Metabolomics: Creating life out of small molecules

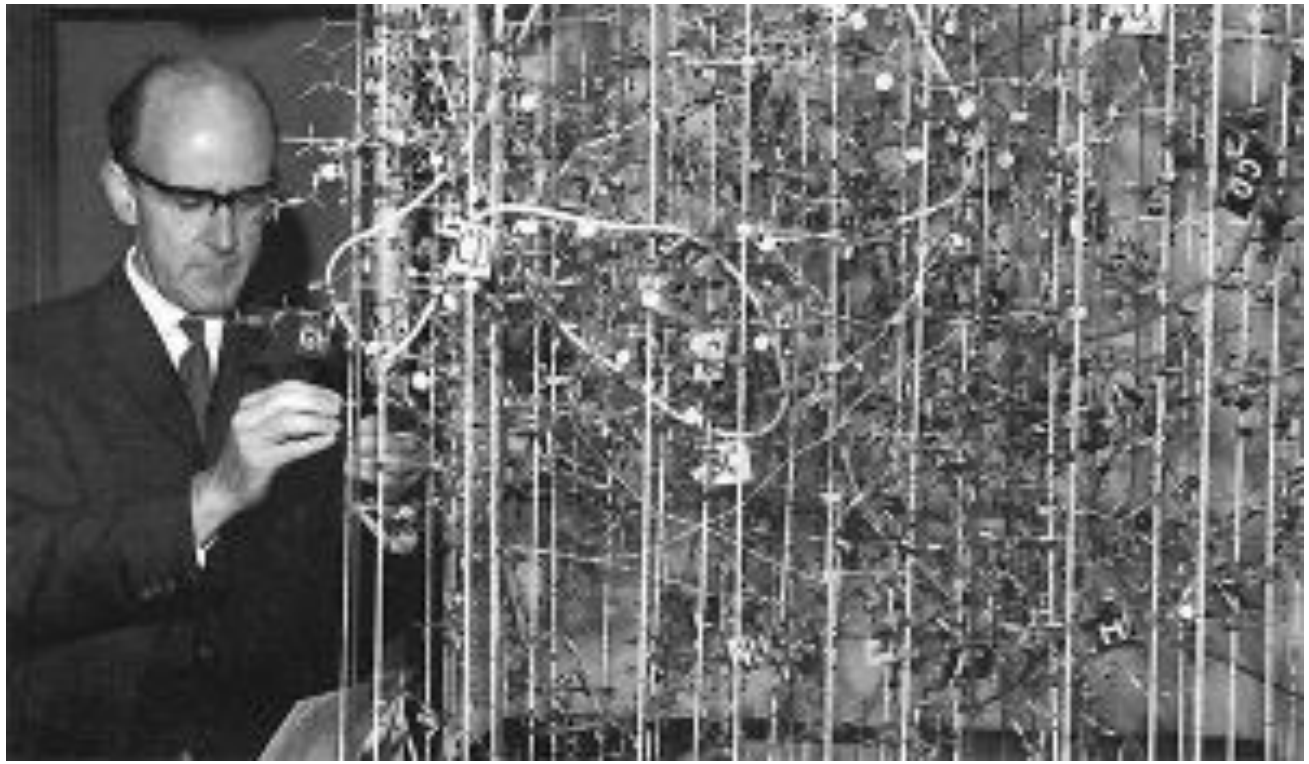
Carlos Cordeiro

MARE

Ciências ULisboa Faculdade de Ciências da Universidade de Lisboa

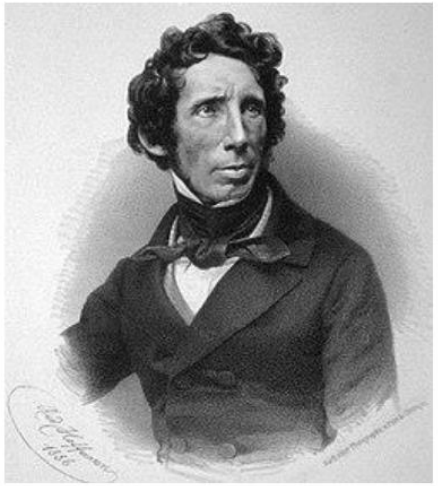
EU FT-ICR MS

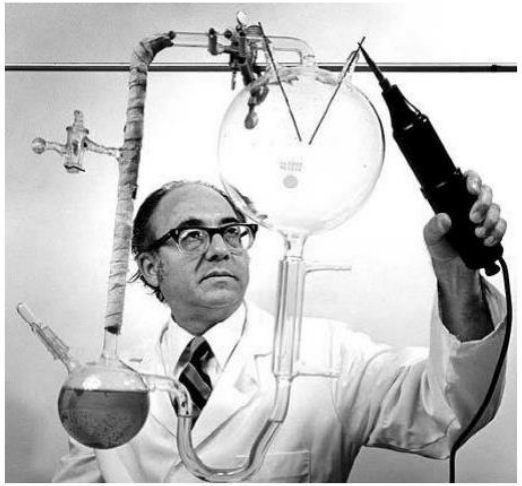
THE ALL IMPORTANT MACROMOLECULES

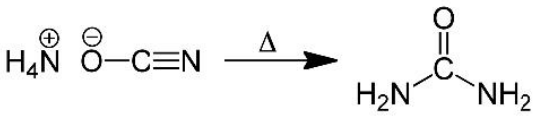


From DNA to RNA to Protein and…

Small molecules!







Building blocs of life

1828

OPEN

Primordial soup was edible: abiotically produced Miller-Urey mixture supports bacterial growth

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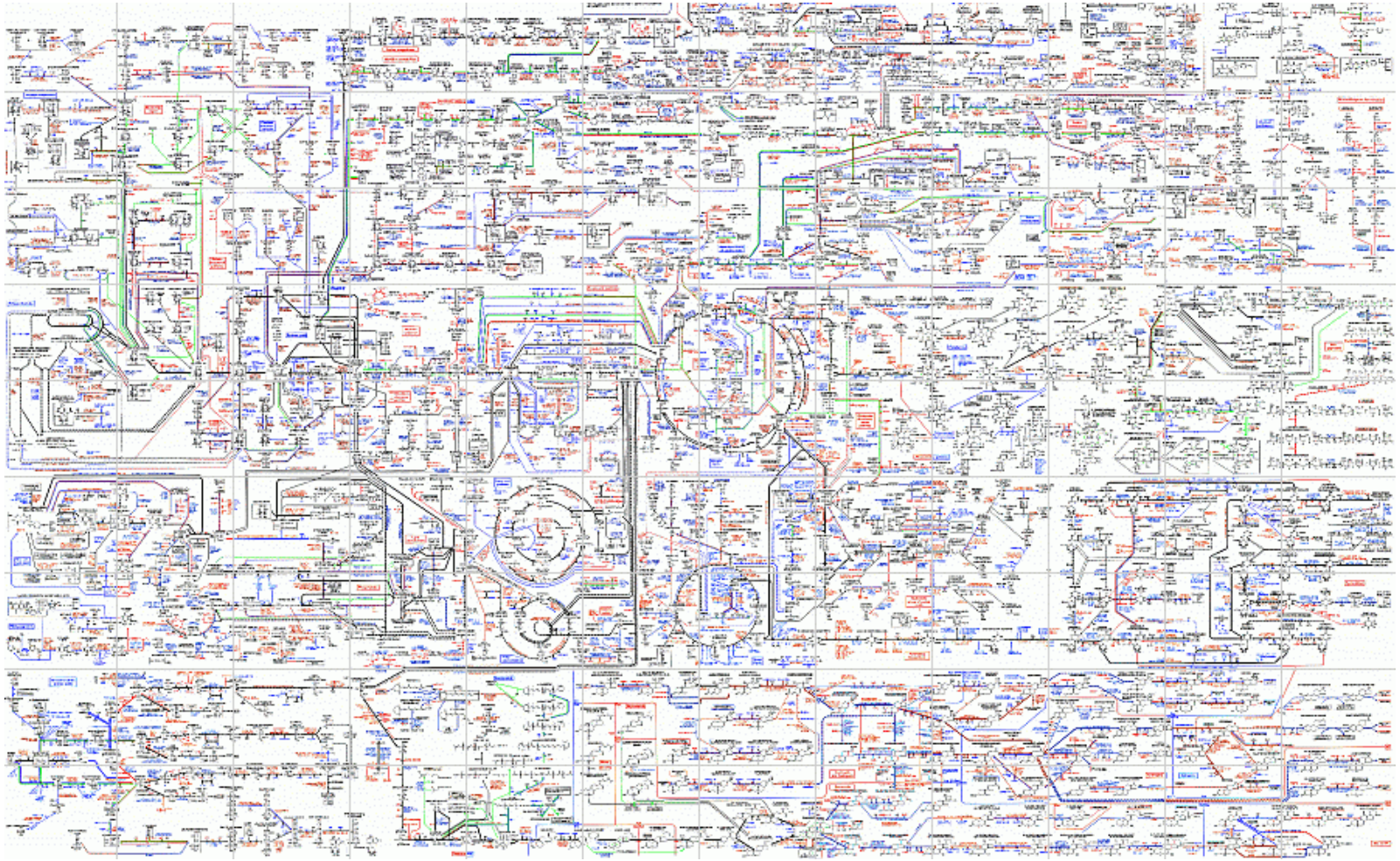
Accepted: 25 August 2015
Published: 28 September 2015

Xueshu Xie[1], Daniel Backman[1], Albert T. Lebedev[2], Viatcheslav B. Artaev[3], Liying Jiang[4], Leopold L. Ilag[4] & Roman A. Zubarev[1]

HOW COMPLEX IS THE METABOLOME?



Biological model

Chemical diversity

Sample preparation

Dynamic range

Databases

Sofwtare

> 200 Kmetabolites

From FT-ICR to MRMS…

> 10.000.000
< 0.2 ppm

CID
ECD/EDD/ETD
SORI-CID
IRMPD
UVPD
BIRD

Any source will fit!
ESI
MALDI

CHEMISTRY vs BIOLOGY

PNAS

Petroleomics: Chemistry of the underworld

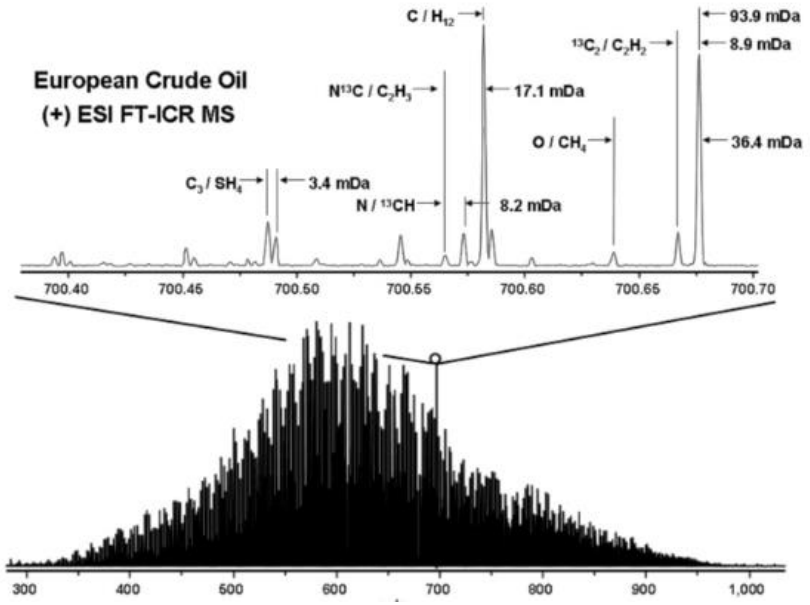
Alan G. Marshall[a,b,1] and Ryan P. Rodgers[a,b,1]

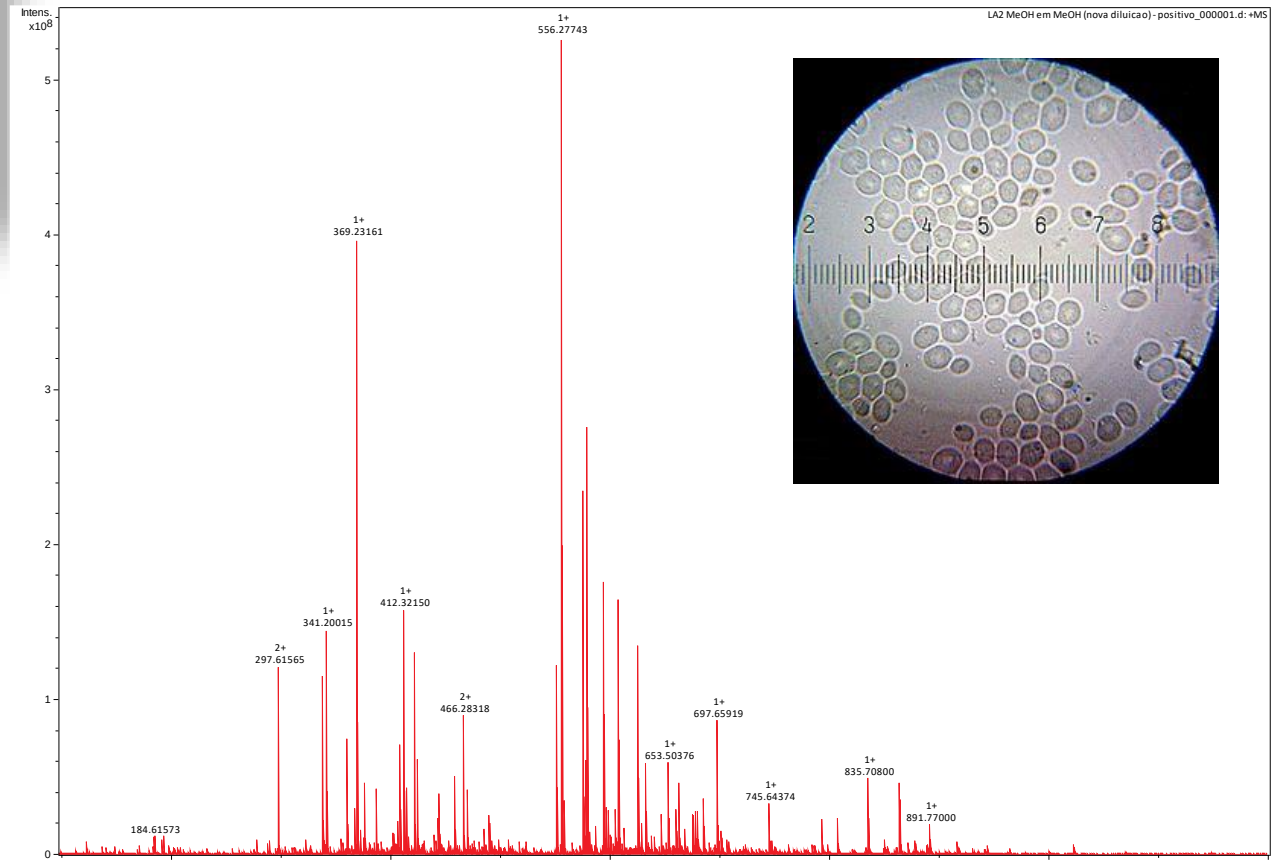
[a]National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, FL 32310-4005; and [b]Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306

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

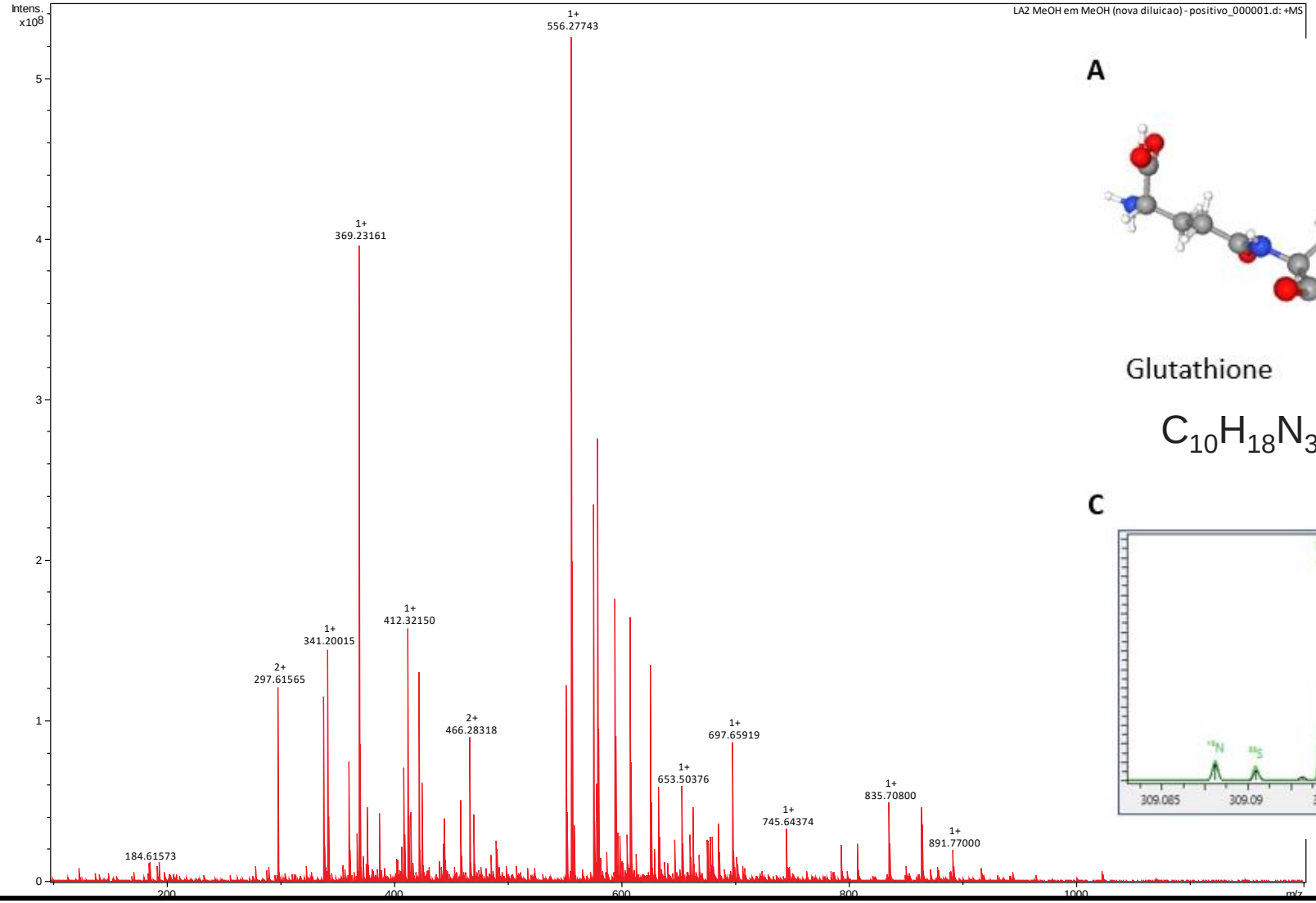
Each different molecular elemental composition—e.g., $C_cH_hN_nO_oS_s$—has a different exact mass. With sufficiently high mass resolving power ($m/\Delta m_{50\%} \sim 400{,}000$, in which m is molecular mass and $\Delta m_{50\%}$ is the mass spectral peak width at half-maximum peak height) and mass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

neutrals. Because many heteroatom-containing components ($N_nO_oS_s$) 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_cH_h), the $N_nO_oS_s$-containing molecules are typically the most

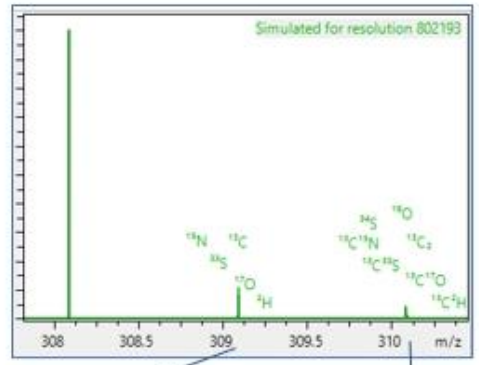


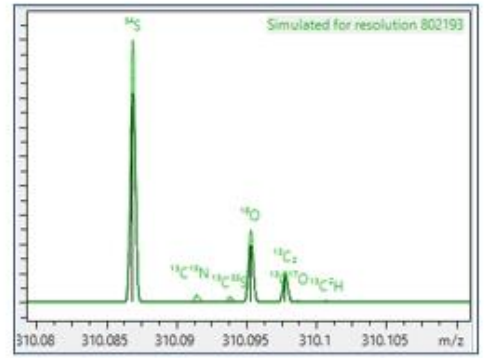


FROM SPECTRUM TO BIOCHEMISTRY

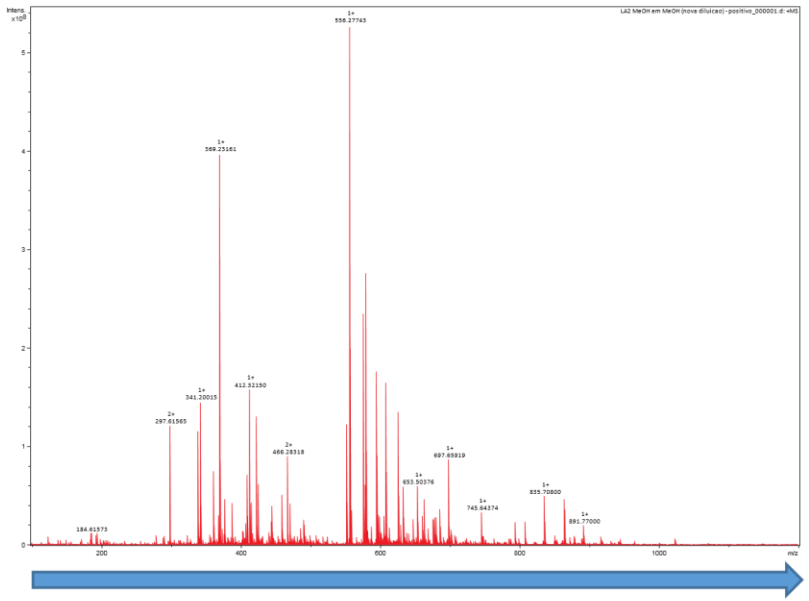


$C_{10}H_{18}N_3O_6S$





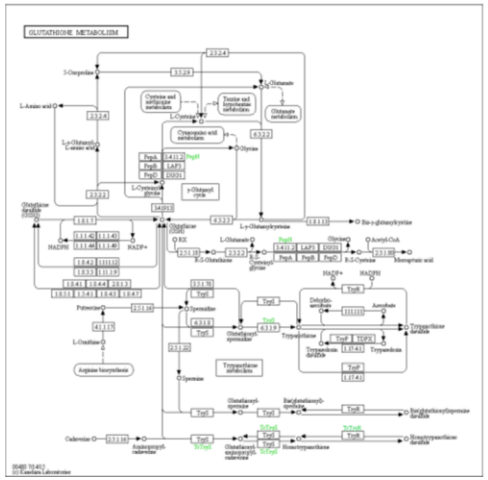
FROM SPECTRUM TO BIOCHEMISTRY



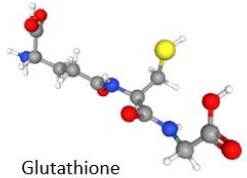
m/z	I
96.99056	278958
97.00244	280155
97.02073	310008
97.05189	268853
97.07603	291872
97.07869	269893
97.11828	285669
97.12189	284327
97.13601	293001
97.13729	268022
97.15587	273592
97.18356	290592
97.19118	291970
97.20449	269770
97.25192	288699
97.2798	287809
97.29958	310364
97.34781	269897
97.35957	280948
97.36091	326947
97.3623	271440
97.36912	290100
97.37229	270598
97.37827	306282
97.38467	294736
97.45464	271385
97.47817	283492
97.49127	297140
97.49986	276791
97.56283	286061
97.59155	269006
97.59562	275327
97.62294	299329

http://masstrix3.helmholtz-muenchen.de/masstrix3/

HMDB02108	158.024620232491	C4H9NO2S	Methylcysteine; S-methyl L-cysteine [thioether]
C05330	158.024620232491	C4H9NO2S	Homocysteine;2-Amino-4-mercaptobutyric acid
C00155	158.024620232491	C4H9NO2S	L-Homocysteine;L-2-Amino-4-mercaptobutyric acid
HMDB00742	158.024620232491	C4H9NO2S	Homocysteine (see KEGG C05330); 2-amino-4-sulfanylbutano...
C15587	159.006755567491	C5H4N4	Purine;Purine base ([M+K39]+)
HMDB01366	159.006755567491	C5H4N4	Purine (see KEGG C00465); 7H-purine [aromatic compound] ...
LMFA01170121	161.044449821241	C6H8O5	Oxoadipic acid; 2-oxo-hexanedioic acid [Dicarboxylic aci...
HMDB00225	161.044449821241	C6H8O5	Oxoadipic acid (see KEGG C00322); 2-oxohexanedioic acid ...
C04287	161.044449821241	C6H8O5	3D-(3,5/4)-Trihydroxycyclohexane-1,2-dione;D-2,3-Diketo-4-d...
C00322	161.044449821241	C6H8O5	2-Oxoadipate;2-Oxoadipic acid
HMDB00398	161.044449821241	C6H8O5	3-Oxoadipic acid (see KEGG C00846); 3-oxo-Hexanedioic ac...
C16159	161.044449821241	C6H8O5	2-Formylglutarate

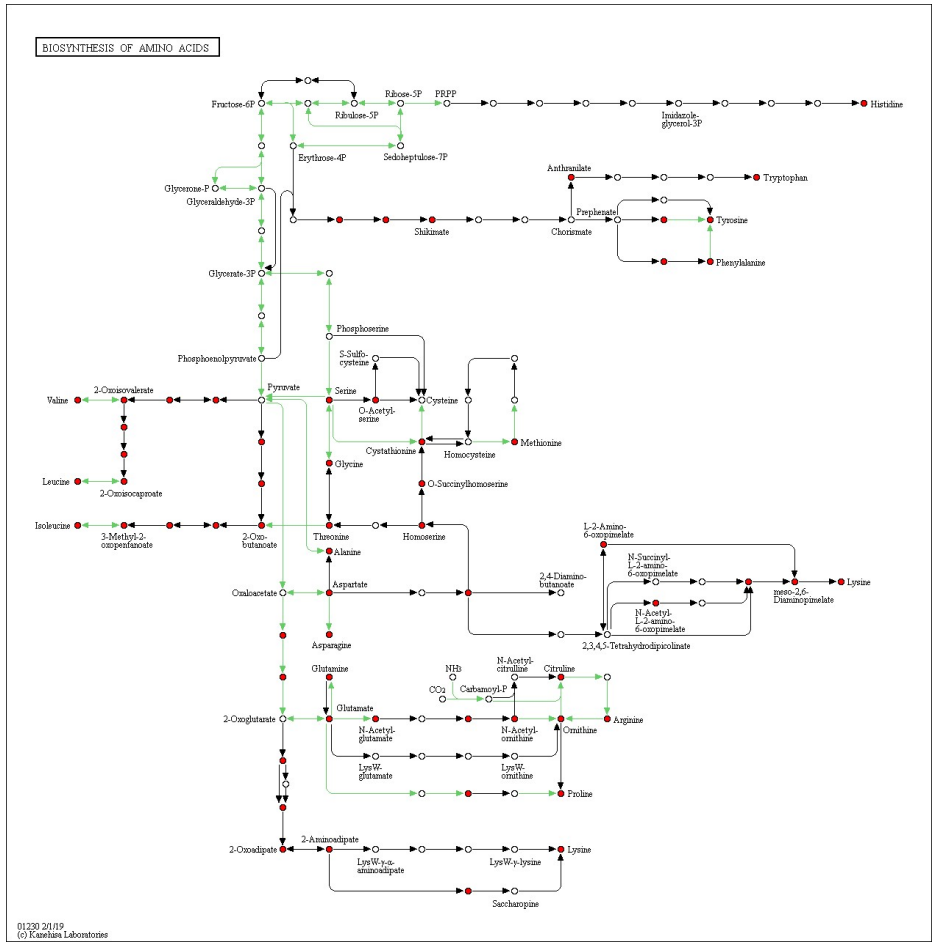


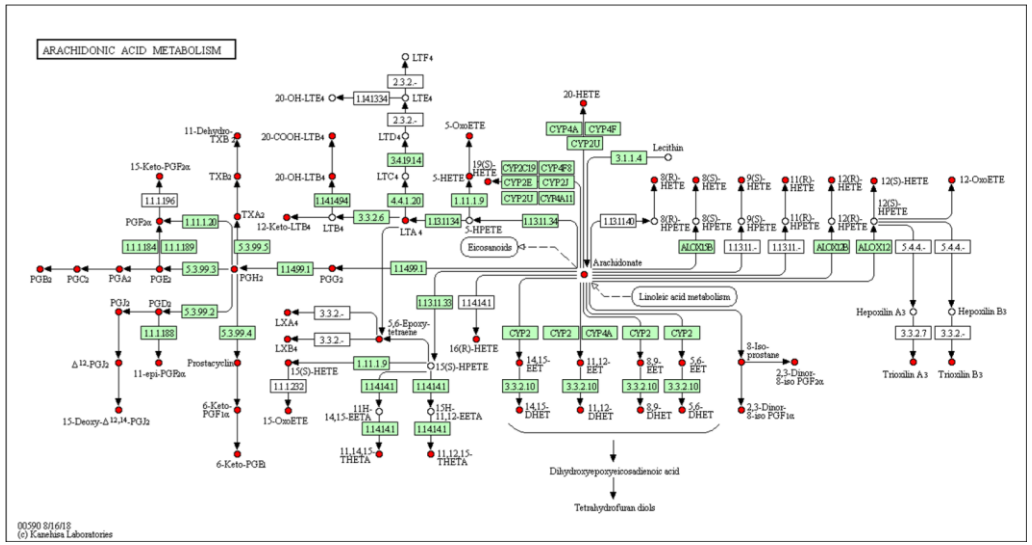
6500



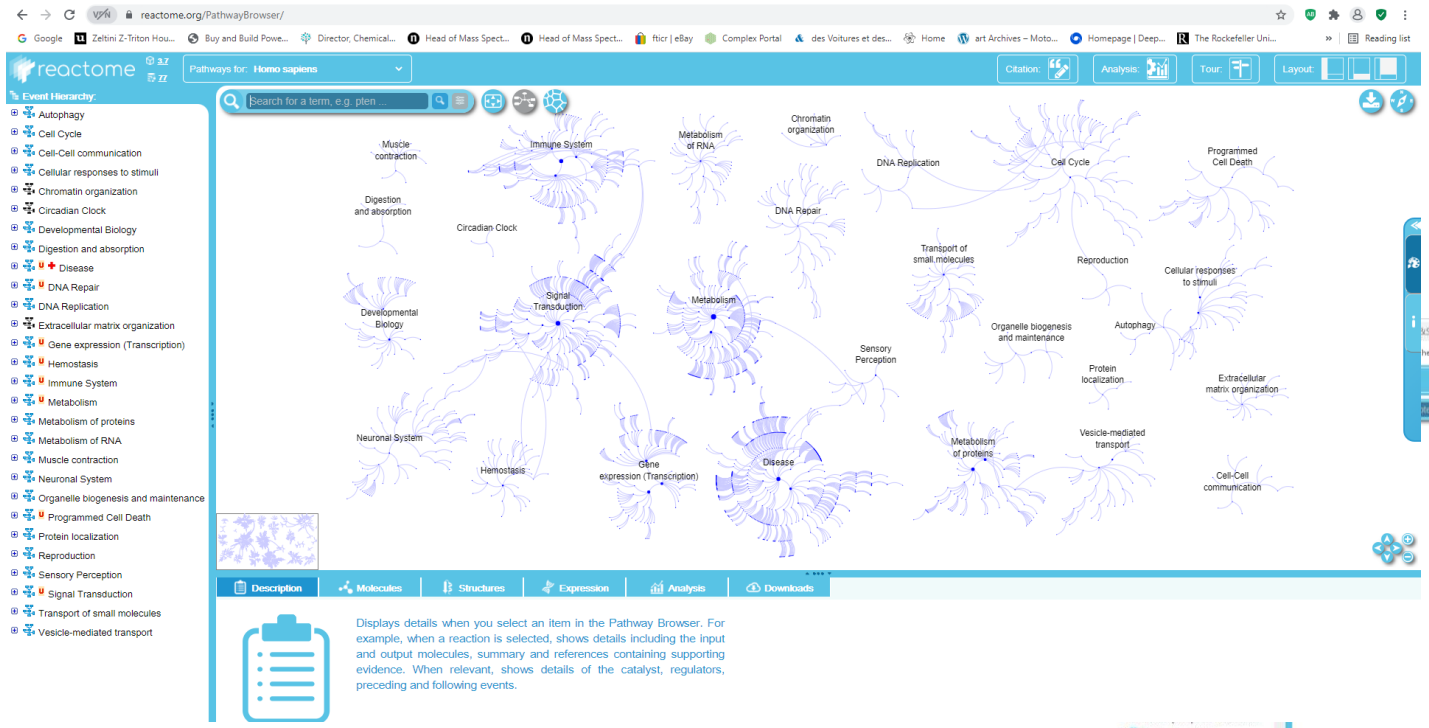
Glutathione

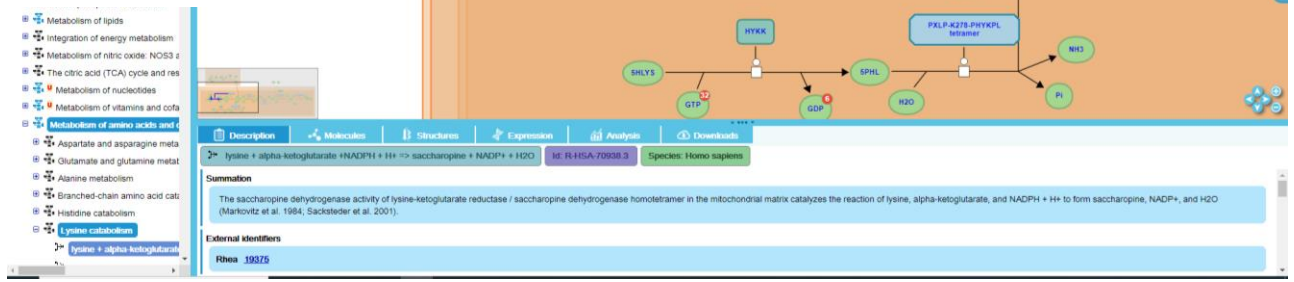
PATHWAY MAPPING





NETWORK ANALYSIS



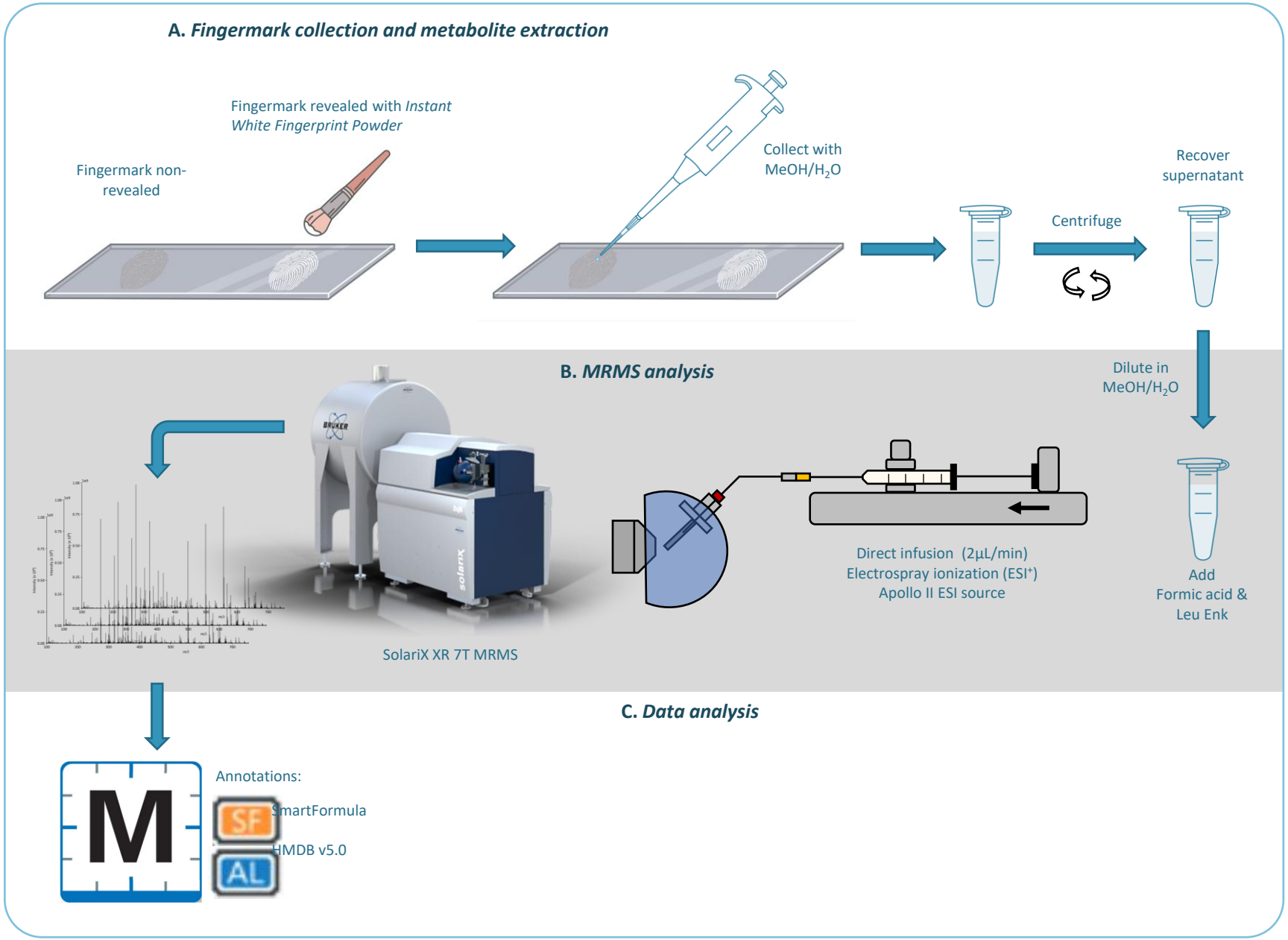


hmdb
The Human Metabolome Database

WE ARE ALL DIFFERENT

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

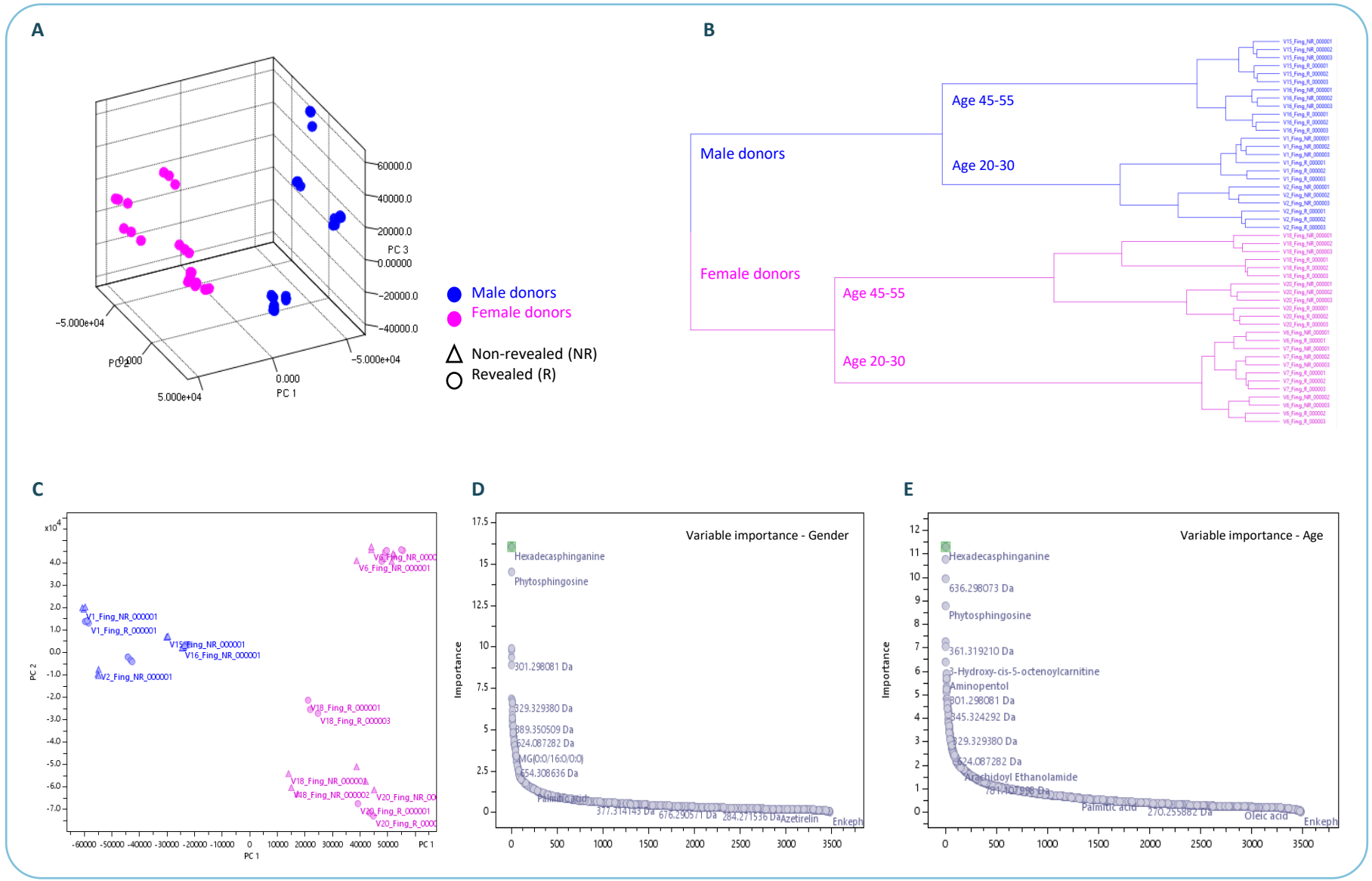
FINGERPRINTING FINGERPRINTS

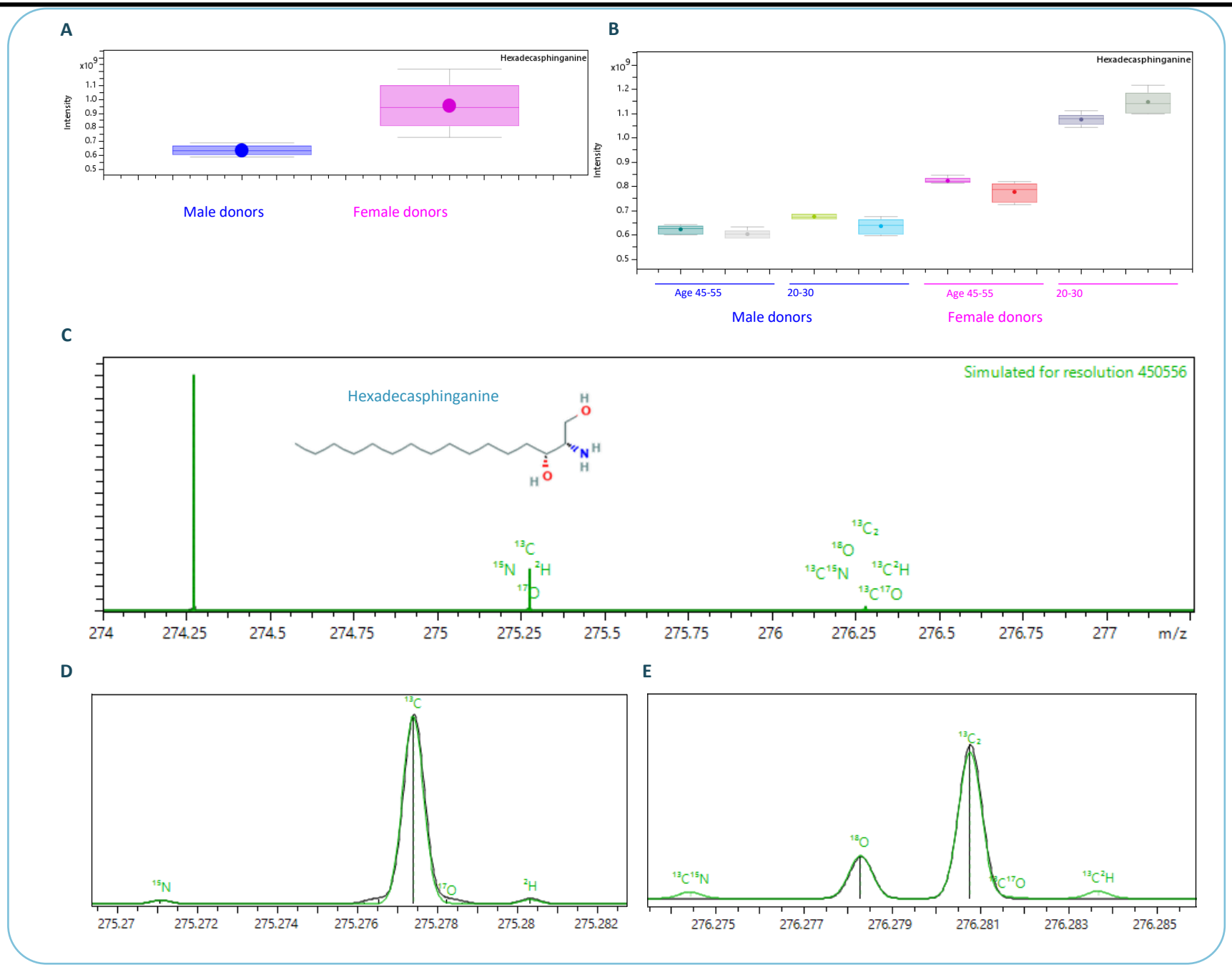

A. Fingermark collection and metabolite extraction
Fingermark revealed with Instant White Fingerprint Powder
Fingermark non-revealed
Collect with MeOH/H2O
Centrifuge
Recover supernatant
Dilute in MeOH/H2O
B. MRMS analysis
Direct infusion (2µL/min)
Electrospray ionization (ESI+)
Apollo II ESI source
Add Formic acid & Leu Enk
SolariX XR 7T MRMS
C. Data analysis
Annotations:
SmartFormula
HMDB v5.0

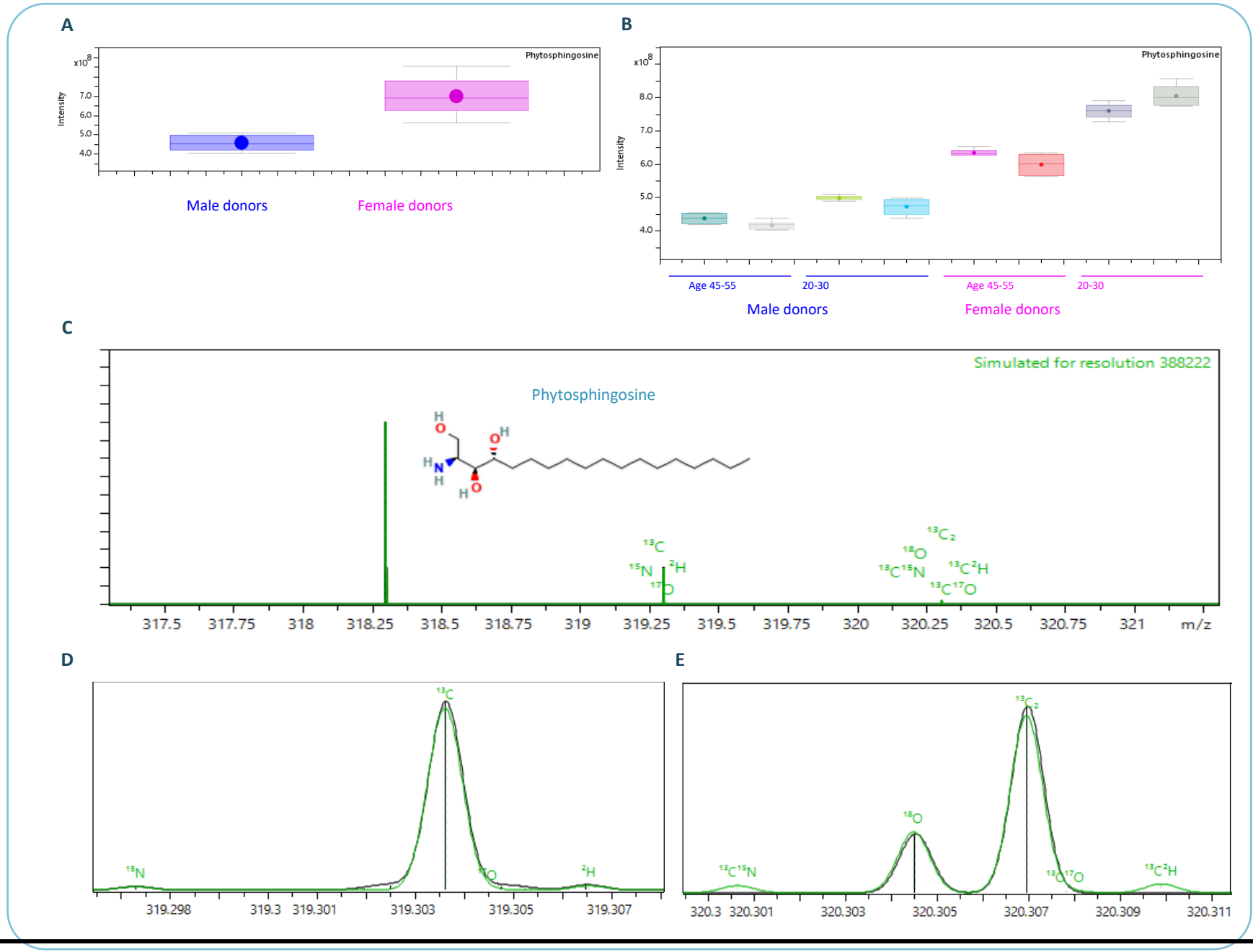
From spectrum to formulas

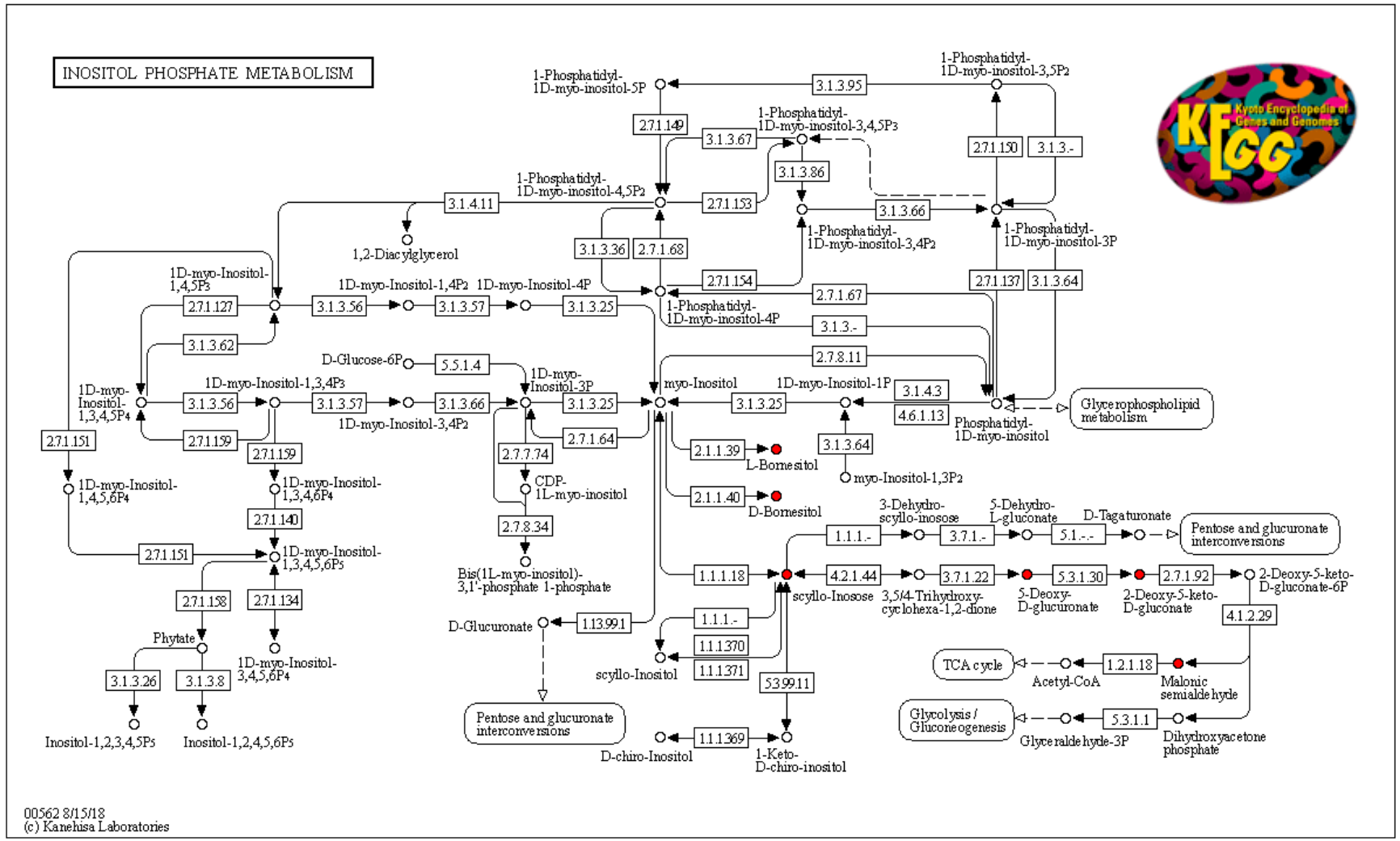
Hypogeic acid	C16H30O2	255,23187	$[M+H^+]$	0,051
Palmitic acid	C16H32O2	257,24752	$[M+H^+]$	0,051
Cyclohexaneundecanoic acid	C17H32O2	269,24751	$[M+H^+]$	0,011
Heptadecanoic acid	C17H34O2	271,26316	$[M+H^+]$	0,011
Linoleic acid	C18H32O2	281,24751	$[M+H^+]$	0,011
Oleic acid	C18H34O2	283,26315	$[M+H^+]$	-0,025
Stearic acid	C18H36O2	285,27881	$[M+H^+]$	0,011

Ab initio calculation (CHONPS... Accurate mass...Isotopic pattern)
Data base annotation (HMDB >200.000)


A
60000.0
40000.0
20000.0
PC 3
0.00000
-20000.0
-40000.0
-5.000e+04
PC 2
0.000
5.000e+04
0.000
PC 1
-5.000e+04
Male donors
Female donors
Non-revealed (NR)
Revealed (R)
B
Male donors
Age 45-55
Age 20-30
Female donors
Age 45-55
Age 20-30
C
PC 2
PC 1
V6_Fing_NR_000001
V1_Fing_NR_000001
V1_Fing_R_000001
V16_Fing_NR_000001
V2_Fing_NR_000001
V18_Fing_R_000001
V18_Fing_R_000003
V18_Fing_NR_000001
V18_Fing_NR_000002
V20_Fing_R_000001
D
Variable importance - Gender
Importance
Hexadecasphinganine
Phytosphingosine
301.298081 Da
329.329380 Da
389.350509 Da
624.087282 Da
MG(0:0/16:0/0:0)
654.308636 Da
Palmitic acid
377.314143 Da
676.290571 Da
284.271536 Da
Azetirelin
Enkeph
E
Variable importance - Age
Importance
Hexadecasphinganine
636.298073 Da
Phytosphingosine
361.319210 Da
3-Hydroxy-cis-5-octenoylcarnitine
Aminopentol
301.298081 Da
345.324292 Da
329.329380 Da
624.087282 Da
Arachidoyl Ethanolamide
Palmitic acid
270.255882 Da
Oleic acid
Enkeph


A
Hexadecasphinganine
x10^9
Intensity
Male donors
Female donors
B
Hexadecasphinganine
Age 45-55
20-30
Age 45-55
20-30
Male donors
Female donors
C
Simulated for resolution 450556
Hexadecasphinganine
274
274.25
274.5
274.75
275
275.25
275.5
275.75
276
276.25
276.5
276.75
277
m/z
D
E
275.27
275.272
275.274
275.276
275.278
275.28
275.282
276.275
276.277
276.279
276.281
276.283
276.285


A
Phytosphingosine
x10^8
Intensity
7.0
6.0
5.0
4.0
Male donors
Female donors
B
Phytosphingosine
x10^8
Intensity
8.0
7.0
6.0
5.0
4.0
Age 45-55
20-30
Age 45-55
20-30
Male donors
Female donors
C
Simulated for resolution 388222
Phytosphingosine
317.5
317.75
318
318.25
318.5
318.75
319
319.25
319.5
319.75
320
320.25
320.5
320.75
321
m/z
D
319.298
319.3
319.301
319.303
319.305
319.307
E
320.3
320.301
320.303
320.305
320.307
320.309
320.311


INOSITOL PHOSPHATE METABOLISM
1-Phosphatidyl-1D-myo-inositol-5P
1-Phosphatidyl-1D-myo-inositol-3,5P2
1-Phosphatidyl-1D-myo-inositol-3,4,5P3
1-Phosphatidyl-1D-myo-inositol-4,5P2
1-Phosphatidyl-1D-myo-inositol-3,4P2
1-Phosphatidyl-1D-myo-inositol-3P
1-Phosphatidyl-1D-myo-inositol-4P
1,2-Diacylglycerol
1D-myo-Inositol-1,4,5P3
1D-myo-Inositol-1,4P2
1D-myo-Inositol-4P
1D-myo-Inositol-1,3,4P3
1D-myo-Inositol-3,4P2
1D-myo-Inositol-3P
D-Glucose-6P
myo-Inositol
1D-myo-Inositol-1P
1D-myo-Inositol-1,3,4,5P4
1D-myo-Inositol-1,4,5,6P4
1D-myo-Inositol-1,3,4,6P4
1D-myo-Inositol-1,3,4,5,6P5
1D-myo-Inositol-3,4,5,6P4
Phytate
Inositol-1,2,3,4,5P5
Inositol-1,2,4,5,6P5
CDP-1L-myo-inositol
Bis(1L-myo-inositol)-3,1'-phosphate 1-phosphate
L-Bornesitol
D-Bornesitol
myo-Inositol-1,3P2
Phosphatidyl-1D-myo-inositol
Glycerophospholipid metabolism
3-Dehydro-scyllo-inosose
5-Dehydro-L-gluconate
D-Tagaturonate
Pentose and glucuronate interconversions
scyllo-Inosose
3,5/4-Trihydroxy-cyclohexa-1,2-dione
5-Deoxy-D-glucuronate
2-Deoxy-5-keto-D-gluconate
2-Deoxy-5-keto-D-gluconate-6P
D-Glucuronate
scyllo-Inosol
TCA cycle
Acetyl-CoA
Malonic semialdehyde
Glycolysis / Gluconeogenesis
Glyceraldehyde-3P
Dihydroxyacetone phosphate
D-chiro-Inositol
1-Keto-D-chiro-inositol
3.1.3.95
2.7.1.149
3.1.3.67
3.1.3.86
2.7.1.150
3.1.3.-
3.1.4.11
2.7.1.153
3.1.3.66
3.1.3.36
2.7.1.68
2.7.1.154
2.7.1.67
2.7.1.137
3.1.3.64
2.7.1.127
3.1.3.56
3.1.3.57
3.1.3.25
3.1.3.62
5.5.1.4
2.7.8.11
3.1.4.3
4.6.1.13
2.7.1.151
2.7.1.159
2.7.1.64
2.7.7.74
2.1.1.39
3.1.3.64
2.1.1.40
2.7.8.34
1.1.1.-
3.7.1.-
5.1.-.-
1.1.1.18
4.2.1.44
3.7.1.22
5.3.1.30
2.7.1.92
4.1.2.29
1.13.99.1
1.1.1.370
1.1.1.371
5.3.99.11
1.2.1.18
5.3.1.1
1.1.1.369
2.7.1.140
2.7.1.158
2.7.1.134
3.1.3.26
3.1.3.8
00562 8/15/18
(c) Kanehisa Laboratories

COMING OUT THIS CHRISTMAS!

The invisible metabolic word of fingermarks revealed by Magnetic Resonance Mass Spectrometry (MRMS)

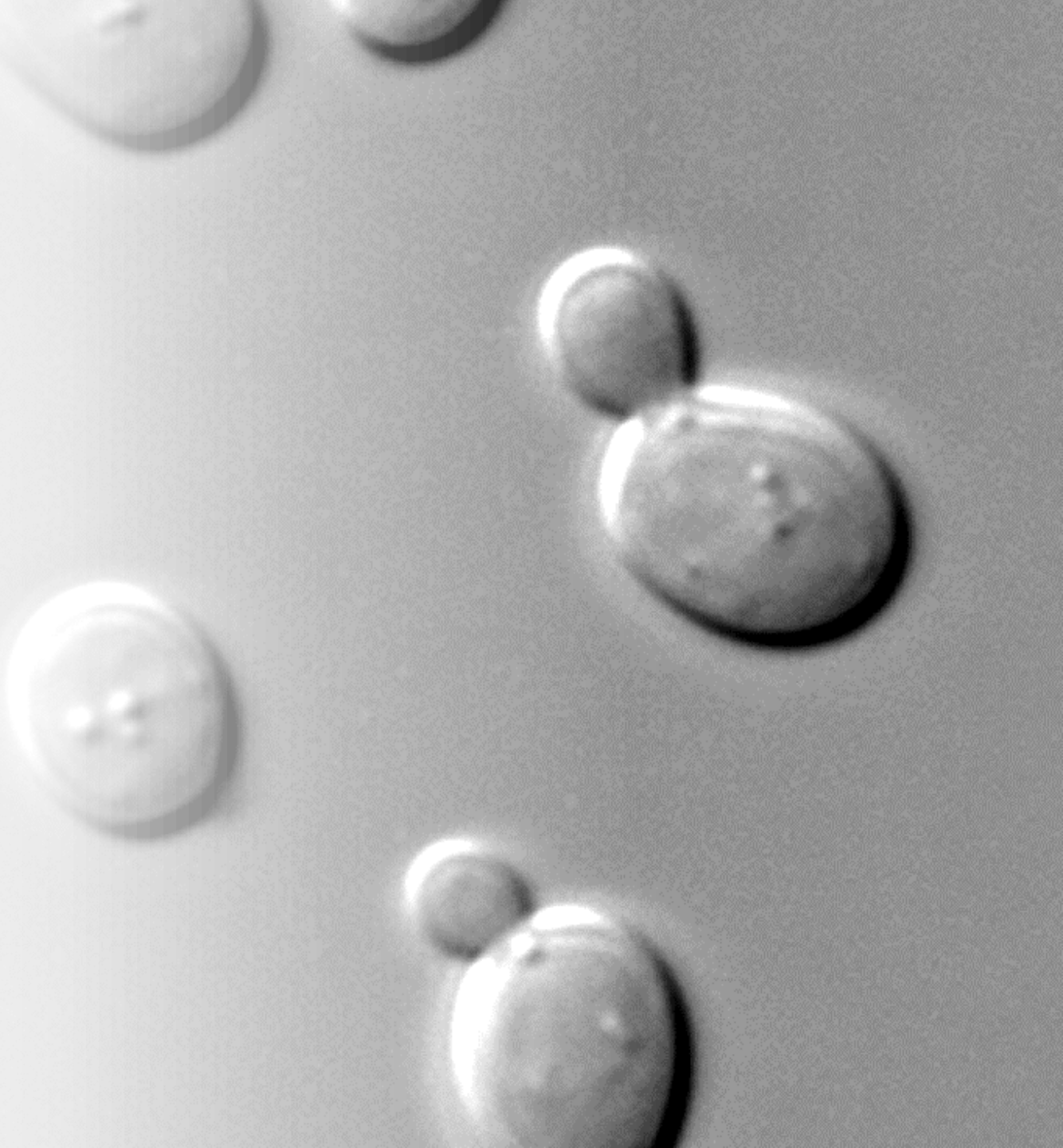
Fingermarks leave behind a plethora of chemical information defined by metabolites, exogenous substances including pharmaceuticals, grooming products, and even drugs of abuse and explosives.

Abstract

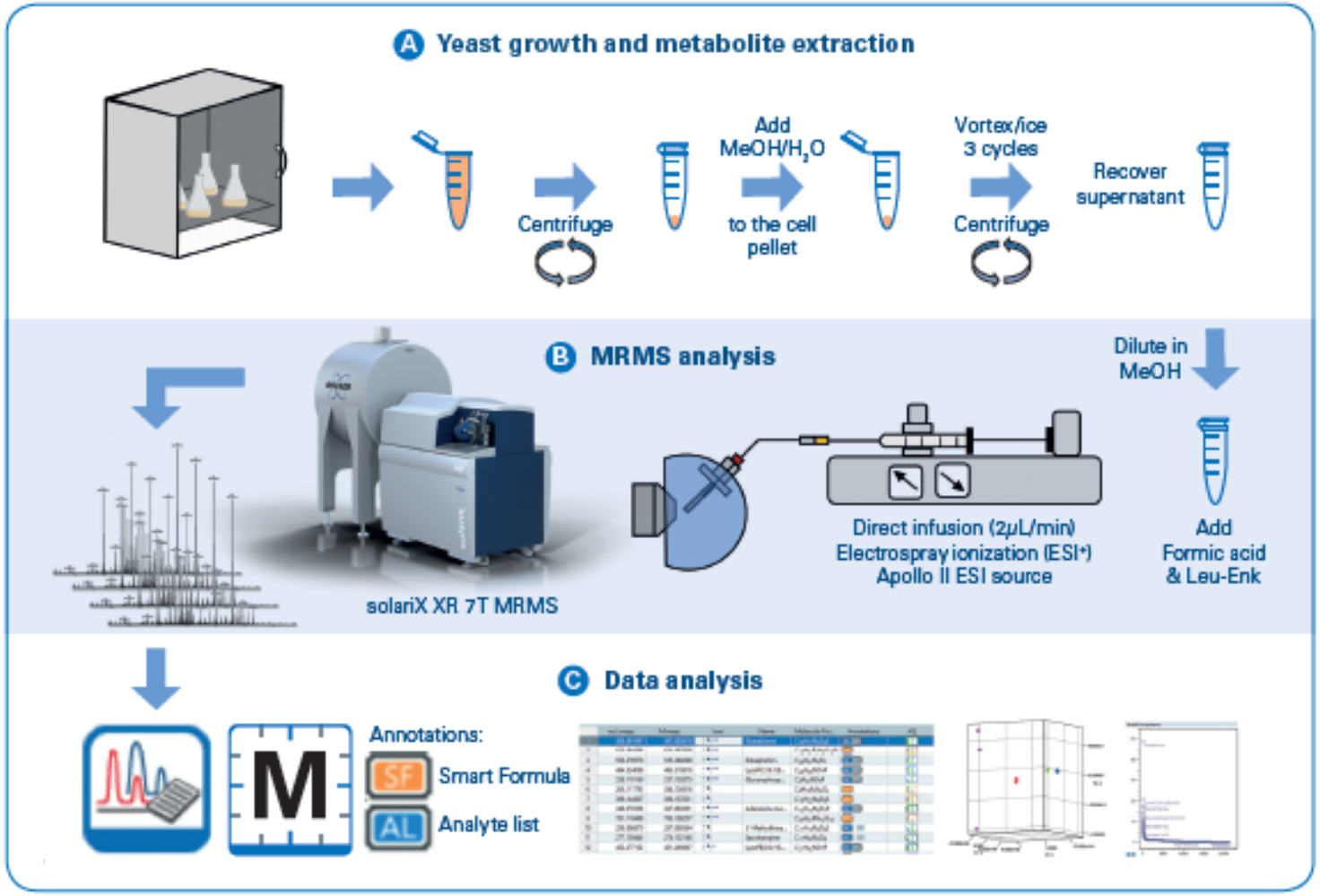
The composition of this extremely complex mixture can be revealed by Magnetic Resonance Mass Spectromtry (MRMS) and the gathered information used for sex and age group identification. Moreover, specific metabolites were found to contribute the most for these differentiations, proving a biochemical foundation linking metabolome to sex and age.

Keywords:
Metabolomics, MRMS, fingerprints, chemical profile

DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS



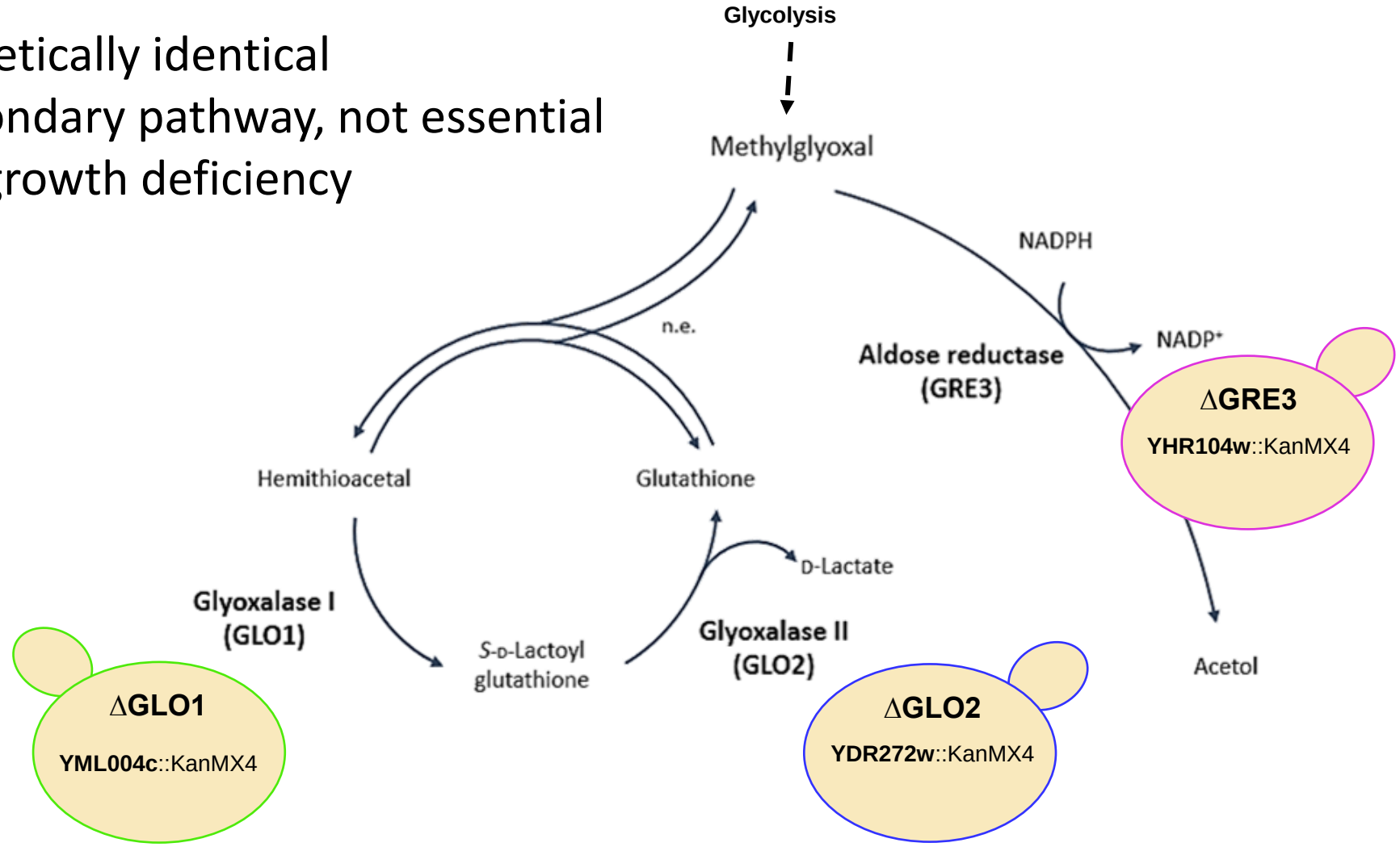
DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS



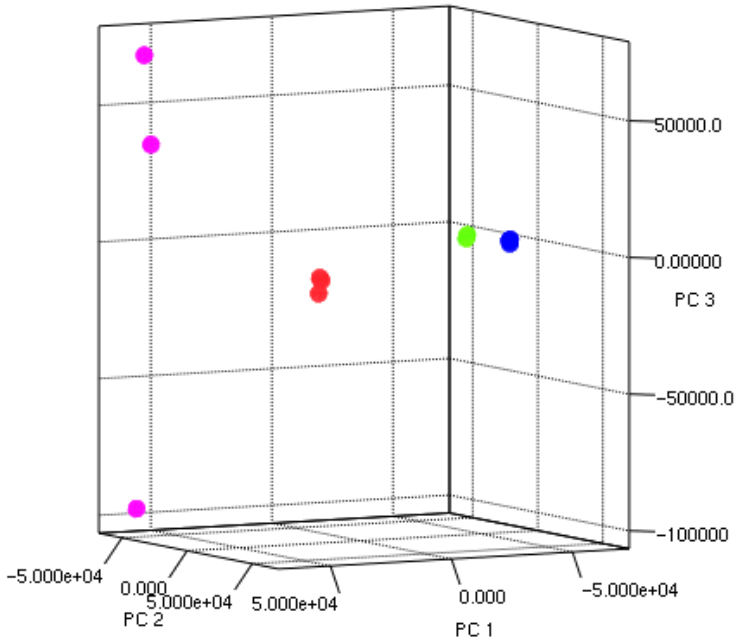
DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS

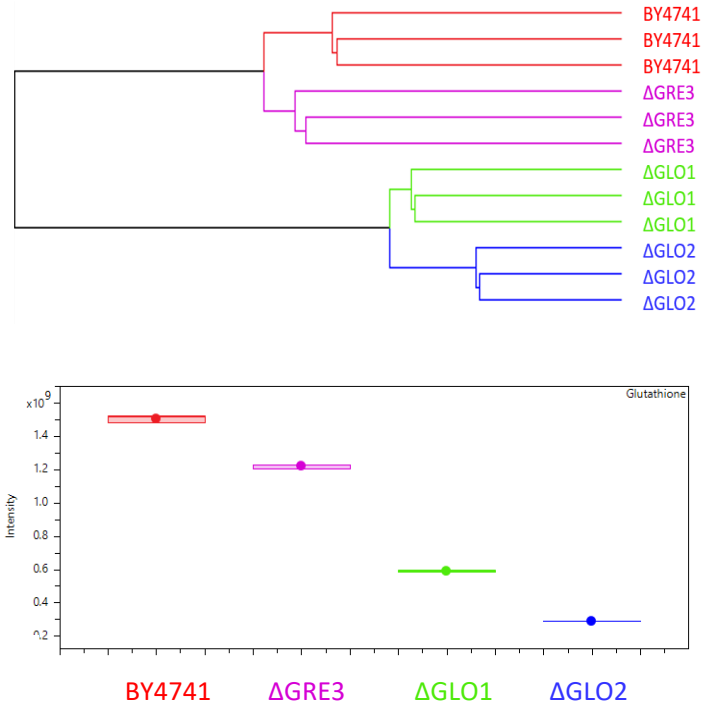
BY4741
MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0

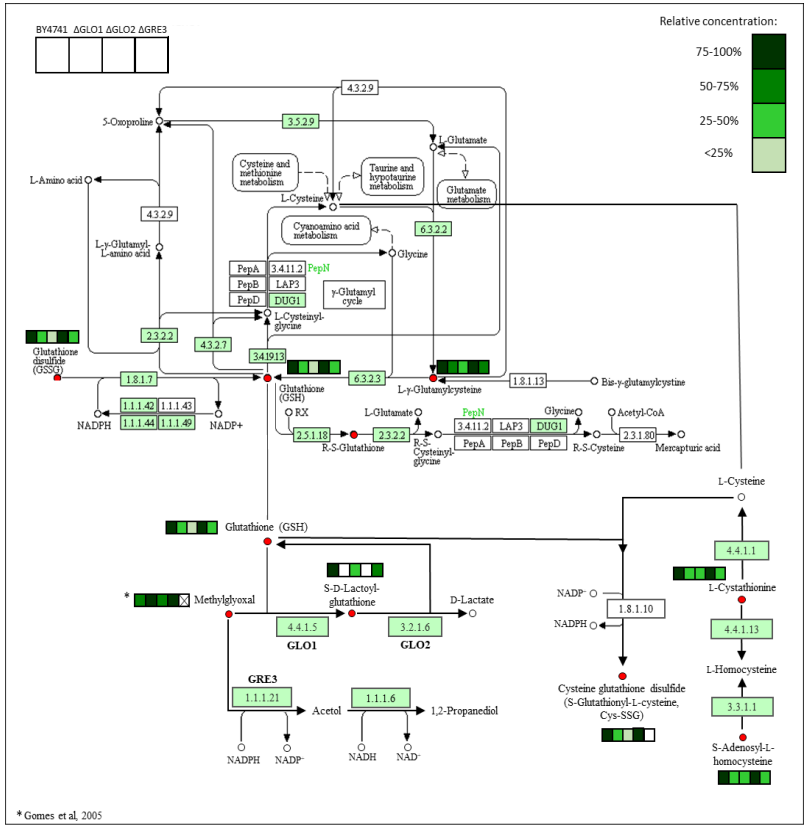
Genetically identical
Secondary pathway, not essential
No growth deficiency



DISCRIMINATION OF SINGLE-GENE YEAST MUTANTS





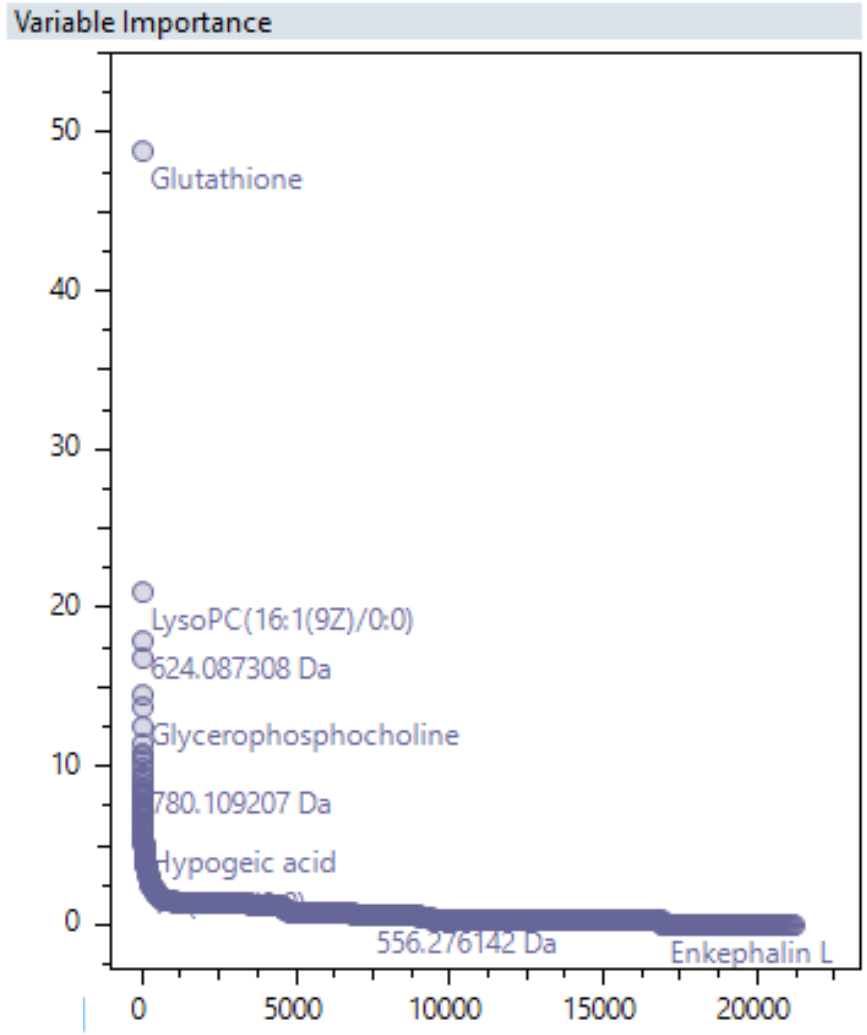


Glutathione metabolism

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

BEYOND GLUTATHIONE

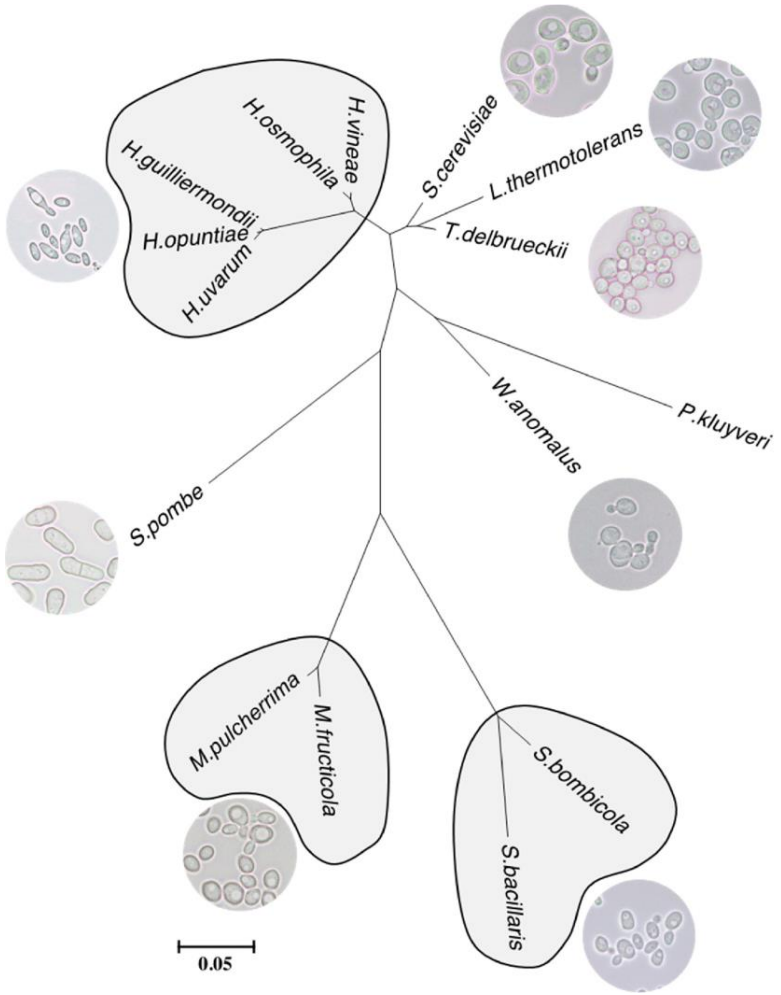
Position	Mass (Da)	Metabolite Name	Molecular Formula	VIP Score
1	307.0838	Glutathione	$C_{10}H_{17}N_3O_6S$	8.417995046
2	493.3168	PC(16:1(9Z)/0:0)	$C_{24}H_{48}NO_7P$	5.993469554
3	624.0873	N/A	$C_{14}H_{28}N_{10}O_{10}S_4$	5.587837143
4	257.1029	Glycerophosphocholine	$C_8H_{20}NO_6P$	4.837523121
5	324.1057	N/A	$C_{12}H_{20}O_{10}$	4.177424151
6	337.3345	N/A	$C_{22}H_{43}NO$	4.153906335
7	254.2246	Hypogeic acid	$C_{16}H_{30}O_2$	4.077281087
8	385.3192	Pentadecanoylcarnitine	$C_{22}H_{43}NO_4$	3.77589036
9	398.1372	N/A	$C_{15}H_{22}N_6O_5S$	3.484675636
10	451.2699	PE(16:1(9Z)/0:0)	$C_{21}H_{42}NO_7P$	3.172850261

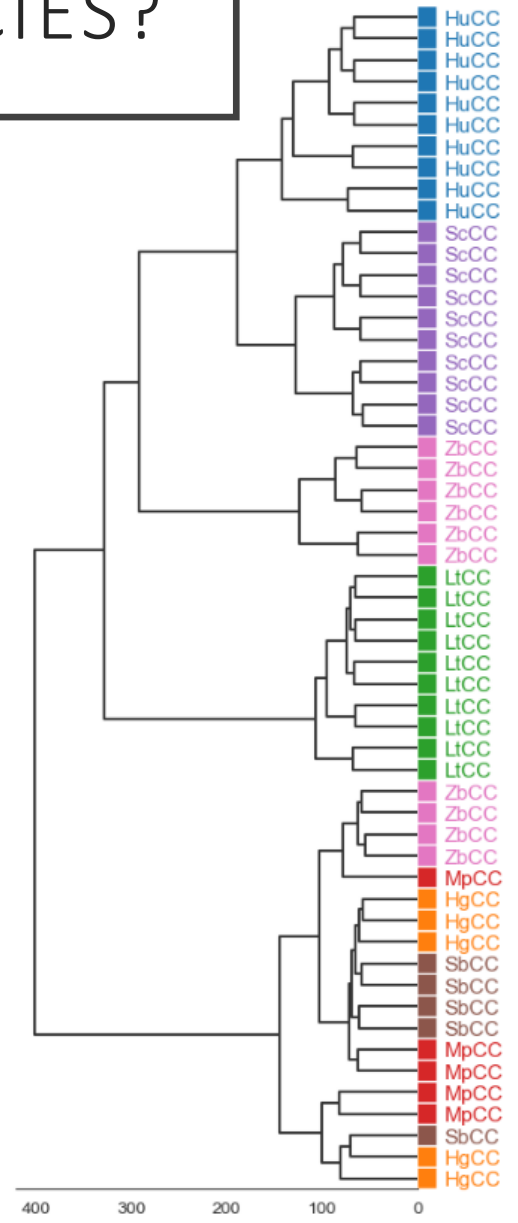


WHAT ABOUT DIFFERENT YEAST SPECIES?

Yeast Species

Hu - ***Hanseniaspora uvarum***
Hg - ***Hanseniaspora guilliermondii***
Lt - ***Lachancea thermotolerans***
Mp - ***Metschnikowia pulcherrima***
Sc - ***Saccharomyces cerevisiae***
Sb - ***Starmerella bacillaris***
Zb - ***Zygosaccharomyces bailii***





LOOKING AT THE BIG PICTURE

1	613
2	192
3	120
4	111
5	57
6	68
7	21
8	49
9	24
10	37
11	20
12	16
13	7
14	15
15	6
16	8
17	12
18	10
19	7
20	12

21	1
22	10
23	4
24	12
25	1
26	5
27	4
28	5
29	2
30	3
31	2
32	2
34	4
36	5
37	1
38	2
39	1
40	1
42	1

44	1
45	1
46	2
50	1
58	1
60	2
62	1
63	1
70	1
78	1
80	1
86	1
95	1
98	1
106	1
107	1
109	1
112	1
168	1
825	1

32438 metabolites, 1493 with annotations

Counts of numbers of possible compounds for each annotated peak

40% Are unique

70% < 5 possible annotations

FUTURE DIRECTION FOR FT-ICR-MS METABOLOMICS

- Development of a scoring method for metabolite annotation
- Validation of annotations (2D-FT-ICR-MS)
- Isomer discrimination (MSMS or ion-mobility)
- Quantification

Metametabolomics

THE TEAM

MARE

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MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

EU FT-ICR MS

RNE
Portuguese Mass Spectrometry Network