Analysis of OMICS data in the context of metabolic networks

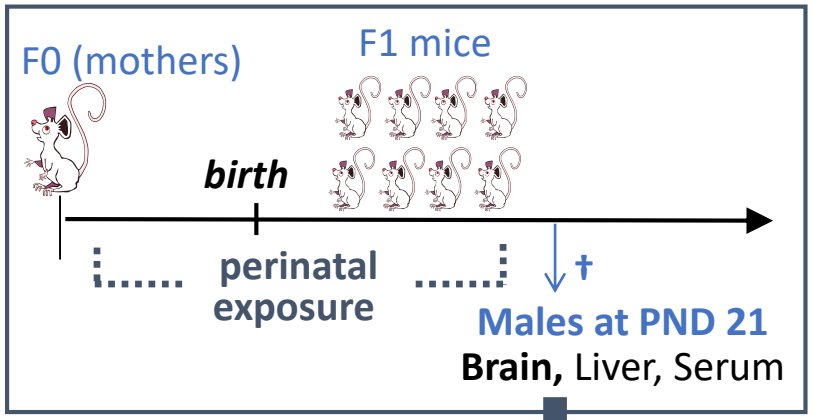
Nathalie Poupin, **MeX, Toxalim**
INRA Toulouse, France
nathalie.poupin@inra.fr

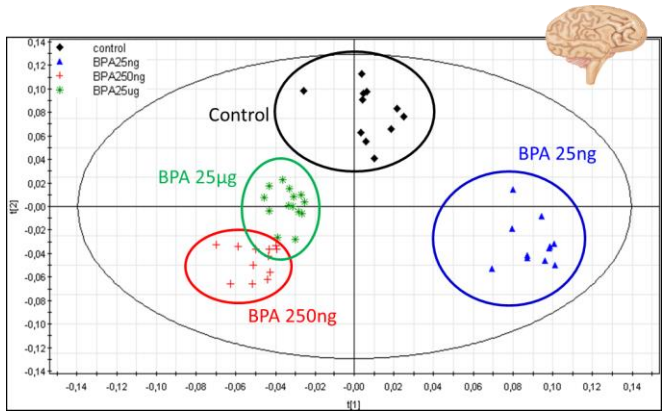
Overview

- **How to make sense of OMICS data**? Why do we need metabolic networks & modelling strategies?
- **Metabolic networks** to gather metabolic knowledge and integrate OMICS data
- Modeling global metabolism – **Graph-based approaches**
- Modeling global metabolism – **Flux-based approaches**

How to interpret metabolic fingerprints?

Impact of perinatal exposure to low doses of bisphenol A

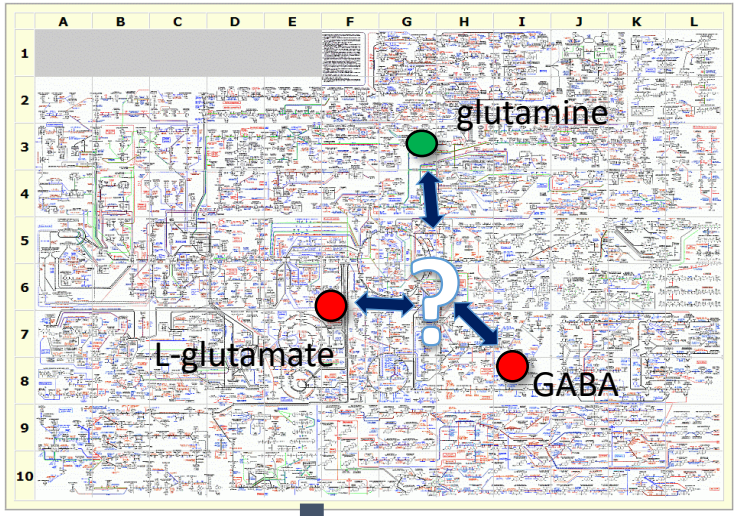




discriminant metabolites
(BPA 25 µg/kg *vs.* ctrl)

Metabolites	Brain PND21
Cholines	↓
Glutamate	↓
Glutamine	↑
Glycine	↑
Aspartic Acid	↑
GABA	↓

What is the biochemistry acting behind the scene?



Use of **metabolic network models** for **contextualization of omic data**

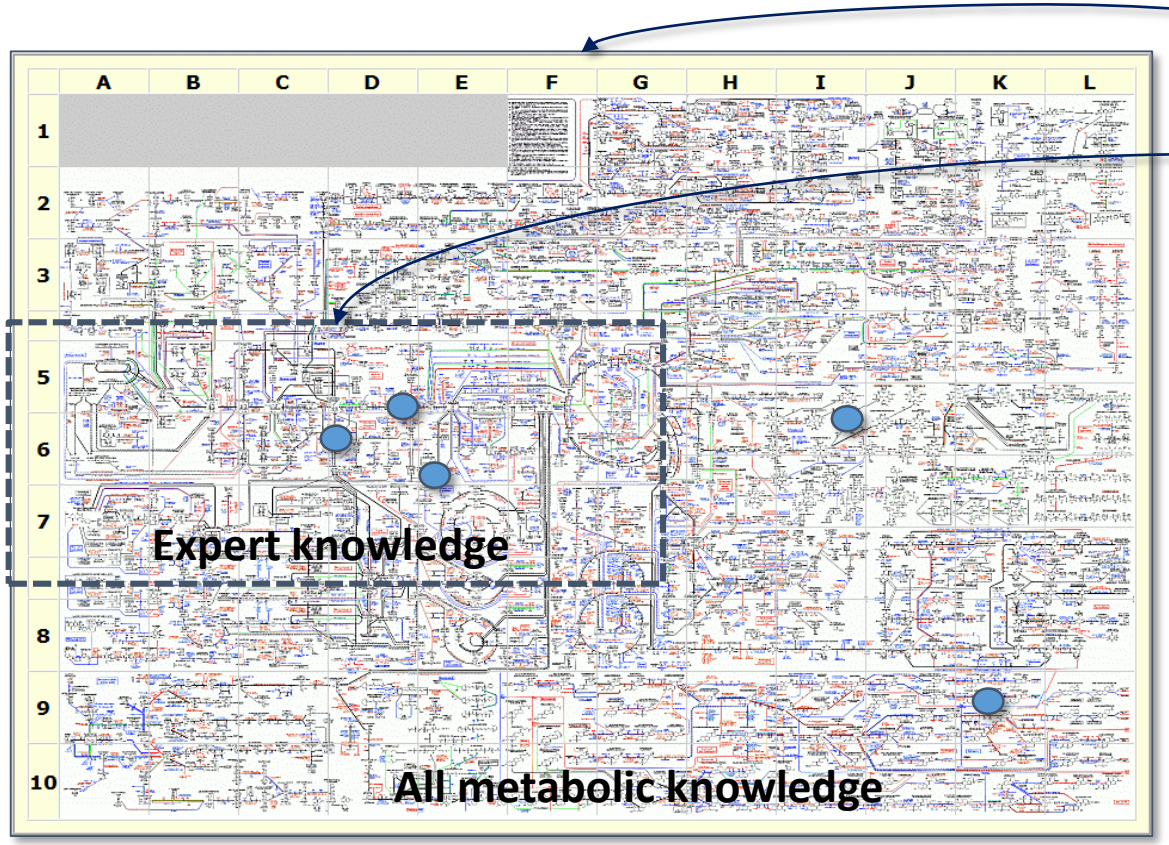
What are the metabolic processes involved?

Cabaton NJ, *et al*. 2013
Environ. Health Perspect. 121:586–93

Untargeted observations require a more holistic analysis

"Rationality is bounded when it falls short of omniscience. And the failures of omniscience are largely failures of knowing all the alternatives, uncertainty about relevant exogenous events, and inability to calculate consequences."

Herbert A. Simon, "Rational decision making in business organizations"
Nobel Memorial Lecture 1978.



Metabolomics data

Metabolic network

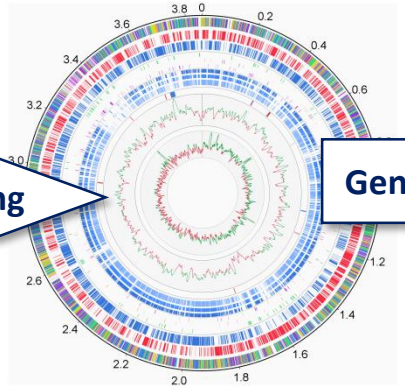
= succession of chemical reactions, whose main functions are to produce energy from available resources, often gathered in metabolic pathways (that perform a specific function or transformation, e.g. glycolysis)

Przytycka TM & Andrews J. (2010). *BMC Biol*. 8: 62.

Genome scale metabolic network reconstructions

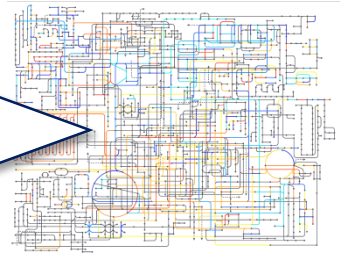
Biochemical reactions known to take place in a target organism & associated genes

Genome sequencing



Genome annotation

Network reconstruction



Model refinement

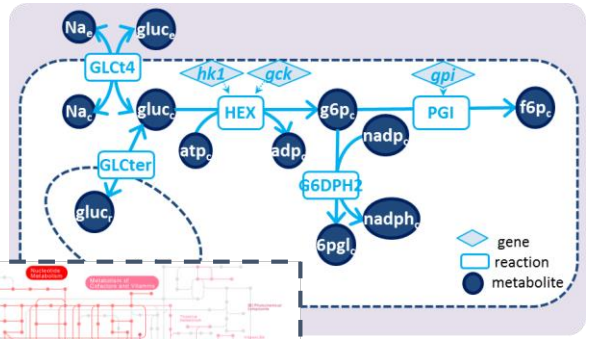
Thiele I, Palsson BØ. Nat. Protoc. 2010; 5:93–121

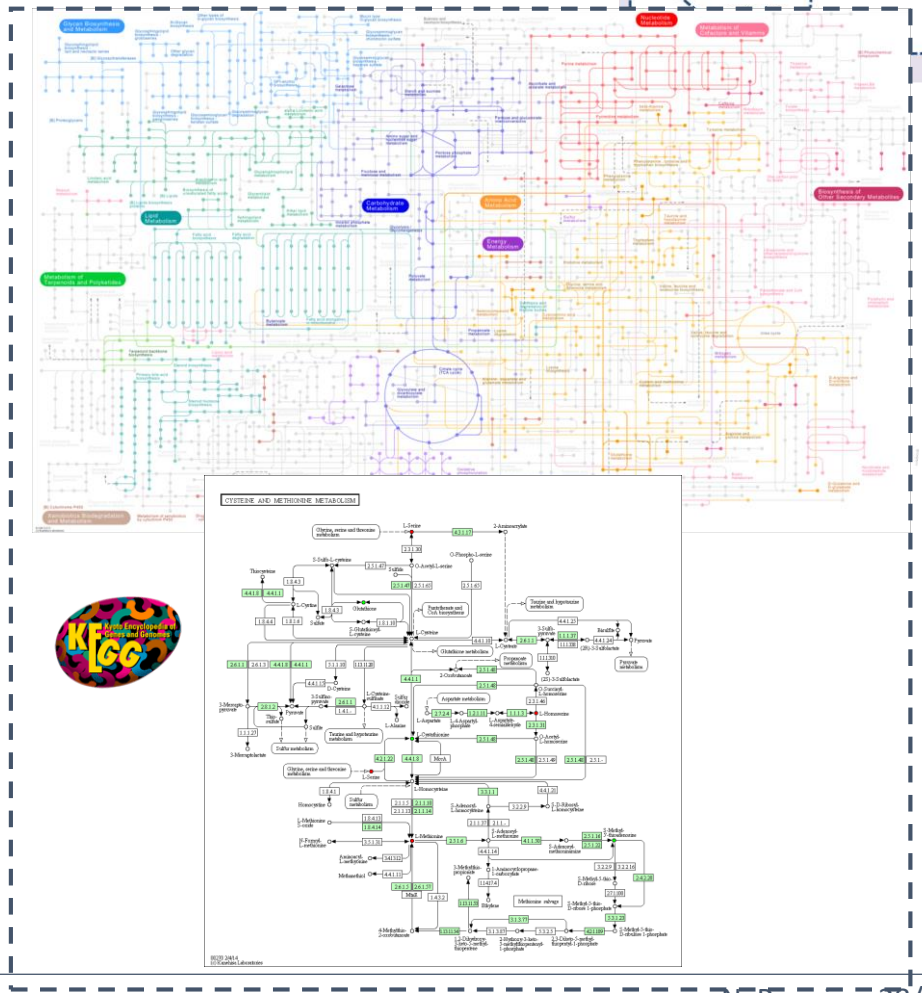
list of genes	list of reactions		list of metabolites
(6524) or (6526)	GLCt4	$Na_e + gluc_e \leftrightarrow Na_c + gluc_c$	$gluc_e$
(3098)	HEX1	$gluc_c + ATP_c \rightarrow ADP_c + g6p_c$	$gluc_c$
(2821)	PGI	$g6p_c \rightarrow f6p_c$	Na_e
(2539)	G6PDH2r	$g6p_c + nadp_c \rightarrow 6pgl_c + nadph_c$	Na_c
	GLCter	$gluc_c \leftrightarrow gluc_r$	ATP_c
			ADP_c
			$g6p_c$
			$f6p_c$
			$6pgl_c$
			$nadp_c$
			$nadph_c$
			$gluc_r$

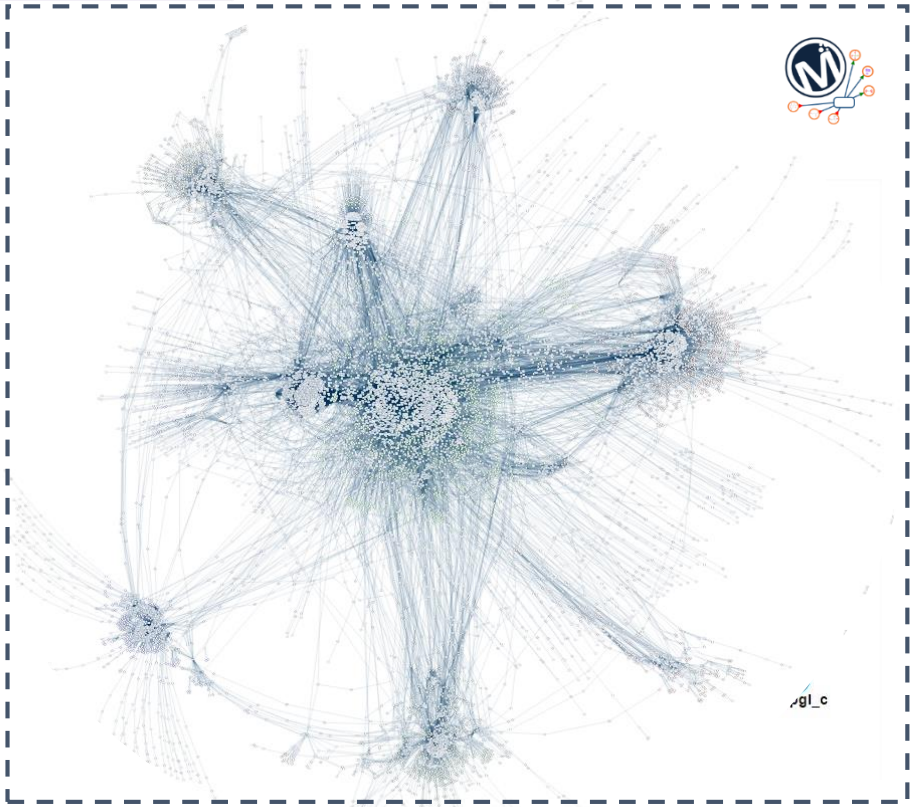
stoichiometric associations

→ mathematically structured knowledge base
→ Generic reconstructions generated from anotated genomes
→ Published for many organisms

Metabolic network representations

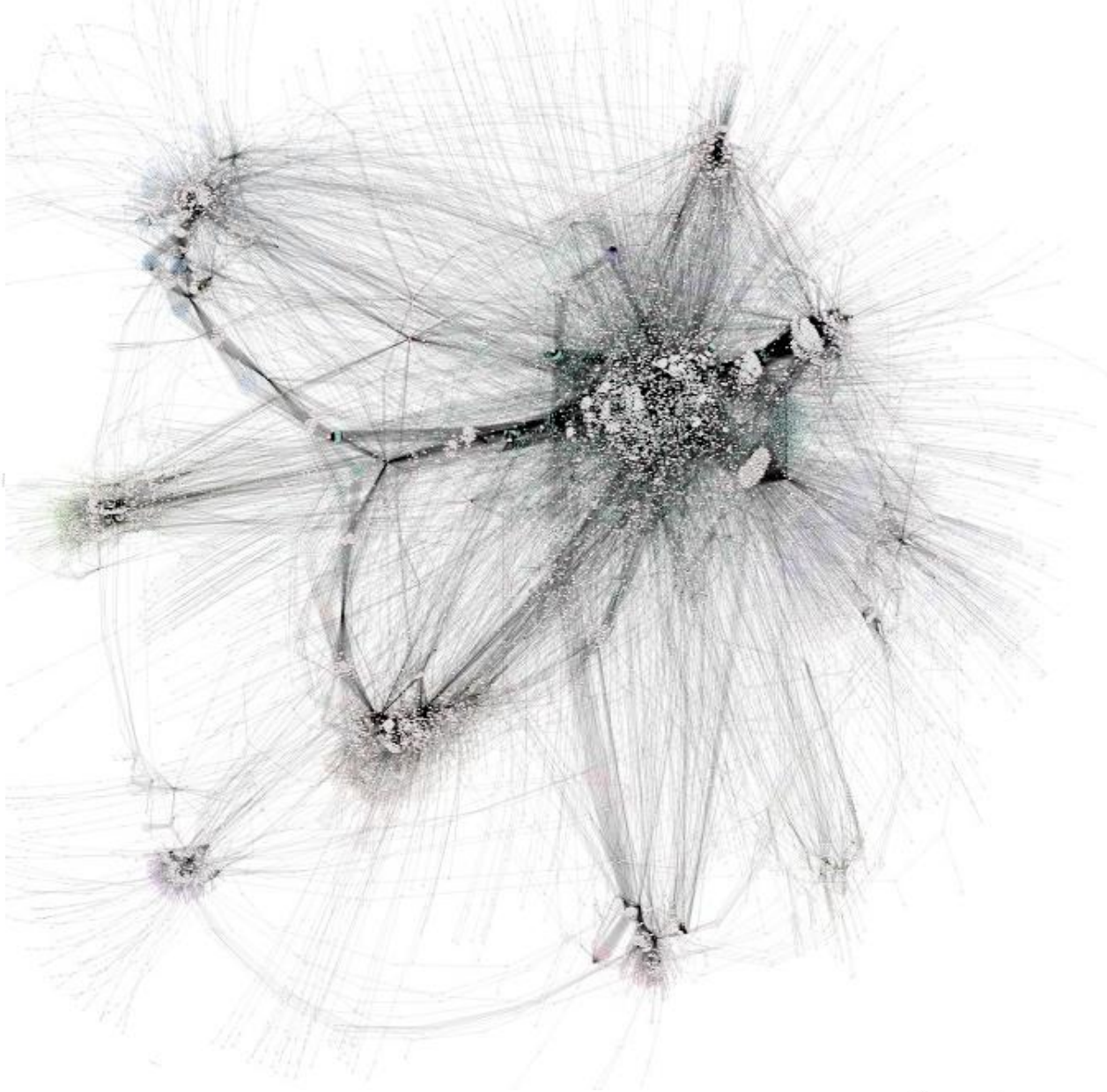






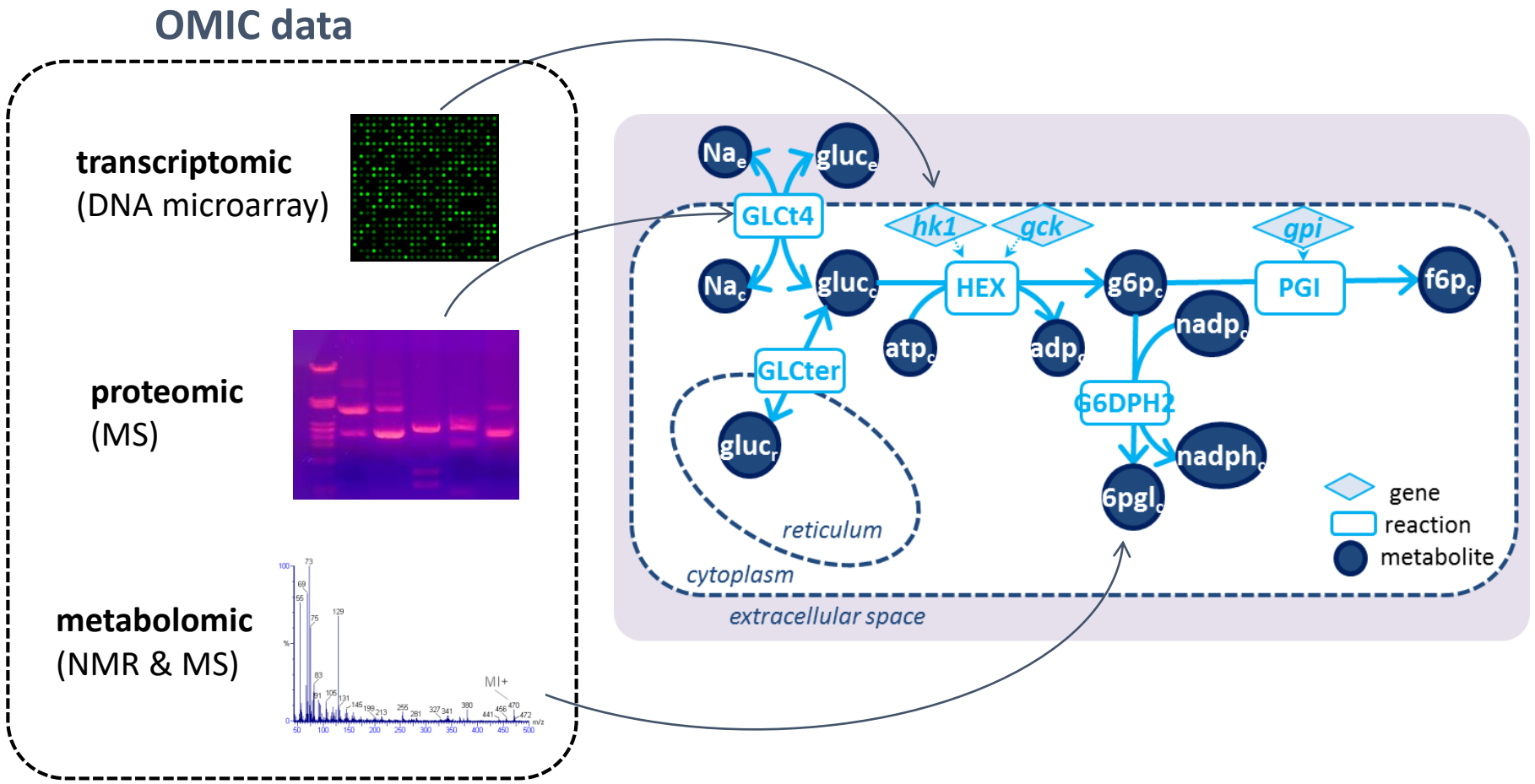
The global Human metabolic network Recon 3D[1]

[1]Brunk E, Sahoo S, Zielinski DC, Altunkaya A, Dräger A *et al*. (2018) *Recon3D enables a three-dimensional view of gene variation in human metabolism.* Nat Biotechnol.



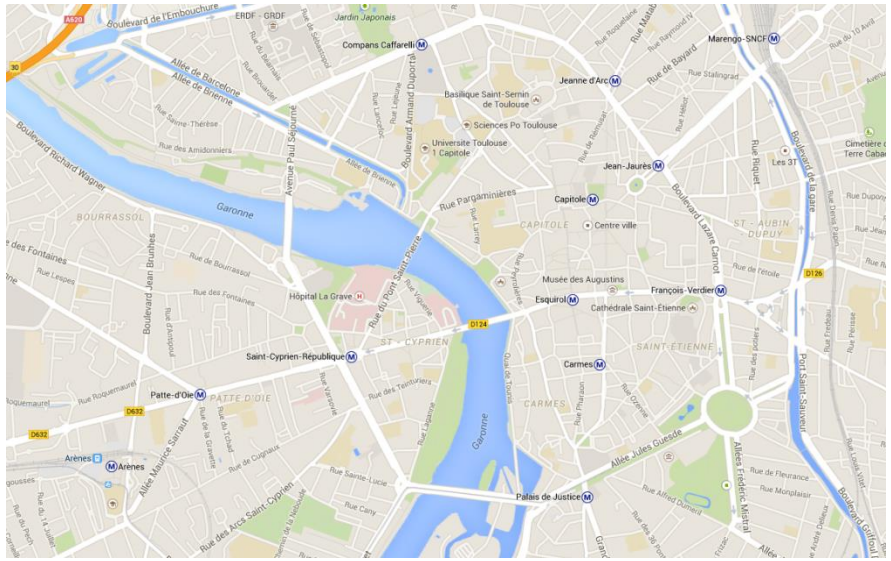
8 399 metabolites (4140 uniques)
13 543 reactions
3 697 genes

Metabolic networks for multi-omic interpretation



Analysing metabolic networks: **Graphs *vs*. fluxes**

Graph based analysis



Flux based analysis

Structural / topological analysis

→ understand the organisation of the system, study some structural properties such as connectivity

Find possible pathways between metabolites of interest

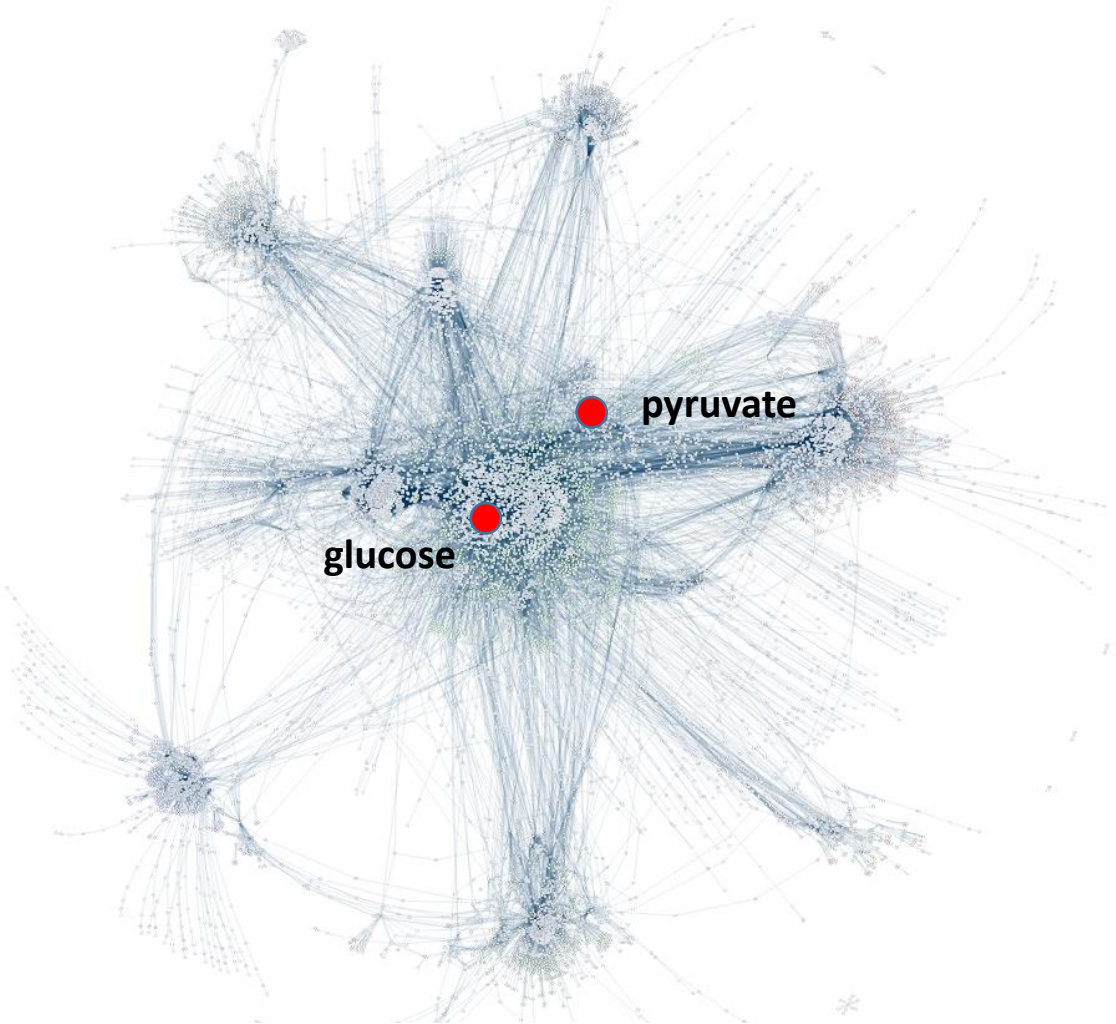
Dynamic / semi-quantitative analysis

→ understand the behavior of the system under specific conditions, identify the most active pathways

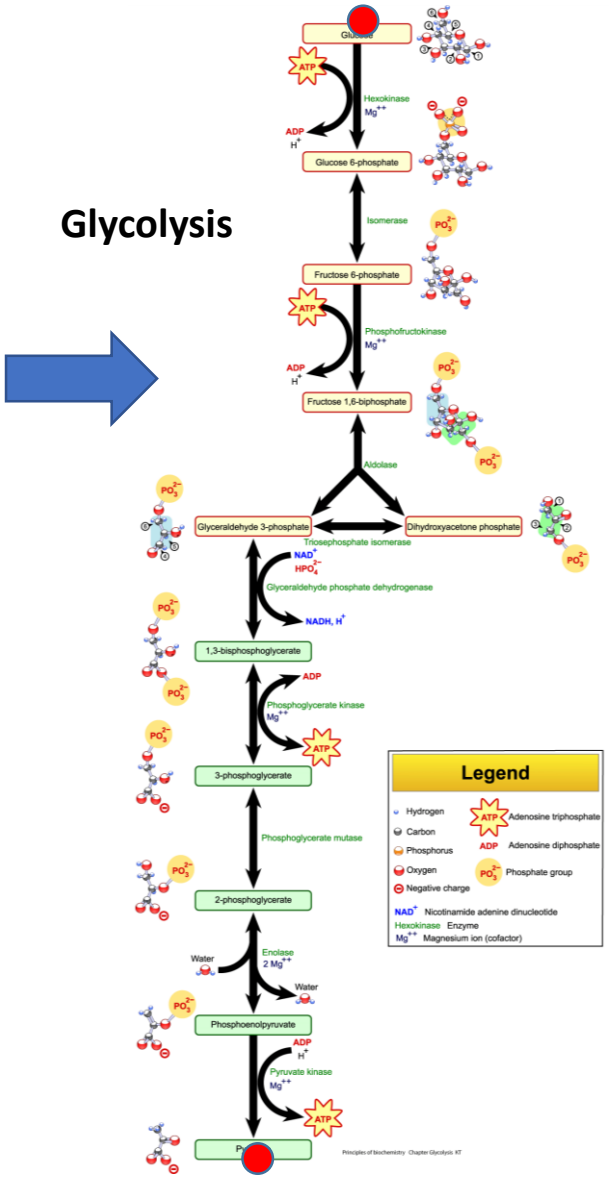
Predict metabolic fluxes, cell growth, drug targets…

Graph-based analysis: looking for metabolic paths

Problem complexity: finding paths from glucose to pyruvate



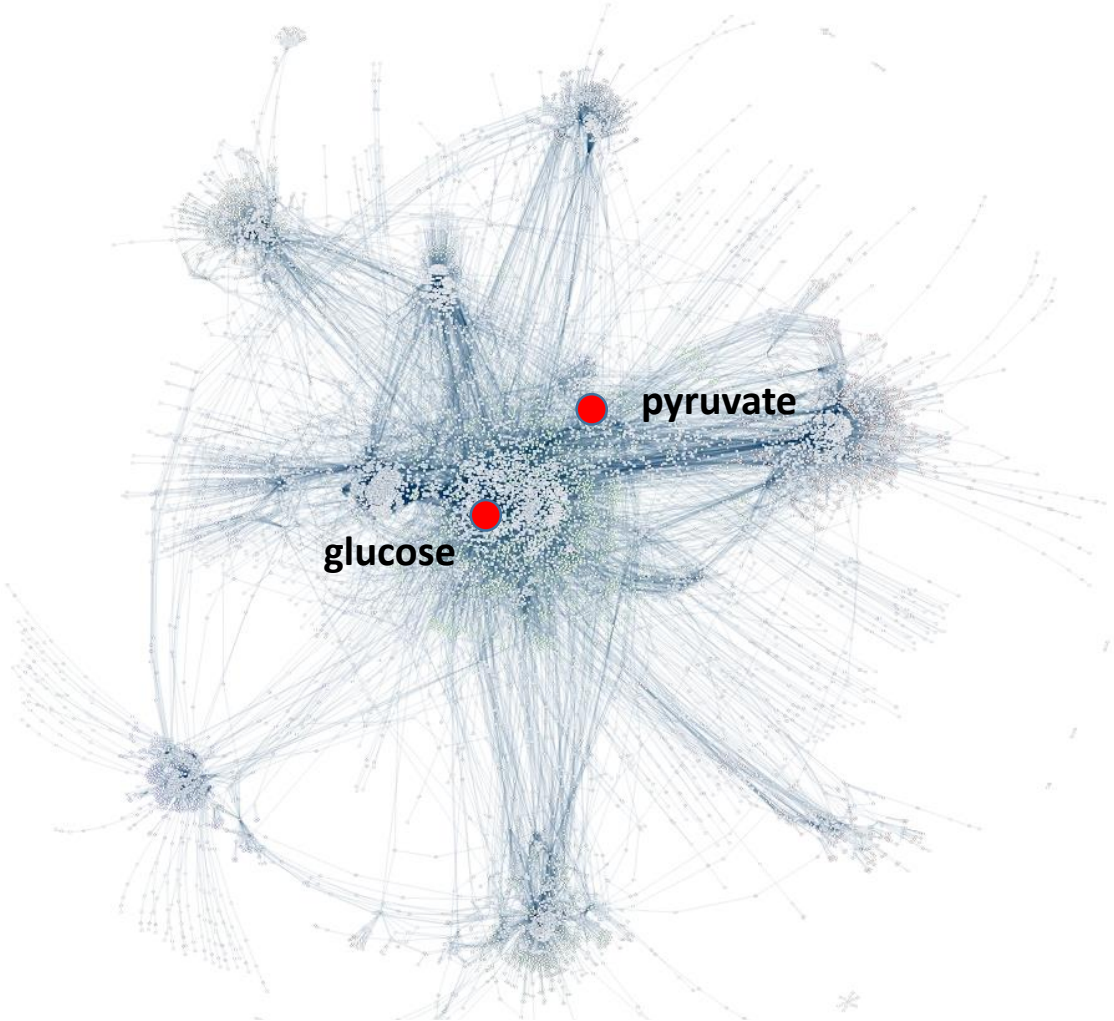
Glycolysis



Metabolic path = succesion of reactions connecting metabolites.

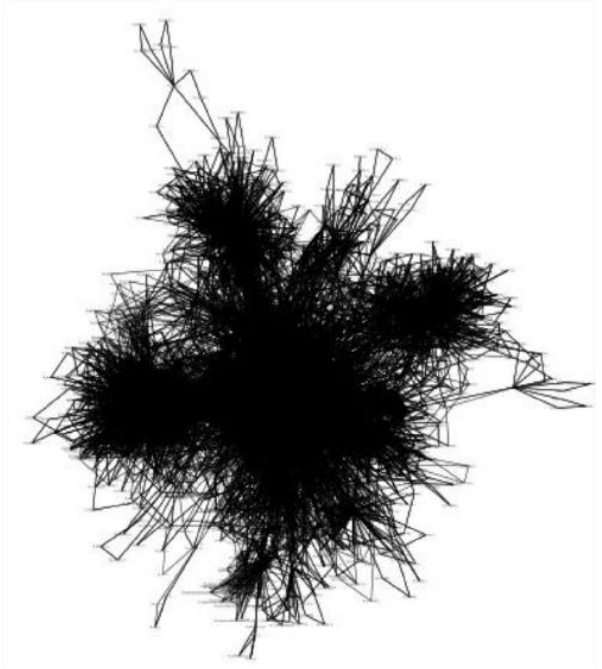
Graph-based analysis: looking for metabolic paths

Problem complexity: finding paths from glucose to pyruvate



Metabolic path = succesion of reactions connecting metabolites.

In the whole network:
500 000 possible paths
between glucose and pyruvate!

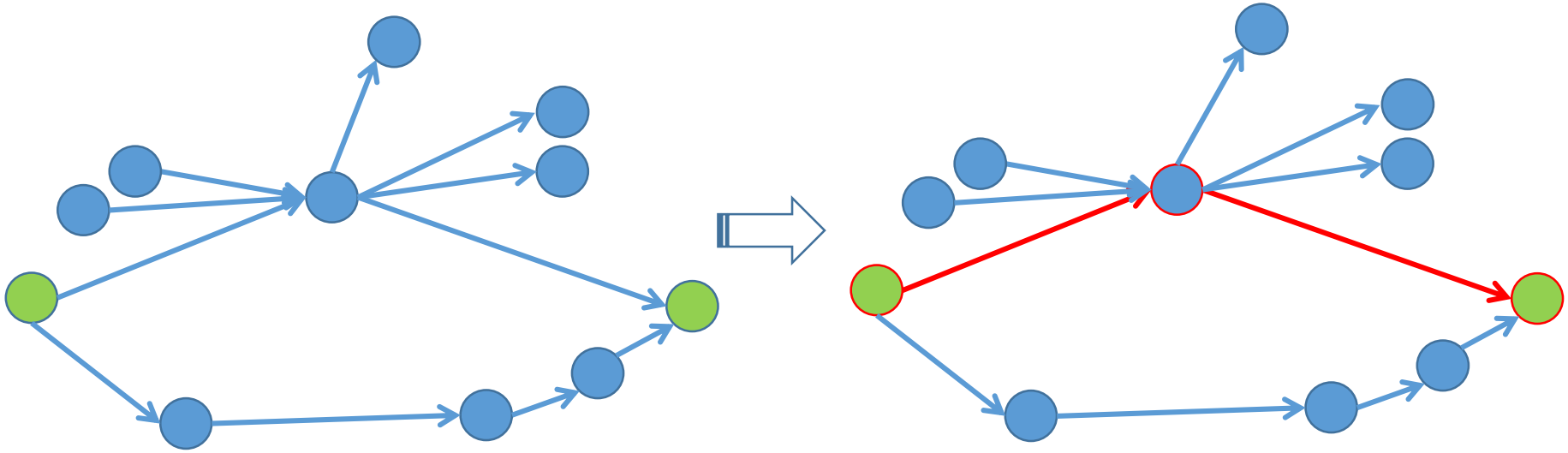


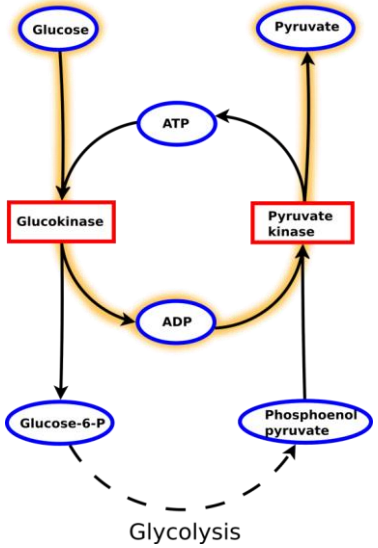
→ need for graph algorithms to compute & select paths between metabolites in the network.

Küffner R, Zimmer R, Lengauer T. Bioinformatics 2000; 16:825–36c

Shortest path *vs.* lightest path

Shortest path

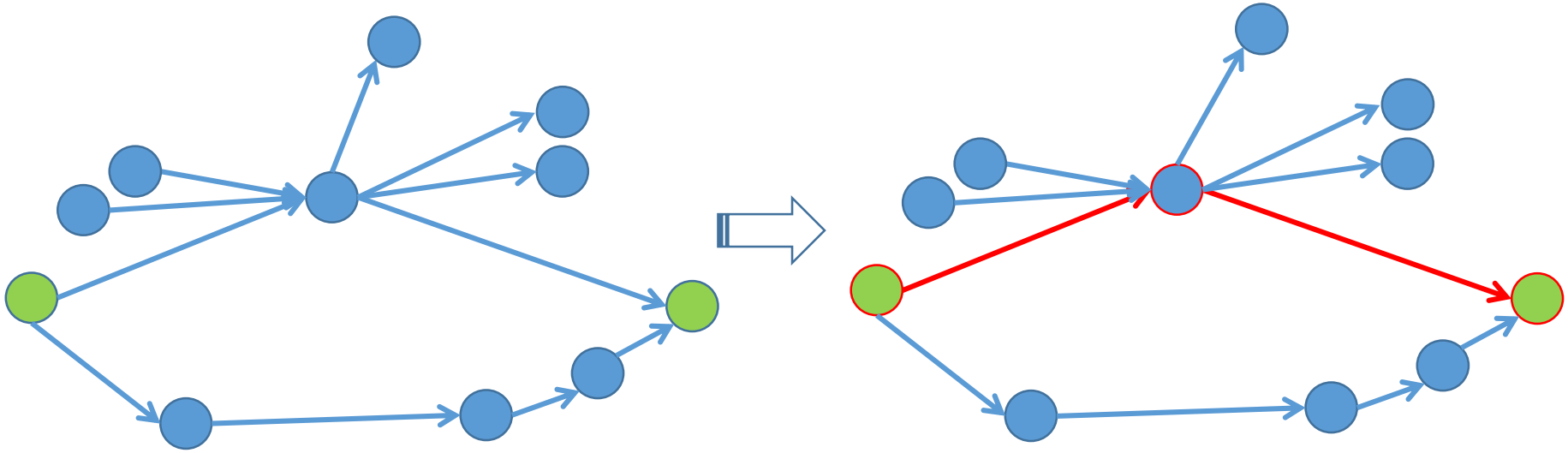




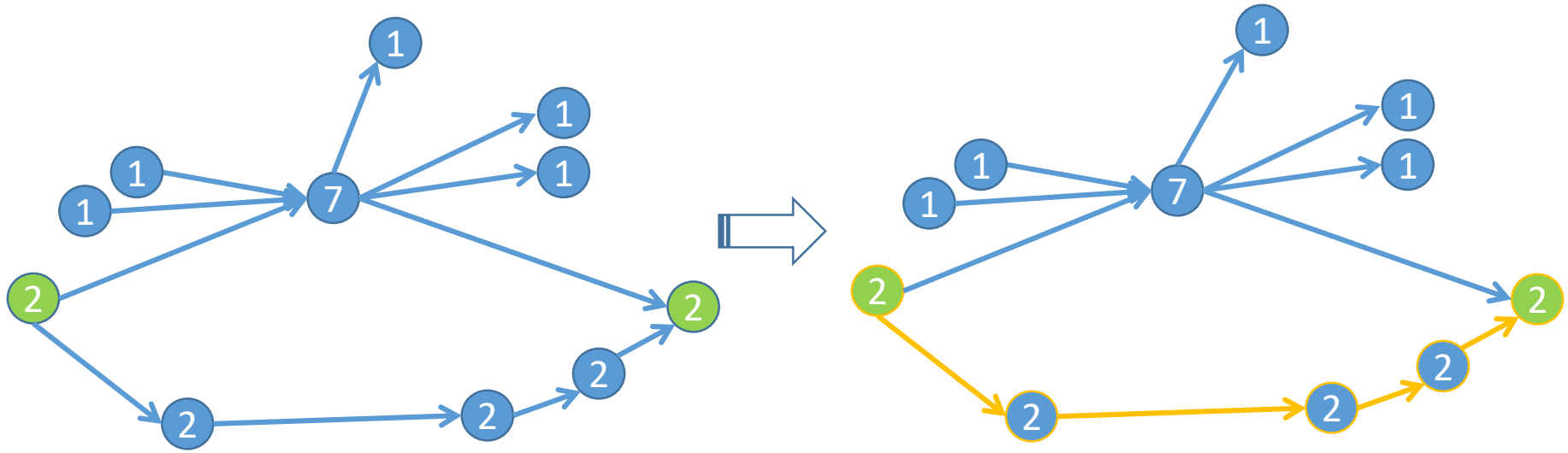
the shortest path is not always the most relevant!

Shortest path *vs.* lightest path

Shortest path



Lightest path

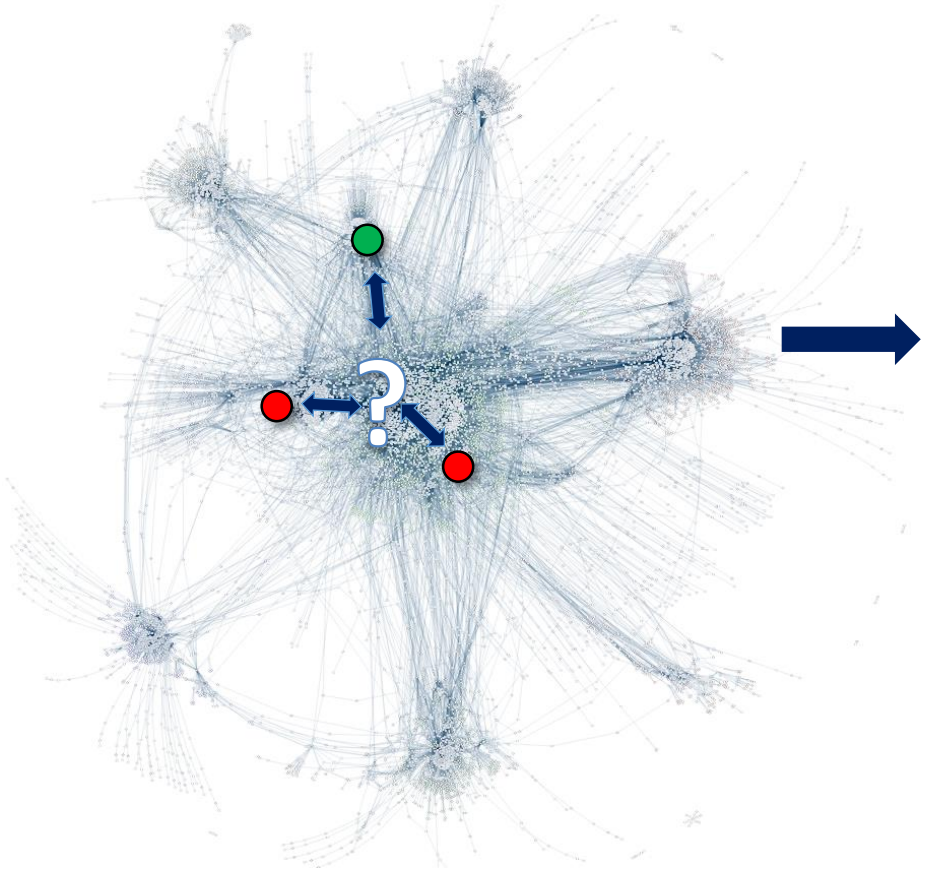


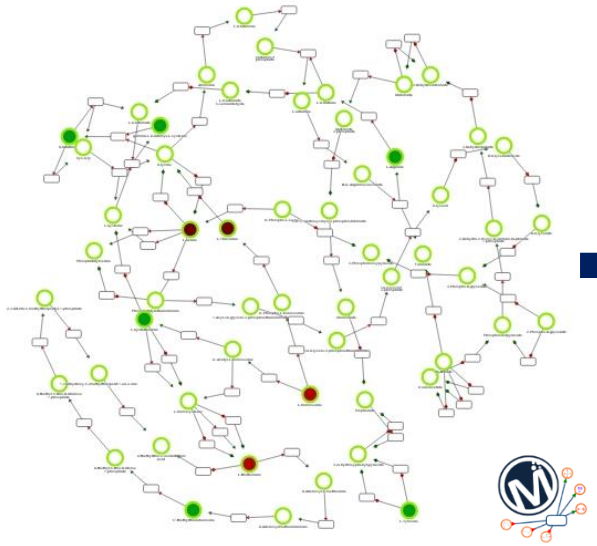
Graph-based analysis: Interpreting metabolic fingerprints

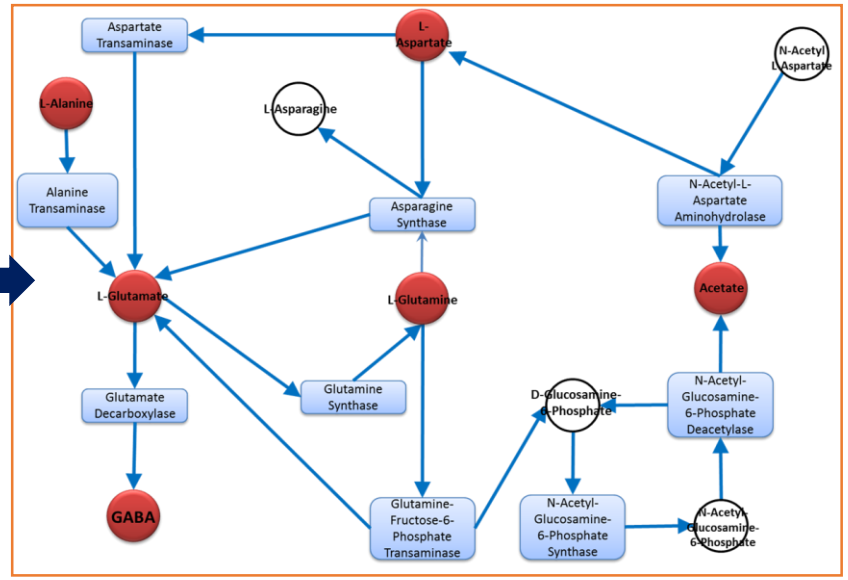
How can we connect metabolites?
Finding the cascade of biochemical reactions that connect modulated metabolites

Sub-network extraction
Graph algorithms & visualization tools to mine these large networks

Mechanistic interpretation
Frainay & Jourdan 2017 Brief. In Bioinformatics







Zalko D, Soto AM, Canlet C, et al. Endocrinology 2016

MetExplore: web server for network analysis of omics data

MetExplore Computational infrastructure for metabolic network analysis

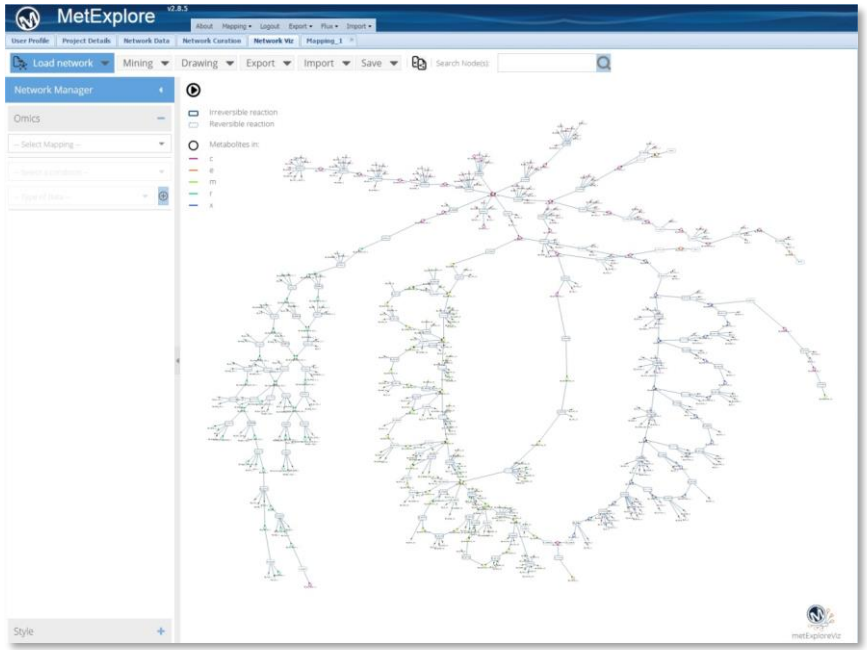
Funding: ANR MetaboHub, H2020 Phenomenal

- Long lasting project established in 2009
- >400 registered users, >350 persons trained, >20 000 visits since 2009
- Shared platform in international projects

L. Cottret et al. *Nucleic Acids Res.*, 2010

www.metexplore.fr

0 € to use it, **11** developers, **182** persons trained, **293** registered users, **841** networks, **13414** visitors

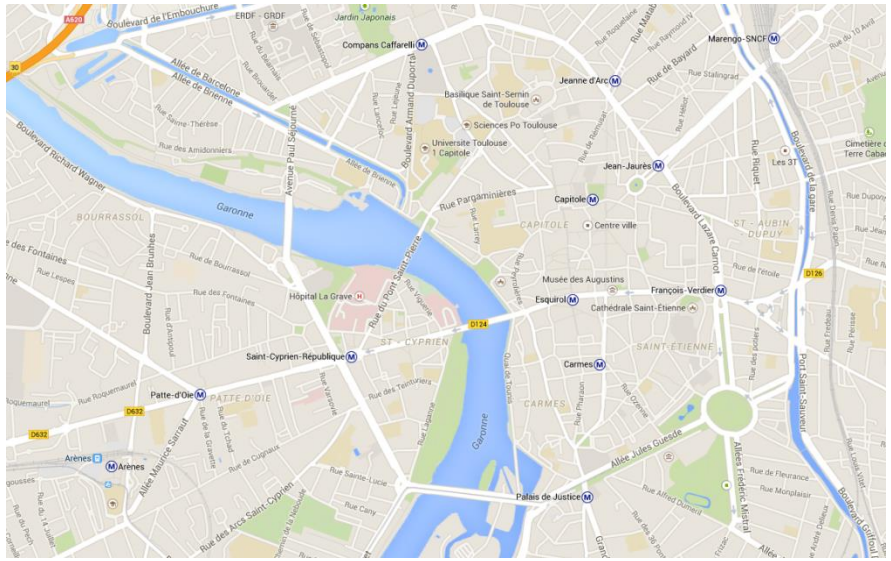


Functions:

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

Graphs *vs.* fluxes

Graph based analysis



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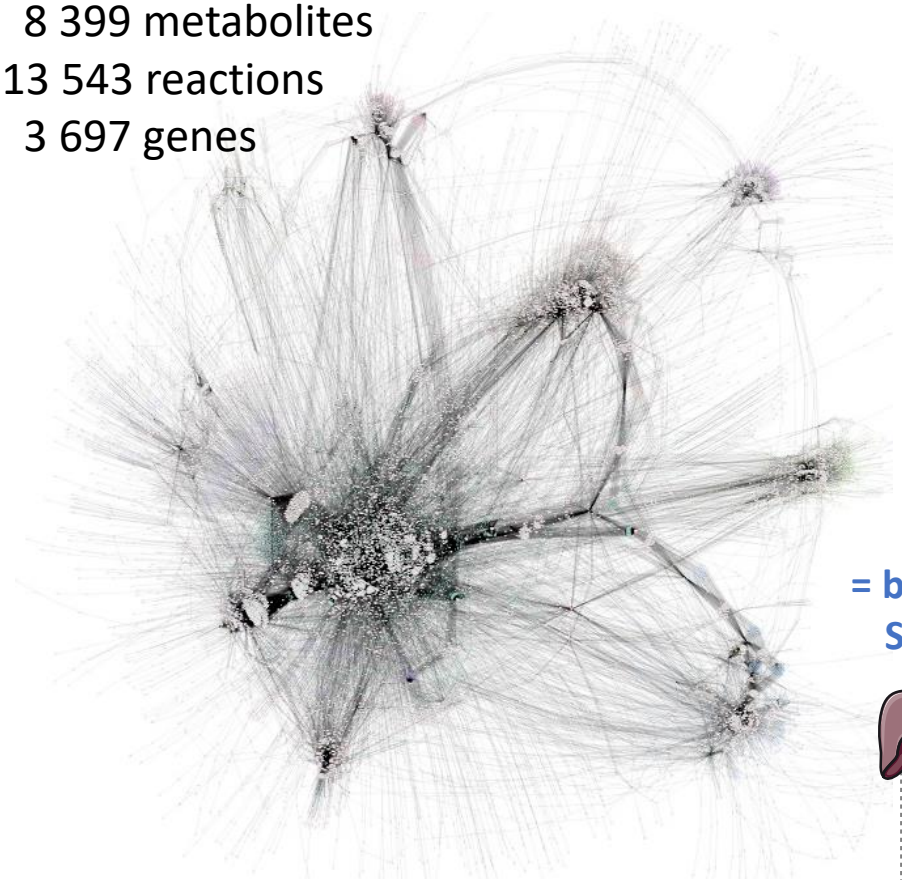
Flux & Constraint-based analysis to contextualize metabolic networks

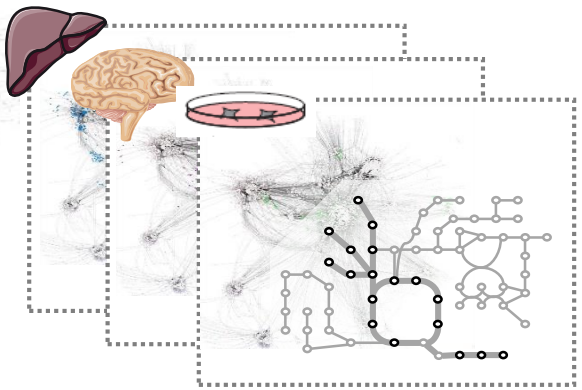
global Human metabolic network Recon3D[1]

8 399 metabolites
13 543 reactions
3 697 genes



→ **generic metabolic capacity**
= an **infinity of possible phenotypes**

CONTEXTUALIZATION
= building TISSUE- or CONDITION-SPECIFIC METABOLIC MODELS



Flux analysis approaches

Objective =
determining the activity of the reactions in a given context

principle = computing fluxes for all reactions in the network

flux *= amount of a metabolite that is synthesized or consumed through a reaction per time unit (mmol. g DW^{-1}. h^{-1})*

1) Which reactions are active?
flux ≠ 0 ⇔ réactions « actives »
flux = 0 ⇔ réactions « inactives »

1 sub-network of active reactions = functional metabolic network for a given condition

2) **Quantification of the activity** of the different metabolic pathways

[1]Brunk E., et al. *Nat Biotechnol*. 2018.

Flux & Constraint-based analysis to contextualize metabolic networks

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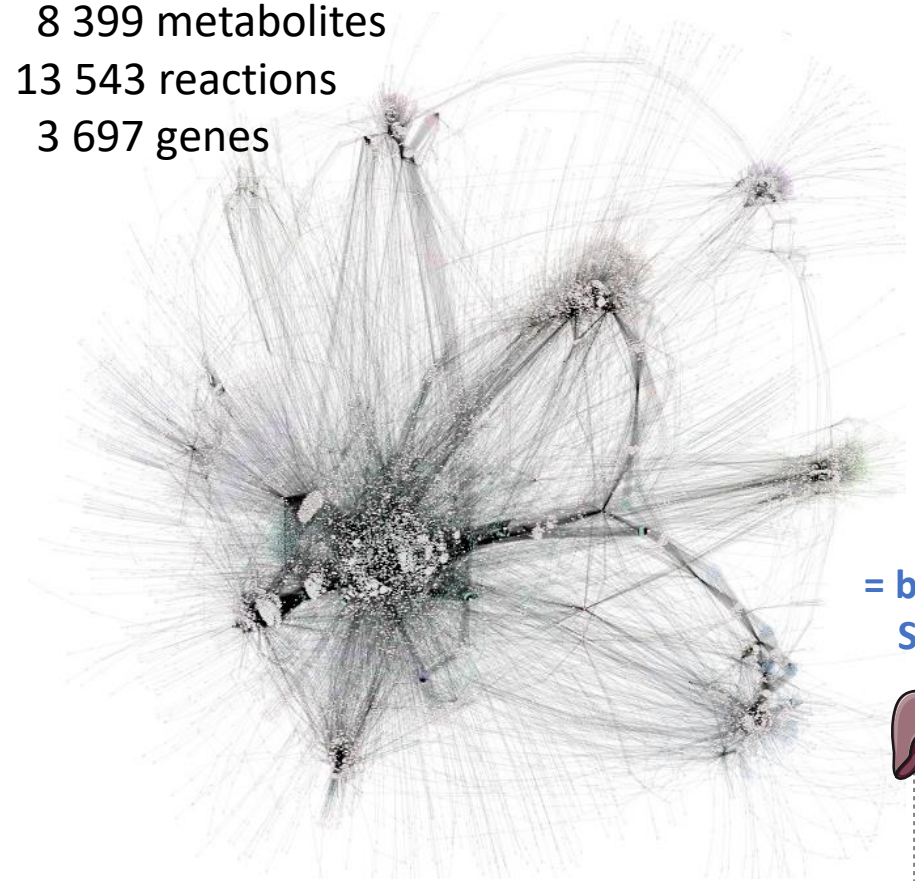

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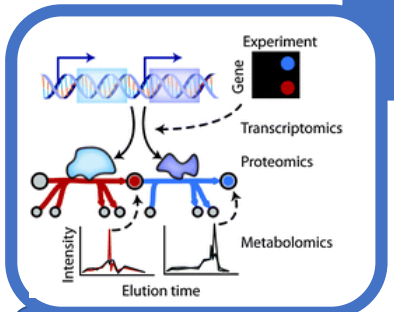
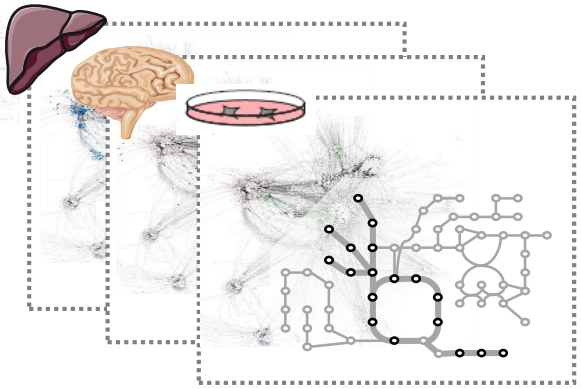
OMICS data



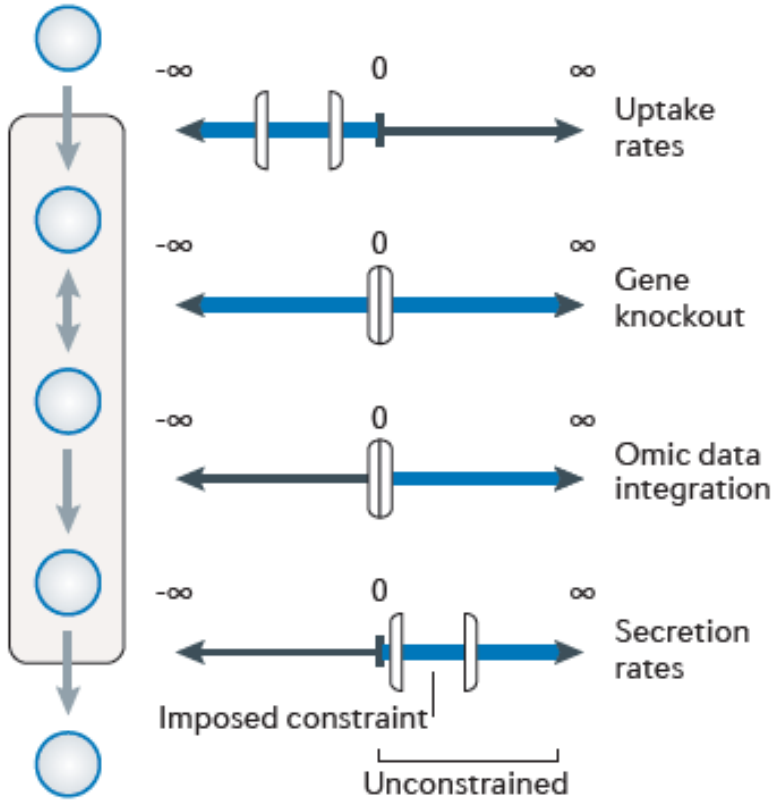
constraints

CONTEXTUALIZATION
= building TISSUE- or CONDITION-SPECIFIC METABOLIC MODELS



Constraint-based modeling

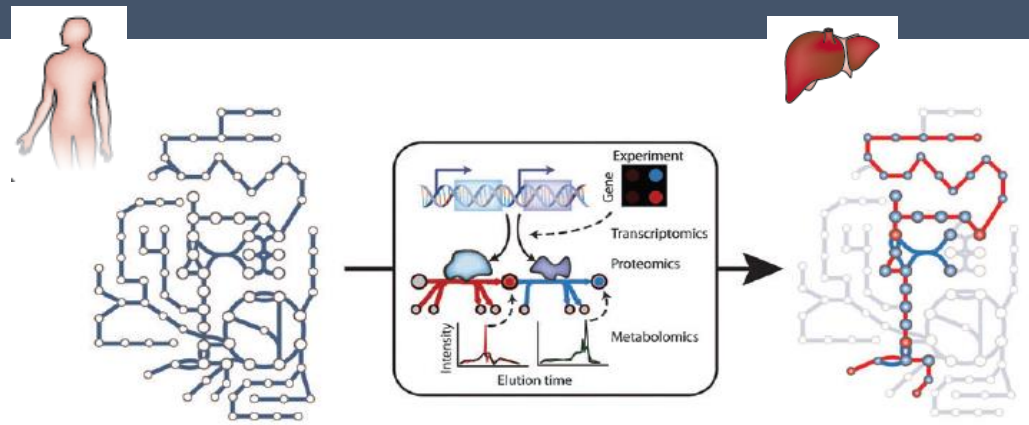
Objective =
putting constraints on reactions fluxes based on omics data



From Bordbar et al., 2014, Nature Reviews

[1]Brunk E., et al. *Nat Biotechnol*. 2018.

Building tissue-specific models



from Hyduke et al., Molecular BioSystem, 2013

computational BIOLOGY

ANALYSIS

Network-based prediction of human tissue-specific metabolism

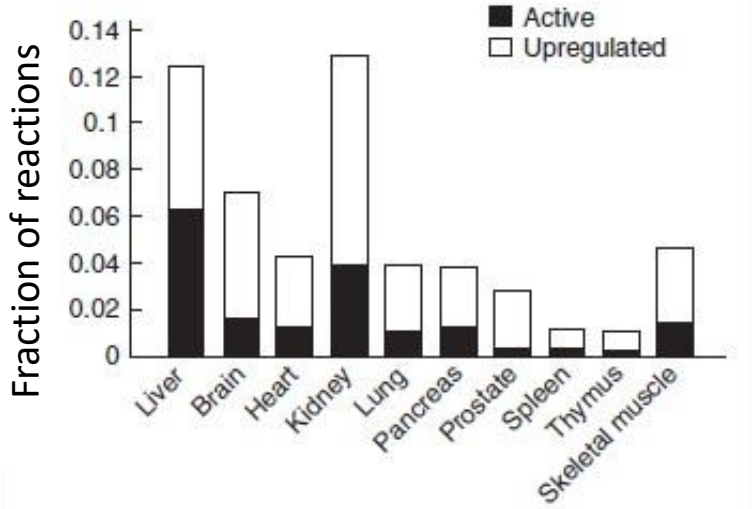
Tomer Shlomi[1,4], Moran N Cabili[1,4], Markus J Herrgård[2], Bernhard Ø Palsson[2] & Eytan Ruppin[1,3]

[1]Shlomi T et al., Nature Biotech., 2008

Gene expression data obtained from different databases

⇩

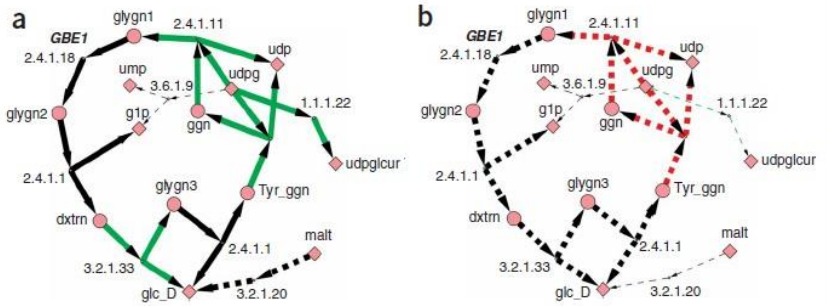
Prediction of activity state for 644 reactions (average 408 / tissue)



Reactions predicted to be active in 10 different tissues

Upregulated = predicted to be active, but not associated with highly expressed gene

liver **spleen**



Subnetwork representing the glycogen metabolism

GBE1 is predicted to be specifically active in liver

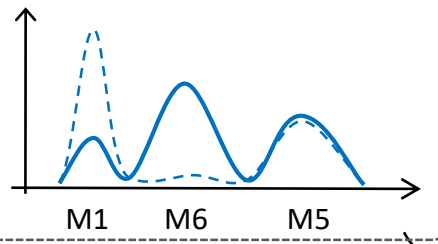
Identification of metabolic modulations from exometabolomics data

1. Converting exometabolomic profiles to uptake/secretion profile

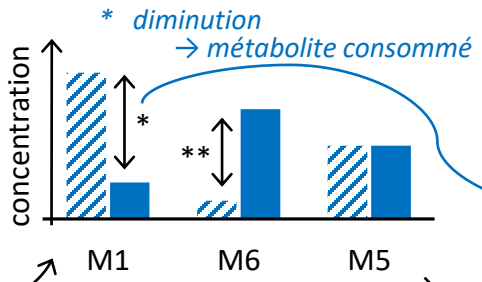
Example : ***In vitro* cell cultures**

Extraction of extracellular medium at 2 time points

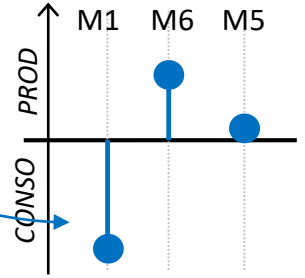
spectres (MS ou RMN) exométabolomique



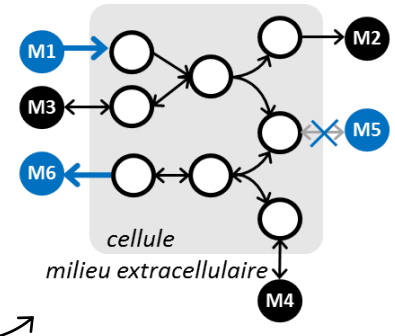
quantification des métabolites



profil de production / consommation des métabolites



calcul des quantités produites / consommées entre t0 et T24h



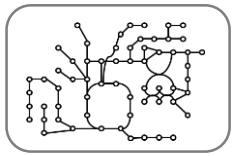
conversion en contraintes in silico

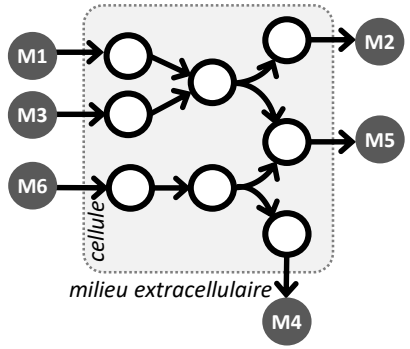
* *diminution* → *consommation* → *import forcé du milieu vers la cellule dans le modèle*
** *augmentation* → *production* → *export forcé du milieu vers la cellule dans le modèle*

Identification of metabolic modulations from exometabolomics data

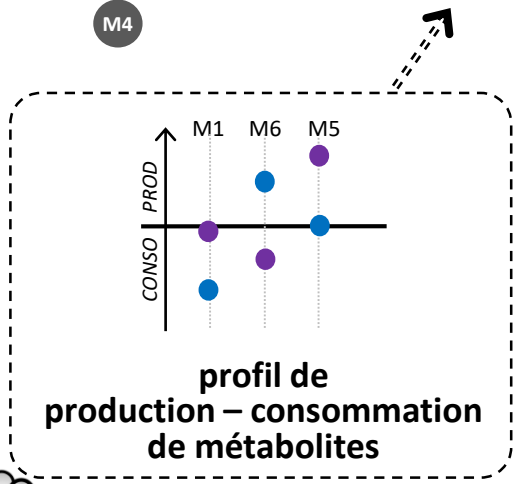
2. Computing intracellular metabolic fluxes & Identifying metabolic modulations

Réseau métabolique humain générique



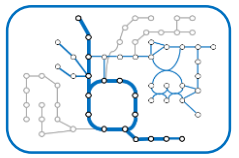


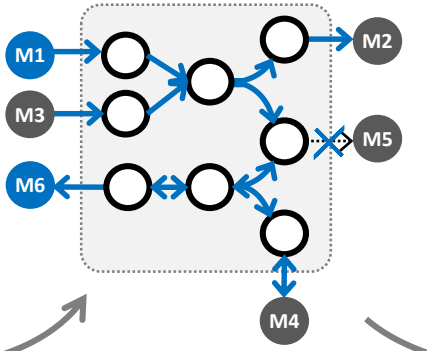
intégration des données d'exométabolomique

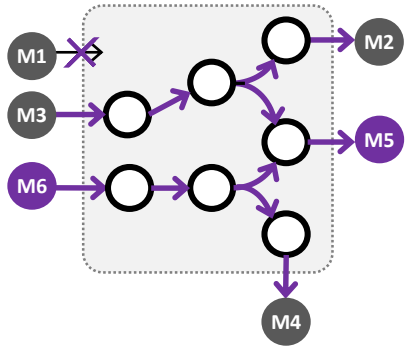


profil de production – consommation de métabolites

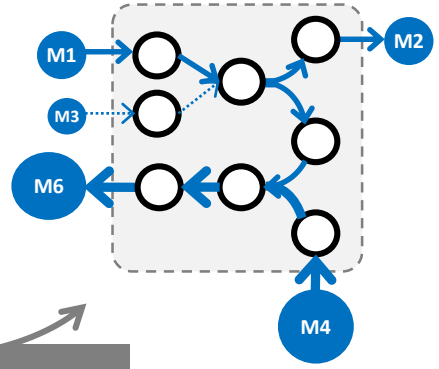
Réseau métabolique condition-spécifique

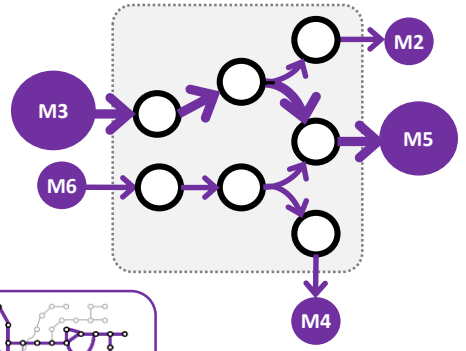


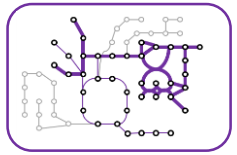


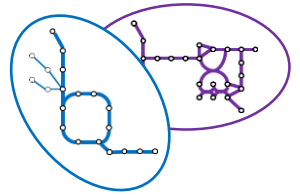


calcul des flux intracellulaires









Identification & comparaison des modulations métaboliques selon les conditions

Example : Revealing metabolic differences between 2 lymphoblastic leukemia cell lines

Aurich et al. Metabolomics. **2014. 11: 603–619.**

Prediction of intracellular metabolic states from extracellular metabolomic data

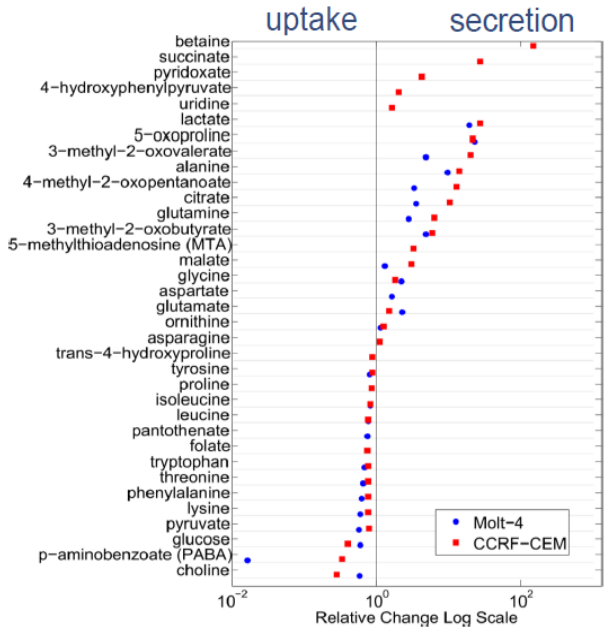
Maike K. Aurich · Giuseppe Paglia · Óttar Rolfsson · Sigrún Hrafnsdóttir · Manuela Magnúsdóttir · Magdalena M. Stefaniak · Bernhard Ø. Palsson · Ronan M. T. Fleming · Ines Thiele

Experimental data

- **2 lymphoblastic leukomia cell lines** (Molt-4 & CCRF-CEM)
- Metabolomic analyses:
  75 extracellular metabolites detected
  2 ≠ time points (2h & 48h)

identified **uptaken / secreted metabolites**

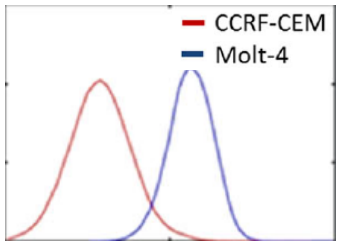
Molt-4 cells	**CCRF-CEM cells**
16 uptaken	15 uptaken
11 secreted	17 secreted



Modeling

computation of intracellular metabolic fluxes distributions

probable metabolic states for each model (probability distributions for fluxes)



probability distribution of flux value for each reaction

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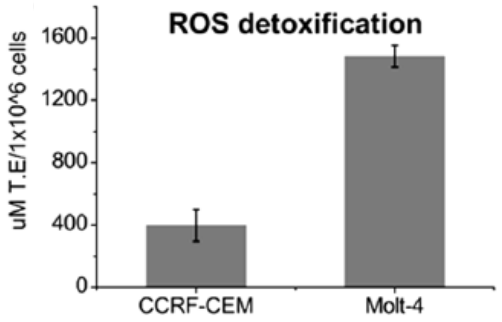
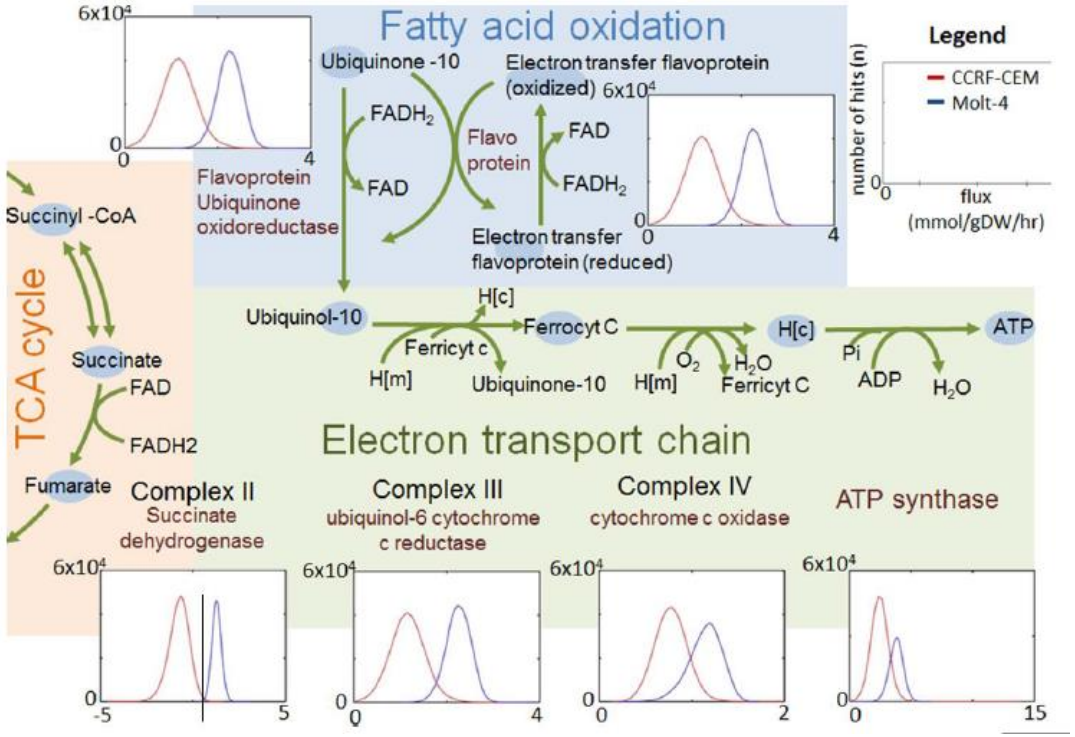
Different use of metabolic pathways predicted by the models

Higher utilization of the oxidative phosphorylation by the Molt-4 model

→ experimentally supported by **increased capacity for ROS detoxification** in Molt-4 cells





Take home messages

- **Genome scale metabolic networks provide an holistic context** …

  … but require **modeling** and **algorithms**

- **Graph-based analyses** are useful to understand the connections between metabolites and reactions
- **Flux-based analyses** can be used to predict the system behavior under specific conditions & predict biomarkers
- **"back-and-forth" are essential between experimentation and model** (for model construction, validation, refinement …)
- **A model is necessarily a simplification of the biological complexity**: the interpretation and extrapolation are limited

"Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful" *George EP Box*

Acknowledgments

MeX Team (Metabolism of Xenobiotics - D. Zalko)

F. Jourdan
M. Chazalviel
F. Vinson
C. Frainay
E. Camenen
L. Cottret

Thank you for your attention!