



Metabolomics for nutrition and toxicology, *in vivo*, *in vitro* and *in silico* studies. Overview of the French metabolomics community

Fabien JOURDAN
INRA Toulouse



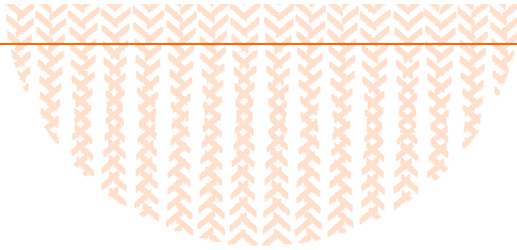
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Outline

- **Metabolomics to study food contaminants**
- **Identifying biomarkers with high resolution mass spectrometry**
- **Metabolic network analysis**
- **Metabolic network reconstruction for cell lines**
- **Metabolomics in France**





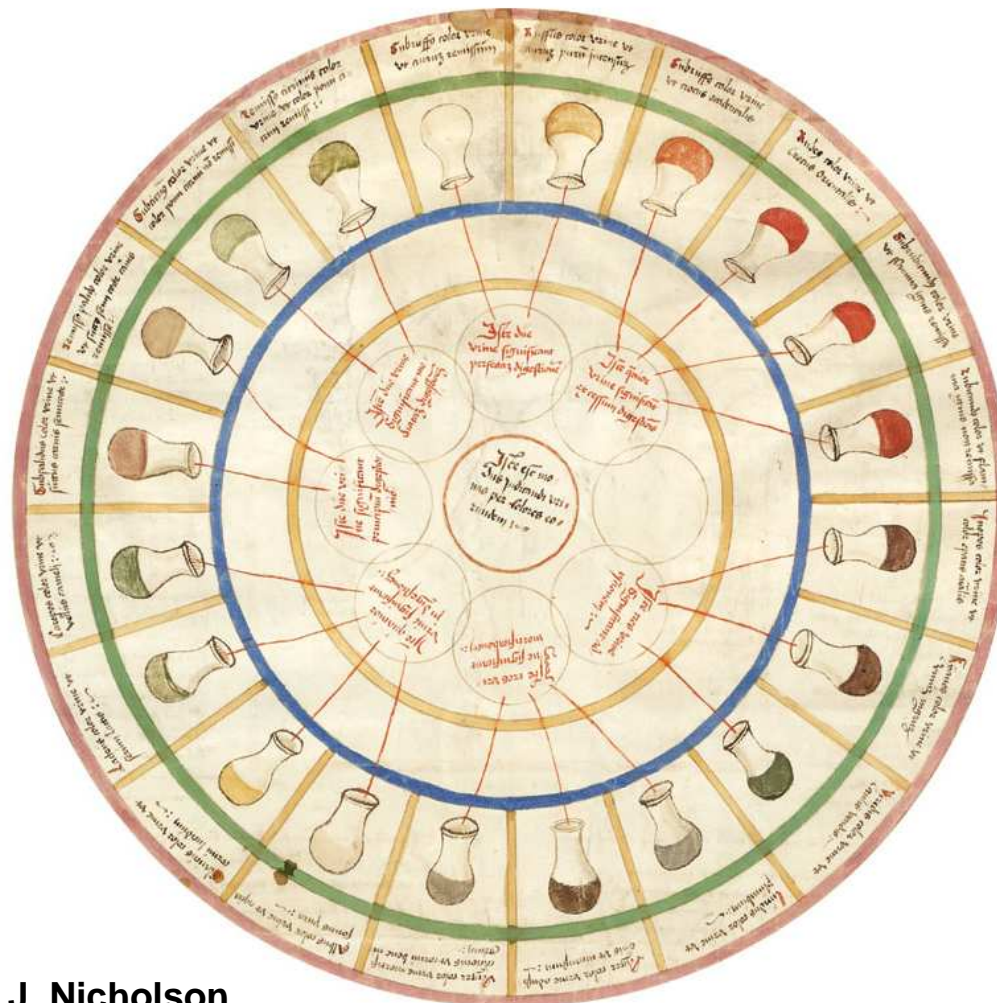
Metabolomics to study food contaminants



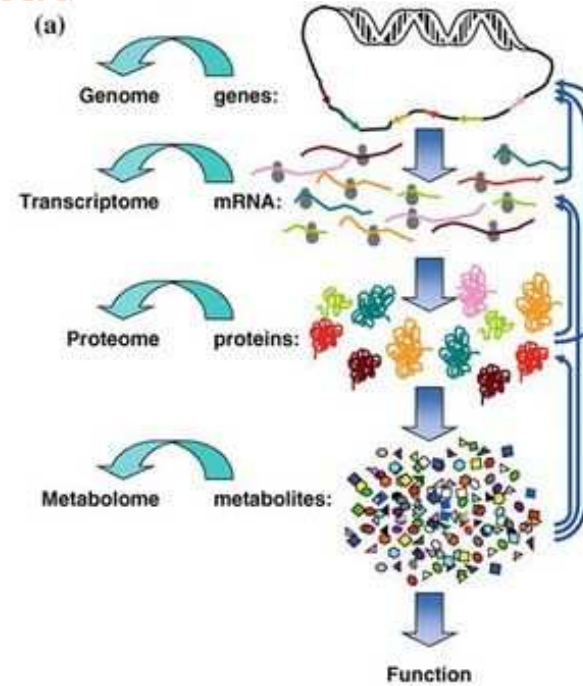
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From genes to metabolites



J. Nicholson



~1500



~15000



~50000

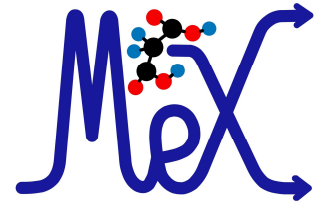
Using the metabolome shift as a phenotypic marker



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Food contaminants



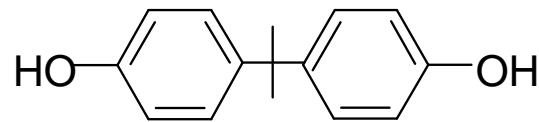
MeX team (D Zalko),
INRA Toulouse

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



Bisphenol A

Prod. ca. 3 Million T/year



Epoxy Resins



Free monomer = model Xeno-estrogen



Effects / human health ?

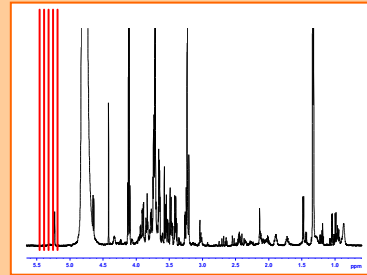


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Fingerprinting

Data acquisition



Spectrum bucketing

Numerical data

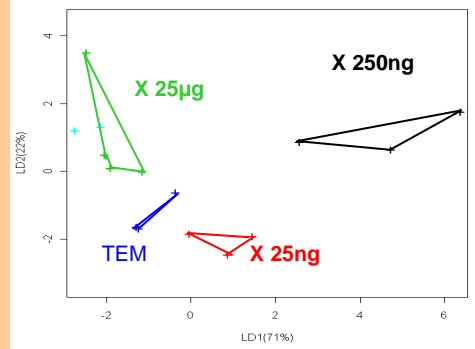
Variable 1 v1,v2...

Variable 2 v1,v2...

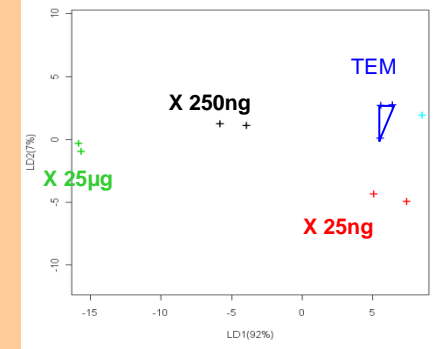
...

Multivariate statistics

Liver



Brain



Discrimination of adult mice exposed *in utero* to low doses of xenobiotics

Biomarkers

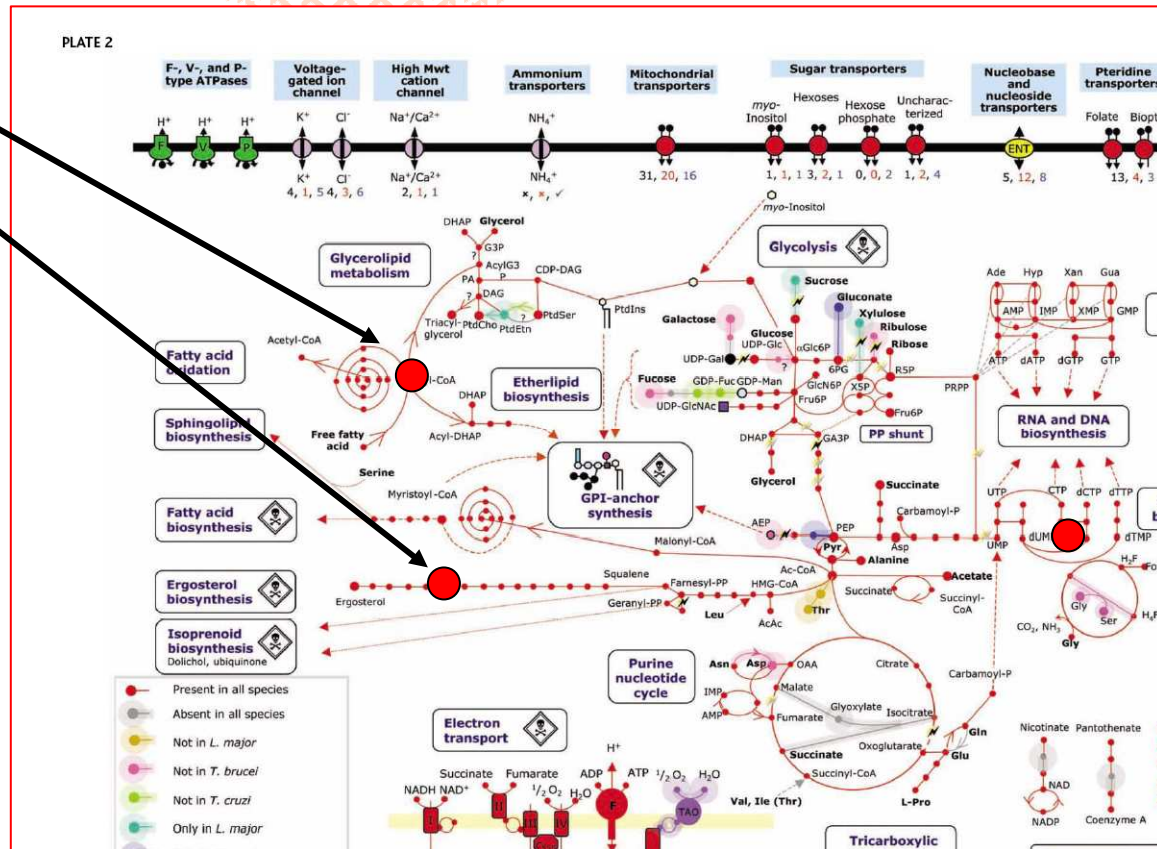
hippurate/tryptophane
valine
lactate
lysine
cétoglutarate/succinate
glucose
TMAO/phénylalanine



From Biomarkers to System Biology

hippurate/tryptophane
valine
lactate
lysine
cétoglutarate/succinate
glucose
TMAO/phénylalanine

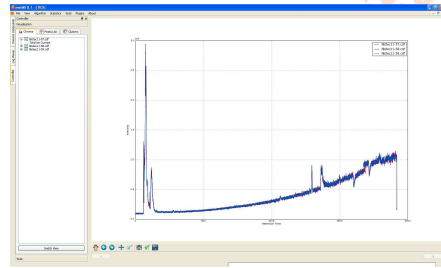
Given a list of biomarkers...



...find the processes involved

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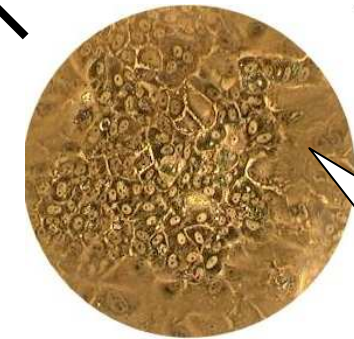
MS raw data

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	364.120629	C14H20N6O6S1	10 pathways Display pathways	1
8-hydroxypurine	CPD-9017	136.03805	C6H4N4O1	0 pathway	1

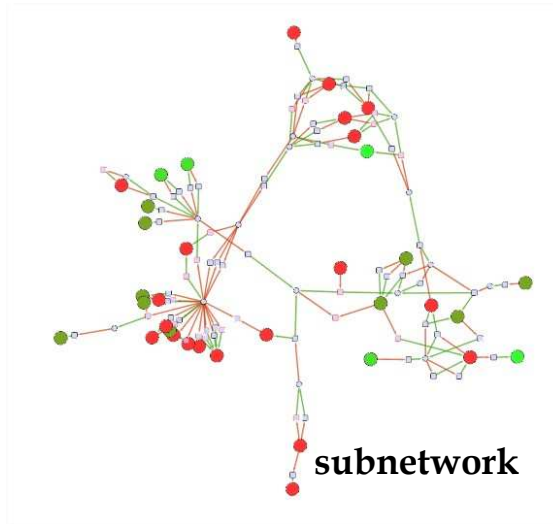
Metabolite list



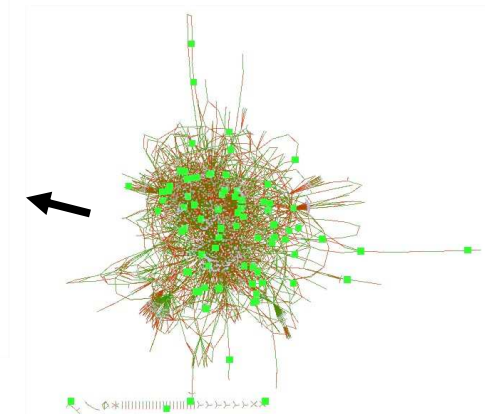
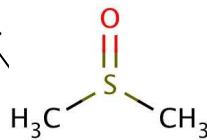
LC-HRMS



HepaRG

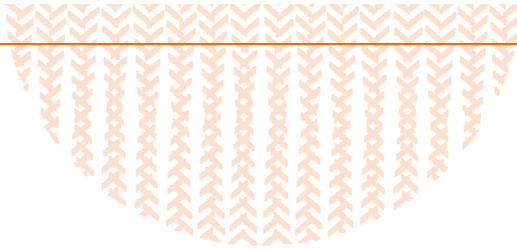


subnetwork



Data in human network





Identifying biomarkers with high resolution mass spectrometry



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High Resolution Mass Spectrometry

NMR

Mass spectrometry

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

High resolution MS :

- FT ICR
- Orbitrap



30

30.049160

30.006100

30.065484

30.010565

CH4N

NO

C2H4

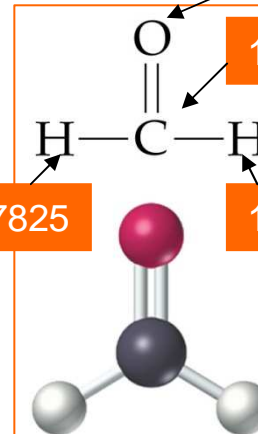
CH2O

15.994915

12.000000

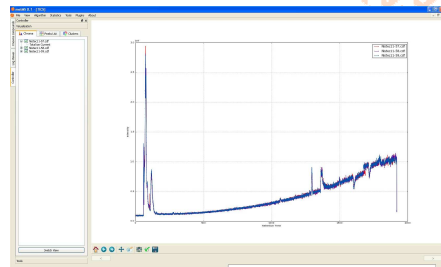
1.007825

1.007825



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MS raw data

Compound Name	Identifier	Mass	Formula	Pathways	sample
(R)-3-hydroxybutanoate	CPD-335	104.047362	C4H8O3	0 pathway	<input type="checkbox"/>
3-hydroxy-isobutyrate	3-HYDROXY-ISOBUTYRATE	104.047362	C4H8O3	1 pathway Display pathways	<input type="checkbox"/>
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8-hydroxypurine	CPD-9017	136.03805	C6H4N4O1	0 pathway	<input type="checkbox"/>

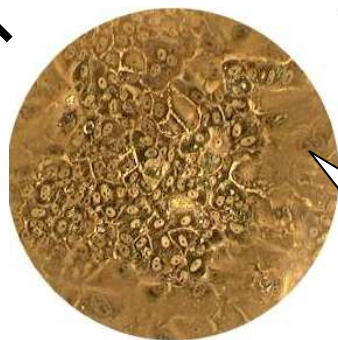
Metabolite list



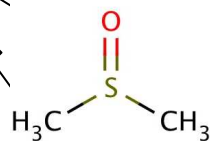
LC-HRMS



Going from raw data to a metabolite list
 Challenge : tenth of thousands of peaks
 among which only few hundreds are relevant



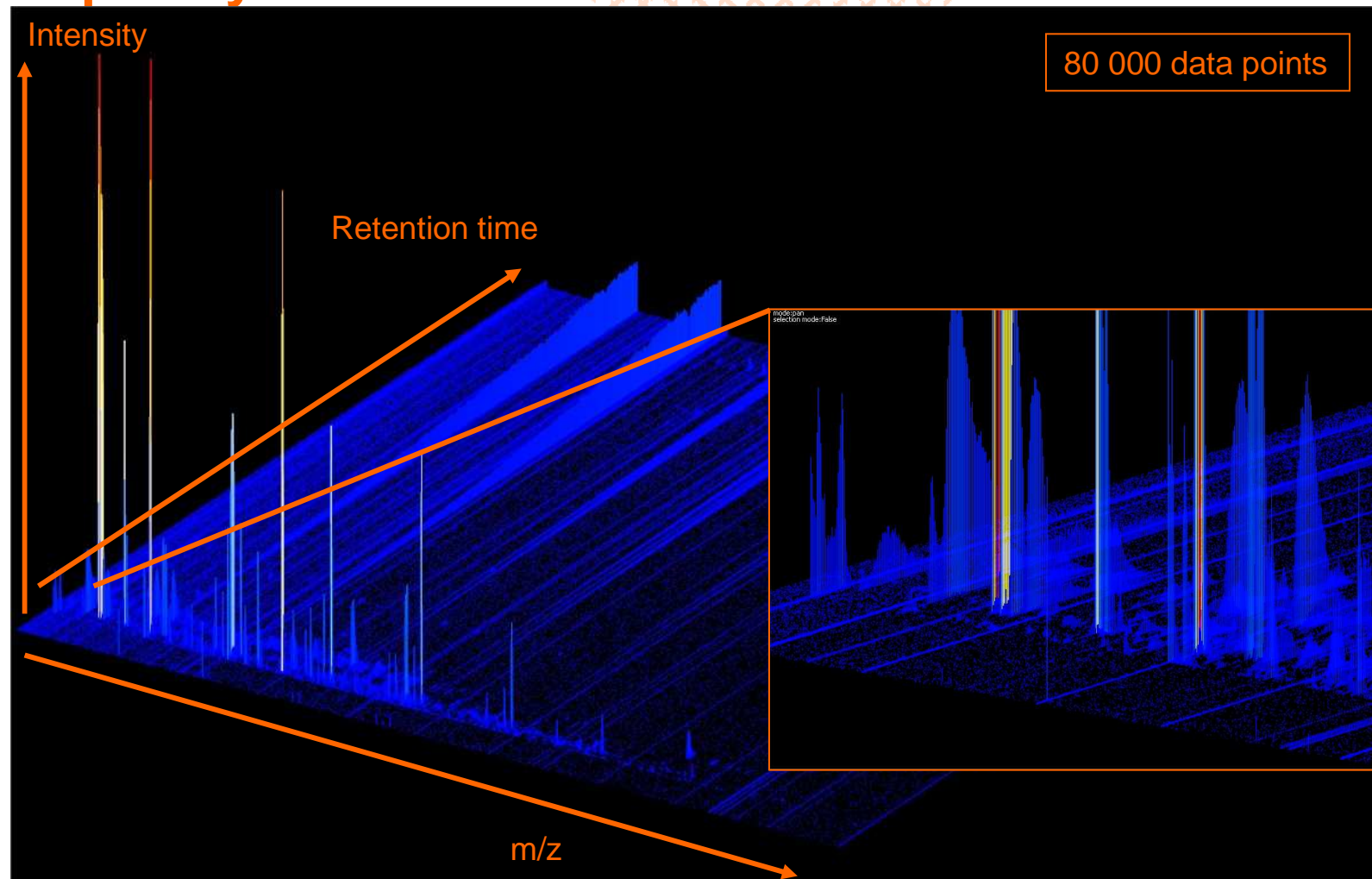
HepaRG



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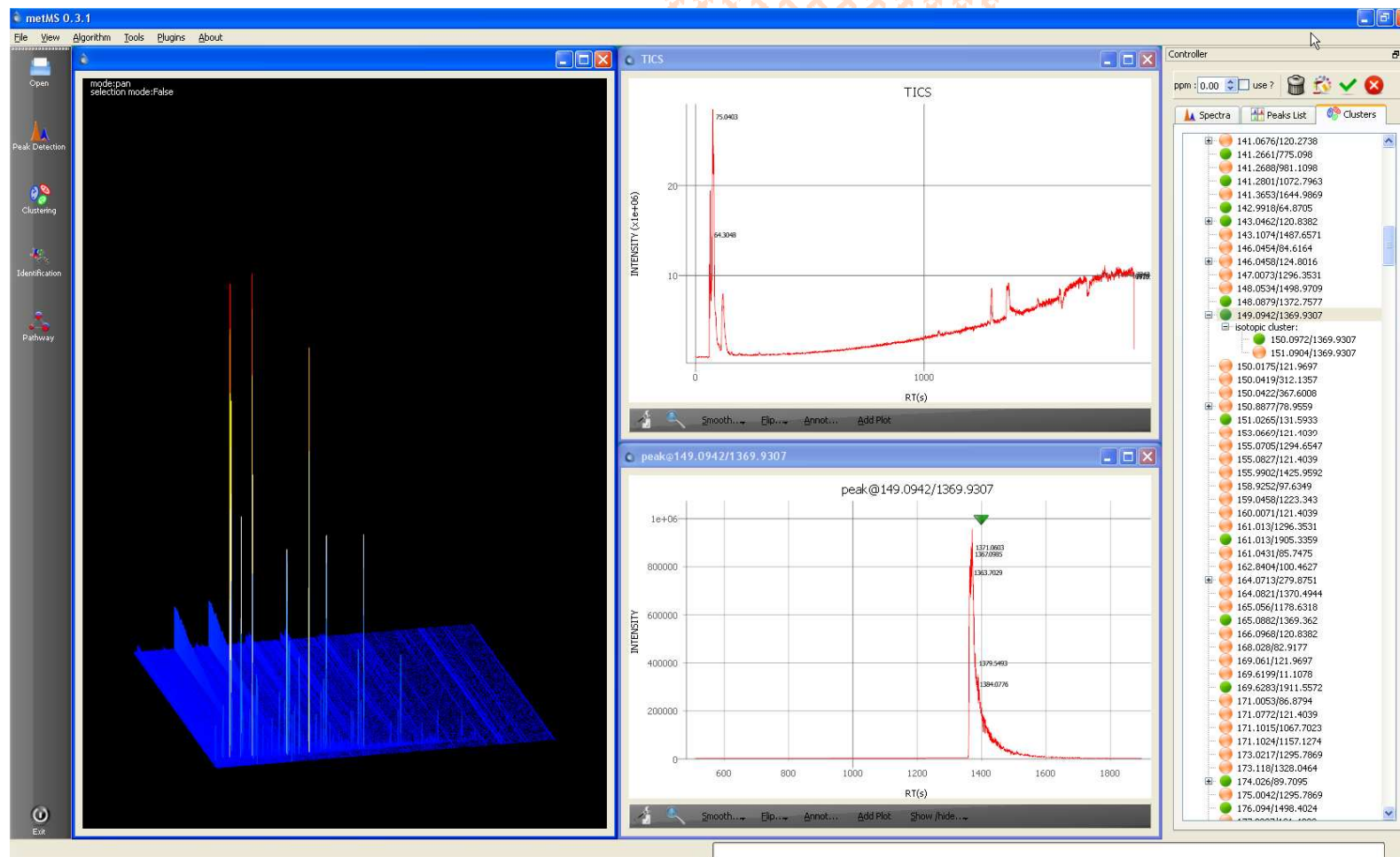
Complexity of LC-HRMS data



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MetMS: implementation of LC-HRMS data treatment

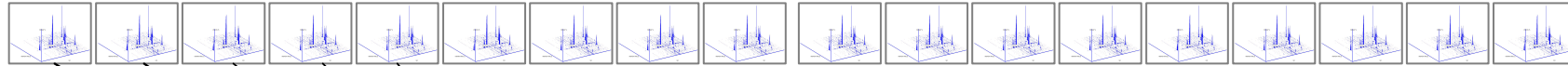


Marc Dubois

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Computational Challenge



18 samples for a condition



Scripps Center For Metabolomics
METLIN: Metabolite and Tandem MS Database

XCMS



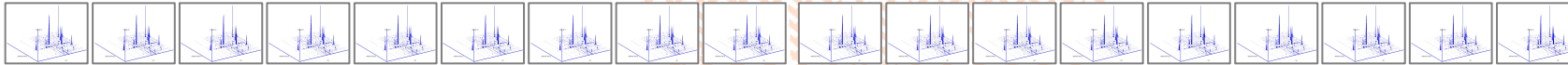
	A	B
1	exact mass	retention time
2	307.063427809286	1014.87168102335
3	403.138561743954	994.002293383756
4	447.068375627694	171.512766016008
5	299.005338647854	713.542363217923
6	305.022719248425	1643.99557163300
7	283.027781167477	1359.31933067860
8	396.105844158039	1194.21002632329
9	217.002878600446	1450.42819933016
10	201.024981090449	1532.99014233025
11	283.027663292645	531.752199938246
12	171.027015142314	1289.31181975929
13	283.027770816323	1177.84505625444
14	283.027830387926	1443.68769151619
15	308.086501554988	1193.79131977766
16	171.026887987536	158.235226597383
17	60.0172695993117	1540.27482811595
18	299.005298963986	650.500998390242
19	305.022469730536	1087.42609034510
20	288.075700357267	107.424259304909
21	463.011248047309	1241.38126586809
22	463.01118039837	1116.90830794752

Applying treatments
on each sample...but
**it can take more than
a day !!**



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18 samples for a condition



Computation is performed on a computer cluster and takes less than an hour



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METLIN: Metabolite and Tandem MS Database



	A	B
1	exact mass	retention time
2	307.063427809286	1014.87168102335
3	403.138561743954	994.002293383756
4	447.068375627694	171.512766016008
5	299.005338647854	713.542363217923
6	305.022719248425	1643.99557163300
7	283.027781167477	1359.31933067860
8	396.105844158039	1194.21002632329
9	217.002878600446	1450.42819933016
10	201.024981090449	1532.99014233025
11	283.027663292645	531.752199938246
12	171.027015142314	1289.31181975929
13	283.02770816323	1177.84505625444
14	283.027830387926	1443.68769151619
15	308.086601554988	1193.79131977766
16	171.026887987536	159.235226597383
17	60.0172695993117	1540.27482611595
18	299.005296963986	650.50099390242
19	305.022469730536	1087.42609034510
20	288.075700357267	107.424259304909
21	463.011248047309	1241.38126586809
22	463.01118038837	1116.92831944752

Data treatment is a challenge in HRMS metabolomics



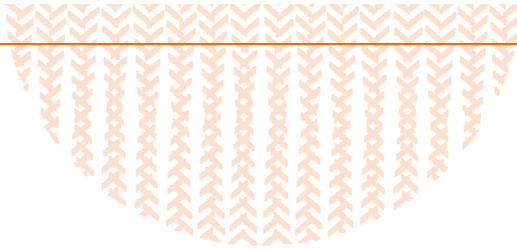
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Output of raw data treatment

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



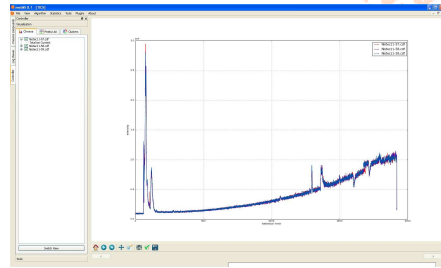


Metabolic Network Analysis



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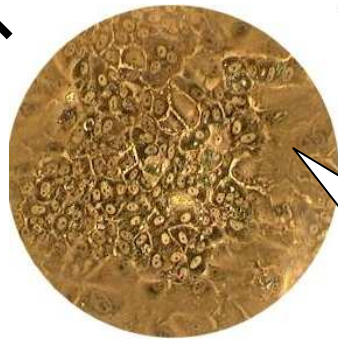
MS raw data

Compound Name	Identifier	Mass	Formula	Pathways	sample
(R)-3-hydroxybutanoate	CPD-335	104.047362	C4H8O3	0 pathway	<input type="checkbox"/>
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S-adenosyl-L-homocysteine	ADENOSYL-HOMO-CYS	364.120629	C14H20N6O6S1	10 pathways Display pathways	<input type="checkbox"/>
8-hydroxyguanine	CPD-9017	136.03805	C5H4N4O1	0 pathway	<input type="checkbox"/>

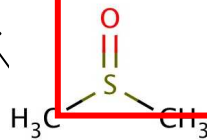
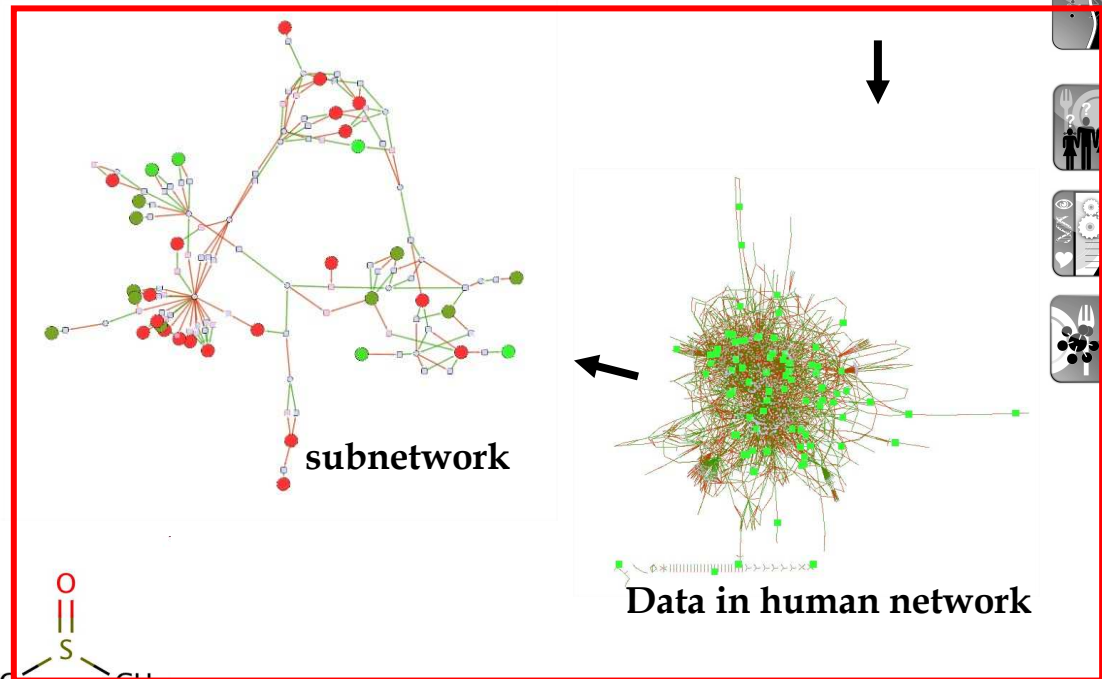
Metabolite list

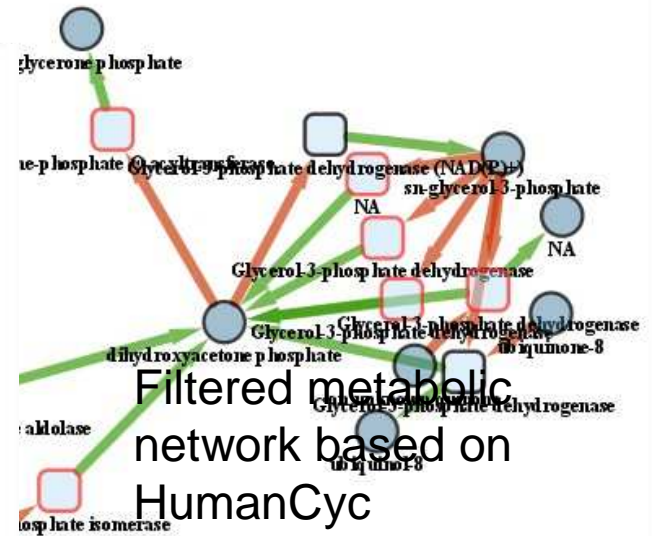
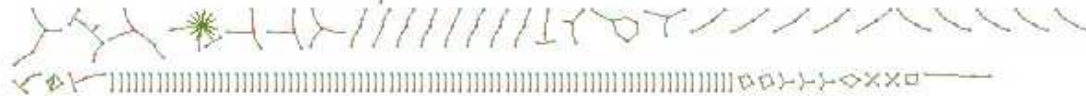
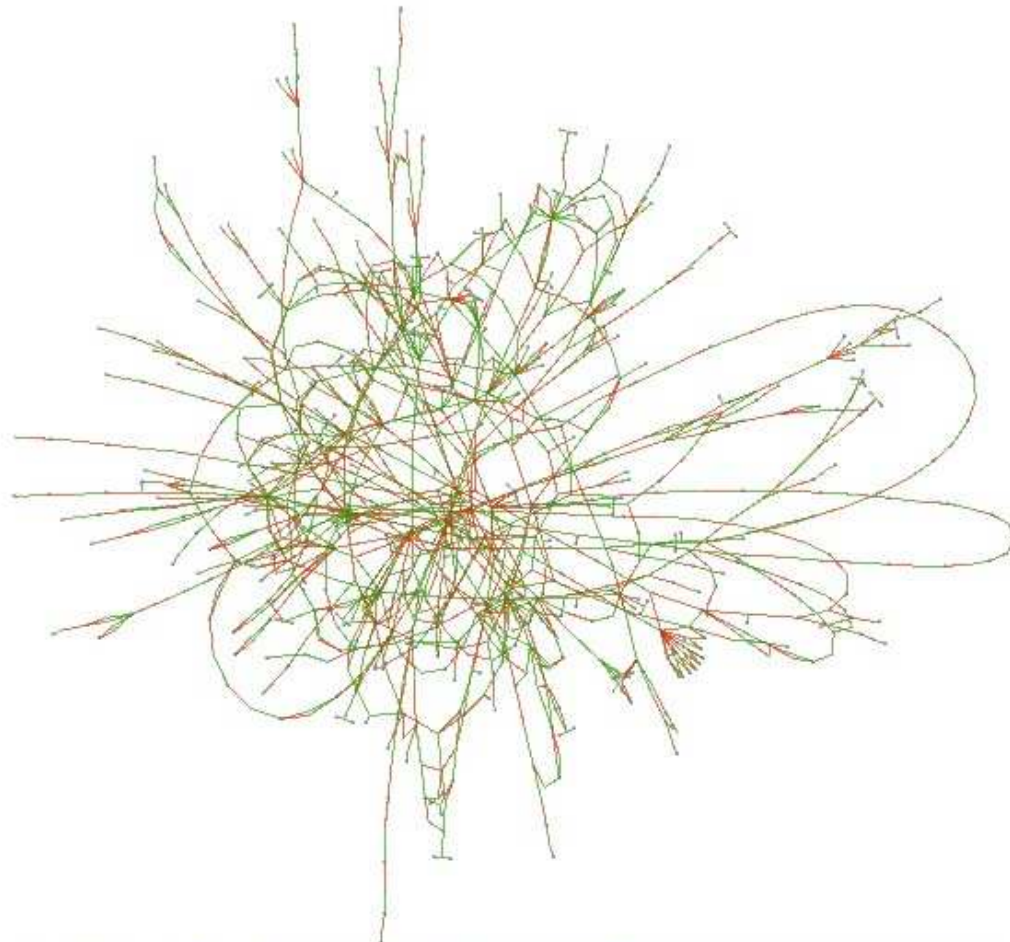


LC-HRMS



HepaRG





Filtered metabolic network based on HumanCyc

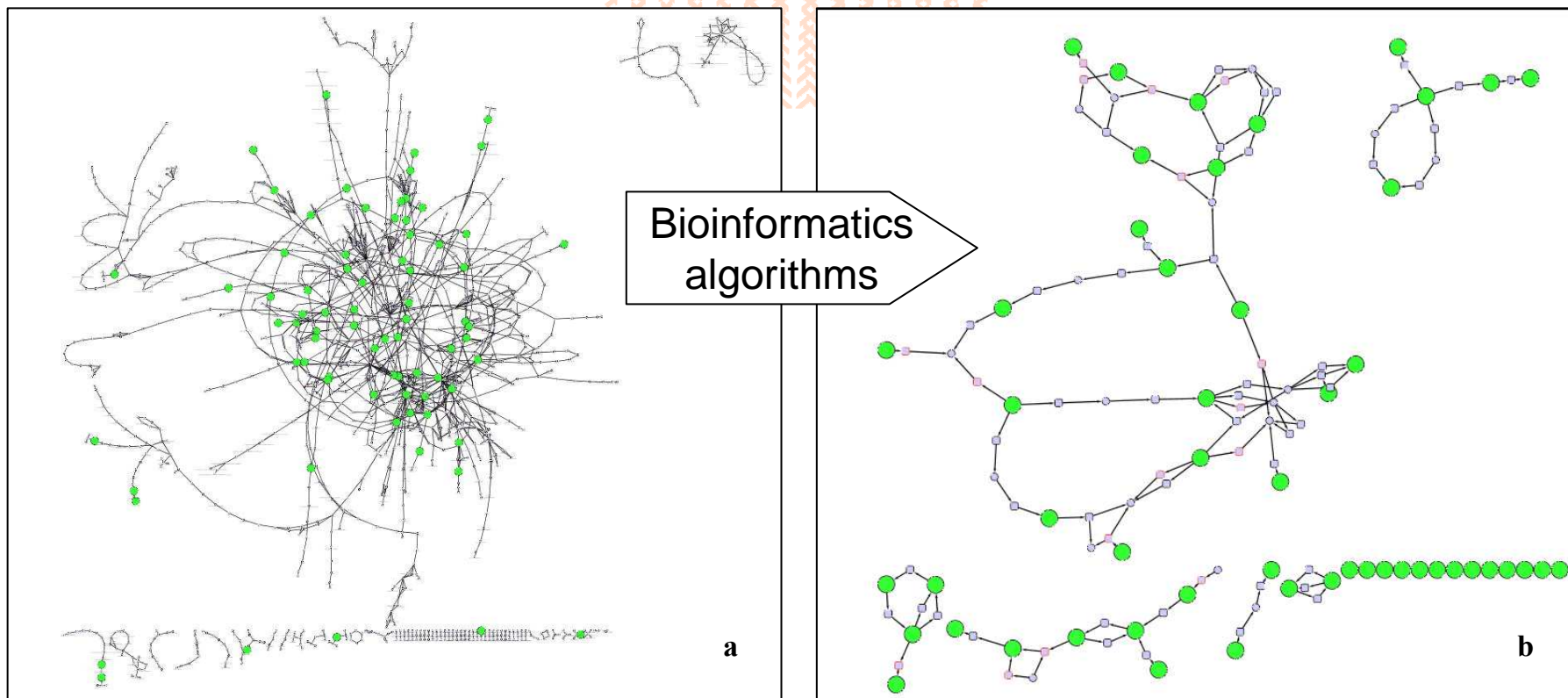
883 reactions

1027 metabolites

kinase (phosphorylating)
 Glycerol-3-phosphate
 aldolase

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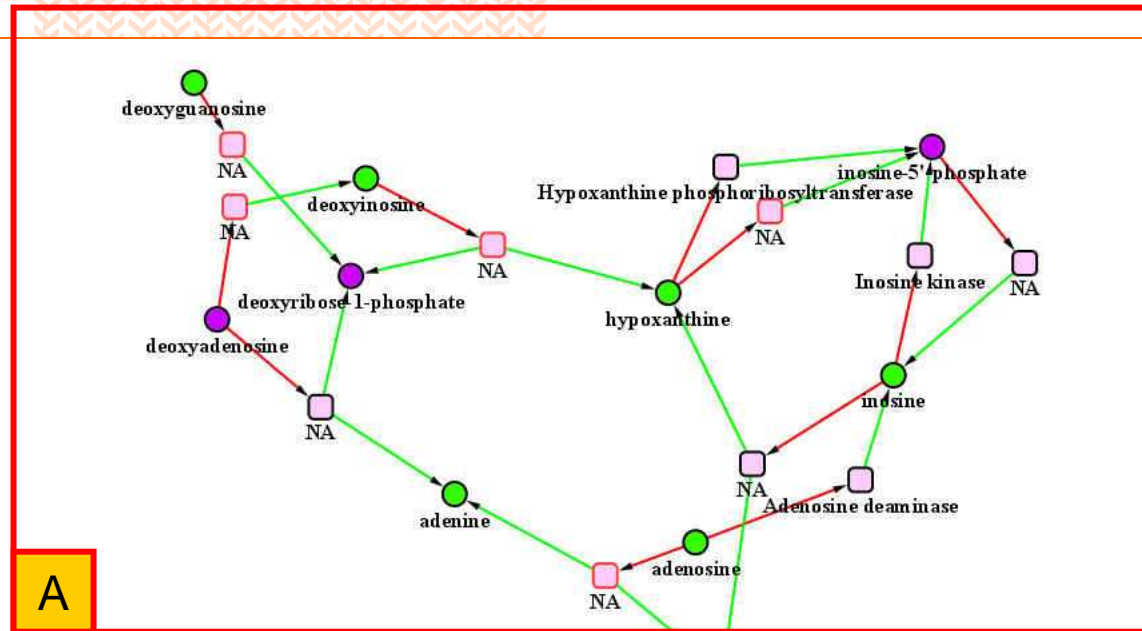




Aim : given a set of metabolites, extracting a “sparser” sub-network containing all the identified compounds.

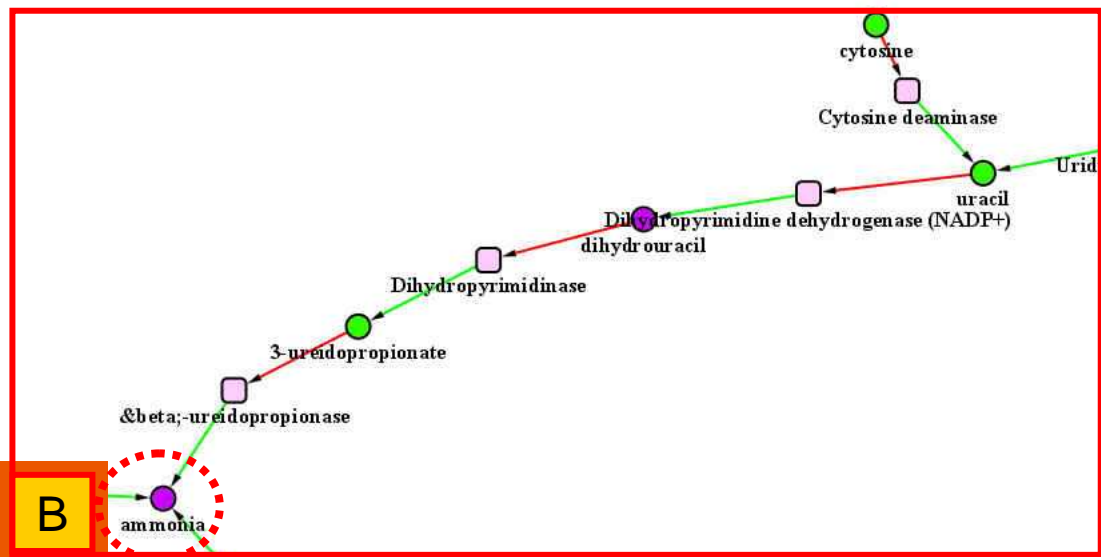
Not all the metabolites are identified, it requires to fill the gaps

Jourdan *et al. Metabolomics*, 2010, 6, 312-321



A

Purine Pathway



B

Pyrimidine Pathway

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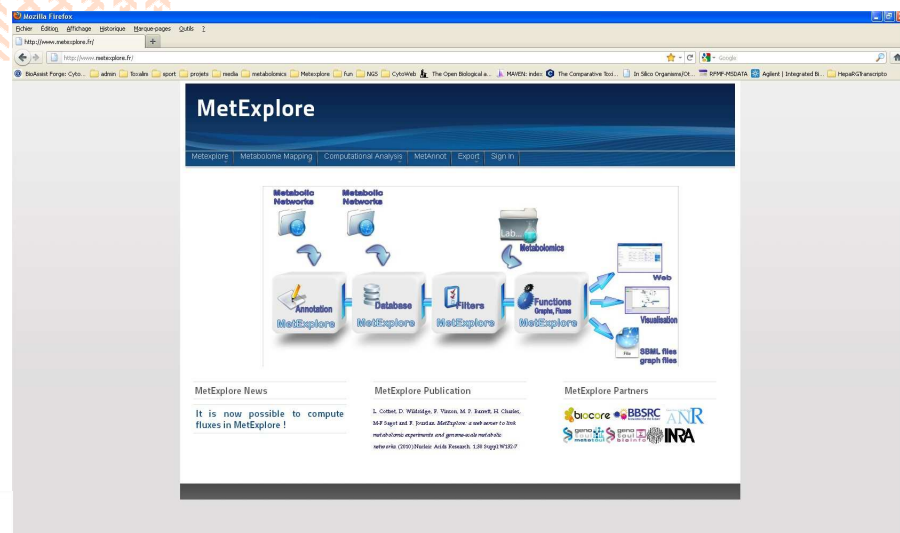
Overview MetExplore

URL: www.metexplore.fr

Registered users:52

Metabolic networks:174

Reactions:19927



W132–W137 *Nucleic Acids Research*, 2010, Vol. 38, Web Server issue
doi:10.1093/nar/gkq312

Published online 5 May 2010

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

Ludovic Cottret^{1,*}, David Wildridge², Florence Vinson¹, Michael P. Barrett²,
Hubert Charles^{3,4}, Marie-France Sagot^{3,5} and Fabien Jourdan¹

¹INRA, UMR1089, Xénobiotiques, F-31000 Toulouse, France, ²Division of Infection and Immunity, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, UK, ³Bamboo Team, INRIA Grenoble-Rhône-Alpes, 38330 Montbonnot Saint-Martin, ⁴UMR203 Biologie Fonctionnelle Insectes et Interactions (BF2I), INRA, INSA-Lyon, Université de Lyon, F-69621 Villeurbanne and ⁵Université de Lyon, F-69000, Lyon; Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne, France

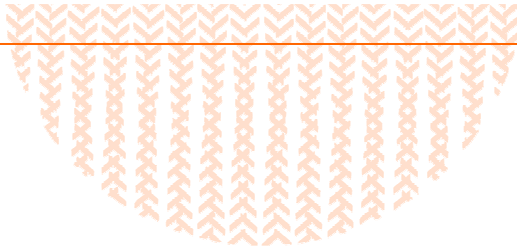
Received January 29, 2010; Revised March 30, 2010; Accepted April 17, 2010



2,437 visits came from 304 cities

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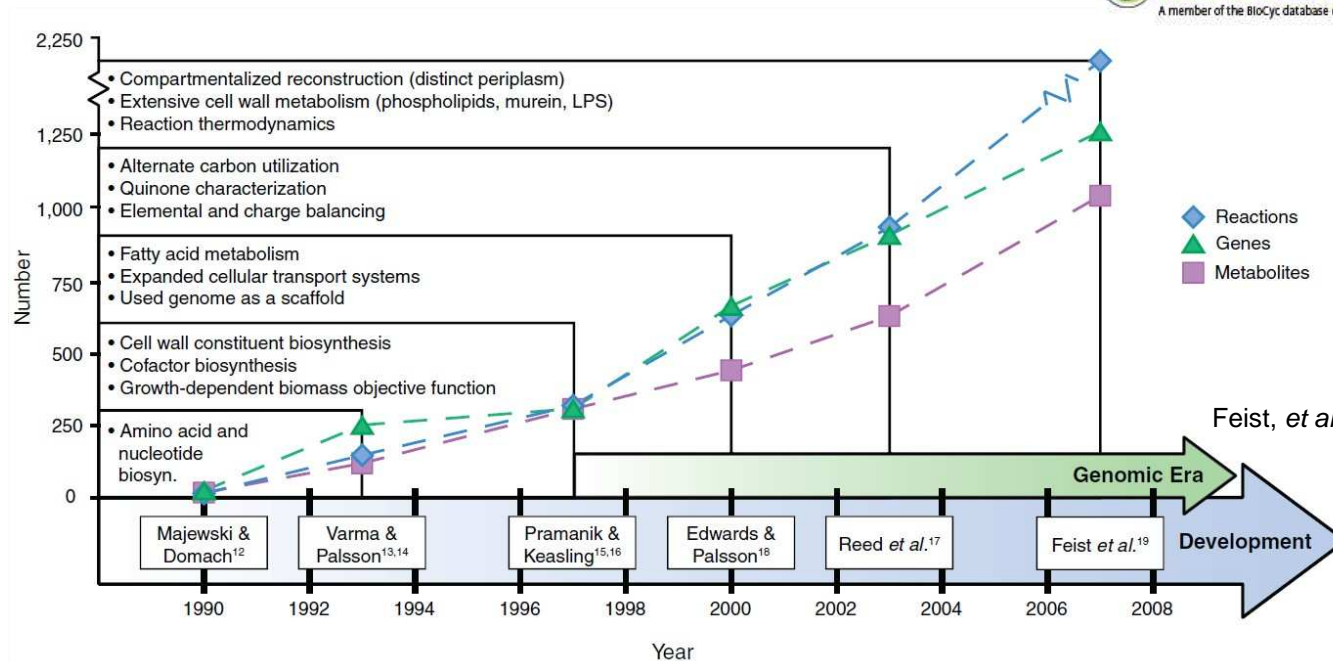
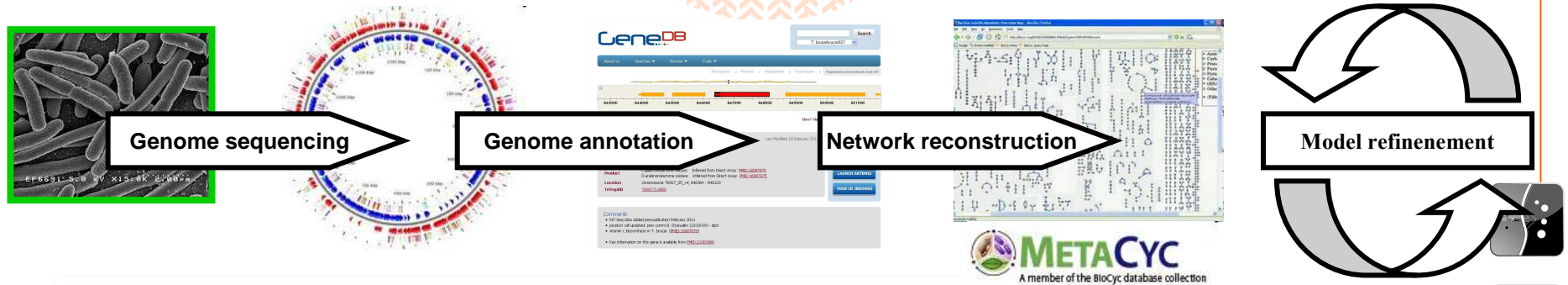
Metabolic network reconstruction for cell lines



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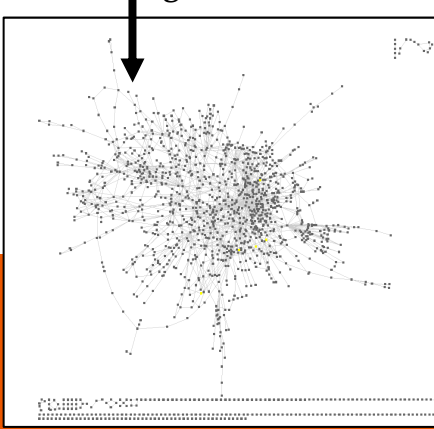
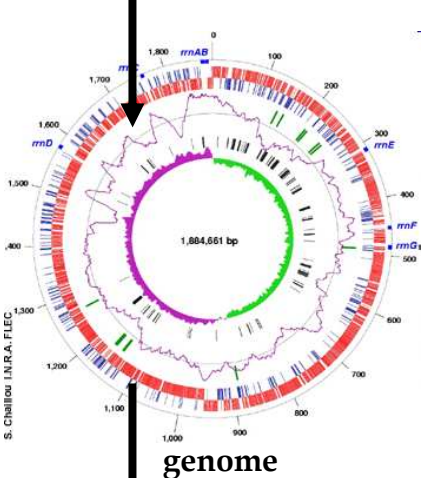
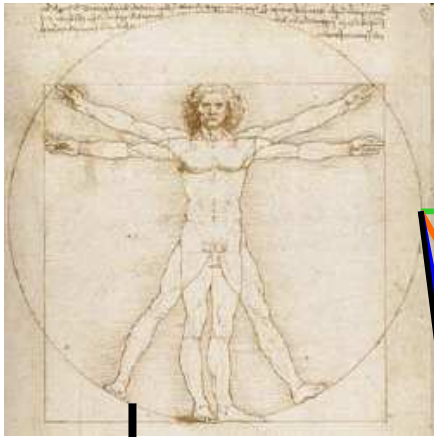
From genome to metabolic networks



Feist, *et al.*. *Nat Biotechnol*, **2008**, 26, 659-667

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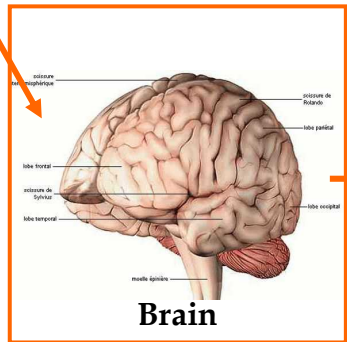
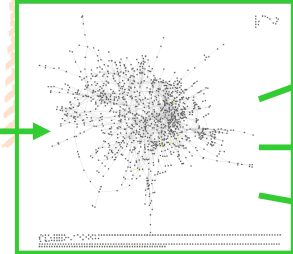




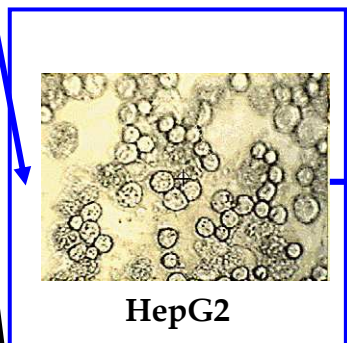
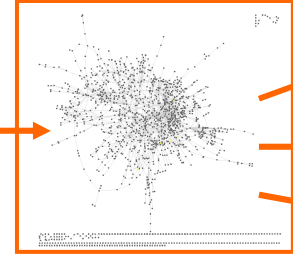
network



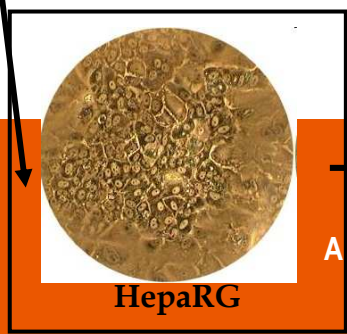
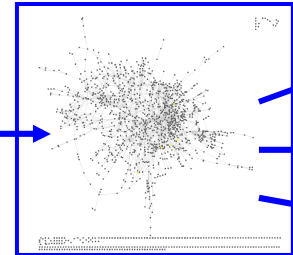
Liver



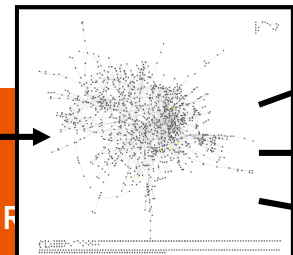
Brain



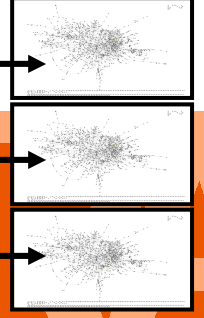
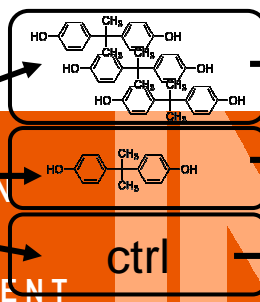
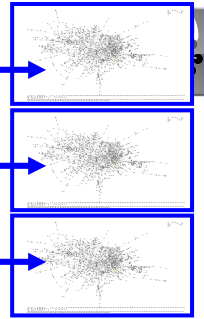
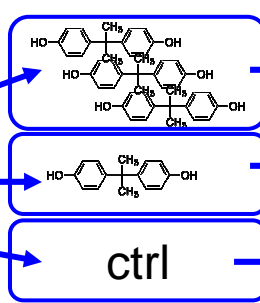
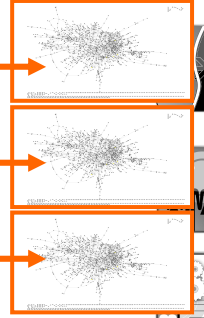
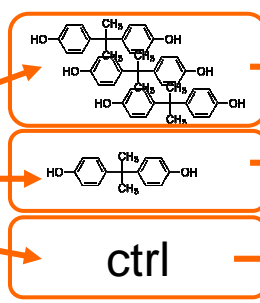
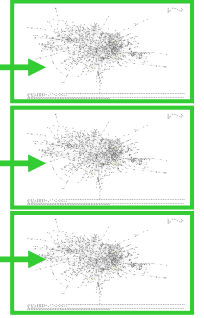
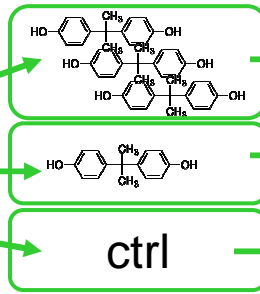
HepG2



HepaRG



Tox (e.g. BPA)



AGE

ENVIRONNEMENT

ATION

Network-based prediction of human tissue-specific metabolism

Tomer Shlomi^{1,4}, Moran N Cabili^{1,4}, Markus J Herrgard², Bernhard O Palsson² & Eytan Ruppin^{1,3}

Direct *in vivo* investigation of mammalian metabolism is complicated by the distinct metabolic functions of different tissues. We present a computational method that successfully describes the tissue specificity of human metabolism on a large scale. By integrating tissue-specific gene- and protein-expression data with an existing comprehensive reconstruction of the global human metabolic network, we predict tissue-specific metabolic activity in ten human tissues. This reveals a central role for post-transcriptional regulation in shaping tissue-specific metabolic activity profiles. The predicted tissue specificity of genes responsible for metabolic diseases and tissue-specific differences in metabolite exchange with biofluids extend markedly beyond tissue-specific differences manifest in enzyme-expression data, and are validated by large-scale mining of tissue-specificity data. Our results establish a computational basis for the genome-wide study of normal and abnormal human metabolism in a tissue-specific manner.

Metabolic network modeling of biological systems involves the analysis and prediction of metabolic flux distributions under diverse physiological and genetic conditions. Traditional modeling techniques are based on mathematical approaches that require detailed information on kinetics and on enzyme and metabolite concentrations^{1,2}. However, a lack of accurate information of kinetic constants and enzyme and metabolite intracellular concentrations limits the current applicability of such methods to small-scale systems. Constraint-based modeling bypasses this hurdle by analyzing the function of large-scale metabolic networks through relying solely on simple physical-chemical constraints³. In recent years, constraint-based modeling has been frequently used to successfully predict various phenotypes of microorganisms, such as their growth rates, rates of nutrient uptake, by-product secretion and the lethality of gene knockouts (see ref. 4 for review).

Despite this progress in applying constraint-based modeling to studying the metabolism of microorganisms, large-scale modeling of human metabolism is still in its infancy. Nonetheless, the emergence of

metabolic diseases such as diabetes and obesity as major sources of morbidity and mortality^{5,6} has stimulated research into human metabolism and its regulation. Metabolic enzymes and their regulators are increasingly considered viable drug targets for these and other conditions^{7,8}. However, in reconstructing human metabolic networks, most of the previous work has focused on characterizing distinct metabolic pathways^{9,10}. Until recently, reconstructions of large-scale human metabolic networks had been performed only for specific cell types and organelles^{11–13}. Although fundamental steps forward, reconstructions of the global human metabolic network based on an extensive evaluation of genomic and bibliomic data (that is, comprehensive assessment of the literature)^{14,15} are not tissue specific. In adapting constraint-based modeling methods from the realm of microorganisms to that of multicellular organisms, one encounters two main hurdles. The first is that different tissues have different metabolic objectives that are not well characterized and remain largely unknown. This is in contrast to modeling microorganisms where a simple objective function (such as maximizing the biomass production rate) can be used together with flux balance analysis⁴ to predict biologically plausible flux distributions. The second major obstacle is the lack of information on tissue-specific metabolite uptake and secretion, which is essential for employing flux balance analysis.

We present a new constraint-based computational method for systematically predicting human tissue-specific metabolic behavior by integrating a genome-scale metabolic network with tissue-specific gene- and protein-expression data. Changes in gene- and protein-expression levels play a major role in controlling tissue-specific metabolic functions^{16–18}, and a strong correlation between gene expression and measured^{19,20} and predicted^{21–24} metabolic fluxes is reported for microorganisms. To account for metabolic flux activity that is not reflected in the expression data (that is, post-transcriptional regulatory effects), we treat tissue-specific variations in enzyme-expression levels not as the final determinants of enzyme activity, but as cues for the likelihood that the enzyme in question supports metabolic flux in its associated reaction(s). Network integration is then used to accumulate these cues into a global, consistent metabolic behavior, which reflects the outcome of putative post-transcriptional regulatory effects. Our method's reliance on enzyme-expression data to infer tissue-specific metabolic flux eliminates the need for a priori knowledge of tissue-specific objective functions and metabolites exchanged by the tissue with biofluids. Instead, the method provides predictions regarding tissue-specific metabolite uptake and secretion.

To examine our method's ability to correctly predict metabolic behavior based on gene-expression data, we first apply it to predicting the metabolic state of the yeast *Saccharomyces cerevisiae* under

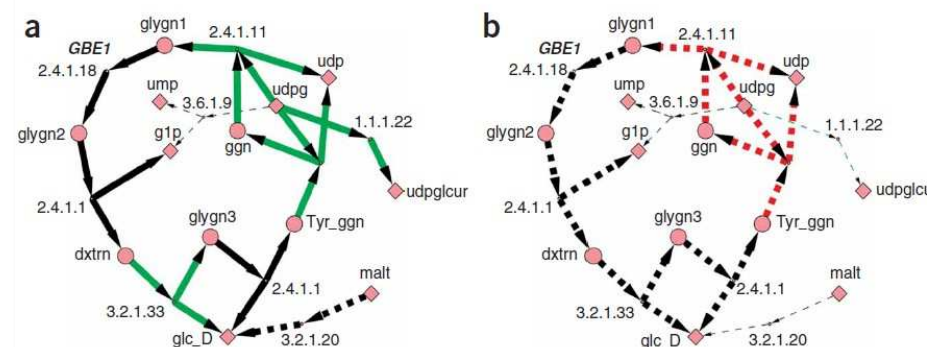
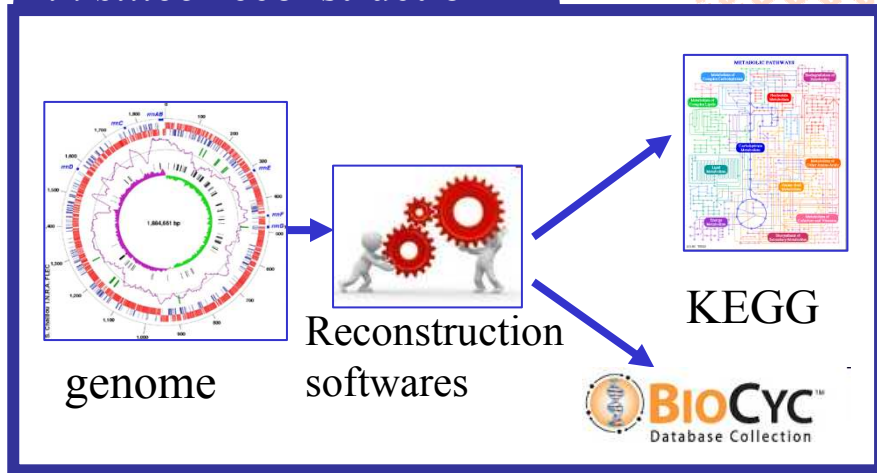


Figure 4 An example of a sub-network of glycogen metabolism. (a,b) The predicted tissue-specific activity of *GBE1* (1,4-alpha-glucan branching enzyme) in the liver (a) and its inactivity in the spleen (b) are illustrated. Nodes

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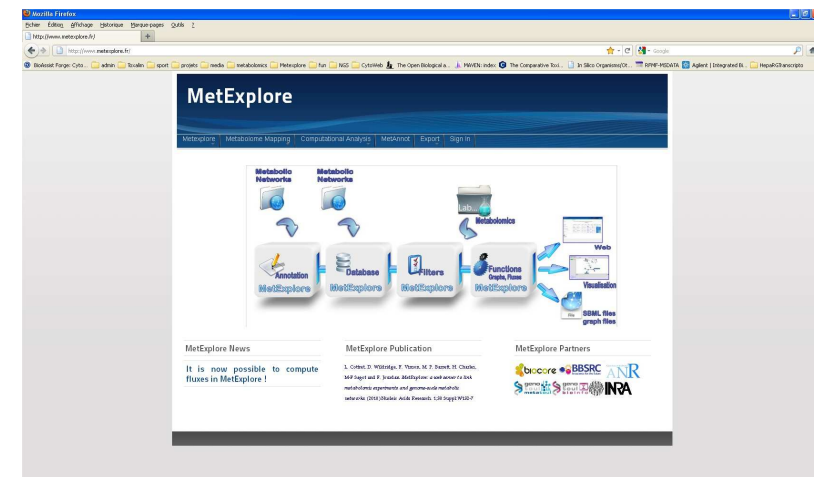
Published online 17 August 2008; doi:10.1038/nbt.1487

in silico reconstruction



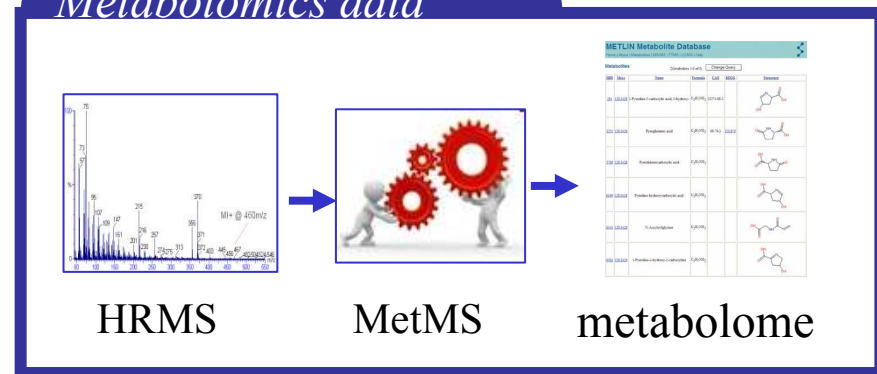
Cell lines annotation pipeline

Data integration

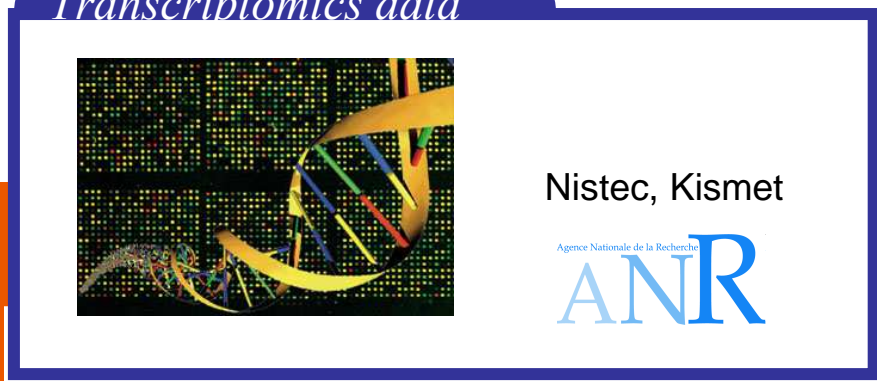


MetExplore: web server the storage, manual annotation and analysis of metabolic networks

Metabolomics data



Transcriptomics data



<http://www.metexplore.fr>

Cottret, L.; Wildridge, D.; Vinson, F.; Barrett, M. P.; Charles, H.; Sagot, M.-F. & Jourdan, F. MetExplore: a web server to link metabolomic experiments and genome-scale metabolic networks. *Nucleic Acids Res*, 2010, 38, W132-W137

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Annotation Project, manual curation

MetExplore - Annotation Jordan is connected

Visualization Annotation BioSource Manager Modify Share Parameters Admin

My BioSource:

Pathways **Reactions** Metabolites Enzymes Proteins Genes

<input type="checkbox"/>	name	dbidentifier	Ec
<input type="checkbox"/>	2-oxoglutarate dehydrogenase	R_AKGDm	1.2.4.2
<input type="checkbox"/>	aconitase	R_ACONT	4.2.1.3
<input type="checkbox"/>	Aconitate hydratase	R_ACONTm	4.2.1.3
<input type="checkbox"/>	ATP-Citrate lyase	R_ACITL	
<input type="checkbox"/>	Citrate lyase	R_CITL	4.1.3.6
<input type="checkbox"/>	citrate synthase	R_CS	
<input type="checkbox"/>	fumarase	R_FUM	4.2.1.2
<input type="checkbox"/>	fumarase, mitochondrial	R_FUMm	4.2.1.2
<input type="checkbox"/>	Isocitrate dehydrogenase (NAD+)	R_ICDHxm	1.1.1.41
<input type="checkbox"/>	isocitrate dehydrogenase (NADP)	R_ICDH	1.1.1.42
<input type="checkbox"/>	Isocitrate dehydrogenase (NADP+)	R_ICDHym	1.1.1.42
<input type="checkbox"/>	Isocitrate dehydrogenase (NADP+)	R_ICDHyp	1.1.1.42
<input type="checkbox"/>	malate dehydrogenase	R_MDH	1.1.1.37
<input type="checkbox"/>	malate dehydrogenase, mitochondrial	R_MDHm	1.1.1.37
<input type="checkbox"/>	succinate dehydrogenase	R_SUCD1m	1.3.99.1
<input type="checkbox"/>	Succinate--CoA ligase (ADP-forming)	R_SUCOASm	6.2.1.5
<input type="checkbox"/>	Succinate--CoA ligase (GDP-forming)	R_SUCOAS1m	6.2.1.4

Select All

Filter

Delete Filter

Citric Acid Cycle

Add/ Delete

Delete Reactions Add Reactions

Compartment ✔

- Cytosol
- EndoplasmicReticulum
- Extraorganism
- GolgiApparatus
- Lysosome
- Mitochondria
- Nucleus
- Peroxisome

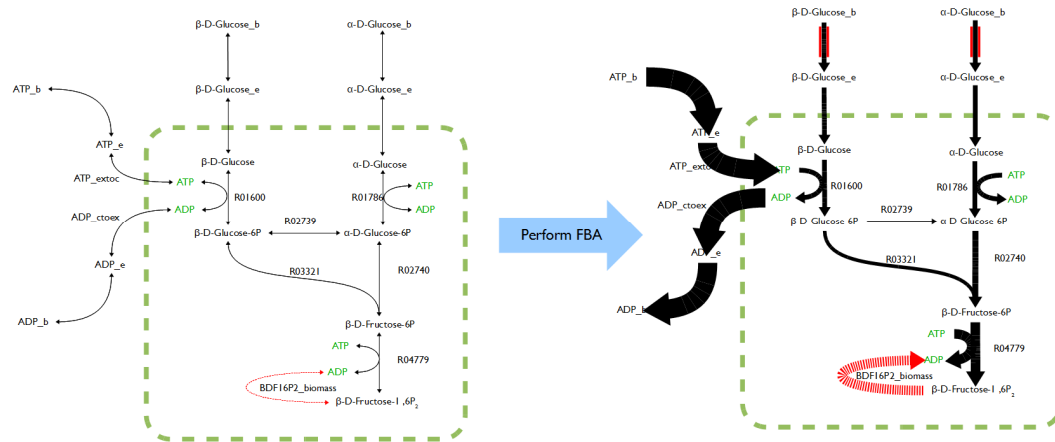


Florence Vinson

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Next step: Metabolic Flux Plasticity



FBA: [2] Fell DA, Small JR: Fat synthesis in adipose tissue. An examination of stoichiometric constraints. *Biochem J* 1986, **238**:781-786
FBA: [3] Orth JD, Thiele I, Palsson B: What is flux balance analysis? *Nat Biotechnol* 2010, **28**:245-248.

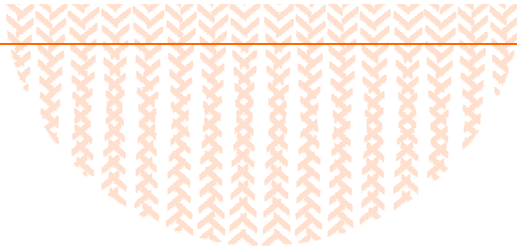
Xenobiotics act on regulation of enzymes leading to changes in flux distribution.

In the case of cancer cells, these fluxes are different from healthy cell lines (Rupin *et al.*)

What are the flux distribution shifts when cells are exposed to xenobiotics?

What are the long time effects?





Metabolomics in France



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French Metabolomics and Fluxomics network

BOARD:

D ROLIN (president, Bordeaux)

C JUNOT (Vice president, Saclay)

C DEBORDE (Secretary, Bordeaux)

JC MARTIN (Treasurer, Marseille)

C CANLET (Toulouse)

AM DELORS (Clermont-Ferrand)

F JOURDAN (Toulouse)

A PARIS (Paris)

JC PORTAIS (Toulouse)

E PUJOS-Guillot (Clermont-Ferrand)



Aims: animation, formation, networking, funding young researchers to attend conferences



National Conference

Date	Attendance	Labs
2005	86	27
2006	107	39
2008	109	44
2010	101	56
2011	136	71

Invited Speakers

J. Nicholson
R. Breitling, M. Oresic

If you want to communicate with us:
Mailing list:
rfmf@listes.inra.fr

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MetaboHub: creating a french metabolomics infrastructure

Developping and providing metabolomics analysis for large scale studies

Three scientific work packages

- Metabolomics (HT, standardisation, target metabolomics)
- Fluxomics (network, synthetic biology)
- Bioinformatics (data management)

Four facilities

- Paris (CEA)
- Bordeaux (INRA)
- Toulouse (INRA)
- Clermont Ferrand (INRA)



Conclusion

- **Metabolomics bioinformatics pipeline in HRMS is a challenging computational problem**
- **Metabolic networks can be used for system biology interpretation of metabolomics data**
- **Genome based reconstructions have to be improved to provide better models**
- **Flux analysis will help to move towards dynamic studies**
- **French metabolomics is animated through the RFMF**
- **National facility project: MetaboHub**



France Metabolomics

INRA Toulouse: Florence Vinson, Marc Dubois, M Dussart, D Zalko (MeX team)

CEA Paris: C Junot

INSA Toulouse: Ludovic Cottret, JC Portais (Metasys team)

International Metabolomics

University of Glasgow: M.P Barrett, D Wildridge

Sanger Institute: F Logan, M Beriman

France Bioinformatics

INRIA Lyon: MF Sagot, V Lacroix, V Acuña, P Milreu, C Klein

University of Évry: E Birmelé

International Bioinformatics

Università degli Studi di Firenze: P Crescenzi, A Marino

Univ. La Sapienza, Rome: A Marchetti-Spaccamela

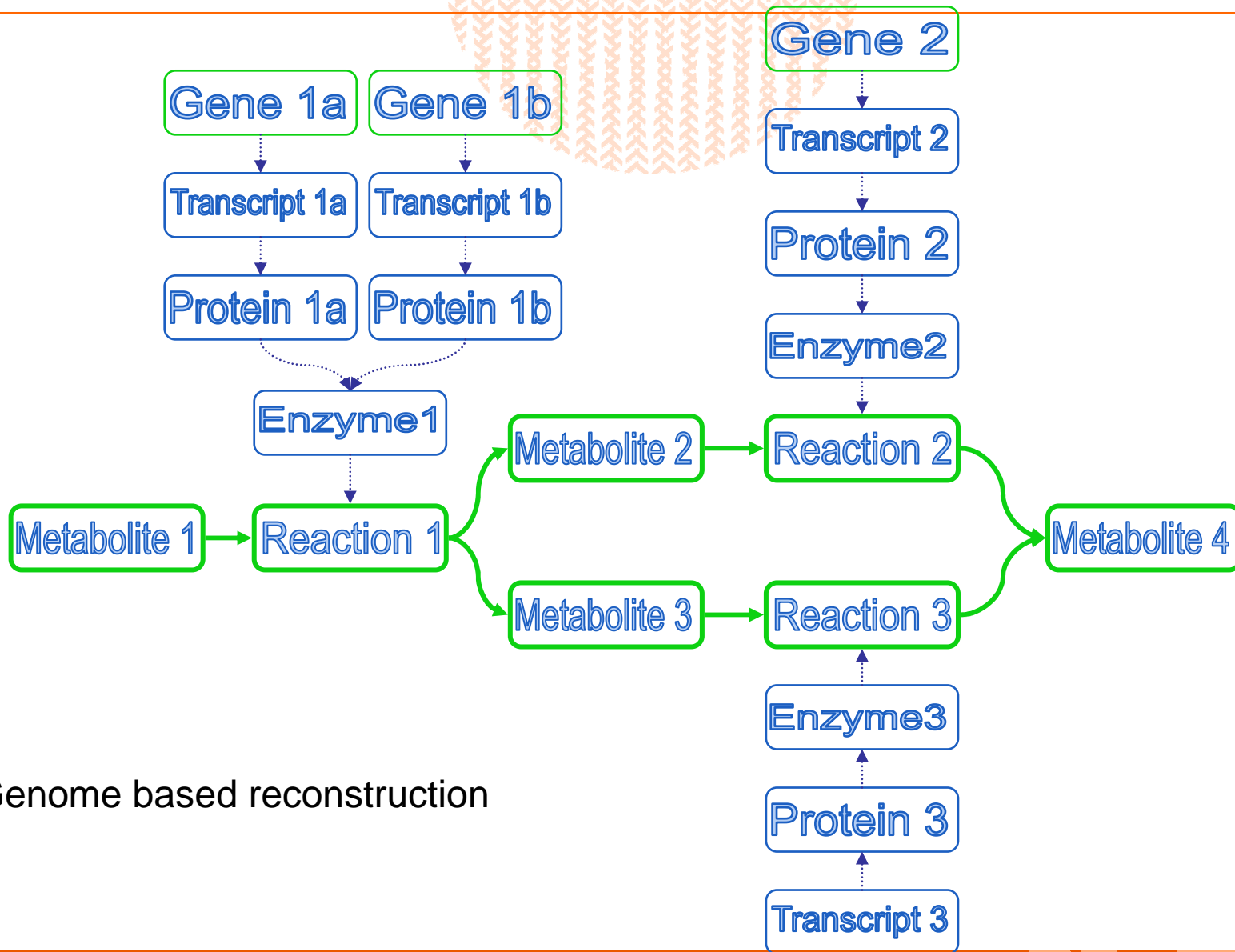
Free Univ. Amsterdam & CWI: L Stougie



And thanks for your attention !

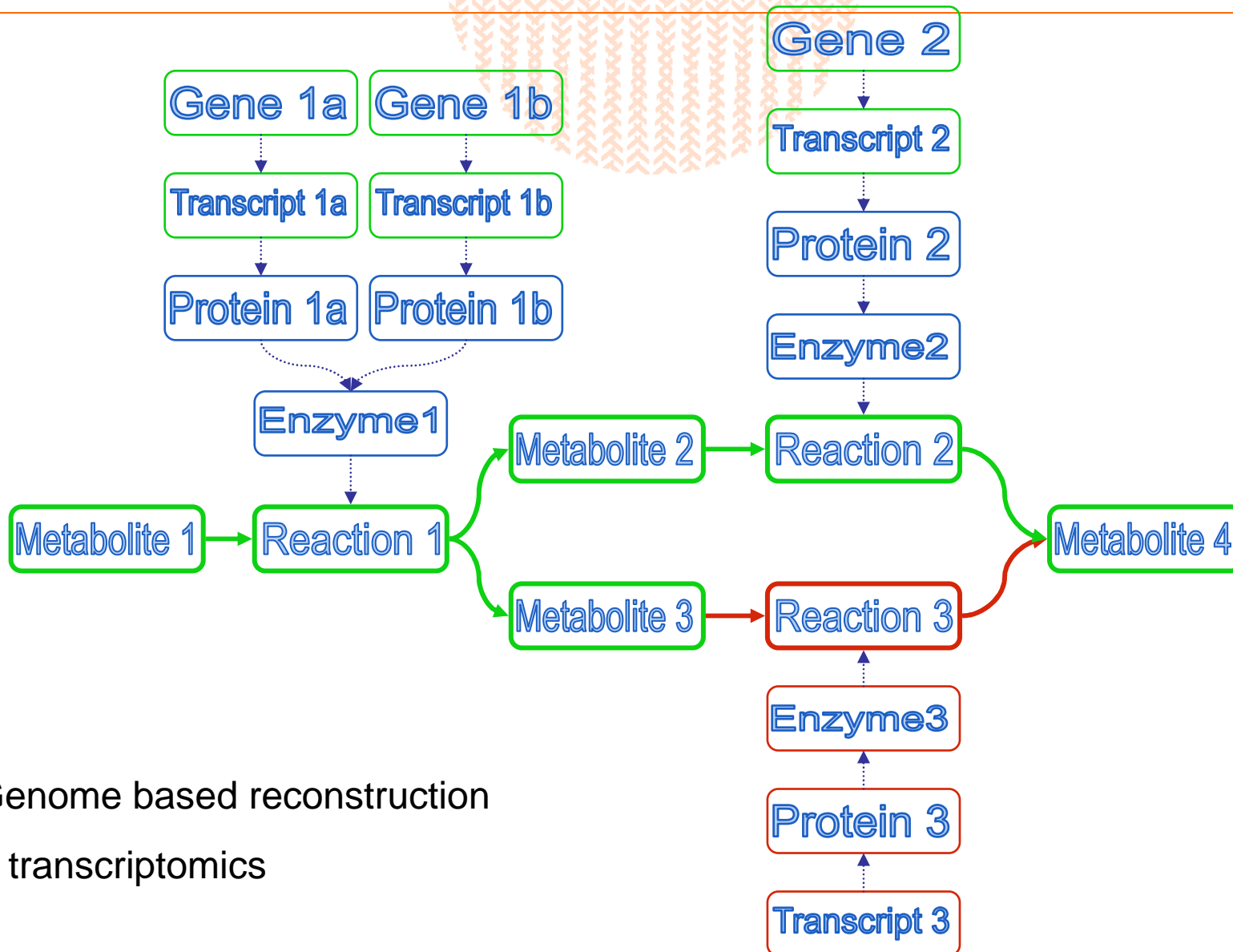
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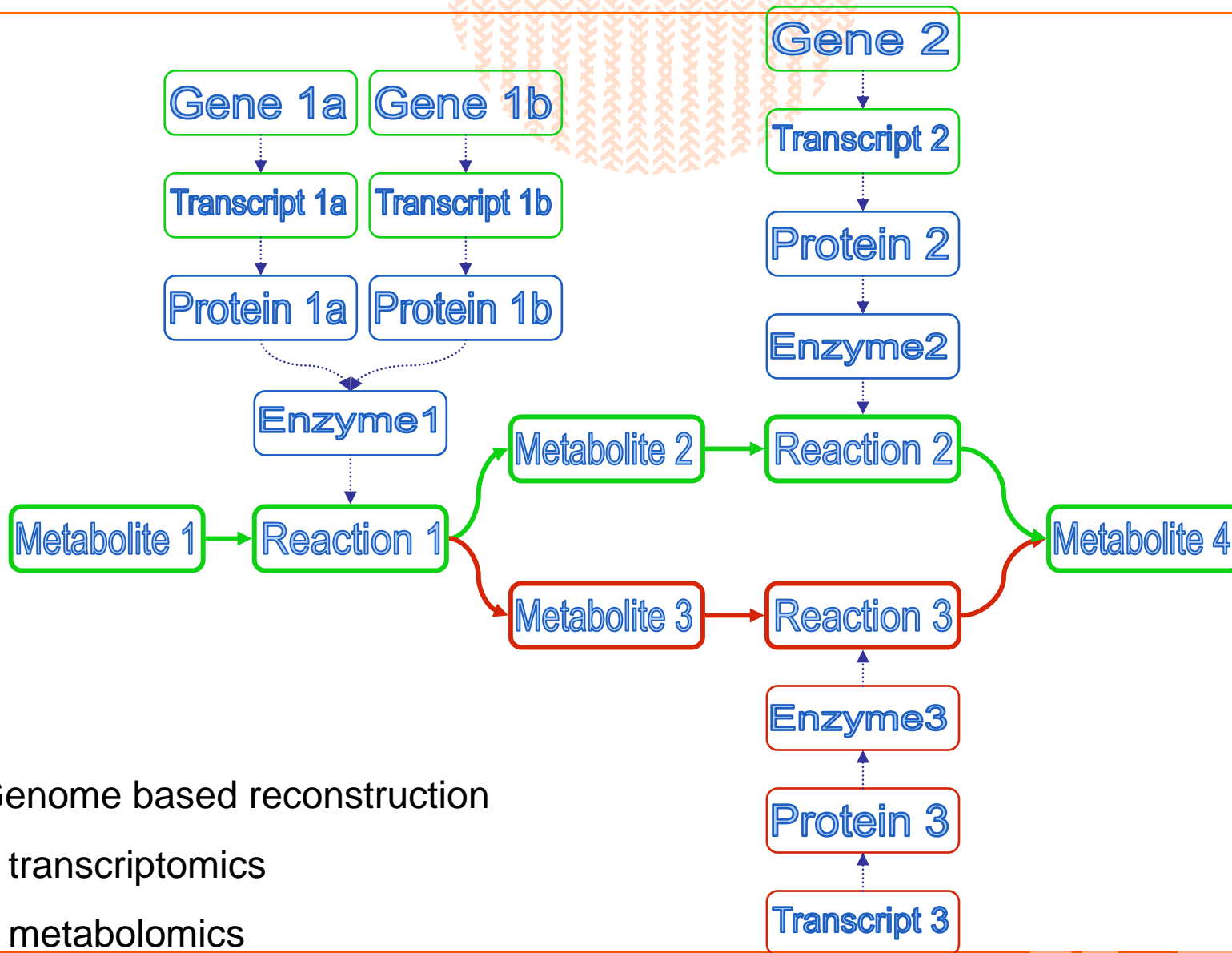


Genome based reconstruction



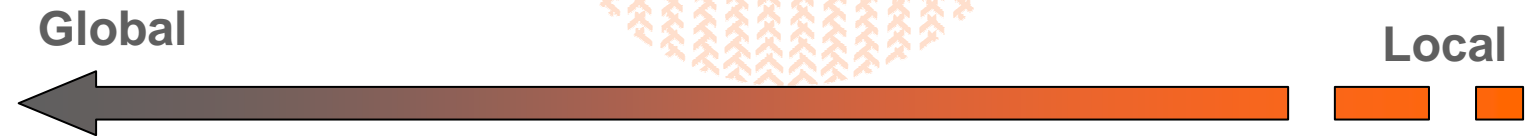


Genome based reconstruction
+ transcriptomics



Genome based reconstruction
 + transcriptomics
 + metabolomics

Where we stand ?



(a) Interaction-based

$$\begin{array}{ccc} & C & \\ A & & B \end{array}$$

Static models
No stoichiometry
No parameters

(b) Constraint-based

$$A + B \rightleftharpoons C$$

Static models
Stoichiometry
No parameters

(c) Mechanism-based

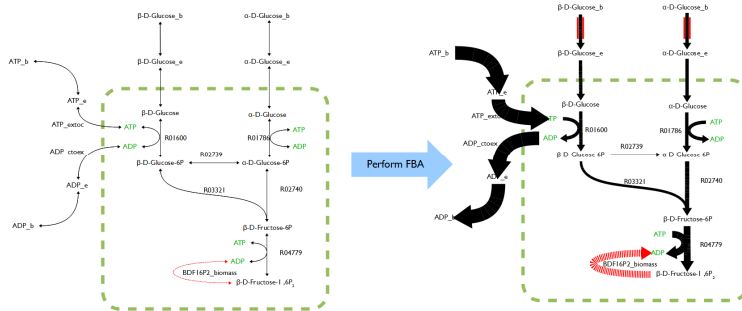
$$A + B \xrightleftharpoons[k_{-1}]{k_1} C$$

Dynamic models
Stoichiometry
Kinetic parameters



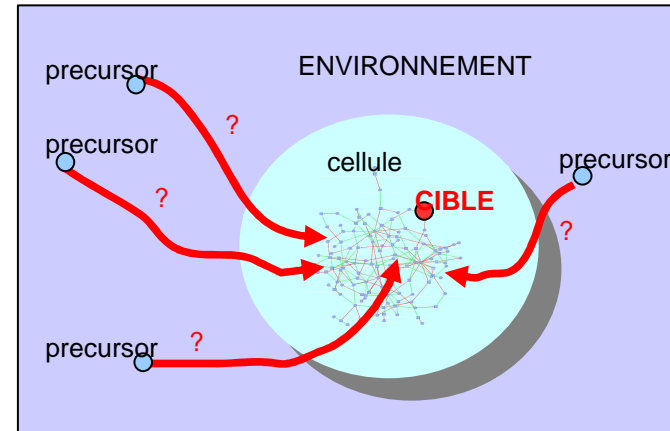
Use of metabolic network models

Synthetic biology



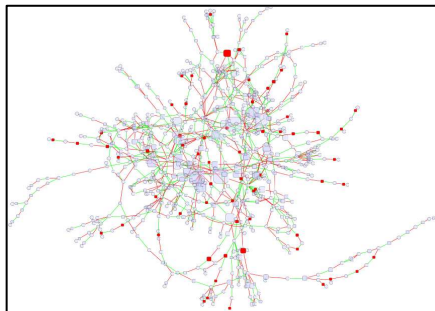
FBA: [2] Fell DA, Small JR: Fat synthesis in adipose tissue. An examination of stoichiometric constraints. *Biochem J* 1986, **238**:781-786
FBA: [3] Orth JD, Thiele I, Palsson B: What is flux balance analysis? *Nat Biotechnol* 2010, **28**:245-248.

Symbiosis



Precursors: [4] Cottret et al. *WABI 08 Proceedings*, 2008, 233-244

Drug target discovery

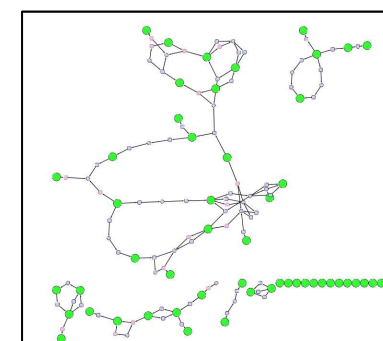
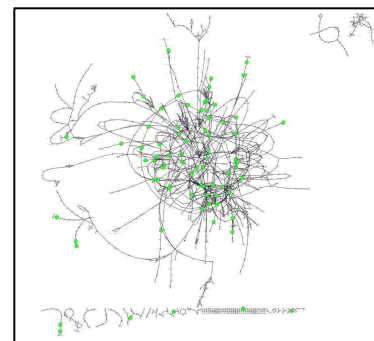


Target enzyme with evidence	EC number	Reference	Enzymes	Chickpea	In human
Juvenile polyoma protein synthase	2.3.1.41	Valler et al. 2003	PF05056	Yes	Yes
Catalase	4.3.3.5	Fraser et al. 1997	PF13_0044	Yes	Yes
Choline transport		Alvarez et al. 1996	PF02026	Yes	No
Chaperone synthase	4.2.3.5	McRobert and McCoskey 2002	MG091_1999	Yes	No
RNA-dependent DNA polymerase	2.7.7.7	Baker Jr et al. 1996	11_00000	Yes	No
Broad (beta)-carboxyl reductase (NADH)	1.1.1.3	Suzuki and Suzuki 2003	MG091_275	Yes	No
Phenylalanyl aminotransferase	2.3.1.21	Chakrabarti et al. 2002	PF13_0482	Yes	Yes
Phenylalanyl phospho transferase	4.1.2.25	Varshavskan et al. 1999	PF14_0425	Yes	Yes
Carnitine diacylglycerol synthase	4.3.2.2	Manojkumar et al. 2002	PF10_0076	Yes	Yes
Histone deacetylase		Datta-Bhatnagar et al. 1996	PF14_0090	Yes	Yes
Hypoxanthine phosphoribosyltransferase	2.4.2.20	Davison et al. 1993	PF10_0221	Yes	Yes
NAD dehydrogenase	1.1.1.205	Vincent et al. 1982	PF10_0202	No	Yes
Lactoylglutathione lyase	4.4.1.5	Thornalley et al. 1994	PF11_0145	Yes	Yes
Lymphophilinase	3.1.1.5	Zakavack et al. 1994	PF02_0005	Yes	No
			PF02_0048	Yes	No
			PF14_0217	Yes	No
			PF14_0218	Yes	No
NADH dehydrogenase (ubiquinone)	1.6.5.3	Kung'u et al. 2002	PF02056	Yes	No
Pyruvate nucleoside phosphorylase	2.6.2.1	Kocik et al. 2002	PF06_0544	Yes	No
Ribonucleoside Reductase	1.1.1.41	Chakrabarti et al. 1993	PF14_0152	Yes	Yes
			PF14_0153	Yes	Yes
RNA Polymerase	2.7.7.6	Lin et al. 2002	24_00000	Yes	Yes
S-adenosyl-L-homocysteine hydrolase	2.3.1.3	Shah et al. 2003	PF02060	No	Yes
S-adenosylmethionine decarboxylase	4.1.1.20	Wright et al. 1991	PF10_0222	Yes	Yes
Sphingomyelinase	3.1.4.2	Hesselt et al. 2002	PF10_0700	Yes	No
Succinate dehydrogenase	1.3.99.1	Sureshram et al. 2000	PF06_0200	No	Yes
			PF10_0224	Yes	Yes
Thiamine nucleoside (NADPH)	1.8.1.8	Kung'u et al. 2002	PF11_0700	No	Yes
Thymidylate synthase	2.1.1.45	Jiang et al. 2000	PF08_0300	Yes	Yes

The reaction and its EC number are given as well as the Protein Identifiers for the corresponding enzymes for enzymatic reactions catalyzed by more than 10 proteins. See <http://www.ebi.ac.uk/Enzyme>. The In Human column denotes whether or not the enzymatic activity for a similar enzyme in human as determined by BLAST alignment with an expectation of less than 0.0001. Of these 24 reactions, 21 (87.5%) were identified as Chokepoints.

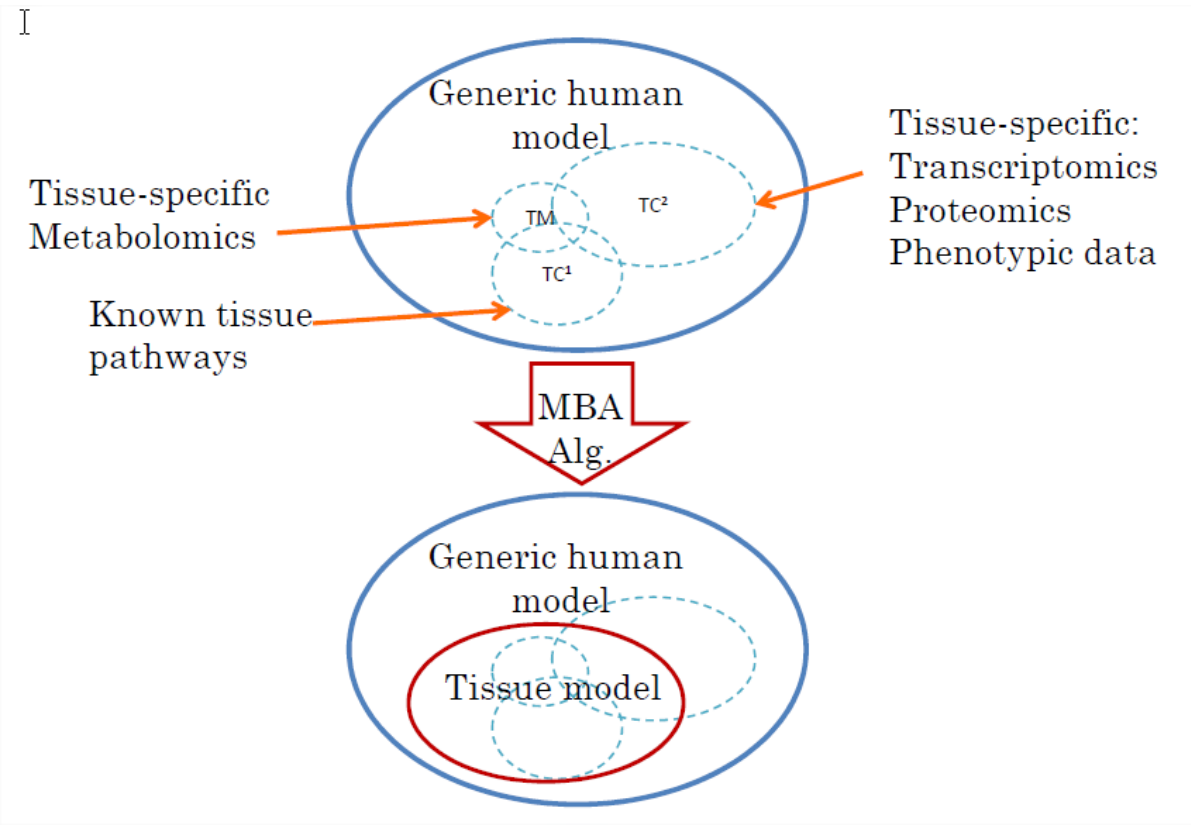
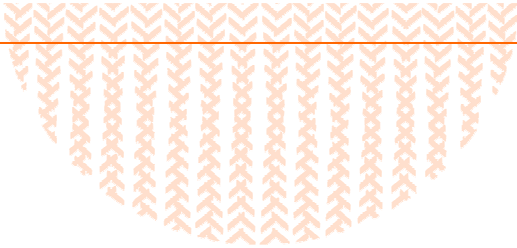
Choke points: [5] Yeh et al. *Genome Res*, 2004, **14**, 917-924

Metabolic effects in toxicology

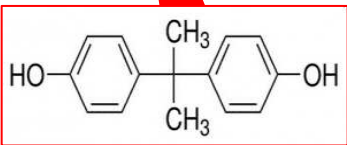
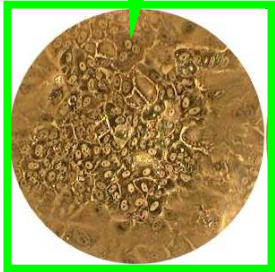
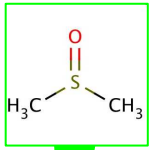


Sub-processes: [6] Antonov et al. *FEBS J*, 2009, **276**, 2084-2094

Sub-processes: [7] Jourdan et al. *Metabolomics*, 2010, **6**(2): 312-321

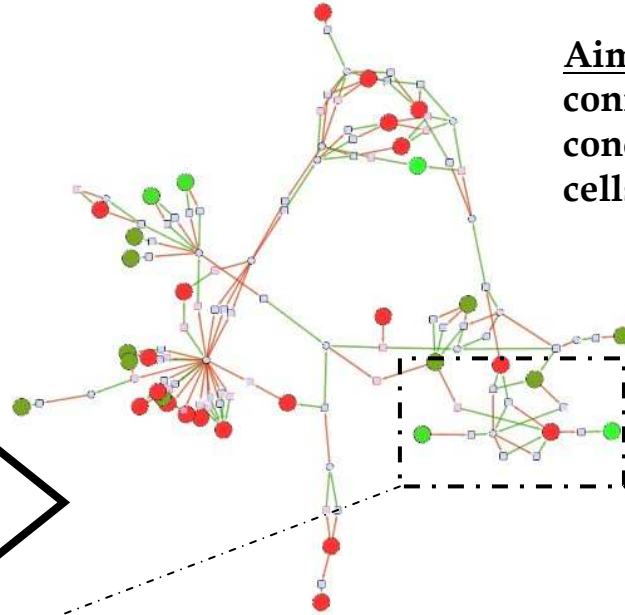


DMSO

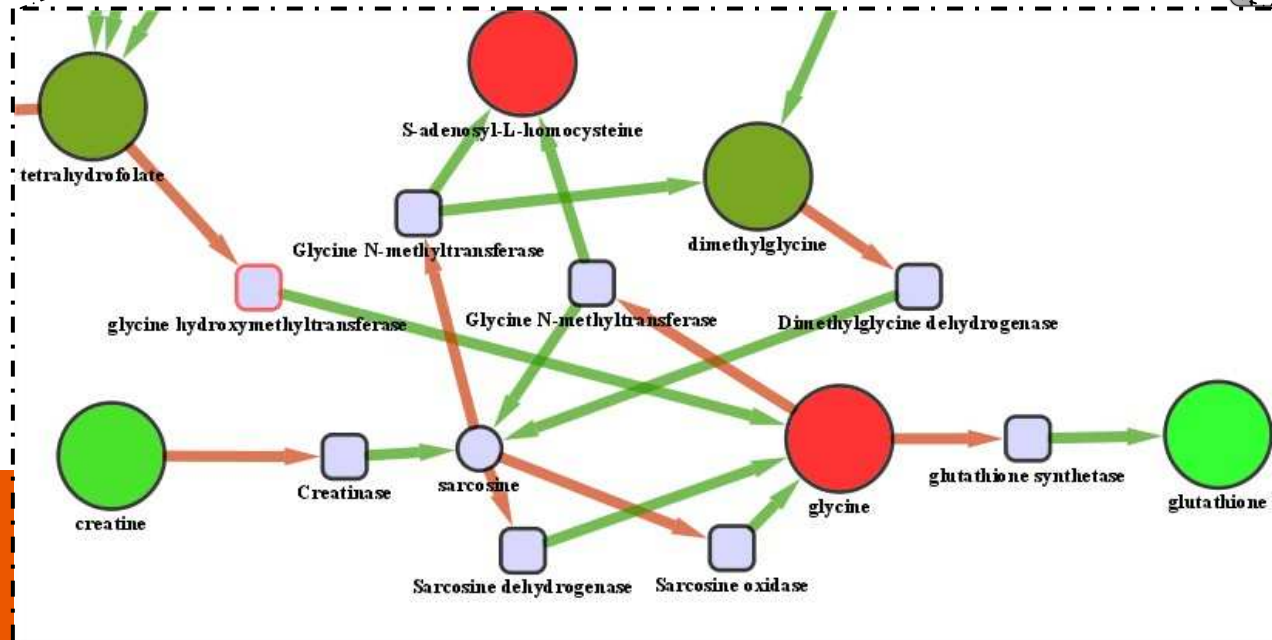


BPA

Network analysis



Aim: understanding which process connect the metabolites which concentrations are affected when cells are exposed to BPA



ENVIRONNEMENT