

Model optimization for Synthetic biology design

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Assistant teacher : Sysbiomics team

Overview :

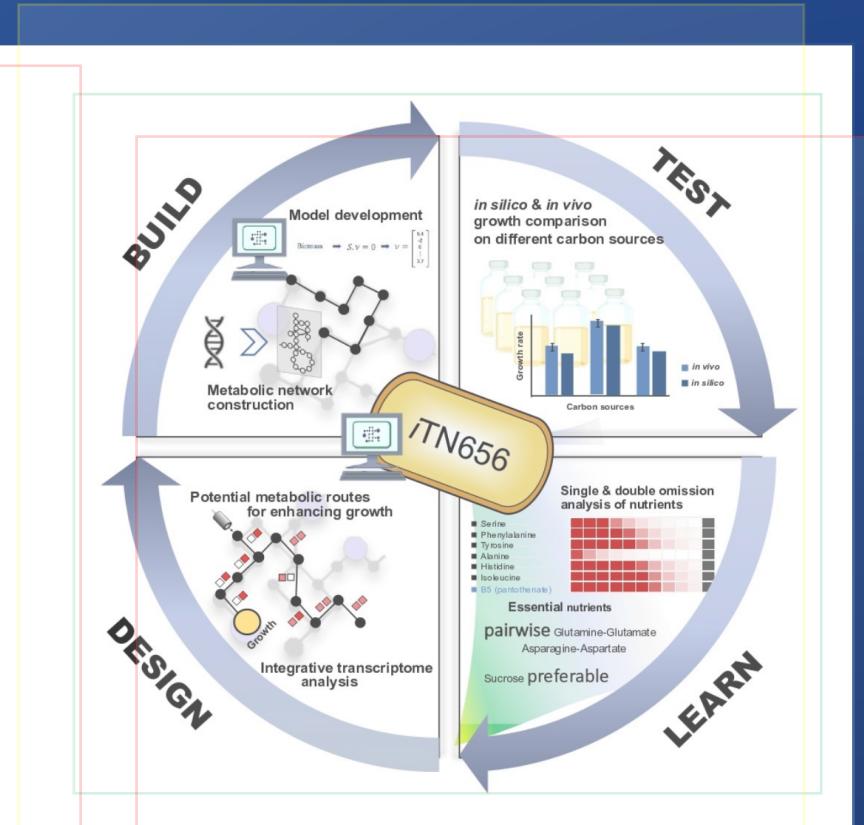
Short lecture - Introduction to genome-scale metabolic modeling and flux balance analysis (FBA)

Hands-on workshop

- Set up community system
- Introduction to the model, *iTN656*
- Import the model
- Define constraints
- Optimize objective function

...TEST...LEARN...DESIGN...

- Model validation
- Identifying the essential/preferable nutrients
- Integrative transcriptomics



Namrak T, Raethong N, Jatuponwiphat T, Nititiprasert S, Vongsangnak W, Nakphaichit M: **Probing Genome-Scale Model Reveals Metabolic Capability and Essential Nutrients for Growth of Probiotic *Limosilactobacillus reuteri* KUB-AC5.** *Biology* 2022, 11:294.

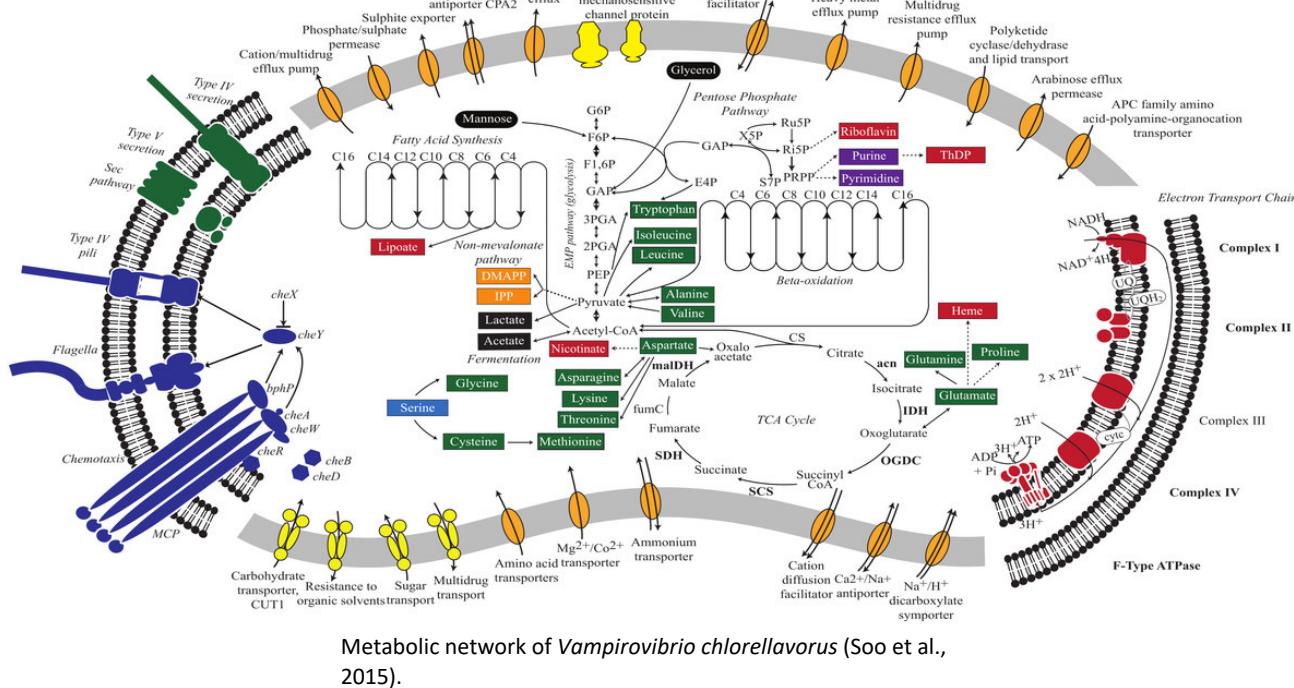
Why Model Metabolism?

Metabolism is a core process of every cell providing the energy (**catabolism**) and building blocks (**anabolism**) for maintaining homeostasis and cellular functions such as:

- Growth: maintenance of a higher rate of anabolism than catabolism
- Response to stimuli: phenotypic changes of the cell caused by single or combined factors
 - Internal factor: several types of molecules (e.g., DNA, RNA) found in the cell.
 - external factor: physical or chemical signals come from outside the cell.

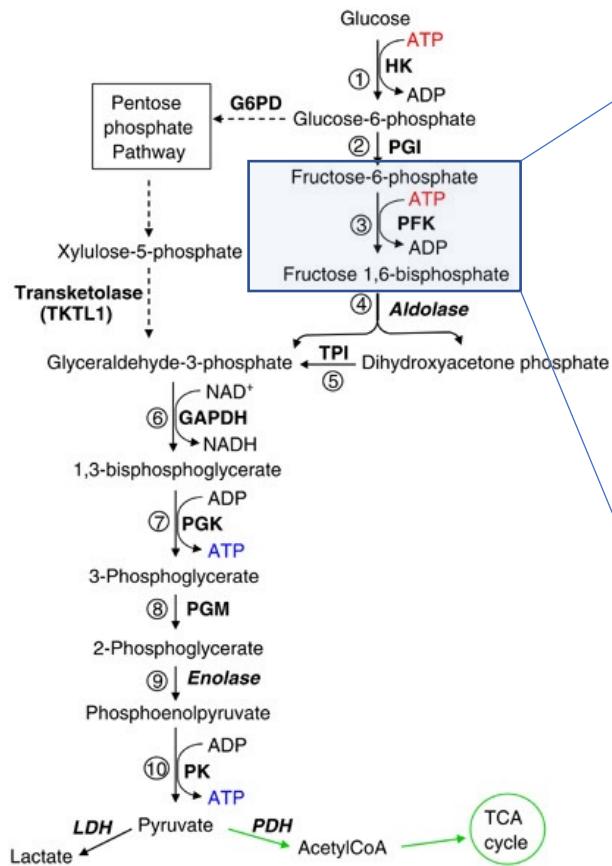
As a result, metabolism plays a central role in elucidating the **genotype-phenotype relation**.

Genome-scale metabolic network



- Explore capabilities of genome-scale (**global**) metabolic network
- How do we go from a network to a model we can stimulate?

Metabolic reaction



Metabolites

Fructose-6-phosphate (F6P),
Fructose 1,6-bisphosphate (FBP)

Enzyme

phosphofructokinase
(PFK, EC 2.7.1.11)

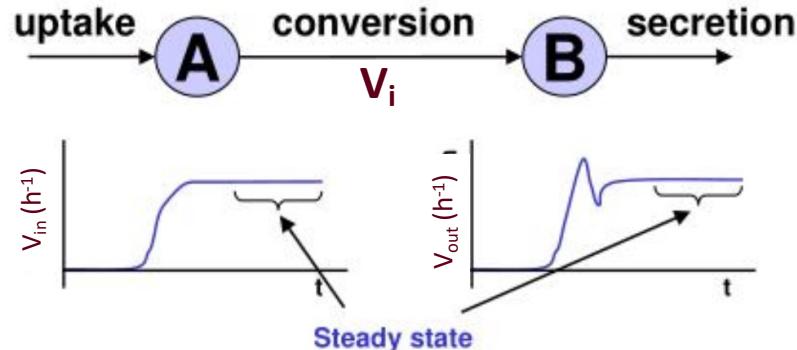
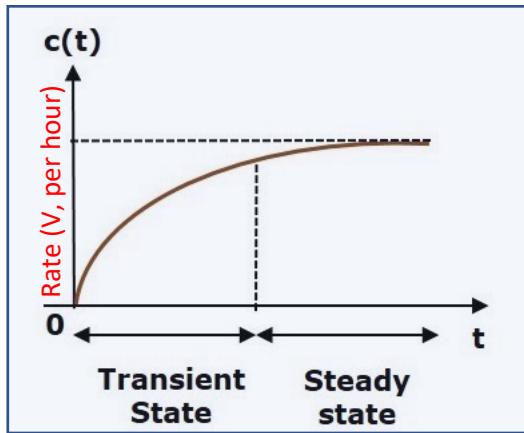
Reaction & Stoichiometry

1 F6P => 1 FBP

Regulation

gene regulation
metabolite regulation

Steady State Assumptions



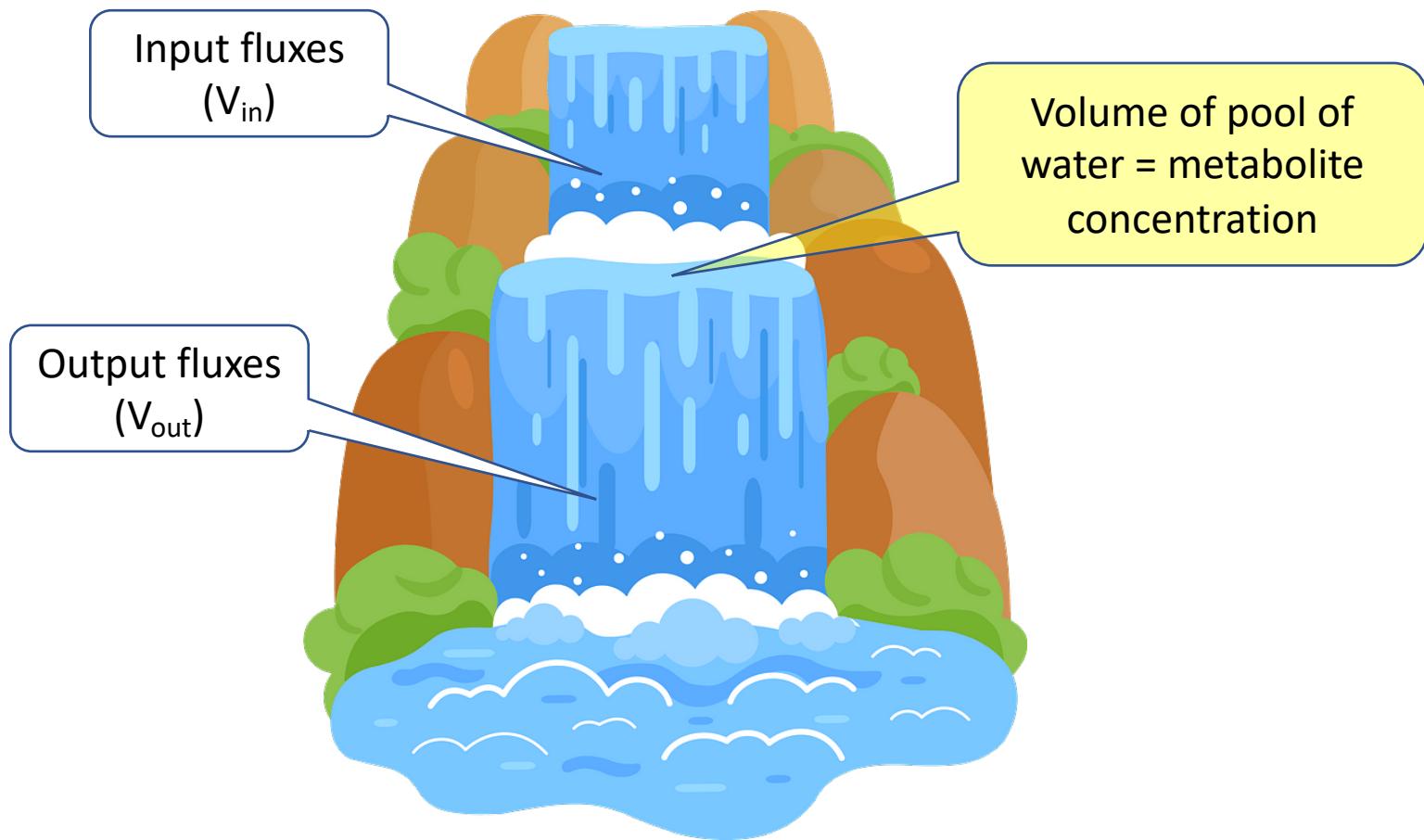
- Dynamics are transient
- At appropriate time-scales and conditions, **metabolism is in steady state**

$$\text{Rate of uptake A } (V_{in}) = \text{Rate of secretion B } (V_{out})$$

Two key implications

- Rate of internal reaction (V_i) is constant.
- Internal metabolite concentration is constant.

Metabolic flux



Reaction Stoichiometries are Universal

The conversion of glucose to glucose 6-phosphate always follows this stoichiometry:



This is **chemistry** not biology.

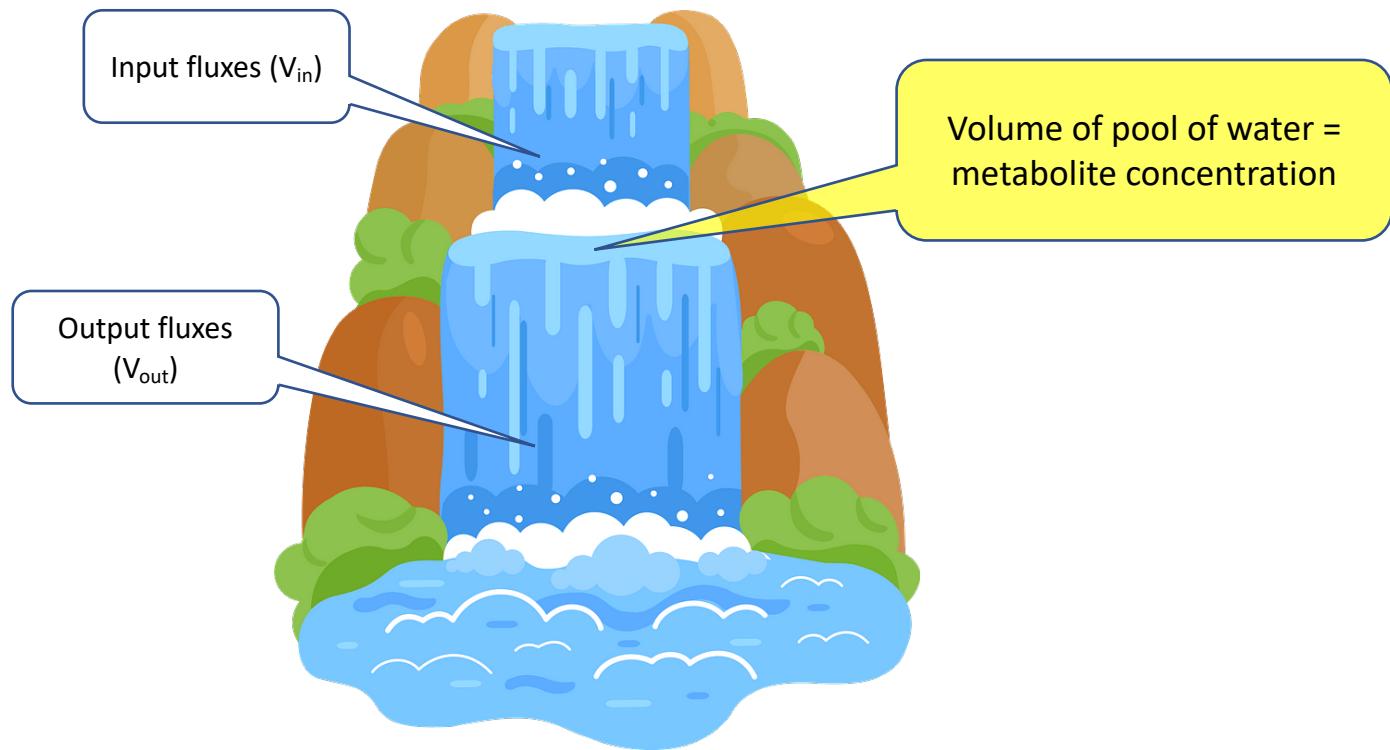
Biology => the **enzymes** catalyzing the reaction

- Enzymes influence rates and kinetics
- Activation energy
- Substrate affinity
- Rate constants



Not required for **steady state** modeling!

Metabolic Flux Analysis



Use **universal reaction Stoichiometries** to predict metabolic network capabilities at **Steady State** ($V_{in} = V_{out}$)

Stoichiometry As Vectors

We can denote the stoichiometry of a reaction by a vector of coefficients.

One coefficient per metabolite:

- Positive if metabolite is produced.
- Negative if metabolite is consumed.

Example:

Metabolites:

A, B, C, D

Reactions:

R1: 2A + B \rightarrow C

R2: C \rightarrow D



Stoichiometry Vectors:

	A	B	C	D
R1	-2	-1	1	0
R2	0	0	-1	1

Stoichiometric Matrix (S)

More example:

Metabolites:

A, B, C, D, E, F, G, ...

Reactions:

R1: ...

R2: ...

R3: $2A + B \rightarrow C$

R4: $C \rightarrow D$

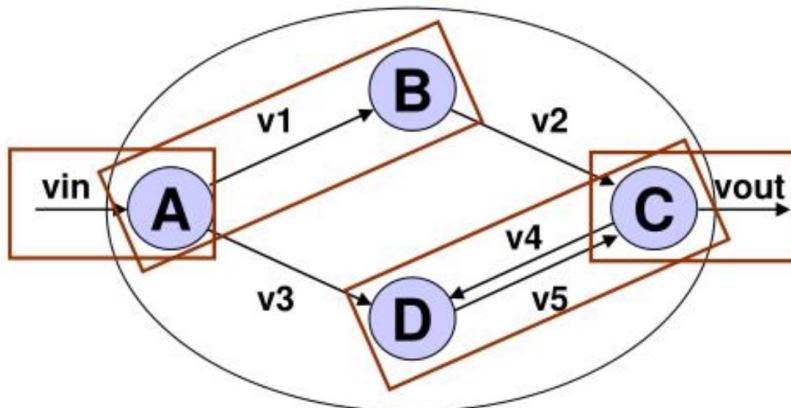
R5: ...

R6: ...



Metabolites	Reactions					
	R1	R2	R3	R4	R5	R6
A	-2	0
B			-1	0		
C			1	-1		
D			0	1		
E			0	0		
F			0	0		
G			0	0		

Simple System



	v1	v2	v3	v4	v5	v _{in}	v _{out}
A	-1	0	-1	0	0	1	0
B	1	-1	0	0	0	0	0
C	0	1	0	-1	1	0	-1
D	0	0	1	1	-1	0	0

Exchange Reactions

We have introduced two new things

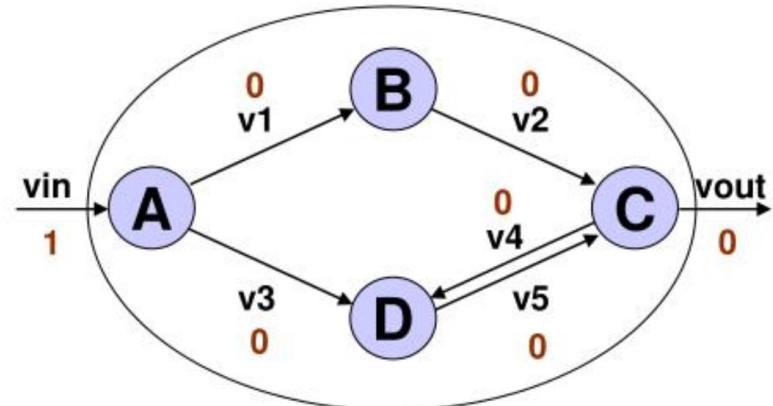
- **Reversible reactions** – are represented by two reactions that proceed in each direction (e.g. v_4 , v_5)
- **Exchange reactions** – allow for fluxes from/into an infinite pool outside the system (e.g. v_{in} and v_{out}). These are frequently the only fluxes experimentally measured.

Calculating changes in concentration

At steady state,

What happens if v_{in} is **1**.
“unit” per hour (h^{-1})

We can calculate changes in
concentration of metabolites with
Stoichiometric Matrix (S).



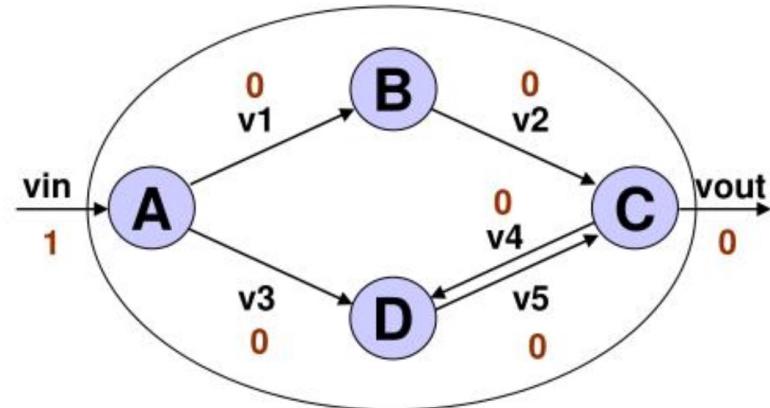
$$\frac{dx}{dt} = S \bullet V$$

Calculating changes in concentration

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What happens if v_{in} is **1**.
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We can calculate changes in concentration of metabolites with Stoichiometric Matrix (S).



V is a vector of fluxes through each reaction

$$\begin{array}{l} \text{dA/dt} \\ \text{dB/dt} \\ \text{dC/dt} \\ \text{dD/dt} \end{array} \begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} = A \left[\begin{array}{ccccc|cc} v1 & v2 & v3 & v4 & v5 & v_{in} & v_{out} \\ -1 & 0 & -1 & 0 & 0 & 1 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & -1 & 1 & 0 & -1 \end{array} \right] \bullet \begin{pmatrix} v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v_{in} \\ v_{out} \end{pmatrix}$$

Then, S^*V is a vector describing the change in concentration of each metabolite per unit time.



Advantages of Stoichiometric Matrix (S)



The stoichiometry of a given reaction is preserved across organisms, while the reaction rates may not be preserved.



Does NOT depend on kinetics or reaction rates.



Depends on gene annotations and mapping from gene to **reactions** and biochemical information.

Genes to Reactions

- Enzyme database
- Indexed by EC number
- **EC numbers** can be assigned to genes by functional annotation:
 - **Blast** to known genes
 - **PFAM** domains

KEGG ENZYME: 1.1.1.363 Help

Entry	EC 1.1.1.363	Enzyme
Name	glucose-6-phosphate dehydrogenase [NAD(P)+]; G6PDH; G6PD; Glc6PD	
Class	Oxidoreductases; Acting on the CH-OH group of donors; With NAD+ or NADP+ as acceptor BRITE hierarchy	
Sysname	D-glucose-6-phosphate:NAD(P)+ 1-oxidoreductase	
Reaction (IUBMB)	D-glucose 6-phosphate + NAD(P)+ = 6-phospho-D-glucono-1,5-lactone + NAD(P)H + H+ [RN:R00835 R10520]	
Reaction (KEGG)	R00835 > R02736; R10520 > R10907 Reaction	
Substrate	D-glucose 6-phosphate [CPD:C00092]; NAD+ [CPD:C00003]; NADP+ [CPD:C00006]	
Product	6-phospho-D-glucono-1,5-lactone [CPD:C01236]; NADH [CPD:C00004]; NADPH [CPD:C00005]; H+ [CPD:C00080]	
Comment	The enzyme catalyses a step of the pentose phosphate pathway. The enzyme from the Gram-positive bacterium Leuconostoc mesenteroides prefers NADP+ while the enzyme from the Gram-negative bacterium Gluconacetobacter xylinus prefers NAD+. cf. EC 1.1.1.49, glucose-6-phosphate dehydrogenase (NADP+) and EC 1.1.1.388, glucose-6-phosphate dehydrogenase (NAD+).	
History	EC 1.1.1.363 created 2013, modified 2015	
Pathway	ec00030 Pentose phosphate pathway ec01100 Metabolic pathways ec01110 Biosynthesis of secondary metabolites ec01130 Biosynthesis of antibiotics	

Online Metabolic Databases

There are several online databases with **curated** and/or **automated EC number** assignments for sequenced genomes such as BiGG, MetaCyc and KEGG.

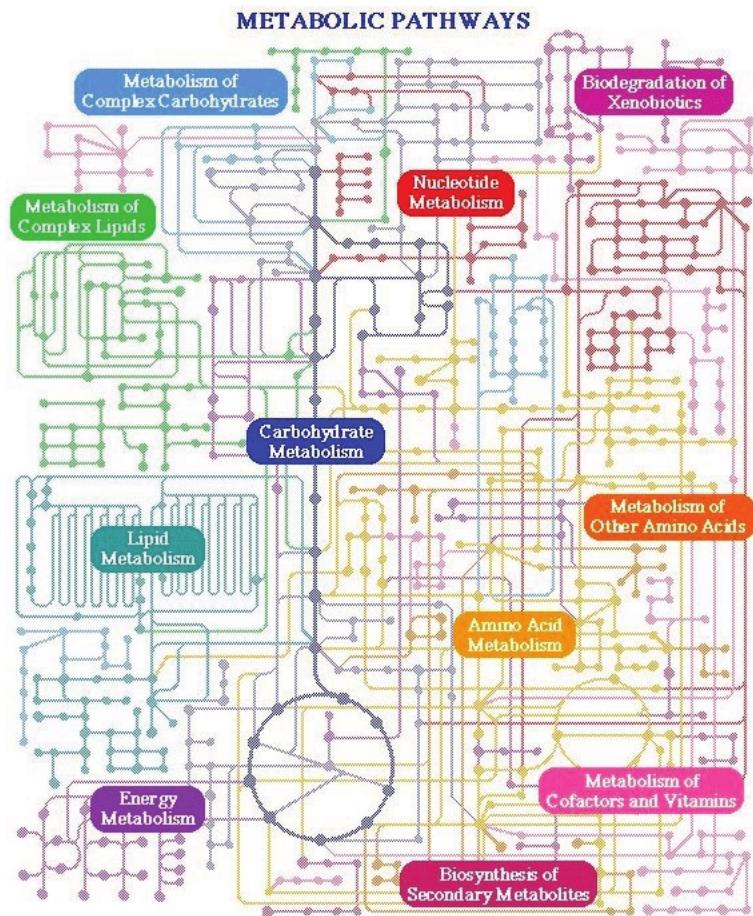
BiGG Models

Search the database by model, reaction, metabolite, or gene ?

Exclude multi-strain models from search

Latest update Version 1.6: Adds 23 new models & more!

[View Models](#) [View Metabolites](#) [View Reactions](#)



From Genomes to the S Matrix

Relationships between
genes-enzymes-reactions

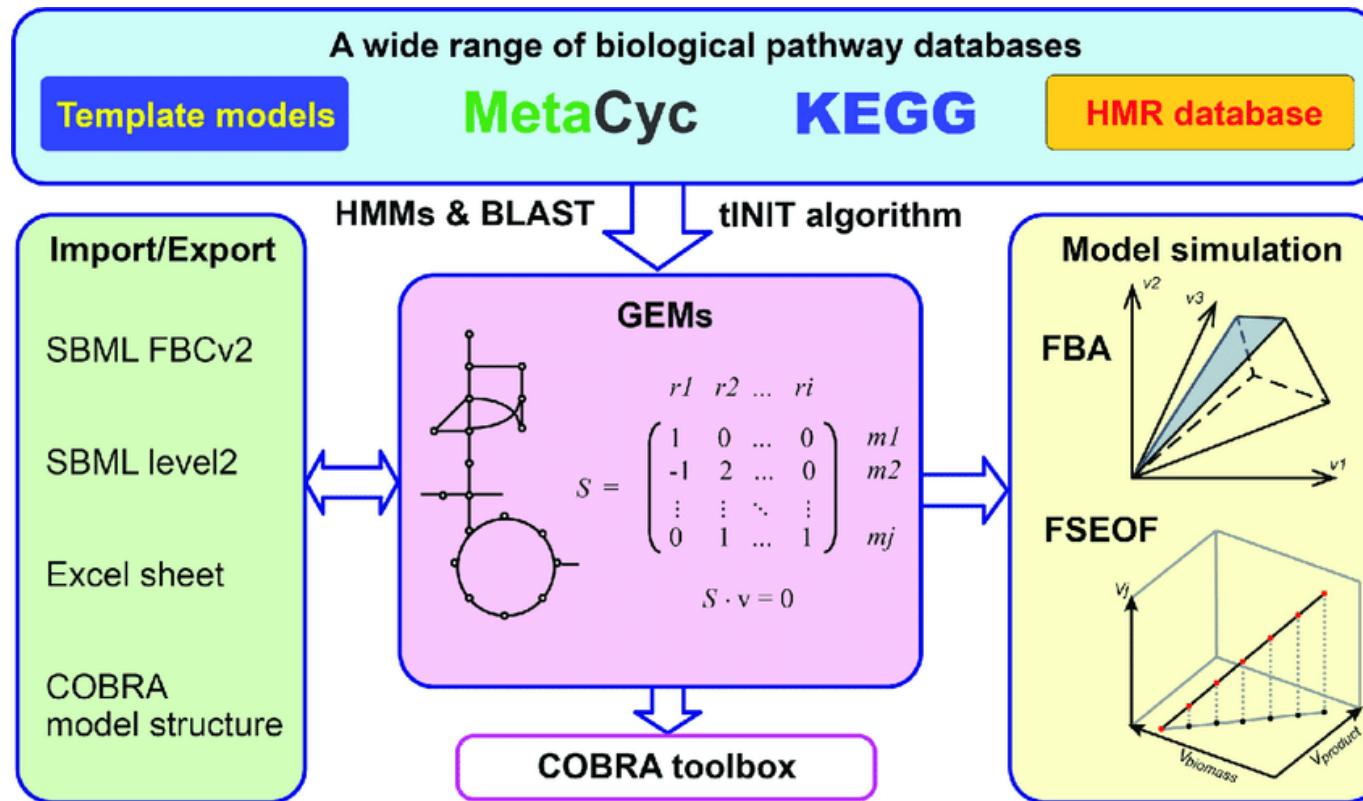
- 1 gene 1 rxn
- 1 gene 1+ rxns
- 1+ genes 1 rxn

The same reaction can be included as multiple roles
(paralogs) see R9, R10.

	Gene A	Gene B	Gene C		Gene D		Gene E	Gene E'
	↓	↓	↓		↓		↓	↓
	Enzyme A	Enzyme B/C			Enzyme D		Enzyme E	Enzyme E'
R1	●	●	●	-1	●	●	●	0
R2								●
R3								●
R4								
R5								
R6								
R7								
R8								
R9								
R10								
A	●	●	●	-1	●	●	●	0
B					0			-1
C					0			0
D					-2			-1
E					0			0
F					0			0
G					1			0
H					0		1	
I	●	●	●	1	●	●	●	0

Same rxn

From Genomes to the GEMs



Genes-enzymes-reactions can be assigned to Genomes by functional annotation and semi-automatic GEM reconstruction using **Template models**:

What Can We Use GEMs?



we can investigate the **metabolic capabilities** of the system.

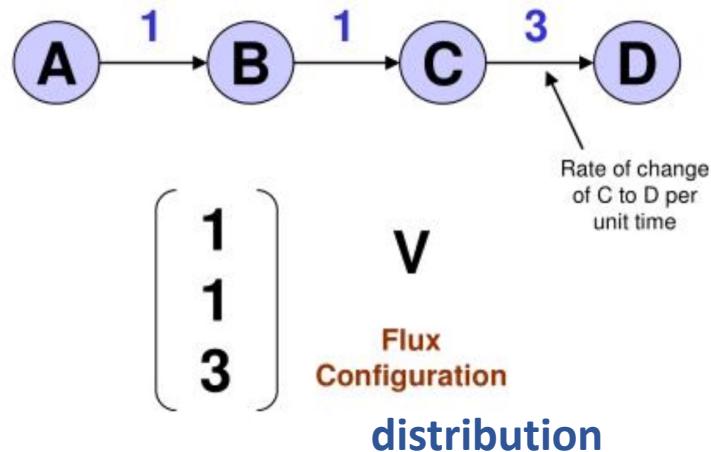


We can determine what combination of fluxes (flux configurations) are possible at **steady state**.

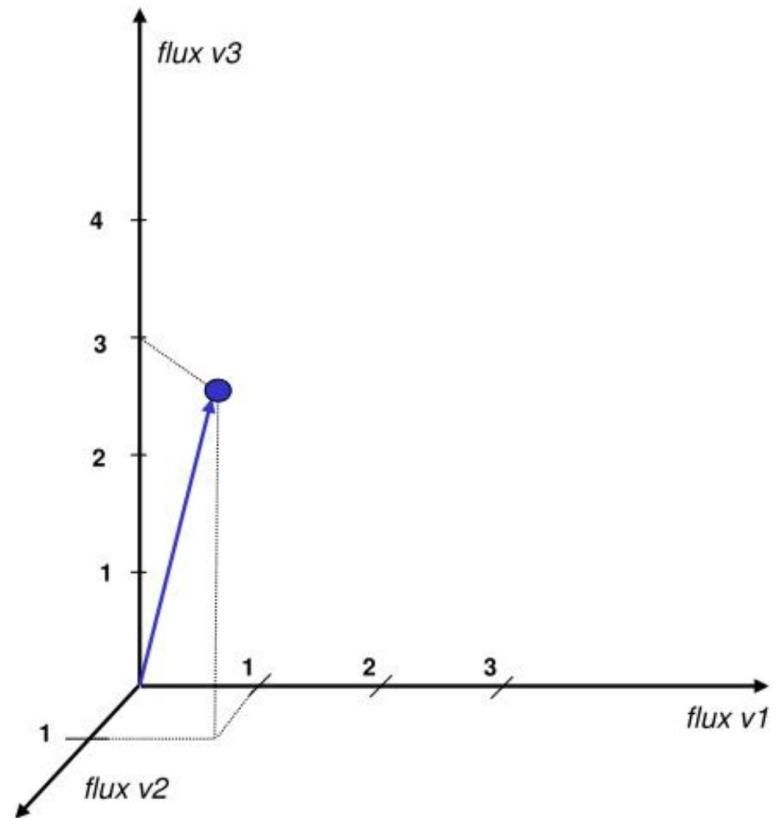
How?

...Flux Balance Analysis (**FBA**)

Flux Configuration, V



We want to know what region of this space contains **feasible fluxes** given our **constraints**.



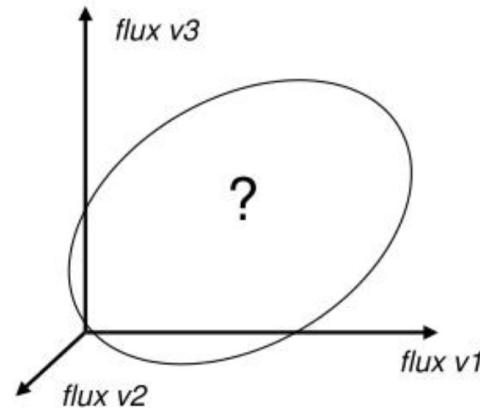
Steady State Constraint

At steady state, metabolite concentrations are constant:

$$0 = \frac{dx}{dt} = S \cdot V \quad v_i \geq 0, \quad i = 1..n^*$$

All steady state flux vectors, V , must satisfy these constraints.

What region do these V live in?



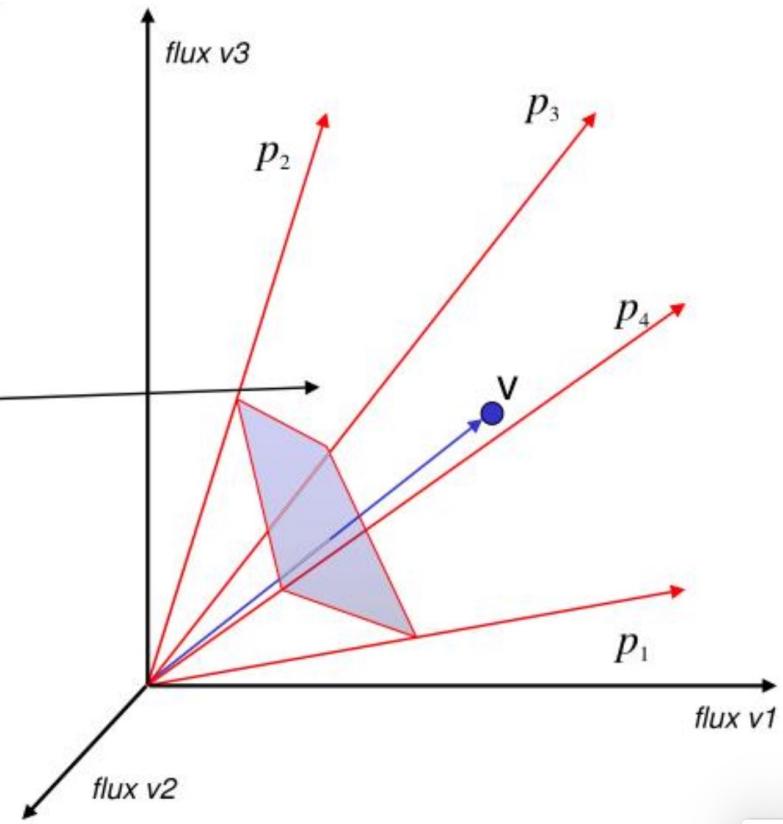
The solution through **convex** analysis
cone

The Flux Cone

Solution is a convex flux cone

Every steady state **flux** vector
is **inside** this **CONE**.

At steady state, the organism
“lives in” here.



Note: Edges/Bounds of the cone are circumscribed by Extreme Pathways.

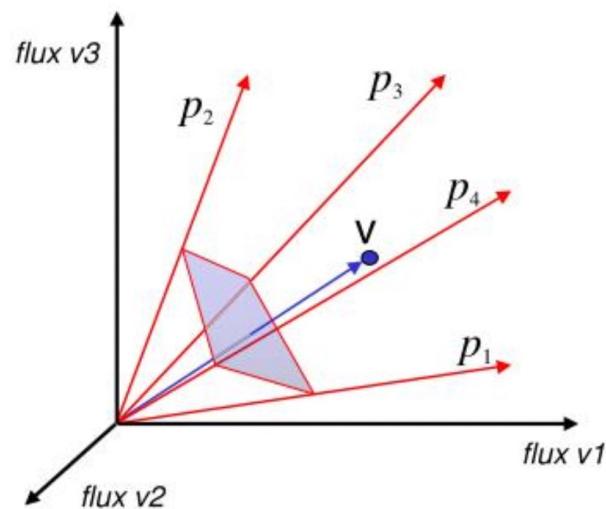
Extreme pathways

Extreme pathways are “fundamental modes” of the metabolic system at steady state.

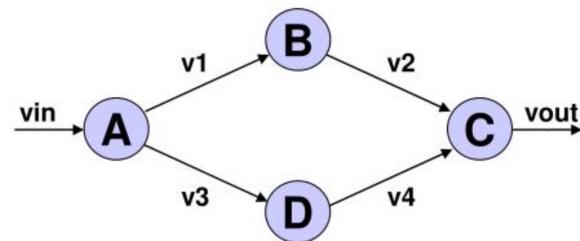
They are steady state flux vectors.

All other steady state flux vectors are non-negative linear combinations.

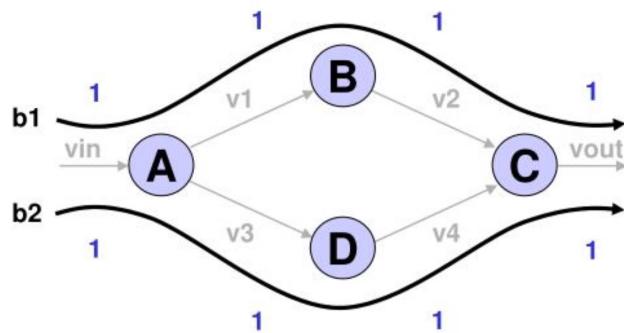
$$V = \sum_i \alpha_i p_i \quad \alpha_i \geq 0$$



Example Extreme Pathways



$$\left[\begin{array}{cccc|cc} & v1 & v2 & v3 & v4 & vin & vout \\ A & -1 & 0 & -1 & 0 & 1 & 0 \\ B & 1 & -1 & 0 & 0 & 0 & 0 \\ C & 0 & 1 & 0 & 1 & 0 & -1 \\ D & 0 & 0 & 1 & -1 & 0 & 0 \end{array} \right]$$

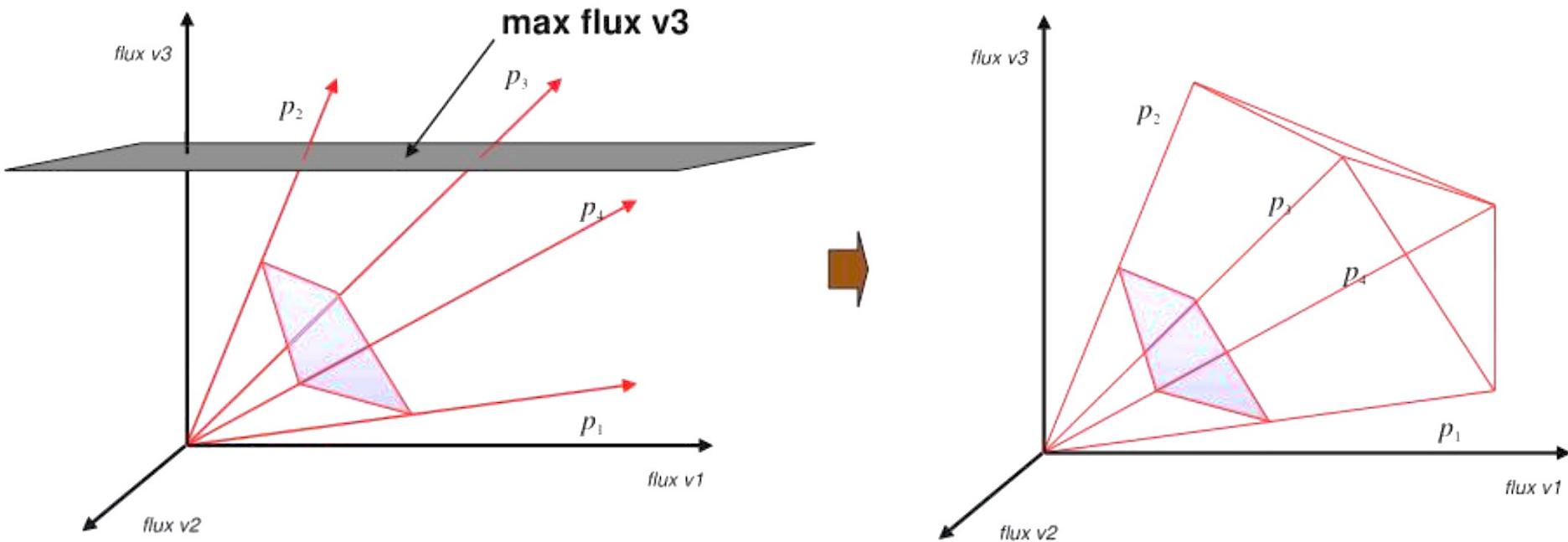


$$\left[\begin{array}{c|c} b1 & b2 \\ \hline 1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ 1 & 1 \\ 1 & 1 \end{array} \right] \quad \begin{array}{ll} v1 & \\ v2 & \\ v3 & \\ v4 & \\ vin & \\ vout & \end{array}$$

All steady state **fluxes configurations** are combinations of these extreme pathways

Capping the Solution Space

- Cone is open ended, but no reaction can have infinite flux.
- Often one can estimate constraints on transfer fluxes (**exchange** reactions):
 - Max glucose **uptake** measured at maximum growth rate
 - Max oxygen **uptake** based on diffusivity equation
- Flux constraints result in constraints on extreme pathways flux.



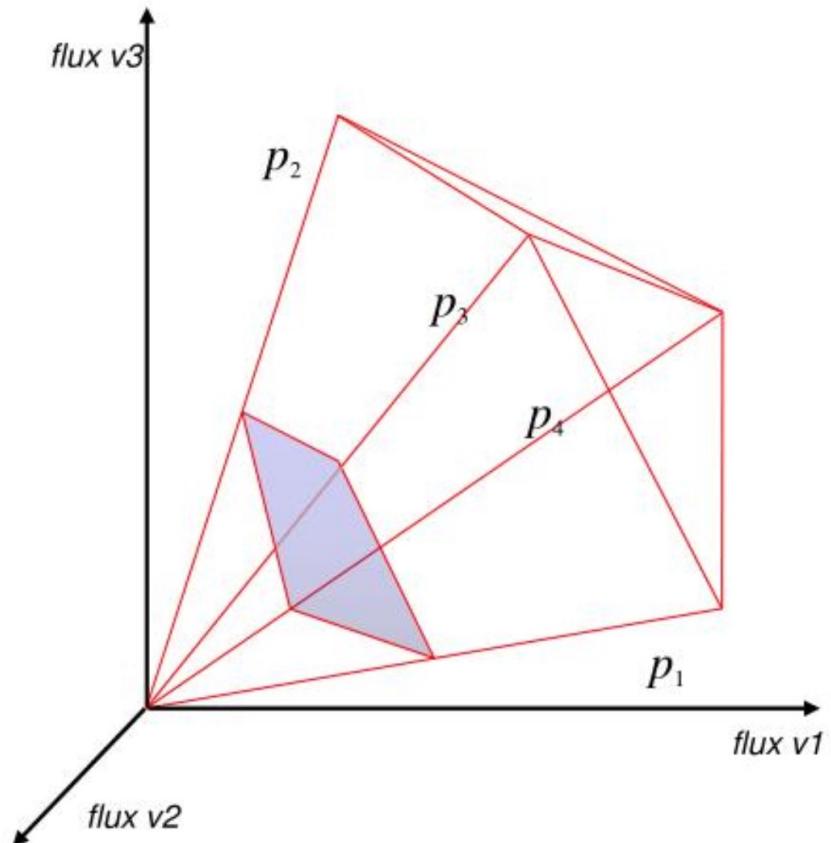
The Constrained Flux Cone

Contains all achievable flux distributions given the constraints:

- *Stoichiometry*
- *Reversibility*
- *Max and Min Fluxes = Lower and Upper bounds*

Only requires:

- *Annotation*
- *Stoichiometry*
- *Small number of flux constraints (small relative to number of reactions) = experimentally measured at maximum growth rate such as glucose uptake*



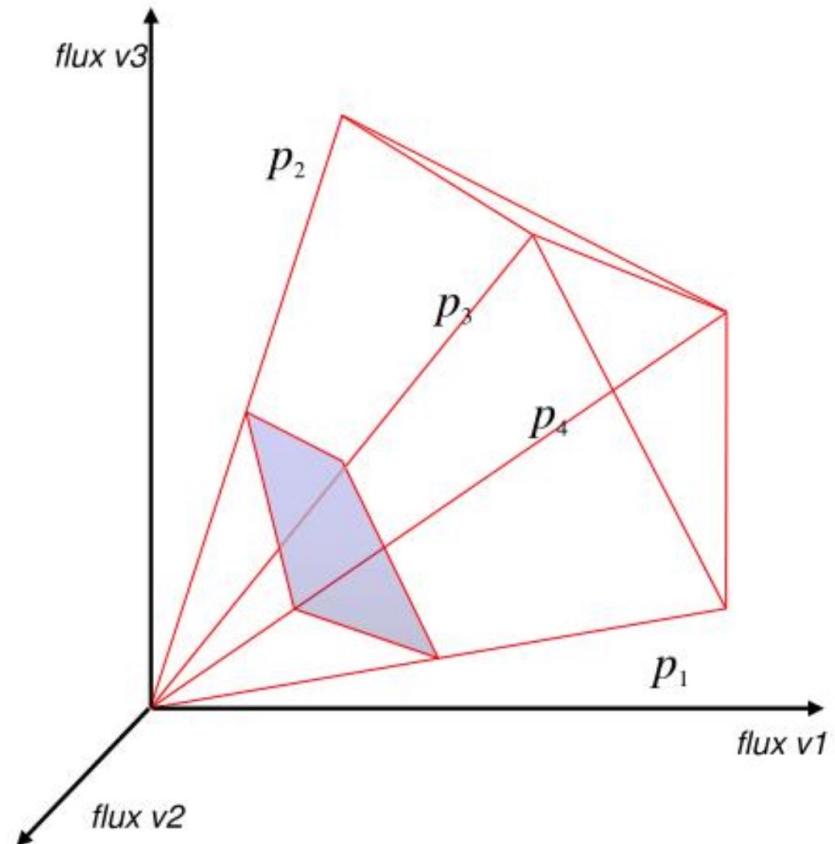
Selecting One Flux Distribution

At any one point in time, organisms have a single flux configuration

How do we select one flux configuration?

We will assume organisms are trying to maximize a “**fitness**” function that is a **function of fluxes**, $F(v)$?

Objective function

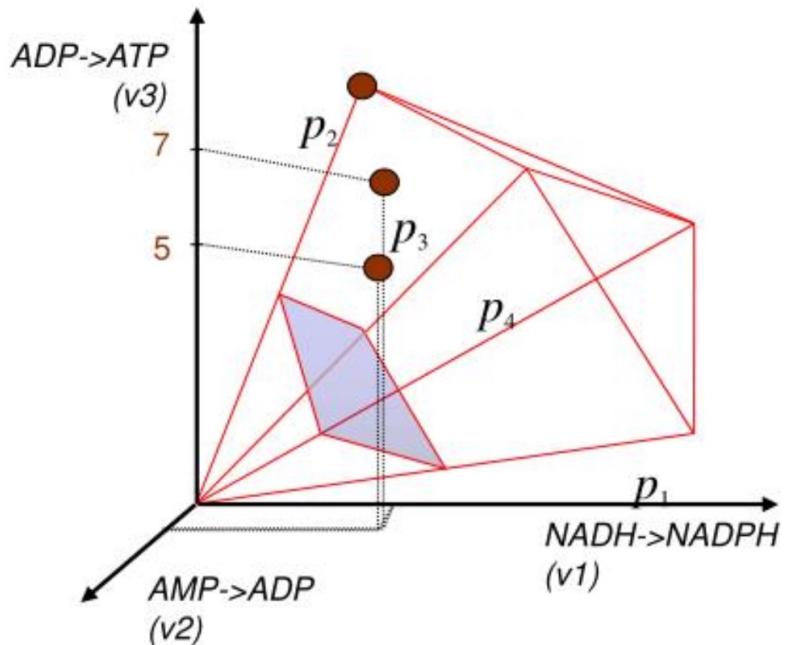


Optimizing Objective Function

Imagine we are trying to optimize ATP production.

Then, a reasonable choice for the **objective function** is $F(V)=v_3$

Objective: find the flux in the cone that maximizes v_3 .



If we choose $F(v)$ to be a linear function of V :

$$F(v) = \sum_i \alpha_i v_i$$

The optimizing flux will always lie on vertex or edge of the cone **Linear Programming**

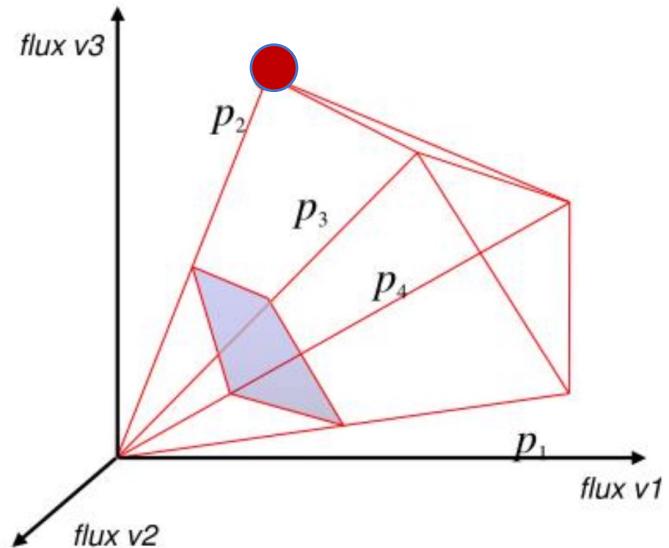
Flux Balance Analysis

Start with stoichiometric matrix or
GEM and constraints:

Chose an Objective function of fluxes to optimize:

$$F(v) = \sum_i \alpha_i v_i$$

Use **linear programming**, we can find a **feasible steady state flux** configuration that **maximizes the F** 

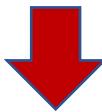


Optimizing *E. coli* Growth

For one gram of *E. coli* **Biomass**,
we need this ratio of metabolites.



Assuming a matched balanced set of metabolite fluxes,
we can formulate the **Biomass** reaction (Z) as
an Objective function for optimizing *E. coli* **Growth**.



Metabolite	(mmol)
ATP	41.257
NADH	-3.547
NADPH	18.225
G6P	0.205
F6P	0.0709
R5P	0.8977
E4P	0.361
T3P	0.129
3PG	1.496
PEP	0.5191
PYR	2.8328
AcCoA	3.7478
OAA	1.7867
AKG	1.0789

$$Z = 41.257v_{ATP} - 3.547v_{NADH} + 18.225v_{NADPH} + 0.205v_{G6P} + 0.0709v_{F6P} \\ + 0.8977v_{R5P} + 0.361v_{E4P} + 0.129v_{T3P} + 1.496v_{3PG} + 0.5191v_{PEP} \\ + 2.8328v_{PYR} + 3.7478v_{AcCoA} + 1.7867v_{OAA} + 1.0789v_{AKG}$$

Flux Balance Analysis Overview

Stoichiometric Matrix
Gene annotation
Enzyme and reaction
catalog

Feasible Space

$$S^*v=0$$

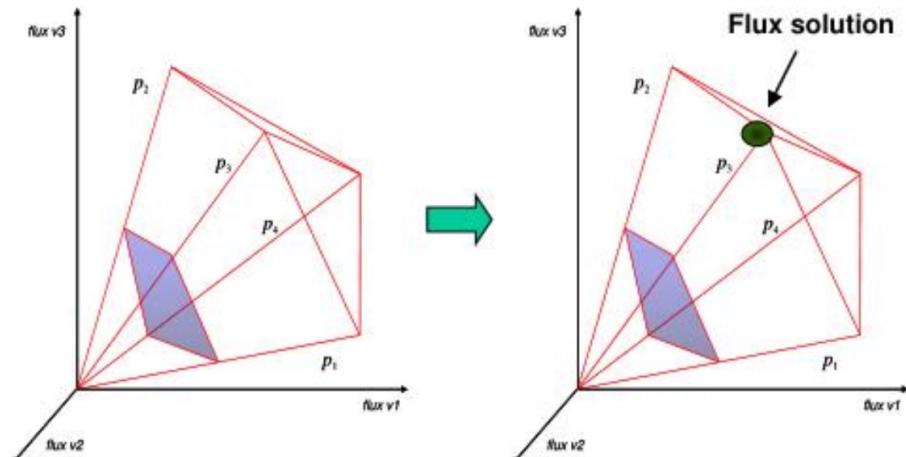
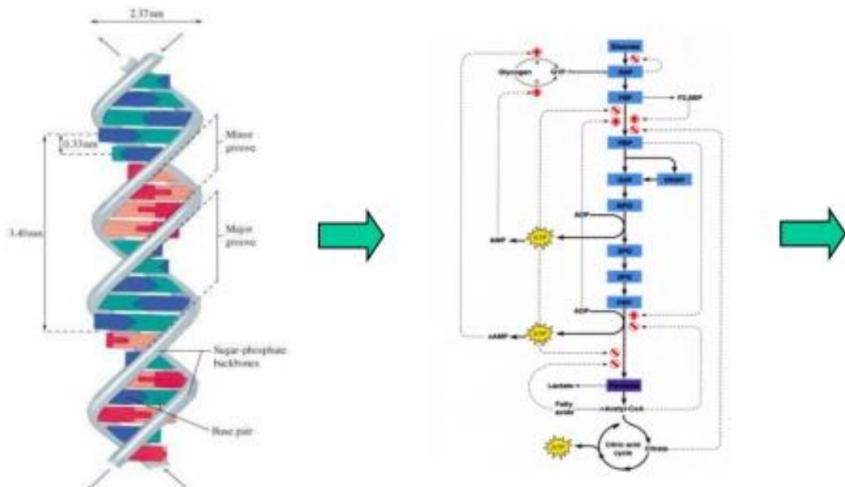
Add constraints:

$$v_i > 0$$

$$\alpha_i > v_i > \beta_i$$

Optimal Flux
Growth objective
 $Z=c^*v$

Solve with linear
programming



Hands-on workshop :

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- Import the model
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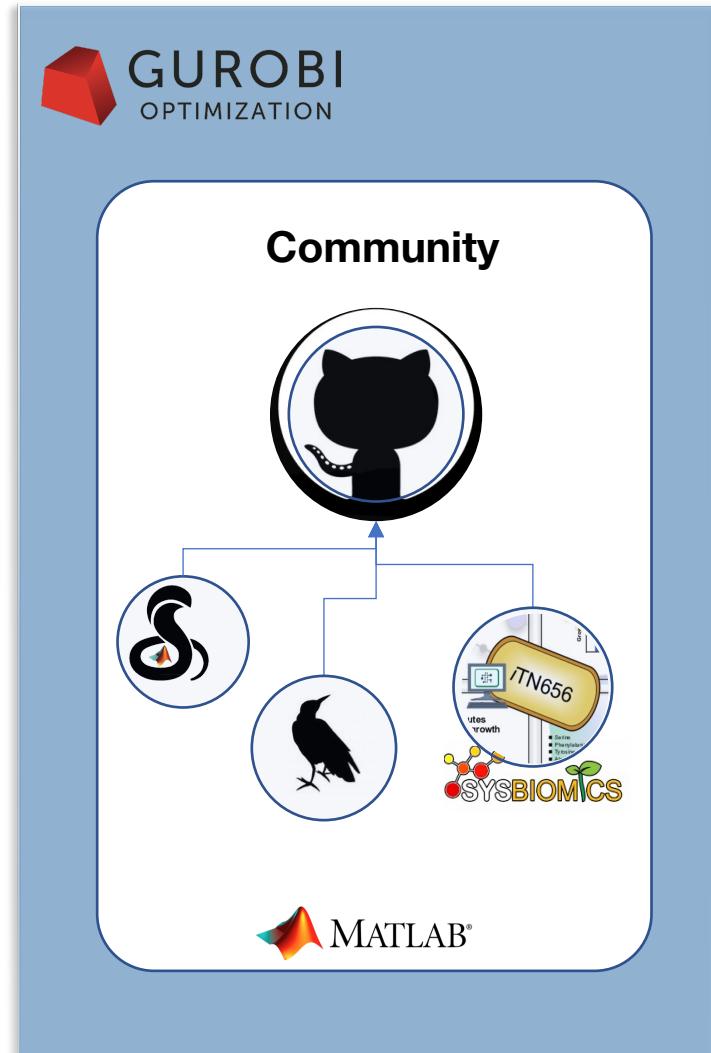
- Model validation
- Identifying the essential/preferable nutrients
- Integrative transcriptomics

Set up community system

To initialize constraint-based reconstruction and analysis toolbox

Genome-scale modeling requires
the community system. This includes:

1. Linear optimization (LP) solver
2. Simulation software
3. Toolboxes and Models



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```
nachonase — python3.7 — 80x24
exec gurobi.sh
Last login: Tue Jul 12 15:52:32 on ttys002
You have new mail.
nachonase@Nachons-MacBook-Air ~ % exec gurobi.sh
Python 3.7.4 (default, Aug 27 2019, 11:27:39)
[Clang 8.0.0 (clang-800.0.42.1)] on darwin
Type "help", "copyright", "credits" or "license" for more information.

-----
Warning: your license will expire in 14 days
-----

Academic license - for non-commercial use only - expires 2022-07-30
Using license file /Users/nachonase/gurobi.lic
Set parameter LogFile to value gurobi.log

Gurobi Interactive Shell (mac64), Version 9.1.2
Copyright (c) 2021, Gurobi Optimization, LLC
Type "help()" for help

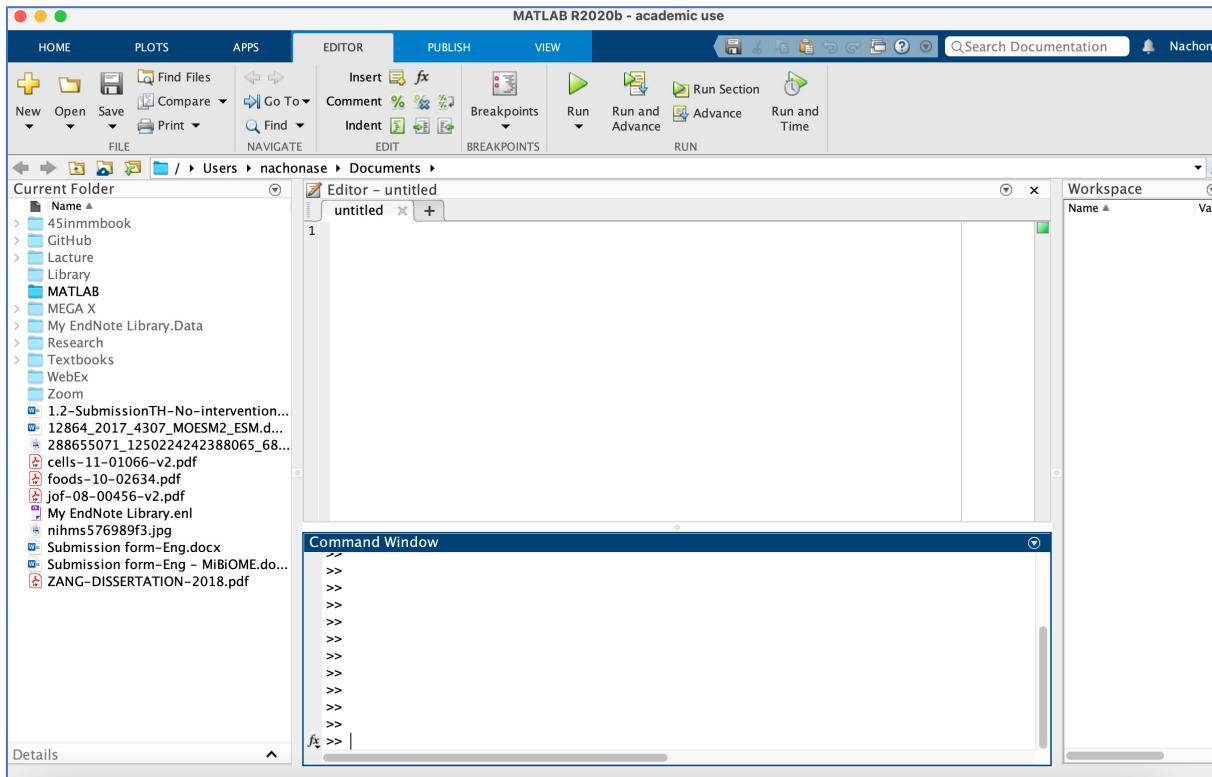
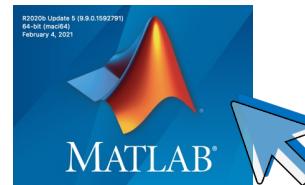
gurobi> |
```

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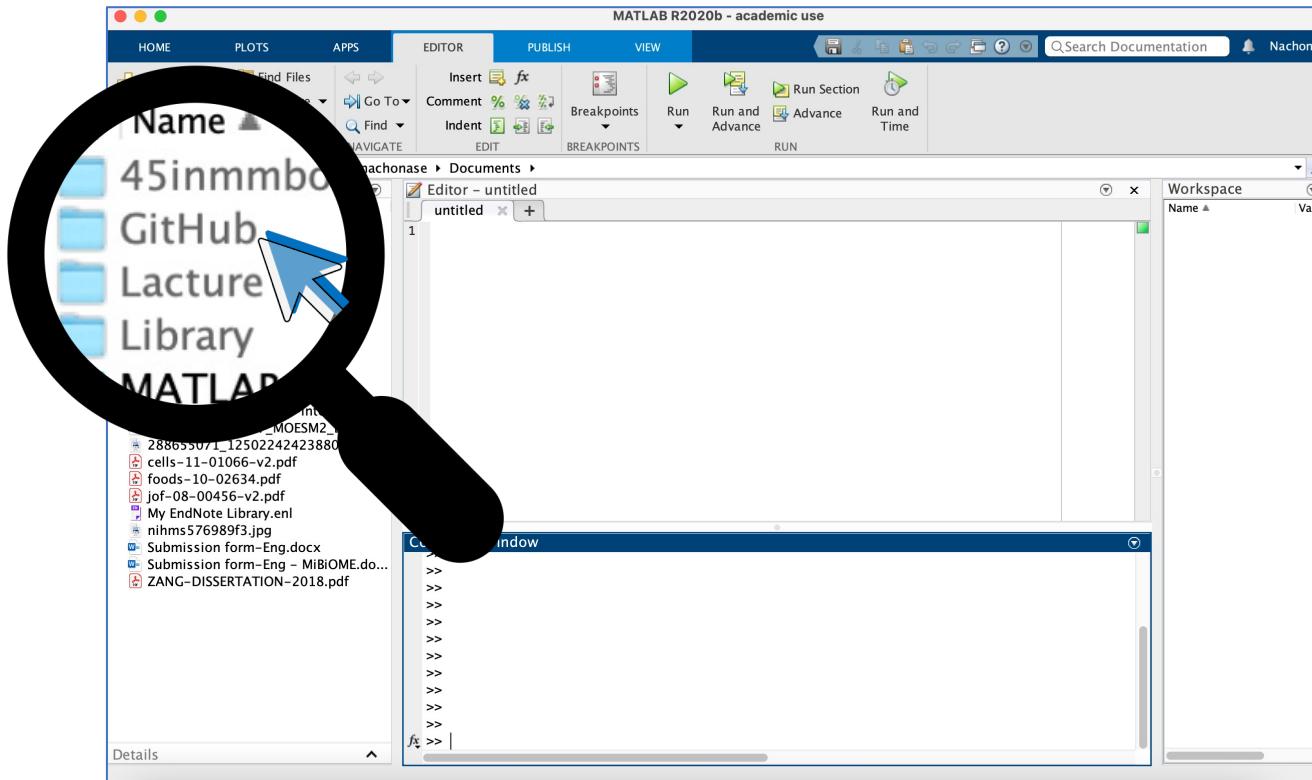


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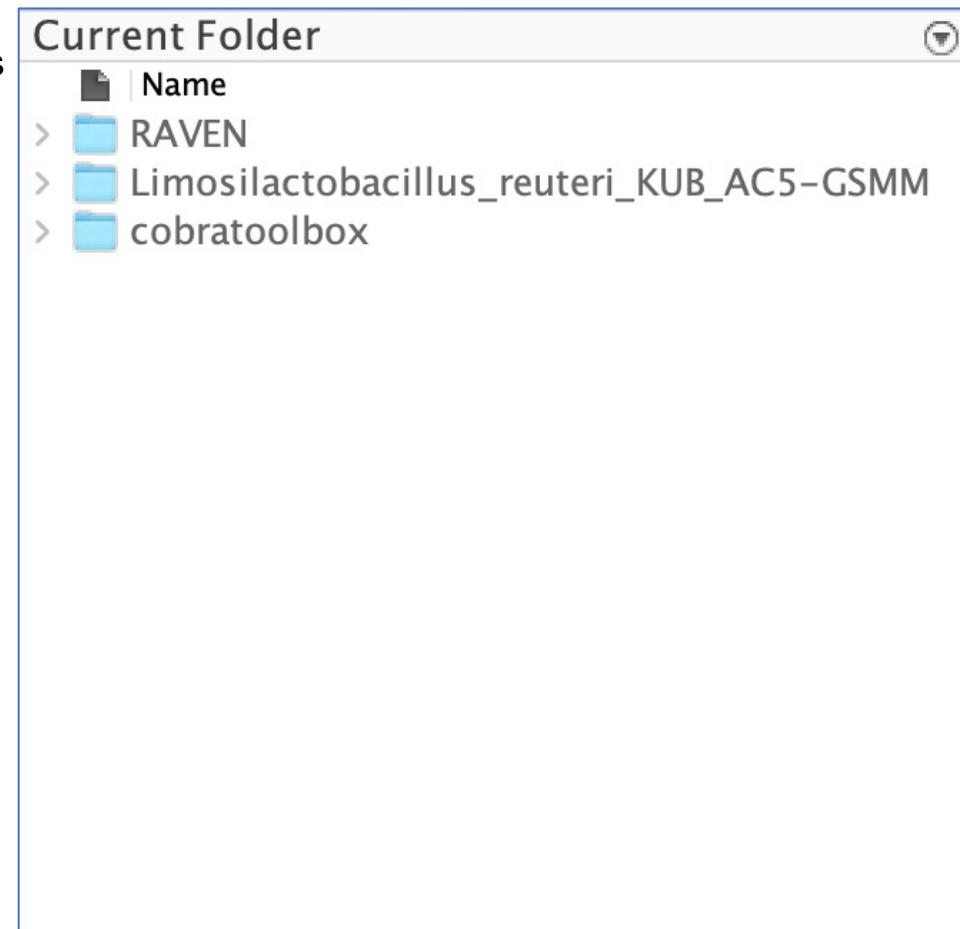
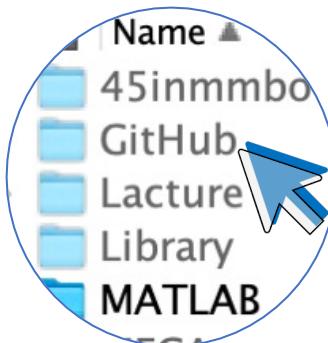


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Add all toolboxes and model to Path !!



RAVEN



Limosilactobacillus_reuteri_KUB_AC5-GSMM



cobratoolbox

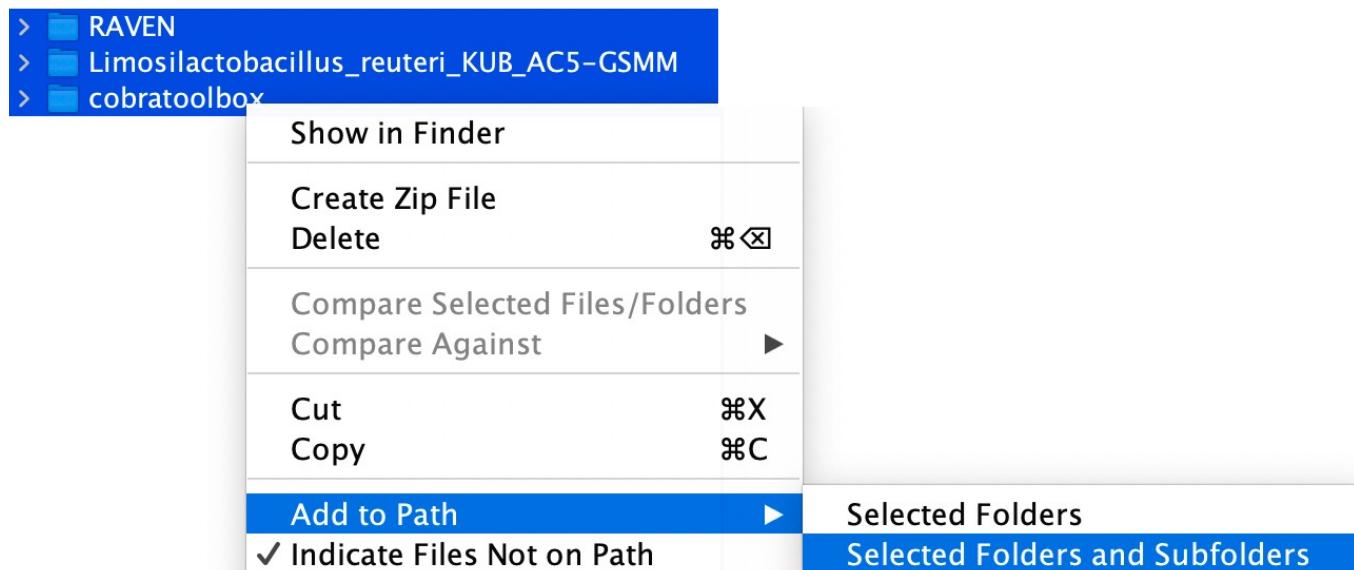
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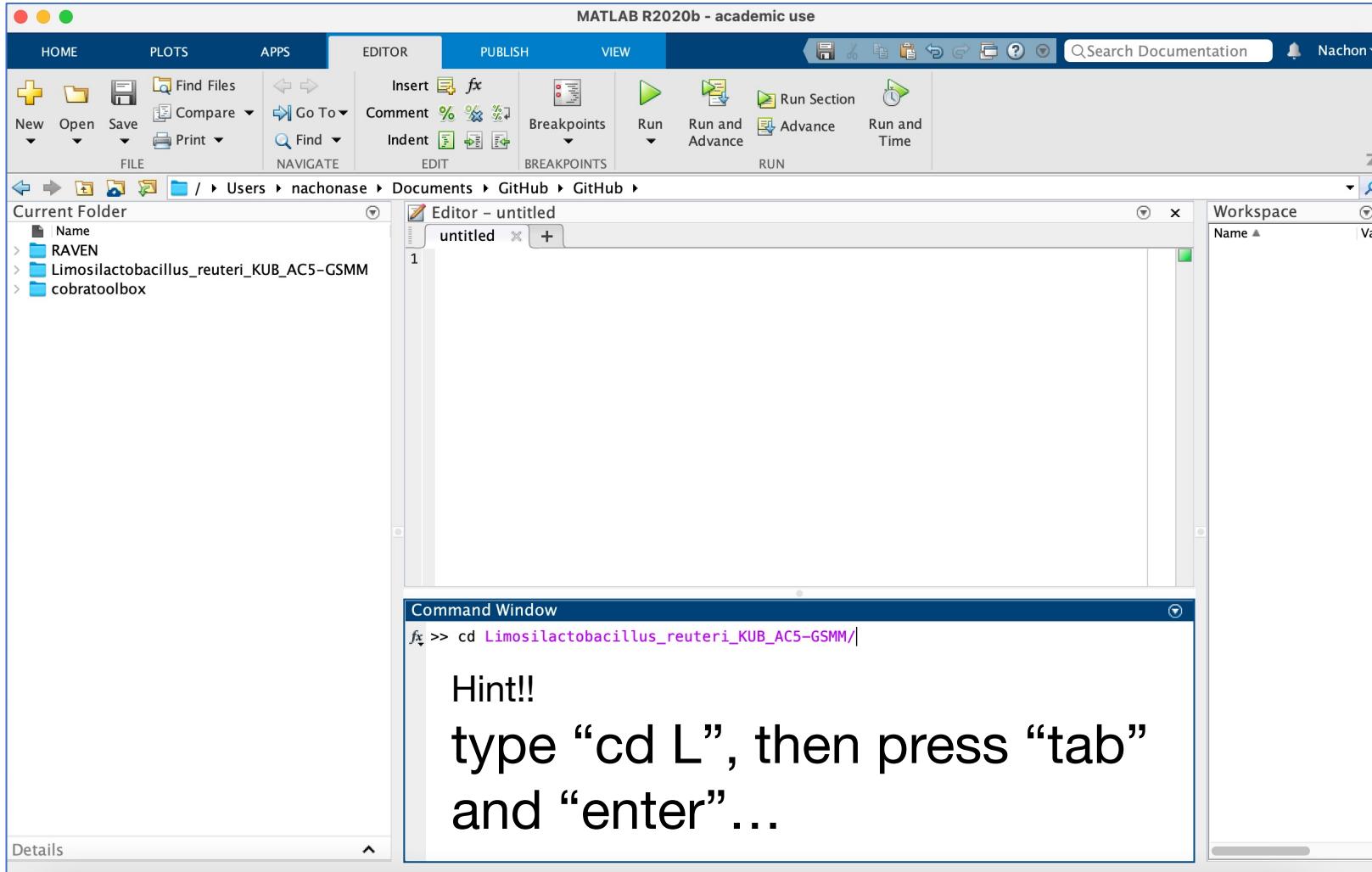
Let get into the model !!

Syntax:

```
>> cd Limosilactobacillus_reuteri_KUB_AC5-GSMM/
```

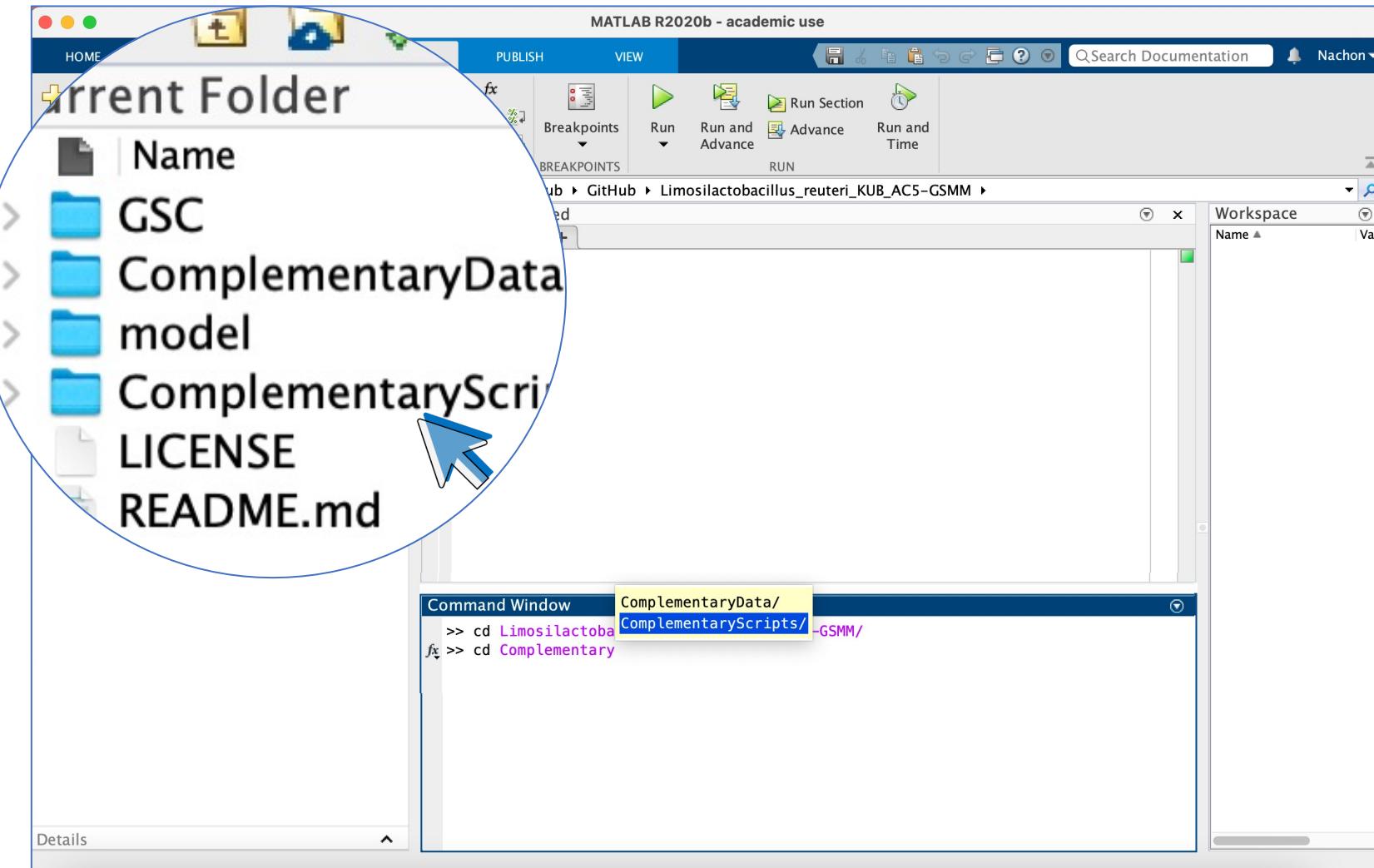
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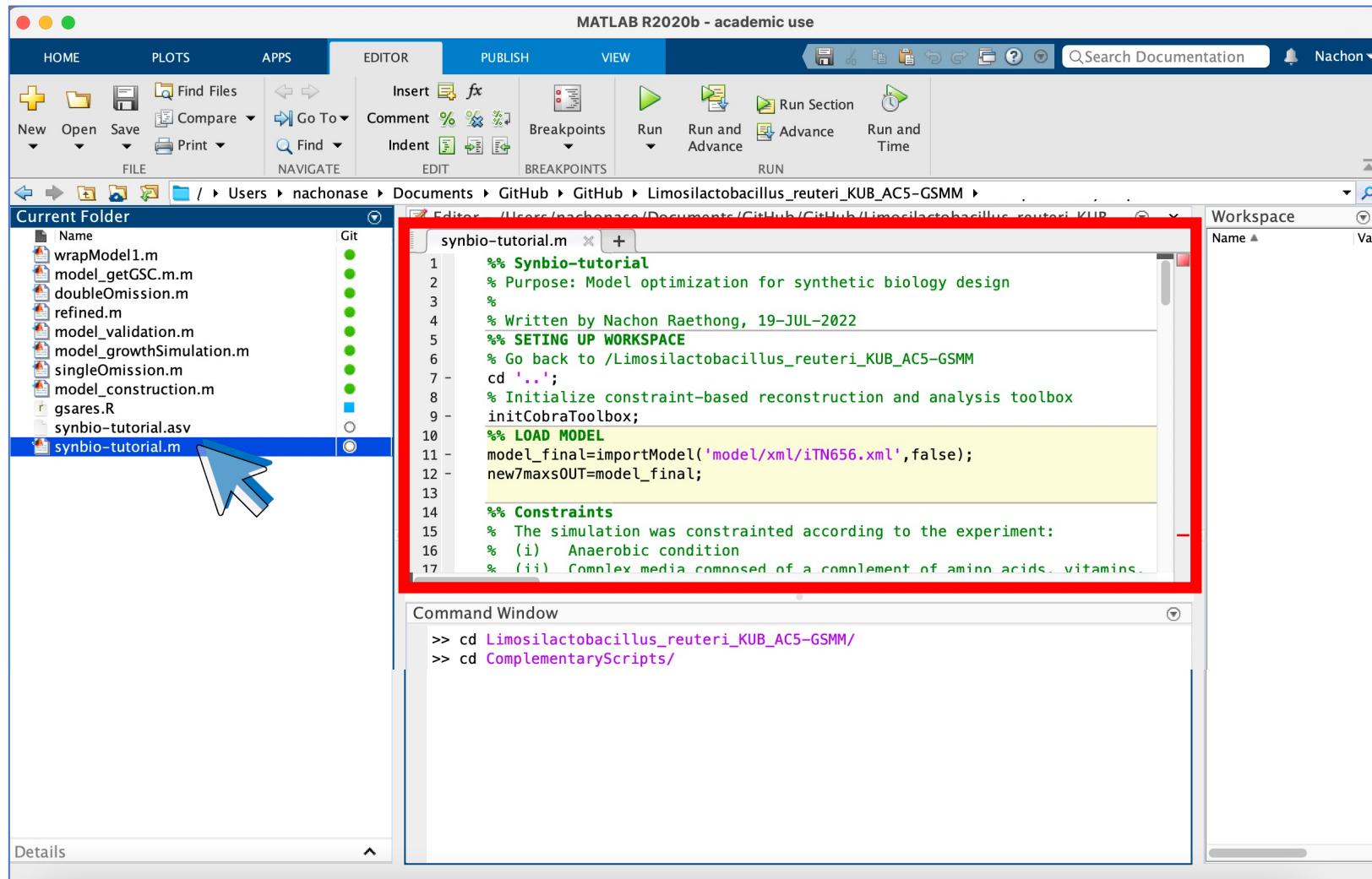
Set up community system

To initialize constraint-based reconstruction and analysis toolbox



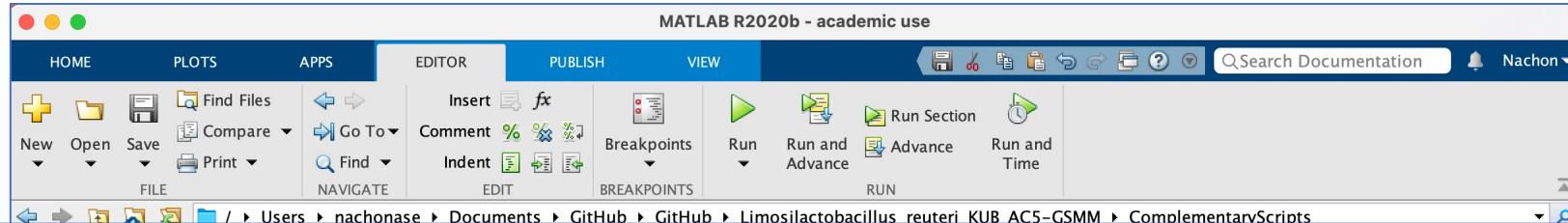
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To initialize constraint-based reconstruction and analysis toolbox



Set up community system

To initialize constraint-based reconstruction and analysis toolbox



Q1: Where are you, now? What is your current folder/workspace?

A:

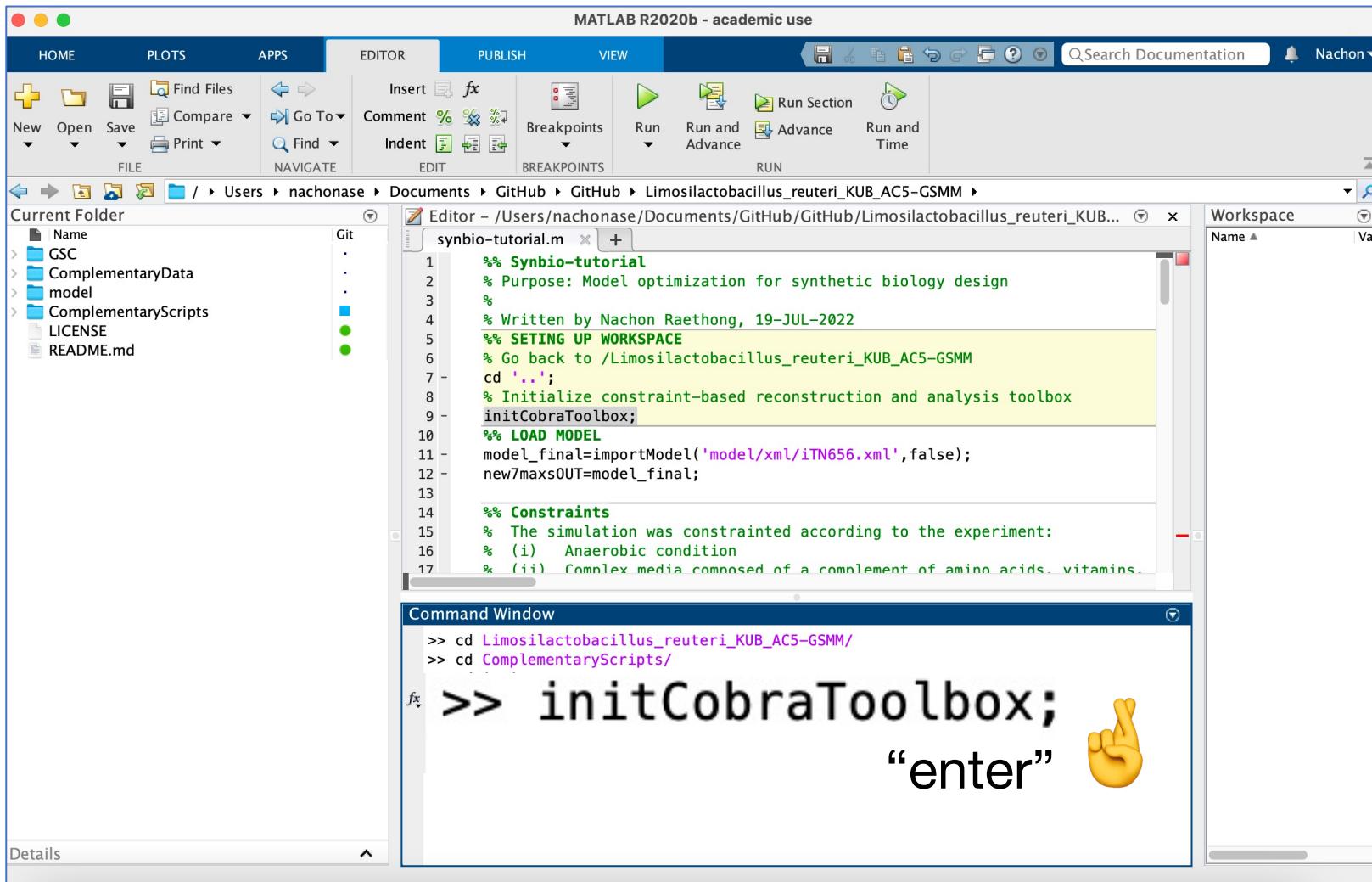
A screenshot of the MATLAB Command Window. The window title is "Command Window". The command history shows:

```
>> cd Limosilactoba
>> cd Complementary
fx>>
```

A context menu is open over the command history, listing options like Select All, Wrap Comments, Comment, Uncomment, Smart Indent, Evaluate Current Section, Insert Section Breaks Around Selection, Insert Text Markup, Function Browser, Function Hints, Code Folding, and Split Screen. The menu also includes keyboard shortcuts such as ⌘A for Select All and ⌘W for Wrap Comments. To the right of the Command Window, there is a "experiment:" pane containing the text "amino_acids_vitamins..".

Set up community system

To initialize constraint-based reconstruction and analysis toolbox



```

COBRA TOOLBOX v1.5.2 | Constraint-Based Reconstruction and Analysis
The COBRA Toolbox - 2017
Documentation: http://opencobra.github.io/cobratoolbox

> Checking if git is installed ... Done.
> Checking if the repository is tracked using git ... Done.
> Checking if curl is installed ... Done.
> Checking if remote can be reached ... Done.
> Initializing and updating submodules ... Done.
> Adding all the files of The COBRA Toolbox ... Done.
> Define CB map output... set to svg.
> Retrieving models ... Done.
> TranslateSBML is installed and working properly.
> Configuring solver environment variables ...
- [*--] ILOG_CPLEX_PATH: /Users/syrra/Applications/IBM/ILOG/CPLEX_Studio1271/cplex/matlab/x86-64_osx
- [*--] GUROBI_PATH: /Library/gurobi702/mac64/matlab
- [*--] TOMLAB_PATH : --> set this path manually after installing the solver ( see instructions )
- [*--] MOSEK_PATH : --> set this path manually after installing the solver ( see instructions )
Done.
> Checking available solvers and solver interfaces ... Done.
> Setting default solvers ... Done.
> Saving the MATLAB path ... Done.
- The MATLAB path was saved in the default location.

> Summary of available solvers and solver interface s

      Support   LP   MILP    QP   MIQP    NLP
-----+-----+-----+-----+-----+-----+
cplex_direct  full     0     0     0     0     -
dqqMinos     full     1     -     -     -     -
glpk          full     1     1     -     -     -
gurobi        full     1     1     1     1     -
ibm_cplex     full     1     1     1     -     -
matlab         full     1     -     -     -     1
mosek          full     0     0     0     -     -
pdco           full     1     -     1     -     -
quadMinos     full     1     -     -     -     1
tomlab_cplex  full     0     0     0     0     -
qpng           experimental     -     -     1     -     -
tomlab_snpt   experimental     -     -     -     -     0
gurobi_mex    legacy    0     0     0     0     -
lindo_old     legacy    0     -     -     -     -
lindo_legacy   legacy    0     -     -     -     -
lp_solve       legacy    1     -     -     -     -
opti           legacy    0     0     0     0     0
-----+-----+-----+-----+-----+-----+
Total          -     8     3     4     1     2

+ Legend: - = not applicable, 0 = solver not compatible or not installed, 1 = solver installed.

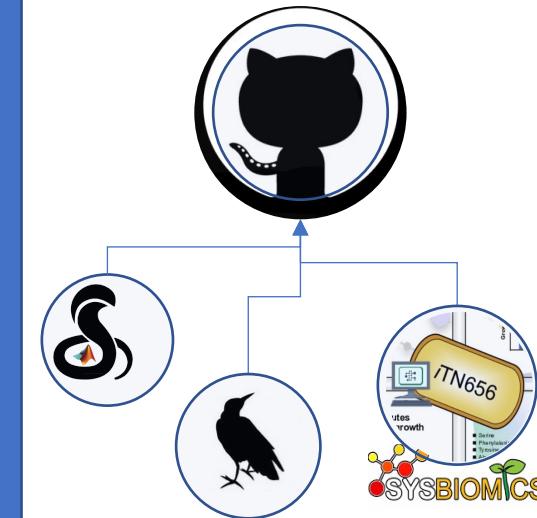
> You can solve LP problems using: 'dqqMinos' - 'glpk' - 'gurobi' - 'ibm_cplex' - 'matlab' - 'pdco' - 'quadMinos' - 'lp_solve'
> You can solve MILP problems using: 'glpk' - 'gurobi' - 'ibm_cplex'
> You can solve QP problems using: 'gurobi' - 'ibm_cplex' - 'pdco' - 'qpng'
> You can solve MIQP problems using: 'gurobi'
> You can solve NLP problems using: 'matlab' - 'quadMinos'

> Checking for available updates ...
> The COBRA Toolbox is up-to-date.

```



Community



MATLAB®

Linear optimization (LP) solver

Introduction to the model, *i*TN656

*i*TN656 is the genome-scale metabolic model of *Limosilactobacillus reuteri* KUB-AC5.



Q2: How many genes, metabolites, compartments and reactions included in the model, *i*TN656 ?

A:

Enter to “model” folder !!

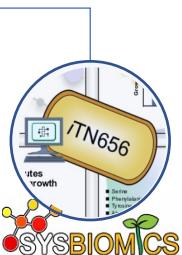
There are 4 different formats of the model:

- *i*TN656.mat
- *i*TN656.txt
- *i*TN656.xlsx
- *i*TN656.xml

Open *i*TN565.xlsx !!

< > Limosilactobacillus_reuteri_KUB_AC5-GSMM	
Name	Date Modified
> .git	23 Apr BE 256
> ComplementaryData	7 Feb BE 256
> ComplementaryScripts	1 May BE 256
> GSC	7 Feb BE 256
LICENSE	7 Feb BE 256
> model	7 Feb BE 256
README.md	7 Feb BE 256

Introduction to the model, iTN656

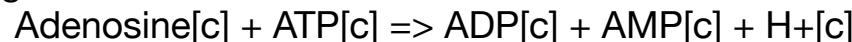


iTN656 is the genome-scale metabolic model of *Limosilactobacillus reuteri* KUB-AC5.
This model includes

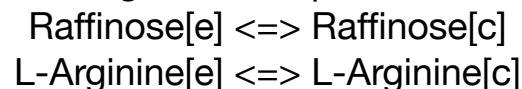
2 compartments (i.e. cytosol and extracellular space),
656 genes,
831 metabolites,
953 metabolic reactions.

There are 4 types of metabolic reactions included in the model:

1) Enzymatic reaction e.g. adenosine kinase:



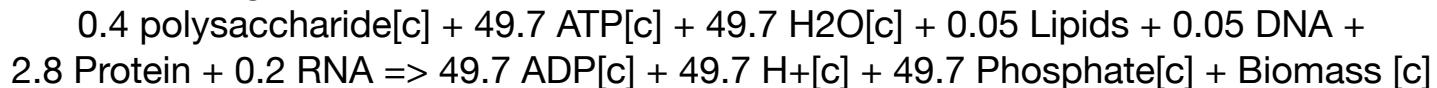
2) Transport reaction e.g. raffinose and arginine transporters:



3) Exchange reaction e.g. choline and arabinose exchanges:



4) Pseudo reaction e.g. biomass formulation:

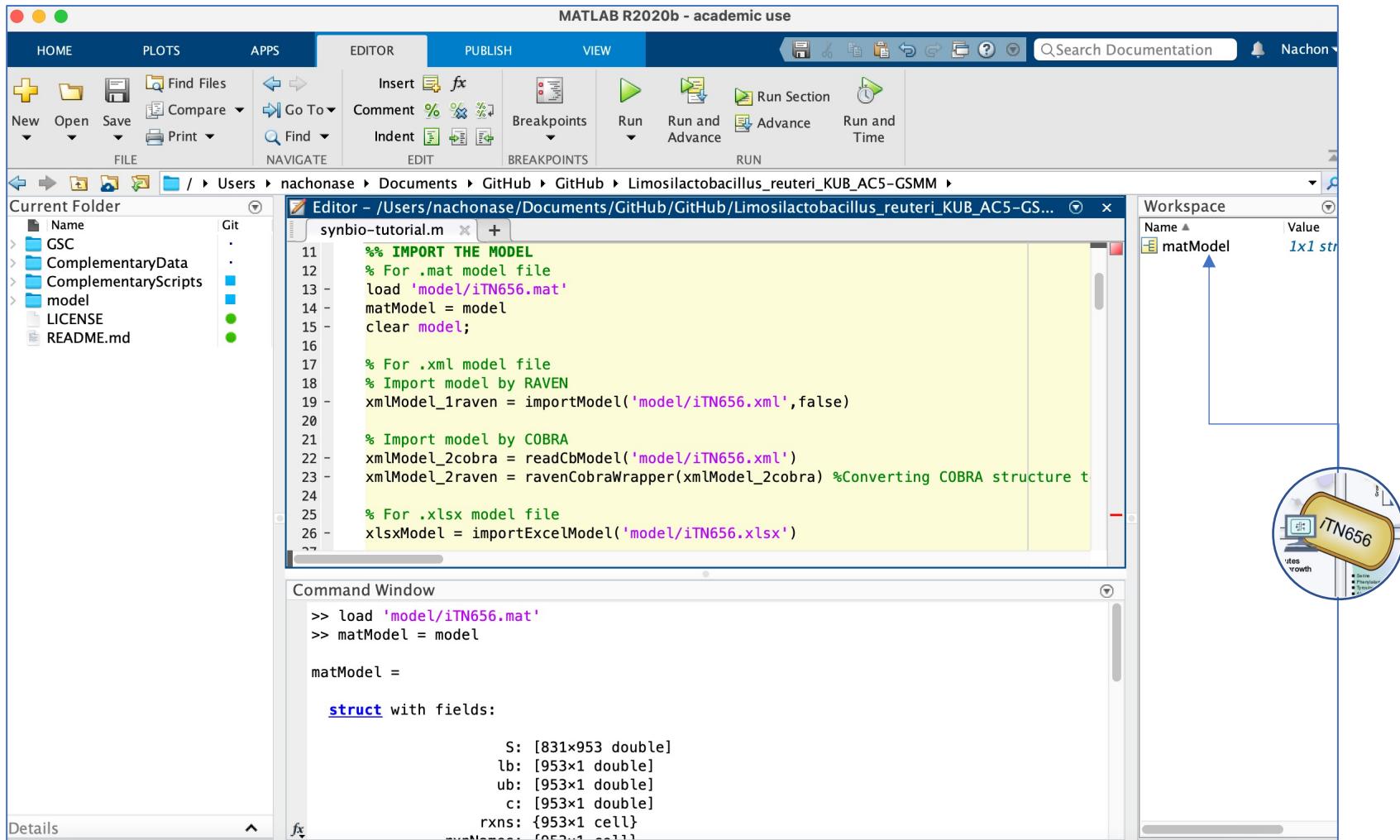


Q3: How many exchange reactions are there in the iTN656 ?

A: ...

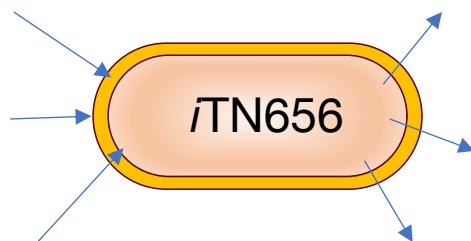
Import the model

At this point we should import the model, *iTN656*. As seen in model folder, there are 4 different formats of the model which can be imported into Matlab by different commands.

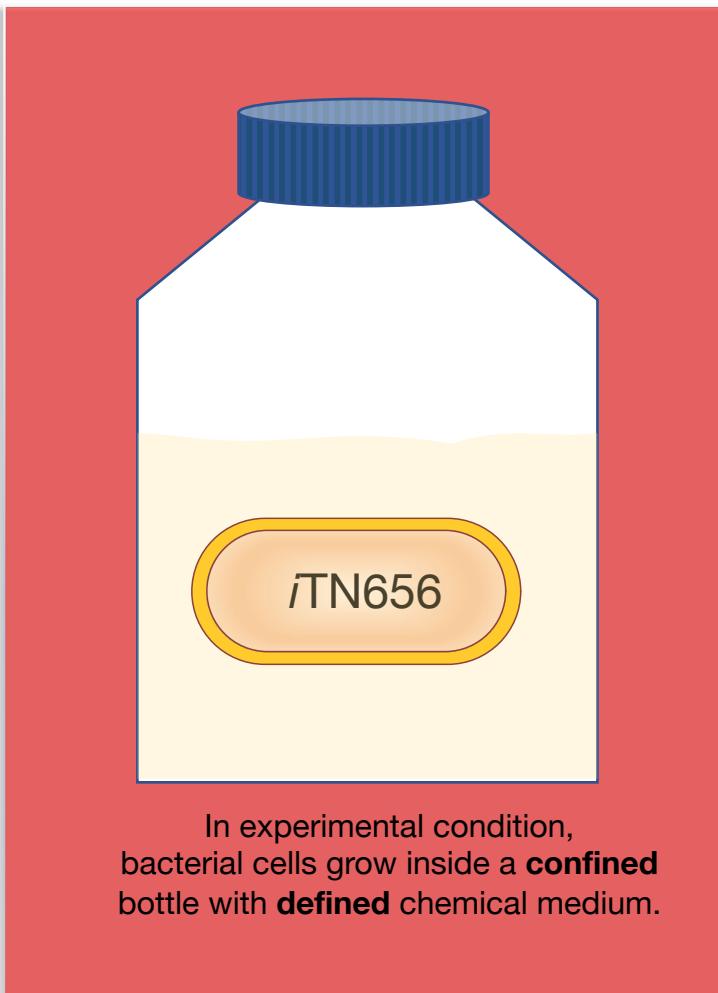
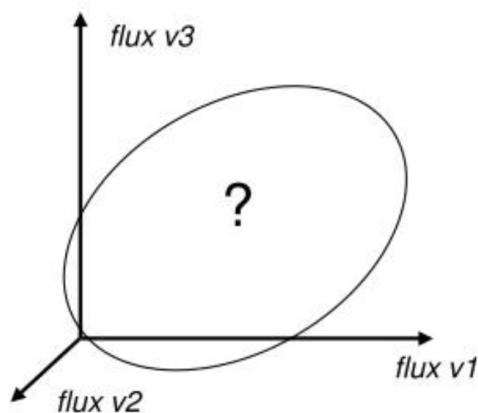


Define constraints

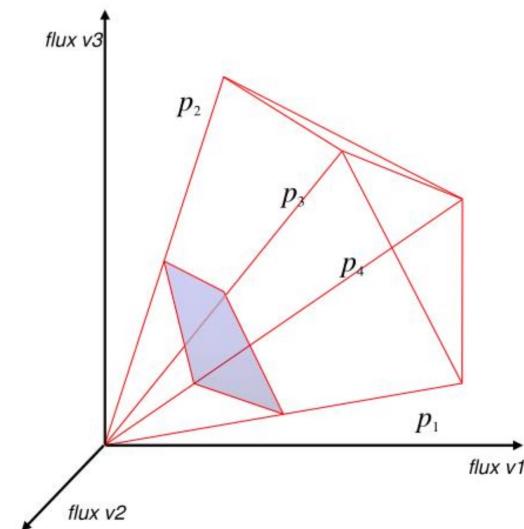
At steady state, metabolite concentrations are constant.



In unconstrained condition,
model can be anything.



In experimental condition,
bacterial cells grow inside a **confined**
bottle with **defined** chemical medium.



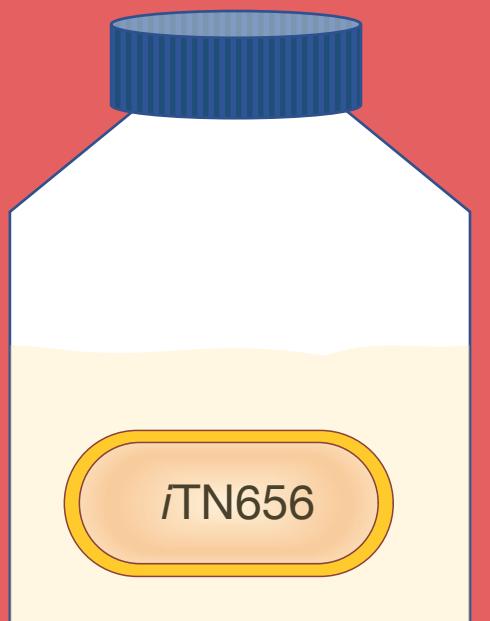
*Small number of flux
constraints (small relative to
number of reactions) =
experimentally measured
at maximum growth rate
such as glucose uptake.*

Define constraints

At steady state, metabolite concentrations are constant.

Thus, the simulation will constrain according to the experimental condition:

- (i) Anaerobic condition
- (ii) Complex medium composed of a complement of carbon source (e.g. glucose), amino acids, vitamins, lipids and ions.



In experimental condition,
bacterial cells grow inside a **confined**
bottle with **defined** chemical medium.

How to constrain the model ?

Often one can estimate constraints on transfer fluxes (**exchange reactions**):

- Limit oxygen uptake based on anaerobic condition
- Limit the uptake rates of glucose, amino acids, vitamins, lipids and ions based on defined chemical medium composition

2.3. Cultivation of *L. reuteri* KUB-AC5

L. reuteri KUB-AC5 obtained from the collection of the Department of Biotechnology, Faculty of Agro-industry, Kasetsart University, Thailand [4]. For inoculum preparation, the cell culture was propagated twice in MRS medium (Difco). For cultivation, the KUB-AC5 containing 10^7 CFU mL $^{-1}$ were suspended in 0.85% NaCl solution with the absorbance 600 nm of 0.5. The medium used for the cultivation was the modified MRS which composed of 5.0 g per liter (g L $^{-1}$) yeast extract, 2.0 g L $^{-1}$ of K₂HPO₄, 1.0 g L $^{-1}$ of (NH₄)₂SO₄, 0.1 g L $^{-1}$ of MgSO₄·7H₂O, 0.05 g L $^{-1}$ of MnSO₄·4H₂O, and 1.0 mL of Tween 80 [27]. The medium was adjusted for pH 6.5 ± 0.2 before autoclaved at 121 °C under 15 pound per square inch (psi) for 15 minutes (min). The individual sterile carbon source solution (i.e., glucose, sucrose, maltose, or lactose) was added to a final concentration of 20 g L $^{-1}$. The medium was inoculated at 2% (v/v).

Define constraints

At steady state, metabolite concentrations are constant.

Thus, the simulation will constrain according to the experimental condition:

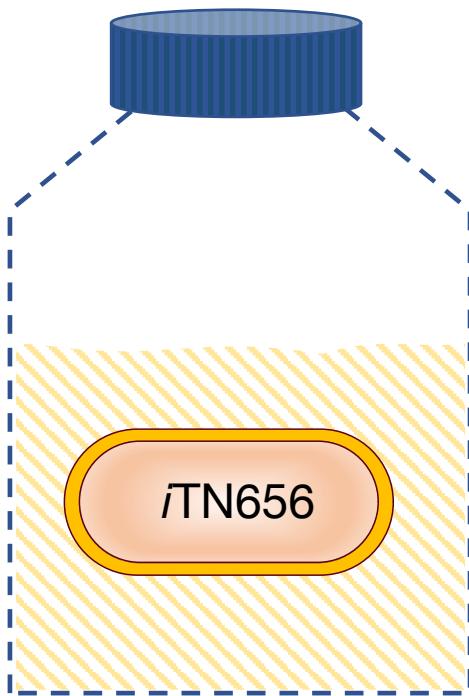
The screenshot shows the MATLAB R2020b interface. The top menu bar includes HOME, PLOTS, APPS, EDITOR (selected), PUBLISH, and VIEW. The toolbar below has buttons for New, Open, Save, Print, Insert, Comment, Indent, Breakpoints, Run, Run and Advance, and Run and Time. The Current Folder browser on the left shows a directory structure with files like GSC, ComplementaryData, ComplementaryScripts, model, LICENSE, and README.md. The Editor window displays a script named 'synbio-tutorial.m' containing MATLAB code. The Workspace browser on the right lists variables and their values:

Name	Value
amino_acids	1x20 cell
i	10
lipid	1x3 cell
matModel	1x1 str
new7maxsOUT	1x1 str
products	1x10 cell
vitamin	1x7 cell
xmlModel_1ra...	1x1 str

```
35 - new7maxsOUT = xmlModel_1raven;
36 - new7maxsOUT = setParam(new7maxsOUT,'lb',{'EXC_BOTH_o2_e'},[-0.00000000001]);
37 -
38 - amino_acids = {'EXC_BOTH_arg_L_e', 'EXC_BOTH_cys_L_e', 'EXC_BOTH_il...e', 'EXC...
39 - for i = 1:numel(amino_acids)
40 -     new7maxsOUT=setParam(new7maxsOUT,'lb',amino_acids(i),[-1]);
41 - end
42 -
43 - lipid= {'EXC_BOTH_hdcea_e', 'EXC_BOTH_ocdcya_e', 'EXC_BOTH_ocdctr_e'};
44 - for i = 1:numel(lipid)
45 -     new7maxsOUT=setParam(new7maxsOUT,'lb',lipid(i),[-1]);
46 - end
47 -
48 - vitamin = {'EXC_BOTH_4abz_e', 'EXC_BOTH_pydam_e', 'EXC_BOTH_thm_e', 'EXC_BOTH_pyda...
49 - for i = 1:numel(vitamin)
50 -     new7maxsOUT=setParam(new7maxsOUT,'lb',vitamin(i),[-0.0001]);
51 - end
52 -
53 -
54 -
55 - products = {'EXC_BOTH_hxan_e' 'EXC_BOTH_xan_e',...
56 - 'EXC_BOTH_gcald_e', 'EXC_BOTH_btd_RR_e',...
57 - 'EXC_BOTH_ac_e', 'EXC_BOTH_lac_D_e',...
58 - 'EXC_BOTH_orot_e','EXC_BOTH_etoh_e',...
59 - 'EXC_BOTH_mal_L_e', 'EXC_BOTH_lac_L_e'};
60 - for i = 1:numel(products)
61 -     new7maxsOUT=setParam(new7maxsOUT,'lb',products(i),[0]);
62 -     new7maxsOUT=setParam(new7maxsOUT,'ub',products(i),[1000]);
63 - end
```

Optimize objective function

In constrained condition, we can formulate the **Biomass reaction** as an **Objective function** for Optimizing KUB-AC5 Growth.



In constrained condition, the uptake rates of oxygen, glucose, amino acids, vitamins, lipids and ions are limited according to the experiment.

Biomass reaction is selected as an **Objective function** for Optimizing KUB-AC5 Growth.

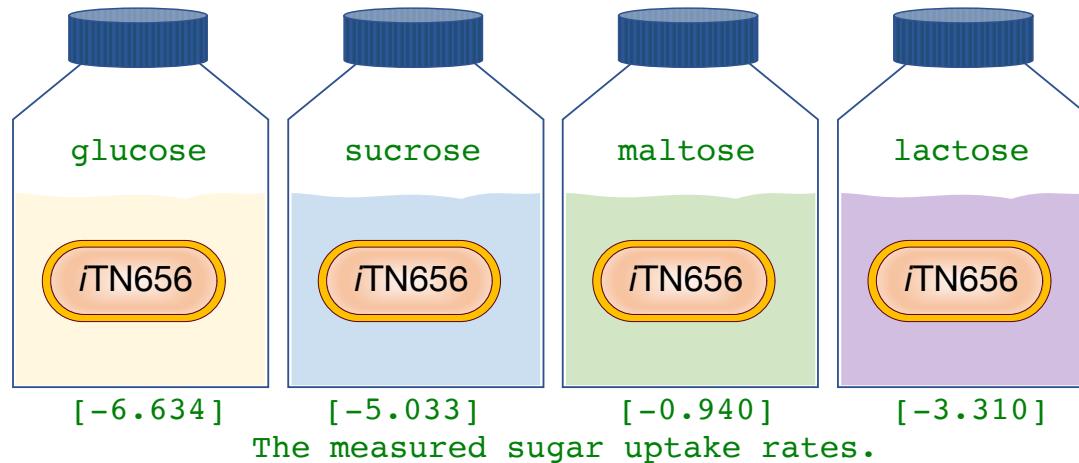
```
%% Optimize objective function
% In constrained condition, the uptake rates of oxygen, glucose,
% amino acids, vitamins, lipids and ions are limited according to
% the experiment. Biomass reaction is selected as an Objective function
% for Optimizing KUB-AC5 Growth.

new7maxs0UTg20 = setParam(new7maxs0UTg20, 'obj', {'AC5_GROWTH'}, [1]);
sol = solveLP(new7maxs0UTg20)
printFluxes(new7maxs0UTg20, sol.x, true, 10^-3)
fprintf(['umax = ' num2str(sol.f*-1) ' per hour'\n']);
```

Q4: Is the growth rate of KUB-AC5 higher than 0.1 per hour ?
A: Yes or No

...TEST...LEARN...DESIGN...

Model validation



Q5: Fill the predicted growth rates and %ERROR for each sugar in the table.

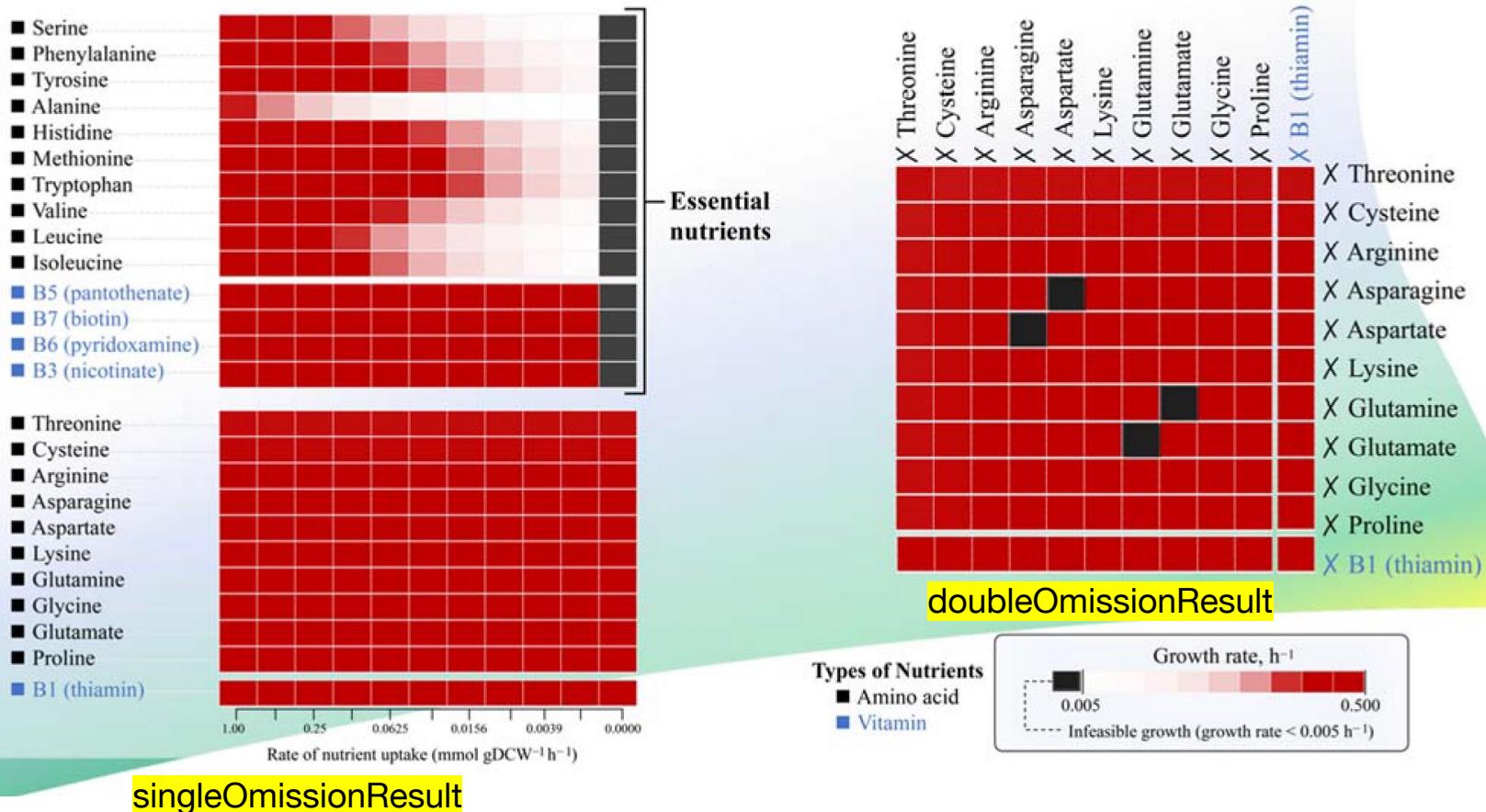
	Sugar uptake rates	<i>In vitro</i> growth	<i>In silico</i> growth	%ERROR
Glucose	6.634 mmol/gDW/h	0.151 h ⁻¹	Xxxx h ⁻¹	xxxx
Sucrose	5.033 mmol/gDW/h	0.247 h ⁻¹	Xxxx h ⁻¹	xxxx
Maltose	0.940 mmol/gDW/h	0.199 h ⁻¹	Xxxx h ⁻¹	xxxx
Lactose	3.310 mmol/gDW/h	0.078 h ⁻¹	Xxxx h ⁻¹	xxxx

Rationale:

As lactobacilli are fastidious microorganisms that have **complex nutritional requirements** for growth including carbohydrates, amino acids, vitamins, nucleotides, and fatty acids thus, optimal media formulation is the important factor for probiotic production in industry. The most common medium for lactic acid bacteria is the DeMan-Rogosa-Sharpe medium, MRS. However, MRS for industrial production would be high cost with unauthorized ingredients. **The optimal design of media composition is hence important with respective to cost effective process in industrial biotechnology.**

...TEST... **LEARN**...DESIGN...

We therefore aim to identify the essential/preferable nutrients for KUB-AC5 growth : using *i*TN656 as a scaffold



Rationale:

To further identify the key metabolic routes for enhancing *L. reuteri* KUB-AC5 growth : The list of differentially expressed genes (**DEGs**) under different carbon sources (e.g., sucrose and glucose) were retrieved and integrated with the iTN656 using **R** package **Piano** (Platform for Integrated Analysis of Omics data).

To explore this, we need to have:

1. **GSC.txt** which is a gene set collection extracted from genome-scale metabolic models
2. **GLS.txt** which is the list of genes, fold changes and p-values.

gene set	gene
Acetyl-CoA	AC5u0009GL000222
Acetyl-CoA	AC5u0009GL000223
Acetyl-CoA	AC5u0009GL000224
Coenzyme A	AC5u0009GL000373
Coenzyme A	AC5u0009GL000410
Coenzyme A	AC5u0009GL000584
Malonyl-CoA	AC5u0009GL000222
Malonyl-CoA	AC5u0009GL000223
Malonyl-CoA	AC5u0009GL000224
Malonyl-CoA	AC5u0009GL000226
Malonyl-CoA	AC5u0009GL000229
Malonyl-CoA	AC5u0009GL001601
Pyridoxamine	AC5u0009GL000270
Pyridoxamine	AC5u0009GL000843
Pyridoxamine	AC5u0009GL001072
Pyridoxamine	AC5u0009GL000581
Pyridoxamine	AC5u0009GL000992
Pyridoxal	AC5u0009GL000270
Pyridoxal	AC5u0009GL000843
Pyridoxal	AC5u0009GL001072
Pyridoxal	AC5u0009GL000581
Pyridoxal	AC5u0009GL001752
Pyridoxal	AC5u0009GL000621
Pyridoxal	AC5u0009GL002175
Thiamin monophosphate	AC5u0009GL000271
Thiamin monophosphate	AC5u0009GL000964
Thiamin monophosphate	AC5u0009GL001481
Thiamin monophosphate	AC5u0009GL002025
Thiamin monophosphate	AC5u0009GL000020

A

gene	p	FC
AC5u0009GL000001	0.06267053	-0.276172798
AC5u0009GL000002	0.000460436	0.231021413
AC5u0009GL000003	0.000440649	0.320823095
AC5u0009GL000004	0.04819742	0.257343849
AC5u0009GL000005	0.233517494	0.134457234
AC5u0009GL000006	0.207951195	0.119844484
AC5u0009GL000007	0.005400812	-0.18291248
AC5u0009GL000008	0.307414784	-0.090896107
AC5u0009GL000009	0.437663584	-0.086403833
AC5u0009GL000010	0.788864951	-0.031147229
AC5u0009GL000011	0.625380381	-0.065283033
AC5u0009GL000012	1.20E-05	-0.616217107
AC5u0009GL000013	0.146296646	0.120848826
AC5u0009GL000014	0.000683922	0.230731016
AC5u0009GL000015	4.27E-07	0.331725652
AC5u0009GL000016	1.67E-06	0.394489031
AC5u0009GL000017	0.984301463	0.004731103
AC5u0009GL000018	0.075260841	-0.146707047
AC5u0009GL000019	3.70E-05	-0.320994585
AC5u0009GL000020	0.066202575	-0.131933684
AC5u0009GL000021	0.513534757	-0.039035518
AC5u0009GL000022	0.448309709	-0.050362723
AC5u0009GL000023	0.245718384	-0.112309851
AC5u0009GL000024	0.022314824	-0.192353316
AC5u0009GL000025	0.102397826	-0.13623832
AC5u0009GL000026	0.486781277	0.064098409
AC5u0009GL000027	0.004946061	0.321935868
AC5u0009GL000028	0.000140521	0.363446611
AC5u0009GL000029	5.07E-06	-1.051283432

B

Q6: Which one is GSC.txt ?
A: A or B

