

Omic data analysis in genome scale metabolic networks

Summer school Multi-omics - Aussois
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07 / 09 / 2023

Collaborators:
- Ludovic Cottret
- Clément Frainay
- Fabien Jourdan
- Nathalie Poupin
- Florence Vinson

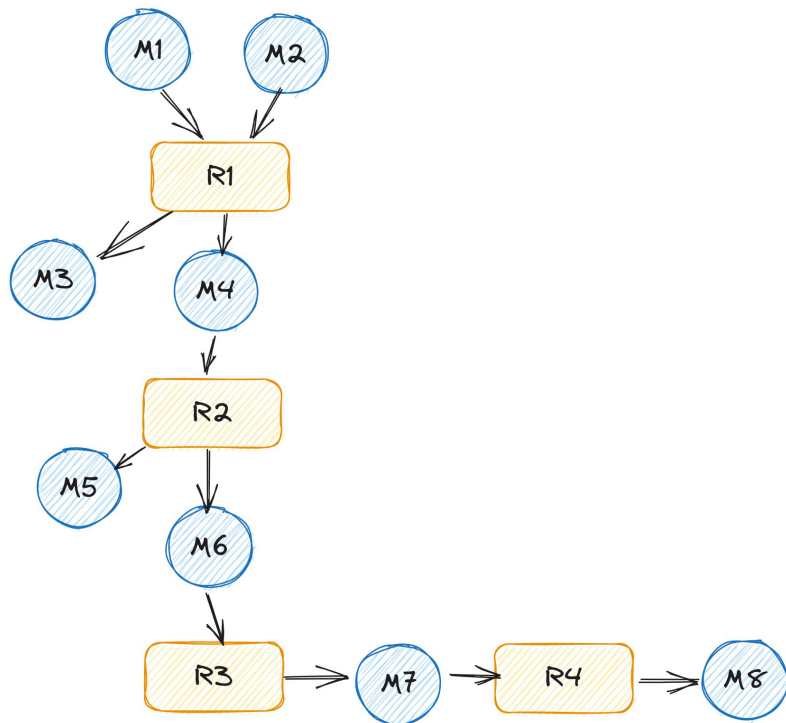


Metabolic networks

A definition of a metabolic network

A metabolic network is:

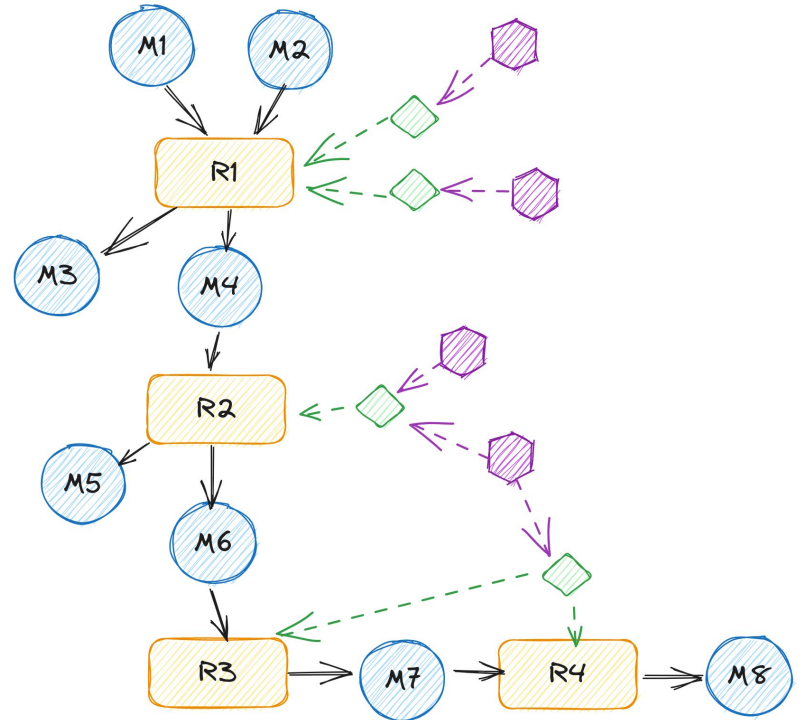
- a set of biochemical reactions linked together by the metabolites that they consume and produce



A definition of a metabolic network

A metabolic network is:

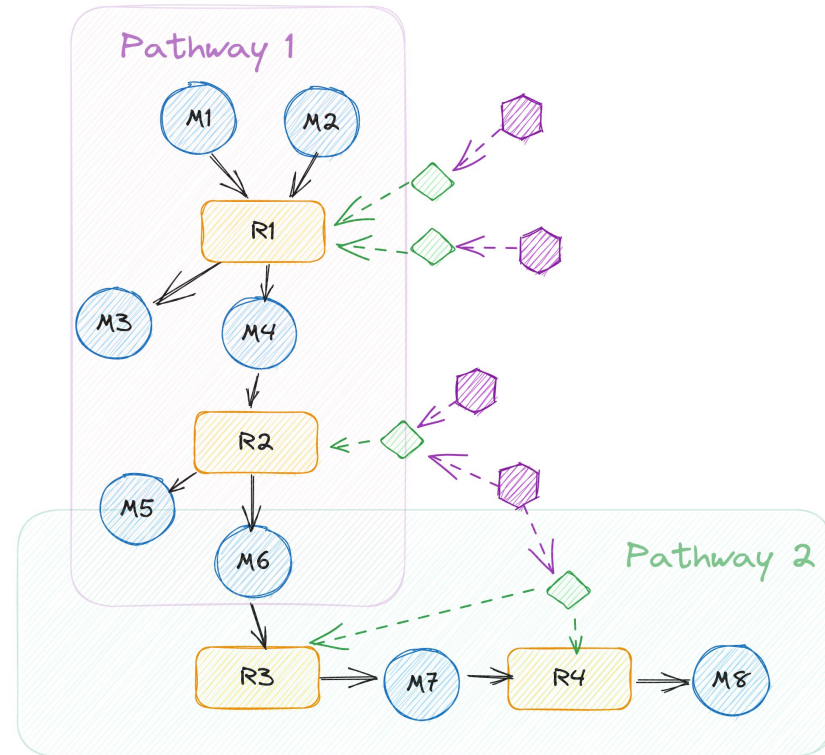
- a set of biochemical reactions linked together by the metabolites that they consume and produce
- the set of the genes that code for the enzymes that catalyse the reactions



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

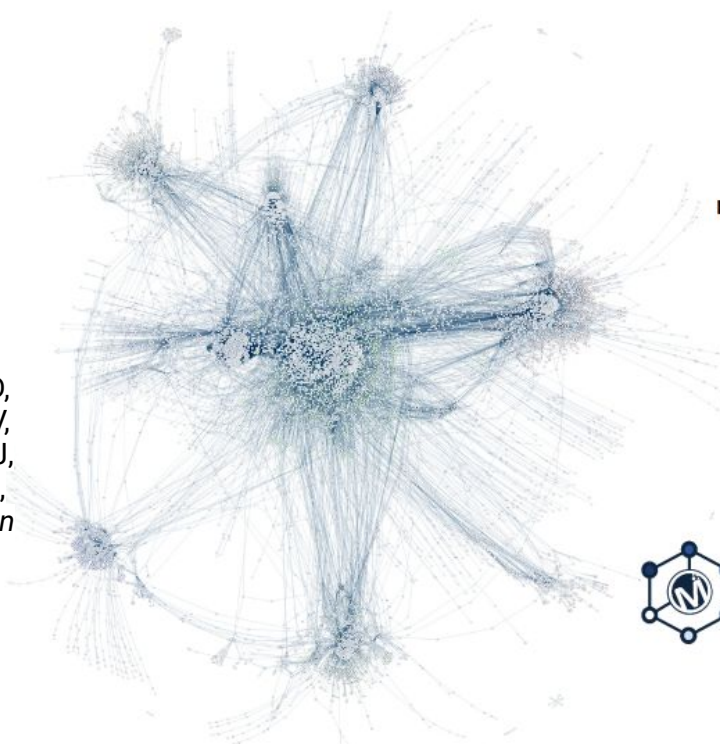
Human-GEM

13024 reactions

8363 metabolites

2920 genes

Robinson JL, Kocabaş 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

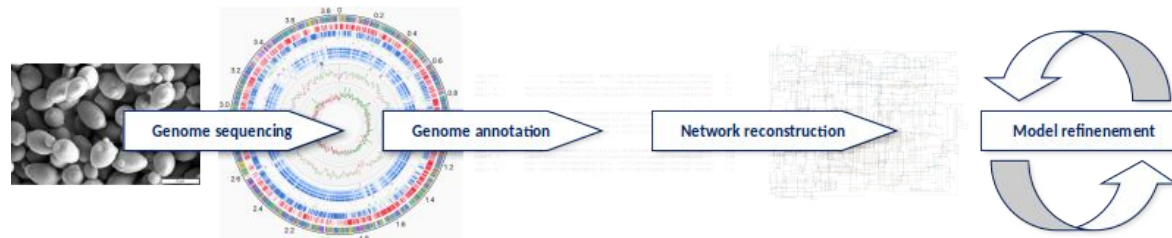
1675 genes

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

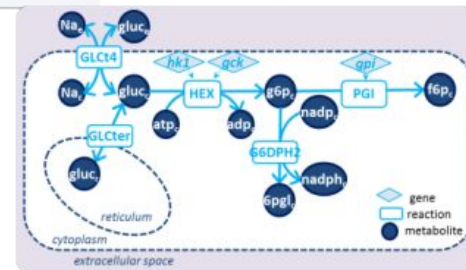
stoichiometric associations

list of metabolites
gluc _e
gluc _c
Na _e
Na _c
ATP _c
ADP _c
g6p _c
f6p _c
6pgl _c
nadp _c
nadph _c
gluc _r

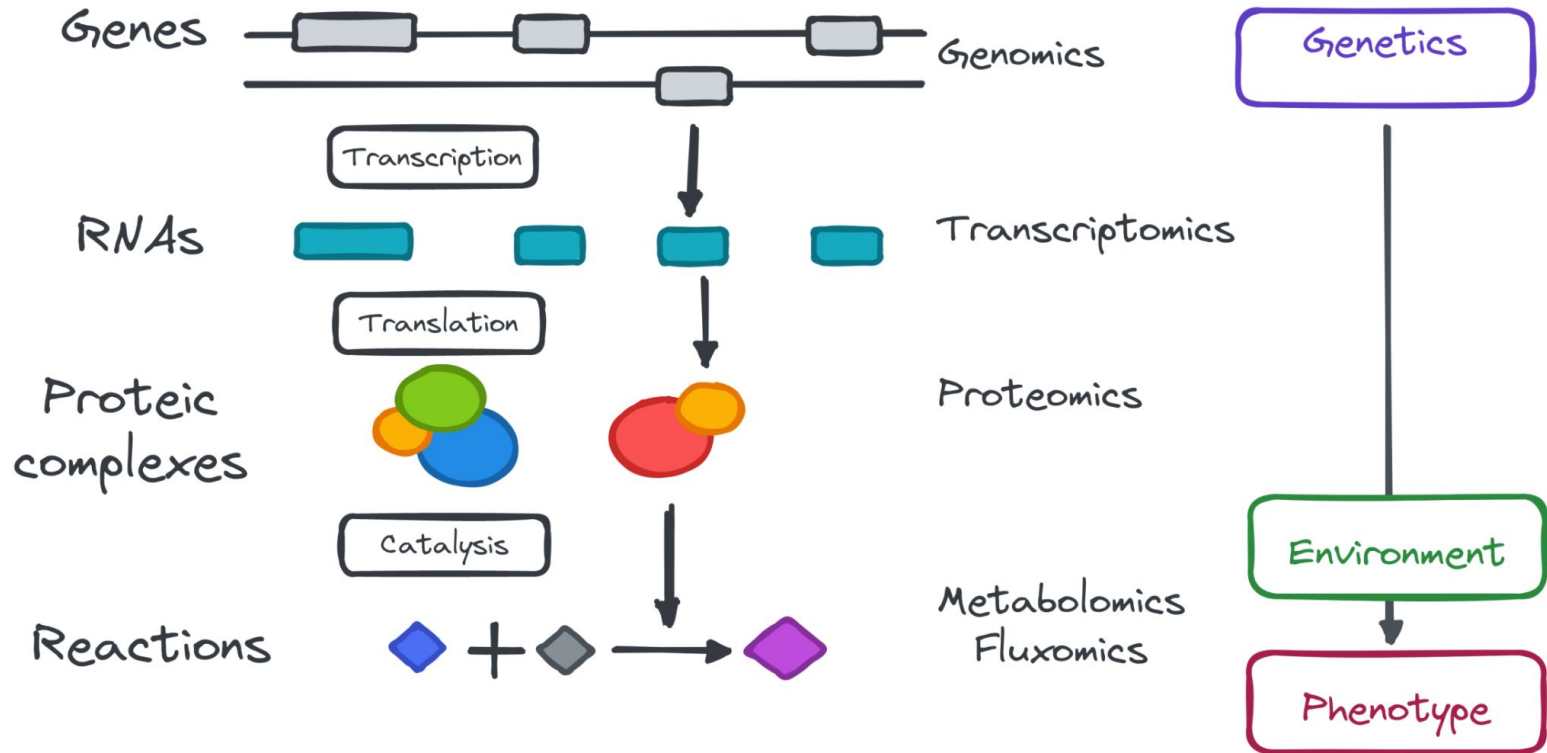
Thiele I, Palsson BØ. A protocol for generating a high-quality genome-scale metabolic reconstruction. Nat. Protoc. 2010; 5:93-121

	list of reactions	list of genes
GLCt4	$\text{Na}_e + \text{gluc}_e \rightleftharpoons \text{Na}_c + \text{gluc}_c$	(6524) or (6526)
HEX1	$\text{gluc}_c + \text{ATP}_c \rightleftharpoons \text{ADP}_c + \text{g6p}_c$	(3098)
PGI	$\text{g6p}_c \rightleftharpoons \text{f6p}_c$	(2821)
G6PDH2r	$\text{g6p}_c + \text{nadp}_c \rightleftharpoons \text{6pgl}_c + \text{nadph}_c$	(2539)
GLCter	$\text{gluc}_c \rightleftharpoons \text{gluc}_r$	

Biochemical reactions known to take place in a target organism & associated genes and metabolites

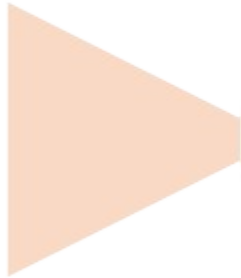


The metabolic network: a context of interpretation for omics data

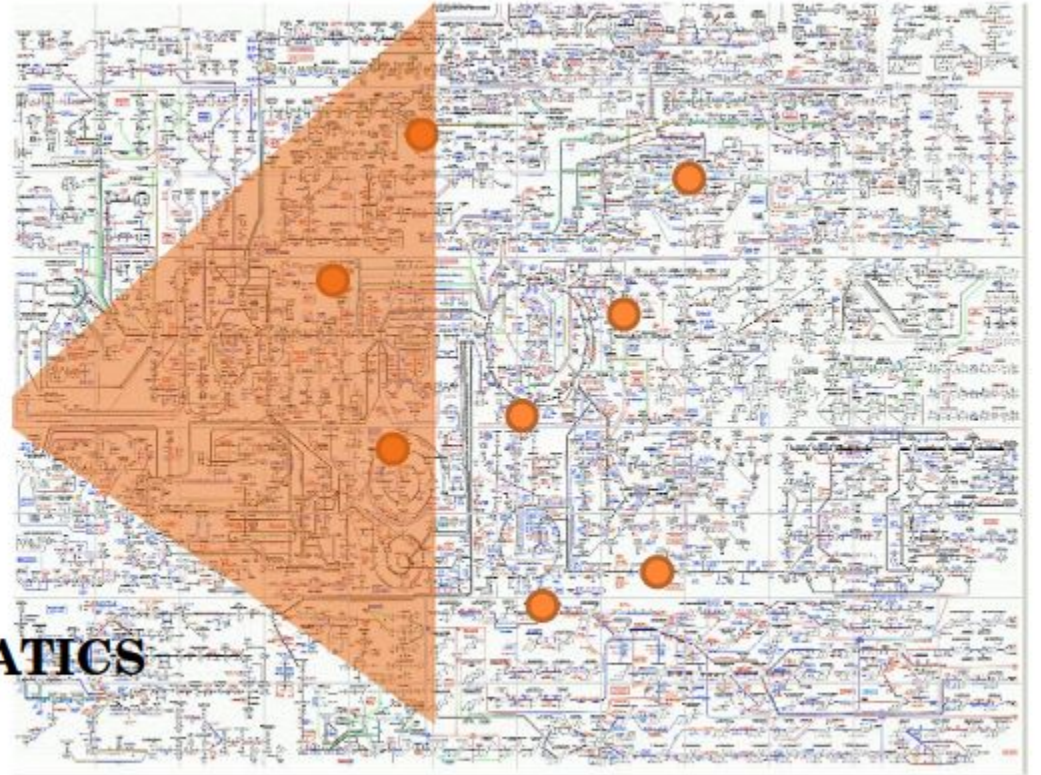


Mapping omics data

OMICS
DATA



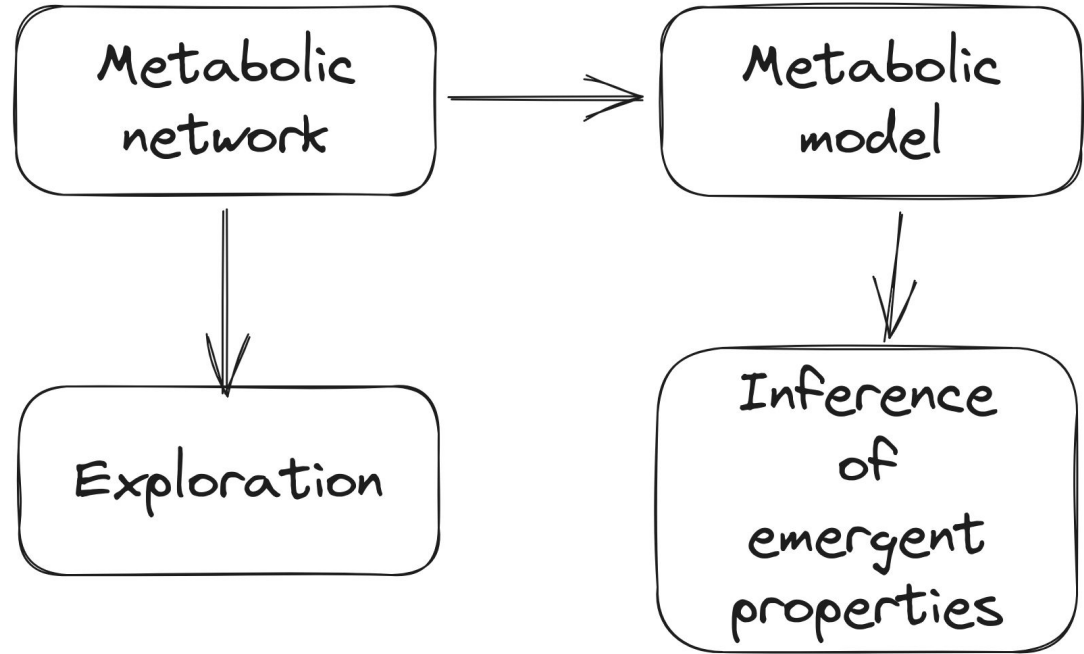
BIOINFORMATICS



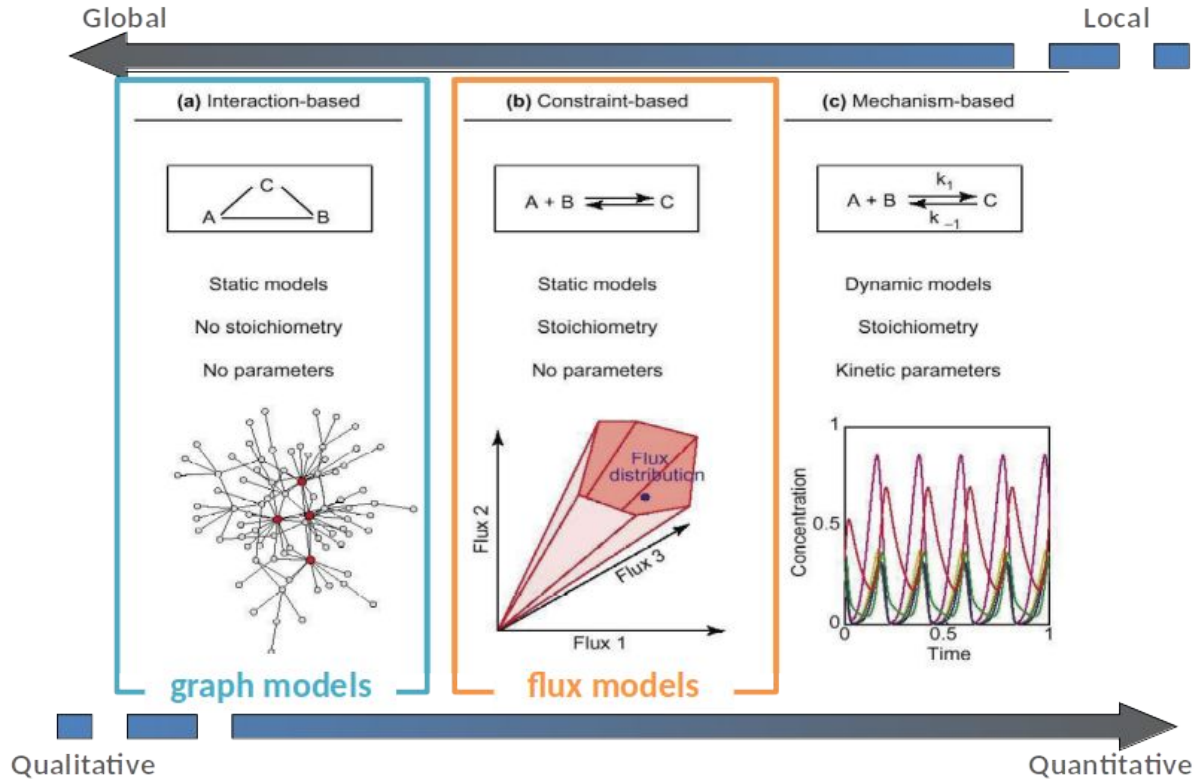
Metabolic networks to metabolic models

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

Metabolic network: textual description

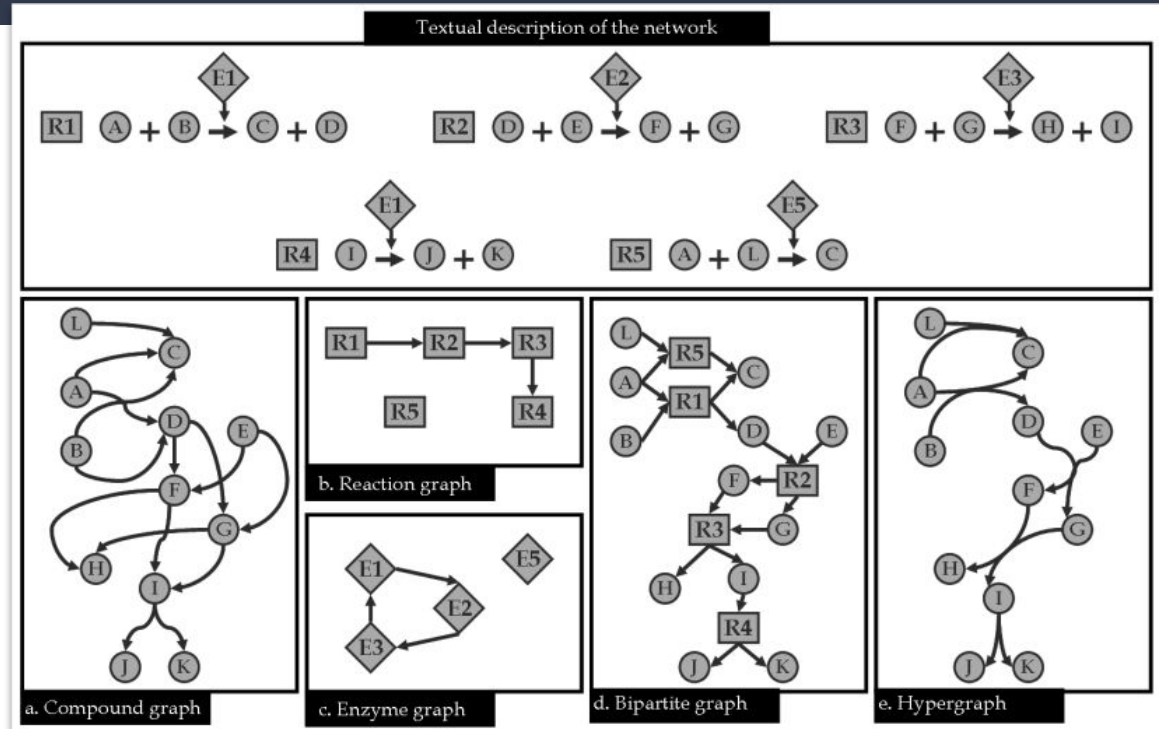


Metabolic models

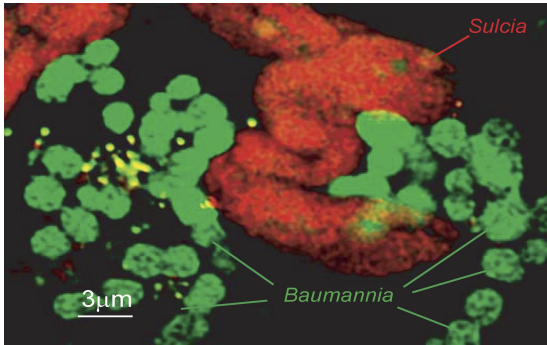


Graph models

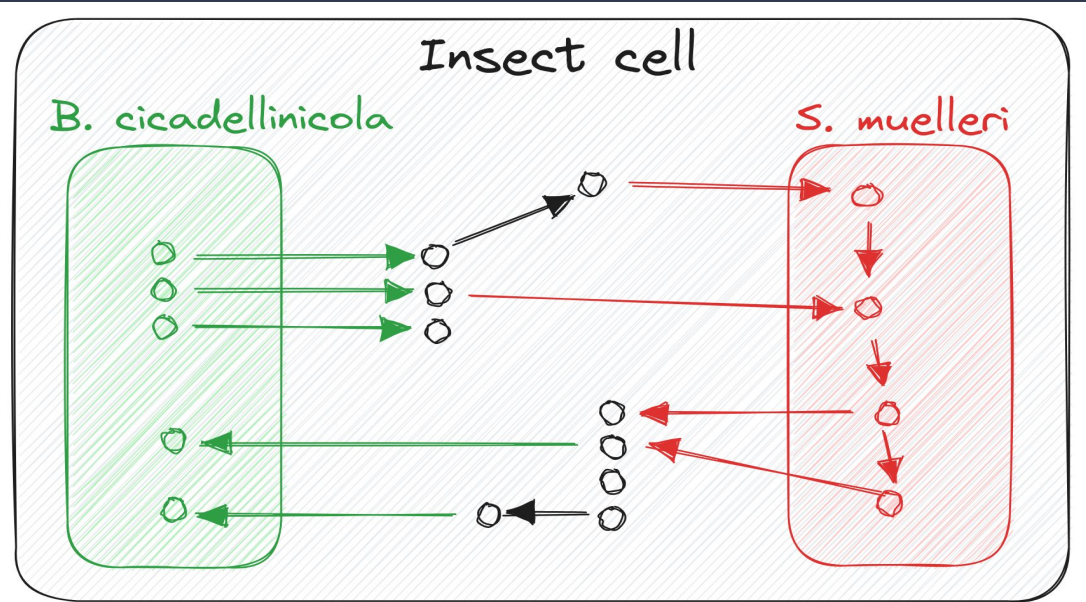
Metabolic graphs are built from the descriptions of the set of reactions that constitute a metabolic network



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

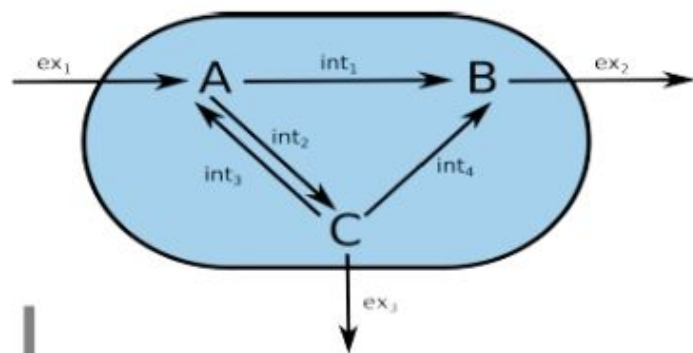
Stoichiometric matrix S: $M * n$

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 _c	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

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

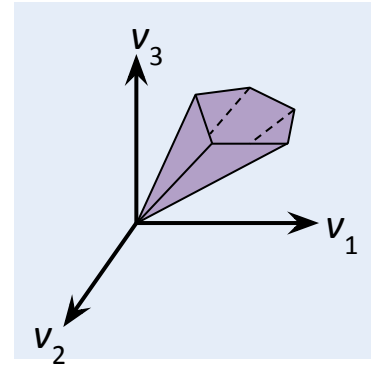
$$\begin{bmatrix} \frac{dA}{dt} \\ \frac{dB}{dt} \\ \frac{dC}{dt} \end{bmatrix} = \begin{bmatrix} -1 & -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & -1 \end{bmatrix} \times \begin{bmatrix} int_1 \\ int_2 \\ int_3 \\ int_4 \\ ex_1 \\ ex_2 \\ ex_3 \end{bmatrix}$$

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

for the network: $\frac{dS}{dt} = \mathbf{S} \cdot \mathbf{v} = \mathbf{0}$ **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: $\mathbf{S} \cdot \mathbf{v} = \mathbf{0}$

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

FBA: Restricting reactions flux bounds

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

in the matrix format:

$$\mathbf{lb} \leq \mathbf{v} \leq \mathbf{ub}$$

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

how to determine the bounds?

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

2. possible constraints:

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

enzymatic capacity: $a < v < b$

for exchange reactions: $v > 0 \leftrightarrow$ secretion

$v < 0 \leftrightarrow$ intake

A linear programming optimization problem

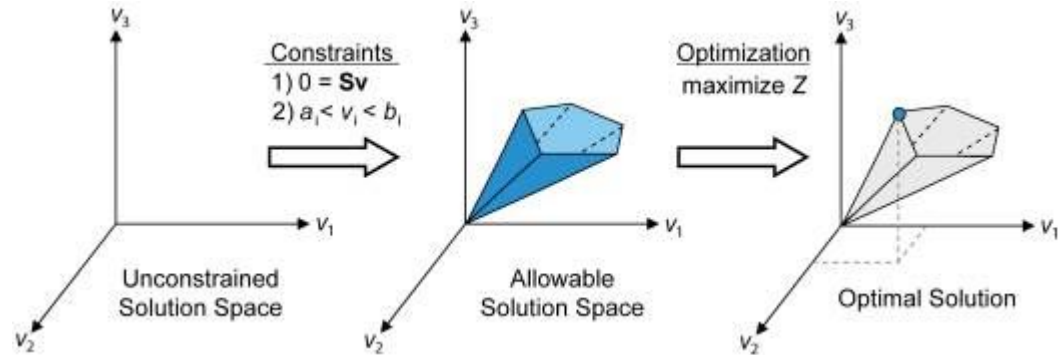
Aim = finding one particular solution in the entire solution space (optimal solution under some conditions)

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

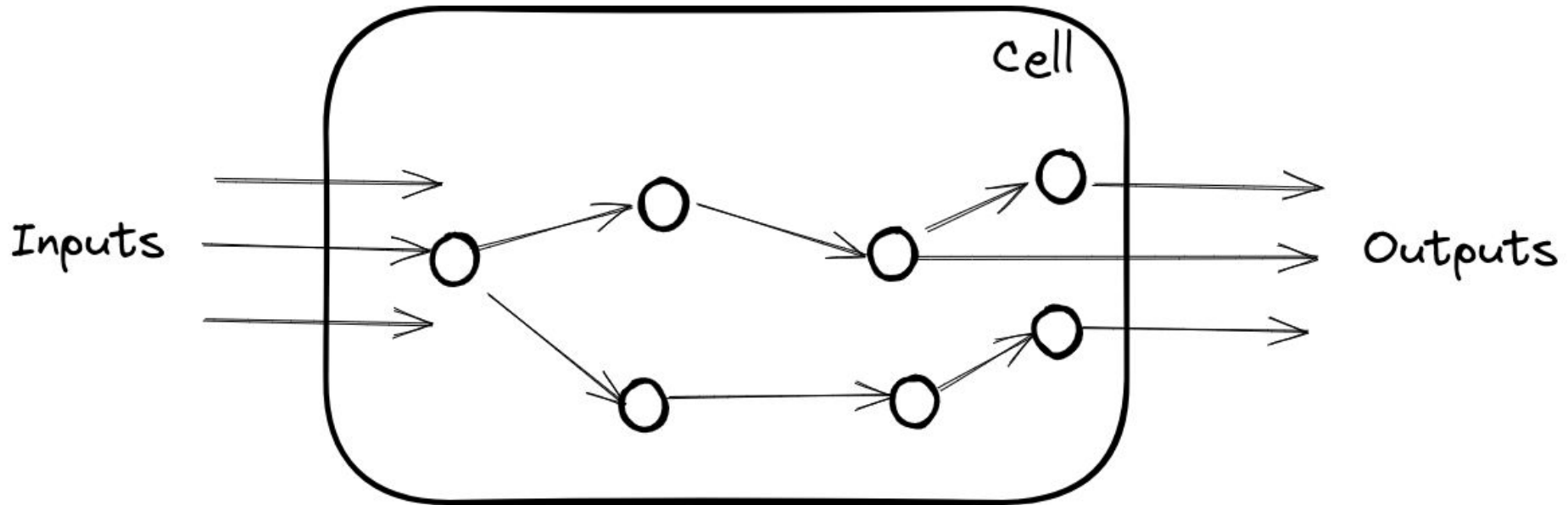
→ **Output of FBA** = particular flux distribution which maximizes or minimizes an objective function

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

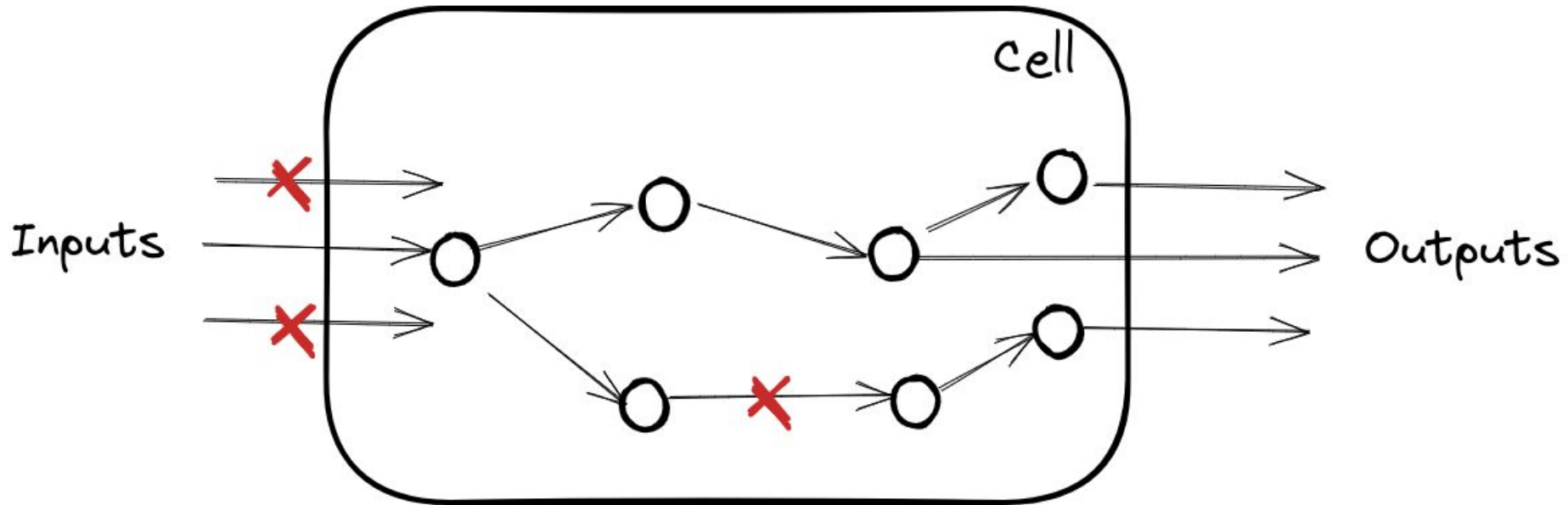
$$\left\{ \begin{array}{l} \text{max: } \mathbf{Z} \\ \text{subject to:} \\ \mathbf{S} \cdot \mathbf{v} = \mathbf{0} \\ \mathbf{lb} \leq \mathbf{v} \leq \mathbf{ub} \end{array} \right.$$



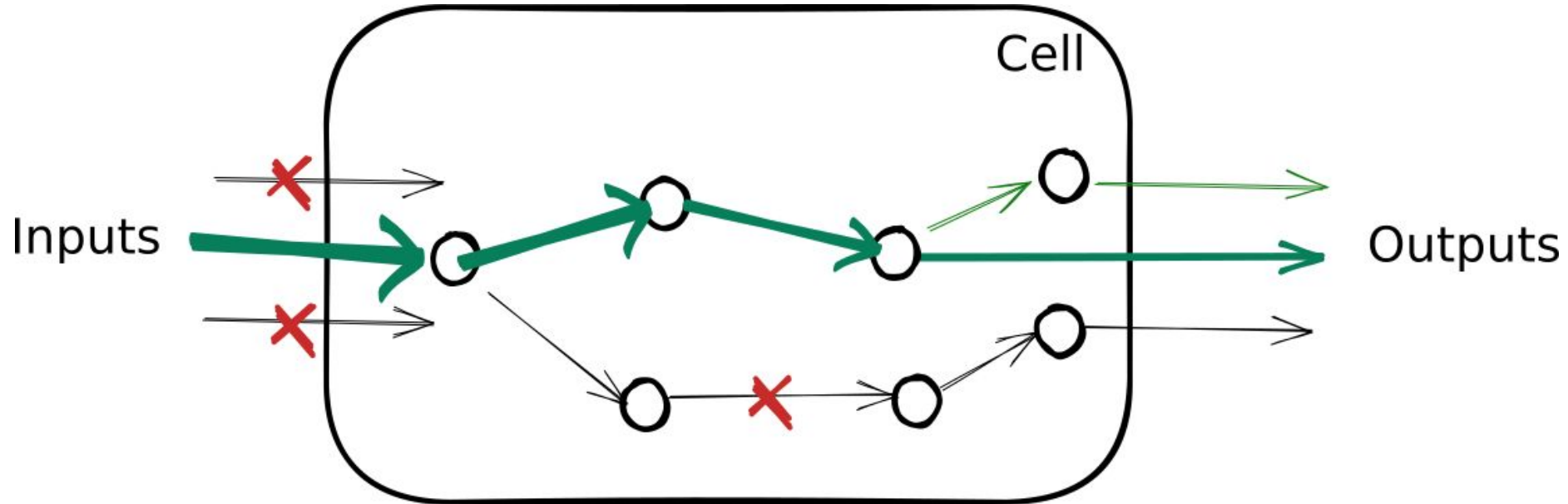
Flux Balance Analysis (FBA)



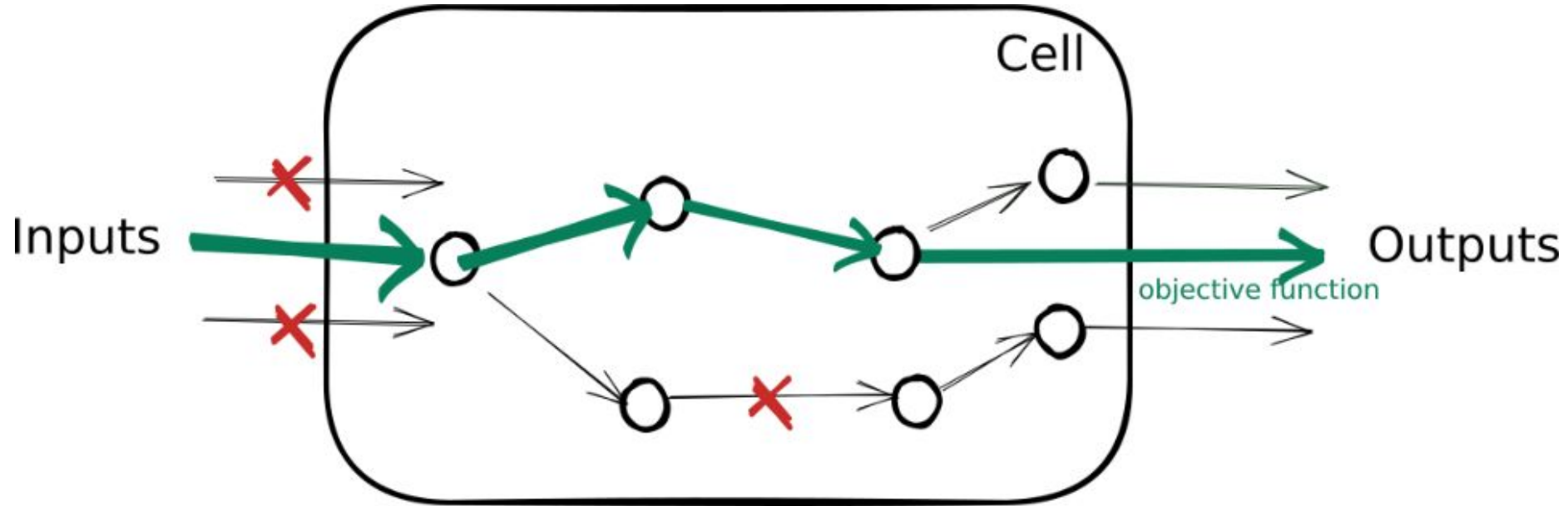
FBA: environmental and genetic constraints



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

W122-W127 Nucleic Acids Research, 2018, Vol. 46, Web Server issue
doi:10.1093/nar/gkx112 Published online 3 May 2018

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

Ludovic Cottret^{1,*}, Hubert Charles^{2,3,†},
Published online 30 April 2018 Nucleic Acids Research, 2018, Vol. 46, Web Server issue W1495-W1502
doi:10.1093/nar/gky201

MetExplore: collaborative edition and exploration of metabolic networks

Ludovic Cottret^{1,*}, Clément Frainay², Maxime Chazalviel^{2,3}, Floréal Cabanettes¹,
Yoann Gloaguen^{4,5,6}, Etienne Camenen², Benjamin Merlet², Stéphanie Houz^{7,8,9},
Jean-Charles Portais^{7,8,9}, Nathalie Poupin², Florence Vinson² and Fabien Jourdan^{1,*}

Bioinformatics, 2017, 30(12), 3113-3123
doi:10.1093/bioinformatics/btx088
Application Date: 15 September 2017
Applications Note



Systems biology

MetExploreViz: web component for interactive metabolic network visualization

Maxime Chazalviel^{1,2}, Clément Frainay³, Nathalie Poupin¹,
Florence Vinson¹, Benjamin Merlet¹, Yoann Gloaguen², Ludovic Cottret⁴
and Fabien Jourdan^{1,*}

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)

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: Expand All Collapse All

	Id	Name	Source Database	Database Type	Nb Reactions	Nb Metabolites	Nb Genes
#		Harpegnathos saltator					
#		Helianthus annuus					
#		Helicobacter pylori					
#		Heliconius melpomene					
#		Homo sapiens					
159	1...	Recon 2.02 Human metabolism global reconstruction (Recon 2) - model - ...	Publication	Others (SBML...)	7440	5063	2191
160	1...	Hsasp	BioCyc	BioCyc	2527	2701	3583
161	2...	Recon 1 Homo. sapiens	Publication	Others (SBML...)	3742	3188	1905
162	2...	Homo sapiens (human) KEGG Genes Database	KEGG Map	KEGG	1869	1560	1417
163	3...	Recon 2.03, enriched with additional database refs, without compartment...	MetExplore	Others (SBML...)	4210	2592	1893
164	3...	HepatoNet1 Gille2010	Publication	Others (SBML...)	2539	1420	0
165	4...	Swainston2016 - Reconstruction of human metabolic network (Recon 2.2)	Publication	Others (SBML...)	7785	6047	1675
166	4...	Recon 2.04 - Human metabolic global reconstruction	VMH	Others (SBML...)	7440	5063	2140
167	4...	iHsa_Ratcon 1	Publication	Others (SBML...)	8264	5620	2315
168	5...	Homo sapiens (human) KEGG Genes Database	KEGG Map	KEGG	1931	1572	1455
169	5...	Recon 2.03 (initial - VMH)	VMH	Others (SBML...)	7440	5063	2191
170	5...	Recon 3D	VMH	SBML	13543	8399	3697
171	5...	Recon3D Flat	MetExplore	SBML	5389	4095	2990
172	5...	Human Metabolic Reaction HMR2.00 (kix)	HMA	Others (SBML...)	8181	6006	3765
173	5...	Human1 (HumanGEM)	Publication	SBML	13097	10073	3628
174	6...	Homo sapiens (human) KEGG Genes Database	KEGG Map	KEGG	2067	1618	1456
175	6...	Human 1.7 (HumanGEM)	SBML	SBML	13082	8378	3625
176	6...	human1.10	humanGEM github	SBML	13078	8370	3625
#		Hordeum vulgare					
#		Hydra vulgaris					
#		Ixodes scapularis					
#		Klebsiella pneumoniae					
#		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

Compartment	Path	Rxn	Met	E. Cplx	G. Prod	Genes
10	99	7785	6047	1815	1675	1675

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
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

geneSymbol	moduleColor	GSsubtype	p_GSsubtype	MM.turquoise	p_MMturquoise	M
UNC119B	black	-0.00370038062939586	0.942836202777498	0.138973665896518	0.00680678957369399	0
XAF1	black	-0.0129288477133737	0.802167070283227	-0.0745674080345198	0.147905398011975	-1
SAMD9L	black	-0.0159104308999613	0.757831880390286	-0.0189579526430889	0.713322891481581	0
IFIT3	black	0.022306669500763	0.66551708768726	-0.0144735267897258	0.779109076821725	-1
IFI27	black	0.0245334233426714	0.634447660871975	-0.058526650347724	0.256336457258585	-1
IFITM3	black	0.0341598242885462	0.507883700542923	0.0194761117738355	0.705846195052202	-1
RSAD2	black	-0.0354389947948108	0.492116338470493	-0.114311033579536	0.0262570034583564	-1
IFIT2	black	0.036981814033086	0.473452708854494	0.0387958031744976	0.452013962333683	0
OAS2	black	0.0391806736477964	0.447536675914385	0.0213275273098786	0.679366143933709	-1
STAT1	black	-0.0534831775338735	0.299675078389574	-0.156046588284105	0.00234619101231982	0
MX1	black	-0.0579761971135094	0.260843826228363	-0.107882685124308	0.0360241085901714	-1
ISG15	black	0.0586330766876939	0.255471241739053	0.00599316081877001	0.907545153934827	-1

Mapping genes

Missing data: few explications

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

MetExplore v2.32.12

About Ormics Toolbox Flux Import Export Login

User Profile Network Data Network Curation Network Viz Mapping

Demo

Upload file (.csv .txt): separator: tab

Consider first row as header of columns:

Mapping name: Mapping

Perform one separate mapping for each column:

Copy/Paste in grid

Object: Gene Feature: Identifier

	Identifie	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

Propagate: without conditions values

Mapping Nb Data: 2050 Nb Data In Network: 223 Nb Mapped: 221

Map Save Mapping in File

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)	
+		Edit		Delete		Curation Votes		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										

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 the Flux Variability Analysis web interface. At the top, there is a navigation bar with tabs for 'User Profile', 'Network Data', 'Network Curation', 'Network Viz', and 'Flux Variability Analysis'. The main content area is divided into several sections:

- 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 (with a 'report reaction table selection' button)
 - objectiveSense:** MIN (selected) and MAX (radio buttons)
 - reactionSet:** (with a 'report reaction table selection' button)
 - secondObjectiveReactions:** (with a 'report reaction table selection' button)
 - secondObjectiveSense:** MIN (selected) and MAX (radio buttons)
 - libertyPercentage:** 0 (input field)
 - ko_genes:** (with a 'report gene table selection' button)
 - ko_reactions:** R_r0744_R_FAOX5OHc_R_rc (with a 'report reaction table selection' button)
- Launch:** A blue button at the bottom.

Filters

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

Propagation of the filters

The screenshot shows a web application interface with a table of HGNC identifiers. The table has columns for Name, Identifier, and Mapping. The Mapping column is further divided into 'identified' and 'condition0'. A search filter is applied to the Name column, and a context menu is open over the table, showing options like 'Filter on selection', 'Delete Filter & Search', and 'help'. The table contains 29 rows of data.

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

Filters: genes mapped

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

- Update data on grids

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 Votes					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)									
Name		Identifier		Nb Reactions		% Reactions with Enz.		Mapping on Gene																																																																							
exact sub-string search		exact sub-string search						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

Be 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

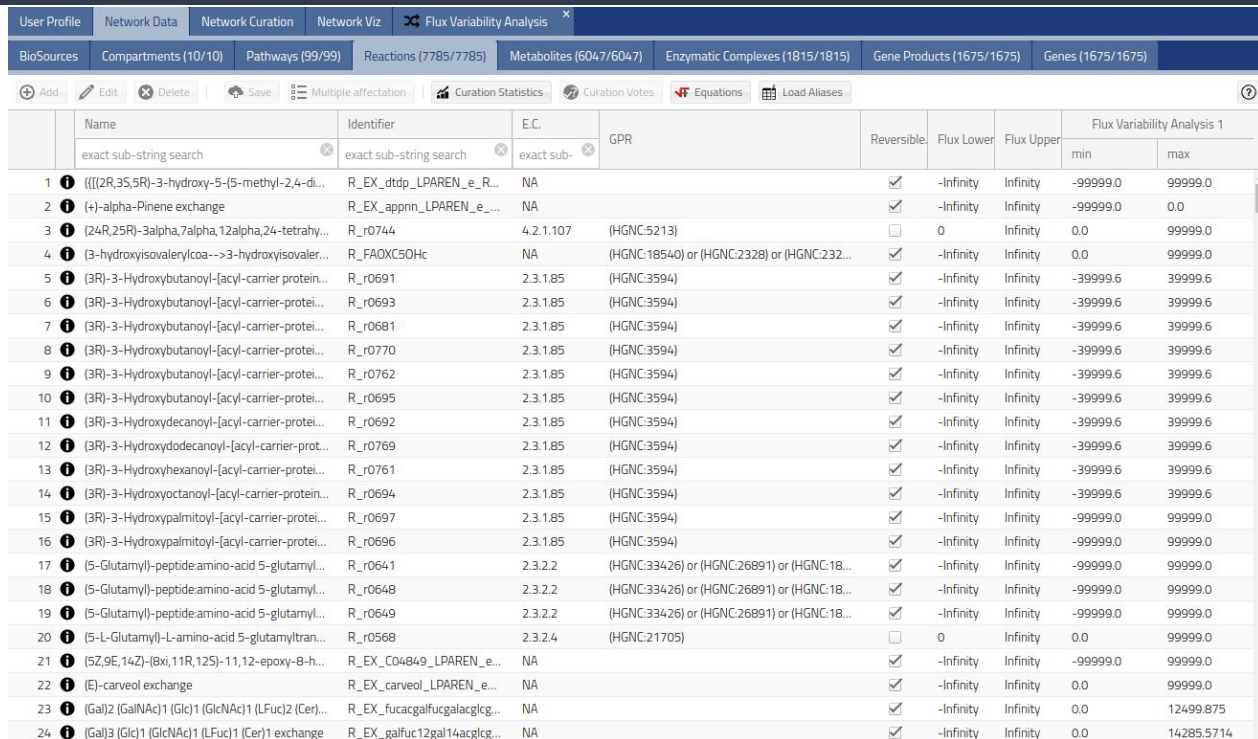
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7386414/>

Filters: FBA result

Only reactions with a non-zero flux value are kept

276 reactions of interest remain

Visualise this sub-network



The screenshot shows a software interface with a top navigation bar containing tabs for 'User Profile', 'Network Data', 'Network Curation', 'Network Viz', and 'Flux Variability Analysis'. Below this is a secondary navigation bar with tabs for 'BioSources', 'Compartments (10/10)', 'Pathways (99/99)', 'Reactions (7785/7785)', 'Metabolites (6047/6047)', 'Enzymatic Complexes (1815/1815)', 'Gene Products (1675/1675)', and 'Genes (1675/1675)'. A toolbar below the navigation bar includes buttons for 'Add', 'Edit', 'Delete', 'Save', 'Multiple affectation', 'Curation Statistics', 'Curation Votes', 'Equations', and 'Load Aliases'. The main content area is a table with columns for 'Name', 'Identifier', 'E.C.', 'GPR', 'Reversible', 'Flux Lower', 'Flux Upper', and 'Flux Variability Analysis 1' (subdivided into 'min' and 'max'). The table lists 24 reactions, each with a status icon, a name, an identifier, an E.C. number, a GPR string, a checkbox for 'Reversible', and numerical values for 'Flux Lower', 'Flux Upper', 'min', and 'max'.

	Name	Identifier	E.C.	GPR	Reversible	Flux Lower	Flux Upper	Flux Variability Analysis 1	
								min	max
1	(((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-di...	R_EX_dtdp_LPAREN_e_R...	NA		<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	99999.0
2	(+)-alpha-Pinene exchange	R_EX_appnn_LPAREN_e_...	NA		<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	0.0
3	(24R,25R)-3alpha,7alpha,12alpha,24-tetrahy...	R_r0744	4.2.1.107	(HGNC:5213)	<input type="checkbox"/>	0	Infinity	0.0	99999.0
4	(3-hydroxyisovalerylcoa-->3-hydroxyisovaler...	R_FAOXC50Hc	NA	(HGNC:18540) or (HGNC:2328) or (HGNC:232...	<input checked="" type="checkbox"/>	-Infinity	Infinity	0.0	99999.0
5	(3R)-3-Hydroxybutanoyl-[acyl-carrier protein...	R_r0691	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
6	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0693	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
7	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0681	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
8	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0770	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
9	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0762	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
10	(3R)-3-Hydroxybutanoyl-[acyl-carrier-protei...	R_r0695	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
11	(3R)-3-Hydroxydecanoyl-[acyl-carrier-protei...	R_r0692	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
12	(3R)-3-Hydroxydodecanoyl-[acyl-carrier-prot...	R_r0769	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
13	(3R)-3-Hydroxyhexanoyl-[acyl-carrier-protei...	R_r0761	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
14	(3R)-3-Hydroxyoctanoyl-[acyl-carrier-protein...	R_r0694	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-39999.6	39999.6
15	(3R)-3-Hydroxypalmitoyl-[acyl-carrier-protei...	R_r0697	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	99999.0
16	(3R)-3-Hydroxypalmitoyl-[acyl-carrier-protei...	R_r0696	2.3.1.85	(HGNC:3594)	<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	99999.0
17	(5-Glutamyl)-peptide-amino-acid 5-glutamyl...	R_r0641	2.3.2.2	(HGNC:33426) or (HGNC:26891) or (HGNC:18...	<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	99999.0
18	(5-Glutamyl)-peptide-amino-acid 5-glutamyl...	R_r0648	2.3.2.2	(HGNC:33426) or (HGNC:26891) or (HGNC:18...	<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	99999.0
19	(5-Glutamyl)-peptide-amino-acid 5-glutamyl...	R_r0649	2.3.2.2	(HGNC:33426) or (HGNC:26891) or (HGNC:18...	<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	99999.0
20	(5-L-Glutamyl)-L-amino-acid 5-glutamyltran...	R_r0568	2.3.2.4	(HGNC:21705)	<input type="checkbox"/>	0	Infinity	0.0	99999.0
21	(5Z,9E,14Z)-[xi,11R,12S)-11,12-epoxy-8-h...	R_EX_C04849_LPAREN_e_...	NA		<input checked="" type="checkbox"/>	-Infinity	Infinity	-99999.0	99999.0
22	(E)-carveol exchange	R_EX_carveol_LPAREN_e_...	NA		<input checked="" type="checkbox"/>	-Infinity	Infinity	0.0	99999.0
23	(Gal)2 (GalNAc)1 (Glc)1 (GlcNAc)1 (LFuc)2 (Cer)...	R_EX_fucacgalfucgalacg...	NA		<input checked="" type="checkbox"/>	-Infinity	Infinity	0.0	12499.875
24	(Gal)3 (Glc)1 (GlcNAc)1 (LFuc)1 (Cer)1 exchange	R_EX_galfuc12gal14acg...	NA		<input checked="" type="checkbox"/>	-Infinity	Infinity	0.0	14285.5714

Visualisation

Compartments:

cytoplasm :



endoplasmic reticulum :



mitochondrion :



peroxisome :



Pathways:

Eicosanoid metabolism :



Fatty acid oxidation :



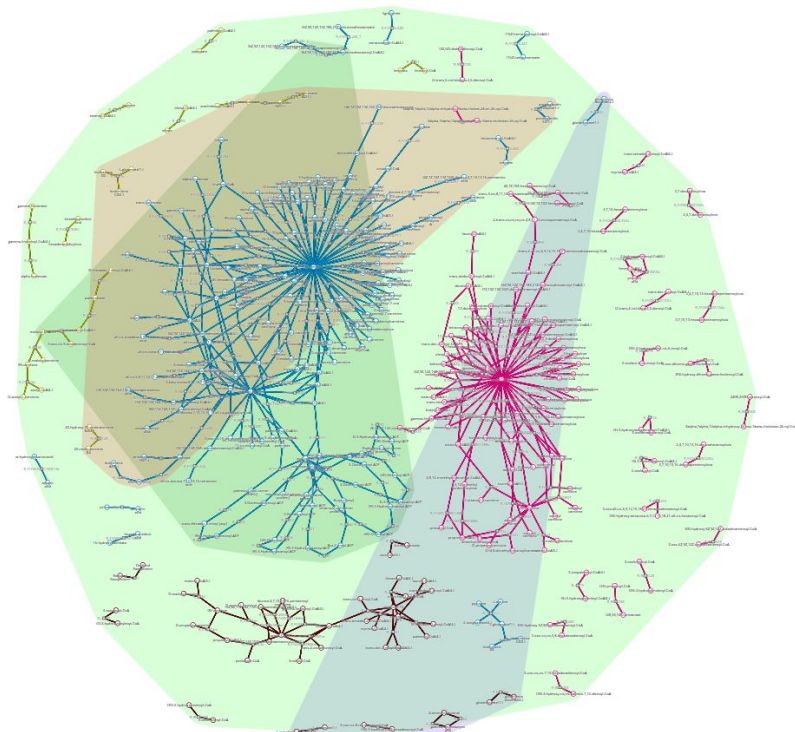
Fatty acid synthesis :



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 displays the MetExplore web interface. The top navigation bar includes 'User Profile', 'Project Details', 'Network Data', 'Network Curation', and 'Network Viz'. The main content area is titled 'Project A-V Splanchnic Postprandemic Created 2018-12-22'. Below this is a 'TODO list' table with columns for Description, Project, User, Limit date, Status, and Priority. A 'BioSources' table is visible at the bottom, listing various sources for the project. The right sidebar contains project configuration options, including 'Selected BioSource', 'Public/Private' settings, and a detailed view of the selected bioresource: 'Homo sapiens (Strain: Liver)'. The bioresource details include a table with columns for Compart, Path, Run, Met, E. Cplx, G. Prod, and Genes, and a list of associated data fields like Name, Organism, Tissue, Cell Type, Strain, Source Database, UR, ID in Database, Version, and Database type.

Compart	Path	Run	Met	E. Cplx	G. Prod	Genes
8	3	2935	1424	850	850	850

Id	Name	Organism	Strain	Source Database...	Database Type	Publication
1	HepatoNet1 with EXons (bAP bACM)	Homo sapiens	Liver	Gile et al., 201...	SBML	
2	HepatoNet1_3D (noND addr)	Homo sapiens	Liver	Gile et al., 201...	SBML	
3	HepatoNet1_3D (noND addr)	Homo sapiens	Liver	Gile et al., 201...	SBML	
4	HepatoNet1 with EXons & AdR for project PPC	Homo sapiens	Liver	Gile et al., + m...	SBML	
5	HepatoNet1_3D (stMED, AdR, cstP)	Homo sapiens	Liver	SBML	SBML	

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



Thanks to MetExplore team



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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/>

