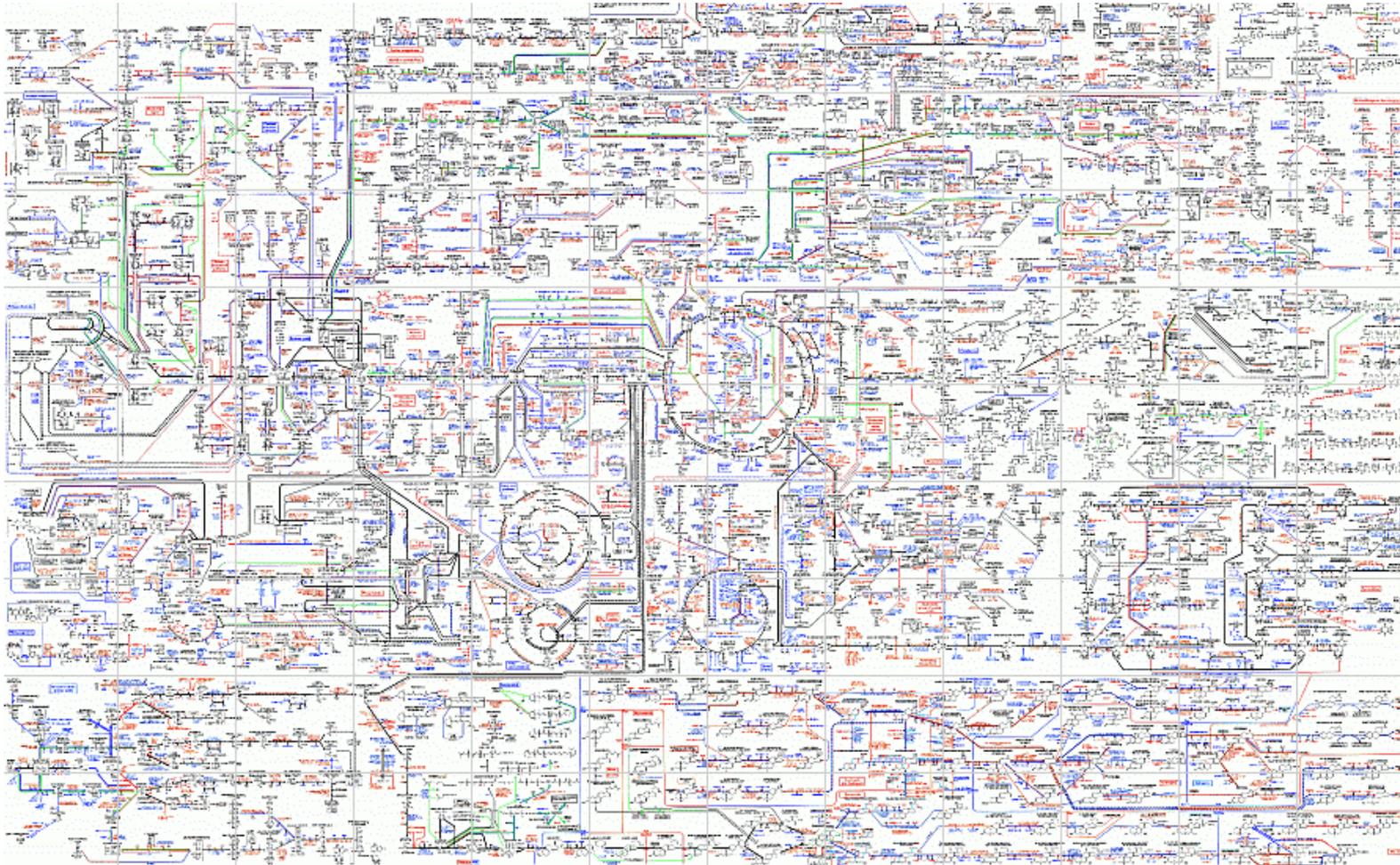


# Metabolomics: Revealing the Biochemical Fingerprints of Life

Carlos Cordeiro



# HOW COMPLEX IS THE METABOLOME?



Biological model

Chemical diversity

Sample preparation

Dynamic range

Databases

Software

# CHEMISTRY vs BIOLOGY

## Petroleomics: Chemistry of the underworld

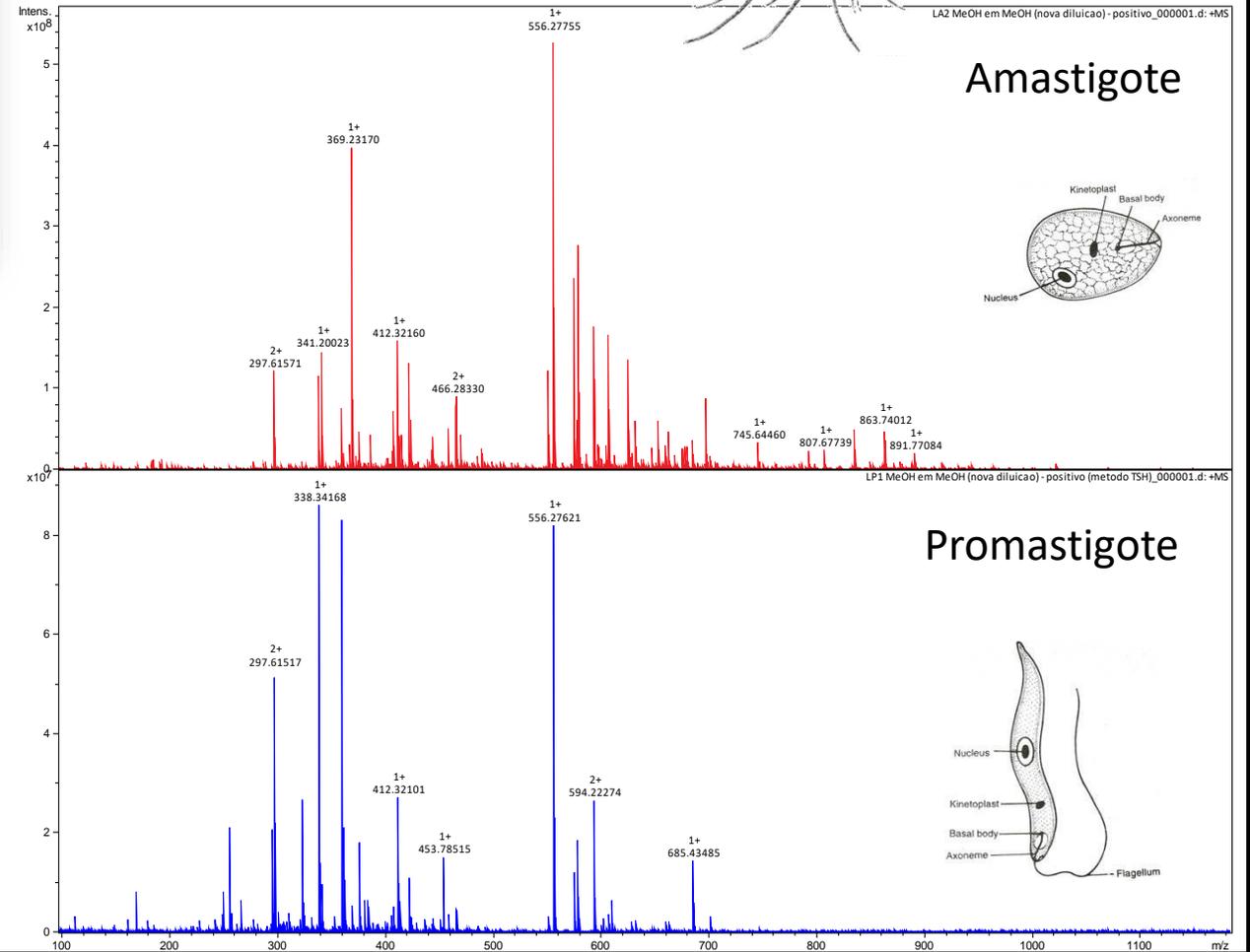
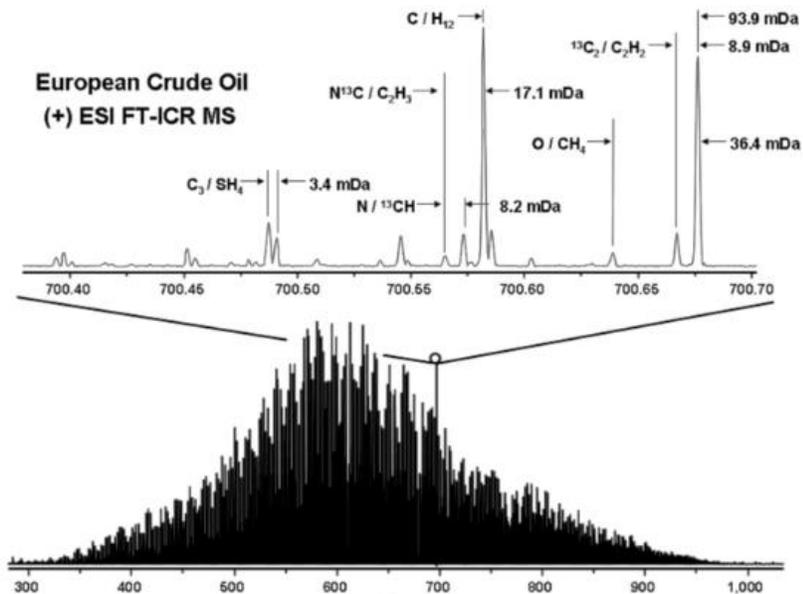
Alan G. Marshall<sup>a,b,1</sup> and Ryan P. Rodgers<sup>a,b,1</sup>

<sup>a</sup>National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, FL 32310-4005; and <sup>b</sup>Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306

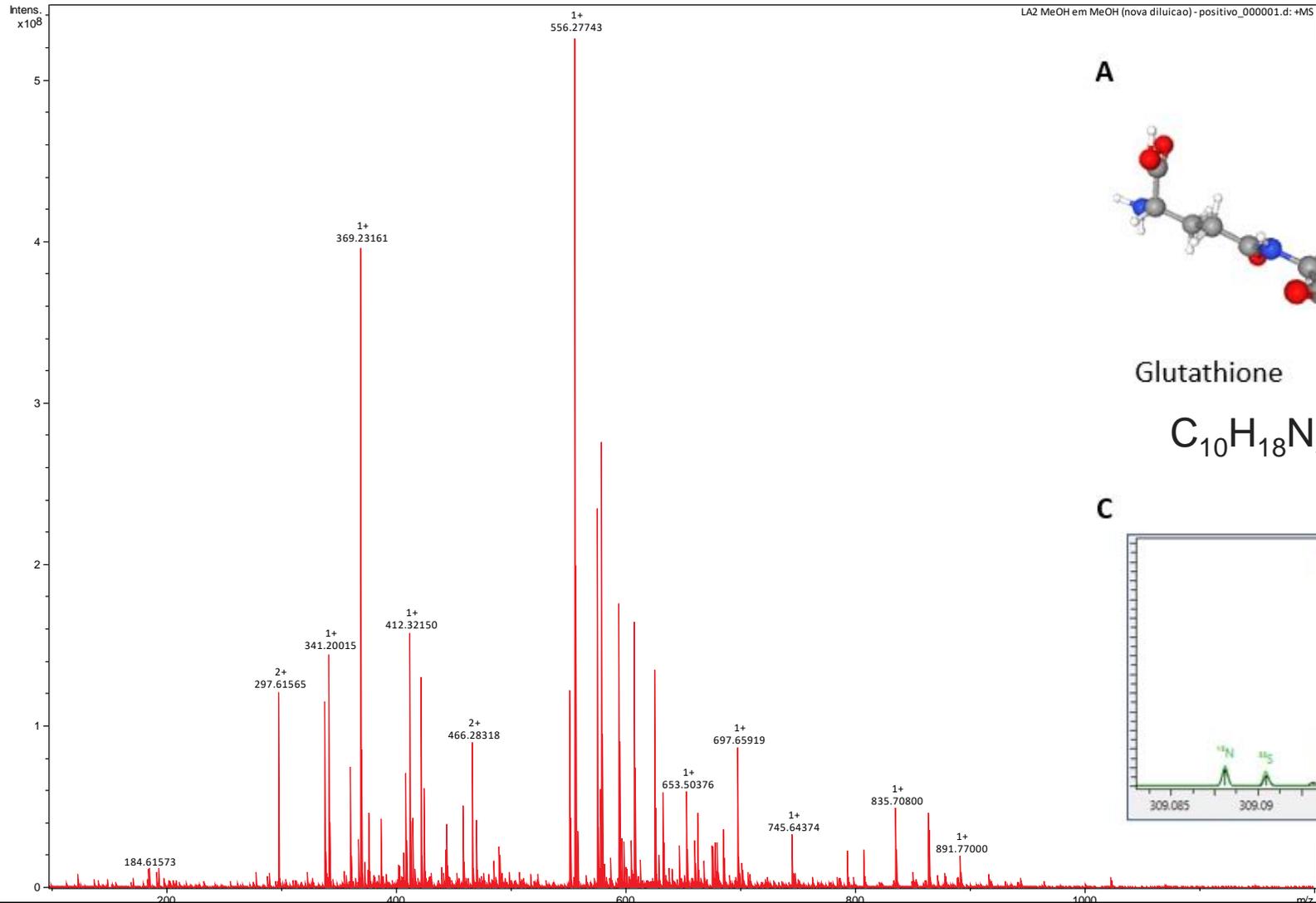
Edited by Fred W. McLafferty, Cornell University, Ithaca, NY, and approved August 14, 2008 (received for review May 24, 2008)

Each different molecular elemental composition—e.g.,  $C_nH_mN_pO_qS_r$ —has a different exact mass. With sufficiently high mass resolving power ( $m/\Delta m_{50\%} \sim 400,000$ , in which  $m$  is molecular mass and  $\Delta m_{50\%}$  is the mass spectral peak width at half-maximum peak height) and mass accuracy ( $< 300$  ppb) up to  $\sim 800$  Da, now routinely available

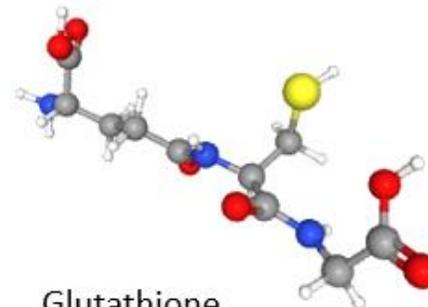
neutrals. Because many heteroatom-containing components ( $N_pO_qS_r$ ) of petroleum are highly polar, ESI is specific and especially efficient in generating their gas-phase ions. Although petroleum crude oils typically contain 90% hydrocarbons ( $C_nH_m$ ), the  $N_pO_qS_r$ -containing molecules are typically the most problematic with respect to pollution, fueling of combustion for



# FROM SPECTRUM TO BIOCHEMISTRY



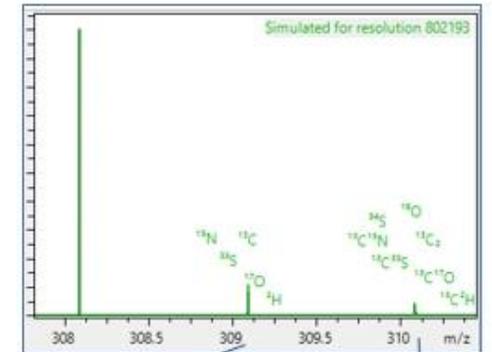
A



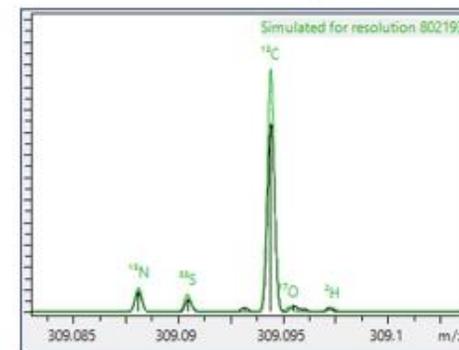
Glutathione



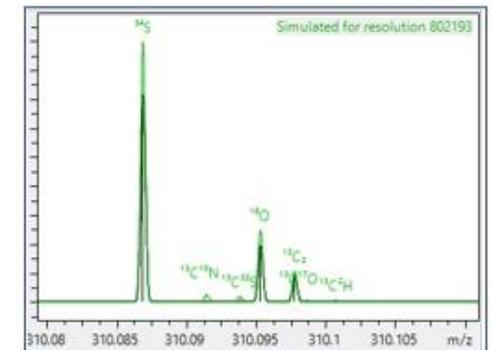
B



C



D







# NETWORK ANALYSIS



reactome.org/PathwayBrowser/

Pathways for: **Homo sapiens**

Search for a term, e.g. pten

Event Hierarchy:

- Autophagy
- Cell Cycle
- Cell-Cell communication
- Cellular responses to stimuli
- Chromatin organization
- Circadian Clock
- Developmental Biology
- Digestion and absorption
- Disease
- DNA Repair
- DNA Replication
- Extracellular matrix organization
- Gene expression (Transcription)
- Hemostasis
- Immune System
- Metabolism
- Metabolism of proteins
- Metabolism of RNA
- Muscle contraction
- Neuronal System
- Organelle biogenesis and maintenance
- Programmed Cell Death
- Protein localization
- Reproduction
- Sensory Perception
- Signal Transduction
- Transport of small molecules
- Vesicle-mediated transport

Network diagram showing interconnected biological processes: Muscle contraction, Immune System, Metabolism of RNA, Chromatin organization, DNA Replication, Cell Cycle, Programmed Cell Death, Digestion and absorption, Circadian Clock, DNA Repair, Transport of small molecules, Reproduction, Cellular responses to stimuli, Developmental Biology, Signal Transduction, Metabolism, Organelle biogenesis and maintenance, Autophagy, Extracellular matrix organization, Neuronal System, Hemostasis, Gene expression (Transcription), Disease, Metabolism of proteins, Vesicle-mediated transport, Cell-Cell communication.

Buttons: Description, Molecules, Structures, Expression, Analysis, Downloads

Displays details when you select an item in the Pathway Browser. For example, when a reaction is selected, shows details including the input and output molecules, summary and references containing supporting evidence. When relevant, shows details of the catalyst, regulators, preceding and following events.

mitochondrial intermembrane space

mitochondrial matrix

Metabolic pathway diagram showing the conversion of L-lysine to succinyl-CoA and then to acetyl-CoA, involving enzymes like AASS tetramer, ALDH7A1 tetramer, and PLP-4278-PHYKPL tetramer. Key molecules include L-Glu, NADPH, NADP+, NAD+, H2O, NADH, H+, Lys, and Succinyl-CoA.

Reaction: Lysine + alpha-ketoglutarate + NADPH + H+ → succinyl-CoA + NADP+ + H2O

Summation: The succinyl-CoA synthetase activity of lysine-ketoglutarate reductase / succinyl-CoA synthetase homodimer in the mitochondrial matrix catalyzes the reaction of lysine, alpha-ketoglutarate, and NADPH + H+ to form succinyl-CoA, NADP+, and H2O (Markovitz et al. 1984; Sacksteder et al. 2001).

External identifiers: Rhea 19875

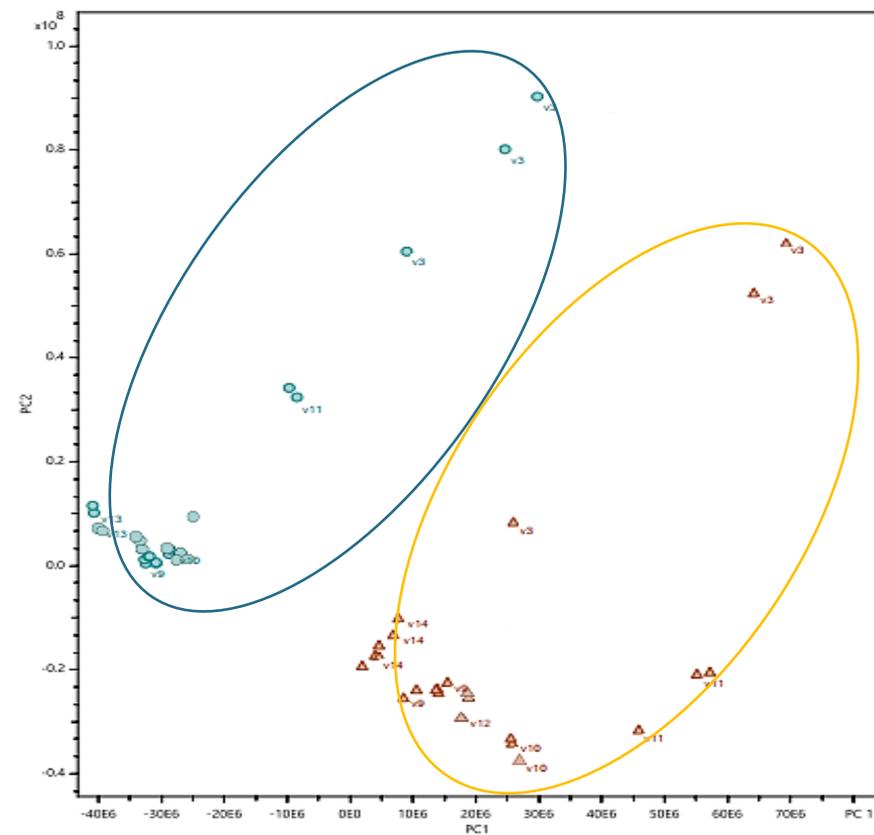
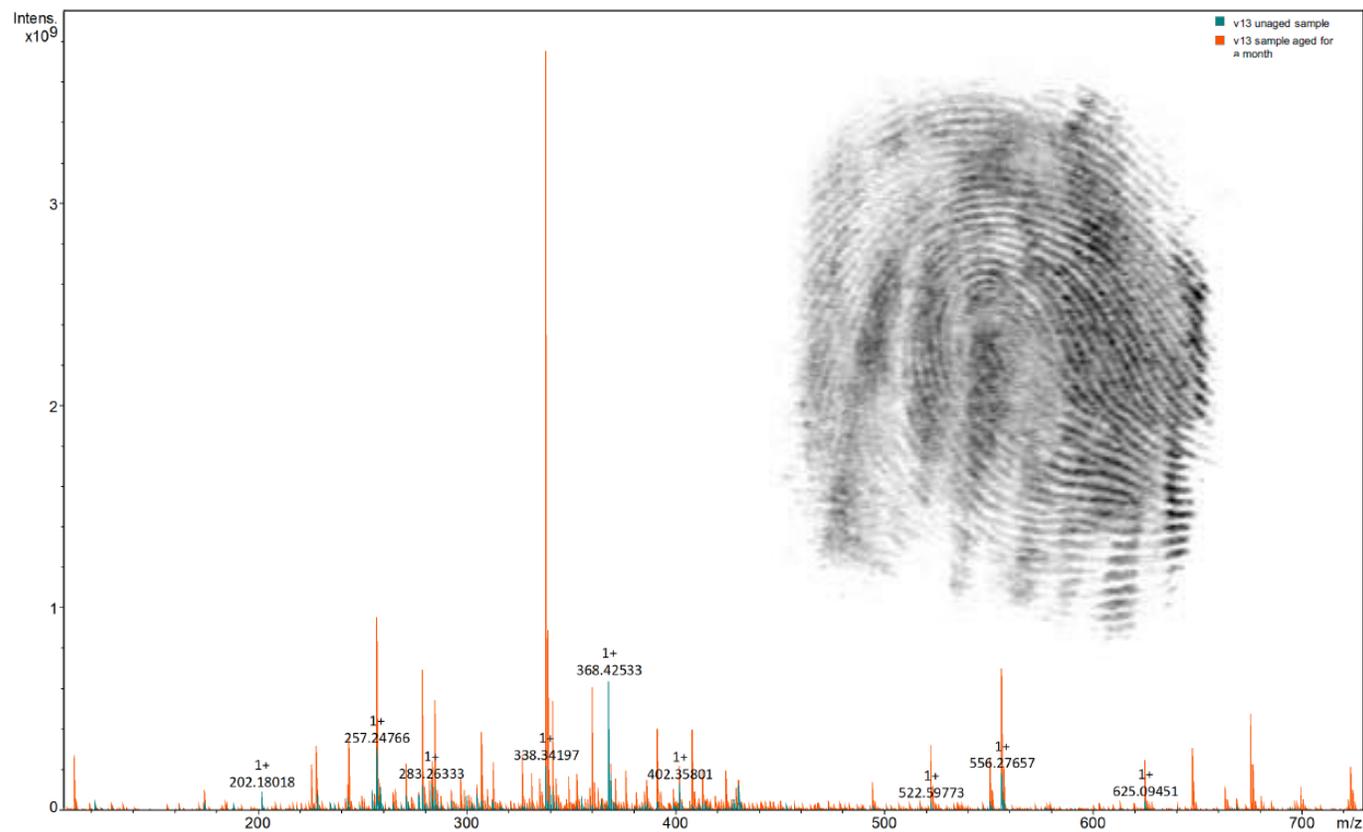
- Metabolism of lipids
- Integration of energy metabolism
- Metabolism of nitric oxide: NOS3 z
- The citric acid (TCA) cycle and res
- Metabolism of nucleotides
- Metabolism of vitamins and cofa
- Metabolism of amino acids and
- Aspartate and asparagine meta
- Glutamate and glutamine meta
- Alanine metabolism
- Branched-chain amino acid cat
- Histidine catabolism
- Lysine catabolism
- Lysine + alpha-ketoglutarate



WE ARE ALL DIFFERENT

EU-FT-ICR-MS  
Advanced Users School  
Lisboa, April 2019

# THE REAL FINGERPRINT





# DISCRIMINATION OF GRAPEVINE GENOTYPES

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# SAMPLE PREPARATION

EuPA Open Proteomics 12 (2016) 4–9



ELSEVIER

Contents lists available at ScienceDirect

EuPA Open Proteomics

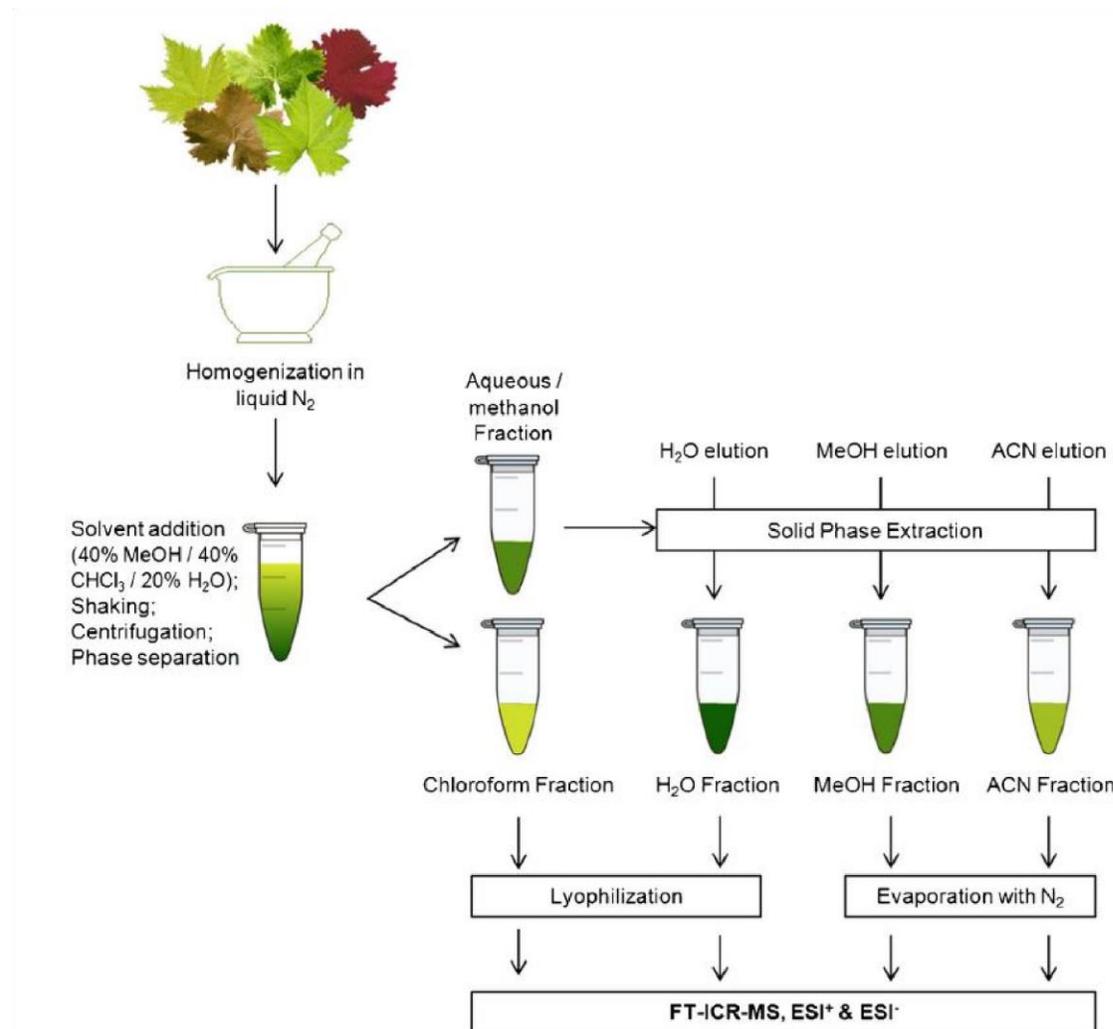
journal homepage: [www.elsevier.com/locate/euprot](http://www.elsevier.com/locate/euprot)

## Metabolite extraction for high-throughput FTICR-MS-based metabolomics of grapevine leaves

Marisa Maia<sup>a,b,c</sup>, Filipa Monteiro<sup>a</sup>, Mónica Sebastiana<sup>a</sup>, Ana Patrícia Marques<sup>b,c</sup>, António E.N. Ferreira<sup>b,c</sup>, Ana Ponces Freire<sup>c</sup>, Carlos Cordeiro<sup>b,c</sup>, Andreia Figueiredo<sup>a,\*\*</sup>, Marta Sousa Silva<sup>b,c,\*</sup>

<sup>a</sup> Biosystems & Integrative Sciences Institute (BioISI), Science Faculty of Lisbon University, 1749-016 Lisbon, Portugal

<sup>b</sup> Laboratório de FTICR e Espectrometria de Massa Estrutural, Faculdade de Ciências da Universidade de Lisboa, Portugal



# DISCRIMINATION OF RESISTANT AND SUSCEPTIBLE GRAPEVINE GENOTYPES



Wild *Vitis* (*V. riparia*)  
Resistant

X



*V. vinifera*  
Susceptible

*The problem*

The **screening process**  
for **disease resistance**  
and **grape quality**  
takes **2-3 years!**

*What producers*  
*want*

New cultivars with  
**good berry quality** and  
a **high degree of**  
**resistance against**  
**downy mildew**

*The goal:*

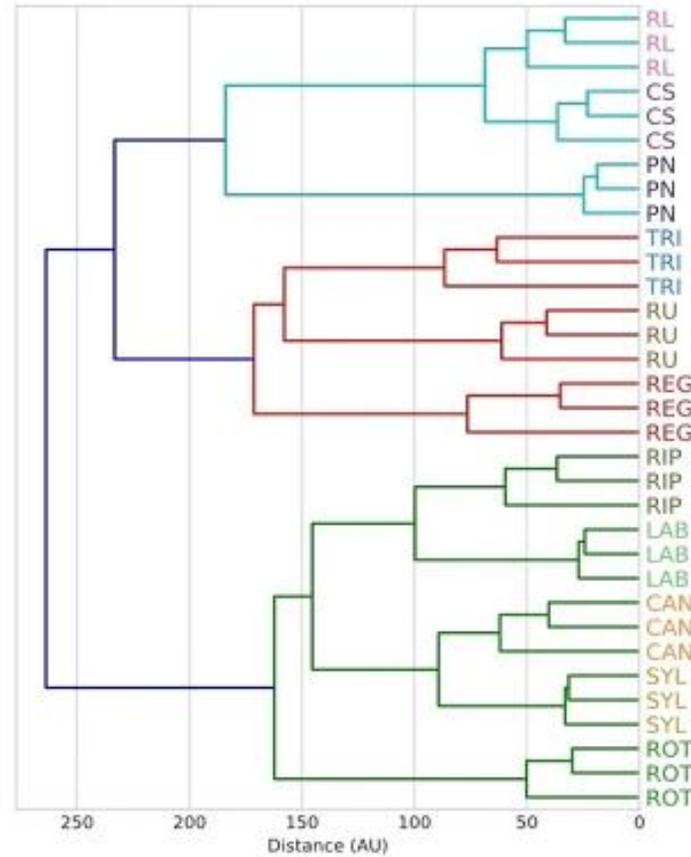
To shorten this time,  
by uncovering **biomarkers**  
for downy mildew resistance

# DISCRIMINATION OF RESISTANT AND SUSCEPTIBLE GRAPEVINE GENOTYPES



Wild *Vitis*, Resistant

Wild *Vitis*  
species



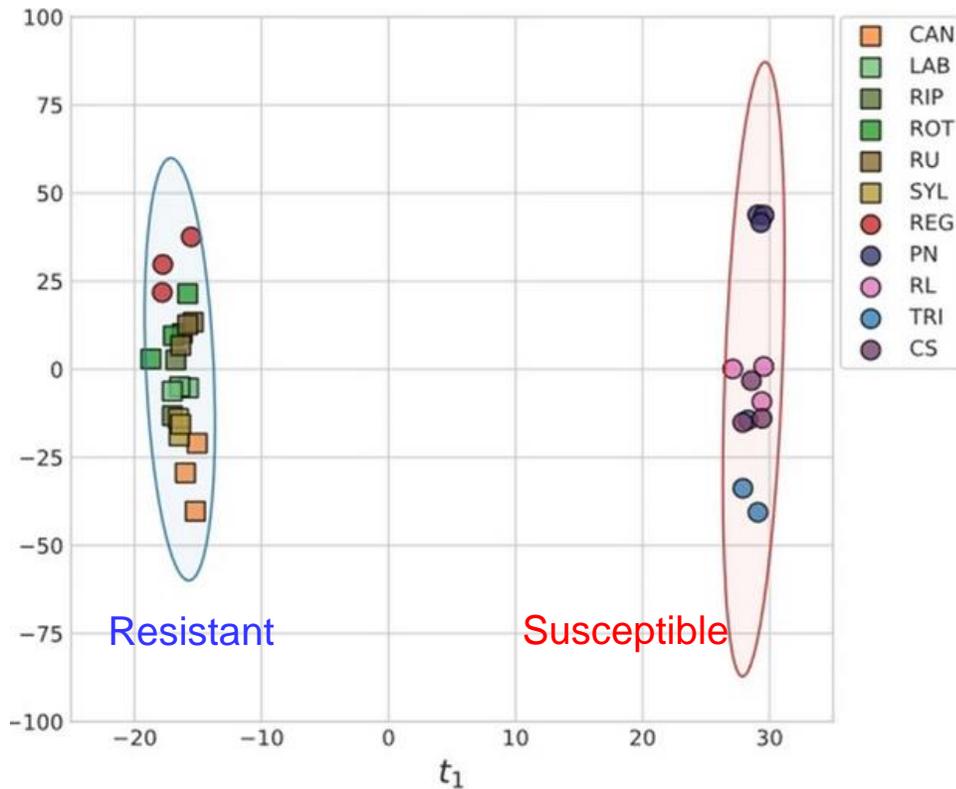
*Vitis vinifera*  
cultivars



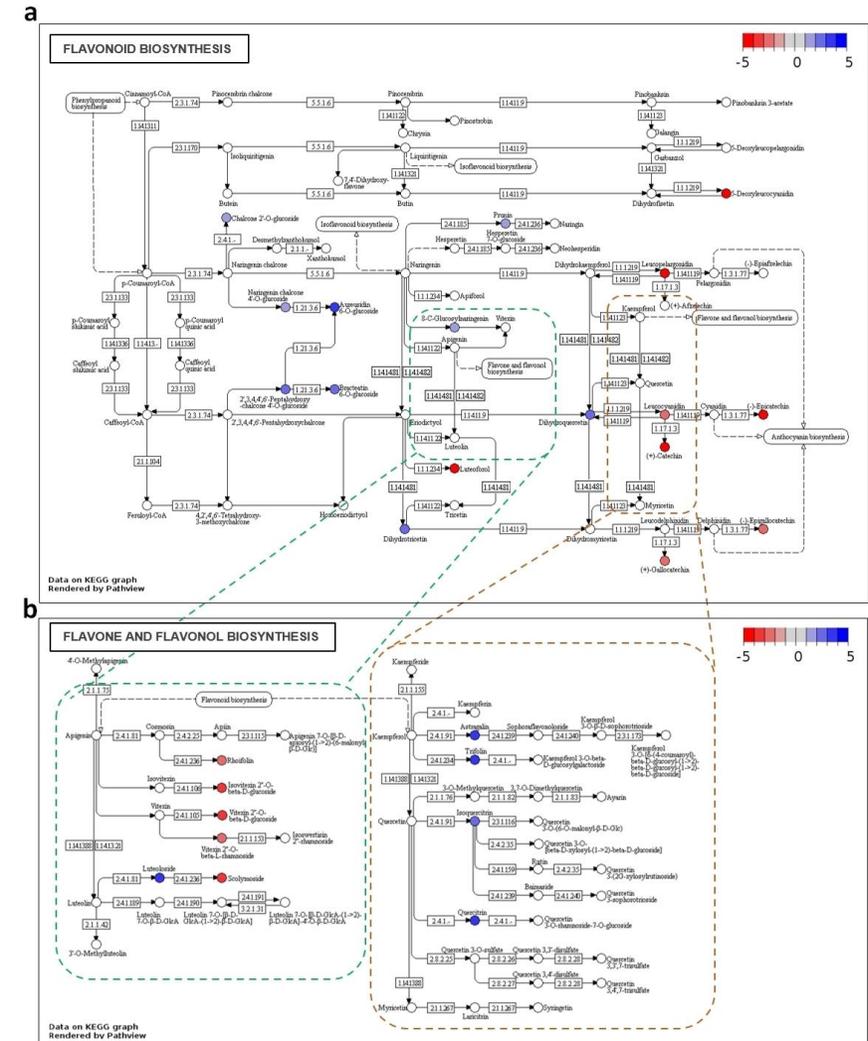
*V. vinifera*, Susceptible

**Separation between *V. vinifera* and wild *Vitis* only based on their metabolic composition**

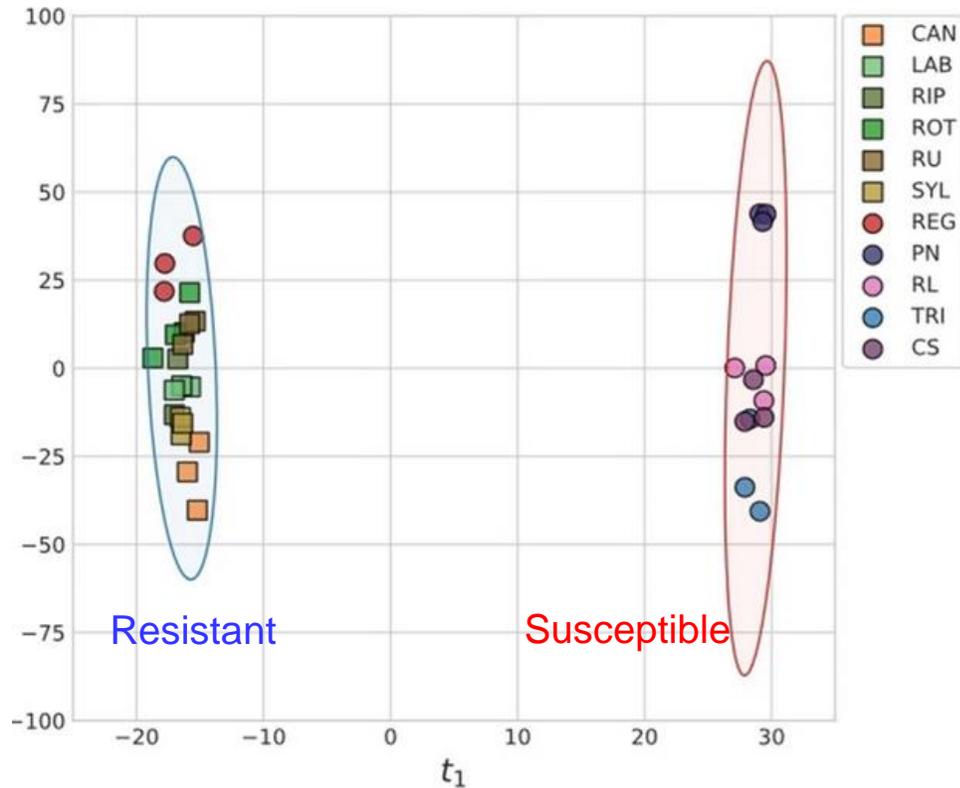
# DISCRIMINATION OF RESISTANT AND SUSCEPTIBLE GRAPEVINE GENOTYPES



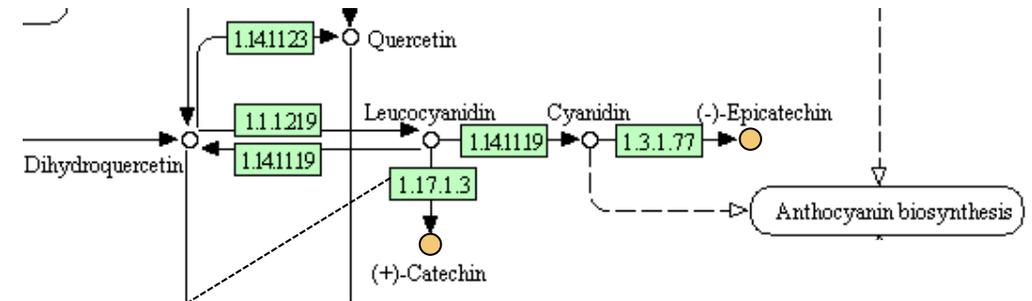
**Discriminating compounds between resistant and susceptible genotypes identified**  
**Activation of different metabolic pathways**



# DISCRIMINATION OF RESISTANT AND SUSCEPTIBLE GRAPEVINE GENOTYPES

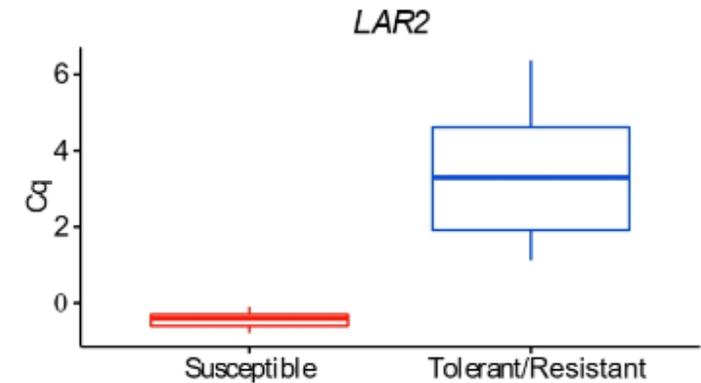


**Catechin emerged as a promising biomarker of susceptibility**



1.17.1.3  
Leucoanthocyanidin reductase 2  
(LAR2)

**LAR2 expression analysis by qPCR**



# DISCRIMINATION OF MALE AND FEMALE WILD GRAPEVINES, *VITIS SYLVESTRIS*



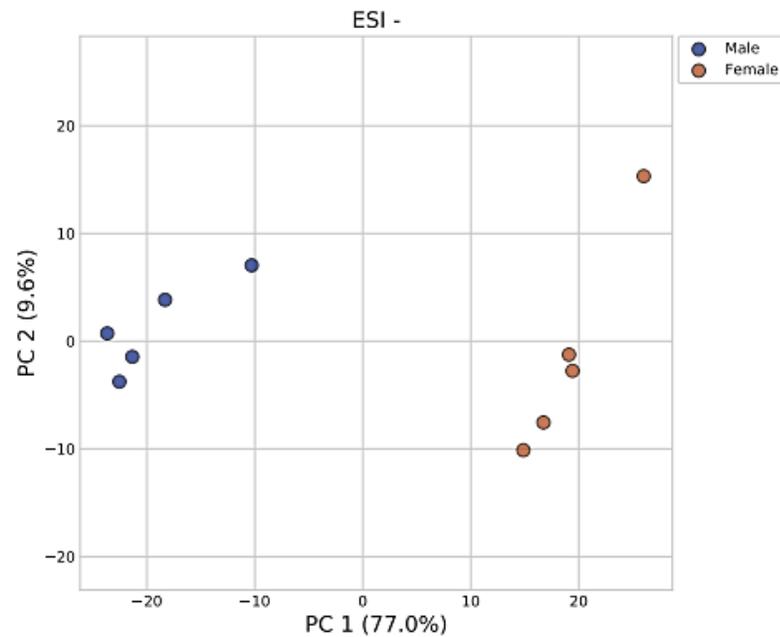
*Vitis vinifera*

Image from:  
Patty Skinkis, Oregon State University

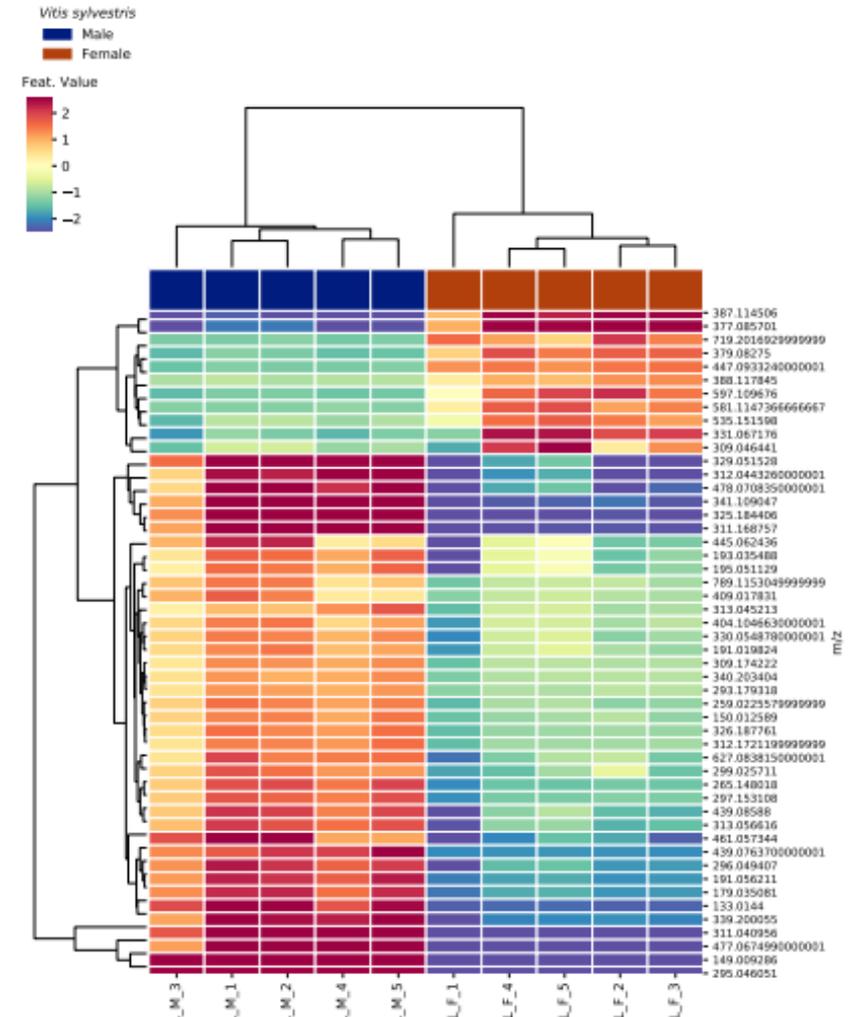
*Vitis sylvestris*

Plant images from  
*Scientific Reports* | (2020) 10:18993  
<https://doi.org/10.1038/s41598-020-76012-6>

# DISCRIMINATION OF MALE AND FEMALE WILD GRAPEVINES, *VITIS SYLVESTRIS*



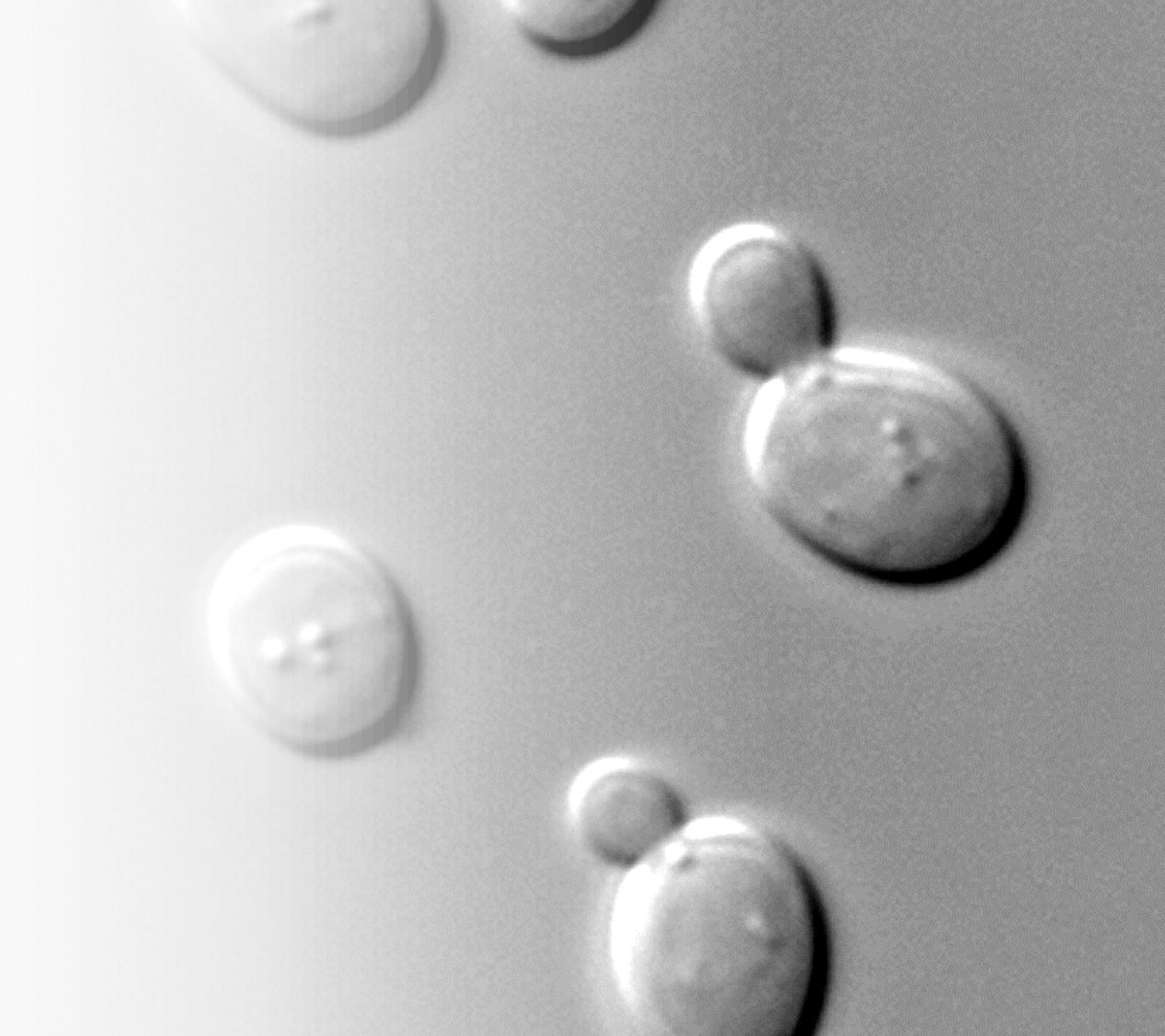
**Discriminating  
compounds  
between male and  
female genotypes  
identified, before  
the appearance of  
flowers**





# DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS

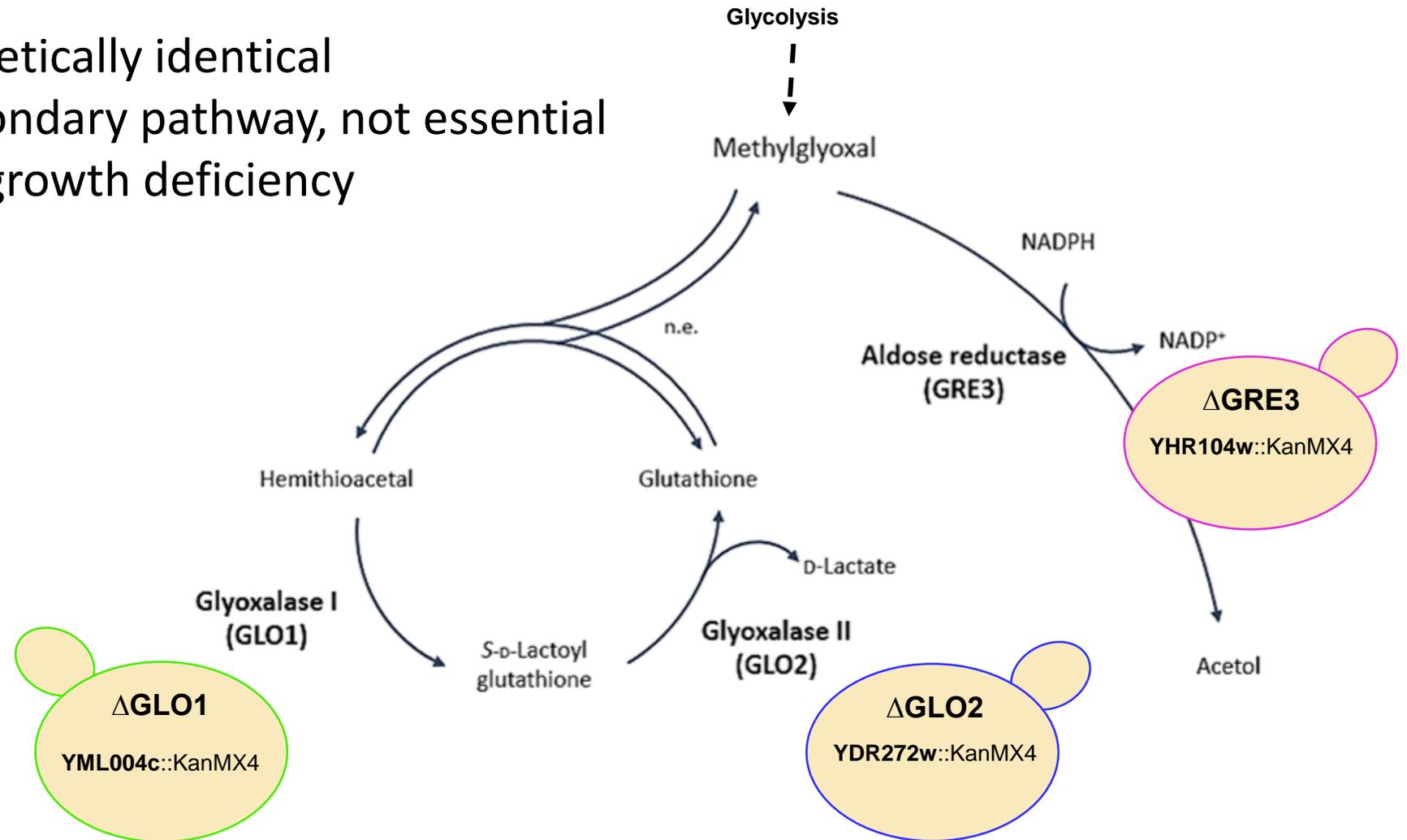
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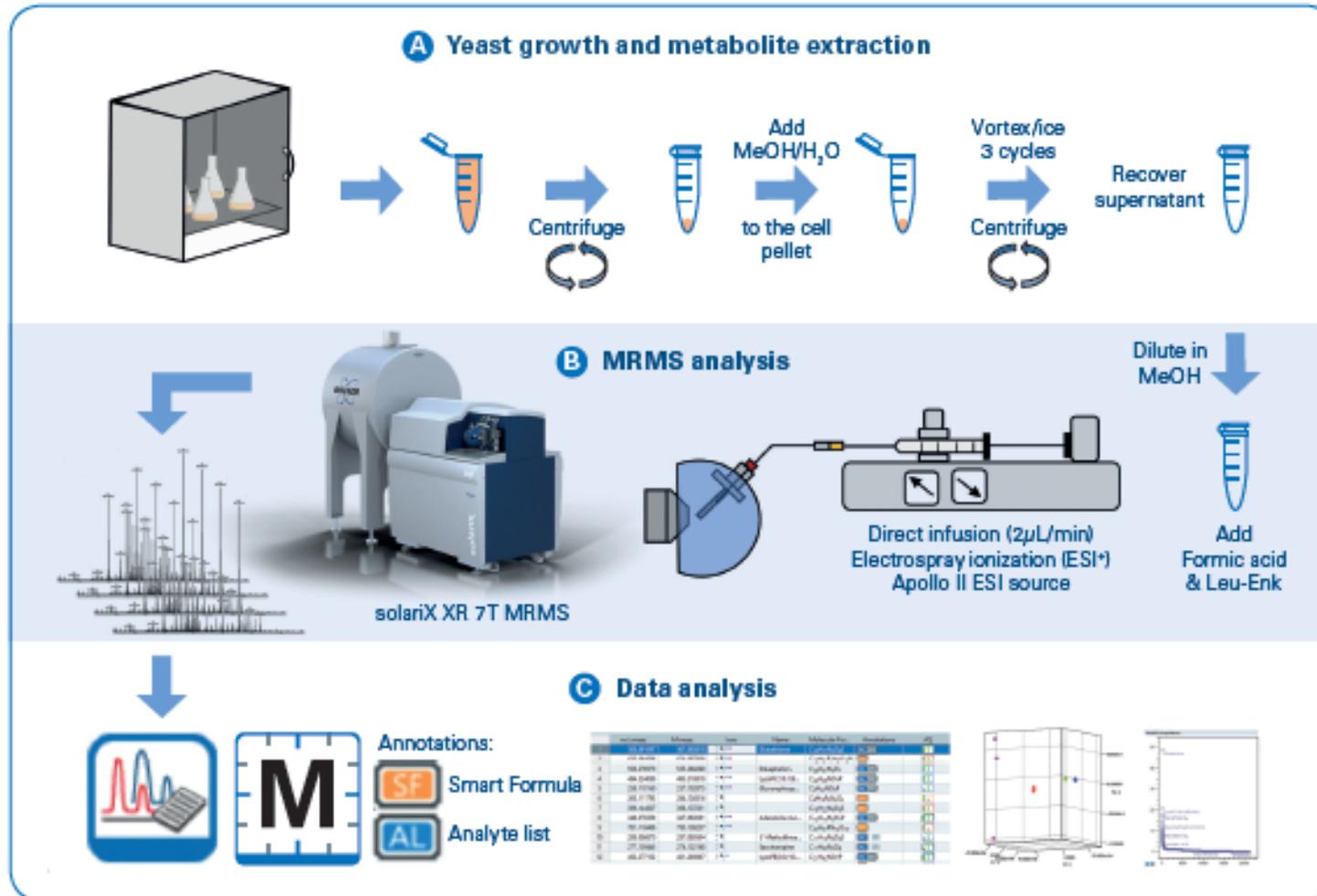
# DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS

**BY4741**  
MATa; his3Δ1;  
leu2Δ0; met15Δ0;  
ura3Δ0

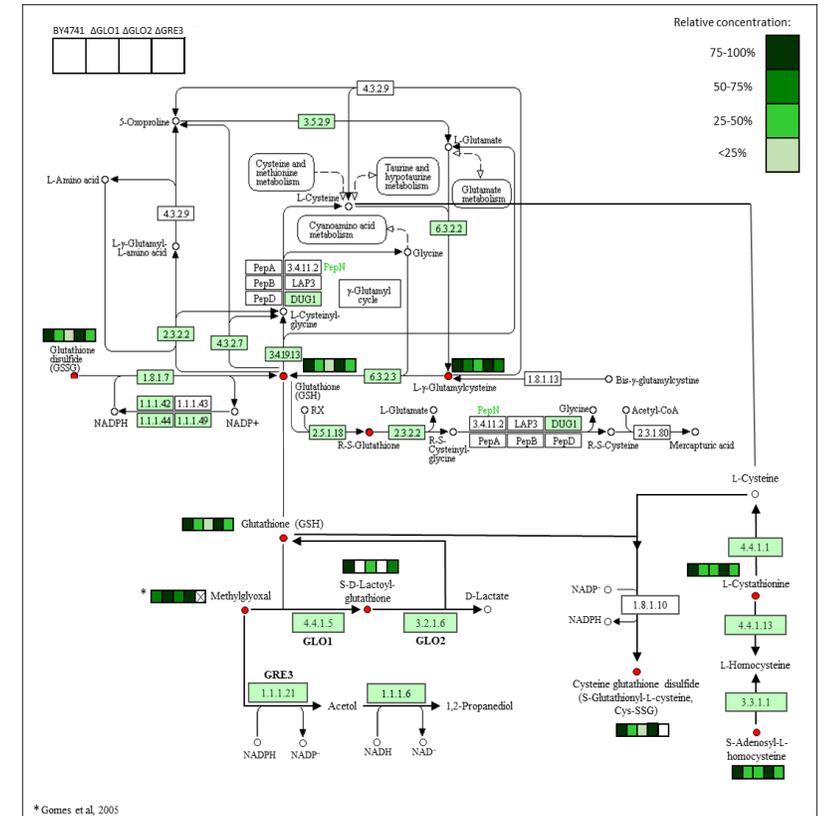
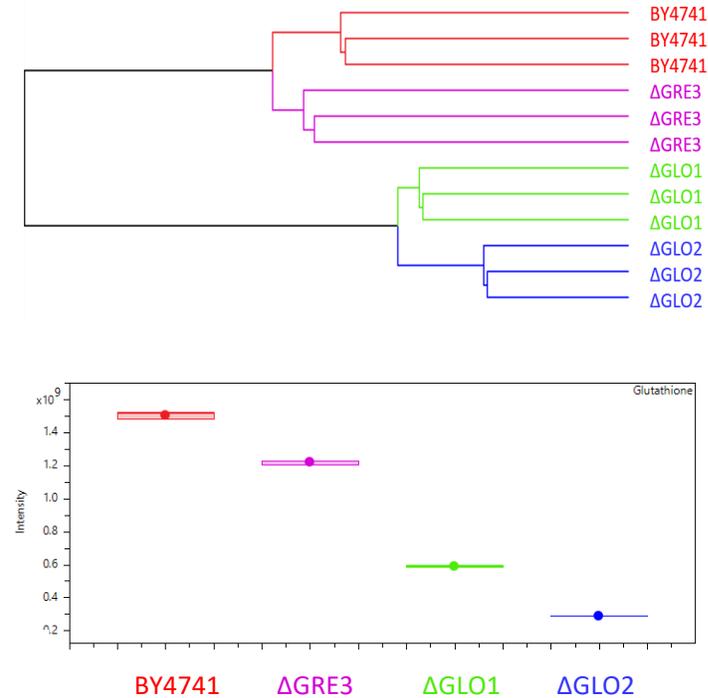
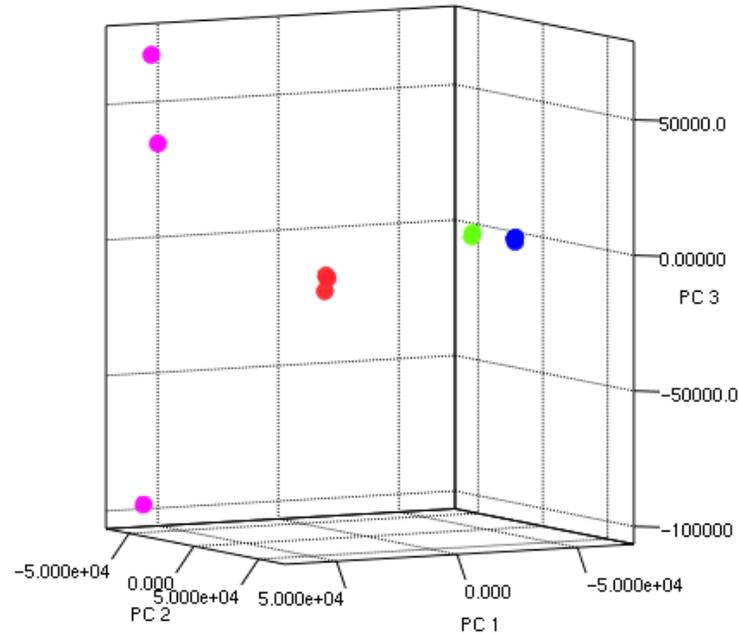
Genetically identical  
Secondary pathway, not essential  
No growth deficiency



# DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS



# DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS



**Discrimination only based on their metabolic composition**  
**Glutathione was the most discriminating compound**  
**Wider functional and cellular effects elucidated**

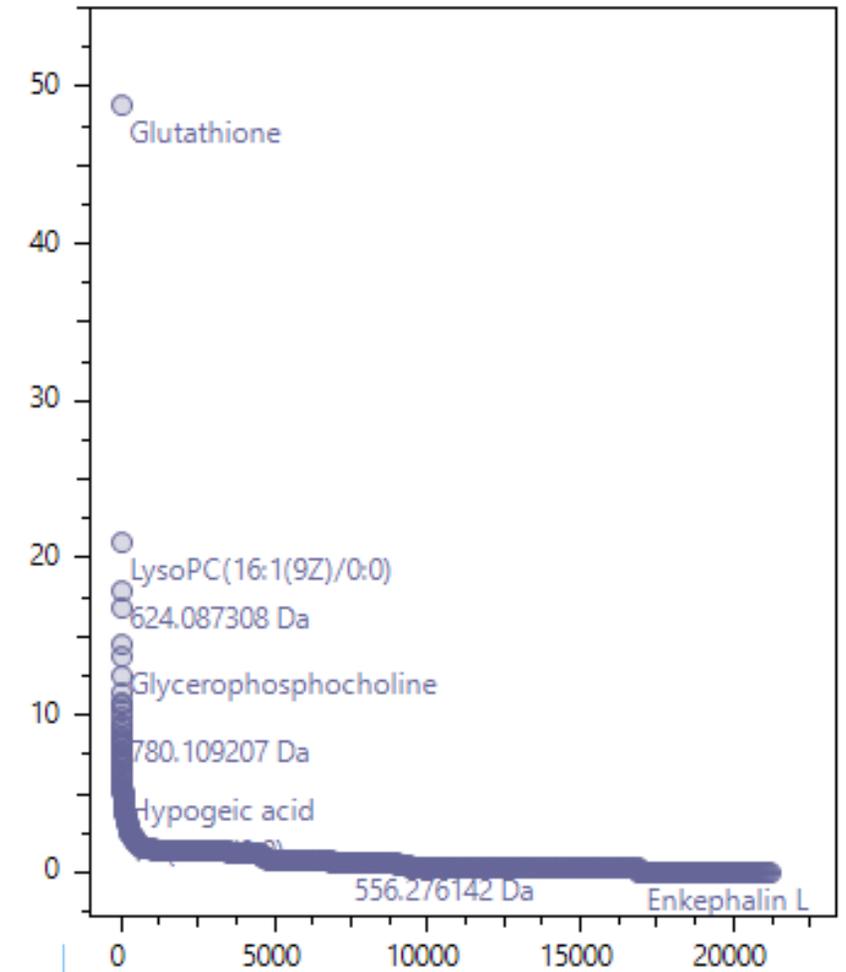
Glutathione metabolism

\* Gomes et al, 2005

# BEYOND GLUTATHIONE

Position	Mass (Da)	Metabolite Name	Molecular Formula	VIP Score
1	307.0838	Glutathione	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S	8.417995046
2	493.3168	PC(16:1(9Z)/0:0)	C <sub>24</sub> H <sub>48</sub> NO <sub>7</sub> P	5.993469554
3	624.0873	N/A	C <sub>14</sub> H <sub>28</sub> N <sub>10</sub> O <sub>10</sub> S <sub>4</sub>	5.587837143
4	257.1029	Glycerophosphocholine	C <sub>8</sub> H <sub>20</sub> NO <sub>6</sub> P	4.837523121
5	324.1057	N/A	C <sub>12</sub> H <sub>20</sub> O <sub>10</sub>	4.177424151
6	337.3345	N/A	C <sub>22</sub> H <sub>43</sub> NO	4.153906335
7	254.2246	Hypogeic acid	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	4.077281087
8	385.3192	Pentadecanoylcarnitine	C <sub>22</sub> H <sub>43</sub> NO <sub>4</sub>	3.77589036
9	398.1372	N/A	C <sub>15</sub> H <sub>22</sub> N <sub>6</sub> O <sub>5</sub> S	3.484675636
10	451.2699	PE(16:1(9Z)/0:0)	C <sub>21</sub> H <sub>42</sub> NO <sub>7</sub> P	3.172850261

Variable Importance



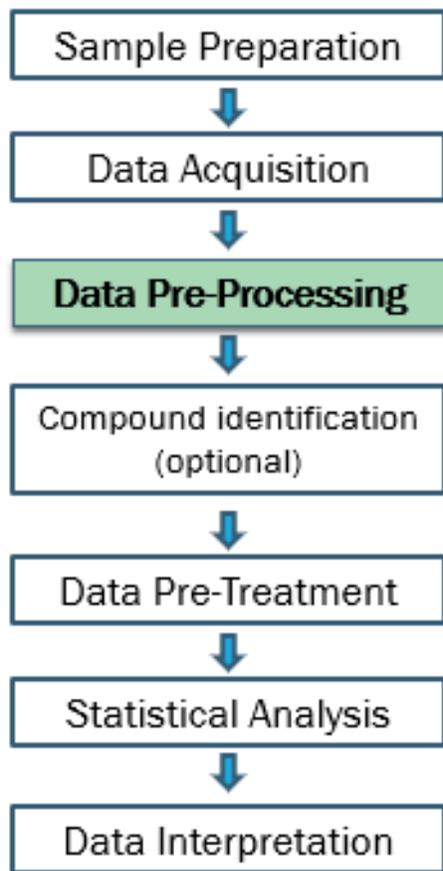


# DEVELOPMENT OF NEW METHODS FOR METABOLOMICS DATA ANALYSIS

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# DEVELOPMENT OF NEW METHODS FOR METABOLOMICS DATA ANALYSIS

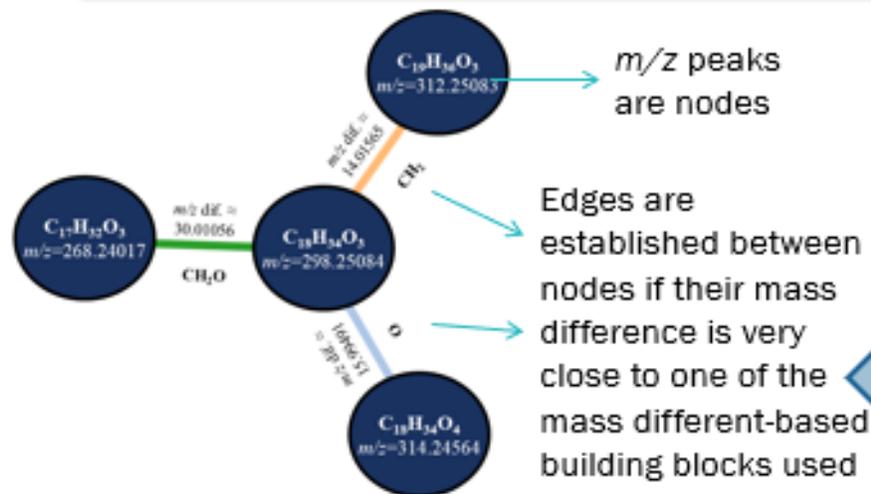


## Binary similarity (BinSim)

Samp./Feat.	A-1	A-2	A-3	B-1	B-2	B-3
x	163	124	189	200	176	223
y	115	108	101	-	98	-
z	-	-	-	165	126	-
w	-	98	103	879	-	-
v	-	-	-	176	215	245

Samp./Feat.	A-1	A-2	A-3	B-1	B-2	B-3
x	1	1	1	1	1	1
y	1	1	1	0	1	0
z	0	0	0	1	1	0
w	0	1	1	1	0	0
v	0	0	0	1	1	1

## Mass Difference Networks (MDiN) construction



Transformations	$\Delta$ Mass (Da)	Reaction Types
O (-NH)	0.984016	Deamination
NH <sub>2</sub> (-O)	1.031634	Transamination
H <sub>2</sub>	2.015650	Hydrogenations / Dehydrogenations
CH <sub>2</sub>	14.015650	Methylations
O	15.994915	Oxygenations / Hydroxylations
H <sub>2</sub> O	18.010565	Condensation / Dehydration / Cyclization
NCH	27.010899	Transfer of a formidoyl group
CO	27.994915	Formylation
CHOH	29.002740	Hydroxymethylation
S	31.972071	Transfer of a -SH group
C <sub>2</sub> H <sub>2</sub> O	42.010565	Acetylation
CONH	43.005814	Transfer of a carbamoyl group
CO <sub>2</sub>	43.989829	Carboxylation / Decarboxylation
SO <sub>2</sub>	79.956815	Sulphation
PO <sub>3</sub> H	79.966331	Phosphorylation

# JOIN US IN LISBON FOR THE 6<sup>TH</sup> SHORT COURSE ON FT-ICR-MS



## EU FT-ICR MS 6<sup>th</sup> Short Course

*Mapping post-translational  
modifications through FT-ICR*

### Where and when

LISBOA, 17 -20 October 2021

Faculdade de Ciências  
Universidade de Lisboa, Portugal

[ciencias.ulisboa.pt](http://ciencias.ulisboa.pt)



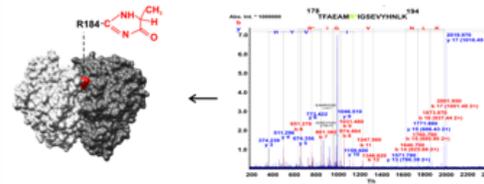
**Contacts:** Carlos Cordeiro  
[cacordeiro@fc.ul.pt](mailto:cacordeiro@fc.ul.pt)

Marta Sousa Silva  
[mfsilva@fc.ul.pt](mailto:mfsilva@fc.ul.pt)



## Course overview

This course will cover the analysis and mapping of protein post-translational modifications using FT-ICR mass spectrometry



Specific topics include:

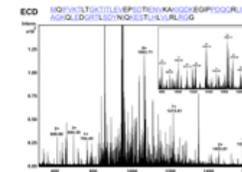
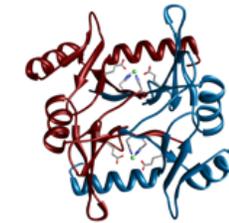
**Sample preparation  
Ionization  
Top-down MS  
Fragmentation methods  
Sequence analysis**

## NO PARTICIPATION FEE!

Travel, accommodation, meals  
and social program included

Up to 20 applicants  
will be selected by the  
Scientific Committee

Tutorial lectures  
Instrument demos  
Hands-on exercises  
Computational data analysis



## Faculty

Carlos  
CORDEIRO

Peter  
O'CONNOR

Petr  
NOVAK

Roman  
ZUBAREV

**Application:** [www.eu-fticr-ms.eu](http://www.eu-fticr-ms.eu)

**DEADLINE FOR APPLICATIONS:**

**June 31<sup>st</sup> 2021**



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 731077

# THE TEAM

