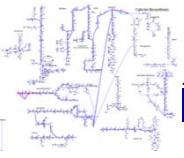
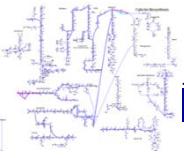


Flux Variability Analysis & Parsimonious Flux Balance Analysis



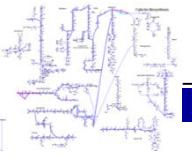
Learning Objectives

- Explain alternate optimal solutions,
- Explain flux variability analysis,
- Explain parsimonious flux balance analysis.



Lesson Outline

- Alternate Optimal Solutions
- Flux Variability Analysis
- Parsimonious FBA



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 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

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

$$\alpha_j \leq v_j^k \leq \beta_j$$

$$1 \leq k \leq n$$

Up to n alternate flux vectors

$\Rightarrow v^1, v^2, \dots, v^n$ 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).



Identifying Alternate Equivalent Optimal Solutions

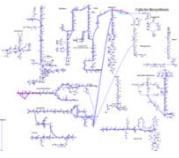
- A function that is provided by the Cobra Toolbox to identify alternate equivalent optimal solutions is called

`enumerateOptimalSolutions(model)`

- In Matlab workspace a new structure called "solutions" is created that contains all the alternate equivalent optimal solutions.
- For large models, this computation will take a long time.

```
% findingOptimalSolutionsSucc.m  
  
clear;  
  
load('ecoli_textbook.mat');  
  
model = changeRxnBounds(model,'EX_o2(e)',-40,'l');  
  
model = changeRxnBounds(model,'EX_glc(e)',0,'l');  
  
model = changeRxnBounds(model,'EX_succ(e)',-20,'l');  
  
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');  
  
% List optimal solutions  
  
changeCobraSolver('glpk','all') % Does not work with gurobi   
  
[solutions] = enumerateOptimalSolutions(model);
```

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 Matlab Screenshot

MATLAB R2014b

findingOptimalSolutionsSucc.m

Alternate Optimal Solution Flux Vectors

Variables - solutions.fluxes

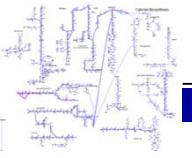
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|----|---------|---------|---------|---|---|---|---|---|
| 1 | 0 | 0 | 0 | | | | | |
| 2 | 0 | 0 | 0 | | | | | |
| 3 | 0 | 0 | 0 | | | | | |
| 4 | 8.1376 | 8.1376 | 8.1376 | | | | | |
| 5 | 8.1376 | 8.1376 | 8.1376 | | | | | |
| 6 | 0 | 0 | 0 | | | | | |
| 7 | 0 | 0 | 0 | | | | | |
| 8 | 7.2312 | 7.2312 | 7.2312 | | | | | |
| 9 | 0 | 0 | 0 | | | | | |
| 10 | 0 | 0 | 0 | | | | | |
| 11 | 8.3900 | 8.3900 | 8.3900 | | | | | |
| 12 | 57.7816 | 57.7816 | 57.7816 | | | | | |
| | 0.0450 | 0.0450 | 0.0450 | | | | | |

Workspace

| Name | Value |
|-----------|------------|
| model | 1x1 struct |
| solutions | 1x1 struct |
| solverOK | 1 |

Command History

```
findingOptimalSo...
4x findingOptimalSo...
findingOptimalSo...
findingOptimalSo...
3x findingOptimalSo...
findingOptimalSo...
findingOptimalSo...
3x findingOptimalSo...
2x FluxVariabilityE...
cho_2014
FluxVariabilityS...
findingOptimalSo...
```



Alternate Optimal Solution Non-zero Flux Values

| Reaction | S1 | S2 | S3 | Lower | Upper | Range |
|--------------|----------|----------|----------|----------|----------|-------|
| 'ACONTa' | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 0 |
| 'ACONTb' | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 0 |
| 'AKGDH' | 7.231221 | 7.231221 | 7.231221 | 7.231221 | 7.231221 | 0 |
| 'ATPM' | 8.39 | 8.39 | 8.39 | 8.39 | 8.39 | 0 |
| 'ATPS4r' | 57.78164 | 57.78164 | 57.78164 | 57.78164 | 57.78164 | 0 |
| Biomass | 0.840134 | 0.840134 | 0.840134 | 0.840134 | 0.840134 | 0 |
| 'CO2t' | -44.2477 | -44.2477 | -44.2477 | -44.2477 | -44.2477 | 0 |
| 'CS' | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 0 |
| 'CYTBD' | 66.55278 | 66.55278 | 66.55278 | 66.55278 | 66.55278 | 0 |
| 'ENO' | -3.49017 | -3.49017 | -3.49017 | -3.49017 | -3.49017 | 0 |
| 'EX_co2(e)' | 44.24767 | 44.24767 | 44.24767 | 44.24767 | 44.24767 | 0 |
| 'EX_h2o(e)' | 30.36754 | 30.36754 | 30.36754 | 30.36754 | 30.36754 | 0 |
| 'EX_h(e)' | -23.1469 | -23.1469 | -23.1469 | -23.1469 | -23.1469 | 0 |
| 'EX_nh4(e)' | -4.58108 | -4.58108 | -4.58108 | -4.58108 | -4.58108 | 0 |
| 'EX_o2(e)' | -33.2764 | -33.2764 | -33.2764 | -33.2764 | -33.2764 | 0 |
| 'EX_pi(e)' | -3.0906 | -3.0906 | -3.0906 | -3.0906 | -3.0906 | 0 |
| 'EX_succ(e)' | -20 | -20 | -20 | -20 | -20 | 0 |
| 'FBA' | -0.83568 | -0.83568 | -0.83568 | -0.83568 | -0.83568 | 0 |
| 'FBP' | 0.835681 | 0.835681 | 0.835681 | 0.835681 | 0.835681 | 0 |

AOS_Example.xlsx

| Reaction | S1 | S2 | S3 | Lower | Upper | Range |
|-----------|----------|----------|----------|----------|----------|----------|
| 'FUM' | 27.23122 | 27.23122 | 27.23122 | 27.23122 | 27.23122 | 0 |
| 'GAPD' | -2.23333 | -2.23333 | -2.23333 | -2.23333 | -2.23333 | 0 |
| 'GLNS' | 0.214822 | 0.214822 | 0.214822 | 0.214822 | 0.214822 | 0 |
| 'GLUDy' | -4.36626 | -4.36626 | -4.36626 | -4.36626 | -4.36626 | 0 |
| 'H2Ot' | -30.3675 | -30.3675 | -30.3675 | -30.3675 | -30.3675 | 0 |
| 'ICDHyr' | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 8.137641 | 0 |
| 'MDH' | 13.56499 | 13.56499 | 20.05742 | 13.56499 | 20.05742 | 6.492425 |
| 'ME1' | 0 | 6.492425 | 0 | 0 | 6.492425 | 6.492425 |
| 'ME2' | 13.66623 | 7.173804 | 7.173804 | 7.173804 | 13.66623 | 6.492425 |
| 'NADH16' | 39.32156 | 39.32156 | 39.32156 | 39.32156 | 39.32156 | 0 |
| 'NADTRHD' | 6.492425 | 0 | 0 | 0 | 6.492425 | 6.492425 |
| 'NH4t' | 4.581084 | 4.581084 | 4.581084 | 4.581084 | 4.581084 | 0 |
| 'O2t' | 33.27639 | 33.27639 | 33.27639 | 33.27639 | 33.27639 | 0 |
| 'PDH' | 11.2863 | 11.2863 | 11.2863 | 11.2863 | 11.2863 | 0 |
| 'PGI' | -0.17223 | -0.17223 | -0.17223 | -0.17223 | -0.17223 | 0 |
| 'PGK' | 2.233329 | 2.233329 | 2.233329 | 2.233329 | 2.233329 | 0 |
| 'PGM' | 3.490169 | 3.490169 | 3.490169 | 3.490169 | 3.490169 | 0 |
| 'Plt2r' | 3.090602 | 3.090602 | 3.090602 | 3.090602 | 3.090602 | 0 |
| 'PPCK' | 3.926283 | 3.926283 | 10.41871 | 3.926283 | 10.41871 | 6.492425 |
| 'PYK' | 0 | 0 | 6.492425 | 0 | 6.492425 | 6.492425 |



Reactions with Changing Flux Identified Through Alternate Optimal Solutions for Growth on Succinate

| Reaction | Minimum Flux (mmol gDW ⁻¹ hr ⁻¹) | Maximum Flux (mmol gDW ⁻¹ hr ⁻¹) |
|----------|--|--|
| 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 |

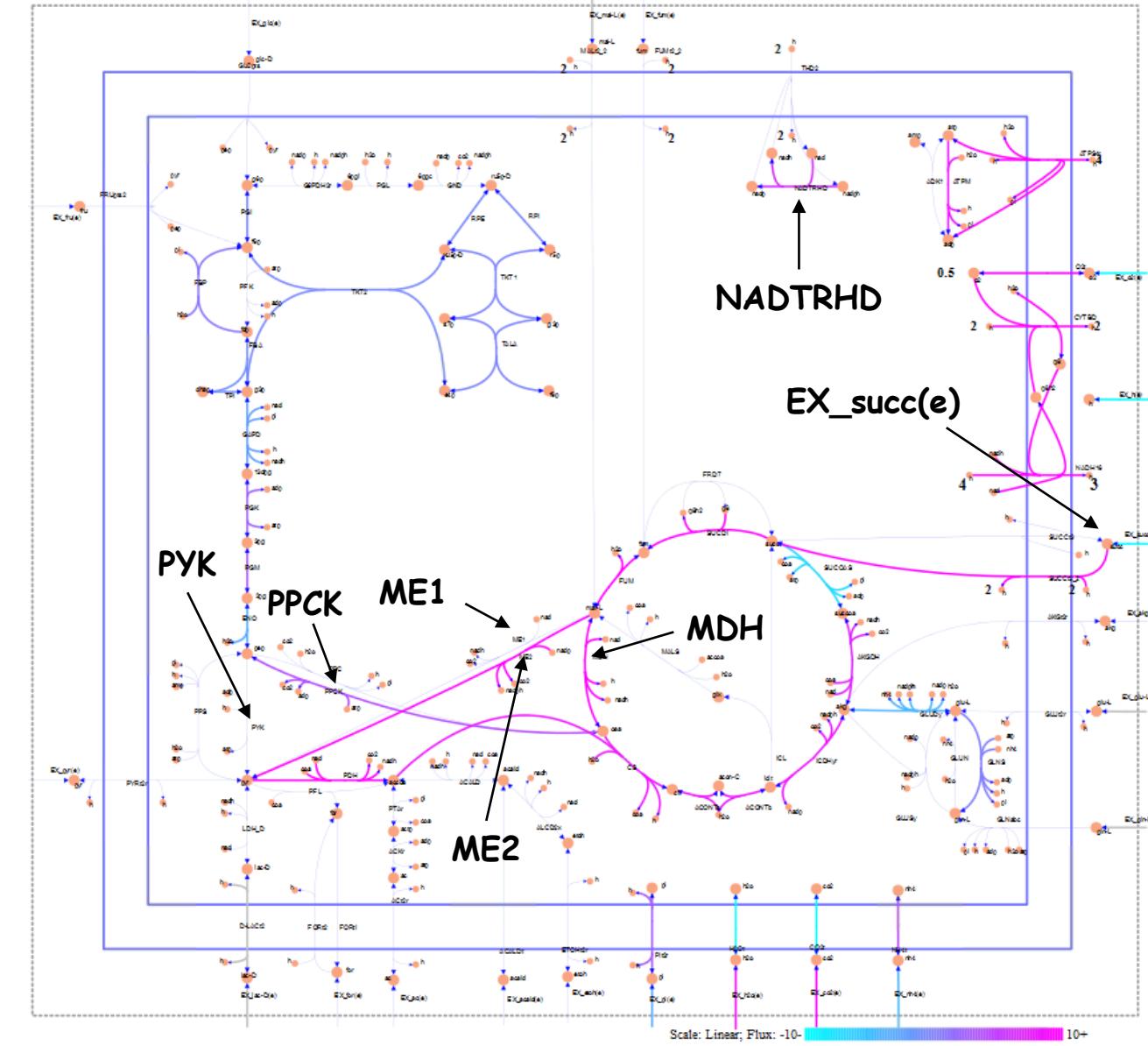
findingOptimalSolutionsSucc.m



Reactions with Changing Flux Identified Through Alternate Optimal Solutions

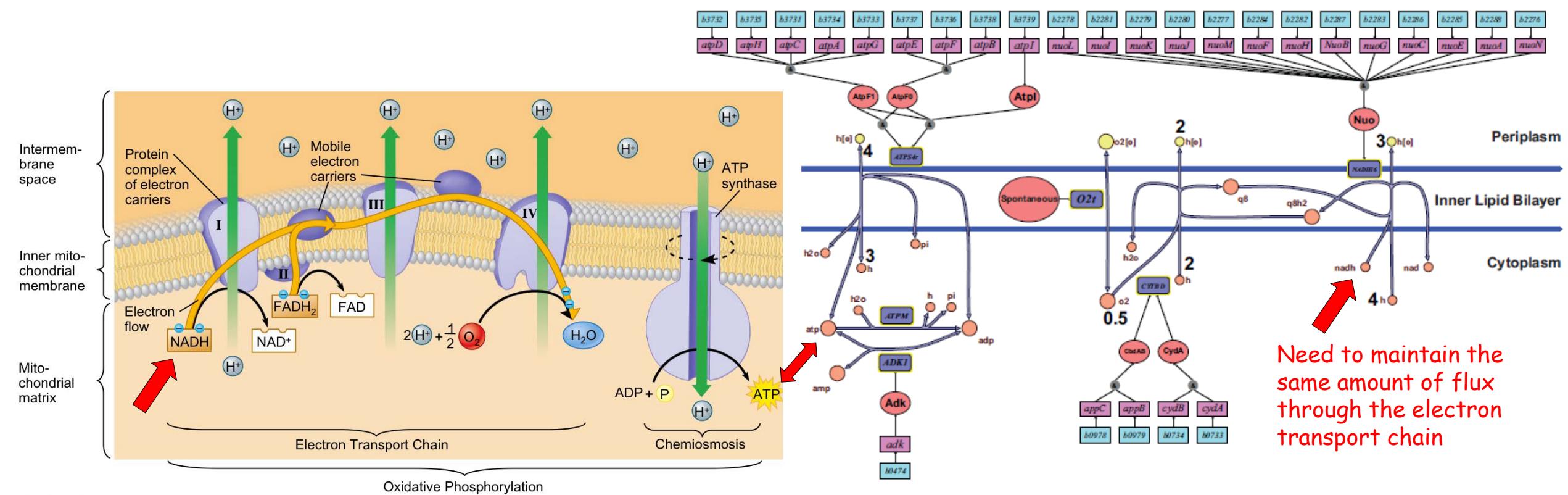
- MDH (malate dehydrogenase)
 - ✓ mal-L + nad \leftrightarrow h + nadh + oaa
- ME1 (malic enzyme (NAD))
 - ✓ mal-L + nad \rightarrow co2 + nadh + pyr
- ME2 (malic enzyme (NADP))
 - ✓ mal-L + nadp \rightarrow co2 + nadph + pyr
- NADTRHD (NAD transhydrogenase)
 - ✓ nad + nadph \rightarrow nadh + nadp
- PPCK (phosphoenolpyruvate carboxykinase)
 - ✓ atp + oaa \rightarrow adp + co2 + pep
- PYK (pyruvate kinase)
 - ✓ adp + h + pep \rightarrow atp + pyr

All reactions are centered around energy and reducing power production



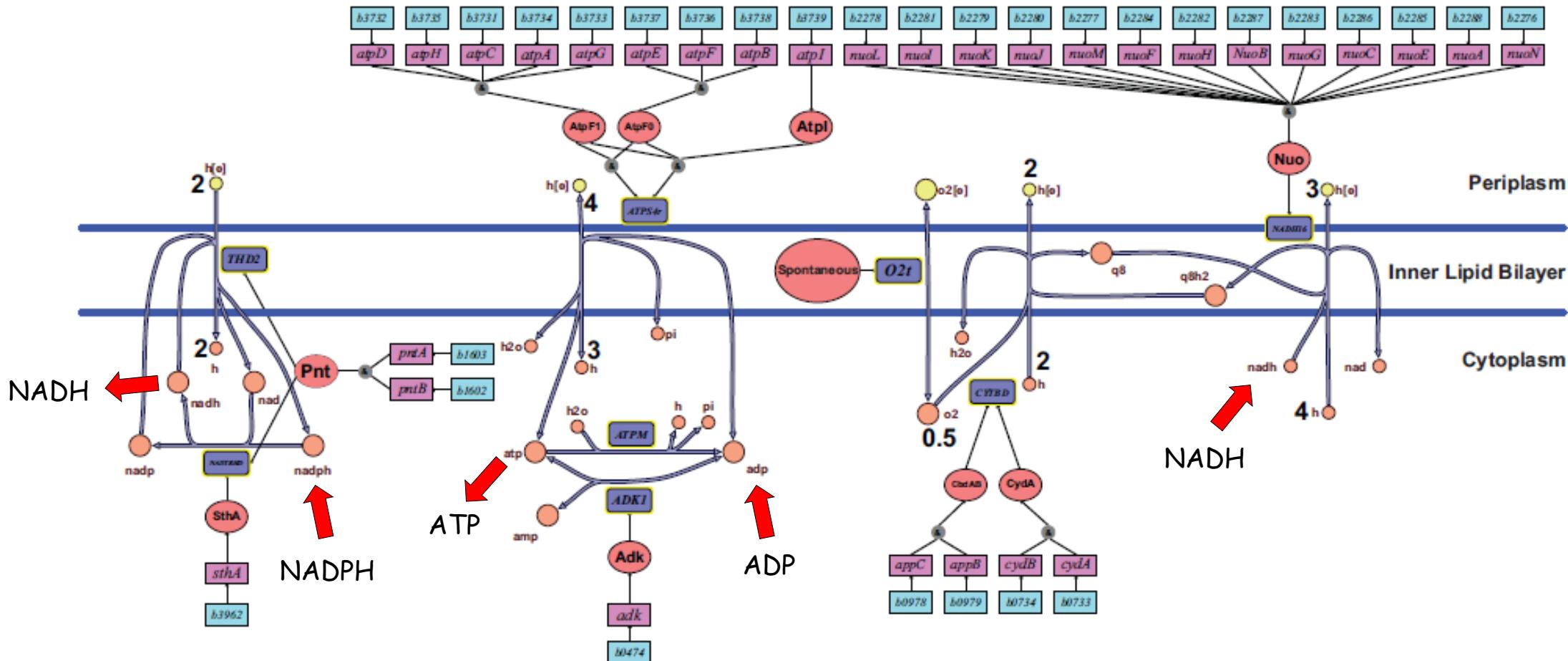


Energy Production: Electron Transport Chain

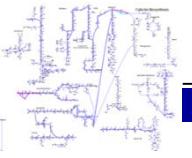




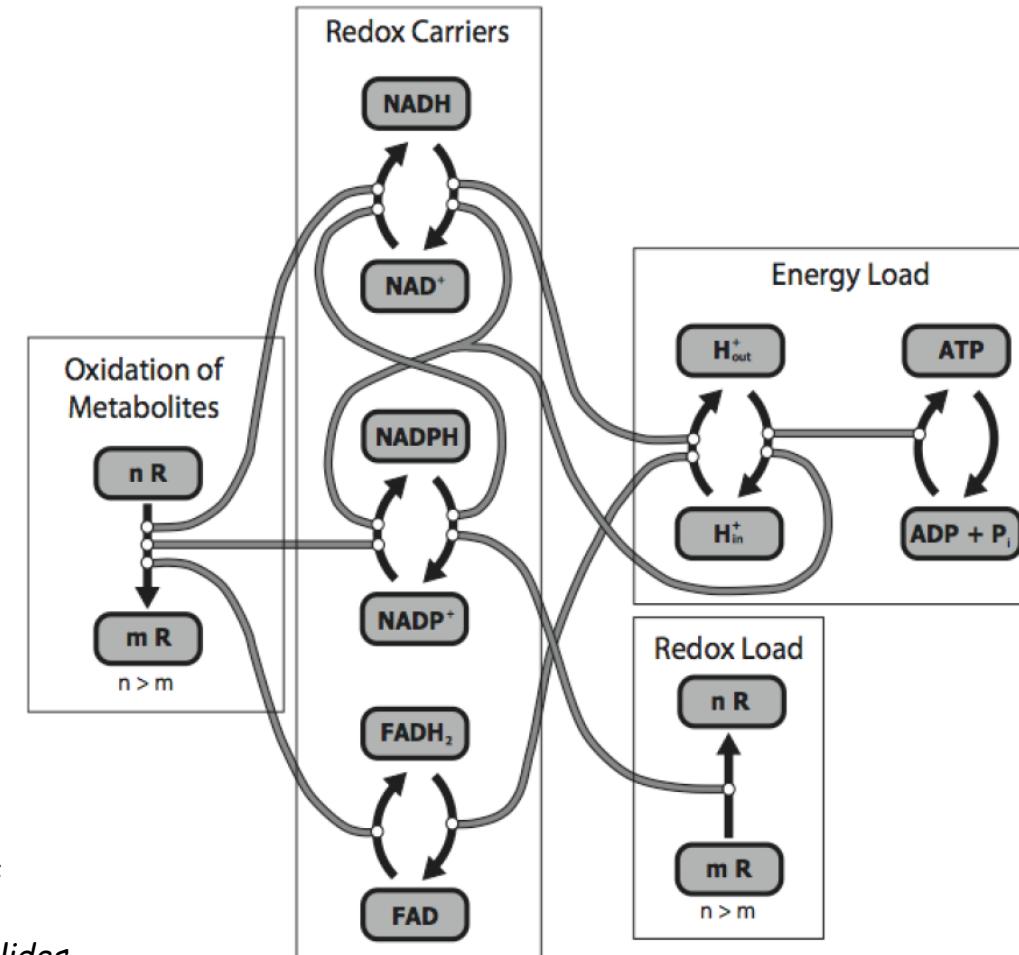
Oxidative Phosphorylation and Transfer of Reducing Equivalents



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



Redox Trafficking in the Core Metabolic Pathways: Cofactor View



B. O. Palsson lectures on Systems Biology: Simulation of Dynamic Network States, Lecture #8.
<http://sbrg.ucsd.edu/Publications/Books/SB2LectureSlides>



Visualizing the Alternate Optimal Solution Flux Vectors

```
% findingOptimalSolutionsSuccVisualize.m
clear;

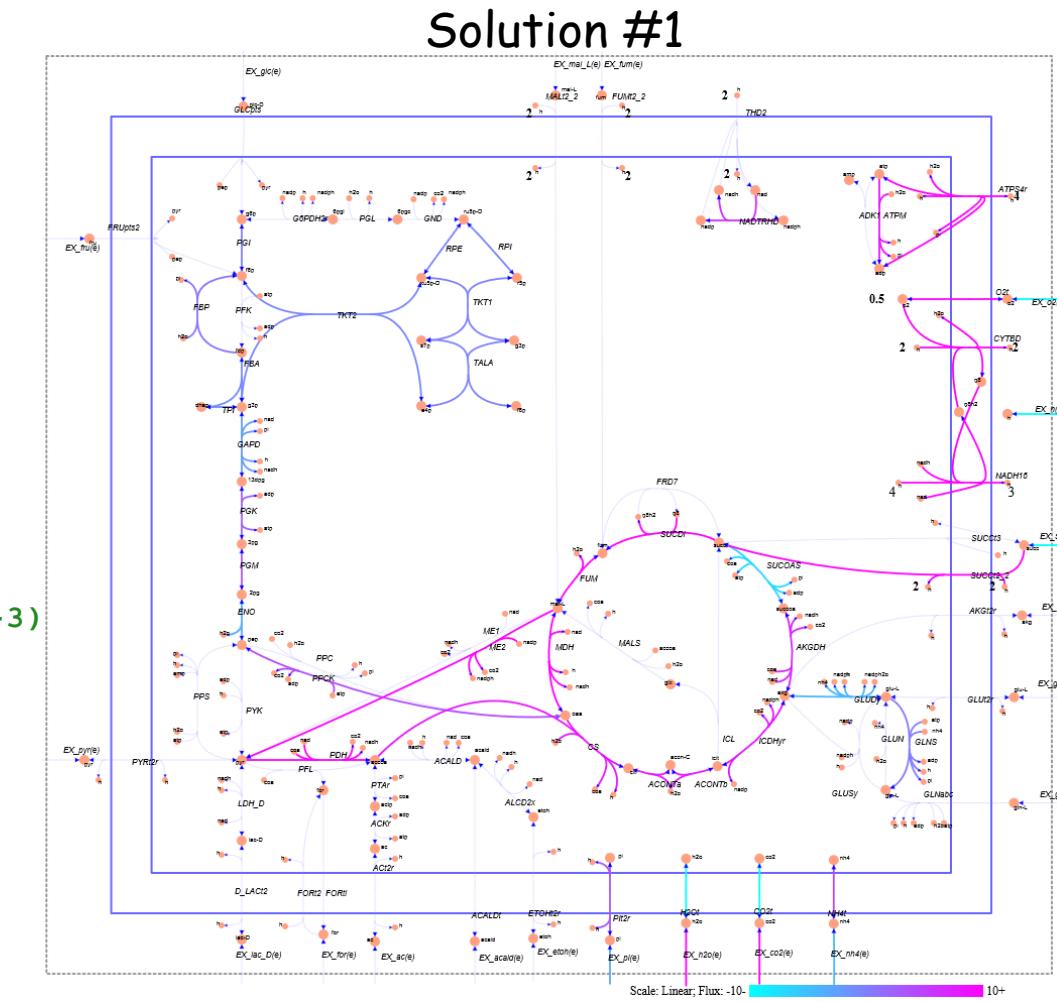
load('ecoli_textbook.mat');
model = changeRxnBounds(model,'EX_glc(e)',0,'l');
model = changeRxnBounds(model,'EX_o2(e)',-40,'l');
model = changeRxnBounds(model,'EX_succ(e)',-20,'l');
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');

% List optimal solutions
changeCobraSolver('glpk','all');

[solutions] = enumerateOptimalSolutions(model);

v = solutions.fluxes(:,1); % Select which vector wanted to be mapped (1-3)
printFluxVector(model, FBAsolution.x, true)

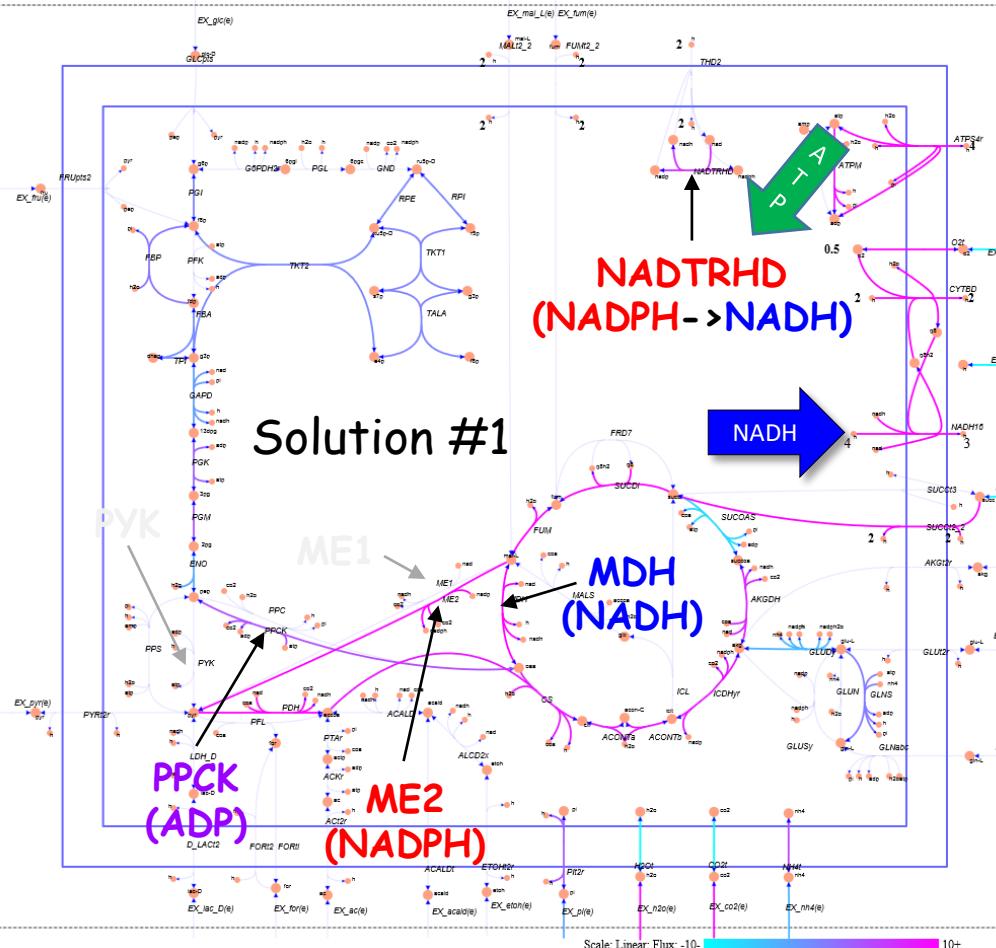
map=readCbMap('ecoli_Textbook_ExportMap');
options.lb = -10;
options.ub = 10;
options.zeroFluxWidth = 0.1;
options.rxnDirMultiplier = 10;
drawFlux(map, model, v, options);
```





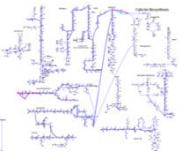
Alternate Optimal Solutions #1

findingOptimalSolutionsSuccVisualize.m



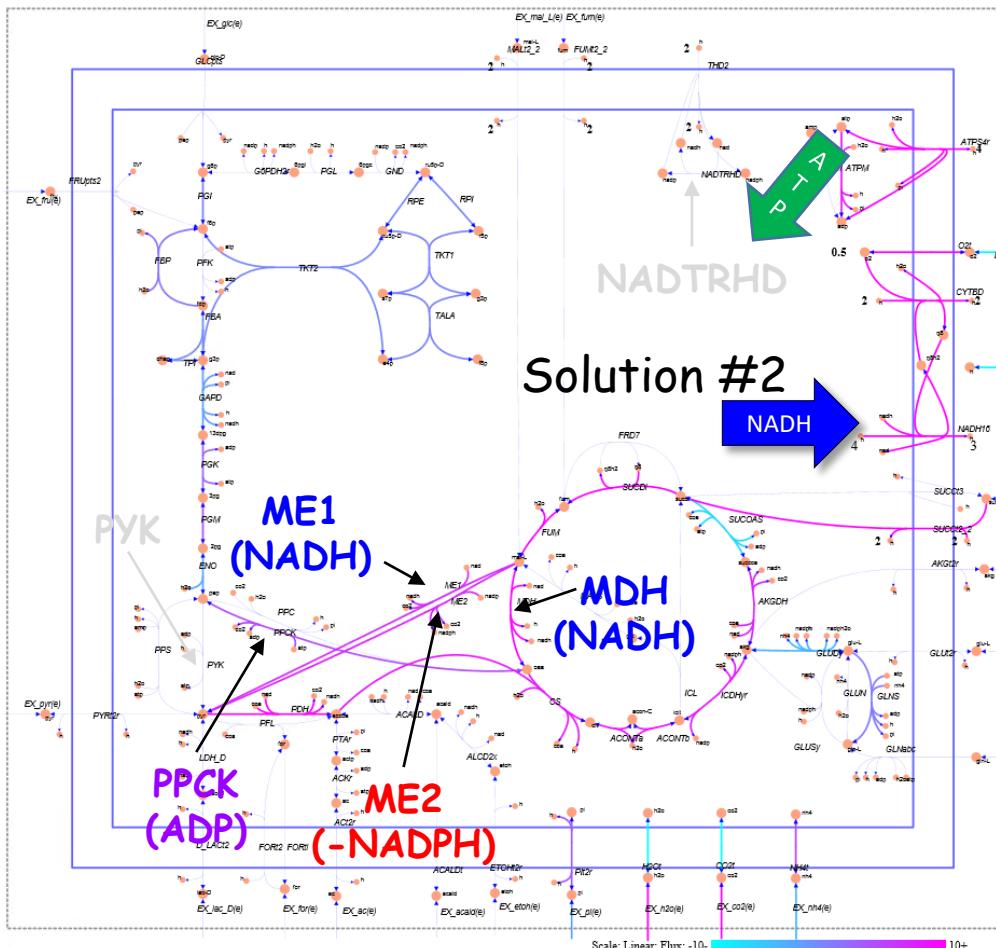
| | | | | | |
|---------------|----------------|----------------|----------------|---------------------|-----------|
| ACONTa | 8.13764 | FBA | -0.835681 | PGM | 3.49017 |
| ACONTb | 8.13764 | FBP | 0.835681 | PIt2r | 3.0906 |
| AKGDH | 7.23122 | FUM | 27.2312 | PPCK | 3.92628 |
| ATPM | 8.39 | GAPD | -2.23333 | RPE | -0.603888 |
| ATPS4r | 57.7816 | GLNS | 0.214822 | RPI | -0.603888 |
| Biomass | 0.840134 | GLUDy | -4.36626 | SUCCt2_2 | 20 |
| CO2t | -44.2477 | H2Ot | -30.3675 | SUCDi | 27.2312 |
| CS | 8.13764 | ICDHyr | 8.13764 | SUCOAS | -7.23122 |
| CYTBD | 66.5528 | MDH | 13.565 | TALA | -0.1503 |
| ENO | -3.49017 | ME2 | 13.6662 | TKT1 | -0.1503 |
| EX_co2(e) | 44.2477 | NADH16 | 39.3216 | TKT2 | -0.453588 |
| EX_h2o(e) | 30.3675 | NADTRHD | 6.49242 | TPI | -0.835681 |
| EX_h(e) | -23.1469 | NH4t | 4.58108 | Other AOS Reactions | |
| EX_nh4(e) | -4.58108 | O2t | 33.2764 | ME1 | 0 |
| EX_o2(e) | -33.2764 | PDH | 11.2863 | PYK | 0 |
| EX_pi(e) | -3.0906 | PGI | -0.172228 | | |
| EX_succ(e) | -20 | PGK | 2.23333 | | |

ATP**ADP****NADH****NADHP**



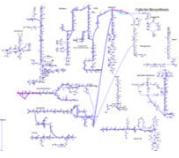
Alternate Optimal Solutions #2

findingOptimalSolutionsSuccVisualize.m



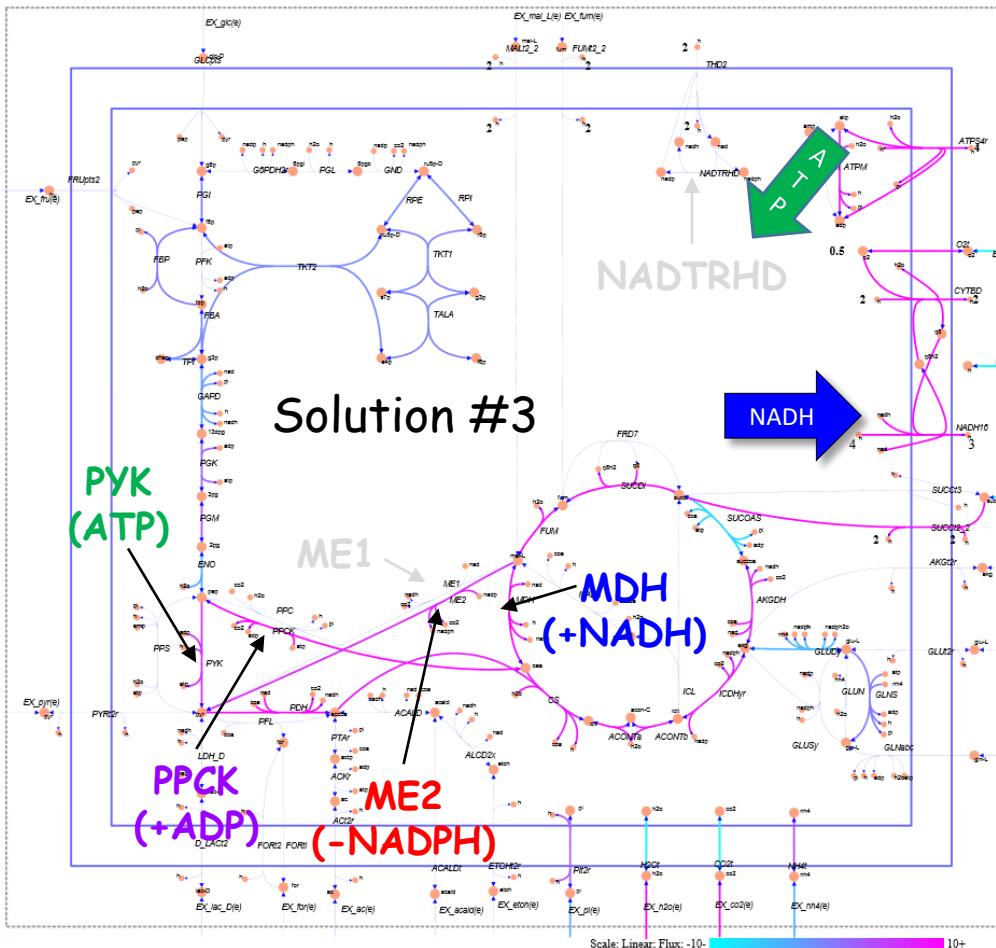
| | | | | | |
|------------|----------|--------|-----------|---------------------|-----------|
| ACONTa | 8.13764 | FBA | -0.835681 | PGM | 3.49017 |
| ACONTb | 8.13764 | FBP | 0.835681 | PIt2r | 3.0906 |
| AKGDH | 7.23122 | FUM | 27.2312 | PPCK | 3.92628 |
| ATPM | 8.39 | GAPD | -2.23333 | RPE | -0.603888 |
| ATPS4r | 57.7816 | GLNS | 0.214822 | RPI | -0.603888 |
| Biomass | 0.840134 | GLUDy | -4.36626 | SUCCt2_2 | 20 |
| CO2t | -44.2477 | H2Ot | -30.3675 | SUCDI | 27.2312 |
| CS | 8.13764 | ICDHyr | 8.13764 | SUCOAS | -7.23122 |
| CYTBD | 66.5528 | MDH | 13.565 | TALA | -0.1503 |
| ENO | -3.49017 | ME1 | 6.49242 | TKT1 | -0.1503 |
| EX_co2(e) | 44.2477 | ME2 | 7.1738 | TKT2 | -0.453588 |
| EX_h2o(e) | 30.3675 | NADH16 | 39.3216 | TPI | -0.835681 |
| EX_h(e) | -23.1469 | NH4t | 4.58108 | Other AOS Reactions | |
| EX_nh4(e) | -4.58108 | O2t | 33.2764 | NADTRHD 0 | |
| EX_o2(e) | -33.2764 | PDH | 11.2863 | PYK 0 | |
| EX_pi(e) | -3.0906 | PGI | -0.172228 | | |
| EX_succ(e) | -20 | PGK | 2.23333 | | |

ATPADPNADHNADHP



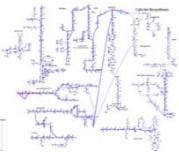
Alternate Optimal Solutions #3

findingOptimalSolutionsSuccVisualize.m



| | | | | | |
|---------------|----------------|---------------|----------------|---------------------|-----------|
| ACONTa | 8.13764 | FBA | -0.835681 | PGM | 3.49017 |
| ACONTb | 8.13764 | FBP | 0.835681 | PIt2r | 3.0906 |
| AKGDH | 7.23122 | FUM | 27.2312 | PPCK | 10.4187 |
| ATPM | 8.39 | GAPD | -2.23333 | PYK | 6.49242 |
| ATPS4r | 57.7816 | GLNS | 0.214822 | RPE | -0.603888 |
| Biomass | 0.840134 | GLUDy | -4.36626 | RPI | -0.603888 |
| CO2t | -44.2477 | H2Ot | -30.3675 | SUCCt2_2 | 20 |
| CS | 8.13764 | ICDHyr | 8.13764 | SUCDi | 27.2312 |
| CYTBD | 66.5528 | MDH | 20.0574 | SUCOAS | -7.23122 |
| ENO | -3.49017 | ME2 | 7.1738 | TALA | -0.1503 |
| EX_co2(e) | 44.2477 | NADH16 | 39.3216 | TKT1 | -0.1503 |
| EX_h2o(e) | 30.3675 | NH4t | 4.58108 | TKT2 | -0.453588 |
| EX_h(e) | -23.1469 | O2t | 33.2764 | TPI | -0.835681 |
| EX_nh4(e) | -4.58108 | PDH | 11.2863 | Other AOS Reactions | |
| EX_o2(e) | -33.2764 | PGI | -0.172228 | NADTRHD | |
| EX_pi(e) | -3.0906 | PGK | 2.23333 | 0 | |
| EX_succ(e) | -20 | | | ME1 | 0 |

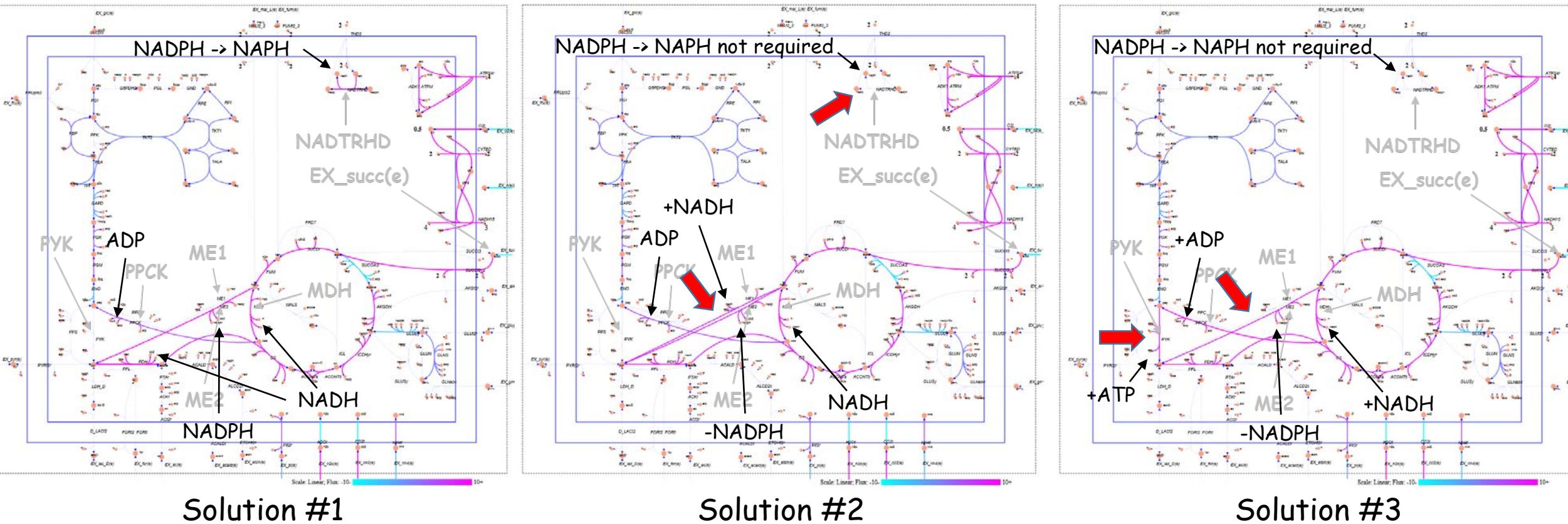
ATP**ADP****NADH****NADHP**



Alternate Optimal Solutions

findingOptimalSolutionsSuccVisualize.m

All three solution produce the same amount of ATP by providing the same amount of NADH for the electron transport chain





Reducing Alternate Optimal Solutions Code

```
% findingOptimalSolutionsSuccVisualizeOne.m
clear; clc;

load('ecoli_textbook.mat');
model = changeRxnBounds(model,'EX_glc(e)',0,'l');
model = changeRxnBounds(model,'EX_o2(e)',-40,'l');
model = changeRxnBounds(model,'EX_succ(e)',-20,'l');
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');

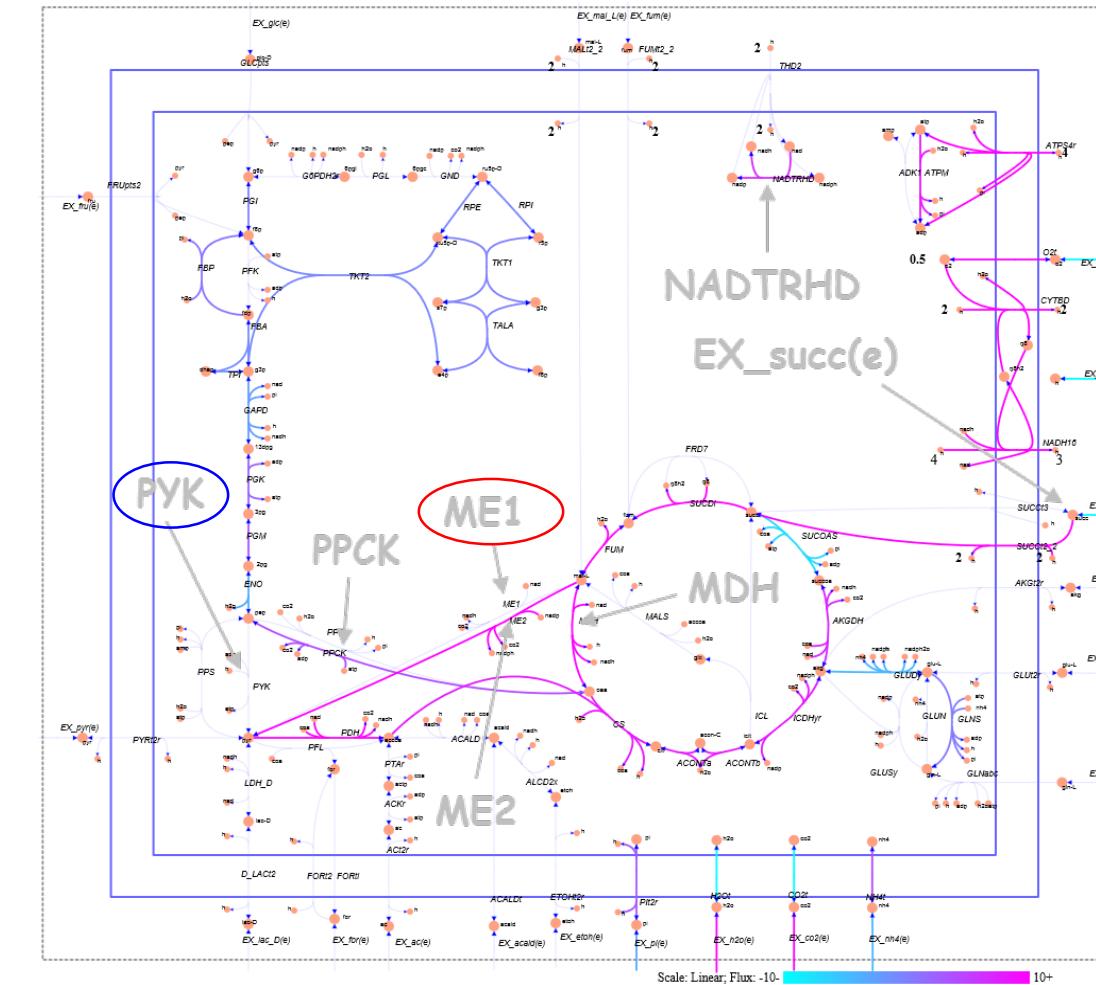
model = changeRxnBounds(model,'ME1',0,'b');
% model = changeRxnBounds(model,'NADTRHD',0,'b');
model = changeRxnBounds(model,'PYK',0,'b');

% List optimal solutions
solverOK = changeCobraSolver('glpk','all');

[solutions] = enumerateOptimalSolutions(model);

v = solutions.fluxes(:,1); % Select which vector wanted to be mapped
(1-3)
printFluxVector(model, v, true)

map=readCbMap('ecoli_Textbook_ExportMap');
options.lb = -10;
options.ub = 10;
options.zeroFluxWidth = 0.1;
options.rxnDirMultiplier = 10;
drawFlux(map, model, v, options);
```



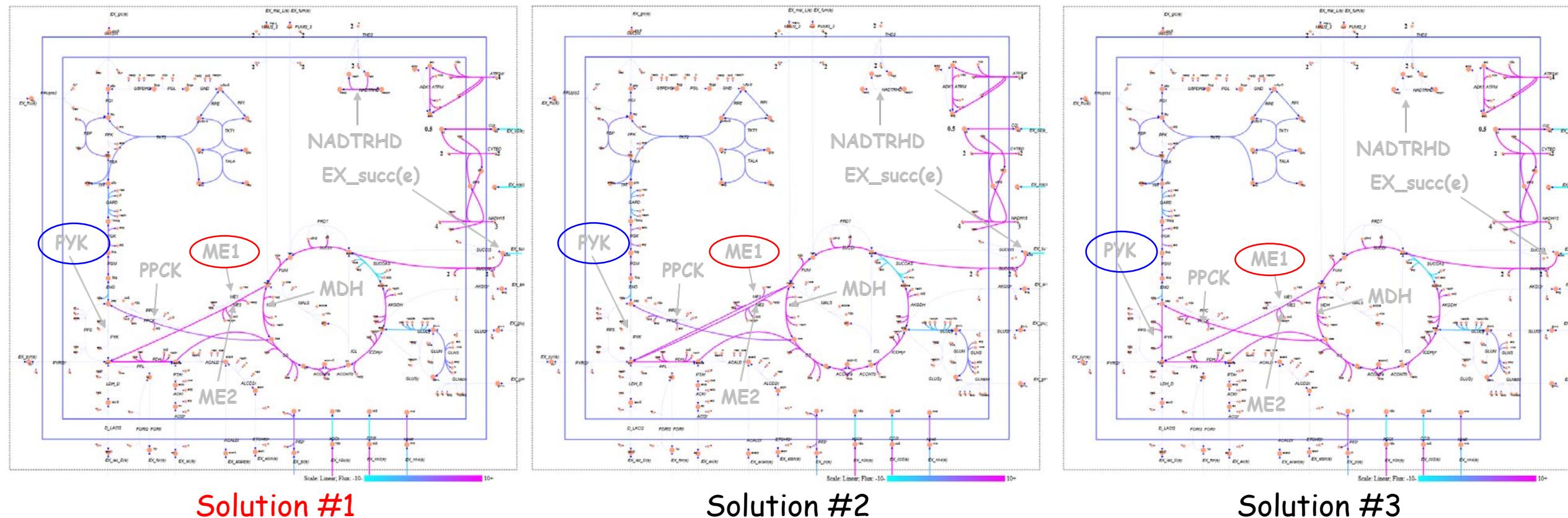
Solution #1

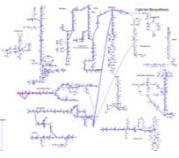


Reducing Alternate Optimal Solutions

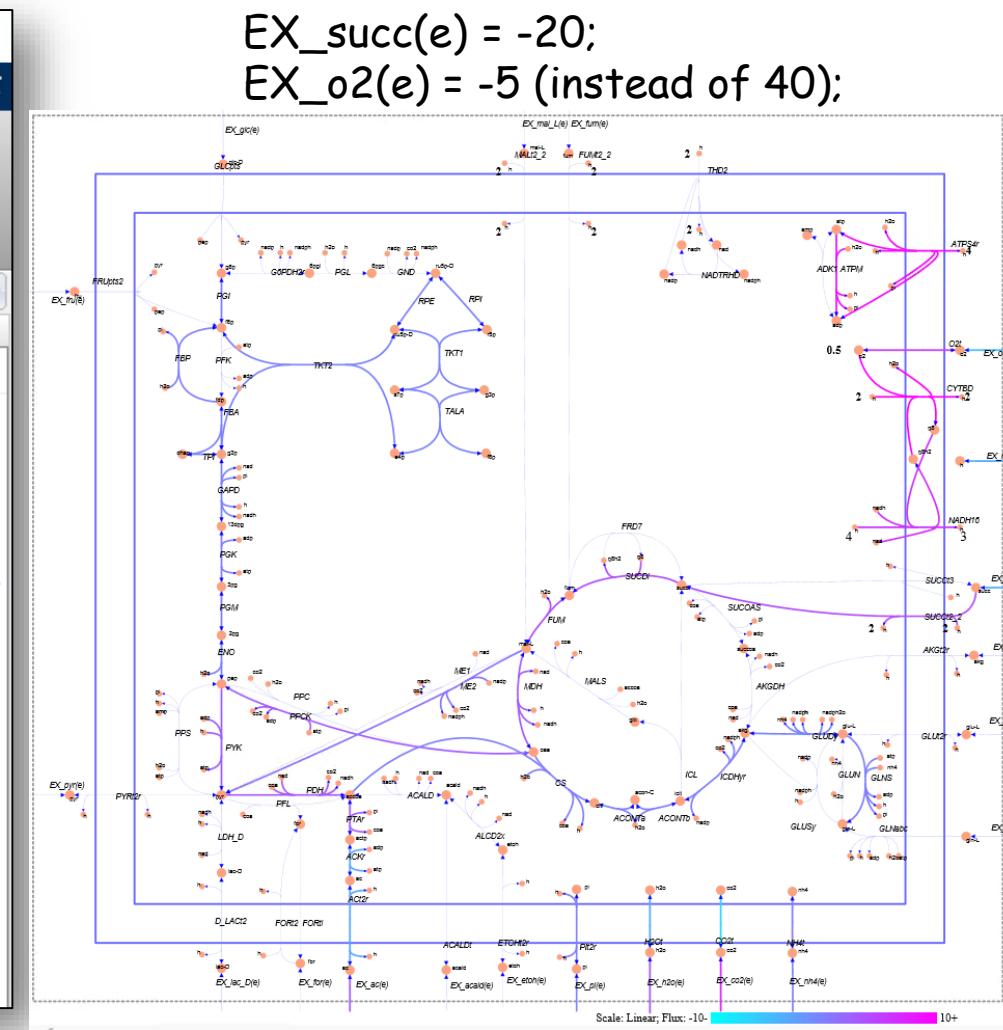
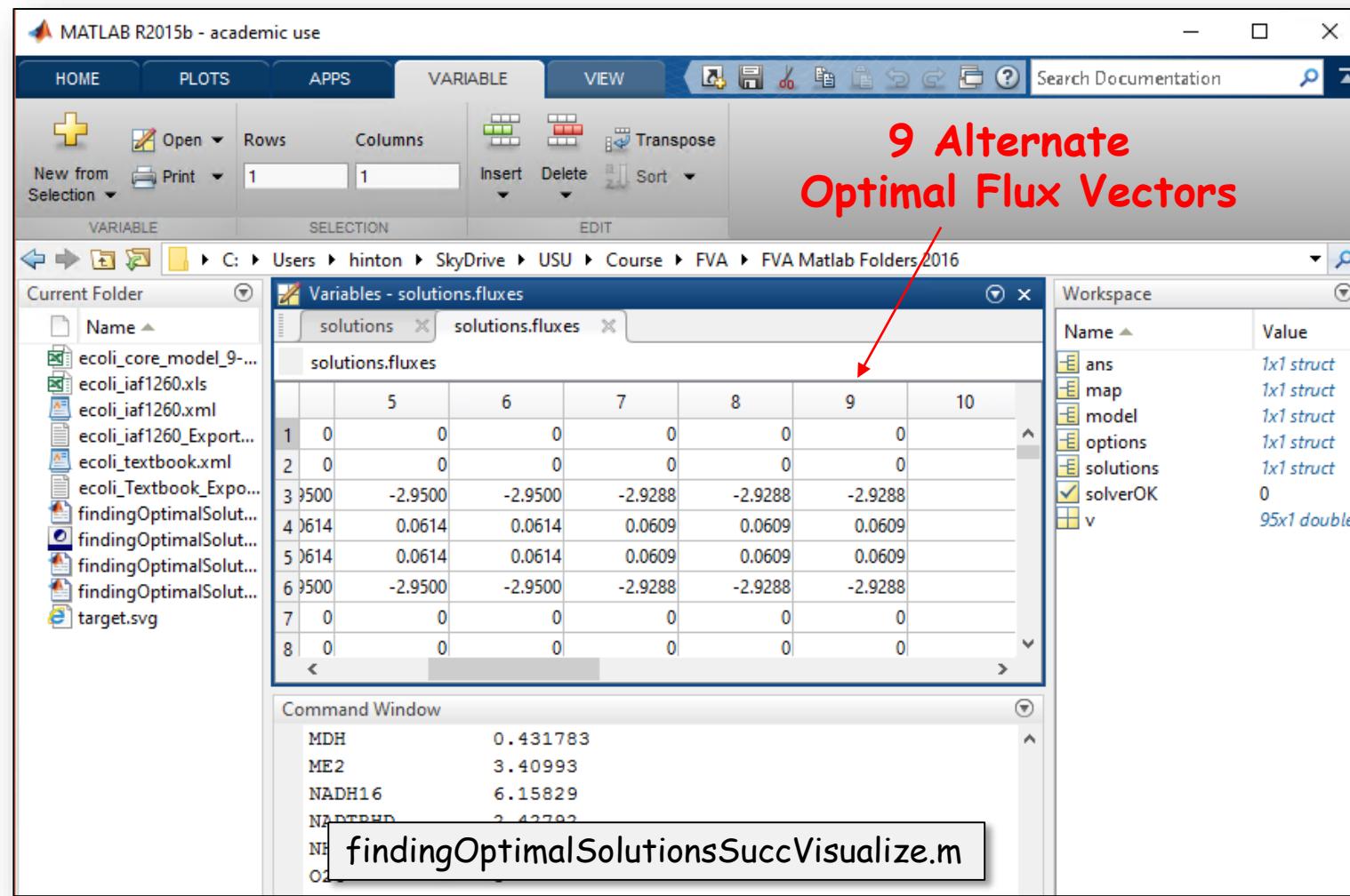
findingOptimalSolutionsSuccVisualizeOne.m

If both ME1 and PYK are set to zero (knocked out), then there will only be one optimal solution (in this simple model)





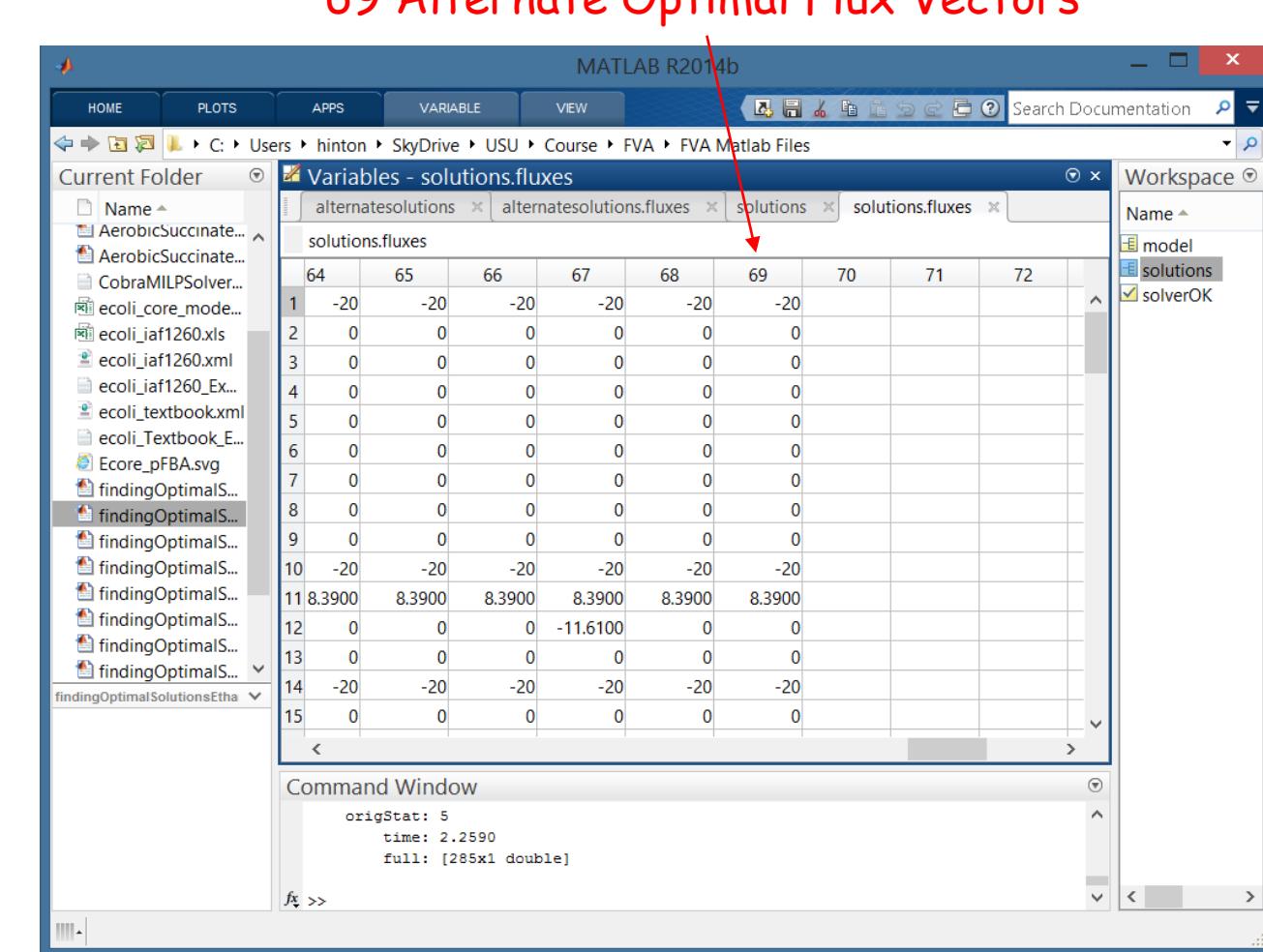
Changing Aerobic Conditions: Alternate Optimal Solutions

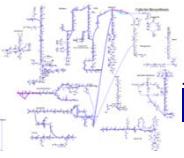




Alternate Optimal Solutions for Ethanol Production

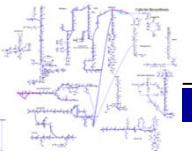
```
% findingOptimalSolutionsEthanol.m  
  
clear;  
  
load('ecoli_textbook.mat');  
  
model = changeRxnBounds(model,'EX_glc(e)',-10,'l');  
model = changeRxnBounds(model,'EX_o2(e)',0,'l');  
  
model = changeObjective(model,'EX_etooh(e)');  
  
% List optimal solutions  
  
solverOK = changeCobraSolver('glpk','all');  
[solutions] = enumerateOptimalSolutions(model);
```





Review Questions

- What are alternate optimal solutions?
- What is the relationship between alternate optimal solutions and a cell's phenotype?
- What are silent phenotypes?
- How can you find the alternate optimal solutions using the Cobra Toolbox?
- How many alternate optimal solutions can there be for a given phenotype?
- How many alternate optimal solutions can there be for a carbon source?
- Do aerobic/anaerobic conditions impact the number alternate optimal solutions?
- Does the choice of objective function impact the number alternate optimal solutions?



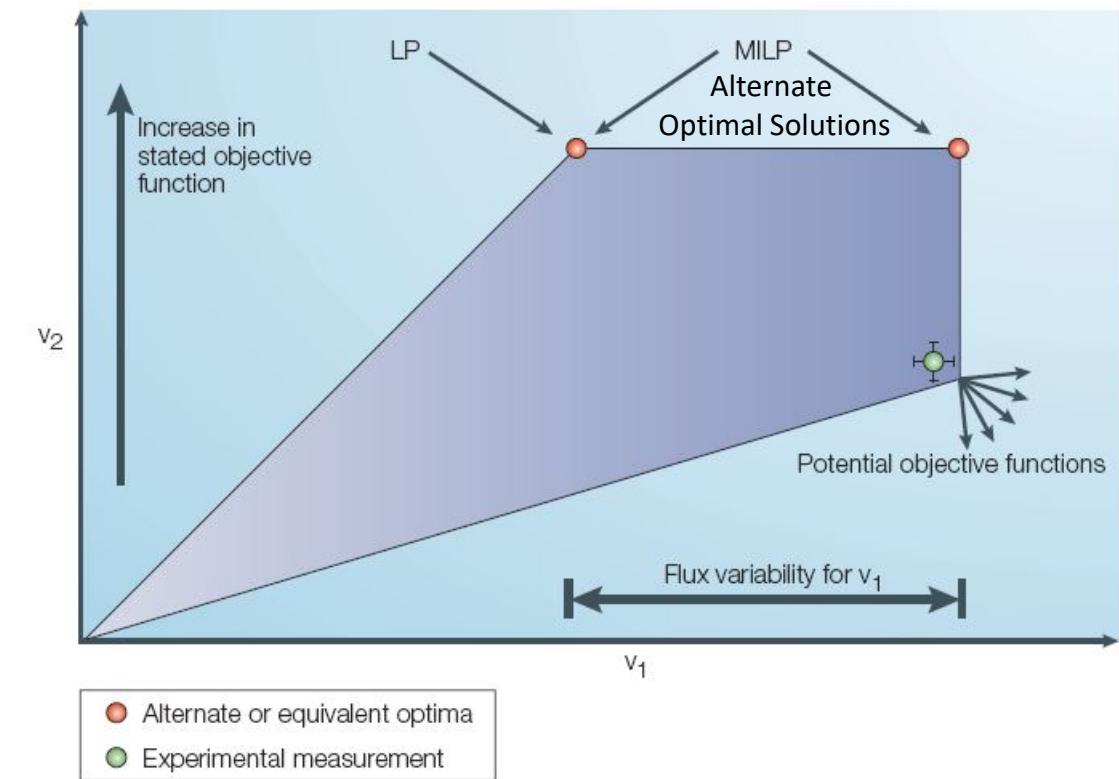
Lesson Outline

- Alternate Optimal Solutions
- • Flux Variability Analysis
- Parsimonious FBA

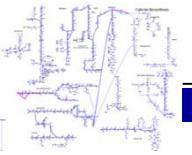


Flux Variability Analysis

- 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.



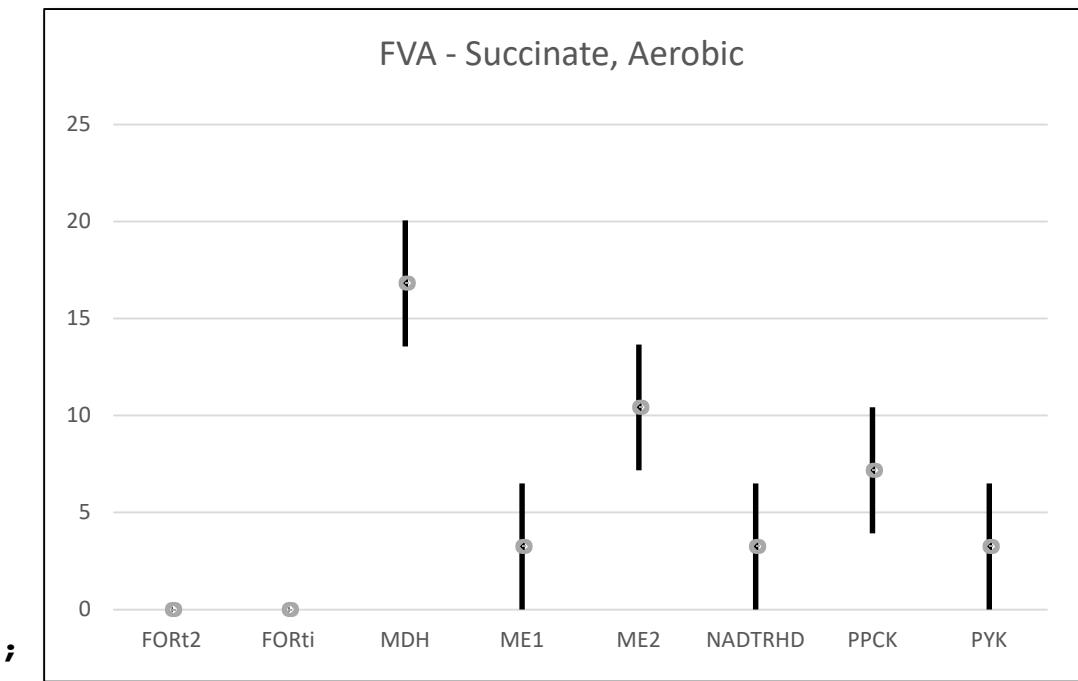
Price, N. D., J. L. Reed, et al. (2004). "Genome-scale models of microbial cells: evaluating the consequences of constraints." *Nature reviews. Microbiology* 2(11): 886-897.



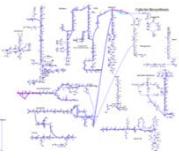
Flux Variability Analysis Example

```
% FluxVariabilitySuccinate.m  
  
% Load model  
  
load('ecoli_textbook.mat');  
  
% Change carbon source from glucose to succinate  
  
model = changeRxnBounds(model,'EX_glc(e)',0,'1');  
model = changeRxnBounds(model,'EX_succ(e)',-20,'1');  
  
% Set optimization objective to Biomass_Ecoli_core_N(w/GAM)_Nmet2  
  
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');  
  
% Perform flux variability analysis  
  
[minFlux,maxFlux]=fluxVariability(model,100,'max',model.rxn, false, false);  
  
% Print flux values  
  
printFluxVector(model, [minFlux, maxFlux], true)
```

Does not allow loops



FVA_Succinate_Aerobic.xlsx



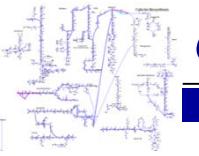
Flux Variability Analysis Example Output (No Loops)

FVA_example1.xlsx

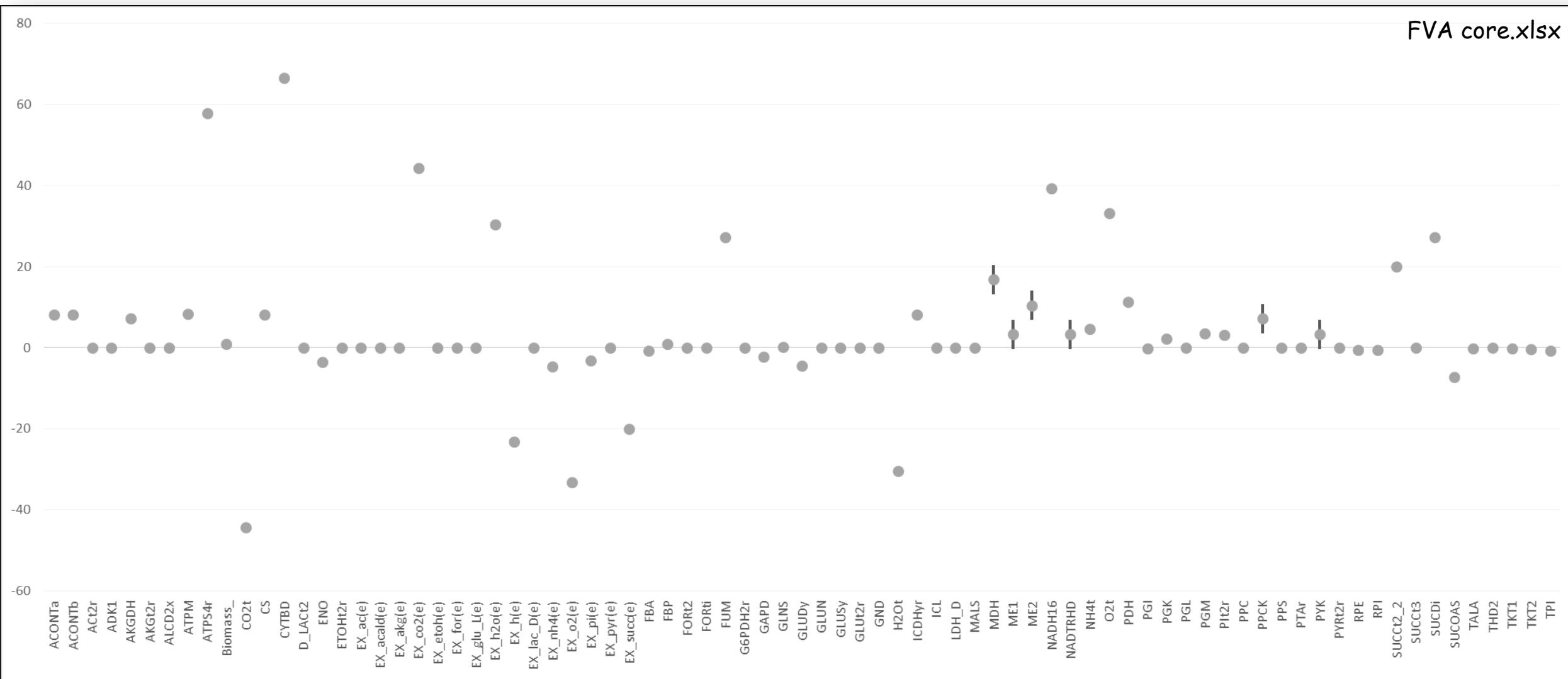
| Reaction | Lower | Upper | Difference |
|-------------|-----------|----------|------------|
| ACALD | -5.07E-06 | 0 | 0 |
| ACALDt | -5.07E-06 | 0 | 0 |
| ACKr | -7.76E-06 | 0 | 0 |
| ACONTa | 8.13763 | 8.13764 | 0 |
| ACONTb | 8.13763 | 8.13764 | 0 |
| ACt2r | -7.76E-06 | 0 | 0 |
| ADK1 | 0 | 3.30E-05 | 0 |
| AKGDH | 7.23119 | 7.23122 | 0 |
| AKGt2r | -3.07E-06 | 0 | 0 |
| ALCD2x | -4.40E-06 | 0 | 0 |
| ATPM | 8.39 | 8.39 | 0 |
| ATPS4r | 57.7816 | 57.7817 | 0 |
| Biomass | 0.840134 | 0.840134 | 0 |
| CO2t | -44.2477 | -44.2477 | 0 |
| CS | 8.13763 | 8.13764 | 0 |
| CYTBD | 66.5528 | 66.5528 | 0 |
| D_LACT2 | -4.25E-06 | 0 | 0 |
| ENO | -3.49018 | -3.49017 | 0 |
| ETOHt2r | -4.40E-06 | 0 | 0 |
| EX_ac(e) | 0 | 7.76E-06 | 0 |
| EX_acald(e) | 0 | 5.07E-06 | 0 |
| EX_akg(e) | 0 | 3.07E-06 | 0 |
| EX_co2(e) | 44.2477 | 44.2477 | 0 |
| EX_etoh(e) | 0 | 4.40E-06 | 0 |
| EX_for(e) | 0 | 2.20E-05 | 0 |
| EX_glu_L(e) | 0 | 2.75E-06 | 0 |
| EX_h2o(e) | 30.3675 | 30.3675 | 0 |
| EX_h(e) | -23.1469 | -23.1469 | 0 |

| Reaction | Lower | Upper | Difference |
|-------------|-----------|-----------|------------|
| EX_lac_D(e) | 0 | 4.25E-06 | 0 |
| EX_nh4(e) | -4.58109 | -4.58108 | 0 |
| EX_o2(e) | -33.2764 | -33.2764 | 0 |
| EX_pi(e) | -3.0906 | -3.0906 | 0 |
| EX_pyr(e) | 0 | 5.07E-06 | 0 |
| EX_succ(e) | -20 | -20 | 0 |
| FBA | -0.8357 | -0.83568 | 0 |
| FBP | 0.835681 | 0.835714 | 0 |
| FORt2 | 0 | 0.000132 | 0.00013187 |
| FORti | 0 | 0.000132 | 0.00013187 |
| FUM | 27.2312 | 27.2312 | 0 |
| G6PDH2r | 0 | 4.40E-05 | 0 |
| GAPD | -2.23334 | -2.23E+00 | 0 |
| GLNS | 0.214822 | 0.214855 | 0 |
| GLUDy | -4.36626 | -4.36623 | 0 |
| GLUN | 0 | 3.30E-05 | 0 |
| GLUSy | 0 | 3.30E-05 | 0 |
| GLUt2r | -2.75E-06 | 0.00E+00 | 0 |
| GND | 0.00E+00 | 4.40E-05 | 0 |
| H2Ot | -30.3675 | -3.04E+01 | 0 |
| ICDHyr | 8.13761 | 8.13764 | 0 |
| ICL | 0 | 3.30E-05 | 0 |
| LDH_D | -4.25E-06 | 0.00E+00 | 0 |
| MALS | 0.00E+00 | 3.30E-05 | 0 |
| MDH | 13.565 | 2.01E+01 | 6.49253 |
| ME1 | 0 | 6.4925 | 6.4925 |
| ME2 | 7.17372 | 13.6663 | 6.49254 |
| NADH16 | 39.3216 | 39.3216 | 0 |

| Reaction | Lower | Upper | Difference |
|----------|-----------|----------|------------|
| NADTRHD | 0 | 6.4925 | 6.4925 |
| NH4t | 4.58108 | 4.58109 | 0 |
| O2t | 33.2764 | 33.2764 | 0 |
| PDH | 11.2863 | 11.2863 | 0 |
| PFK | 0 | 3.30E-05 | 0 |
| PFL | 0 | 2.20E-05 | 0 |
| PGI | -0.17227 | -0.17223 | 0 |
| PGK | 2.23333 | 2.23334 | 0 |
| PGL | 0 | 4.40E-05 | 0 |
| PGM | 3.49017 | 3.49018 | 0 |
| PIt2r | 3.0906 | 3.0906 | 0 |
| PPC | 0 | 3.30E-05 | 0 |
| PPCK | 3.92625 | 10.4188 | 6.49254 |
| PPS | 0 | 3.30E-05 | 0 |
| PTAr | 0 | 7.76E-06 | 0 |
| PYK | 0 | 6.4925 | 6.4925 |
| PYRt2r | -5.07E-06 | 0 | 0 |
| RPE | -0.60389 | -0.60386 | 0 |
| RPI | -0.6039 | -0.60389 | 0 |
| SUCCt2_2 | 20 | 20 | 0 |
| SUCCt3 | 0 | 4.40E-05 | 0 |
| SUCDi | 27.2312 | 27.2312 | 0 |
| SUCOAS | -7.23122 | -7.23119 | 0 |
| TALA | -0.1503 | -0.15029 | 0 |
| THD2 | 0 | 6.59E-05 | 0 |
| TKT1 | -0.1503 | -0.15029 | 0 |
| TKT2 | -0.45359 | -0.45357 | 0 |
| TPI | -0.8357 | -0.83568 | 0 |



Flux Variability Analysis Example

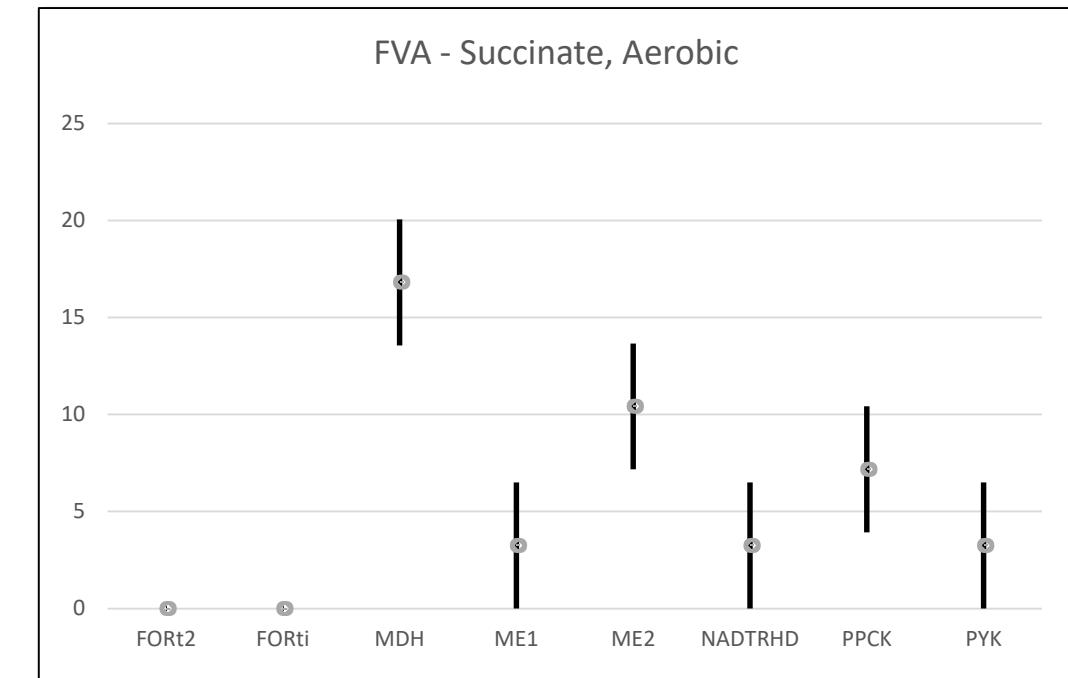




Flux Variability Analysis Example (Continued)

| Reaction | Minimum Flux (mmol gDW ⁻¹ hr ⁻¹) | Maximum Flux (mmol gDW ⁻¹ hr ⁻¹) |
|----------|--|--|
| 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 |

Variable Reactions For Growth On Succinate
(Same as Alternate Optimal Solutions Example)

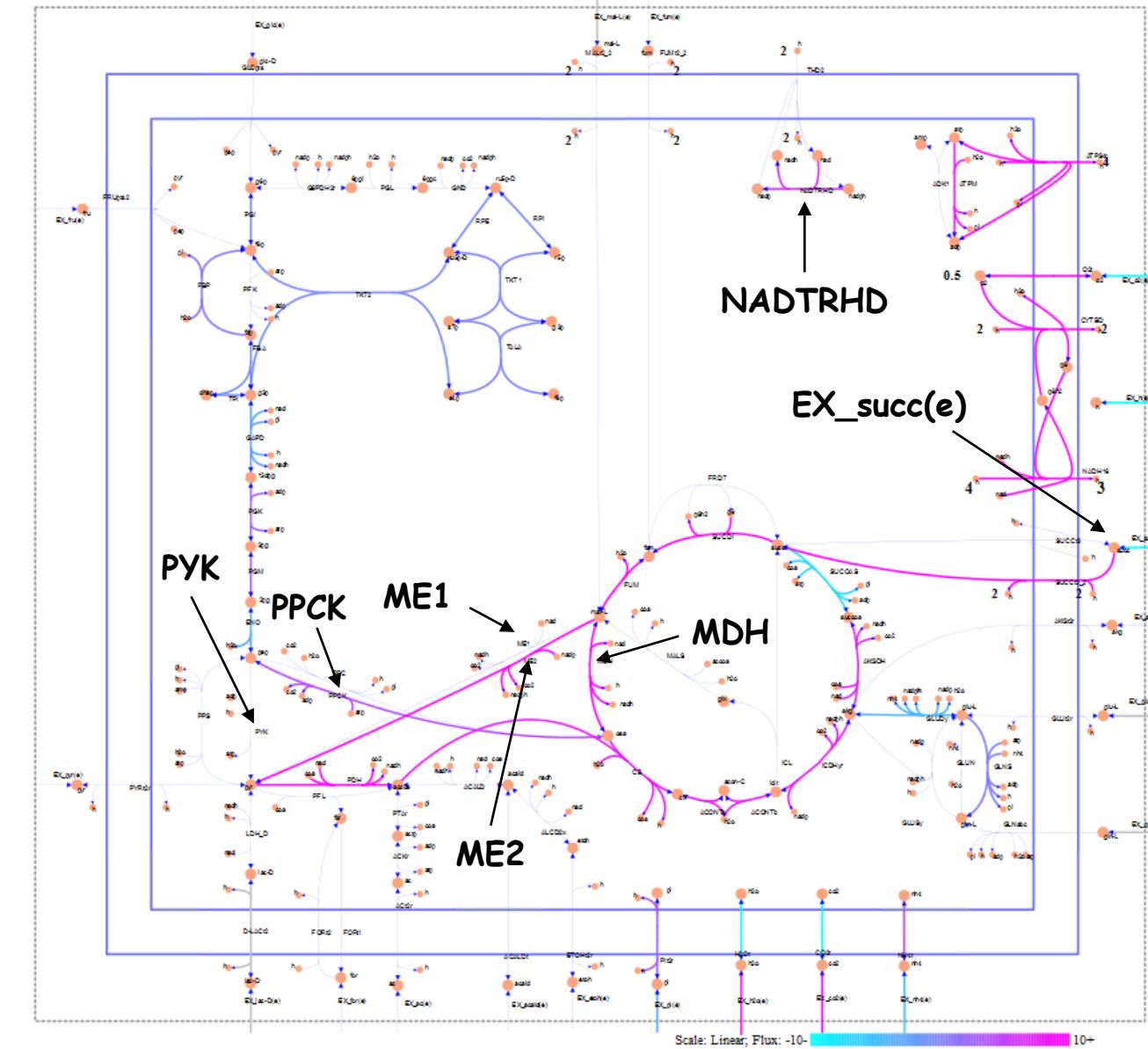


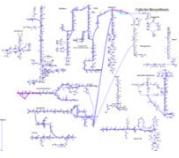


Fluxes Identified Through Flux Variability Analysis

(AerobicSuccinateBioMass.m)

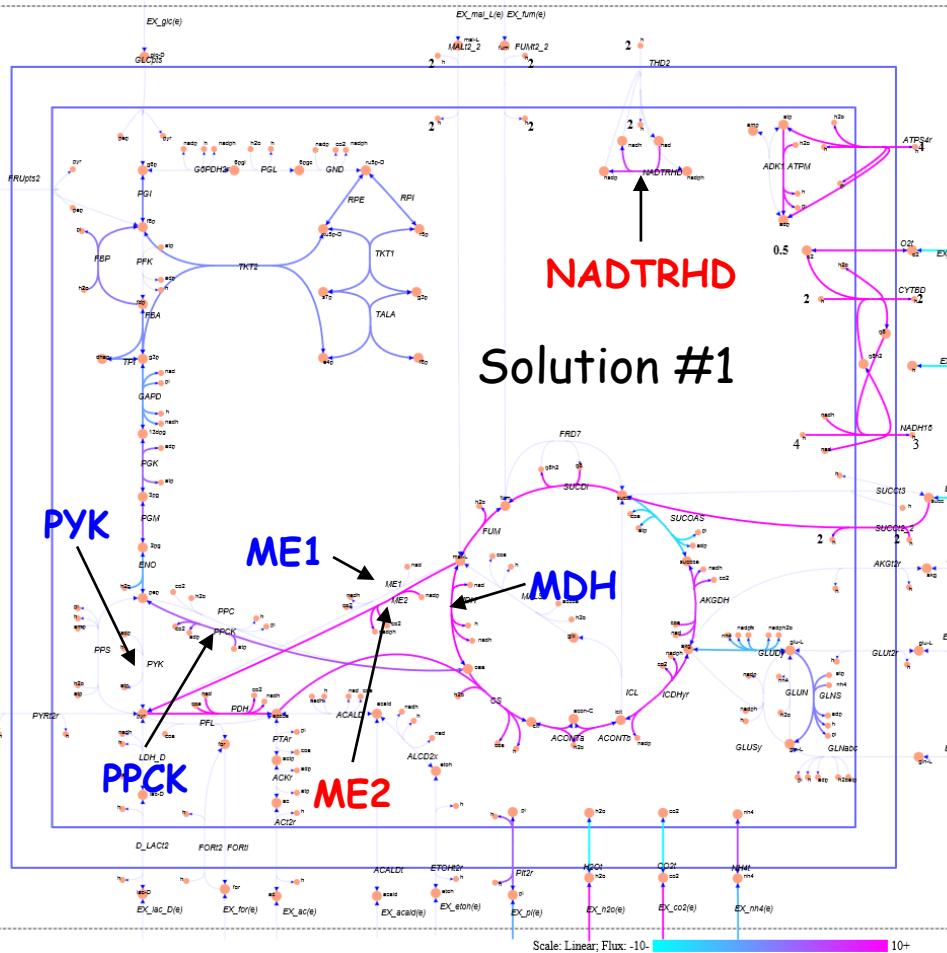
- MDH (malate dehydrogenase)
- ME1 (malic enzyme (NAD))
- ME2 (malic enzyme (NADP))
- NADTRHD (NAD transhydrogenase)
- PPCK (phosphoenolpyruvate carboxykinase)
- PYK (pyruvate kinase)





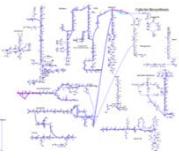
Flux Variability Analysis #1

findingOptimalSolutionsSuccVisualize.m



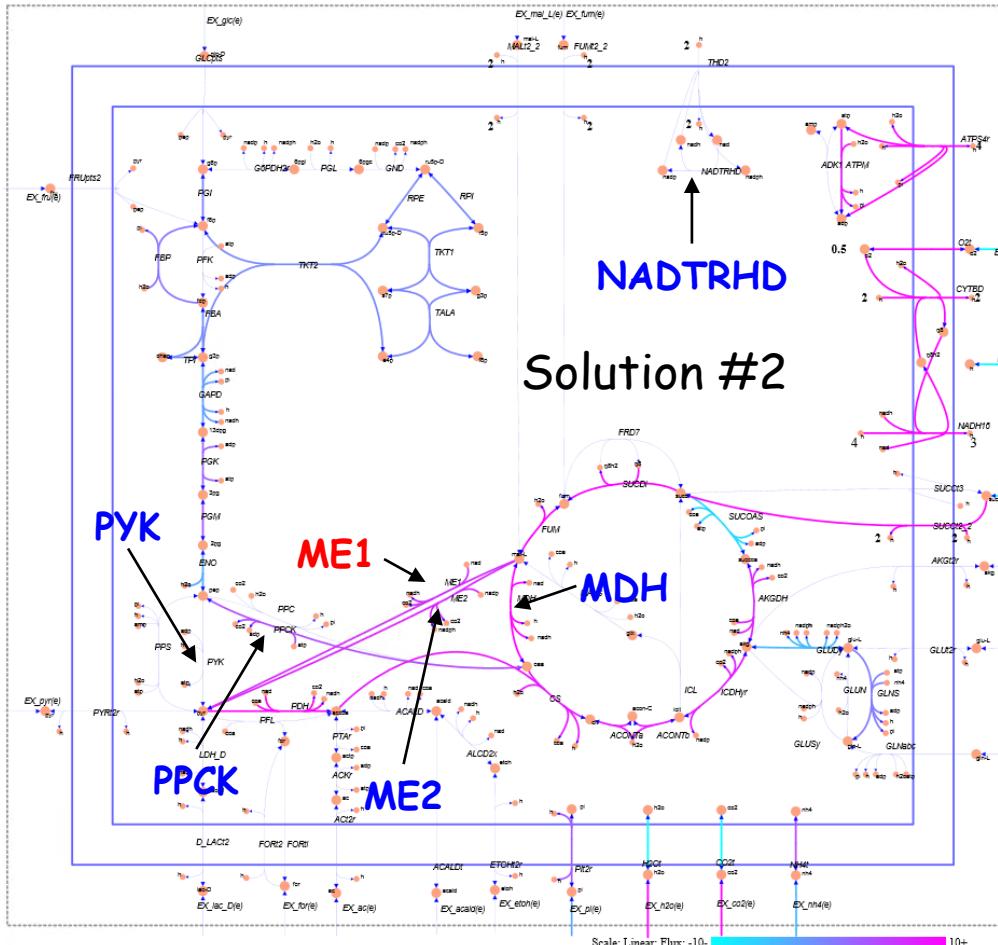
| | | | | | |
|------------|----------|---------|-----------|---------------------|-----------|
| ACONTa | 8.13764 | FBA | -0.835681 | PGM | 3.49017 |
| ACONTb | 8.13764 | FBP | 0.835681 | PIt2r | 3.0906 |
| AKGDH | 7.23122 | FUM | 27.2312 | PPCK | 3.92628 |
| ATPM | 8.39 | GAPD | -2.23333 | RPE | -0.603888 |
| ATPS4r | 57.7816 | GLNS | 0.214822 | RPI | -0.603888 |
| Biomass | 0.840134 | GLUDy | -4.36626 | SUCCt2_2 | 20 |
| CO2t | -44.2477 | H2O† | -30.3675 | SUCDi | 27.2312 |
| CS | 8.13764 | ICDHyr | 8.13764 | SUCOAS | -7.23122 |
| CYTBD | 66.5528 | MDH | 13.565 | TALA | -0.1503 |
| ENO | -3.49017 | ME2 | 13.6662 | TKT1 | -0.1503 |
| EX_co2(e) | 44.2477 | NADH16 | 39.3216 | TKT2 | -0.453588 |
| EX_h2o(e) | 30.3675 | NADTRHD | 6.49242 | TPI | -0.835681 |
| EX_h(e) | -23.1469 | NH4† | 4.58108 | | |
| EX_nh4(e) | -4.58108 | O2† | 33.2764 | Other FVA Reactions | |
| EX_o2(e) | -33.2764 | PDH | 11.2863 | ME1 | 0 |
| EX_pi(e) | -3.0906 | PGI | -0.172228 | PYK | 0 |
| EX_succ(e) | -20 | PGK | 2.23333 | | |

FVA Upper Bound; Lower Bound.



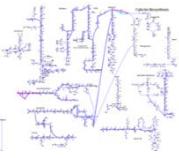
Flux Variability Analysis #2

findingOptimalSolutionsSuccVisualize.m



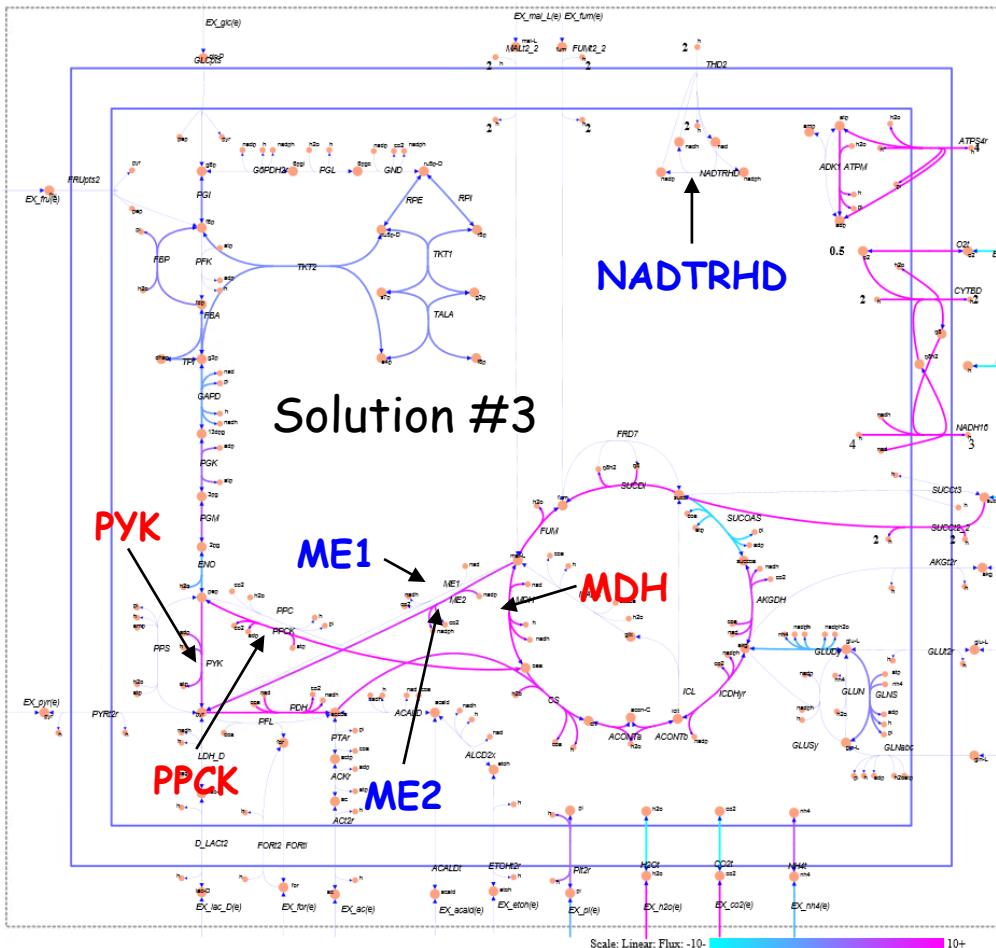
| | | | | | |
|------------|----------|--------|-----------|---------------------|-----------|
| ACONTa | 8.13764 | FBA | -0.835681 | PGM | 3.49017 |
| ACONTb | 8.13764 | FBP | 0.835681 | PIt2r | 3.0906 |
| AKGDH | 7.23122 | FUM | 27.2312 | PPCK | 3.92628 |
| ATPM | 8.39 | GAPD | -2.23333 | RPE | -0.603888 |
| ATPS4r | 57.7816 | GLNS | 0.214822 | RPI | -0.603888 |
| Biomass | 0.840134 | GLUDy | -4.36626 | SUCCt2_2 | 20 |
| CO2t | -44.2477 | H2Ot | -30.3675 | SUCDi | 27.2312 |
| CS | 8.13764 | ICDHyr | 8.13764 | SUCOAS | -7.23122 |
| CYTBD | 66.5528 | MDH | 13.565 | TALA | -0.1503 |
| ENO | -3.49017 | ME1 | 6.49242 | TKT1 | -0.1503 |
| EX_co2(e) | 44.2477 | ME2 | 7.1738 | TKT2 | -0.453588 |
| EX_h2o(e) | 30.3675 | NADH16 | 39.3216 | TPI | -0.835681 |
| EX_h(e) | -23.1469 | NH4t | 4.58108 | | |
| EX_nh4(e) | -4.58108 | O2t | 33.2764 | Other FVA Reactions | |
| EX_o2(e) | -33.2764 | PDH | 11.2863 | NADTRHD 0 | |
| EX_pi(e) | -3.0906 | PGI | -0.172228 | PYK | 0 |
| EX_succ(e) | -20 | PGK | 2.23333 | | |

FVA Upper Bound; Lower Bound.



Flux Variability Analysis #3

findingOptimalSolutionsSuccVisualize.m



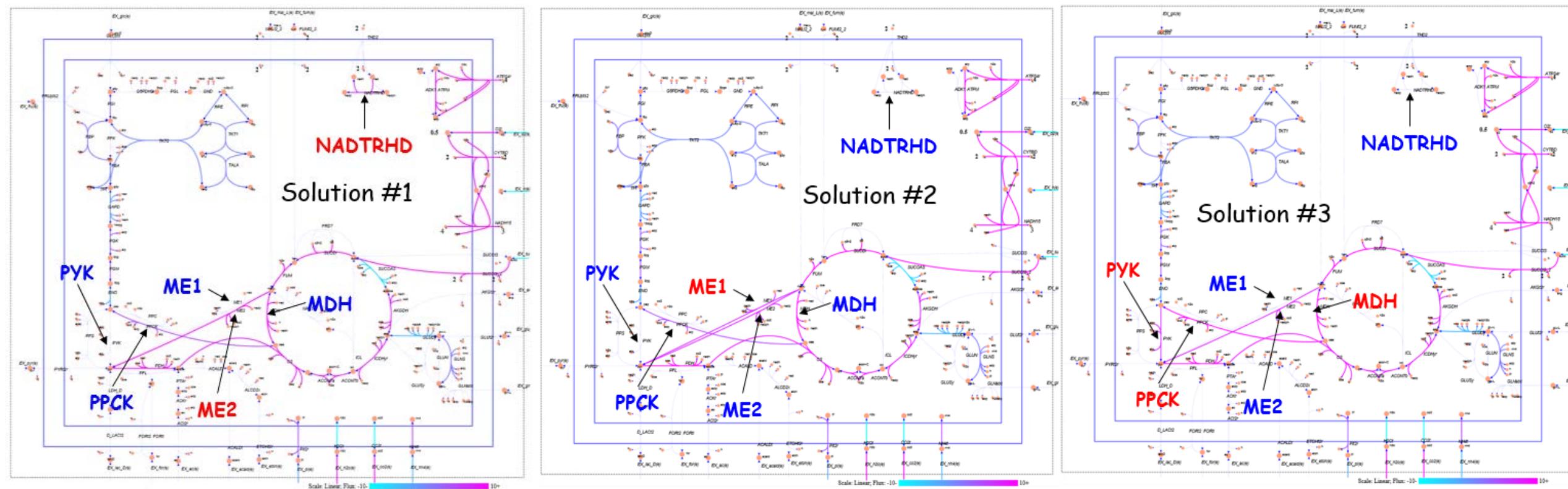
| | | | | | |
|------------|----------|--------|-----------|---------------------|-----------|
| ACONTa | 8.13764 | FBA | -0.835681 | PGM | 3.49017 |
| ACONTb | 8.13764 | FBP | 0.835681 | PIt2r | 3.0906 |
| AKGDH | 7.23122 | FUM | 27.2312 | PPCK | 10.4187 |
| ATPM | 8.39 | GAPD | -2.23333 | PYK | 6.49242 |
| ATPS4r | 57.7816 | GLNS | 0.214822 | RPE | -0.603888 |
| Biomass | 0.840134 | GLUDy | -4.36626 | RPI | -0.603888 |
| CO2t | -44.2477 | H2Ot | -30.3675 | SUCCt2_2 | 20 |
| CS | 8.13764 | ICDHyr | 8.13764 | SUCDi | 27.2312 |
| CYTBD | 66.5528 | MDH | 20.0574 | SUCOAS | -7.23122 |
| ENO | -3.49017 | ME2 | 7.1738 | TALA | -0.1503 |
| EX_co2(e) | 44.2477 | NADH16 | 39.3216 | TKT1 | -0.1503 |
| EX_h2o(e) | 30.3675 | NH4t | 4.58108 | TKT2 | -0.453588 |
| EX_h(e) | -23.1469 | O2t | 33.2764 | TPI | -0.835681 |
| EX_nh4(e) | -4.58108 | PDH | 11.2863 | | |
| EX_o2(e) | -33.2764 | PGI | -0.172228 | Other FVA Reactions | |
| EX_pi(e) | -3.0906 | PGK | 2.23333 | NADTRHD 0 | |
| EX_succ(e) | -20 | | | ME1 0 | |

FVA Upper Bound; Lower Bound.



Three Alternate Optimal Solutions

All three solution produce the same amount of NADH for the electron transport chain



FVA Upper Bound; Lower Bound.

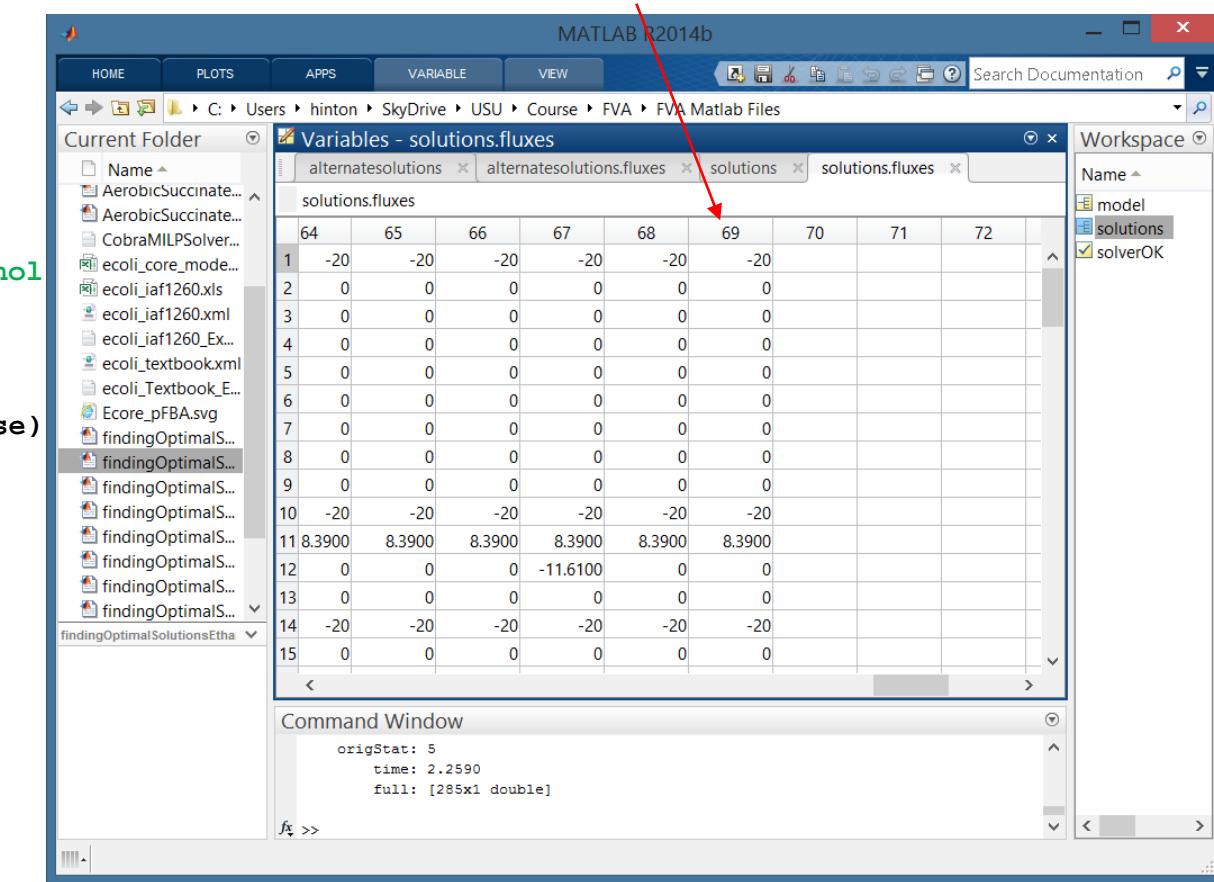


Flux Variability Analysis for Maximum Ethanol Production

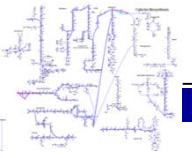
```
% FluxVariabilityEthanol.m
clear; clc;
% Load the E.coli core model
load('ecoli_textbook.mat');
model = changeRxnBounds(model,'EX_glc(e)',0,'1');
model = changeRxnBounds(model,'EX_succ(e)',-20,'1');
model = changeObjective(model,'EX_etoh(e)'); % Optimize for maximum ethanol

% Perform flux variability analysis
[minFluxL,maxFluxL]=fluxVariability(model,100,'max',model.rxns,false,false)

% Print flux values
Difference = abs(maxFlux - minFlux);
FluxDifference = Difference;
n = length(Difference);
for i=1:n % Set small values of flux to zero
    if Difference(i) < 0.0001
        FluxDifference(i) = 0;
    end
end
printFluxVector(model, [minFlux, maxFlux, FluxDifference])
```

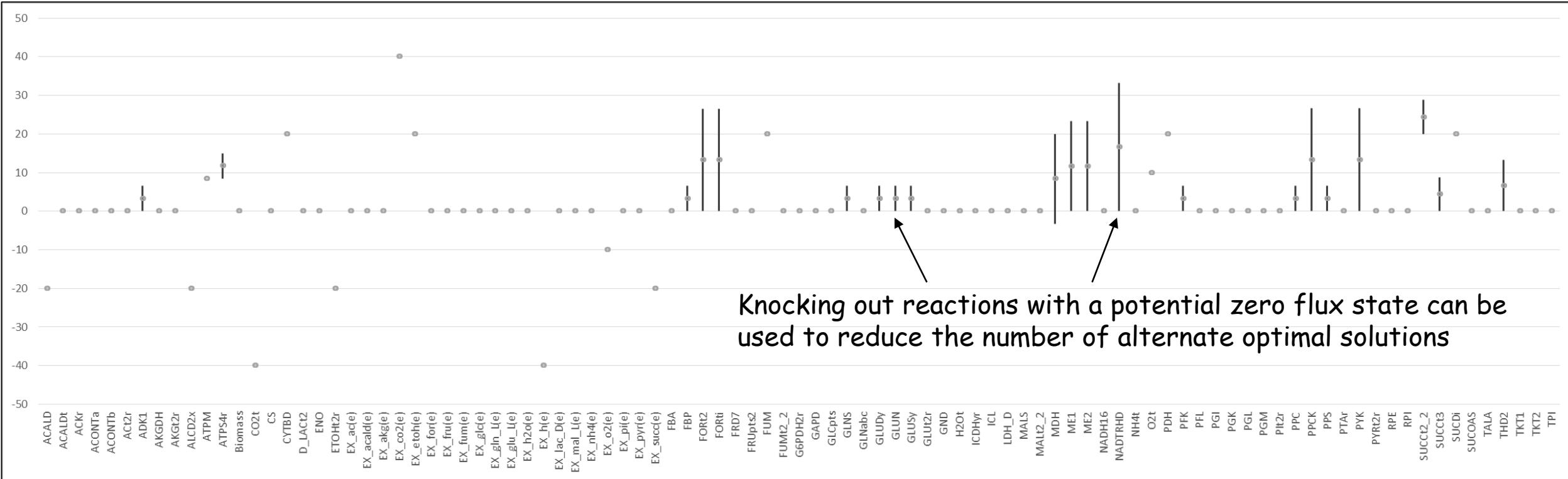


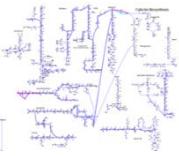
findingOptimalSolutionsEthanol.m



FVA Chart for Ethanol Production

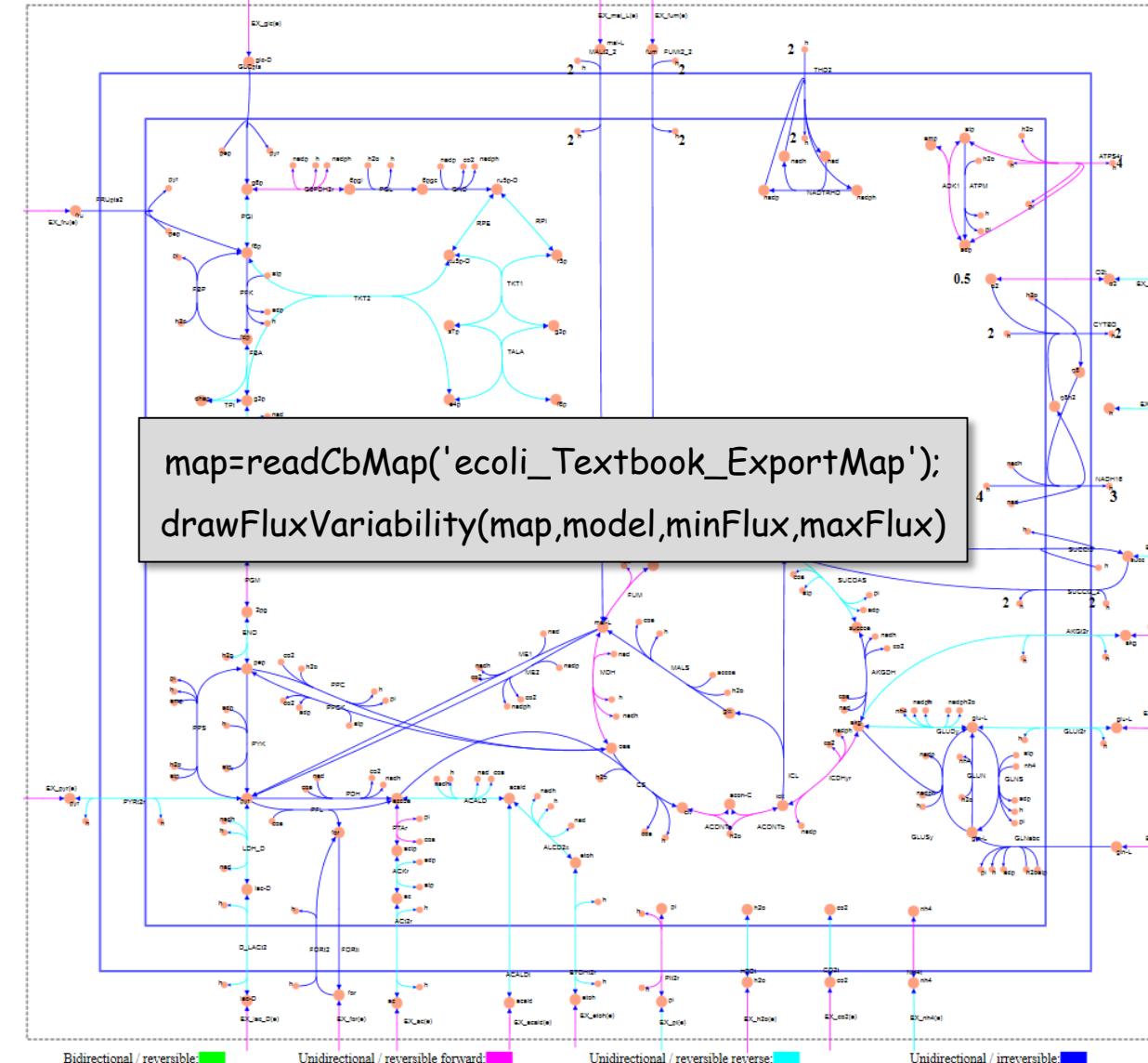
FVA Ethanol Production.xlsx





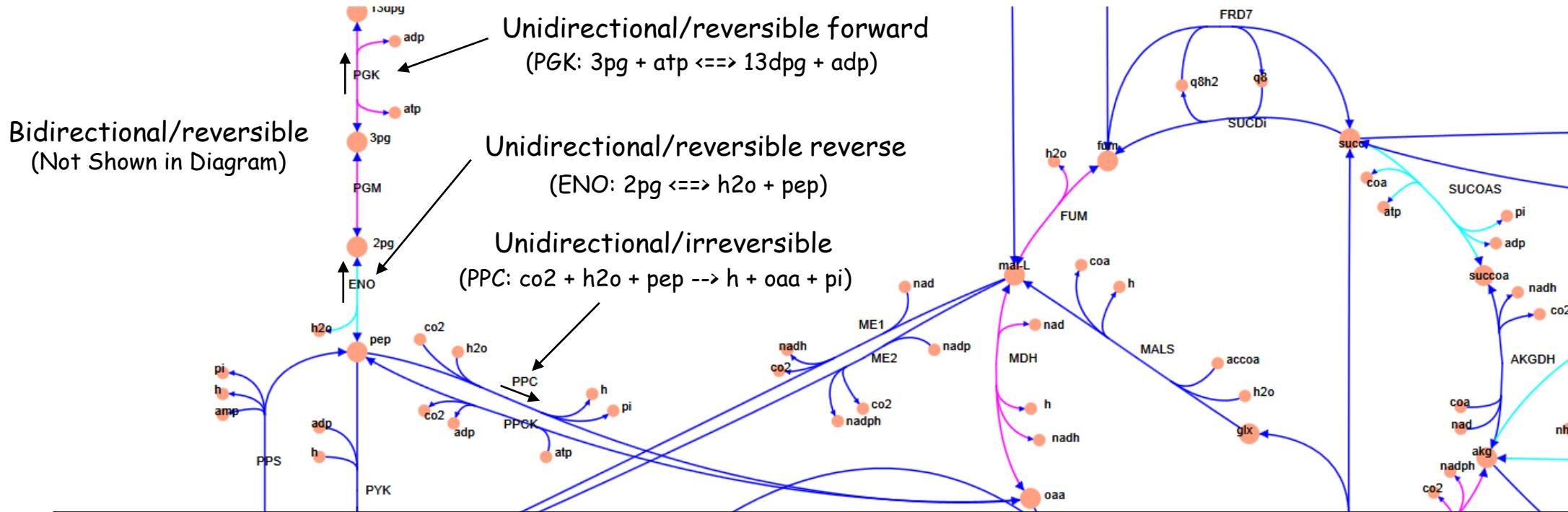
Flux Variability Map

FluxVariabilitySuccinate.m

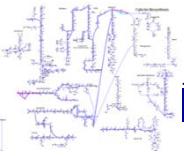




Flux Variability Map - Close-up



- Bidirectional/reversible = calculated flux change is bidirectional and changes directions/Stoichiometry is reversible
- Unidirectional/reversible forward = calculated flux change is unidirectional in direction of listed Stoichiometry/Stoichiometry is reversible
- Unidirectional/reversible reverse = calculated flux change is unidirectional in opposite direction of listed Stoichiometry/Stoichiometry is reversible
- Unidirectional/irreversible = calculated flux change is unidirectional/Stoichiometry is irreversible



FVA Classifications

FVA can be used to classify the reactions in a metabolic network. Assuming a biomass production rate greater than 90% of the optimal growth rate;

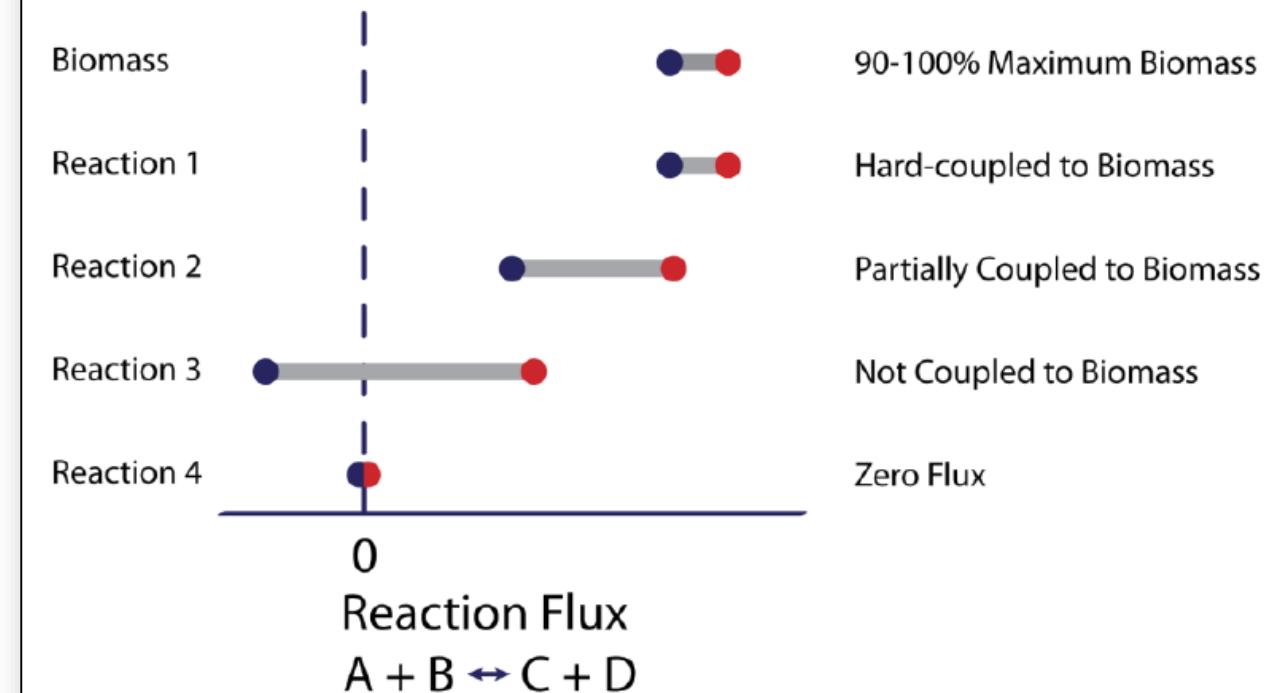
- A reaction was classified as "**Hard-coupled to biomass**" if the flux varied exactly with biomass production.
- "**Partially coupled to biomass**" included reactions that were required to have a non-zero flux, but were more flexible in the range.
- Reactions were classified as "**Not coupled to biomass**" if they could have a zero or non-zero flux while maintaining 90% biomass.
- Reactions were considered "**zero flux**" if they could maintain a flux in other conditions, but could not in growth conditions.

Flux Variability Analysis (FVA)

Step 1: Set Biomass production to at least 90% of optimum

Step 2: Find minimum (●) and maximum (●) flux for each reaction

Step 3: Classify each reaction according to allowable flux span



Lewis, N. E., K. K. Hixson, et al. (2010). "Omic data from evolved *E. coli* are consistent with computed optimal growth from genome-scale models-Supplementary Material." Molecular Systems Biology 6: 390.



FVA Classifications

```
% FVASuccinateClassificationSimple.m
clear; clc;

% Input the E.coli core model
load('ecoli_textbook.mat');

% Change carbon source from glucose to succinate
model = changeRxnBounds(model,'EX_glc(e)',0,'l');
model = changeRxnBounds(model,'EX_succ(e)',-20,'l');

% Set optimization objective to Biomass_Ecoli_core_N(w/GAM)_Nmet2
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');

% Perform flux variability analysis classification
[minFlux,maxFlux]=fluxVariability(model,90,'max',model.rxnss,false,false);

BioMassID = findRxnIDs(model, 'Biomass_Ecoli_core_N(w/GAM)_Nmet2');
BiomassRatio = minFlux(BioMassID)/maxFlux(BioMassID);
```

A red arrow points from the line 'minFlux, maxFlux = fluxVariability(model, 90, 'max', model.rxnss, false, false);' to the line 'BioMassID = findRxnIDs(model, 'Biomass_Ecoli_core_N(w/GAM)_Nmet2');

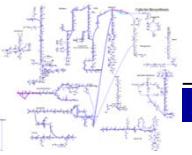
```
% Find hard-coupled reactions
HCReactions = {};
n = length(maxFlux);
j = 1;
for i=1:n
    if (minFlux(i) == BiomassRatio*maxFlux(i)) && (maxFlux(i) > 0)
        HCReactions(j) = model.rxnss(i);
        j = j+1;
    end
end
HardCoupledReactions = transpose(HCReactions)
```

```
% Find partially-coupled reactions
PCReactions = {};
n = length(maxFlux);
j = 1;
for i=1:n
    if (minFlux(i) > 0 ) && (minFlux(i) < BiomassRatio*maxFlux(i))
        PCReactions(j) = model.rxnss(i);
        j = j+1;
    end
end
PartiallyCoupledReactions = transpose(PCReactions)

% Find not-coupled reactions
NCReactions = {};
n = length(maxFlux);
j = 1;
for i=1:n
    if (minFlux(i) <= 0 ) && (minFlux(i) < maxFlux(i))
        NCReactions(j) = model.rxnss(i);
        j = j+1;
    end
end
NotCoupledReactions = transpose(NCReactions)

% Find zero-flux reactions
ZFRxns = {};
n = length(maxFlux);
j = 1;
for i=1:n
    if (minFlux(i) == 0 ) && (maxFlux(i) == 0)
        ZFRxns(j) = model.rxnss(i);
        j = j+1;
    end
end
ZeroFluxReactions = transpose(ZFRxns)
```

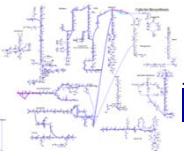
FVASuccinateClassificationSimple.m



FVA Classifications for Succinate Growth

| Hard-Coupled Reactions | Partially-Coupled Reactions | Not-Coupled Reactions | Zero-flux Reactions | |
|-------------------------------------|---|--|---|--|
| 'Biomass_Ecoli_core_N(w/GAM)_Nmet2' | 'ACONTa' 'ACONTb' 'ATPS4r' 'Biomass' 'CS' 'CYTBD' 'EX_co2(e)' 'EX_h2o(e)' 'FBP' 'FUM' 'GLNS' 'ICDHyr' 'MDH' 'NADH16' 'NH4t' 'O2t' 'PDH' 'PGK' 'PGM' 'PIt2r' 'SUCCt2_2' 'SUCDi' | 'ACALD' 'ACALDT' 'ACKr' 'ACt2r' 'ADK1' 'AKGDH' 'AKGt2r' 'ALCD2x' 'CO2t' 'D_LACT2' 'ENO' 'ETOHt2r' 'EX_ac(e)' 'EX_acald(e)' 'EX_akg(e)' 'EX_etoeh(e)' 'EX_for(e)' 'EX_glu_L(e)' 'EX_h(e)' 'EX_lac_D(e)' 'EX_nh4(e)' 'EX_o2(e)' 'EX_pi(e)' | 'EX_pyr(e)' 'EX_succ(e)' 'FBA' 'FORT2' 'FORTi' 'G6PDH2r' 'GAPD' 'GLUDy' 'GLUN' 'GLUSy' 'GLUT2r' 'GND' 'H2Ot' 'ICL' 'LDH_D' 'MALS' 'ME1' 'ME2' 'NADTRHD' 'PFK' 'PFL' 'PGI' 'PGL' | 'EX_fru(e)' 'EX_fum(e)' 'EX_glc(e)' 'EX_gln_L(e)' 'EX_mal_L(e)' 'FRD7' 'FRUpts2' 'FUMt2_2' 'GLCpts' 'GLNabc' 'MALt2_2' |

FVA_SuccinateClassification.m



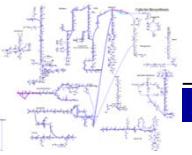
Review Questions

- What is flux variability analysis?
- What is the relationship between the value of the objective function and the flux values calculated through flux variability analysis?
- How is flux variability analysis related to alternate optimal flux vectors?
- How can you implement flux variability analysis using the Cobra Toolbox?
- Does flux variability analysis identify the specific alternate optimal solutions?
- What is the value of knowing which reactions carry flux, which reactions carry no flux, and which reactions span a range of flux values?
- Explain the different FVA classifications; hard-coupled, partially-coupled, not-coupled, and no-flux reactions?



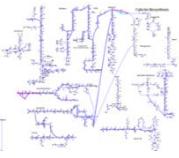
Lesson Outline

- Alternate Optimal Solutions
- Flux Variability Analysis
- • Parsimonious FBA



singleRxnDeletion, singleGeneDeletion

- A cobra toolbox function that performs single reaction or gene deletion (knockout) analysis
 - ✓ `[grRatio,grRateKO,grRateWT,hasEffect,delRxns,hasEffect] = singleRxnDeletion(model)`
 - ✓ `[grRatio,grRateKO,grRateWT,delRxns,hasEffect] = singleGeneDeletion(model)`
- grRatio - Computed growth rate ratio between the model with a deleted reaction/gene and the original model without any deletions
- grRateKO - Growth rate of model with a reaction deletion/gene (1/h)
- grRateWT - Growth rate of the original model (1/h)
- hasEffect - Does a reaction deletion/gene affect anything
- delRxn - Deleted reactions/genes
- fluxSolution - FBA/MOMA/IMOMA fluxes for models with reaction/gene deletions
- Typically, if the grRatio is below a certain tolerance, tol, then the reaction/gene is categorized as essential



Essential Reactions

Essential reactions, metabolic genes necessary for *in silico* growth in the given media;

```
% EssentialReactions.m
clear; clc;

% Load the E.coli core model
load('ecoli_textbook.mat');

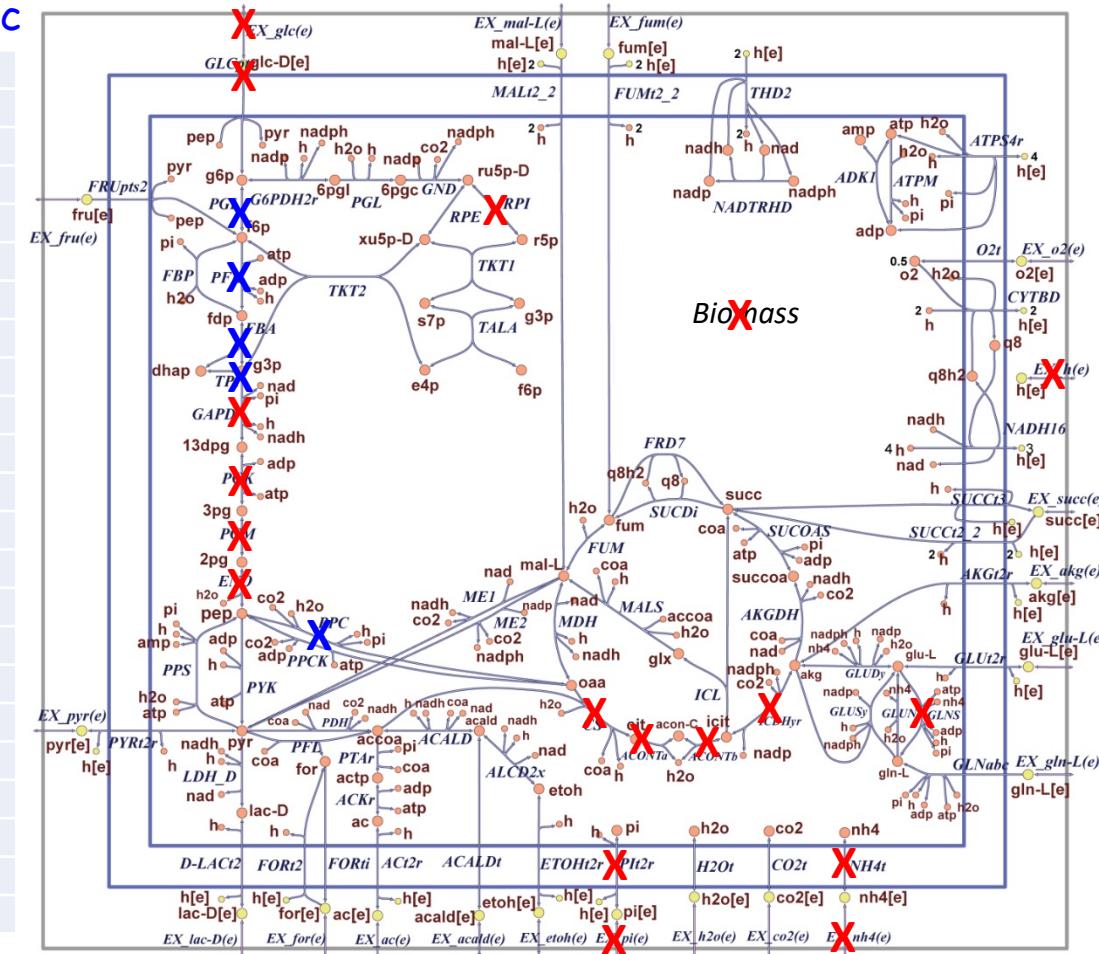
model = changeRxnBounds(model,'EX_glc(e)',-10,'1');
model = changeRxnBounds(model,'EX_o2(e)',-30,'1');

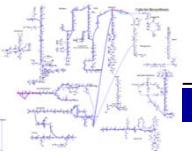
tol = 1e-6; % Growth rate lower limit
RxnRatio = singleRxnDeletion(model);
RxnRatio(isnan(RxnRatio))=0; % Replace NaN with 0
EssentialRxns = model.rxns(RxnRatio<tol)
```

*RxnRatio = Computed growth rate ratio between deletion strain and wild type

Aerobic Anaerobic

| | |
|-------------|-------------|
| 'ACONTa' | 'ACONTa' |
| 'ACONTb' | 'ACONTb' |
| 'Biomass' | 'Biomass' |
| 'CS' | 'CS' |
| 'ENO' | 'ENO' |
| 'EX_glc(e)' | 'EX_glc(e)' |
| 'EX_h(e)' | 'EX_h(e)' |
| 'EX_nh4(e)' | 'EX_nh4(e)' |
| 'EX_pi(e)' | 'EX_pi(e)' |
| 'GAPD' | 'FBA' |
| 'GLCpts' | 'GAPD' |
| 'GLNS' | 'GLCpts' |
| 'ICDHyr' | 'GLNS' |
| 'NH4t' | 'ICDHyr' |
| 'PGK' | 'NH4t' |
| 'PGM' | 'PPK' |
| 'PIt2r' | 'PGK' |
| 'RPI' | 'PPK' |
| | 'RPI' |
| | 'TPP' |





Essential Genes

Essential genes, metabolic genes necessary for *in silico* growth in the given media;

```
% EssentialGenes.m
clear;clc;

% Load the E.coli core model
load('ecoli_textbook.mat');

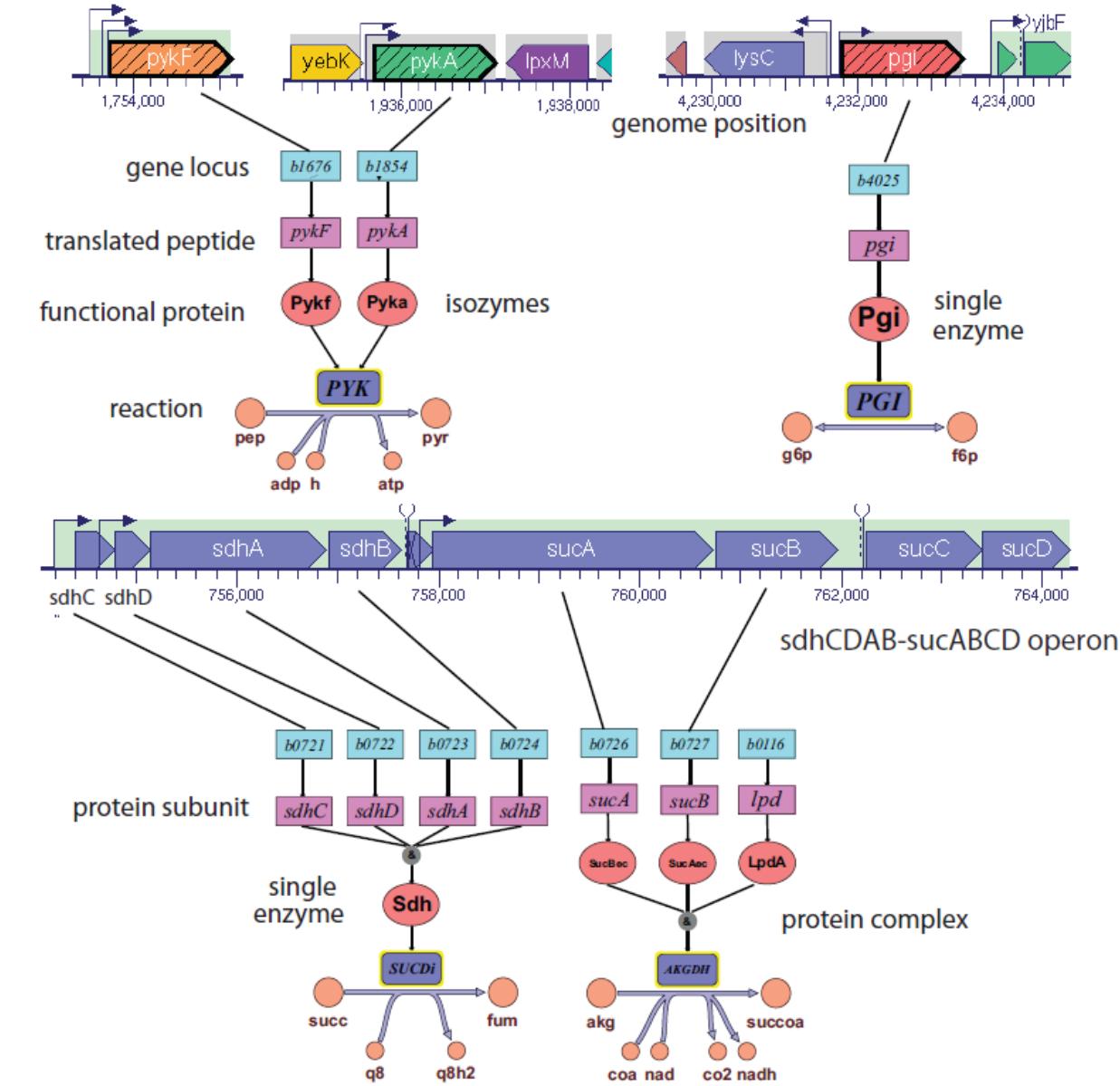
model = changeRxnBounds(model,'EX_glc(e)',-10,'l');
model = changeRxnBounds(model,'EX_o2(e)',-30,'l');

tol = 1e-6; % Growth rate lower limit
grRatio = singleGeneDeletion(model);
grRatio(isnan(grRatio))=0;
EssentialGenes = model.genes(grRatio<tol)

% List reactions associated with genes
[results ListResults] = findRxnsFromGenes(model, EssentialGenes);
```

| Aerobic |
|---------|
| 'b0451' |
| 'b0720' |
| 'b1136' |
| 'b1779' |
| 'b2415' |
| 'b2416' |
| 'b2779' |
| 'b2926' |

| Anaerobic |
|-----------|
| 'b0451' |
| 'b0720' |
| 'b1136' |
| 'b1779' |
| 'b2415' |
| 'b2416' |
| 'b2779' |
| 'b2926' |
| 'b3919' |
| 'b3956' |
| 'b4025' |

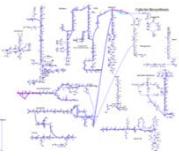




Parsimonious FBA

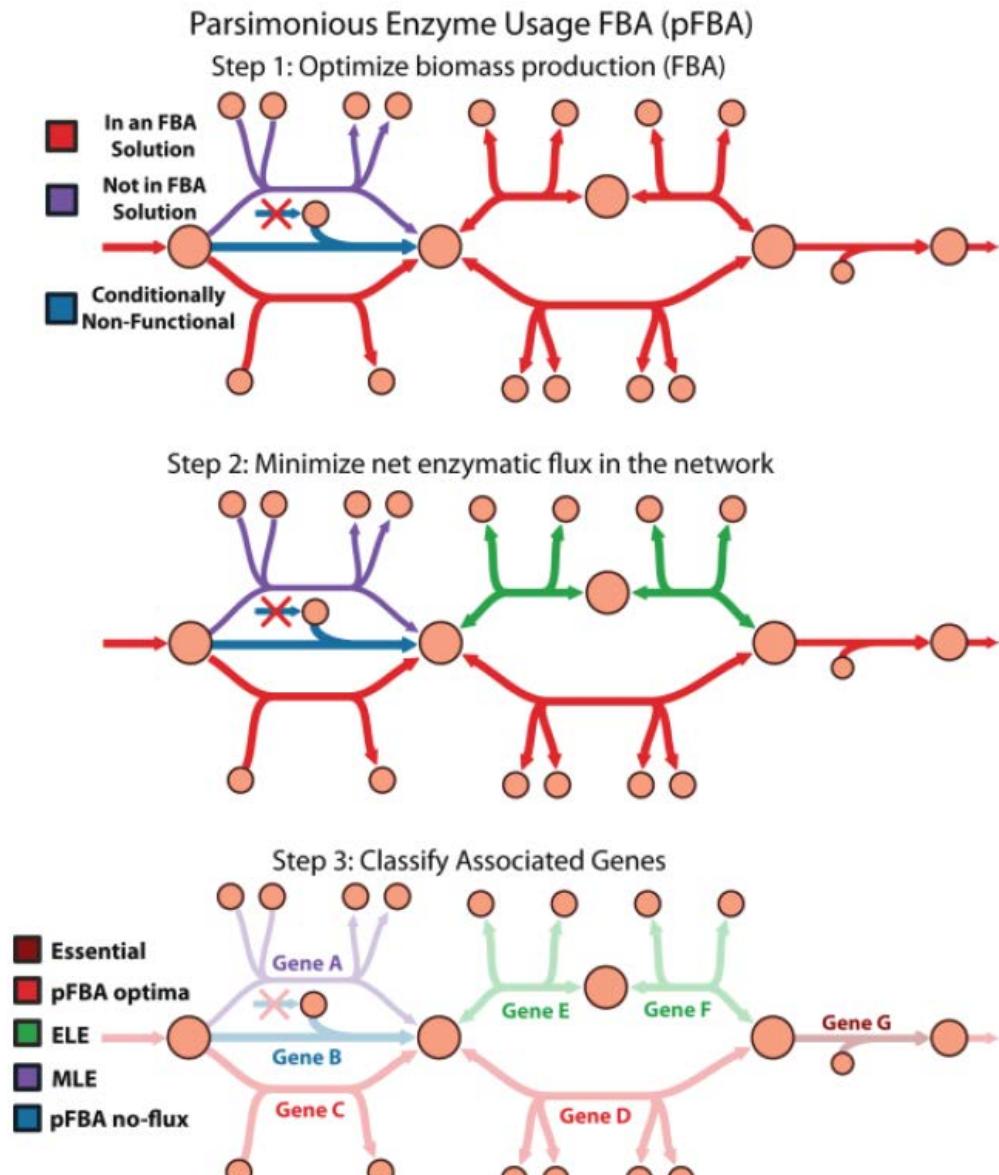
- Flux parsimony - minimize the total material flow required to achieve an objective.
- The underlying assumption is that, under growth pressure, there is a selection for strains that can process the growth substrate the most rapidly and efficiently while using the minimum amount of enzyme.
- Genes are classified into six categories:
 1. essential genes, metabolic genes necessary for *in silico* growth in the given media;
 2. pFBA optima, non-essential genes contributing to the optimal growth rate and minimum gene-associated flux;
 3. enzymatically less efficient (ELE), genes requiring more flux through enzymatic steps than alternative pathways that meet the same predicted growth rate;
 4. metabolically less efficient (MLE), genes requiring a growth rate reduction if used;
 5. pFBA no-flux, genes that are unable to carry flux in the experimental conditions; and
 6. Blocked, genes that are only associated with the reactions that cannot carry a flux under any condition ("blocked" reactions).
- A map showing the category of each gene can be created.

Lewis, N. E., K. K. Hixson, et al. (2010). "Omic data from evolved *E. coli* are consistent with computed optimal growth from genome-scale models." Molecular Systems Biology 6: 390.

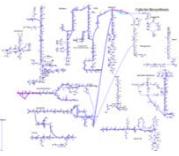


Parsimonious Enzyme Usage

- Gene A, classified as MLE, represents an enzyme that uses a suboptimal co-factor to catalyze a reaction, thereby reducing the growth rate if used.
- Gene B, classified as pFBA no-flux, cannot carry a flux in this example since it is unable to take up or produce a necessary precursor metabolite.
- Genes E and F in this example require two different enzymes to catalyze the same transformation which Gene D can do alone; therefore they are classified as ELE.
- Gene G is essential, since its removal will stop the flux through all pathways.
- Genes C and D represent the most efficient (topologically and metabolically) pathway and therefore are part of the pFBA optima.



Lewis, N. E., K. K. Hixson, et al. (2010). "Omic data from evolved *E. coli* are consistent with computed optimal growth from genome-scale models." *Molecular Systems Biology* 6: 390.



Parsimonious FBA Example

pFBA_Ecoli_Core.m (Aerobic)

```
% pFBA_Ecoli_Core.m

clear;

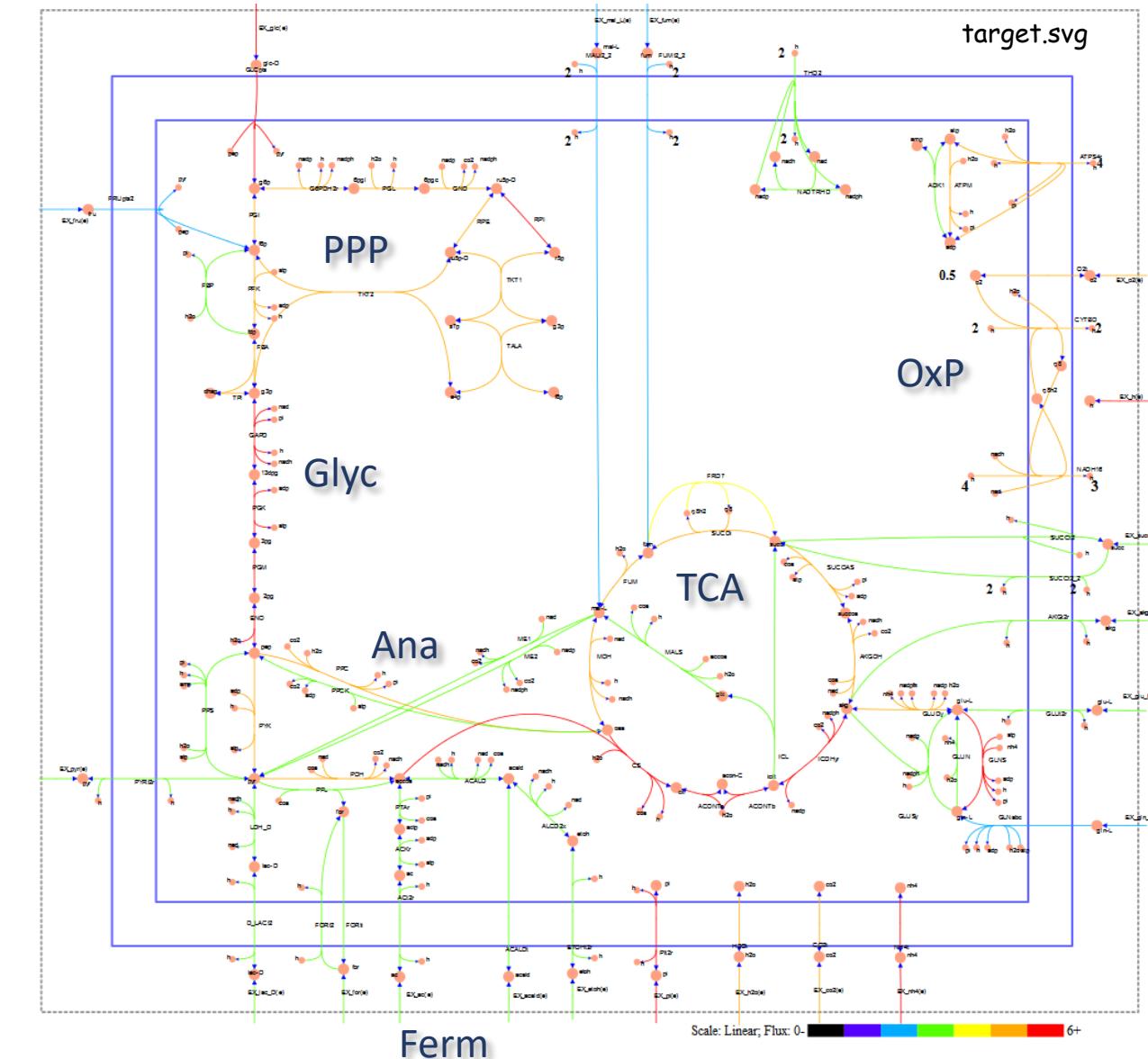
model=readCbModel('ecoli_textbook');

model = changeRxnBounds(model,'EX_glc(e)',-10,'1');
model = changeRxnBounds(model,'EX_o2(e)',-0 or -30,'1');

map=readCbMap('ecoli_Textbook_ExportMap');

[GeneClasses RxnClasses modelIrrevFM] = pFBA(model,
    'geneoption',0, 'tol',1e-7)
```

Red = Essential reactions,
Orange = pFBA optima reaction
Yellow = ELE reactions,
Green = MLE reactions,
Blue = zero flux reactions,
Purple = blocked reactions,
Black = not classified





pFBA Classification

pFBA_Ecoli_Core.m (Anaerobic)

The screenshot shows the MATLAB environment with two windows open. On the left is the 'Variables - RxnClasses' window, which displays a 1x1 struct with 6 fields: Essential_Rxns, pFBAOpt_Rxns, ELE_Rxns, MILE_Rxns, ZeroFlux_Rxns, and Blocked_Rxns. On the right is the 'Workspace' window, which lists several variables: GeneClasses, map, model, modellrevFM, and RxnClasses.

1. Essential genes, metabolic reactions/genes necessary for *in silico* growth in the given media;
2. pFBA optima, non-essential reactions/genes contributing to the optimal growth rate and minimum gene-associated flux;
3. Enzymatically Less Efficient (ELE), reactions/genes requiring more flux through enzymatic steps than alternative pathways that meet the same predicted growth rate;

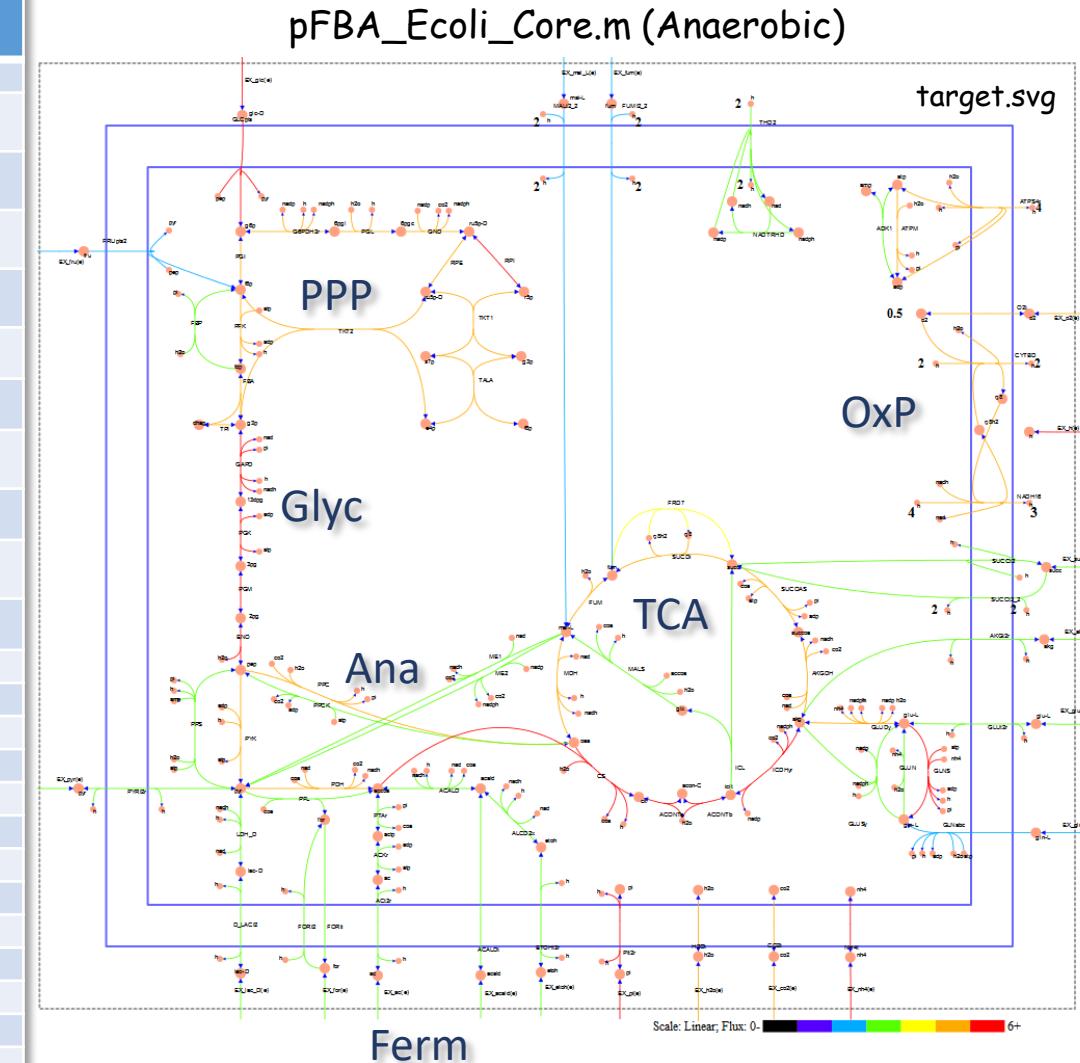
4. Metabolically Less Efficient (MLE), reactions/genes requiring a growth rate reduction if used;
5. pFBA no-flux, reactions/genes that are unable to carry flux in the experimental conditions; and
6. Blocked, reactions/genes that are only associated with the reactions that cannot carry a flux under any condition ("blocked" reactions).

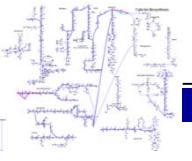
| Essential | | pFBA Optima | | Enzymatically Less Efficient | | Metabolically Less Efficient | | | pFBA No-flux | Blocked |
|-------------|----------|--------------|-------------|------------------------------|---------------|------------------------------|------------|---------------|--------------|---------|
| 'ACONTa' | 'GLNS' | 'ACALD' | 'EX_h2o(e)' | 'FRD7' | 'ACALDt' | 'FORT2' | 'ME2' | 'CYTBD' | | |
| 'ACONTb' | 'ICDHyr' | 'ACKr' | 'FORTl' | 'SUCDI' | 'ADK1' | 'FUM' | 'NADH16' | 'EX_fru(e)' | | |
| Biomass' | 'NH4t' | 'ACt2r' | 'GLUDY' | | 'AKGDH' | 'G6PDH2r' | 'NADTRHD' | 'EX_fum(e)' | | |
| 'CS' | 'PFK' | 'ALCD2x' | 'H2Ot' | | 'AKGt2r' | 'GLUN' | 'PDH' | 'EX_gln_L(e)' | | |
| 'ENO' | 'PGI' | 'ATPM' | 'PFL' | | 'D_LACT2' | 'GLUSy' | 'PGL' | 'EX_mal_L(e)' | | |
| 'EX_glc(e)' | 'PGK' | 'ATPS4r' | 'PTAR' | | 'EX_acald(e)' | 'GLUt2r' | 'PPCK' | 'EX_o2(e)' | | |
| 'EX_h(e)' | 'PGM' | 'CO2t' | 'PYK' | | 'EX_akg(e)' | 'GND' | 'PPS' | 'FRUpts2' | | |
| 'EX_nh4(e)' | 'Plt2r' | 'ETOHt2r' | 'RPE' | | 'EX_glu_L(e)' | 'ICL' | 'PYRt2r' | 'FUMt2_2' | | |
| 'EX_pi(e)' | 'PPC' | 'EX_ac(e)' | 'TALA' | | 'EX_lac_D(e)' | 'LDH_D' | 'SUCCt2_2' | 'GLNabc' | | |
| 'FBA' | 'RPI' | 'EX_co2(e)' | 'THD2' | | 'EX_pyr(e)' | 'MALS' | 'SUCCt3' | 'MALt2_2' | | |
| 'GAPD' | 'TPI' | 'EX_etoh(e)' | 'TKT1' | | 'EX_succ(e)' | 'MDH' | 'SUCAOS' | 'O2t' | | |
| 'GLCpts' | | 'EX_for(e)' | 'TKT2' | | 'FBP' | 'ME1' | | | | |



Parsimonious FBA Data

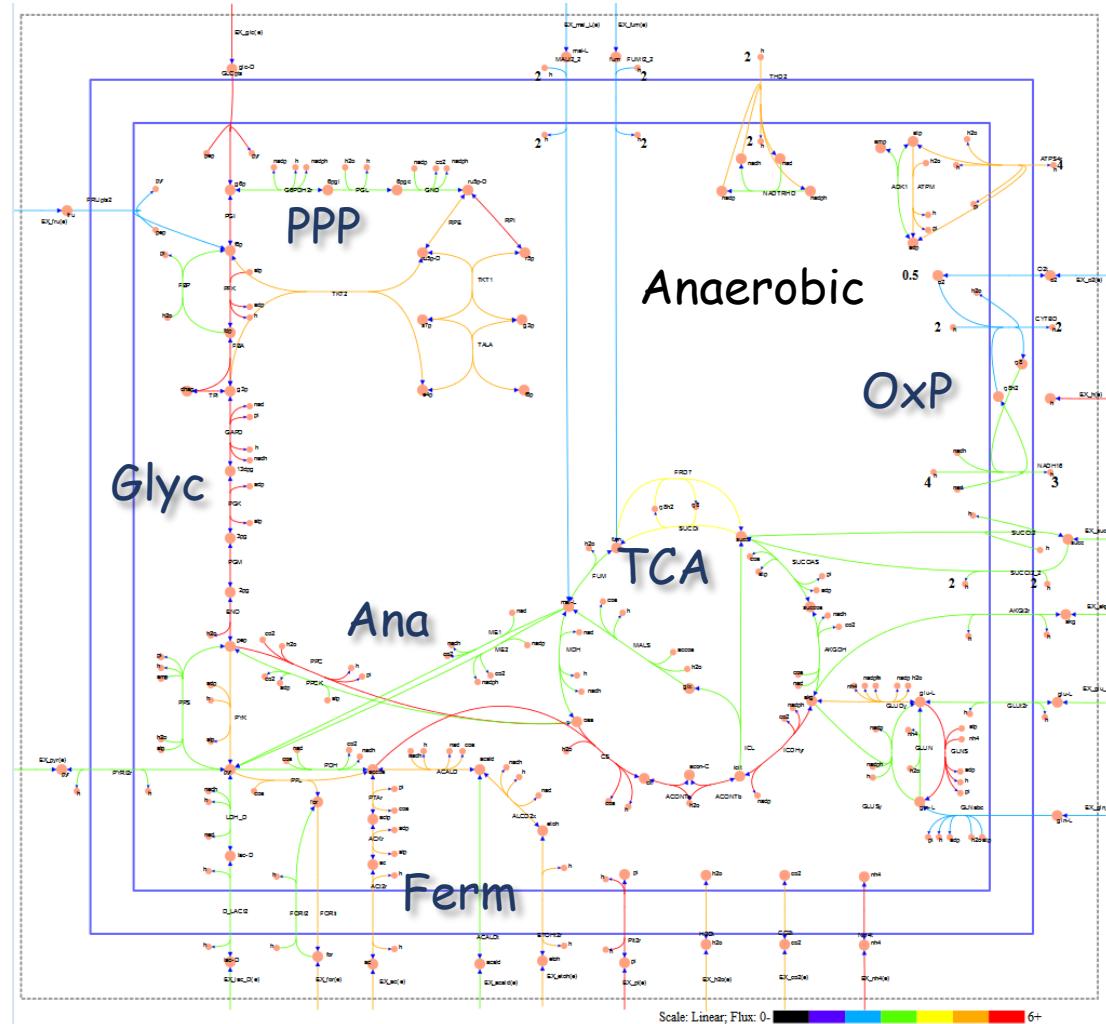
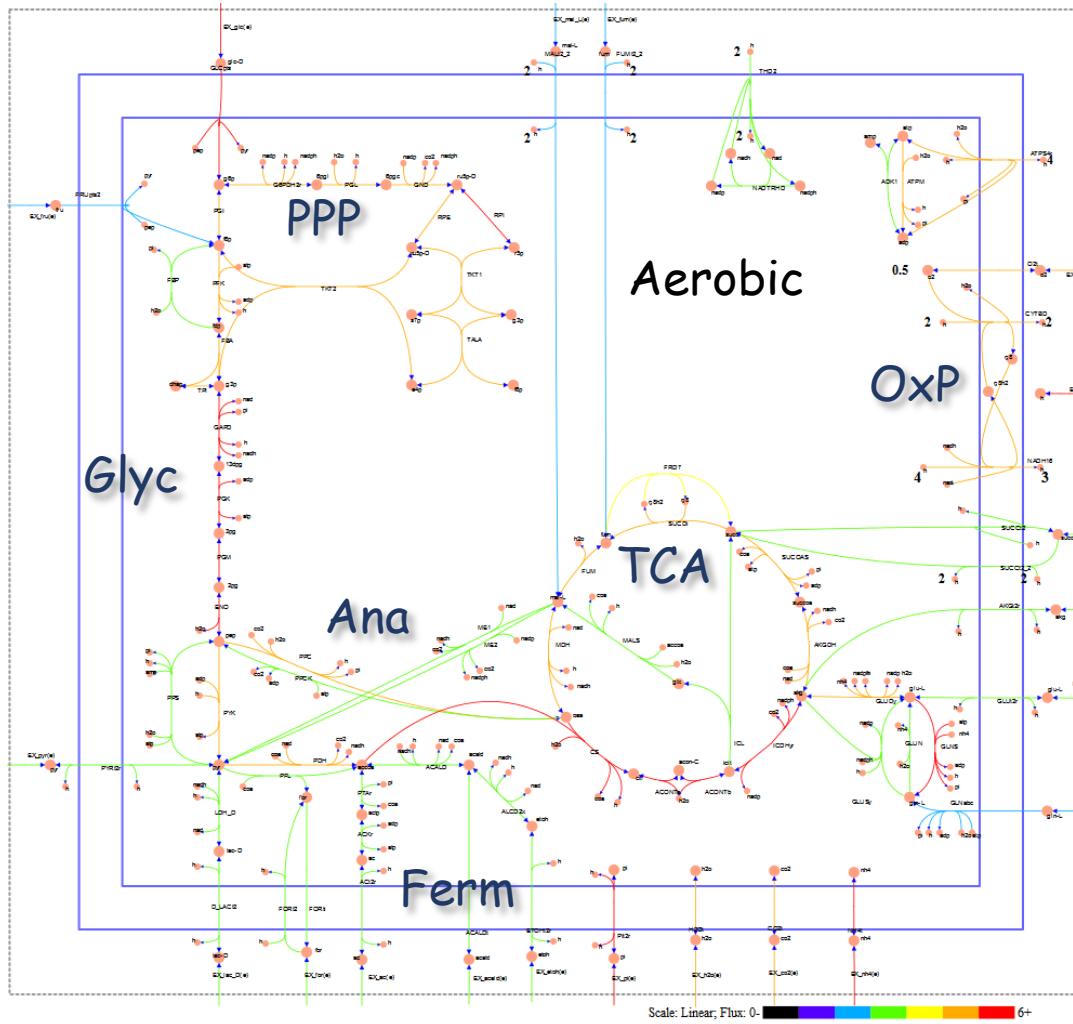
| Essential | pFBA Optima | Enzymatically Less Efficient | Metabolically Less Efficient | | pFBA No-flux | Blocked |
|-------------|--------------|------------------------------|------------------------------|------------|---------------|---------|
| 'ACONTa' | 'ACALD' | 'FRD7' | 'ACALDT' | 'ME2' | 'CYTBD' | |
| 'ACONTb' | 'ACKr' | 'SUCDi' | 'ADK1' | 'NADH16' | 'EX_fru(e)' | |
| Biomass' | 'ACT2r' | | 'AKGDH' | 'NADTRHD' | 'EX_fum(e)' | |
| 'CS' | 'ALCD2x' | | 'AKGt2r' | 'PDH' | 'EX_gln_L(e)' | |
| 'ENO' | 'ATPM' | | 'D_LACT2' | 'PGL' | 'EX_mal_L(e)' | |
| 'EX_glc(e)' | 'ATPS4r' | | 'EX_acald(e)' | 'PPCK' | 'EX_o2(e)' | |
| 'EX_h(e)' | 'CO2t' | | 'EX_akg(e)' | 'PPS' | 'FRUpts2' | |
| 'EX_nh4(e)' | 'ETOHt2r' | | 'EX_glu_L(e)' | 'PYRt2r' | 'FUMt2_2' | |
| 'EX_pi(e)' | 'EX_ac(e)' | | 'EX_lac_D(e)' | 'SUCCt2_2' | 'GLNabc' | |
| 'FBA' | 'EX_co2(e)' | | 'EX_pyr(e)' | 'SUCCt3' | 'MALt2_2' | |
| 'GAPD' | 'EX_etoh(e)' | | 'EX_succ(e)' | 'SUCOAS' | 'O2t' | |
| 'GLCpts' | 'EX_for(e)' | | 'FBP' | | | |
| 'GLNS' | 'EX_h2o(e)' | | 'FORt2' | | | |
| 'ICDHyr' | 'FORti' | | 'FUM' | | | |
| 'NH4t' | 'GLUDy' | | 'G6PDH2r' | | | |
| 'PFK' | 'H2Ot' | | 'GLUN' | | | |
| 'PGI' | 'PFL' | | 'GLUSy' | | | |
| 'PGK' | 'PTAr' | | 'GLUT2r' | | | |
| 'PGM' | 'PYK' | | 'GND' | | | |
| 'PIt2r' | 'RPE' | | 'ICL' | | | |
| 'PPC' | 'TALA' | | 'LDH_D' | | | |
| 'RPI' | 'THD2' | | 'MALS' | | | |
| 'TPI' | 'TKT1' | | 'MDH' | | | |
| | 'TKT2' | | 'ME1' | | | |





Parsimonious FBA Maps

pFBA_Ecoli_Core.m

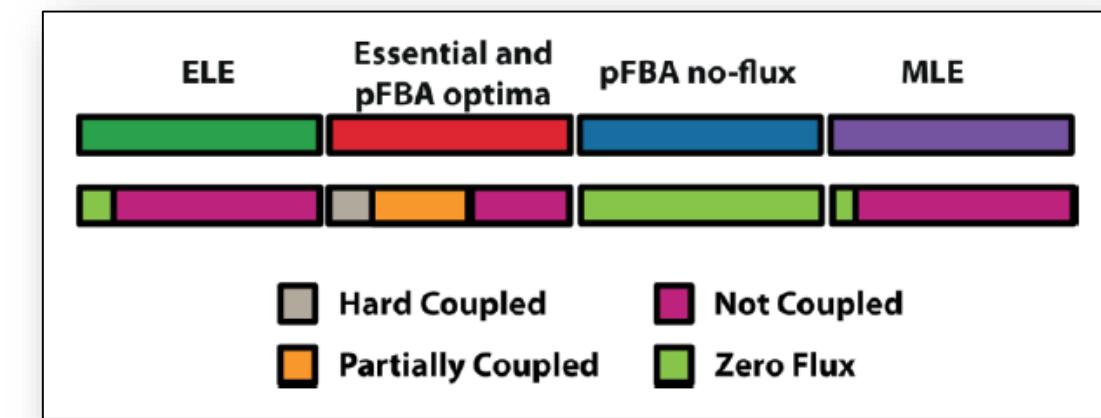




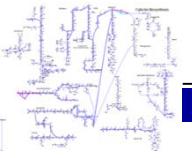
pFBA vs. FVA Reaction Classes

The pFBA genes were mapped to the FVA reaction classes.

- The hard-coupled and partially-coupled reactions were all associated with the essential and pFBA optima genes, and the
- pFBA no-flux genes were all within the FVA zero-flux reactions.
- FVA Zero Flux reactions were identified in the other pFBA classes since some Zero-Flux reactions are catalyzed by genes which may be active for alternative, functional reactions.



Lewis, N. E., K. K. Hixson, et al. (2010). "Omic data from evolved *E. coli* are consistent with computed optimal growth from genome-scale models-Supplementary Material." *Molecular Systems Biology* 6: 390.



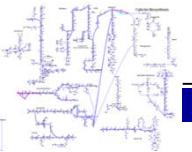
Review Questions

- Why do they call it parsimonious flux balance analysis?
- What are essential genes/reactions?
- What are pFBA optima genes/reactions?
- What are enzymatically less efficient (ELE) genes/reactions?
- What are metabolically less efficient genes/reactions?
- What are pFBA no-flux genes/reactions?
- What are blocked genes/reactions?
- What is the difference between pFBA optima genes/reactions, enzymatically less efficient (ELE) genes/reactions and metabolically less efficient (MLE), genes/reactions?
- How can you implement parsimonious flux balance analysis using the Cobra Toolbox
- How can parsimonious flux balance analysis be used to metabolically engineer a cell?



Lesson Outline

- Alternate Optimal Solutions
- Flux Variability Analysis
- Parsimonious FBA



New Cobra Toolbox Functions

```
% Changing solver type
changeCobraSolver('glpk','all')

% Finding alternate optimal solutions
[solutions] = enumerateOptimalSolutions(model); % Finding alternate optimal solutions

% Flux Variability Analysis
[minFlux,maxFlux]=fluxVariability(model,100,'max',model.rxns,false,false); % Flux Variability Analysis

% Single reaction deletion
[grRatio,grRateKO,grRateWT,hasEffect,delRxns,hasEffect] = singleRxnDeletion(model)

% Single gene deletion
[grRatio,grRateKO,grRateWT,delRxns,hasEffect] = singleGeneDeletion(model)

% Parsimonious Flux Balance Analysis
[GeneClasses RxnClasses modelIrrevFM] = pFBA(model,'geneoption',0, 'tol',1e-7)
```