

Métabolomique-Fluxomique

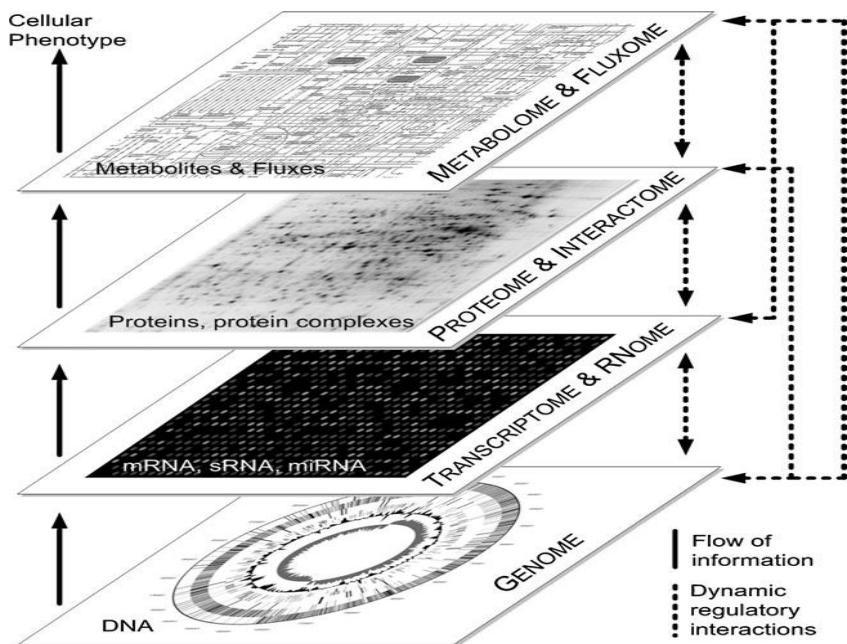
Réseaux métaboliques

Fabien Jourdan

An introduction to metabolomics

1. Introduction
2. Concepts & methods in metabolomics
 1. The world of small molecules
 2. Analytical platforms for metabolomics
 3. Different approaches
 1. Chemometrics
 2. Metabolomics
 3. Fluxomics
3. MetaToul: Toulouse metabolomics & fluxomics facilities

Comprehensive analysis of metabolic networks

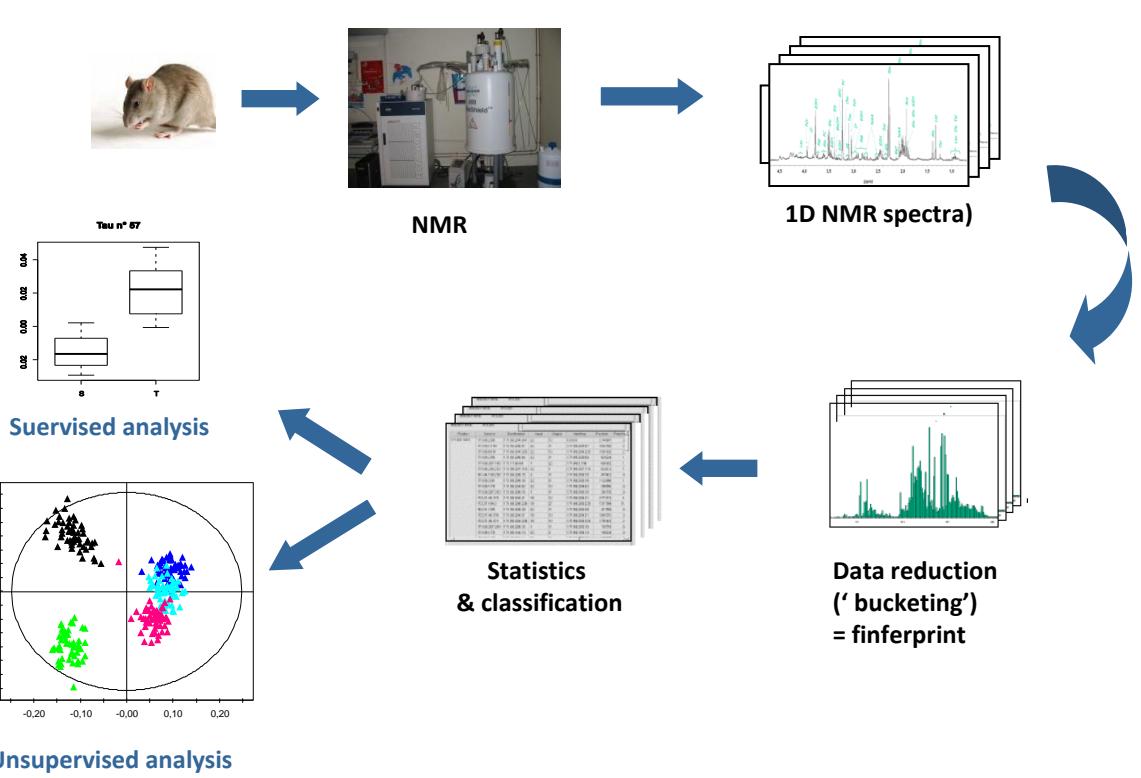


- Readout of the metabolic network
- Response to genetic/environmental perturbations
- Systems biology (& synthetic biology)
- Identification of key regulated sites in networks
- Investigation of gene function
- Phenotyping: next to any observable phenotype: diagnostics, functional genomics....

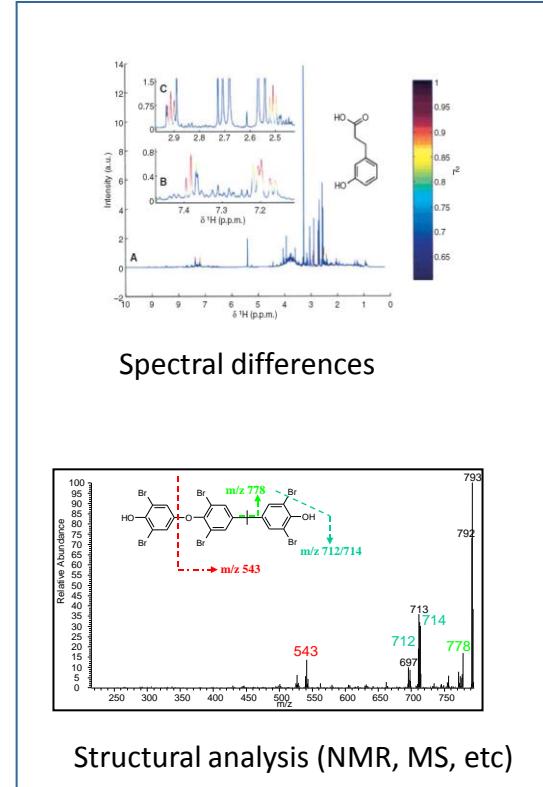
Objectives:

1. Classification of samples based on biochemical fingerprints & statistical analysis
2. Measurement of global metabolic perturbations

Metabolic fingerprinting or profiling by NMR (MS) + statistical analyses



Biomarker identification





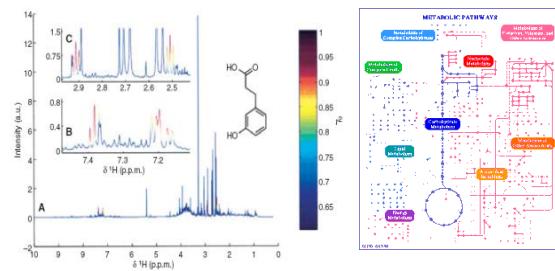
Health
Personalized medicine



Biotechnology



Pharmacology



Basic & Applied Microbiology



Nutrition & agrofood industry



Agronomy

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Metabolome

Stephen Oliver (1998, UK)

- Metabolome : “the set of all low-molecular weight compounds synthesized by an organism”.

Oliver Fiehn (Germany, 2002)

- Metabolomics (Strict definition): comprehensive analysis to identify and quantify all metabolites of a biological system.

The metabolome include peptides, lipids, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, minerals, food additives, drugs, toxins, pollutants etc that biological systems ingest, metabolize, catabolize or come into contact with.

How large is the metabolome ?



~1500



~15000



~50000*

(*currently 40153 in HMDB)

Database contents

Database	Nb of entries
PubChem	32,000,000
Chemical Abstracts (chemlist)	308,000
Dictionary of Natural Products	170,000
Human Metabolome Database (HMDB)	40,153
KEGG	17,101
BioCyc	10,965

Single species: rice

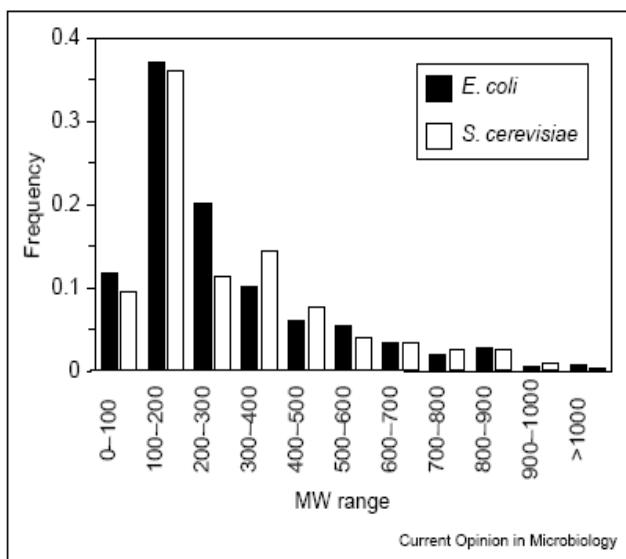
Table 1. Number of small molecules found in *oryza sativa* by database search.

Database	Oryzae compounds
Compound DBs	
PubChem/PubMed	>3000
CAS	>1400
Beilstein	554
SetupX	268
Dr. Duke	201
DNP	55
KNAPSAcK DB	48
Ortholog/Pathway DBs	
KEGG	3661
RiceCyc	1500
Reactome	396
Patent DBs	
IBM Patent Search	9780

The table reports all organic metabolites with possible organism mix-ups including bacterial and fungal n
doi:10.1371/journal.pone.0005440.t001

See: <http://www.metabolomicssociety.org/databases>

Molecular mass range



80% of hydrophilic metabolites have a molecular mass ≤ 600 (*E. coli*, *S. cerevisiae*)

Physico-chemical diversity

➤ Comparison with transcriptomics/proteomics

ADN/ARN: 4 bases

Proteins: 20 amino-acids

Common physico-chemical properties

Extraction/analysis: easiest automation

➤ Metabolites

Number $\geq 150\,000$ in Nature (most are unknown)

Most of them not identified

broad chemical diversity

High turn-over rates (<sec)

Dynamic range:

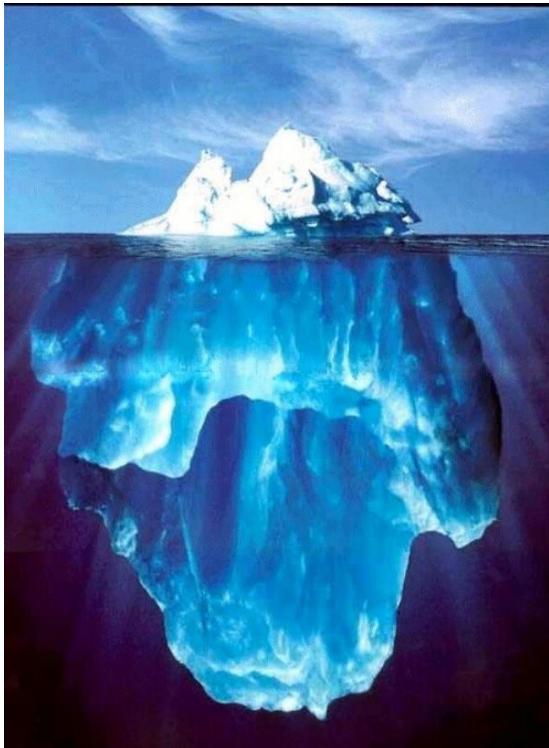
Major constituents (sugars, etc.): 10-100 mM

Minor constituents (vitamins, etc.): pM



The metabolome analysis relies on combinations of approaches

Dynamic range of the metabolome (concentration range)



$\sim 0,1 \text{ M}$

(major components, carbon
sources, sugars, etc)

9 decades

= dynamic range of the metabolome in a
single sample

(the dynamic range of a MS detector is 4-5
decades)

$\sim 10^{-10} \text{ M}$

(minor components, bioeffective
compounds: signal molecules, etc)

The timescales of the metabolome are short

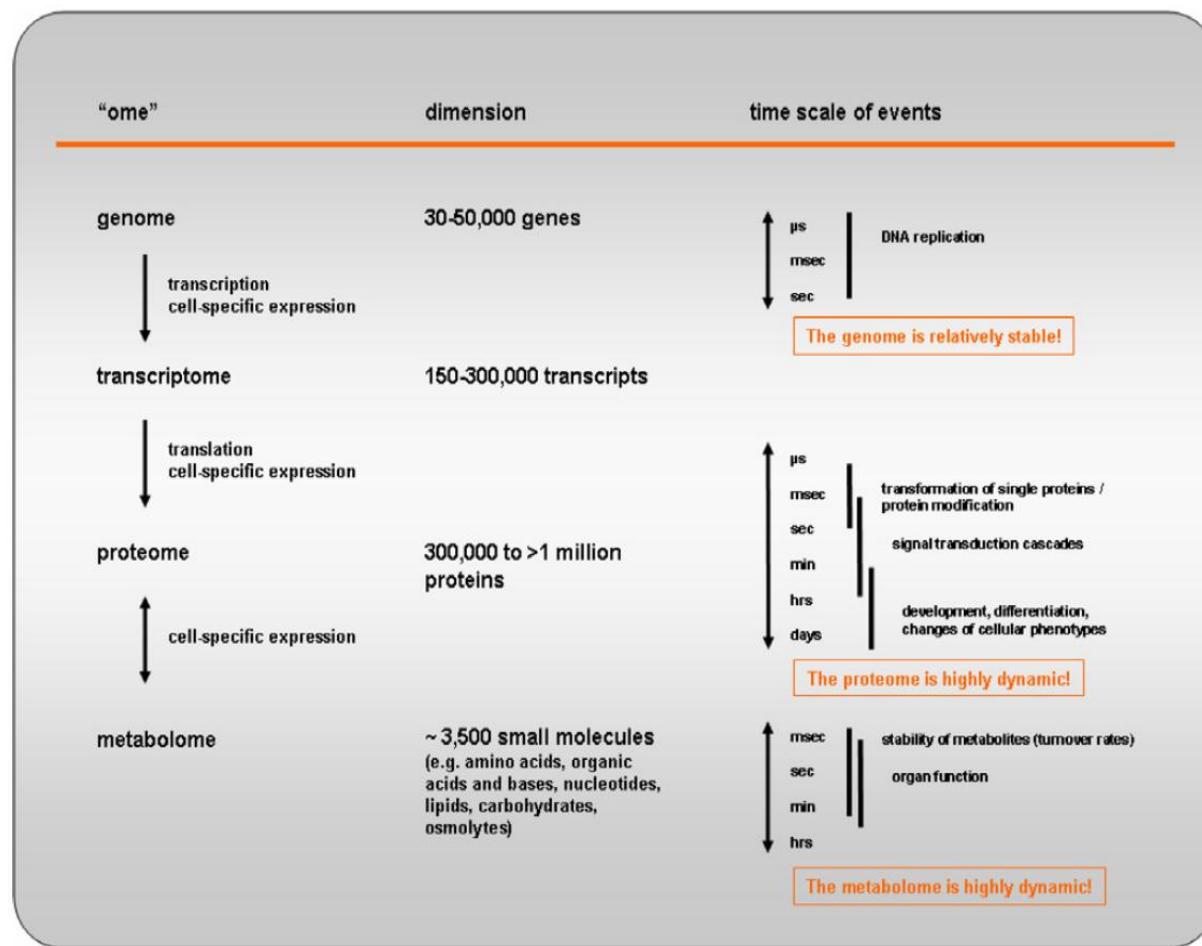


Figure 2. Complexity of the different "omic" levels with respect to quantitative dimensions and time-scales of processes within an organism (time-scale for proteome adapted from [41]).

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NMR & MS are the main platforms for metabolomics

NMR



Advantages

- Easy sample preparation (biofluids)
- Analysis of complex mixtures without separation or a priori consideration
- Detailed structural information
- Multi-nuclear: ^1H , ^{13}C , ^{31}P , ^{15}N , etc
- Isotopic analysis (stable isotopes)
- in vivo /in situ analysis (non invasive & non destructive)
- (Can be coupled to LC)
- Robust, reliable (HT analysis)

Drawbacks

- Limited sensitivity (micro to nano-moles):
access to major metabolites only
- Cost of equipments
(analysis not necessarily expensive)
- Dynamic range

MS



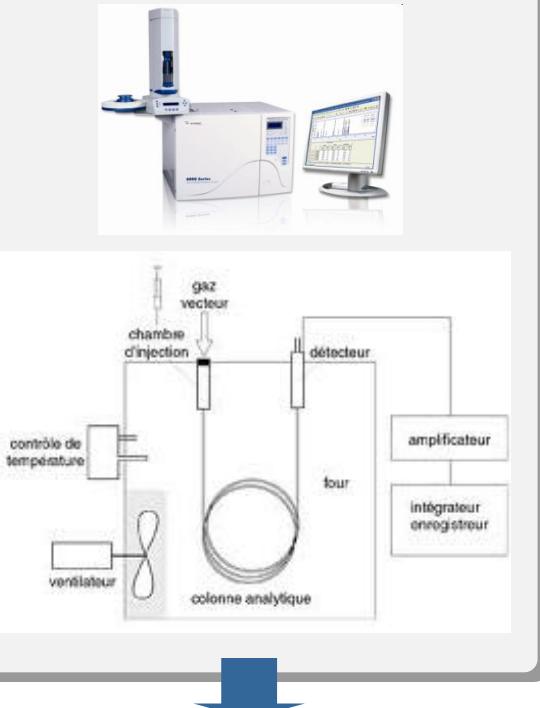
Advantages

- Mass information (identification)
- Flexibility brought by the various types of MS detectors
- High sensitivity (10s attomole)
- Isotopic analysis (stable/radioactive)
- Complex mixtures: need for separation: GC/LC/CE

Drawbacks

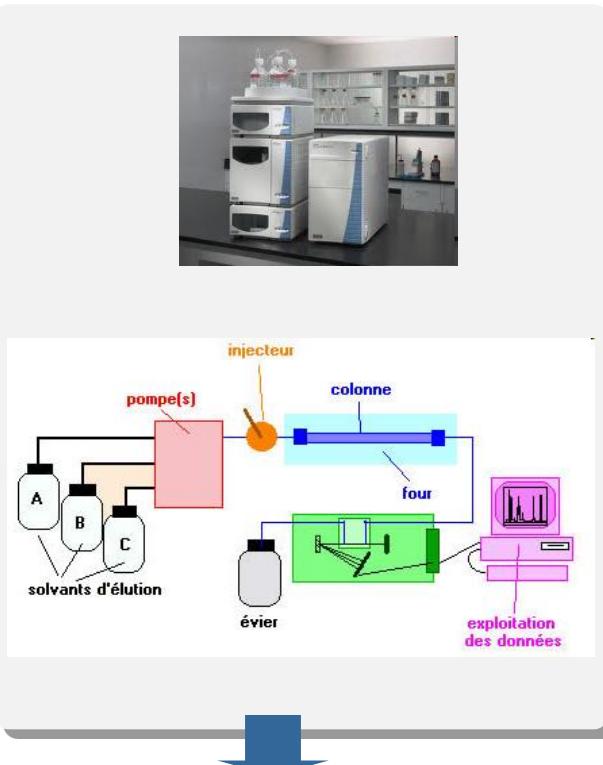
- Complex mixtures: need for separation: GC/LC/CE
- (Cost of equipments)
- Lack of robustness for fingerprinting
- Lack of reproducibility between equipments (LC-MS)

Gaz chromatography



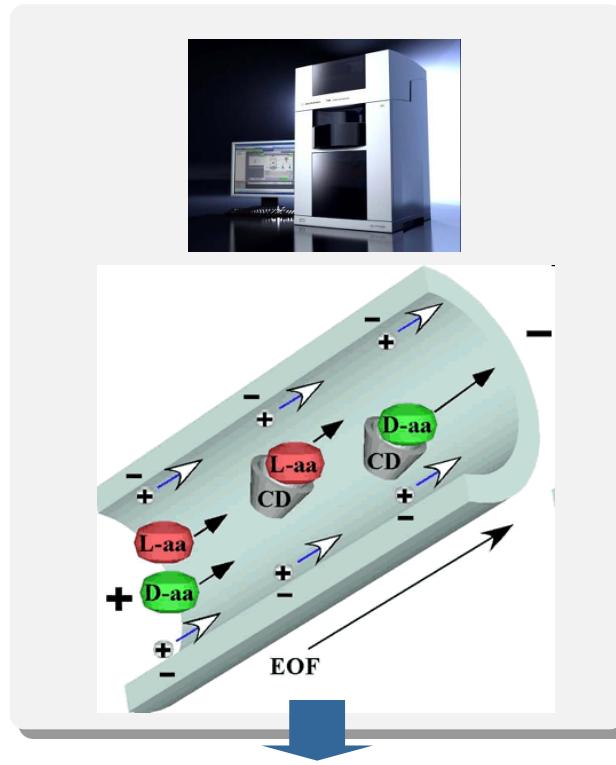
Volatile compounds
(~20% of known compounds)

Liquid Chromatography



Soluble compounds
(~80% of known compounds)

Capillary electrophoresis



Soluble ionic compounds

Combining analytical platforms: improved metabolome

coverage

frontiers in
PLANT SCIENCE

TECHNOLOGY REPORT
published: 10 February 2012
doi: 10.3389/fpls.2012.00015



Metabolomics as a hypothesis-generating functional genomics tool for the annotation of *Arabidopsis thaliana* genes of “unknown function”

Stephanie M. Quanbeck¹, Libuse Brachova¹, Alexis A. Campbell¹, Xin Guan¹, Ann Perera¹, Kun He², Seung Y. Rhee², Preeti Bais³, Julie A. Dickerson³, Philip Dixon⁴, Gert Wohlgemuth⁵, Oliver Fiehn⁵, Lenore Barkan⁶, Iris Lange⁶, B. Markus Lange⁶, Insuk Lee⁷, Diego Cortes⁸, Carolina Salazar⁹, Joel Shuman¹⁰, Vladimir Shulaev⁹, David V. Huhman¹¹, Lloyd W. Sumner¹¹, Mary R. Roth¹², Ruth Welti¹², Hilal Ilarslan¹³, Eve S. Wurtele¹³ and Basil J. Nikolau^{1*}



Analysis of *A. thaliana* metabolome

Table 1 | Summary of metabolites/compounds identified by the analytical laboratories in the *Arabidopsis* Metabolomics Consortium.

Analytical platform	Profiling Laboratory	Number of metabolites chemically annotated	Number of metabolites with unknown chemical annotation	Total number of metabolites
GC-TOFMS	Fiehn	196	419	615
UHPLC-QTOFMS	Sumner	176	157	333
Glycerolipids	Welti	159	0	159
Fatty acids	Nikolau	59	112	171
Cuticular waxes	Nikolau	37	25	62
Phytosterols/tocopherols	Lange	11	17	28
Chlorophylls/carotenoids	Lange	6	3	9
CE-MS	Shulaev	36	36	72
LC-MS	Shulaev	57	10	67
Total		737	779	1516

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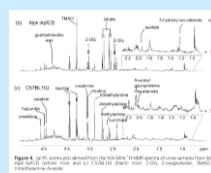
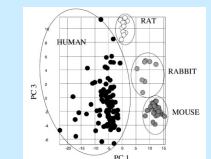
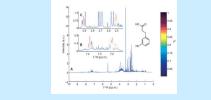
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Chemometrics

Classification of samples based on biochemical fingerprints and statistical analysis & biomarker discovery

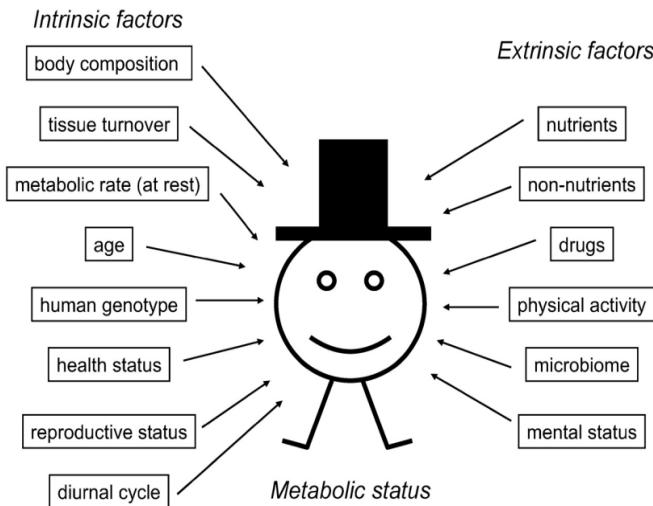
Pioneering work from **Jeremy Nicholson (Imperial College, London, UK), late 90'**

Objectives	Methods	
1. High-throughput Phenotyping (Fast tools for biochemical analysis)	- Metabolic fingerprints (NMR, MS) - Metabolic profiles (quantitative)	 Figure 4 Panel (a) shows 1H NMR spectra of urine from healthy volunteers and patients with metabolic diseases. Panel (b) shows 13C NMR spectra of the same samples. The spectra are overlaid with chemical structures of the metabolites: alpha-Acid glycoprotein, creatinine, citrate, and trimethylamine.
2. Revealing perturbations	- comparative analysis (up to cohorts) - Statistical analysis: classification, unsupervised or supervised approaches	 Figure 5 PCA score plot showing the separation of four animal models (HUMAN, RAT, RABBIT, MOUSE) based on their metabolic profiles. The plot has PC 1 on the x-axis and PC 3 on the y-axis. Data points are colored by species: HUMAN (red), RAT (blue), RABBIT (green), and MOUSE (yellow).
3. Biomarker discovery (diseases, intoxication, nutritional states, etc.)	- Conventional structural analysis (NMR, RMN, etc.) - Spectral differences (e.g. STOCSY)	 Figure 6 1H NMR spectrum of a sample showing a complex multiplet at approximately 3 ppm. A color-coded 2D NMR correlation map is overlaid, showing correlations between different peaks. A 1D 1H NMR spectrum is also shown below the 2D map.

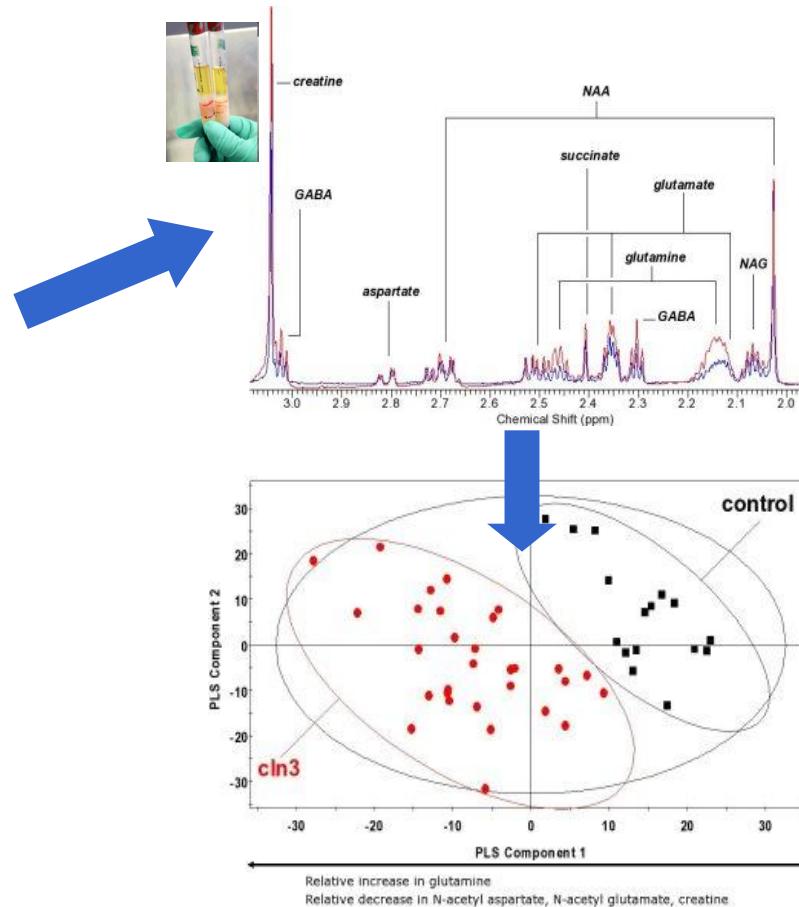
'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data.

Nicholson et al. *Xenobiotica*. 1999 29:1181-9.

Factors influencing the metabolic status

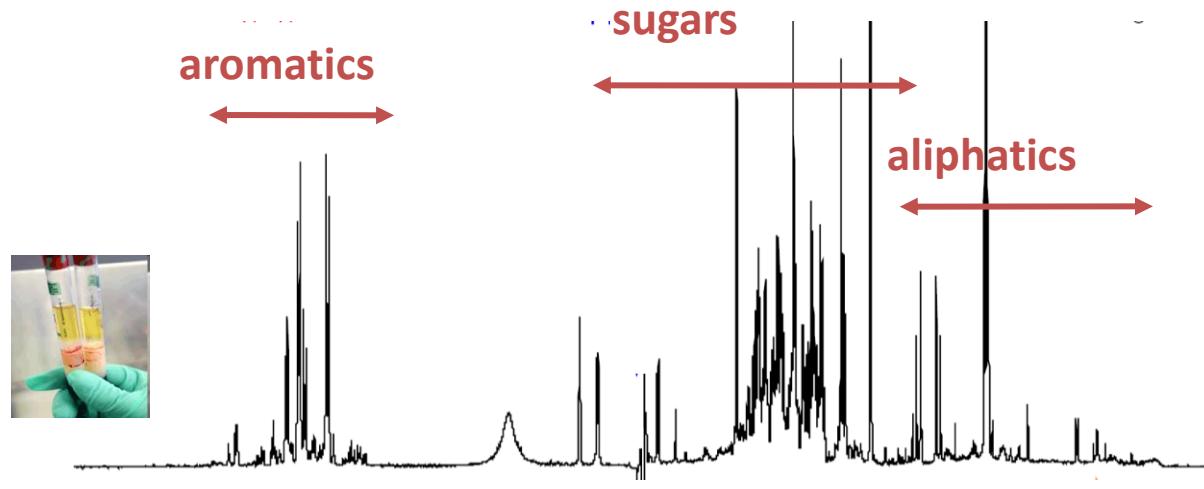


The metabolic status is highly sensitive to genome, environment and behaviour variations
= a good probe for the assessment of perturbations



NMR-based metabolic fingerprinting & profiling: Detecting occurring (major) metabolites without a priori considerations

NMR



1H-NMR spectrum of a urine sample

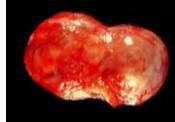
Samples



- Biofluids
Urine, blood, plasma, LCR, etc..
Cultivation media, exsudates (plants), etc



« Easy to collect and require limited sample preparation for NMR



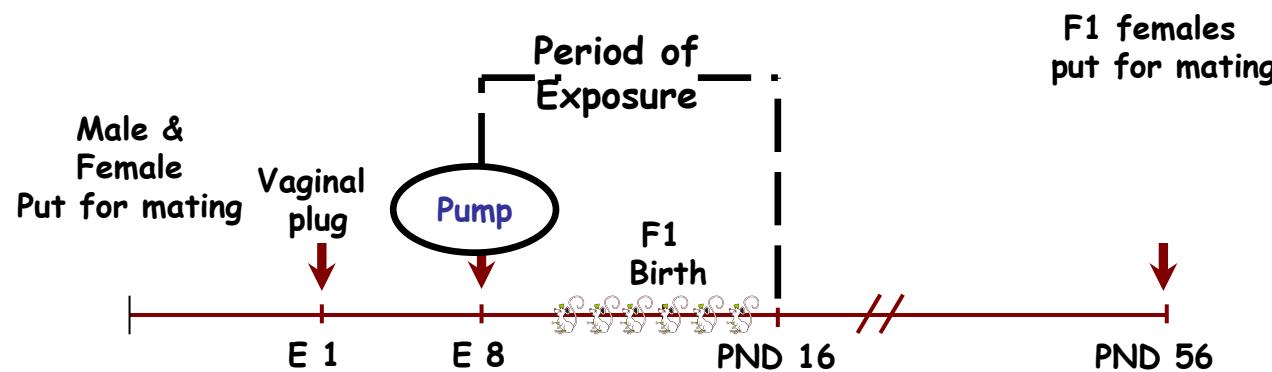
- Tissues, biopsies
in situ analysis (HR-MAS NMR)



- Extracts
tissular or cellular extracts
microorganisms, plants, animal, etc.

Partenaires: D. Zalko, TOXALIM
A. Soto, Tufts Univ. School of Med., Boston

Perinatal exposure of CD1 mice to BPA



10 animals per group

Treatment:
Control (50% DMSO)
25 ng BPA/kg BW/d
250 ng BPA/ kg BW /d
25 µg BPA/ kg BW /d

10 ng DES/ kg BW /d (positif control)

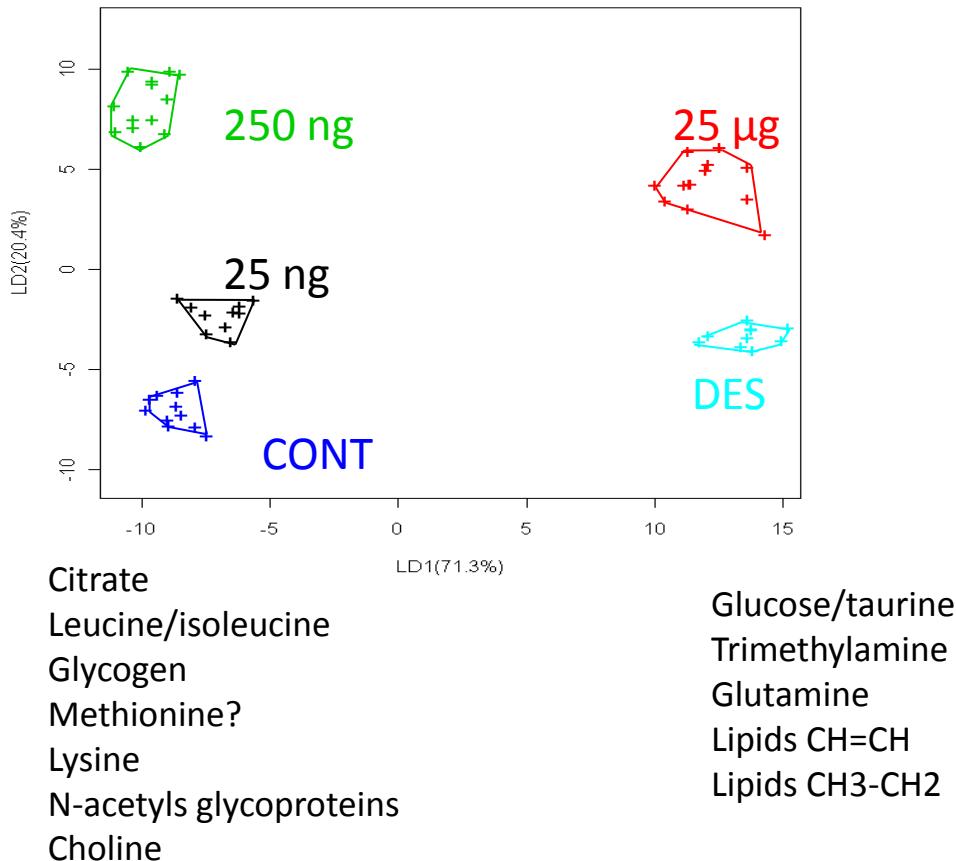
Daily tolerable
dose (DJR) :
50 µg /kg/day

Factorial Discriminant Analysis

F1 mice serum at 21 jours

Alanine
 Glucose
 Creatine
 Taurine
 Threonine

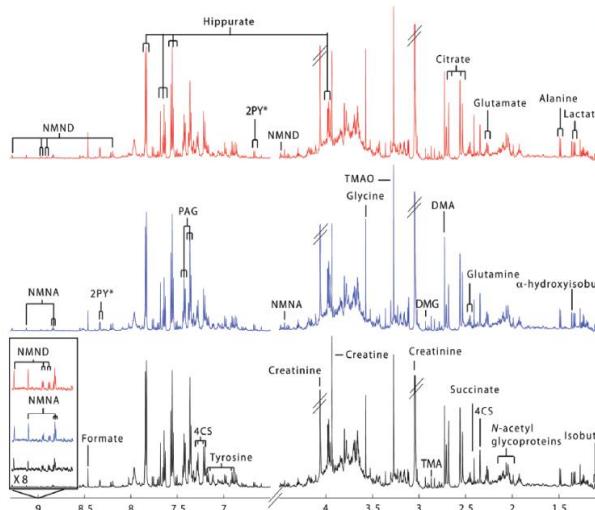
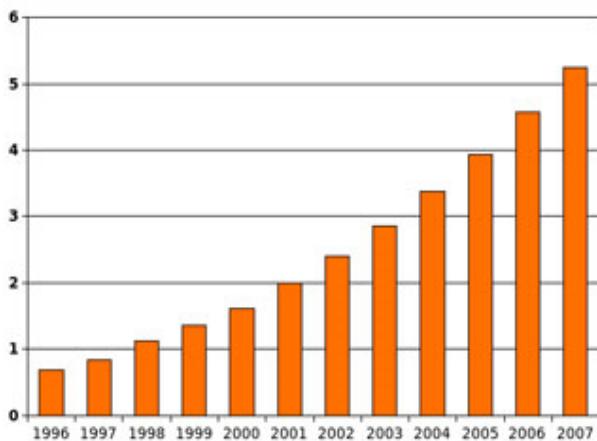
 Glutamate/pyruvate
 Glycogen
 Leucine/isoleucine



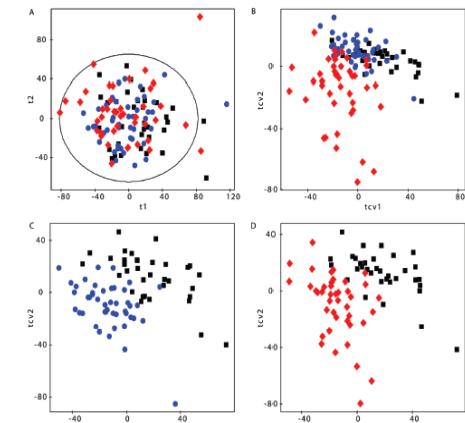
Urinary Metabolic Phenotyping Differentiates Children with Autism from Their Unaffected Siblings and Age-Matched Controls

Journal of
proteome
research 2010

Ivan K. S. Yap,[†] Manya Angley,^{†,‡} Kirill A. Veselkov,[†] Elaine Holmes,[†] John C. Lindon,[†] and Jeremy K. Nicholson*,[†]



Median 600 MHz profiles of urines from autistic patients, siblings, and controls



Statistical analysis (PCA) of urine profiles

Increase of autism prevalence in the U.S.A.
(nb/1000 inhabitants)

Urinary Metabolic Phenotyping Differentiates Children with Autism from Their Unaffected Siblings and Age-Matched Controls

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Ivan K. S. Yap,[†] Manya Angley,^{†,‡} Kirill A. Veselkov,[†] Elaine Holmes,[†] John C. Lindon,[†] and Jeremy K. Nicholson^{*,†}

Covariance profiles (STOCSY)

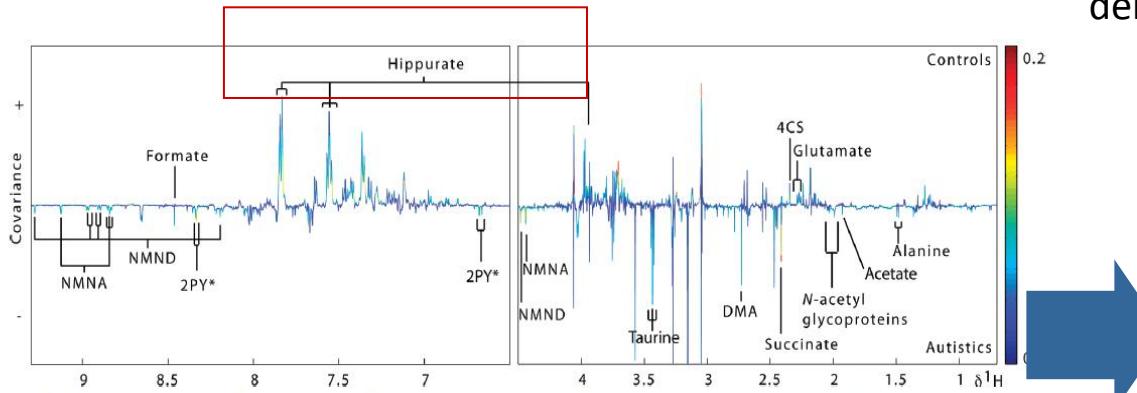


Figure 3. O-PLS-DA coefficients plot showing differences in urinary profiles between controls and autistics.

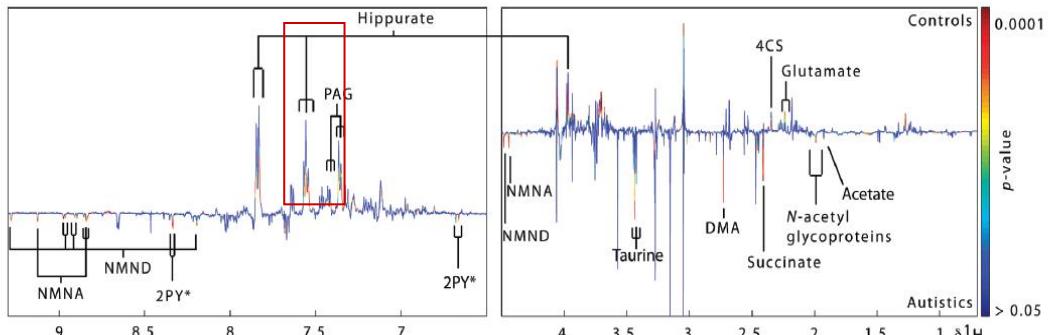
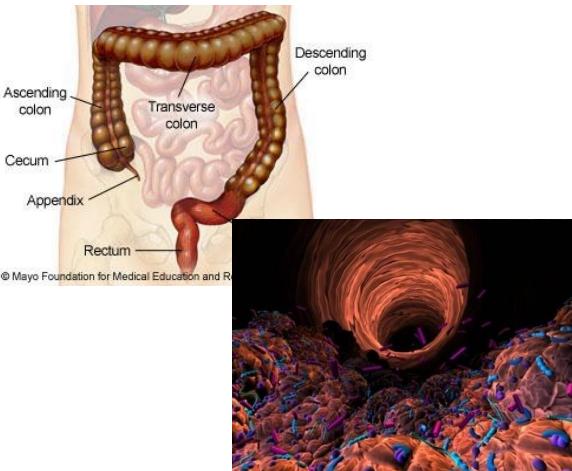


Figure 4. Covariance plot showing the color-coded significance of the urinary metabolite profiles calculated using the permutation test between the control and autistic groups. The small red-colored peak at $\delta 1.27$ is tentatively assigned to β -hydroxyisovalerate and this was found subsequently not to be significantly different between subject groups.

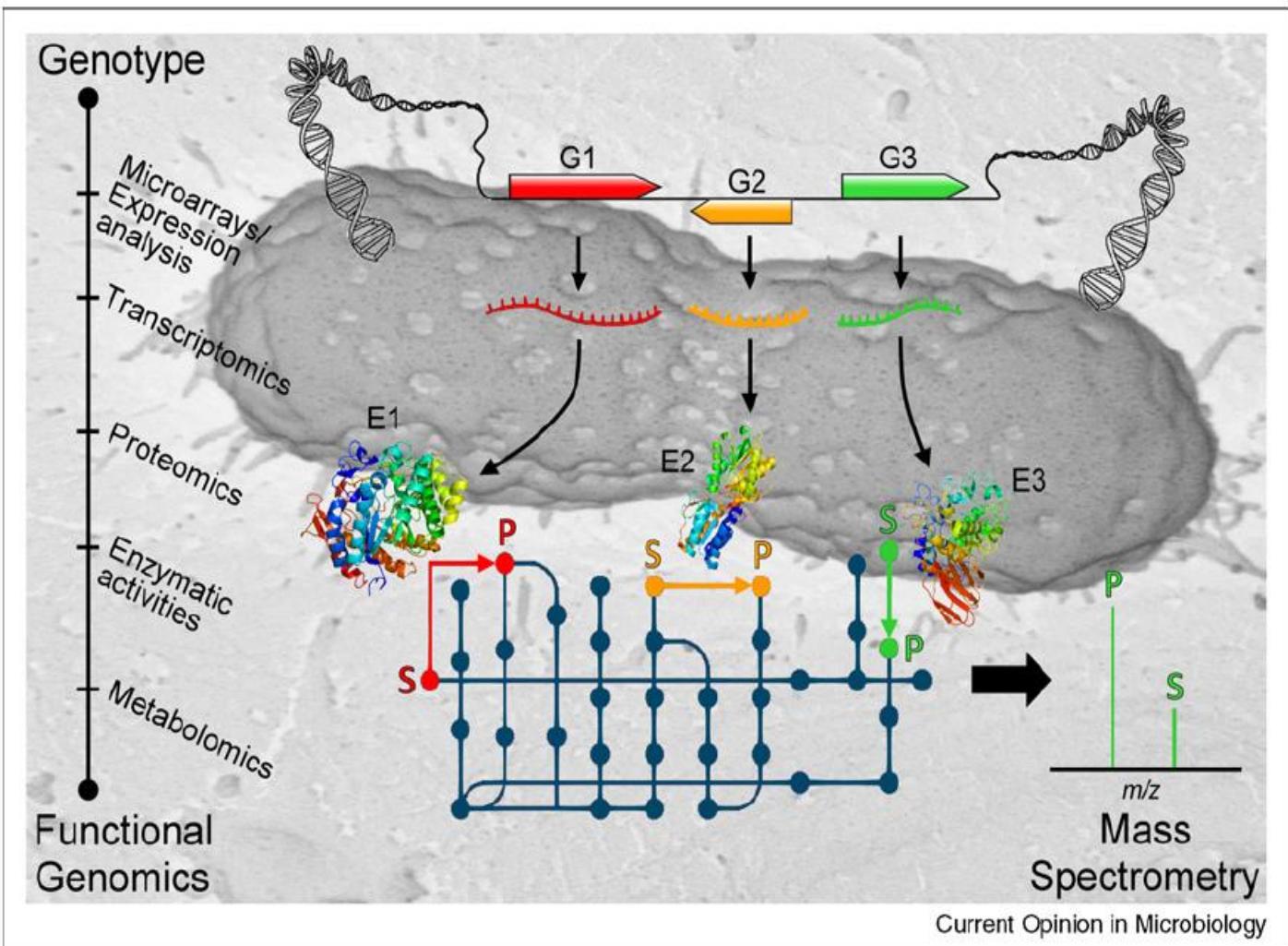
Hippurate and phenylacetylglutamine (PAG) are derived from precursors synthesized by gut organisms



Link with increase in clostridia species within gut microflora ?

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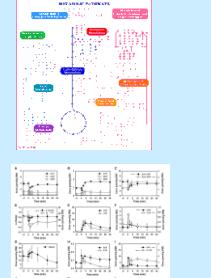
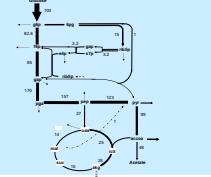
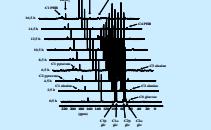


Functional genomics (comprehensive metabolome analysis)

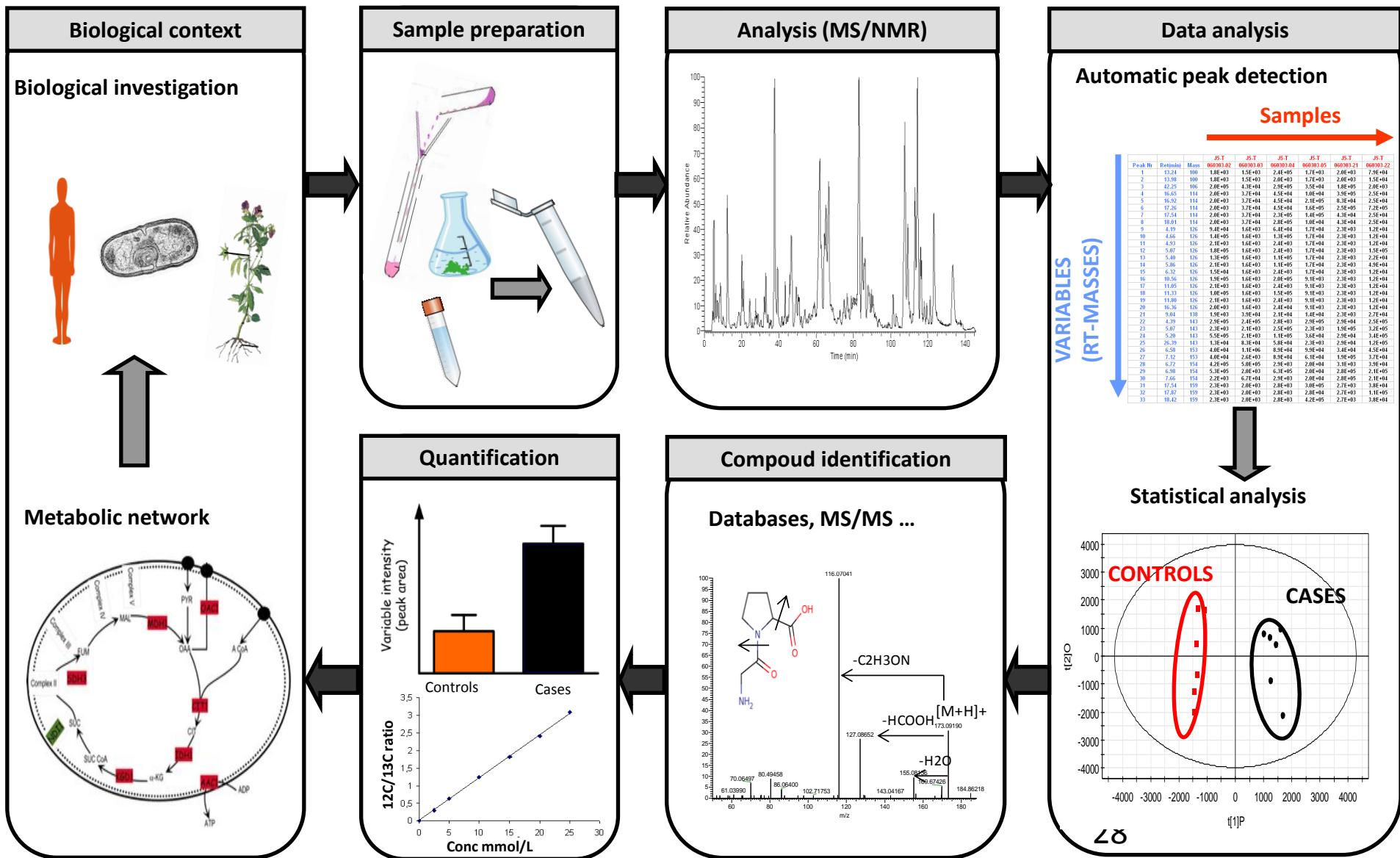
Metabolomics: identify/quantify the metabolome

(O. Fiehn, early 00's: large scale metabolite analysis)

Fluxomics: quantify the activity of metabolic reactions

Objectives	Tools	
1. Identify the metabolome - metabolites - reactions	<ul style="list-style-type: none">- targeted metabolomics- global metabolomics (the whole metabolome)- isotopic studies (^{13}C, ^{15}N, etc.): identification of metabolites or reactions- Metabolic network reconstruction	
2. Quantify the metabolome - metabolites - reactions	<ul style="list-style-type: none">- Quantitative metabolomics (IDMS, etc.)- ^{13}C-fluxomics- Flux calculation tools (mathematical models)	
3. Metabolic response to genetic or environmental perturbations	<ul style="list-style-type: none">- Comparative analysis- <i>in vivo</i> / <i>in situ</i> NMR	

The metabolomics workflow



Sampling for Metabolome Analysis of Microorganisms

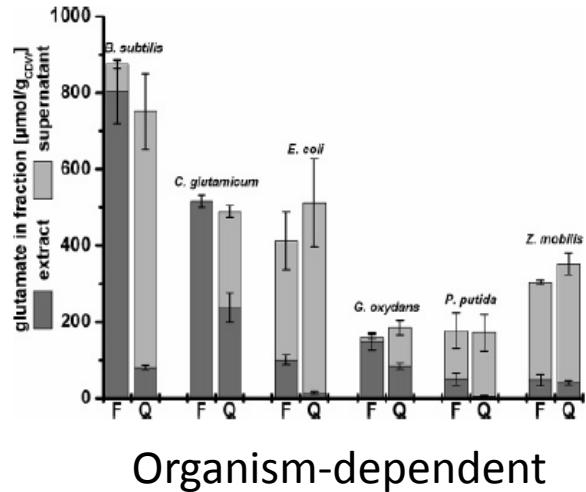
Christoph J. Bolten,[†] Patrick Kiefer,^{‡,†} Fabien Letisse,^{‡,§} Jean-Charles Portais,^{‡,§} and Christoph Wittmann^{*,†}

Collection & Quenching in 3-4 sec

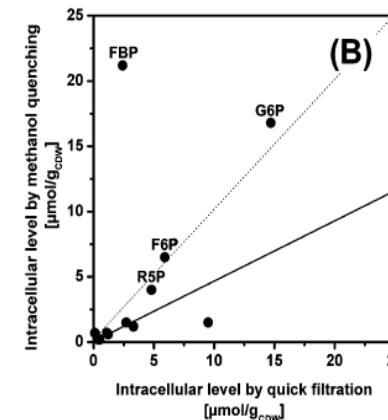


Time-dependent:
immediate blockage of
metabolic activity

Various extraction methods



Organism-dependent



compound-dependent

& storage !

Even with *E. coli* there is currently no truly reliable metabolite sampling method !!

The challenge: identification of compounds in (highly) complex mixtures

LC/MS spectrum

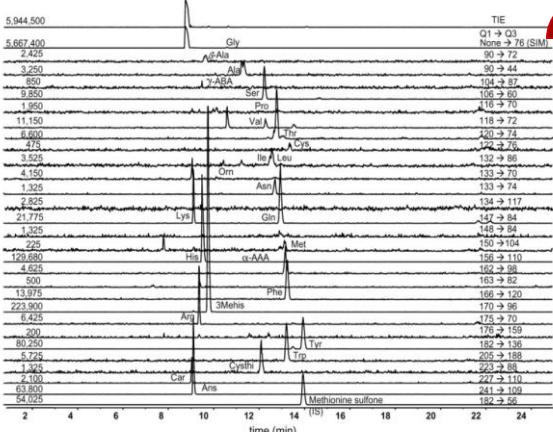


Figure 4. Electropherogram of amino acids in human urine by CE-ESI-MS/MS. The human urine was diluted with Milli-Q water (1:5). The numbers in the left corner of each trace are the abundances associated with the highest peak in the electropherogram for TIE and each m/z , and the numbers in the right are m/z ions of Q1 (protonated precursor ion) and Q3 (product ion) in MRM for each analyte. Other conditions as in Fig. 2.

Formula ?

$C_aH_bO_cN_dS_eP_f\dots$

An accurate mass does not give a unique formula

Table 2: Limits for unique formula assignment at certain levels of mass accuracy [ppm]. Above the listed mass ranges multiple formula findings cumulate. The CAS database sometimes reports D instead of H and radicals and ions as substances. Molgen was used with lowest element valence values. Formulas must contain C and H out of elements CHNSOP

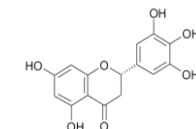
ppm	mass range < [Da]	example compound 1	example compound 2	CAS Hits formula 1	CAS Hits formula 2	MOLGEN formula 1	MOLGEN formula 2
0.1	185.9760	CH ₂ N ₂ O ₉	C ₄ H ₁₁ PS ₃	0	6	7116	1116
0.5	138.0000	C ₄ H ₂ N ₄ S	C ₃ H ₈ O ₂ P ₂	27	0	247932	353
1	126.0000	C ₂ H ₈ O ₂ P ₂	C ₃ H ₂ N ₄ S	1	27	2852	24928
2	126.0000	C ₂ H ₈ O ₂ P ₂	C ₃ H ₂ N ₄ S	1	27	2852	24928
3	126.0000	C ₂ H ₈ O ₂ P ₂	C ₃ H ₂ N ₄ S	1	27	2852	24928
4	95.9981	C ₃ H ₂ N ₂ P	CH ₄ O ₃ S	0	17	522	14
5	95.9981	C ₃ H ₂ N ₂ P	CH ₄ O ₃ S	0	17	522	14
6	95.9981	C ₃ H ₂ N ₂ P	CH ₄ O ₃ S	0	17	522	14
7	95.9981	C ₃ H ₂ N ₂ P	CH ₄ O ₃ S	0	17	522	14
8	95.9981	C ₃ H ₂ N ₂ P	CH ₄ O ₃ S	0	17	522	14
9	95.9981	C ₃ H ₂ N ₂ P	CH ₄ O ₃ S	0	17	522	14
10	93.9911	CH ₂ O ₅	C ₂ H ₆ S ₂	1	22	9	5
20	77.9788	CH ₂ O ₂ S	CH ₄ P ₂	13	20	9	4

A formula does not give a unique compound

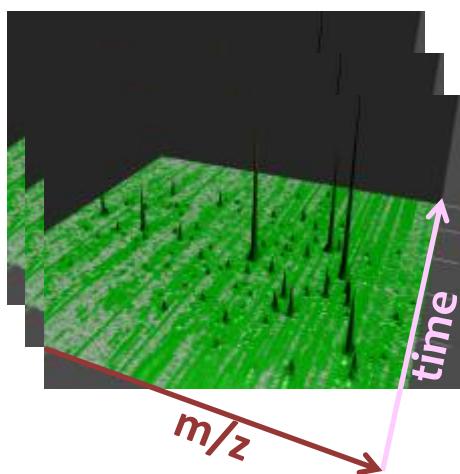
Table 1: Example of a molecular formula search for $C_{15}H_{12}O_7$ in different chemical databases. Search date: July 2007

Database name	Compounds found	Total database entries
Chemical Abstracts (CAS)	181	24,000,000
Beilstein Database (MDL)	166	8,000,000
Dictionary of Natural Products (DNP)	129	170,000
PubChem (NIH)	19	800,000
Available Chemicals Directory (MDL)	6	400,000
ChemIDplus (NIH)	6	370,000
KEGG (Kyoto University)	3	13,000
NIST05 (NIST mass spectral database)	2	163,000
MOLGEN molecular isomer generator (allowing 2 benzene groups; 1 ether group, 1 keto group; 5 hydroxy groups)		788,000

Structure ?



Molecular phenotyping: from raw data to identification



n raw files

$\sim 10^2$ Mo/sample

Signal processing

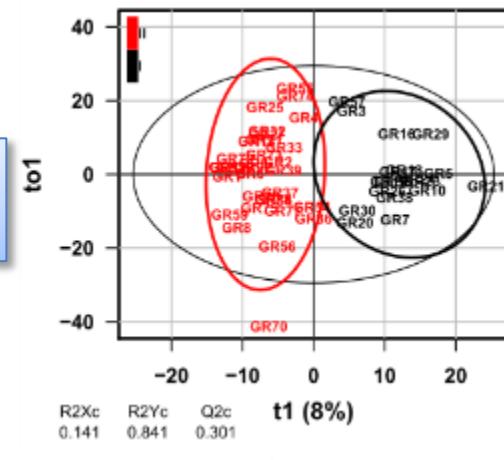
p variables

<i>m/z</i>	<i>rt</i>	Db_001	...	Db_078
96.0090	93.9263	10461	...	19857
96.9219	48.9327	1100122	...	1196290
96.9600	66.0438	5353164	...	7670637
...
999.6504	614.4241	5054	...	53641
999.6609	614.4223	19093	...	43504
999.6714	614.4268	14117	...	25030

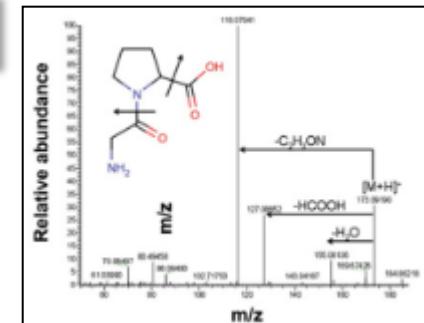
$\sim 10^2$ Mo/experiment

Statistical analysis

n samples



Identification



Existing platforms for untargeted metabolomics



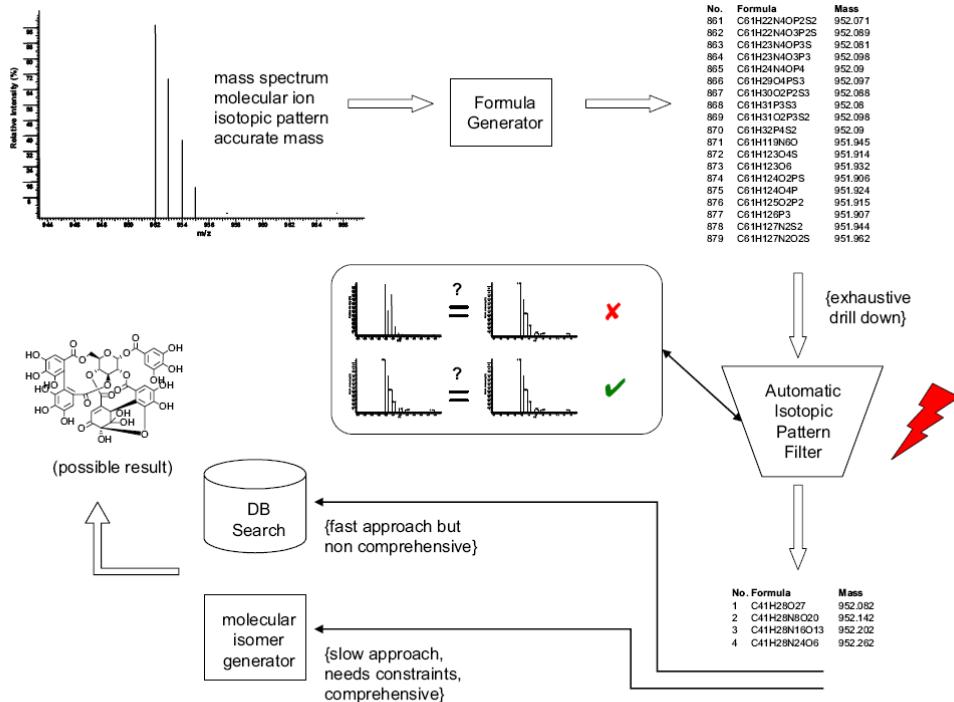
MetaboAnalyst



		MeltDB metedb.cebitec.uni-bielefeld.de Kessler <i>et al.</i> (2013)	MetaboAnalyst www.metaboanalyst.ca Xia <i>et al.</i> (2012)	XCMS Online xcmsonline.scripps.edu Tautenhahn <i>et al.</i> (2012)
Data processing	NMR			
	GC-MS	GC ²		
	LC-MS			
Statistical analysis			time-series	
Functional enrichment analysis				
Metabolic pathway analysis				
Workflow management				
Database		Mass decomposition	HMDB	METLIN

Metabolite annotation is a major challenge in metabolomics

Setting-up global strategies for metabolite annotation

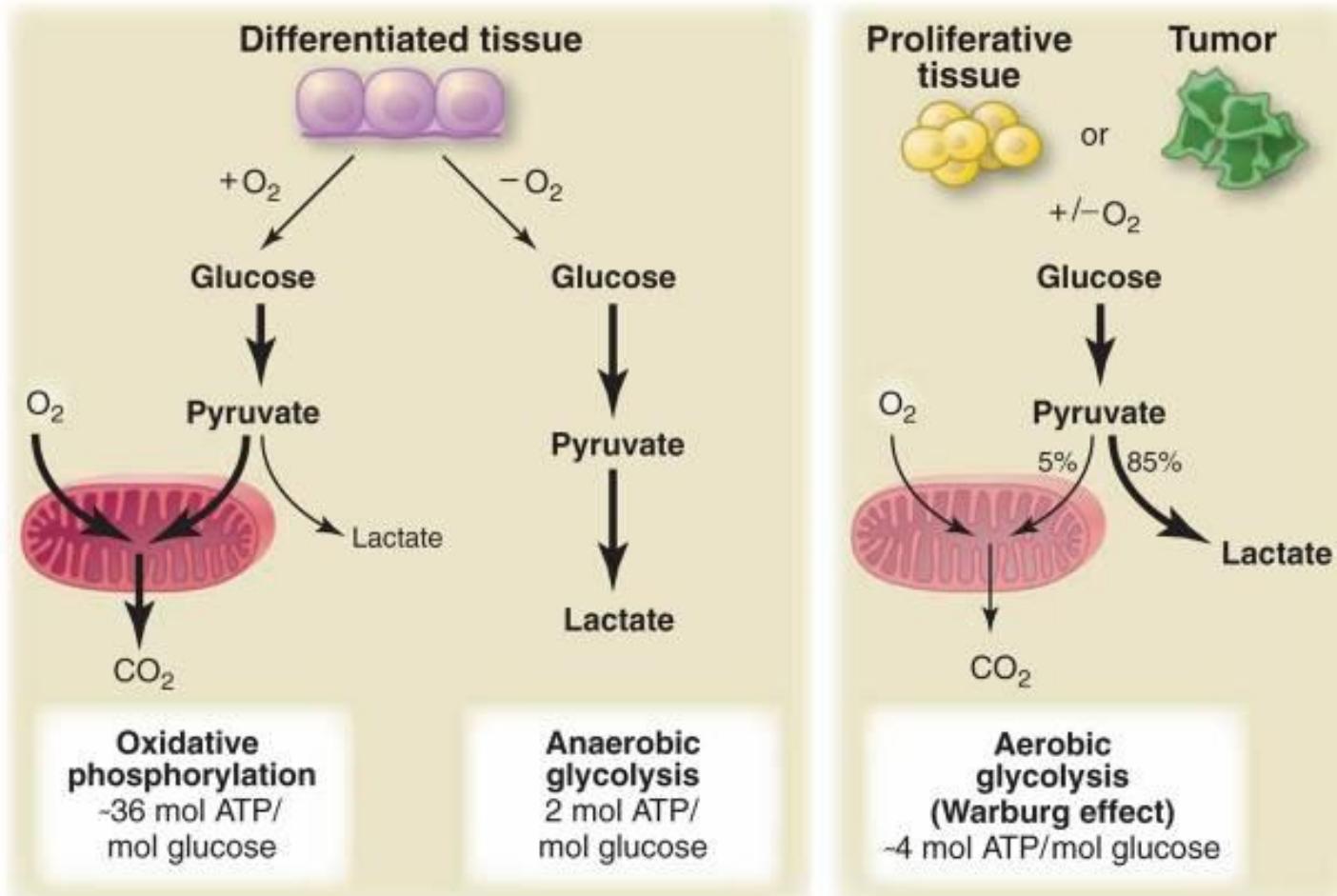


Complementary investigations

- Reduction of candidates
 - Basic chemistry rules
 - Exploitation of the isotopic cluster
 - Complementary approaches
 - H/D exchange
 - Derivatization
 - Isotope labelling approaches for identification
 - Confirmation (pure compound)

Figure 2 Metabolite annotation schema based on mass spectrometric calculation of elemental compositions and subsequent database queries

Kind & Fiehn, BMC Bioinformatics 2006

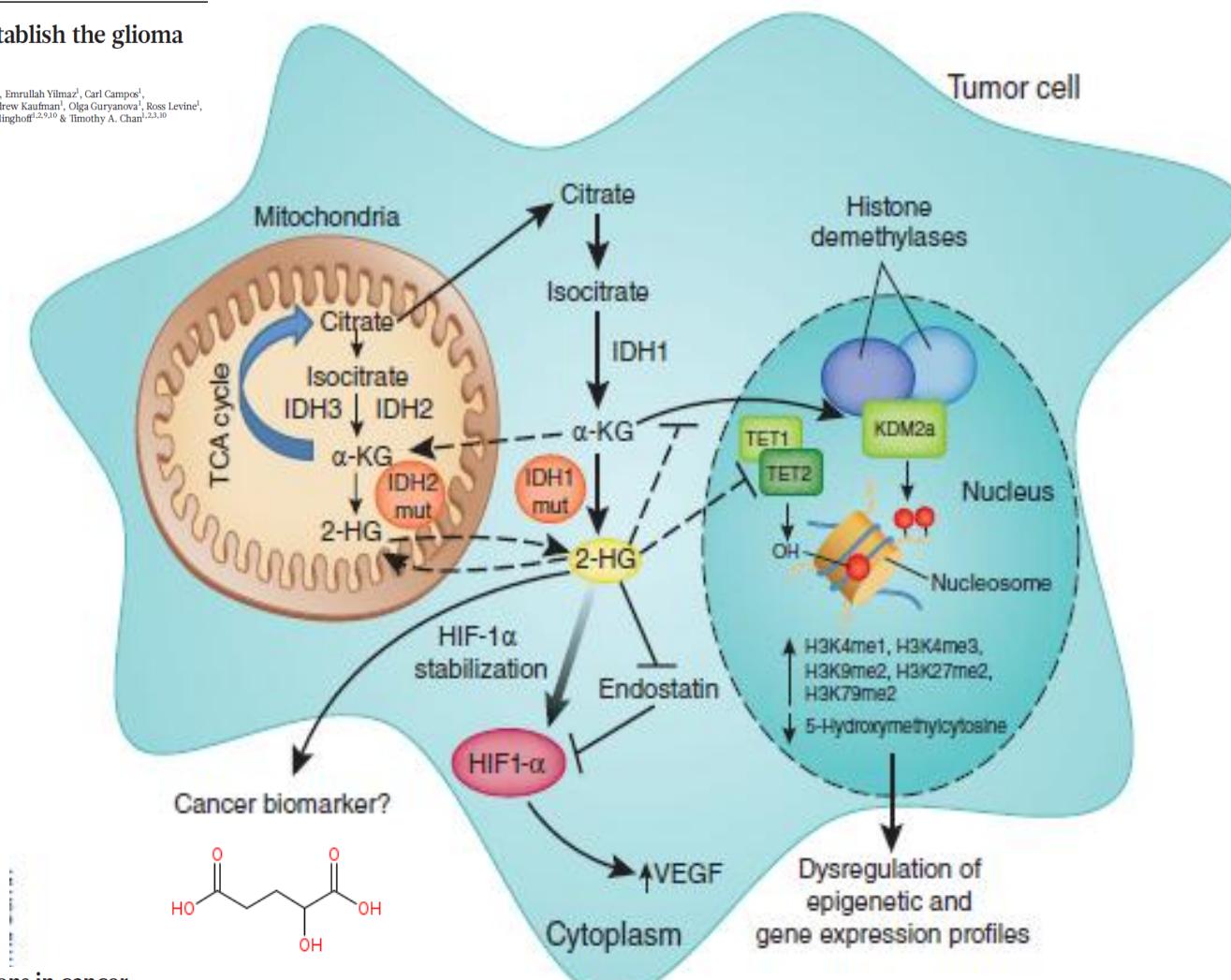


LETTER

doi:10.1038/nature10866

IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype

Sevin Turcan^{1*}, Daniel Rohle^{1,2*}, Anuj Goenka^{1,3*}, Logan A. Walsh¹, Fang Pang¹, Emrullah Yilmaz¹, Carl Campos¹, Armida W. M. Fabius¹, Chao Lu^{4,5}, Patrick S. Ward^{4,5}, Craig B. Thompson⁴, Andrew Kaufman¹, Olga Guryanova¹, Ross Levine¹, Adriana Heguy¹, Agnes Viale⁶, Luc G. T. Morris^{1,7}, Jason T. Huse^{1,8}, Ingo K. Melinghoff^{1,2,9,10} & Timothy A. Chan^{1,2,3,10}



Metabolism unhinged: IDH mutations in cancer

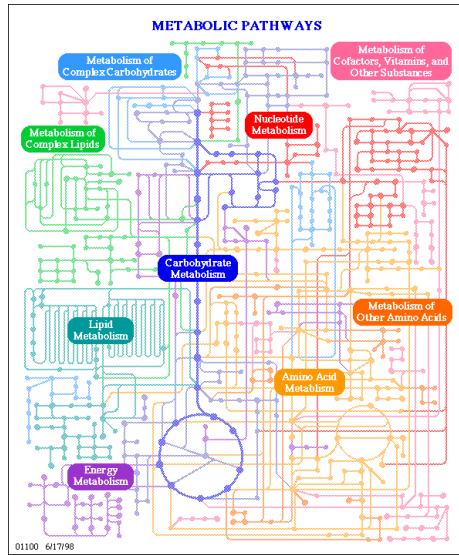
John R Prensner & Arul M Chinnaianyan

Recently characterized *IDH1* and *IDH2* mutations in leukemia and glioblastoma have introduced a fascinating cancer-specific role for metabolic genes essential to cellular respiration. Studies also link aberrant *IDH1* and *IDH2* activity to an altered metabolic profile, an observation that may have broad implications for both cancer epigenetics and clinical management of disease.

An introduction to metabolomics

1. Introduction
2. Concepts & methods in metabolomics
 1. The world of small molecules
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 3. Different approaches
 1. Chemometrics
 2. Metabolomics
 3. **Fluxomics**
3. MetaToul: Toulouse metabolomics & fluxomics facilities

Metabolism: not just metabolites, but also biochemical reactions that are tightly interconnected



Hundreds or thousands of reactions

Network organisation

Metabolic behaviour:

- Individual properties
- Collective properties

Ex: *E. coli* central metabolism

89 metabolites

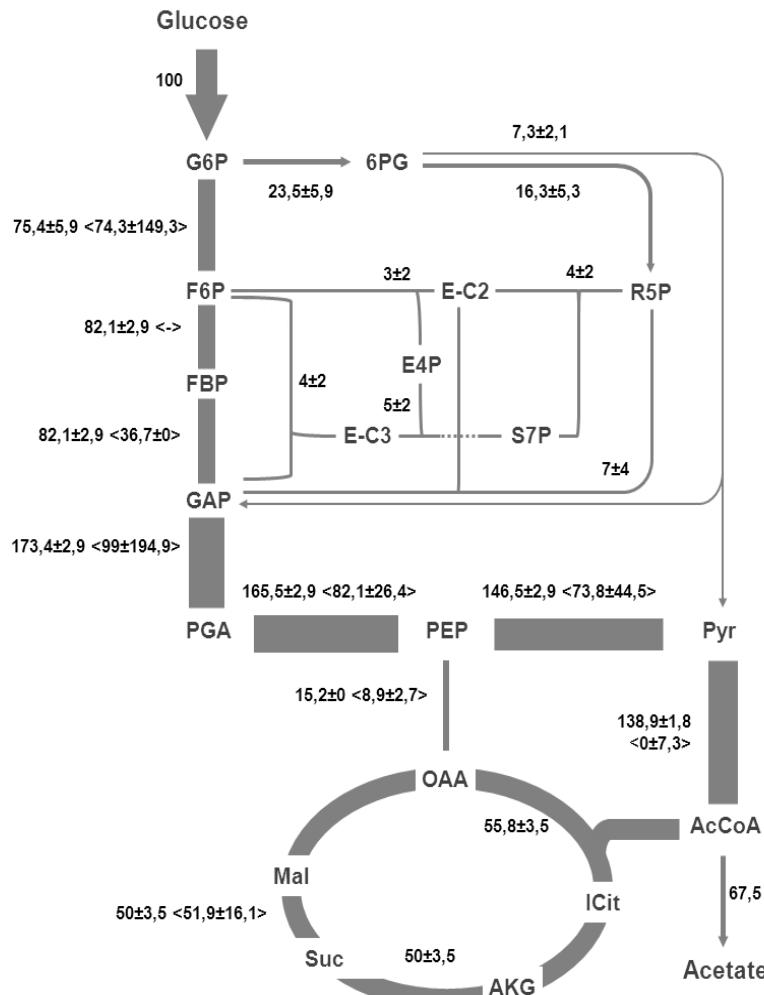
110 reactions

43 279 elementary flux modes

Basic Questions

- Can we analyze the operation of the metabolism at the network level ?
- How and to which extent functioning relates to structure (topology) ?
- General/design principles of functioning ?
- System-level mechanisms of regulation/adaptation ?
- Behaviour prediction: biotechnology, drug targeting ?

- Gives the distribution of intracellular carbon (& energy) fluxes *in vivo*
- Represents the actual (contextual) activity of the metabolic network



Metabolic network ccccc

- Identification of (novel) pathways, enzymes
- Linear pathways, cycles, reaction reversibility
- Compartmentation, channeling

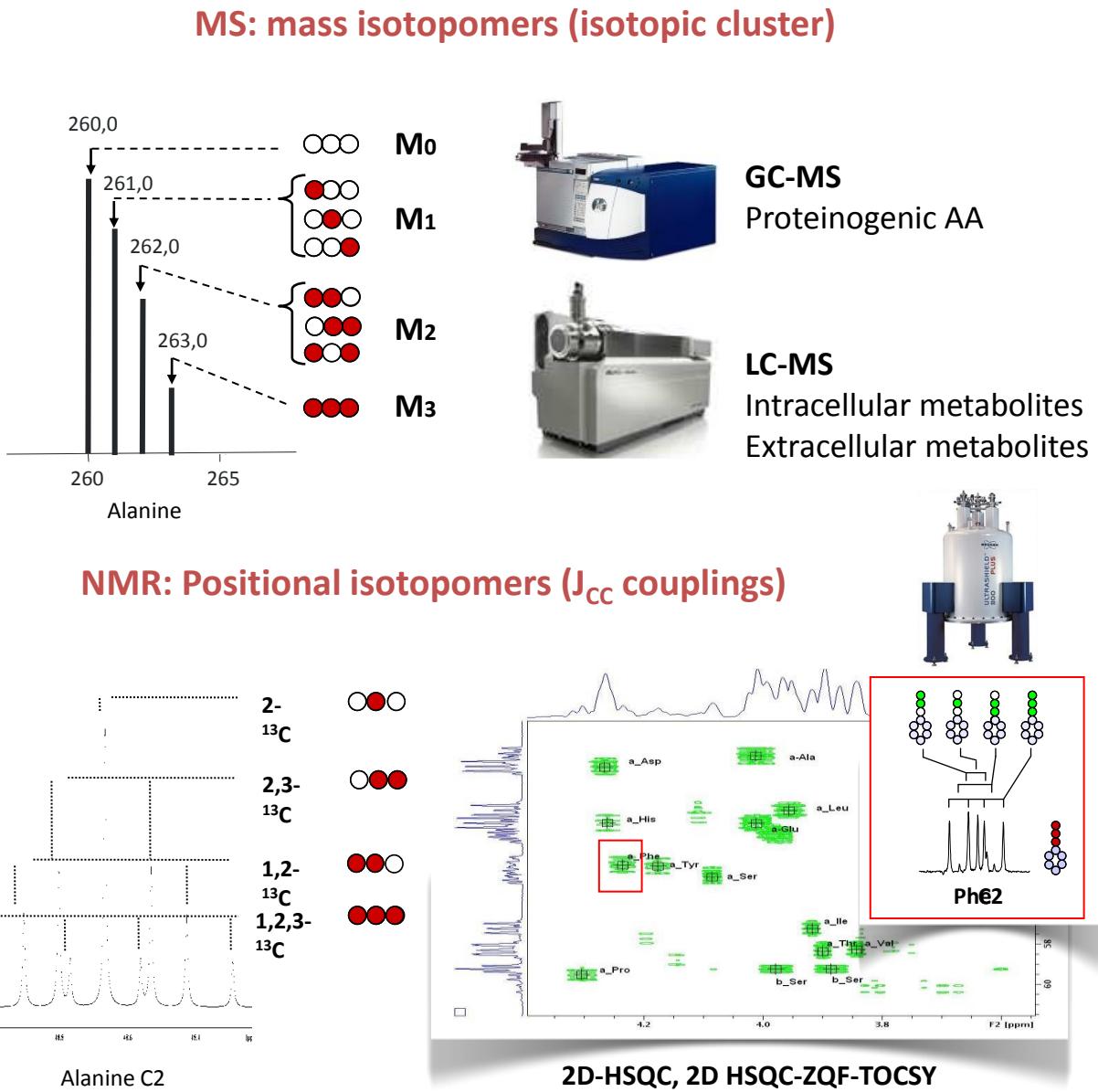
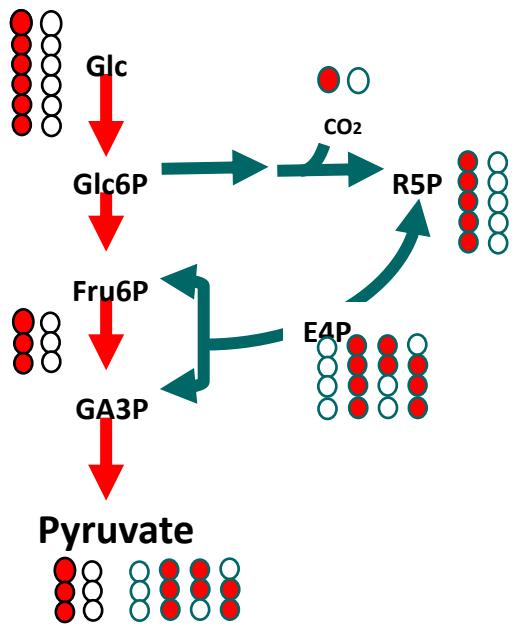
Quantification of pathway activity

- Carbon fluxes
- Energy fluxes (ATP, redox)
- Response to environmental / genetic modifications

Applications

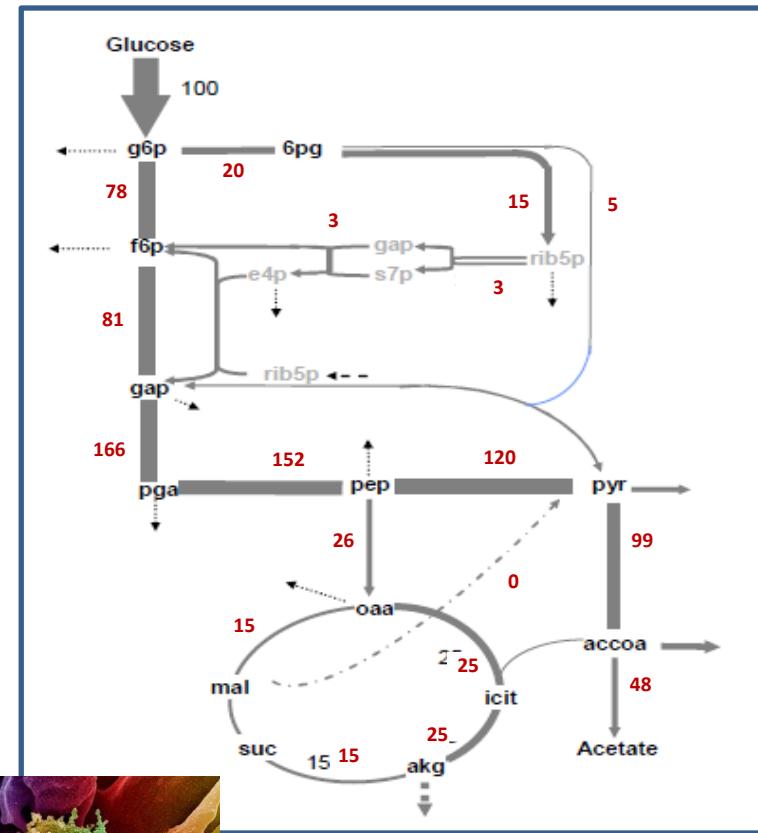
- Microorganisms, plant cells & tissues, animal cells
- Systems biology
- Biotechnology
- Synthetic biology
- Pharmacology, drug targeting





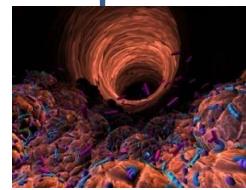
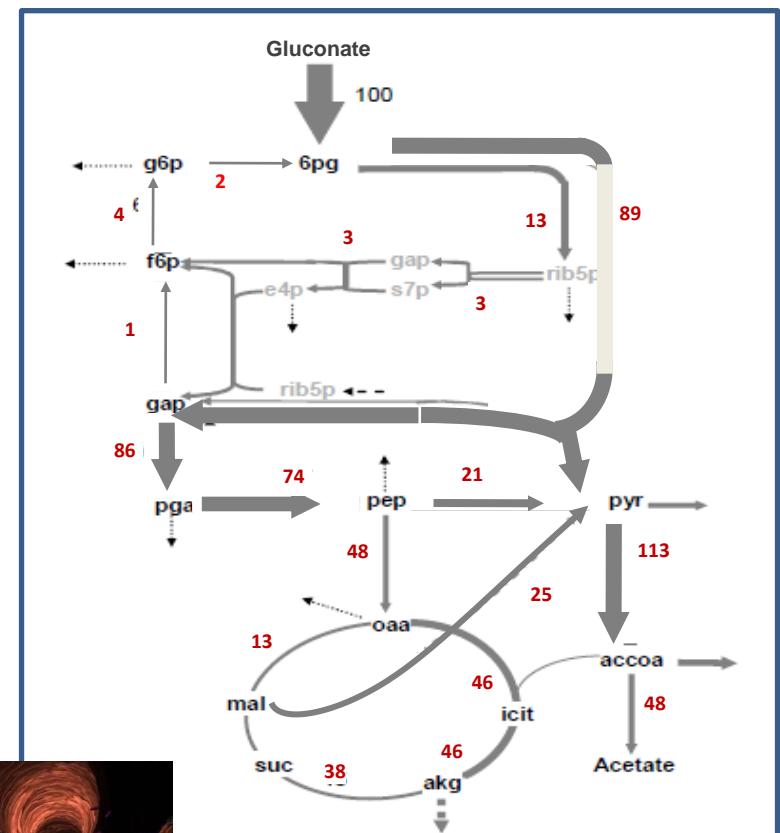
Carbon nutrition in (the real life of) *E. coli*

Glucose

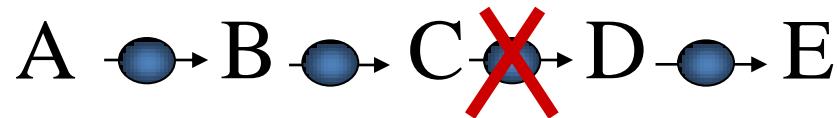


in vitro context

Gluconate



in vivo context



E. coli Yeast

No compensation

Lethal phenotype

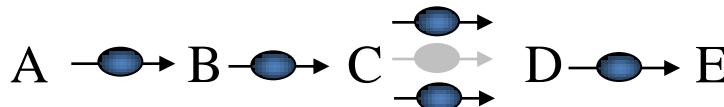
Very few genes in *E.coli* (<10)



(+) (?)

Enzyme Redundancy

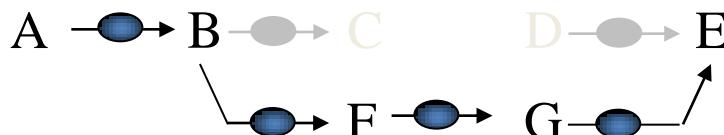
Local mechanisms



++

Alternative pathways

System-level mechanisms
New metabolic processes

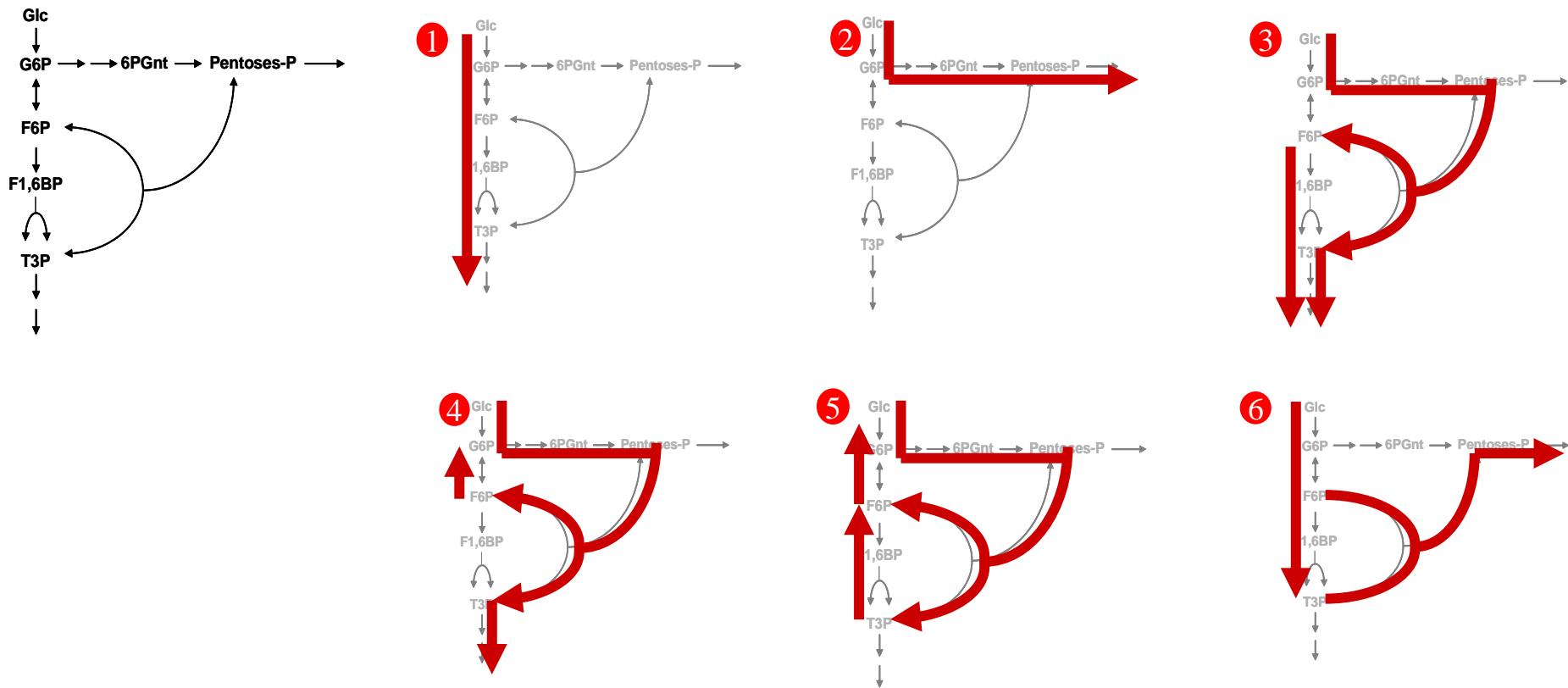


++ +

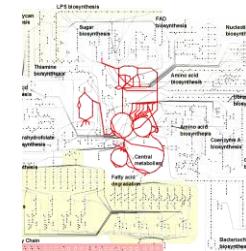
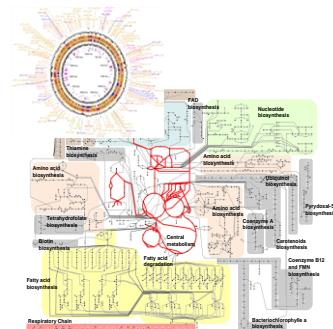
Elementary flux mode (EFM) analysis

EFM: Minimal set of enzymes that could operate at steady state with all irreversible reactions proceeding in the appropriate direction. ‘Elementary’ means nondecomposable.

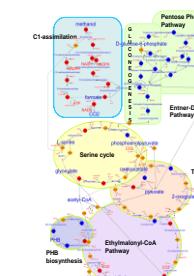
Schuster et al. 1999



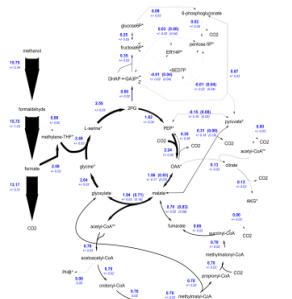
Example: methylotrophic growth in *M. extorquens*



¹³C-Methanol
Experiments
(3 biological replicates)

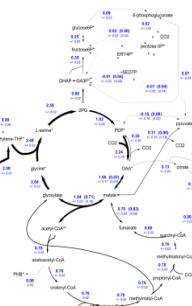
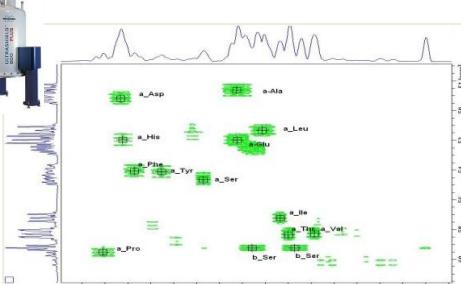
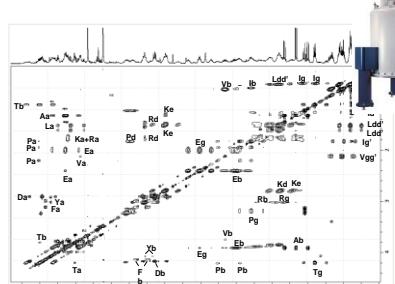
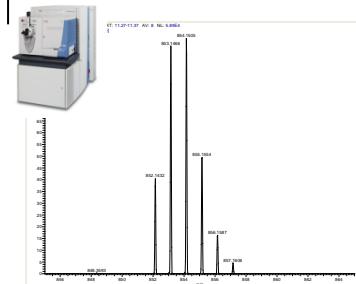


Functional topology
Metabolic pathway analysis



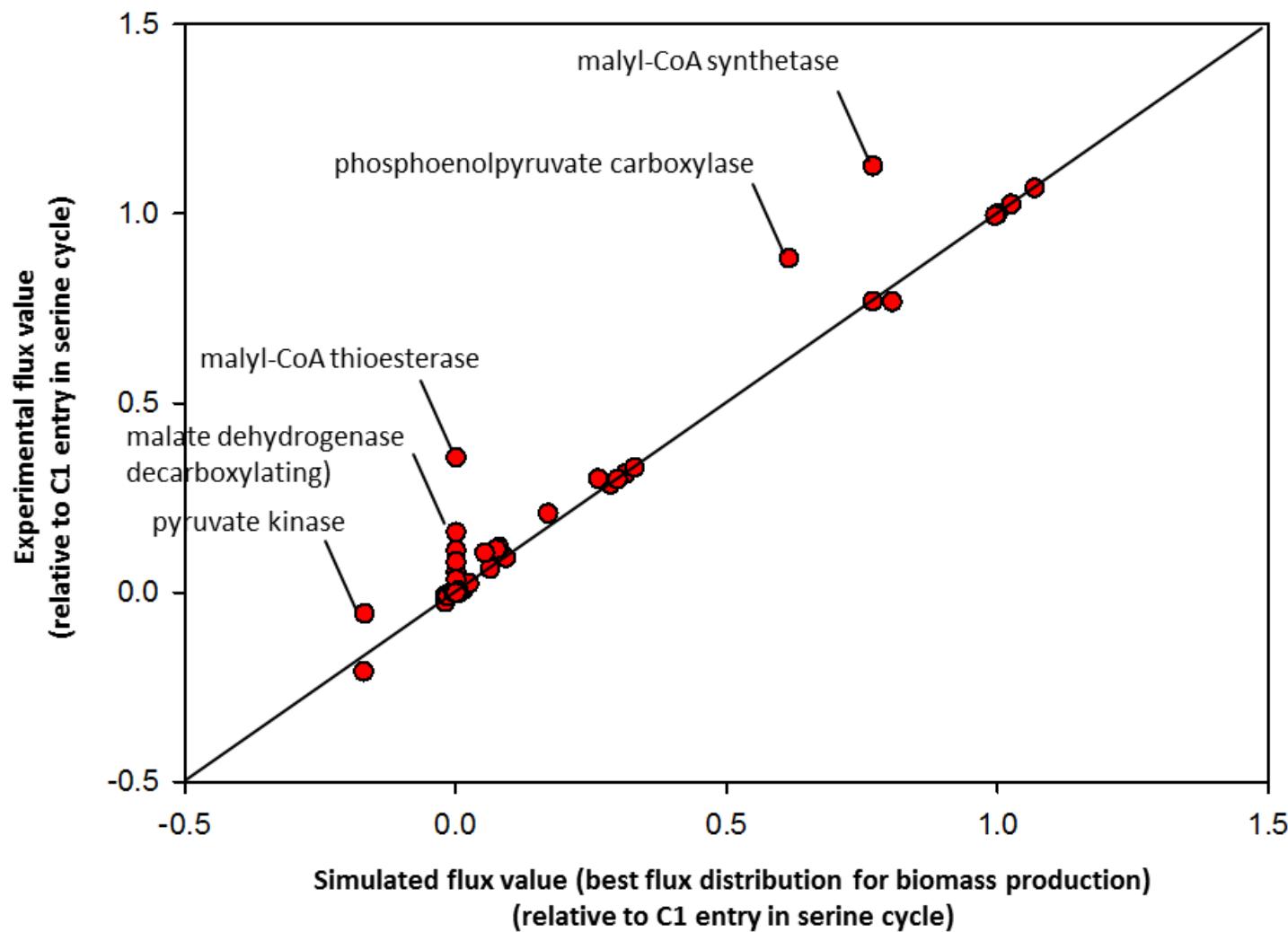
Simulation of
Flux distribution

Mass & positional isotopomers

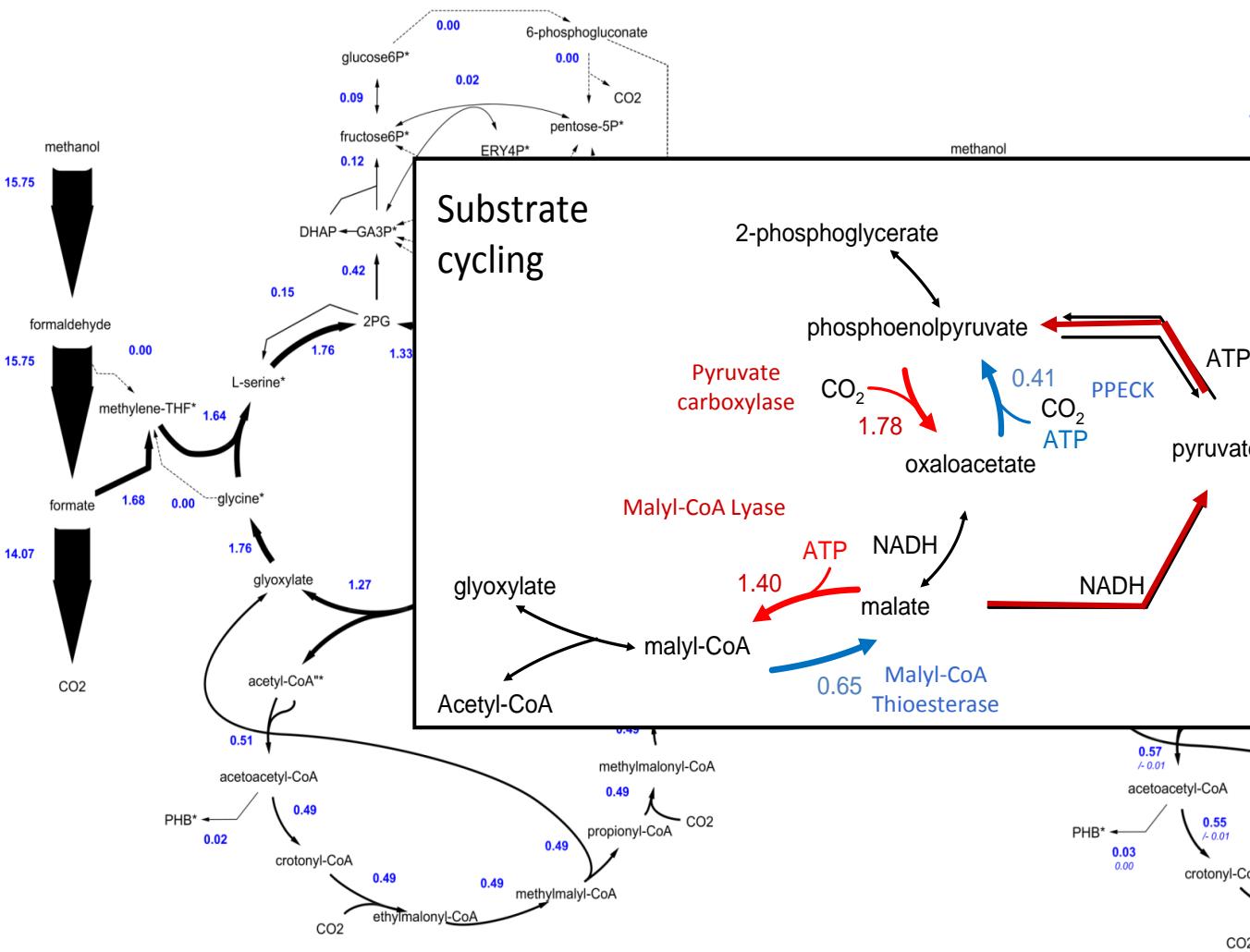


Experimental
Flux distribution

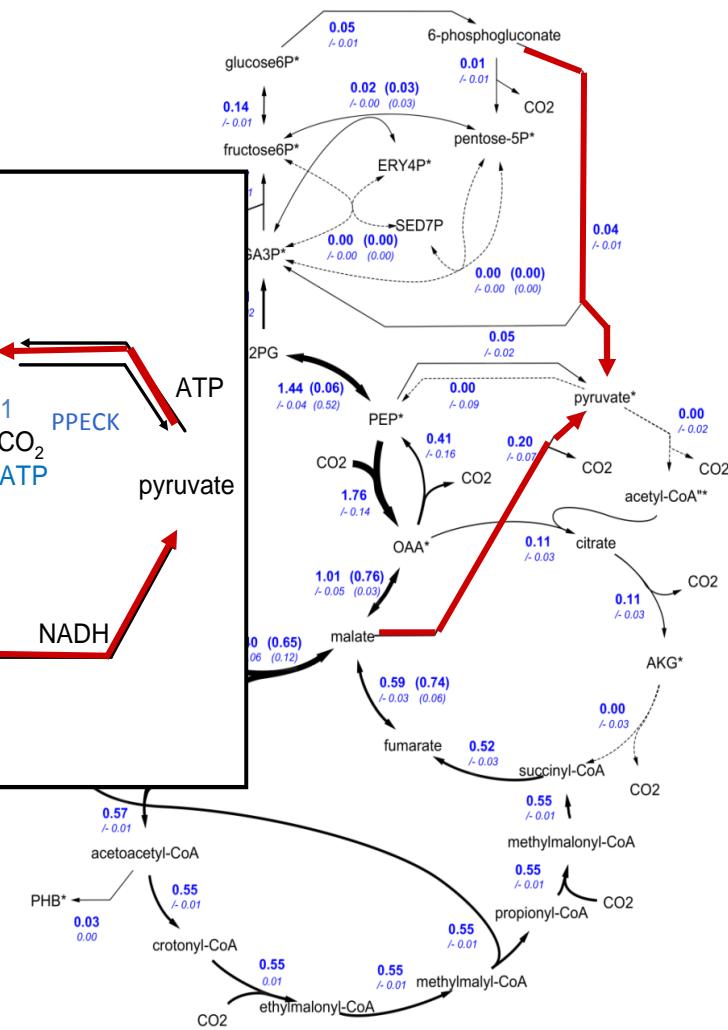
167 isotopomer data
/ replicate



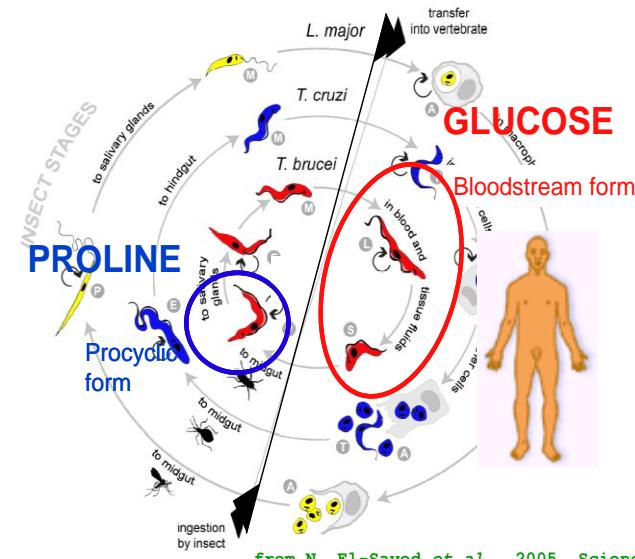
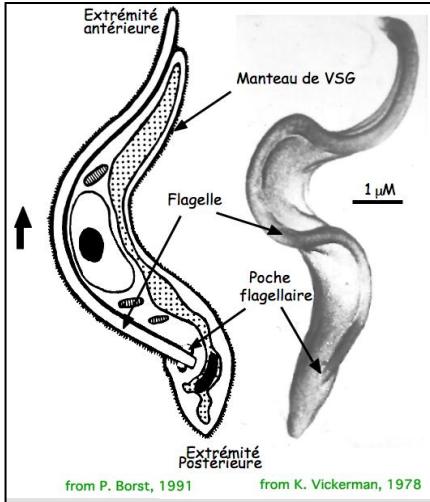
Flux distribution optimal for growth (*in silico* calculations using EFMs & FBA)



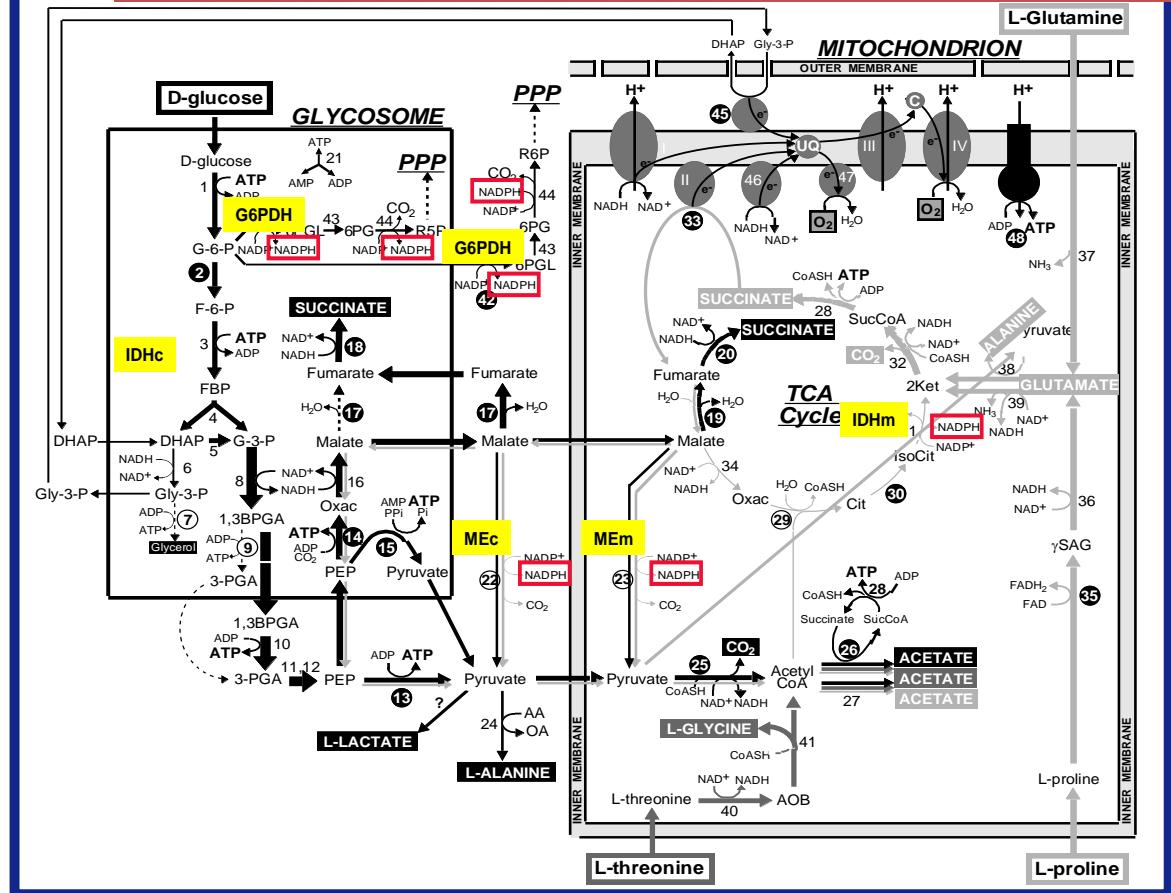
Observed flux distribution (¹³C-fluxomics)



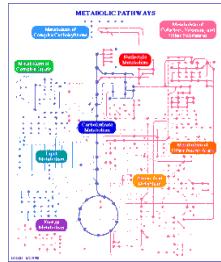
The causal agent of the sickness disease



Redox balances / network topology / adaptation to carbon nutrition



Ebikeme et al. J Biol Chem. 2010
Millerloux et al. J. Biol. Chem. 2012
Allman et al. J. Biol. Chem. 2013



- Tools are available for the functional analysis of large metabolic networks
 - In silico approaches
 - Experimental approaches: metabolomics, fluxomics, in vivo NMR, enzymomics, etc
- Global properties of metabolic networks
 - Large-scale organisation
 - Role of network topology
 - Metabolic homeostasis ensured by local /global mechanisms
 - Counteracting measures & alternative pathways
 - Optimality ?
- Future developments
 - High-throughput methods
 - Modelling of combined metabolic & regulatory networks
 - Dynamics of metabolic adaptation

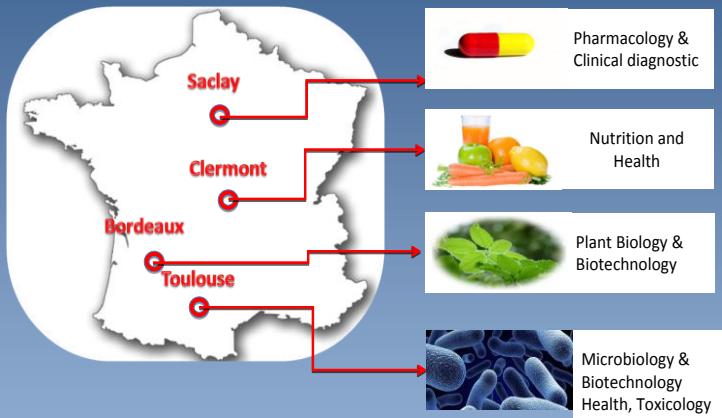
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MetaboHUB: French National Infrastructure for Metabolomics & Fluxomics

An infrastructure for metabolomics dedicated to Innovation,
Training and Technology transfer.

MetaboHUB: Bringing together 4 outstanding metabolomics platforms



MetaboHUB: a core facility with a wide range of analytical tools and competences a common ground of expertise and complementary application domains



Coordinator: D. Rolin (Bordeaux)

Total budget 45 ME

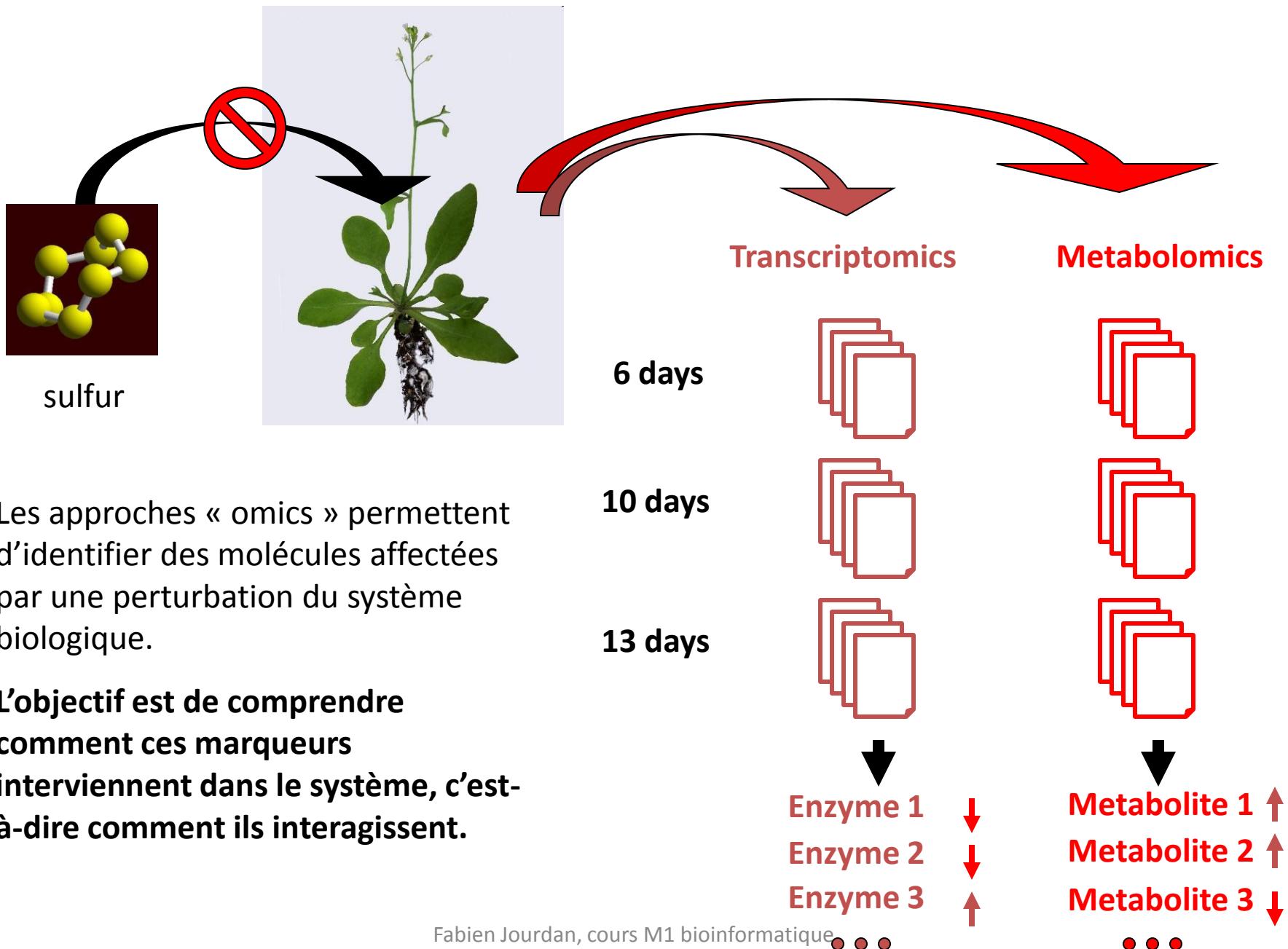
Funding : 10,0 ME

Launched in 2013

Main objectives:

- 1- Gather in a coordinated infrastructure, unique in France, four outstanding metabolomics centres supported by laboratories for state-of the heart R&D
- 2- Create a shared infrastructure bringing together the various technologies required for the characterization of metabolomes, open to the research communities and industrial companies
- 3- Strengthen the attractiveness and competitiveness of France through increased innovation thanks to developments made available to the whole community
- 4- Deliver state of the art technological developments, a comprehensive scientific cooperation, training and mentoring to the whole community

Exploitation des données de métabolomique



Flavanoids

Tryptophan

G3P

Serine

Glycine

O-Acetylserine

...

Cysteine

Glutathione

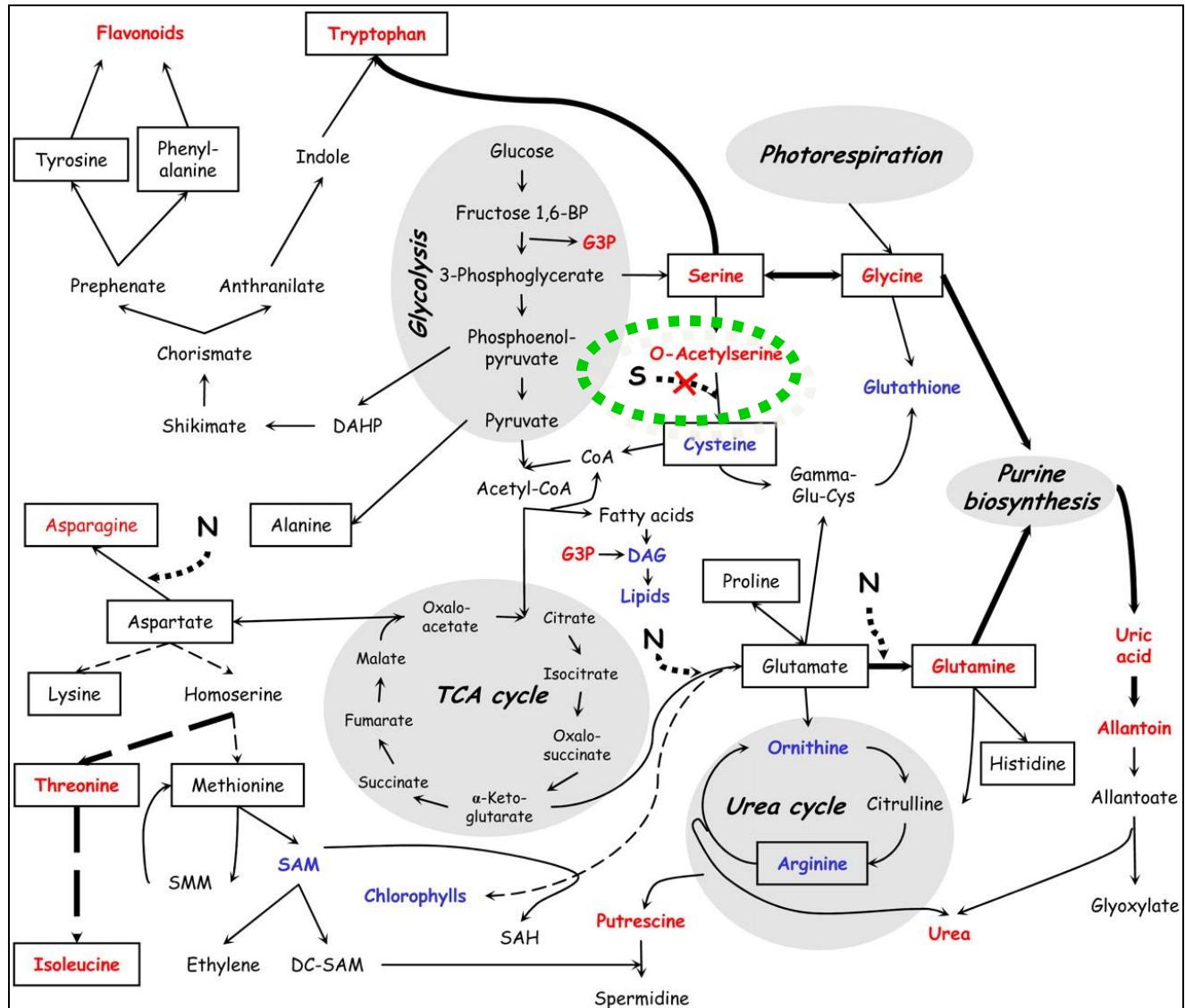
DAG

Lipids

SAM

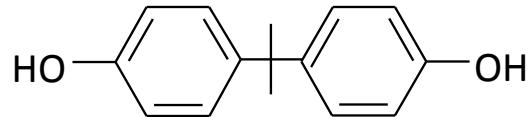
Chlorophylls

...



Reconstruire l'« histoire » métabolique à partir de ces données

- Develop novel and efficient strategies to address modern toxicological challenges linked with food contaminants
 - Chronic & low dose exposures issues
 - Exposure during critical periods of the development



Polycarbonates



Prod. ca. 3 Million T/year

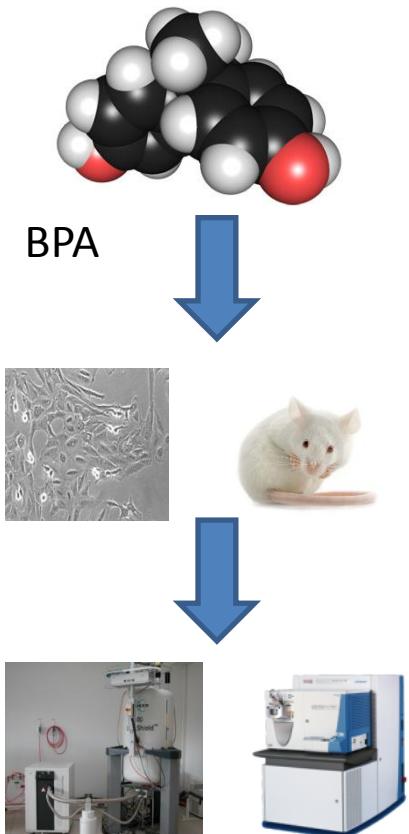
Epoxy Resins

Free monomer = model Xeno-estrogen

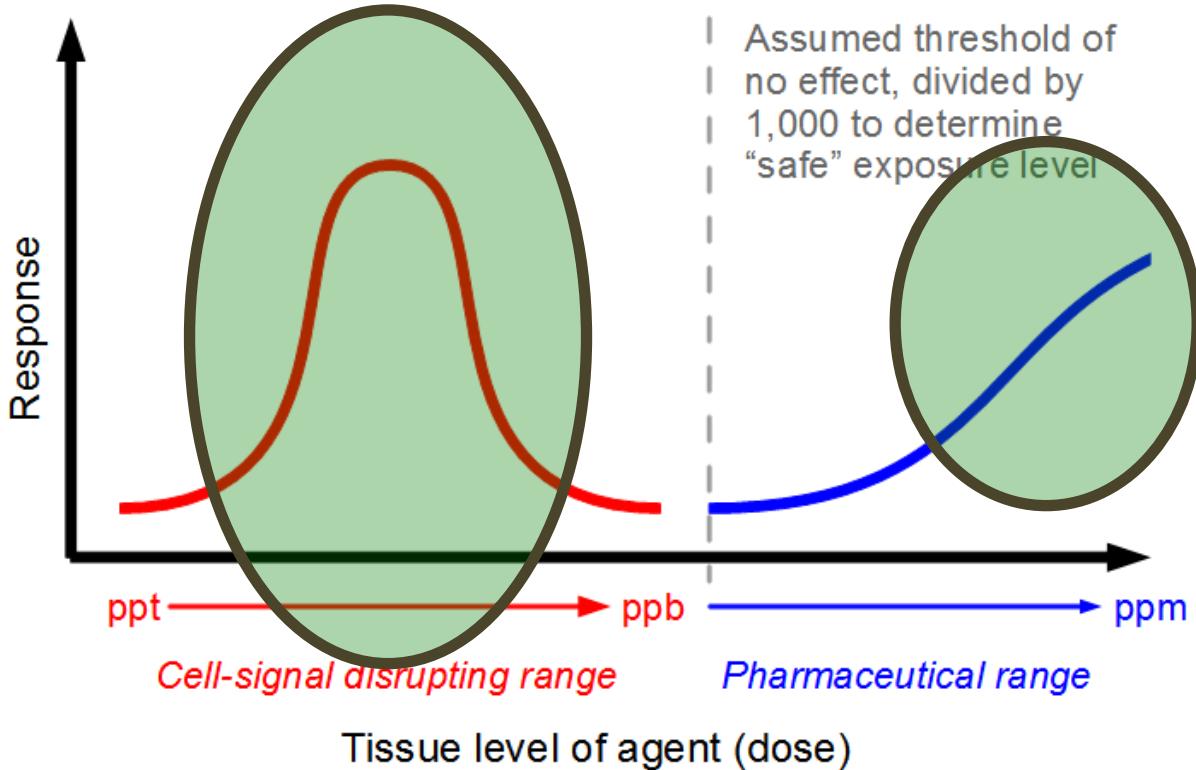


Effects / human health ?

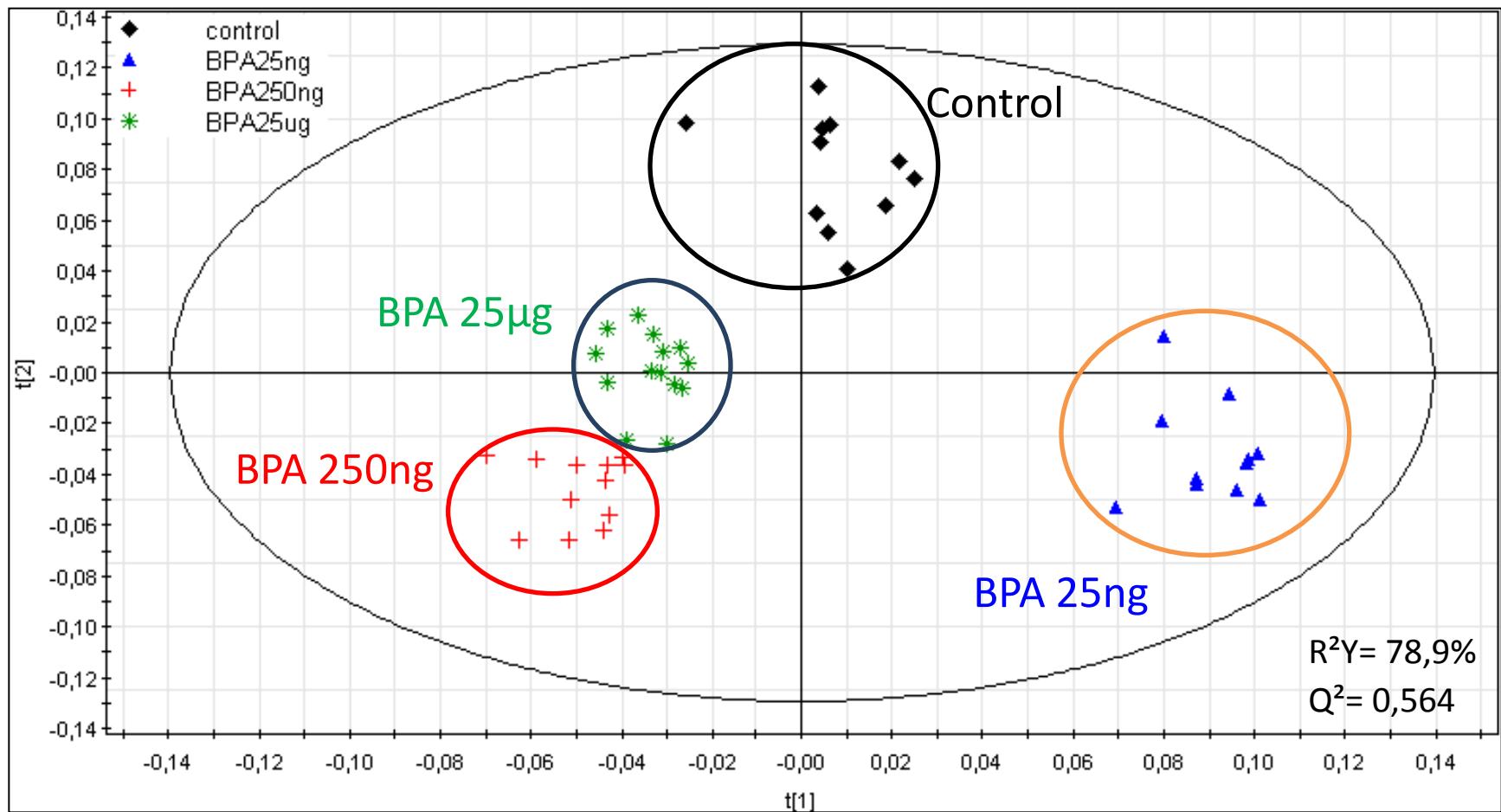
Metabolomics and food toxicology



Inverted-U dose-response curve for some endocrine disrupting chemicals

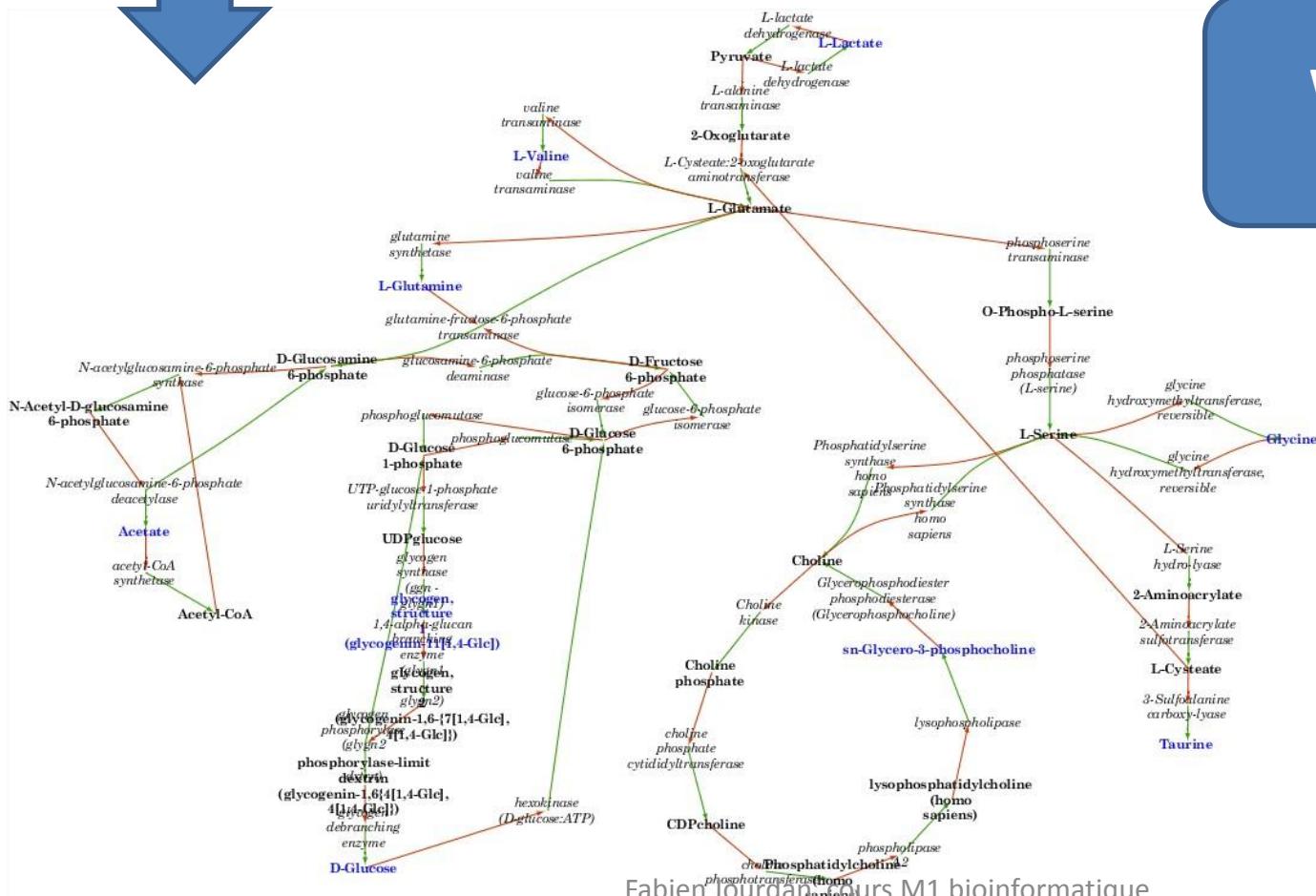


Low doses of bisphenol-A disrupt the metabolome in perinatally exposed CD-1 mice. Cabaton et al. 2013 EHP



Brain, males, PND21

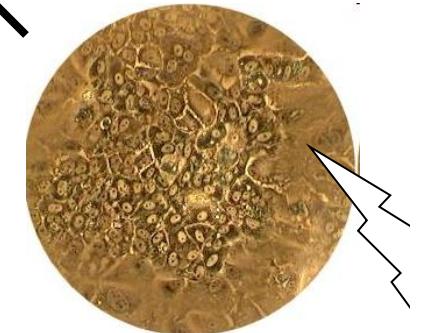
Metabolite (Recon)	Strain	Xenobioti	Pvalue	DMSO/10-6	DMSO/10-9	DMSO/10-12	DMSO/10-15	50nM	250nM
M_g3pc_c	HepaRg	BPA	3,20E-07			HepaRG_BPA			
M_g3pc_c	HepaRg	Aflatoxine	8,70E-10					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_ac_c	HepaRg	Aflatoxine	4,20E-04					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_glc_DASH_D_c	HepaRg	BPA	0,0292			HepaRG_BPA			
M_glc_DASH_D_c	HepaRg	Aflatoxine	9,50E-08					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_gly_c	HepaRg	BPA	8,40E-09			HepaRG_BPA			
M_gly_c	HepaRg	Aflatoxine	4,40E-05					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_glygn1_c	HepaRg	Aflatoxine	3,90E-07					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_gln_DASH_L_c	HepaRg	Aflatoxine	7,00E-07					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_lac_DASH_L_c	HepaRg	Aflatoxine	7,80E-07					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_lys_DASH_L_c	HepaRg	Aflatoxine	1,30E-05					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_val_DASH_L_c	HepaRg	Aflatoxine	3,10E-07					HepaRG_Aflatoxine	HepaRG_Aflatoxine
M_taur_c	HepaRg	BPA	0,0364			HepaRG_BPA			
M_taur_c	HepaRg	Aflatoxine	3,20E-07					HepaRG_Aflatoxine	HepaRG_Aflatoxine



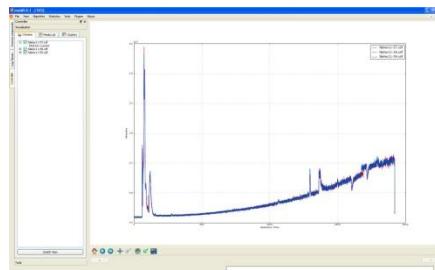
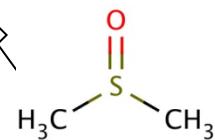
What is the biology behind?



LC-HRMS



HepaRG

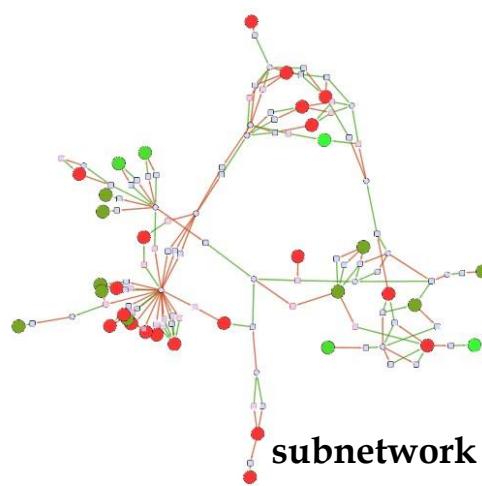


MS raw data

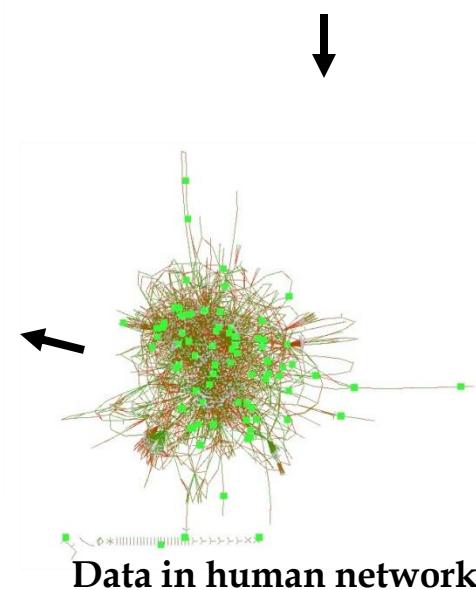


Compound Name	Identifier	Mass	Formula	Pathways	sample
(R)-3-hydroxybutanoate	CPD-335	104.047362	C4H8O3	0 pathway	1
3-hydroxy-isobutyrate	3-HYDROXY-ISOBUTYRATE	104.047362	C4H8O3	1 pathway Display pathways	1
L-isoleucine	ILE	131.094606	C6H13N1O2	7 pathways Display pathways	1
L-leucine	LEU	131.094606	C6H13N1O2	4 pathways Display pathways	1
S-adenosyl-L-homocysteine	ADENOSYL-HOMO-CYS	384.120629	C14H20N6O5S1	10 pathways Display pathways	1
8-hydroxopurine	CPD-9017	136.03805	C5H4N4O1	0 pathway	1

Metabolite list



subnetwork



Data in human network

Metabolomics technology

Mass spectrometry

- LC-MS
- GC-MS
- CE- MS

NMR

- highly quantitative

High Resolution Mass Spectrometry

- FT ICR
- Orbitrap



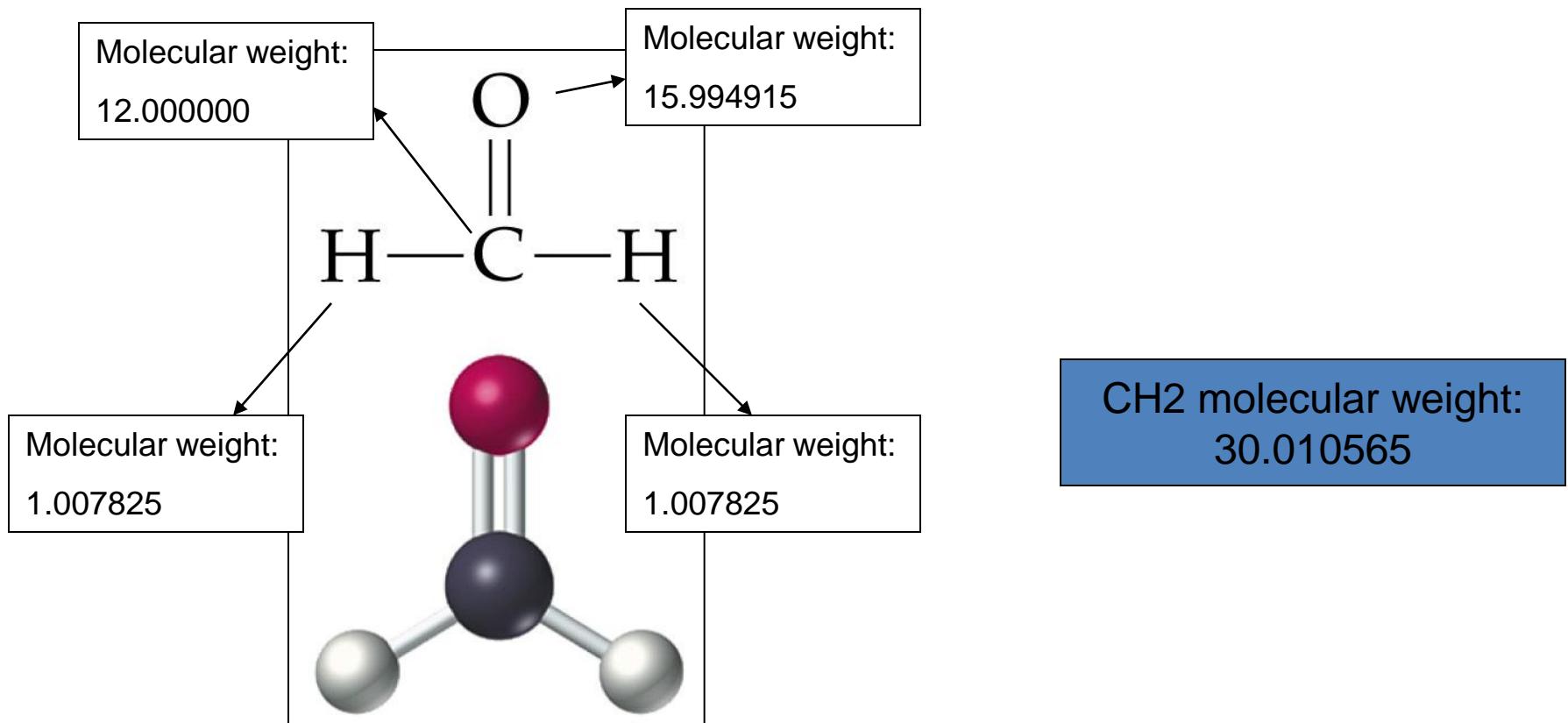
180

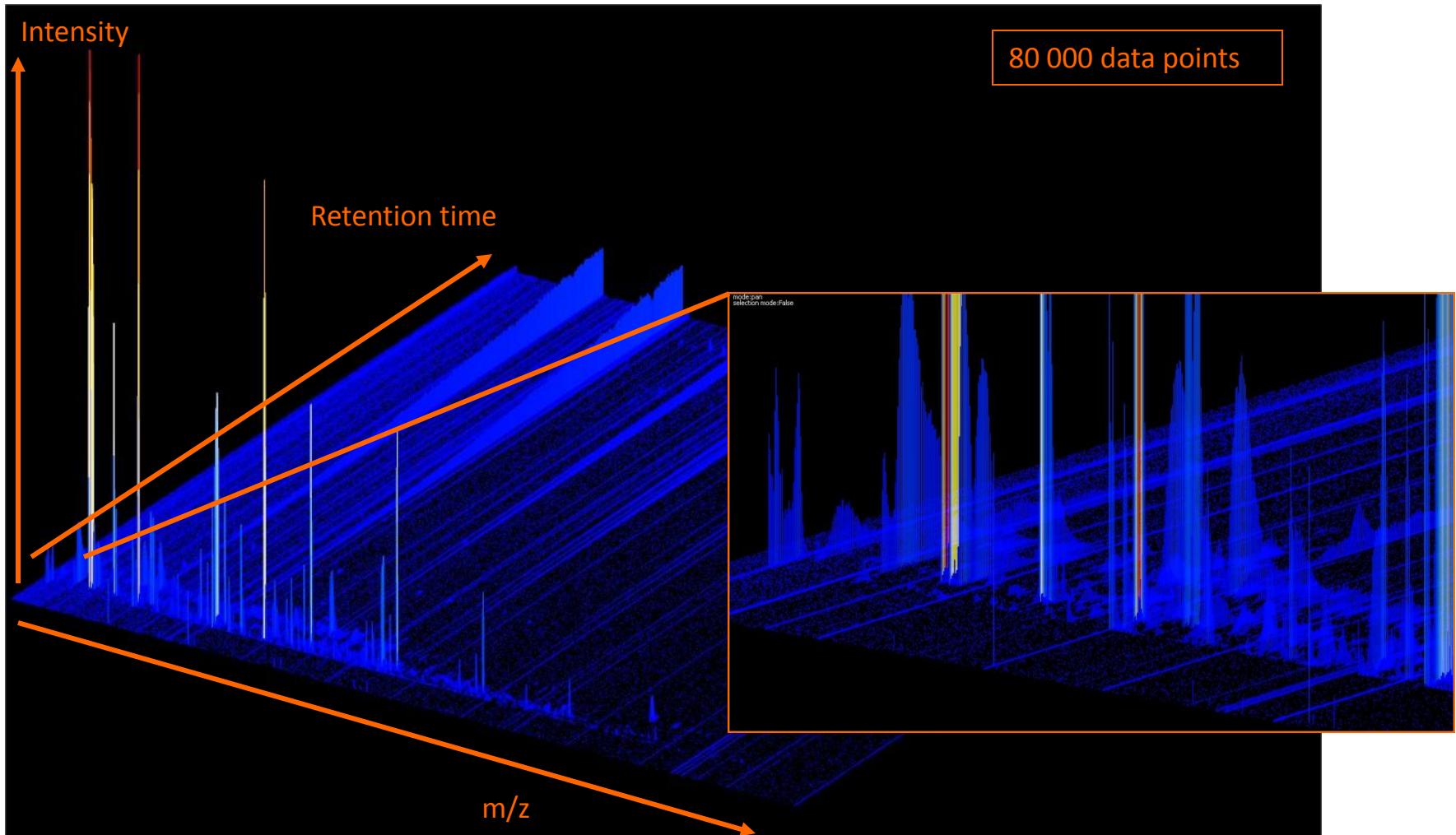
Hundreds of PubChem hits

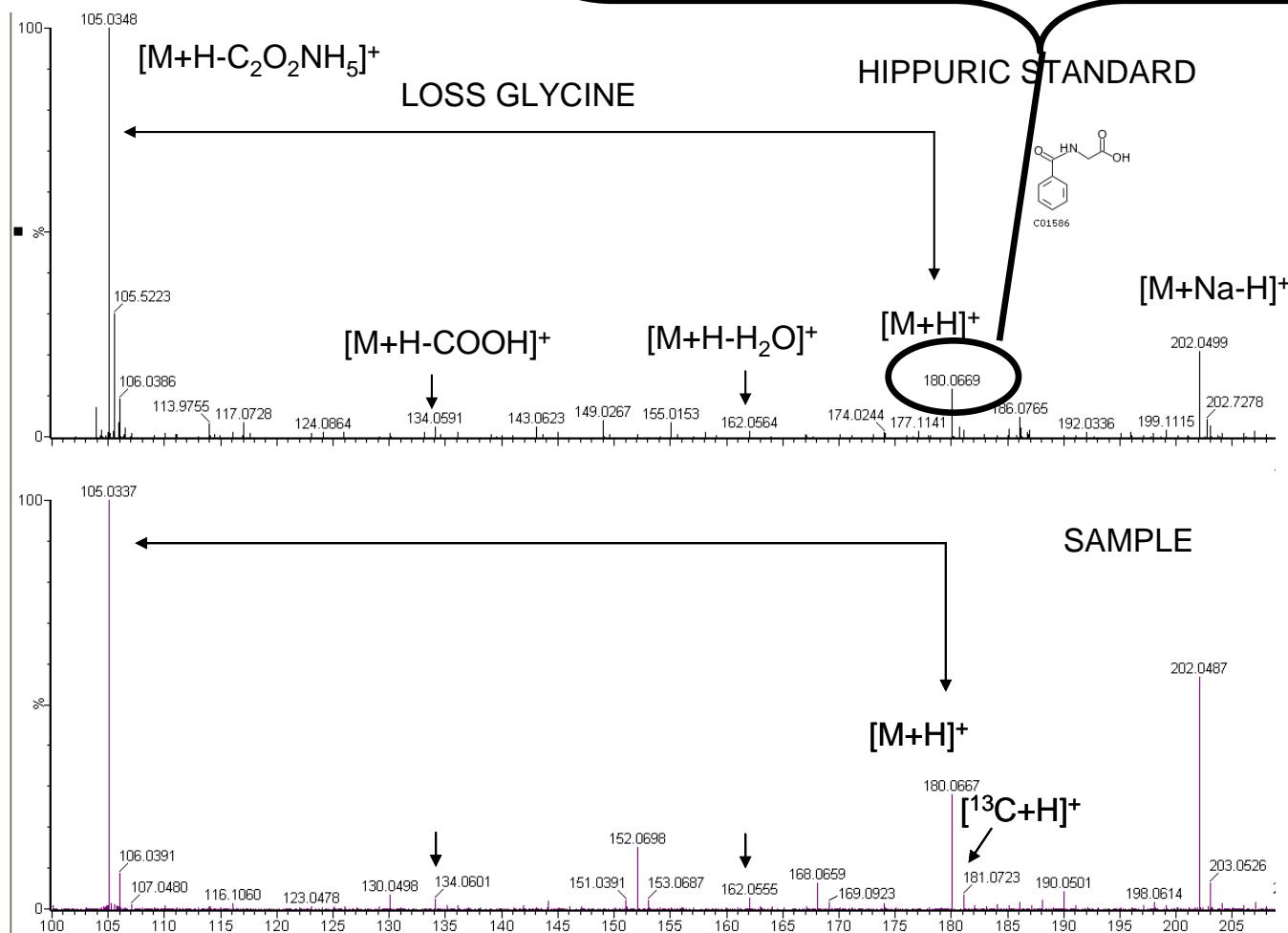
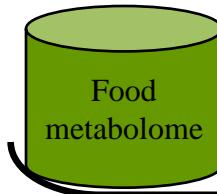
180.1562

Glucose

Molecular weight of a molecule

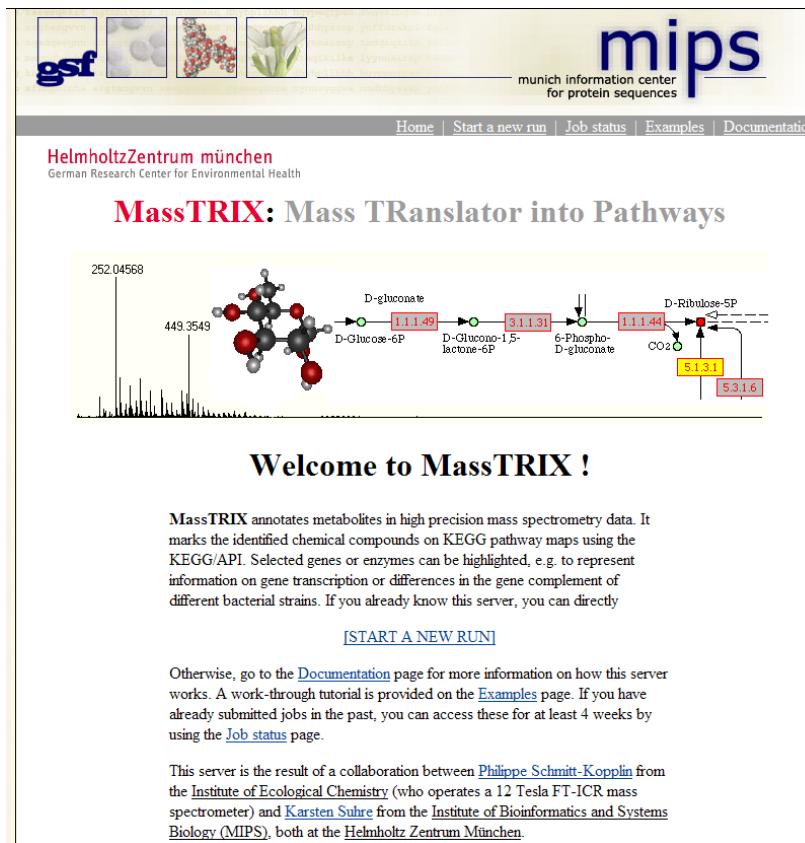


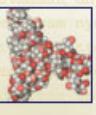




Masstrix

- <http://mips.gsf.de/proj/metabolomics/>





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for protein sequences

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MassTRIX: Mass TRanslator into Pathways

Start a new job

Data upload

Enter a peak list (mass/intensity pairs) [help](#)

[example data loaded, [click here to clear this page](#)]

Upload a peak list

[Parcourir...](#)

or paste your data into the field below

```
149.28542      1691728.4
150.00182      1034193.2
153.74312      1168253.2
154.53576      2268302.6
154.97517      7257750
156.01244      2497666.5
156.96744      8085036
157.08700      1217346.6
157.12337      2142021.5
158.02247      2644522.8
158.96554      1432960.3
.....
```

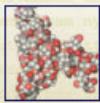
Parameters

Scan mode [help](#) negative ionisation (correct for H⁺ loss)

Max. error [help](#) 1.0 ppm

Database [help](#) KEGG with isotopes (default)

Organism [help](#) *Saccharomyces cerevisiae*



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MassTRIX: Mass TRanslator into Pathways

List of existing jobs

To access a private job, enter its id below

Click on the job id that you wish to explore

ID (recent jobs first)	job description	owner	status
EXAMPLE_Yeast	Yeast cell extract, exp. growth in YPD	MassTRIX	finished
08093011231522468	Yeast cell extract, exp. growth in YPD	testuser	spooled
08092916394418773	W_NF C tot	unknown	finished
08092913444918649	W_F_3L	unknown	finished
0809291309409569	M31 control pellet	JOCE	finished
0809291308459432	M8_control pellet	JOCE	finished
08092911195021956	W_F_2L	unknown	finished
080926130627987	NNK	NNK	finished
0809260522167847	Yeast cell extract, exp. growth in YPD	testuser	finished
0809251540337274	ires vs soylow	unknown	finished
0809251539026619	iresist vs ctl	unknown	finished
08092514493030356	70s	70s	finished
08092511310115582	W_F	unknown	finished



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MassTRIX: Mass TRanslator into Pathways

JobID: [EXAMPLE Yeast](#)

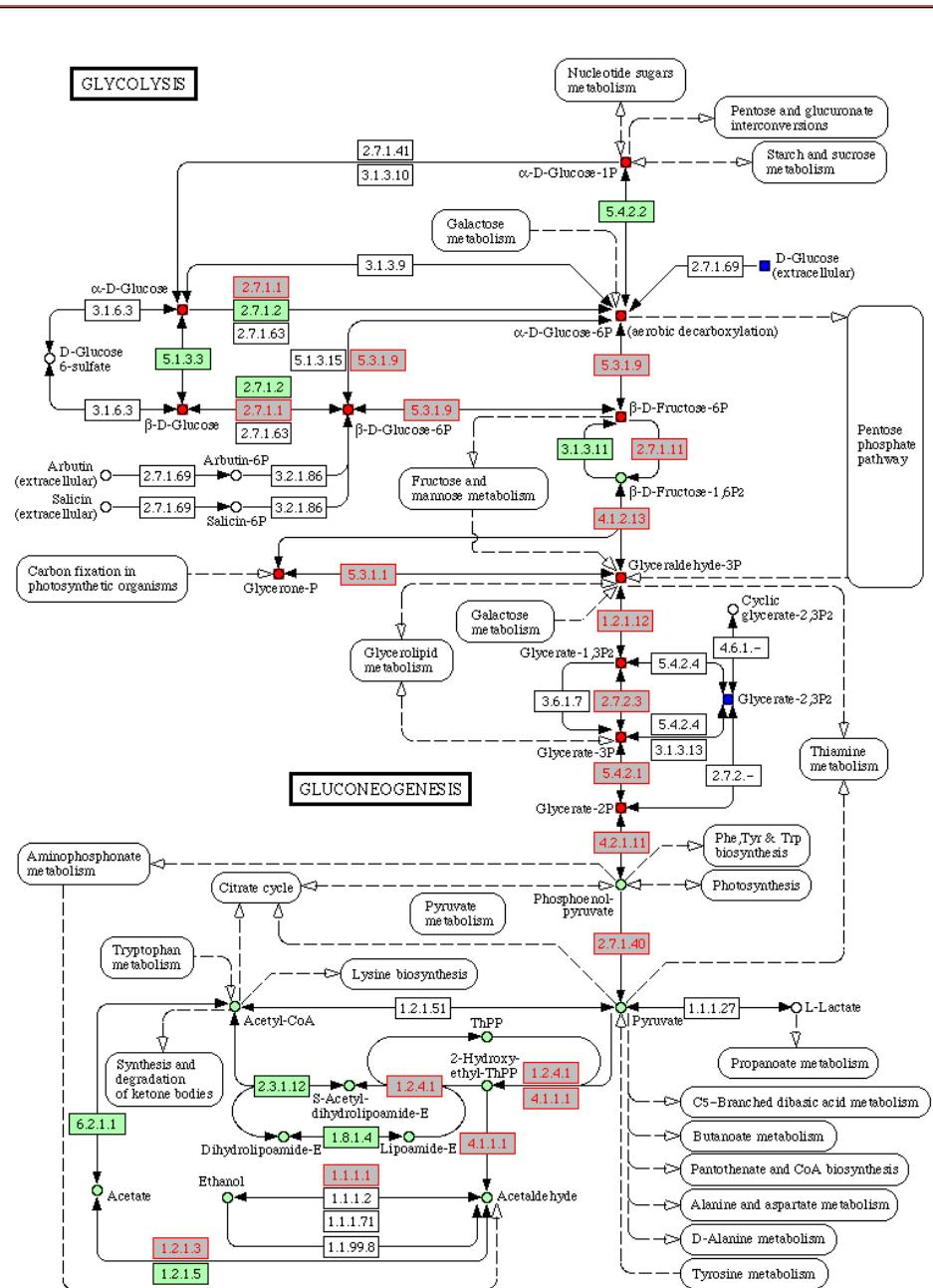
*** Yeast cell extract, exp. growth in YPD ***

sce Saccharomyces cerevisiae

[Pathways](#) | [Compounds](#) | [Error plot](#) | [Log file](#) | [Input data](#)

PATHWAYS

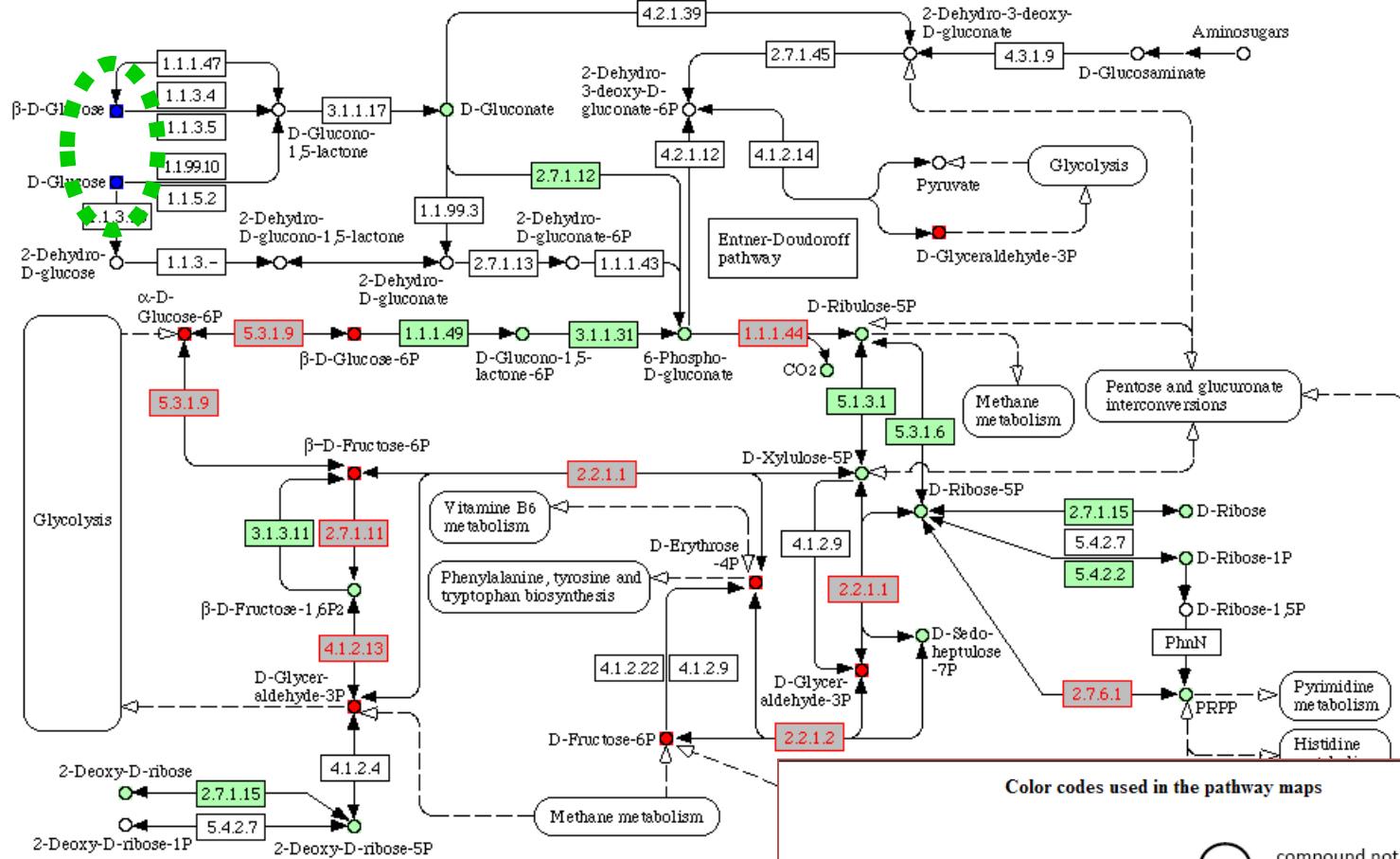
Map	KEGG Pathway	N ^{map}	N ^{org}	N ^{id}	N ^{orgid}
sce00010	Glycolysis / Gluconeogenesis	32	23	13	11
sce00020	Citrate cycle (TCA cycle)	22	20	0	0
sce00030	Pentose phosphate pathway	34	20	8	6
sce00040	Pentose and glucuronate interconversions	53	12	6	1
sce00051	Fructose and mannose metabolism	46	18	17	10
sce00052	Galactose metabolism	41	27	21	15
sce00061	Fatty acid biosynthesis	36	36	1	1
sce00062	Fatty acid elongation in mitochondria	27	12	1	0
sce00071	Fatty acid metabolism	51	28	3	1
sce00072	Synthesis and degradation of ketone bodies	6	3	0	0
sce00100	Biosynthesis of steroids	78	29	16	5
sce00120	Bile acid biosynthesis	42	15	3	1
sce00130	Ubiquinone biosynthesis	35	12	0	0
sce00190	Oxidative phosphorylation	16	16	0	0
sce00220	Urea cycle and metabolism of amino groups	42	29	4	3
sce00230	Purine metabolism	91	61	7	6
sce00240	Pyrimidine metabolism	60	35	6	5
sce00251	Glutamate metabolism	29	19	1	1



Color codes used in the pathway maps

- | | | | |
|----------------|------------------------------------------|--|--------------------------------------------------|
| 5.4.2.1 | enzyme not annotated in organism | | compound not identified, not present in organism |
| 5.4.2.2 | enzyme annotated in organism | | compound not identified, present in organism |
| 5.4.2.3 | tagged enzyme, not annotated in organism | | compound identified, not present in organism |
| 5.4.2.4 | tagged enzyme, annotated in organism | | compound identified, present in organism |
| 5.4.2.5 | ambiguous compound | | compound ambiguous, e.g. multiple compounds |

PENTOSE PHOSPHATE PATHWAY



00030 3/23/06

Color codes used in the pathway maps

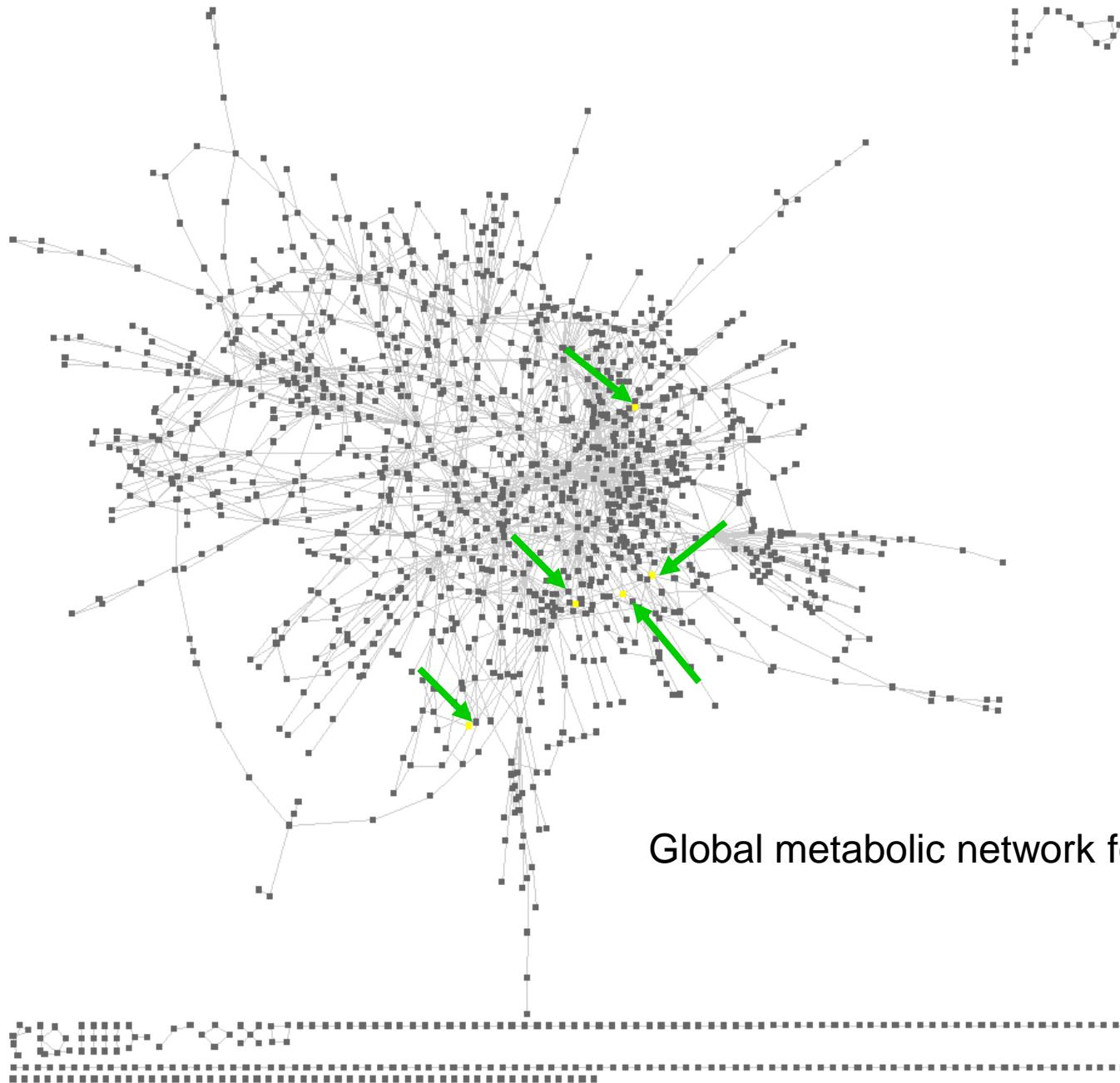
- Compound not identified, not present in organism (white circle)
- Compound not identified, present in organism (light green circle)
- Compound identified, not present in organism (blue circle)
- Compound identified, present in organism (red circle)
- Tagged enzyme, not annotated in organism (yellow box)
- Tagged enzyme, annotated in organism (pink box)
- Compound ambiguous, e.g. multiple compounds (pink circle)

Fabien Jourdan

UPS. 2014

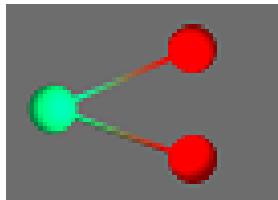
Approche globale

- Certains processus métaboliques peuvent faire appel à plusieurs voies.
- L'objectif est de proposer des méthodes permettant d'analyser ces données dans le réseau et non dans les voies.

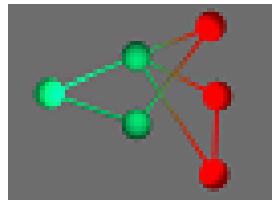


Approche par voisinage

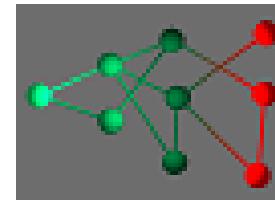
- Partir de chaque élément sélectionner et construire le réseau de proche en proche



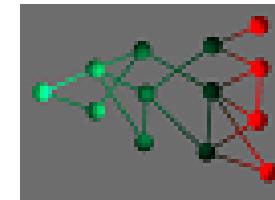
Distance 1



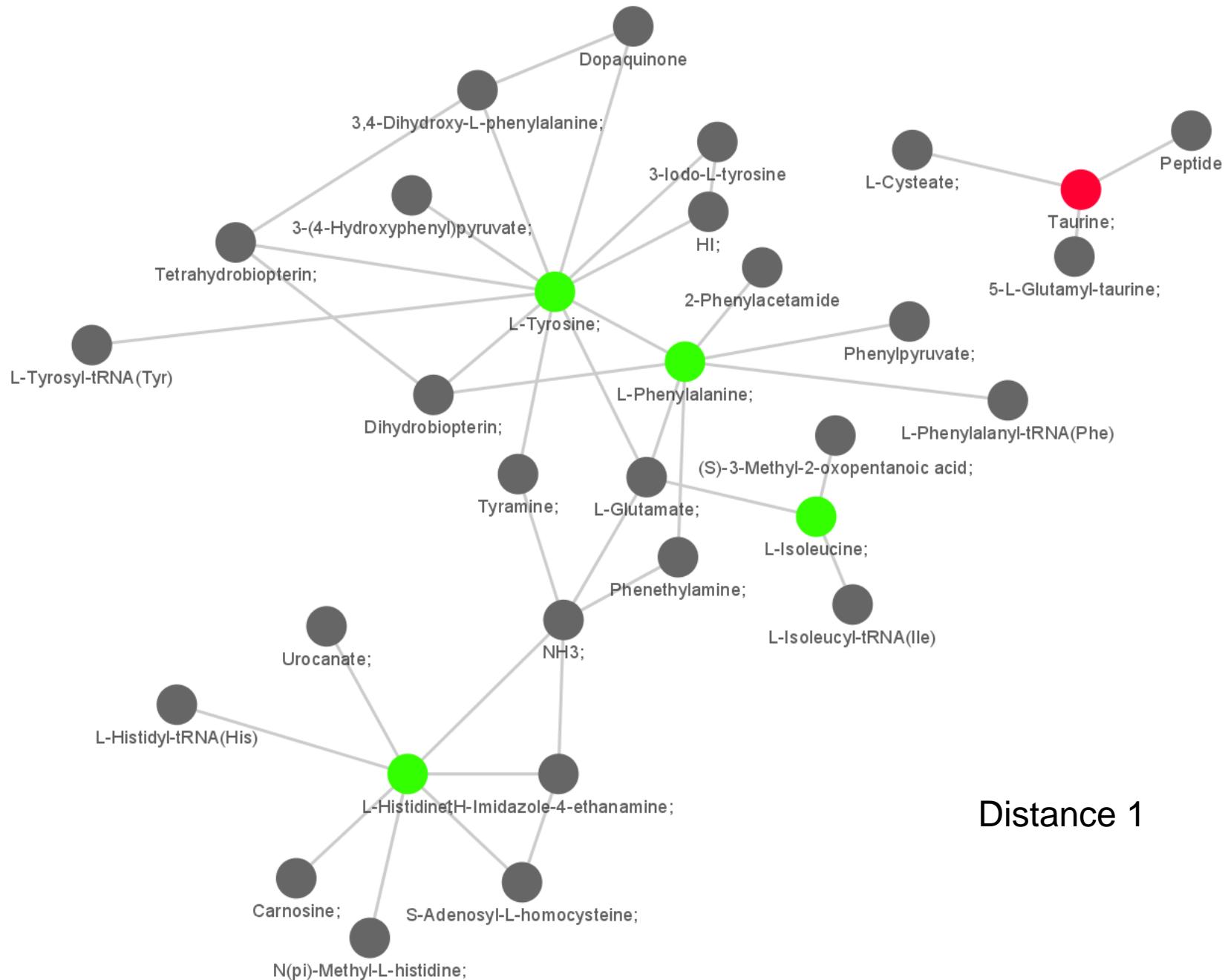
Distance 2

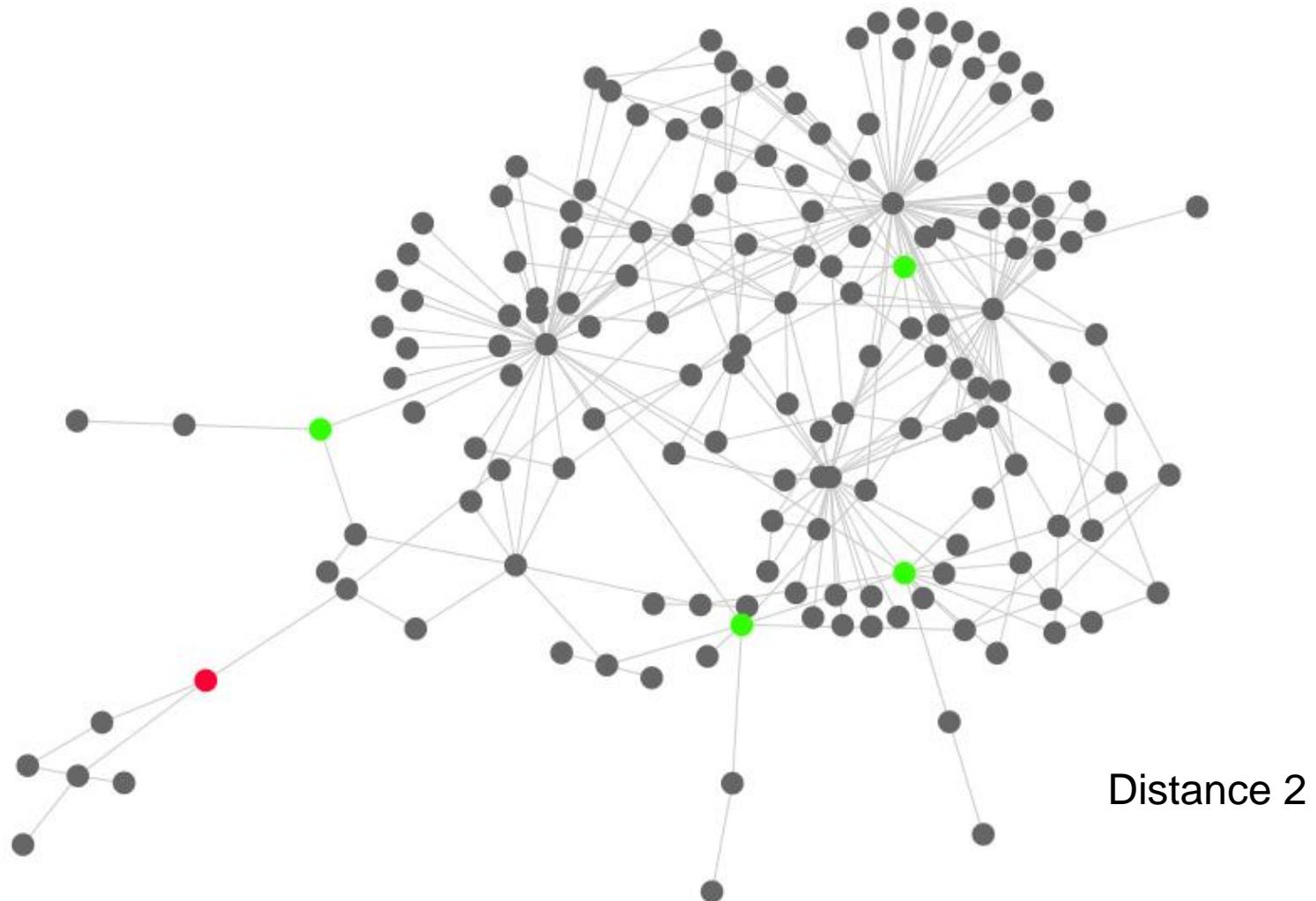


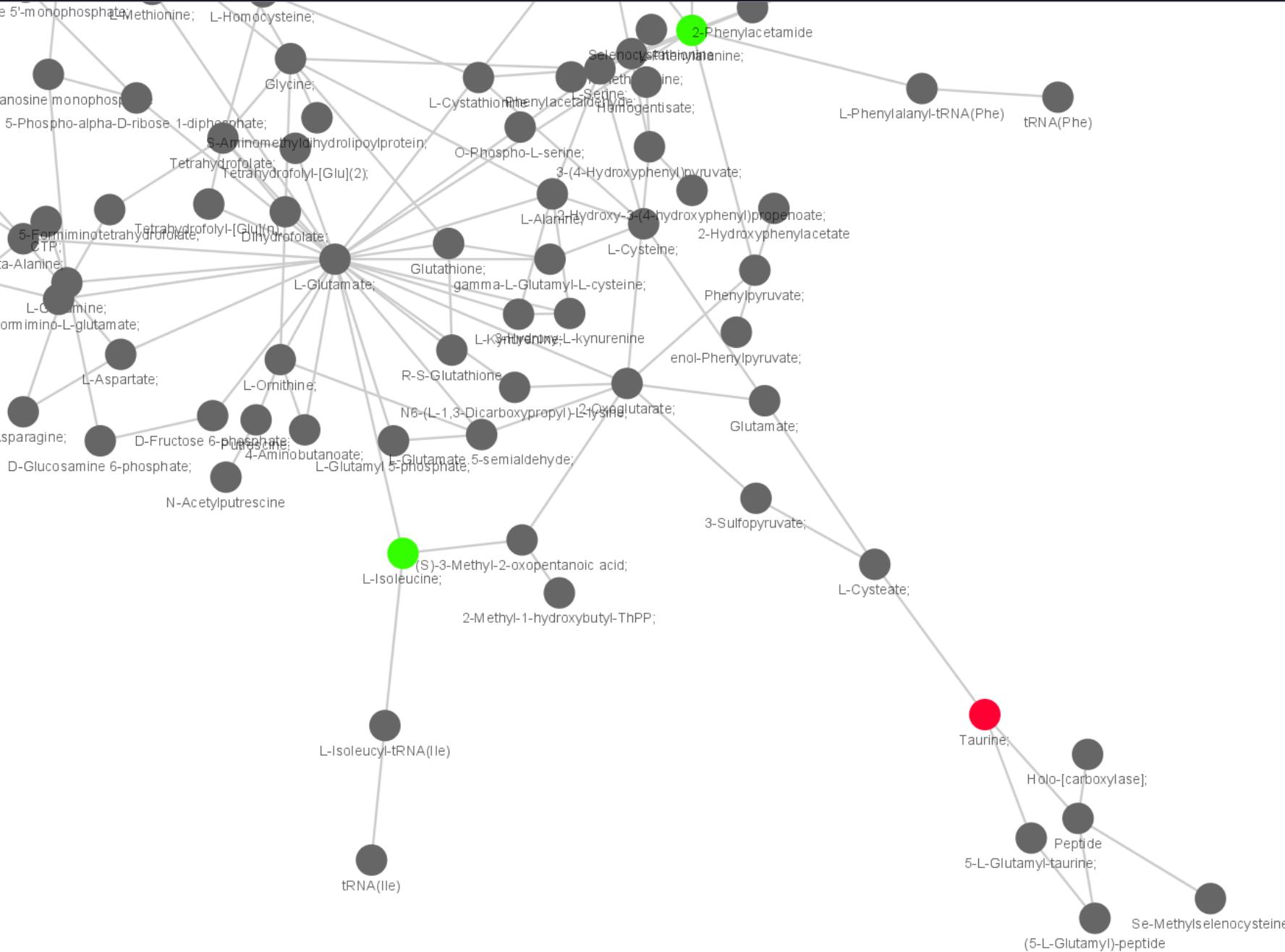
Distance 3



Distance 4

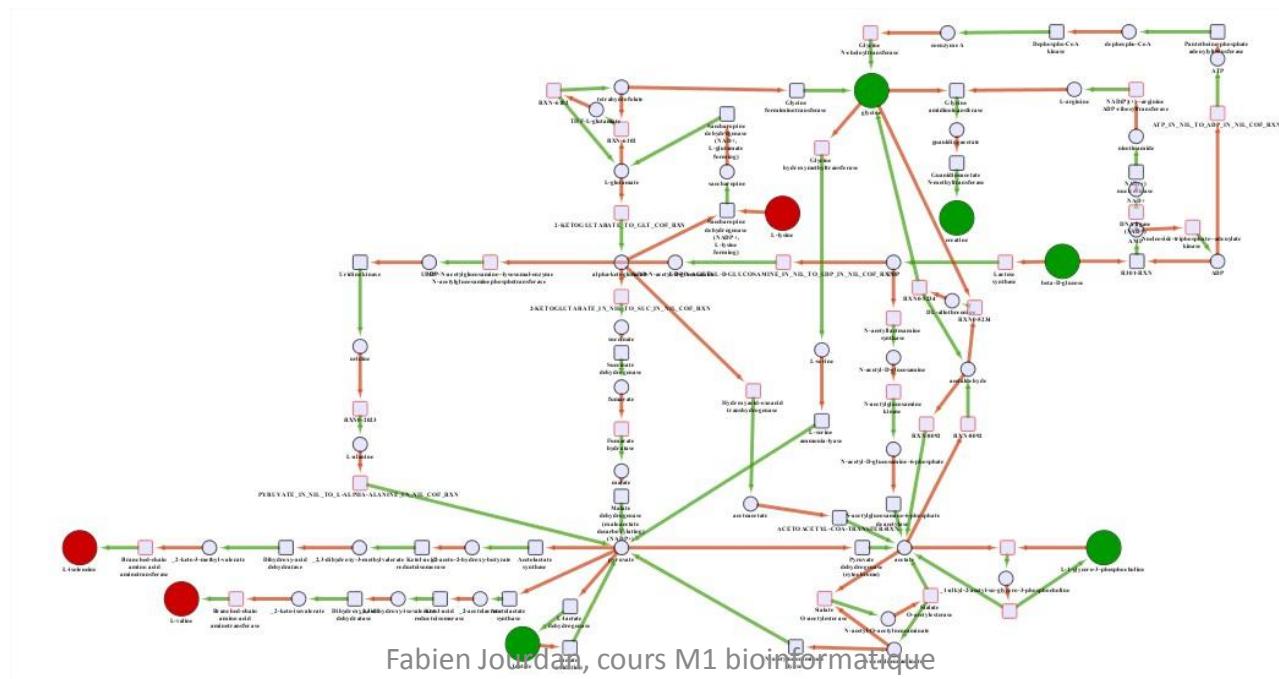






Moyens pour identifier les sous réseaux

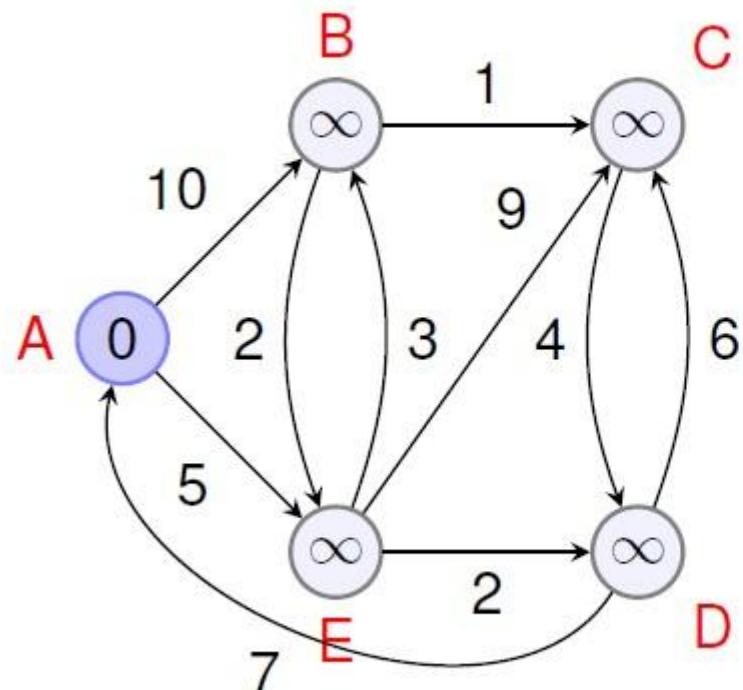
- Chemins les plus courts entre chaque paire de métabolites
 - Faire l'union de ces chemins



Shortest path

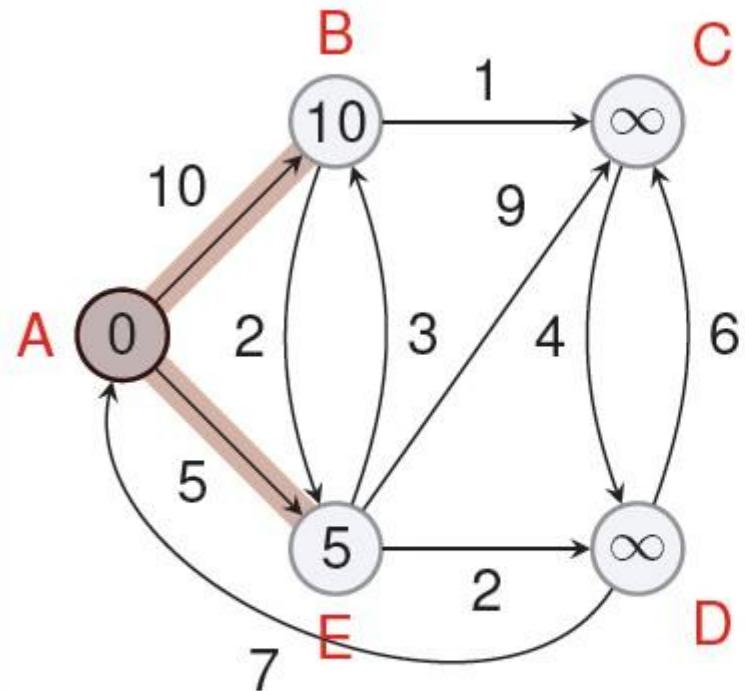
- Trouver le plus court chemin entre deux métabolites.
- Exemple de solution : algorithme de Dijkstra

On se place au sommet de plus petit poids, ici le sommet A.



A	B	C	D	E
0	∞	∞	∞	∞
•	•	•	•	•
•	•	•	•	•
•	•	•	•	•

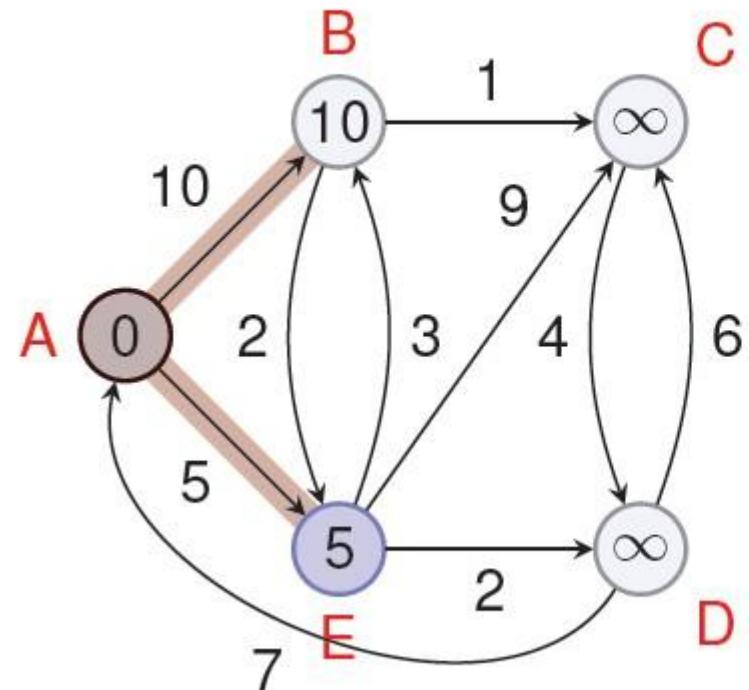
On étudie chacune des arêtes partant du sommet choisi.



A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	5_A
•				
•				
•				
•				

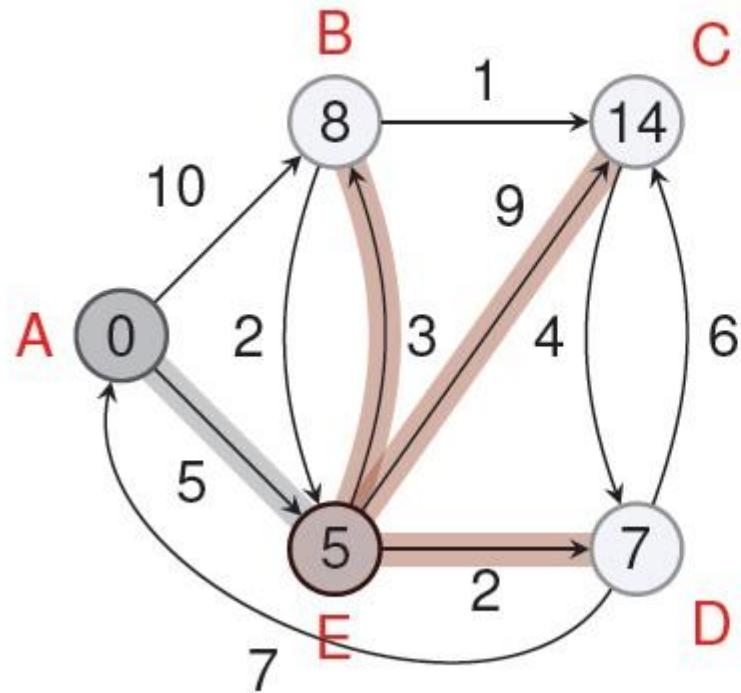
Dans les colonnes, on mets la distance à A , et le sommet d'où l'on vient.

On se place de nouveau au sommet de plus petit poids, ici E .

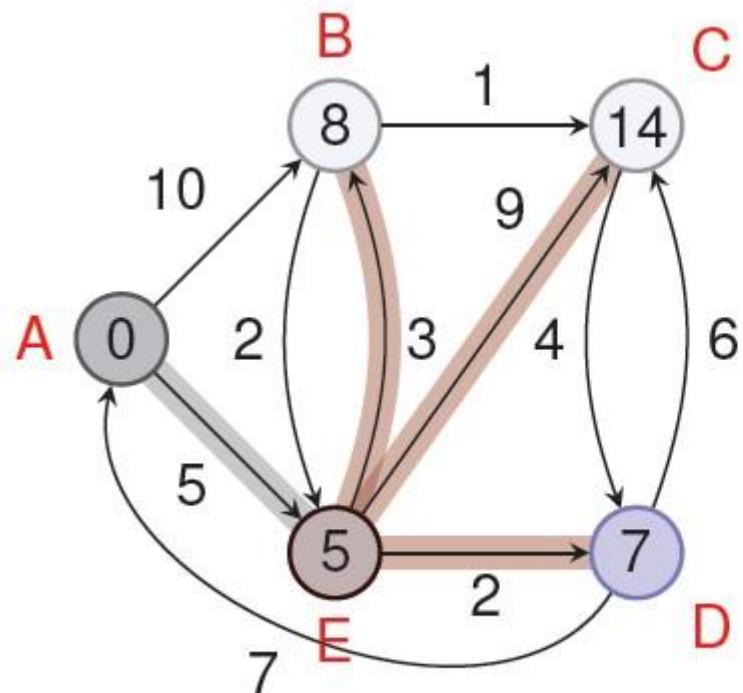


A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	∞
•	•	∞	∞	∞
•	•	•	∞	5_A
•	•	•	•	•

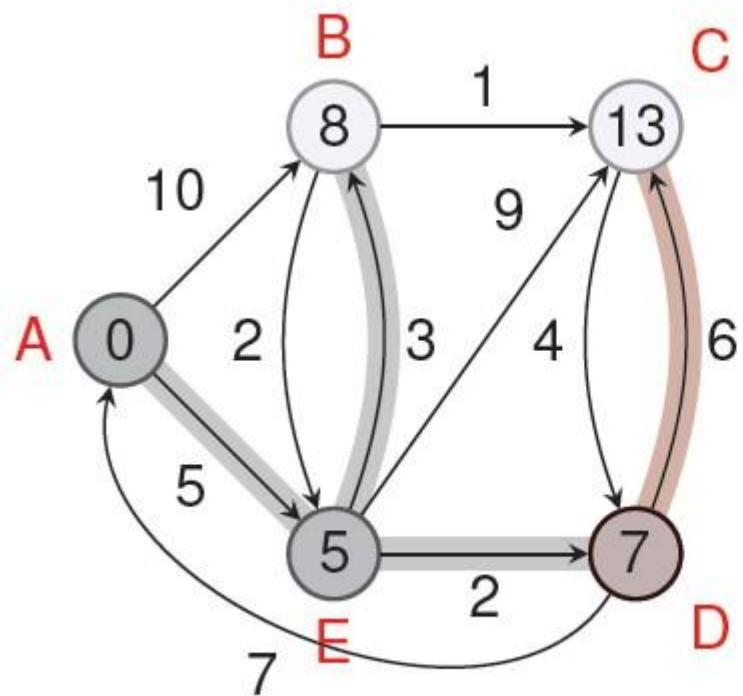
Et ainsi de suite.



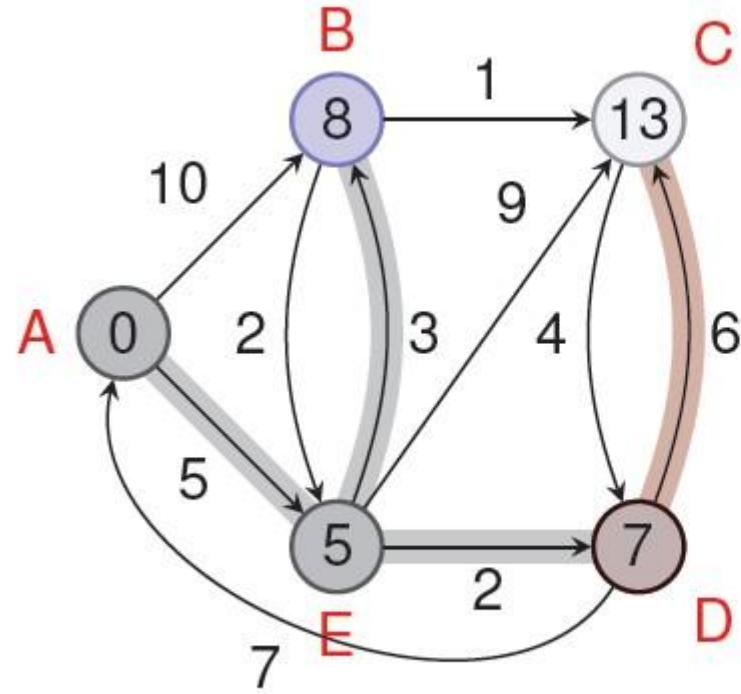
A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	5_A
•	8_E	14_E	7_E	•
•				•
•				•



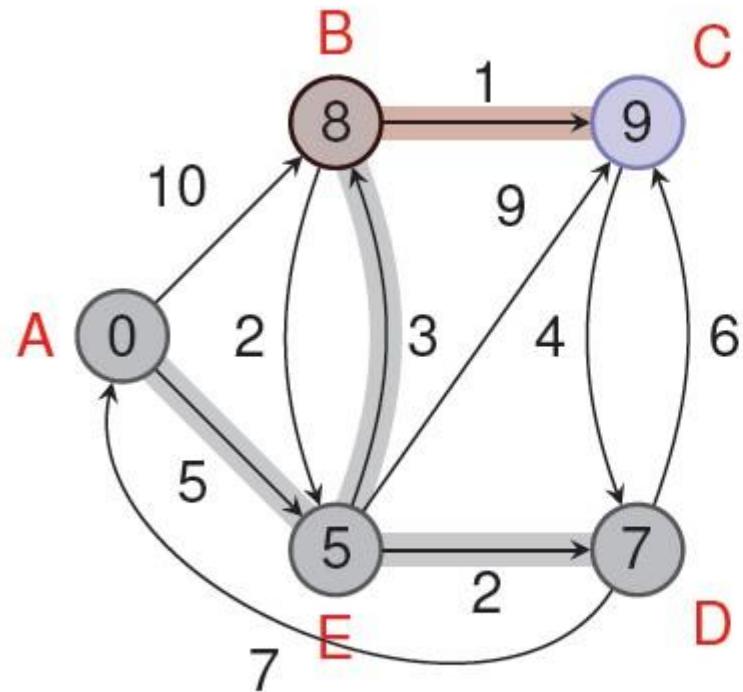
A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	5_A
•	8_E	14_E	7_E	•
•			•	•
•			•	•



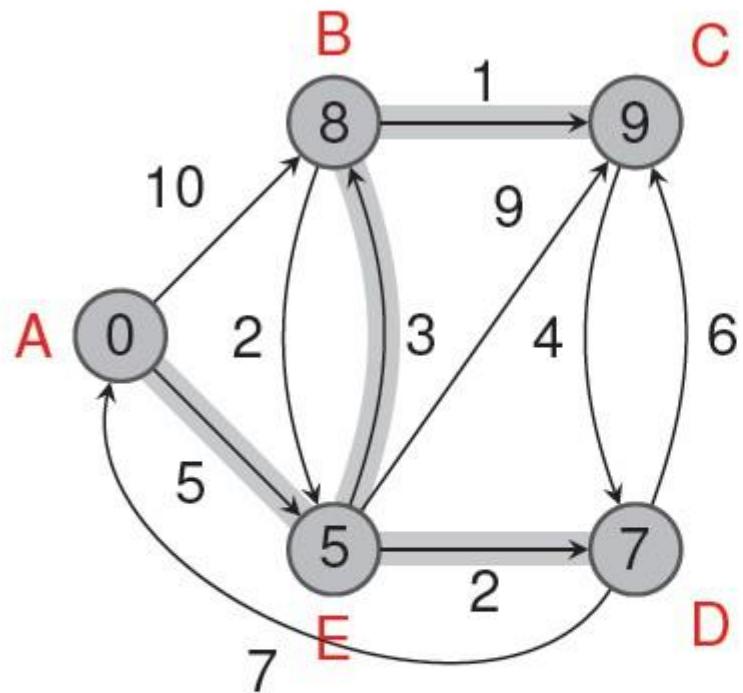
A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	5_A
•	8_E	14_E	13_D	•
•	8_E		•	•
•			•	•
•			•	•



A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	5_A
•	8_E	14_E	7_E	•
•	8_E	13_D	•	•
•	•	•	•	•
•	•	•	•	•



A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	5_A
•	8_E	14_E	7_E	•
•	8_E	13_D	•	•
•	•	9_B	•	•
•	•		•	•



A	B	C	D	E
0	∞	∞	∞	∞
•	10_A	∞	∞	5_A
•	8_E	14_E	7_E	•
•	8_E	13_D	•	•
•	•	9_B	•	•
•	•	•	•	•

Si l'on ne considère que les flèches soulignées, on obtient un *arbre*, un graphe sans cycle.

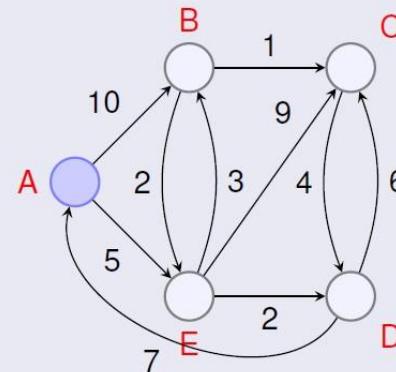
Algorithme de Dijkstra

```

1  function Dijkstra(Graph, source):
2      for each vertex v in Graph:           // Initializations
3          dist[v] := infinity ;            // Unknown distance function from source to v
4          previous[v] := undefined ;       // Previous node in optimal path from source
5      end for ;
6      dist[source] := 0 ;                  // Distance from source to source
7      Q := the set of all nodes in Graph ; // All nodes in the graph are unoptimized - thus are in Q
8      while Q is not empty:              // The main loop
9          u := vertex in Q with smallest distance in dist[] ;
10         if dist[u] = infinity:
11             break ;                    // all remaining vertices are inaccessible from source
12         end if ;
13         remove u from Q ;
14         for each neighbor v of u:      // where v has not yet been removed from Q.
15             alt := dist[u] + dist_between(u, v) ;
16             if alt < dist[v]:          // Relax (u,v,a)
17                 dist[v] := alt ;
18                 previous[v] := u ;
19                 decrease-key v in Q;   // Reorder v in the Queue
20             end if ;
21         end for ;
22     end while ;
23     return dist[] ;
24 end Dijkstra.

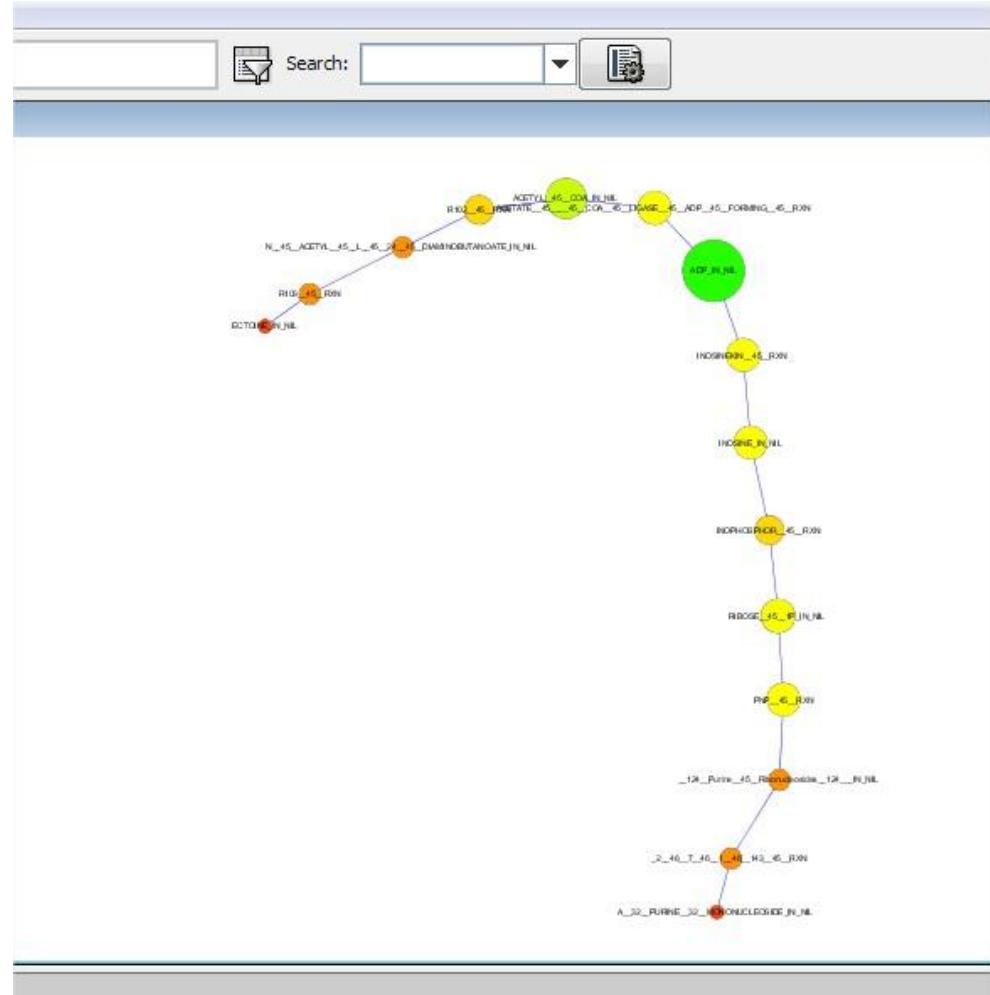
```

Cherchons les plus courts chemins d'origine A dans ce graphe:



Shortest path

- Trouver le plus court chemin entre deux métabolites.
 - Exemple de solution : algorithme de Dijkstra
 - MAIS on obtient des chemins qui passent par des métabolites de fort degrés pas nécessairement informatifs d'un point de vue biologique.



Lightest path

- Objectif : éviter les chemins contenant des métabolites « hubs »
- Prendre en compte le degré des métabolites pour calculer un chemin
- Possibilité de pondérer en fonction de la consommation d'énergie

doi:10.1016/j.jmb.2005.09.079

J. Mol. Biol. (2006) 356, 222–236



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Inferring Meaningful Pathways in Weighted Metabolic Networks

Fabien Jourdan, cours M1 Bioinformatique
Didier Croes¹, Fabian Couche¹, Shoshana J. Wodak^{1,2*}
UPS² and Jacques van Helden^{1*}

Adaptez l'algorithme de Dijkstra

```
1 function Dijkstra(Graph, source):
2     for each vertex v in Graph:           // Initializations
3         dist[v] := infinity ;             // Unknown distance function from source to v
4         previous[v] := undefined ;        // Previous node in optimal path from source
5     end for ;
6     dist[source] := 0 ;                  // Distance from source to source
7     Q := the set of all nodes in Graph ; // All nodes in the graph are unoptimized - thus are in Q
8     while Q is not empty:              // The main loop
9         u := vertex in Q with smallest distance in dist[] ;
10        if dist[u] = infinity:
11            break ;                    // all remaining vertices are inaccessible from source
12        end if ;
13        remove u from Q ;
14        for each neighbor v of u:      // where v has not yet been removed from Q.
15            alt := dist[u] + dist_between(u, v) ;
16            if alt < dist[v]:          // Relax (u,v,a)
17                dist[v] := alt ;
18                previous[v] := u ;
19                decrease-key v in Q;   // Reorder v in the Queue
20            end if ;
21        end for ;
22    end while ;
23    return dist[] ;
24 end Dijkstra.
```

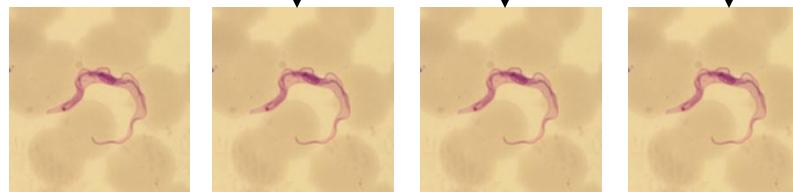
Utilisation de la métabolomique pour la reconstruction

Trypanosoma Brucei

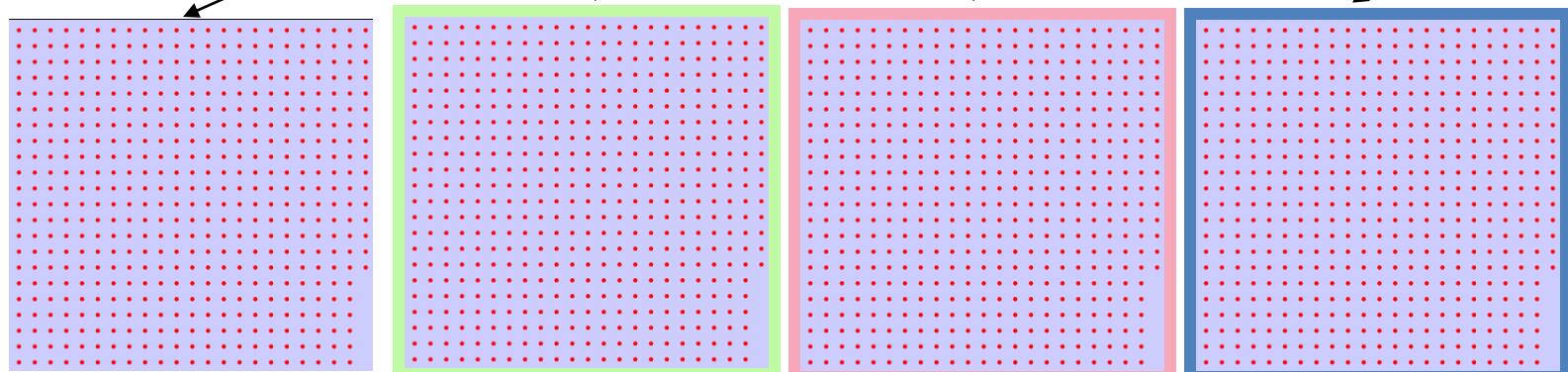
Drug A

Drug B

Drug C



High
resolution
metabolome



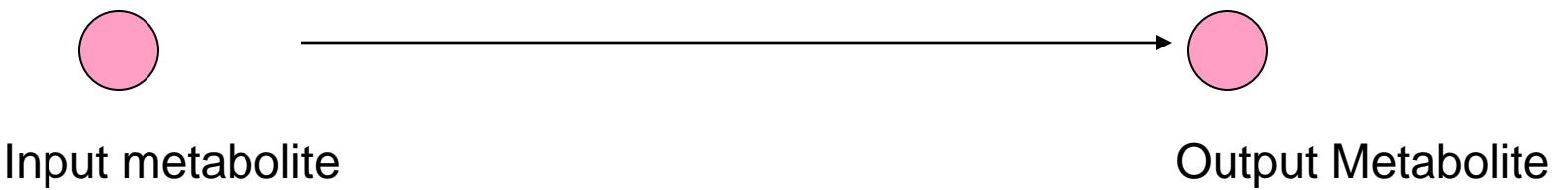
Networks



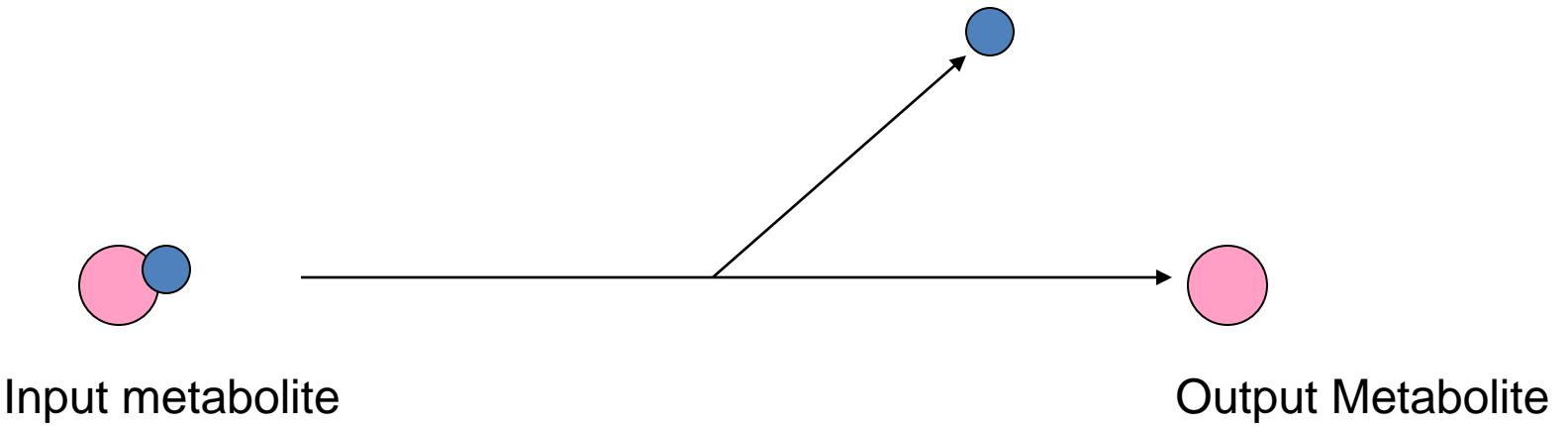
Ab initio method

Breitling R, Ritchie S, Goodenowe D, Stewart ML, Barrett MP (2006): **Ab initio prediction of metabolic networks using Fourier Transform Mass Spectrometry data.** *Metabolomics* 2: 155–164.

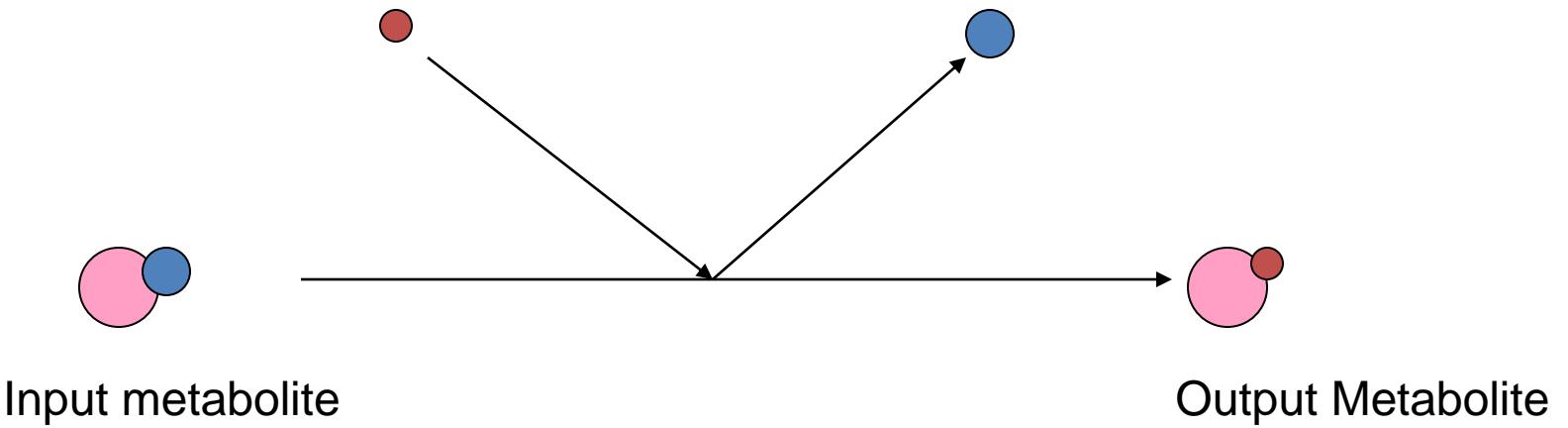
Most metabolic reactions are like that:



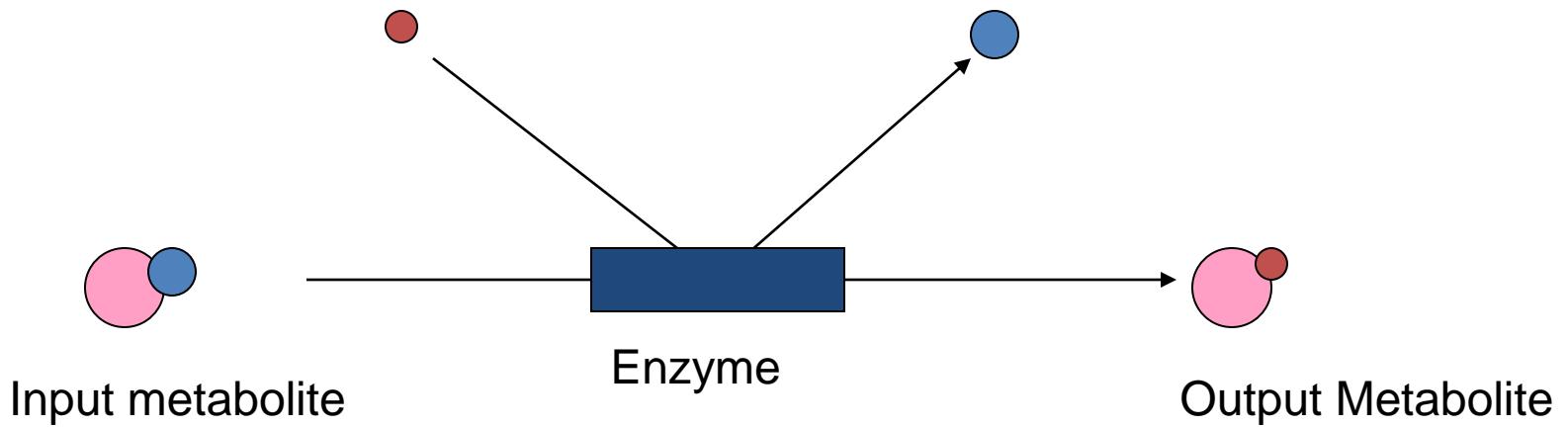
Most metabolic reactions are like that:



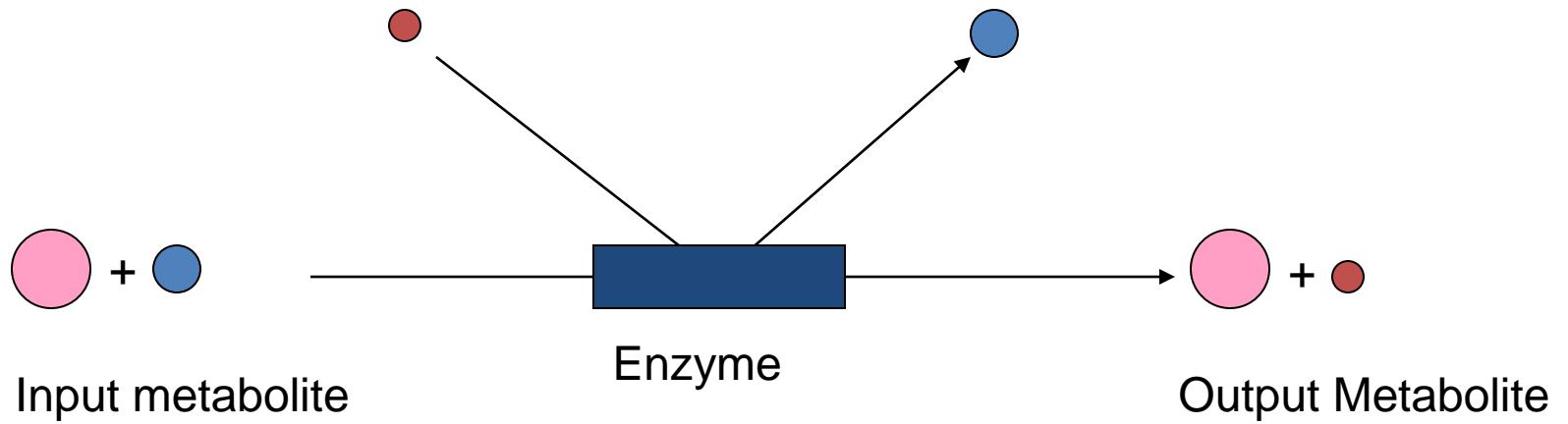
Most metabolic reactions are like that:



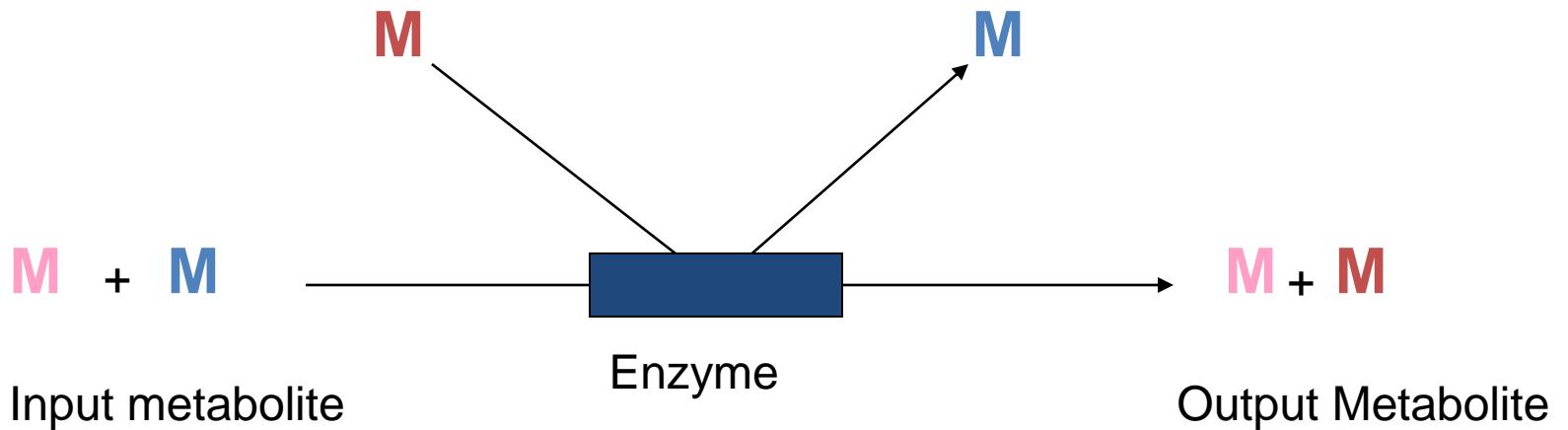
Most metabolic reactions are like that:



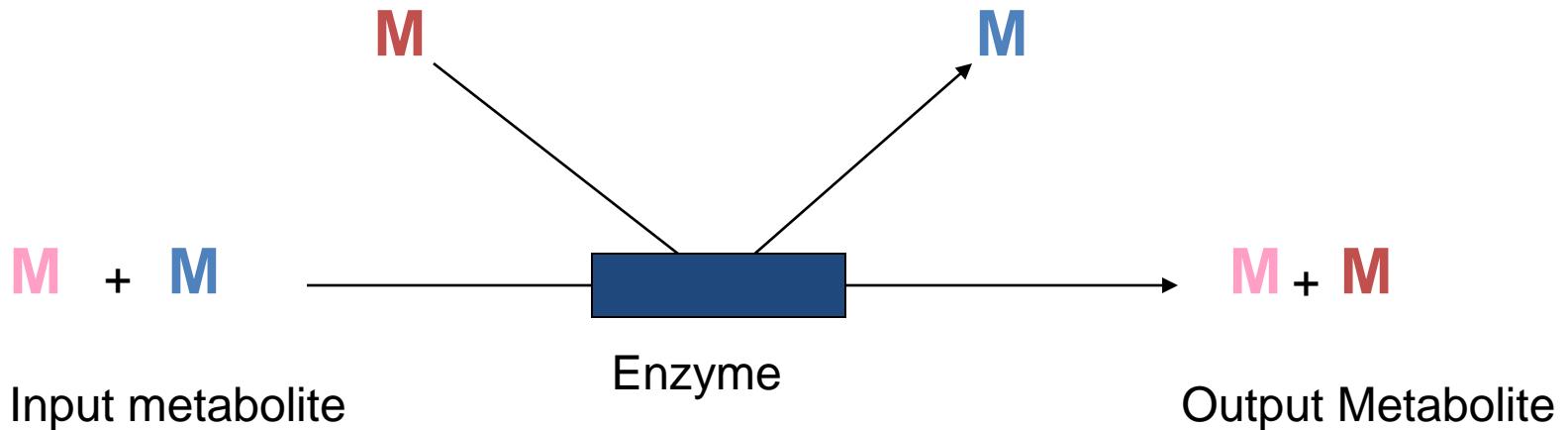
Most metabolic reactions are like that:



Most metabolic reactions are like that:

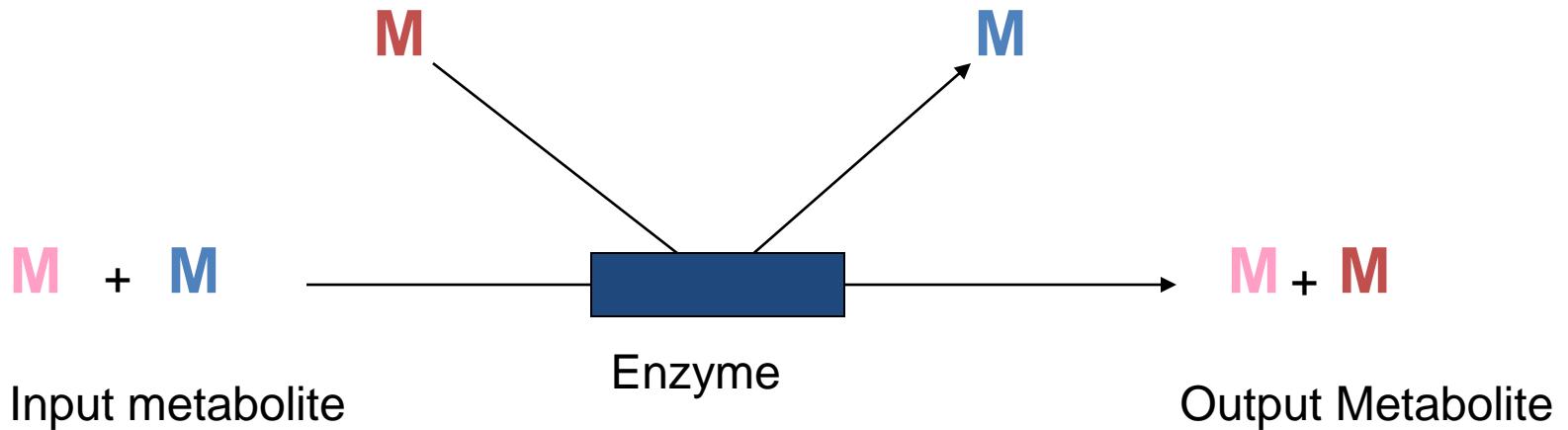


Most metabolic reactions are like that:



$$\text{Output Metabolite} = \text{Input metabolite} + M - M$$

Most metabolic reactions are like that:



$$\text{Output Metabolite} = \text{Input metabolite} + \boxed{\text{M} - \text{M}}$$

Transformation function

Transformations

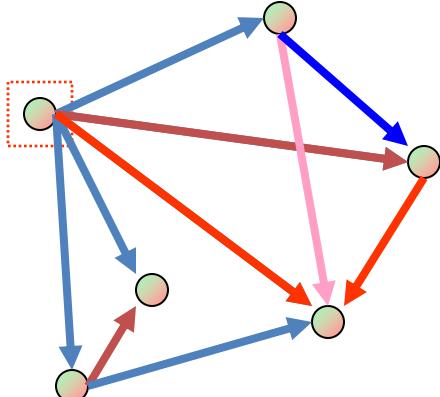
Transformation label	Formula	Mass
Isomeric		0
Alanine	C3H5NO	71.0371138
Arginine	C6H12N4O	156.101111
Asparagine	C4H6N2O2	114.042928
Aspartic Acid	C4H5NO3	115.026943
Cysteine	C3H5NOS	103.009186
Cystine	C6H10N2O3S2	222.013286
Glutamic Acid	C5H7NO3	129.042593
Glutamine	C5H8N2O2	128.058578
Glycine	C2H3NO	57.0214638
Histidine	C6H7N3O	137.058912
Isoleucine	C6H11NO	113.084064
Leucine	C6H11NO	113.084064
Lysine	C6H12N2O	128.094963
Methionine	C5H9NOS	131.040486
Phenylalanine	C9H9NO	147.068414
Proline	C5H7NO	97.0527639
Serine	C3H5NO2	87.0320285
Threonine	C4H7NO2	101.047679
Tryptophan	C11H10N2O	186.079313
Tyrosine	C9H9NO2	163.063329
Valine	C5H9NO	99.068414
acetotacetate (-H2O)	C4H4O2	84.0211294
acetone (-H)	C3H5O	57.0340398
adenylate (-H2O)	C10H12N5O6P	329.052522
biotinyl (-H)	C10H15N2O3S	243.080339
biotinyl (-H2O)	C10H14N2O2S	226.0776
carbamoyl P transfer (-H2PO4)	CH2ON	44.0136387
co-enzyme A (-H)	C21H34N7O16P3S	765.099566
co-enzyme A (-H2O)	C21H33N7O15P3S	748.096826
glutathione (-H2O)	C10H15N3O5S	289.073243
Glucuronic Acid (-H2O)	C6H8O6	176.032088
monosaccharide (-H2O)	C6H10O5	162.052824
trisaccharide (-H2O)	C18H30O15	486.158471
erythrose (-H2O)	C4H8O4	102.031695
transamination (-O)	NH4	2.039459
isoprene addition (-H)		
malonyl group (-H2O)		
palmitoylation (-H2O)		
pyridoxal phosphate (-H2O)		
urea addition (-H)		
adenine (-H)		
adenosine (-H2O)		
Adenosine 5'-diphosphate (-H2O)		
Adenosine 5' monophosphate (-H2O)		
cytidine 5' diphosphate (-H2O)		
cytidine 5' monophosphate (-H2O)		
cytosine (-H)		
Guanosine 5-diphosphate (-H2O)		
Guanosine 5-monophosphate (-H2O)		
guanine (-H)		
guanosine (-H2O)		
deoxythymidine 5' diphosphate (-H2O)		
thymidine (-H2O)		
thymine (-H)		
thymidine 5' monophosphate (-H2O)		
uridine 5' diphosphate (-H2O)		
uridine 5' monophosphate (-H2O)		
uracil (-H)		
uridine (-H2O)		
acetylation (-H)		
acetylation (-H2O)		
C2H2		
Carboxylation		
CHO2		
condensation/dehydration		
diphosphate		
ethyl addition (-H2O)		
Formic Acid (-H2O)		
glyoxylate (-H2O)		
hydrogenation/dehydrogenation		
hydroxylation (-H)		
Inorganic Phosphate		
ketol group (-H2O)		
methanol (-H2O)		
phosphate		
primary amine		
pyrophosphate		
secondary amine		
sulfate (-H2O)		
tertiary amine		
C6H10O5		
C6H10O6		
D-Ribose (-H2O) (ribosylation)		
disaccharide (-H2O)		
glucose-N-Phosphate (-H2O)		

C5H7	67.0547753
C3H2O3	86.000394
C16H30O	238.229666
C8H8N05P	229.014011
CH3N2O	59.0245378
C5H4N5	134.04667
C10H11N5O3	249.086189
C10H13N5O9P2	409.018854
C10H12N5O6P	329.052522
C9H13N3O10P2	385.007621
C9H12N3O7P	305.041288
C4H4N3O	110.035437
C10H13N5O10P2	425.013769
C10H12N5O7P	345.047436
C5H4N5O	150.041585
C10H11N5O4	265.081104
C10H14N2O10P2	384.012372
C10H12N2O4	224.079707
C5H5N2O2	125.035102
C10H13N2O7P	304.046039
C9H12N2O11P2	385.991636
C9H11N2O8P	306.025304
C4H3N2O2	111.019452
C9H10N2O5	226.058972
C2H3O2	59.0133044
C2H2O	42.0105647
C2H2	26.0156501
CO2	43.9898293
CHO2	44.9976543
H2O	18.0105647
H3O6P2	160.94049
C2H4	28.0313001
CO	27.9949146
C2O2	55.9898293
H2	2.01565007
O	15.9949146
P	30.9737634
C2H2O	42.0105647
CH2	14.0156501
HPO3	79.9663324
NH2	16.0187241
PP	61.9475268
NH	15.010899
SO3	79.9568157
N	14.003074
C6H10O5	162.052824
C6H10O6	178.047738
C5H8O4	132.042259
C12H20O11	340.100562
C6H11O8P	242.019156

Ab initio method

Breitling R, Ritchie S, Goodenowe D, Stewart ML, Barrett MP (2006): **Ab initio prediction of metabolic networks using Fourier Transform Mass Spectrometry data.** *Metabolomics* 2: 155–164.

Alanine	C3H5NO	71.037113835
Arginine	C6H12N4O	156.101111124
Asparagine	C4H6N2O2	114.042927522
Aspartic Acid	C4H5NO3	115.026943115
Cysteine	C3H5NOS	103.009185635



Algorithm:

For each mass m

 For each transformation T

 For each other mass m'

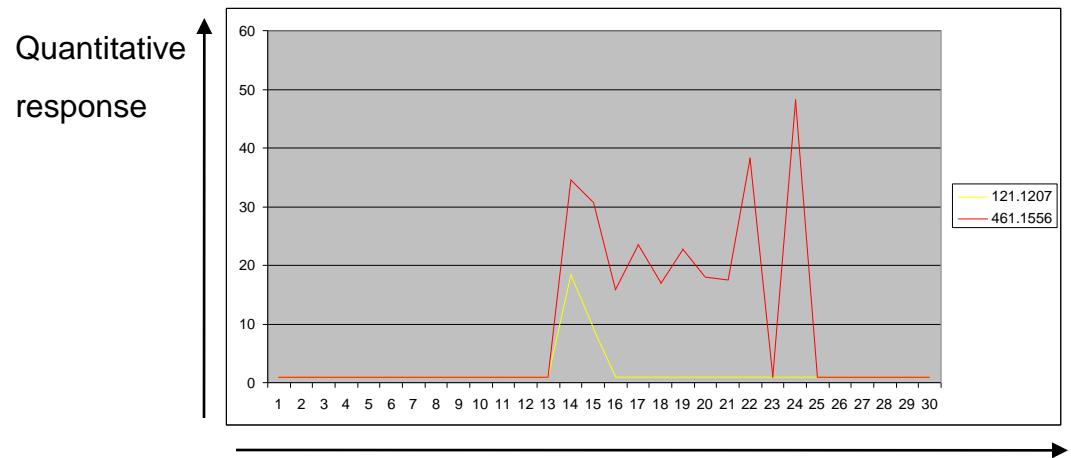
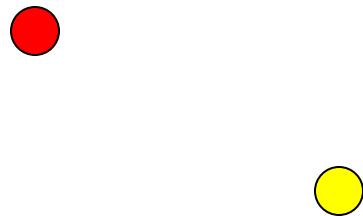
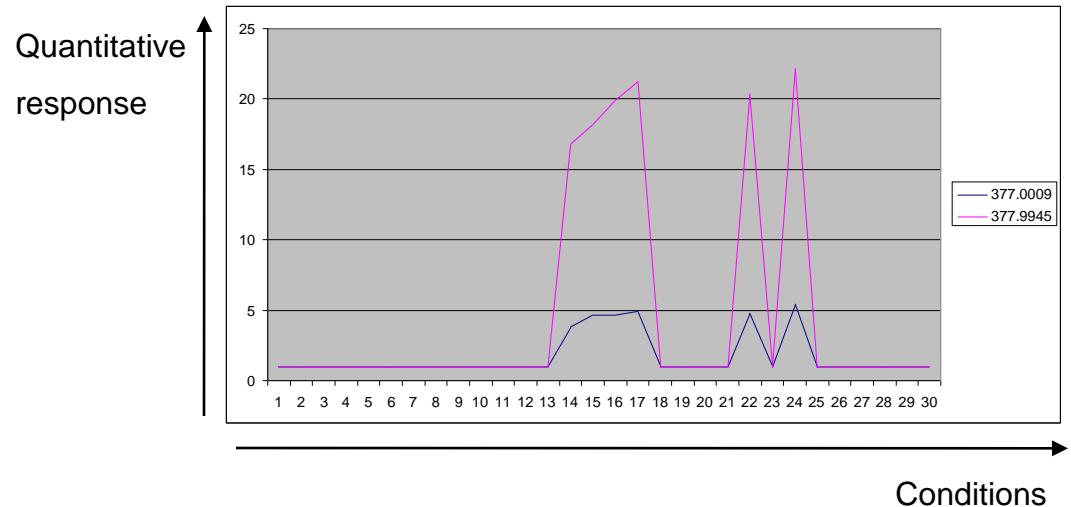
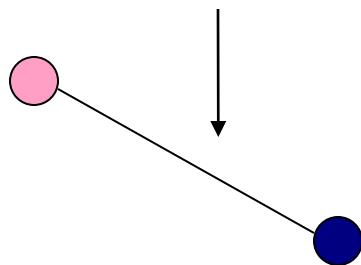
 If($T(m)=m'$)

 Add an edge between m and m'

Méthode des corrélations

- Avec les méthodes telles que la RMN ou la masse on peut avoir les réponses quantitatives pour chaque variables
- Ces valeurs correspondent à l'aire sous le pic

“high correlation”





Discovery of meaningful associations in genomic data using partial correlation coefficients

Alberto de la Fuente*, Nan Bing†, Ina Hoeschele and Pedro Mendes

Virginia Polytechnic Institute and State University, Virginia Bioinformatics Institute,
1880 Pratt Drive, Blacksburg, Virginia, 24061 USA

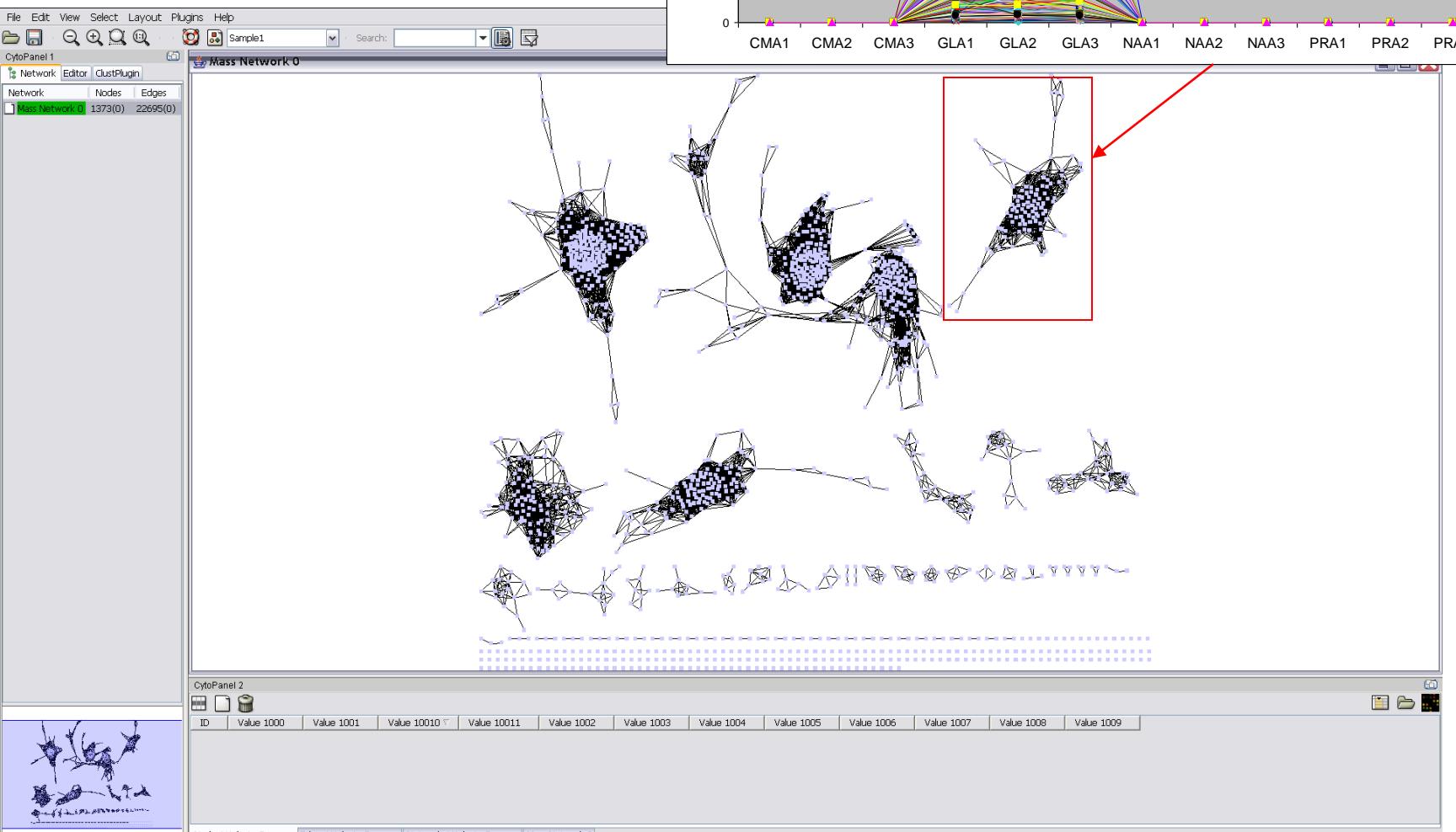
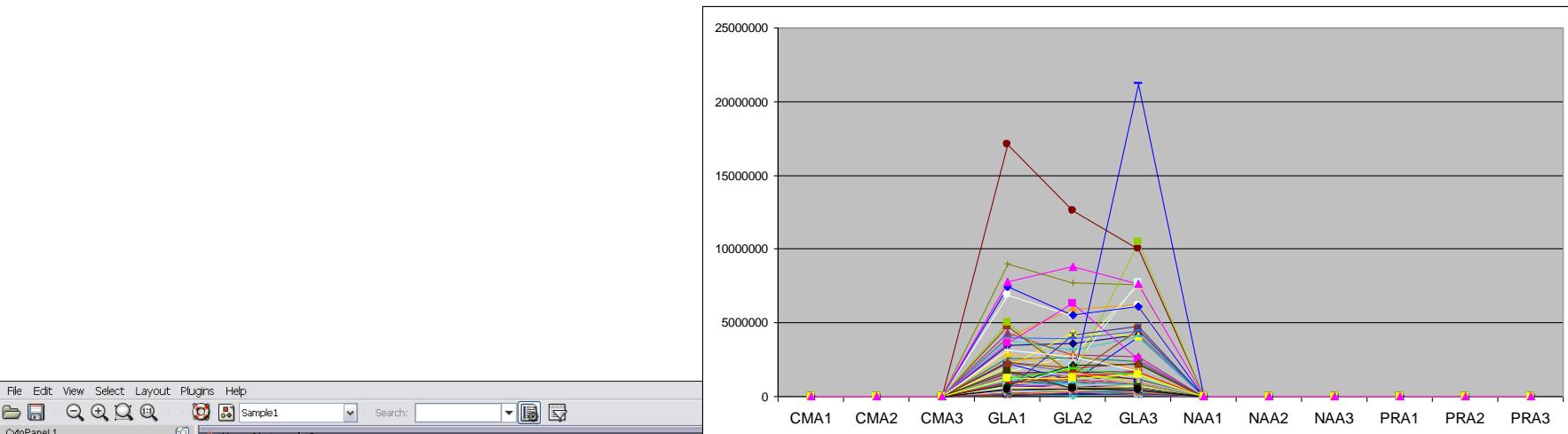
Received on June 2, 2004; revised on July 15, 2004; accepted on July 24, 2004

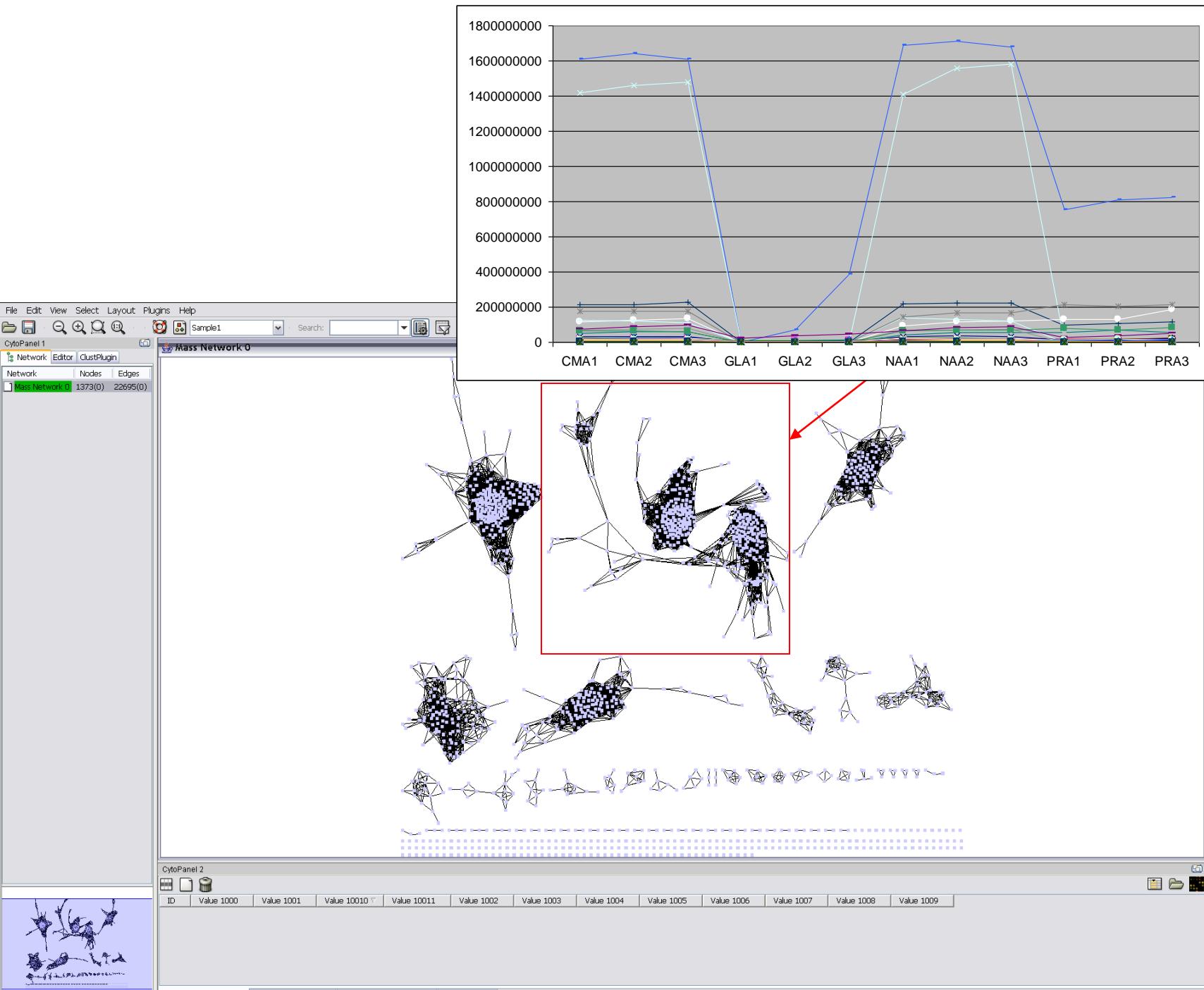
Advance Access publication July 29, 2004

$$\text{zeroth-order correlation: } r_{xy} = \frac{\text{cov}(x,y)}{\sqrt{\text{var}(x)\text{var}(y)}} \quad (1)$$

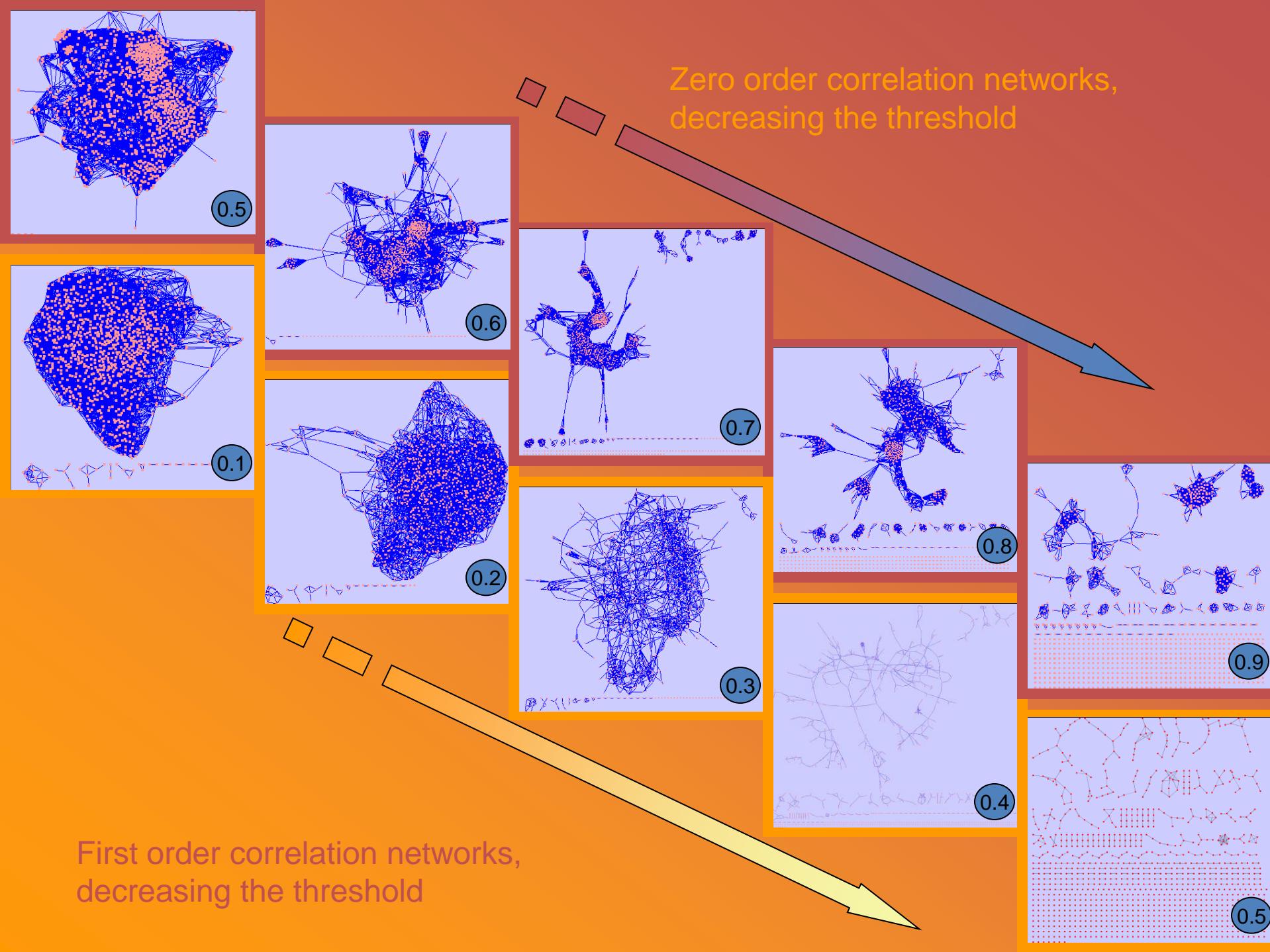
$$\text{first-order correlation: } r_{xy.z} = \frac{r_{xy} - r_{xz}r_{yz}}{\sqrt{(1 - r_{xz}^2)(1 - r_{yz}^2)}} \quad (2)$$

$$\text{second-order correlation: } r_{xy.zq} = \frac{r_{xy.z} - r_{xq.z}r_{yq.z}}{\sqrt{(1 - r_{xq.z}^2)(1 - r_{yq.z}^2)}} \quad (3)$$





Zero order correlation networks,
decreasing the threshold



First order correlation networks,
decreasing the threshold

Dessiner les réseaux métaboliques

Visualizing metabolic networks

- Applying a classical graph drawing algorithm to the network.
- Generally a version of force based algorithms

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SBML Layout Viewer

This site takes an SBML File and renders it according to the SBML Layout Extension, or the JDesigner annotation. An experimental support for CellDesigner Model annotations (Annotation Version 2.5) has been added.

SBML File to visualize:

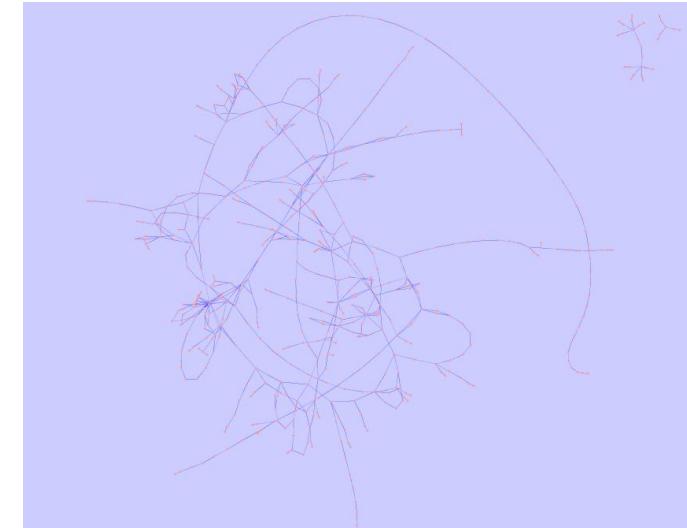
Image Format:

Some experimental settings:
Gravity: 15
Edge Length: 50
 Use Magnetism
 Use Boundary
 Use Grid

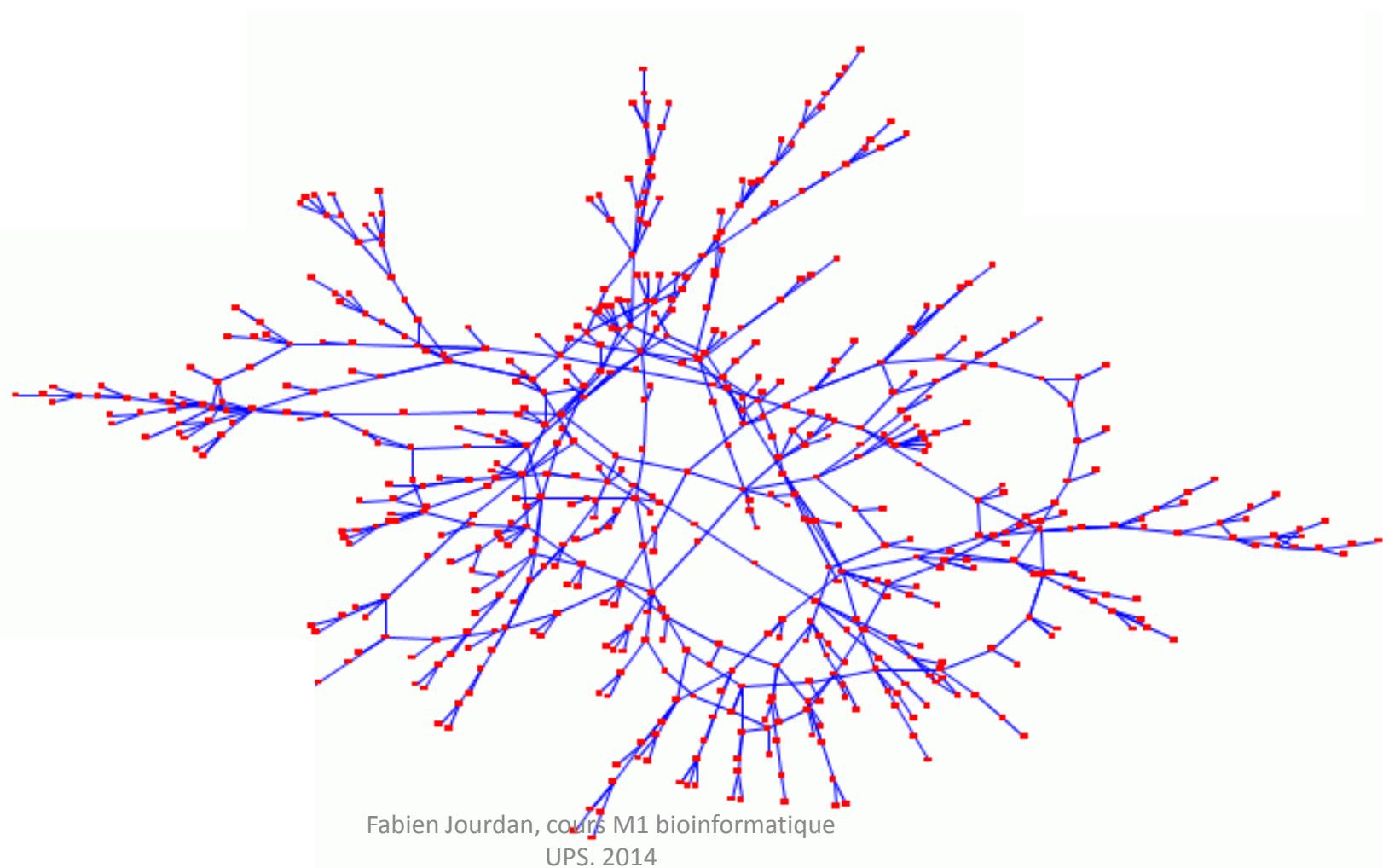
Original Size | Scaled x2 | 640x480 | 1024x768 | SVG Export (experimental)



SBML viewer: <http://sbw.kgi.edu/Layout/>



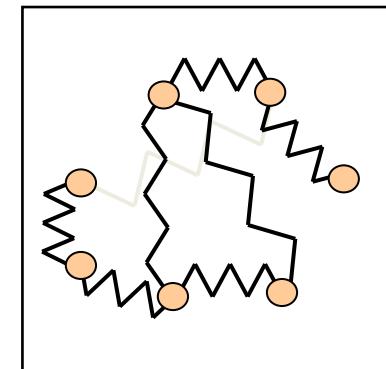
Dessin et analogie physique



Fabien Jourdan, cours M1 bioinformatique
UPS. 2014

Dessin et analogie physique

- On assimile le graphe à un système de particules chargées (masses et ressorts)
 - Elles se repoussent l'une l'autre
 - Les arêtes introduisent des forces d'attraction
- L'algorithme de dessin simule le système physique, les forces, et déplacent les particules (masses)



Dessin et analogie physique

- Le système doit se stabiliser
 - Une configuration stable correspond à un « beau dessin »
- Qualité
 - Bonne répartition des sommets, longueur des arêtes « uniformes »
 - Bonne séparation des composantes plus fortement connectées
 - Intuitif, facile à implémenter (programme)

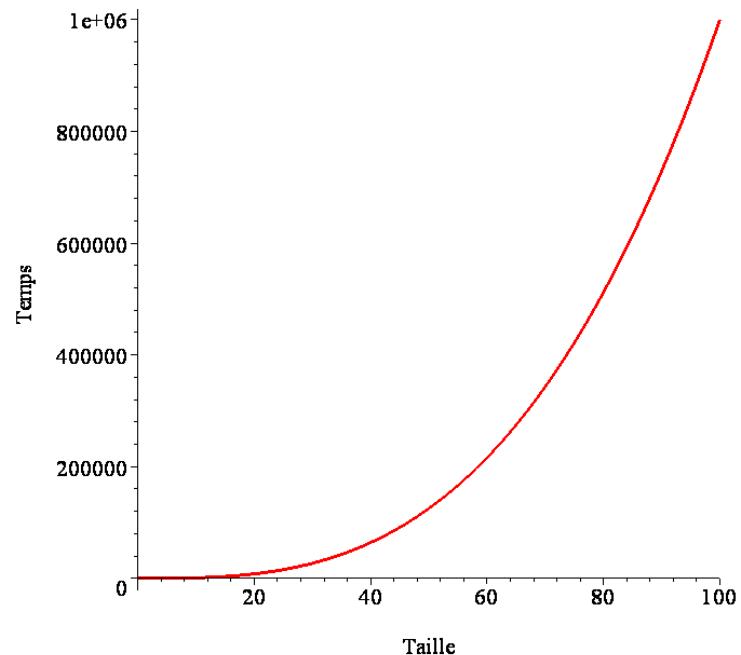
L'algorithme

- On choisit une position de départ aléatoire
- Pour chaque sommet v
 - On calcule un vecteur de force F_v qui modifiera sa position
 - On parcourt tous les sommets et on applique une force de répulsion
 - On parcourt ses voisins et on applique une force d'attraction

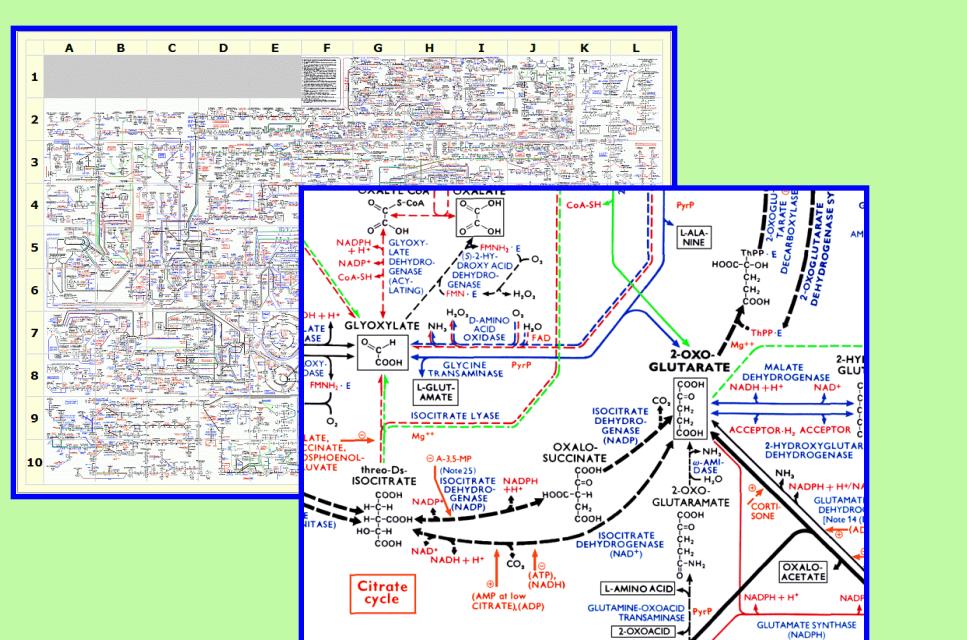
On répète $O(N)$ fois

Dessin et analogie physique

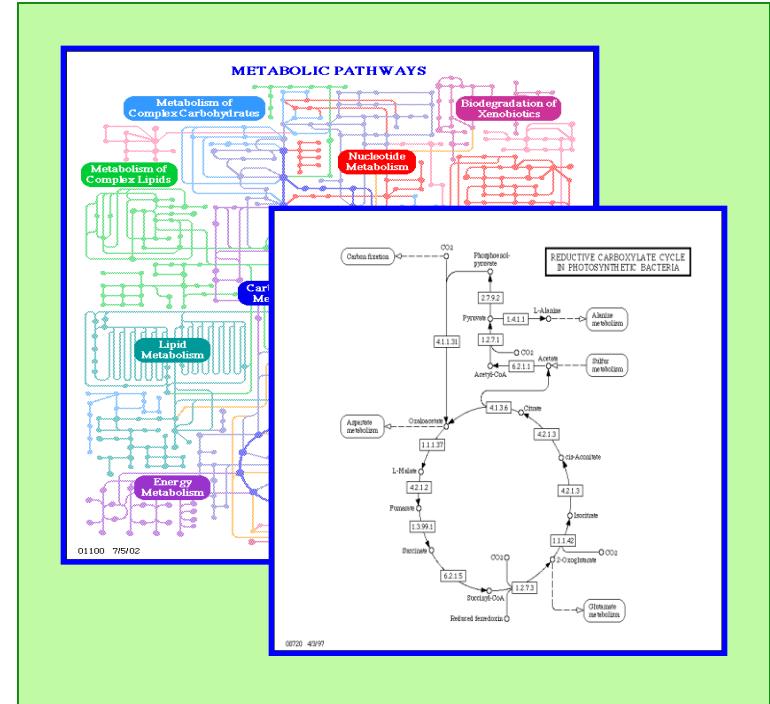
- Défauts
 - Non déterministe
 - Complexité algorithmique élevée (difficile montée en charge)
 - Sensibilité du paramétrage



Manual representations



Boehringer Posters, <http://www.expasy.org>



KEGG



ELSEVIER

BioSystems 47 (1998) 1–7



On representation of metabolic pathways

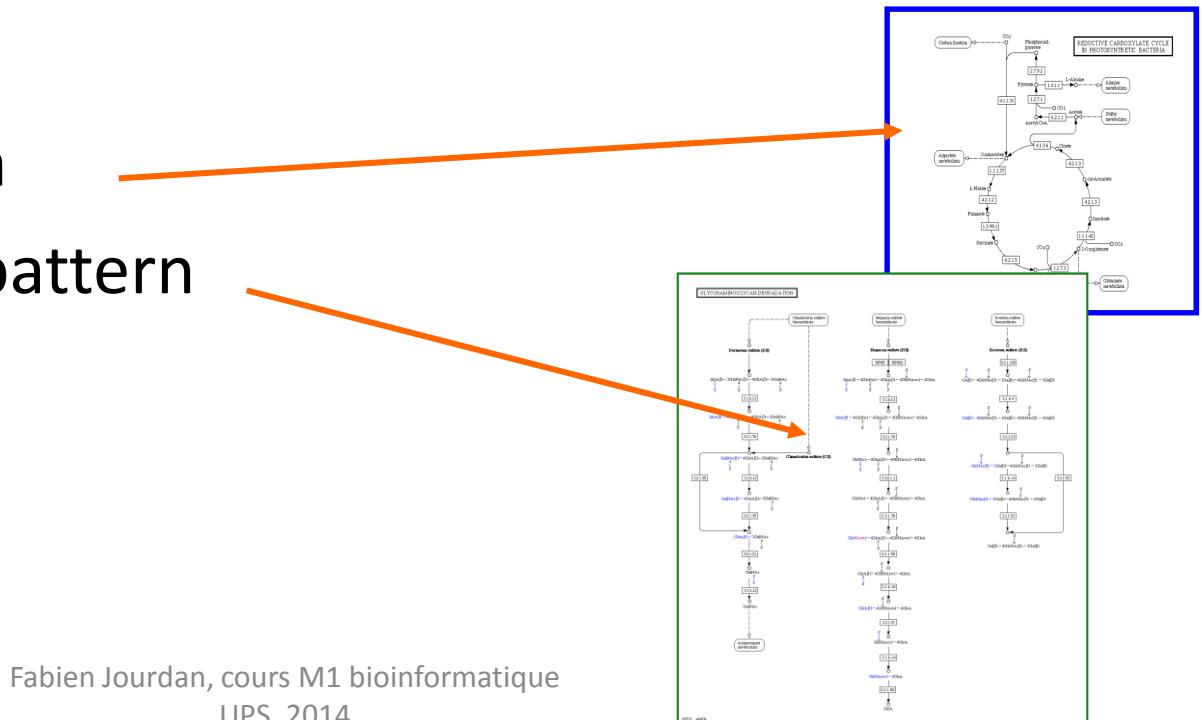
Gerhard Michal *
Kreuzeckstr. 19, D-82327 Tutzing, Germany

Fabien Jourdan, cours M1 bioinformatique
UPS. 2014

And all the text-books....

Importance of manual representations

- Biologists study metabolism from “text-book representation”
- They associate graph patterns to metabolic processes:
 - Cyclic pattern
 - Hierarchical pattern



Metabolic network drawing and constraints

Using metabolic pathways as building blocks of representations

Overview of the *E. coli* Metabolic Map

This diagram provides a schematic of all pathways of *E. coli* metabolism in the ecocyc database. Nodes represent metabolites, with shape indicating class of metabolite (see key); lines represent reactions. Move the mouse over a metabolite icon to identify it; click on a metabolite icon to navigate to the pathway.

- Instructions
- Pathway Tools query page
- Oomics Viewer Paint omics data onto this diagram

A legend on the right side identifies metabolite classes: Amino Acids, Carbohydrates, Proteins, Purines, Pyrimidines, Cotactors, tRNAs, Other, and (Filled) Phosphorylated.

Reactome - a curated knowledgebase of biological pathways

The data displayed is for Homo sapiens. Use the menu to change this species.

Reaction → Experimentally confirmed reaction → Manually inferred reaction → Electronically inferred reaction → Linked reactions

Apoptosis	Cell Cycle Checkpoints	Cell Cycle, Mitotic	DNA Repair
DNA Replication	Electron Transport Chain	Gene Expression	Hemostasis
HIV Infection	Influenza Infection	Immune System Signaling	Insulin receptor mediated signaling
Integration of pathways involved in energy metabolism	Lipid metabolism	Maintenance of Telomeres	Metabolism of amino acids and related nitrogen-containing molecules
Metabolism of glucose, other sugars, and ethanol	Notch Signaling Pathway	Nucleotide metabolism	Oxidative decarboxylation of pyruvate and TCA cycle
Post-translational modification of proteins	TGF-beta signaling	Transcription	Translation
mRNA Processing	Xenobiotic metabolism		

About Reactome
The Reactome project is a collaboration among Cold Spring Harbor Laboratory, The European Bioinformatics Institute, and The Gene Ontology Consortium to develop a curated resource of core pathways and reactions in human biology. The information in this database is authored by biological researchers with expertise in their fields, maintained by the Reactome editorial staff, and cross-referenced to several databases (NCBI, UniProt, the Human Genome Project, KEGG (C. elegans and Drosophila), ChEBI, PubMed and GO). In addition to curated human events, inferred orthologous events in 22 non-human species including mouse, rat, chicken, zebrafish, worm, fly, yeast, two plants and *E. coli* are also available.

Reactome is a free on-line resource, and Reactome software is open-source. However, please take note of our disclaimer.

News and Notes

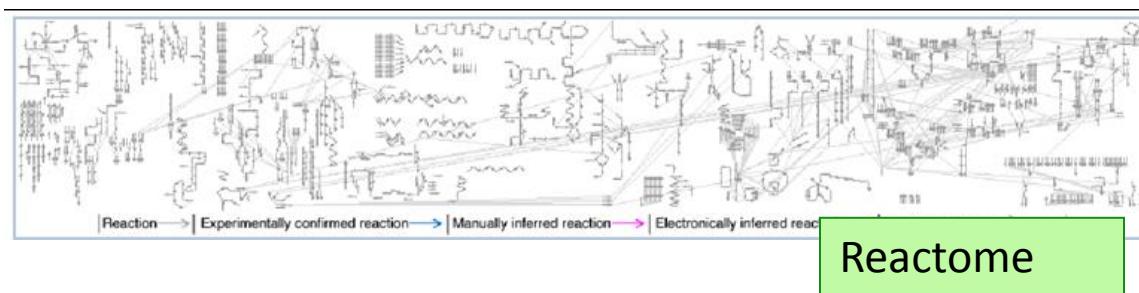
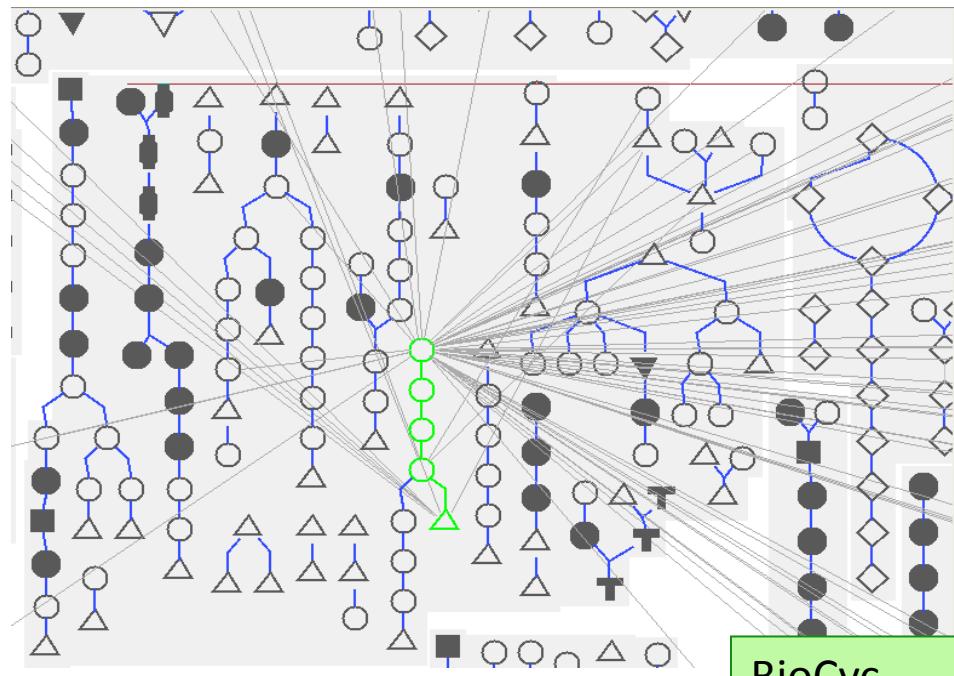
- August 2, 2008: Version 1.0 Released
New modules include the **signaling cascade** mediated by **Toll-like receptor 2**, **Maintenance of Telomeres**, **Entry and Binding** under **HIV Life Cycle** and the pathways of **Complement Activation**. Reactions and pathways chosen by the user are now highlighted prominently in the reaction map at the top of each web page. A new web display feature allows the user to choose the focus species annotations – curated (for human) and electronic (for other species) for other species. **Stable identifiers** have been introduced to facilitate the tracking of data objects over successive releases of Reactome. Updated release statistics and the Editorial Calendar are available. Click here to [contact us](#).
- More...

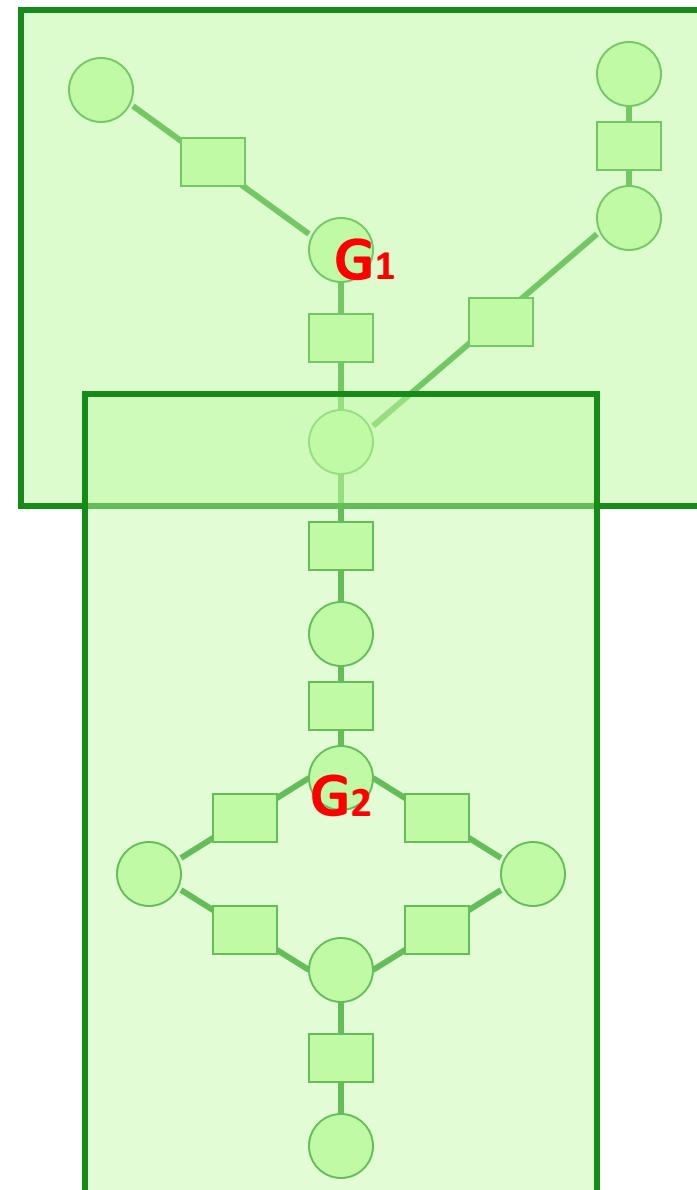
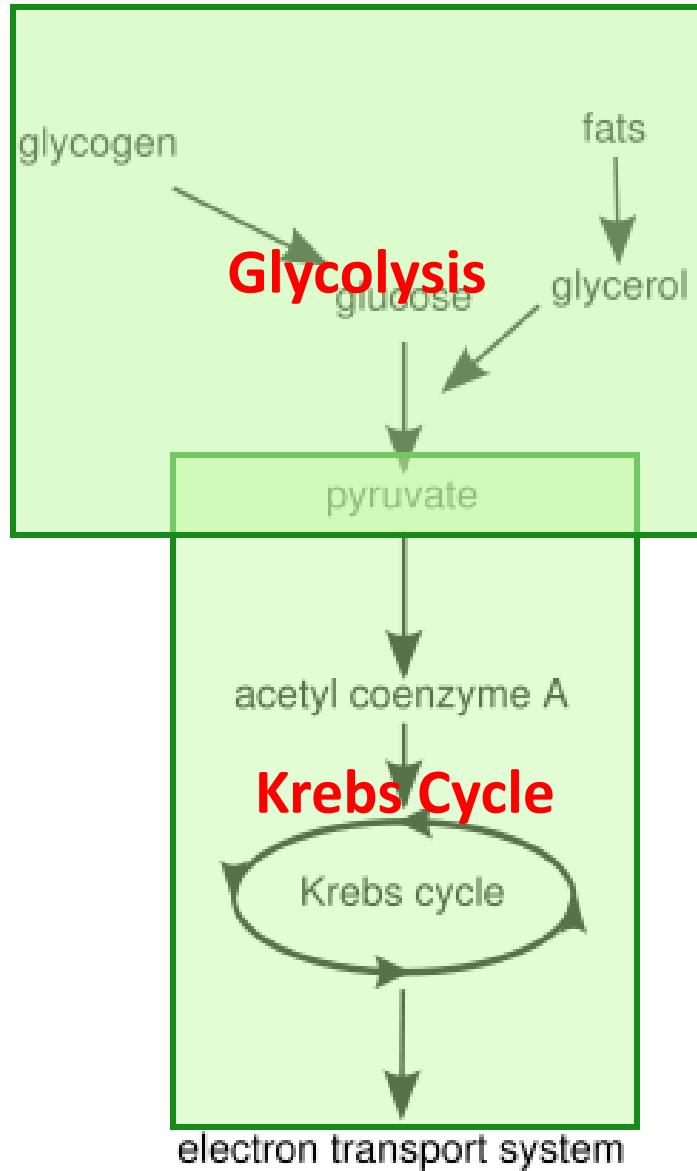
Fabien Jourdan, cours M1 bioinformatique
UPS. 2014

Reactome

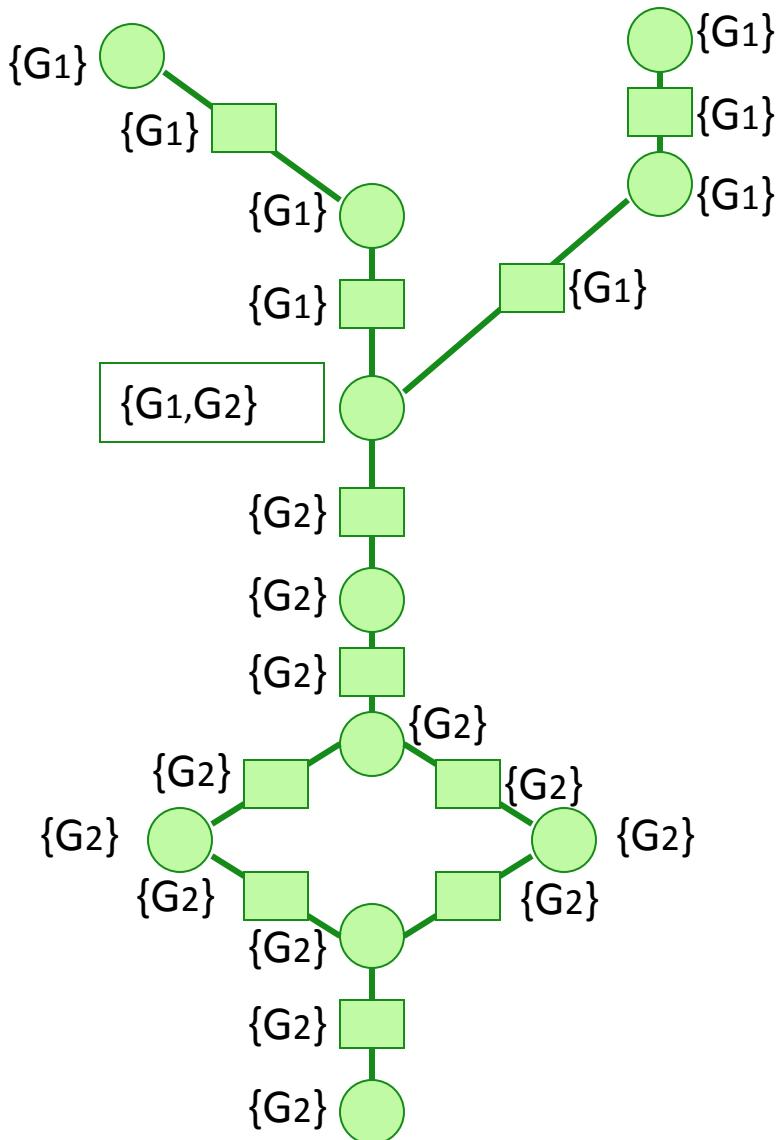
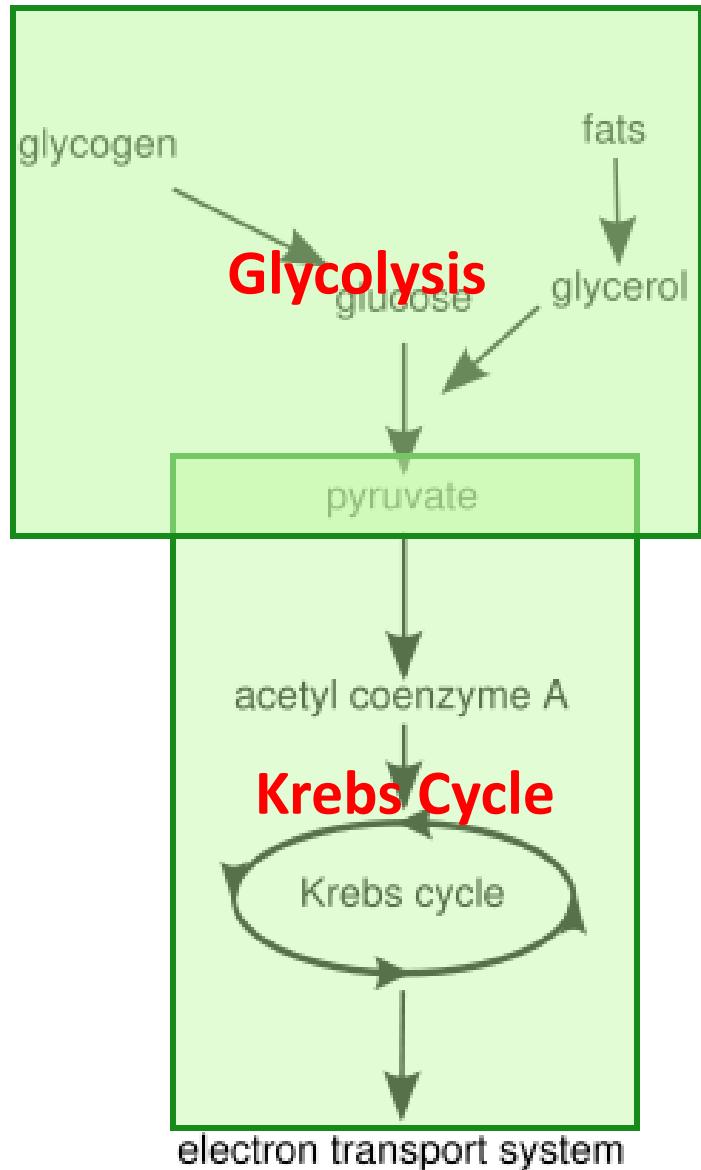
Limitations

- It is hard to show the link between pathways
- Their solution is to only show links going out of a metabolic pathway.





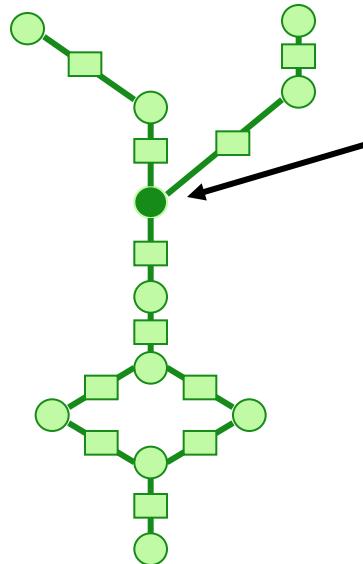
Glycolysis and Krebs Cycle are two metabolic pathways.



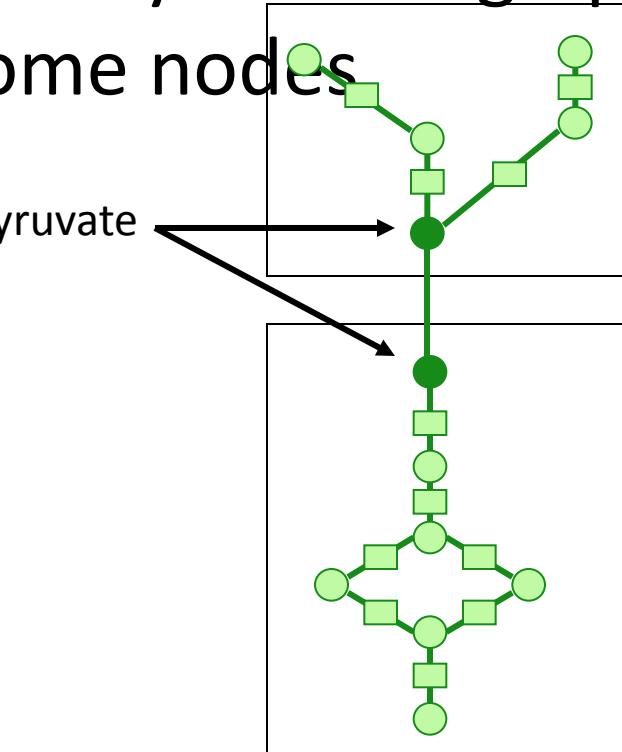
Glycolysis and Krebs Cycle are two metabolic pathways.

Node duplication

- Considering each pathway as a subgraph implies duplicating some nodes



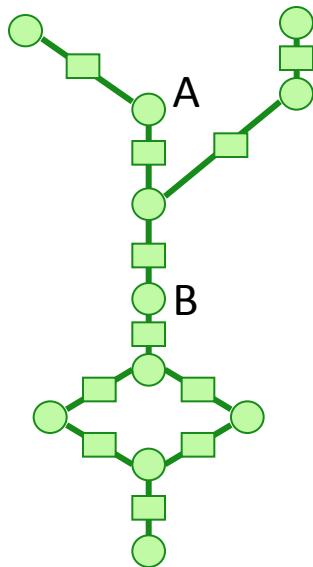
In the network



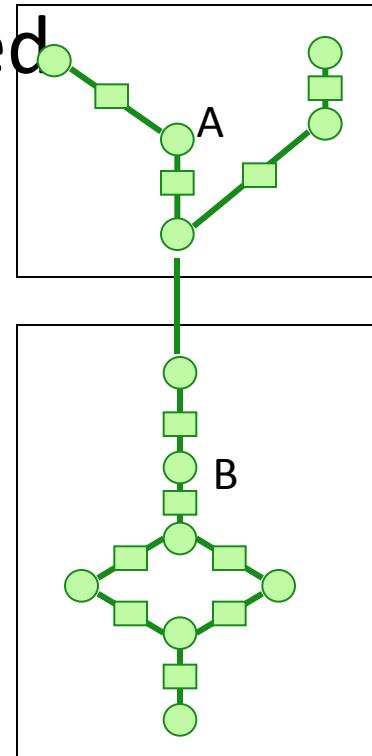
In BioCyc and Reactome

Problem with duplication

- The topology is not preserved



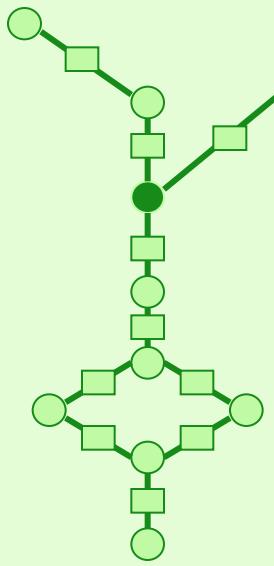
In the network $d(A,B)=4$



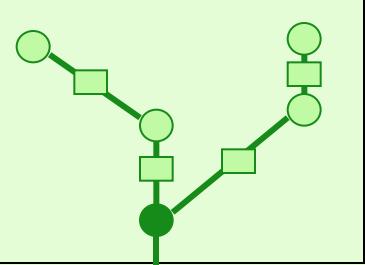
In BioCyc and Reactome $d(A,B)=5$

Constraints

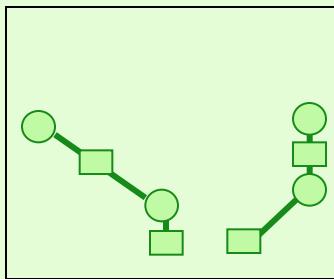
- Representing nodes of the same metabolic pathway close to each other.
- Following text-book drawing conventions.
- Not duplicating nodes.



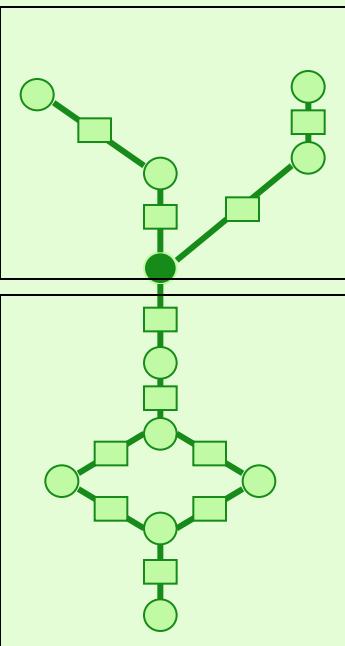
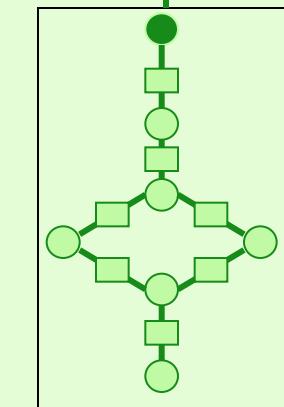
In the network



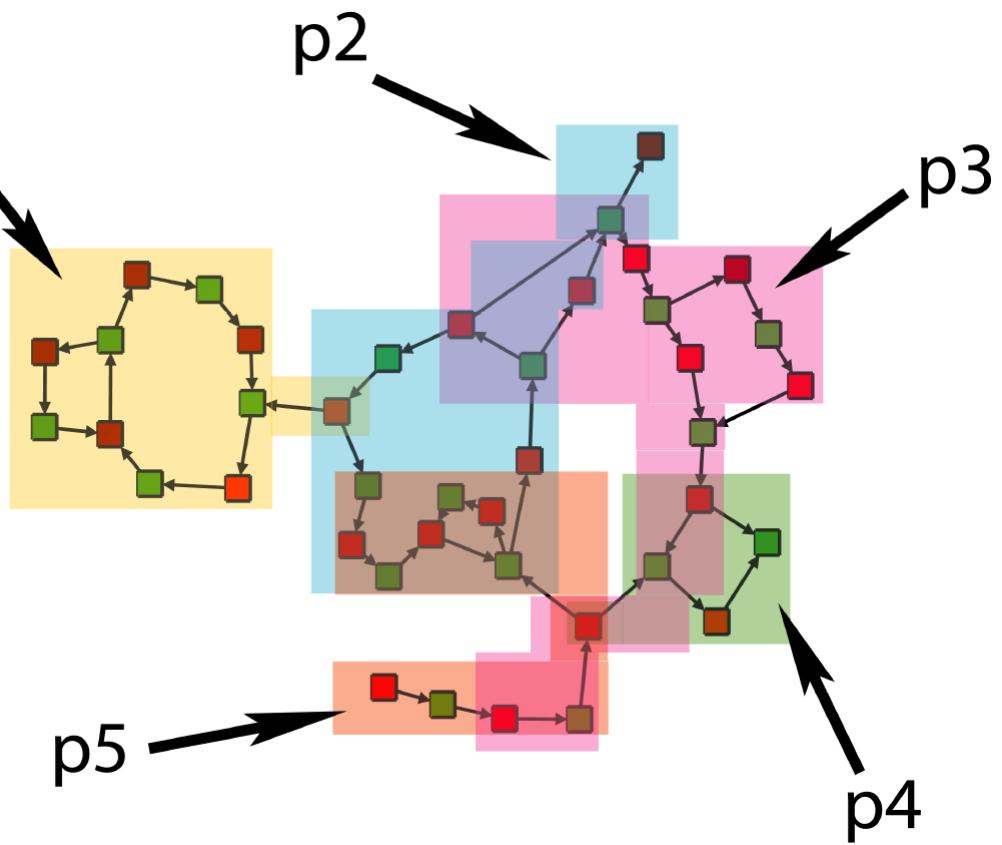
In BioCyc and Reactome



No duplication

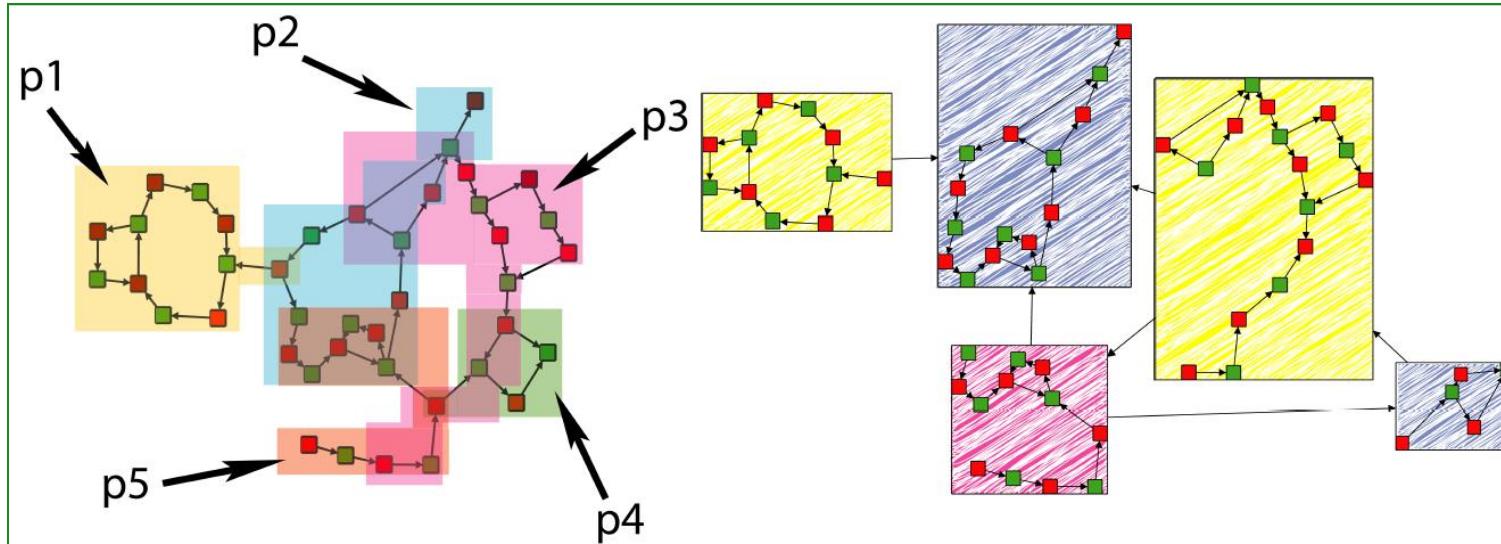


- When a node is shared by two or more pathways, we will have to choose within which pathway it will be drawn.



A network containing
five pathways

Which pathway are we
going to draw entirely?

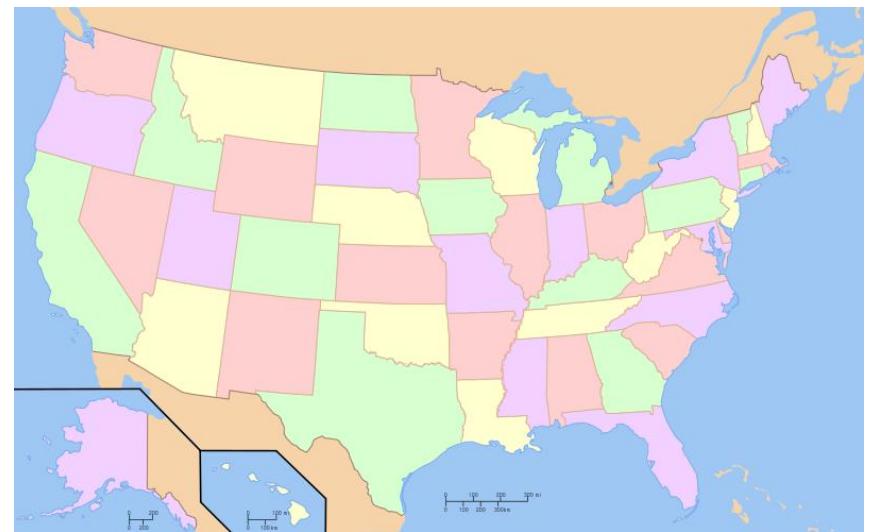


1. We build the dependency graph.
 - In this graph we want to choose as many large clusters as possible.
 - It is the same as solving the **maximum independent subset** which is an NP-Complete problem.
2. To solve it we use the **Welsh and Powel** algorithm.

Graph Coloring

Coloring in general

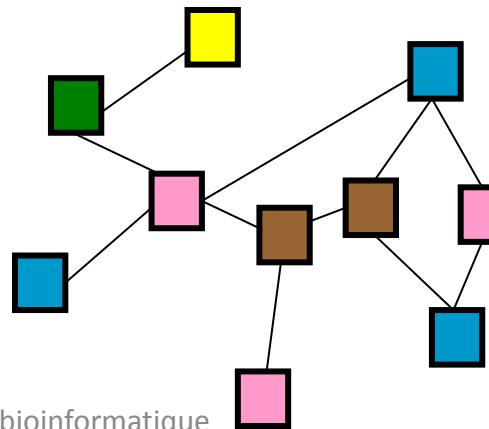
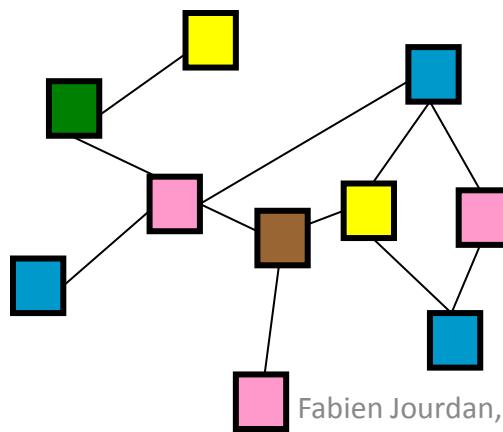
- In mathematics, the **four color theorem**, or the **four color map theorem** states that, given any separation of a plane into contiguous regions, producing a figure called a *map*, no more than four colors are required to color the regions of the map so that no two adjacent regions have the same color.



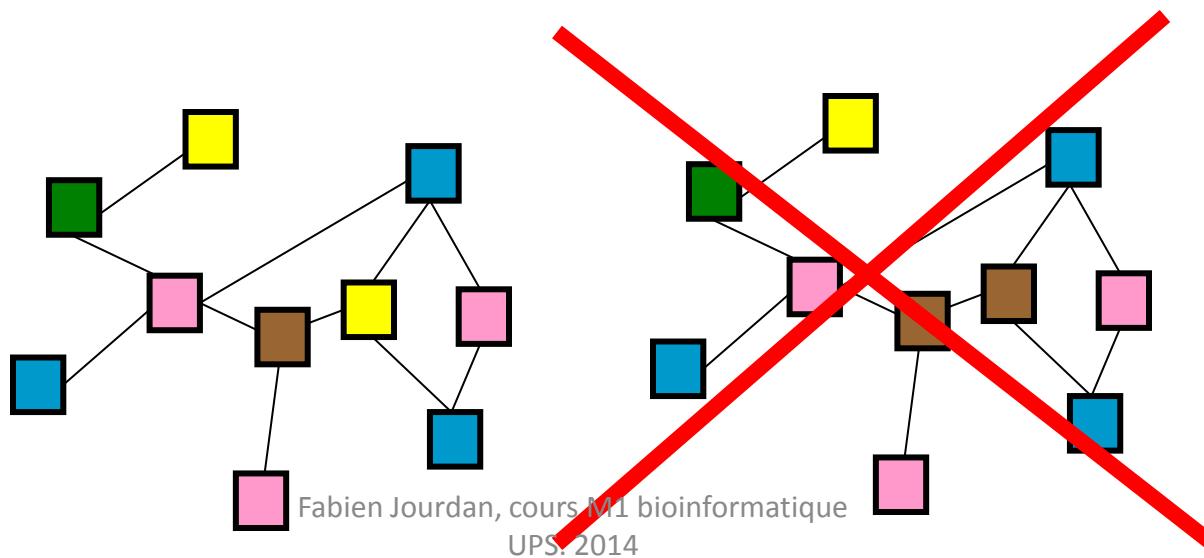
Simple question...long story

- The Four Color Problem dates back to **1852** when Francis Guthrie, while trying to color the map of counties of England noticed that four colors sufficed. He asked his brother Frederick if it was true that any map can be colored using four colors in such a way that adjacent regions (i.e. those sharing a common boundary segment, not just a point) receive different colors. Frederick Guthrie then communicated the conjecture to DeMorgan. The first printed reference is due to Cayley in 1878.
- A year later the first 'proof' by Kempe appeared; its incorrectness was pointed out by Heawood 11 years later. Another failed proof is due to Tait in 1880; a gap in the argument was pointed out by Petersen in 1891. Both failed proofs did have some value, though. Kempe discovered what became known as Kempe chains, and Tait found an equivalent formulation of the Four Color Theorem in terms of 3-edge-coloring.
- The next major contribution came from Birkhoff whose work allowed Franklin in 1922 to prove that the four color conjecture is true for maps with at most 25 regions. It was also used by other mathematicians to make various forms of progress on the four color problem. We should specifically mention Heesch who developed the two main ingredients needed for the ultimate proof - reducibility and discharging. While the concept of reducibility was studied by other researchers as well, it appears that the idea of discharging, crucial for the unavoidability part of the proof, is due to Heesch, and that it was he who conjectured that a suitable development of this method would solve the Four Color Problem.
- This was confirmed by Appel and Haken in **1976**, when they published their proof of the Four Color Theorem

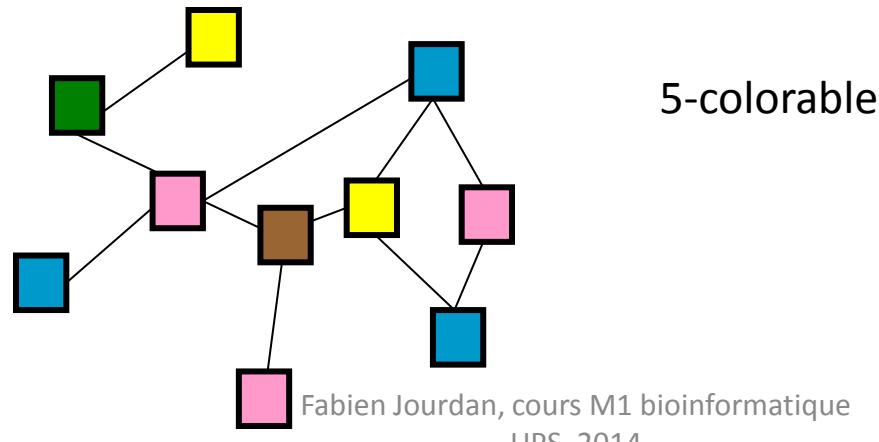
- A graph is said to be k vertex colorable (or k -colorable) if it is possible to assign one color from a set of k colors to each vertex such that no two adjacent vertices have the same color.



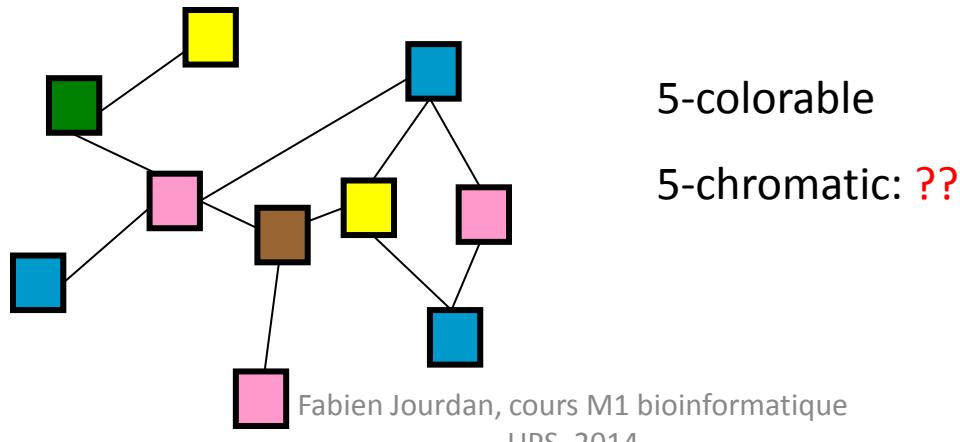
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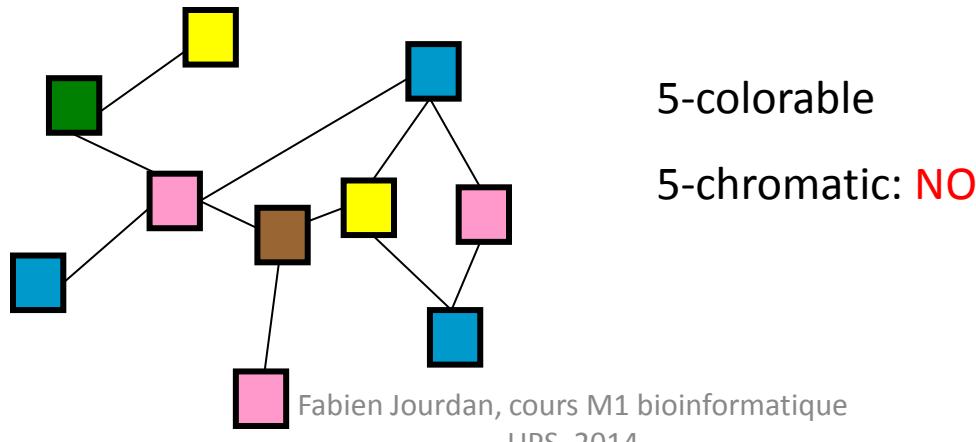
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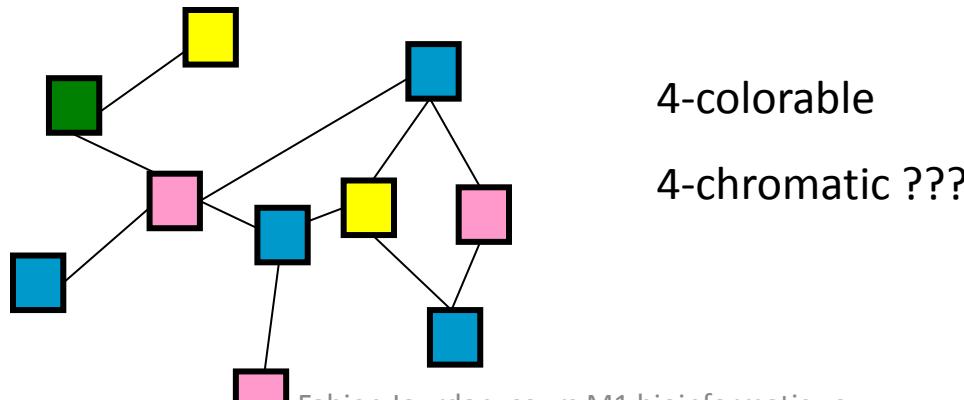
- If the graph G is k -colorable but not $(k-1)$ -colorable, we say that G is a k -chromatic graph and that its chromatic number $\chi(G)$ is k .



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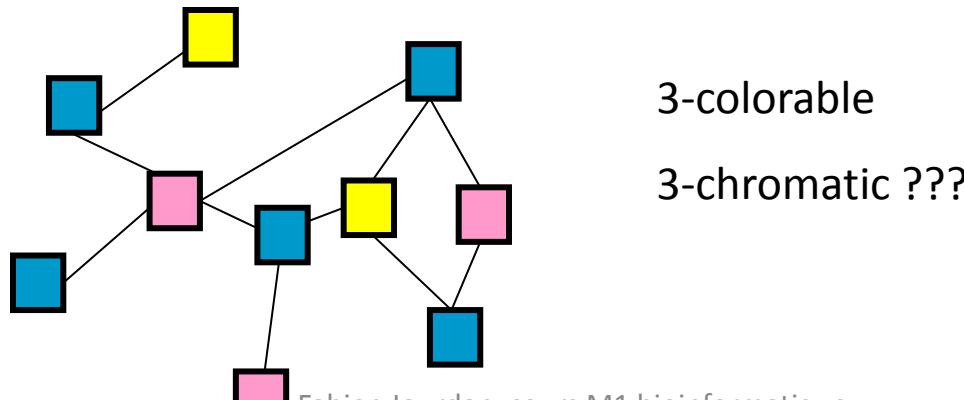
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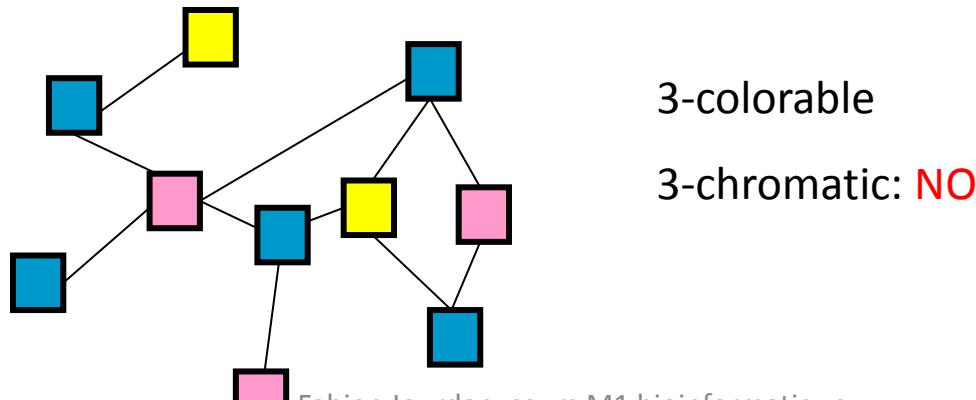
4-colorable

4-chromatic ???

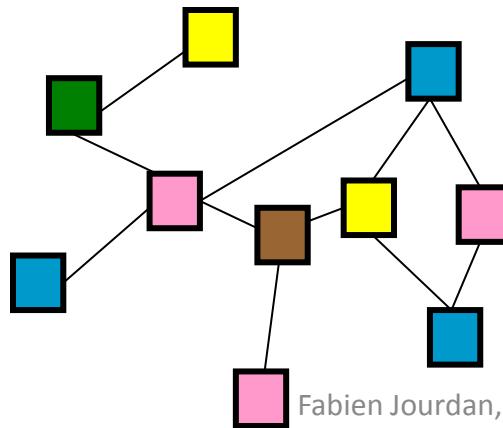
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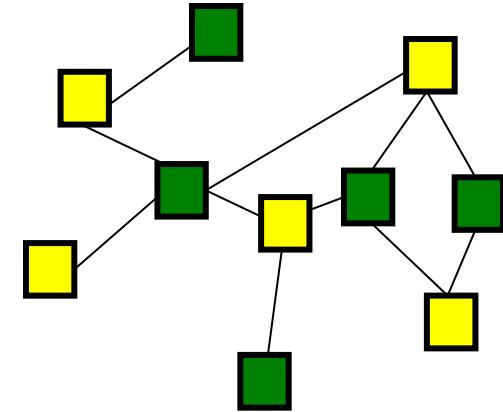
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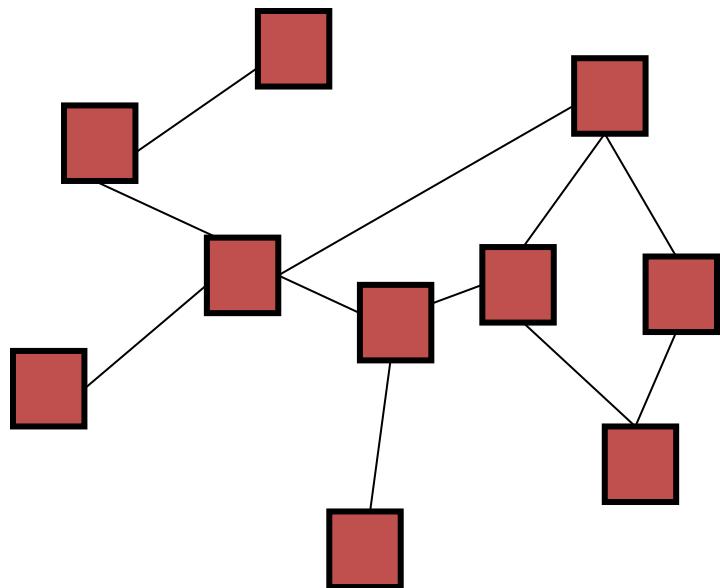
5-colorable
2-chromatic



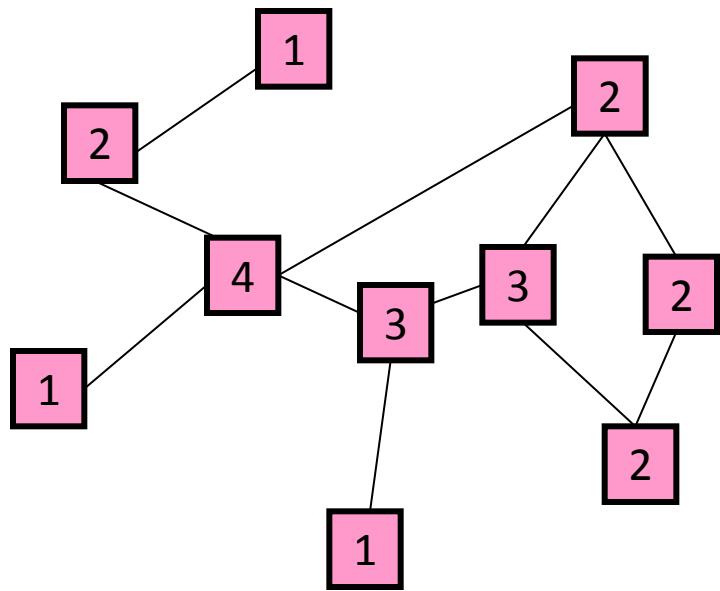
Algorithme de Welsh Powel

- Repérer le degré de chaque sommet.
- Ranger les sommets par ordre de degrés décroissants. (dans certains cas plusieurs possibilités)
- Attribuer au premier sommet (A) de la liste une couleur.
- Suivre la liste en attribuant la même couleur au premier sommet (B) qui ne soit pas adjacent à (A).
- Suivre (si possible) la liste jusqu'au prochain sommet (C) qui ne soit adjacent ni à A ni à B.
- Continuer jusqu'à ce que la liste soit finie.
- Prendre une deuxième couleur pour le premier sommet (D) non encore colorié de la liste.
- Répéter les opérations 4 à 6.
- Continuer jusqu'à avoir colorié tous les sommets.

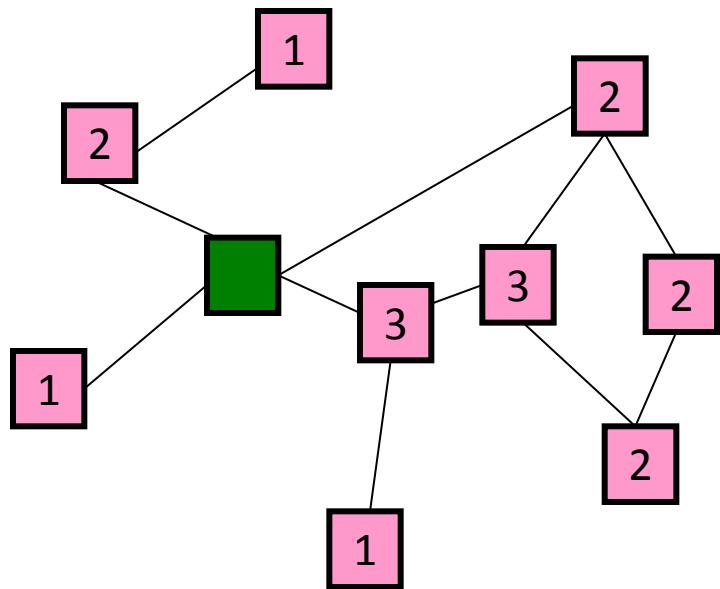
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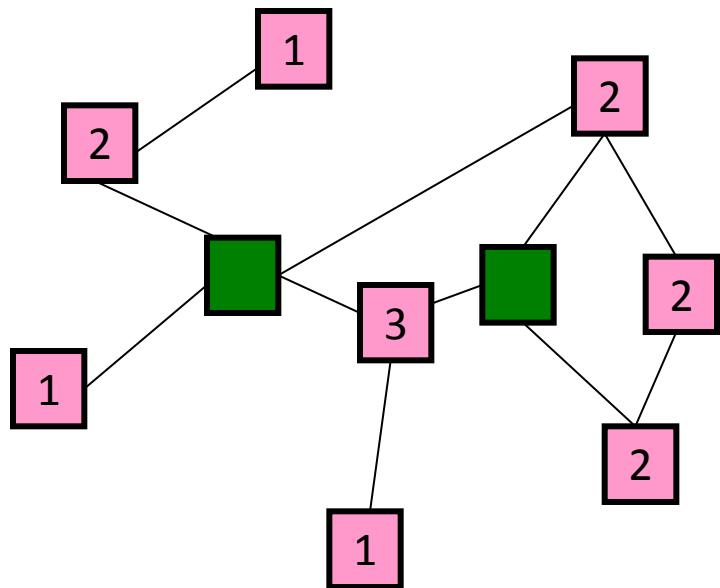
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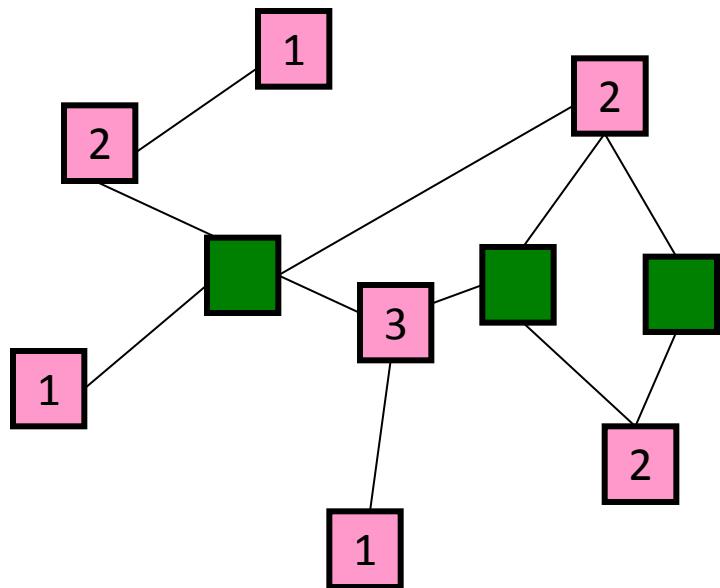
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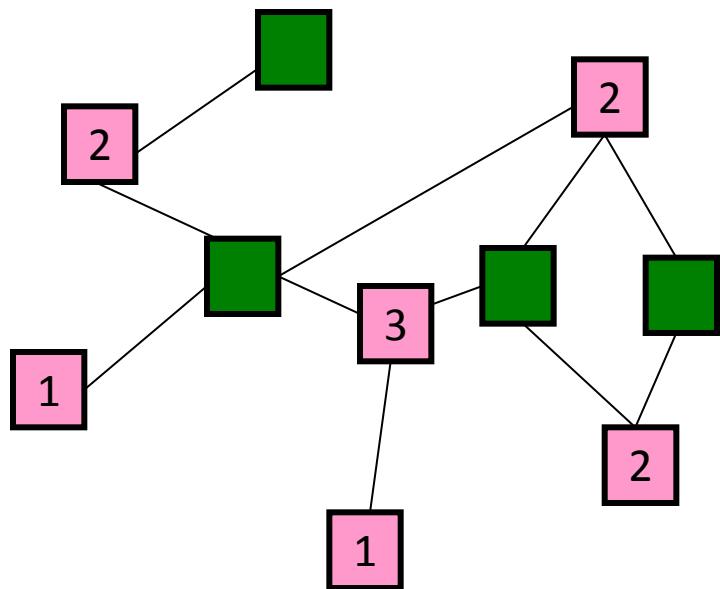
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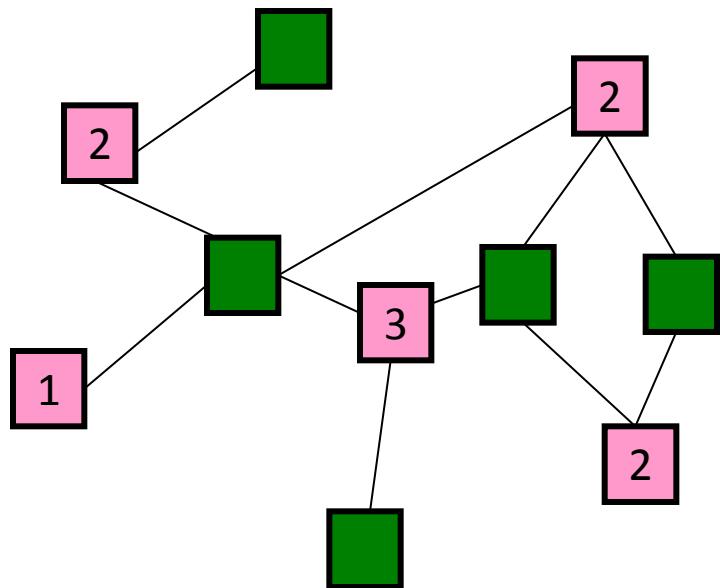
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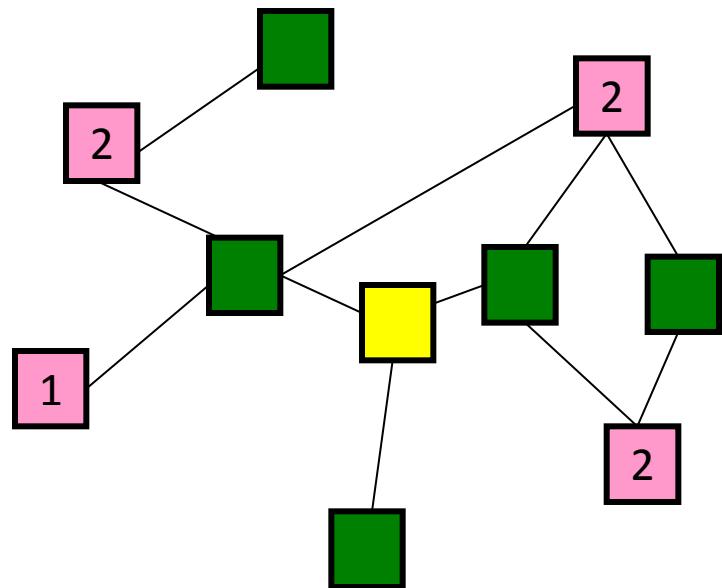
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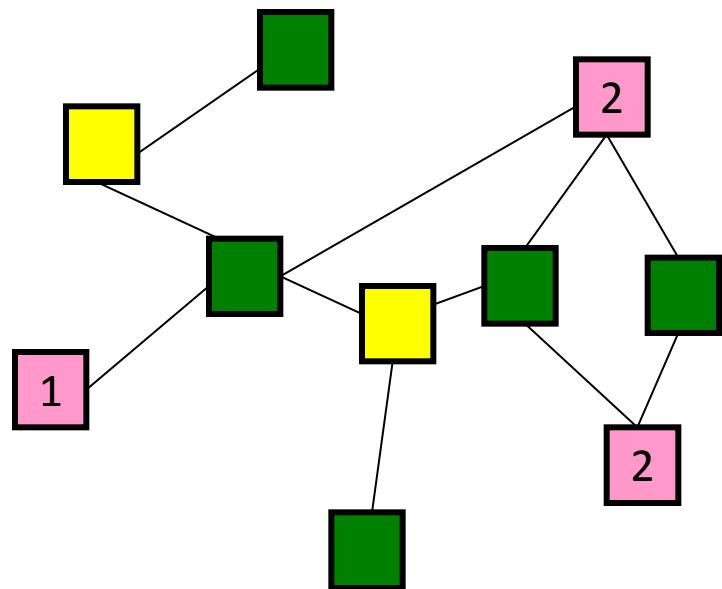
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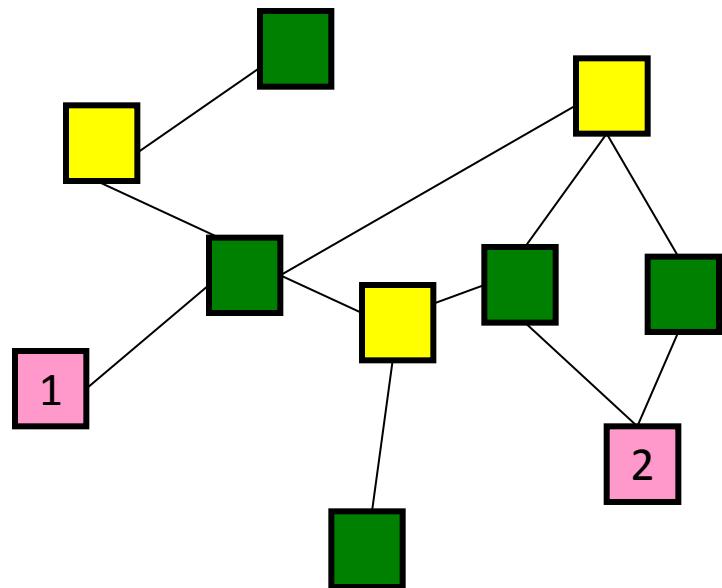
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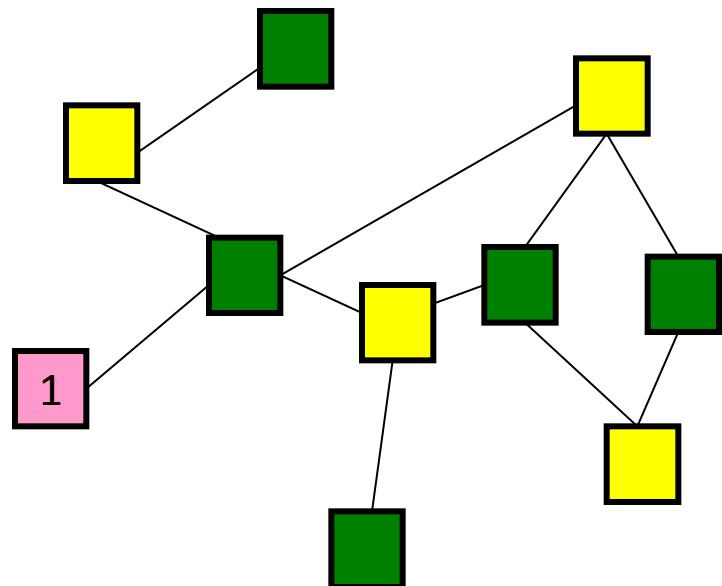
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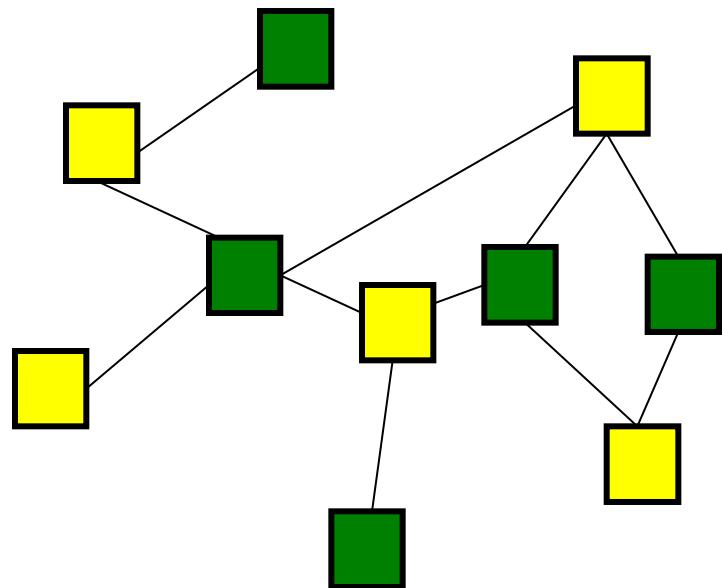
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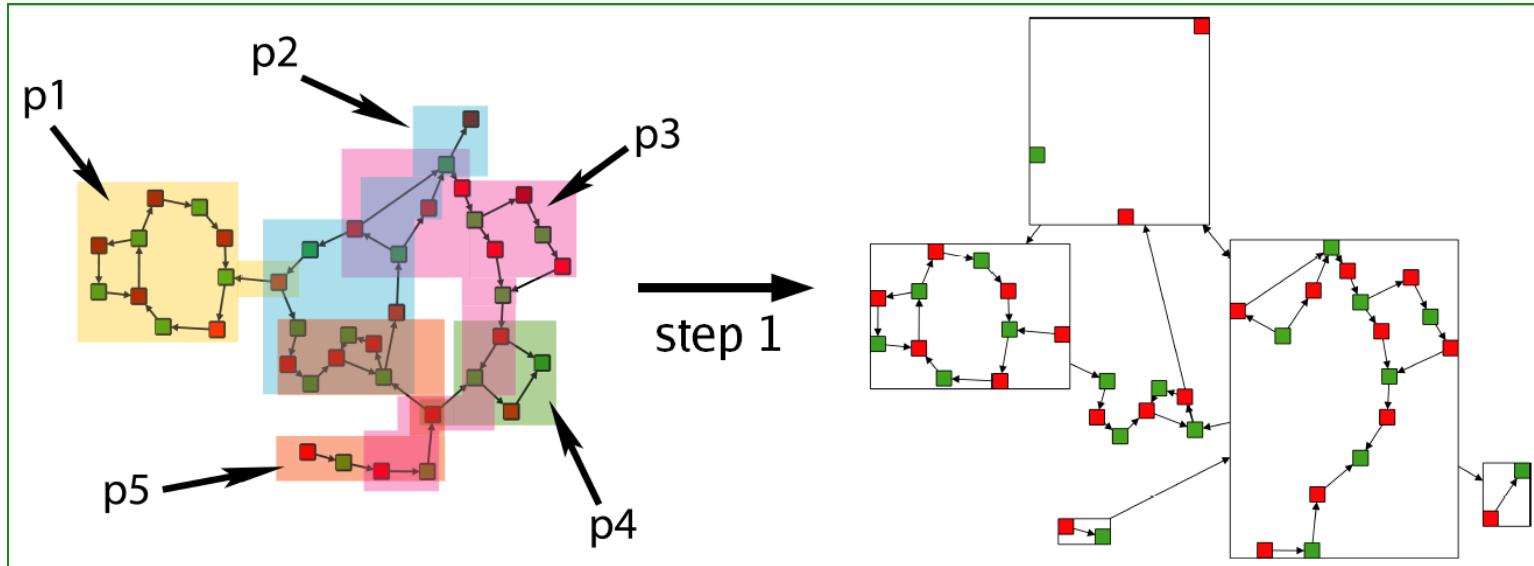


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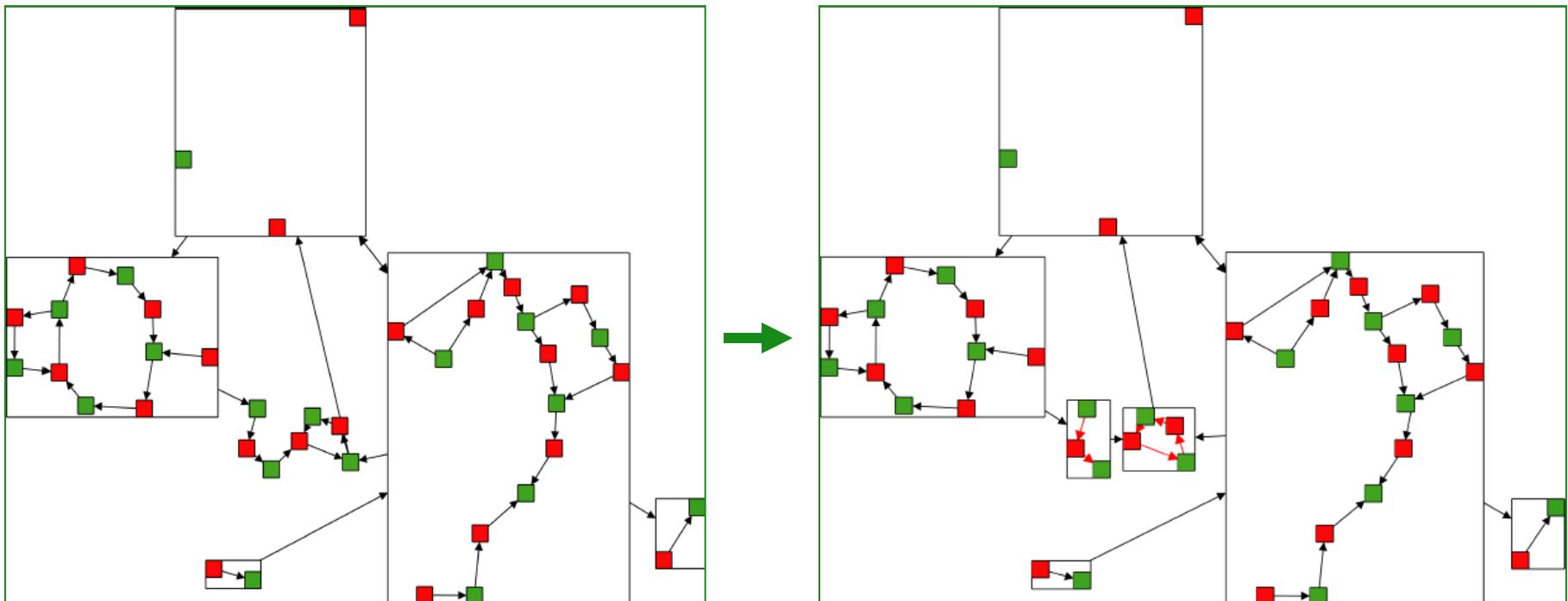
Graph Coloring

- Given a graph, find a node coloring such that two nodes with the same color are not neighbors.
- Finding a color set such that the number of color is minimum is an NP-Complete problem.

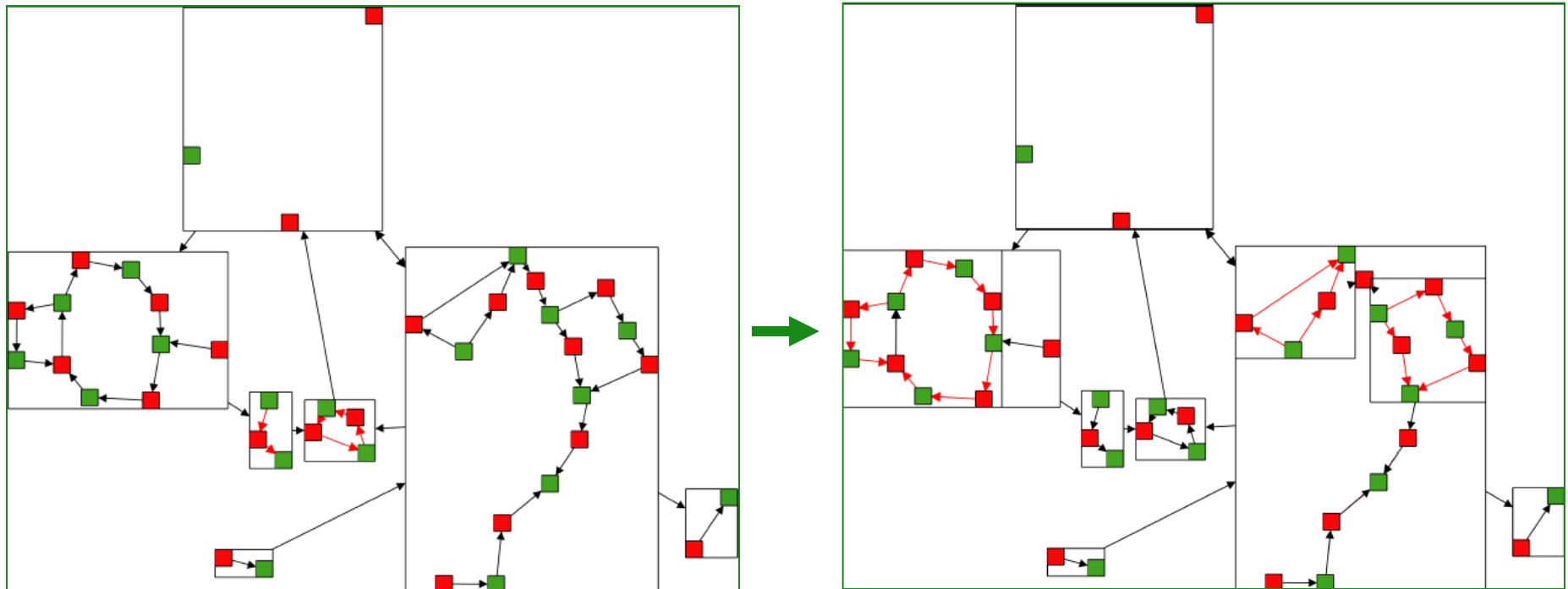


1. We build the dependency graph.
 - In this graph we want to choose as many large clusters as possible.
 - It is the same as solving the **maximum independent subset** which is an NP-Complete problem.
2. To solve it we use the **Welsh and Powel** algorithm.
3. We choose the color (set of clusters) containing the **maximum number of nodes**.

- Next step of the algorithm consists in finding circular and linear patterns between metanodes.



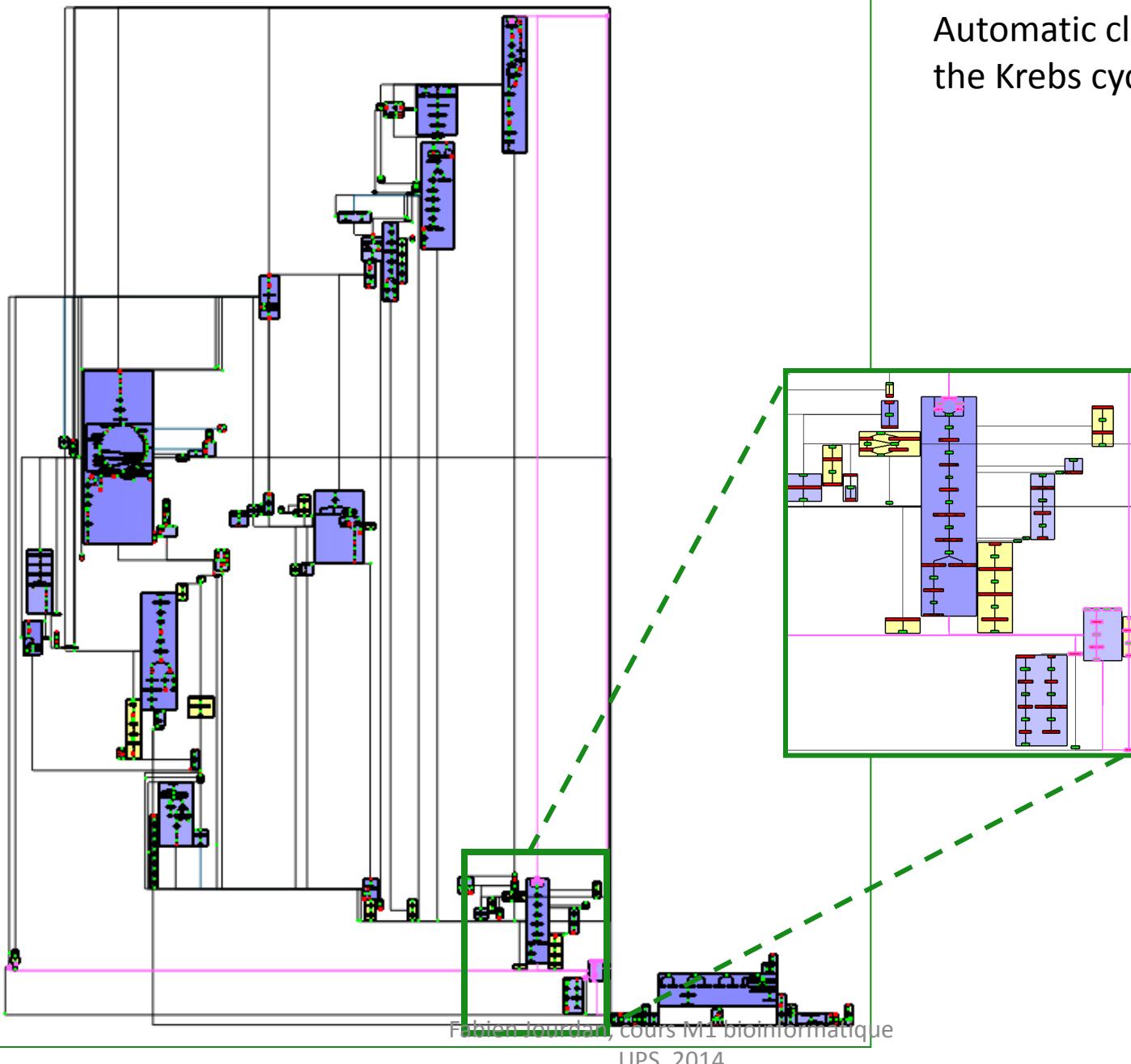
- Then we detect cycles and chains in metanodes.

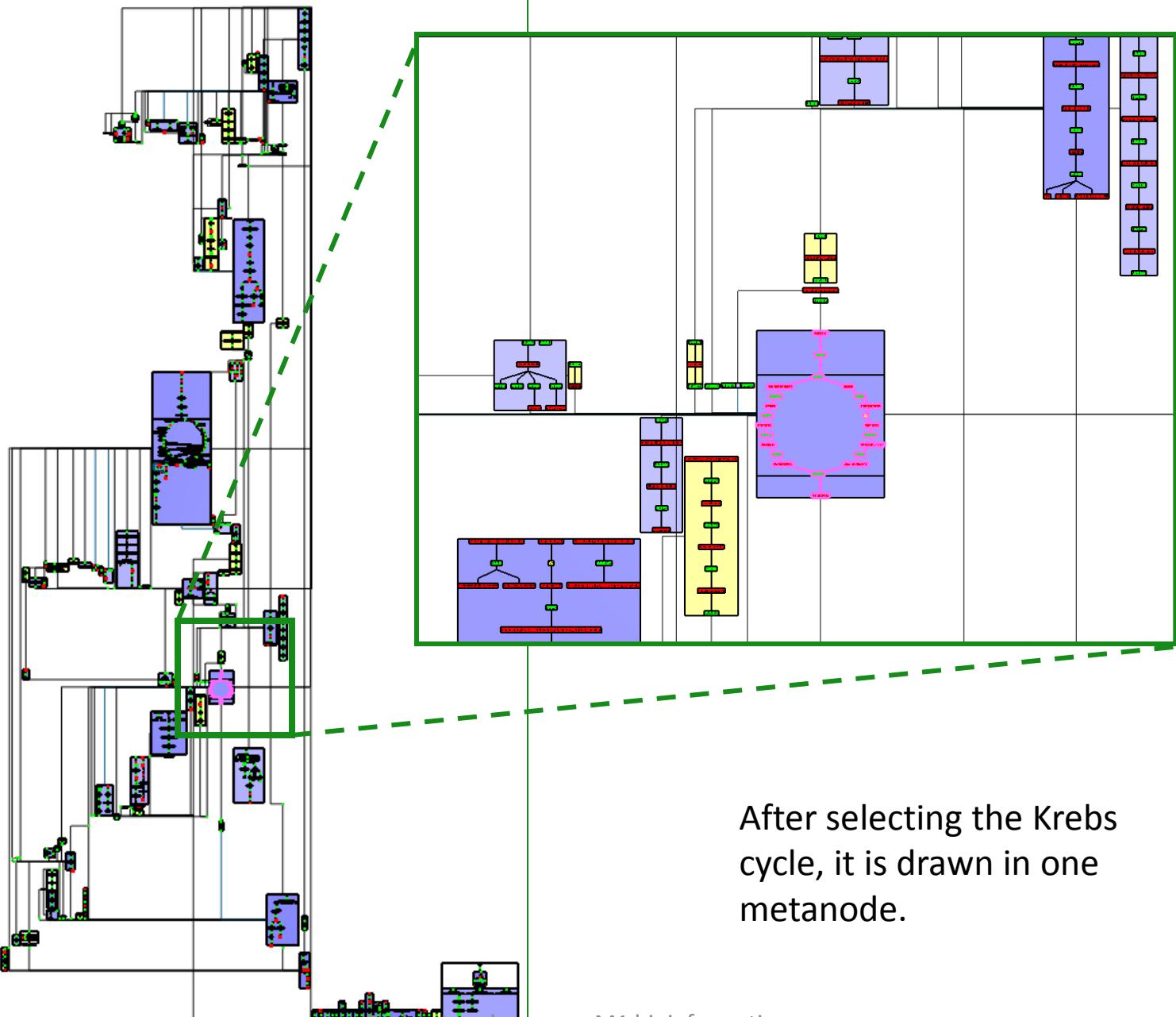


Semi-automatic clustering

- Since the clustering is automatic, important pathways can be split.
- With MetaViz it is possible to constrain this algorithm by selecting pathways that will have to be carefully drawn.

Automatic clustering will split the Krebs cycle.





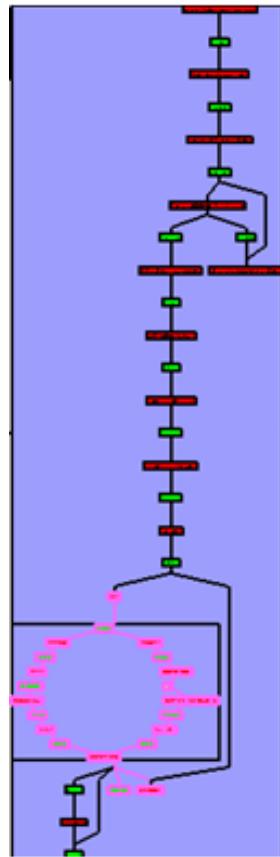
After selecting the Krebs cycle, it is drawn in one metanode.

Drawing clusters and quotient graph

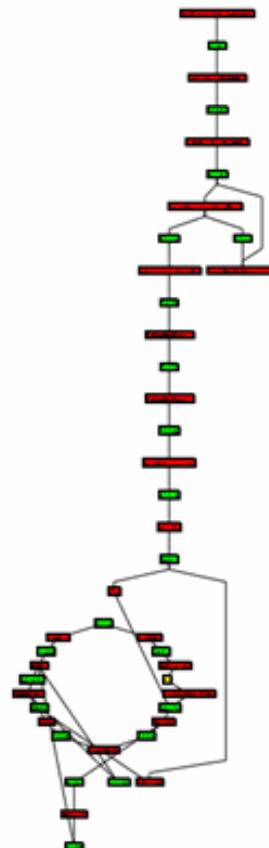
Drawing algorithms

- For clusters:
 - Hierarchical drawing (Auber et al. 2001)
 - Circular drawing
- For quotient graph
 - Planar drawing: Mixed model algorithm
(Gutwenger and Mutzel 1998)

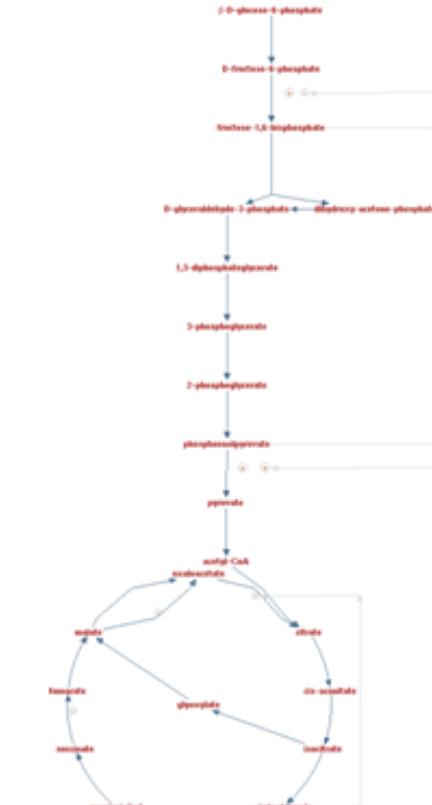
Inside Metanodes



a



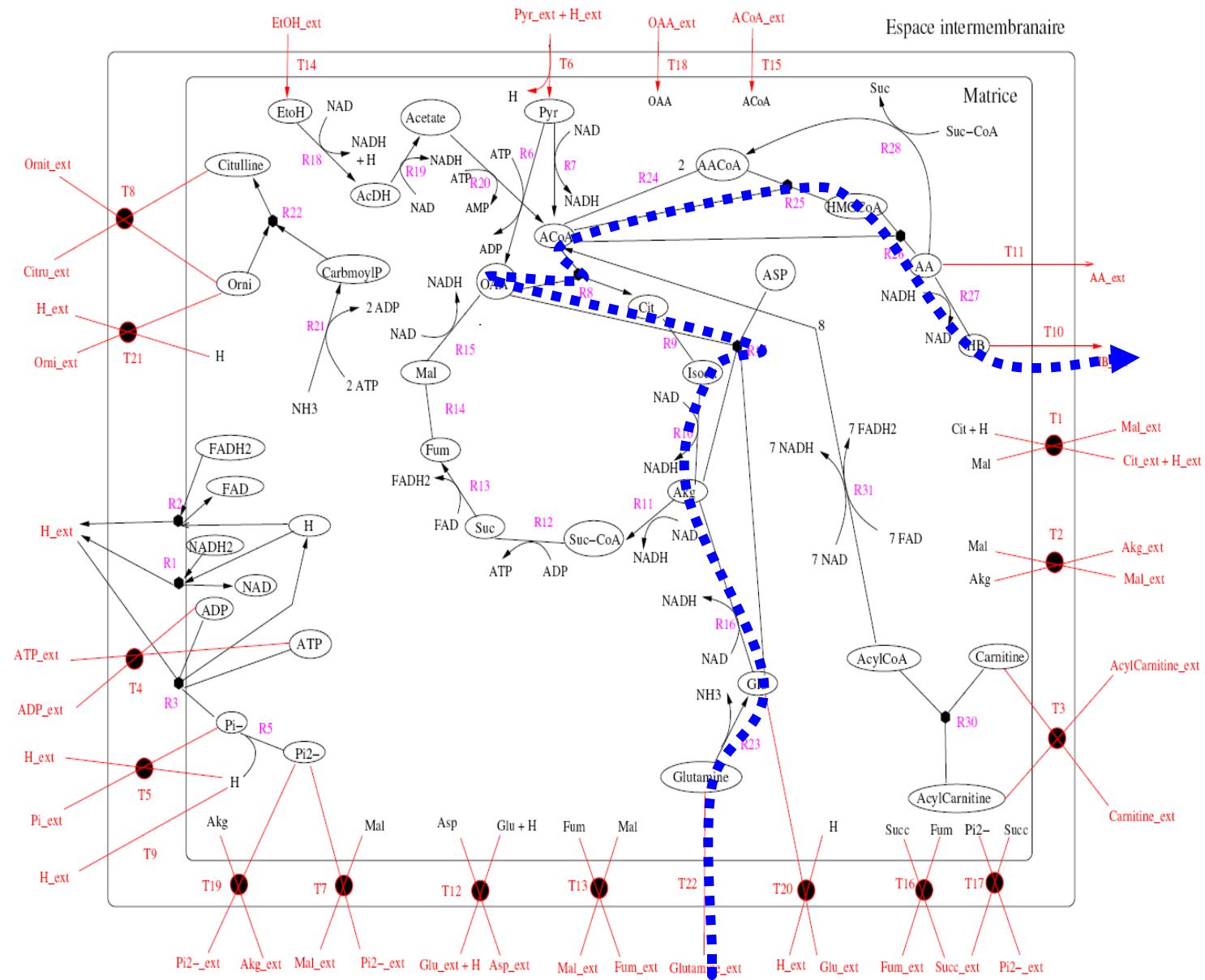
b



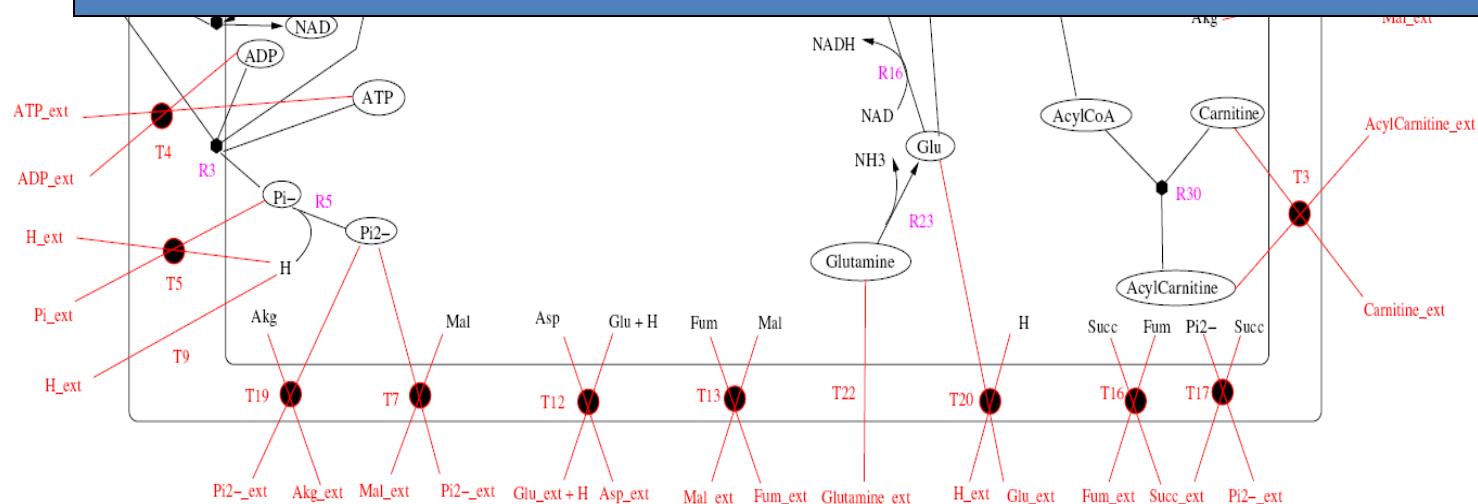
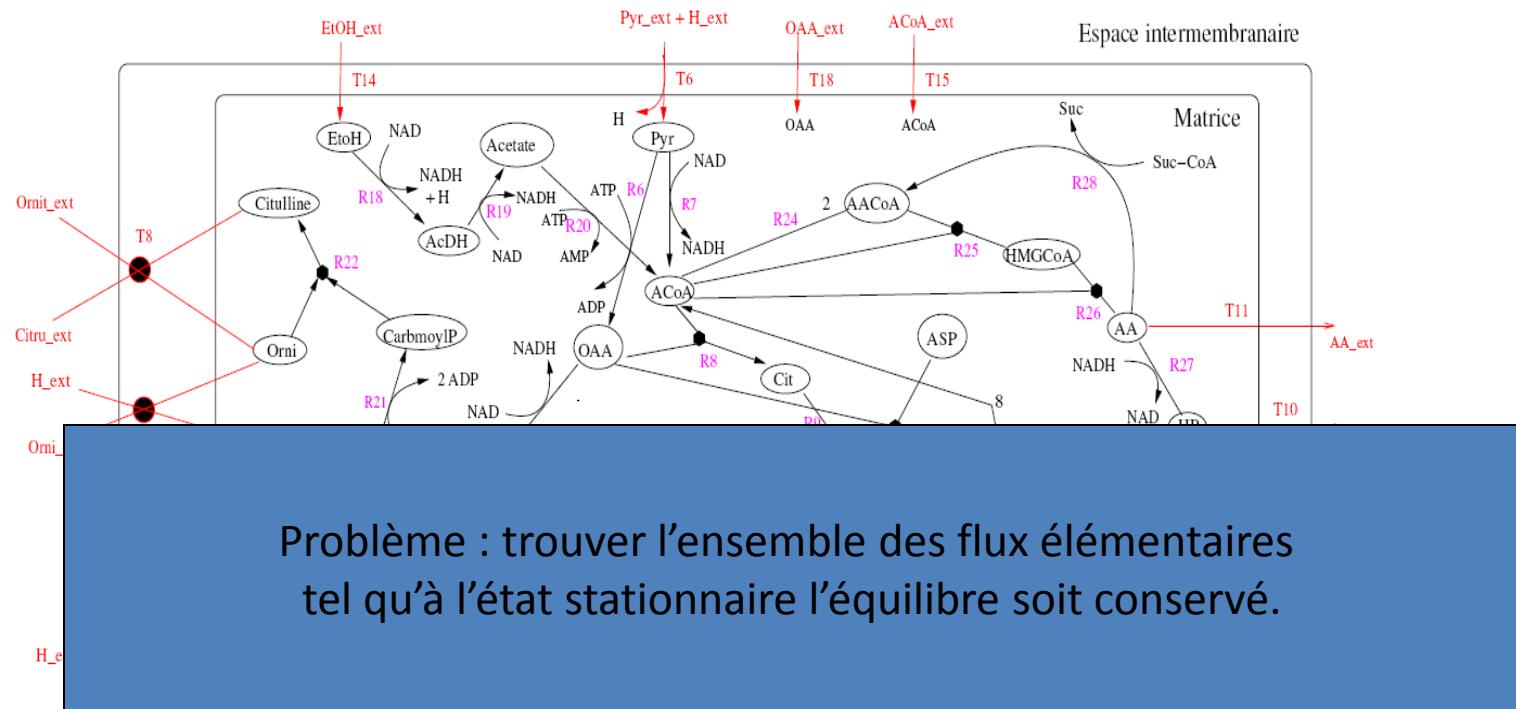
c

Modes élémentaires

Modèle général du métabolisme mitochondrial



Modèle général du métabolisme mitochondrial



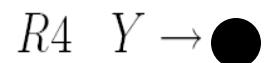
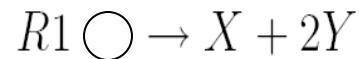
Matrice de stœchiométrie

Définition 4 Soit un réseau métabolique contenant m métabolites internes et r réactions arbitrairement orientées, la **matrice de stœchiométrie** $N = (n_{ij})_{1 \leq i \leq m, 1 \leq j \leq r}$ associée est définie tel que : $\forall i \in \llbracket 1; m \rrbracket \forall j \in \llbracket 1; r \rrbracket$,

$$n_{ij} = \begin{cases} a & \text{si la réaction } j \text{ produit } a \text{ molécules de } i \text{ et } a \in \mathbb{Q}^+. \\ -a & \text{si la réaction } j \text{ consomme } a \text{ molécules de } i \text{ et } a \in \mathbb{Q}^+. \\ 0 & \text{sinon.} \end{cases}$$

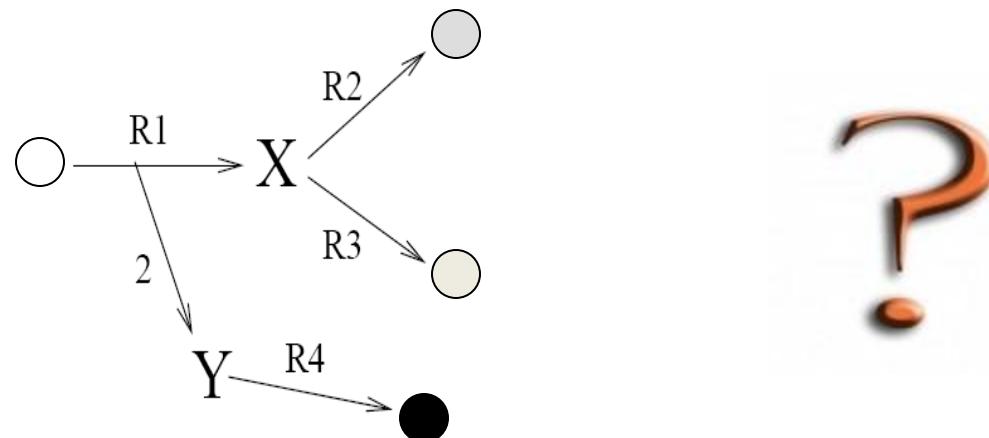
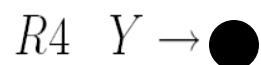
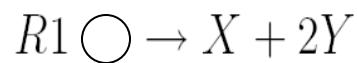
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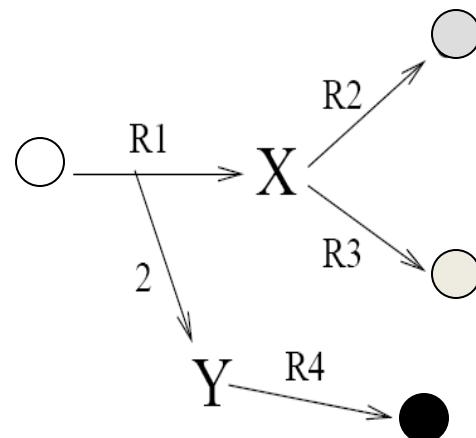
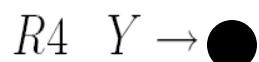
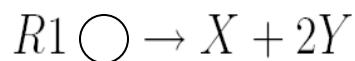
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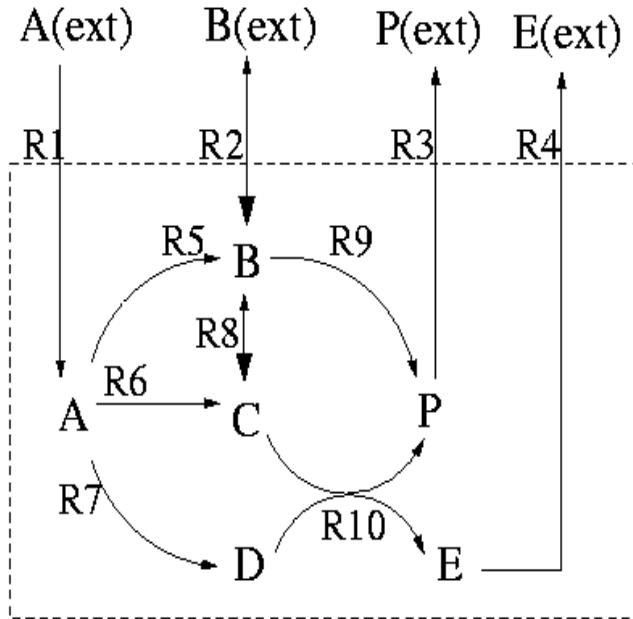
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$$N = \begin{pmatrix} R1 & R2 & R3 & R4 \\ 1 & -1 & -1 & 0 \\ 2 & 0 & 0 & -1 \end{pmatrix} \begin{matrix} X \\ Y \end{matrix}$$

Construction du modèle

- Système
- Métabolites
 - Internes $m=6$
 - Externes
- Flux (réactions +transports):
 $q=10$



- Stœchiométrie :

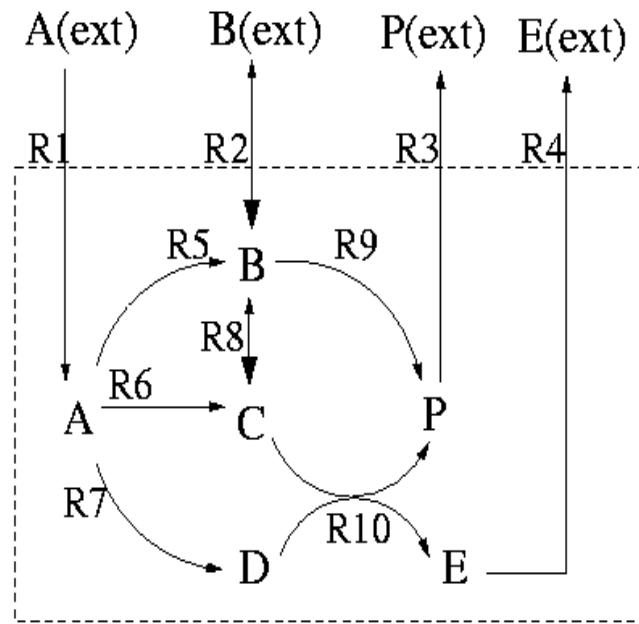
$$\mathbf{N} = \begin{pmatrix} 1 & 0 & 0 & 0 & -1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & -1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix} \begin{matrix} \leftarrow A \\ \leftarrow B \\ \leftarrow C \\ \leftarrow D \\ \leftarrow E \\ \leftarrow P \end{matrix}$$

Problème

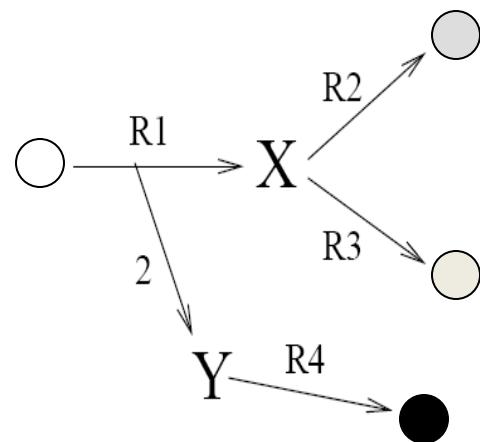
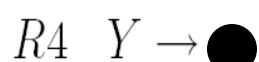
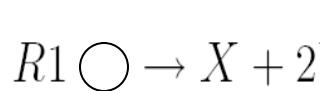
- On peut obtenir l'information de réversibilité sur les réactions
- Mais la matrice ne permet pas de dissocier les réactions réversibles et irréversibles
- La solution est de scinder la réaction en deux lignes
- Mais alors on agrandit significativement la matrice

Construction du modèle

- Flux (réactions +transports):
 $q=10$
 - $\text{Rev}=\{R2, R8\}$
 - $\text{Irrev}=\{R1, R3, R4, R5, R6, R7, R9, R10\}$



- $Irr(N) = \{i \in \llbracket 1; r \rrbracket : v_i \geq 0\}$: l'indice des réactions qui pour des raisons thermodynamique ou autre doivent être positives.
- $Rev(N)$: l'indice des réactions qui n'ont pas de contraintes de signes.



$$N = \begin{pmatrix} R1 & R2 & R3 & R4 \\ 1 & -1 & -1 & 0 \\ 2 & 0 & 0 & -1 \end{pmatrix} \begin{matrix} X \\ Y \end{matrix}$$

$Irr(N)=\{1,2,3,4\}$

Définition 5 Un flux à l'état stationnaire $V = (v_1, \dots, v_r)^t \in \mathbb{R}^r$ d'un réseau représenté par une matrice N est faisable si

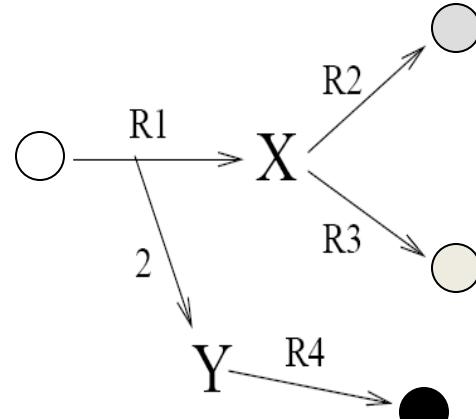
$$\forall i \in Irr(N), v_i \geq 0. \quad (2.3)$$

$$R1 \text{ } \bigcirc \rightarrow X + 2$$

$$R2 \text{ } X \rightarrow \bigcirc$$

$$R3 \text{ } X \rightarrow \textcolor{lightgray}{\bigcirc}$$

$$R4 \text{ } Y \rightarrow \bullet$$



$$N = \begin{pmatrix} R1 & R2 & R3 & R4 \\ 1 & -1 & -1 & 0 \\ 2 & 0 & 0 & -1 \end{pmatrix} \begin{matrix} X \\ Y \end{matrix}$$

$$Irr(N) = \{1, 2, 3, 4\}$$

$$V = (1, 2, 1, 0)$$

OUI

$$V = (1, 2, -1, -2)$$

NON

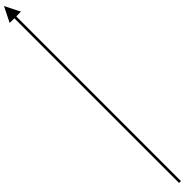
$$V = (1, 1, 0, 2)$$

OUI

Définition 7 Un vecteur $e = (e_1, \dots, e_r)^t \in \mathbb{R}^r$ est un mode élémentaire de flux (efm) pour «elementary flux mode») s'il vérifie les conditions suivantes :

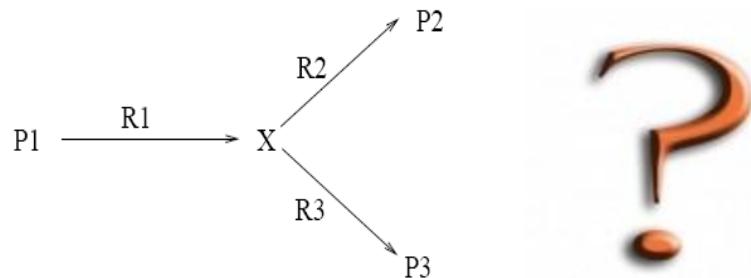
1. $N_e = 0$. (état stationnaire)
2. Pour chaque indice j d'une réaction irréversible $e_j \geq 0$. (faisabilité)
3. Pour chaque état stationnaire faisable e' de N ,
 $\text{supp}(e') \subseteq \text{supp}(e) \Rightarrow \exists \alpha \in \mathbb{R} \text{ tel que } e' = \alpha e$. (minimalité)

Le flux ne doit pas être la combinaison linéaire
de deux autres efm.



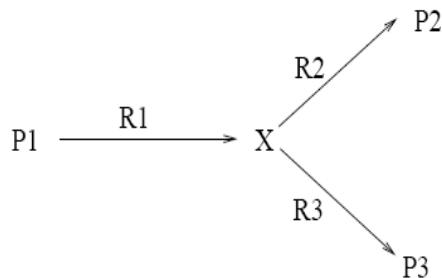
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$$N = \begin{pmatrix} 1 & -1 & -1 \end{pmatrix}$$

$$\mathbf{v}=(-1,-1,0)$$

$$\mathbf{v}=(1,0,1)$$

$$\mathbf{v}=(0,1,1)$$

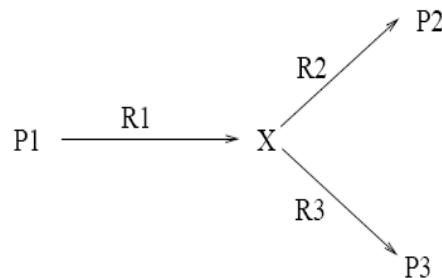
$$\mathbf{v}=(1,1,0)$$

$$\mathbf{v}=(2,1,1)$$



Définition 7 Un vecteur $e = (e_1, \dots, e_r)^t \in \mathbb{R}^r$ est un mode élémentaire de flux (efm) pour «elementary flux mode») s'il vérifie les conditions suivantes :

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$$N = \begin{pmatrix} 1 & -1 & -1 \end{pmatrix}$$

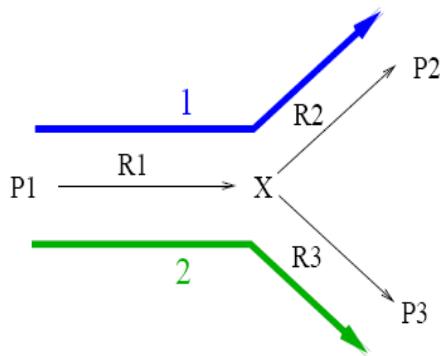
$V=(-1,-1,0)$ non efm

$V=(1,0,1)$ efm

$V=(0,1,1)$ non efm

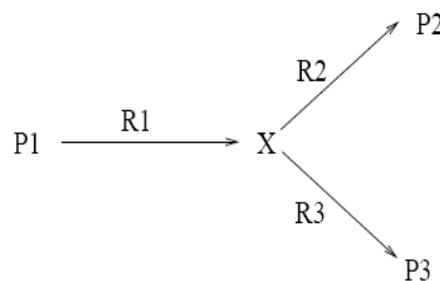
$V=(1,1,0)$ efm

$V=(2,1,1)$ non efm



Définition 7 Un vecteur $e = (e_1, \dots, e_r)^t \in \mathbb{R}^r$ est un mode élémentaire de flux (efm) pour «elementary flux mode») s'il vérifie les conditions suivantes :

1. $Ne = 0$. (état stationnaire)
2. Pour chaque indice j d'une réaction irréversible $e_j \geq 0$. (faisabilité)
3. Pour chaque état stationnaire faisable e' de N ,
 $\text{supp}(e') \subseteq \text{supp}(e) \Rightarrow \exists \alpha \in \mathbb{R}$ tel que $e' = \alpha e$. (minimalité)



$$N = (1 \ -1 \ -1)$$

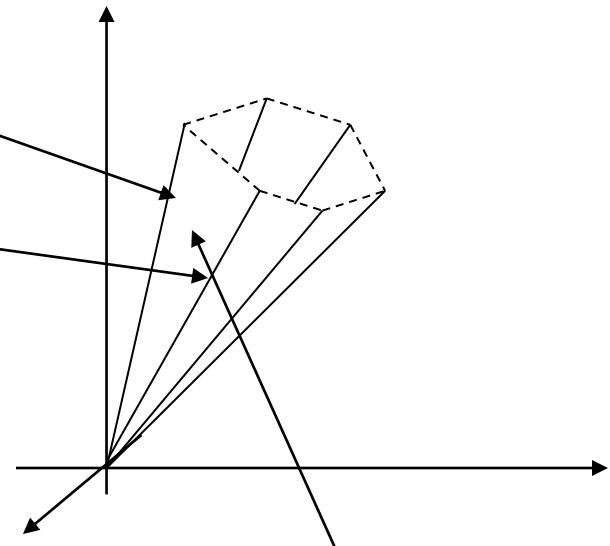
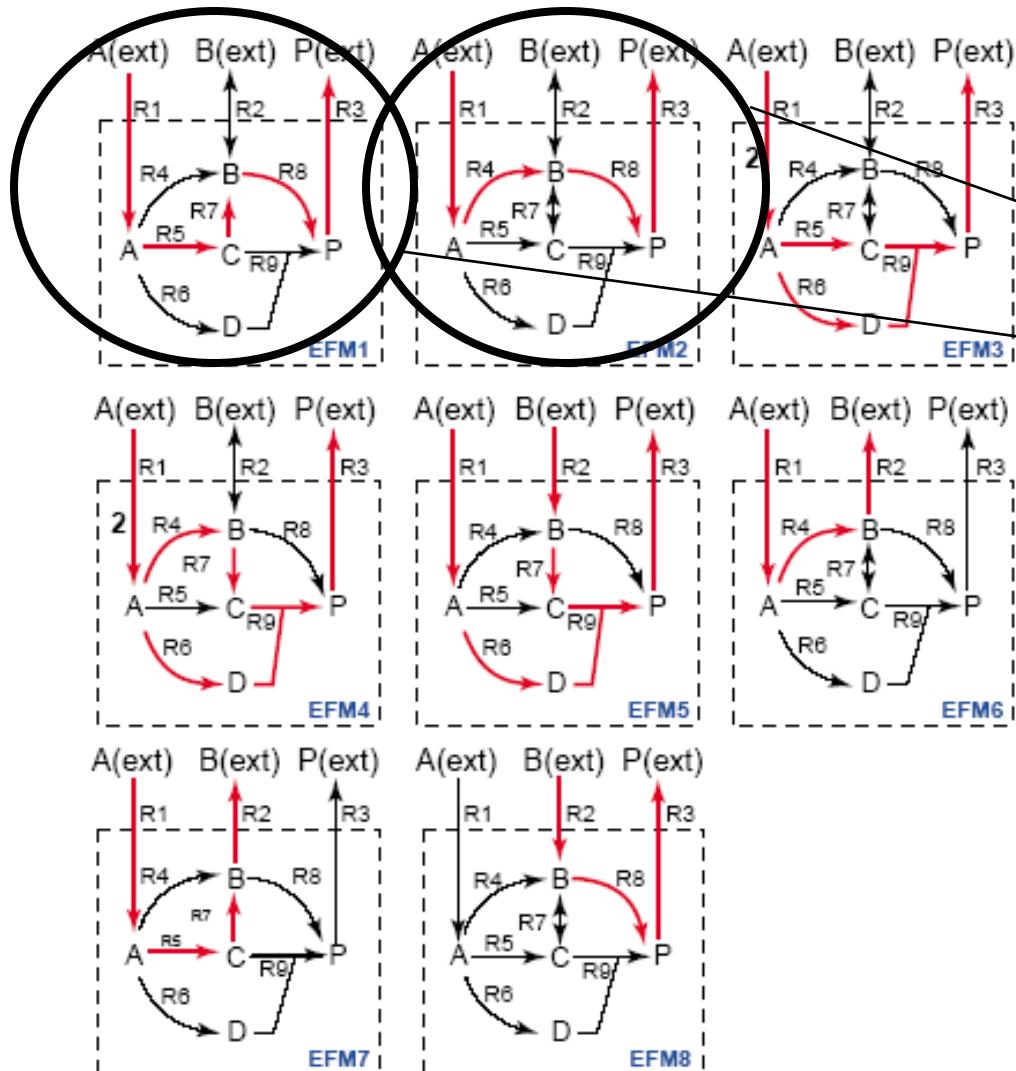
$V=(-1,-1,0)$ non efm

$V=(1,0,1)$ efm

$V=(0,1,1)$ non efm

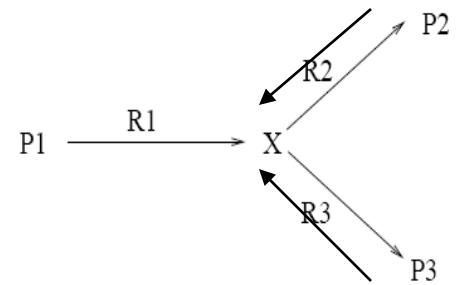
$V=(1,1,0)$ efm

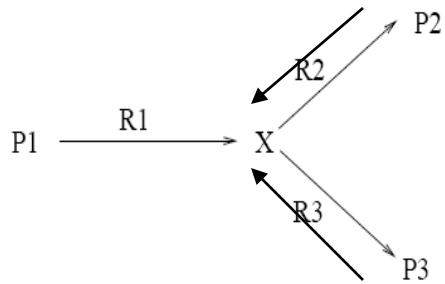
$V=(2,1,1)$ non efm



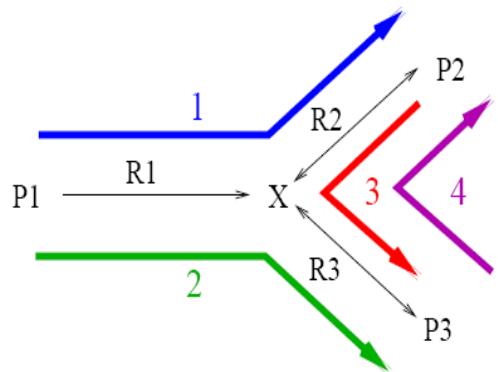
ensemble des
solutions optimales
pour P/A

(Klamt, 03)





Quand on est en présence de réactions irréversibles et réversibles on dissocie les deux ensembles qu'on analyse de manière indépendante

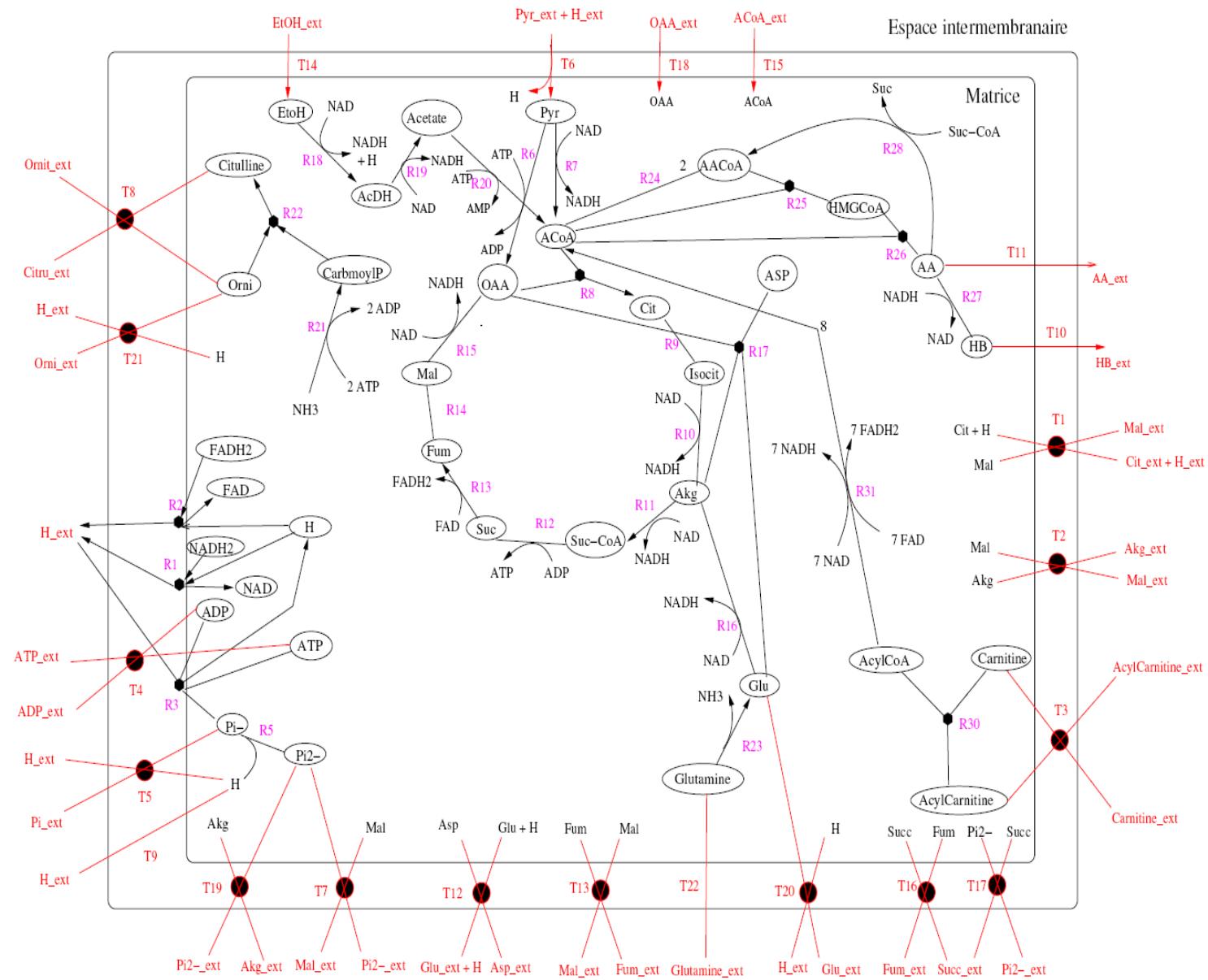


Logiciels	auteurs
YANA [64] (JAVA)	R. Schwarz
METATOOL [42] (C)	Th. Pfeiffer
EMPATH (SmallTalk)	J. Woods
METAFLUX (MAPLE)	K. Mauch
FluxAnalyzer [33] (MATLAB)	S. Klamt
ScrumPy [46] (Python)	M. Poolman

Exemple

- Modèle : métabolisme mitochondrial
- Question : Modes élémentaires du métabolisme mitochondrial dans différents tissus
 - Muscle
 - Foie
 - Levure
- Résultats et figures extraits de la thèse de Sabine Pérès

Modèle général du métabolisme mitochondrial



Tissus	Muscle	Foie	Levure
Nombre de réactions	37	44	40
Nombre de réactions irréversible	12	16	11
Nombre de métabolites internes	29	34	32
Nombre de métabolites externes	21	25	25
Connectivité moyenne	4,2	3,47	3,9
Nombre d' <i>efms</i>	6 190	4 226	4 637
Nombre maximal d' <i>efms</i> possibles	$5,76 \cdot 10^9$	$2,85 \cdot 10^{13}$	$4,42 \cdot 10^{11}$
Longueur minimale d'un <i>efm</i>	2	2	2
Longueur maximale d'un <i>efm</i>	23	24	22
Longueur moyenne d'un <i>efm</i>	17,7	16,7	15,3

TAB. 3.1 – Caractéristiques des réseaux et des *efms* des trois tissus

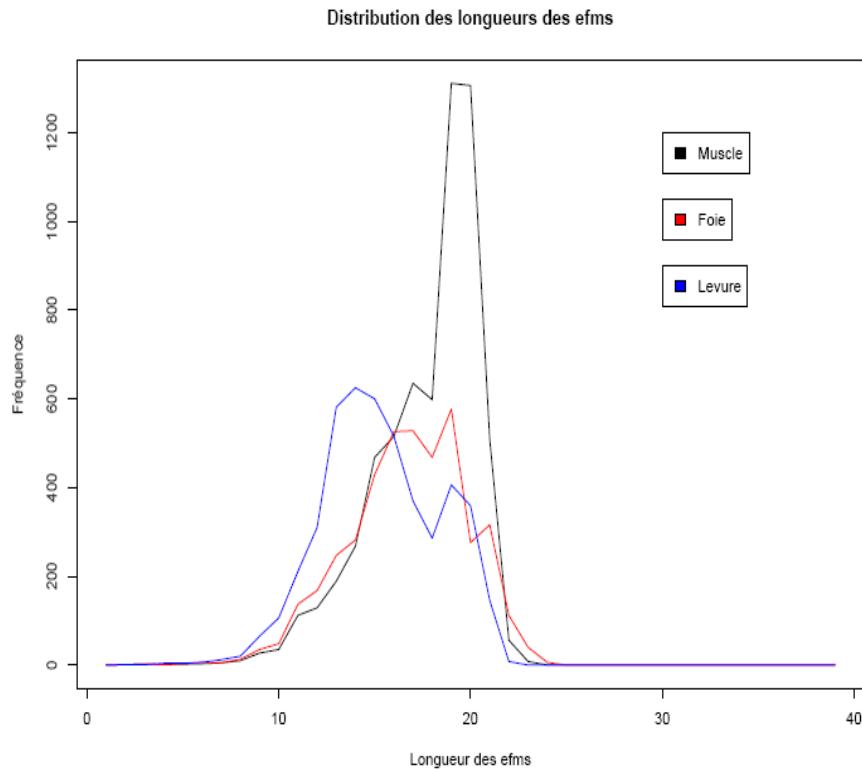
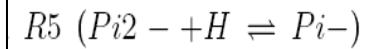


FIG. 3.8 – Distribution de la longueur des *efms*. Les abscisses représentent la longueur des *efms* et les ordonnées représentent le nombre d'*efms* correspondant.

Muscle		Foie		Levure	
Réaction	%	Réaction	%	Réaction	%
R5	84.29	R5	81.16	R5	86.58
R8	83.19	T6	72.29	R15	64.78
R13	80.22	R13	64.69	R3	62.30
R11	80.22	R12	64.69	T6	59.19
T6	76.63	R11	64.69	R12	58.39
R6	74.36	R6	62.25	R11	58.39
R3	66.00	T4	57.69	T4	57.90
T11	61.74	T10	56.81	R8	57.29
T4	60.77	R27	56.81	R1	51.52
T7	60.43	T7	56.17	R6	50.78
T10	60.03	R3	55.41	T16	48.43
R27	60.03	T11	55.18	T18	48.28
R28	58.91	R15	54.42	T5	47.31
R24	58.91	R8	47.65	T17	44.94
R9	54.57	T19	47.01	R9	44.12
R10	54.57	R32	46.07	R10	44.12
T5	53.21	R2	45.17	T7	41.92
R15	50.42	T5	44.98	T9	40.71
T1	50.19	R9	41.64	T19	40.56
R32	48.20	R10	41.64	T12	39.76
T19	47.72	T2	34.99	R17	39.76
R12	47.38	T12	34.69	T13	37.24
R2	46.73	R17	34.69	R14	37.24
T13	40.12	T3	34.47	T2	36.89
R14	40.12	R31	34.47	T1	36.64
R1	38.09	R30	34.47	T14	35.97
T9	37.26	T1	33.81	R4	35.97
T2	36.18	T13	32.37	R20	35.97
T12	34.89	R14	32.37	R19	35.97
R17	34.89	R7	30.12	R18	35.97
T20	16.41	T9	28.18	R2	33.03
R16	16.41	R16	28.13	R13	33.03
T3	15.73	R1	26.07	R7	21.88
R31	15.73	R26	18.26	T20	15.59
R30	15.73	R25	18.26	R16	15.59
R7	11.58	R24	18.26	T8	0
R4	0	T2	14.43	T3	0
		R23	14.43	T15	0
		T20	13.72	T11	0
		T8	12.42	T10	0
		R22	12.42		
		R21	12.42		



Réaction non spécifique à la mitochondrie mais faisant partie de la production d'ATP



TAB. 3.2 – Réactions les plus fréquentes des trois tissus

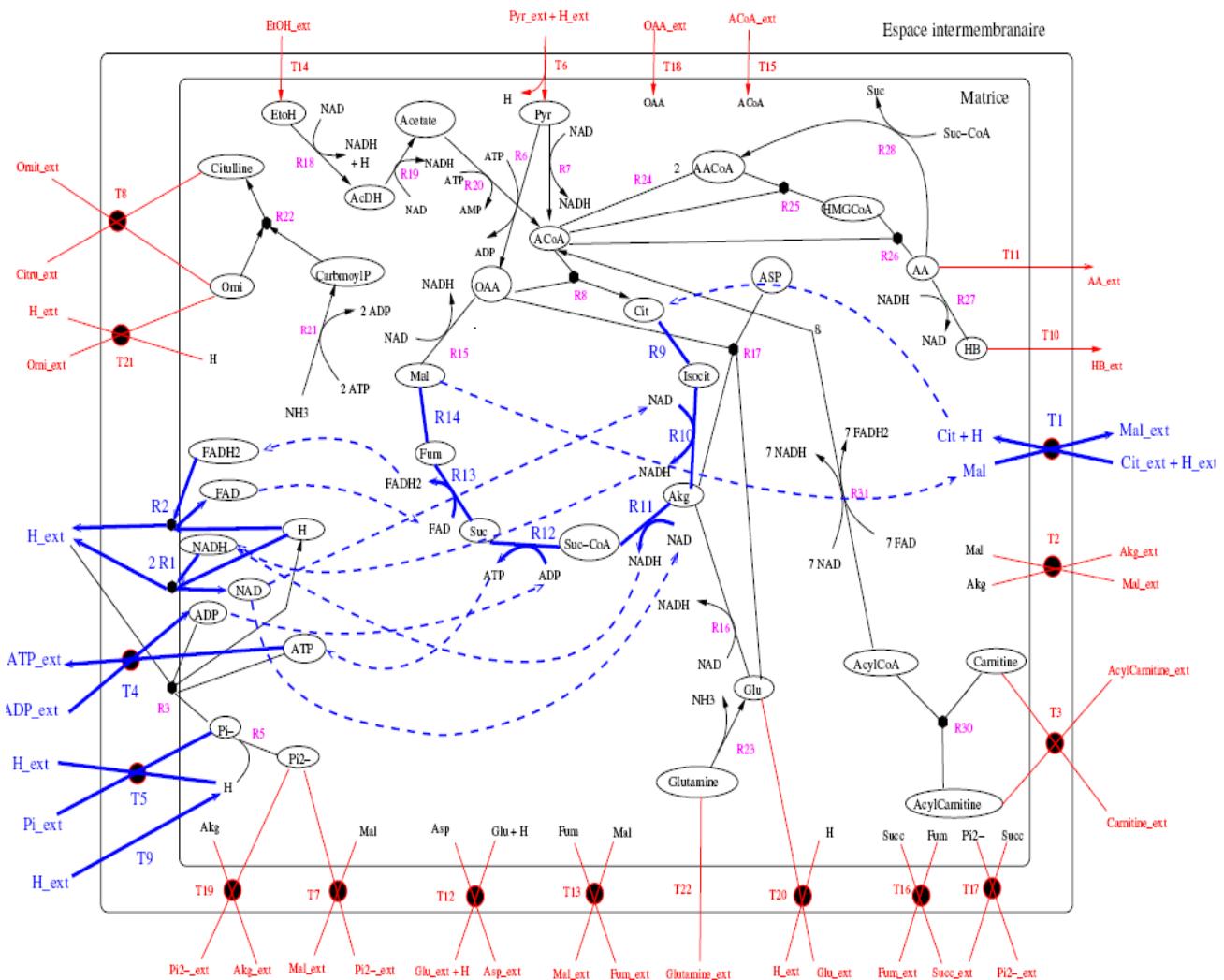


FIG. 3.11 – Synthèse d'ATP par le cycle de Krebs.