Large Genome-Scale Metabolic Models
Learning Objectives

Each student should be able to:

• Describe the progress made in the evolution of *E.coli* metabolic models.

• Describe the metabolic model of the cyanobacteria *Synechocystis sp. PCC6803*.

• Describe the metabolic models of the yeast *Saccharomyces cerevisiae*.

• Describe the metabolic models of the algae *Chlamydomonas reinhardtii*.

• Describe the metabolic models of *Homo sapiens*. 
Lesson Outline

• Overview

• Prokaryotes
  ✓ *Escherichia coli* (Bacteria)
  ✓ *Synechocystis sp. PCC6803* (Cyanobacteria)

• Eukaryotes
  ✓ *Saccharomyces cerevisiae* (Yeast)
  ✓ *Chlamydomonas reinhardtii* (Algae)
  ✓ *Homo sapiens* (Human)
Phylogenetic Coverage of Genome-scale Network Reconstructions

A GENome scale Network Reconstructions (GENREs) serves as a structured knowledge base of established biochemical facts, while a GENome scale Models (GEMs) is a model which supplements the established biochemical information with additional (potentially hypothetical) information to enable computational simulation and analysis.

Expansion of Metabolic Networks and Global Reactome Coverage Over Time

## BIGG Models

[http://bigg.ucsd.edu/models](http://bigg.ucsd.edu/models)

<table>
<thead>
<tr>
<th>BIGG ID</th>
<th>Organism</th>
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<th>Reactions</th>
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## Other Models – Bacteria, Archea, Eukaryota

[http://sbrg.ucsd.edu/InSilicoOrganisms/OtherOrganisms](http://sbrg.ucsd.edu/InSilicoOrganisms/OtherOrganisms)

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<tr>
<th>Organism</th>
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<th>Metabolites</th>
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<th>Compartments</th>
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Utah State University  Lesson: Large Metabolic Models

BIE 5500/6500
Lesson Outline

- Overview
- Prokaryotes
  - Escherichia coli (Bacteria)
    - Synechocystis sp. PCC6803 (Cyanobacteria)
- Eukaryotes
  - Saccharomyces cerevisiae (Yeast)
  - Chlamydomonas reinhardtii (Algae)
  - Homo sapiens (Human)
Escherichia coli

- *Escherichia coli* is a Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria.

- The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.

http://en.wikipedia.org/wiki/Escherichia_coli
The Iterative Reconstruction and History of the *E. Coli* Metabolic Network

E. coli Strain Reconstructions

- Escherichia coli 042
- Escherichia coli 536
- Escherichia coli 55989
- Escherichia coli ABU 83972
- Escherichia coli APEC O1
- Escherichia coli ATCC 8739
- Escherichia coli B str. REL606
- Escherichia coli BL21(DE3) AM946981
- Escherichia coli BL21(DE3) BL21-Gold(DE3)pLysS AG
- Escherichia coli BL21(DE3) CP001509
- Escherichia coli BW2952
- Escherichia coli CFT073
- Escherichia coli DH1
- Escherichia coli DH1 ME8569
- Escherichia coli E24377A
- Escherichia coli E21a
- Escherichia coli ETEC H10407
- Escherichia coli HS
- Escherichia coli IAI1
- Escherichia coli IAI39
- Escherichia coli IHE3034
- Escherichia coli KO11FL
- Escherichia coli LF82
- Escherichia coli NA114
- Escherichia coli O103:H2 str. 12009
- Escherichia coli O111:H- str. 11128
- Escherichia coli O127:H6 str. E2348/69
- Escherichia coli O157:H7 EDL933
- Escherichia coli O157:H7 str. EC4115
- Escherichia coli O157:H7 str. Sakai
- Escherichia coli O157:H7 str. TW14359
- Escherichia coli O26:H11 str. 11368
- Escherichia coli O55:H7 str. CB9615
- Escherichia coli O83:H1 str. NRG 857C
- Escherichia coli S88
- Escherichia coli SE11
- Escherichia coli SE15
- Escherichia coli SMS-3-5
- Escherichia coli str. K-12 substr. DH10B
- Escherichia coli str. K-12 substr. MG1655
- Escherichia coli str. K-12 substr. W3110
- Escherichia coli UM146
- Escherichia coli UMN026
- Escherichia coli UMNK88
- Escherichia coli UTI89
- Escherichia coli W
- Escherichia coli W CP002185
- Escherichia coli K-12 MG1655

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  ✓ *Homo sapiens* (Human)
Synechocystis sp. PCC6803 (Cyanobacteria)

- *Synechocystis* is a genus of cyanobacteria primarily represented by the strain *Synechocystis* sp. *PCC6803*.
- *Synechocystis* sp. *PCC6803* lives in freshwater and is capable of both phototrophic growth by oxygenic photosynthesis in sunlight and heterotrophic growth by glycolysis and oxidative phosphorylation during dark periods.
- It is able to effectively anticipate transitions of light and dark phases by using a circadian clock.
- *Synechocystis* sp. *PCC 6803* was the first photosynthetic organism that the entire genome sequence was determined.

http://synechocystis.asu.edu/
Chemiosmotic Theory


Chemiosmotic theory states that energy-transducing membranes (i.e., bacterial membranes, mitochondrial and chloroplast membranes) pump protons across the membrane, thereby generating an electrochemical gradient of protons across the membrane (proton motive force, $\Delta p$) that can be used to do useful work when the protons return across the membrane to the lower potential.
The proton motive force is the sum of electrical energy (membrane potential) and chemical energy (concentration gradient).

\[
\Delta p = \Delta \Psi + \frac{RT}{F} \ln \frac{[H^+]_{in}}{[H^+]_{out}} \quad V
\]

or

\[
\Delta p = \Delta \Psi (mV) + 60 \Delta \rho H \quad mV
\]

with

\[
\Delta G = yF \Delta p \quad J
\]

where \( y \) is equal to the number of moles of protons.
Energy Storage

- Concentration Gradient
- Membrane Potential
- Light
- Chemical Energy
- Cell Membrane
- ΔG
- ADP
- ATP
- NADP+
- NADPH
- Equilibrium Displacement
- Reducing Agents
Mitochondrial Respiratory Chain
(Oxidative Phosphorylation)

http://www.tokresource.org/tok_classes/biobiobio/biomenu/cell_respiration/c8_9x16_chemiosmosis.jpg
ORGANIZATION OF THE THYLAKOID MEMBRANE

1. \( \text{Light} \rightarrow \text{Photosystem II} \)
2. \( 2 \text{H}^+ \rightarrow 2 \text{H}^+ \)
3. \( \text{NADP}^+ \rightarrow \text{NADPH} + \text{H}^+ \)

STROMA (Low \( \text{H}^+ \) concentration)

THYLAKOID SPACE (High \( \text{H}^+ \) concentration)

LUMEN

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Biology, Campbell & Reese, 7ed
Figure 5: Schematic overview of a cyanobacterial cell. Indicated are: thylakoid membranes (green) that contain photosynthetic and respiratory complexes and that separate the cytoplasm from the lumen; the cytoplasmic membrane (yellow) that separates the cytoplasm from the periplasm; and the outer membrane and cell wall.

http://synechocystis.asu.edu/index.htm

Figure 6: Transmission electron micrograph of a dividing *Synechocystis* cell illustrating thylakoid membranes (arrowheads) that occur along the periphery of the cytoplasm. Scale bar = 400 nm.
Spectra of Photosynthetic Organisms

“Molecular Mechanisms of Photosynthesis,”
R. E. Blankenship, Figure 1.1

Photosynthetically active radiation spectral region
(400 nm ≤ λ ≤ 700 nm)

No organisms utilize light with λ > 1000 nm
Electron Flow for Oxygenc Photosynthesis

$P_{680} = P_{700} =$ Chlorophyll $a$
$\text{Pheo} =$ Pheophytin
$\text{PQ} =$ Plastoquinone
$b_{a}f =$ Cytochrome $b_{a}f$

$A_{0} =$ Chlorophyll
$A_{1} =$ Phylloquinone
$\text{FeS} =$ Iron-sulfur centers
$\text{Fd} =$ Ferredoxin
$\text{Pc} =$ Plastocyanin
$\text{OEC} =$ Oxygen Evolving Complex

Simplified Model of Photosynthetic Components

(Proton numbers assume two photons)
Constraint-based Metabolic Reconstructions & Analysis

H. Scott Hinton, 2016

Lesson: Large Metabolic Models

Photosynthesis Genes
Synechocystis sp. PCC6803

http://www.genome.jp/kegg-bin/show_pathway?org_name=syn&mapno=00195&mapscale=1.0&show_description=show
Cyanobacteria Phycobilisome Genes

Synechocystis sp. PCC6803

Phycobilisome (Cyanobacteria, Red algae)

http://www.genome.jp/kegg/pathway/map/map00196.html
Photosynthesis Subsystem for iJN678

Nogles_Photosynthesis.m
Modeling of the oxidative phosphorylation and photosynthetic pathways included in iJN678.
Constraint-based Metabolic Reconstructions & Analysis

H. Scott Hinton, 2016

Lesson: Large Metabolic Models

Light reactions

H₂O

Light

Calvin cycle

CO₂

NADP⁺

ADP

+ Pₗ

RuBP

3-Phosphoglycerate

G3P

Starch (storage)

Amino acids
Fatty acids

Sucrose (export)

Chloroplast

Photosystem II
Electron transport chain
Photosystem I

O₂

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Biology, Campbell & Reese, 7ed
Autotrophic Conditions

Glycogen biosynthesis

Pentose phosphate pathway

Calvin cycle

TCA cycle

CO$_2$

O$_2$

H. Scott Hinton, 2016

Constraint-based Metabolic Reconstructions & Analysis

Lesson: Large Metabolic Models

Utah State University

BIE 5500/6500
Heterotrophic Conditions

Heterotrophic Conditions

Glucose ➔ Pentose phosphate pathway

Glycolysis/Gluconeogenesis

TCA cycle

Growth

Nogales_Heterotropic_plots.m

Nogales_PPPA.m
Mixotrophic Conditions

Mixotrophic Conditions

Glucose (>-5)

Pentose phosphate pathway

Calvin cycle

TCA cycle

Glucose Dominant

Nogales_Mixotropic_plots.m

Constraint-based Metabolic Reconstructions & Analysis

H. Scott Hinton, 2016

Lesson: Large Metabolic Models

O₂

CO₂

Glycolysis/Gluconeogenesis

GAPDH 65.64

PGK 65.77

PGM 170.9/211

GALU 2.69

PGMT 2.69

PGK 97.3/85.1

PGI 100/100

HEX1 100/100

E1p 100/100

FBP 100/100

F6P 100/100

Glucose (>) 5

Mixotrophic Conditions

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)

PGK (mmol/g DW-hr)
Oxygen Robustness Analysis

**Autotrophic**

'EX_photon(e)' > -100
'EX_glc(e)' > 0
'EX_o2(e)' > -20
'EX_hco3(e)' > 0
'EX_co2(e)' > -100

Objective = 'Ec_biomass_SynAuto'

**Heterotrophic**

'EX_glc(e)' > -10
'EX_o2(e)' > -20
'EX_hco3(e)' > -3.7
'EX_co2(e)' > -100

Objective = 'Ec_biomass_SynHetero'

**Mixotrophic**

'EX_photon(e)' > -100
'EX_glc(e)' > -10
'EX_o2(e)' > -20
'EX_hco3(e)' > -3.7
'EX_co2(e)' > -100

Objective = 'Ec_biomass_SynMixo'
**CO₂ Robustness Analysis**

**Autotrophic**
- \( 'EX\_photon(e)' > -100 \)
- \( 'EX\_glc(e)' > 0 \)
- \( 'EX\_o2(e)' > -20 \)
- \( 'EX\_hco3(e)' > 0 \)
- \( 'EX\_co2(e)' > -100 \)
- Objective = \( 'Ec\_biomass\_SynAuto' \)

**Heterotrophic**
- \( 'EX\_glc(e)' > -10 \)
- \( 'EX\_o2(e)' > -20 \)
- \( 'EX\_hco3(e)' > -3.7 \)
- \( 'EX\_co2(e)' > -100 \)
- Objective = \( 'Ec\_biomass\_SynHetero' \)

**Mixotrophic**
- \( 'EX\_photon(e)' > -100 \)
- \( 'EX\_glc(e)' > -10 \)
- \( 'EX\_o2(e)' > -20 \)
- \( 'EX\_hco3(e)' > -3.7 \)
- \( 'EX\_co2(e)' > -100 \)
- Objective = \( 'Ec\_biomass\_SynMixo' \)
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  ✓ *Saccharomyces cerevisiae* (Yeast)
  ✓ *Chlamydomonas reinhardtii* (Algae)
  ✓ *Homo sapiens* (Human)
Saccharomyces cerevisiae (Yeast)

• *Saccharomyces cerevisiae* is a species of yeast. It is perhaps the most useful yeast, having been instrumental to winemaking, baking, and brewing since ancient times.
• It is believed to have been originally isolated from the skin of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-color fruits such as plums; it exists among the waxes of the cuticle).
• It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model bacterium.
• It is the microorganism behind the most common type of fermentation. *S. cerevisiae* cells are round to ovoid, 5–10 μm in diameter.
• It reproduces by a division process known as budding.

http://en.wikipedia.org/wiki/Saccharomyces_cerevisiae
**Yeast Model**

*(S. cerevisiae iND750)*
### iND750 Reactions (8 compartments)

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<th>Rxn description</th>
<th>Formula</th>
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<td>Starch and Sucrose Metabolism</td>
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<td>Exo-1,3-beta-glucan glucohydrolase</td>
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<td>Starch and Sucrose Metabolism</td>
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<td>13GS</td>
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<td>$udpg[c] \rightarrow 13BDgcn[c] + h[c] + udp[c]$</td>
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<td>2DDA7Ptm</td>
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</tr>
<tr>
<td>2OHPH_5tn</td>
<td>2-Octaprenyl-6-hydroxyphenol nuclear transport</td>
<td>$2ohph_5[c] \leftrightarrow 2ohph_5[n]$</td>
<td>Transport, Nuclear</td>
</tr>
<tr>
<td>2OMPH_5tn</td>
<td>2-Octaprenyl-6-methoxyxrenol mitochondrial transport</td>
<td>$2omph_5[c] \leftrightarrow 2omph_5[m]$</td>
<td>Transport, Mitochondrial</td>
</tr>
<tr>
<td>2OMPH_5tn</td>
<td>2-Octaprenyl-6-methoxyxrenol nuclear transport</td>
<td>$2omph_5[c] \leftrightarrow 2omph_5[n]$</td>
<td>Transport, Nuclear</td>
</tr>
<tr>
<td>2OXOADPtm</td>
<td>2-oxoadipate transport out of mitochondria via diffusion</td>
<td>$2oxoadp[m] \rightarrow 2oxoadp[c]$</td>
<td>Transport, Mitochondrial</td>
</tr>
<tr>
<td>34HPPOR</td>
<td>4-Hydroxyphenylpyruvate:oxygen oxidoreductase</td>
<td>$34hpp[c] + o2[c] \rightarrow \text{co2[c] + hgentis[c]}$</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>34HPPt2m</td>
<td>3-(4-hydroxyphenyl)pyruvate mitochondrial transport via proton symport</td>
<td>$34hpp[c] + h[c] \leftrightarrow 34hpp[m] + h[m]$</td>
<td>Transport, Mitochondrial</td>
</tr>
<tr>
<td>34HPPt2p</td>
<td>3-(4-hydroxyphenyl)pyruvate peroxisomal transport via proton symport</td>
<td>$34hpp[c] + h[c] \leftrightarrow 34hpp[x] + h[x]$</td>
<td>Transport, Peroxisomal</td>
</tr>
<tr>
<td>3C3HMPtm</td>
<td>2-Isopropylmalate transport, diffusion, mitochondrial</td>
<td>$3c3hmp[c] \leftrightarrow 3c3hmp[m]$</td>
<td>Transport, Mitochondrial</td>
</tr>
<tr>
<td>3C4MOPtm</td>
<td>3-Carboxy-4-methyl-2-oxopentanoate transport, diffusion, mitochondrial</td>
<td>$3c4mop[c] \leftrightarrow 3c4mop[m]$</td>
<td>Transport, Mitochondrial</td>
</tr>
<tr>
<td>3DSPHR</td>
<td>3-Dehydrosphinganine reductase</td>
<td>$3dphgn[c] + h[c] + nadph[c] \rightarrow \text{nadp[c] + sphgn[c]}$</td>
<td>Sphingolipid Metabolism</td>
</tr>
<tr>
<td>3HAO</td>
<td>3-hydroxyanthranilate 3,4-dioxygenase</td>
<td>$3hanthr[c] + o2[c] \rightarrow \text{cmusa[c] + h[c]}$</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>3MOBtm</td>
<td>3-methyl-2-oxobutanoate transport, diffusion, mitochondrial</td>
<td>$3mob[c] \leftrightarrow 3mob[m]$</td>
<td>Transport, Mitochondrial</td>
</tr>
</tbody>
</table>
iND750 Phenotype Phase Plane Analysis

% S_cerevisiae_PPPA.m
clear;

% Enter key parameters
model=readCbModel('S_cerevisiae_iND750');
model = changeRxnBounds(model,'EX_glc(e)',-10,'l');
model = changeRxnBounds(model,'EX_o2(e)',-20,'l');

% Set objective function
model = changeObjective(model,'biomass_SC4_bal');

[growthRates,shadowPrices1,shadowPrices2]=phenotypePhasePlane(model,'EX_glc(e)','EX_o2(e)',50,10,20);
iND750 multiProduction Envelope

% S_cerevisiae_ProductionEnvelope.m

% Input the E.coli core model
model = readCbModel('S_cerevisiae_iND750');

% Set key variables
model = changeRxnBounds(model, 'EX_glc(e)', -10, 'l');
model = changeRxnBounds(model, 'EX_o2(e)', -10, 'l');
model = changeObjective(model, 'biomass_SC4_bal');

deletions = {};
lineColor = 'b';
biomassRxn = 'biomass_SC4_bal';
geneDelFlag = false;
nPts = 50;

% Show only growth coupled metabolites
[biomassValues, targetValues] = multiProductionEnvelope(model, deletions, biomassRxn, false, 20, false);
iND750 dynamicFBA

% S_cerevisiae_dFBA.m
clear;

% Input the E.coli core model
model=readCbModel('S_cerevisiae_iND750');

% Set key variables
model = changeRxnBounds(model,'EX_glc(e)',-10,'l');
model = changeRxnBounds(model,'EX_o2(e)',-10,'l');
model = changeObjective(model,'biomass_SC4_bal');

% Set-up for one variables for dynamicFBA
substrateRxns = {'EX_ala_L(e)','EX_glc(e)'};
initConcentrations = [0,20];
initBiomass = .01;
timeStep = .25; nSteps = 100;
plotRxns = {'EX_ala_L(e)','EX_etoh(e)','EX_glc(e)'};

[concentrationMatrix,excRxnNames,timeVec,biomassVec] = ...
    dynamicFBA(model,substrateRxns,initConcentrations,initBiomass,timeStep,nSteps,plotRxns);

% Plot labels
subplot(1,2,1); title('Biomass Concentration'); xlabel('Time(h)'); ylabel('Concentration (g/L)');
subplot(1,2,2); title('Substrate Concentrations'); xlabel('Time(h)'); ylabel('Concentrations (mmol/L)');
The Development of Metabolic Modeling in *Saccharomyces Cerevisiae* since 1995

**Workflow of the Consensus Process**

Consensus Cellular Compartments of Saccharomyces Cerevisiae (Yeast 4.0)


<table>
<thead>
<tr>
<th>Compartment</th>
<th>Reactions</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm</td>
<td>835</td>
<td>590</td>
</tr>
<tr>
<td>Extracellular</td>
<td>15</td>
<td>158</td>
</tr>
<tr>
<td>Golgi</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Mitochondrion</td>
<td>188</td>
<td>235</td>
</tr>
<tr>
<td>Nucleus</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td>Endoplasmic reticulum (ER)</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Vacuole</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Peroxisome</td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td>Mitochondrial membrane</td>
<td>142</td>
<td>0</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>311</td>
<td>0</td>
</tr>
<tr>
<td>Peroxisomal membrane</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>ER membrane</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Vacuolar membrane</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Golgi membrane</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Nuclear membrane</td>
<td>26</td>
<td>0'</td>
</tr>
</tbody>
</table>
## Using Yeast 5.0

```plaintext
writeCbModel(model,'text','Yeast5')
```

### Constraint-based Metabolic Reconstructions & Analysis

H. Scott Hinton, 2016

---

<table>
<thead>
<tr>
<th>Rxn name</th>
<th>Rxn description</th>
<th>Formula</th>
<th>Gene-reaction association</th>
<th>Reversible</th>
<th>LB</th>
<th>UB</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>r_0001</td>
<td>(R)-lactate:ferricytochrome-c 2-oxidoreductase</td>
<td>s_0025 + 2 s_0709 ↔ 2 s_0710 + s_1399</td>
<td>(YDL174C and YEL039C) or (YDL174C and YJR048W) or (YEL039C and YEL071W) or (YEL071W and YJR048W)</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0002</td>
<td>(R)-lactate:ferricytochrome-c 2-oxidoreductase</td>
<td>s_0027 + 2 s_0709 ↔ 2 s_0710 + s_1401</td>
<td>(YDL178W and YEL039C) or (YDL178W and YJR048W)</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0003</td>
<td>(R,R)-butanediol dehydrogenase</td>
<td>s_0035 + s_1198 ↔ s_0020 + s_0794 + s_1203</td>
<td>YAL060W</td>
<td>1</td>
<td>-1000</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0004</td>
<td>(S)-lactate:ferricytochrome-c 2-oxidoreductase</td>
<td>s_0063 + 2 s_0709 ↔ 2 s_0710 + s_1399</td>
<td>(YEL039C and YML054C) or (YJR048W and YML054C)</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0005</td>
<td>1,3-beta-glucan synthase</td>
<td>s_1543 ↔ s_0001 + s_0794 + s_1538</td>
<td>(YGR032W or YLR342W)</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0006</td>
<td>1,6-beta-glucan synthase</td>
<td>s_1543 ↔ s_0004 + s_0794 + s_1538</td>
<td>(YGR143W or YPR159W)</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0007</td>
<td>1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase</td>
<td>s_0077 ↔ s_0312</td>
<td>YIL020C</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0008</td>
<td>1-acyl-sn-glycerol-3-phosphate acyltransferase</td>
<td>s_0081 + s_0379 ↔ s_0530 + 4 s_0795 + s_1332</td>
<td>YOR175C</td>
<td>1</td>
<td>-1000</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0009</td>
<td>1-acyl-sn-glycerol-3-phosphate acyltransferase</td>
<td>s_0082 + s_0380 ↔ s_0531 + 4 s_0798 + s_1334</td>
<td>YCR034W or YDL052C</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0010</td>
<td>1-phosphatidylinositol-3-phosphate 5-kinase</td>
<td>s_0104 + s_0440 ↔ s_0099 + s_0400 + s_0802</td>
<td>YFR019W</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0011</td>
<td>1-phosphatidylinositol-3-phosphate 5-kinase</td>
<td>s_0100 + s_0434 ↔ s_0095 + s_0394</td>
<td>YFR019W</td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>r_0012</td>
<td>1-pyrroline-5-carboxylate dehydrogenase</td>
<td>s_0119 + 2 s_0807 + s_1200 ↔ s_0799 + s_0993 + s_1205</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

Yeast 5.0 Phenotype Phase Plane Analysis

% Yeast5_PPPA.m
clear;
glucose = {'r_1714'}; oxygen = 'r_1992'; biomass = 'r_2110';

% Enter key parameters
model=readCbModel('Yeast_metabolic_network_model');
model = changeRxnBounds(model,glucose,-10,'l'); % Glucose
model = changeRxnBounds(model,oxygen,-10,'l'); % Oxygen

% Set objective function
model = changeObjective(model,biomass);

[growthRates,shadowPrices1,shadowPrices2]=phenotypePhasePlane(model,'r_1714','r_1992',50,10,20);

title('Yeast 5.0 Phenotype Phase Plane Analysis');
xlabel('Growth-rate (1/hr)'); ylabel('Oxygen (mmol/g DW-hr)'); zlabel('Glucose (mmol/g DW-hr)');
% Yeast5_ProductionEnvelope.m

clear;
glucose = 'r_1714'; oxygen = 'r_1992'; biomass = 'r_2110'; ethanol = 'r_1761';

% Enter key parameters
model=readCbModel('Yeast_metabolic_network_model');
model = changeRxnBounds(model,glucose,-10,'l'); % Glucose
model = changeRxnBounds(model,oxygen,-10,'l'); % Oxygen

% Set objective function
model = changeObjective(model,biomass);

deletions = {};
lineColor = 'b';
targetRxn = ethanol;
biomassRxn = biomass;
geneDelFlag = false;
nPts = 50;

figure(1)
[biomassValues,targetValues] = productionEnvelope(model,deletions,lineColor,targetRxn,biomassRxn,geneDelFlag,nPts);
xlabel('Biomass (mmol/g DW-hr)')
ylabel('Ethanol (mmol/g DW-hr)')
Lesson Outline

• Overview

• Prokaryotes
  ✓ *Escherichia coli* (Bacteria)
  ✓ *Synechocystis sp. PCC6803* (Cyanobacteria)

• Eukaryotes
  ✓ *Saccharomyces cerevisiae* (Yeast)
  ✓ *Chlamydomonas reinhardtii* (Algae)
  ✓ *Homo sapiens* (Human)
**Chlamydomonas reinhardtii**  
(Algae)

*Chlamydomonas reinhardtii* is a single-cell green alga about 10 micrometres in diameter that swims with two flagella. It has a cell wall made of hydroxyproline-rich glycoproteins, a large cup-shaped chloroplast, a large pyrenoid, and an "eyespot" that senses light.

Although widely distributed worldwide in soil and fresh water, *C. reinhardtii* is used primarily as a model organism in biology in a wide range of subfields. When illuminated, *C. reinhardtii* can grow in media lacking organic carbon and chemical energy sources, and can also grow in the dark when supplied with organic carbon. *C. reinhardtii* is also of interest in the biopharmaceuticals field and the biofuel field, as a source of hydrogen.

Chlamydomonas reinhardtii (Algae)

- The model alga Chlamydomonas reinhardtii has been developed to study diverse biological processes from photosynthesis to phototaxis.
- The genome-scale C. reinhardtii GEM (iRC1080) has been developed by Roger L. Chang et al that accounts for 1080 genes, associated with 2190 reactions and 1068 unique metabolites, and encompasses 83 subsystems distributed across 10 compartments.
- Defining effective spectral bandwidths associated with each photon-utilizing reaction enables the network to model growth under different light sources via stoichiometric representation of the spectral composition of emitted light.
- The potential optical inputs include prism reactions for 11 distinct light sources, covering most sources that have been used in published studies for algal and plant growth including solar light, various light bulbs, and LEDs.

Chlamydomonas reinhardtii

Eyespot Apparatus

- The eyespot apparatus (or stigma) is a photoreceptive organelle found in the flagellate or (motile) cells of green algae and other unicellular photosynthetic organisms such as euglenids. It allows the cells to sense light direction and intensity and respond to it by swimming either towards the light (positive phototaxis) or away from the light (negative phototaxis).
- Eyespot-mediated light perception helps the cells in finding an environment with optimal light conditions for photosynthesis.
- Eyespots are the simplest and most common "eyes" found in nature, composed of photoreceptors and areas of bright orange-red pigment granules.
- Signals relayed from the eyespot photoreceptors result in alteration of the beating pattern of the flagella, generating a phototactic response.

Light Harvesting Complexes

- All chlorophyll-based photosynthetic organisms contain some form of light-gathering systems (antenna). These systems function to absorb light and then transfer the energy from the light to an energy trap such as a reaction center.

- Antenna pigments are typically arranged in well-defined, three-dimensional structures, so that only a few energy transfer steps will be required to connect any two pigments in the array.
Energy Funnel

• More distal parts of the antenna system maximally absorb photons at shorter wavelengths than the pigments that are close to the reaction center.

• With each transfer, a small amount of energy from the initial higher energy photon, is lost as heat.

• For the funneling arrangement to work, there must be both a spatial and an energetic ordering of the antenna pigments,
  • the short-wavelength absorbing pigments must be farthest from the reaction center.
  • the longer-wavelength absorbing pigments must be the closer to the reaction center.

• These accessory pigments provide a broader coverage of the solar spectrum than is possible with just chlorophylls.

• Most of the energy is transferred by either non-radiative Förster resonance energy transfer or exciton coupling (for shorter distances).

Forms Of Energy Transfer

- Dipole-Dipole Coupling
- Forster Resonance Energy Transfer

- Shared Delocalized States
- Exciton Coupled Energy Transfer

- Electron Changes Molecule
- Electron Energy Transfer

M1 = Molecule #1, M2 = Molecule #2
Photosynthetic Pigments

- Cartenoids are extended polyenes that absorb light between 400 and 500 nm, and are responsible for most red and yellow colors of fruits and flowers, as well as the fall color of leaves.
- Luteins are accessory pigments in plants.
- Phycoerythrobilin and phycocyanobilin are primary pigments in cyanobacteria and red algae.

http://www.biochem.arizona.edu/classes/bioc462/462b/Miesfeld/Photosynthesis.html
Pigments Energy Transfer

http://plantphys.info/plant_physiology/lightrxn.shtml

Photosystem II
chlorophyll b
P450
lutein
P470
zeaxanthin
P480
β-carotene
P500
lycopene
P510
chlorophyll b
P650
chlorophyll a
P680

In each energy transfer some energy is lost as heat: 2nd law of thermodynamics.

But enough energy is passed to P680 to eject an electron to the electron transport system.

to: ETS

from: H₂O

e⁻
e⁻
Chlorophyll

"Molecular Mechanisms of Photosynthesis," R. E. Blankenship, Figure 4.2
Chlorophyll Biosynthesis

http://www.genome.ad.jp/dbget-bin/get_pathway?org_name=ko&mapno=00860
Spectral Bandwidths for Photon-utilizing Reactions

- **Photosystem I**: The absorbance spectrum for the photosystem I-light harvesting complex I supercomplex (PSI-LHCI) includes both red and blue spectral ranges. The resulting effective spectral bandwidths for PSI were from 406 to 454 nm, with maximum absorbance at 437 nm \((\text{photon}_{437}[u])\), and from 662 to 691 nm, with maximum absorbance at 680 nm \((\text{photon}_{680}[u])\). -- Thylakoid Lumen

- **Photosystem II**: The absorbance spectrum for the photosystem II-light harvesting complex II supercomplex (PSII-LHCII) includes both red and blue spectral ranges. The effective spectral bandwidths for PSII were from 378 to 482 nm, with maximum absorbance at 438 nm \((\text{photon}_{438}[u])\), and from 659 to 684 nm, with maximum absorbance at 673 nm \((\text{photon}_{673}[u])\). -- Thylakoid Lumen

- **Protochlorophyllide photoreductase and divinylprotochlorophyllide photoreductase**: Two distinct spectral ranges of light can transform protochlorophyllide into chlorophyllide. The result was two effective spectral ranges: the first effective spectral bandwidth was from 608 to 666 nm, with maximum activity at 646 nm \((\text{photon}_{646}[h])\), and the second was from 417 to 472 nm, with maximum activity at 450 nm \((\text{photon}_{450}[h])\). -- Chloroplast (biosynthesis of chlorophyll)

- **Vitamin D3 synthesis**: The activity spectrum for this spontaneous reaction was taken from published models. The resulting effective spectral bandwidth was from 281 to 306 nm, with maximum activity at 298 nm \((\text{photon}_{298}[c])\). -- Cytosol

- **Rhodopsin photoisomerase**: The activity spectrum for rhodopsin photoisomerase encodes two distinct phototactic rhodopsin proteins (CSRA and CSRB) that require one and two photons, respectively. The resulting effective spectral bandwidth was from 451 to 526 nm, with a median activity at 490 nm \((\text{photon}_{490}[s])\). -- Eyespot

Prism Reactions for 11 Different Light Sources

• **Solar, lithosphere:** The ASTM G173 spectrum of sunlight measured from Earth’s ground level. ([http://rredc.nrel.gov/solar/spectra/am1.5](http://rredc.nrel.gov/solar/spectra/am1.5))

• **Solar, exosphere:** Spectral irradiance data measured on October 16, 2009 from NASA’s SORCE satellite project (Harder et al, 2000).

• **Soft white incandescent bulb:** Spectral irradiance of an Airam 60 W soft white incandescent light ([http://www.mv.helsinki.fi/aphalo/photobio/lamps.html](http://www.mv.helsinki.fi/aphalo/photobio/lamps.html)).

• **Warm white fluorescent tube:** The relative intensity spectrum in arbitrary units for a Sunbrite 18 W warm white fluorescent light ([http://www.ledmuseum.org](http://www.ledmuseum.org)).

• **Cool white fluorescent tube:** Spectral irradiance of a Sylvania 215 W high output cool white fluorescent ([http://www.mv.helsinki.fi/aphalo/photobio/lamps.html](http://www.mv.helsinki.fi/aphalo/photobio/lamps.html)).

• **Metal halide lamp:** The spectral irradiance of a General Electric MVR 250 metal halide lamp with a clear polycarbonate filter ([http://www.mv.helsinki.fi/aphalo/photobio/lamps.html](http://www.mv.helsinki.fi/aphalo/photobio/lamps.html)).

• **High pressure sodium lamp:** Spectral irradiance of a Sylvania LU 250 high pressure sodium lamp with a clear polycarbonate filter ([http://www.mv.helsinki.fi/aphalo/photobio/lamps.html](http://www.mv.helsinki.fi/aphalo/photobio/lamps.html)).

• **Growth room:** The Spectral irradiance of a Conviron growth room with fluorescent level 3 and incandescent level 3 ([http://www.mv.helsinki.fi/aphalo/photobio/lamps.html](http://www.mv.helsinki.fi/aphalo/photobio/lamps.html)).

• **White LED:** Spectral irradiance of a Hewlett Packard HLMP-CW31 white LED ([http://www.mv.helsinki.fi/aphalo/photobio/lamps.html](http://www.mv.helsinki.fi/aphalo/photobio/lamps.html)).

• **653 nm peak red LED array:** The spectrum of a red LED with peak intensity at 653 nm ([http://mo-www.harvard.edu/Java/MiniSpectroscopy.html](http://mo-www.harvard.edu/Java/MiniSpectroscopy.html)).

• **674 nm peak red LED:** Spectral irradiance of a Quantum Devices QDDH68002 red LED with peak intensity at 674 nm ([http://www.mv.helsinki.fi/aphalo/photobio/lamps.html](http://www.mv.helsinki.fi/aphalo/photobio/lamps.html)).
### Components Associated with PRISM Spectra

<table>
<thead>
<tr>
<th>Name</th>
<th>PRISM Components</th>
<th>LB</th>
<th>UB</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRISM_red_LED_array_653nm</td>
<td>photonVis[e] --&gt; photon646[h] + photon673[u] + photon680[u]</td>
<td>96.62811</td>
<td>96.62811</td>
</tr>
</tbody>
</table>
Constraint-based Metabolic Reconstructions & Analysis

H. Scott Hinton, 2016

Lesson: Large Metabolic Models

"PRISM_solar_exo" PRISM Spectrum

Photosynthesis

\[ \text{photonVis}[e] \rightarrow \text{photon298}[c] + \text{photon437}[u] + \text{photon438}[u] + \text{photon450}[h] + \text{photon490}[s] + \text{photon646}[h] + \text{photon673}[u] + \text{photon680}[u] \]
“PRISM_red_LED_674nm” PRISM Spectrum

\[\text{photonVis}[e] \rightarrow \text{photon298}[c] + \text{photon437}[u] + \text{photon438}[u] + \text{photon450}[h] + \text{photon490}[s] + \text{photon646}[h] + \text{photon673}[u] + \text{photon680}[u]\]

Photosynthesis
Photosynthesis in iRC1080

Photosystem II

Photosystem I

Algae_Photosynthesis.m
Photosynthetic Model Simulation Results

A. \( \text{O}_2 \) photoevolution under solar light. Simulated (blue line) and experimentally measured (green dots) \( \text{O}_2 \) evolution are compared.

B. Photosynthetic growth under red LED light. Simulations were performed using the 653-nm prism reaction, and experimentally grown culture was exposed to 660 nm LED light. Simulated (blue line) and experimentally measured (green dots) growth are compared.

C. Efficiency of light utilization. The minimum photon flux required for maximum-simulated growth (bottom), biomass yield (middle), and energy conversion efficiency (top) are presented for 11 light sources derived from measured spectra and for the designed growth-efficient LED.

### Light and Dark-regulated Reaction Constraints

<table>
<thead>
<tr>
<th>Description</th>
<th>Reaction Abbreviation(s)</th>
<th>Constraint (mmol/gDW/h)</th>
<th>Derivation</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_solar_litho</td>
<td>646.07</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_solar_exo</td>
<td>417.59</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_incandescent_60W</td>
<td>15.94</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_fluorescent_warm_18W</td>
<td>8.10</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_fluorescent_cool_215W</td>
<td>44.63</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_metal_halide</td>
<td>17.53</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_high_pressure_sodium</td>
<td>36.15</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_growth_room</td>
<td>58.47</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_white_LED</td>
<td>4.59</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_red_LED_array_653nm</td>
<td>96.63</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Effective incident photon flux</td>
<td>PRISM_red_LED_674nm</td>
<td>3.65</td>
<td>From data</td>
<td>Light</td>
</tr>
<tr>
<td>Maximum proton uptake rate</td>
<td>EX_h(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum water uptake rate</td>
<td>EX_h2o(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum phosphate uptake rate</td>
<td>EX_pi(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum ammonia uptake rate</td>
<td>EX_nh4(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum nitrate uptake rate</td>
<td>EX_no3(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum sulfate uptake rate</td>
<td>EX_so4(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum ferrous ion uptake rate</td>
<td>EX_fe2(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum ferric ion uptake rate</td>
<td>EX_fe3(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum magnesium ion uptake rate</td>
<td>EX_mg2(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum sodium ion uptake rate</td>
<td>EX_na1(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum oxygen uptake rate</td>
<td>EX_o2(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum carbon dioxide uptake rate</td>
<td>EX_co2(e)</td>
<td>-11.16</td>
<td>From data</td>
<td>Autotrophic</td>
</tr>
<tr>
<td>Maximum acetate uptake rate</td>
<td>EX_ac(e)</td>
<td>-10</td>
<td>Assumed</td>
<td>Heterotrophic</td>
</tr>
<tr>
<td>Non-growth-associated ATP maintenance</td>
<td>ATPM(NGAM)</td>
<td>0.183</td>
<td>From data</td>
<td>Minimal</td>
</tr>
<tr>
<td>Maximum oxygen photoevolution rate</td>
<td>DM_o2(u)</td>
<td>8.28</td>
<td>From data</td>
<td>Autotrophic</td>
</tr>
<tr>
<td>Maximum starch degradation rate</td>
<td>STARCH300DEGRA;STARCH300DEGRB</td>
<td>4.35E-05</td>
<td>From data</td>
<td>Aerobic, light</td>
</tr>
<tr>
<td>Maximum starch degradation rate</td>
<td>STARCH300DEGR2A;STARCH300DEGR2B</td>
<td>1.15E-04</td>
<td>From data</td>
<td>Aerobic, dark</td>
</tr>
<tr>
<td>Maximum starch degradation rate</td>
<td>STARCH300DEGRA;STARCH300DEGRB</td>
<td>6.53E-05</td>
<td>From data</td>
<td>Anaerobic, light</td>
</tr>
<tr>
<td>Maximum starch degradation rate</td>
<td>STARCH300DEGR2A;STARCH300DEGR2B</td>
<td>1.72E-04</td>
<td>From data</td>
<td>Anaerobic, dark</td>
</tr>
</tbody>
</table>

Key Operating Constraints

```matlab
model=readCbModel('IRC1080');
% model = changeRxnBounds(model,'EX_ac(e)',0,'l');
% model = changeRxnBounds(model,'EX_starch(h)',0,'l');

% Set unwanted photon spectrums to zero
model = changeRxnBounds(model,'PRISM_solar_litho',0,'b');
model = changeRxnBounds(model,'PRISM_solar_exo',0,'b');
model = changeRxnBounds(model,'PRISM_incandescent_60W',0,'b');
model = changeRxnBounds(model,'PRISM_fluorescent_warm_18W',0,'b');
model = changeRxnBounds(model,'PRISM_fluorescent_cool_215W',0,'b');
model = changeRxnBounds(model,'PRISM_metal_halide',0,'b');
model = changeRxnBounds(model,'PRISM_high_pressure_sodium',0,'b');
model = changeRxnBounds(model,'PRISM_growth_room',0,'b');
model = changeRxnBounds(model,'PRISM_white_LED',0,'b');
% model = changeRxnBounds(model,'PRISM_red_LED_array_653nm',0,'b');
model = changeRxnBounds(model,'PRISM_red_LED_674nm',0,'b');
model = changeRxnBounds(model,'PRISM_design_growth',0,'b');

% Set unwanted biomass functions to zero
% model = changeRxnBounds(model,'Biomass_Chlamy_auto',0,'b'); % autotrophic (light only)
model = changeRxnBounds(model,'Biomass_Chlamy_mixo',0,'b'); % mixotrophic (light + acetate + starch)
model = changeRxnBounds(model,'Biomass_Chlamy_hetero',0,'b'); % heterotrophic (acetate + starch)
```

Alternate carbon sources

Only one light source should be allowed to be active. Set the others to zero

Disable unused biomass functions
% Algae_PPPA.m

clear;
changeCobraSolver('glpk','all');
% Enter key parameters
model=readCbModel('iRC1080');
model = changeRxnBounds(model,'EX_ac(e)',0,'l');
model = changeRxnBounds(model,'EX_starch(h)',0,'l');
model = changeRxnBounds(model,'PRISM_solar_exo',0,'l');

% Set unwanted photon spectrums to zero
...
% Set unwanted biomass functions to zero
% model = changeRxnBounds(model,'Biomass_Chlamy_auto',0,'b'); % autotrophic
model = changeRxnBounds(model,'Biomass_Chlamy_mixo',0,'b'); % mixotrophic
model = changeRxnBounds(model,'Biomass_Chlamy_hetero',0,'b'); % heterotrophic

% Set objective function
model = changeObjective(model,'Biomass_Chlamy_auto');

[growthRates,shadowPrices1,shadowPrices2]=phenotypePhasePlane(model,'EX_co2(e)','EX_photonVis(e)',50,10,400);
iRC1080 Photosynthetic Ethanol Production Capability

```matlab
% Algae_RA.m
clear;

% Enter key parameters
model = readCbModel('iRC1080');
model = changeRxnBounds(model,'EX_ac(e)',-0,'l');
model = changeRxnBounds(model,'EX_starch(h)',0,'l');
model = changeRxnBounds(model,'PRISM_solar_litho',0,'l');

% Set unwanted photon spectrums to zero
...

% Set unwanted biomass functions to zero
% model = changeRxnBounds(model,'Biomass_Chlamy_auto',0,'b'); % autotrophic
model = changeRxnBounds(model,'Biomass_Chlamy_mixo',0,'b'); % mixotrophic
model = changeRxnBounds(model,'Biomass_Chlamy_hetero',0,'b'); % heterotrophic

% Set objective function
model = changeObjective(model,'Biomass_Chlamy_auto');
[controlFlux, objFlux] = robustnessAnalysis(model,'EX_etoh(e)',100);
```
Lesson Outline

• Overview

• Prokaryotes
  ✓ *Escherichia coli* (Bacteria)
  ✓ *Synechocystis sp. PCC6803* (Cyanobacteria)

• Eukaryotes
  ✓ *Saccharomyces cerevisiae* (Yeast)
  ✓ *Chlamydomonas reinhardtii* (Algae)
  ✓ *Homo sapiens* (Human)
'Google Map' of Human Metabolism

Human Model

(H. sapiens Recon 1)

- In 2007, the genome-scale reconstruction of the global human metabolic network, Recon 1, was published by Duarte et al.
- Recon 1 accounts for 1496 open reading frames, 2004 proteins, 2712 metabolites and 3311 metabolic reactions.
- The network is mass- and charge balanced and fully compartmentalized, accounting for the cytoplasm, nucleus, mitochondria, lysosome, peroxisome, Golgi apparatus and endoplasmic reticulum.
- Recon1 can be transformed into global metabolic reconstructions of similar mammals (e.g. Mus Musculus).
- Recon 1 is available through the BIGG database.

Recon 1 Cell-specific Reconstructions

• Human brain

• Liver

• Kidney

• Aveolar macrophage

• Red Blood Cells
Recon 2 is a consensus metabolic reconstruction integrating metabolic information from five different resources:

- Recon 1, a global human metabolic reconstruction (Duarte et al, PNAS, 104(6), 1777-1782, 2007)
- EHMN, Edinburgh Human Metabolic Network (Hao et al., BMC Bioinformatics 11, 393, 2010)
- HepatoNet1, a liver metabolic reconstruction (Gille et al., Molecular Systems Biology 6, 411, 2010),
- Ac/FAO module, an acylcarnitine/fatty acid oxidation module (Sahoo et al., Molecular bioSystems 8, 2545-2558, 2012),
- A human small intestinal enterocytes reconstruction (Sahoo and Thiele, submitted).

Additionally, more than 370 transport and exchange reactions were added, based on a literature review.
## Comparison of Recon 1 and Recon 2

<table>
<thead>
<tr>
<th>Property</th>
<th>Recon 1</th>
<th>Recon 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of reactions</td>
<td>3,744</td>
<td>7,440</td>
</tr>
<tr>
<td>Total number of metabolites</td>
<td>2,766</td>
<td>5,063</td>
</tr>
<tr>
<td>Number of unique metabolites</td>
<td>1,509</td>
<td>2,626</td>
</tr>
<tr>
<td>Number of metabolites in extracellular space</td>
<td>404</td>
<td>642</td>
</tr>
<tr>
<td>Number of metabolites in cytoplasm</td>
<td>995</td>
<td>1,878</td>
</tr>
<tr>
<td>Number of metabolites in mitochondrion</td>
<td>393</td>
<td>754</td>
</tr>
<tr>
<td>Number of metabolites in nucleus</td>
<td>95</td>
<td>165</td>
</tr>
<tr>
<td>Number of metabolites in endoplasmic reticulum</td>
<td>235</td>
<td>570</td>
</tr>
<tr>
<td>Number of metabolites in peroxisome</td>
<td>143</td>
<td>435</td>
</tr>
<tr>
<td>Number of metabolites in lysosome</td>
<td>217</td>
<td>302</td>
</tr>
<tr>
<td>Number of metabolites in Golgi apparatus</td>
<td>284</td>
<td>317</td>
</tr>
<tr>
<td>Number of transcripts</td>
<td>1,905</td>
<td>2,194</td>
</tr>
<tr>
<td>Number of unique genes</td>
<td>1,496</td>
<td>1,789</td>
</tr>
<tr>
<td>Number of subsystems</td>
<td>90</td>
<td>99</td>
</tr>
<tr>
<td>Number of Exchange Reactions</td>
<td>405</td>
<td>642</td>
</tr>
</tbody>
</table>

Recon 2-based Models

Adrenal gland, glandular cells
Appendix, glandular cells
Appendix, lymphoid tissue
Bone marrow, hematopoietic cells
Breast, glandular cells
Bronchus, respiratory epithelial cells
Cerebellum, cells in granular layer
Cerebellum, cells in molecular layer
Cerebellum, Purkinje cells
Cerebral cortex, glial cells
Cerebral cortex, neuronal cells
cervix, uterine, glandular cells
cervix, uterine, squamous epithelial cells
Colon, glandular cells
duodenum, glandular cells
epididymis, glandular cells
Esophagus, squamous epithelial cells
Fallopian tube, glandular cells
gall bladder, glandular cells
Heart muscle, myocytes
Hippocampus, glial cells
Hippocampus, neuronal cells
Kidney, cells in glomeruli
Kidney, cells in tubules
Lateral ventricle, neuronal cells
Liver, bile duct cells
Liver, hepatocytes
Lung, macrophages
Lung, pneumocytes
Lymph node, germinal center cells
Lymph node, non-germinal center cells
Nasopharynx, respiratory epithelial cells
Ovarian squamous epithelial cells
Ovary, ovarian stroma cells
Pancreas exocrine glandular cells
Pancreas, islets of Langerhans
Parathyroid gland, glandular cells
Placenta, decidual cells
Placenta, trophoblastic cells
Prostate, glandular cells
recon2
recon2_model.xml
recon2_spreadsheet.xls
rectum, glandular cells
salivary gland, glandular cells
seminal vesicle, glandular cells
Skeletal muscle, myocytes
Skin, epidermal cells
Small intestine, glandular cells
Smooth muscle, smooth muscle cells
Spicen, cells in red pulp
Spleen, cells in white pulp
Stomach, lower glandular cells
Stomach, upper glandular cells
Testis, cells in seminiferous ducts
Thyroid, Leydig cells
Thyroid gland, glandular cells
tonsil, germinal center cells
tonsil, non-germinal center cells
tonsil, squamous epithelial cells
Urinary bladder, urothelial cells
Uterus, post-menopause cells in endometrial stroma
Uterus, post-menopause, glandular cells
Uterus, pre-menopause cells in endometrial stroma
Uterus, pre-menopause, glandular cells
Vagina, squamous epithelial cells
Vulva, anal skin, epidermal cells

Recon 2 Cell-specific Reconstructions

• Using Recon 2

• Kidney

• Membrane Transporters
Four Major Applications of Recon Models

I) Utilizing high-throughput data, Recon models can be tailored to cell and tissue-specific networks. The process has been done both algorithmically and manually.

II) Similarly, Recon models have been transformed into other mammalian reconstructions, particularly *M. musculus*. The high overlap of homologous genes in Recon models with similar mammals allows for reconstructing accurate mammalian models quickly.

III) High-throughput data can be interpreted by mapping the data onto Recon model’s metabolic network backbone. This process has been done to study pathological and drug-treated states.

IV) Recon models can be used to simulate and predict phenotypes, providing biological clues to physiology and pathology as well as guiding experimental design.

Challenge When Working with the Recon 2 Model

- Recon 2 represents a “common environment” that is not specific to any tissues or cell type. There is really no such thing as a “common environment.”
- Many of the specific cell models that can be download with Recon 2 are not complete.
- Most of the exchange reaction have been opened up to allow uptake which will give an inaccurate growth rate.
- The environmental conditions for the cell will need to be understood before the exchange constraints can be set.
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  ✓ *Escherichia coli* (Bacteria)
  ✓ *Synechocystis sp. PCC6803* (Cyanobacteria)

• Eukaryotes
  ✓ *Saccharomyces cerevisiae* (Yeast)
  ✓ *Chlamydomonas reinhardtii* (Algae)
  ✓ *Homo sapiens* (Human)
Reflective Questions

1. What is the difference between a GENRE and a GEM?
2. What is the purpose of having many different E.coli metabolic models?
3. What is the difference between the photosynthesis systems of Synechocystis sp. PCC6803 and Chlamydomonas reinhardtii?
4. What is the purpose of the proton motive force?
5. What is the difference between photosystem I and photosystem II?
6. What is the difference between autotrophic, heterotrophic, and mixotrophic conditions?
7. How was the consensus process used to created the Yeast 4 and Yeast 5.0 models?
8. What is the purpose of the extensive photosynthesis system included in Chlamydomonas reinhardtii?
9. How can Recon 2 be effectively used?