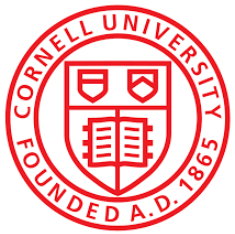


Modeling Biological Systems With Cybernetic Control Laws and Steady State Flux Distributions

Master of Science Exam
Department of Chemical and Biomolecular Engineering
Kritika Kashyap
Committee Member : Jeffrey D. Varner (Chair)
Matthew DeLisa

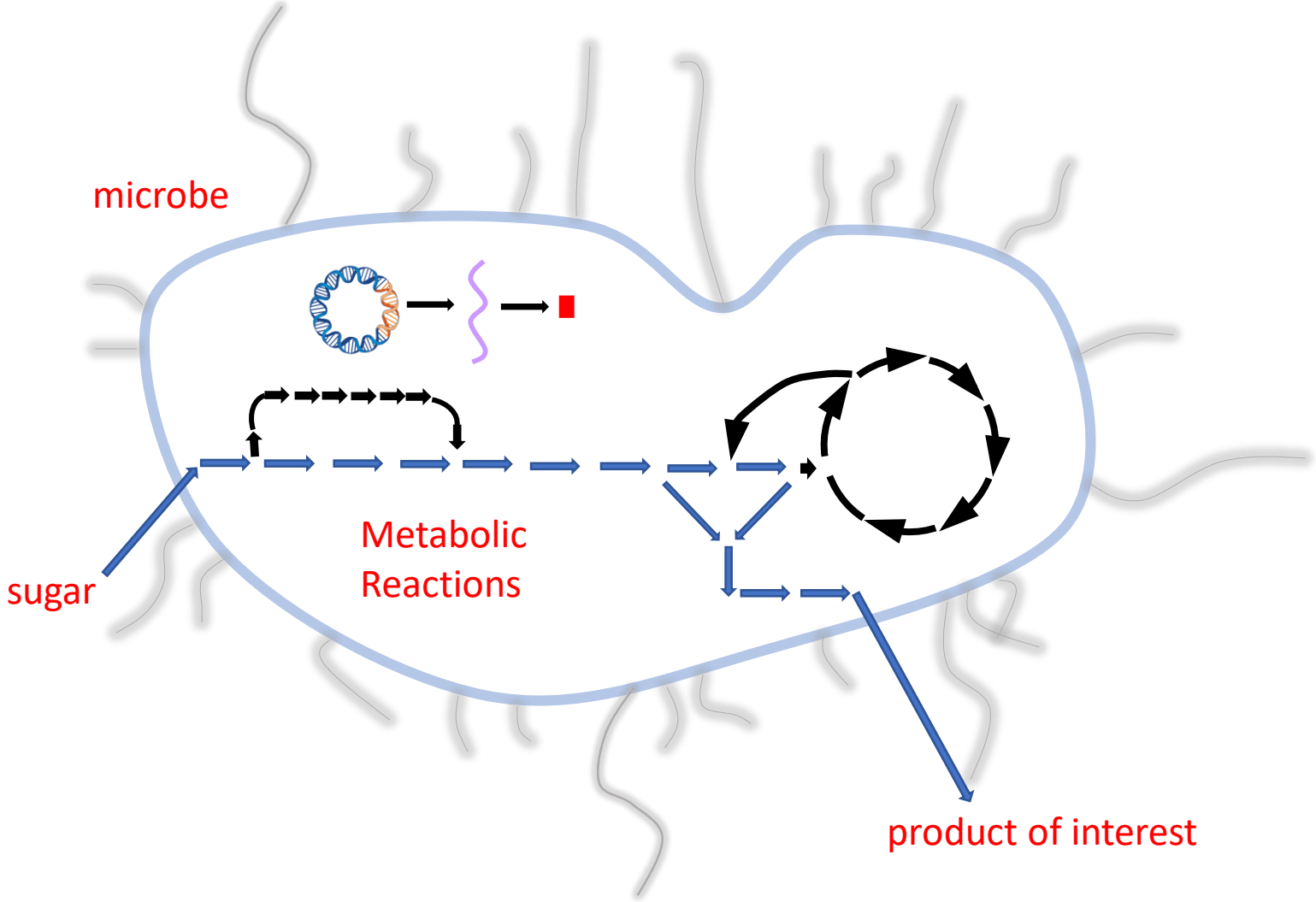
Overview

Review Basic Concepts

Model of *Escherichia coli* (*E. coli*) cells

Model of *Chinese Hamster Ovary* (*CHO*) cells

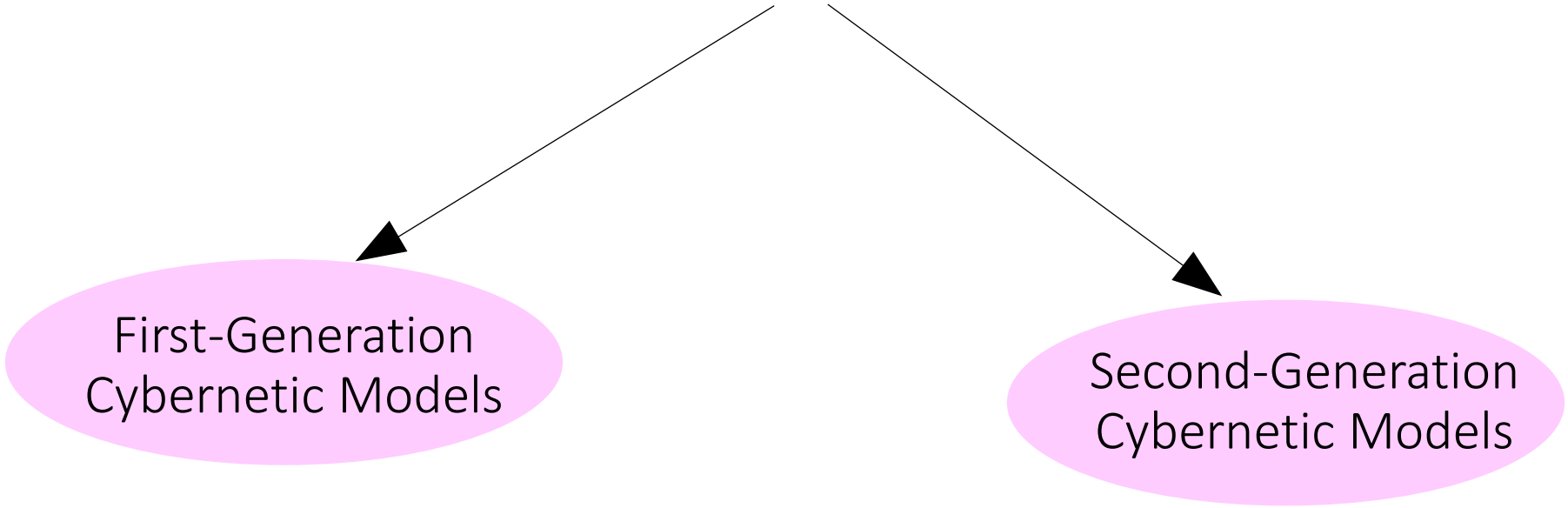
Motivation : Metabolic Engineering of Organisms



Cybernetic Models

1. Assumption : The ultimate goal of an organism is to grow and survive

2. A cell will act, sense, compare itself to its goal and self correct its inner functions until it can successfully achieve its goal



First-Generation Cybernetic Models

$$\min T$$

$$\frac{dx}{dt} = f(x,u)$$

$$\sum_{j=0}^{m} u_j = 1$$

mid 1980's

Ramkrishna introduces cybernetic modeling

1985

Dhurjati et al.

1986

Kompala et al.

objective *max growth*

$p_j = r_j$

$u_j = \frac{p_j}{\sum p_j}$

$v_j = \frac{p_j}{max\ p_j}$

1988

Turner et al.

induced enzyme synthesis

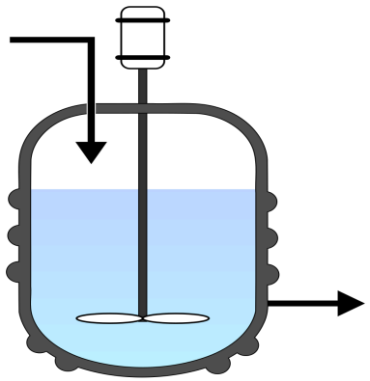
constitutive enzyme synthesis

1997

Narang et al.

1991

Baloo et al.



Second-Generation Cybernetic Models

1996

Straight et al.

1999

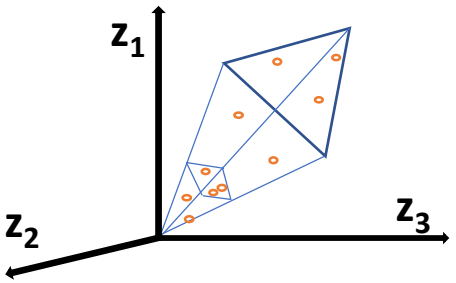
Varner et al.

Local Control

Global Control

2007

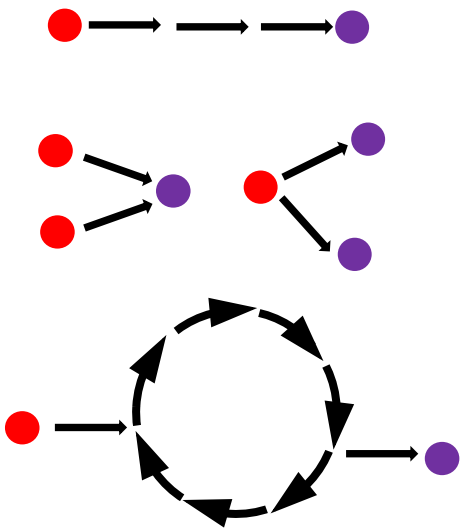
Young et al.



Flux Analysis

2008

Kim et al.



What are elementary flux modes?

$$\sum_{j=1}^{R} S_{ij}\, z_j = 0$$

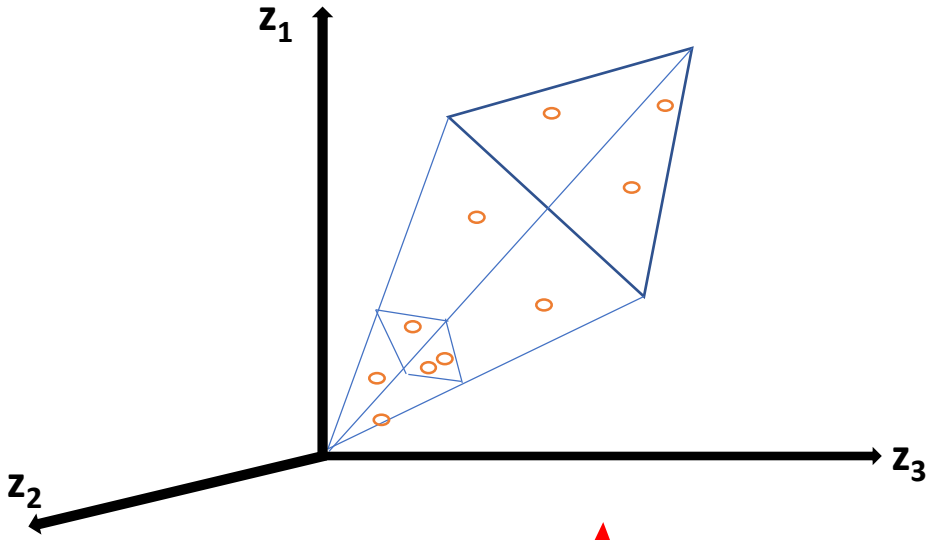
for all i = 1,..,M

j = 1,…,R

(Steady state assumption)

constraints

$lb_j \le z_j \le ub_j$



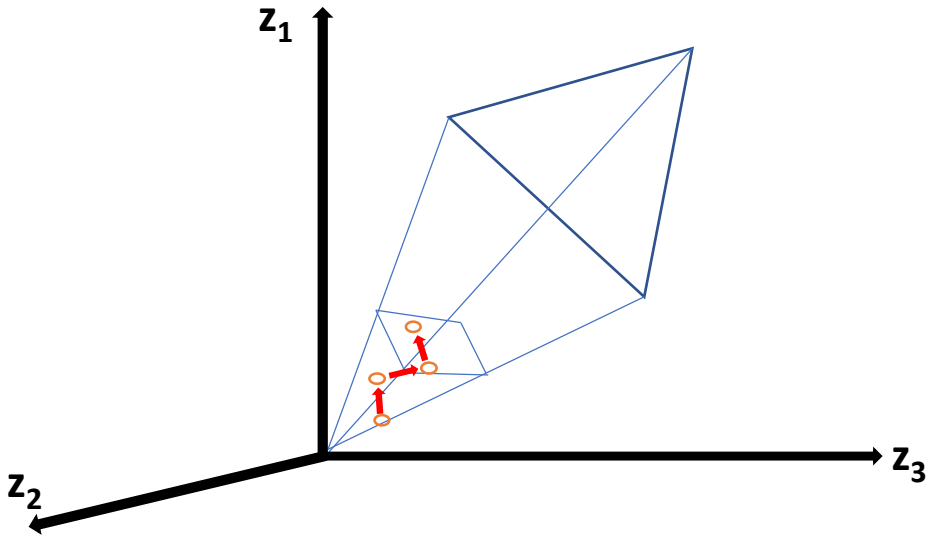
Computationally Expensive

Mathematically Challenging

Steady State Flux Analysis

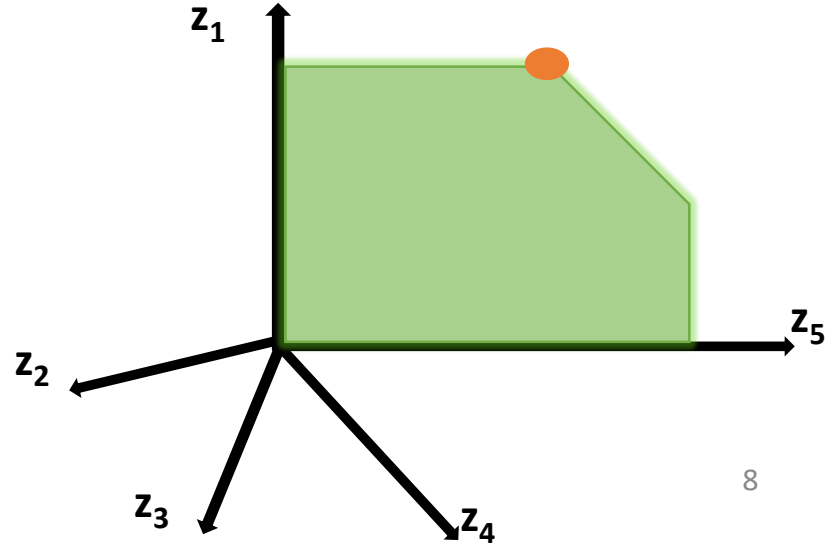
Markov-Chain Monte-Carlo

UnBiased Flux Analysis



Flux Balance Analysis

Biased Flux Analysis



Flux Balance Analysis

Intracellular fluxes are difficult to measure

Extracellular fluxes are relatively easier to measure and are used to constrain the solution space

Mathematical Formulation

$$max\ c^T z$$

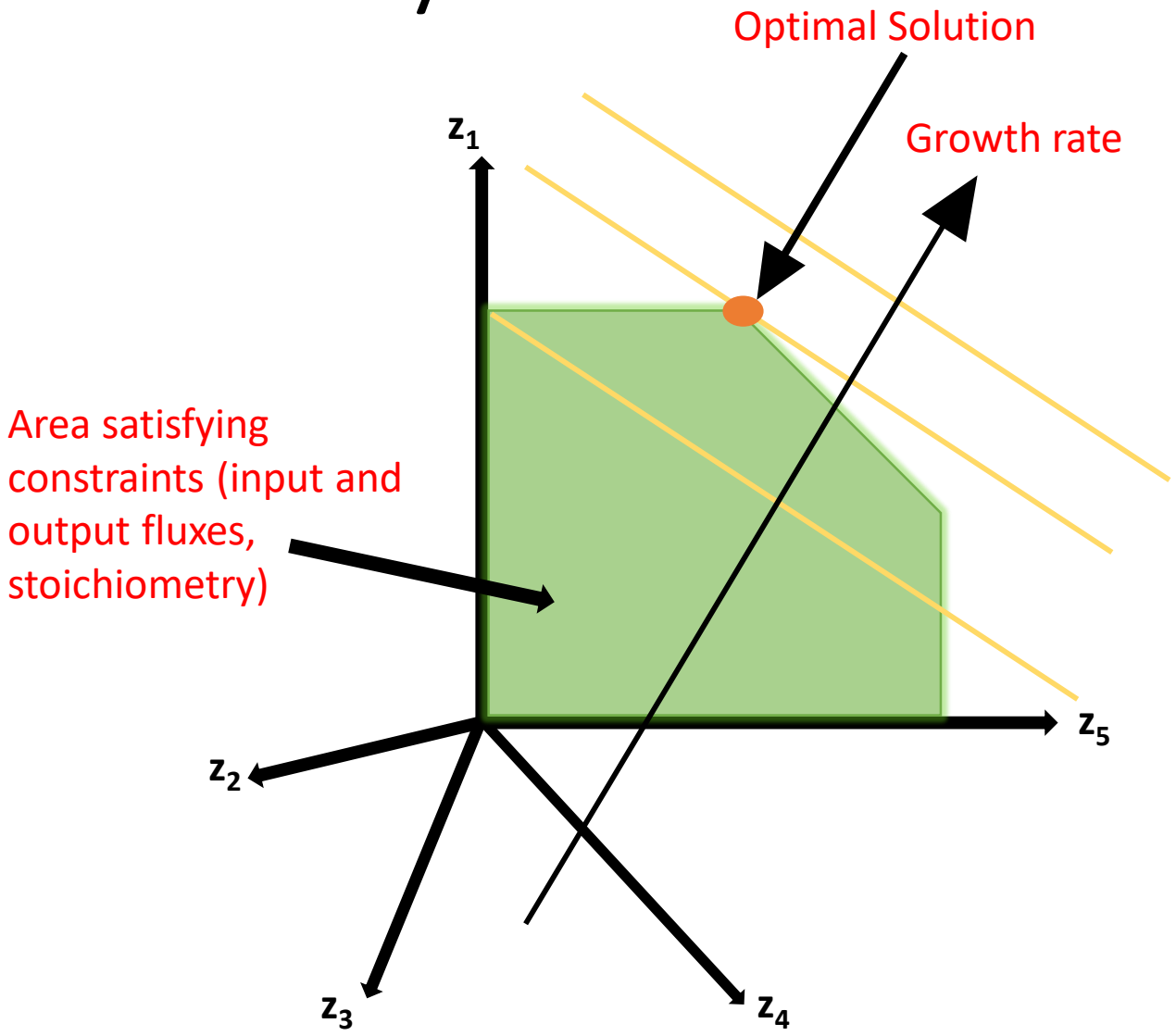
$$\sum_{j=1}^{R} S_{ij}\ z_j = 0 \qquad \text{for all } i = 1,..,M$$

$$j = 1,\ldots,R$$

(Steady state assumption)

constraints

$lb_j \le z_j \le ub_j$



Markov-Chain Monte-Carlo Sampling

1. Implement following constraints to get the solution space :

$$\sum_{j=1}^{R} S_{ij}\, z_j = 0 \quad \text{for all } i = 1,..,M$$

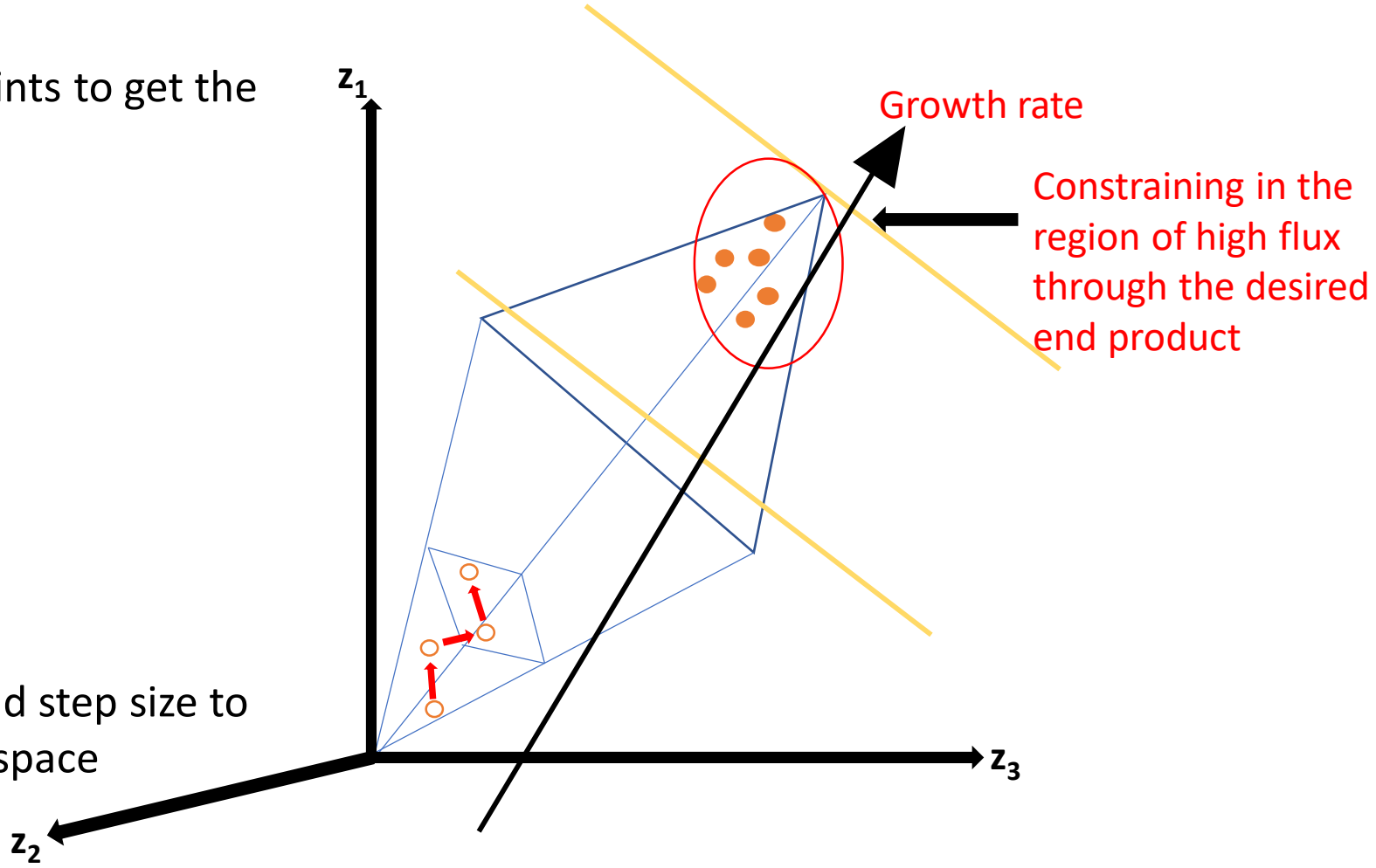
$$j = 1,\ldots,R$$

(Steady state assumption)

constraints

$lb_j \le z_j \le ub_j$

2. Select an initial point
3. Choose a random direction and step size to reach next point in the solution space



FBA vs Markov-Chain Monte-Carlo

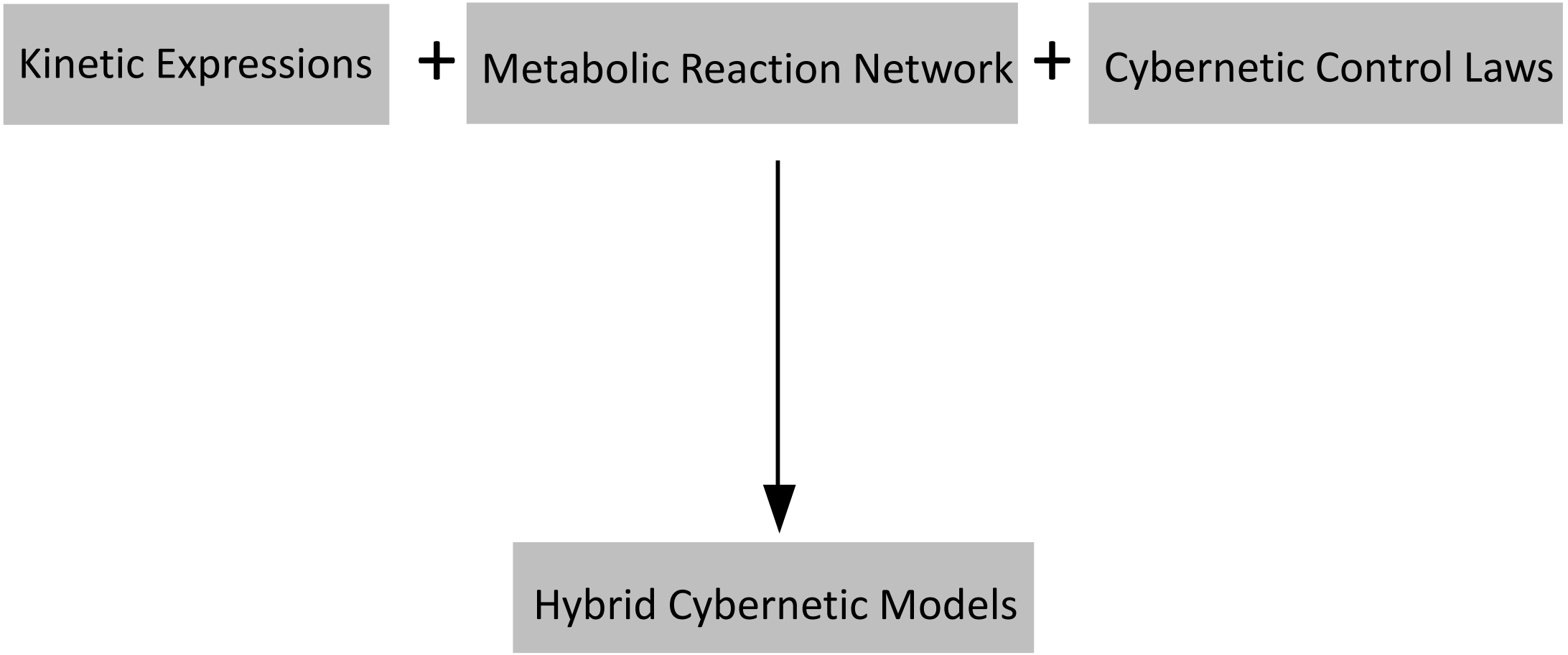
Flux Balance Analysis

1. Assumes an objective function like growth maximization
2. Artificial objective functions are used to generate multiple flux distributions
3. Solution time is within seconds

Markov-Chain Monte-Carlo

1. No growth maximization assumption
2. A sample of feasible flux distribution is obtained
3. Solution time ranges in minutes to an hour

Hybrid Cybernetic Models



Contribution

Central Carbon stoichiometry (*E. coli*)	+	Constraints on extracellular fluxes	+	Flux Balance Analysis	+	Cybernetic Control Laws	+ Kinetic Rate Expressions	= Hybrid Cybernetic Model with FBA
Central Carbon stoichiometry (*E. coli*)	+	Constraints on extracellular fluxes	+	Markov-Chain Monte-Carlo	+	Cybernetic Control Laws	+ Kinetic Rate Expressions	= Hybrid Cybernetic Model with MCMC
Genome scale stoichiometry (*CHO-K1*)	+	Constraints on extracellular fluxes	+	Flux Balance Analysis	+	Cybernetic Control Laws	+ Kinetic Rate Expressions	= Hybrid Cybernetic Model with FBA
Genome scale stoichiometry (*CHO-K1*)	+	Constraints on extracellular fluxes	+	Markov-Chain Monte Carlo	+	Cybernetic Control Laws	+ Kinetic Rate Expressions	= Hybrid Cybernetic Model with MCMC

Overview

Review Basic Concepts

Model of *Escherichia coli* (*E. coli*) cells

Model of *Chinese Hamster Ovary* (*CHO*) cells

Hybrid Cybernetic Models

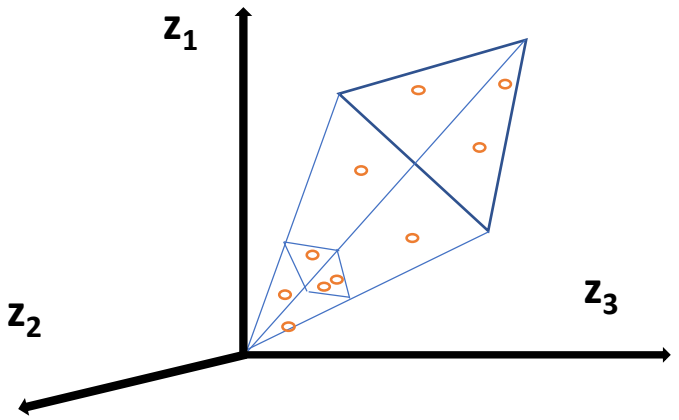
Rate Expressions

$$r_{M,l} = k_{M,l}\, e_l \frac{s_l}{K_l + s_l}$$

$$r_{E,l} = k_{e,l} \frac{s_l}{K_l + s_l}$$

Cybernetic Control Laws

$$u_l = \frac{z_{s,l}\, r_{M,l}}{\sum_{l=1}^{L} z_{s,l}\, r_{M,l}}$$



$$v_l = \frac{z_{s,l}\, r_{M,l}}{\max_{l=1,..,L} z_{s,l}\, r_{M,l}}$$

The E. coli System

Model Equations and Assumptions

Batch fermentation of E. coli has been simulated. A reaction network of 118 reactions and 62 metabolites was used. Dynamics within the central carbon metabolism were the focus. Cell death has been ignored.

Specific growth rate for each mode is given as

$$r_{G,l} = z_{biomass,l}\, r_{M,l}$$

Total growth rate of the system is written as

$$\mu = \sum_{l=1}^{L} r_{G,l}\, v_l$$

The abundance of extracellular species is given as

$$\frac{ds_i}{dt} = \sum_{j=1}^{R}\sum_{l=1}^{L} S_{i,j}\, z_j\, r_{M,l}\, v_l\, c$$

Pseudoenzyme levels are governed by

$$\frac{de_l}{dt} = \alpha_l + r_{E,l}\, u_l - (\beta_l + \mu)\, e_l$$

Cell mass is governed by

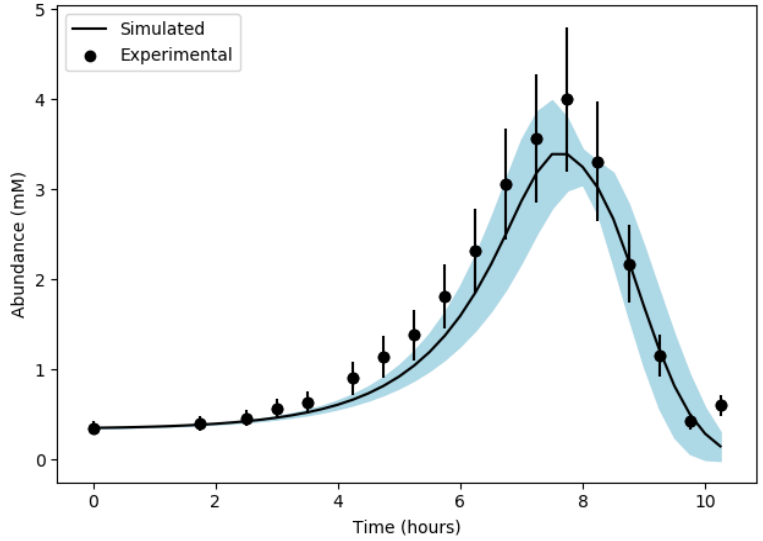
$$\frac{dc}{dt} = \mu\, c$$

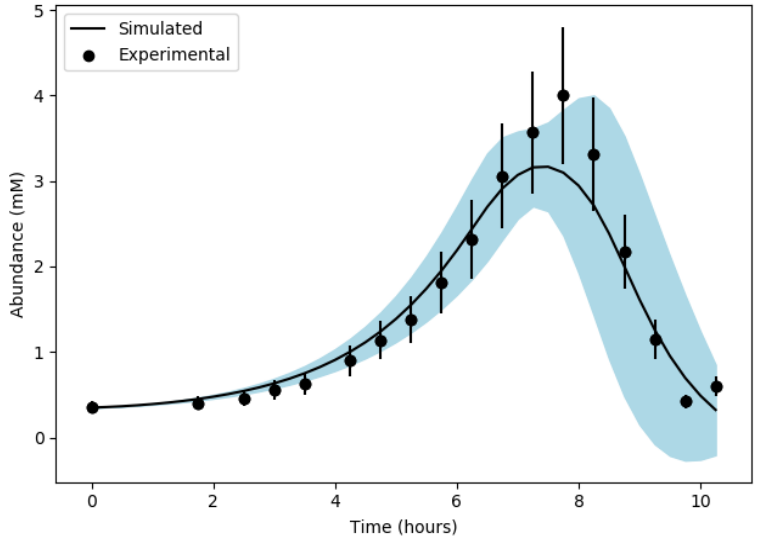
Results

HCM-FBA

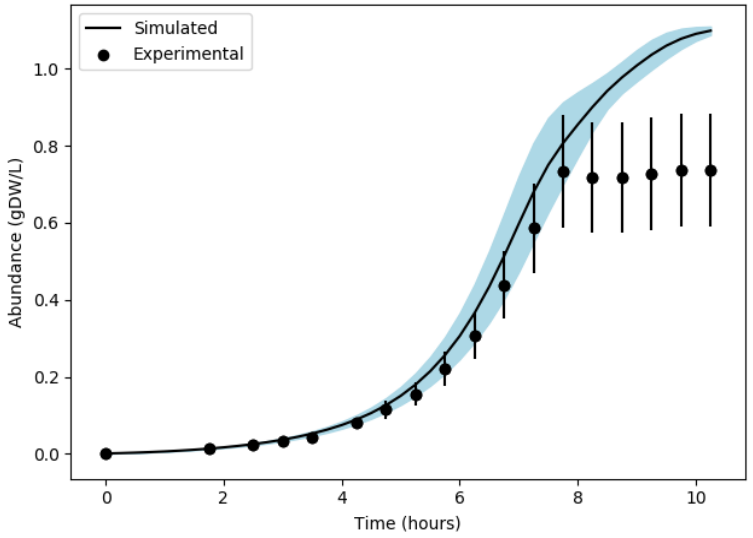
HCM-MCMC

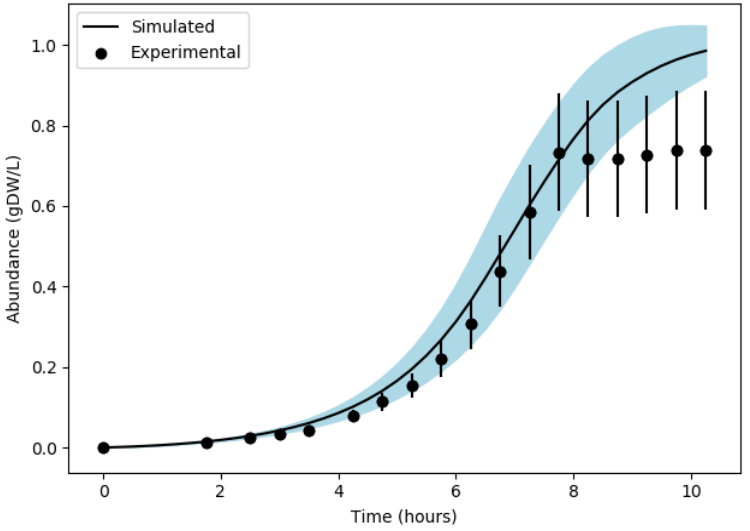
Acetate



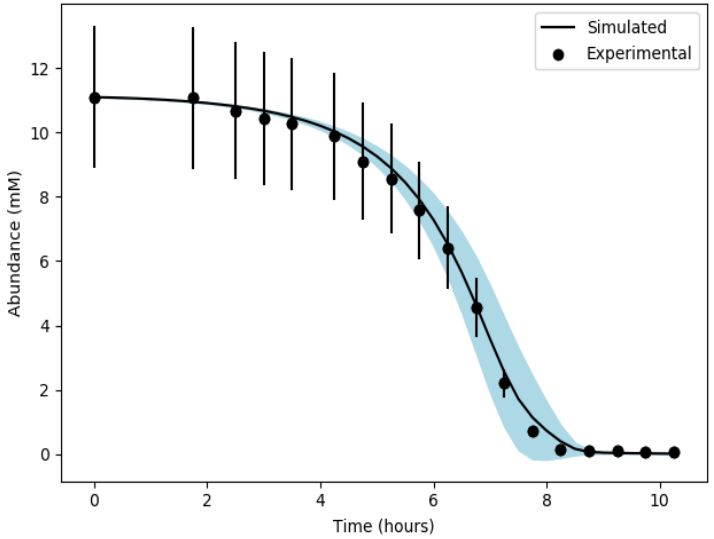


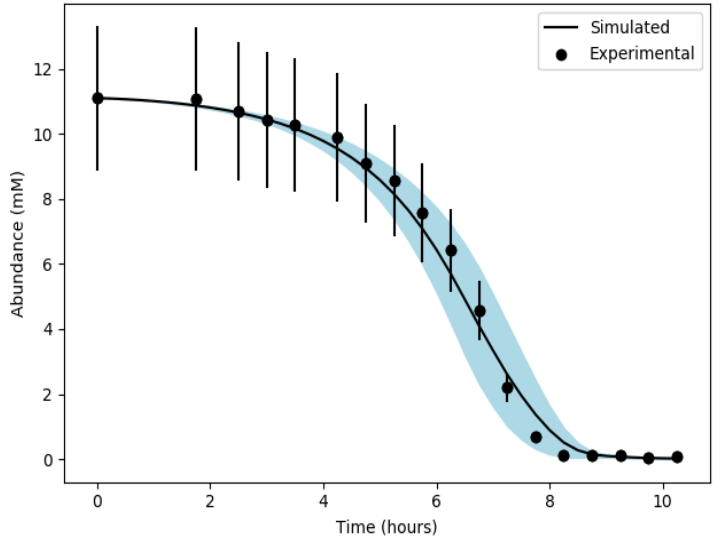
Biomass





Glucose





Varma, A., & Palsson, B. O. (1994)

Summary

1. 29 flux modes were generated using FBA and MCMC sampling

2. The framework selects glucose as the preferred substrate

3. There is a clear switch in substrate utilization when glucose is exhausted. Acetate uptake begins at this time

4. HCM-MCMC has no performance penalty compared to HCM-FBA

5. HCM-MCMC offers a better solution strategy than HCM-FBA by eliminating the need to define an objective function that might not be biologically relevant

Overview

Review Basic Concepts

Model of *Escherichia coli* (*E. coli*) cells

Model of *Chinese Hamster Ovary* (*CHO*) cells

Chinese Hamster Ovary System

Model Equations and Assumption

Fed-batch based kinetics are performed for *CHO-K1* cells has been simulated. A reaction network of 4723 reactions and 2773 metabolites was used. Cell death has been ignored. Volumetric flow rate will affect dynamics of this system.

Feed stream enters the reactor at a volumetric flow rate F

$$F = \frac{dV}{dt}$$

Rate of dilution can be written as

$$D = \frac{F}{V_o + Ft}$$

The abundance of extracellular species is given as

$$\frac{ds_i}{dt} = D(s_f - s) + \sum_{j=1}^{R}\sum_{l=1}^{L} S_{i,j}\, z_{i,j}\, r_{M,l}\, v_l\, c$$

Pseudoenzyme levels are governed by

$$\frac{de_l}{dt} = \alpha_l + r_{E,l}\, u_l - (\beta_l + \mu)\, e_l$$

Cell mass is governed by

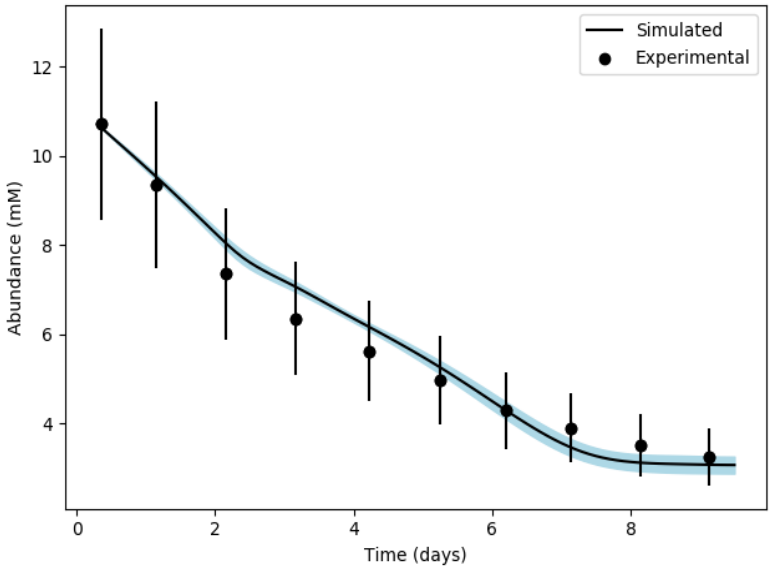
$$\frac{dc}{dt} = (\mu - D)\, c$$

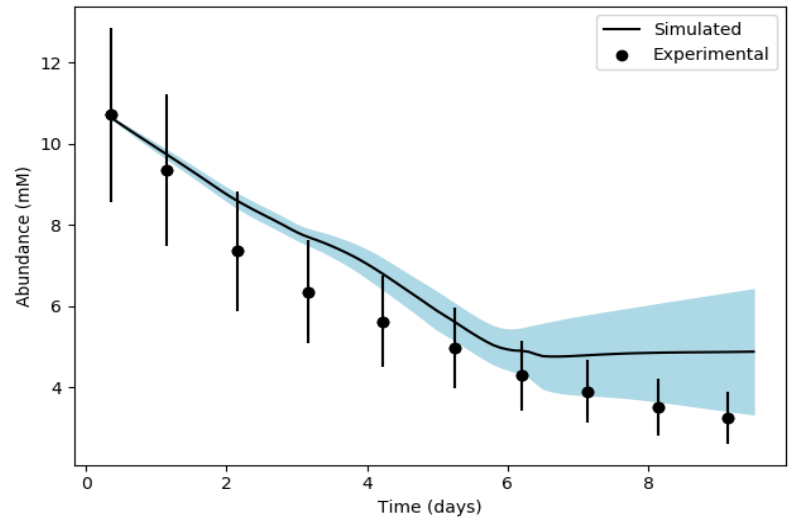
Results

HCM-FBA

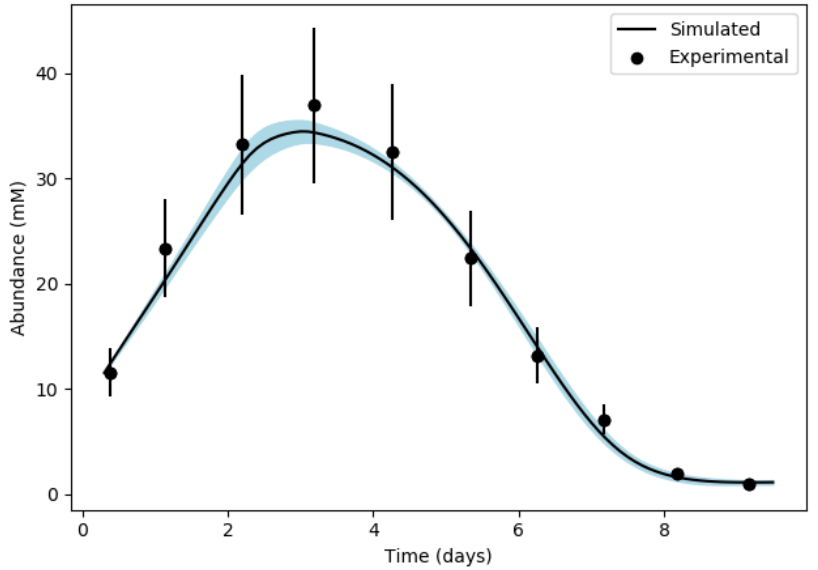
HCM-MCMC

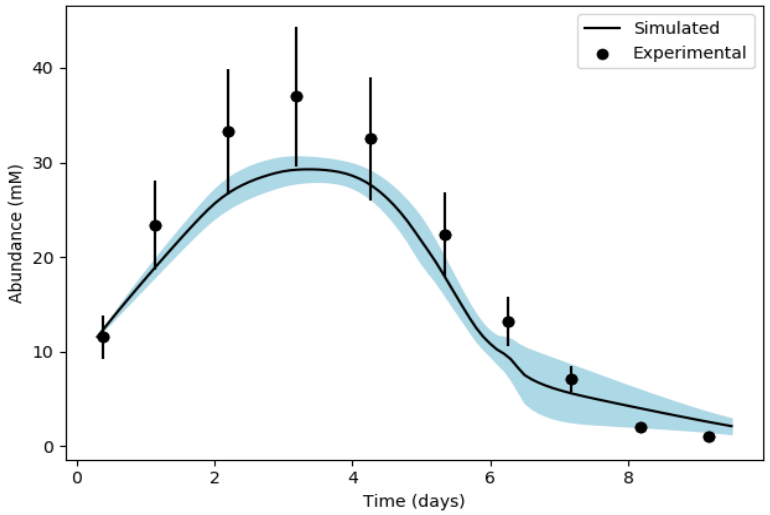
Serine



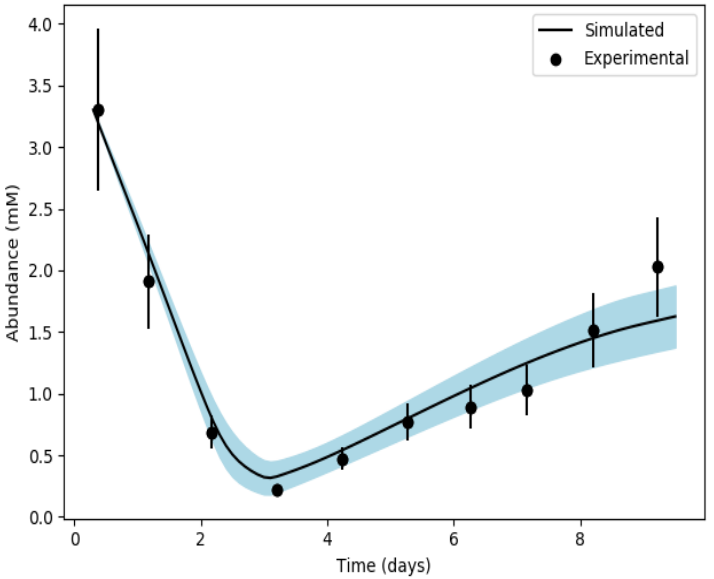


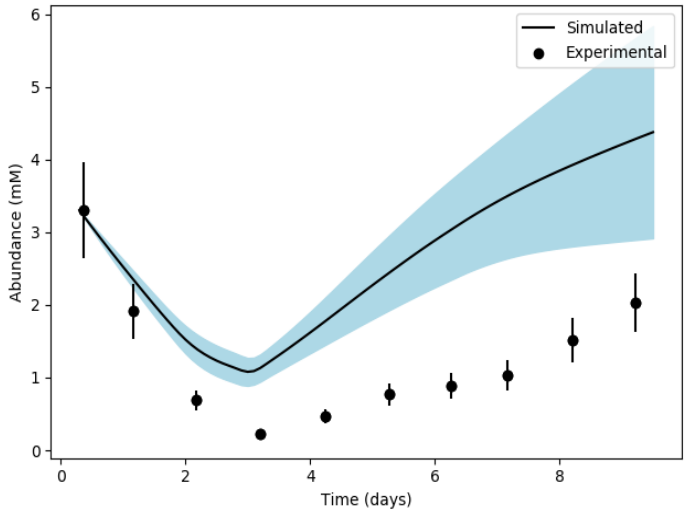
Lactate





Glutamine





Nolan, R.P., & Lee, Kyongbum. (2011)

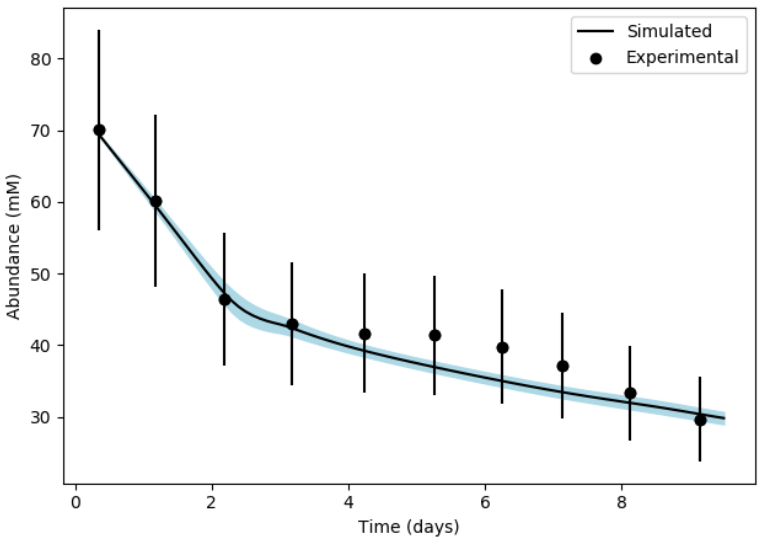
Results

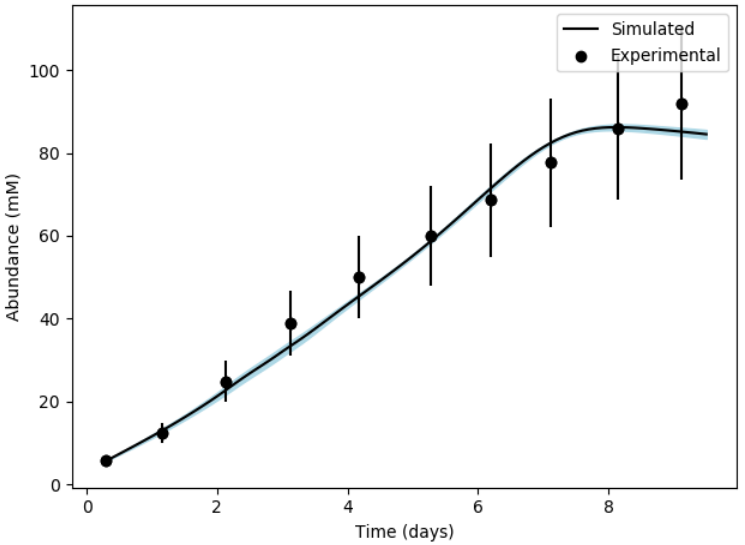
Glucose

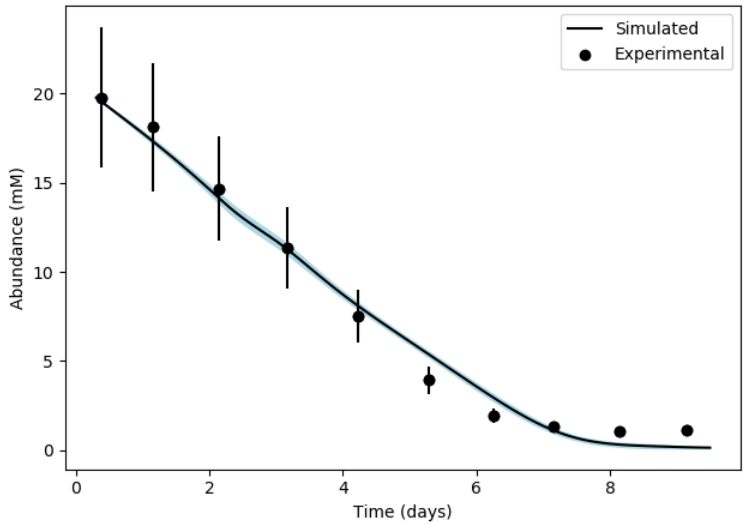
Biomass

Asparagine

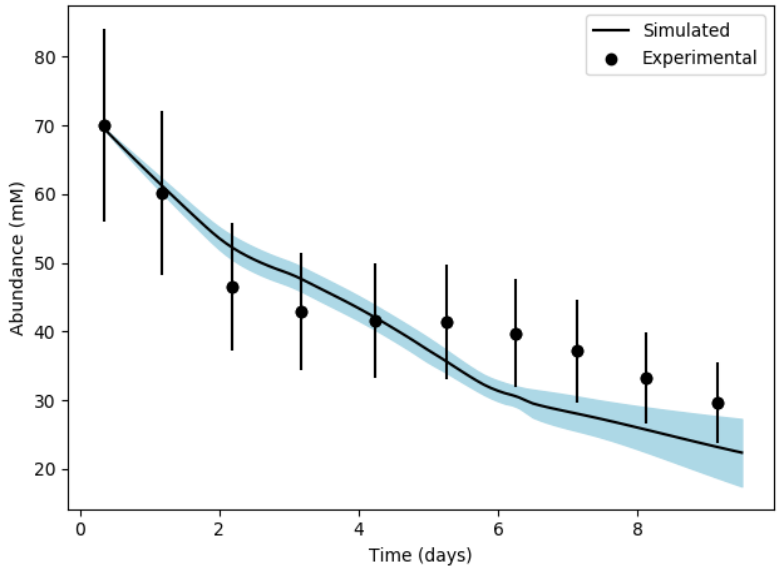
HCM-FBA

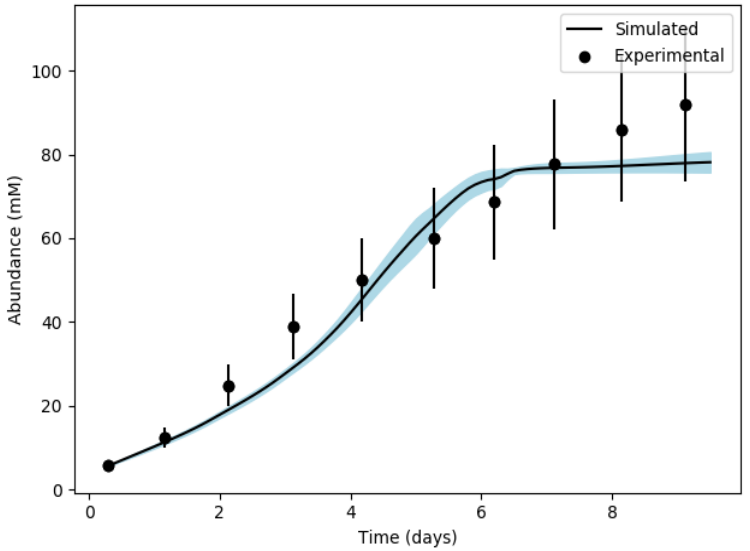


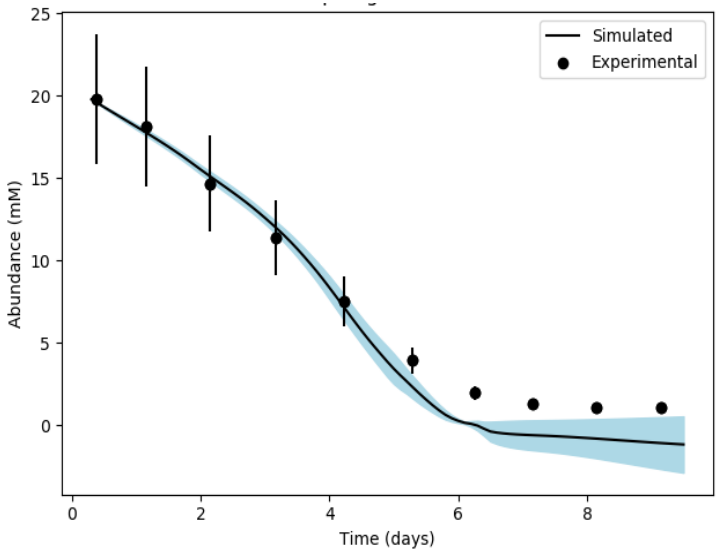




HCM-MCMC







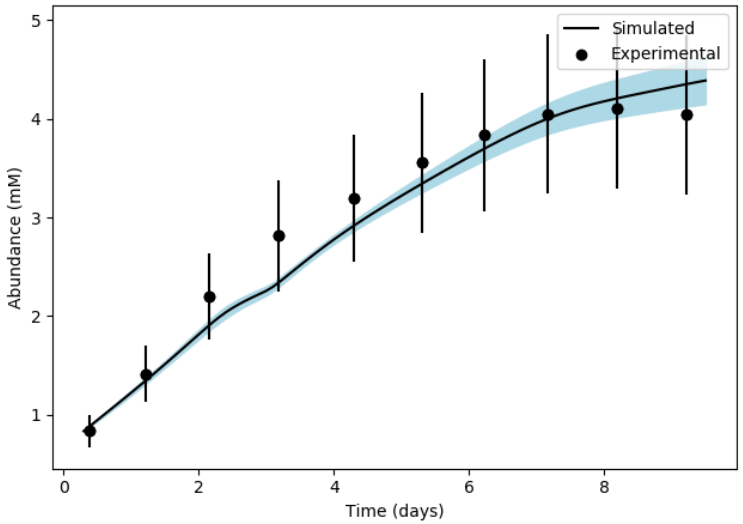
Nolan, R.P., & Lee, Kyongbum. (2011)

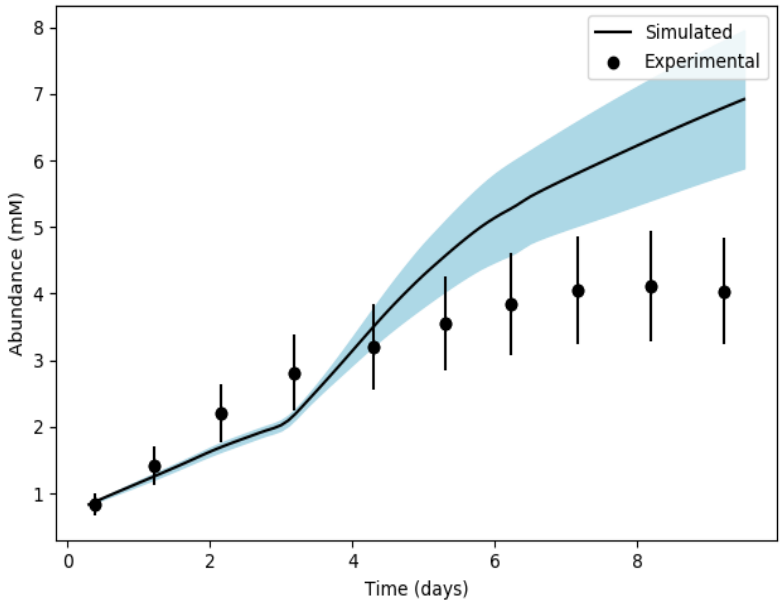
Results

HCM-FBA

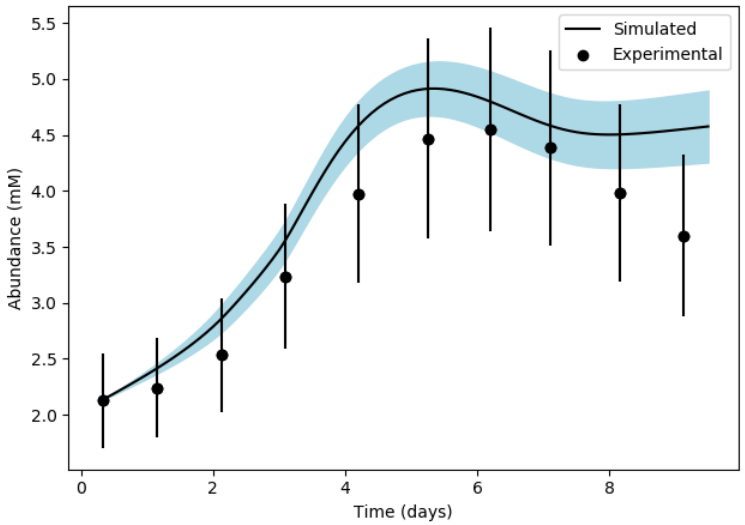
HCM-MCMC

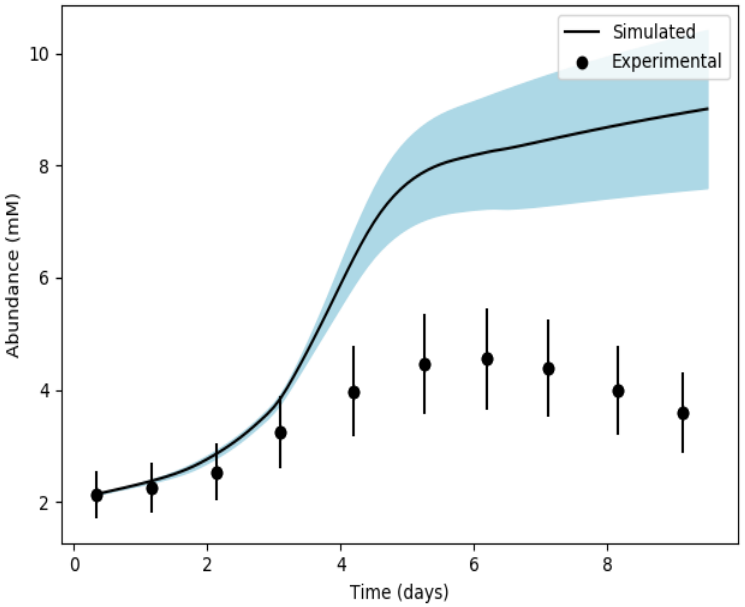
Glutamate



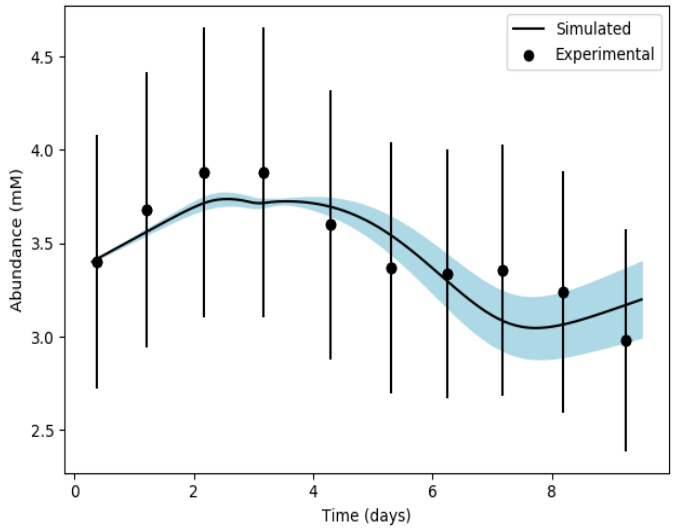


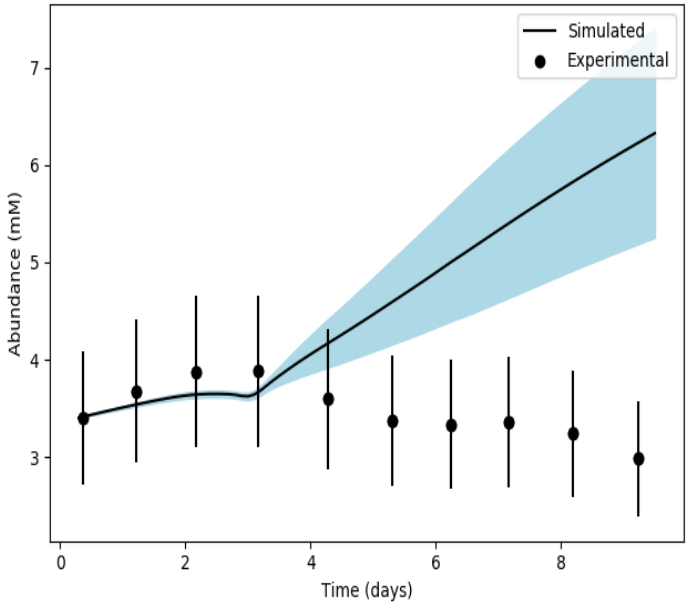
Aspartate





Glycine





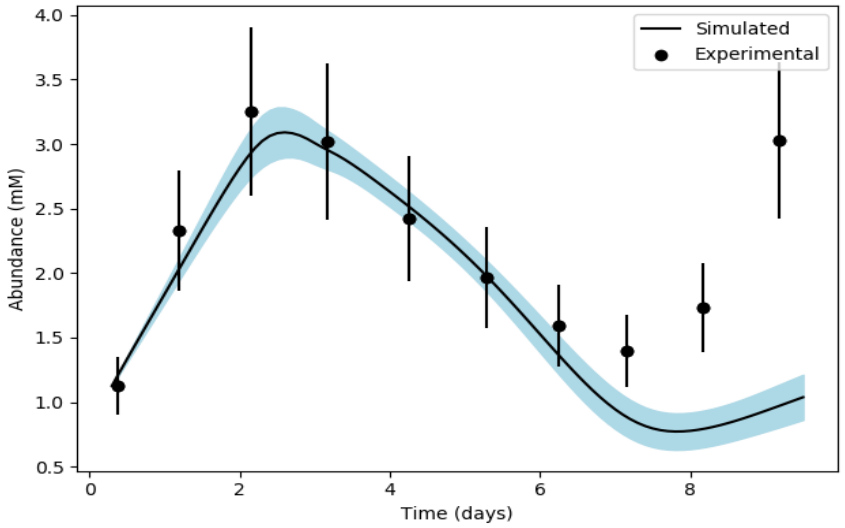
Nolan, R.P., & Lee, Kyongbum. (2011)

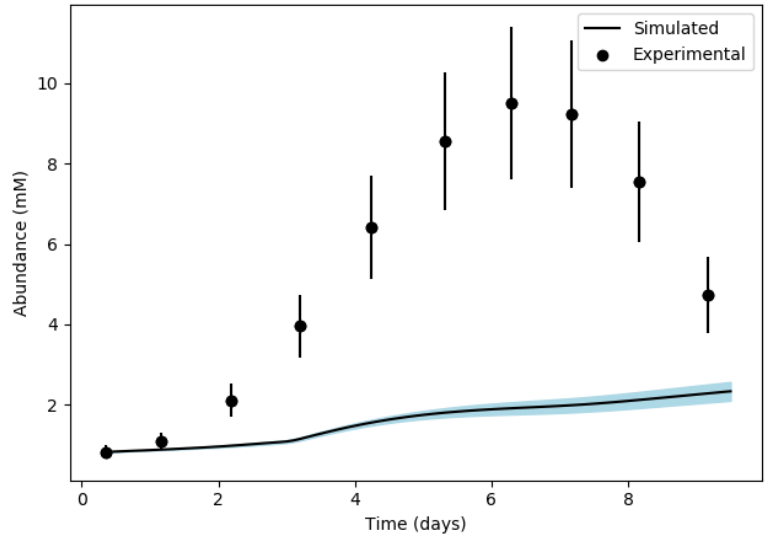
Results

Ammonia

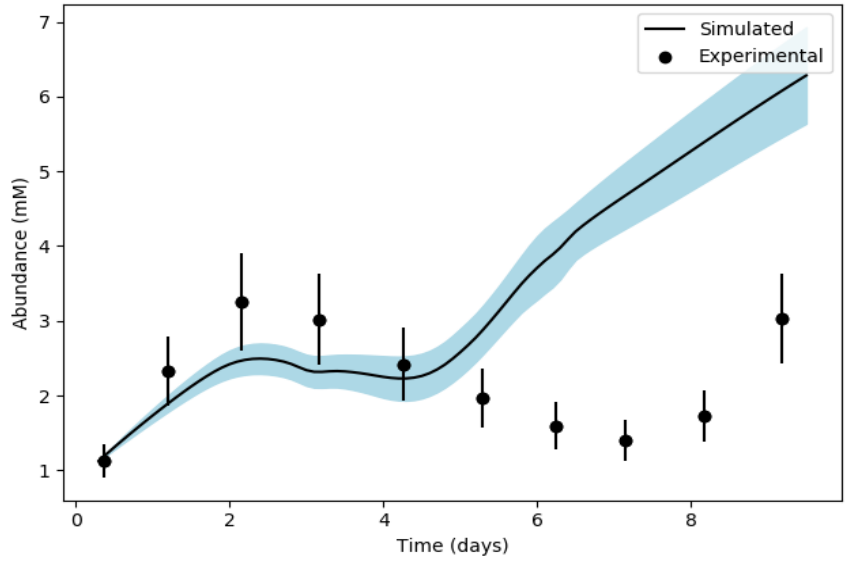
Alanine

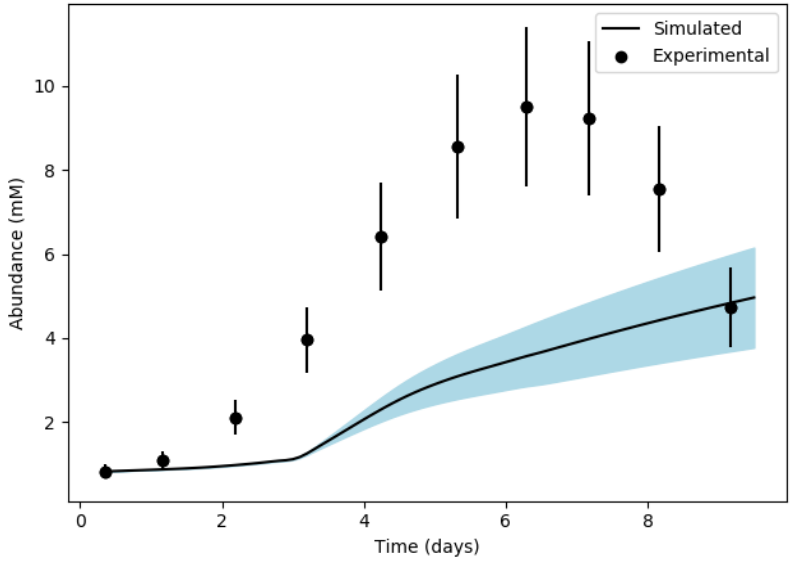
HCM-FBA





HCM-MCMC





Nolan, R.P., & Lee, Kyongbum. (2011)

Summary

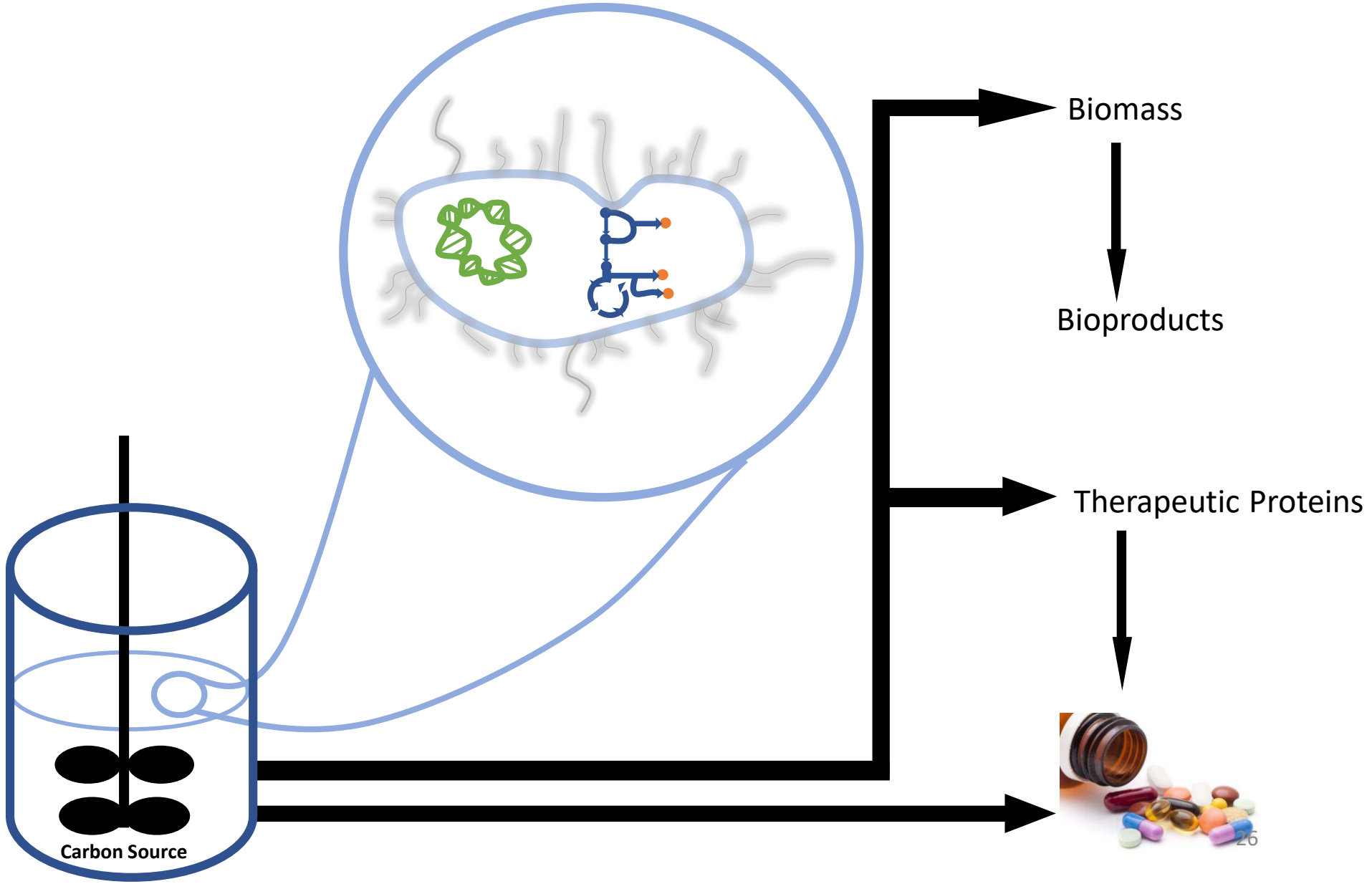
1. 3 flux modes have been used to describe dynamics

2. *E. coli* shows single-substrate kinetics. *CHO-K1* demonstrates multi-substrate kinetics

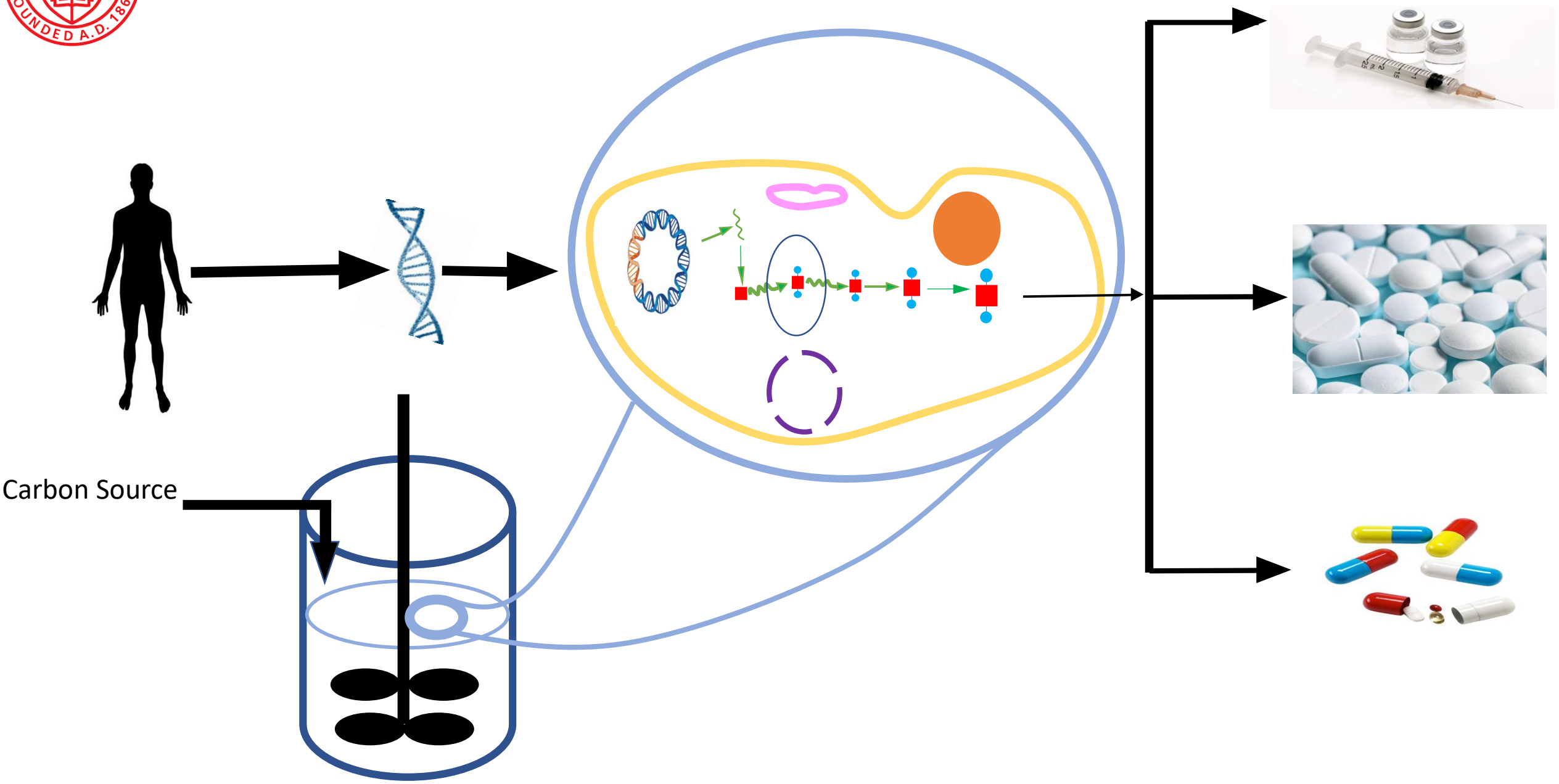
3. *E. coli* shows sequential uptake pattern. *CHO-K1 demonstrates simultaneous uptake pattern*

4. *Glutamine, glucose, lactate, asparagine and serine are important for biomass production*

Future Work : Implementing New Age Biology



Future Work : Producing glycosylated proteins



Acknowledgement

Prof. Varner

Prof. DeLisa

Dr. Michael Vilkhovoy

All members of Varner lab