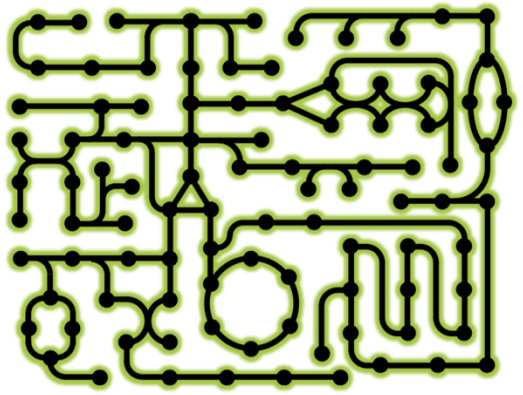

GEMs

Concepts & Developments

NBIS Omics Integration and Systems Biology workshop
October 2024, Lund University

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Science for Life Laboratory (SciLifeLab)
Chalmers University of Technology
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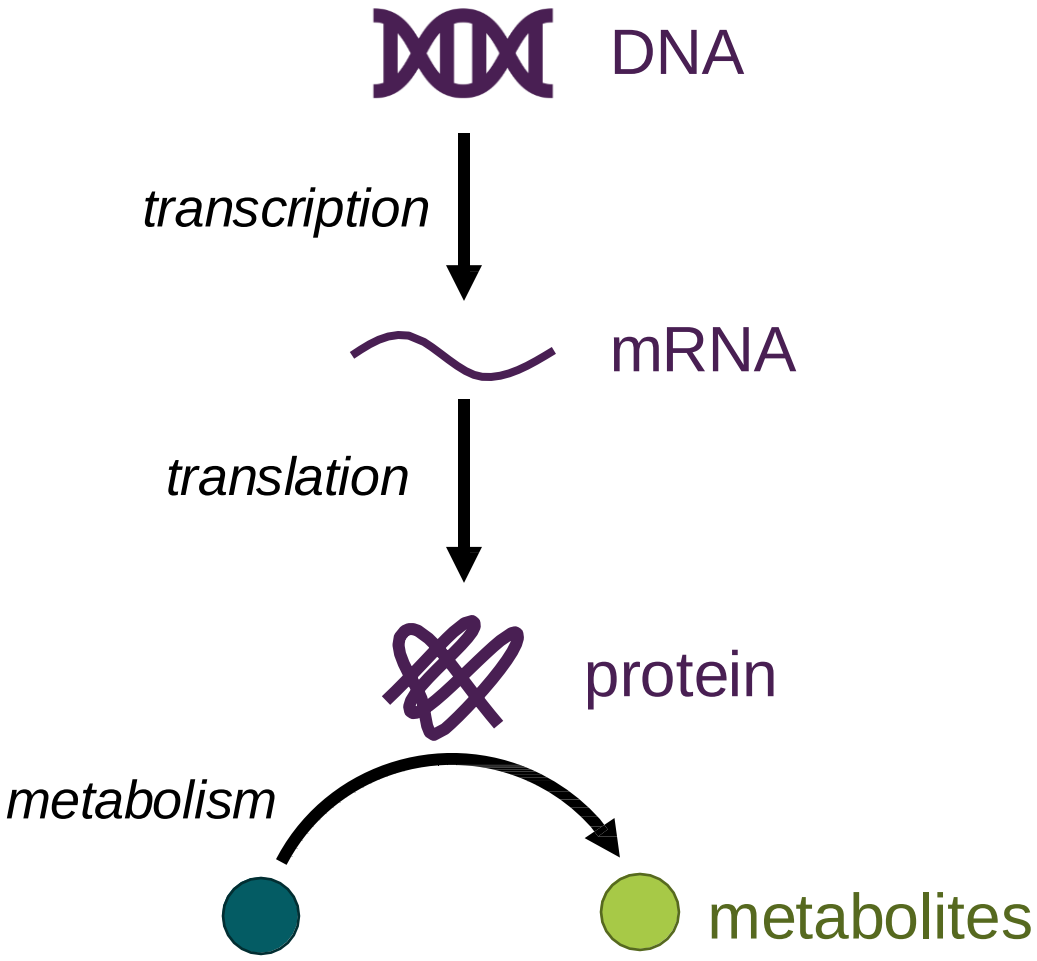
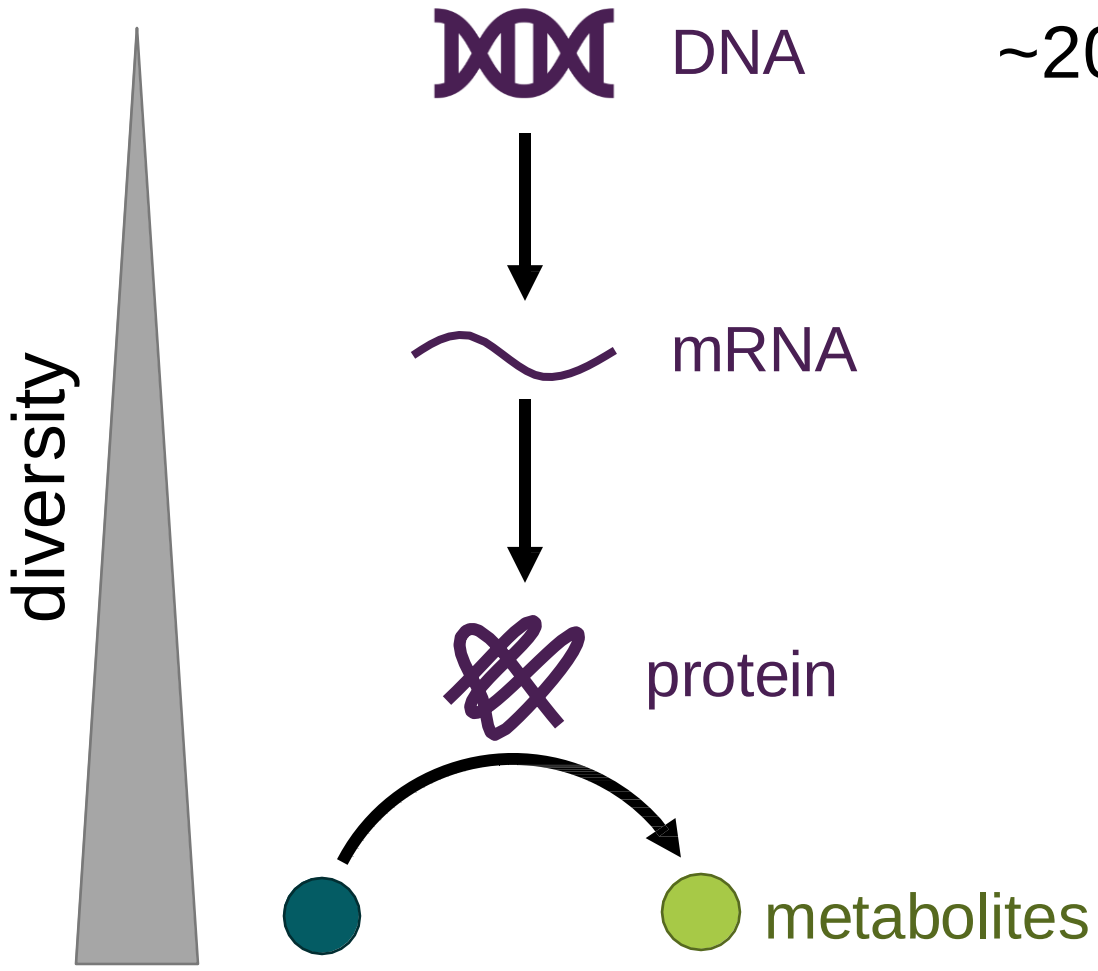
These slides are adapted from previous course materials created by **Jonathan Robinson**. Some updates have been made for this iteration.

Background



Metabolism provides the **energy** and **building blocks** necessary to sustain life.

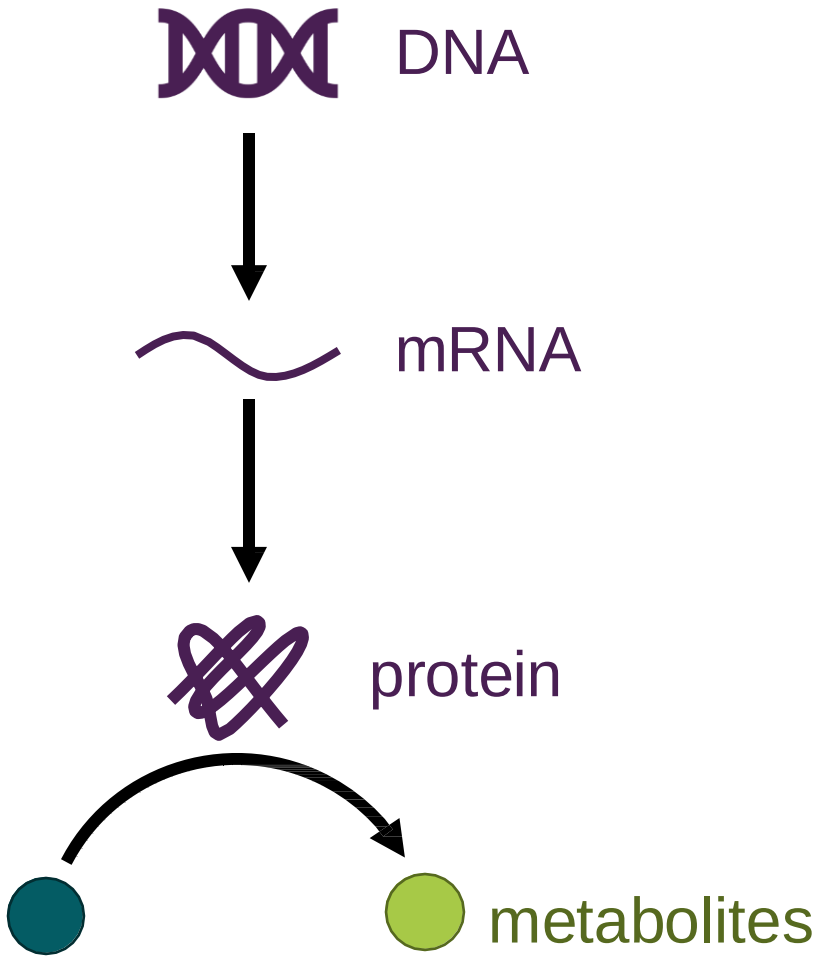
Background



~20,000 genes
(protein-coding)

>100,000 metabolites

Background



stable

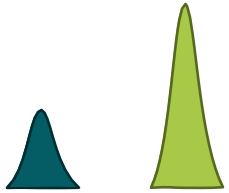
relatively stable

stable

ranges from stable
to very unstable

Background

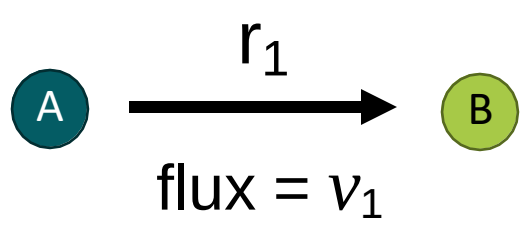
We can generally measure metabolite concentrations



…but what is often important is the flow or **flux** of metabolites through the reactions.

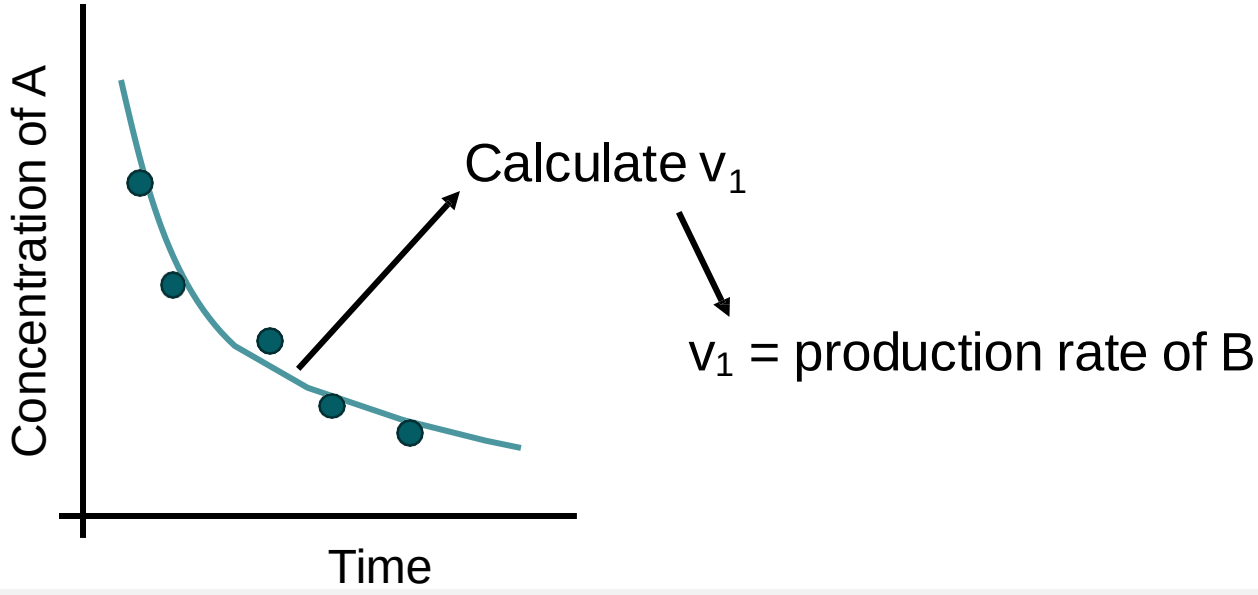
Background

Assume that we want to know the production rate of **B**, but can only measure the concentration of **A**

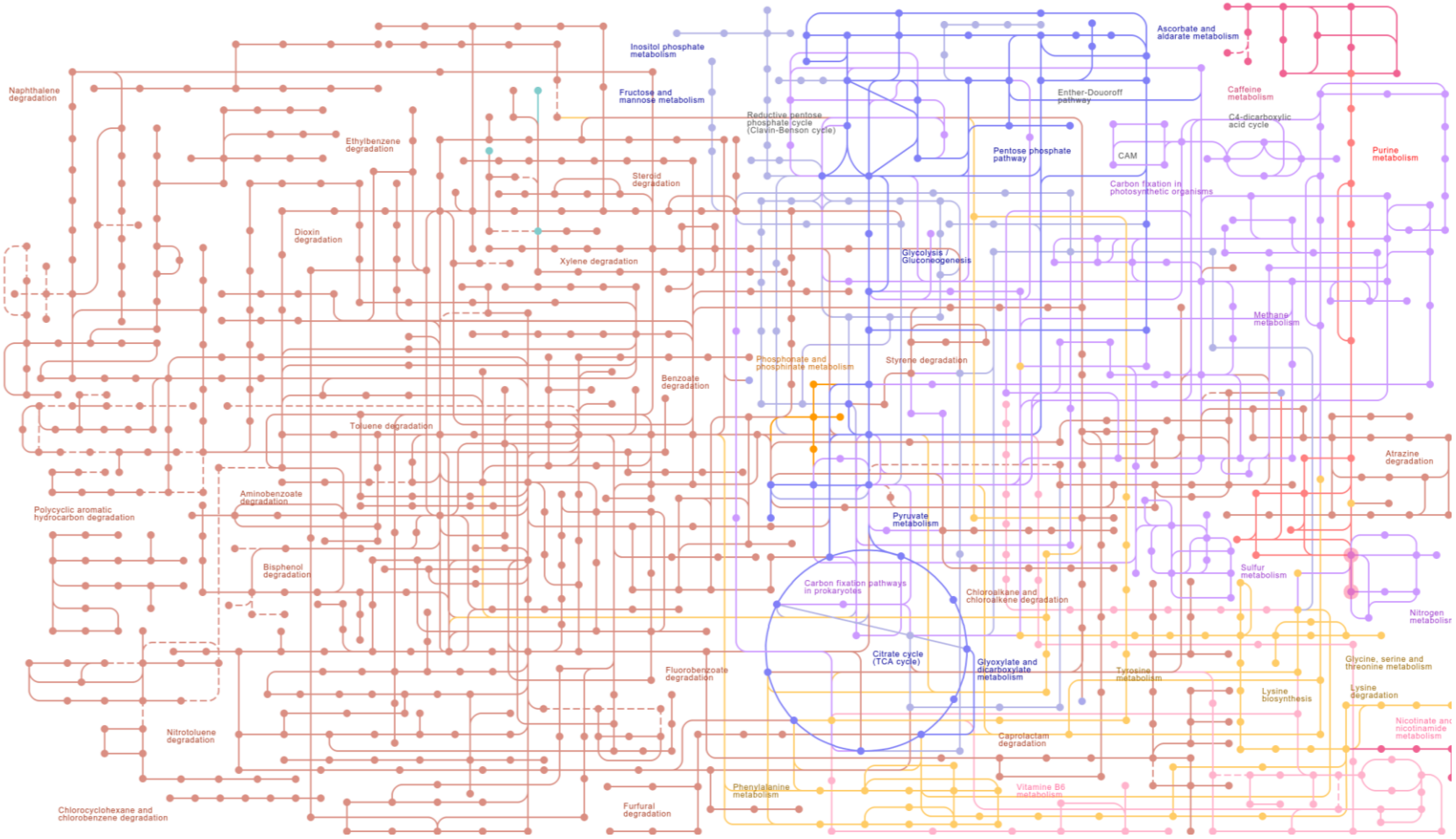


$$\frac{dA}{dt} = -v_1$$

$$\frac{dB}{dt} = v_1$$

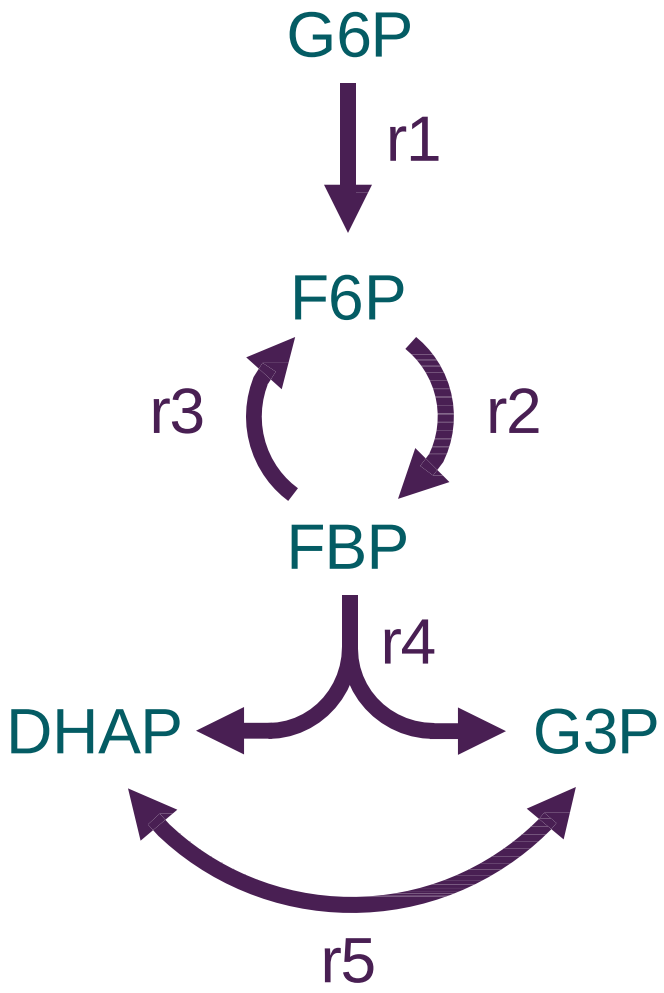


Background



Biological networks are much more interconnected and comlicated…

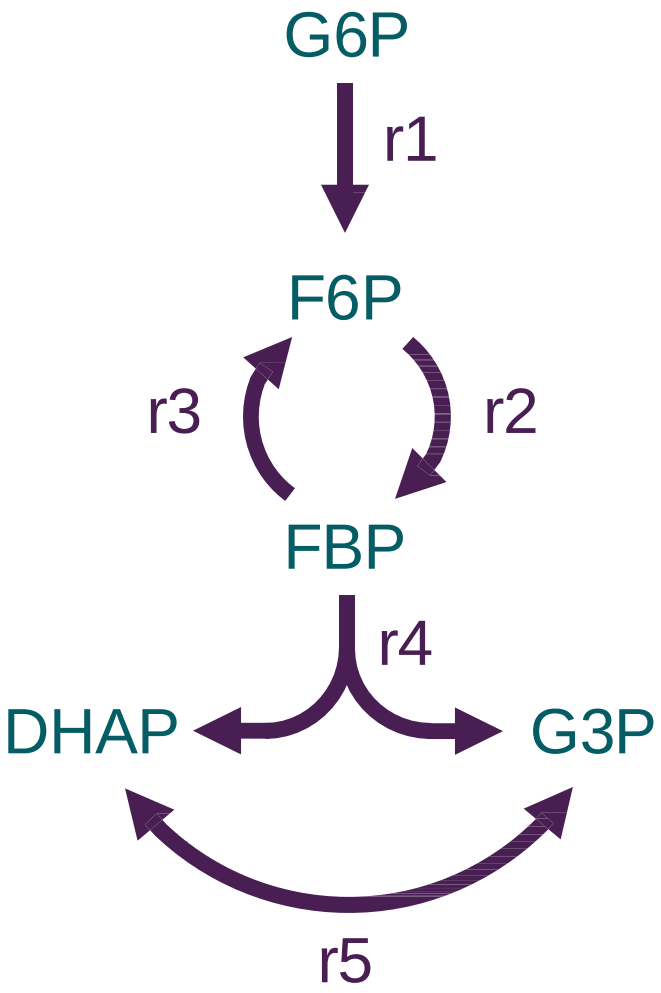
The Stoichiometric Matrix



Reactions

Metabolites	r1
G6P	**−1**
F6P	**1**
FBP	0
DHAP	0
G3P	0

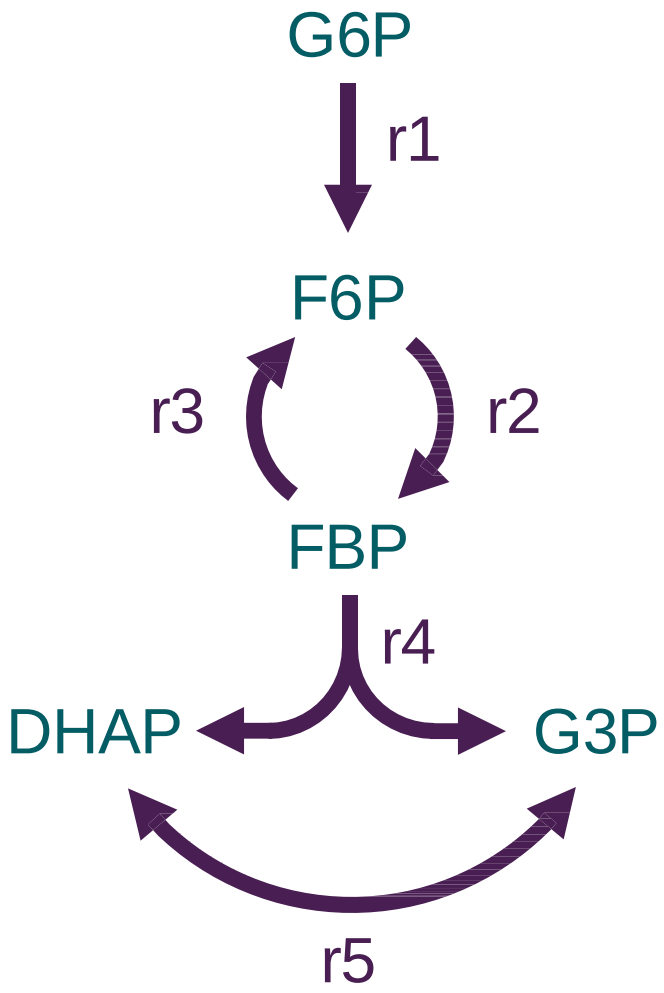
The Stoichiometric Matrix



Reactions

Metabolites	r1	r2
G6P	**−1**	0
F6P	**1**	**−1**
FBP	0	**1**
DHAP	0	0
G3P	0	0

The Stoichiometric Matrix



Reactions

Metabolites	r1	r2	r3	r4	r5
G6P	**−1**	0	0	0	0
F6P	**1**	**−1**	**1**	0	0
FBP	0	**1**	**−1**	**−1**	0
DHAP	0	0	0	**1**	**−1**
G3P	0	0	0	**1**	**1**

S

Genome-scale model (GEM)

Chemical formula
Charge
InChI code
Other external IDs
...

									...
				…	…	…	…	…	**Other IDs**
				…	…	…	…	…	**Name**
KEGG ID	**Compartment**	**Name**	**Symbol**	r1	r2	r3	r4	r5	**Symbol**
C00668	cytosol [c]	glucose 6-phosphate	G6P	**−1**	0	0	0	0	
C00085	cytosol [c]	fructose 6-phosphate	F6P	**1**	**−1**	**1**	0	0	
C00354	cytosol [c]	fructose-1,6-bisphosphate	FBP	0	**1**	**−1**	**−1**	0	
C00111	cytosol [c]	dihydroxyacetone phosphate	DHAP	0	0	0	**1**	**−1**	
C00118	cytosol [c]	glyceraldehyde 3-phosphate	G3P	0	0	0	**1**	**1**	
			...						

Genome-scale model (GEM)

Genes (symbol)	**Proteins** (UniProt ID)
GPI	P06744
n/a	
FBP1, FBP2	P09467, O00757
ALDOA, ALDOB, ALDOC	P04075, P05062, P09972
TPI1	P60174

Transcript IDs

GO Terms

Orthologs

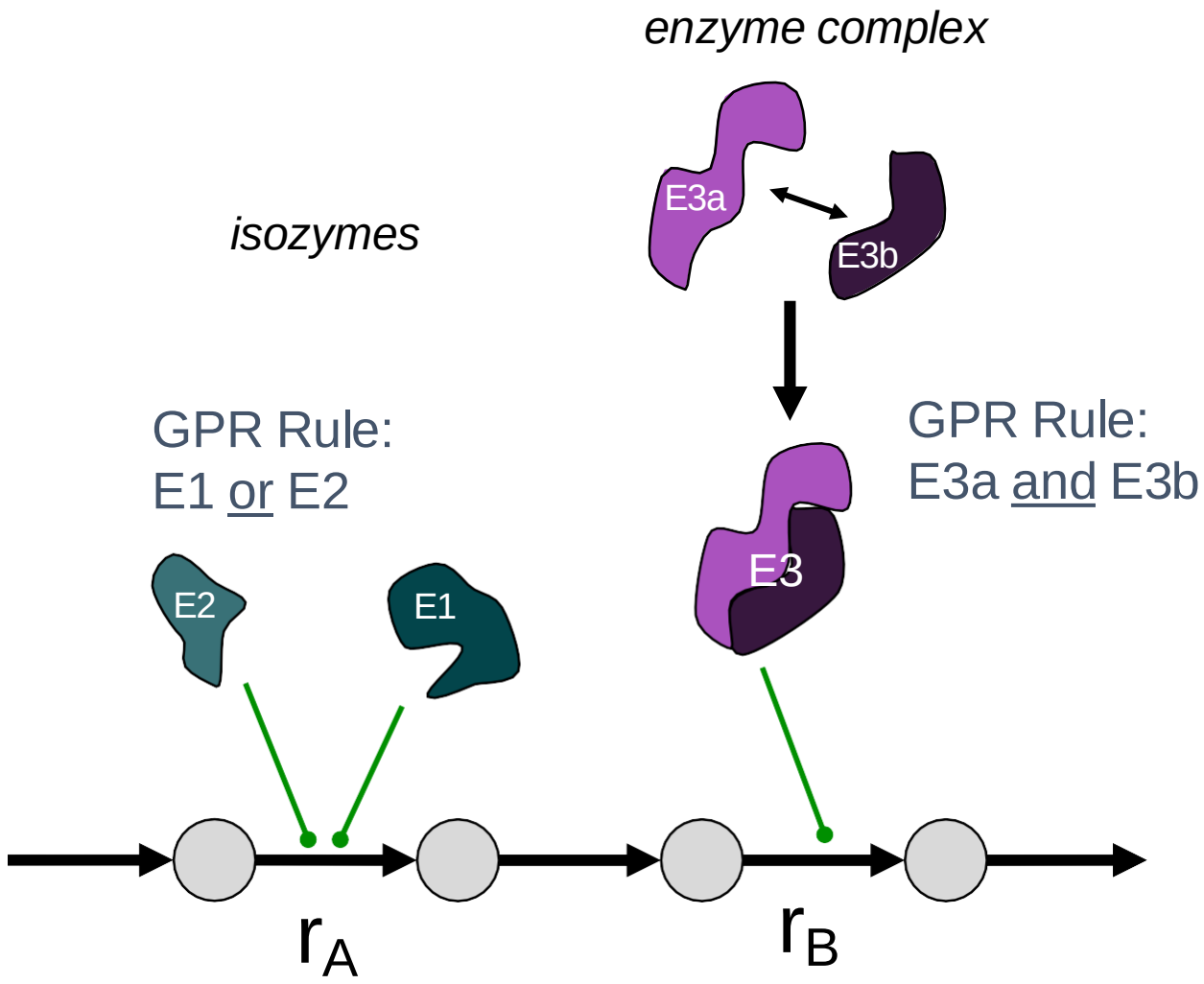
…

Symbol	r1	r2	r3	r4	r5
G6P	**−1**	0	0	0	0
F6P	**1**	**−1**	**1**	0	0
FBP	0	**1**	**−1**	**−1**	0
DHAP	0	0	0	**1**	**−1**
G3P	0	0	0	**1**	**1**

Reactions are linked to genes that encode the enzymes that catalyze the reaction.

These associations are often called “gene-protein rules” (GPR rules)

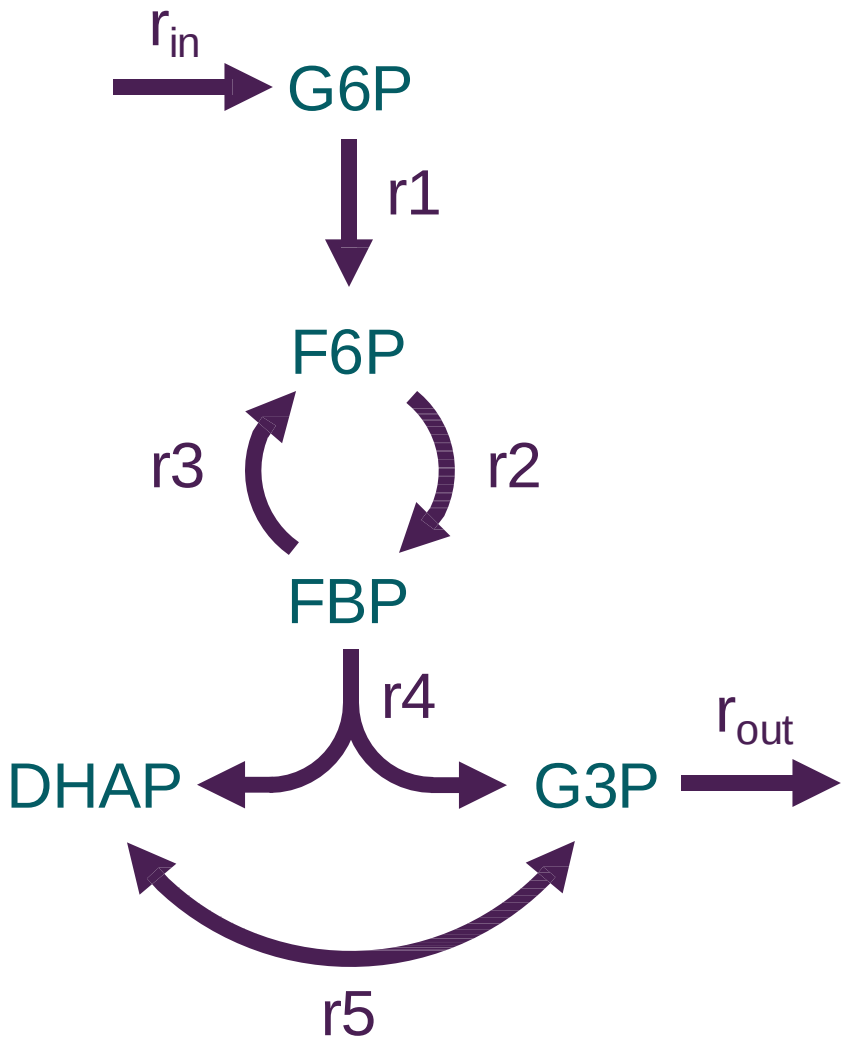
GPR Rules



GPR Rules enable more accurate simulation of gene inactivation/knock-out

Knockout	Effect
E1	none
E2	none
E1 + E2	rA inactive
E3a	rB inactive
E3b	rB inactive
E3a + E3b	rB inactive

Flux Balance Analysis (FBA)

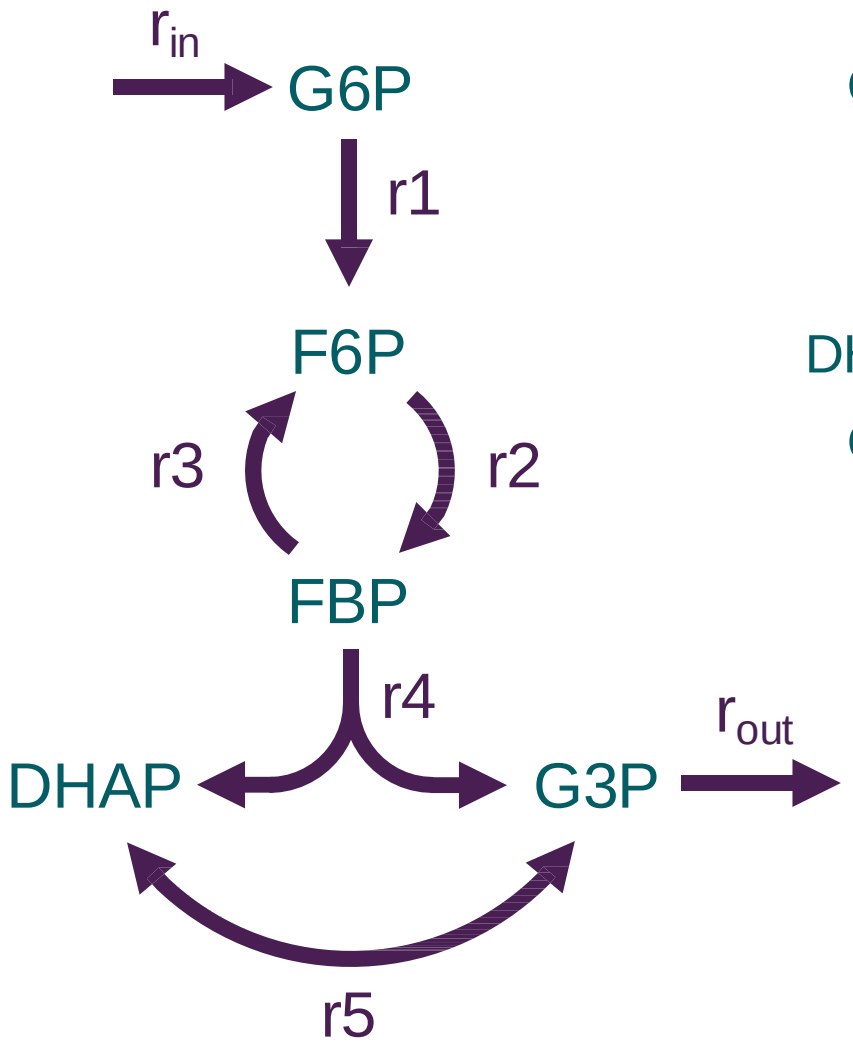


FBA seeks to calculate the reaction **fluxes** (***v***) of a network

The calculation is based on the **conservation of mass**: it cannot be created or destroyed

$$\frac{dX}{dt} = v_{produce} - v_{consume}$$

Flux Balance Analysis (FBA)

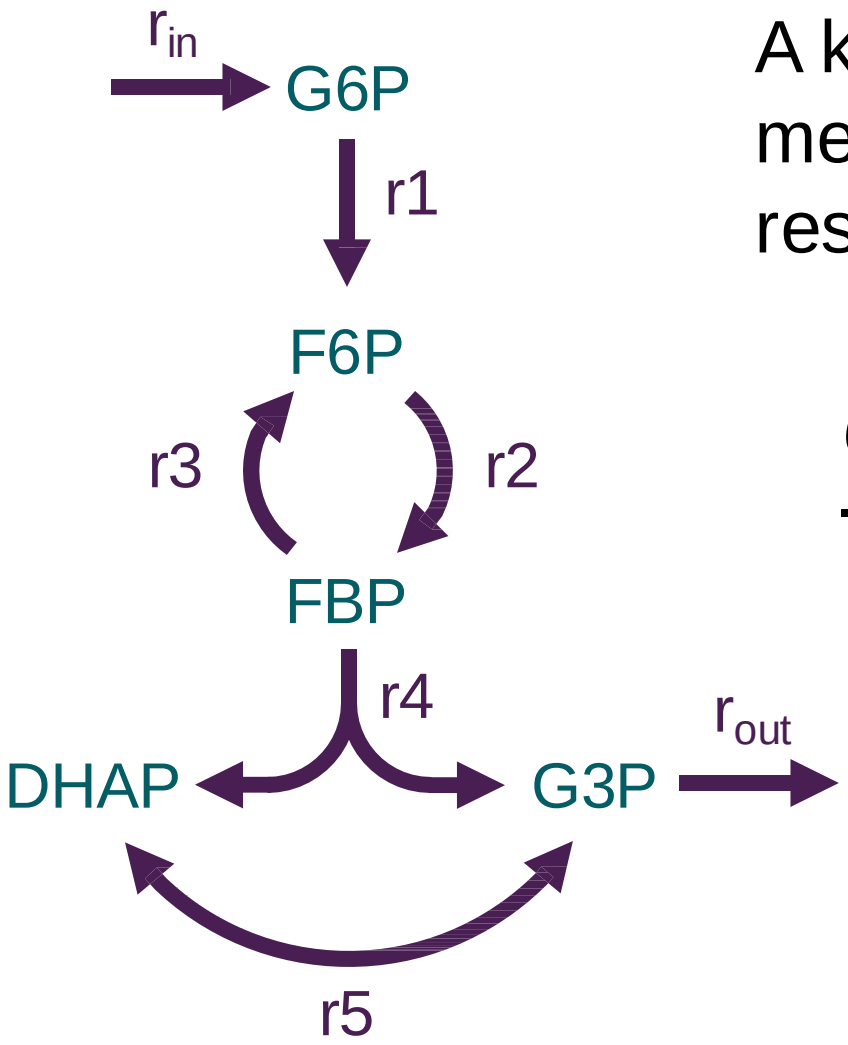


$$
\begin{array}{c} \\ \text{G6P} \\ \text{F6P} \\ \text{FBP} \\ \text{DHAP} \\ \text{G3P} \end{array}
\begin{array}{c}
\begin{array}{ccccccc} \text{r1} & \text{r2} & \text{r3} & \text{r4} & \text{r5} & r_{in} & r_{out} \end{array} \\
\begin{pmatrix}
-1 & 0 & 0 & 0 & 0 & 1 & 0 \\
1 & -1 & 1 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 0 & 1 & 1 & 0 & -1
\end{pmatrix}
\end{array}
\times
\begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_{in} \\ v_{out} \end{pmatrix}
=
\begin{pmatrix} \text{dG6P/dt} \\ \text{dF6P/dt} \\ \text{dFBP/dt} \\ \text{dDHAP/dt} \\ \text{dG3P/dt} \end{pmatrix}
$$

$$\frac{d[G6P]}{dt} = -v_{1} + v_{in}$$

$$\frac{d[G3P]}{dt} = v_4 + v_5 - v_{out}$$

Flux Balance Analysis (FBA)



A key assumption to FBA is **steady state**: metabolite concentrations are **constant** with respect to time!

$$\frac{dX}{dt} = v_{produce} - v_{consume} = 0$$

This assumption allows us to **ignore enzyme kinetics**, thus eliminating **many** unknown parameters

Flux Balance Analysis (FBA)

$$
\begin{array}{c} \\ G6P \\ F6P \\ FBP \\ DHAP \\ G3P \end{array}
\begin{array}{c}
\begin{array}{ccccccc} r1 & r2 & r3 & r4 & r5 & r_{in} & r_{out} \end{array} \\
\begin{pmatrix}
-1 & 0 & 0 & 0 & 0 & 1 & 0 \\
1 & -1 & 1 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 0 & 1 & 1 & 0 & -1
\end{pmatrix}
\end{array}
\times
\begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_{in} \\ v_{out} \end{pmatrix}
=
\begin{pmatrix} dG6P/dt \\ dF6P/dt \\ dFBP/dt \\ dDHAP/dt \\ dG3P/dt \end{pmatrix}
=
\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}
$$

Now we can solve it as a system of linear equations:

$$S \cdot v = 0$$

So we can calculate / estimate fluxes.

Flux Balance Analysis (FBA)

We can further constrain the solution space by limiting reaction fluxes based on their reversibility:

Irreversible reactions

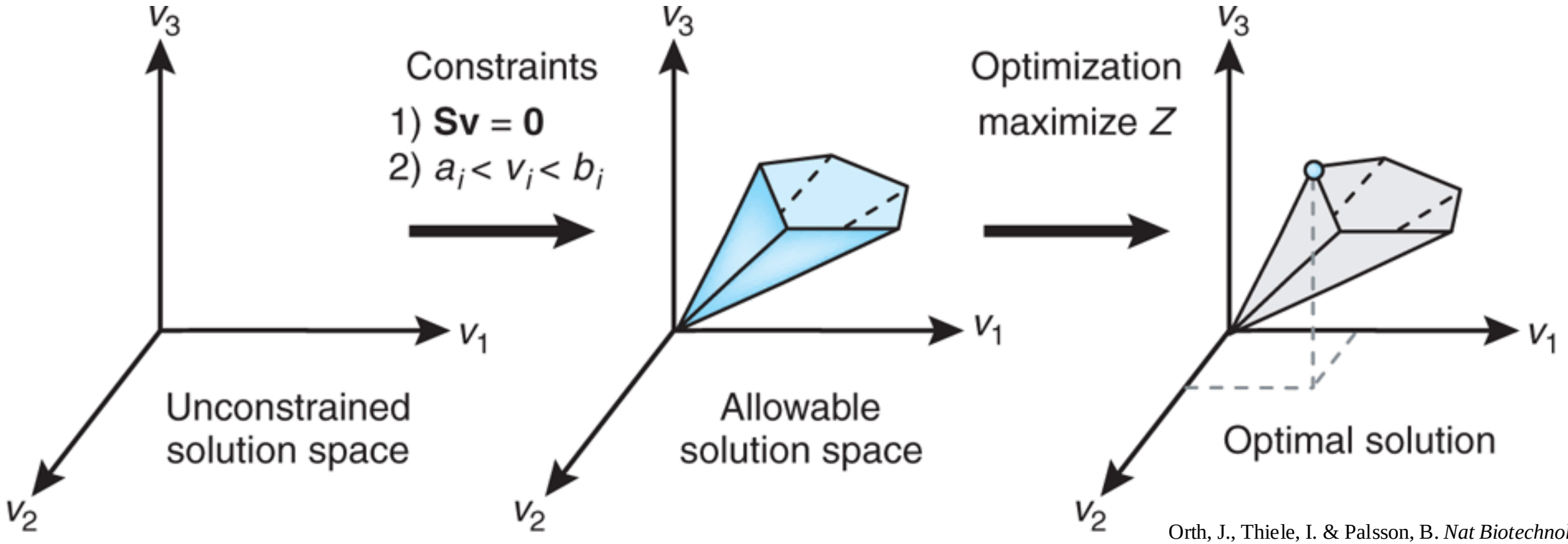
$0 \leq v \leq ub$

Reversible reactions

$lb \leq v \leq ub$

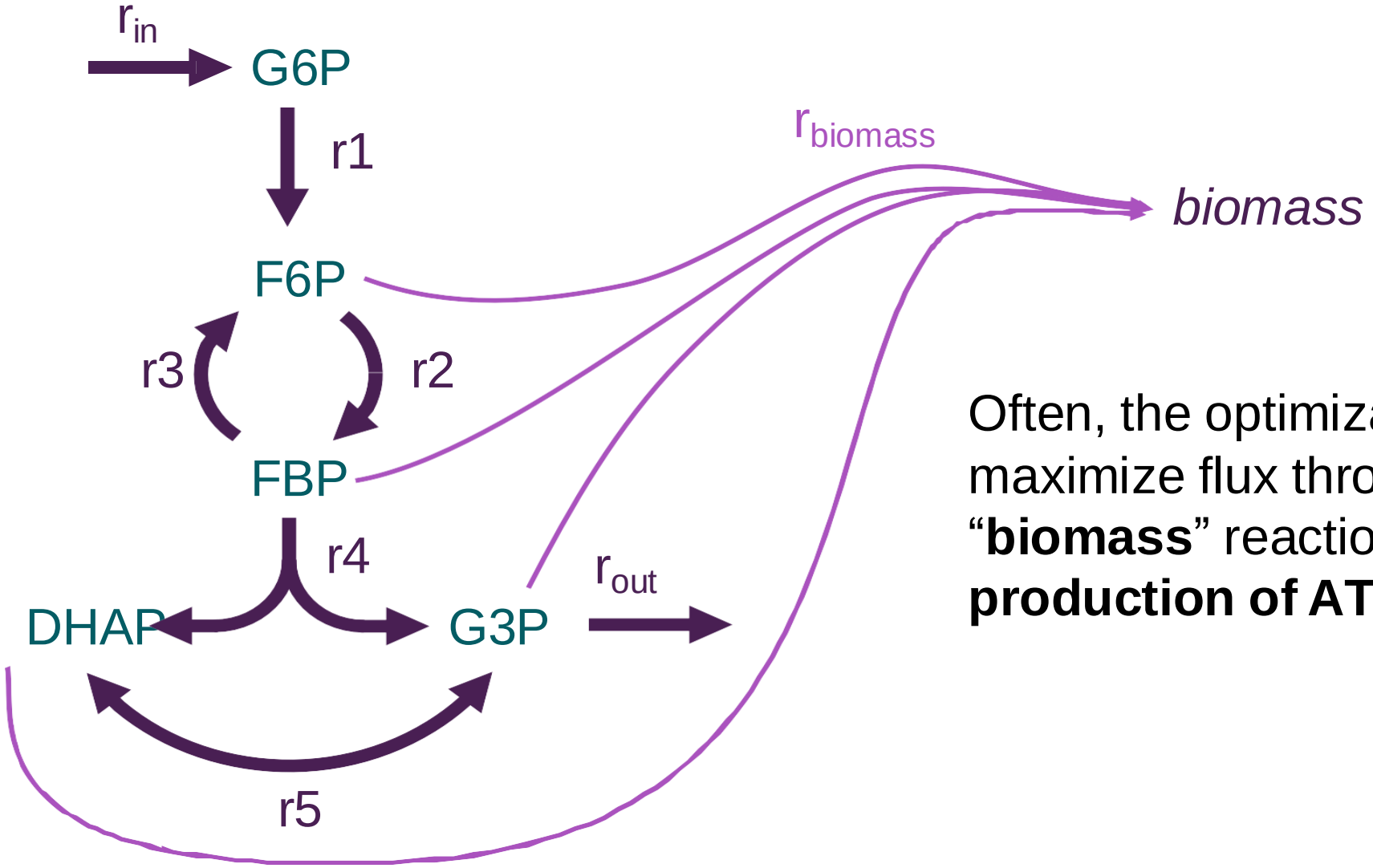
Flux Balance Analysis (FBA)

Since the problem is still **under-defined**, FBA uses linear **optimization** to identify a solution that maximizes (or minimizes) some **objective**



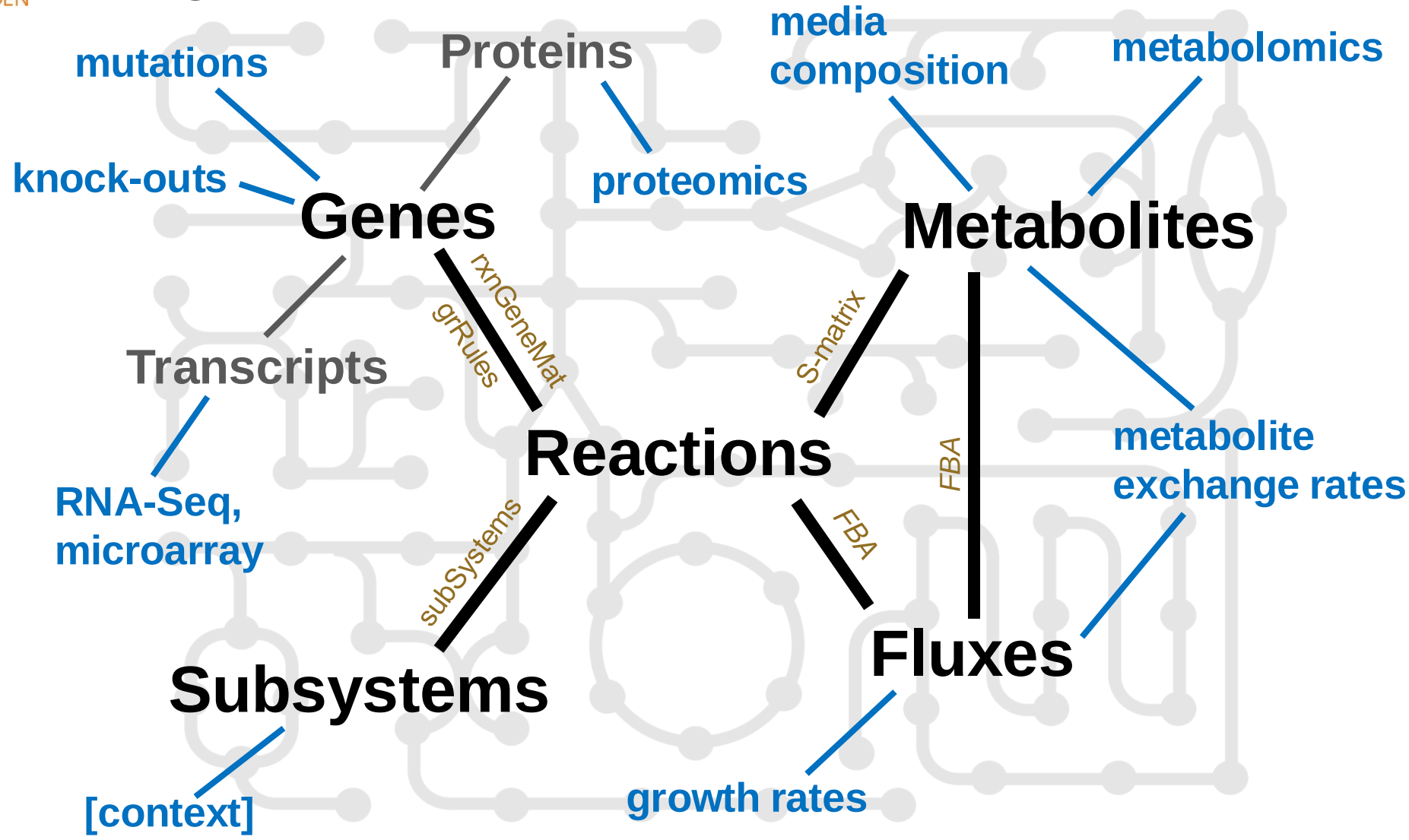
Orth, J., Thiele, I. & Palsson, B. *Nat Biotechnol* (2010).

Flux Balance Analysis (FBA)



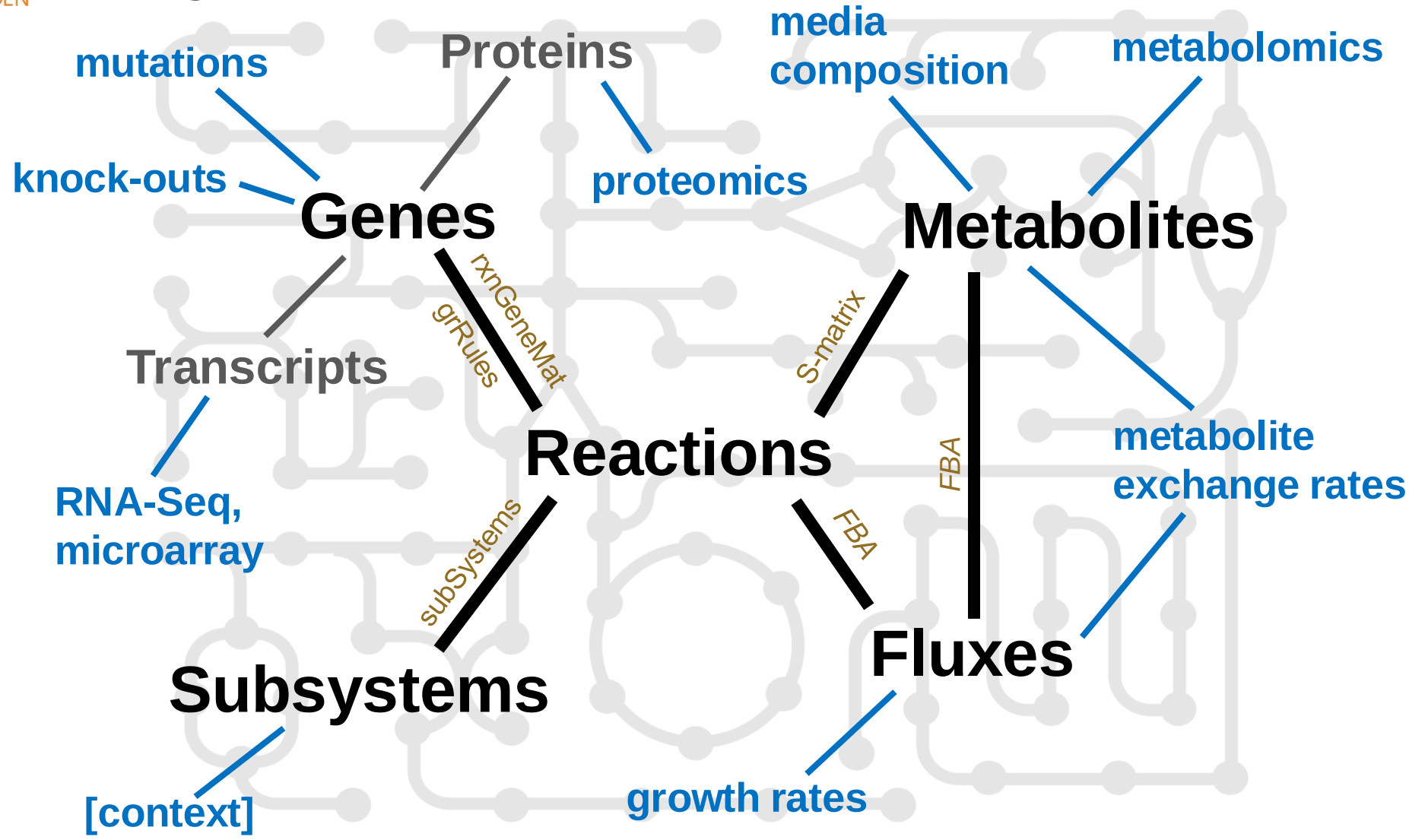
Often, the optimization objective is to maximize flux through an artificial "**biomass**" reaction, or to maximize **production of ATP**.

Genome-scale metabolic models (GEMs) for data integration



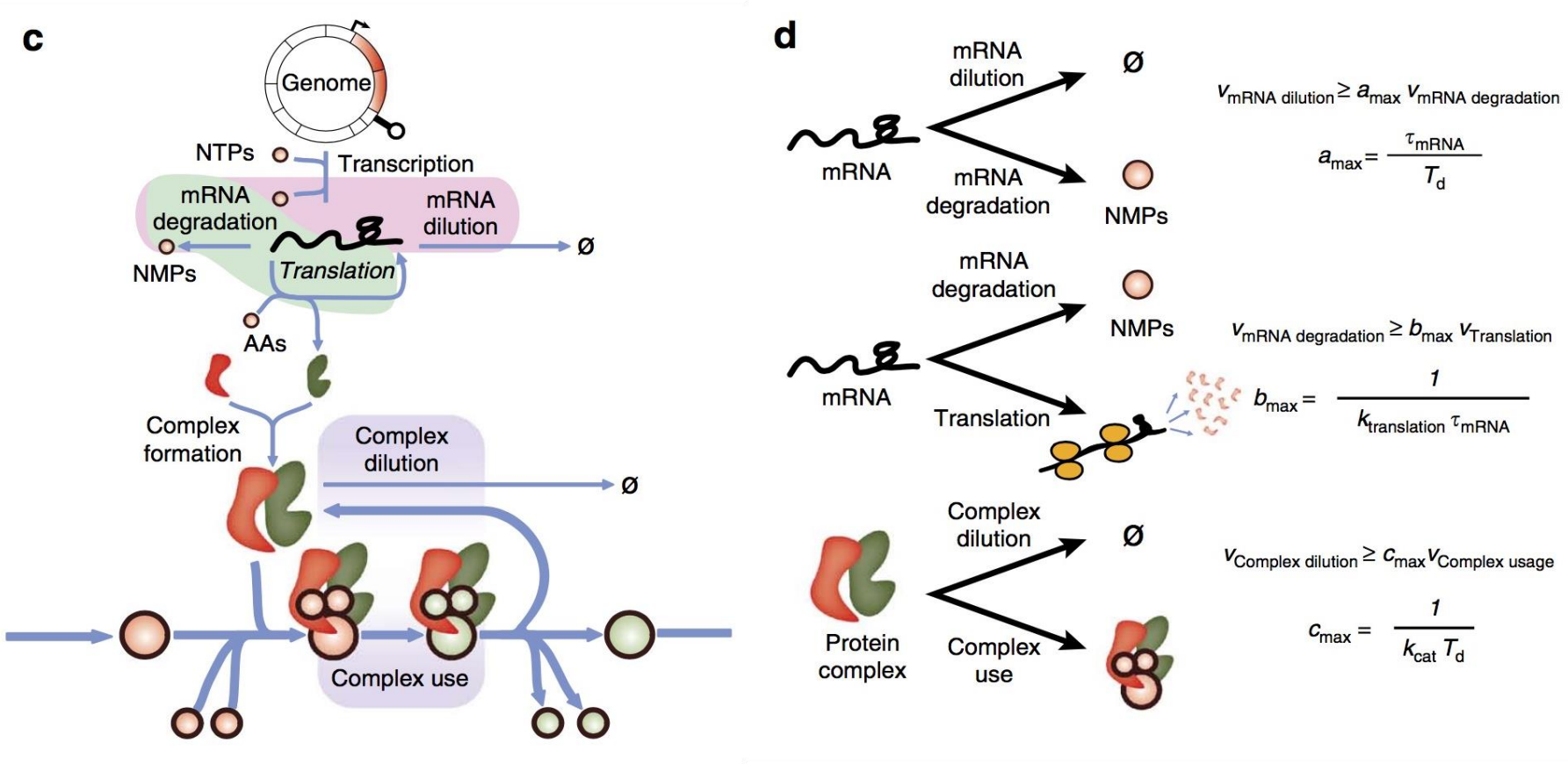
Can GEMs serve as a scaffold for integrating & studying diverse types of (omics) data?

Genome-scale metabolic models (GEMs) for data integration



Metabolism and macromolecular expression (ME) model

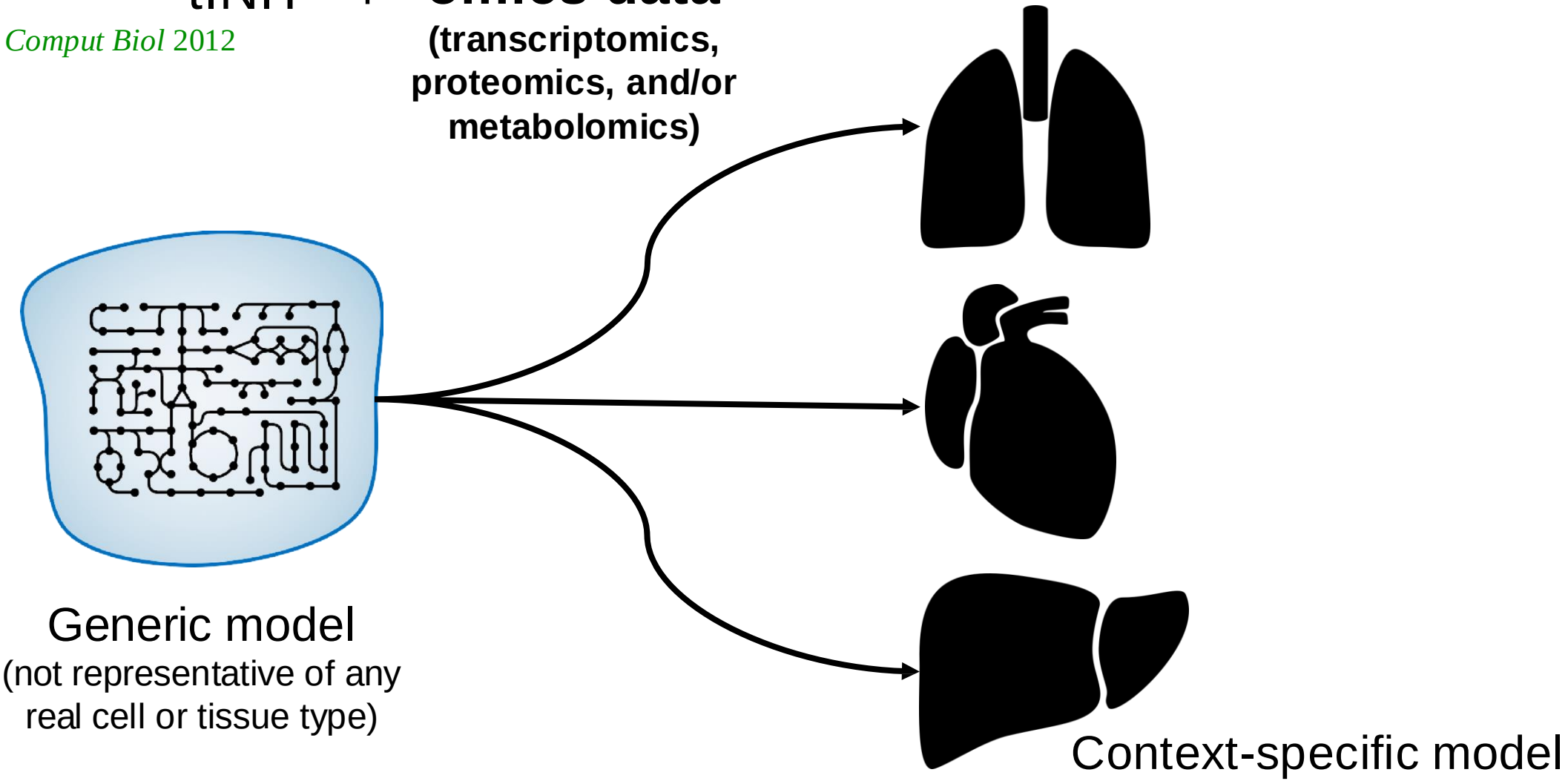
J Lerman et al, Nat. Commun. 2012



GEM contextualization

tINIT + **omics data**
(transcriptomics, proteomics, and/or metabolomics)

R. Ågren, et al. *PLoS Comput Biol* 2012



Generic model
(not representative of any real cell or tissue type)

Context-specific model

GEM contextualization

INIT (Integrative Network Inference for Tissues)

R. Ågren, et al. *PLoS Comput Biol* 2012

- Uses proteomic, transcriptomic, and/or metabolomic data
- An optimization is performed to
  - maximize the number of high-confidence (high expression) reactions that are “on”
  - minimize the number of low-confidence (low-expression) reactions that are “on”
- **All reactions in the final model must be able to carry flux**
- **Metabolites are allowed to accumulate** during the optimization
  - An additional term in the algorithm maximizes the number of “present” metabolites that can be produced
  - Distinction of which metabolites should be “present” are based on literature or data (e.g., metabolomics)

$$\max\left(\sum_{i\in R} w_i y_i + \sum_{j\in M} x_j\right)$$

$$S\vec{v} = \vec{b}$$

$$|v_i| \le 1000 y_i$$

$$|v_i| + 1000(1 - y_i) \ge \varepsilon$$

$$v_i \ge 0, i \in irreversible\ rxns$$

$$b_j \le 1000 x_i$$

$$b_j + 1000(1 - x_i) \ge \varepsilon$$

$$b_j \ge 0$$

$$x_j = 1, j \in present$$

$$y_i, x_j \in \{0,1\}$$

$$w_{i,j} = 5\log\left(\frac{Signal_{i,j}}{Average_i}\right)$$

GEM contextualization

tINIT1 (Task-driven Integrative Network Inference for Tissues)

R. Ågren, et al. *Mol Syst Biol* 2014

- Identical formulation as INIT, with added steps
  - INIT does not necessarily yield simulation-ready models
- User defines a series of metabolic tasks that the model must perform
- Reactions that are required for these tasks are identified
  - A requirement that these reactions are active is included as an additional constraint in the optimization
- A follow-up evaluation of each task is performed
  - If a task fails, a gap-filling algorithm is used to enable task completion

Metabolic Tasks

Rephosphorylation of nucleoside triphosphates
- Aerobic rephosphorylation of ATP from glucose
- Aerobic rephosphorylation of GTP
- Aerobic rephosphorylation of CTP
- Aerobic rephosphorylation of UTP

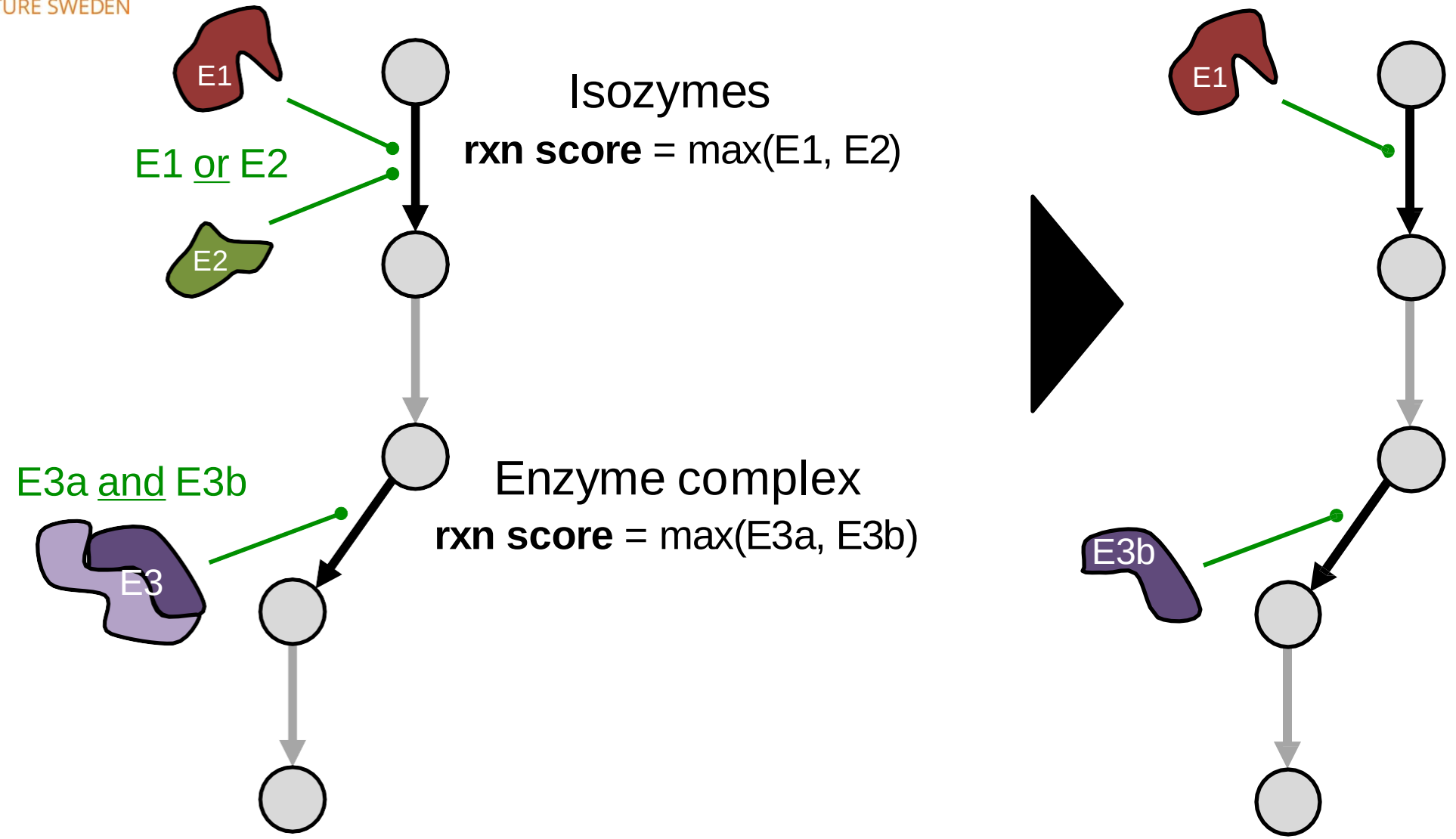
De novo synthesis of nucleotides
- ATP de novo synthesis
- CTP de novo synthesis
- GTP de novo synthesis
- UTP de novo synthesis
- dATP de novo synthesis
- dCTP de novo synthesis
- dGTP de novo synthesis
- dTTP de novo synthesis

Uptake of essential amino acids
- Histidine uptake
- Isoleucine uptake
- Leucine uptake
- Lysine uptake
- Methionine uptake
- Phenylalanine uptake
- Threonine uptake
- Tryptophan uptake
- Valine uptake

De novo synthesis of key intermediates
- Glycerate 3-phosphate de novo synthesis
- Mitochondrial acetyl-CoA de novo synthesis
- Mitochondrial AKG de novo synthesis
- Erythrose 4-phosphate de novo synthesis
- Fructose 6-phosphate de novo synthesis

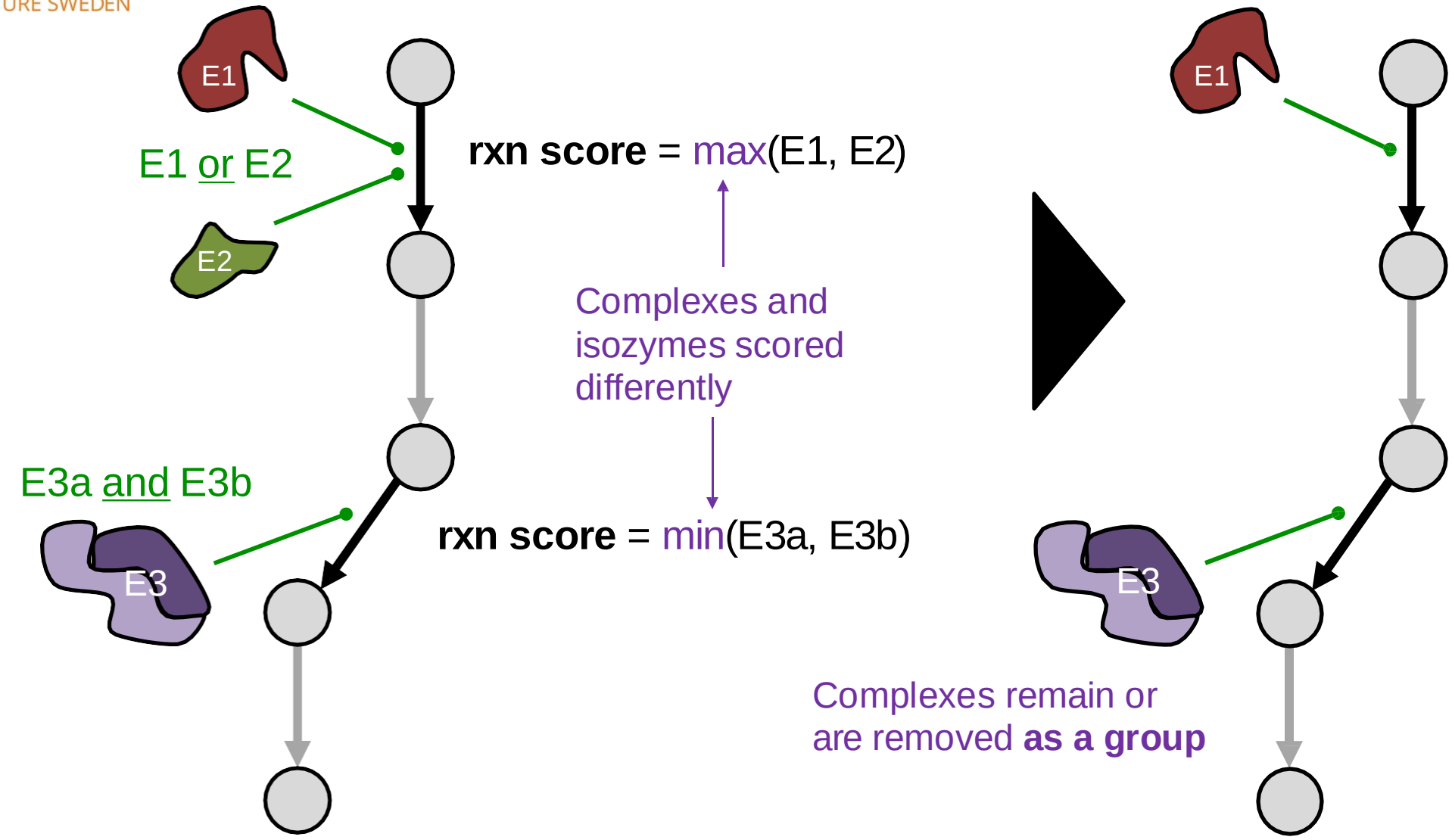
GEM contextualization

tINIT1 (Task-driven Integrative Network Inference for Tissues)



GEM contextualization

tINIT2 (Task-driven Integrative Network Inference for Tissues)



Enzyme-constrained GEMs

- Should any reaction have bounds up to $+\infty$?
- Should these 2 pathways have reactions with the same bounds?

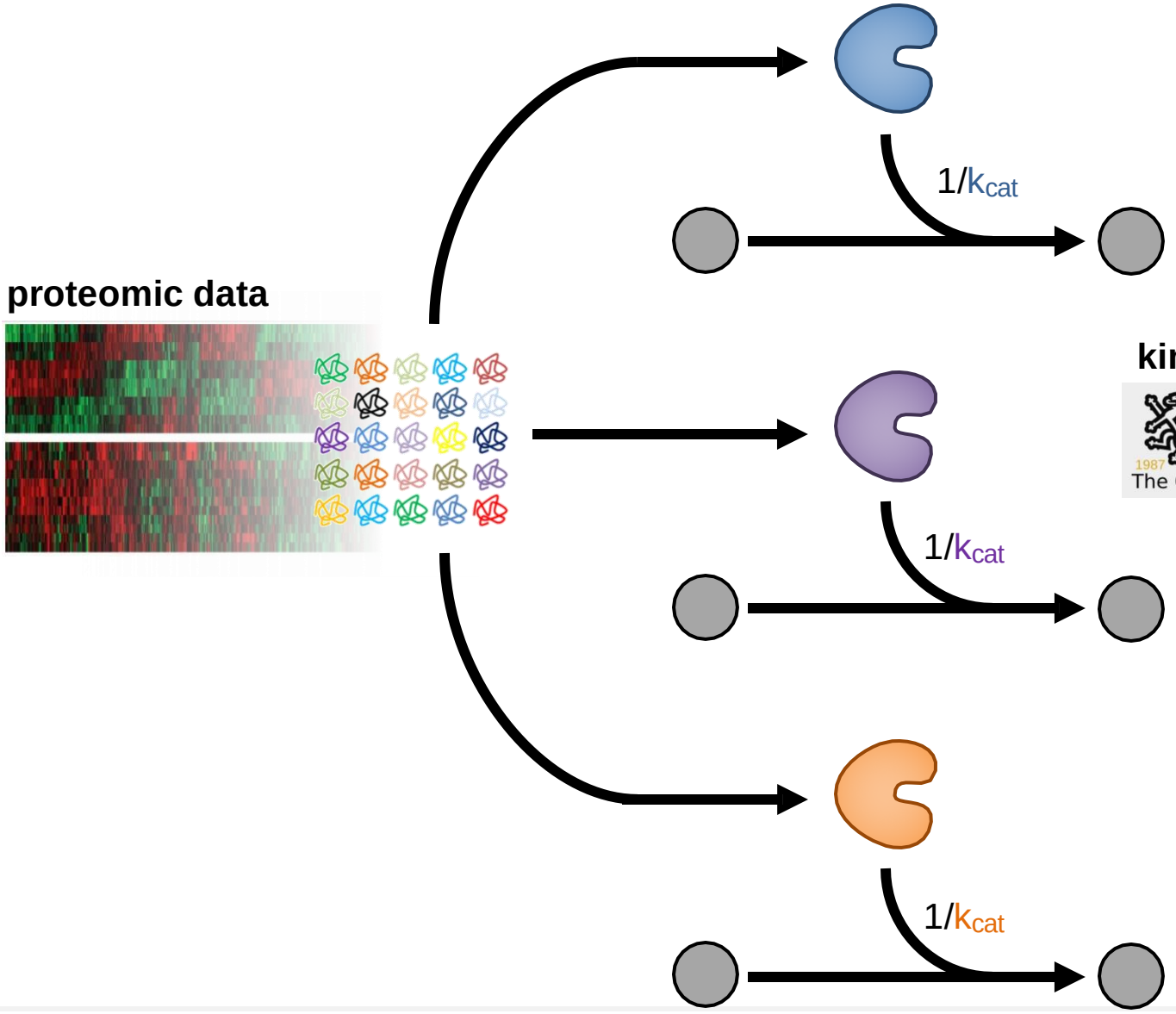
Relationship between enzyme and reaction:

Flux of reaction (from FBA) → $v \leq k_{cat}[E]$ ← Concentration of enzyme (from absolute proteomics)

Turnover number (from databases) ↑

However: No simple implementation for connecting proteomics to GEMs…

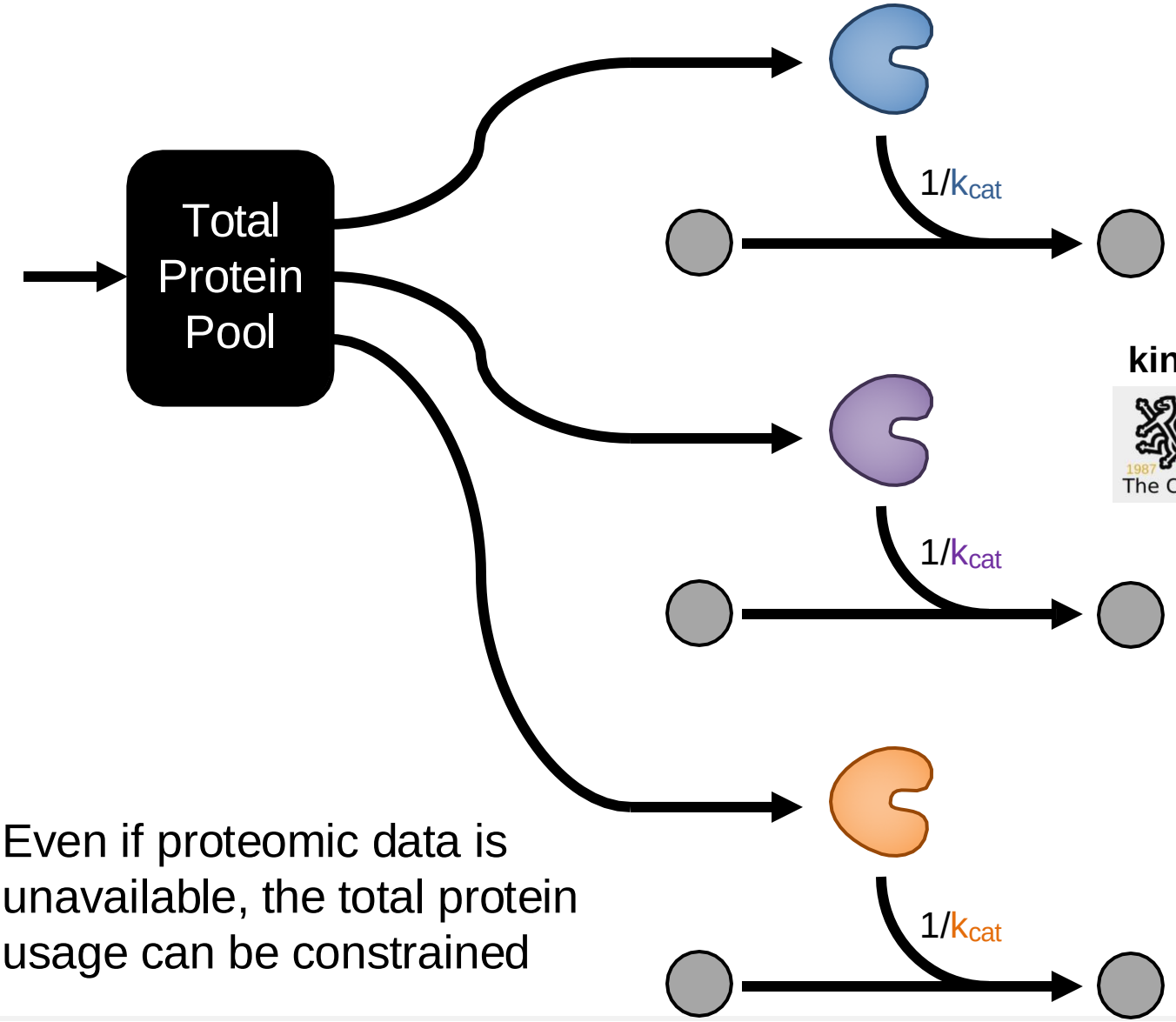
Enzyme-constrained GEMs



kinetic data (k_{cat} values)

BRENDA
1987 2017
The Comprehensive Enzyme Information System

Enzyme-constrained GEMs



kinetic data (k_{cat} values)

BRENDA 1987 2017
The Comprehensive Enzyme Information System

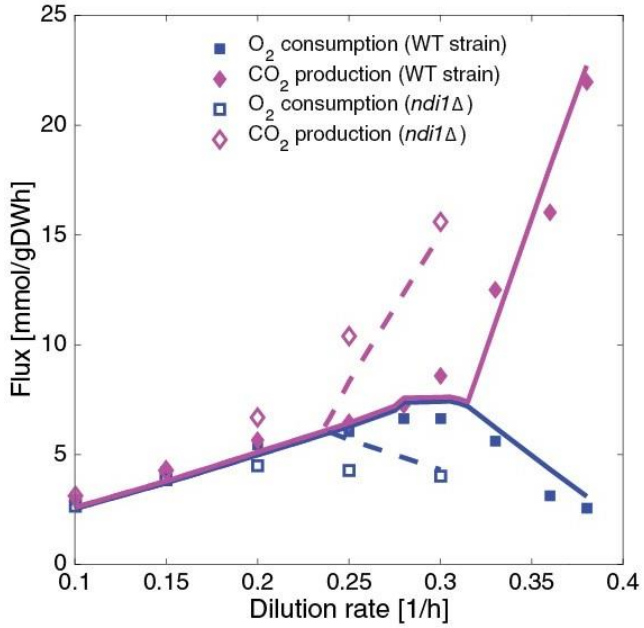
Even if proteomic data is unavailable, the total protein usage can be constrained

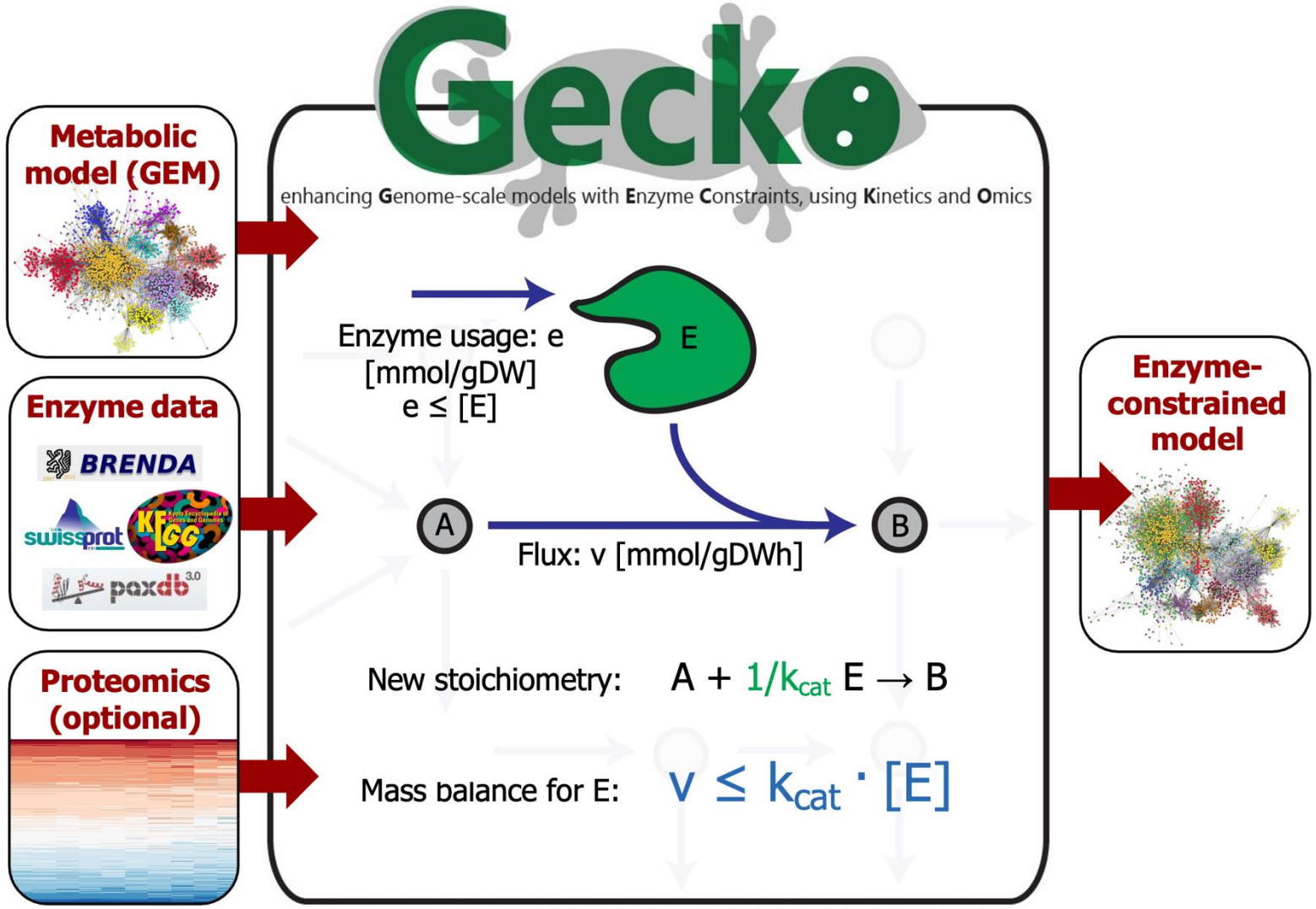
Enzyme-constrained GEMs

B. Sánchez, et al *Mol Syst Biol* 2017

Applications:

- Improving predictions
- Integrating proteomics data into GEMs

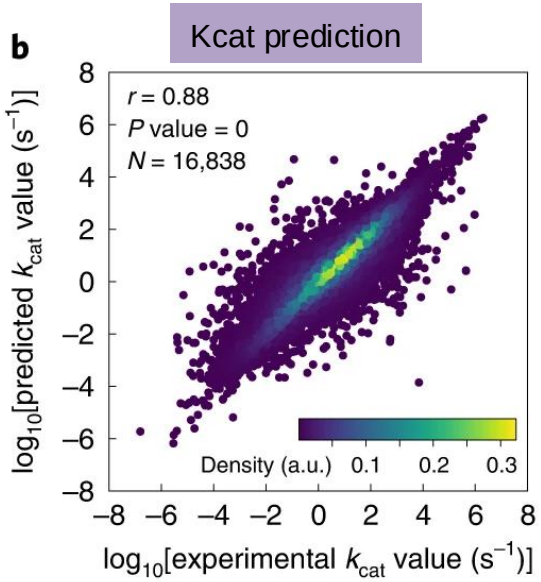




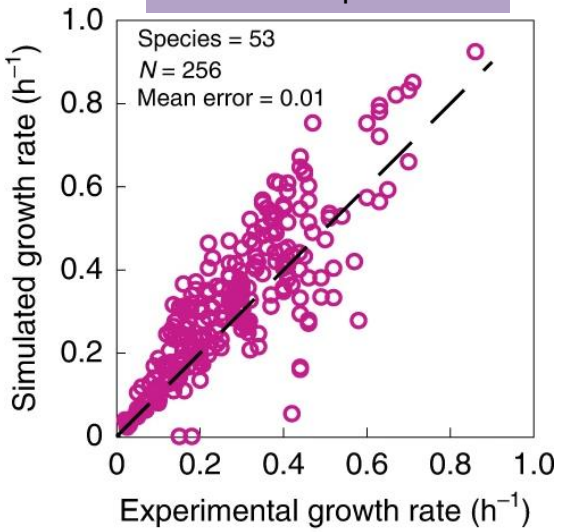
Predicting K_{cat} for ecGEM parameterization

Li F, et al *Nat Cat 2022*

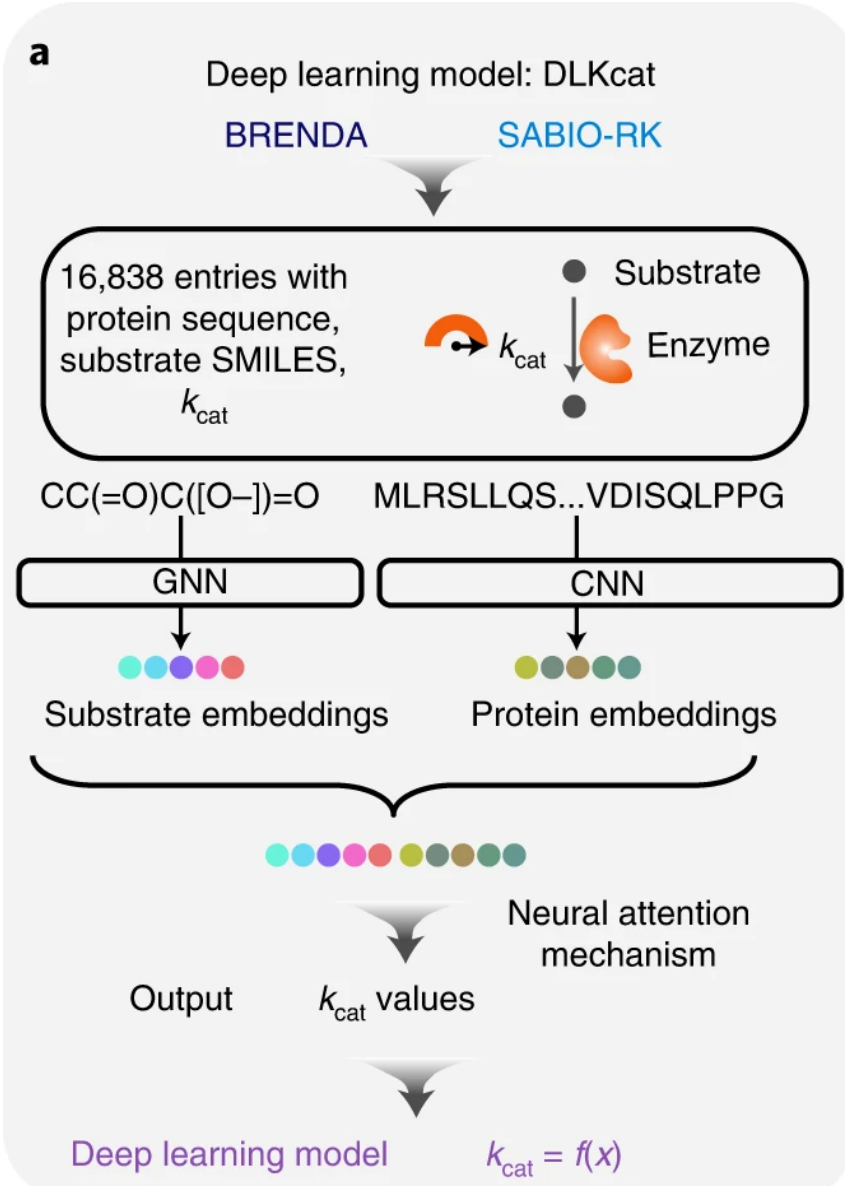
- Experimentally measured k_{cat} data are sparse and noisy
- Deep learning approach (DLKcat) for high-throughput k_{cat} prediction for metabolic enzymes
- They designed a Bayesian pipeline to parameterize enzyme-constrained genome-scale metabolic models from predicted k_{cat} values



Kcat prediction



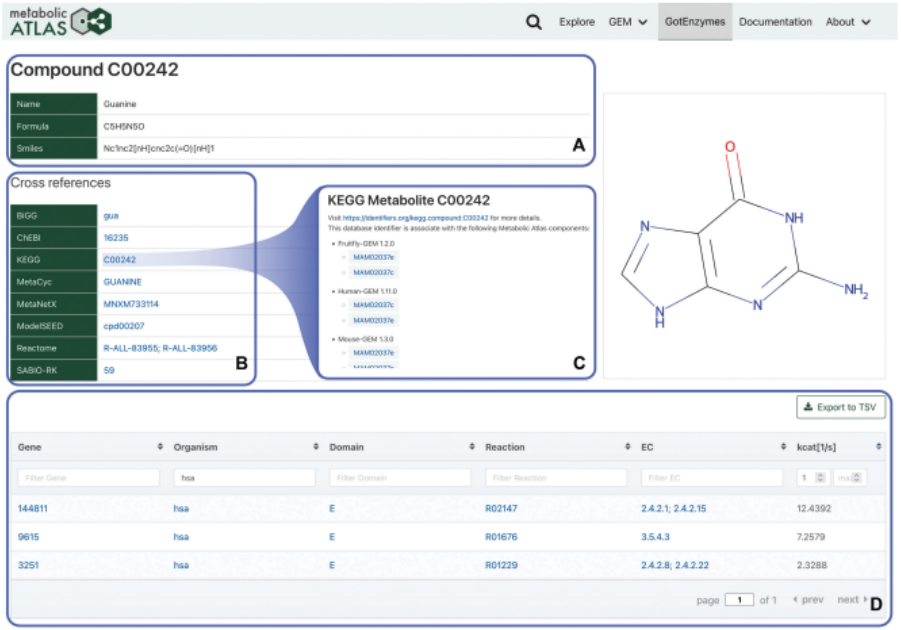
Growth rate prediction



Predicting k_{cat} for ecGEM parameterization

Li F, et al *Nucleic Acids Res, 2023*

- Enzyme performance can be quantitatively described by parameters such as enzyme turnover number kcat and Michaelis constant K_M.
- The ratio kcat/Km is a measure of enzyme efficiency, combining both the affinity for the substrate and the rate of catalysis. It is often used as a benchmark for comparing the performance of different enzymes.
- **GotEnzymes** provides a comprehensive database with enzyme parameter predictions available at https://metabolicatlas.org/gotenzymes.



$$v = \frac{V_{max}[S]}{K_m+[S]} \xrightarrow{\text{Vmax=Kcat[E]total}} v = \frac{Kcat[E]total[S]}{K_m+[S]}$$

Michaelis-Menten equation

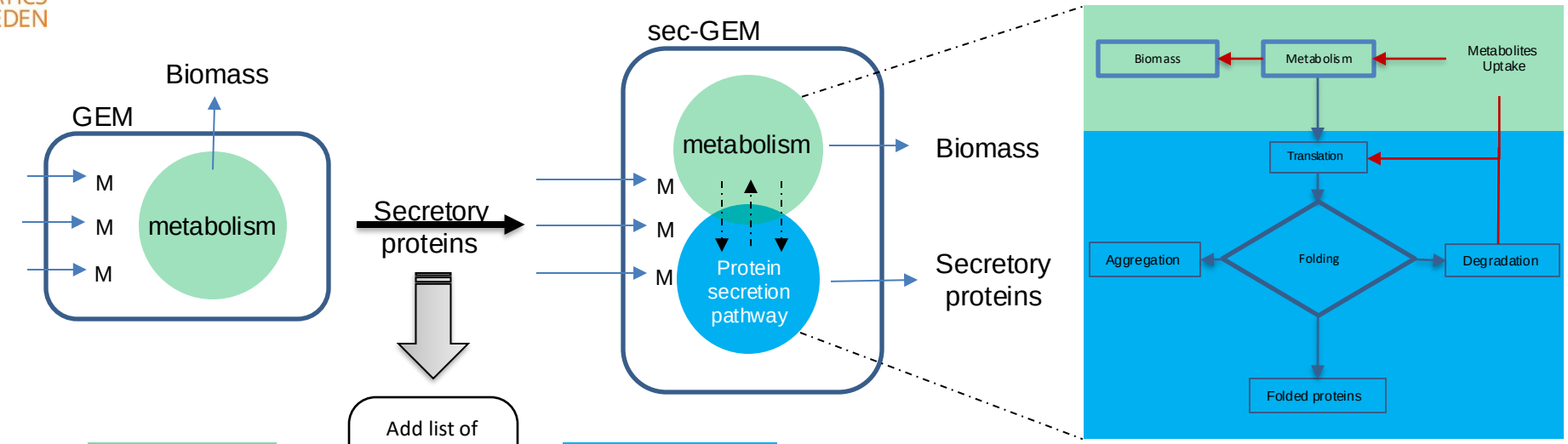
Experimental measurement / DLKcat prediction

Proteomics data / whole protein constrain

Experimental measurement / GotEnzymes prediction

More accurate predictions

Extending the coverage of GEMs: secGEM



Human1

Sec-GEM

Add list of reactions for the selected protein to model

Gap Filling the model for production of added reactions

input a secretory protein

Template reactions

HumanSec

PSIM

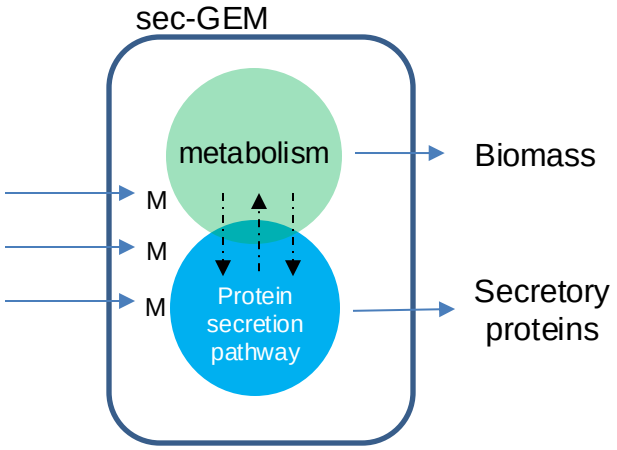
Generating specific reactions for the protein

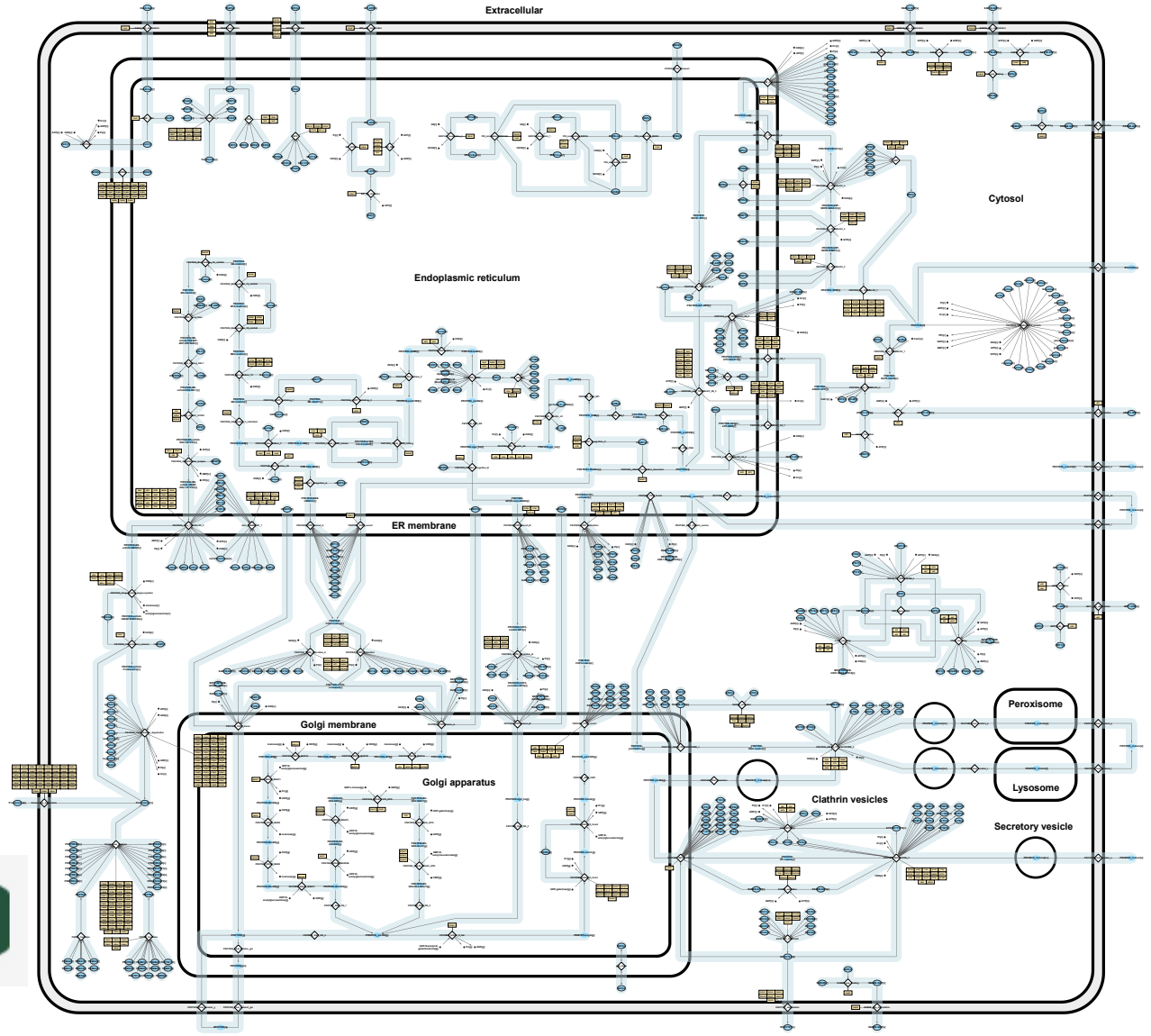
Extract information from Protein Specific Information Matrix

	Protein Specific Information Matrix					
	L	NG	OG	DSB	TMD	...
P_i	340	2	9	1	0	...
...	...	...	...	...	...	...

Saghaleyni, et al *Cell Rep, 2022*

Extending the coverage of GEMs: secGEM





Integrating Single cell transcriptomics into GEMs

Single-cell omics analysis with genome-scale metabolic modeling

J Gustafsson. Et al
Current Opinion in Biotechnology, 2024

Generation and analysis of context-specific genome-scale metabolic models derived from single-cell RNA-Seq data

J Gustafsson. Et al
PNAS, 2023

Johan Gustafsson

Postdoctoral Fellow, Broad institute, USA

Talk Title: *Generation of context-specific genome-scale metabolic models using single-cell RNA-Seq data*

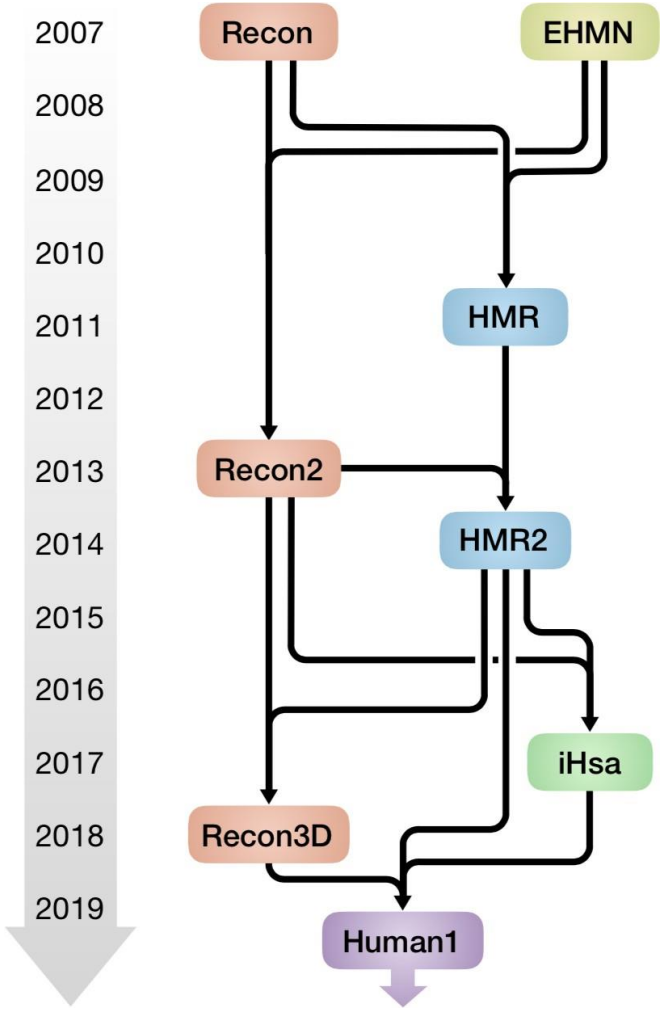
Time: October 17, 13:00 – 14:15 CET online on zoom

Link to Talk: BIG talk event, Link, pass:spd996

Description of the Talk:
The metabolic networks in cells vary across tissues and cell types, and to accurately model the metabolism of cells, the full generic metabolic network defined in the genome needs to be reduced to a context-specific network representing the network expressed specifically in the cells of interest. Single-cell RNA-Seq promises to provide the information needed for such a reduction, but noise in the form of data sparsity is a challenge. Here, we present methods to handle data sparsity and estimate the uncertainty of modeling results.

About the Speaker:
Johan is an expert in modeling cancer metabolism and analyzing single-cell RNA/DNA sequencing data, aiming to uncover vulnerabilities in cancer. With a background in both computer science and biochemistry, Johan has completed a PhD in metabolic modeling at Chalmers University of Technology and now works as a postdoc in the Getz lab at the Broad Institute, focusing on CLL/Richter's syndrome and hypoxia in solid tumors.

Human GEMs



Genome-scale models of human metabolism

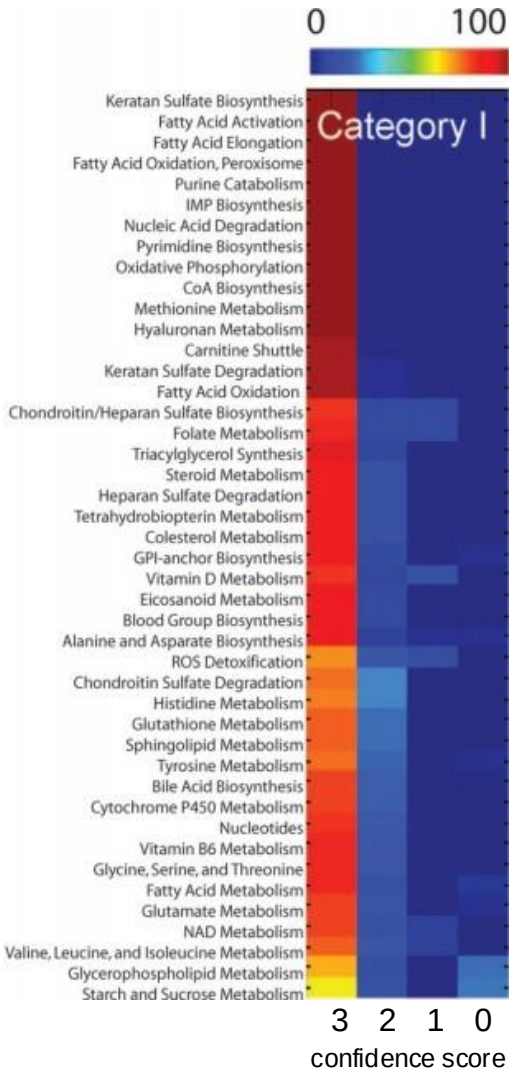
- Began with Recon1 and EHMN (Edinburgh human metabolic network)
- Followed by the first generation of the Human Metabolic Reaction (HMR) model
- A few years later new versions Recon2 and HMR2 were published
- Then Recon3D model improved the annotations.
- The most recent human GEM is Human 1.

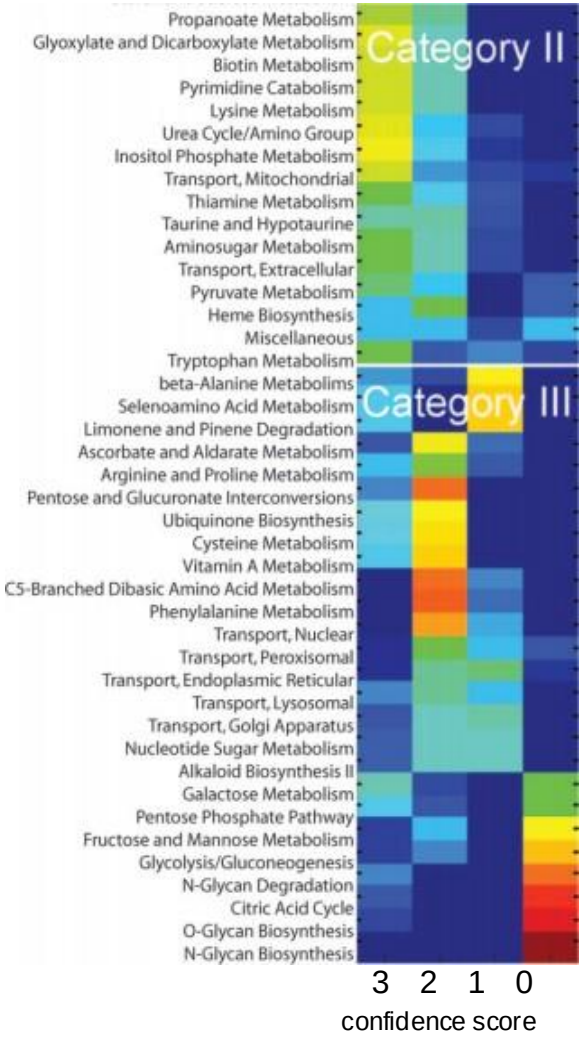
Human GEMs

Recon1

N.C. Duarte, et al. *PNAS* 2007

- Included intracellular **compartments** and exchange
- References and **confidence scores** were provided for each model component
- Highlighted the large differences in characterization of each pathway
  - Category I, II, and III
- Integrated transcriptomic data from gastric bypass patients with the model
  - Gene fold-changes before/after surgery
  - Mapped to network and **visually** identified regions of coordinated expression change



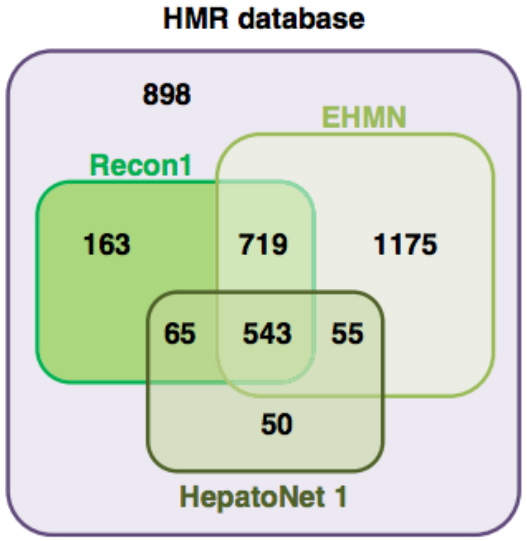


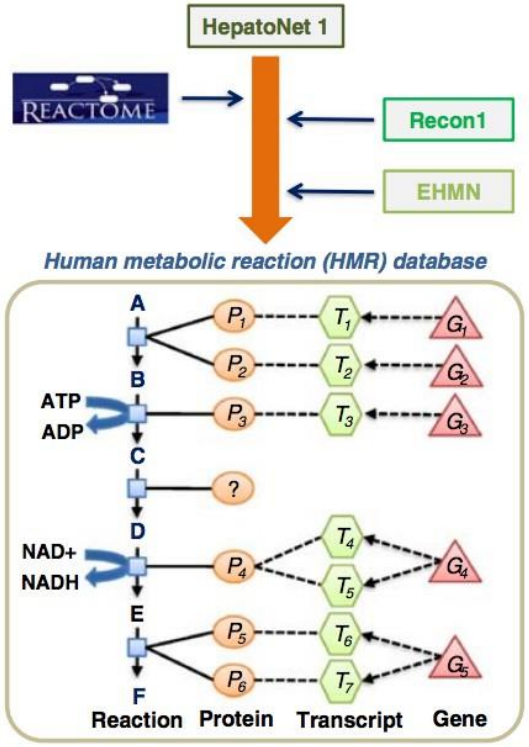
Human GEMs

HMR (Human Metabolic Reaction) **model**

R. Ågren et al. *PLoS Comput Biol* 2012
A. Mardinoglu, et al. *Mol Syst Biol* 2013

- Initially formulated as more of a database than a model
- Merged Recon1 and EHMN with other databases (HumanCyc and KEGG)
- Focused on metabolites and reactions with standard identifiers (KEGG, InChI, etc.)
- HMR was integrated with healthy tissue and cancer proteomics and transcriptomics to generate tissue- and cancer-specific models
  - Developed the INIT algorithm to perform the omics data integration



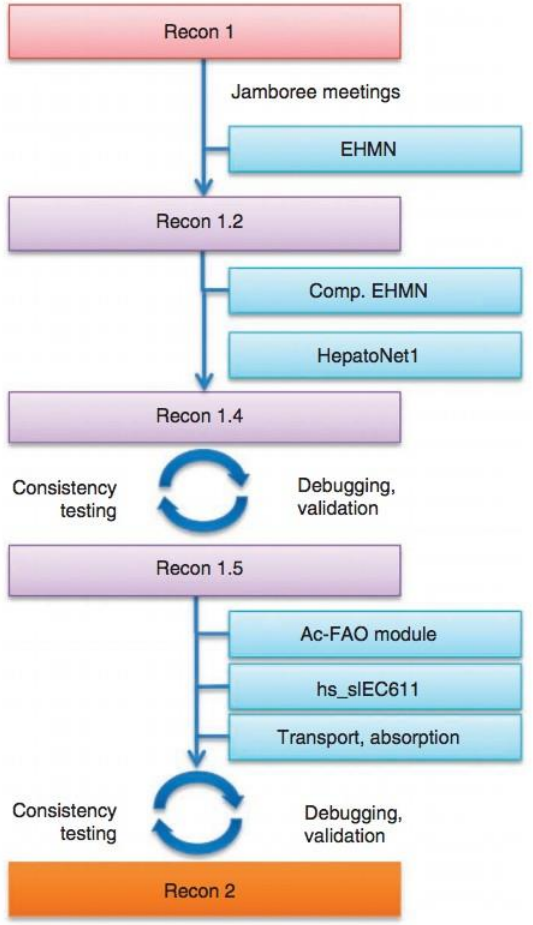


Human GEMs

Recon2

I. Thiele, et al. *Nat Biotechnol* 2013

- Aimed to develop a consensus reconstruction, combining a few previous models
- Used the model to predict biomarkers for inborn errors of metabolism (IEM)
  - Constrained reactions catalyzed by affected enzymes and identified significantly altered exchange reaction fluxes
  - Recon2 outperformed Recon1
- Generated 65 cell type-specific GEMs using HPA expression data (with iMAT)
  - Compared structures (reaction content)
  - 25% of the models could generate biomass



Recon 1

In silico \ *In vivo*	Up	Down
Up	24	1
Down	16	5

Accuracy = 63%
$P = 0.054$

Recon 2

In silico \ *In vivo*	Up	Down
Up	66	5
Down	18	10

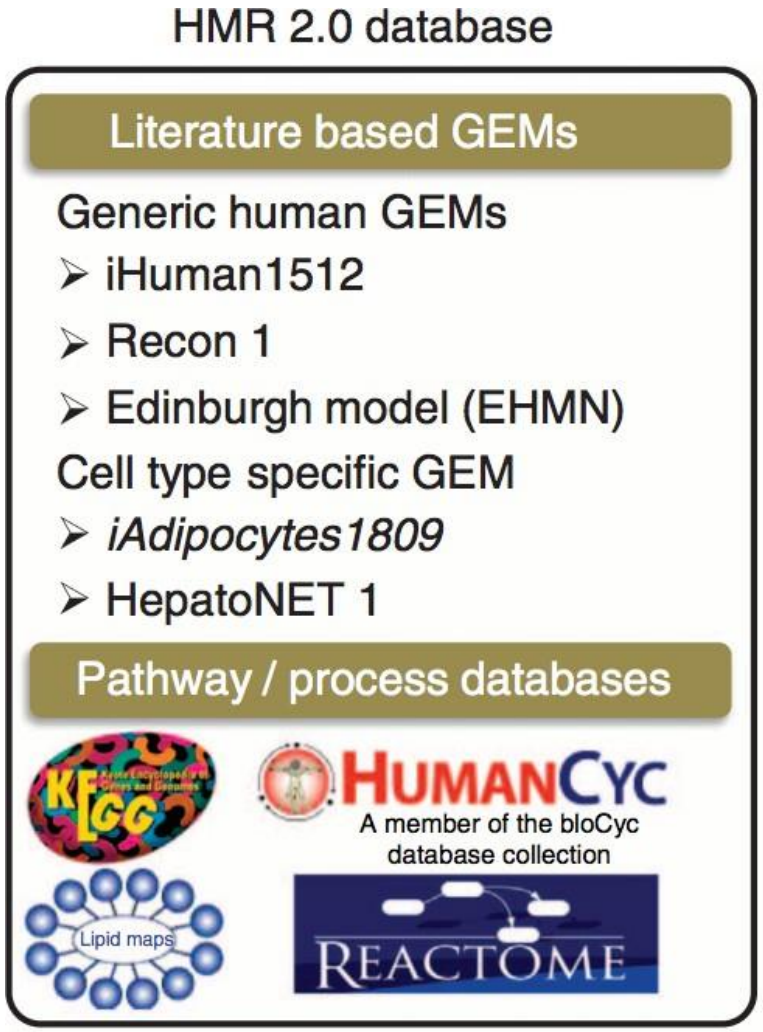
Accuracy = 77%
$P = 7.9*10^{-4}$

Human GEMs

HMR2 (Human Metabolic Reaction) **model**

A. Mardinoglu, et al. *Nat Commun* 2014

- Incorporated extensive lipid metabolism
- Improved reaction-gene associations
  - However, all genes are still assumed to encode isozymes for their associated reactions
- HMDB, Lipid Map, KEGG, and ChEBI identifiers were assigned to metabolites
- KEGG IDs and EC numbers were assigned to reactions
- Also included genes and reactions in Recon2

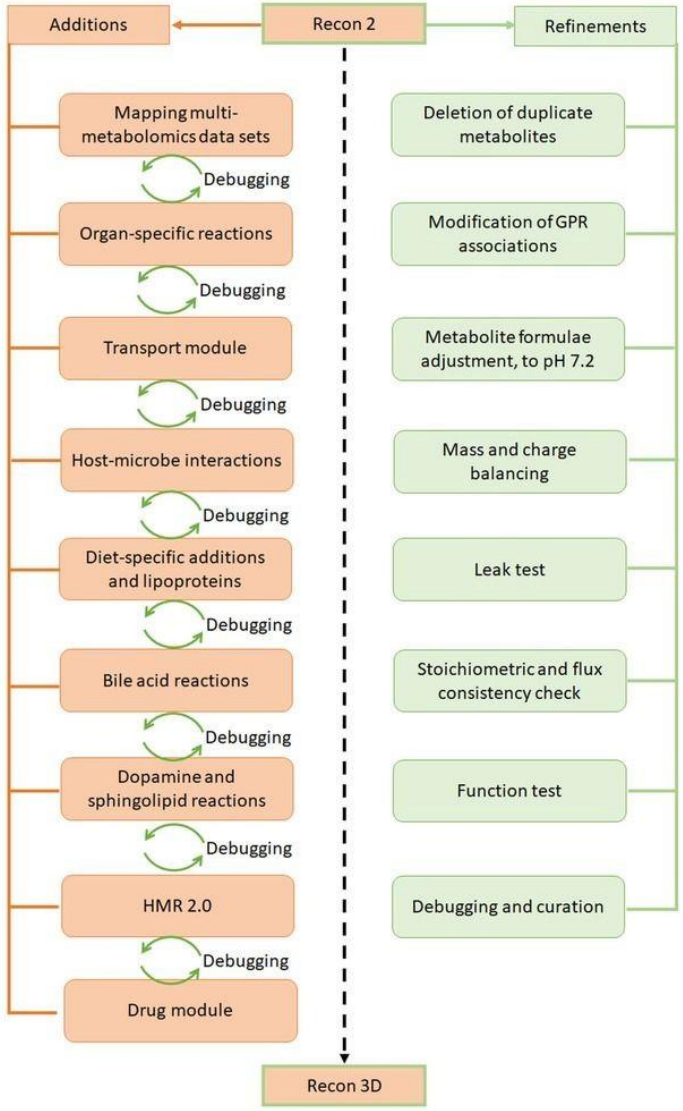


Human GEMs

Recon3D

E. Brunk, et al. *Nat Biotechnol* 2018

- Expanded Recon2 by incorporating other models/networks
  - e.g., HMR2 and drug metabolism
- Curated and fixed many errors present in Recon2
- Added 3D metabolite and protein structure data
- A separate “database version” and “model version” exist
  - The database version contains all the reactions and information, but is not properly balanced.
  - The model version is suitable for simulation purposes (e.g., FBA).

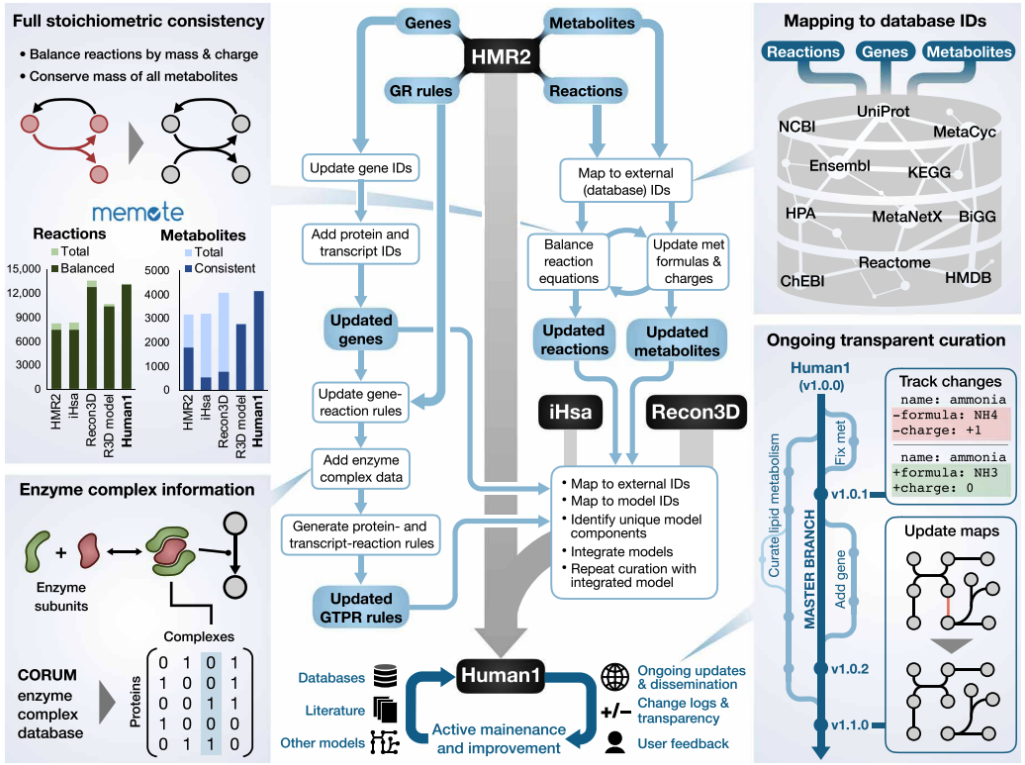


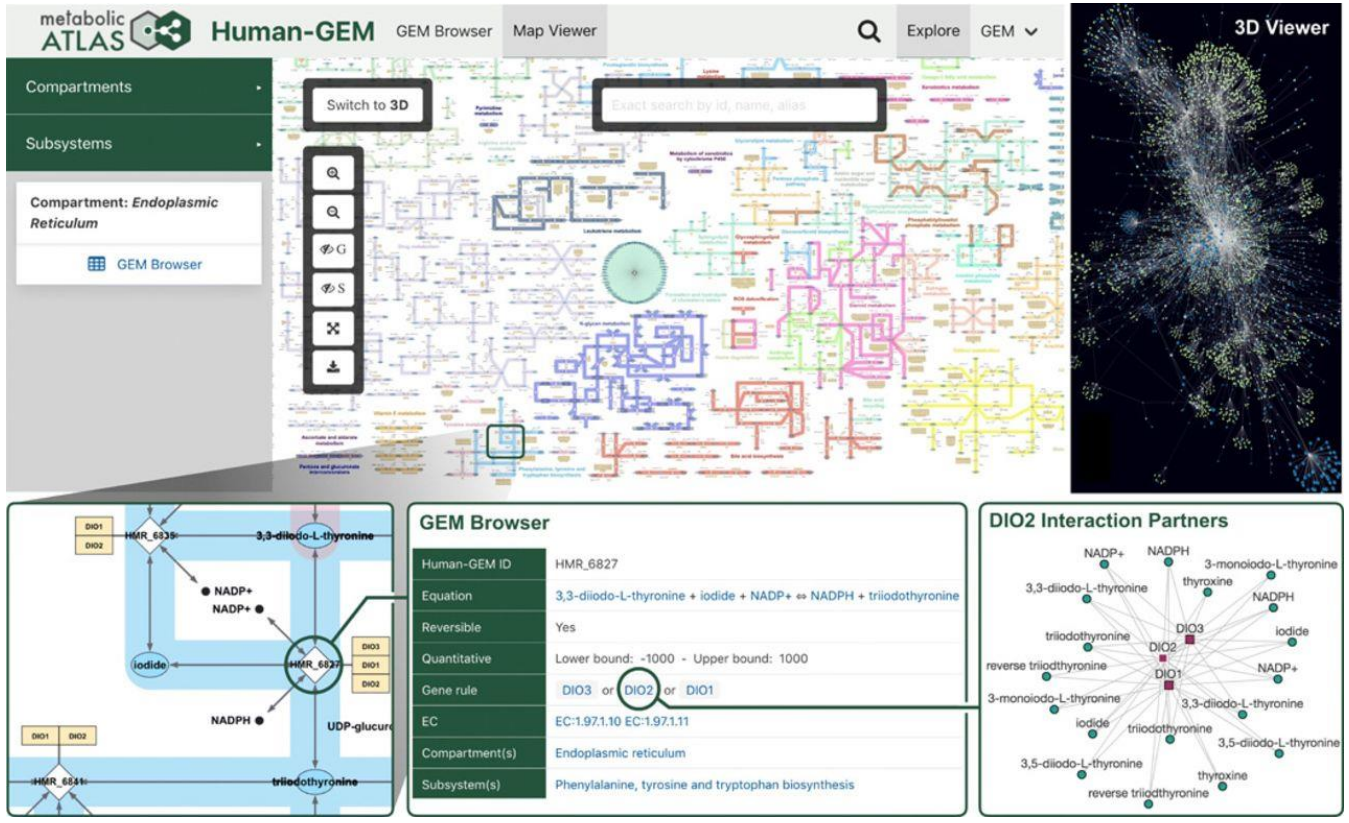
Human GEMs

Human 1

Robinson J, et al. *Science Signalling, 2021*

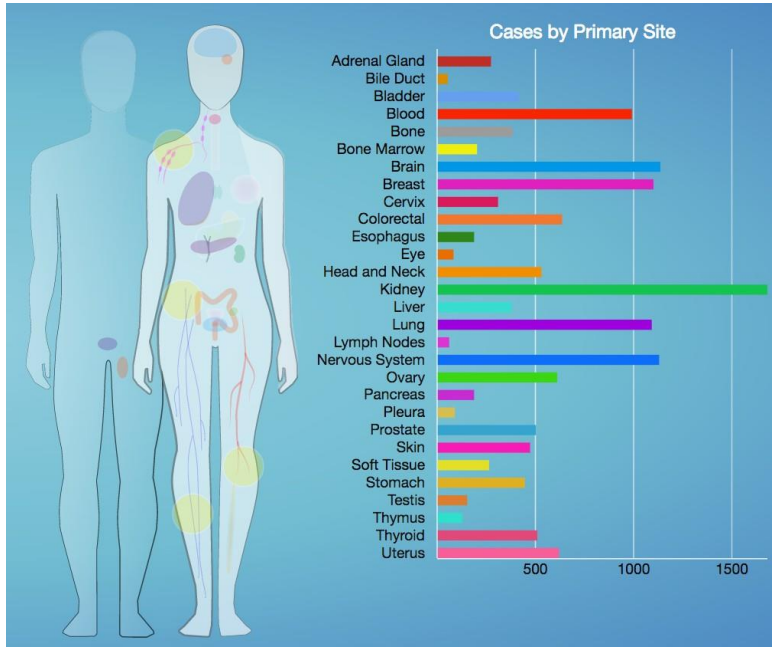
- Extensive curation

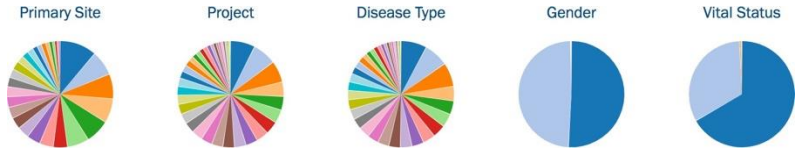




GEM-based comparison of transcriptomes

NIH THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute



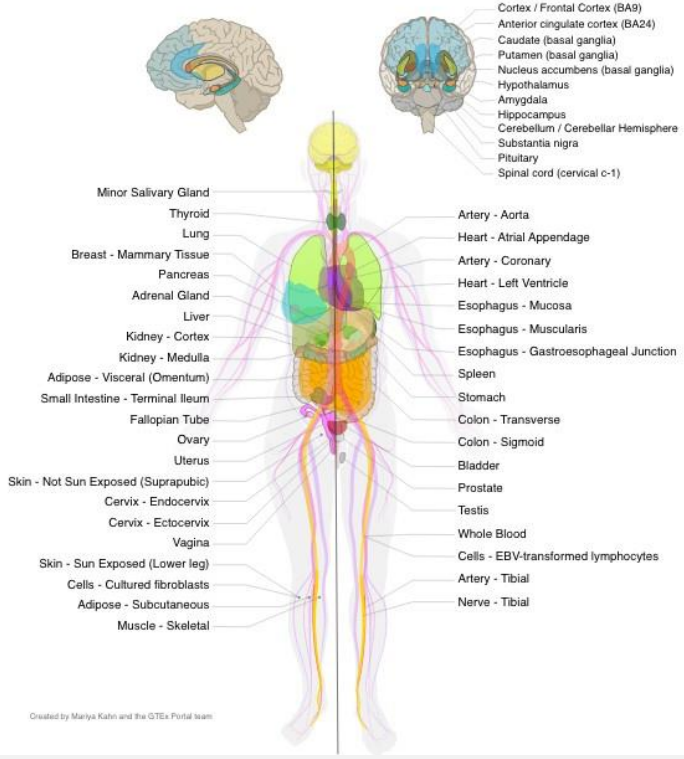


GTEx Portal

Home | Datasets | Expression | QTLs & Browsers | Sample Data | Do

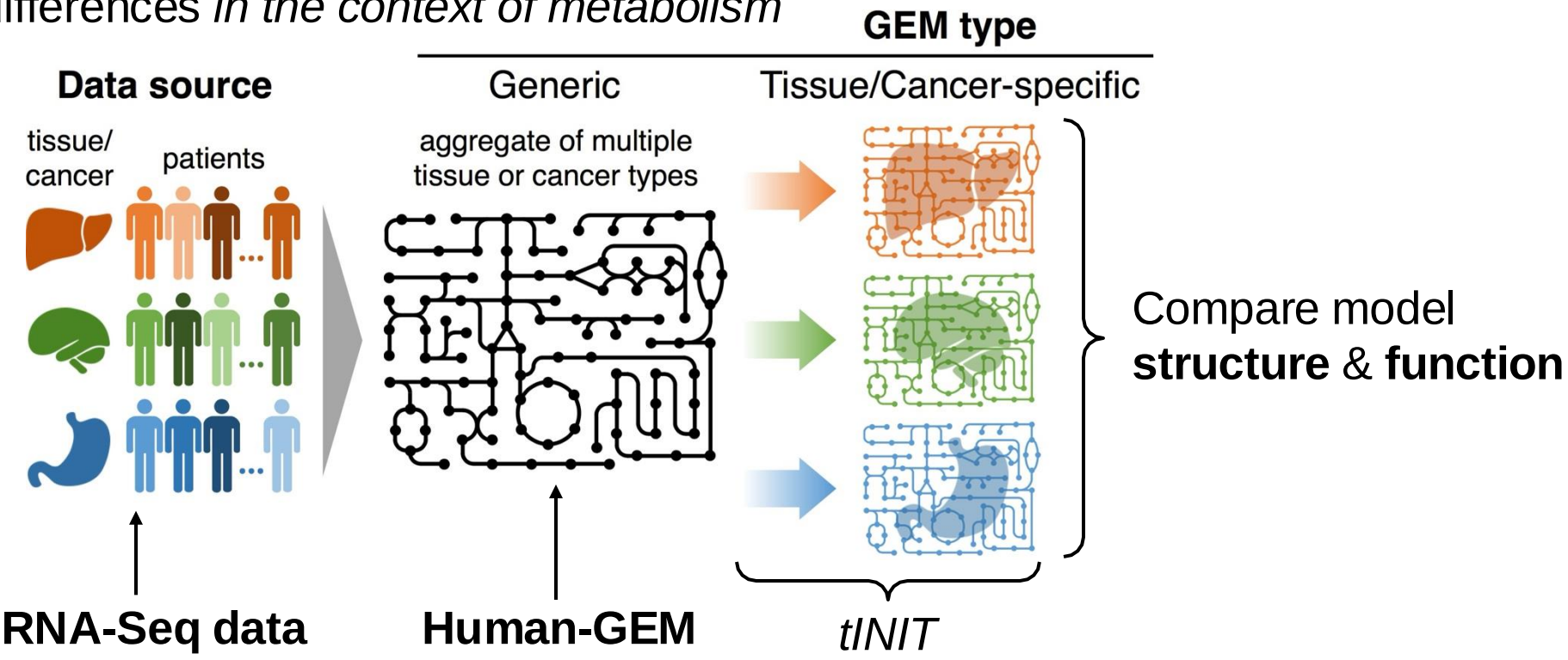
Tissue Sampling Sites

This page provides a visual representation of the biospecimen source sites (BSSs) for the collection of tissue from postmortem/organ procurement cases for the Genotype-Tissue Expression (GTEx) project.
The full documentation on tissue collection procedures can be found on the GTEx Tissue Harvesting Work Instruction.



GEM-based comparison of transcriptomes

Objective: To investigate healthy and tumor tissue transcriptomic differences *in the context of metabolism*



- TCGA
  - 33 tumor types
  - 23 paired-normal tissue types
- GTEx
  - 30 healthy tissue types

GEM-based comparison of transcriptomes

Comparison of model **structures**

Reaction	Lung Tumor	Lung Paired	Lung Healthy	Brain Tumor	Brain Paired	…
rxn1	1	0	1	1	1	
rxn2	0	1	1	1	1	
rxn3	0	0	0	0	0	
rxn4	0	1	0	1	0	
rxn5	1	1	0	1	1	
rxn6	1	0	0	1	0	
rxn7	0	0	1	1	0	
⋮						⋱

Model **contains** reaction

Model **missing** reaction

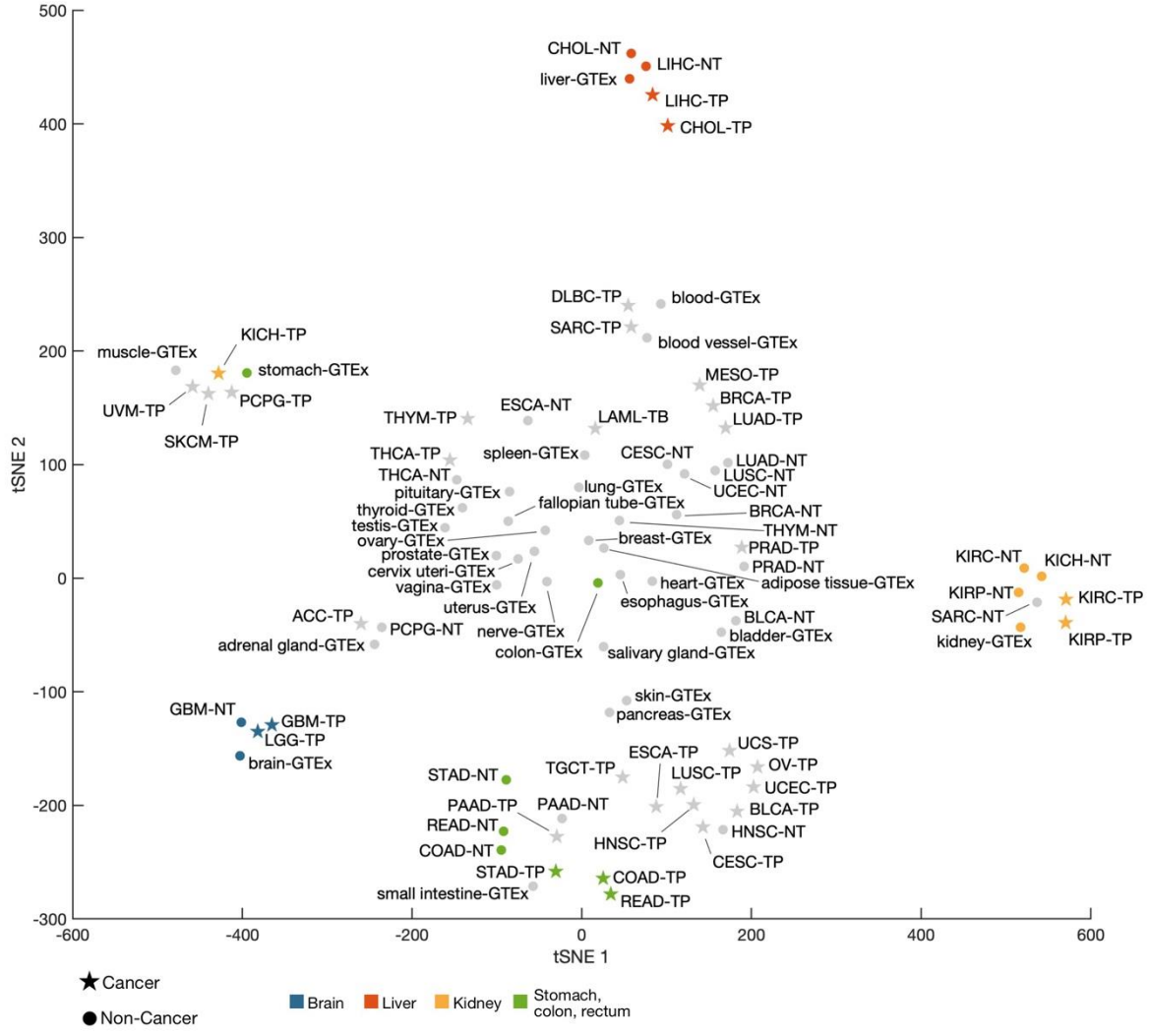
GEM-based comparison of transcriptomes

Comparison of model **structures**

Subsystem	Reaction	Lung Tumor	Lung Paired	Lung Healthy	Brain Tumor	Brain Paired	…
TCA cycle	rxn1	1	0	1	1	1	
TCA cycle	rxn2	0	1	1	1	1	
Glycolysis	rxn3	0	0	0	0	0	
TCA cycle	rxn4	0	1	0	1	0	
Fatty acid oxidation	rxn5	1	1	0	1	1	
Carnitine shuttle	rxn6	1	0	0	1	0	
Glycolysis	rxn7	0	0	1	1	0	
⋮							⋱

GEM-based comparison of transcriptomes

tSNE of model reaction content matrix



Take home Messages

- Developing GEMs is an **iterative process**.
- GEMs can serve as a **scaffold for integrating & studying diverse types of (omics) data** (but needs **formulation** into GEMs concept).
- GEMs are **simulation based and (FBA)** and depending on the objective functions can provide deeper insights into metabolism.
- GEMs enables the analysis of omics data but in the **context of metabolism**.
- I only covered some models and algorithms that are more interesting for me, but there are many other…