Flux Variability Analysis

Learning Objectives

- Explain alternate optimal solutions,
- Explain flux variability analysis,
- Demonstrate the ability to use of flux variability analysis.

Lesson Outline

- Alternate Optimal Solutions
- Flux Variability Analysis
- FVA Examples

Phenotypes

- Phenotype = A phenotype (from Greek phainein, meaning "to show", and typos, meaning "type") is the composite of an organism's **observable** characteristics or traits, such as its morphology, development, biochemical or physiological properties, phenology, behavior, and products of behavior.
- Silent phenotypes have the same overall external cellular function but are based on different underlying reaction networks.

https://en.wikipedia.org/wiki/Phenotype

Alternate Equivalent Optimal Solutions

- The flux distributions calculated by FBA are often not unique. In many cases, it is necessary for a biological system to achieve the same objective value by using alternate equivalent optimal pathways, creating phenotypically different alternate optimal solutions (silent phenotypes).
- Requires the Mixed Integer Linear Programming (MILP) solver
- For large models there can be a very large number of alternate equivalent optimal solutions.

Same objective value for all alternate flux vectors

Maximize the objective function

$$Z = \sum_i c_i v_i^k = \mathbf{c} \cdot \mathbf{v}^k$$

with the following constraints

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

$$\alpha_j \le v_j^k \le \beta_j$$

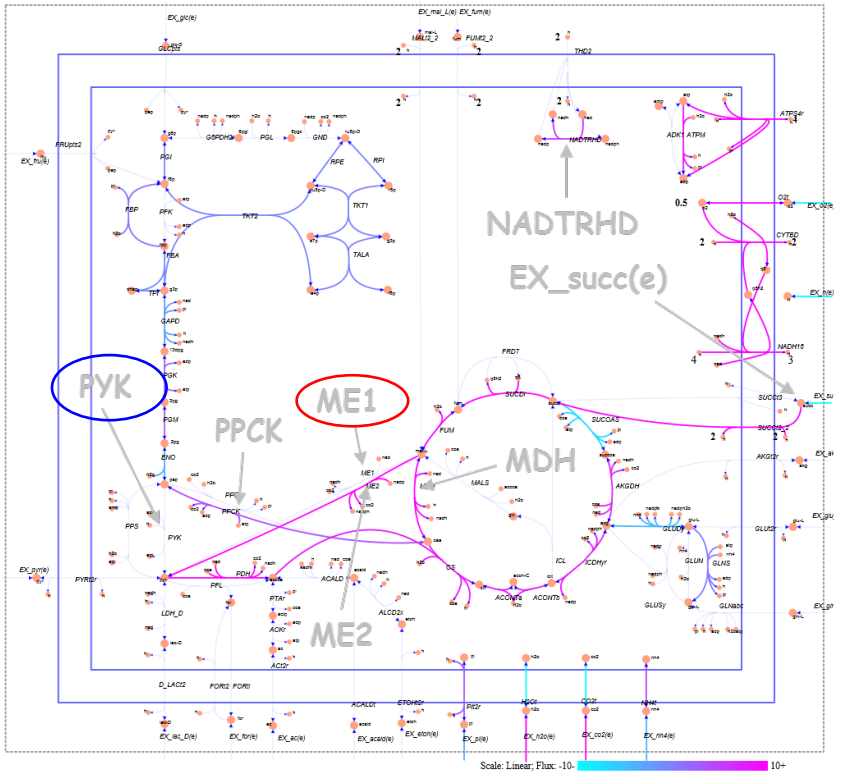
$$1 \le k \le n$$

Up to n alternate flux vectors

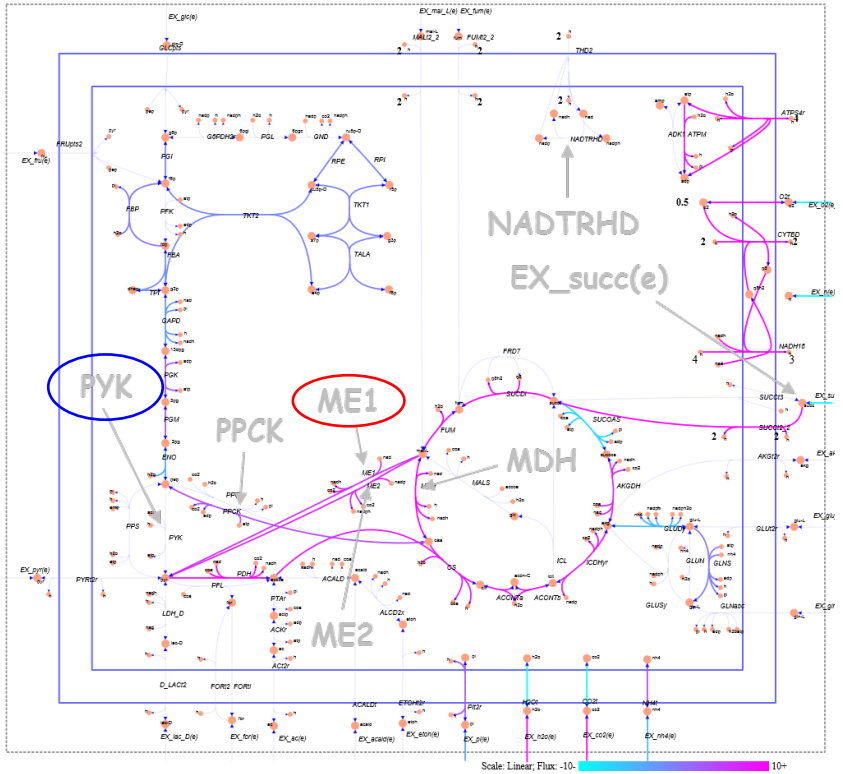
$\Rightarrow$ $v^1, v^2, \ldots, v^n$ all have the same value of Z

Reed, J. L. & Palsson, B. Ø. Genome-scale in silico models of *E. coli* have multiple equivalent phenotypic states: assessment of correlated reaction subsets that comprise network states. Genome Res. 14, 1797–1805 (2004).

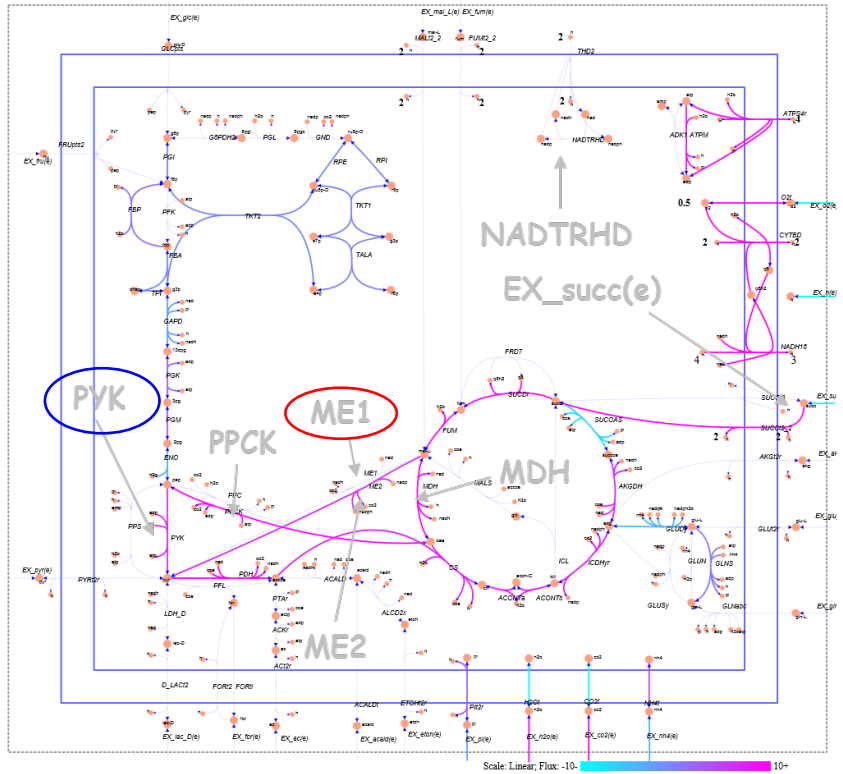
Alternate Optimal Solutions



Solution #1



Solution #2



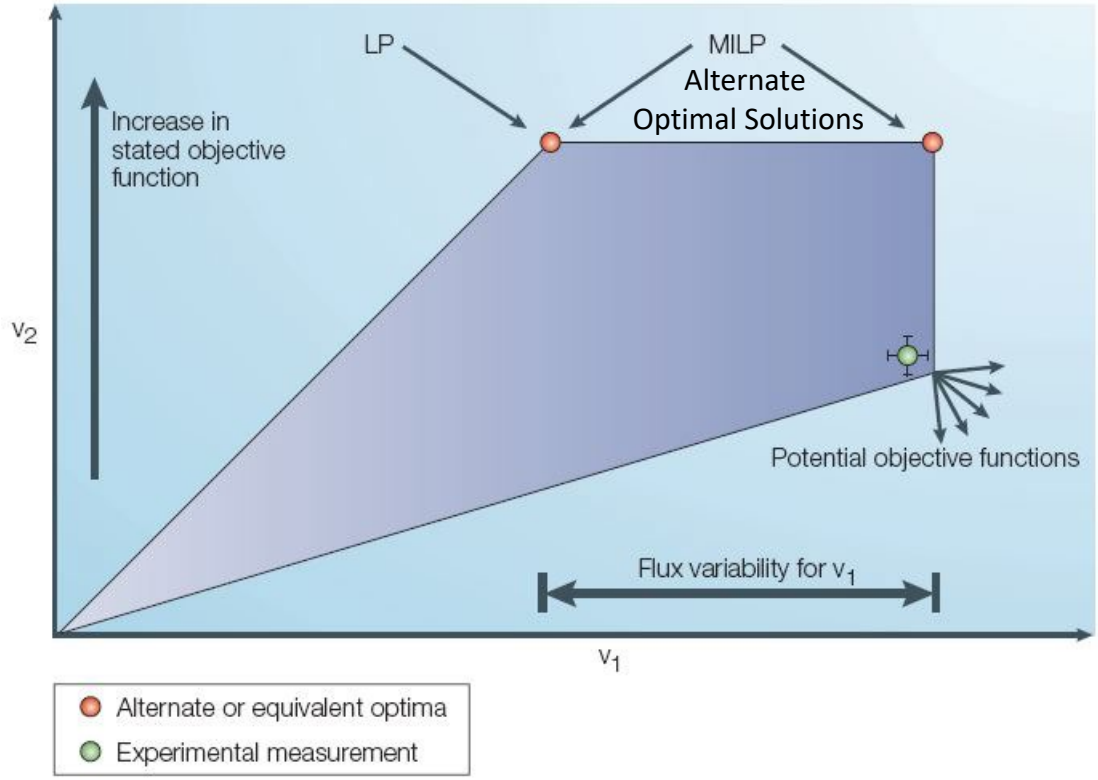
Solution #3

Lesson Outline

- Alternate Optimal Solutions
- Flux Variability Analysis
- FVA Examples

Flux Variability Analysis

- This method identifies the allowable range of flux values through a given reaction by finding the maximum and minimum possible fluxes through the particular reaction for a given **maximum objective value**.
- All reactions under test have the same objective value
- This analysis method begins by finding the optimal value of the objective function for a given set of constraints and then optimizes for the minimum and maximum flux values for each reaction (*1 + 2n* optimizations where *n* is the number of reactions).
- A method that can be used to identify alternate optimal pathways.

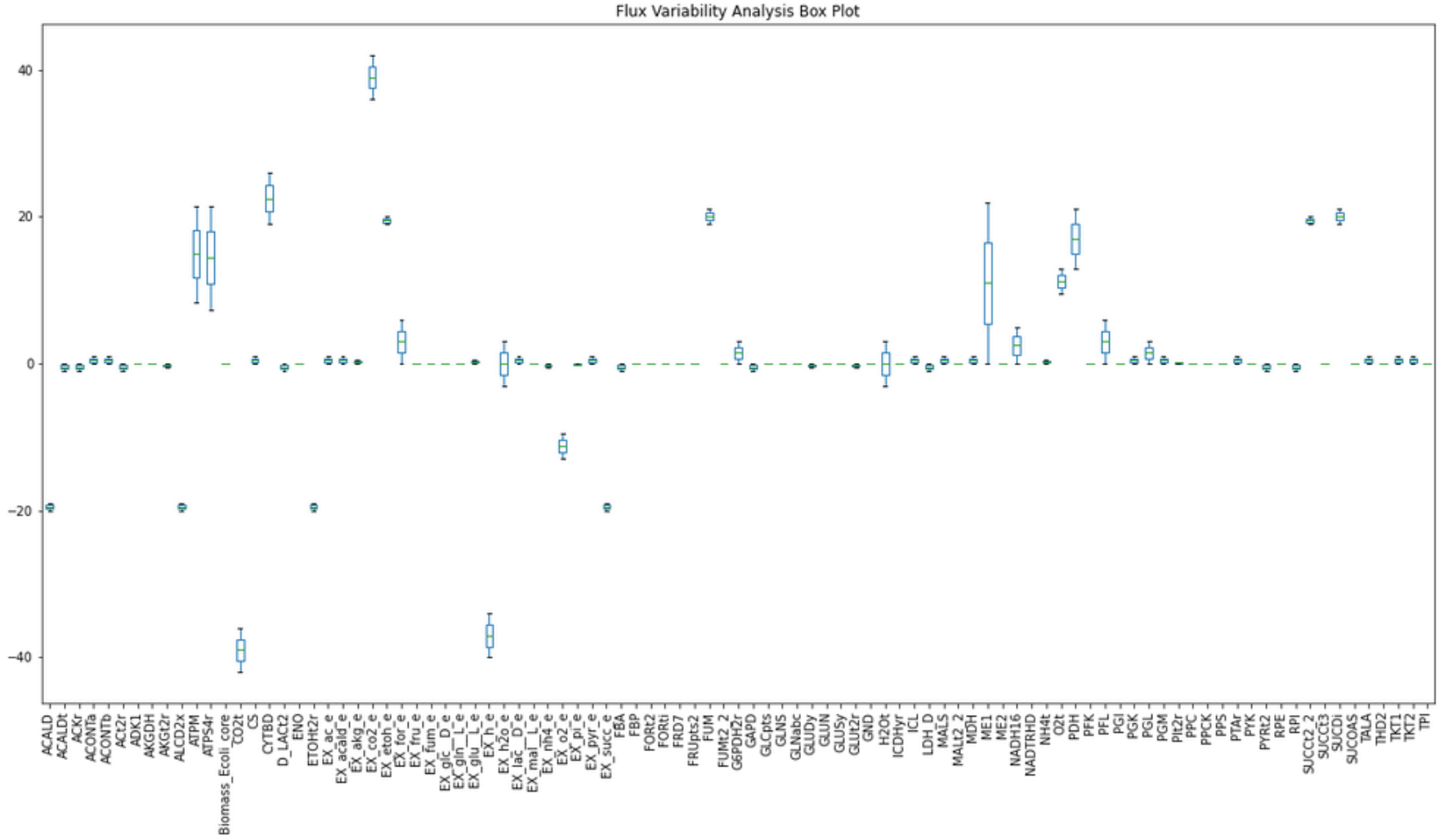


Price, N. D., J. L. Reed, et al. (2004). "Genome-scale models of microbial cells: evaluating the consequences of constraints." Nature reviews. Microbiology 2(11): 886-897.

FVA Data

	minimum	maximum
ACALD	-1.204692	0.000000
ACALDt	-1.204692	0.000000
ACKr	-1.842471	0.000000
ACONTa	6.685508	8.730759
ACONTb	6.685508	8.730759
ACt2r	-1.842471	0.000000
ADK1	0.000000	5.780234
AKGDH	0.039160	7.869660
AKGt2r	-0.728419	0.000000
ALCD2x	-1.044067	0.000000
ATPM	8.390000	16.220500

FVA Box Plot


Flux Variability Analysis Box Plot
40
20
0
−20
−40
ACALD
ACALDt
ACKr
ACONTa
ACONTb
ACt2r
ADK1
AKGDH
AKGt2r
ALCD2x
ATPM
ATPS4r
Biomass_Ecoli_core
CO2t
CS
CYTBD
D_LACt2
ENO
ETOHt2r
EX_ac_e
EX_acald_e
EX_akg_e
EX_co2_e
EX_etoh_e
EX_for_e
EX_fru_e
EX_fum_e
EX_glc__D_e
EX_gln__L_e
EX_glu__L_e
EX_h_e
EX_h2o_e
EX_lac__D_e
EX_mal__L_e
EX_nh4_e
EX_o2_e
EX_pi_e
EX_pyr_e
EX_succ_e
FBA
FBP
FORt2
FORti
FRD7
FRUpts2
FUM
FUMt2_2
G6PDH2r
GAPD
GLCpts
GLNS
GLNabc
GLUDy
GLUN
GLUSy
GLUt2r
GND
H2Ot
ICDHyr
ICL
LDH_D
MALS
MALt2_2
MDH
ME1
ME2
NADH16
NADTRHD
NH4t
O2t
PDH
PFK
PFL
PGI
PGK
PGL
PGM
Pit2r
PPC
PPCK
PPS
PTAr
PYK
PYRt2
RPE
RPI
SUCCt2_2
SUCCt3
SUCDi
SUCOAS
TALA
THD2
TKT1
TKT2
TPI

Lesson Outline

- Alternate Optimal Solutions
- Flux Variability Analysis
- FVA Examples

FVA Lecture Examples

FVA_Lecture_Examples.ipynb

Maximum Growth FVA

Set Python environment and load the model

In [1]:
```
import cobra.test
import pandas as pd
pd.set_option('display.max_rows', 500)
model = cobra.test.create_test_model("textbook")
#model = cobra.io.load_matlab_model('./e_coli_core.mat')
```

```
Academic license - for non-commercial use only - expires 2022-10-10
Using license file C:\Users\hinton\gurobi.lic
```

Set the simulation conditions

In [2]:
```
from cobra.flux_analysis import flux_variability_analysis
model.reactions.EX_glc__D_e.lower_bound = -0 # Must be set to zero if not a carbon source
model.reactions.EX_succ_e.lower_bound = -20
#model.reactions.ME1.bounds = [6.49242,6.49242]
#model.reactions.ME1.bounds = [0,0]
```

Run flux variability analysis

In [3]:
```
fva=flux_variability_analysis(model, model.reactions,loopless = True, fraction_of_optimum=1.0)
fva.round(3)
```

Bordbar *et al. BMC Systems Biology* 2011, **5**:110
http://www.biomedcentral.com/1752-0509/5/110

RESEARCH ARTICLE Open Access

iAB-RBC-283: A proteomically derived knowledge-base of erythrocyte metabolism that can be used to simulate its physiological and patho-physiological states

Aarash Bordbar, Neema Jamshidi and Bernhard O Palsson*

Bordbar, A., N. Jamshidi, et al. (2011). "iAB-RBC-283: A proteomically derived knowledge-base of erythrocyte metabolism that can be used to simulate its physiological and patho-physiological states." BMC systems biology 5: 110.

iAB_RBC_283 Model Attributes

iAB_RBC_283_Model_Attributes.ipynb

Set the 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.flux_analysis import flux_variability_analysis
import matplotlib.pyplot as plot
from cobrapy_bigg_client import client
pd.set_option('display.max_rows', 1000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
```

Load the red blood cell model iAB_RBC_283.json

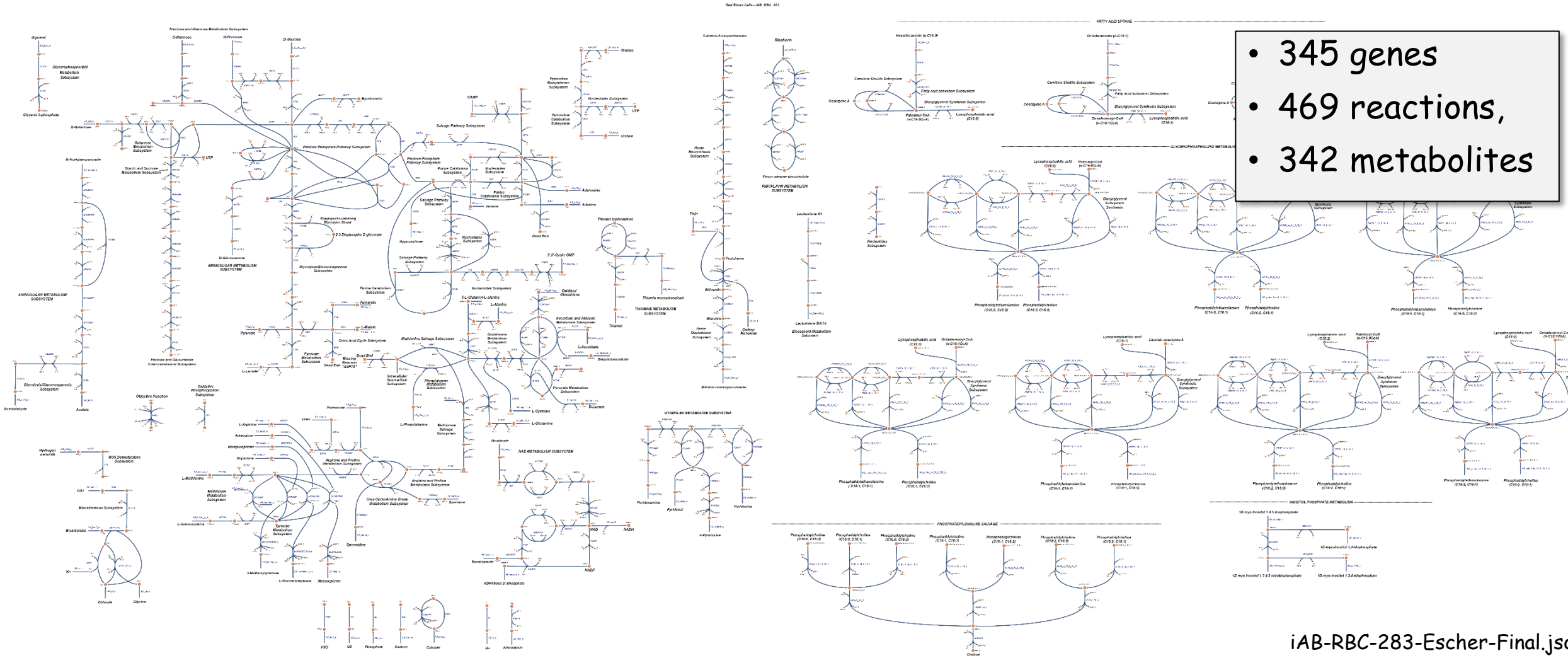
In [2]:
```
model = client.download_model('iAB_RBC_283', save=False) # Loading the model to the simulation
```

```
Set parameter Username
Academic license - for non-commercial use only - expires 2022-10-10
```

Model Attribute Summary

In [3]:
```
model
```

Escher Map: iAB_RBC_283.metabolism

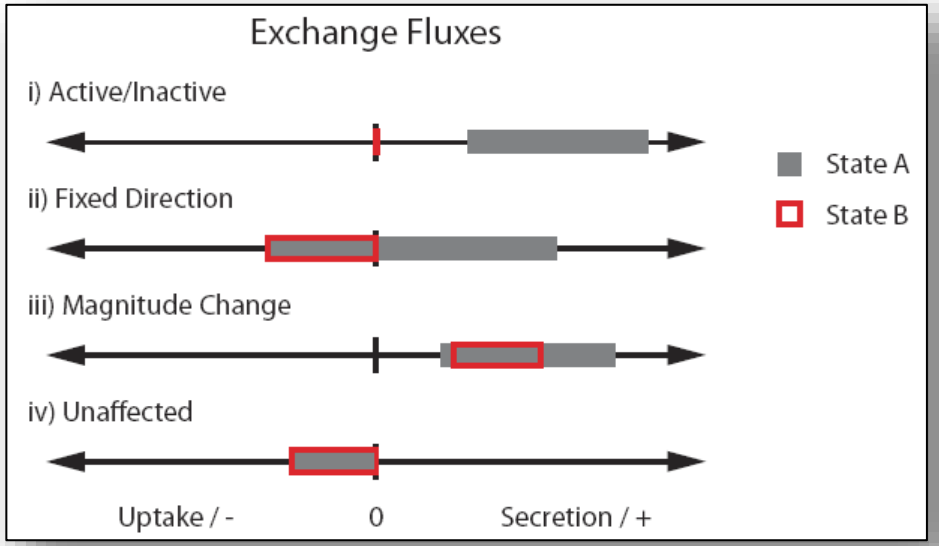


- 345 genes
- 469 reactions,
- 342 metabolites

iAB-RBC-283-Escher-Final.json

Analyzing iAB-RBC-283 as a Functional Biomarker

- The Morbid Map from the Online Mendelian Inheritance in Man (OMIM) and the DrugBank were downloaded from their respective databases. The enzyme names in iAB-RBC-283 were cross-referenced against the database entries to determine morbid single nucleotide polymorphisms (SNPs) in erythrocyte proteins and drugs with protein targets in the erythrocyte. The morbid SNPs that did not have sole pathological effects in the erythrocyte were classified using the Merck Manual.
- Just as FVA can be used to assess the function of a network under a particular set of constraints, it can also be used to assess the changes in function and thus has applications for characterizing disease states and identifying biomarkers. When simulating a morbid SNP or a drug inhibited enzyme, the lower and upper bound constraints on the affected reaction is set to zero. FVA is then used to characterize the exchange reactions under morbid SNP or drug treated conditions and then compared to the normal state.
- A reaction was considered to be confidently altered if the change in the minimum or maximum flux was 40% of the total flux span. The flux span is defined as the absolute difference between the original (unperturbed) maximum and minimum fluxes.



There are four major differences that can occur for an exchange reaction in two different states: i) the reaction is either active (non-zero minimum or maximum flux) or inactive (zero minimum and maximum flux), ii) the exchange becomes fixed in one direction (uptake or secretion only), iii) there is a magnitude change in exchange, iv) the reaction is unaffected and is the same for both states.

Bordbar, A., N. Jamshidi, et al. (2011). "iAB-RBC-283: A proteomically derived knowledge-base of erythrocyte metabolism that can be used to simulate its physiological and patho-physiological states." BMC systems biology 5: 110.

OMIM Database

OMIM®
Online Mendelian Inheritance in Man®
An Online Catalog of Human Genes and Genetic Disorders
Updated 12 June 2015

Search

Advanced Search : OMIM, Clinical Synopses, Gene Map
Need help? : Example Searches, OMIM Search Help, OMIM Tutorial
Mirror sites : us-east.omim.org, europe.omim.org

http://omim.org/

Amberger J, Bocchini CA, Scott AF, Hamosh A: McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res 2009, , 37 Database: D793-6.

OMIM Advanced Search

Search OMIM... Search

Sort by: Relevance Date updated Date created Entries per page: 10

Search in:
- Mim Number
- Title
- Text
- Allelic Variants
- Clinical Synopsis
- Contributors

Only Records With:
- Allelic Variants
- Clinical Synopsis
- Gene Map Locus

Only Records without:
- Gene Map Locus

MIM Number Prefix:
- * gene with known sequence
- + gene with known sequence and phenotype
- # phenotype description, molecular basis known
- % mendelian phenotype or locus, molecular basis unknown
- none - other, mainly phenotypes with suspected mendelian basis

Chromosome:
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 X Y

Mitochondrial Autosomal Unknown

Created From: YYYY/MM/DD To: present
Updated From: YYYY/MM/DD To: present

Note: Entries created before June 2, 1986 have a creation date of June 2, 1986.

Clear Search

Detected SNPs and FVA Results for SNP Perturbations

OMIM	Location	Pathology	Gene Protein Name
115500	11p13	Acatalasemia (3)	CAT
108961	9p21-p12	Acromesomelic dysplasia, Maroteaux type, 602875 (3)	NPR2, ANPRB, AMDM
608958	20q13.11	Adenosine deaminase deficiency, partial, 102700 (3)	ADA
609712	1q21	Adenosine triphosphate, elevated, of erythrocytes, 102900 (3)	PKLR, PK1
180297	6p21.1-p11	Anemia, hemolytic, Rh-null, regulator type, 268150 (3)	RHAG, RH50A
606224	7p15-p14	Anemia, hemolytic, due to UMPH1 deficiency, 266120 (3)	NT5C3, UMPH1, PSN1
608313	6q23	Argininemia, 207800 (3)	ARG1
173335	6q22-q23	Arterial calcification, generalized, of infancy, 208000 (3)	ENPP1, PDNP1, NPPS, M6S1, PCA1, ARHR2
311850	Xq22-q24	Arts syndrome, 301835 (3)	PRPS1, CMTX5, DFNX1, DFN2
600650	1p32	CPT II deficiency, lethal neonatal, 608836 (3)	CPT2
600528	11q13	CPT deficiency, hepatic, type IA, 255120 (3)	CPT1A
600650	1p32	CPT deficiency, hepatic, type II, 600649 (3)	CPT2
154550	15q22-qter	Carbohydrate-deficient glycoprotein syndrome, type Ib, 602579 (3)	MPI, PMI1
182500			
311850			
607672			
600179			6, RCD2
601785			
612732			
609414	2q35	Corneal fleck dystrophy, 121850 (3)	PIKFYVE, PIP5K3
191740	2q37	Crigler-Najjar syndrome, type I, 218800 (3)	UGT1A1, UGT1, GNT1, BILIQTL1
191740	2q37	Crigler-Najjar syndrome, type II, 606785 (3)	UGT1A1, UGT1, GNT1, BILIQTL1
311850	Xq22-q24	Deafness, X-linked 1, 304500 (3)	PRPS1, CMTX5, DFNX1, DFN2

OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. OMIM is authored and edited at the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, under the direction of Dr. Ada Hamosh. Its official home is omim.org.

Affected Protein	ADA	AHCY	AK1
Affected Reaction	ADA	AHC	ADK1
Varying Exchanges	EX_ade(e)	EX_3moxtyr(e)	EX_35cgmp(e)
	EX_hxan(e)	EX_adrnl(e)	EX_3moxtyr(e)
	EX_nh4(e)	EX_dopa(e)	EX_adrnl(e)
		EX_hcyst-L(e)	EX_camp(e)
		EX_mepi(e)	EX_chol(e)
		EX_met-L(e)	EX_dopa(e)
		EX_normete-L(	EX_etha(e)
		EX_nrpphr(e)	EX_glyc(e)
			EX_hcyst-L(e)
			EX_hdca(e)
			EX_lnlc(e)
			EX_mepi(e)
			EX_met-L(e)
			EX_nac(e)
			EX_ncam(e)
			EX_normete-L(e)
			EX_nrpphr(e)
			EX_ocdcea(e)
			EX_orot(e)
			EX_spmd(e)
			EX_sprm(e)
			EX_thm(e)
			EX_thmmp(e)
			EX_uri(e)

A reaction was considered to be confidently altered if the change in the minimum or maximum flux was 40% of the total flux span. The flux span is defined as the absolute difference between the original (unperturbed) maximum and minimum fluxes.

Bordbar, A., N. Jamshidi, et al. (2011). "iAB-RBC-283: A proteomically derived knowledge-base of erythrocyte metabolism that can be used to simulate its physiological and patho-physiological states." BMC systems biology 5: 110.

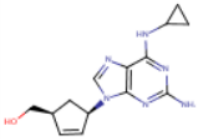
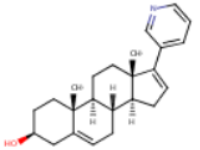
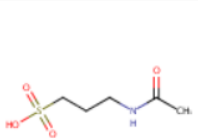
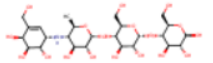
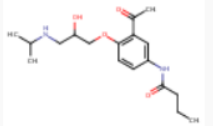
DrugBank Database

DrugBank Version 4.2

The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. The database contains 7759 drug entries including 1600 FDA-approved small molecule drugs, 160 FDA-approved biotech (protein/peptide) drugs, 89 nutraceuticals and over 6000 experimental drugs. Additionally, 4282 non-redundant protein (i.e. drug target/enzyme/transporter/carrier) sequences are linked to these drug entries. Each DrugCard entry contains more than 200 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data.

http://www.drugbank.ca/

Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M: DrugBank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res 2008, , 36 Database: D901-6.

DrugBank ID	Name	Weight	Structure	Categories	Therapeutic Indication
DB01048 136470-78-5	Abacavir	286.3323 $C_{14}H_{18}N_6O$		Anti-HIV Agents / Reverse Transcriptase Inhibitors / Nucleoside and nucleotide reverse transcriptase inhibitors	For the treatment of HIV-1 infection, in combination with other antiretroviral agents.
DB05812 154229-19-3	Abiraterone	349.509 $C_{24}H_{31}NO$		Other hormone antagonists and related agents	Used in combination with prednisone for the treatment of metastatic, castration-resistant prostate cancer.
DB00659 77337-76-9	Acamprosate	181.21 $C_5H_{11}NO_4S$		Alcohol Deterrents / Drugs used in alcohol dependence	For the maintenance of abstinence from alcohol in patients with alcohol dependence who are abstinent at treatment initiation
DB00284 56180-94-0	Acarbose	645.6048 $C_{25}H_{43}NO_{18}$		Alpha glucosidase inhibitors	For treatment and management of diabetes type II (used in combination therapy as a second or third line agent)
DB01193 37517-30-9	Acebutolol	336.4259 $C_{18}H_{28}N_2O_4$		Antihypertensive Agents / Sympathomimetics / Adrenergic beta-1 Receptor Antagonists / Anti-Arrhythmia Agents / Beta blocking agents, selective	For the management of hypertension and ventricular premature beats in adults.

RBC_FVA_Drugs.ipynb

Drug Impacts on Erythrocytes

Set the 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.flux_analysis import flux_variability_analysis
import matplotlib.pyplot as plot
from cobrapy_bigg_client import client
pd.set_option('display.max_rows', 1000)
pd.set_option('display.width',1000)
pd.set_option('display.max_colwidth',None)
```

Download the red blood cell model iAB_RBC_283.json from the BIGG database to insure it is the latest version

In [2]:
```
model = client.download_model('iAB_RBC_283', save=False) # Loading the model to the simulation
```

```
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Using license file C:\Users\hinton\gurobi.lic
```

The model summary using the default conditions of the model

In [3]:
```
model.summary()
```

Lesson Outline

- Alternate Optimal Solutions
- Flux Variability Analysis
- FVA Examples

Reflective Questions

1. What are alternate optimal solutions?
2. What is the relationship between alternate optimal solutions and a cell's phenotype?
3. What are silent phenotypes?
4. How many alternate optimal solutions can there be for a given phenotype?
5. How many alternate optimal solutions can there be for a carbon source?
6. Do aerobic/anaerobic conditions impact the number alternate optimal solutions?
7. Does the choice of objective function impact the number alternate optimal solutions?
8. What is flux variability analysis?
9. What is the relationship between the value of the objective function and the flux values calculated through flux variability analysis?
10. How is flux variability analysis related to alternate optimal flux vectors?
11. Does flux variability analysis identify the specific alternate optimal solutions?
12. What is the value of knowing which reactions carry flux, which reactions carry no flux, and which reactions span a range of flux values?

References

1. Reed, J. L. & Palsson, B. Ø. Genome-scale in silico models of *E. coli* have multiple equivalent phenotypic states: assessment of correlated reaction subsets that comprise network states. Genome Res. 14, 1797–1805 (2004).
2. Price, N. D., J. L. Reed, et al. (2004). "Genome-scale models of microbial cells: evaluating the consequences of constraints." Nature reviews. Microbiology 2(11): 886-897.
3. Bordbar, A., N. Jamshidi, et al. (2011). "iAB-RBC-283: A proteomicallyderived knowledge-base of erythrocyte metabolism that can be used to simulate its physiological and patho-physiological states." BMC systems biology 5: 110.
4. AmbergerJ, BocchiniCA, Scott AF, HamoshA: McKusick'sOnline Mendelian Inheritance in Man (OMIM). Nucleic Acids Res 2009, , 37 Database: D793-6.
5. WishartDS, Knox C, Guo AC, Cheng D, ShrivastavaS, TzurD, GautamB, HassanaliM: DrugBank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res 2008, , 36 Database: D901-6.
6. Mahadevan, R. and C. H. Schilling (2003). "The effects of alternate optimal solutions in constraint-based genome-scale metabolic models." Metabolic engineering 5(4): 264-276
7. Phalakornkule, C. et al. A MILP-based flux alternative generation and NMR experimental design strategy for metabolic engineering. Metab. Eng. 3, 124–137 (2001).
8. Lee, S., Phalakornkule, C., Domach, M. M. & Grossmann, I. E. Recursive MILP model for finding all the alternate optima in LP models for metabolic networks.Comp. Chem. Eng. 24, 711–716 (2000).