

Omics data analysis in genome scale metabolic networks

Summer school Multi-omics - Aussois

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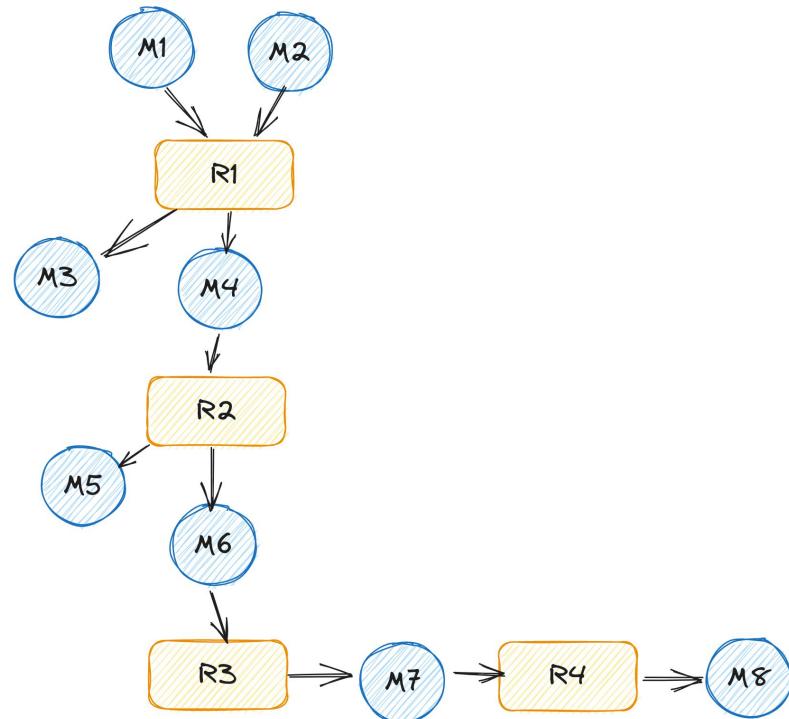


Metabolic networks

A definition of a metabolic network

A metabolic network is:

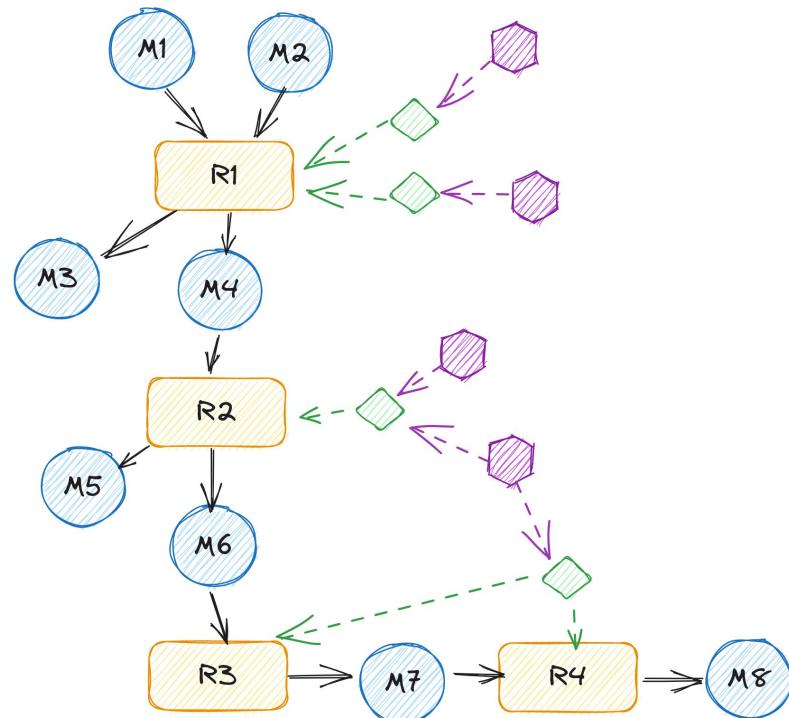
- a set of biochemical reactions linked together by the metabolites that they consume and produce



A definition of a metabolic network

A metabolic network is:

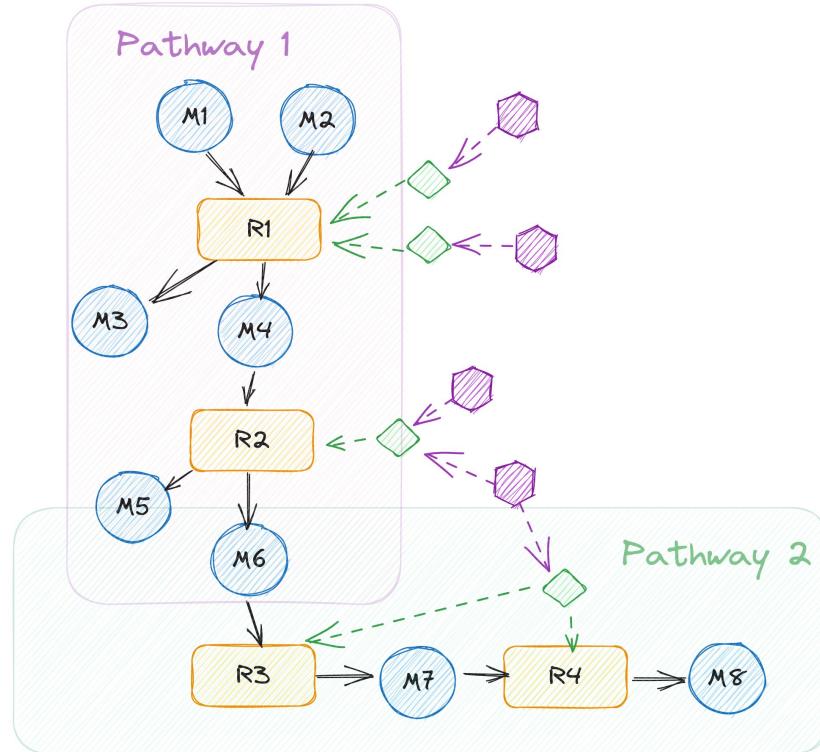
- a set of biochemical reactions linked together by the metabolites that they consume and produce
- the set of the genes that code for the enzymes that catalyse the reactions



A definition of a metabolic network

A metabolic network is:

- a set of biochemical reactions linked together by the metabolites that they consume and produce
- the set of the genes that code for the enzymes that catalyse the reactions
- the set of pathways where the reactions are involved

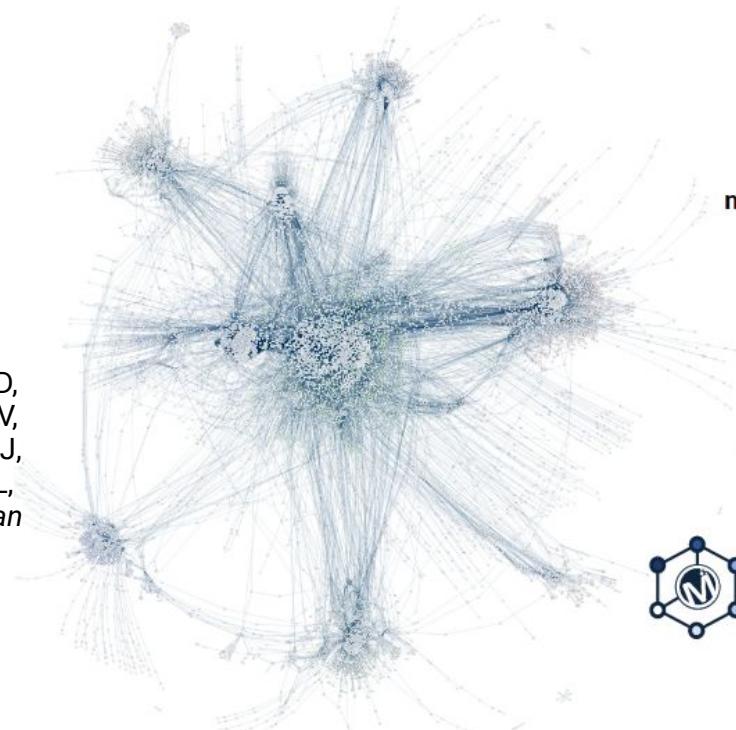


A definition of a genome-scale metabolic network

A metabolic network known to take place in
a target organism

Human-GEM
13024 reactions
8363 metabolites
2920 genes

Robinson JL, Kocabas P, Wang H, Cholley PE, Cook D, Nilsson A, Anton M, Ferreira R, Domenzain I, Billa V, Limeta A, Hedin A, Gustafsson J, Kerkhoven EJ, Svensson LT, Palsson BO, Mardinoglu A, Hansson L, Uhlén M, Nielsen J, 2020. *An atlas of human metabolism*. Science signaling



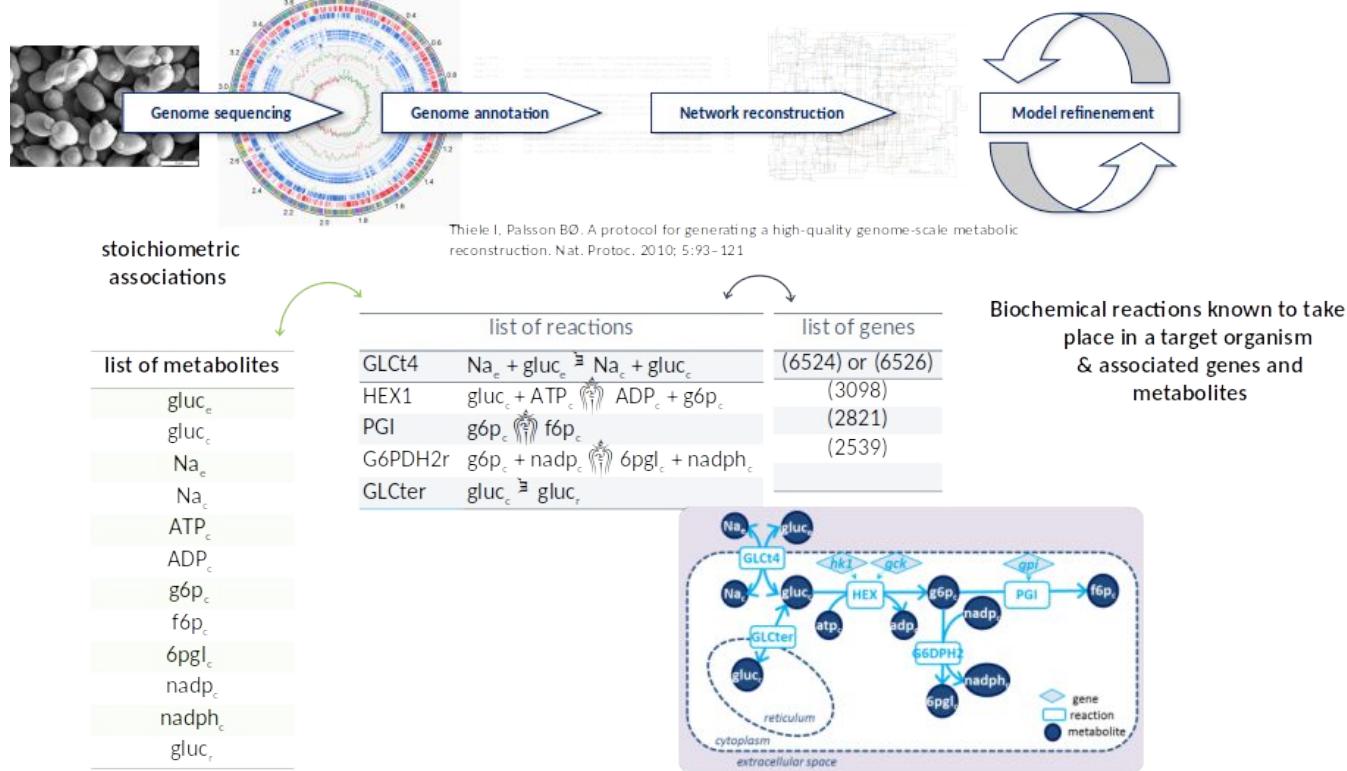
**human genome-scale
metabolic reconstruction Recon2.2**
7785 reactions
2652 metabolites
1675 genes
Swainston N. et al. Metabolomics. 2016.



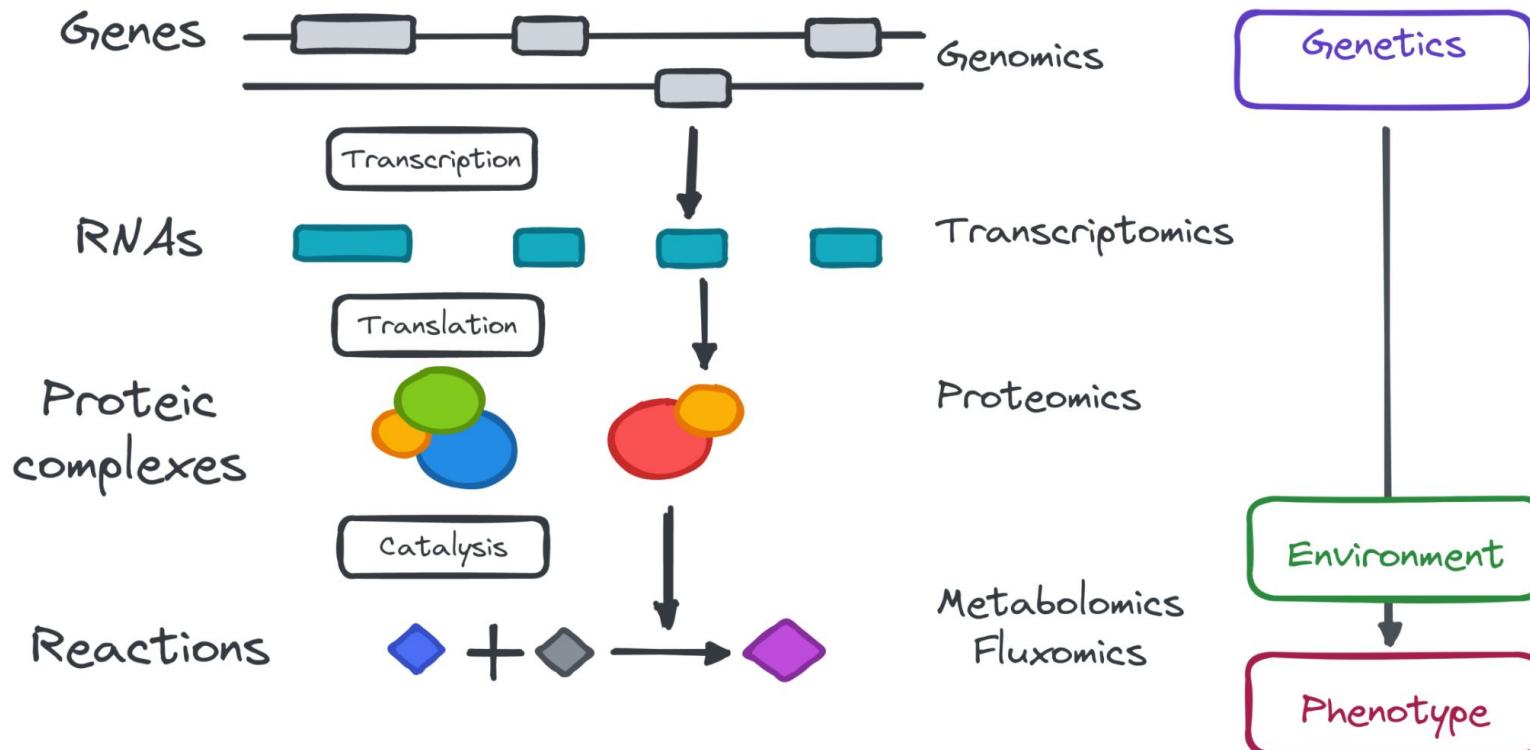
Genome-scale metabolic reconstructions

Build from its genome annotation.

- Infer catalytic activities from comparisons between sequences of target genes and genes of model organisms
- Deduced list of reactions that can potentially take place in the target organism
- Associated metabolites from reactions

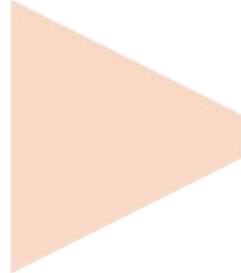


The metabolic network: a context of interpretation for omics data

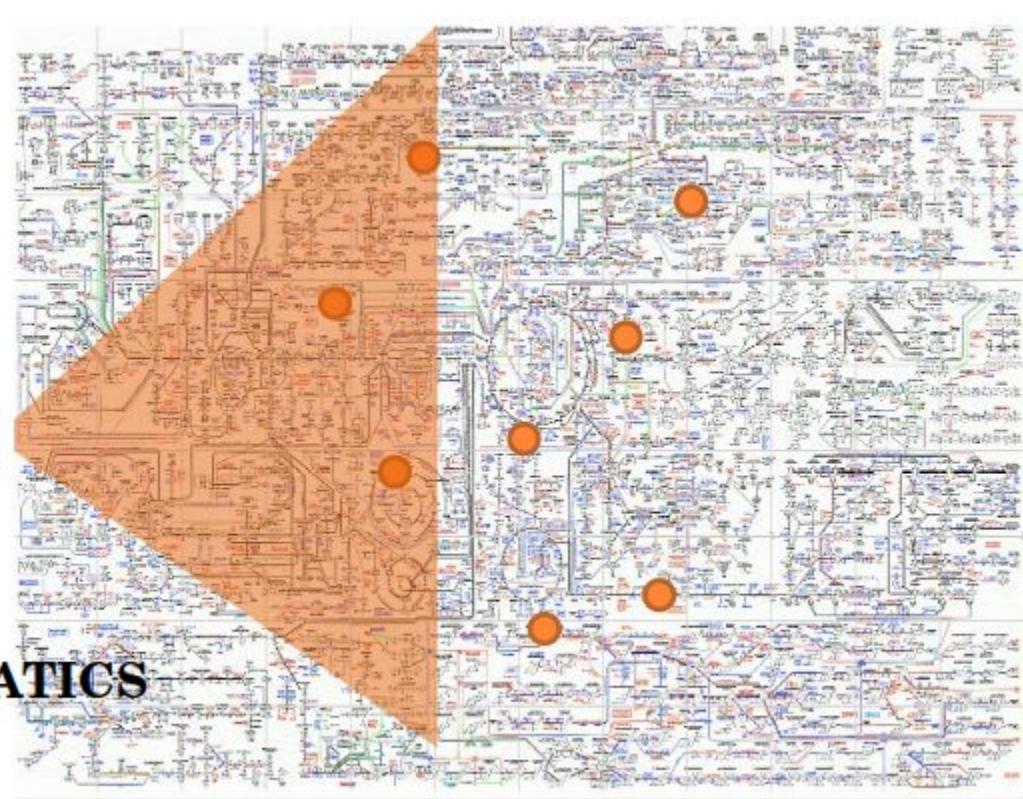


Mapping omics data

OMICS
DATA



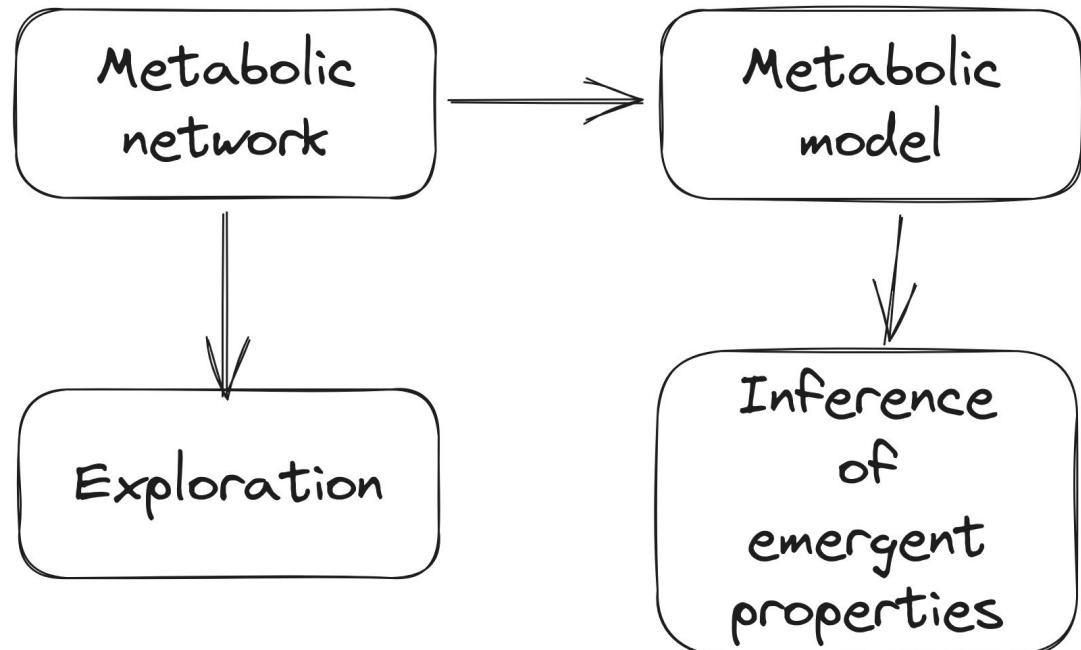
BIOINFORMATICS



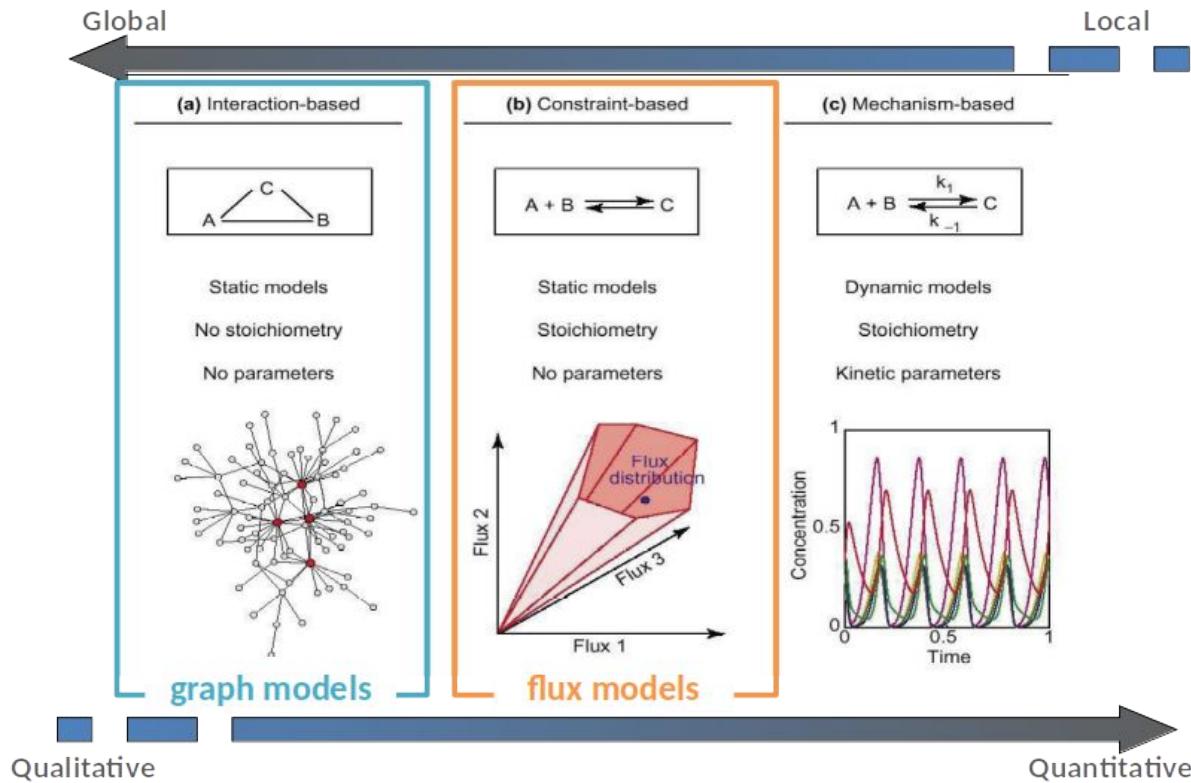
Metabolic networks to metabolic models

The behavior of the whole system cannot be deduced from the analysis of its individual components

Metabolic network: textual description

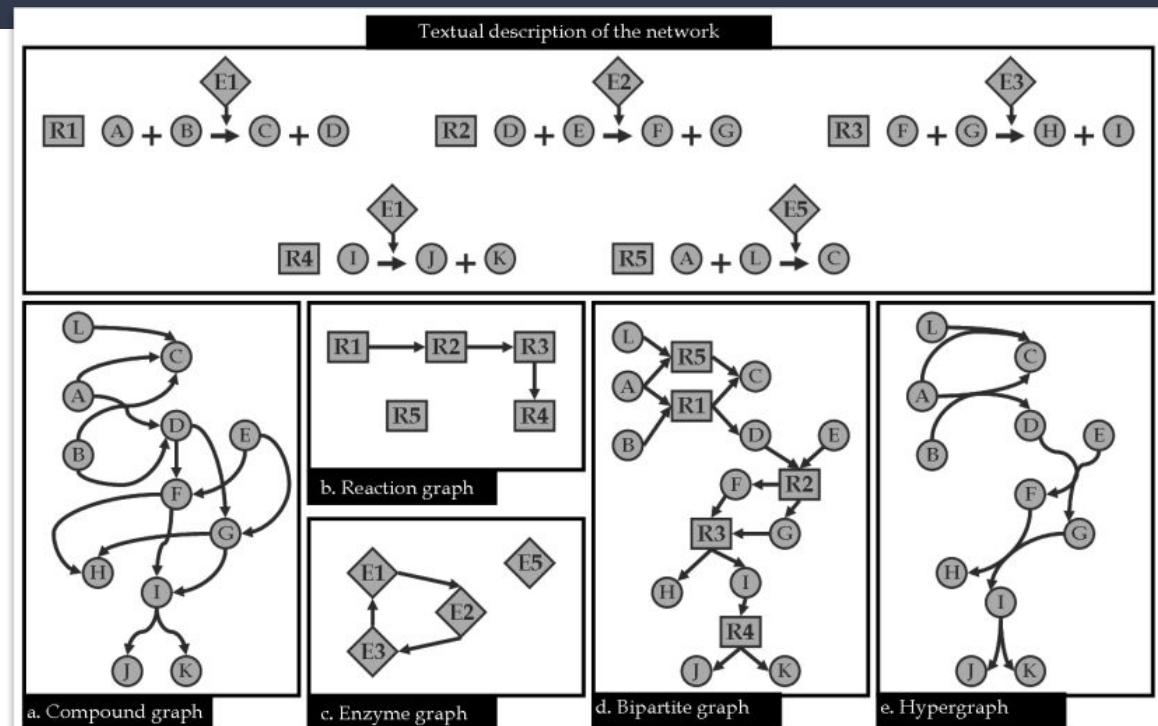


Metabolic models



Graph models

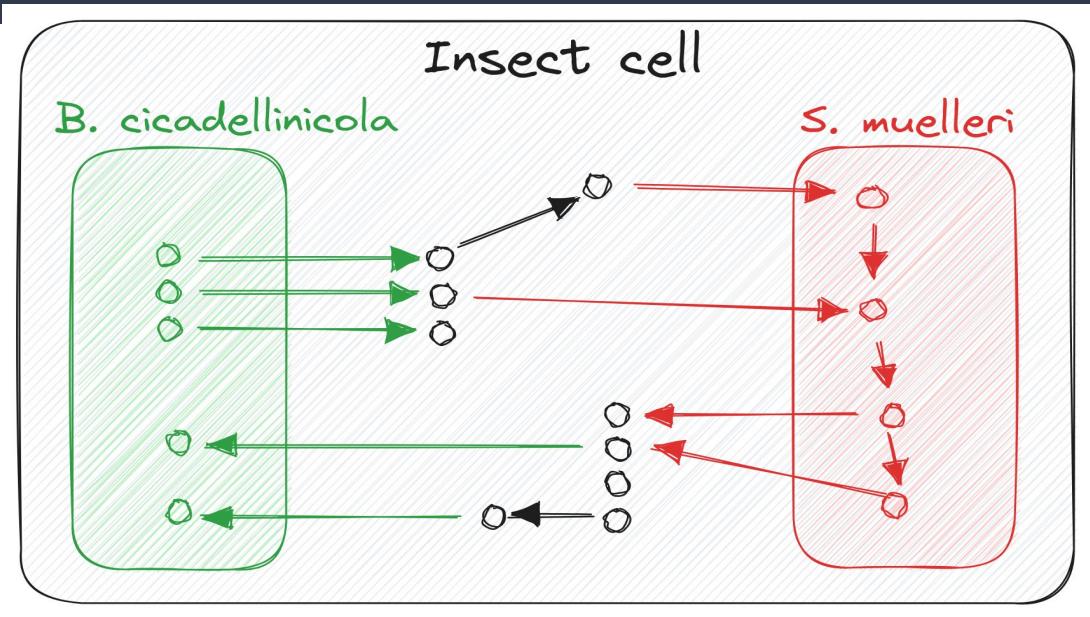
Metabolic graphs are built from the descriptions of the set of reactions that constitute a metabolic network



Graph models to analyse metabolic interactions



Wu D et al. (2006) **Metabolic Complementarity and Genomics of the Dual Bacterial Symbiosis of Sharpshooters.** PLOS Biology 4(6): e188.



Cottret L, Milreu PV, Acuña V, Marchetti-Spaccamela A, Stougie L, Charles H, Sagot MF. **Graph-based analysis of the metabolic exchanges between two co-resident intracellular symbionts, Baumannia cicadellinicola and Sulcia muelleri, with their insect host, Homalodisca coagulata.** PLoS Comput Biol. 2010 Sep 2;6(9):e1000904..

Flux Balance Analysis (FBA)

Based on Genome-scale metabolic network

Aim to describe the biological phenotype

In mathematical terms

- **Compute flux distributions**

Flux = **rate** of synthesis / consumption of a metabolite in a reaction
unit = mmol. g DW⁻¹. h⁻¹

Flux distribution = **flux values for all reactions** in the model

FBA: Mathematical representation

Mathematical representation of metabolic reactions

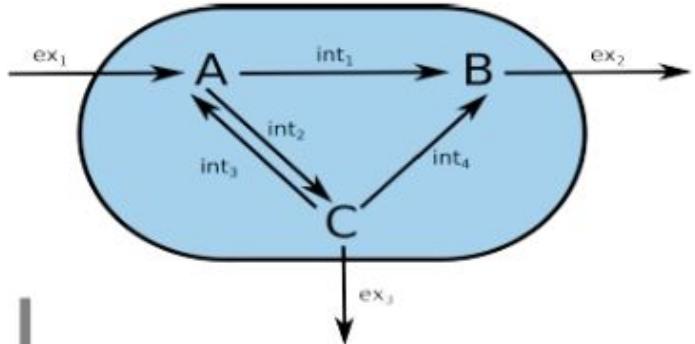
Stoichiometric matrix S: M * n

M = compounds in the metabolic network

n = reactions in the metabolic network

Entries = stoichiometric coefficients of each reactions

	GLCt4	HEX	PGI	G6DH2	GLCter
gluc _e	-1	0	0	0	0
gluc _c	+1	-1	0	0	-1
Na _e	-1	0	0	0	0
Na _c	+1	0	0	0	0
ATP _c	0	-1	0	0	0
ADP _c	0	+1	0	0	0
g6p _c	0	+1	-1	-1	0
f6p _c	0	0	+1	0	0
6pgl _c	0	0	0	+1	0
nadp _c	0	0	0	-1	0
nadph _e	0	0	0	+1	0
gluc _r	0	0	0	0	+1



ex_1, ex_2, ex_3 : exchange fluxes

$int_1 - int_4$: internal fluxes

Mass
balance
equations

$$\frac{dA}{dt} = -int_1 - int_2 + int_3 + ex_1$$

$$\frac{dB}{dt} = int_1 + int_4 - ex_2$$

$$\frac{dC}{dt} = int_2 - int_3 - int_4 - ex_3$$

$$\begin{bmatrix} \frac{dA}{dt} \\ \frac{dB}{dt} \\ \frac{dC}{dt} \end{bmatrix}$$

$$= \begin{bmatrix} -1 & -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & -1 \end{bmatrix} \times$$

$$\begin{bmatrix} int_1 \\ int_2 \\ int_3 \\ int_4 \\ ex_1 \\ ex_2 \\ ex_3 \end{bmatrix}$$

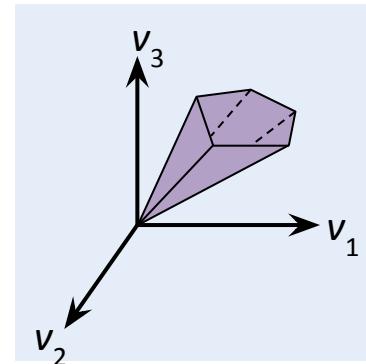
Stoichiometric
matrix (S)

Flux
vector (v)

FBA: Applying constraints

- The **stoichiometric matrix** and the **reconstruction** give a **structure for possible fluxes**, but **not all flux distributions are actually feasible in a given context**.
- **Biological functions are governed by constraints** (Organisms exist in a resource-scarce environment → survival thus depends on best utilization of resources to survive & grow)

→ **the imposition of constraints limits computable phenotypes to the relevant biological plausible ones.**



FBA: The steady-state hypothesis

hypothesis = the time constants characterizing metabolic transients are typically very rapid compared to the time constants of cell growth, so that we consider a steady-state behavior for all system metabolites

for each metabolite i:

$$\frac{dS_i}{dt} = 0 \Leftrightarrow \sum v_{R_{synthesis}} = \sum v_{R_{degradation}}$$

for the network:

$$\frac{dS}{dt} = S \cdot v = 0$$

Mass balance equations

$$\frac{dA}{dt} = -int_1 - int_2 + int_3 + ex_1$$
$$\frac{dB}{dt} = int_1 + int_4 - ex_2$$
$$\frac{dC}{dt} = int_2 - int_3 - int_4 - ex_3$$



Steady state:

$$-int_1 - int_2 + int_3 + ex_1 = 0$$
$$int_1 + int_4 - ex_2 = 0$$
$$int_2 - int_3 - int_4 - ex_3 = 0$$

Matrix form: $S \cdot v = 0$

$$\begin{bmatrix} int_1 \\ int_2 \\ int_3 \\ int_4 \\ ex_1 \\ ex_2 \\ ex_3 \end{bmatrix} = 0$$
$$\begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & -1 \end{bmatrix} \times \begin{bmatrix} int_1 \\ int_2 \\ int_3 \\ int_4 \\ ex_1 \\ ex_2 \\ ex_3 \end{bmatrix} = 0$$

FBA: Restricting reactions flux bounds

for each reaction R_j : $v_{j,\min} \leq v_j \leq v_{j,\max}$

in the matrix format:

$$lb \leq v \leq ub$$

avec $lb = [v_{1,\min}; \dots; v_{j,\min}; \dots; v_{n,\min}]$
et $ub = [v_{1,\max}; \dots; v_{j,\max}; \dots; v_{n,\max}]$

how to determine the bounds?

1. by default $lb = -\text{Inf}$
 $ub = +\text{Inf}$

2. possible constraints:

thermodynamic: $0 < v < +\text{inf}$

enzymatic capacity: $a < v < b$

for exchange reactions: $v > 0 \leftrightarrow \text{secretion}$
 $v < 0 \leftrightarrow \text{intake}$

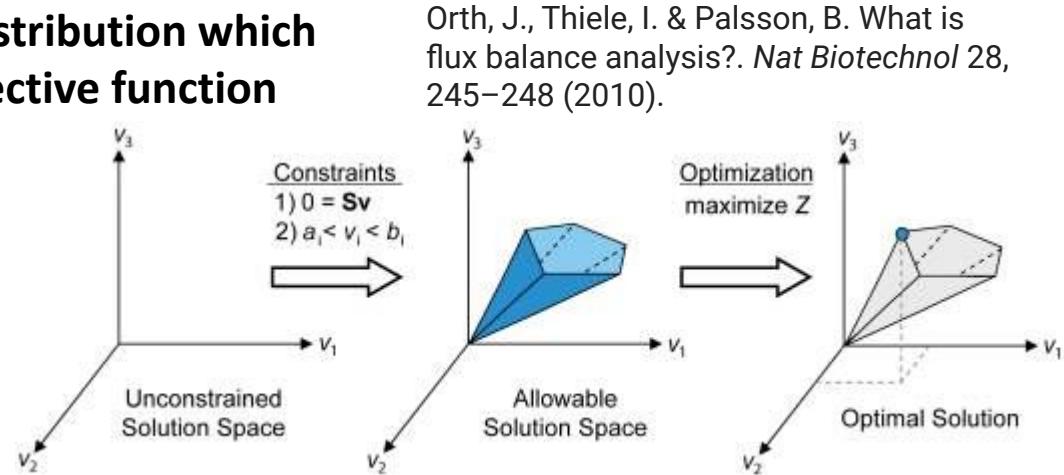
A linear programming optimization problem

Aim = finding one particular solution in the entire solution space (optimal solution under some conditions)

Concept = the cell functions in an optimal metabolic state (e.g. optimal growth under given conditions)

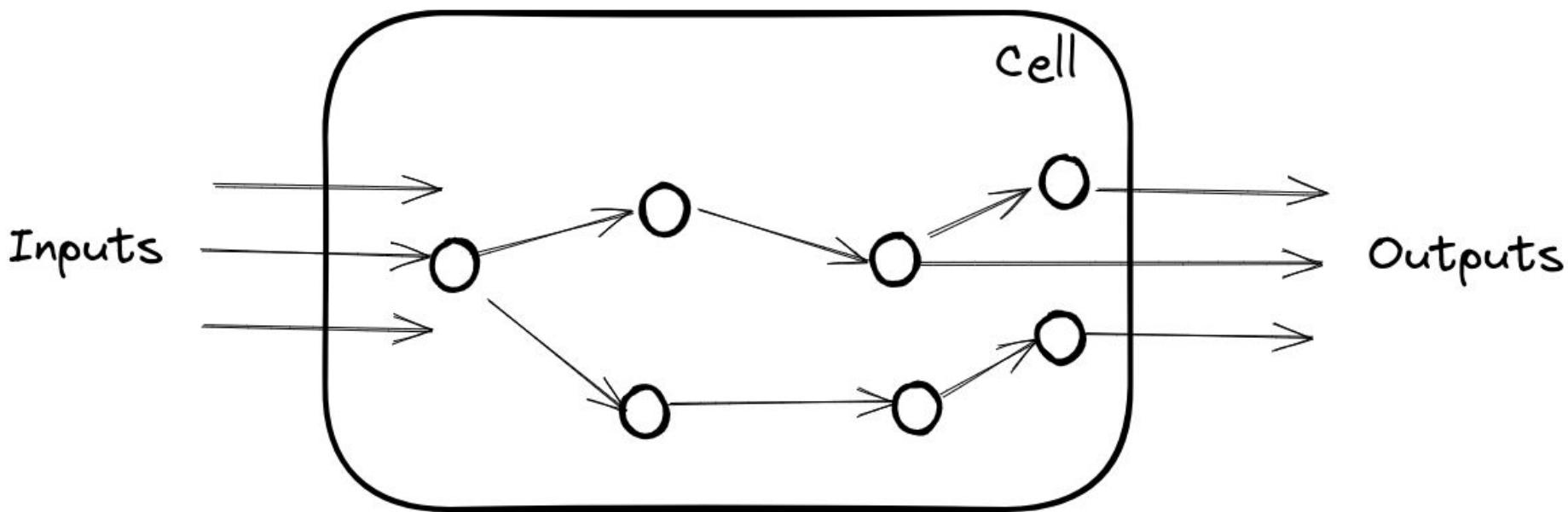
→ **Output of FBA** = particular flux distribution which maximizes or minimizes an objective function

$$\left\{ \begin{array}{l} \text{max: } Z \\ \text{subject to:} \\ S \cdot v = 0 \\ lb \leq v \leq ub \end{array} \right.$$

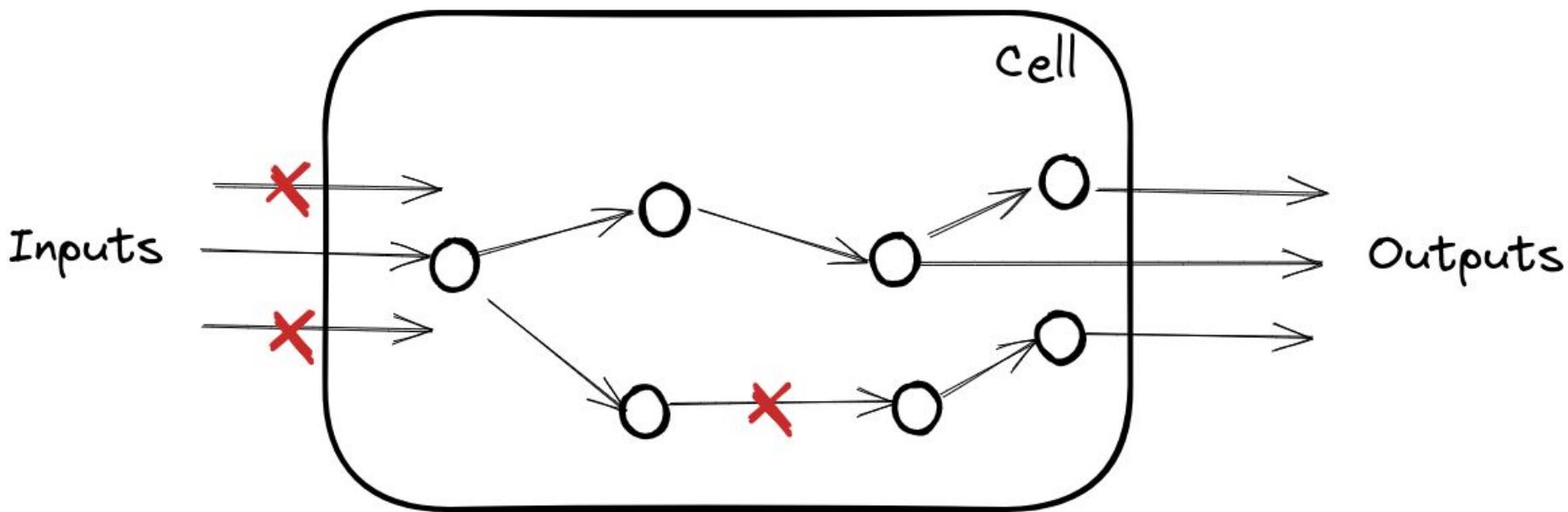


Orth, J., Thiele, I. & Palsson, B. What is flux balance analysis?. *Nat Biotechnol* 28, 245–248 (2010).

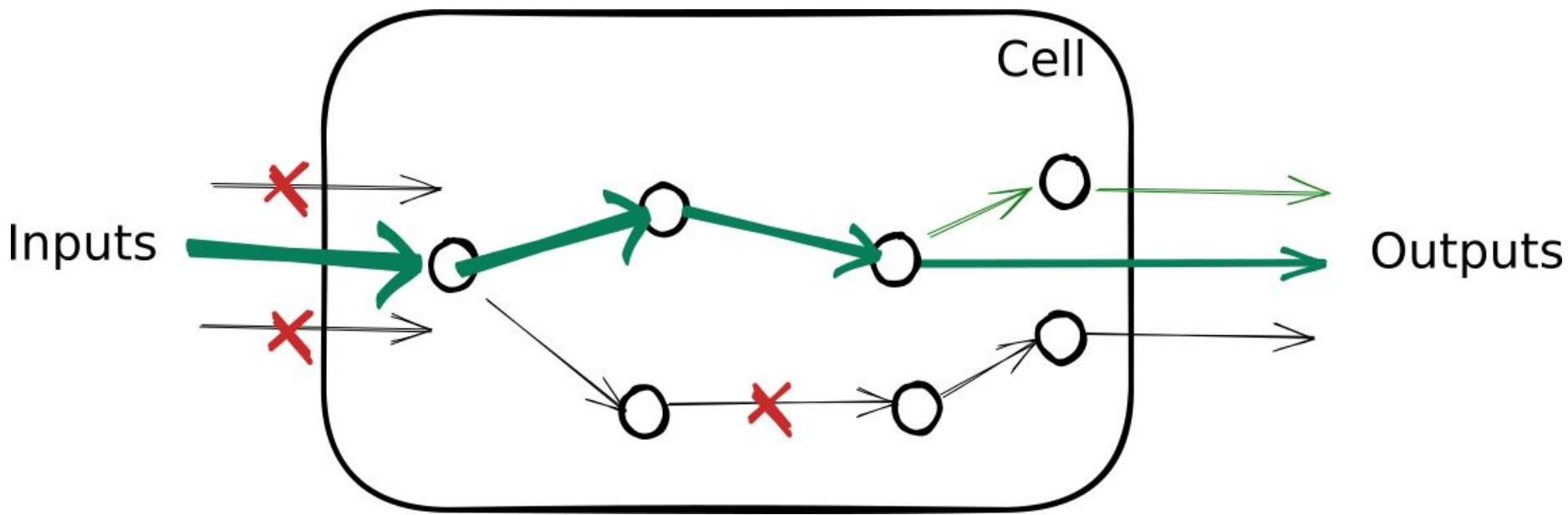
Flux Balance Analysis (FBA)



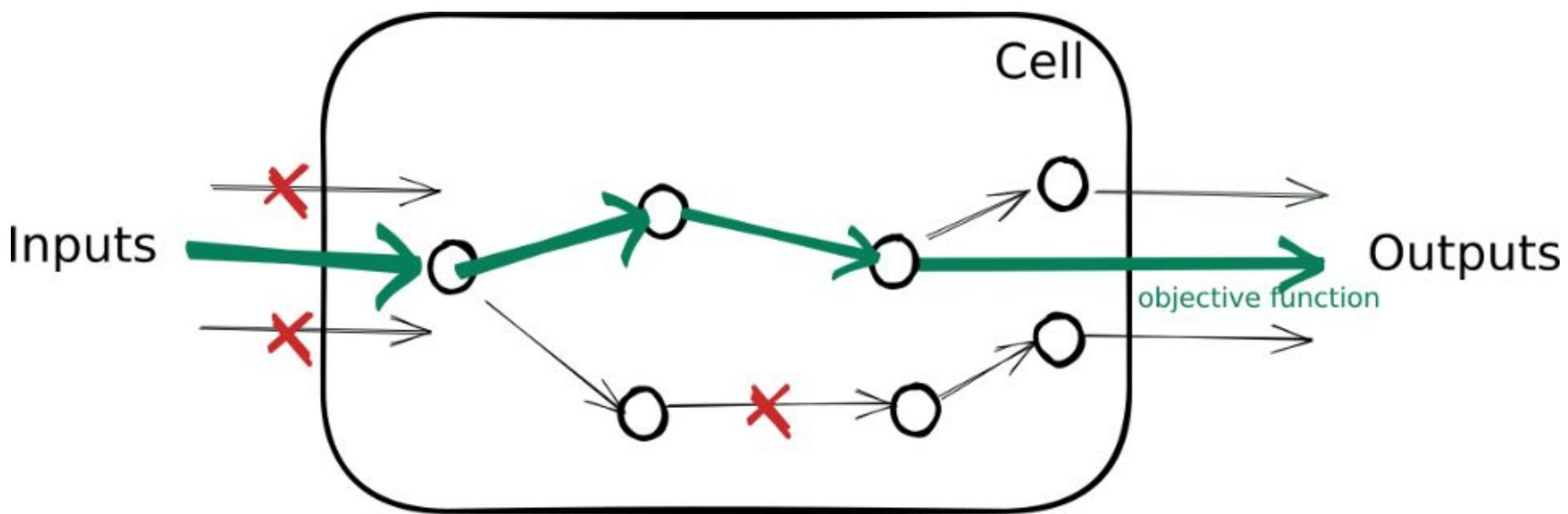
FBA: environmental and genetic constraints



FBA: environmental and genetic constraints



FBA: optimise an objective function



Flux Balance Analysis

- Optimize biomass or production of metabolites of interest
- Analysis of environmental, enzymatic or genetic perturbations (e: *in silico* gene deletions)
- Generate sub-networks from transcriptomics data
- **Just one of many solutions**

Use case with MetExplore: mRNA mapping for BRCA context

MetExplore

MetExplore Computational infrastructure for metabolic network analysis

Funding: ANR MetaboHub, H2020 Phenomenal

- Long lasting project established in 2009

- 842 registered users, >540 persons trained, >20 000 visits since 2009

- > 1300 networks

- Publications:

Cottret et al (2018). Nucleic Acids Research

Chazalviel et al (2017). Bioinformatics

→>140 citations



- Involved in several national and EU grants
- 1 industrial partner (MedDay pharma)



Ludovic Cottret
IR INRAE



Florence Vinson
IE INRAE



Marion Liotier
CDD IE MetaboHub



Website: <http://www.metexplore.fr/>



- Database of metabolic networks
- Collaborative annotation of metabolic networks
- Import of omics data
- Visualization of metabolic networks
- Flux Balance Analysis
- Sub-network extraction (graph based computations)

W20-W21 Nucleic Acids Research, 2018, Vol. 38, Web Server issue
doi:10.1093/nar/gky302

MetExplore: a web server to link metabolomic experiments and genome-scale metabolic networks

Ludovic Cottret^{1,*},
Hubert Charles^{2,*}

Published online 3 May 2018

Nucleic Acids Research, 2018, Vol. 46, Web Server issue W495–W502
doi:10.1093/nar/gky302

MetExplore: collaborative edition and exploration of metabolic networks

Ludovic Cottret^{1,2}, Clément Frainay², Maxime Chazalviel^{2,3}, Floréal Cabanettes¹, Yoann Gloaguen^{4,5,6}, Étienne Camenier², Benjamin Merlet¹, Stéphanie Heuz^{7,8,9}, Jean-Charles Portais^{7,8,9}, Nathalie Poupin², Florence Vinson¹ and Fabien Jourdan^{7,2}

Published online 30 April 2018
doi:10.1093/nar/gky302

Systems biology

MetExploreViz: web component for interactive metabolic network visualization

Maxime Chazalviel^{1,2}, Clément Frainay¹, Nathalie Poupin¹, Florence Vinson¹, Benjamin Merlet¹, Yoann Gloaguen², Ludovic Cottret⁴ and Fabien Jourdan^{1,*}



Select a BioSource

BioSource:

metabolic network built for a strain, a cell line or a specific condition

Recon2.2 Swainston 2016 - Reconstruction of human metabolic network

MetExplore v2.32.12

About Omics Toolbox Flux Import Export Login

User Profile Network Data Network Curation Network Viz

BioSources Compartments (10/10) Pathways (99/99) Reactions (7785/7785) Metabolites (6047/6047) Enzymatic Complexes (1815/1815) Gene Products (1675/1675) Genes (1675/1675)

You can change the grouping option here: Group Table Group by:

	ID	Name	Source Database	Database Type	Nb Reactions	Nb Metabolites	Nb Genes
1	Harpegnathos saltator						
2	Helianthus annuus						
3	Helicobacter pylori						
4	Heliconius melpomene						
5	Homo sapiens						
159	1...	Recon 2.02 Human metabolism global reconstruction (Recon 2) - model - ...	Publication	Others (SBML,...)	7440	5063	2191
160	1...	Hsap	BioCyc	BioCyc	2527	2701	3583
161	1...	Recon 1 Homo_sapiens	Publication	Others (SBML,...)	3742	3188	1905
162	1...	Homo sapiens (human) KEGG Genes Database	KEGG Map	KEGG	1869	1560	1417
163	1...	Recon 2.03, enriched with additional database refs, without compartment...	MetExplore	Others (SBML,...)	4210	2592	1893
164	1...	HepatoNet1 Gille2010	Publication	Others (SBML,...)	2539	1420	0
165	1...	Swainston2016 - Reconstruction of human metabolic network (Recon 2.2)	Publication	Others (SBML,...)	7785	6047	1675
166	1...	Recon 2.04 - Human metabolic global reconstruction	VMH	Others (SBML,...)	7440	5063	2140
167	1...	iHsa_Ratcon1	Publication	Others (SBML,...)	8264	5620	2315
168	1...	Homo sapiens (human) KEGG Genes Database	KEGG Map	KEGG	1931	1572	1455
169	1...	Recon 2.03 (initial - VMH)	VMH	Others (SBML,...)	7440	5063	2191
170	1...	Recon 3D	VMH	SBML	13543	8399	3697
171	1...	Recon3D Flat	MetExplore	SBML	5389	4095	2990
172	1...	Human Metabolic Reaction HMR2.00 (xlsx)	HMA	Others (SBML,...)	8181	6006	3765
173	1...	Human1 (HumanGEM)	Publication	SBML	13097	10073	3628
174	1...	Homo sapiens (human) KEGG Genes Database	KEGG Map	KEGG	2067	1618	1456
175	1...	Human_1.7 (HumanGEM)	SBML	SBML	13082	8378	3625
176	1...	ihsan1.10	humanGEM github	SBML	13078	8370	3625
177		Hordeum vulgare					
178		Hydra vulgaris					
179		Ixodes scapularis					
180		Klebsiella pneumoniae					
181		Lactobacillus casei					

Selected BioSource
Public: Homo sapiens (Strain: Global) (Source: Publication, Version: Recon2.2)
Private: -- Select private BioSource --
Homo sapiens (Strain: Global) (Source: Publication, Version: Recon2.2)
copy this BioSource
Compart Path Rxn Met E.Cpx G.Prod Genes
BioSource Data
MetExplore Id: 4311
Name: Swainston2016 - Reconstruction of human metabolic network (Recon 2.2)
Organism: Homo sapiens
Tissue: global
Cell Type:
Strain: Global
Source Database: Publication
URL:
Id in Database: MODEL1603150001
Version: Recon2.2
Database type: Others (SBML,...)
Publication
Swainston N et al., 2016
Cart
Jobs
Filters

Matching identifiers

Convert geneSymbol to HGNC, Ensembl, ...

Many online tools to convert:

<https://www.genenames.org/tools/multi-symbol-checker/>

MetExplore v2.32.12

About Omics Toolbox Flux Import Export Login

User Profile Network Data Network Curation Network Viz

BioSources Compartments (10/10) Pathways (99/99) Reactions (7785/7785) Metabolites (6047/6047) Enzymatic Complexes (1815/1815) Gene Products (1675/1675) Genes (1675/1675)

Add Edit Delete Curation Votes Load Aliases

Name	Identifier
exact sub-string search	exact sub-string search
1 HGNC:10006	HGNC:10006
2 HGNC:1027	HGNC:1027
3 HGNC:10293	HGNC:10293
4 HGNC:10297	HGNC:10297
5 HGNC:10451	HGNC:10451
6 HGNC:10452	HGNC:10452
7 HGNC:1047	HGNC:1047
	HGNC:1048
	HGNC:10536
	HGNC:10540
	HGNC:10545
	HGNC:10547
	HGNC:10571
	HGNC:10606
	HGNC:1062
	HGNC:1063
	HGNC:10680
	HGNC:10681
	HGNC:10682
	HGNC:10683
	HGNC:10691
	HGNC:10761
22 HGNC:10701	
23 HGNC:108	HGNC:108
24 HGNC:10817	HGNC:10817
25 HGNC:10818	HGNC:10818
26 HGNC:10850	HGNC:10850
27 HGNC:10852	HGNC:10852
28 HGNC:10856	HGNC:10856
29 HGNC:10860	HGNC:10860
30 HGNC:10862	HGNC:10862
31 HGNC:10863	HGNC:10863

Mapping genes

**Missing data: few
explanations**

- Identifiers
- Incomplete network
- **Not metabolism
genes**

Screenshot of the MetExplore v2.32.12 interface, specifically the "Mapping" tab.

The interface includes a top navigation bar with links for About, Omics, Toolbox, Flux, Import, Export, and Login. Below the navigation is a sub-navigation bar with tabs for User Profile, Network Data, Network Curation, Network Viz, and Mapping (which is active).

On the left, there is a "Demo" button and a file upload section with fields for "Upload file (.csv .txt)" and "separator: tab". There are also checkboxes for "Consider first row as header of columns" and "Perform one separate mapping for each column".

The main area features a "Copy/Paste in grid" table. The table has columns for "Identifier" and "Feature". The data is as follows:

	Identifier	Feature
1	false	HGNC:16488
2	false	HGNC:30932
3	false	HGNC:1349
4	false	HGNC:5411
5	false	HGNC:5397
6	false	HGNC:5414
7	false	HGNC:30908
8	false	HGNC:5409

Below the table, there is a "Propagate:" dropdown menu set to "without conditions values".

At the bottom, there are buttons for "Map" and "Save Mapping in File".

At the very bottom, status information is displayed: "Mapping Nb Data: 2050 Nb Data In Network: 223 Nb Mapped: 221".

Mapping genes

Mapping data appear on grids

Ordering data on genes grid by condition (subModules)

- Missing group 1 (black color)

BioSources		Compartments (10/10)		Pathways (99/99)		Reactions (7785/7785)		Metabolites (6047/6047)		Enzymatic Complexes (1815/1815)		Gene Products (1675/1675)		Genes (1675/1675)	
		Add	Edit	Delete	Curation Votes	Load Aliases									?
Name				Identifier				Mapping				X			
exact sub-string search				exact sub-string search				identified ↓				condition0			
1	HGNC:1027			HGNC:1027				true				3			
2	HGNC:10571			HGNC:10571				true				5			
3	HGNC:1063			HGNC:1063				true				8			
4	HGNC:10860			HGNC:10860				true				2			
5	HGNC:10862			HGNC:10862				true				4			
6	HGNC:10872			HGNC:10872				true				2			
7	HGNC:10909			HGNC:10909				true				3			
8	HGNC:10911			HGNC:10911				true				4			
9	HGNC:10922			HGNC:10922				true				8			
10	HGNC:10923			HGNC:10923				true				5			
11	HGNC:10924			HGNC:10924				true				2			
12	HGNC:10937			HGNC:10937				true				9			
13	HGNC:10938			HGNC:10938				true				8			
14	HGNC:10941			HGNC:10941				true				2			
15	HGNC:10942			HGNC:10942				true				8			
16	HGNC:10952			HGNC:10952				true				2			
17	HGNC:10962			HGNC:10962				true				2			
18	HGNC:10969			HGNC:10969				true				8			
19	HGNC:11005			HGNC:11005				true				9			
20	HGNC:11007			HGNC:11007				true				7			
21	HGNC:11023			HGNC:11023				true				4			
22	HGNC:11033			HGNC:11033				true				4			
23	HGNC:11041			HGNC:11041				true				8			
24	HGNC:11055			HGNC:11055				true				8			
25	HGNC:11056			HGNC:11056				true				5			
26	HGNC:11057			HGNC:11057				true				9			
27	HGNC:11063			HGNC:11063				true				9			
28	HGNC:11065			HGNC:11065				true				2			
29	HGNC:11066			HGNC:11066				true				8			
30	HGNC:11177			HGNC:11177				true				2			

Flux Variability Analysis

Non-exhaustive list of metabolites signatures
(oncometabolites)

- From this list of metabolites, we extract reactions that produce or consume these metabolites

Mishra P, Ambs S. Metabolic Signatures of Human Breast Cancer. *Mol Cell Oncol.* 2015

Hypothesis: cancer cell seeks to facilitate its proliferation by increasing its production of biomass

- Optimize biomass reaction for Flux Variability Analysis
- KO reactions

The screenshot shows a user interface for 'Flux Variability Analysis'. At the top, there are tabs: User Profile, Network Data, Network Curation, Network Viz, and Flux Variability Analysis (which is highlighted). Below the tabs, there are several input fields and dropdown menus:

- Description: Computes the minimum and the maximum flux for each reaction that allows the objective function to be optimal.
- Analysis title: Flux Variability Analysis.
- Standard Parameters:
 - objectiveReactions: R_biomass_reaction (dropdown menu, report reaction table selection button).
 - objectiveSense: MIN (radio button) selected, MAX (radio button) available.
- reactionSet: (dropdown menu, report reaction table selection button).
- secondObjectiveReactions: (dropdown menu, report reaction table selection button).
- secondObjectiveSense: MIN (radio button) selected, MAX (radio button) available.
- libertyPercentage: 0 (input field).
- ko_genes: (dropdown menu, report gene table selection button).
- ko_reactions: R_r0744,R_FAOC5OHc,R_c (dropdown menu, report reaction table selection button).

At the bottom right is a 'Launch' button.

Filters

Propagation of the filters

Interlinked grids: filtering one affects the content of the other ones

BioSources	Compartments (10/10)	Pathways (99/99)	Reactions (7785/7785)	Metabolites (6047/6047)	Enzymatic Complexes (1815/1815)	Gene Products (1675/1675)	Genes (1675/1675)
+ Add Edit X Delete Curation Votes Load Aliases							
	Name	Identifier		Mapping			X
	exact sub-string search	exact sub-string search		identified ↓			condition0
1	HGNC-1027	HGNC-1027		true			3
2	HGNC-10571	HGNC-10571		true			5
3	HGNC-1063	HGNC-1063		true			8
4	HGNC-10860	HGNC-10860		true			2
5	HGNC-10862	HGNC-10862		true			4
6	HGNC-10872	HGNC-10872		true			2
7	HGNC-10909	HGNC-10909		true			3
8	HGNC-10911	HGNC-10911		true			4
9	HGNC-10922	HGNC-10922		true			8
10	HGNC-10923	HGNC-10923		true			5
11	HGNC-10924	HGNC-10924		true			2
12	HGNC-10937	HGNC-10937		true			9
13	HGNC-10938	HGNC-10938		true			8
14	HGNC-10941	HGNC-10941		true			2
15	HGNC-10942	HGNC-10942		true			8
16	HGNC-10952	HGNC-10952		true			2
17	HGNC-10962	HGNC-10962		true			2
18	HGNC-10969	HGNC-10969		true			8
19	HGNC-11005	HGNC-11005		true			9
20	HGNC-11007	HGNC-11007		true			7
21	HGNC-11023	HGNC-11023		true			4
22	HGNC-11033	HGNC-11033		true			4
23	HGNC-11041	HGNC-11041		true			8
24	HGNC-11055	HGNC-11055		true			8
25	HGNC-11056	HGNC-11056		true			5
26	HGNC-11057	HGNC-11057		true			9
27	HGNC-11063	HGNC-11063		true			9
28	HGNC-11065	HGNC-11065		true			2
29	HGNC-11066	HGNC-11066		true			8

Filters: genes mapped

BioSources Compartments (9/10) Pathways (78/99) Reactions (1269/7785) Metabolites (1629/6047) Enzymatic Complexes (263/1815) Gene Products (221/1675) Genes (221/1675)			
<input type="button" value="Add"/> <input type="button" value="Edit"/> <input type="button" value="Delete"/> <input type="button" value="Curation Votes"/> <input type="button" value="Load Aliases"/>			
Name		Identifier	Mapping
	exact sub-string search	exact sub-string search	identified ↓ condition0
1	HGNC:1027	HGNC:1027	true 3
2	HGNC:10571	HGNC:10571	true 5
3	HGNC:1063	HGNC:1063	true 8
4	HGNC:10860	HGNC:10860	true 2
5	HGNC:10862	HGNC:10862	true 4
6	HGNC:10872	HGNC:10872	true 2
7	HGNC:10909	HGNC:10909	true 3
8	HGNC:10911	HGNC:10911	true 4
9	HGNC:10922	HGNC:10922	true 8
10	HGNC:10923	HGNC:10923	true 5
11	HGNC:10924	HGNC:10924	true 2
12	HGNC:10937	HGNC:10937	true 9
13	HGNC:10938	HGNC:10938	true 8
14	HGNC:10941	HGNC:10941	true 2
15	HGNC:10942	HGNC:10942	true 8
16	HGNC:10952	HGNC:10952	true 2
17	HGNC:10962	HGNC:10962	true 2
18	HGNC:10969	HGNC:10969	true 8
19	HGNC:11005	HGNC:11005	true 9
20	HGNC:11007	HGNC:11007	true 7
21	HGNC:11023	HGNC:11023	true 4
22	HGNC:11033	HGNC:11033	true 4
23	HGNC:11041	HGNC:11041	true 8
24	HGNC:11055	HGNC:11055	true 8
25	HGNC:11056	HGNC:11056	true 5
26	HGNC:11057	HGNC:11057	true 9
27	HGNC:11063	HGNC:11063	true 9
28	HGNC:11065	HGNC:11065	true 2
29	HGNC:11066	HGNC:11066	true 8
30	HGNC:11177	HGNC:11177	true 2

Pathways enrichment

On mapping: automatique
pathways enrichment with p-value
and corrected p-value

With correction, 4 pathways have
been identified:

- Fatty acid oxidation
- Fatty acid synthesis
- Eicosanoid metabolism
- Glutamate metabolism

Filters data on this 4 pathways

BioSources		Compartments (9/10)		Pathways (78/99)		Reactions (1269/7785)		Metabolites (1629/6047)		Enzymatic Complexes (263/1815)		Gene Products (221/1675)		Genes (221/1675)		
		Add	Edit	Delete	Curation Statistics	Votes										
	Name	Identifier		Nb Reactions	% Reactions with Enz.	Coverage	Nb of Mapped	p-value	Bonferroni corre	BH-corrected p-						
1	Fatty acid oxidation	Fatty acid oxidation		809	78 %	24.14	21	3.13e-3	(2.44e-1)	(1.25e-1)						
2	Fatty acid synthesis	Fatty acid synthesis		118	60 %	41.18	7	3.82e-3	(2.98e-1)	(1.25e-1)						
3	Eicosanoid metabolism	Eicosanoid metabolism		252	62 %	24.66	18	4.83e-3	(3.76e-1)	(1.25e-1)						
4	Glutamate metabolism	Glutamate metabolism		15	93 %	35	7	1.07e-2	(8.37e-1)	(2.09e-1)						
5	Transport, extracellular	Transport, extracellular		1472	79 %	14.05	34	3.68e-1	(1.00e+0)	(7.96e-1)						
6	Nucleotide interconversion	Nucleotide interconversion		177	93 %	12.93	15	5.79e-1	(1.00e+0)	(8.86e-1)						
7	Inositol phosphate metabolism	Inositol phosphate metabolism		64	65 %	18.33	11	1.57e-1	(1.00e+0)	(6.13e-1)						
8	Glycolysis/gluconeogenesis	Glycolysis/gluconeogenesis		40	100 %	12.99	10	5.75e-1	(1.00e+0)	(8.86e-1)						
9	Valine, leucine, and isoleucine metabolism	Valine, leucine, and isoleucine m...		41	85 %	27.03	10	1.77e-2	(1.00e+0)	(2.76e-1)						
10	Sphingolipid metabolism	Sphingolipid metabolism		83	91 %	18	9	2.05e-1	(1.00e+0)	(6.38e-1)						
11	Pyruvate metabolism	Pyruvate metabolism		30	83 %	22.5	9	7.06e-2	(1.00e+0)	(4.99e-1)						
12	O-glycan synthesis	O-glycan synthesis		15	73 %	30.43	7	2.40e-2	(1.00e+0)	(2.67e-1)						
13	Miscellaneous	Miscellaneous		86	69 %	9.09	7	9.02e-1	(1.00e+0)	(1.05e+0)						
14	Cholesterol metabolism	Cholesterol metabolism		57	82 %	24.14	7	7.68e-2	(1.00e+0)	(4.99e-1)						
15	Arginine and Proline Metabolism	Arginine and Proline Metabolism		39	74 %	22.58	7	1.03e-1	(1.00e+0)	(5.07e-1)						
16	Pyrimidine catabolism	Pyrimidine catabolism		35	82 %	25	7	6.53e-2	(1.00e+0)	(4.99e-1)						
17	Glyoxylate and dicarboxylate metabolism	Glyoxylate and dicarboxylate met...		15	73 %	23.33	7	8.94e-2	(1.00e+0)	(5.37e-1)						
18	Triacylglycerol synthesis	Triacylglycerol synthesis		13	100 %	21.43	6	1.54e-1	(1.00e+0)	(6.13e-1)						
19	Glycerophospholipid metabolism	Glycerophospholipid metabolism		66	77 %	8.45	6	9.24e-1	(1.00e+0)	(1.05e+0)						
20	Tryptophan metabolism	Tryptophan metabolism		68	70 %	11.54	6	7.03e-1	(1.00e+0)	(9.79e-1)						
21	Bile acid synthesis	Bile acid synthesis		125	75 %	13.95	6	5.10e-1	(1.00e+0)	(8.75e-1)						
22	Fructose and mannose metabolism	Fructose and mannose metaboli...		25	80 %	20	6	1.95e-1	(1.00e+0)	(6.60e-1)						
23	Tyrosine metabolism	Tyrosine metabolism		117	70 %	8.2	5	9.22e-1	(1.00e+0)	(1.05e+0)						
24	Vitamin C metabolism	Vitamin C metabolism		16	25 %	35.71	5	2.79e-2	(1.00e+0)	(2.73e-1)						
25	Propanoate metabolism	Propanoate metabolism		13	61 %	22.73	5	1.54e-1	(1.00e+0)	(6.13e-1)						
26	Histidine metabolism	Histidine metabolism		16	68 %	21.74	5	1.77e-1	(1.00e+0)	(6.27e-1)						
27	Urea cycle	Urea cycle		68	63 %	20	5	2.26e-1	(1.00e+0)	(6.63e-1)						
28	N-glycan synthesis	N-glycan synthesis		81	40 %	16.67	4	3.92e-1	(1.00e+0)	(7.85e-1)						
29	Purine catabolism	Purine catabolism		36	77 %	14.29	4	5.16e-1	(1.00e+0)	(8.75e-1)						
30	Starch and sucrose metabolism	Starch and sucrose metabolism		32	84 %	16	4	4.24e-1	(1.00e+0)	(8.03e-1)						

Data exploration

By checking scientific literature, we can find some articles that confirm the results found:

- Monaco ME. Fatty acid metabolism in breast cancer subtypes. *Oncotarget*. 2017

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5438746/#:~:text=Evidence%20indicates%20that%20proteins%20involved,invasion%20of%20breast%20cancer%20cells.>

- Fazzari, J., Lin, H., Murphy, C. et al. Inhibitors of glutamate release from breast cancer cells; new targets for cancer-induced bone-pain. *Sci Rep.* 2015

<https://www.nature.com/articles/srep08380#:~:text=Breast%20cancer%20cells%20secrete%20high,advanced%2Dstage%20breast%20cancer%20patients.>

- Wang D, Dubois RN. Eicosanoids and cancer. *Nat Rev Cancer*. 2010

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2898136/>

- Yi H, Talmon G, Wang J. Glutamate in cancers: from metabolism to signaling. *J Biomed Res*. 2019

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7386414/>

Filters: FBA result

Only reactions with a non-zero flux value are kept

276 reactions of interest remain

Visualise this sub-network

Flux Variability Analysis															
BioSources		Compartments (10/10)		Pathways (99/99)		Reactions (7785/7785)		Metabolites (6047/6047)		Enzymatic Complexes (1815/1815)		Gene Products (1675/1675)		Genes (1675/1675)	
		Add	Edit	Delete	Save	Multiple affectation	Curation Statistics	Curation Votes	Equations	Load Aliases					
											Reversible	Flux Lower	Flux Upper	Flux Variability Analysis 1	
		Name	Identifier	E.C.	GPR						min	max			
		exact sub-string search	exact sub-string search	exact sub.											
1	1	(((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-di...	R_EX_dtddp_LPAREN_e_R...	NA							-Infinity	Infinity	-99999.0	99999.0	
2	1	(+)-alpha-Pinene exchange	R_EX_appnn_LPAREN_e_...	NA							-Infinity	Infinity	-99999.0	0.0	
3	1	(24R,25R)-3alpha,7alpha,12alpha,24-tetrahy...	R_r0744	4.2.1.107	(HGNC:5213)						0	Infinity	0.0	99999.0	
4	1	(3-hydroxyisovalerylcoa-->3-hydroxyisovaler...	R_FAOC5OHc	NA	(HGNC:18540) or (HGNC:2328) or (HGNC:232...						-Infinity	Infinity	0.0	99999.0	
5	1	(3R)-3-Hydroxybutanoyl-[acyl-carrier protein...	R_r0691	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
6	1	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0693	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
7	1	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0681	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
8	1	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0770	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
9	1	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0762	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
10	1	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0695	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
11	1	(3R)-3-Hydroxydecanoyl-[acyl-carrier-protei...	R_r0692	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
12	1	(3R)-3-Hydroxypalmitoyl-[acyl-carrier-protei...	R_r0769	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
13	1	(3R)-3-Hydroxyhexanoyl-[acyl-carrier-protei...	R_r0761	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
14	1	(3R)-3-Hydroxyoctanoyl-[acyl-carrier-protein...	R_r0694	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-39999.6	39999.6	
15	1	(3R)-3-Hydroxypalmitoyl-[acyl-carrier-protei...	R_r0697	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-99999.0	99999.0	
16	1	(3R)-3-Hydroxypalmitoyl-[acyl-carrier-protei...	R_r0696	2.3.1.85	(HGNC:3594)						-Infinity	Infinity	-99999.0	99999.0	
17	1	(5-Glutamyl)-peptide:amino-acid 5-glutamyl...	R_r0641	2.3.2.2	(HGNC:3426) or (HGNC:26891) or (HGNC:18...						-Infinity	Infinity	-99999.0	99999.0	
18	1	(5-Glutamyl)-peptide:amino-acid 5-glutamyl...	R_r0648	2.3.2.2	(HGNC:3426) or (HGNC:26891) or (HGNC:18...						-Infinity	Infinity	-99999.0	99999.0	
19	1	(5-Glutamyl)-peptide:amino-acid 5-glutamyl...	R_r0649	2.3.2.2	(HGNC:3426) or (HGNC:26891) or (HGNC:18...						-Infinity	Infinity	-99999.0	99999.0	
20	1	(5-L-Glutamyl)-L-amino-acid 5-glutamyltran...	R_r0568	2.3.2.4	(HGNC:21705)						0	Infinity	0.0	99999.0	
21	1	(5Z,9E,14Z)-(8x,11R,12S)-11,12-epoxy-8-h...	R_EX_C04849_LPAREN_e...	NA							-Infinity	Infinity	-99999.0	99999.0	
22	1	(E)-carvedol exchange	R_EX_carvedol_LPAREN_e...	NA							-Infinity	Infinity	0.0	99999.0	
23	1	(Gal)2 (GalNAc)1 (Glc)1 (GlcNAc)1 (LFuc)2 (Cer)...	R_EX_fucacgalfugalacglcg...	NA							-Infinity	Infinity	0.0	12499.875	
24	1	(Gal)3 (Glc)1 (GlcNAc)1 (LFuc)1 (Cer)1 exchange	R_EX_galfuc12gal14aclgcg...	NA							-Infinity	Infinity	0.0	14285.5714	

Visualisation

Compartments:

cytoplasm :



endoplasmic reticulum :



mitochondrion :



peroxisome :



Pathways:

Eicosanoid metabolism :



Fatty acid oxidation :



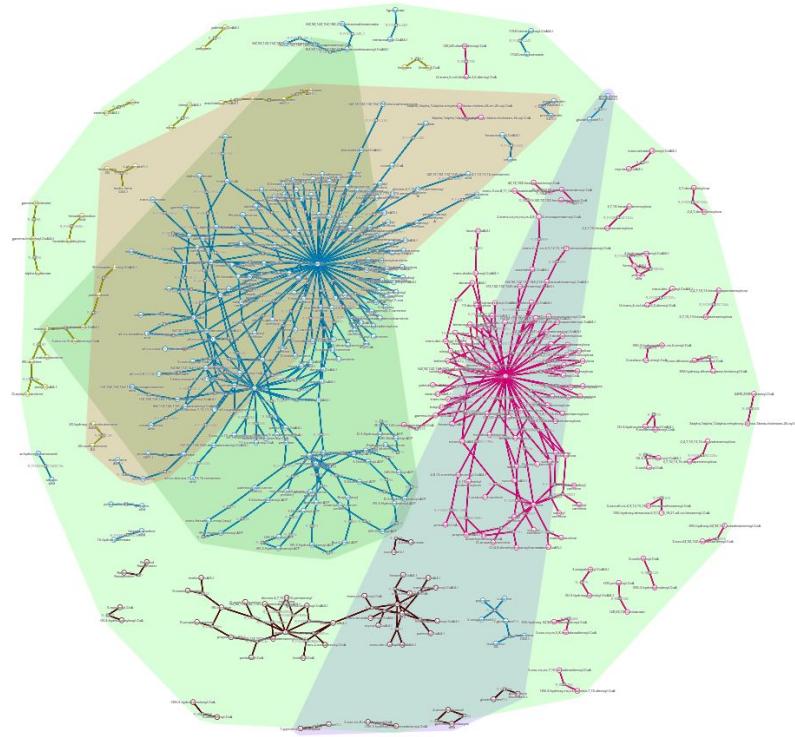
Fatty acid synthesis :



Glutamate metabolism :



- Link
- ↔ Reversible link
- Reaction
- Metabolites



Data exploration

Link between carnitine and cancer development

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9300951/>

Zhang J, Wu G, Zhu H, Yang F, Yang S, Vuong AM, Li J, Zhu D, Sun Y, Tao W. Circulating Carnitine Levels and Breast Cancer: A Matched Retrospective Case-Control Study. *Front Oncol.* 2022

But, we have still the four pathways ...

Hypothesis

- We have succeeded in highlighting the link between genes and four pathways potentially involved in the development of cancer
- By optimising biomass production, we have shown that the flux that pass through these four pathways
- We can therefore identify a sub-network of interest for the study of breast cancer development, and consequently, a list of metabolites to monitor

To go further with MetExplore

Project creation and collaboration

MetExplore allows users, after login, to create a project and add some collaborators.

On this project you have few possibilities like:

- Import your own network with SBML file, from KEGG DB or MetExplore XML file
- Cure your networks (add, edit or delete data)
- Manage your project (TODO list, comments, history, etc...)

The screenshot shows the MetExplore software interface. At the top, there's a navigation bar with links for User Profile, Project Details, Network Data, Network Curation, and Network Viz. Below the navigation bar, the main title is "Projet A-V Splanchnic Postprandial" with a note "(Created 2018-12-22)".

The central area is titled "TODO list" and contains a table with columns: Description, Project, User, Limit date, Status, and Priority. There are no items listed in this table.

On the right side, there's a detailed sidebar for "Selected BioSource". It includes sections for "Public" (with a dropdown menu), "Private" (with a dropdown menu), and "Project" (which is currently selected). Under "Project", it shows "Homo sapiens (Strain: Liver) [Source: Gille et al., + modifs NP, Version: HepatoNet1_10r1]".

Below this, there are tabs for "BioSources", "Comments", "History", "Description", and "Users". The "BioSources" tab is active and displays a table of biosources:

ID	Name	Organism	Strain	Source Database	Database Type	Publication
1...	HepatoNet1 with EXnrN (bP b4M)	Homo sapiens	Liver	Gille et al., 201...	SBML	
2...	HepatoNet1 IO (noID addr)	Homo sapiens	Liver	Gille et al., 201...	SBML	
3...	HepatoNet1_30 (noID addr)	Homo sapiens	Liver	Gille et al., 201...	SBML	
4...	HepatoNet1 with EXnrN & AdDr for project PPrc	Homo sapiens	Liver	Gille et al., + m...	SBML	
5...	HepatoNet1 IO (strMED, AdDr, cstP)	Homo sapiens	Liver	SBML	SBML	

At the bottom of the sidebar, there are sections for "MetExplore Data", "Organism", "Tissue", "Cell Type", "Strain", "Source Database", "URL", "Id in Database", "Version", and "Database type".

Take home messages

Take home messages

- Genome-scale metabolic network reconstruction allows to explore metabolism and to map omics data
- Metabolic networks offer a context to interpret omics data
- Graph models is able to infer complex behaviours of metabolic networks alone or in interaction
- MetExplore offers facilities to build, explore, visualise and model the metabolic networks
- MetExplore is part of a wider tool ecosystem

Useful links

- MetExplore website:
<https://metexplore.toulouse.inrae.fr/>
- MetExplore documentation:
<https://metexplore.toulouse.inrae.fr/metexplore-doc/>
- MetExplore tutorial:
<https://metexplore.pages.mia.inra.fr/metexplore-training/>



Swiss Institute of
Bioinformatics

Thanks to organizers



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CDD IE ANR



Marion Liotier
CDD IE MetaboHub

Thanks to MetExplore team



Contact: contact-metexplore@inrae.fr

Supplementary data and analysis

PAM50 genes list: signatures for breast cancer subtypes
convert to ensembl with <https://biit.cs.ut.ee/gprofiler/convert>

12 genes mapped in humanGEM

25 reactions on 9 pathways including pyrimidine metabolism -> Thioredoxin and its oxydation

Link between this metabolite and cancer progression and metastasis

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3835076/>

