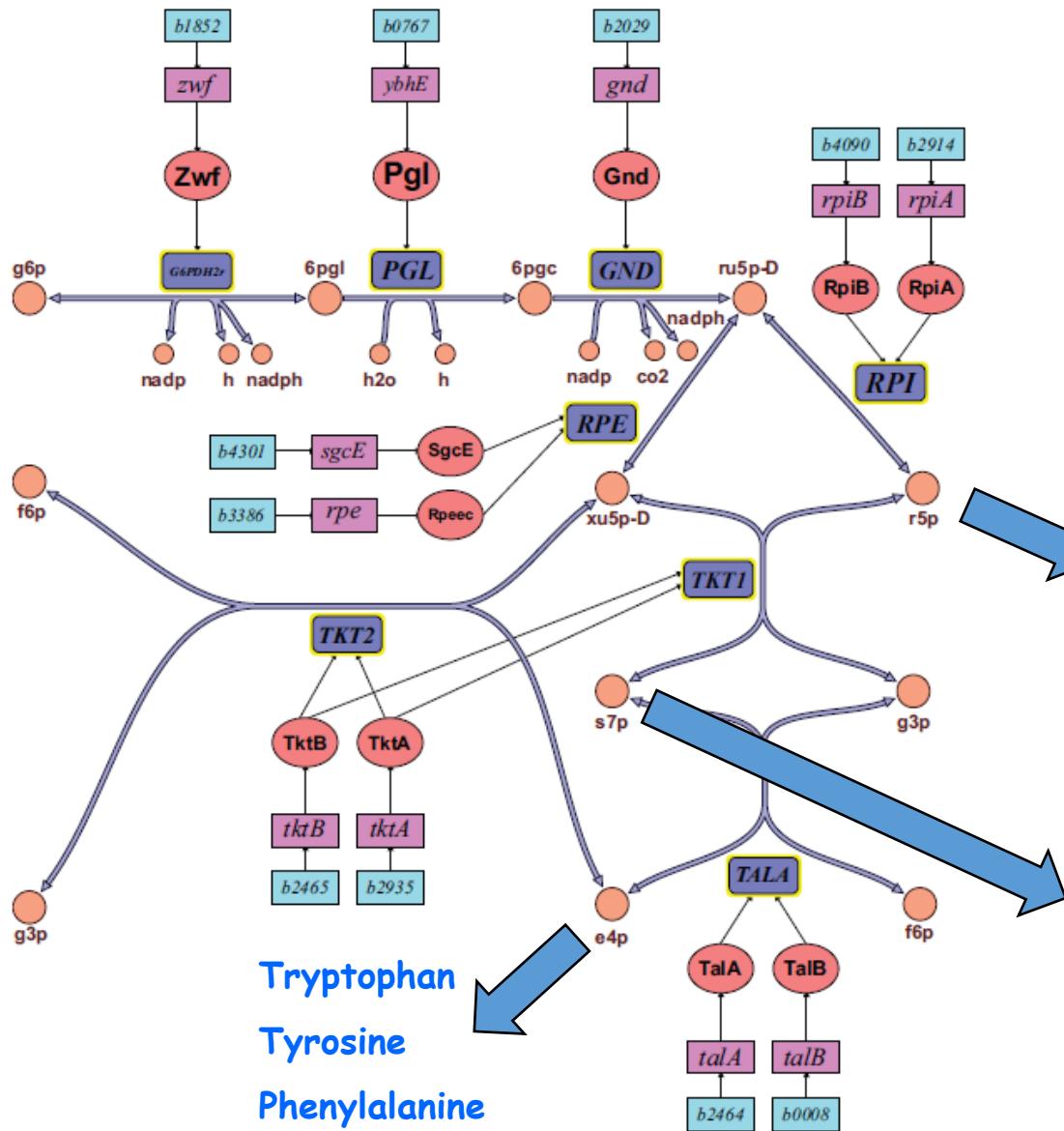


Pentose Phosphate Pathway

E.coli Core Model



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



Biosynthetic Precursors (Pentose Phosphate Pathway)

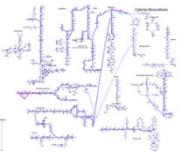
Histidine

Purines (ATP, GTP, dATP, dGTP)

Pyrimidines (UTP, CTP, dCTP, dTTP)

Heptose in Lipopolysaccharides (Endotoxin)

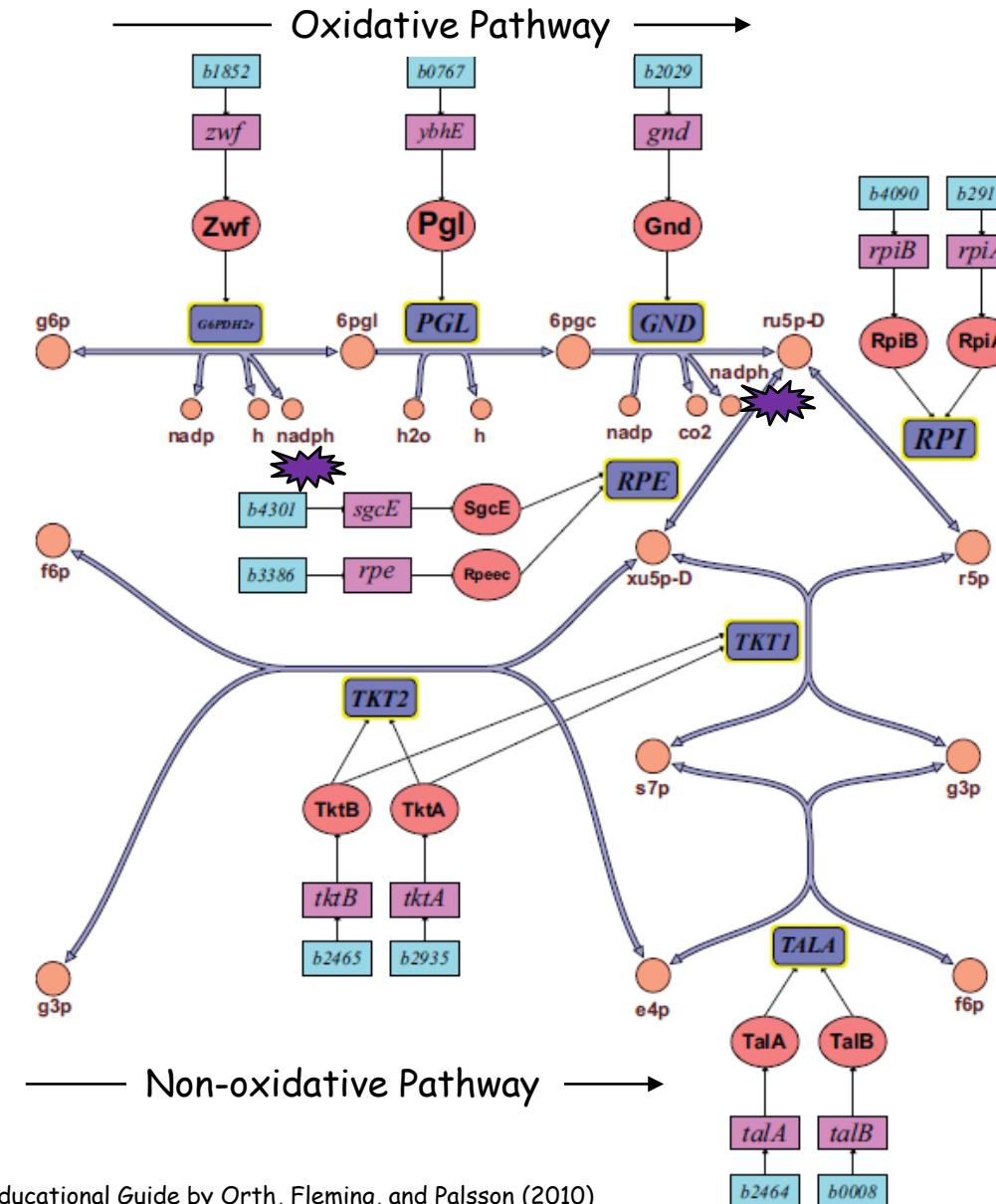
Reconstruction and Use of Microbial Metabolic Networks: the Core *Escherichia coli* Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)



Pentose Phosphate Shunt (Pentose Phosphate Pathway)

The pentose phosphate pathway generates NADPH and provides the 5-carbon (alpha-D-ribose-5-phosphate, "r5p"), 4-carbon biosynthetic precursors (D-erythrose-4-phosphate, "e4p"), and the 7-carbon, sedoheptulose-7-phosphate.

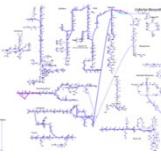
There are two distinct phases in the pathway. The first is the **oxidative phase**, in which NADPH is generated, and the second is the **non-oxidative** synthesis of 5-carbon and 4-carbon precursors.





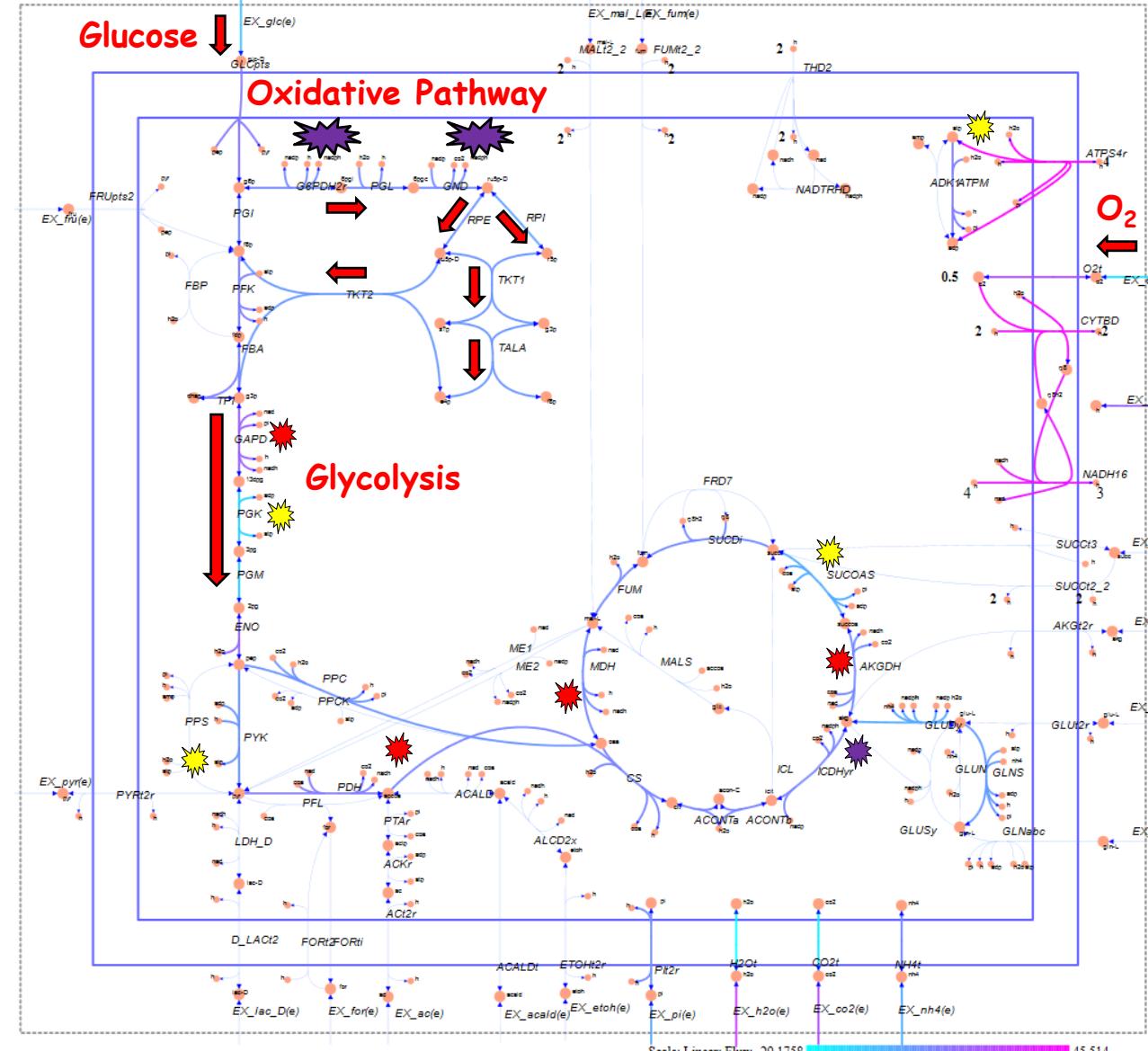
Pentose Phosphate Pathway Subsystem Reactions

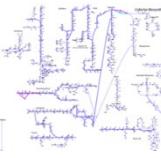
Reaction ID	Reaction Name	Reaction Formula
PGL	6-phosphogluconolactonase	6pgl_c + h2o_c --> 6pgc_c + h_c
RPE	Ribulose 5-phosphate 3-epimerase	ru5p__D_c <=> xu5p__D_c
RPI	Ribose-5-phosphate isomerase	r5p_c <=> ru5p__D_c
TALA	Transaldolase	g3p_c + s7p_c <=> e4p_c + f6p_c
TKT1	Transketolase	r5p_c + xu5p__D_c <=> g3p_c + s7p_c
TKT2	Transketolase	e4p_c + xu5p__D_c <=> f6p_c + g3p_c
G6PDH2r	Glucose 6-phosphate dehydrogenase	g6p_c + nadp_c <=> 6pgl_c + h_c + nadph_c
GND	Phosphogluconate dehydrogenase	6pgc_c + nadp_c --> co2_c + nadph_c + ru5p__D_c



Aerobic Conditions Carbon Source: Glucose

ATP =
 NADPH =
 NADH =



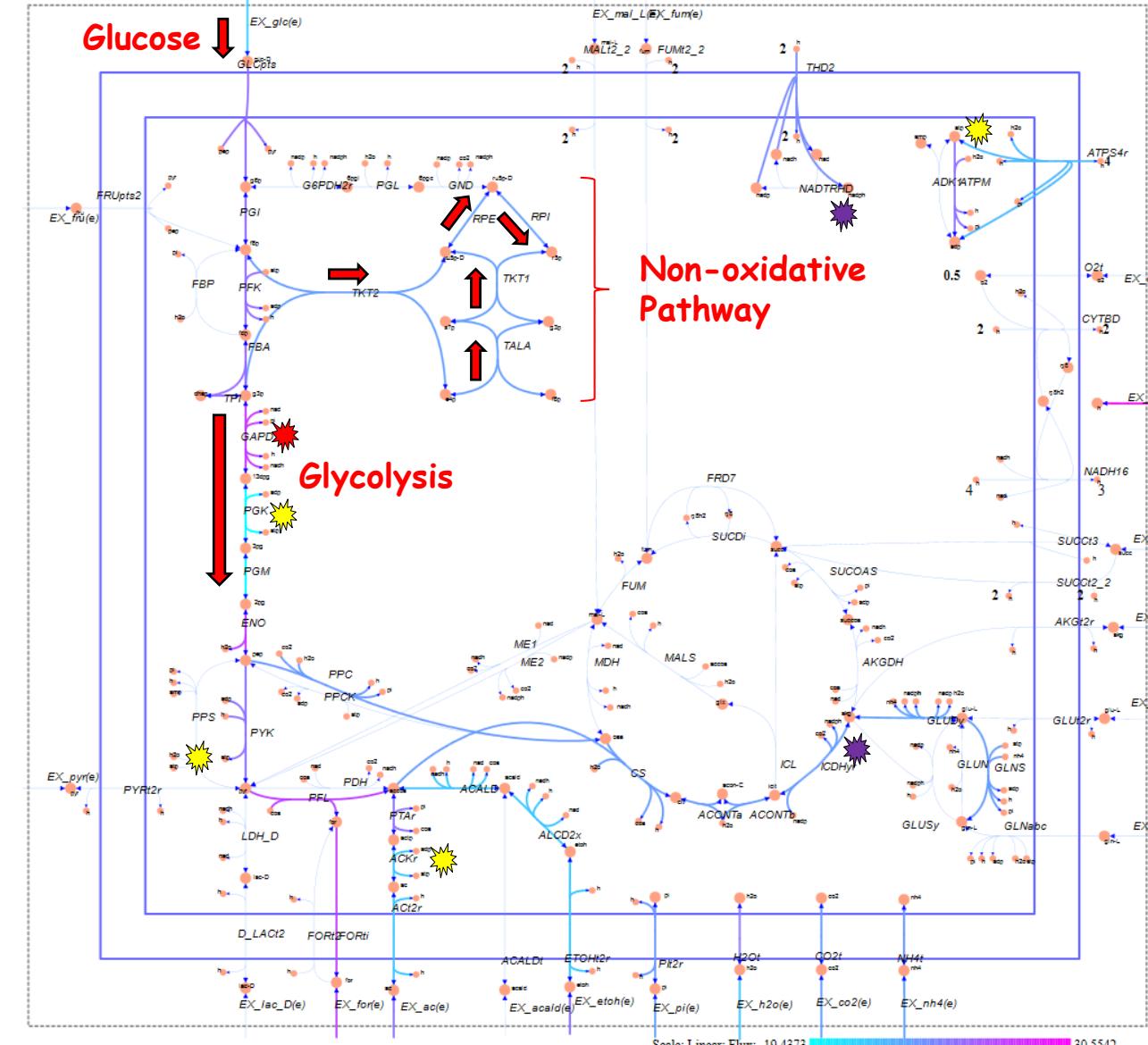


Anaerobic Conditions Carbon Source: Glucose

ATP =

NADPH =

NADH =



AnaerobicGlucoseBioMass.ipynb



Pentose Phosphate Pathway Subsystem

Pentose_Phosphate_pathway_subsystem.ipynb

Set the model environment

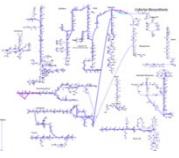
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```
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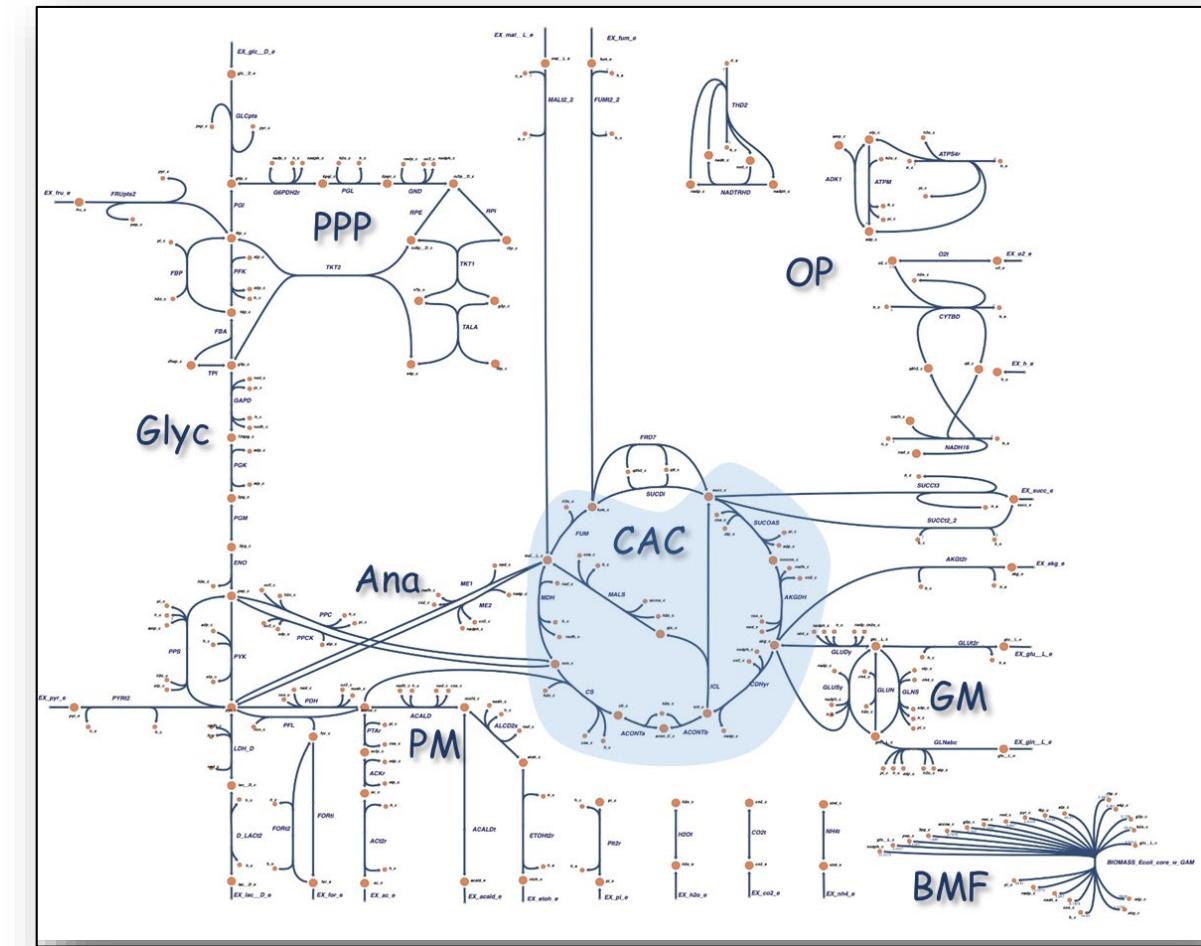
The primary purpose of the pentose phosphate pathway (PPP) is to provide the 4-, 5- and 7-carbon precursors for the cell and produce nadph_c. The 4-, 5- and 7-carbon precursors include D-erythrose-4-phosphate (e4p_c), alpha-D-ribose-5-phosphate, (r5p_c), and sedoheptulose-7-phosphate (s7p_c), respectively. The nadph_c is produced in the oxidative pathway by glucose-6-phosphate dehydrogenase (G6PDH2r) and phosphogluconate dehydrogenase (GND).

The location of the reactions associated with the PPP are shown below on the *E.coli* core map in Figure 1.



E.coli Core Model Subsystems

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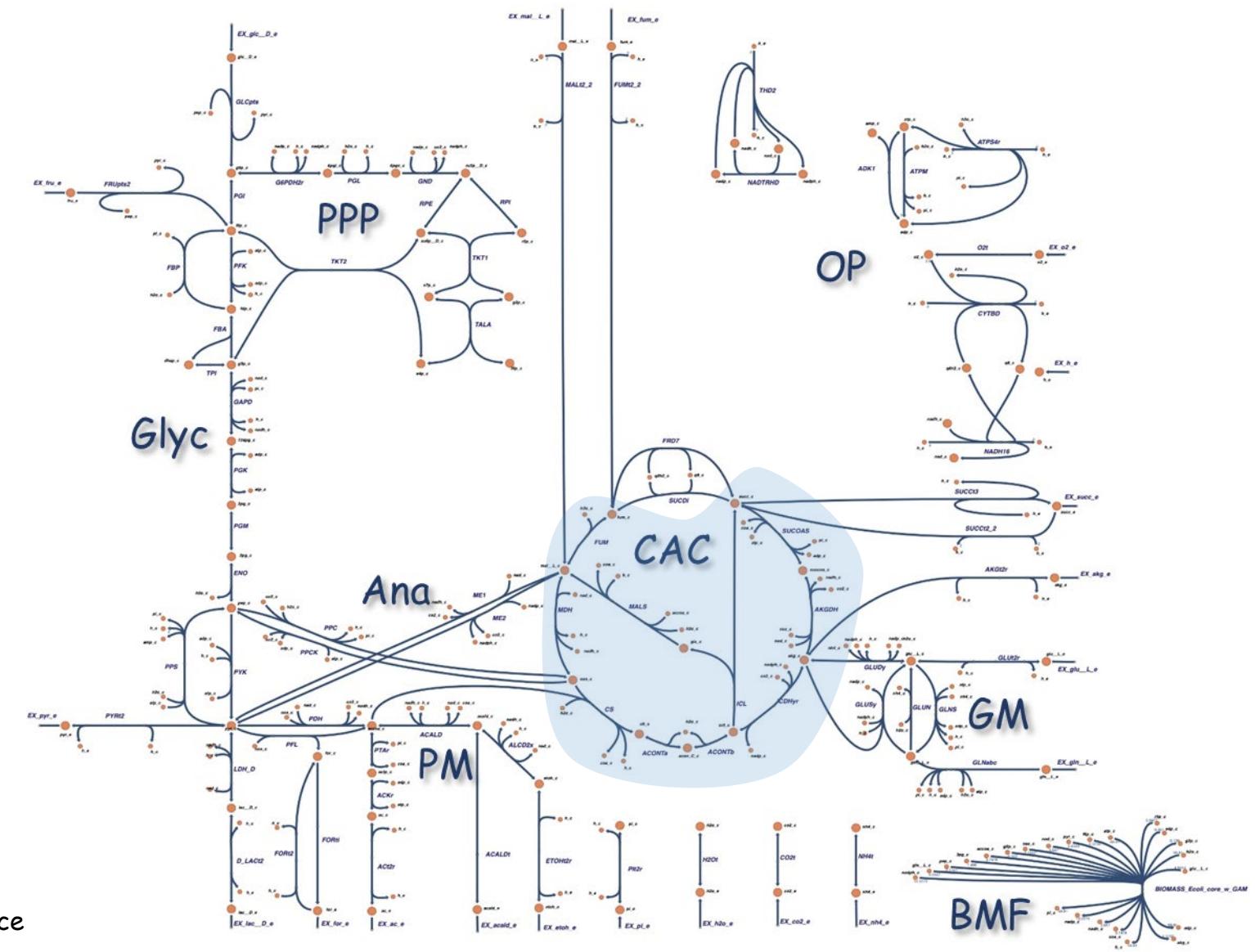


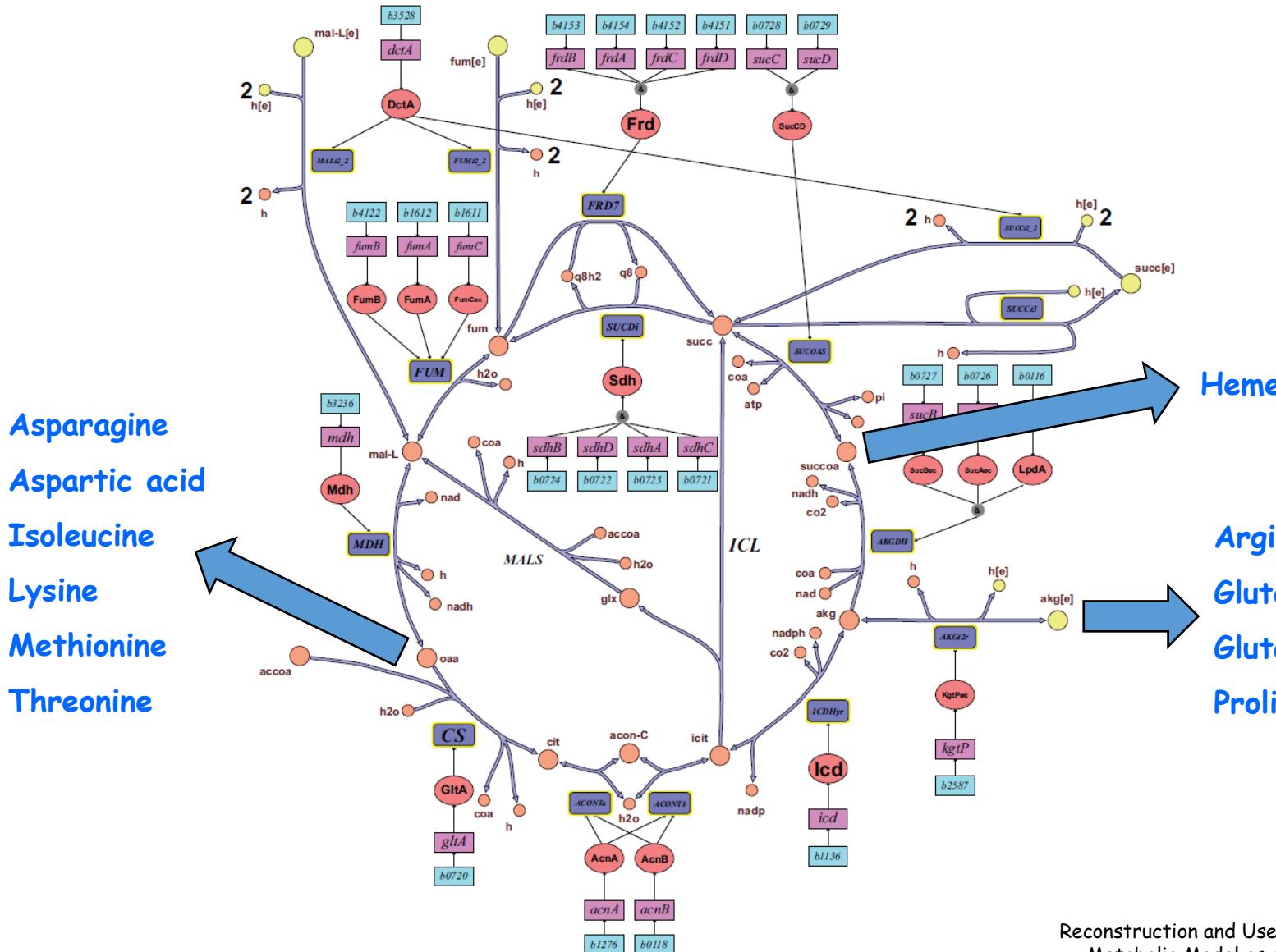


Citric Acid Cycle (CAC)

E.coli Core Model

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





CAC Biosynthetic Precursors

Asparagine
Aspartic acid
Isoleucine
Lysine
Methionine
Threonine

Heme
Arginine
Glutamine
Glutamic acid
Proline

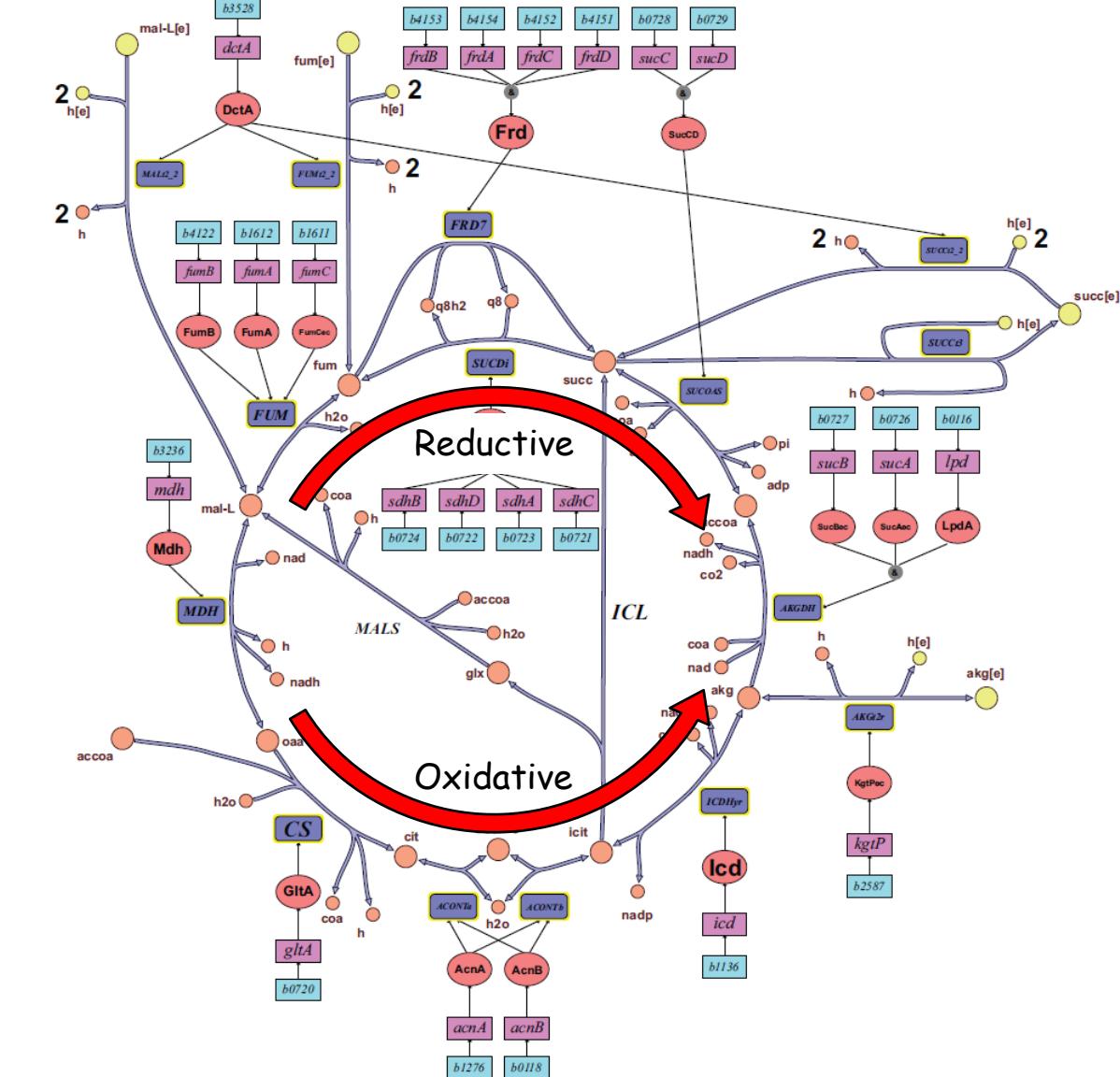
Reconstruction and Use of Microbial Metabolic Networks: the Core *Escherichia coli* Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)



Citric Acid Cycle

The oxidative pathway of the CAC runs counterclockwise in the lower part of the cycle, from oxaloacetate, oaa, through 2-oxoglutarate, akg. Continuing counterclockwise from 2-oxoglutarate, the full tricarboxylic acid cycle can totally oxidize acetyl-CoA, but is only functional during aerobic growth on acetate or fatty acids.

Under anaerobic conditions, the CAC functions not as a cycle, but as two separate pathways. The **oxidative pathway**, the counterclockwise lower part of the cycle, still forms the precursor 2-oxoglutarate. The **reductive pathway**, the clockwise upper part of the cycle, can form the precursor succinyl-CoA.

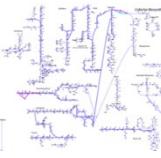


Reconstruction and Use of Microbial Metabolic Networks: the Core *Escherichia coli* Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)



CAC Metabolites & Reactions

Reaction ID	Reaction Name	Reaction Formula
ACONTa	Aconitase (half-reaction A, Citrate hydro-lyase)	cit_c <=> acon_C_c + h2o_c
ACONTb	Aconitase (half-reaction B, Isocitrate hydro-lyase)	acon_C_c + h2o_c <=> icit_c
AKGDH	2-Oxoglutarate dehydrogenase	akg_c + coa_c + nad_c --> co2_c + nadh_c + succoa_c
CS	Citrate synthase	accoa_c + h2o_c + oaa_c --> cit_c + coa_c + h_c
SUCOAS	Succinyl-CoA synthetase (ADP-forming)	atp_c + coa_c + succ_c <=> adp_c + pi_c + succoa_c
FUM	Fumarase	fum_c + h2o_c <=> mal__L_c
ICDHyr	Isocitrate dehydrogenase (NADP)	icit_c + nadp_c <=> akg_c + co2_c + nadph_c
MDH	Malate dehydrogenase	mal__L_c + nad_c <=> h_c + nadh_c + oaa_c

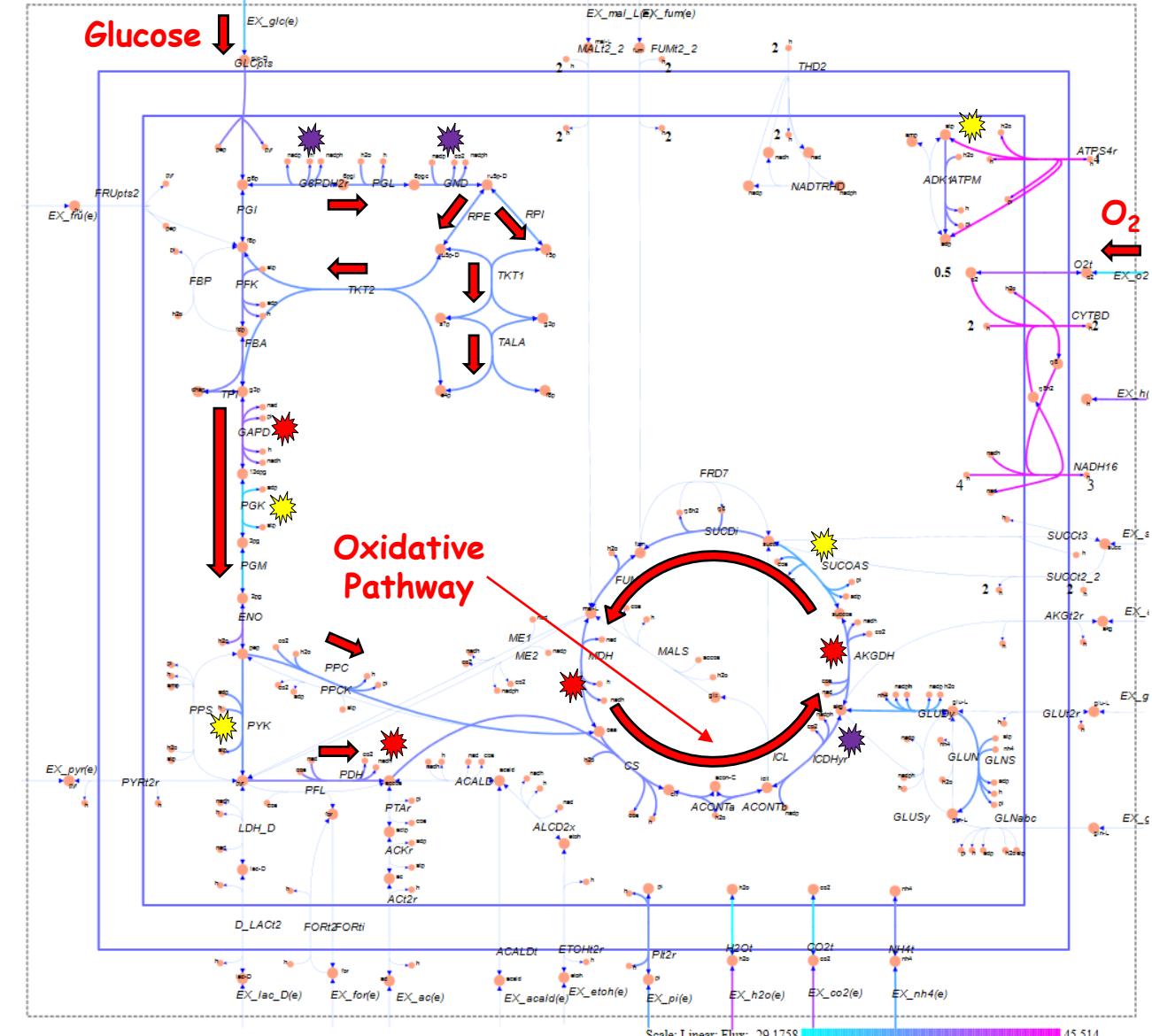


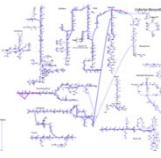
Aerobic Conditions Carbon Source: Glucose

ATP =

NADPH =

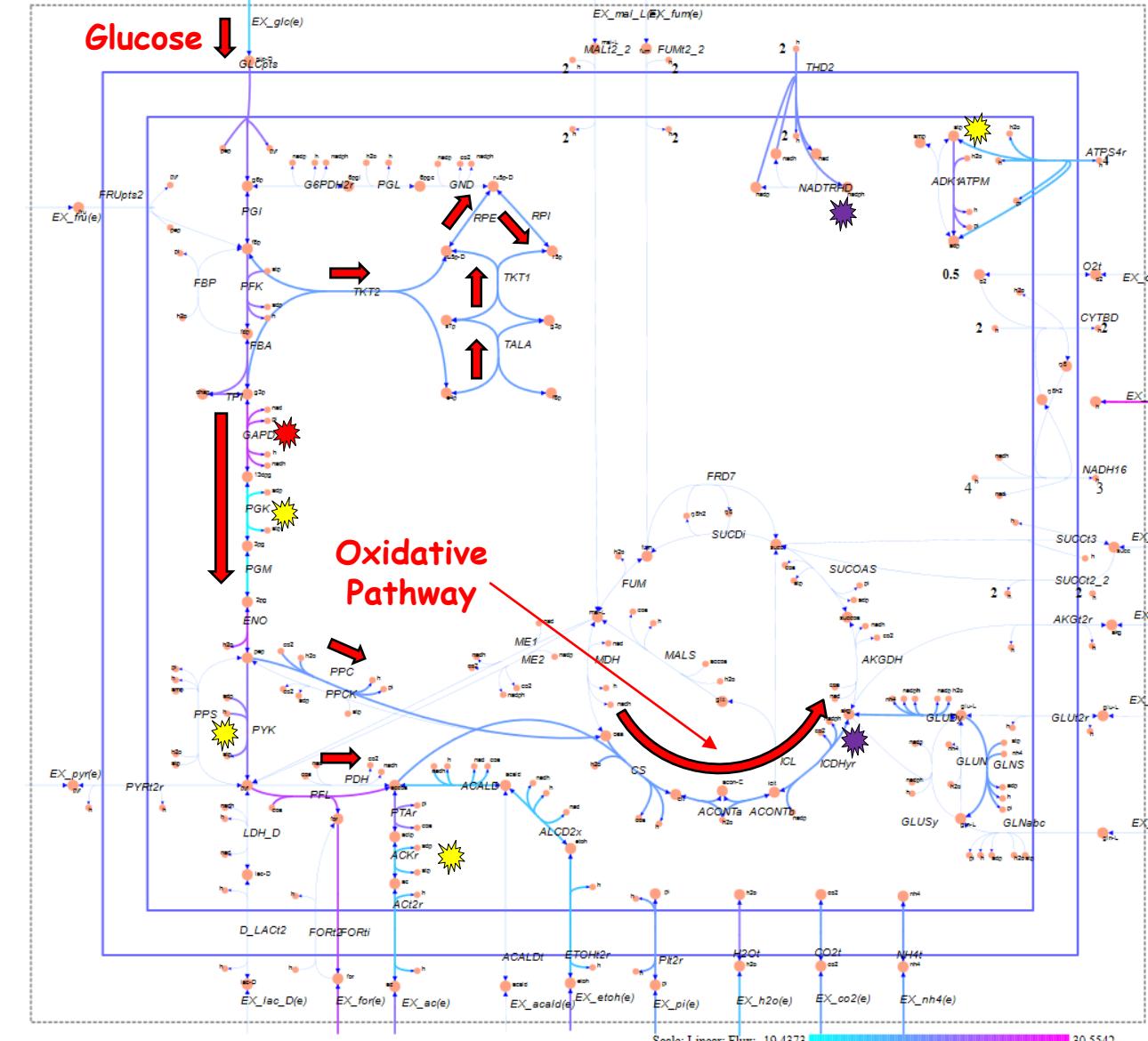
NADH =





Anaerobic Conditions Carbon Source: Glucose

ATP =
 NADPH =
 NADH =



AnaerobicGlucoseBioMass.ipynb



Citric Acid Cycle Subsystem

CAC_subsystem.ipynb

Set the model environment

```
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pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
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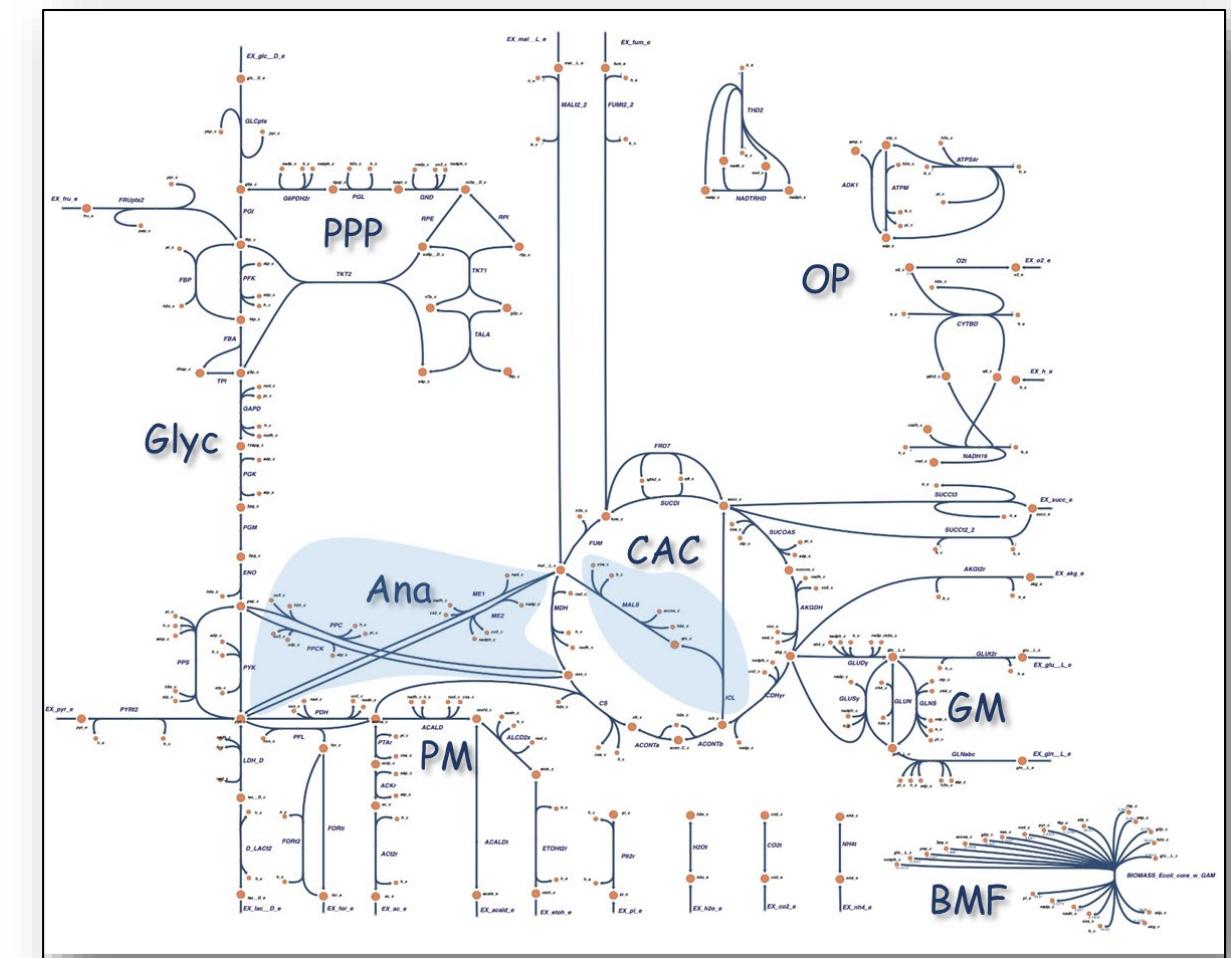
```
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Using license file C:\Users\hinton\gurobi.lic
```

The citric acid cycle (CAC) subsystem or the tricarboxylic acid (TCA) cycle supports a variety of cellular functions depending on the environment. Under aerobic conditions the CAC operates in a counter-clockwise direction using acetyl-CoA as a substrate to produce three cellular precursors, reducing power nadh_c and nadph_c, cellular energy atp_c through substrate phosphorylation, and carbon dioxide (co2_c). While in the anaerobic condition, only part of the CAC will be used to produce two of the three precursors and the reducing power nadph_c. The location of the CAC subsystem is shown on the following *E.coli* core map (Figure 1).



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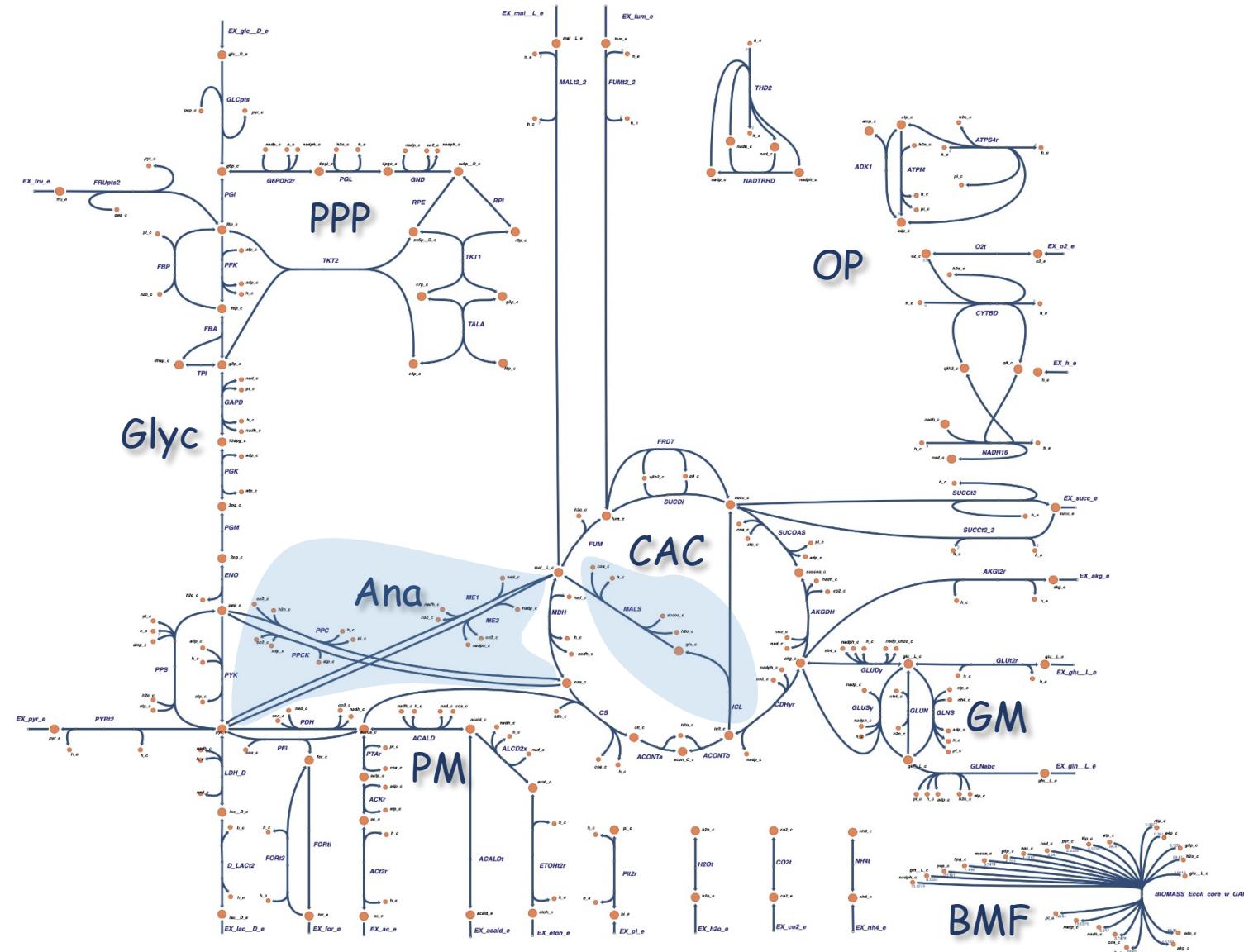
Anaplerotic Reactions Subsystem

E.coli Core Model

The glyoxylate cycle and gluconeogenic reactions allow *E. coli* to grow on 3-carbon (pyruvate) and 4-carbon compounds (malate, fumarate, succinate) by avoiding the loss of carbon to carbon dioxide from the CAC, providing a pathway for generation of glycolytic intermediates from CAC intermediates, and reversing the carbon flux through glycolysis to produce essential precursors for biosynthesis.

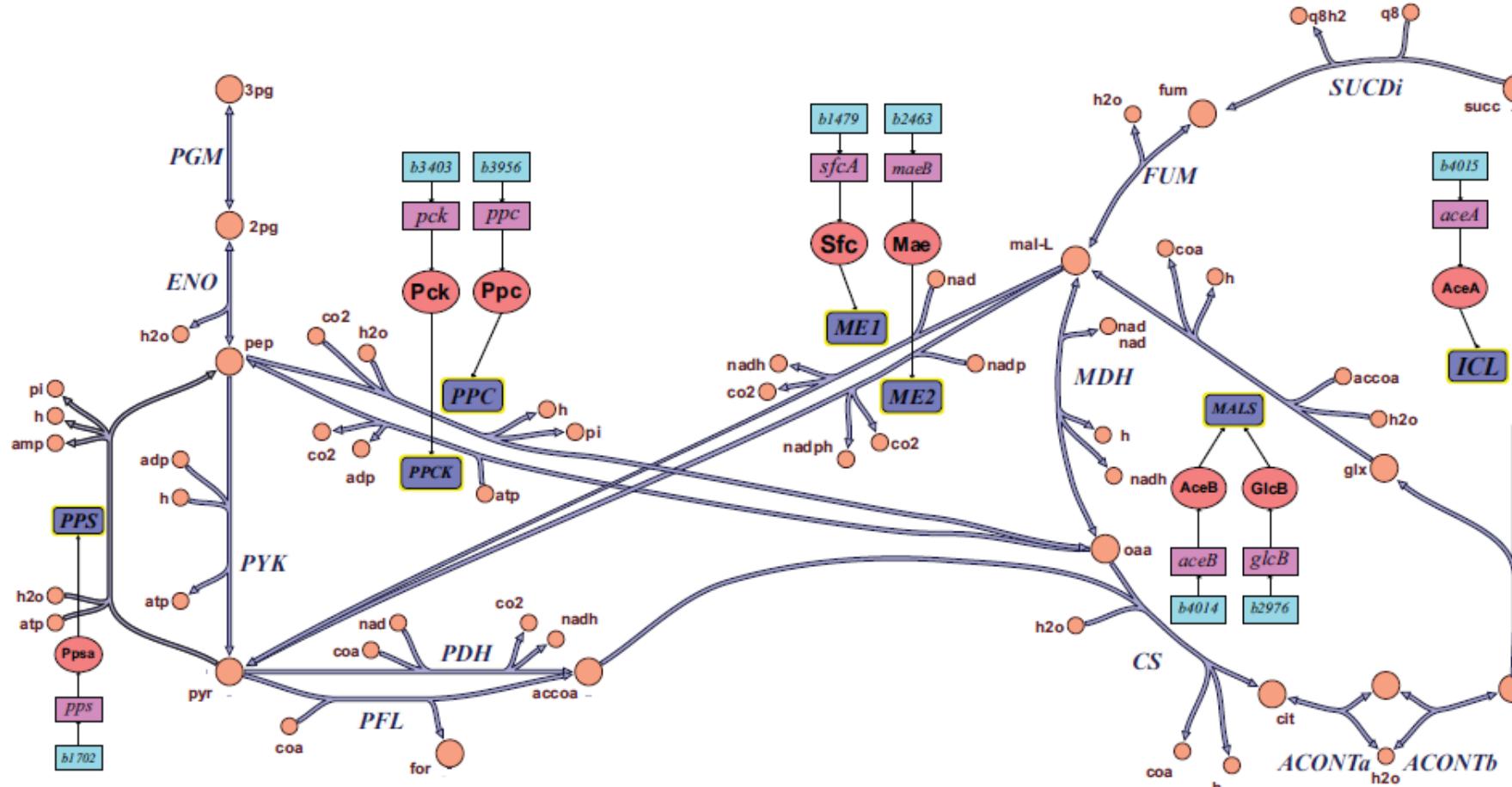
Anaplerotic reactions replenish CAC intermediates drained of for biosynthesis

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





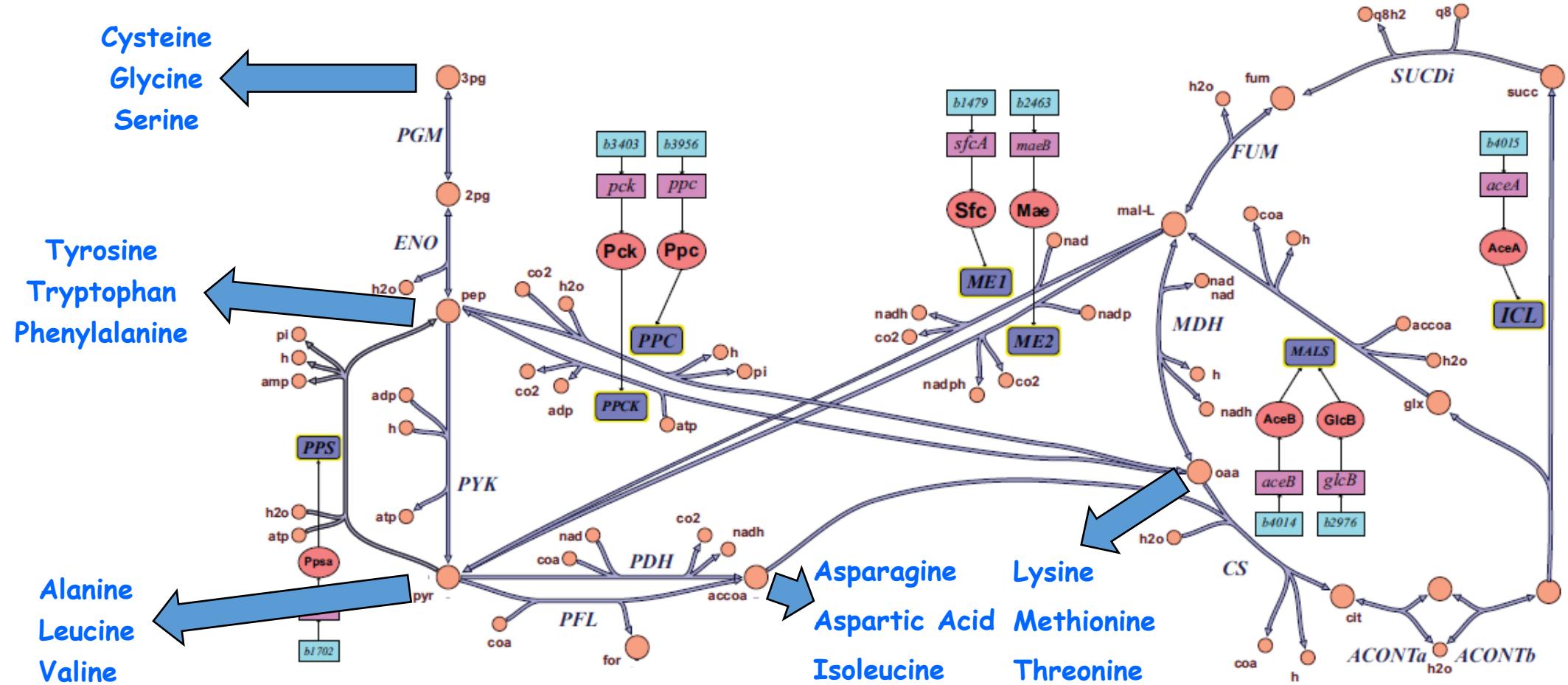
Anaplerotic Reactions Subsystems



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



Biosynthetic Precursors for Anaplerotic Reactions Subsystem

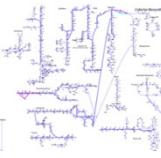


Reconstruction and Use of Microbial Metabolic Networks: the Core *Escherichia coli* Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)



Anaplerotic Reactions Subsystem Reactions

Reaction ID	Reaction Name	Reaction Formula
PPC	Phosphoenolpyruvate carboxylase	co2_c + h2o_c + pep_c --> h_c + oaa_c + pi_c
PPCK	Phosphoenolpyruvate carboxykinase	atp_c + oaa_c --> adp_c + co2_c + pep_c
ICL	Isocitrate lyase	icit_c --> glx_c + succ_c
MALS	Malate synthase	accoa_c + glx_c + h2o_c --> coa_c + h_c + mal__L_c
ME1	Malic enzyme (NAD)	mal__L_c + nad_c --> co2_c + nadh_c + pyr_c
ME2	Malic enzyme (NADP)	mal__L_c + nadp_c --> co2_c + nadph_c + pyr_c



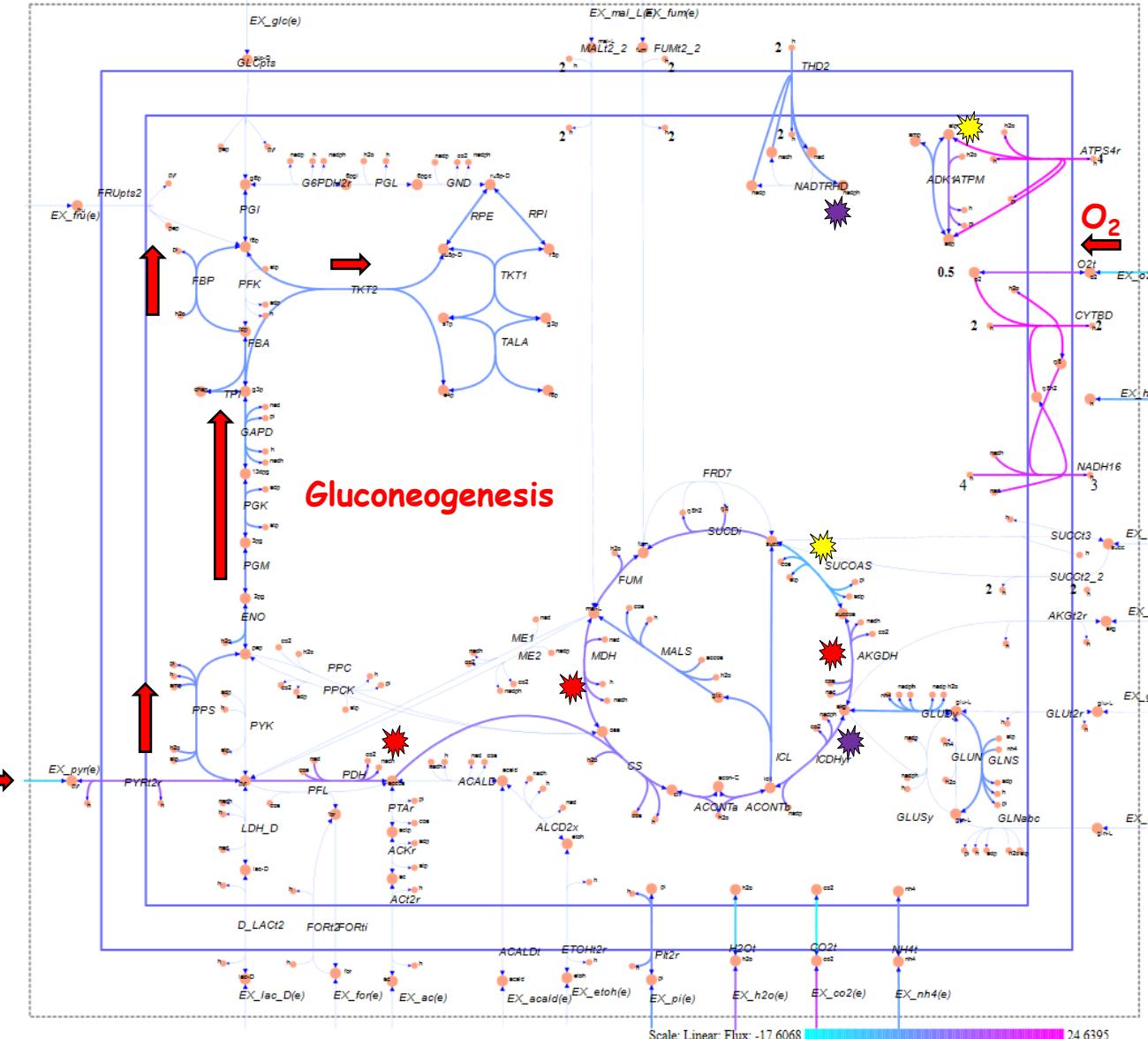
Aerobic Conditions Carbon Source: Pyruvate

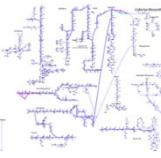
Pyruvate →

ATP = ☀

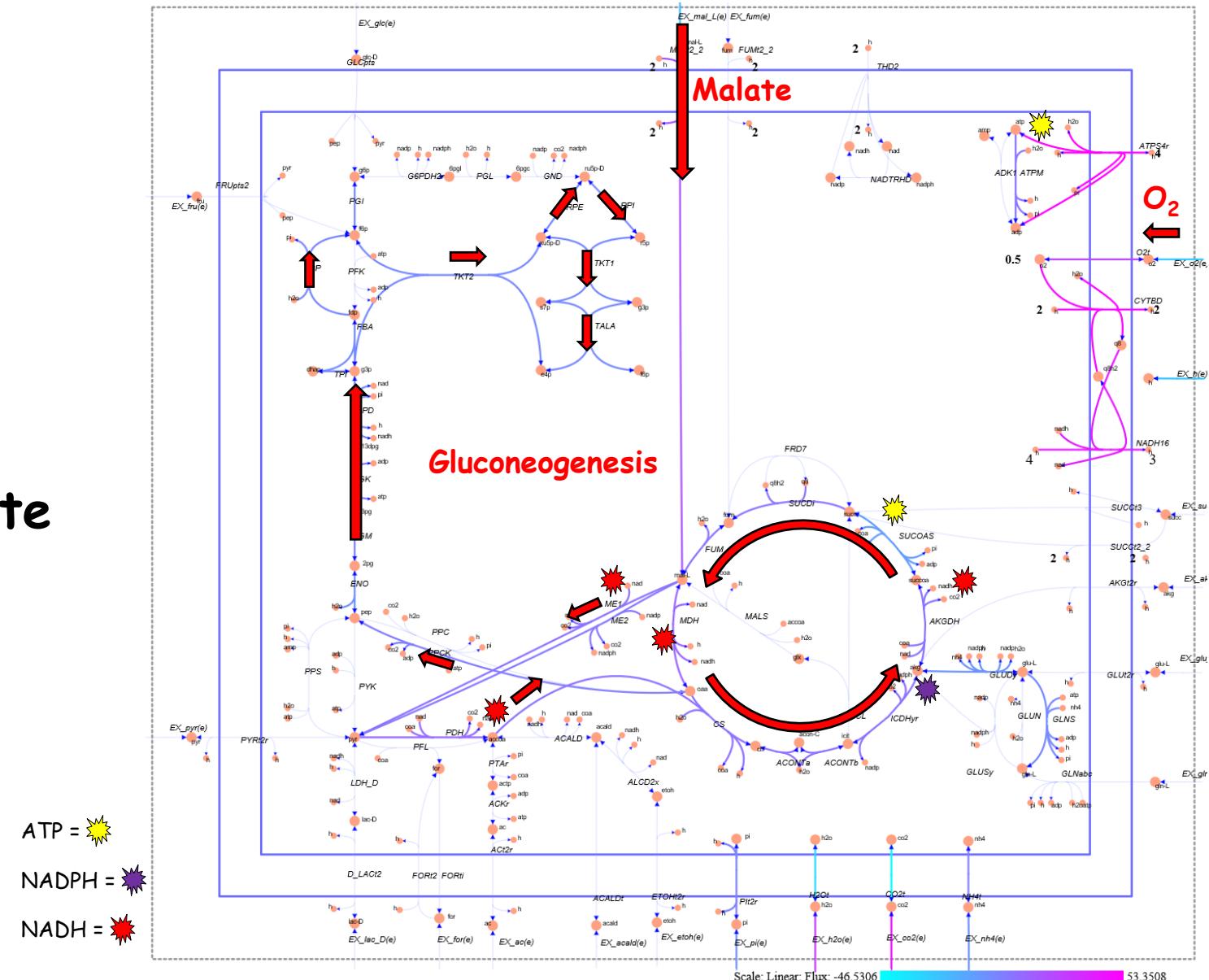
NADPH = ⚡

NADH = ⚡





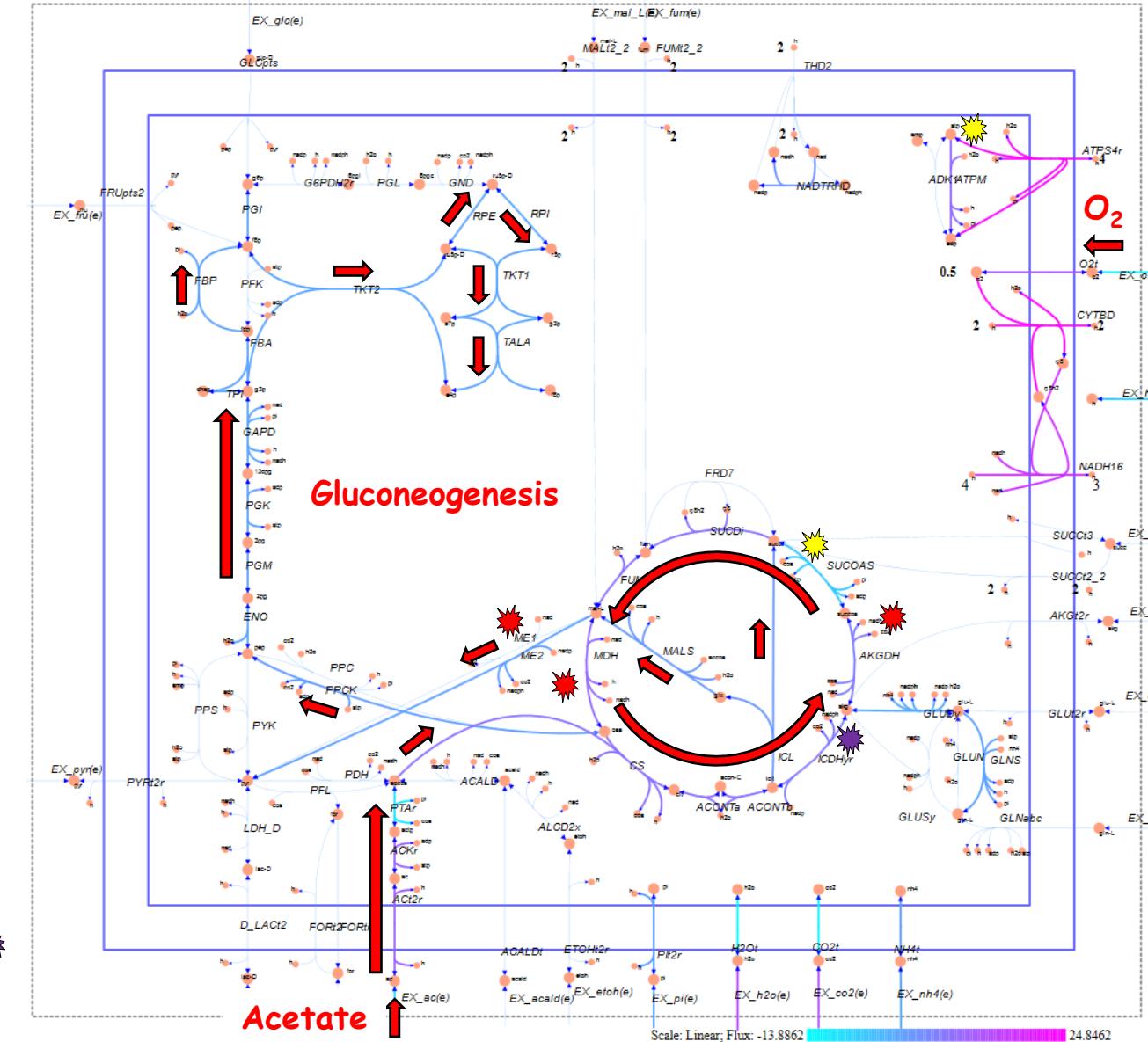
Aerobic Conditions Carbon Source: Malate





Aerobic Conditions Carbon Source: Acetate

ATP =
 NADPH =
 NADH =





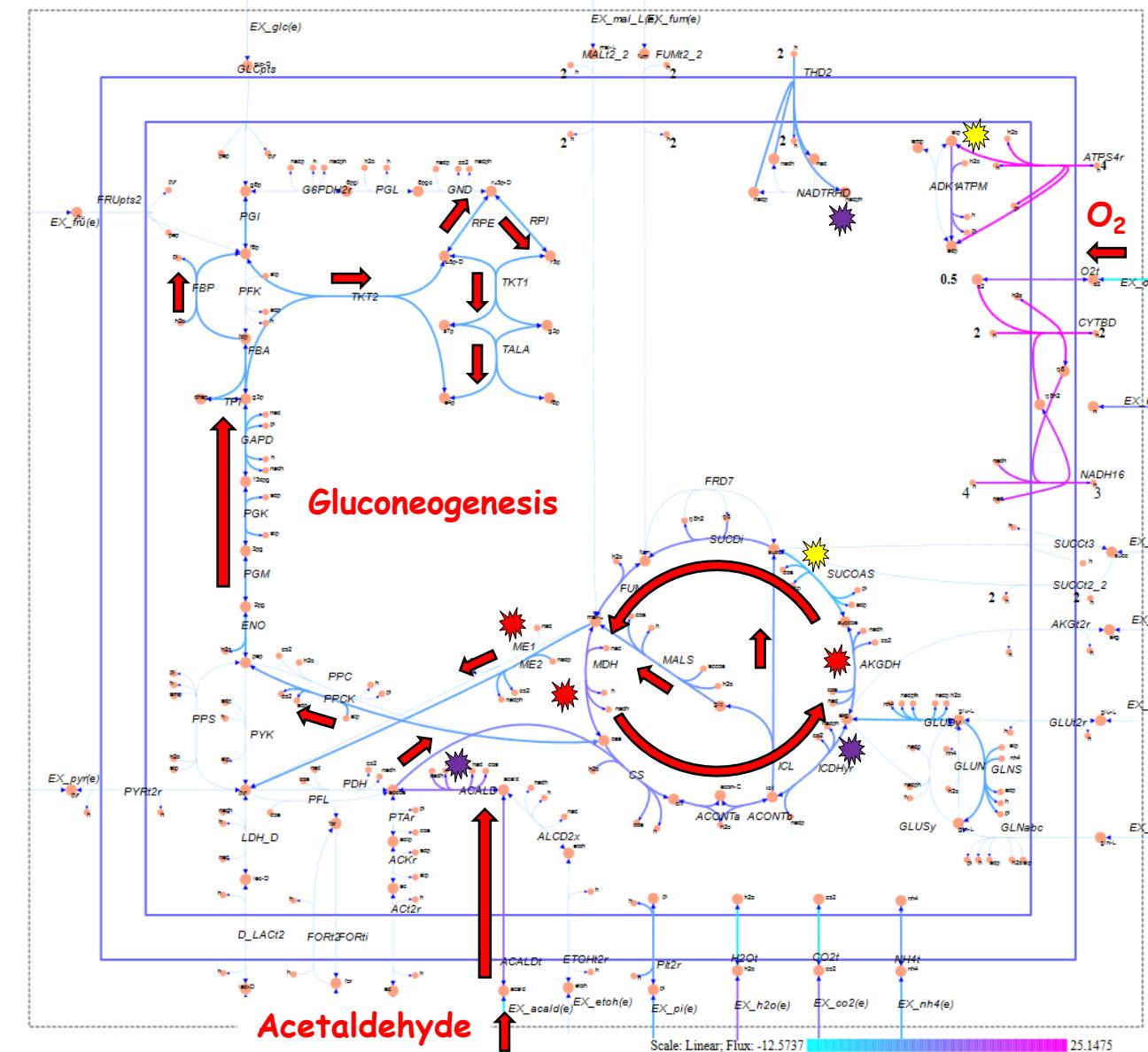
**Aerobic Conditions
Carbon Source: Acetaldehyde**

ATP =

NADPH =

NADH =

AerobicAcetaldehydeBioMass.ipynb



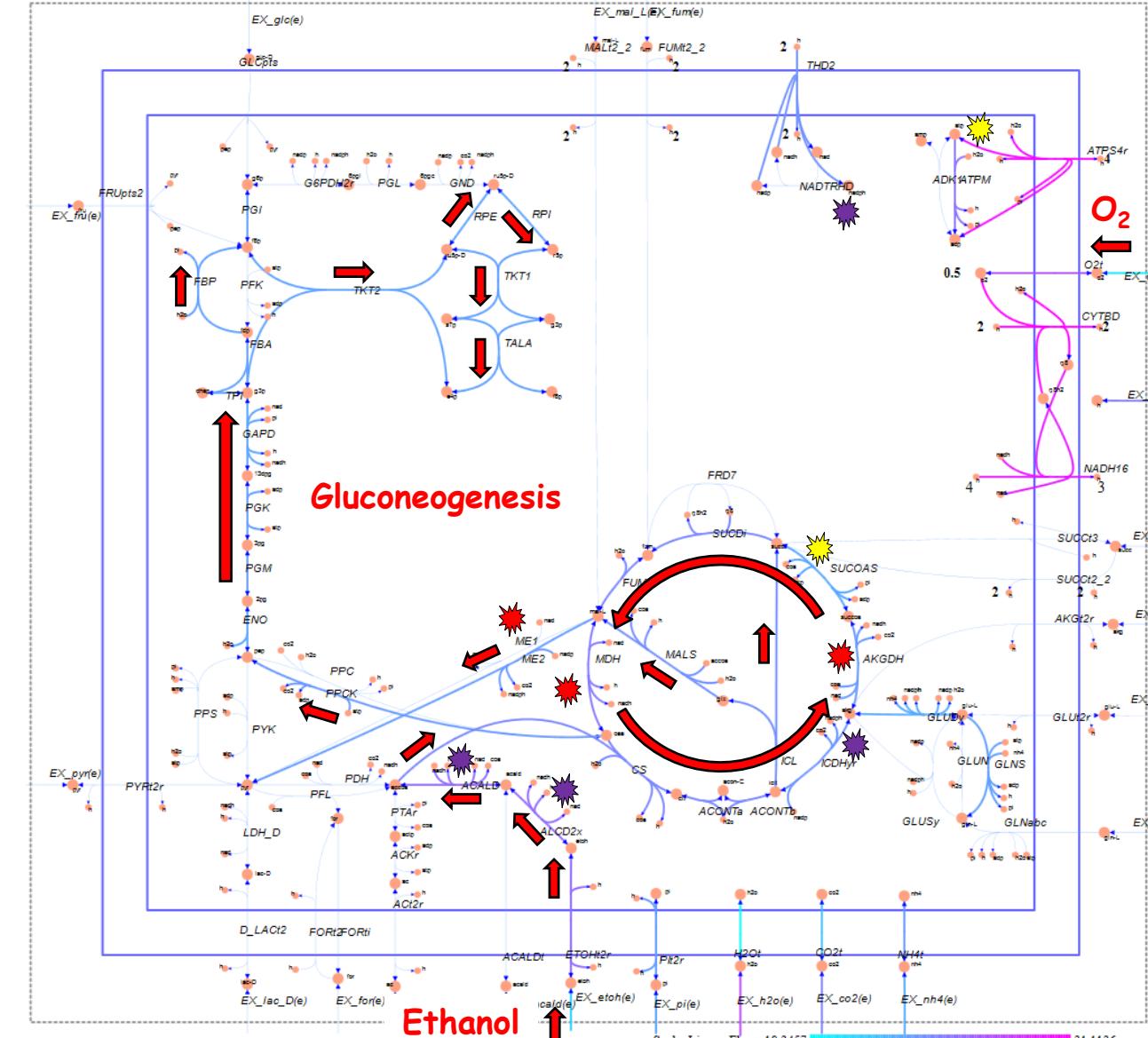


Aerobic Conditions Carbon Source: Ethanol

ATP =

NADPH =

NADH =





Anaplerotic Reactions Subsystem

Anaplerotic_Reactions_Subsystem.ipynb

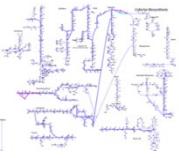
Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 5000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory
```

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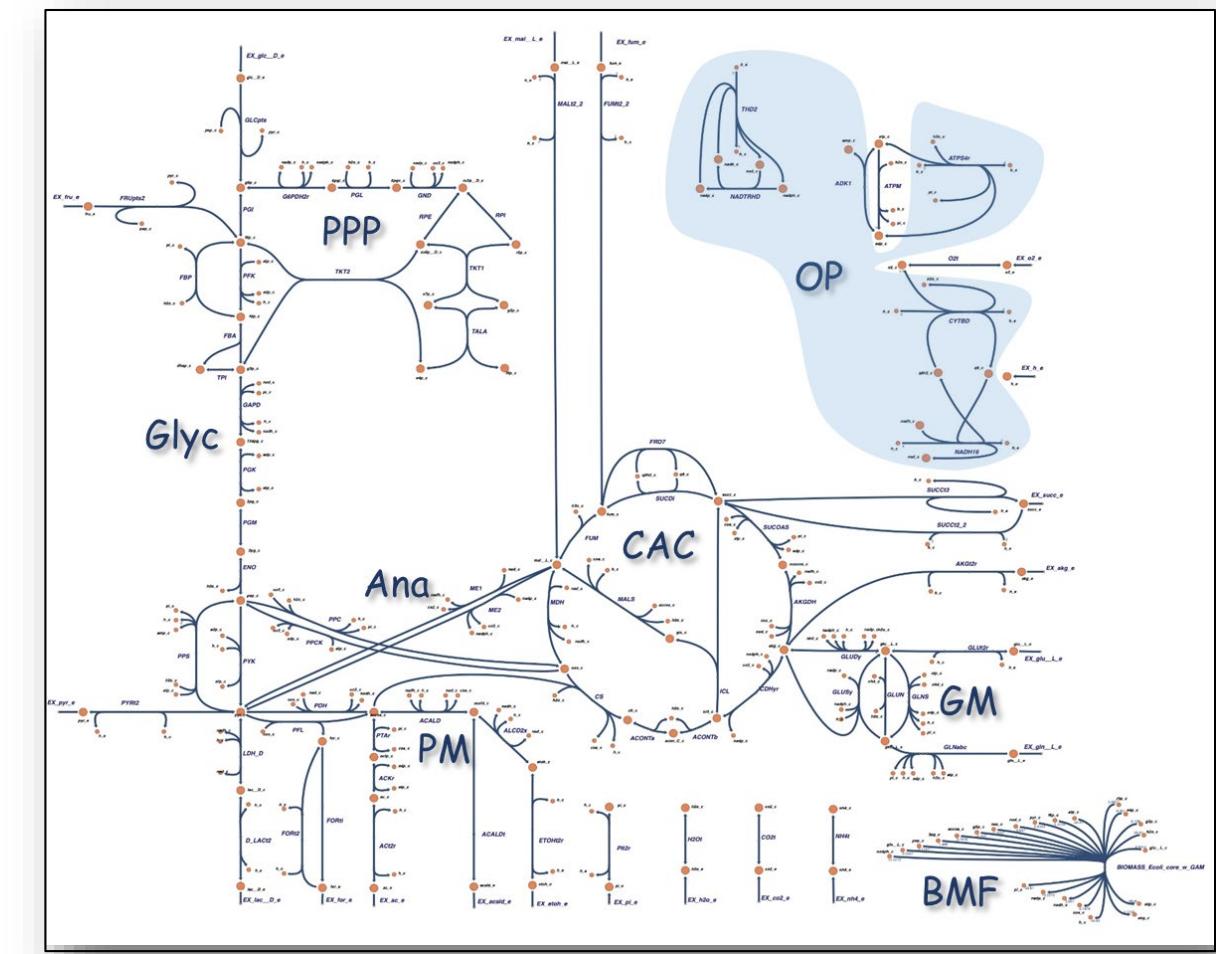
The glyoxylate cycle and gluconeogenic reactions are necessary to allow *E. coli* to grow on 3-carbon (pyruvate) and 4-carbon compounds (malate, fumarate, and succinate). This occurs by avoiding the loss of carbon to carbon dioxide in the TCA cycle (glyoxylate cycle), providing a pathway for generation of glycolytic intermediates from TCA intermediates (anaplerotic reactions), and reversing the carbon flux through glycolysis (gluconeogenesis) to produce essential precursors for biosynthesis.

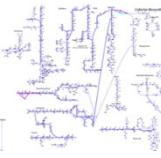
The location of the glyoxylate cycle, gluconeogenesis, and anaplerotic reactions on the *E. coli* core map is shown in Figure 1 below.



E.coli Core Model Subsystems

- Component Parts of the *E. coli* Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
- Citric Acid Cycle
- Anaplerotic Reactions
- **Oxidative Phosphorylation**
- Pyruvate Metabolism
- Glutamate Metabolism
- Biomass and Maintenance Functions
- Inorganic Ion Transport and Metabolism
- Transport, Extracellular
- Extracellular Exchange

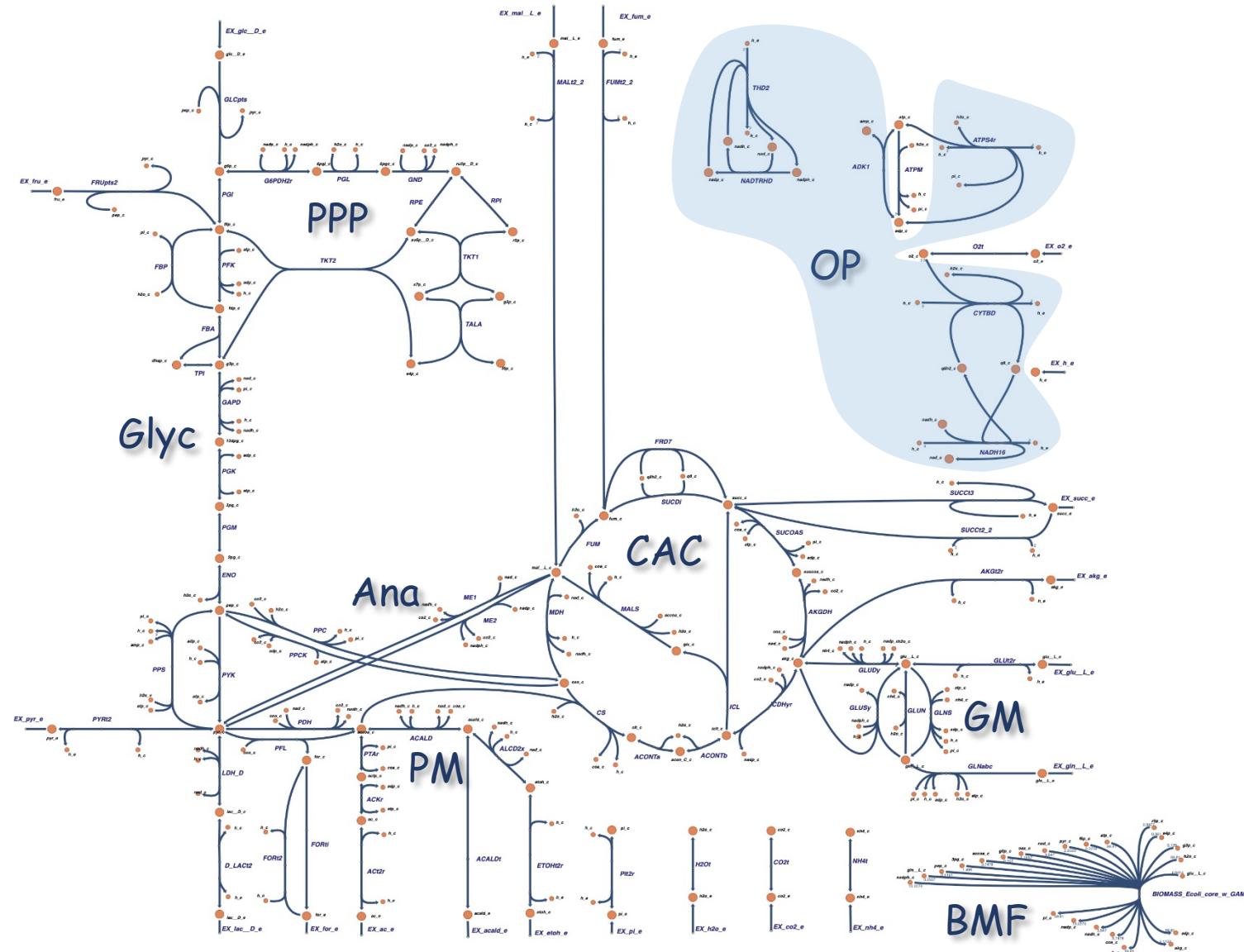


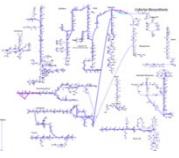


Oxidative Phosphorylation Subsystem

E. coli Core Model

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





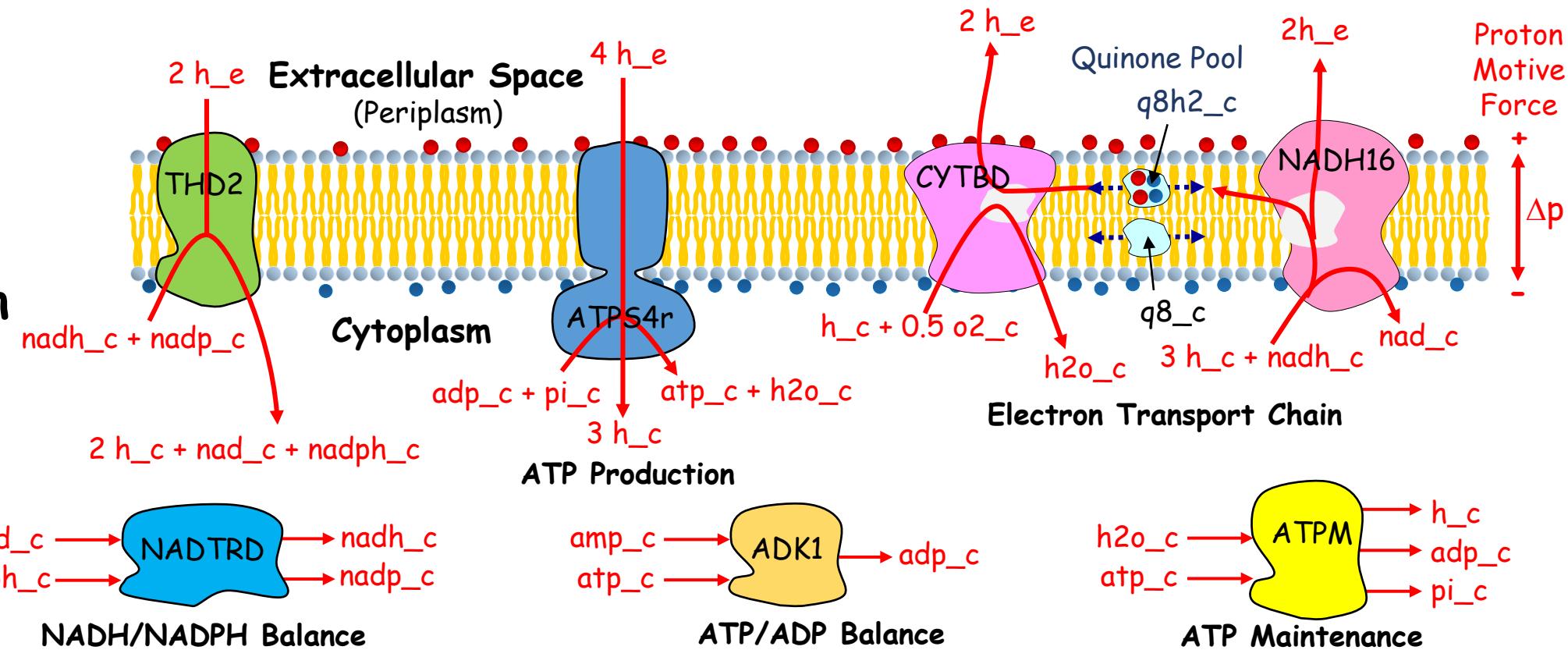
Oxidative Phosphorylation Subsystem

- Energy is required to drive endergonic processes such as biosynthesis, polymerization, active transport of substrate into the cell against concentration gradients, maintaining internal pH, and motility. There are two main mechanisms for the production of energy, **substrate level phosphorylation**, and the **electron transport chain**.
- Substrate level phosphorylation is where ATP is formed by a reaction between ADP and a phosphorylated intermediate of a fueling pathway. Examples include: phosphoglycerate kinase, PGK, and pyruvate kinase, PYK, in glycolysis, and succinyl-CoA synthetase, SUCOAS, in the tricarboxylic acid cycle. Each molecule of glucose can potentially lead to the net generation of four molecules of ATP.
- The electron transport chain which produces the bulk of the cell's ATP under aerobic conditions. Mitchell's chemiosmotic theory describes the mechanism by which electron transport is coupled to the generation of ATP. The electron transport chain translocates protons, H^+ , from the cytoplasm, across the cytoplasmic membrane into the periplasmic space. Since the cytoplasmic membrane is effectively impermeable to protons and hydroxyl ions, OH^- , this establishes a difference in concentration of protons, and a difference in electrical charge, across the cytoplasmic membrane. This thermodynamic potential difference gives rise to a **proton motive force** which can be utilized to drive a myriad of endergonic reactions, such as synthesis of high energy currency metabolites, such as ATP.

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



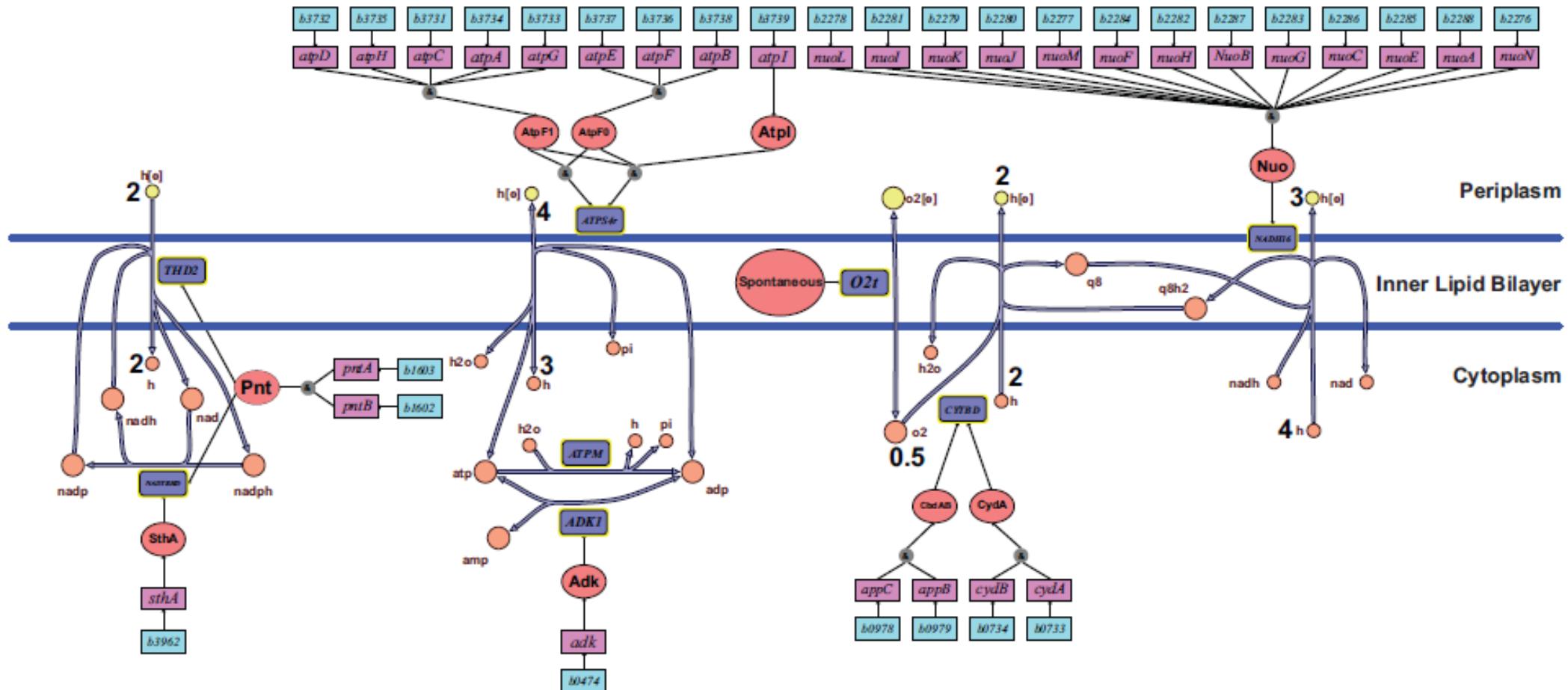
Oxidative Phosphorylation Subsystem



ADK1	Adenylate kinase	$amp_c + atp_c \leftrightarrow 2 adp_c$	ATP/ADP Balance
ATPM	ATP maintenance requirement	$atp_c + h2o_c \rightarrow adp_c + h_c + pi_c$	ATP Maintenance
ATPS4r	ATP synthase (four protons for one ATP)	$adp_c + 4 h_e + pi_c \leftrightarrow atp_c + h2o_c + 3 h_c$	ATP Production
CYTBD	Cytochrome oxidase bd (ubiquinol-8: 2 protons)	$2 h_c + 0.5 o2_c + q8h2_c \rightarrow h2o_c + 2 h_e + q8_c$	Electron Transport Chain
NADH16	NADH dehydrogenase (ubiquinone-8 & 3 protons)	$4 h_c + nadh_c + q8_c \rightarrow 3 h_e + nad_c + q8h2_c$	Electron Transport Chain
NADTRHD	NAD transhydrogenase	$nad_c + nadph_c \rightarrow nadh_c + nadp_c$	NADH/NADPH Balance
THD2	NAD(P) transhydrogenase	$2 h_e + nadh_c + nadp_c \rightarrow 2 h_c + nad_c + nadph_c$	NADH/NADPH Balance



Oxidative Phosphorylation Subsystem

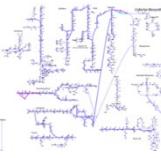


Reconstruction and Use of Microbial Metabolic Networks: the Core *Escherichia coli* Metabolic Model as an Educational Guide by Orth, Fleming, and Palsson (2010)



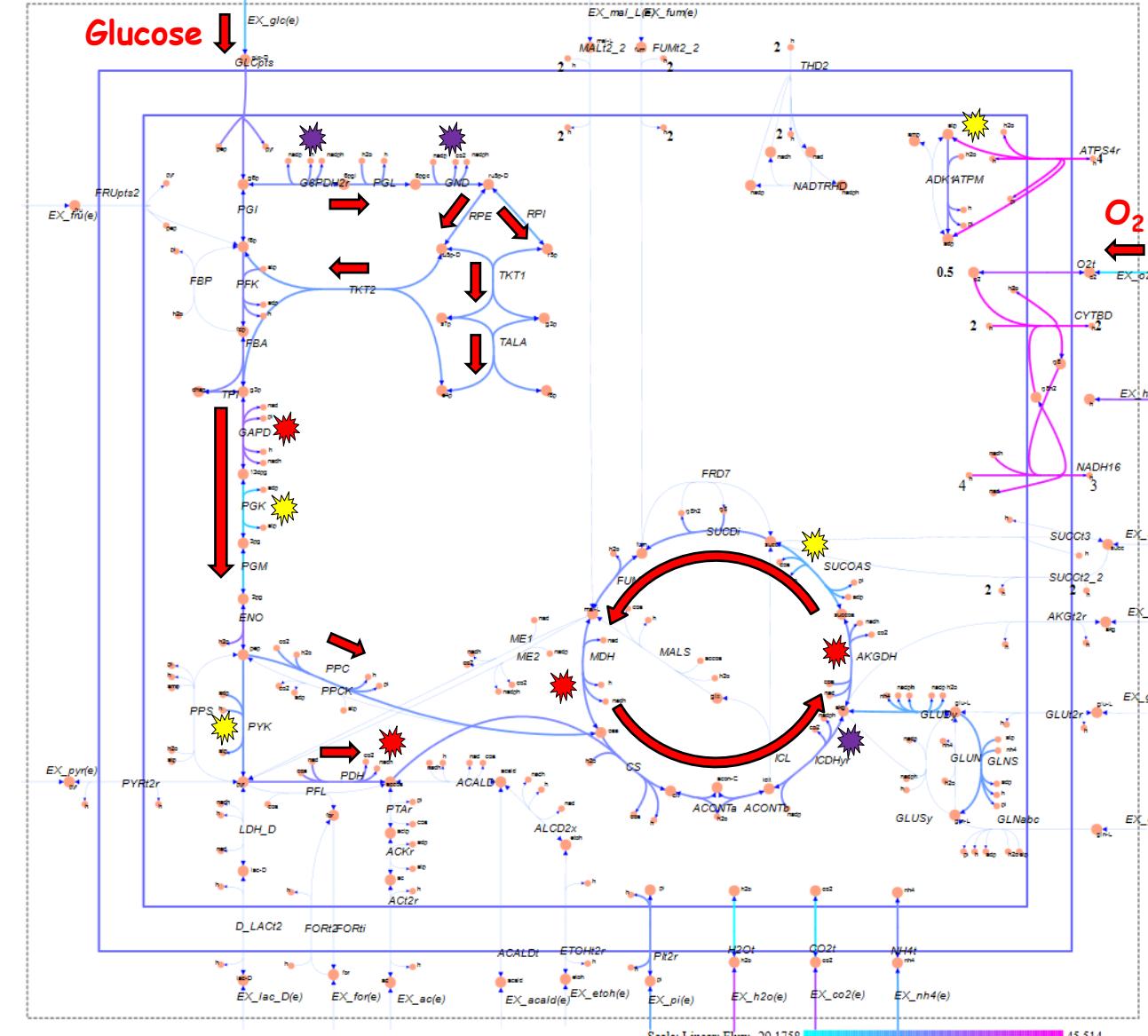
Oxidative Phosphorylation Subsystem Reactions

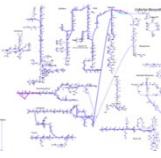
Reaction ID	Reaction Name	Reaction Formula
ADK1	Adenylate kinase	amp_c + atp_c <=> 2.0 adp_c
ATPS4r	ATP synthase (four protons for one ATP)	adp_c + 4.0 h_e + pi_c <=> atp_c + h2o_c + 3.0 h_c
CYTBD	Cytochrome oxidase bd (ubiquinol-8: 2 protons)	2.0 h_c + 0.5 o2_c + q8h2_c --> h2o_c + 2.0 h_e + q8_c
SUCDi	Succinate dehydrogenase (irreversible)	q8_c + succ_c --> fum_c + q8h2_c
THD2	NAD(P) transhydrogenase	2.0 h_e + nadh_c + nadp_c --> 2.0 h_c + nad_c + nadph_c
FRD7	Fumarate reductase	fum_c + q8h2_c --> q8_c + succ_c
NADH16	NADH dehydrogenase (ubiquinone-8 & 3 protons)	4.0 h_c + nadh_c + q8_c --> 3.0 h_e + nad_c + q8h2_c
NADTRHD	NAD transhydrogenase	nad_c + nadph_c --> nadh_c + nadp_c



Aerobic Conditions Carbon Source: Glucose

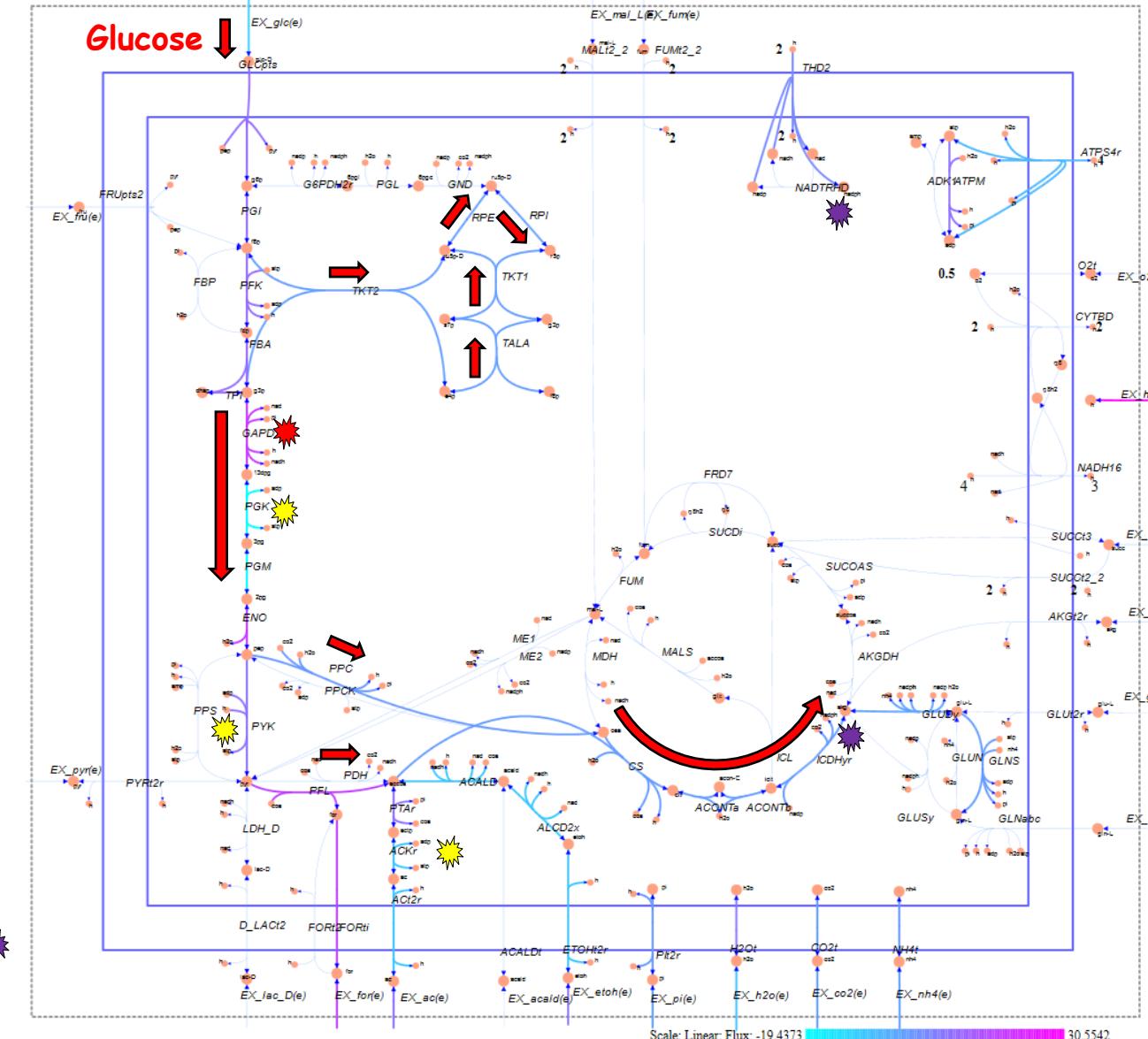
ATP =
 NADPH =
 NADH =





Anaerobic Conditions Carbon Source: Glucose

ATP =
 NADPH =
 NADH =





Oxidative Phosphorylation Subsystem

Oxidative_Phosphorylation_Subsystem.ipynb

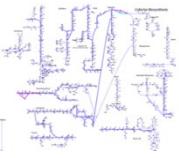
Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 5000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory

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```

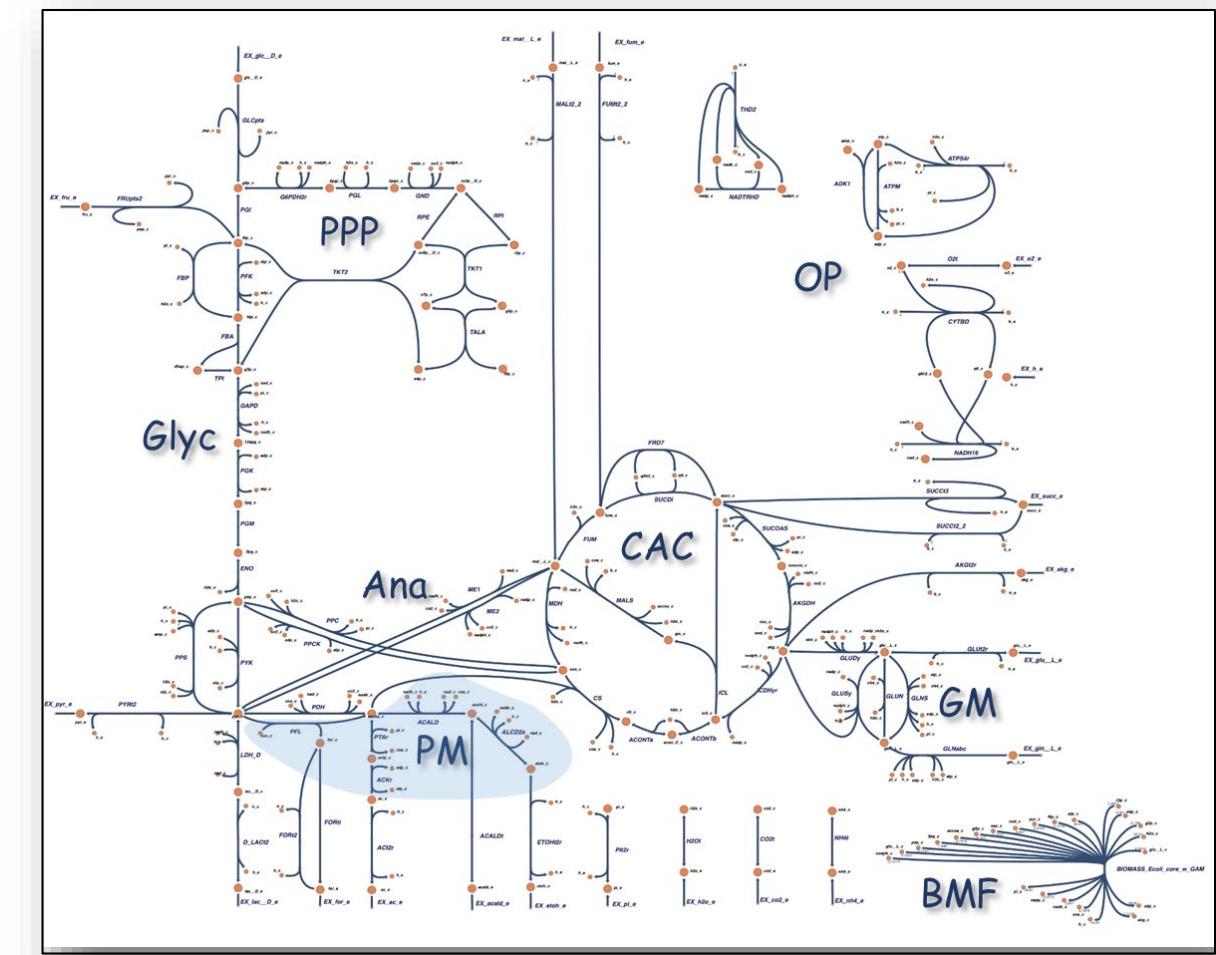
Energy Production & Management

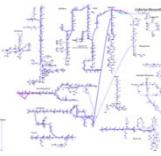
Perhaps the most important requirement of an operational cell is the production and management of energy and reducing power. There are two main mechanisms available within the E.coli core model for the production of ATP (atp_c) energy: 1) substrate level phosphorylation, and 2) oxidative phosphorylation through the use of the electron transport chain. Substrate level phosphorylation occurs when specific metabolic pathways within the cell are net producers of energy. In these cases, atp_c is formed by a reaction between ADP (adp_c) and a phosphorylated intermediate within the pathway. In the core model this occurs in the glycolysis pathway with both phosphoglycerate kinase (PGK), and pyruvate kinase (PYK), and in the tricarboxylic acid cycle with succinyl-CoA synthetase (SUCOAS). Through these substrate level phosphorylation enzymes each molecule of glucose can potentially add four molecules to the total cellular flux of atp_c. The second mechanism for energy generation is oxidative phosphorylation through the electron transport chain, which under aerobic conditions, produces the bulk of the cell's atp_c. In the simple core model, the electron transport chain is used to transport protons (h_c) from the cytoplasm across the cytoplasmic membrane into the extracellular space (periplasmic space in actual cells) to create a proton-motive force which drives ATP synthase (ATPS4r) to produce atp_c.



E. coli Core Model Subsystems

- Component Parts of the *E. coli* Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
- Citric Acid Cycle
- Anaplerotic Reactions
- Oxidative Phosphorylation
- ➡ • Pyruvate Metabolism
- Glutamate Metabolism
- Biomass and Maintenance Functions
- Inorganic Ion Transport and Metabolism
- Transport, Extracellular
- Extracellular Exchange

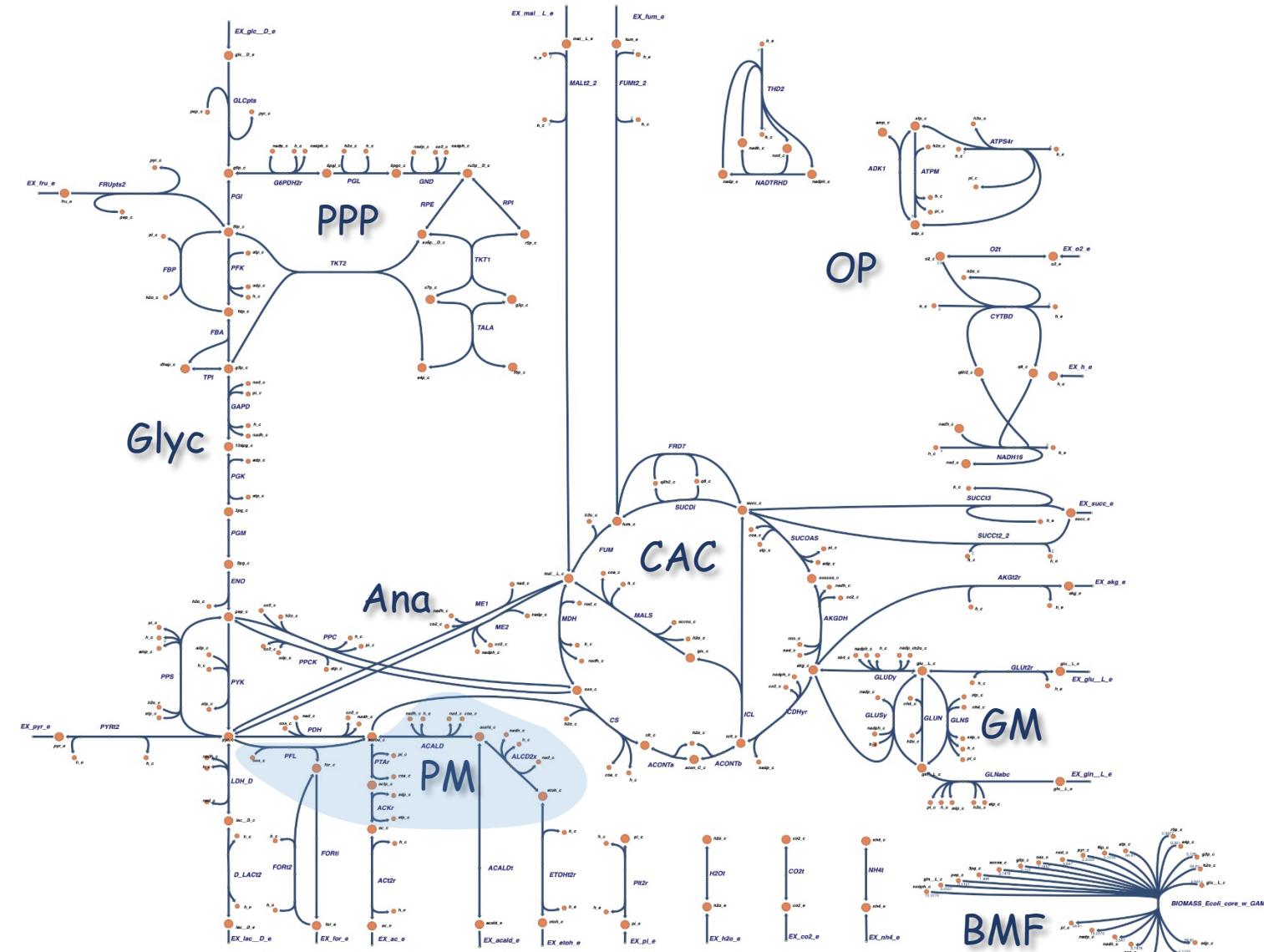




Pyruvate Metabolism

Fermentation is the process of extracting energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor (not oxygen), which is usually an organic compound.

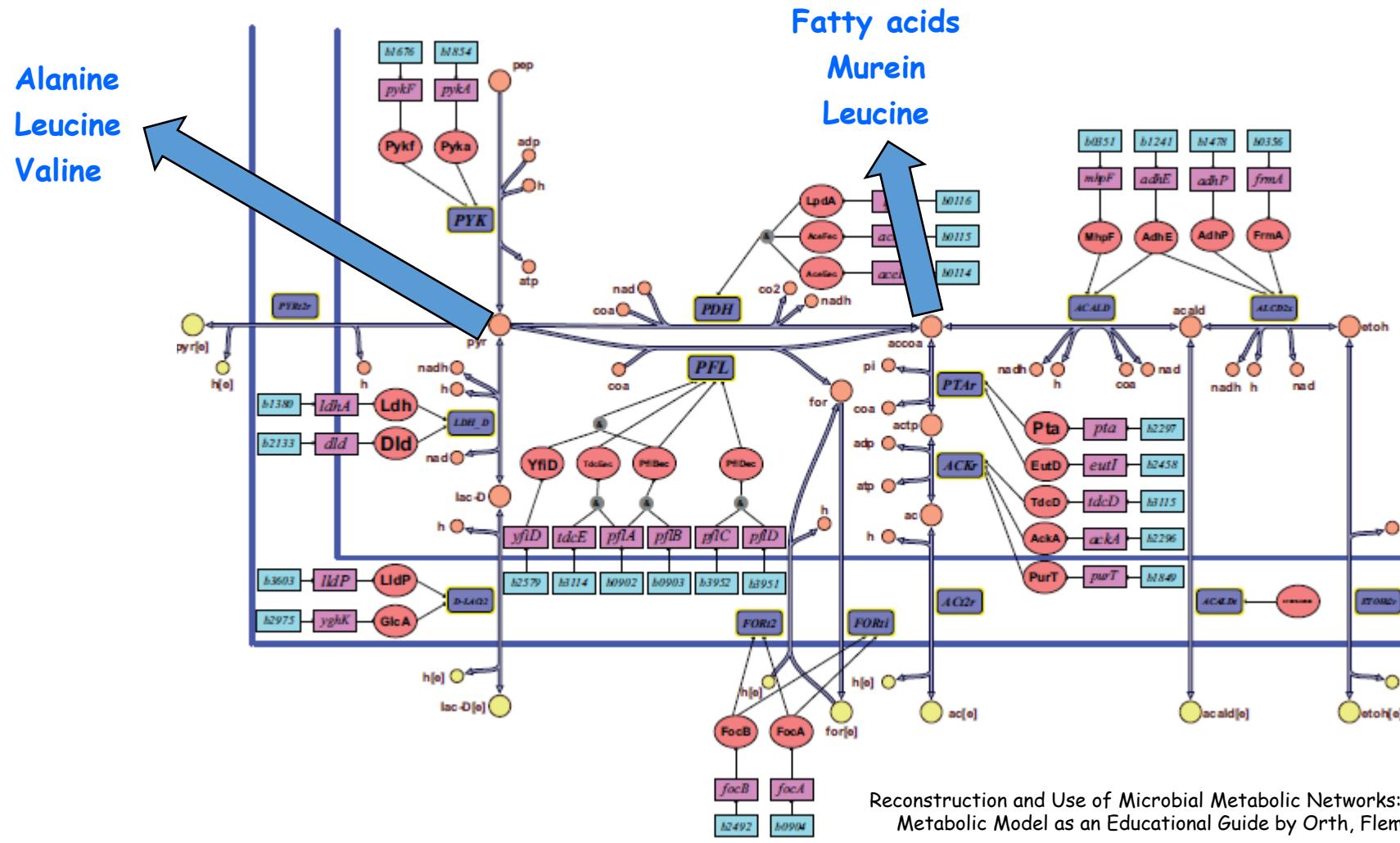
Glycolysis results in the net production of 2 ATP per glucose by substrate level phosphorylation, but this is low compared to 17.5 ATP per glucose for aerobic respiration. The substrates of fermentation are typically sugars, so during fermentative growth, each cell must maintain a large magnitude flux through glycolysis to generate sufficient ATP to drive the constitutive biosynthesis, polymerization, and assembly reactions required for growth. This necessitates a large magnitude efflux of fermentative end products since there is insufficient ATP to assimilate all carbon as biomass. Approximately 10% of carbon substrate is assimilated due to the poor energy yield of fermentation.

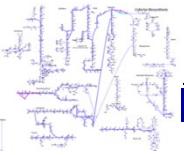


Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" *Nature biotechnology* 28(3): 245-248.



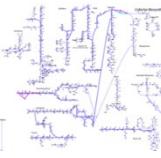
Pyruvate Metabolism Subsystem





Pyruvate Metabolism Subsystem Reactions

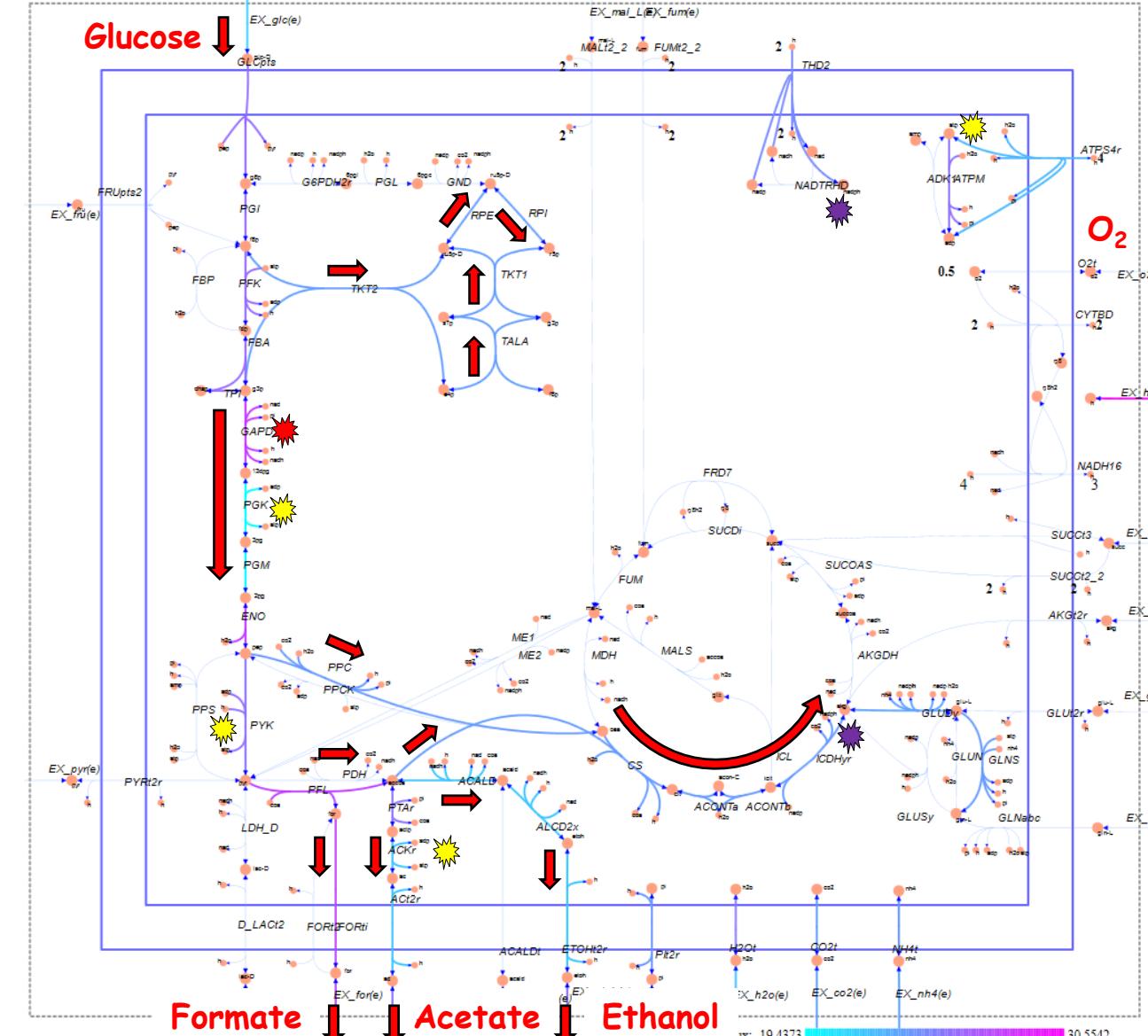
Reaction ID	Reaction Name	Reaction Formula
PFL	Pyruvate formate lyase	coa_c + pyr_c --> accoa_c + for_c
ACALD	Acetaldehyde dehydrogenase (acetylating)	acald_c + coa_c + nad_c <=> accoa_c + h_c + nadh_c
ALCD2x	Alcohol dehydrogenase (ethanol)	etoh_c + nad_c <=> acald_c + h_c + nadh_c
ACKr	Acetate kinase	ac_c + atp_c <=> actp_c + adp_c
PTAr	Phosphotransacetylase	accoa_c + pi_c <=> actp_c + coa_c
LDH_D	D-lactate dehydrogenase	lac__D_c + nad_c <=> h_c + nadh_c + pyr_c



Mixed Acid Fermentation

Anaerobic Conditions
Carbon Source: Glucose

ATP =
NADPH =
NADH =





Pyruvate Metabolism Subsystem

Pyruvate_Metabolism_Subsystem.ipynb

Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 5000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory

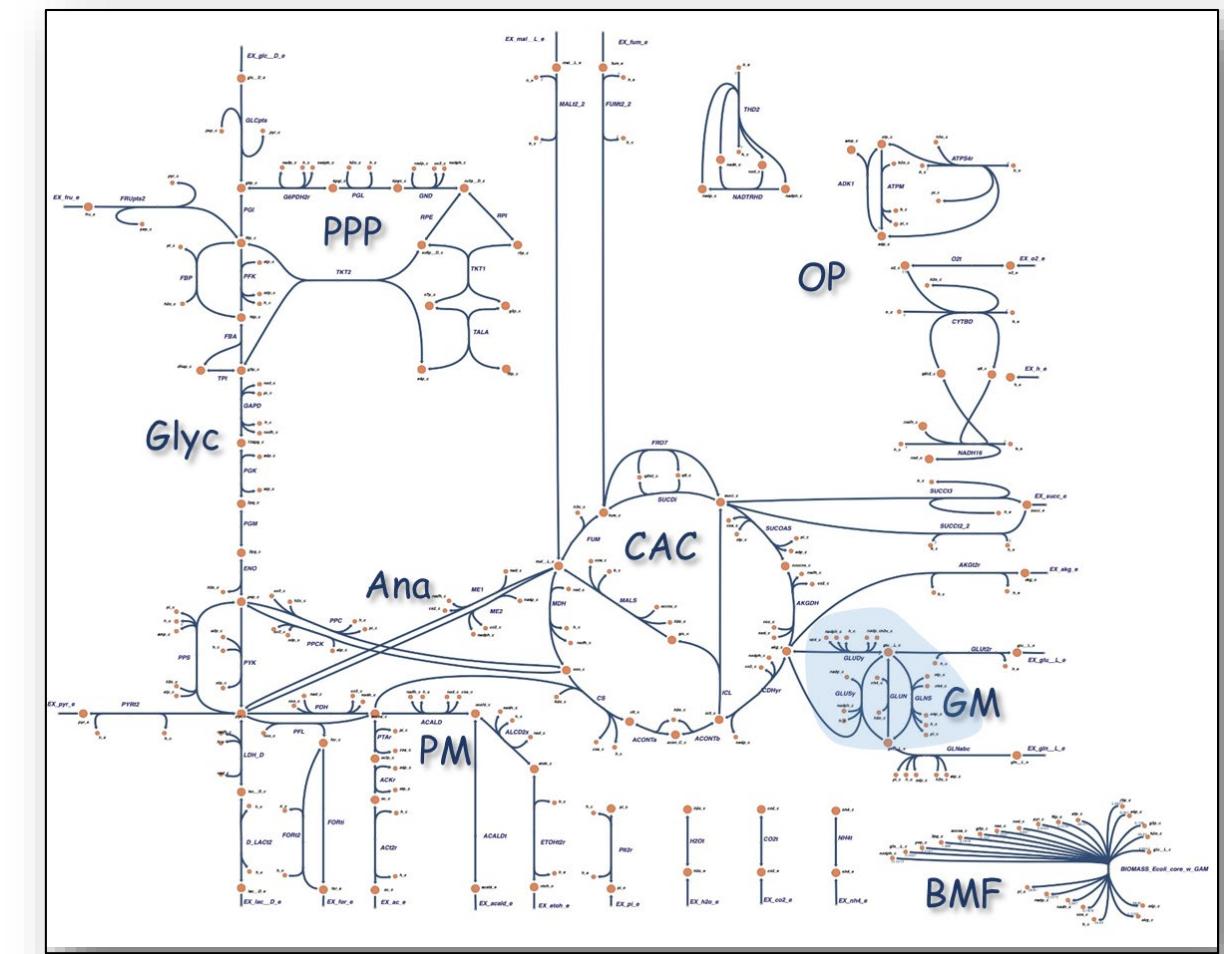
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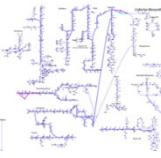
The pyruvate metabolism subsystem contains the reactions required for fermentation. Fermentation is the process of extracting energy from the oxidation of organic compounds without oxygen. The location of the fermentation reactions on the *E.coli* core map are shown in the Figure 1.



E. coli Core Model Subsystems

- Component Parts of the *E. coli* Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
- Citric Acid Cycle
- Anaplerotic Reactions
- Oxidative Phosphorylation
- Pyruvate Metabolism
- Glutamate Metabolism
- Biomass and Maintenance Functions
- Inorganic Ion Transport and Metabolism
- Transport, Extracellular
- Extracellular Exchange





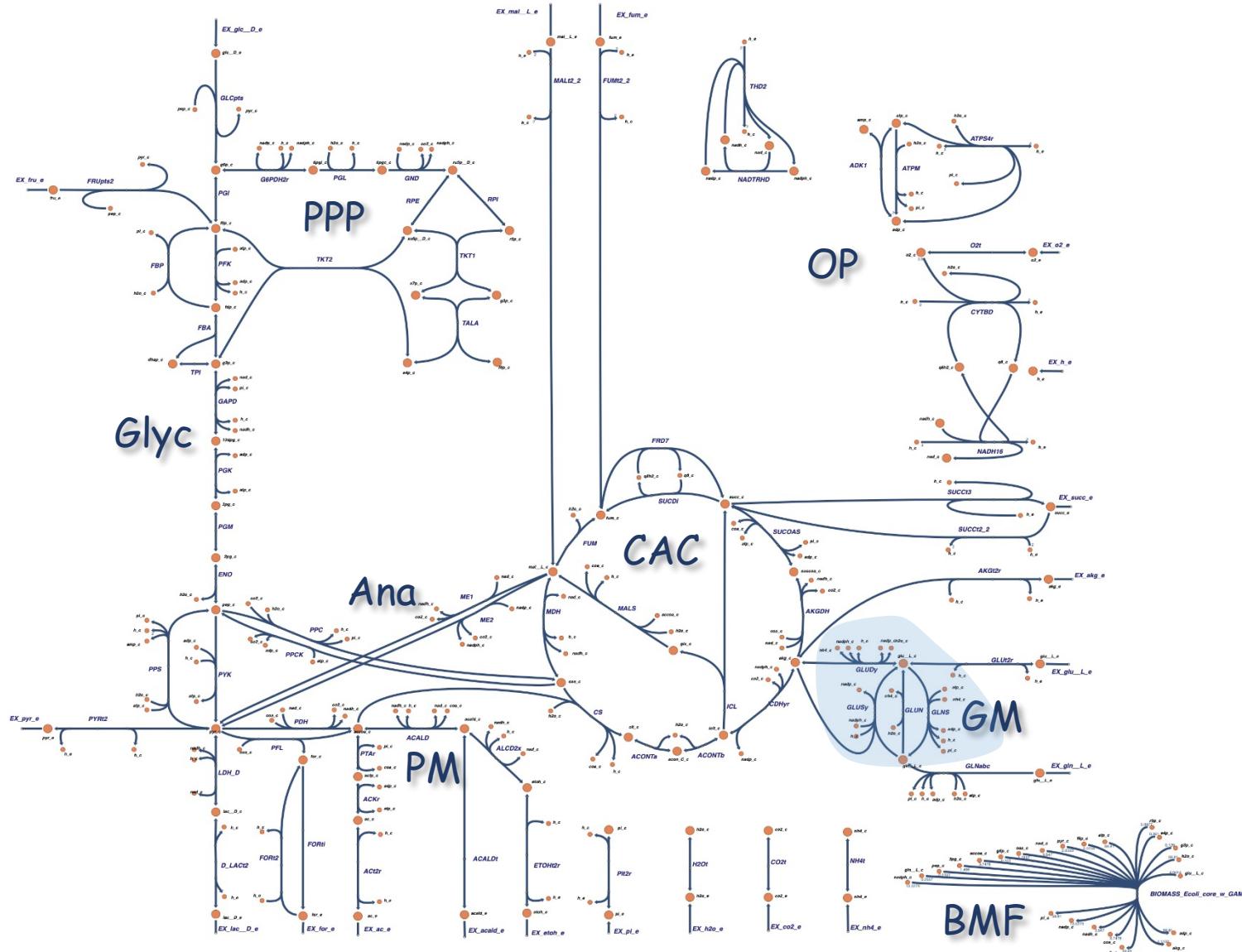
Glutamate Metabolism Subsystem

E.coli Core Model

Nitrogen is the fourth most abundant atom in *E. coli* and enters the cell either by ammonium ion uptake, NH₄⁺, or as a moiety within organic molecules, such as glutamine or glutamate.

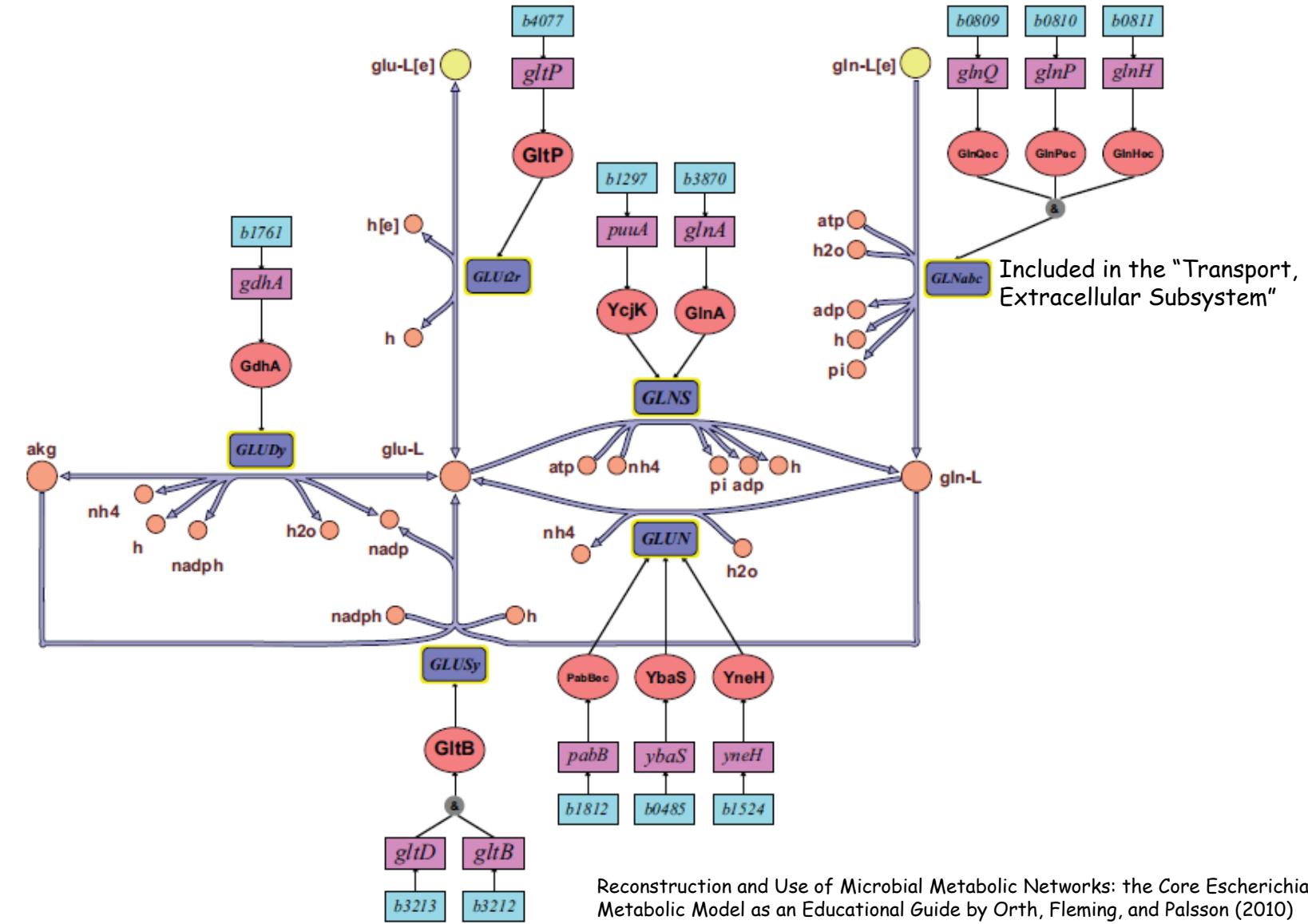
The *E.coli* core model covers the pathways between 2-oxoglutarate, L-glutamate, and L-glutamine.

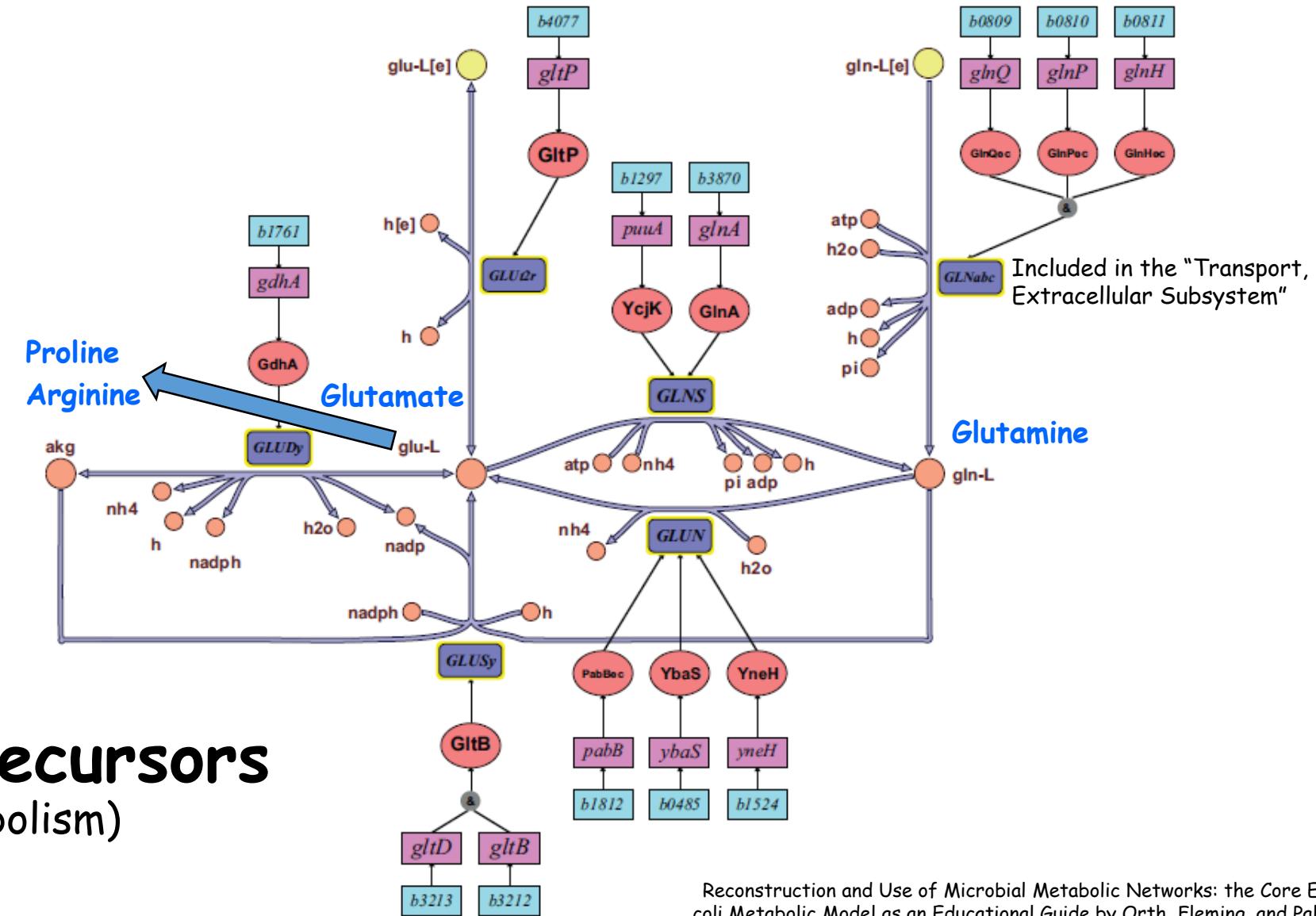
Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" *Nature biotechnology* 28(3): 245-248.





Glutamate Metabolism Subsystem





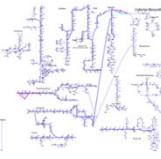
Biosynthetic Precursors (Glutamate Metabolism)

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



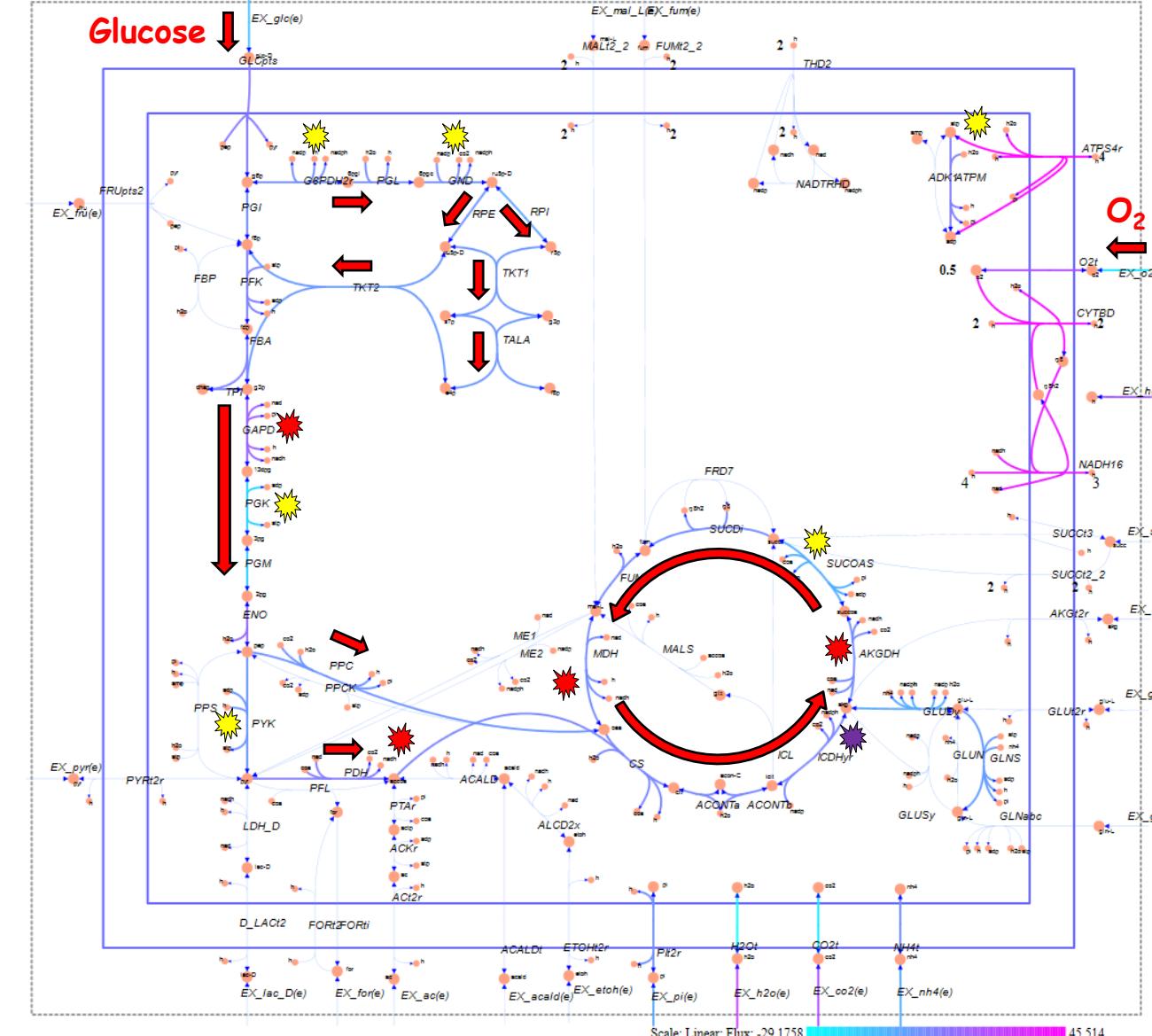
Glutamate Metabolism Subsystem Reactions

Reaction ID	Reaction Name	Reaction Formula
GLNS	Glutamine synthetase	atp_c + glu__L_c + nh4_c --> adp_c + gln__L_c + h_c + pi_c
GLUDy	Glutamate dehydrogenase (NADP)	glu__L_c + h2o_c + nadp_c <=> akg_c + h_c + nadph_c + nh4_c
GLUN	Glutaminase	gln__L_c + h2o_c --> glu__L_c + nh4_c
GLUSy	Glutamate synthase (NADPH)	akg_c + gln__L_c + h_c + nadph_c --> 2.0 glu__L_c + nadp_c



Aerobic Conditions Carbon Source: Glucose

ATP =
 NADPH =
 NADH =



AerobicGlucoseBioMass.ipynb



Glutamate Metabolism Subsystem

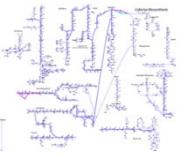
Glutamate_Metabolism_Subsystem.ipynb

Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 5000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory
```

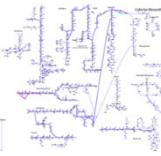
```
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```

The glutamate metabolism subsystem is often referred to as the nitrogen metabolism. Nitrogen enters the cell as either ammonium ion (nh_4_c), or as a moiety within glutamine (glu-L_c) or glutamate (gln-L_c). The *E.coli* core model covers the pathways between 2-oxoglutarate, L-glutamate, and L-glutamine. The location of the glutamate metabolism subsystem reactions on the *E.coli* core map is shown in Figure 1.



E.coli Core Model Subsystems

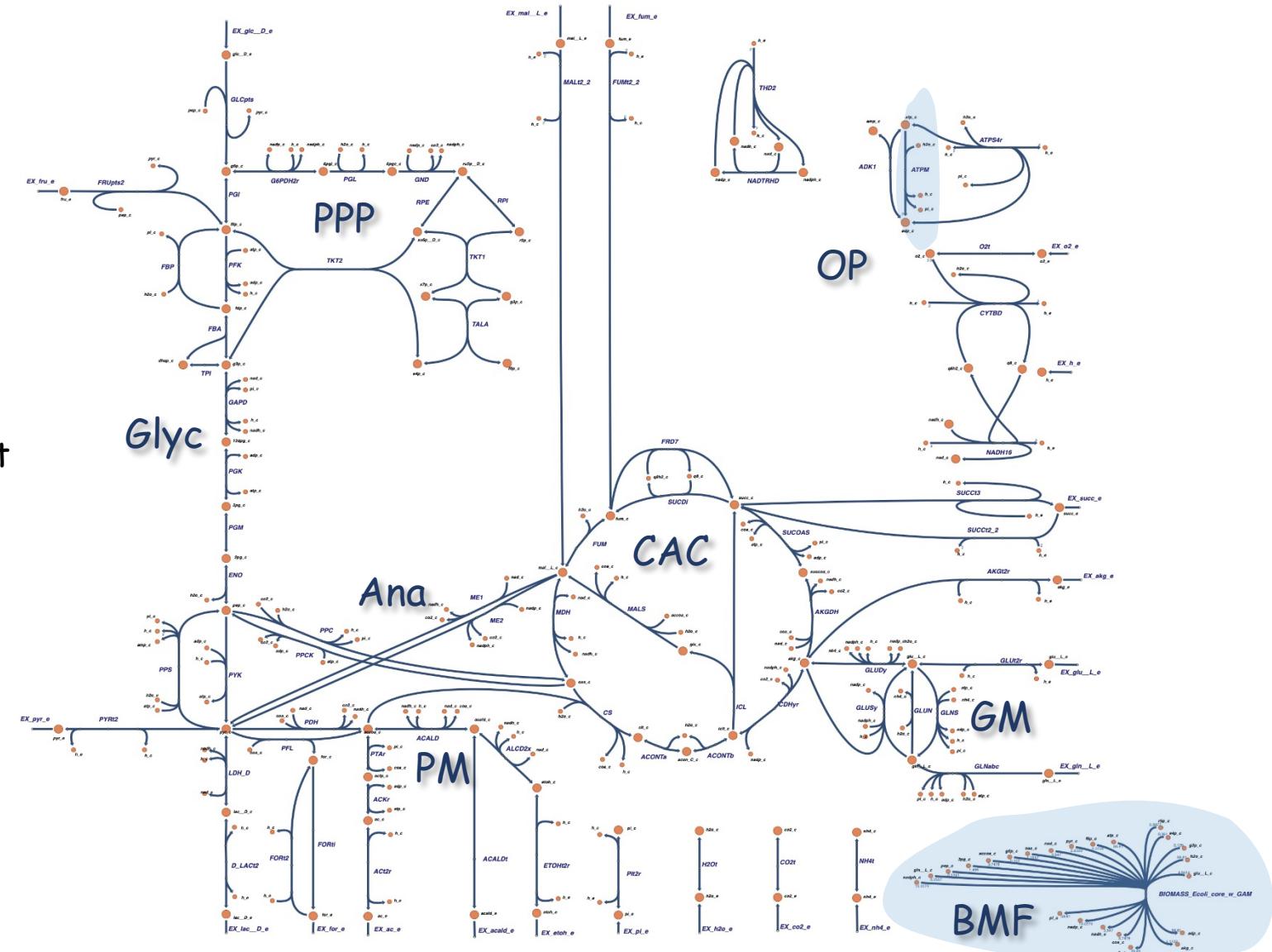
- Component Parts of the *E. coli* Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
- Citric Acid Cycle
- Anaplerotic Reactions
- Oxidative Phosphorylation
- Pyruvate Metabolism
- Glutamate Metabolism
- Biomass and Maintenance Functions
- Inorganic Ion Transport and Metabolism
- Transport, Extracellular
- Extracellular Exchange



Biomass and Maintenance Functions Subsystems

E.coli Core Model

The Biomass and Maintenance Functions Subsystem includes two synthetic reactions that are not found in normal cells. The purpose of these reactions is to help simulate the energy and resource needs of a cell. The location of these two reactions is shown in blue in the following figure.



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

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



Biomass_Maintenance_Functions_Subsystem.ipynb

Biomass and Maintenance Functions Subsystem

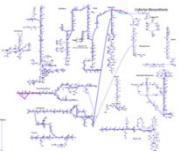
Set the model environment

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In [1]: import cobra.test
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import pandas as pd
import escher
from escher import Builder
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pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory
```

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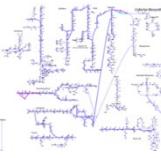
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The Biomass and Maintenance Functions Subsystem includes two synthetic reactions that are not found in normal cells. The purpose of these reactions is to help simulate the energy and resource needs of a cell. The location of these two reactions is shown in blue in the following figure.



E.coli Core Model Subsystems

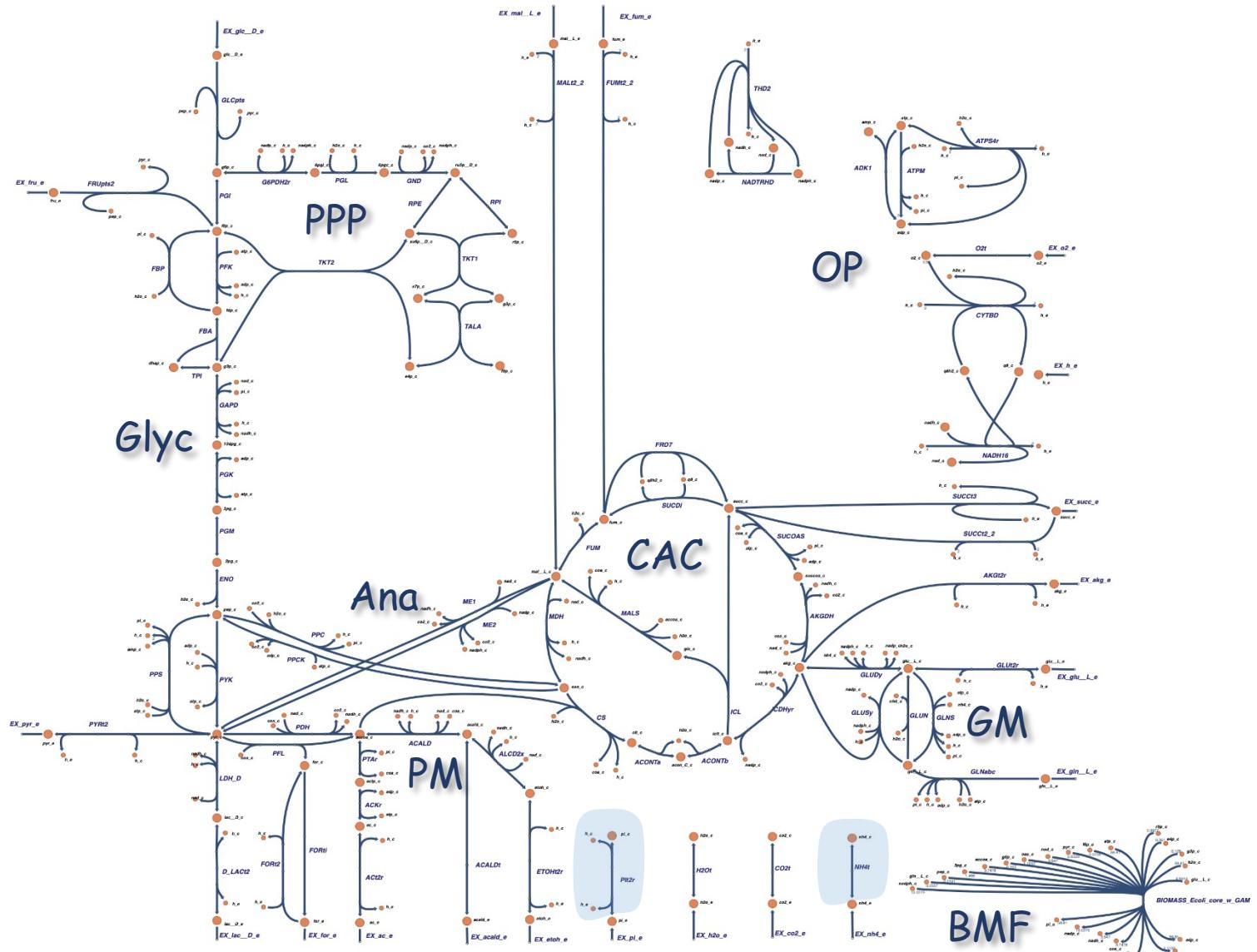
- Component Parts of the *E. coli* Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
- Citric Acid Cycle
- Anaplerotic Reactions
- Oxidative Phosphorylation
- Pyruvate Metabolism
- Glutamate Metabolism
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- Inorganic Ion Transport and Metabolism
- Transport, Extracellular
- Extracellular Exchange



Inorganic Ion Transport and Metabolism Subsystem

This subsystem manages the uptake and secretion of inorganic ions. The *E.coli* core model supports two inorganic ions: phosphate ($\text{pi}_e \leftrightarrow \text{pi}_c$) and ammonium ($\text{nh}_4_e \leftrightarrow \text{nh}_4_c$).

Reaction ID	Reaction Name	Reaction Formula
PIt2r	Phosphate reversible transport via symport	$\text{h}_e + \text{pi}_e \leftrightarrow \text{h}_c + \text{pi}_c$
NH4t	Ammonia reversible transport	$\text{nh}_4_e \leftrightarrow \text{nh}_4_c$





Inorganic_Ion_Transport_Matabolism.ipynb

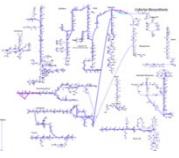
Inorganic Ion Transport and Metabolism

Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 5000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory
```

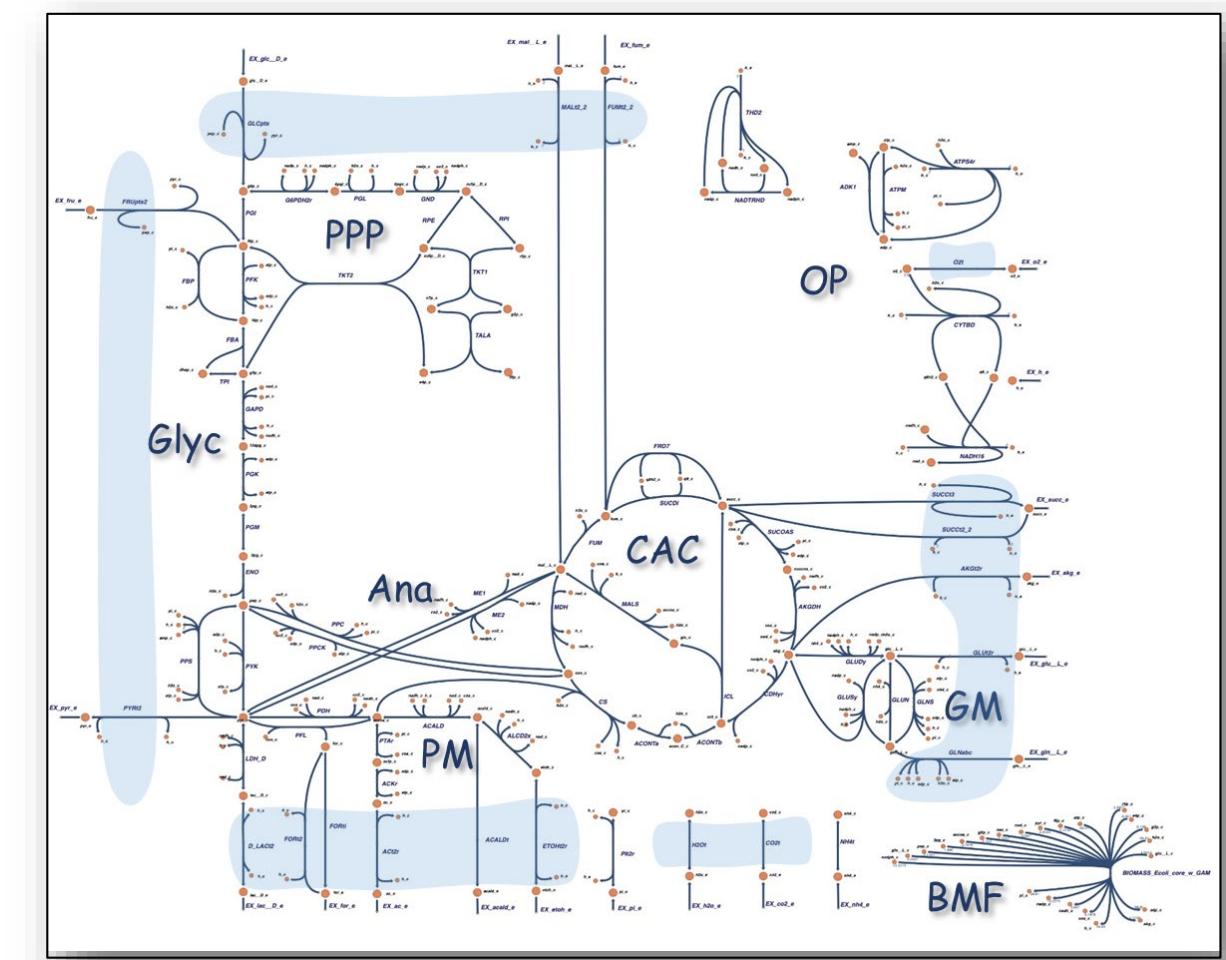
```
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```

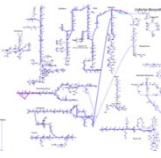
This subsystem manages the uptake and secretion of inorganic ions. The *E.coli* core model supports two inorganic ions; phosphate ($\text{pi}_e \leftrightarrow \text{pi}_c$) and ammonium ($\text{nh4}_e \leftrightarrow \text{nh4}_c$).



E.coli Core Model Subsystems

- Component Parts of the E. coli Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
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- Anaplerotic Reactions
- Oxidative Phosphorylation
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- Glutamate Metabolism
- Biomass and Maintenance Functions
- Inorganic Ion Transport and Metabolism
- Transport, Extracellular
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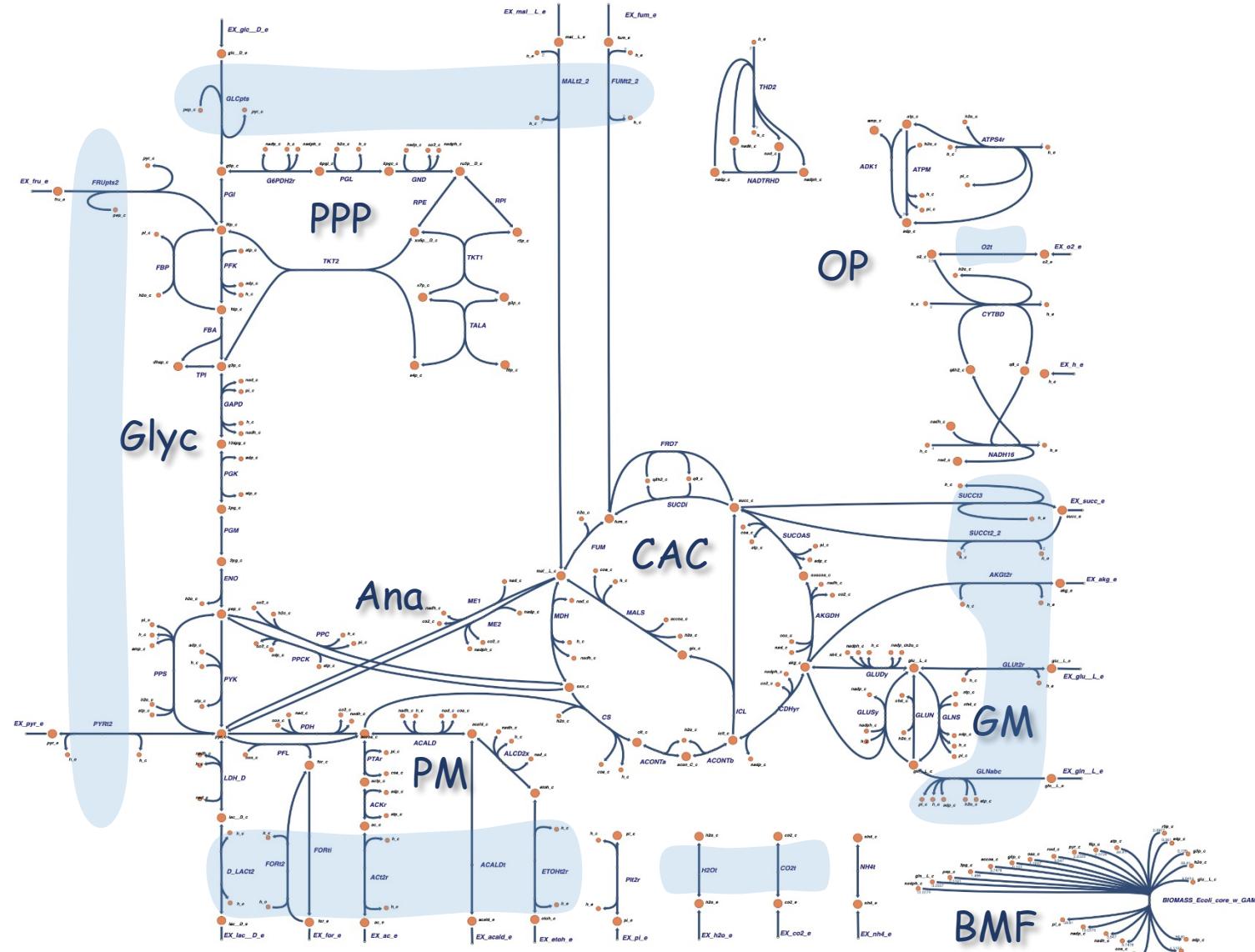




Transport, Extracellular Subsystem *E.coli* Core Model

This subsystem manages the uptake and secretion of organic metabolites. The type of transport reactions supported by the *E.coli* core model include

Reaction Type	Suffix	Example
ABC transporter	-abc	GLNabc
Transport reactions	-t	ACALDt
Reversible reactions	-r	GLUT2r
Phosphotransferase reactions	-pts	GLCpts





Transport, Extracellular Subsystem Reactions

Reaction ID	Reaction Name	Reaction Formula
AKGt2r	2 oxoglutarate reversible transport via symport	akg_e + h_e <=> akg_c + h_c
ACALdt	Acetaldehyde reversible transport	acald_e <=> acald_c
ACt2r	Acetate reversible transport via proton symport	ac_e + h_e <=> ac_c + h_c
PYRt2	Pyruvate transport in via proton symport	h_e + pyr_e <=> h_c + pyr_c
CO2t	CO2 transporter via diffusion	co2_e <=> co2_c
SUCCt2_2	Succinate transport via proton symport (2 H)	2.0 h_e + succ_e --> 2.0 h_c + succ_c
D_LACt2	D lactate transport via proton symport	h_e + lac_D_e <=> h_c + lac_D_c
SUCCt3	Succinate transport out via proton antiport	h_e + succ_c --> h_c + succ_e
ETOHt2r	Ethanol reversible transport via proton symport	etoh_e + h_e <=> etoh_c + h_c
FORt2	Formate transport in via proton symport	for_e + h_e --> for_c + h_c
FORt	Formate transport via diffusion	for_e <-- for_c
FRUpts2	Fructose transport via PEP:Pyr PTS (f6p generating)	fru_e + pep_c --> f6p_c + pyr_c
FUMt2_2	Fumarate transport via proton symport (2 H)	fum_e + 2.0 h_e --> fum_c + 2.0 h_c
GLCpts	D-glucose transport via PEP:Pyr PTS	glc_D_e + pep_c --> g6p_c + pyr_c
GLNabc	L-glutamine transport via ABC system	atp_c + gln_L_e + h2o_c --> adp_c + gln_L_c + h_c + pi_c
GLUt2r	L glutamate transport via proton symport reversible	glu_L_e + h_e <=> glu_L_c + h_c
H2Ot	H2O transport via diffusion	h2o_e <=> h2o_c
MALt2_2	Malate transport via proton symport (2 H)	2.0 h_e + mal_L_e --> 2.0 h_c + mal_L_c
O2t	O2 transport diffusion	o2_e <=> o2_c



Transport_Extracellular_Subsystem.ipynb

Transport, Extracellular Subsystem

Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 5000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory

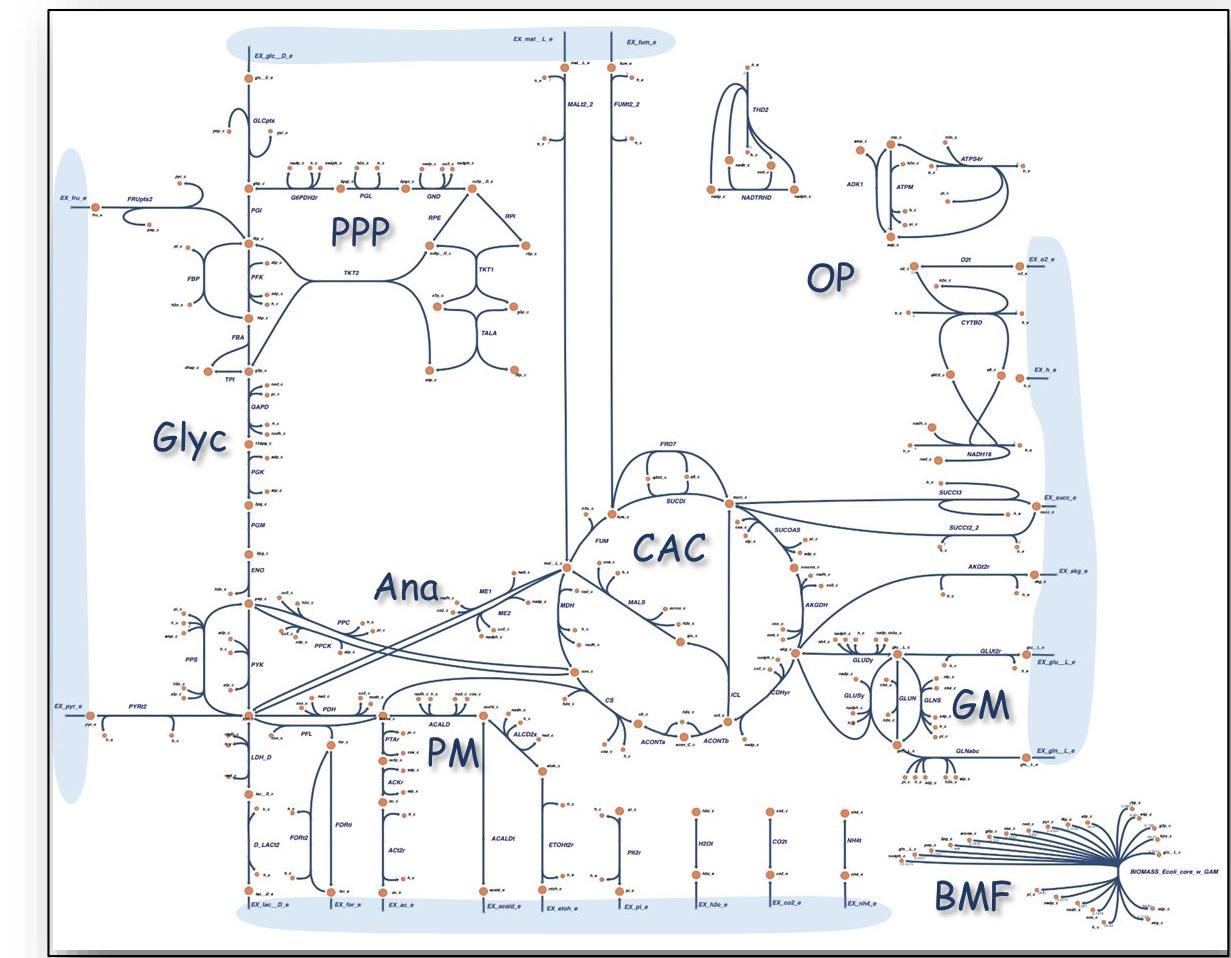
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```

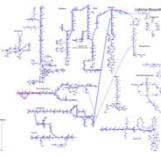
This subsystem manages the uptake and secretion of organic metabolites. The Transport, Extracellular Subsystem includes all the transport reactions of the model as shown below



E.coli Core Model Subsystems

- Component Parts of the *E. coli* Core Model
- Glycolysis/Gluconeogenesis
- Pentose Phosphate Pathway
- Citric Acid Cycle
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- Pyruvate Metabolism
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- Inorganic Ion Transport and Metabolism
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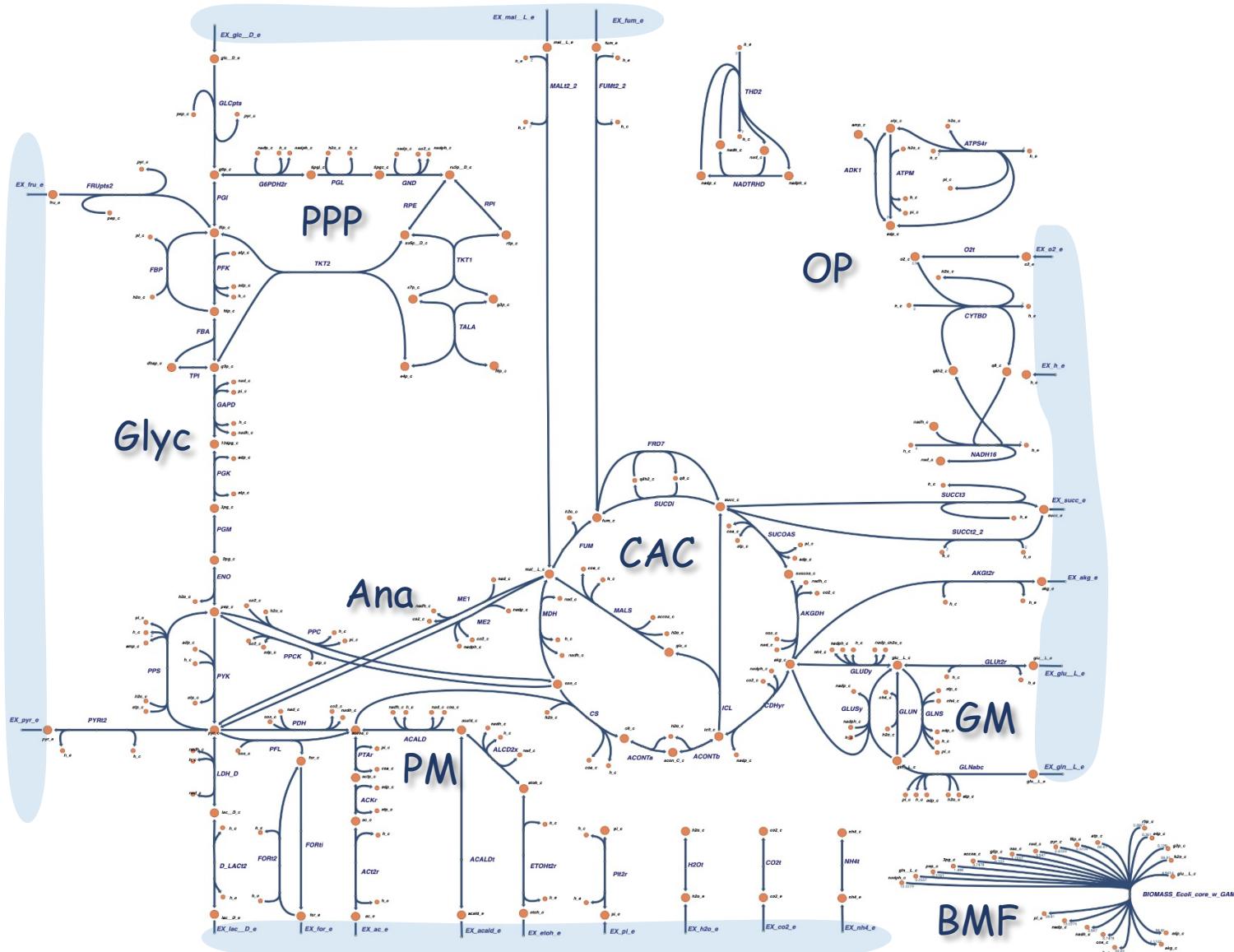




Extracellular Exchange Subsystem

E.coli Core Model

The Extracellular Exchange Subsystem includes all the exchange reactions of the *E.coli* core model.





Extracellular Exchange Subsystem

Reaction ID	Reaction Name	Reaction Formula
EX_ac_e	Acetate exchange	ac_e -->
EX_acald_e	Acetaldehyde exchange	acald_e -->
EX_akg_e	2-Oxoglutarate exchange	akg_e -->
EX_co2_e	CO2 exchange	co2_e <=>
EX_etoh_e	Ethanol exchange	etoh_e -->
EX_for_e	Formate exchange	for_e -->
EX_fru_e	D-Fructose exchange	fru_e -->
EX_fum_e	Fumarate exchange	fum_e -->
EX_glc_D_e	D-Glucose exchange	glc_D_e <=>
EX_gln_L_e	L-Glutamine exchange	gln_L_e -->
EX_glu_L_e	L-Glutamate exchange	glu_L_e -->
EX_h_e	H+ exchange	h_e <=>
EX_h2o_e	H2O exchange	h2o_e <=>
EX_lac_D_e	D-lactate exchange	lac_D_e -->
EX_mal_L_e	L-Malate exchange	mal_L_e -->
EX_nh4_e	Ammonia exchange	nh4_e <=>
EX_o2_e	O2 exchange	o2_e <=>
EX_pi_e	Phosphate exchange	pi_e <=>
EX_pyr_e	Pyruvate exchange	pyr_e -->
EX_succ_e	Succinate exchange	succ_e -->



Extracellular_Exchange_Subsystem.ipynb

Extracellular Exchange Subsystem

Set the model environment

```
In [1]: import cobra.test
import pandas as pd
import numpy as np
import pandas as pd
import escher
from escher import Builder
from cobra.sampling import sample
import matplotlib.pyplot as plt
pd.set_option('display.max_rows', 5000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
model = cobra.io.load_json_model('./e_coli_core.json') # Model must be in the same directory
```

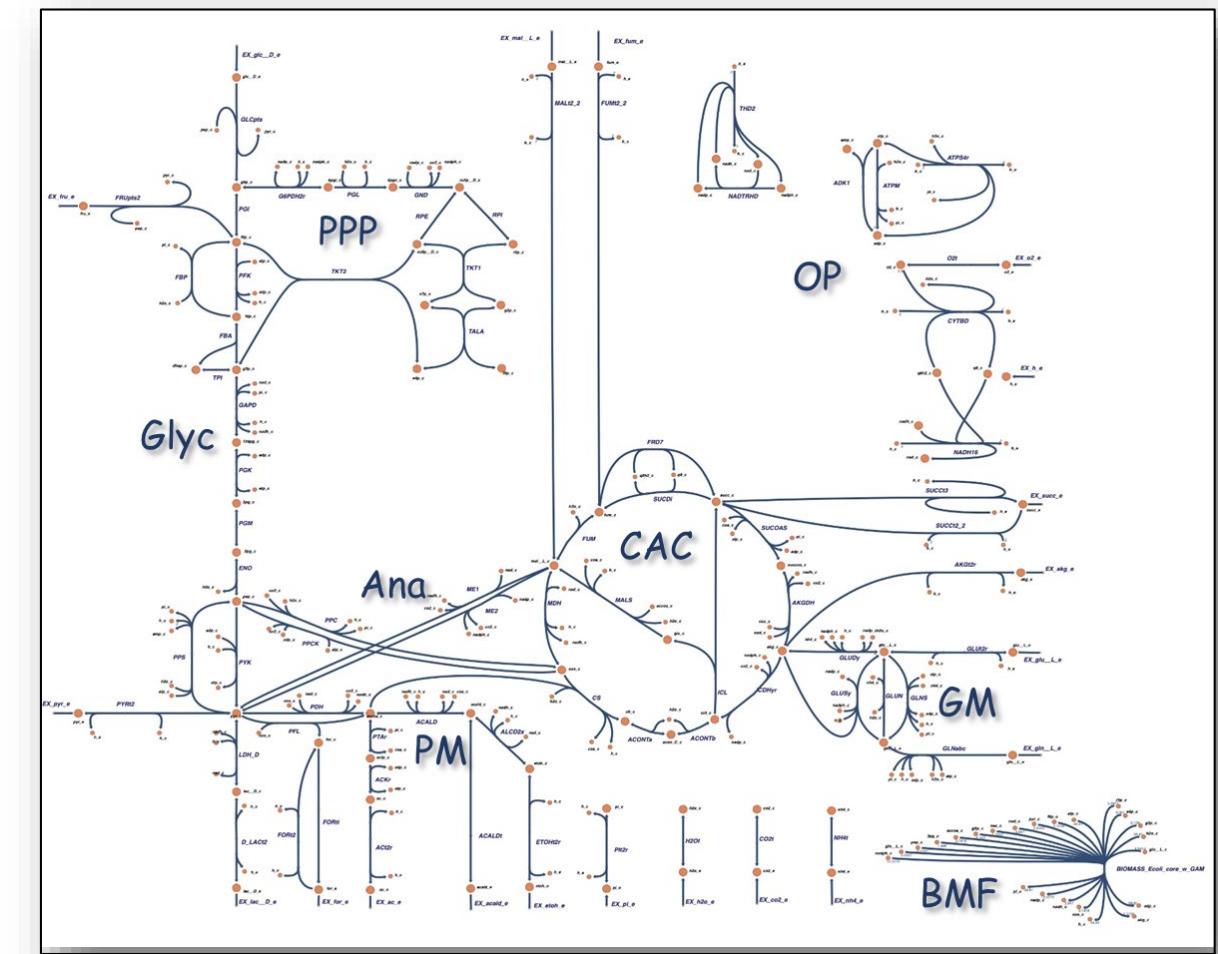
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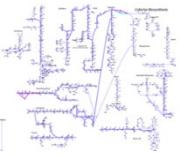
The Extracellular Exchange Subsystem includes all the exchange reactions of the model as shown below



E.coli Core Model Subsystems

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Reflective Questions

1. What is the difference between glycolysis and gluconeogenesis?
2. What reactions make-up the glycolysis pathway?
3. What metabolites are created in the glycolysis pathway?
4. What is the final metabolite created by the glycolysis pathway?
5. What are the biosynthetic precursors created by the glycolysis pathway?
6. What are the biosynthetic precursors created by the pentose phosphate pathway?
7. What is the difference between the oxidative and non-oxidative pathways of the pentose phosphate pathway?
8. What reactions make-up the pentose phosphate pathway?
9. What metabolites are created in the pentose phosphate pathway?
10. What are the different names for the CAC cycle?
11. What are the biosynthetic precursors created by the CAC cycle?
12. What is the oxidative pathway in the CAC cycle?
13. What reactions make-up the CAC cycle?
14. What metabolites are created in the CAC cycle?
15. What is the anapleurotic pathway?
16. What is the glyoxylate cycle?
17. What reactions make-up the anapleurotic pathway and the glyoxylate cycle?
18. What metabolites are created in the anapleurotic pathway and the glyoxylate cycle?
19. What reactions make-up the core models oxidative phosphorylation pathway and electron transfer chain?
20. What metabolites are created in the core models oxidative phosphorylation pathway?
21. What reactions make-up the pyruvate metabolism (fermentation) pathways?
22. What metabolites are created in the pyruvate metabolism pathways?
23. What are the biosynthetic precursors created by the glutamate metabolism (nitrogen) metabolism?
24. What reactions make-up the glutamate metabolism?
25. What metabolites are created in the glutamate metabolism?
26. What is the purpose of the objective function?
27. What is the purpose of the biomass reaction?
28. What is the purpose of the "inorganic ion transport and metabolism" subsystem?
29. What is the purpose of the "transport, external" subsystem?
30. What is the purpose of the "extracellular exchange" subsystem?
31. What are the units of flux in the COBRA models?
32. What is the purpose of the Pentose Phosphate Shunt?



References

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

Jan Koolman and Klaus-Heinrich Roehm, "Color Atlas of Biochemistry", 2nd Edition, 2005.