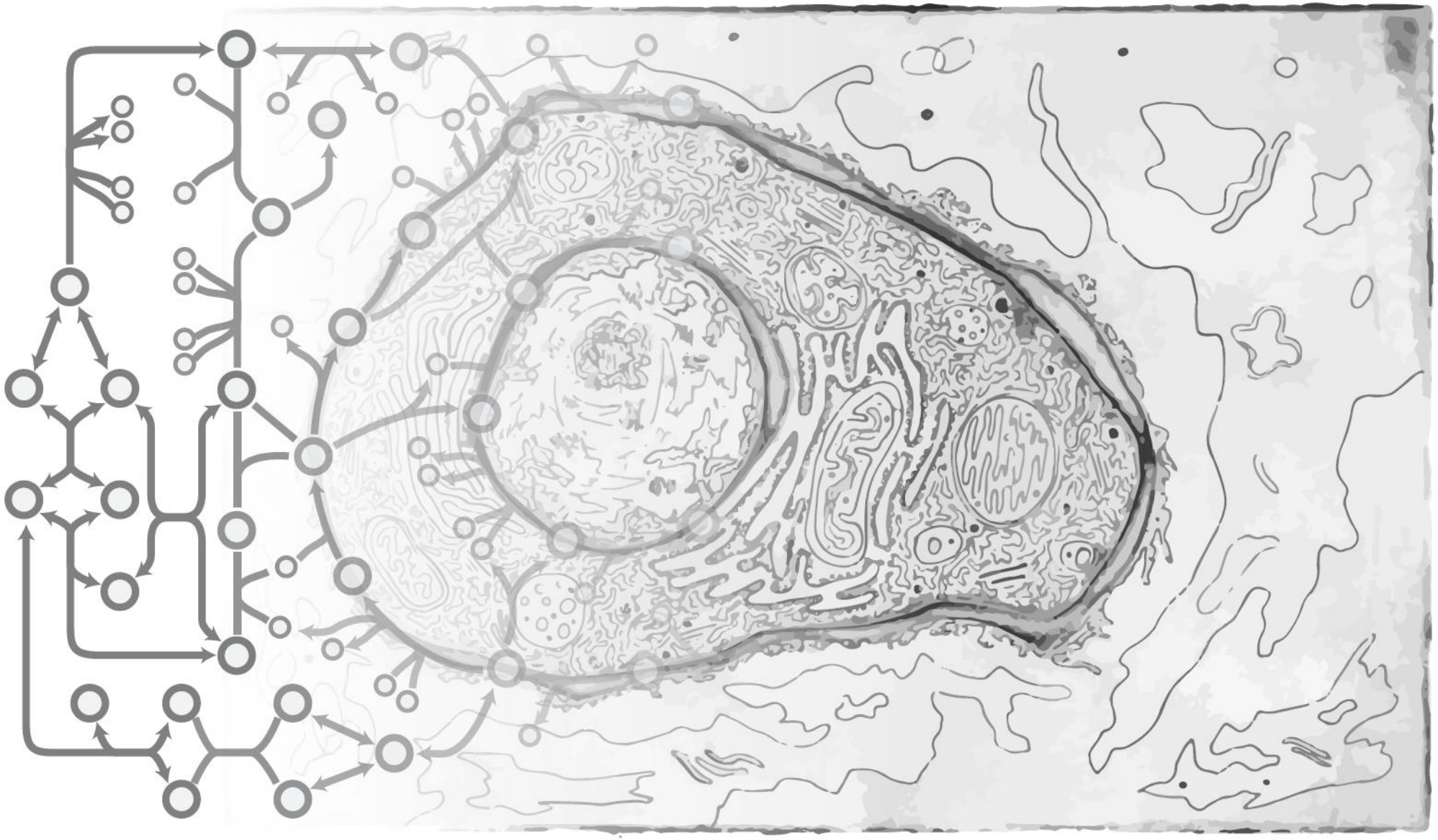
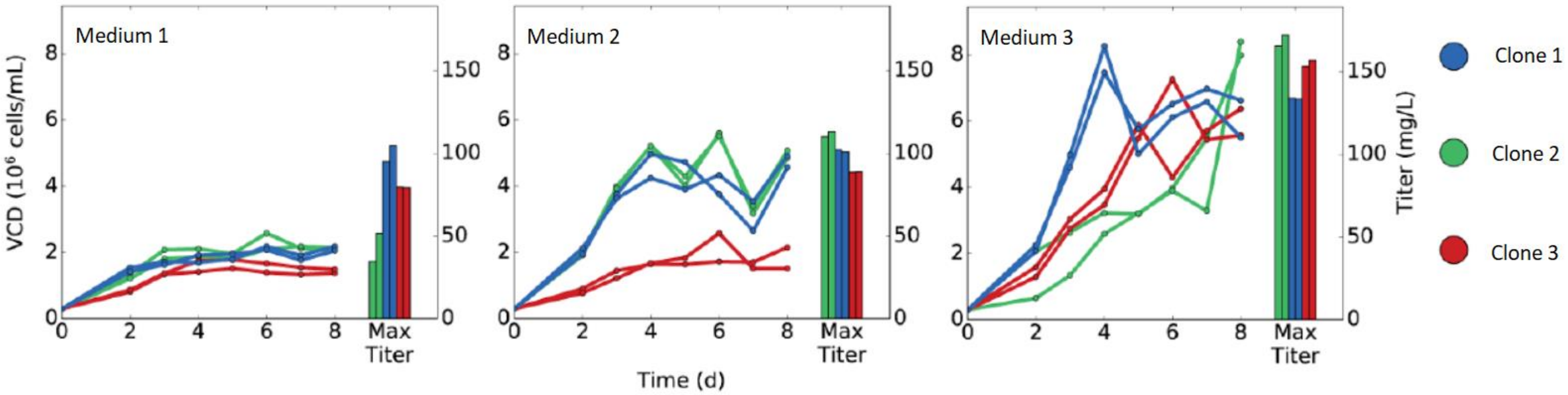


RESOURCES FOR METABOLIC MODELING IN MAMMALIAN CELL CULTURE

Nathan E. Lewis, PhD
Depts of Pediatrics and Bioengineering
University of California, San Diego
May 5, 2019

MODULATING CELL METABOLISM: THE EASIEST WAY TO IMPACT CELL GROWTH AND TITER



HOW INDIVIDUAL METABOLITES IMPACT MAMMALIAN CELL CULTURE

Necessary nutrients

Metabolites providing biomass precursors

- Sugars
- Amino acids

Metabolites impacting enzyme function

- Vitamins and cofactors
- Metal ions

Additives that do other things

- Hormones
- Anti-foam

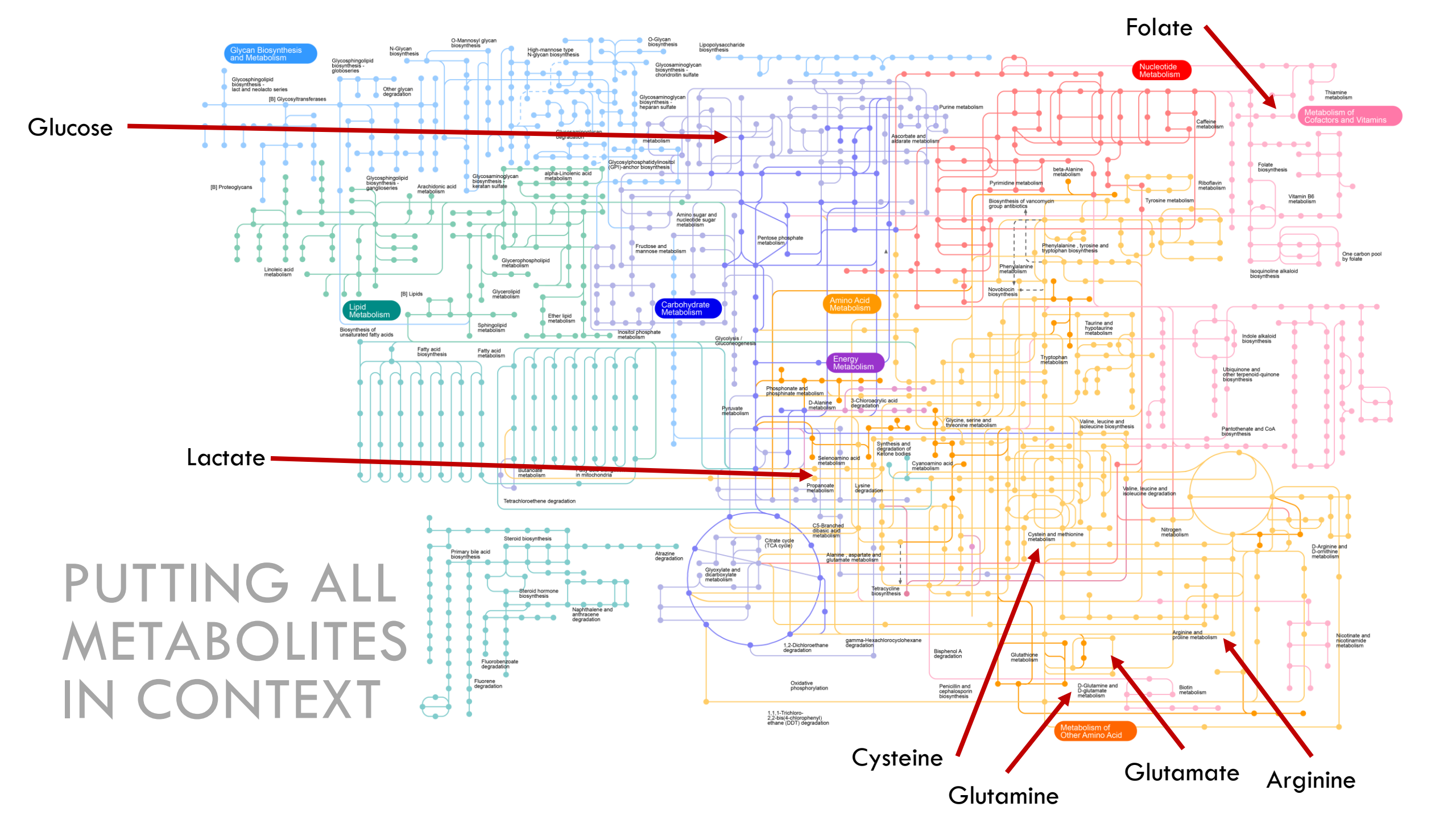
Detrimental metabolites

Substrate competition

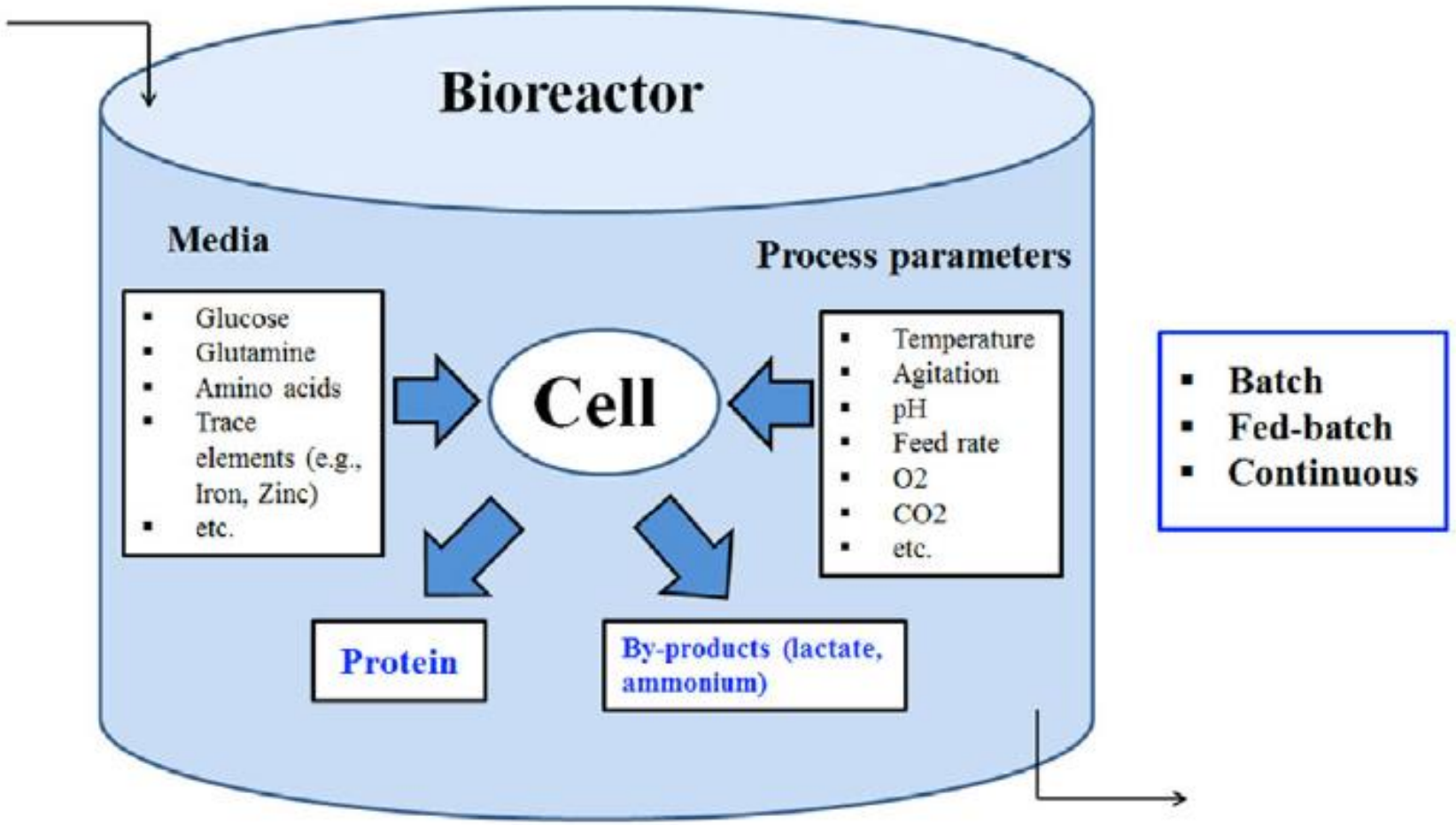
- Imbalanced amino acids competing for transporters
- Sugar nucleotides competing for glycan substrates

Toxic molecules inhibiting growth

- Lactate
- Ammonia
- Excess salts and other osmolites
- Amino acid pathway intermediates



PUTTING ALL METABOLITES IN CONTEXT

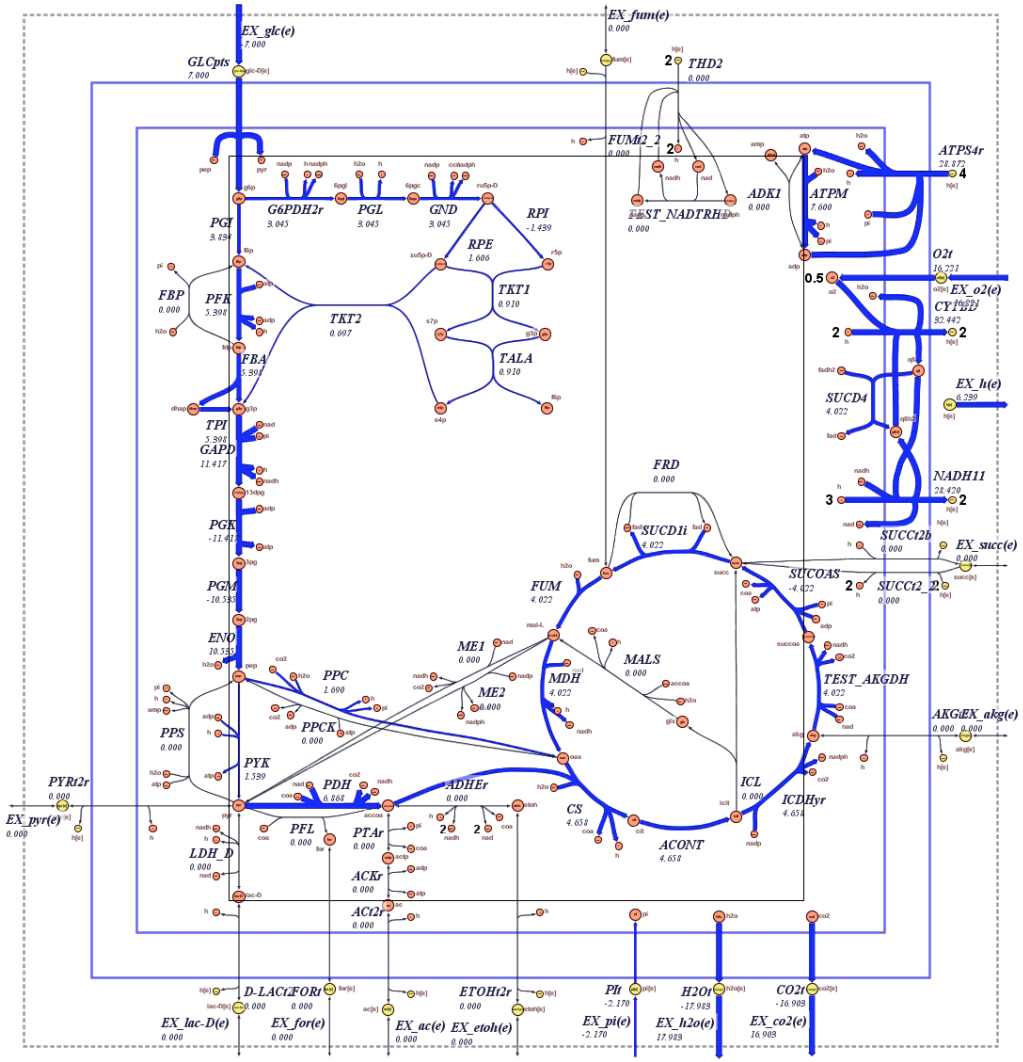


Goals:

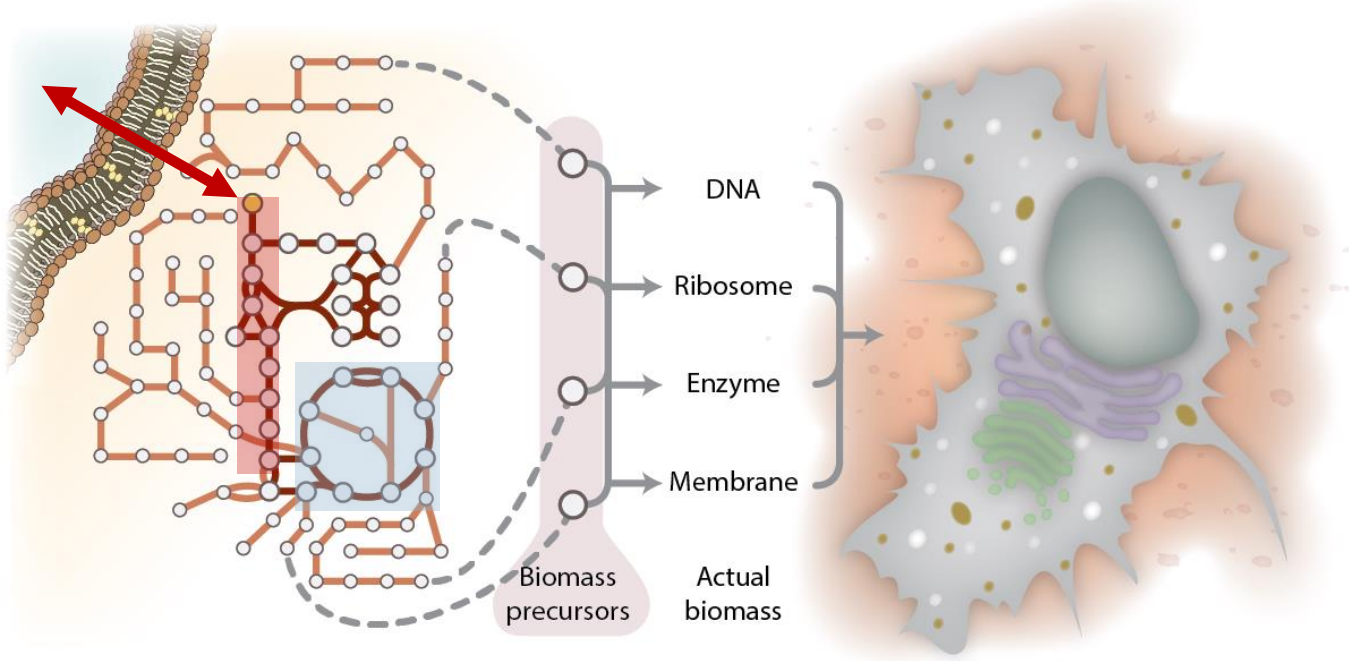
High cell concentration and viability
High specific productivity
High/maintain product quality

Huang, et al., Biotech Bioeng, 2017

A METABOLIC MAP RESOURCE THAT ALLOWS YOU TO SIMULATE



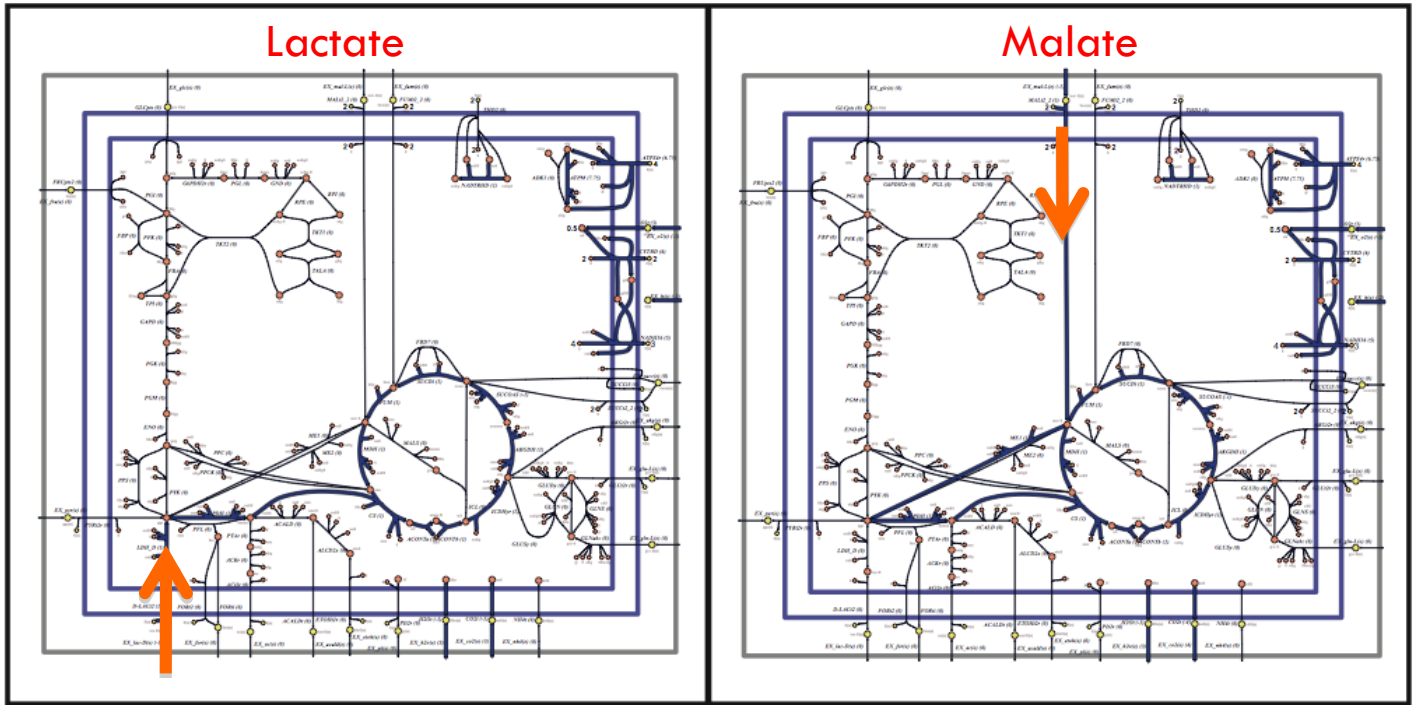
Exo-metabolomics
(spent media analysis)
- Maximum metabolic fluxes in cells

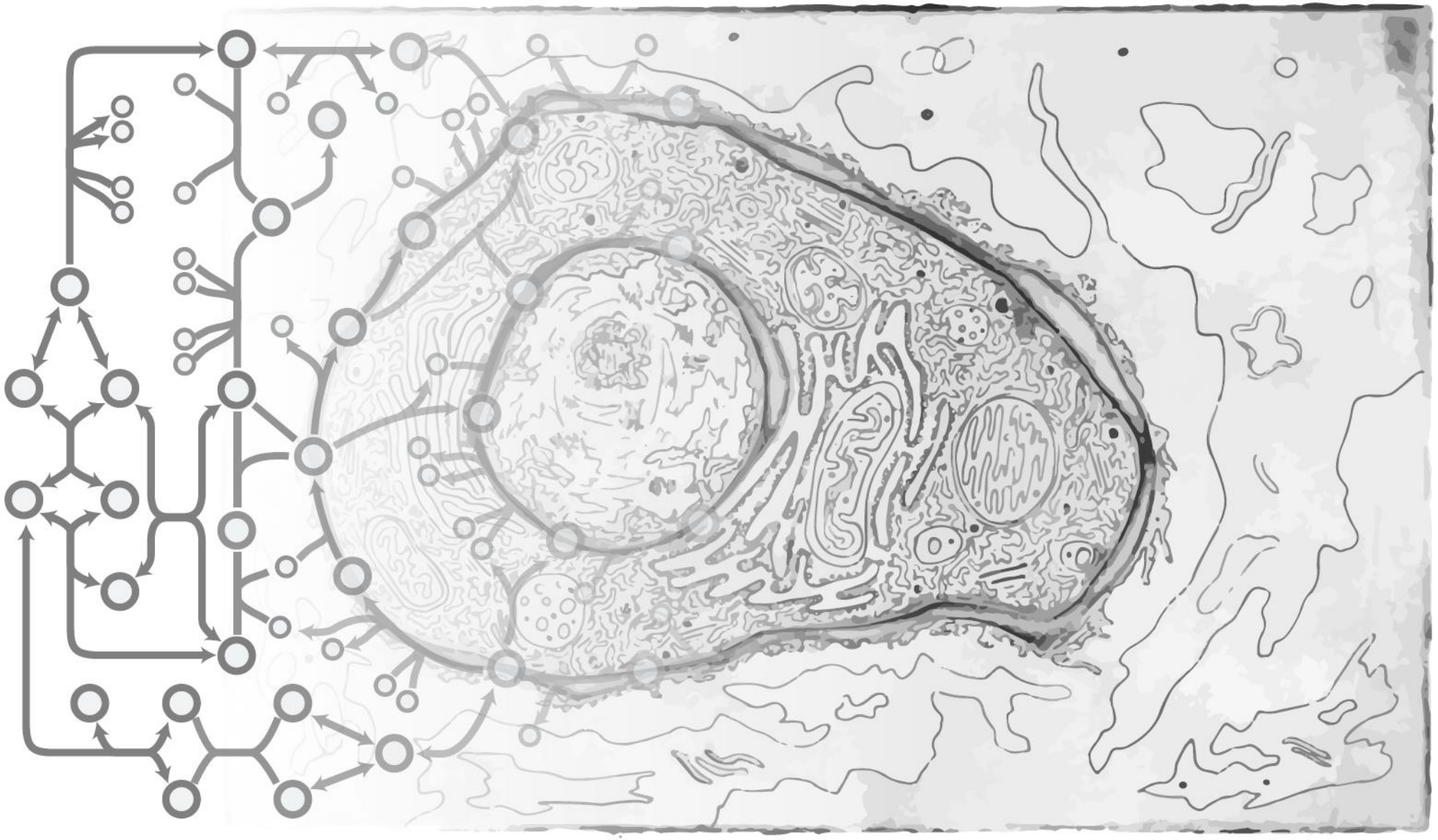


Transcriptomics, Proteomics, etc.
- Expression level of pathways

Endo-metabolomics
- Metabolite changes in cell, may not relate to flux
- May impact regulation

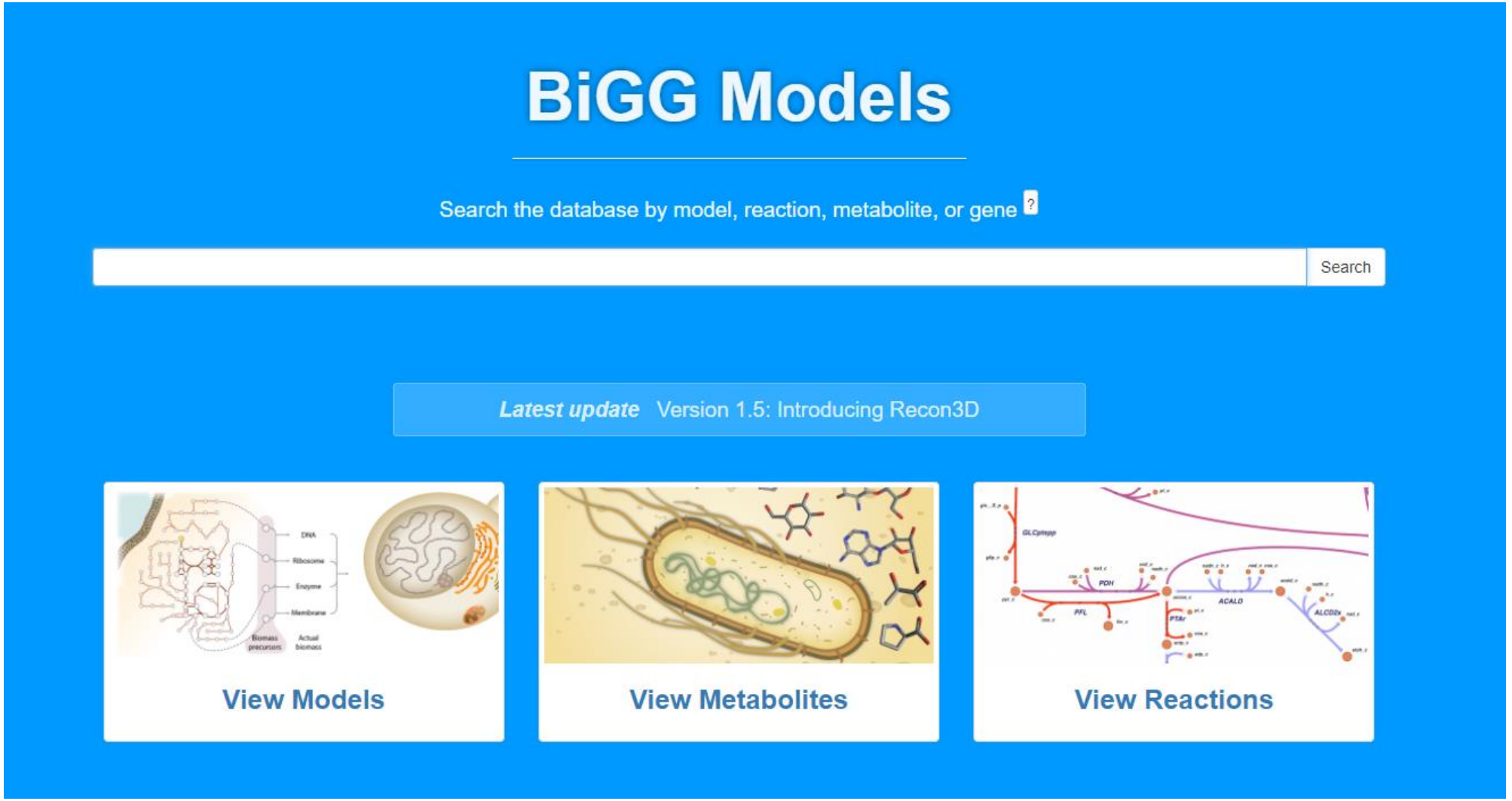
SIMULATING METABOLIC FLUXES UNDER DIFFERENT GROWTH CONDITIONS





WHERE CAN YOU FIND THE CHO METABOLIC MODEL?

HTTP://BIGG.UCSD.EDU – A RESOURCE FOR SEARCHING THE CHO METABOLIC PATHWAYS



CHOgenome.org

Genomic Data Resources

News

Updated CH assembly and CH/CHO-K1 annotations

May 1, 2019

We are excited to announce that the significantly improved Chinese hamster genome, CH PICR (GCF_00366804.1), along with its 2018 RefSeq annotation is now online. In addition, an updated RefSeq annotation for the CHO-K1 cell line is now available. Both can be searched (gene search and BLAST) and viewed on JBrowse. More information on the updated Chinese hamster genome can be found here.

New mRNA Expression Browser (Beta)

June 7, 2017

The browser (beta version) for the visualization of mRNA expression from CHO-K1 cells is now online. The data is from several published DNA-microarray or RNA-Seq experiments. The tutorial on how to use this browser can be found

Genomic Data Resources

This web page contains links to relevant publications and public data sets. Links to next generation sequencing raw data in the Sequence Read Archive (SRA) are provided when available. Journal access may be limited to subscribed users.

Click here to access the original CH and CHO genome data files that are hosted in our own *Cricetulus griseus* File Archive.

Genome

Description	Title	Reference
CHO cell genome-scale metabolism model with 1,766 genes, 6,663 reactions, and integrated -omics data findings	A Consensus Genome-scale Reconstruction of Chinese Hamster Ovary Cell Metabolism	Hefzi H et al. Cell Systems (2016) 3, 434-443. Zipped CHO, CHO-K1, CHO-S, and CHO-DG44 cell line-specific models [.ZIP file]
A chromosome sorting approach was used to facilitate genome assembly from short-read sequences of the Chinese hamster genome	Chinese hamster genome sequenced from sorted chromosomes	Brinkrolf K et al. Nature Biotechnol (2013) 31, 694-695
Draft genomic sequence of the Chinese hamster and resequencing of CHO-K1, DG44, and CHO-K cell lines	Genomic landscapes of Chinese hamster ovary cell lines as revealed by the Cricetulus griseus draft genome	Lewis N et al. Nature Biotechnol (2013) 31, 759-765
Generation of a physical chromosome map of the CHO-DG44 cell line.	Construction of BAC-based physical map and analysis of chromosome rearrangement in Chinese hamster ovary cell lines.	Cao Y et al. Biotechnol Bioeng (2012) 109, 1357-1367 CHO BAC library sequences
Draft genomic sequence and analysis of the	The genomic sequence of the Chinese	Xu X et al. Nature Biotechnol (2011) 29, 735–

Metabolic Engineering 51 (2019) 9–19

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/meteng

Application of a curated genome-scale metabolic model of CHO DG44 to an industrial fed-batch process

Cyrielle Calmels[a,b], Andréa McCann[a,c], Laetitia Malphettes[a], Mikael Rørdam Andersen[b,*]

[a] Department of Upstream Process Sciences, UCB Pharma, Chemin du Foriest 1, 1420 Braine-l'Alleud, Belgium
[b] Department of Biotechnology and Biomedicine, Technical University of Denmark, Søltofts Plads 223, 2800 Kgs, Lyn
[c] Mass Spectrometry Laboratory, University of Liège, Allée du six aout, Liège, Belgium

Cell Systems

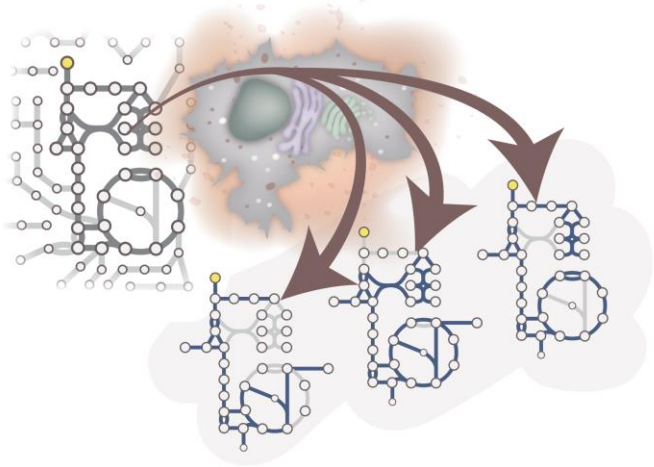
Volume 4, Issue 5, 24 May 2017, Pages 530-542.e6

Article

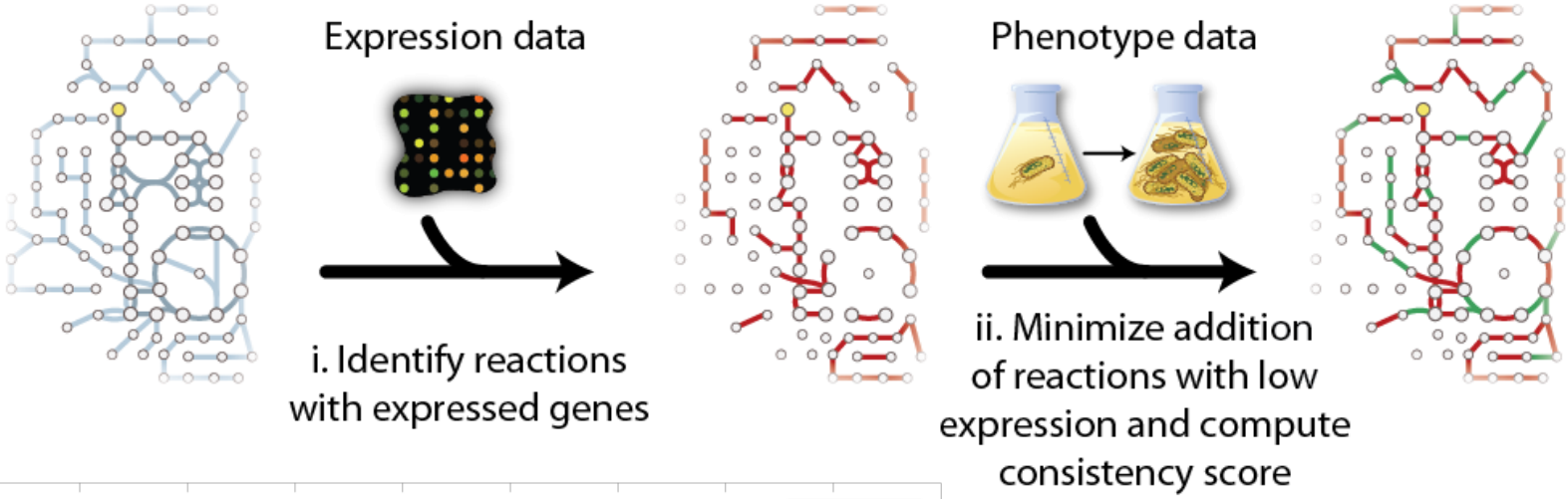
Mammalian Systems Biotechnology Reveals Global Cellular Adaptations in a Recombinant CHO Cell Line

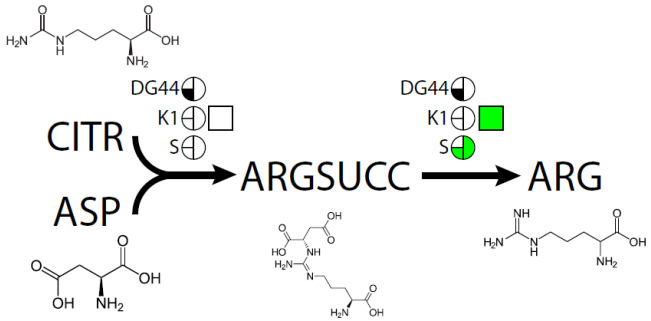
Faraaz Noor Khan Yusufi [1, 6], Meiyappan Lakshmanan [1, 6], Ying Swan Ho [1, 6], Bernard Liat Wen Loo [1], Pramila Ariyaratne [2], Yuansheng Yang [1], Say Kong Ng [1], Tessa Rui Min Tan [1], Hock Chuan Yeo [1, 3], Hsueh Lee Lim [1], Sze Wai Ng [1], Ai Ping Hiu [1], Chung Ping Chow [1], Corrine Wan [1], Shuwen Chen [1], Gavin Teo [1], Gao Song [2], Ju Xin Chin [1] ... Dong-Yup Lee [1, 3, 7]

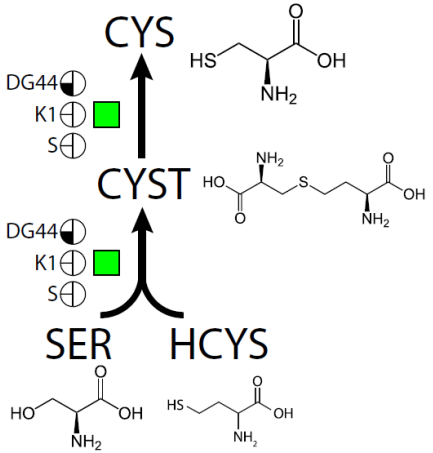
CELL LINE SPECIFIC MODELS

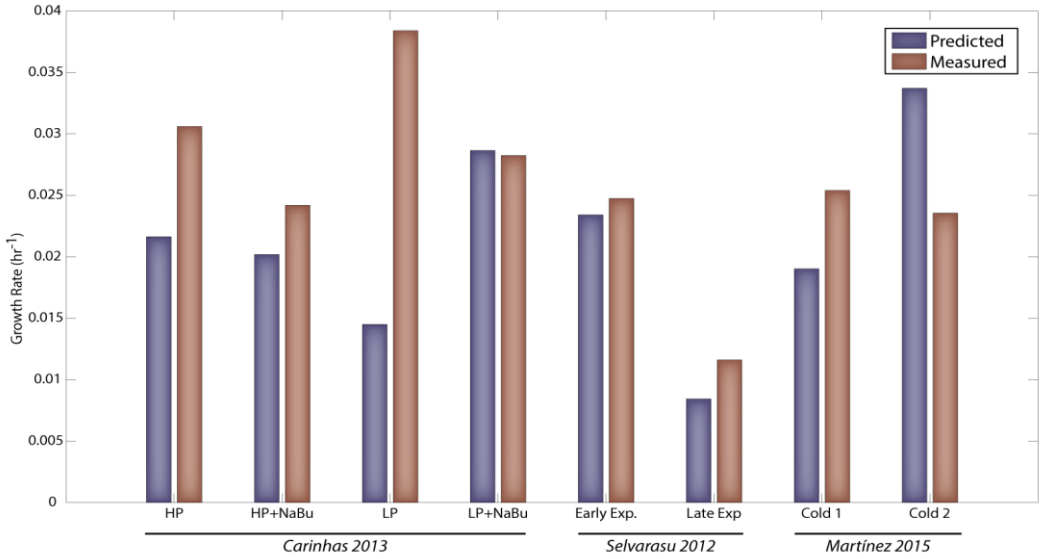


Cell line specific models

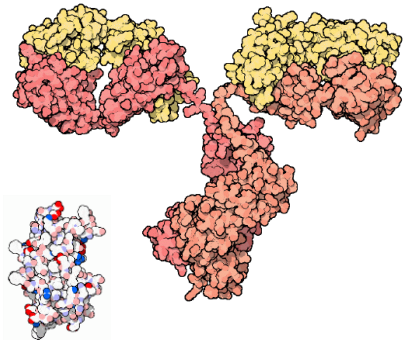








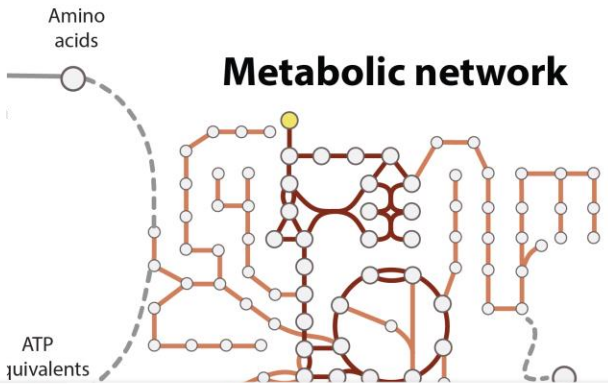
Opdam, et al., Cell Systems, 2017
Richelle, et al. bioRxiv, 2018
Richelle, et al. PLoS Comp Bio, 2019
Joshi, bioRxiv, 2019
Laurent, et al. Nat Protocols, 2019
Hefzi, et al., Cell Systems, 2016



AN EXTENDED FRAMEWORK FOR MODELING PROTEIN SECRETION

Jahir Gutierrez

Amir Feizi



Cold Spring Harbor Laboratory

bioRxiv

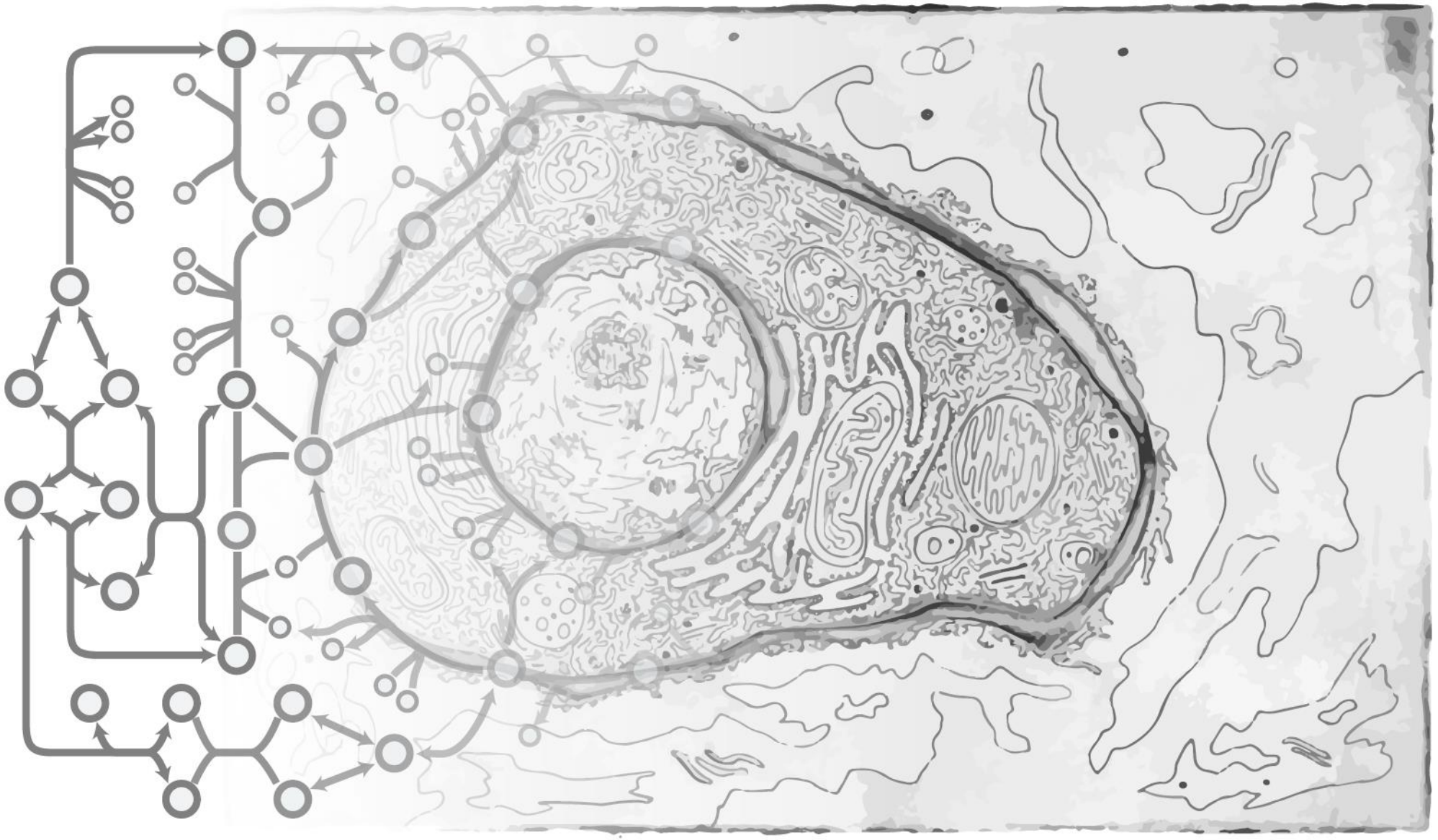
Genome-scale reconstructions of the mammalian secretory pathway predict metabolic costs and limitations of protein secretion

Jahir M. Gutierrez, Amir Feizi, Shangzhong Li, Thomas B. Kallehauge, Hooman Hefzi, Lise M. Grav, Daniel Ley, Deniz Baycin Hizal, Michael J. Betenbaugh, Bjorn Voldborg, Helene Faustrup Kildegaard, Gyun Min Lee, Bernhard O. Palsson, Jens Nielsen, Nathan E. Lewis

261 machinery proteins

Product-specific needs: Nutrients and machinery

Gutierrez, Feizi, et al. bioRxiv, 2018



HOW DO I USED A CHO METABOLIC MODEL?

openCOBRA

Open-source, community-developed code base for COnstraint-Based Reconstruction and Analysis.

RUNNING SIMULATIONS:
COBRA TOOLBOX (MATLAB)
COBRAPY (PYTHON)

ALSO:
SYBIL (R)
OPTFLUX (JAVA)

Packages

The COBRA Toolbox

The COnstraint-Based Reconstruction and Analysis (COBRA) Toolbox written in MATLAB.

COBRApy

COBRApy is a package for constraint-based modeling of biological networks written in Python.

COBRA.jl

High-level, high-performance, constraint-based reconstruction and analysis in Julia.

_computational BIOLOGY

PRIMER

GETTING STARTED

What is flux balance analysis?

Jeffrey D Orth, Ines Thiele & Bernhard Ø Palsson

Flux balance analysis is a mathematical approach for analyzing the flow of metabolites through a metabolic network. This primer covers the theoretical basis of the approach, several practical examples and a software toolbox for performing the calculations.

nature protocols

PROTOCOL UPDATE

https://doi.org/10.1038/s41596-018-0098-2

Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0

Laurent Heirendt[1,24], Sylvain Arreckx[1,24], Thomas Pfau[2], Sebastián N. Mendoza[3,4], Anne Richelle[5], Almut Heinken[1], Hulda S. Haraldsdóttir[1], Jacek Wachowiak[1], Sarah M. Keating[6], Vanja Vlasov[1], Stefania Magnusdóttir[1], Chiam Yu Ng[7], German Preciat[1], Alise Žagare[1], Siu H. J. Chan[7], Maike K. Aurich[1], Catherine M. Clancy[1], Jennifer Modamio[1], John T. Sauls[8], Alberto Noronha[1], Aarash Bordbar[9], Benjamin Cousins[10], Diana C. El Assal[1], Luis V. Valcarcel[11], Iñigo Apaolaza[11], Susan Ghaderi[1], Masoud Ahookhosh[1], Marouen Ben Guebila[1], Andrejs Kostromins[12], Nicolas Sompairac[13], Hoai M. Le[1], Ding Ma[14], Yuekai Sun[15], Lin Wang[7], James T. Yurkovich[16], Miguel A. P. Oliveira[1], Phan T. Vuong[1], Lemmer P. El Assal[1], Inna Kuperstein[13], Andrei Zinovyev[13], H. Scott Hinton[17], William A. Bryant[18], Francisco J. Aragón Artacho[19], Francisco J. Planes[11], Egils Stalidzans[12], Alejandro Maass[3,4], Santosh Vempala[10], Michael Hucka[20], Michael A. Saunders[14], Costas D. Maranas[7], Nathan E. Lewis[5,21], Thomas Sauter[2], Bernhard Ø. Palsson[16,22], Ines Thiele[1] and Ronan M. T. Fleming[1,23*]

Received: 3 October 2016 | Revised: 7 June 2017 | Accepted: 12 July 2017

DOI: 10.1002/bit.26384

REVIEW

WILEY BIOTECHNOLOGY BIOENGINEERING

Quantitative intracellular flux modeling and applications in biotherapeutic development and production using CHO cell cultures

Zhuangrong Huang[1] | Dong-Yup Lee[2,3] | Seongkyu Yoon[1]

The COBRA Toolbox

Search docs

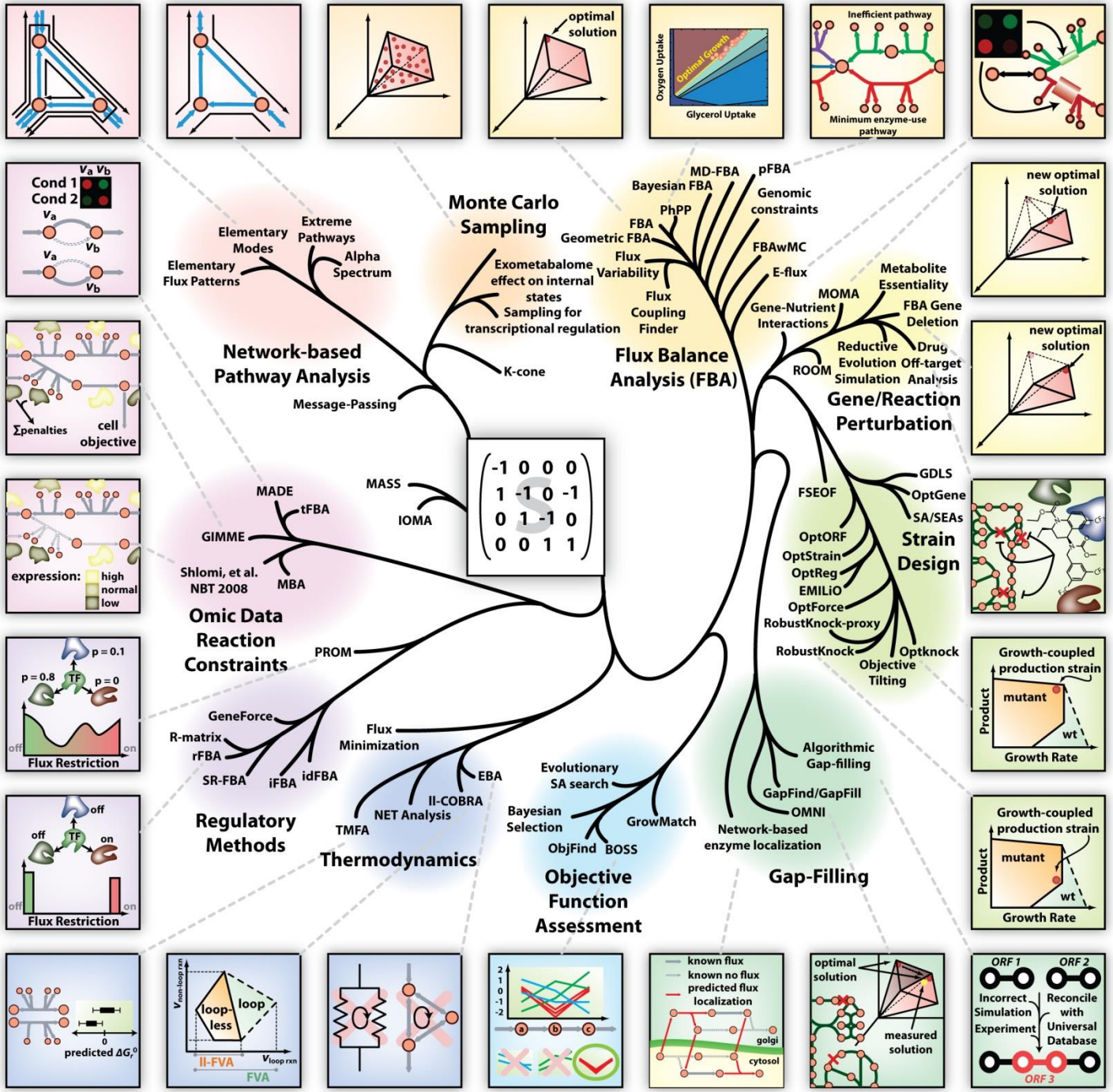
Home
Installation
Functions
Tutorials
Analysis
Base
Data integration
Design
Reconstruction
Visualization
How to contribute
How to cite
Support

Tutorials

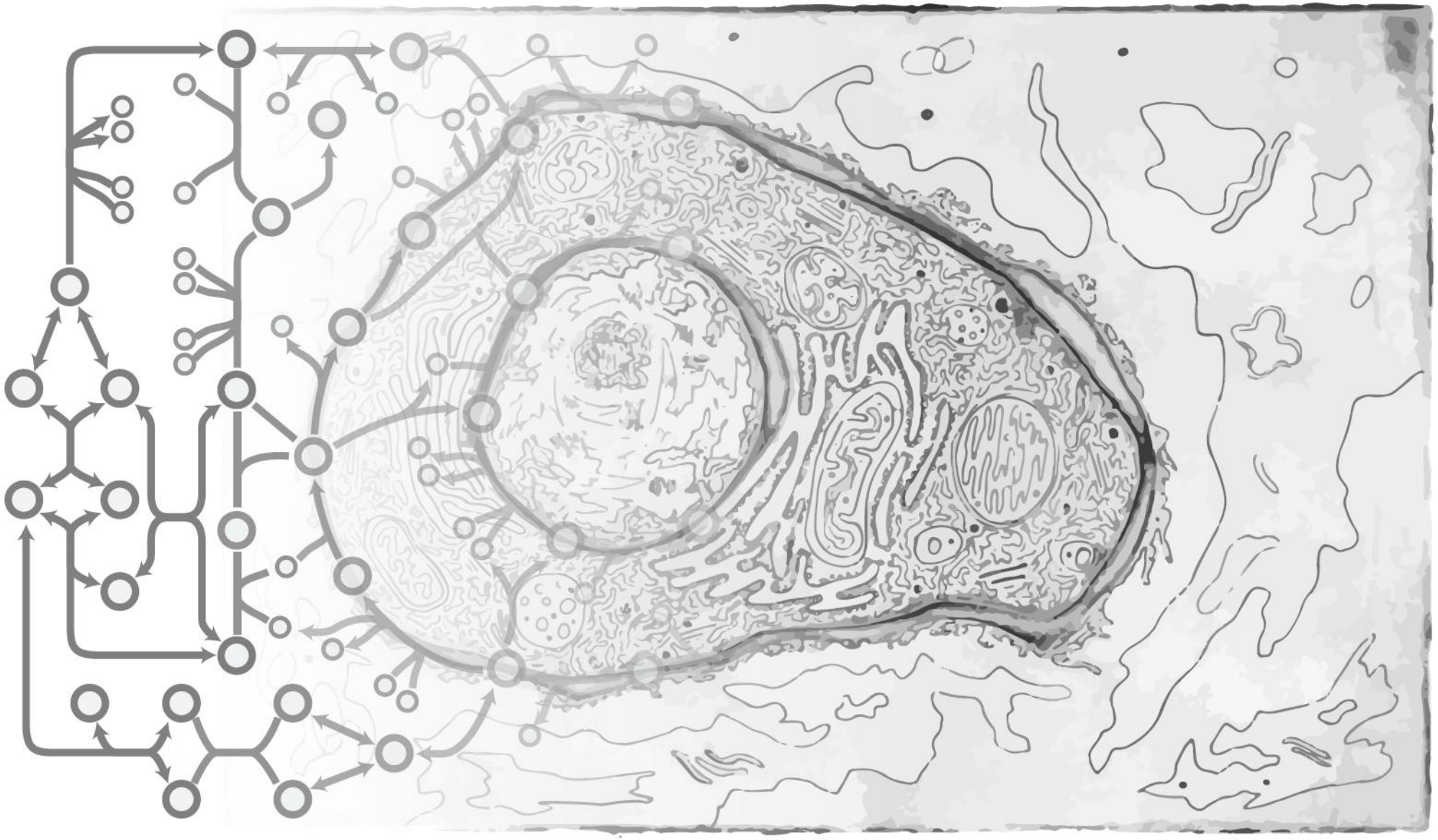
Analysis

- A step-by-step guide to parsimoneous enzyme usage Flux Balance Analysis - pFBA
- Analyze Steady-State Community COBRA Models
- Atomically resolve a metabolic reconstruction
- Browse Networks in the Matlab Command Window Using surfNet
- Computation and analysis of microbe-microbe metabolic interactions
- Computation and analysis of rescued lethal gene deletions in a host-microbe model
- Creation and simulation of personalized microbiota models through metagenomic data integration
- Determining MinSpan vectors of COBRA model
- Flux Balance Analysis (FBA)
- Flux Variability analysis (FVA)
- genetic Minimal Cut Sets - gMCS
- Proton shuttle testing with sparse flux balance analysis
- Reaction essentiality across multiple models
- Relaxed Flux Balance Analysis: Recon 3
- Relaxed Flux Balance Analysis: Toy model
- Simulation of growth of human gut microbes on different diets
- Sparse Flux Balance Analysis
- Sparse flux balance analysis test for a minimial stoichiometrically balanced cycle involving ATP

The toolbox of constraint-based methods for computational modeling goes far beyond FBA… now >200 methods



Lewis, et al. Nature Reviews Microbiology, 2012



HOW DO I VISUALIZE SIMULATIONS OR DATA ON A METABOLIC MODEL?

Escher

https://escher.github.io/#/

Apps | Virtual EMS | Save to Mendeley

GitHub | Docs | What's new?

ESCHER

Build, share, and embed visualizations of metabolic pathways

Filter by organism

All

Map

Glycolysis TCA PPP (RECON1)

Model (Optional)

RECON1

Tool

Builder

Options

- [ ] Scroll to zoom (instead of scroll to pan)
- [ ] Never ask before reloading

Load map

12:50 AM

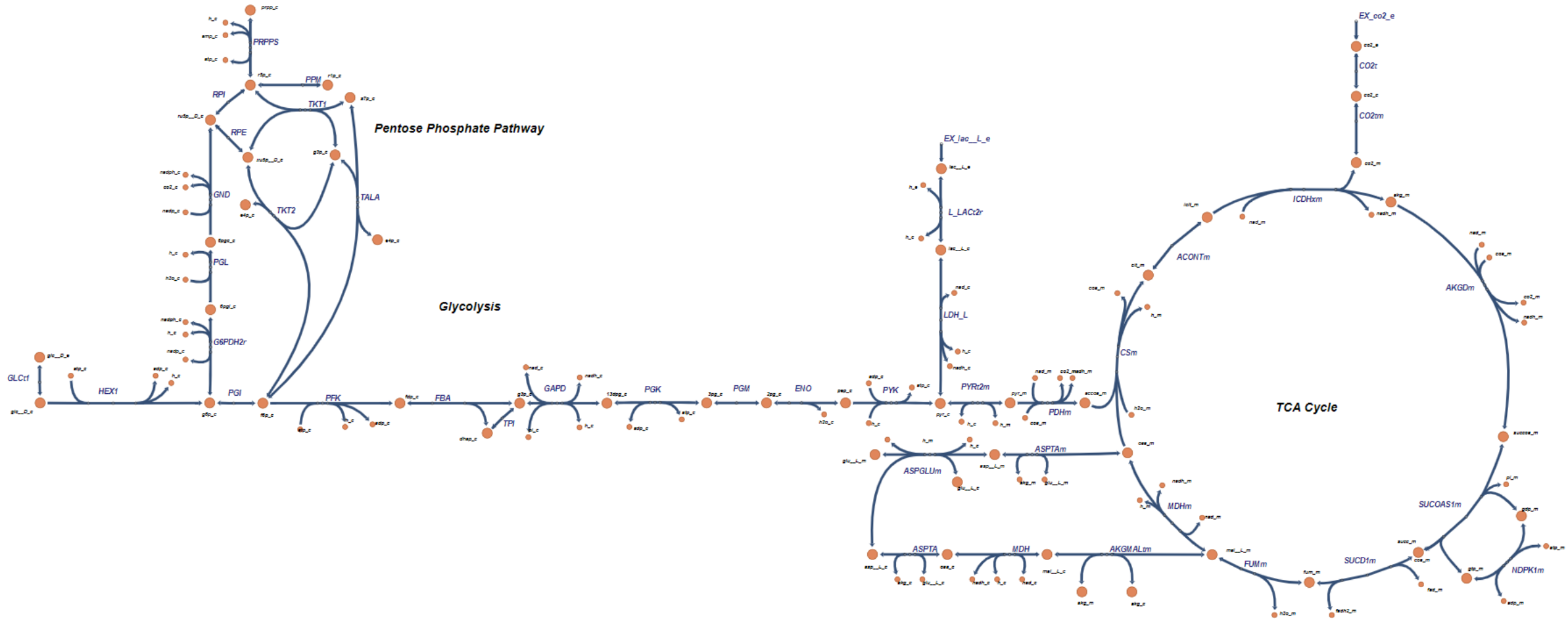
Escher

Escher

https://escher.github.io/#/app?map=RECON1.Glycolysis%20TCA%20PPP&tool=Builder&model=RECON1

Apps Virtual EMS Save to Mendeley

Map Model Data Edit View ?



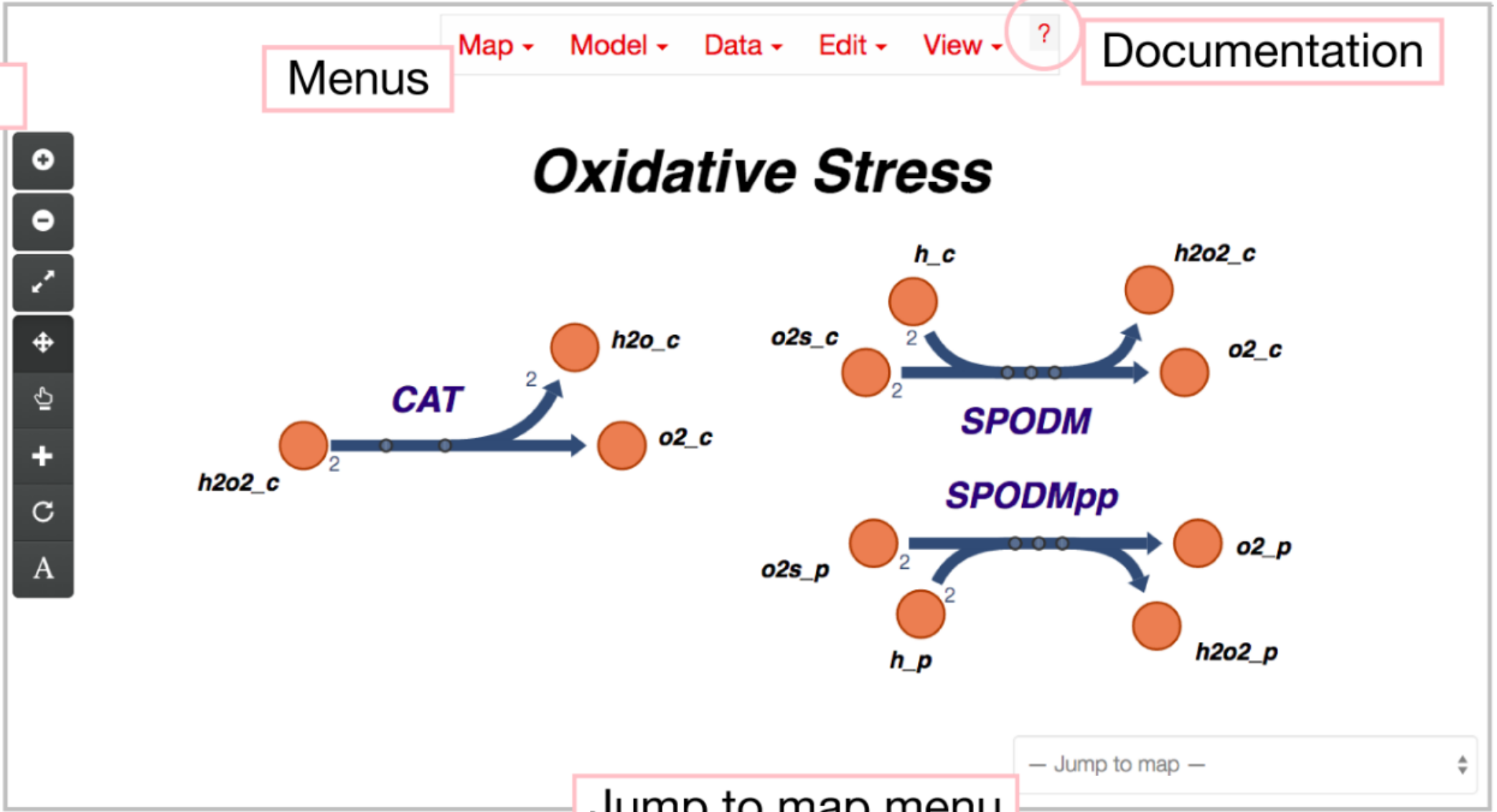
Glycolysis TCA PPP (RECON1)

12:51 AM

Button bar

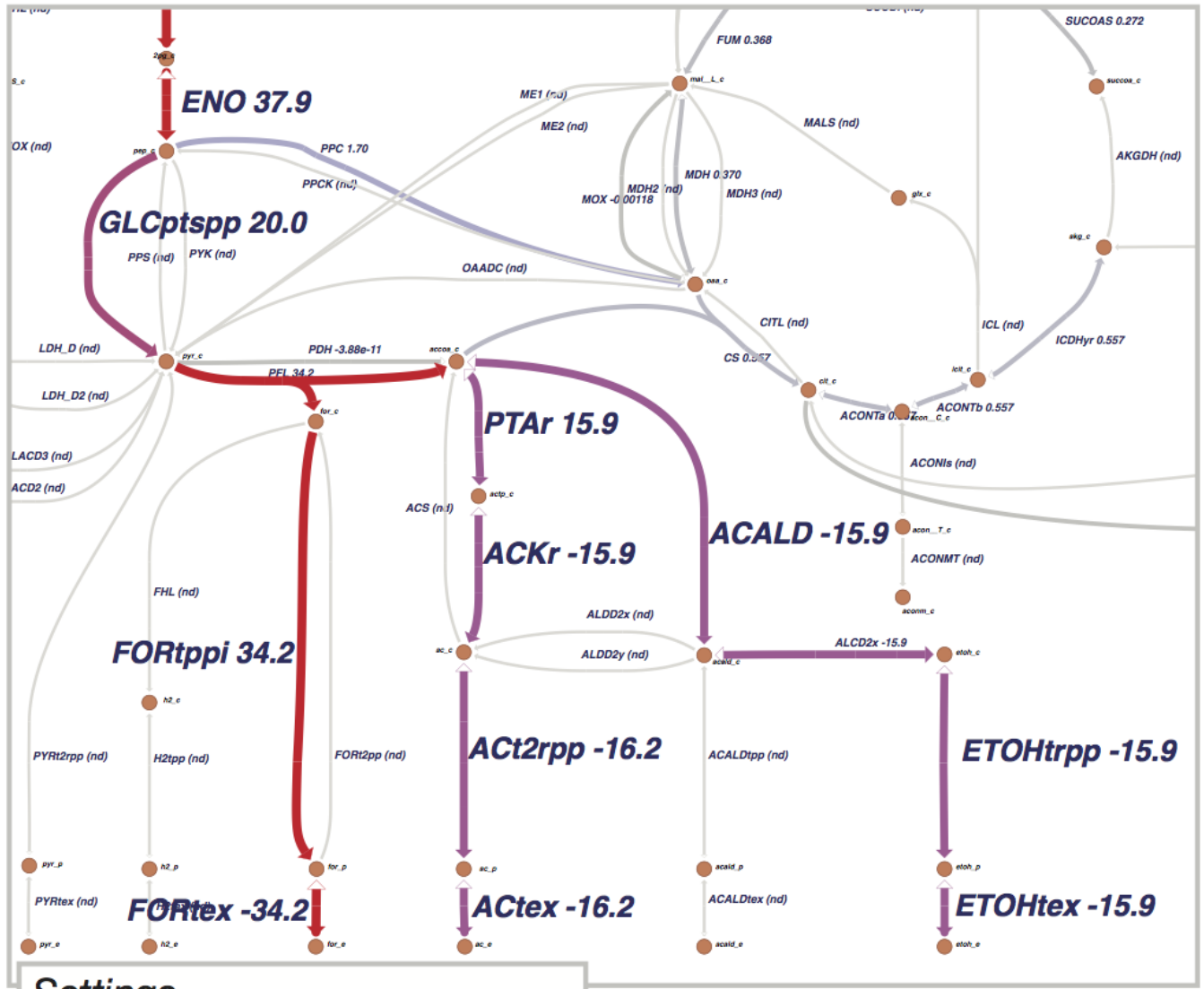
Menus

Documentation



Jump to map menu

Visualizing reaction flux



Settings

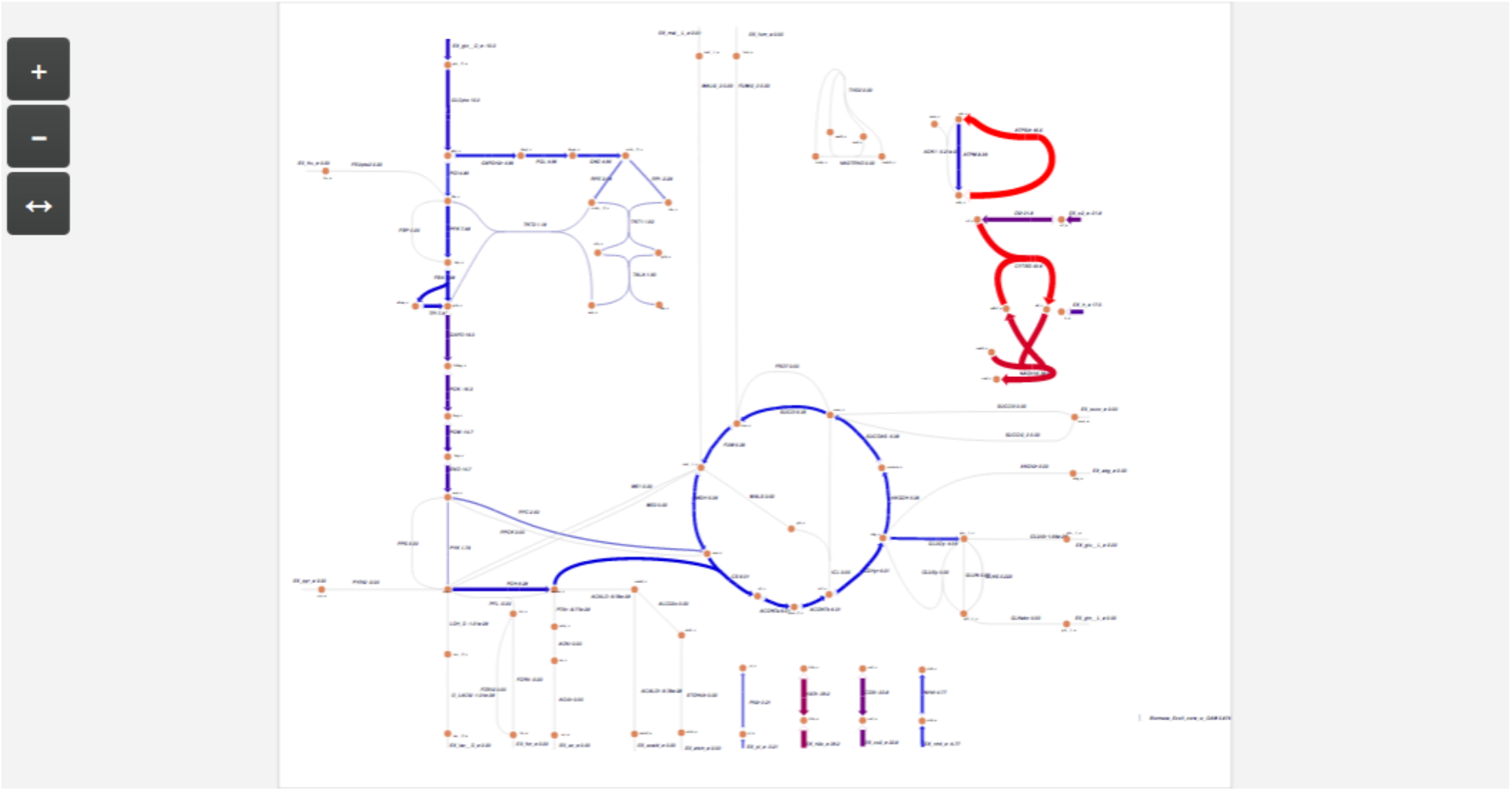
✓ Hide secondary metabolites

✓ Absolute value for reactions

ESCHER AND IPYTHON

In [10]:
```
b = escher.Builder(map_name='e_coli_core.Core metabolism',
                   reaction_data=solution.x_dict,
                   # color and size according to the absolute value
                   reaction_styles=['color', 'size', 'abs', 'text'],
                   # change the default colors
                   reaction_scale=[{'type': 'min', 'color': '#cccccc', 'size': 4},
                                   {'type': 'mean', 'color': '#0000dd', 'size': 20},
                                   {'type': 'max', 'color': '#ff0000', 'size': 40}],
                   # only show the primary metabolites
                   hide_secondary_metabolites=True)
b.display_in_notebook()
```

Out[10]:



SUMMARY

Metabolic modeling elucidates the inner workings of metabolism in a cell

Genome-scale metabolic models exist for the Chinese hamster and others

- http://CHOgenome.org/
- http://bigg.ucsd.edu/
- https://www.metanetx.org/

Models can be trimmed to focus on cell lines (or even subsets of pathways)

Simulation tools exist in several languages: Matlab, Python, R, Java, Julia, etc.

Visualization tools can be deployed using web-based tools or others

- http://escher.github.io/

These slides can be downloaded at http://lewislab.ucsd.edu