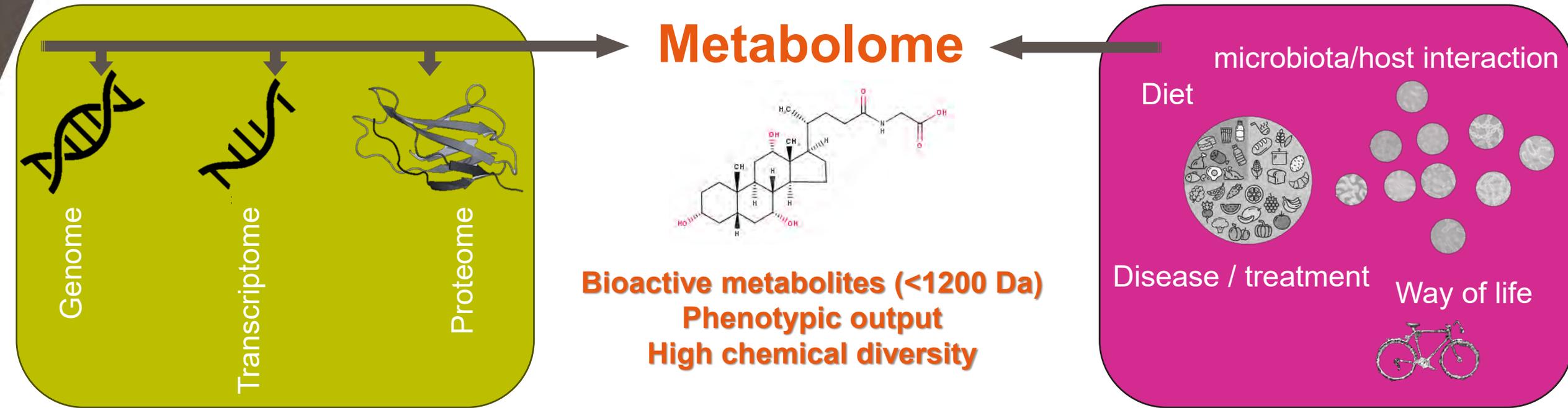




Metabolomics at Gustave Roussy

sylvere.durand@gustaveroussy.fr

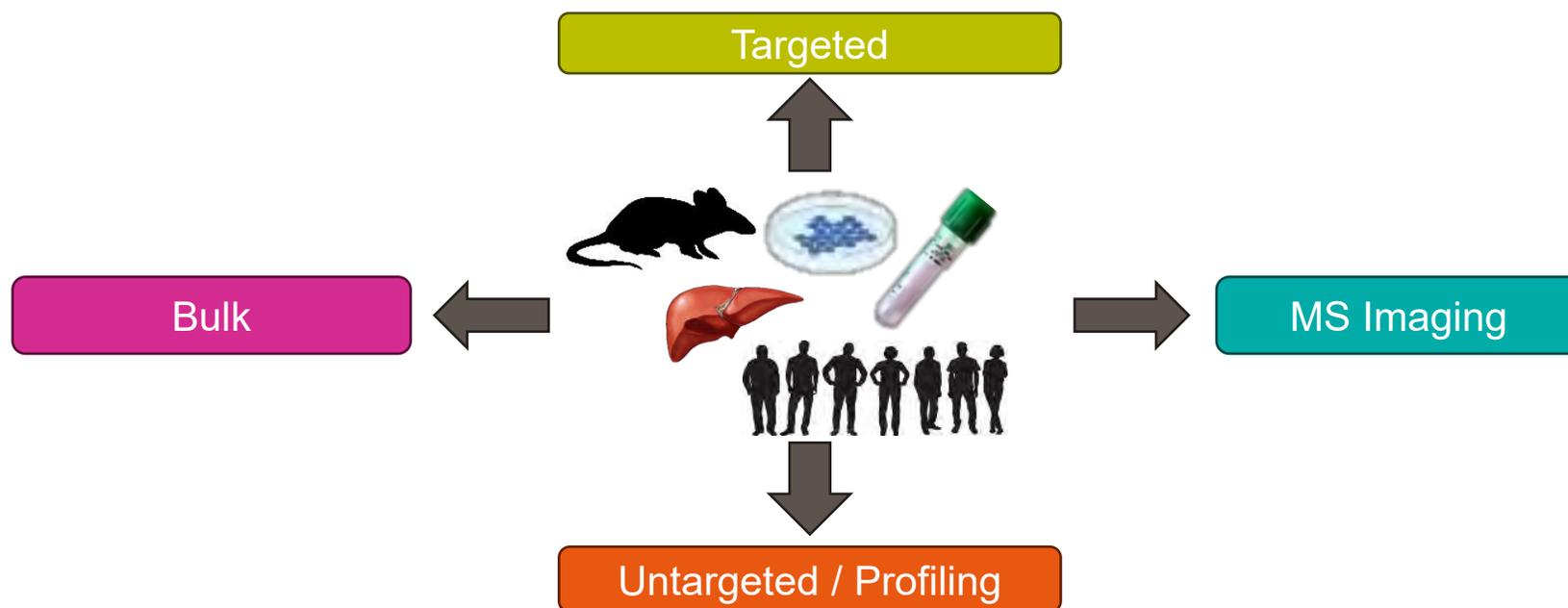
> >> **Metabolome, end result of the “life process”, but not only**



- **Metabolome** can be monitored by several technics (Seahorse, ELISA kits, MS)
- **Metabolomics by Mass Spectrometry**: direct measurements of metabolites extracted
- Wherever are metabolites could be an application for metabolomics

>> One metabolomics, different approaches

- Applications to **pre-clinical** (mice) and **clinical** (human cohorts) projects
- Both **Intracellular & extracellular** samples
 - Suspended or adherent **cell lines** (WT,KO...); supernatant/medium of cells or bacteria cultures
 - All **tissues**: liver, heart, muscle, tumor/biopsy, kidney spleen, hypothalamus, hippocampus, spinal cords...
 - **Gut** contents: ileum, colon, feces
 - **Biofluids**: Serum / plasma (HepLi), tumor fluids, bone marrow fluid



>> Targeted analysis: a straightforward workflow



UHPLC QQQ 6470

MS analyses



UHPLC Orbitrap qExactive

**SFCA/KB/TCA/Aas/
Glycolysis (T1)**
Acetic/butyric/
propionic +10 (C5-
C10) Fas + Ketone
Bodies + AA + TCA +
small organics acids

Bile acids (T4)
Unconjugated
Tauro/Glyco
Conjugated

Coenzyme met. (T2)
CoA derivatives
NAD / NADH
NADP / NADPH
FAD
NMN
GnPs/AnPs/CnPs

NFPA (T5)
Ornithine
Spermidine
Spermine
Cadaverine
Putrescine
(+Acetylated)
SAM/SAH

**Sugar and
phosphates (T2b)**
Glycolysis/PPP
NAD / NADH
NADP / NADPH
FAD, NMN
GnPs/AnPs/CnPs

Lipidomics (T6)
Cer
DG/MG/TG
PC/PI/PG/PA/PS
SM
Sphingosine1P
LPC/LPE/LPI
Ubiquinone

Polyamines (T3)
Ornithine
Spermidine
Spermine
Cadaverine
Putrescine
(+Acetylated)
SAM/SAH

Metabolic fluxes (Fx)
Each method could
be endorsed by
equivalent fluxomic
analysis

**Widely Pseudo-target
(P1)**
Carnitines
Fatty acids
GSH/GSSH
Small peptides...

Full profiling



30 mg



2 M cells

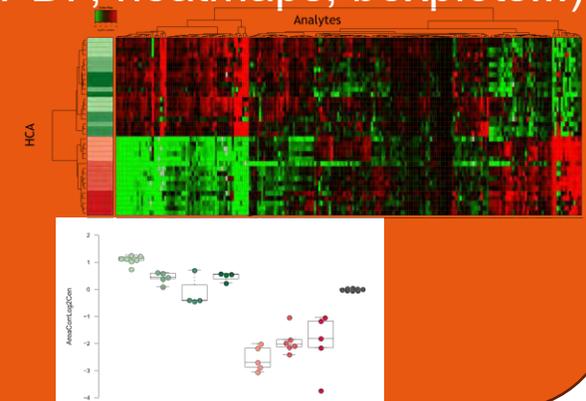


25/50 µL

**Homogenization
Protein precipitation**

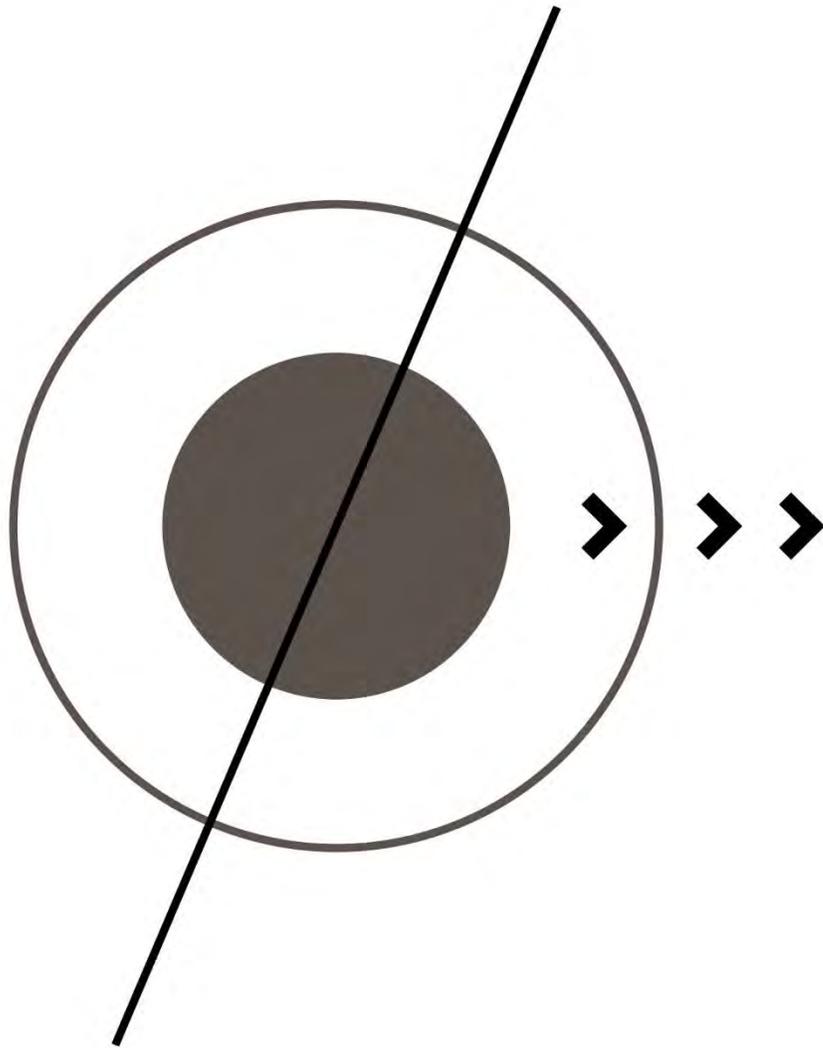
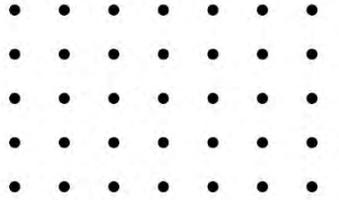


**ready-to-use output (XLS,
PDF, heatmaps, boxplots...)**



From one mere sample, 150-400 metabolites in one single output

TCA, bile acids, polyamines, amino-acids, nucleot(s)ides, nucleosides phosphates, vitamins, short chain fatty acids, ketone bodies, free fatty acids, carnitines, phospholipids, cofactors, phenols, CoAs

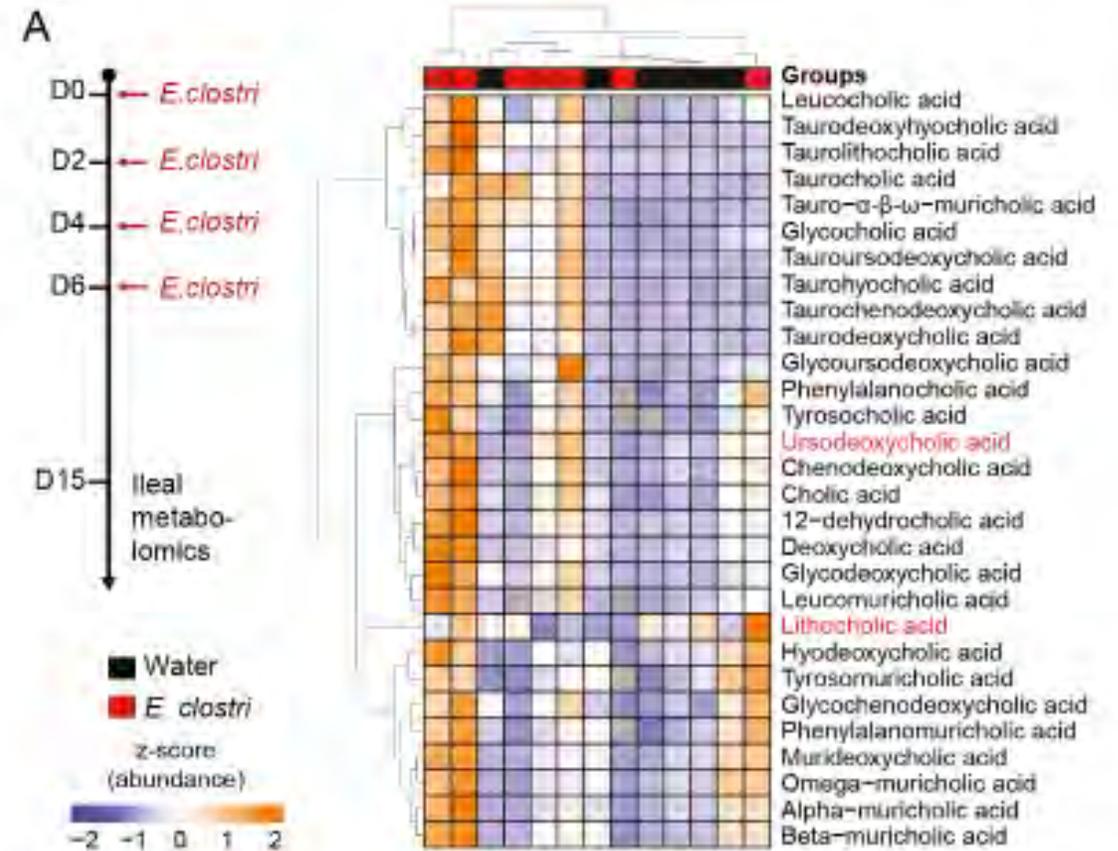


Bulk metabolomics

>>> Bulk analysis, some concretes

- 6 mice per condition
- 30 mg of ileal tissue per mouse
- Widely target by LC/MS
- Bile acids (targeted method) figured out to be interesting

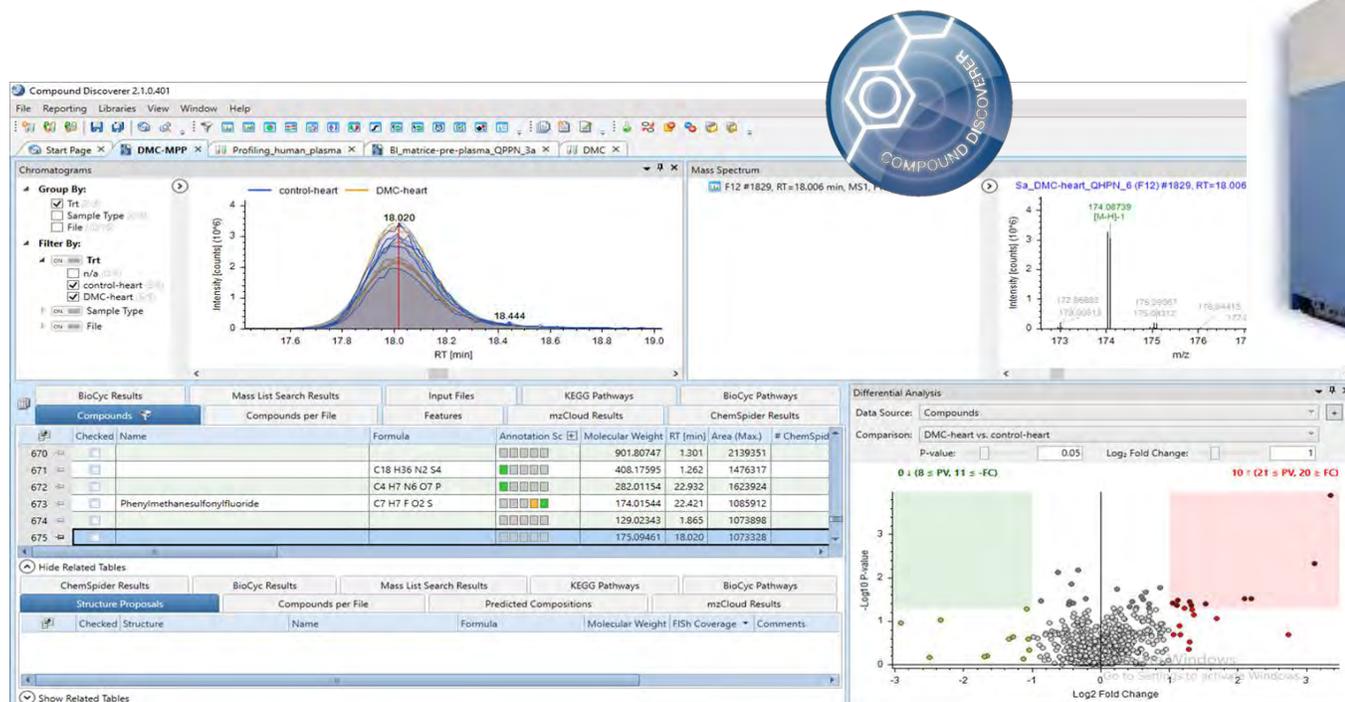
- Extraction of new bile acids ions signal from the profiling acquisition to complete the targeted analysis



Fidelle *et al.*; *Science*; 2023

Targeted or profiling analysis?

- **Profiling**: searching unk/novelties/putatives into full scan MS data
- **Strength**: 1000s of metabolites in one method, for novelties (drug fate), fingerprints (heatmaps, PCA), retro-active data treatment + identification
- **Weakness**: no straightforward for identification, not all pathways represented

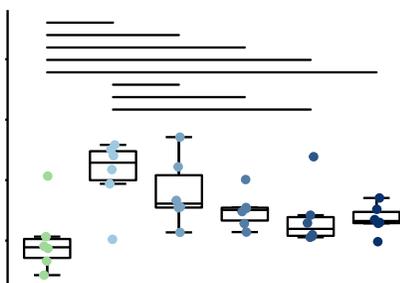


U3000 / Orbitrap HRMS Thermo

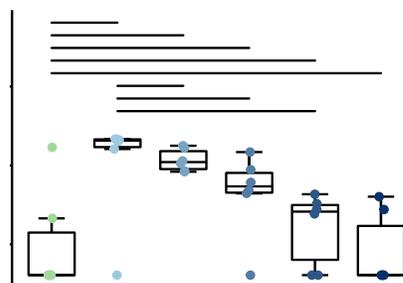
>> Profiling for drug fate: 3,4-dimethylchalcone

• Liver

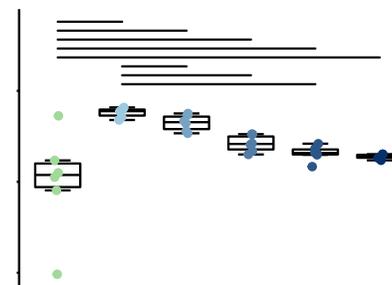
• Plasma



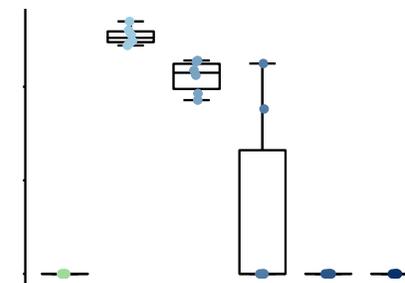
drug



drug metabolite



drug



drug metabolite

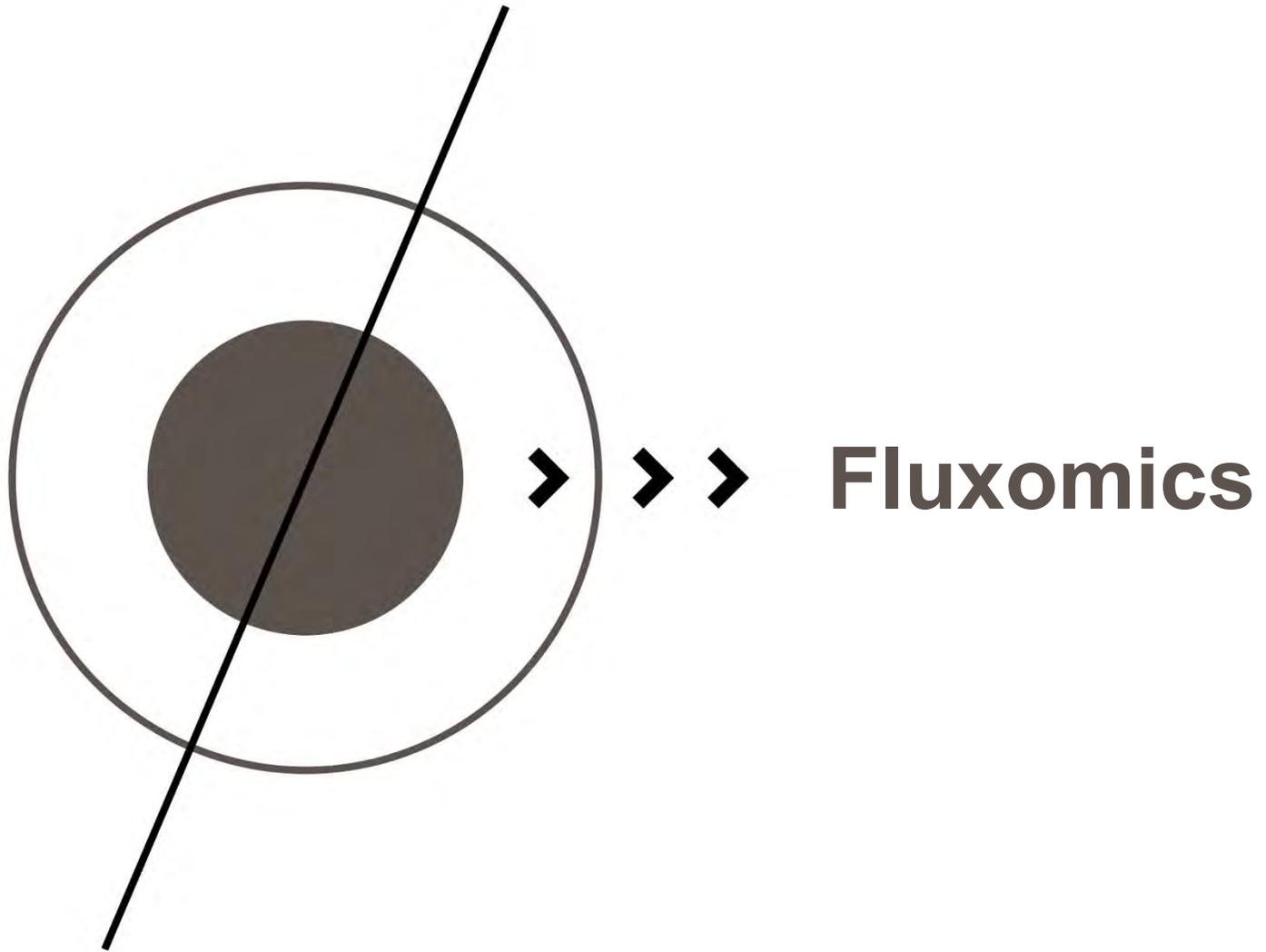
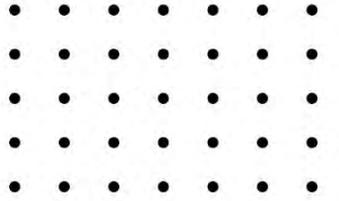
Unpublished data

In silico calculated transformation, and profiling of the corresponding m/z (observed transformation)

Observed transformation	Dealkylated
3,4-Dimethoxychalcone_Dehydration-Reduction	TRUE
3,4-Dimethoxychalcone_Dehydration-Reduction	FALSE
3,4-Dimethoxychalcone_NA	TRUE
3,4-Dimethoxychalcone_Reduction	TRUE
3,4-Dimethoxychalcone_Reduction	TRUE
3,4-Dimethoxychalcone_Reduction	TRUE
3,4-Dimethoxychalcone_Reduction-Sulfation	TRUE
3,4-Dimethoxychalcone_Desaturation-Oxidation-Glutamine-Conjugation	FALSE
3,4-Dimethoxychalcone_GSH-Conjugation-2	TRUE
3,4-Dimethoxychalcone_GSH-Conjugation-2	FALSE

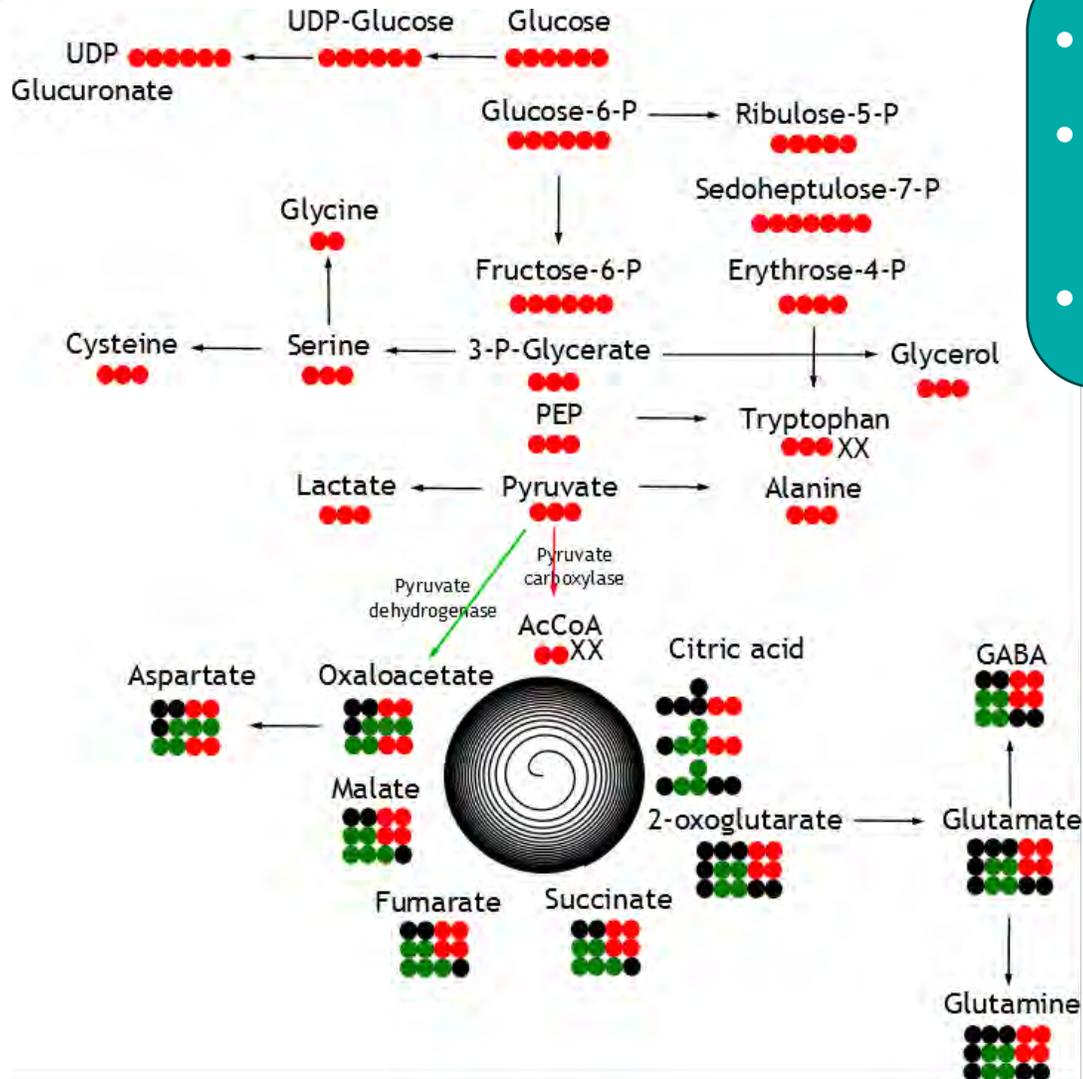


Cerrato *et al.*; *Cell Death Dis.*; 2023



Fluxomics

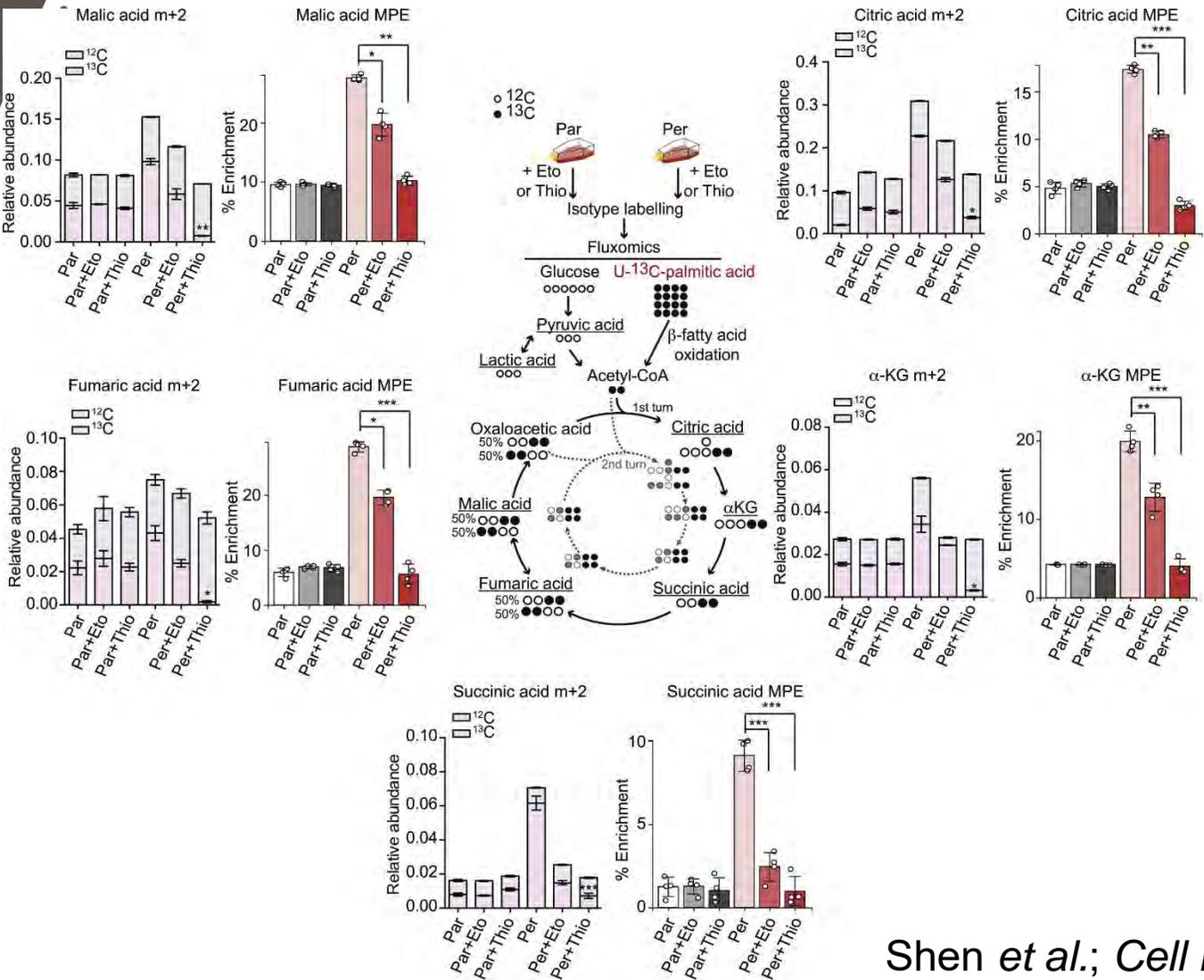
>> Fluxomics: labelling principle



- Nutrient labelled with **stable isotope** (^{13}C , ^2H)
- Glucose, glutamine, spermidine, arginine, methionine, palmitic acid...
- **Red/green: labelled; black: unlabelled**

>>> Fluxomics for dynamic experiments

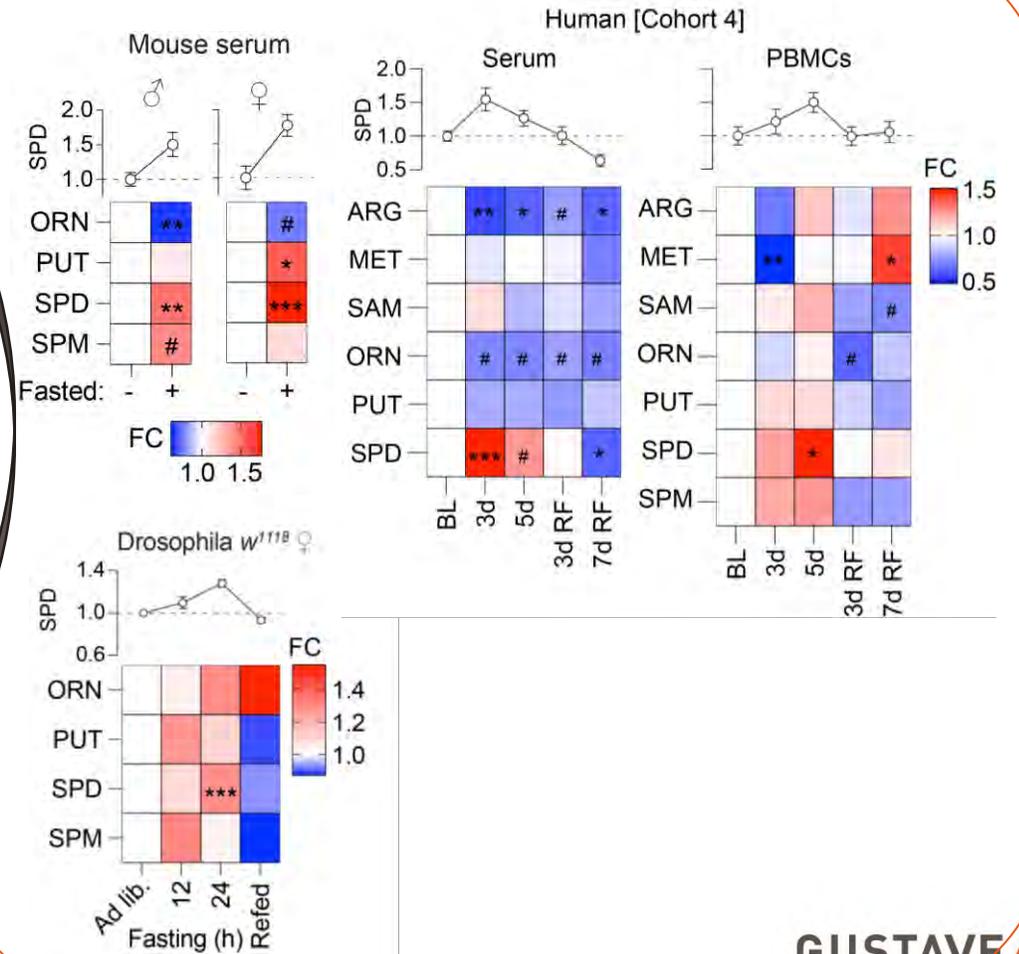
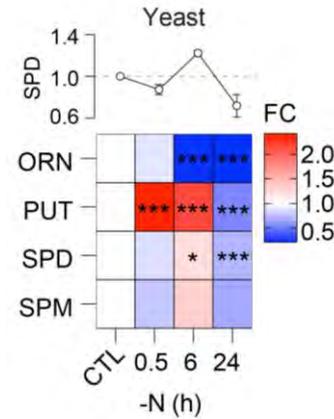
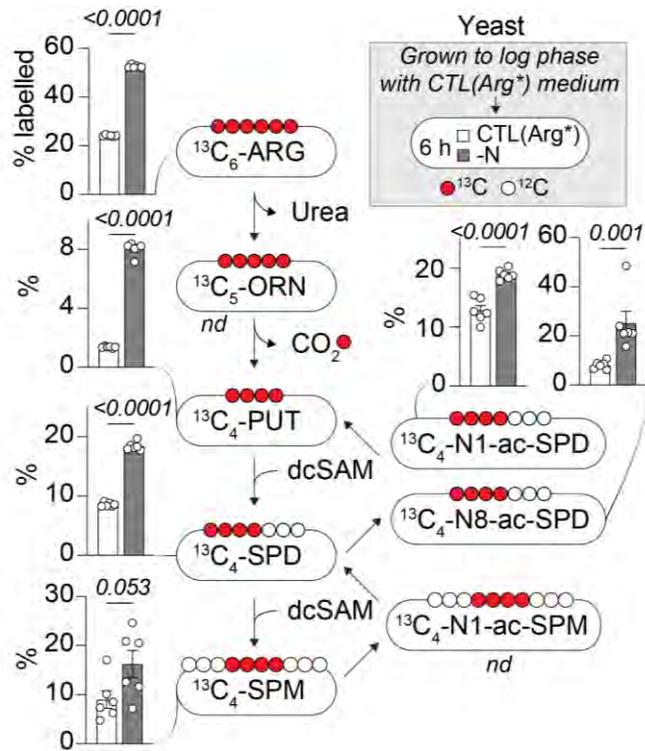
- Labelled glucose (white dots) or palmitic acid (black dots) as nutrients
- Ratios in barplots point out absorption of nutrient into the metabolomic pathway
- Information on the activity of the pathway



Shen et al.; Cell reports; 2020

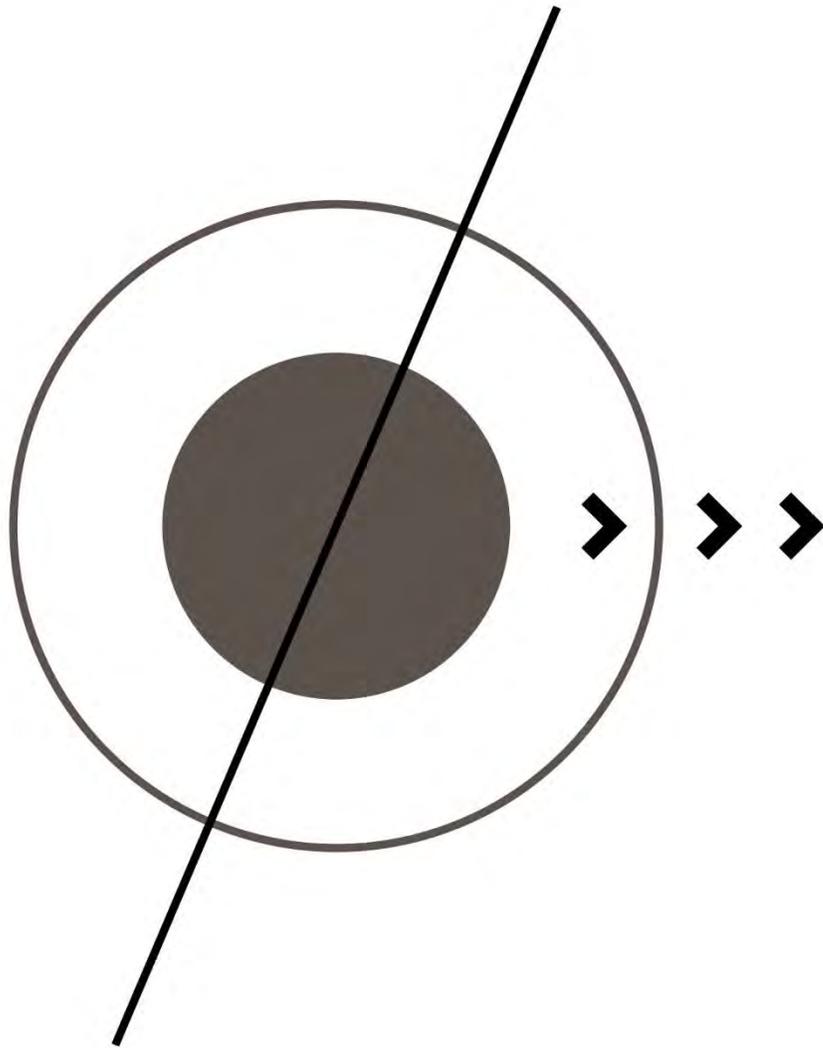
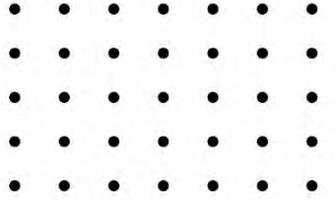
>> Fluxomics to emphasize bulk analysis

- Comparison of polyamines from multiple models **fasting**



- Fluxomics performed on yeast (comparisons arginine labelled with methionine labelled)

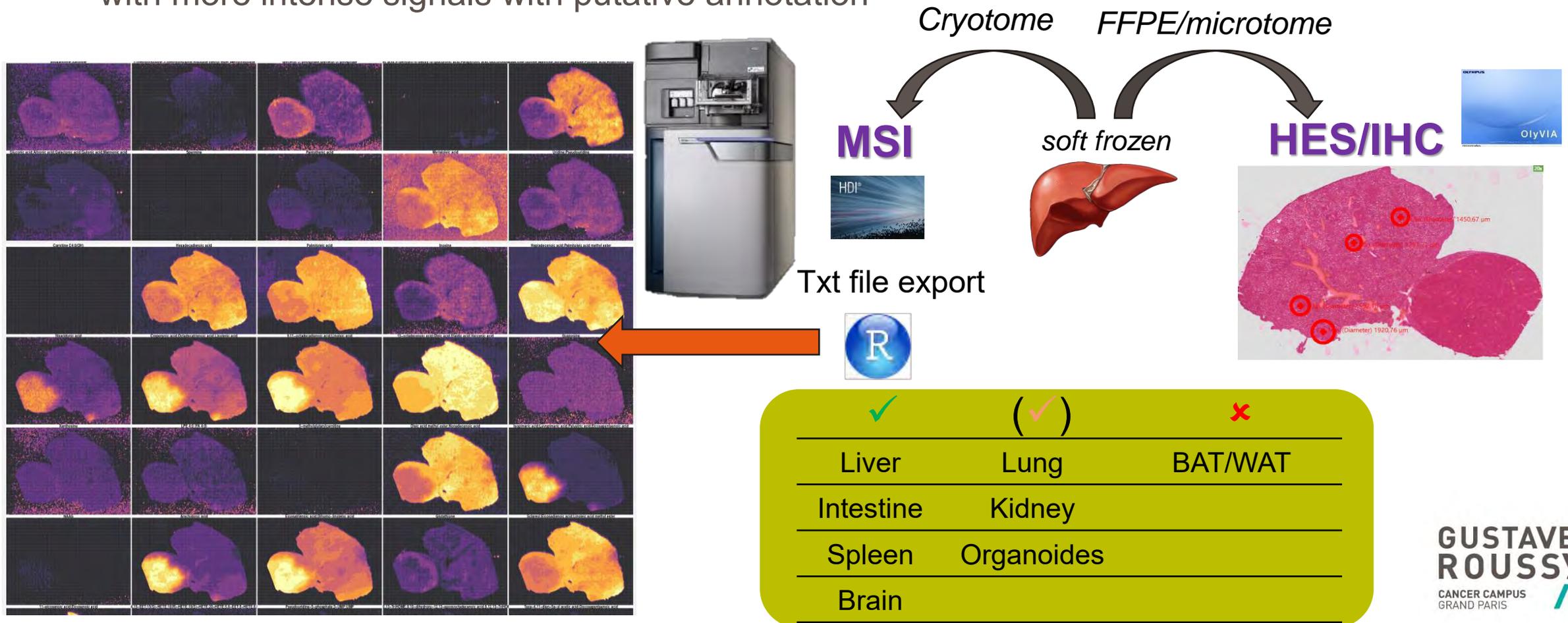
S. Hofer *et al.*, *in press*



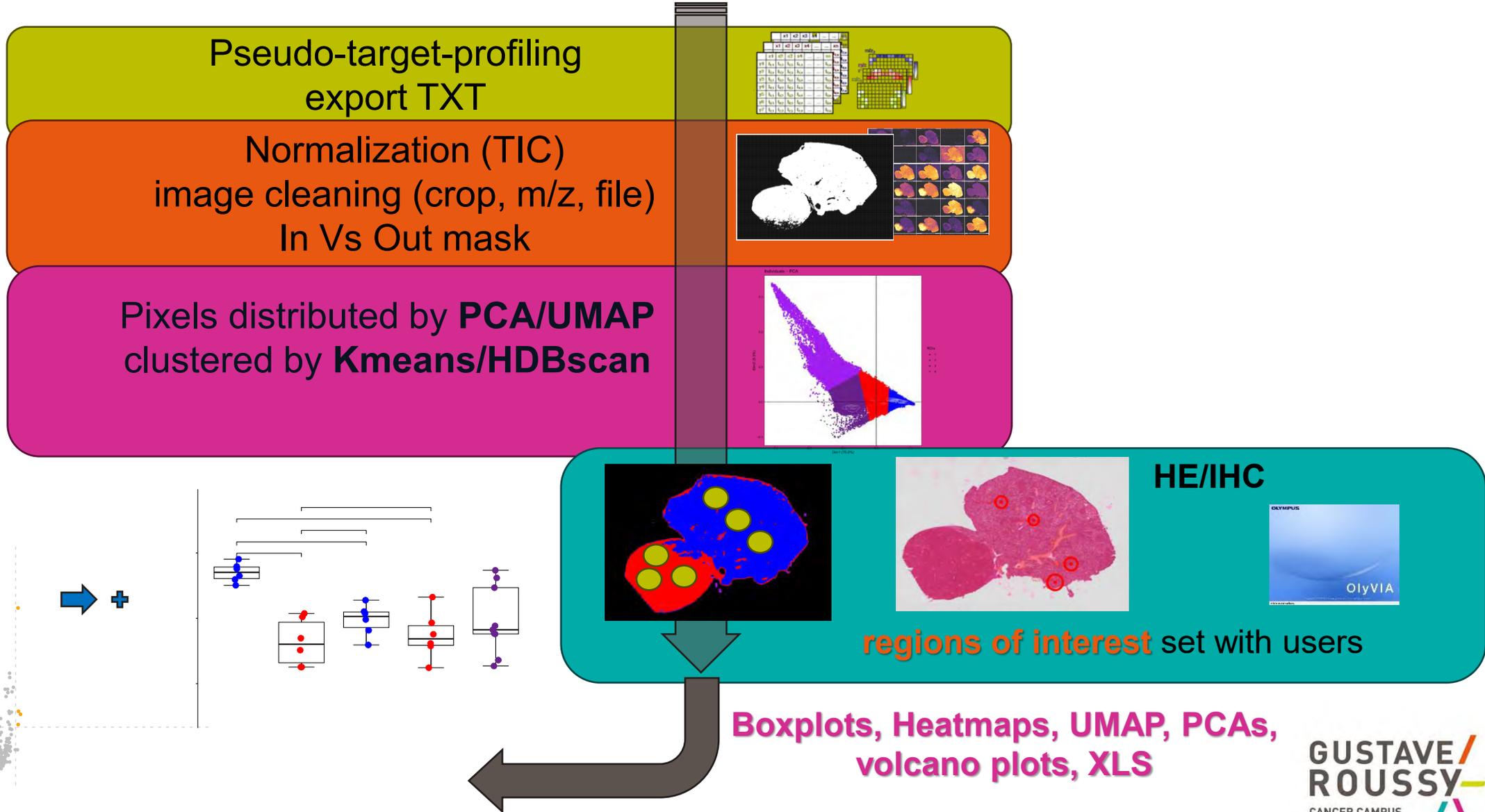
MS Imaging

>> MS Imaging by DESI: acquisition and multiplexing

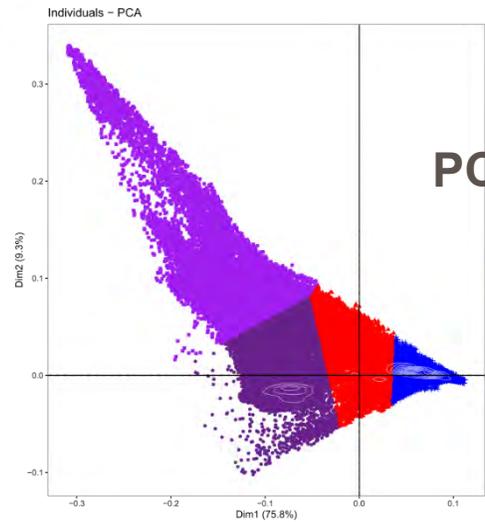
- **Spatial diversity** in organs, one pixel = one mass spectrum ($50 \mu\text{m}^2$)
- **Multiplexing** with IHC (GR *Pathologie Expérimentale et TRANslationnelle* platform)
- **Pseudo-profiling** pseudo-target of metabolites of interest + 1000 unknown metabolites with more intense signals with putative annotation



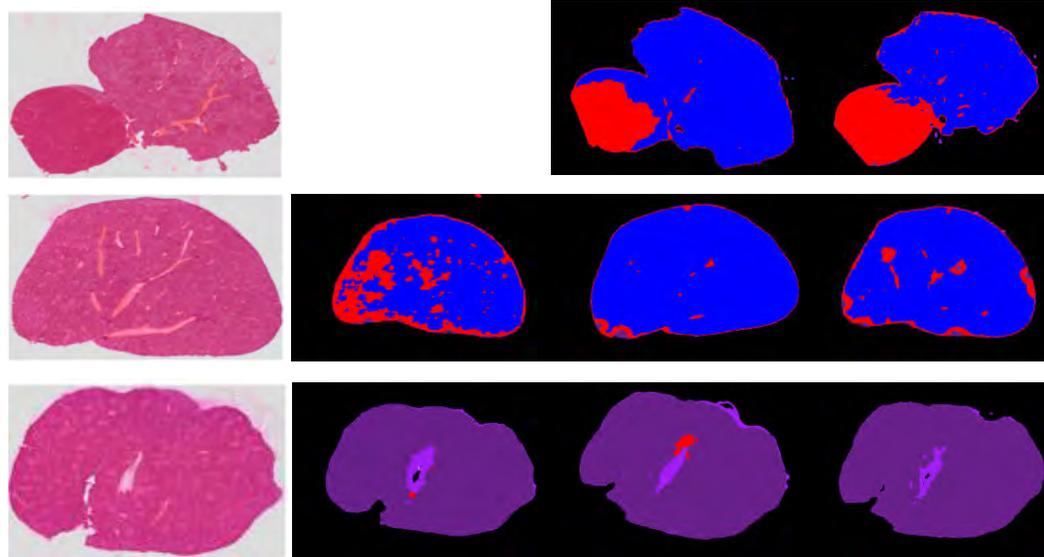
>> MS Imaging by DESI: outputs



>> MSI, some concretes



PCA to set ROIs



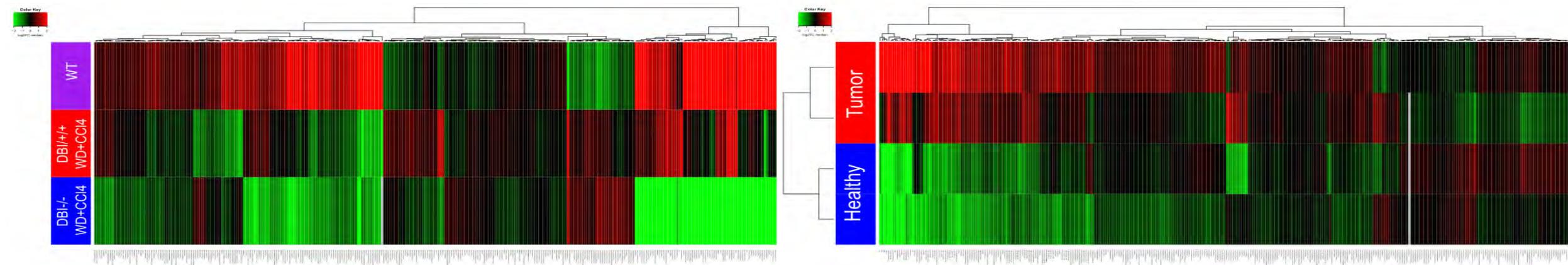
WD + CCI4-Ctrl
(Acbp +/+)

WD + CCI4-acbp ko
(Acbp -/-)

Liver WT control

Sijing Li *et al.*, *In press*

Data representation from ROIs



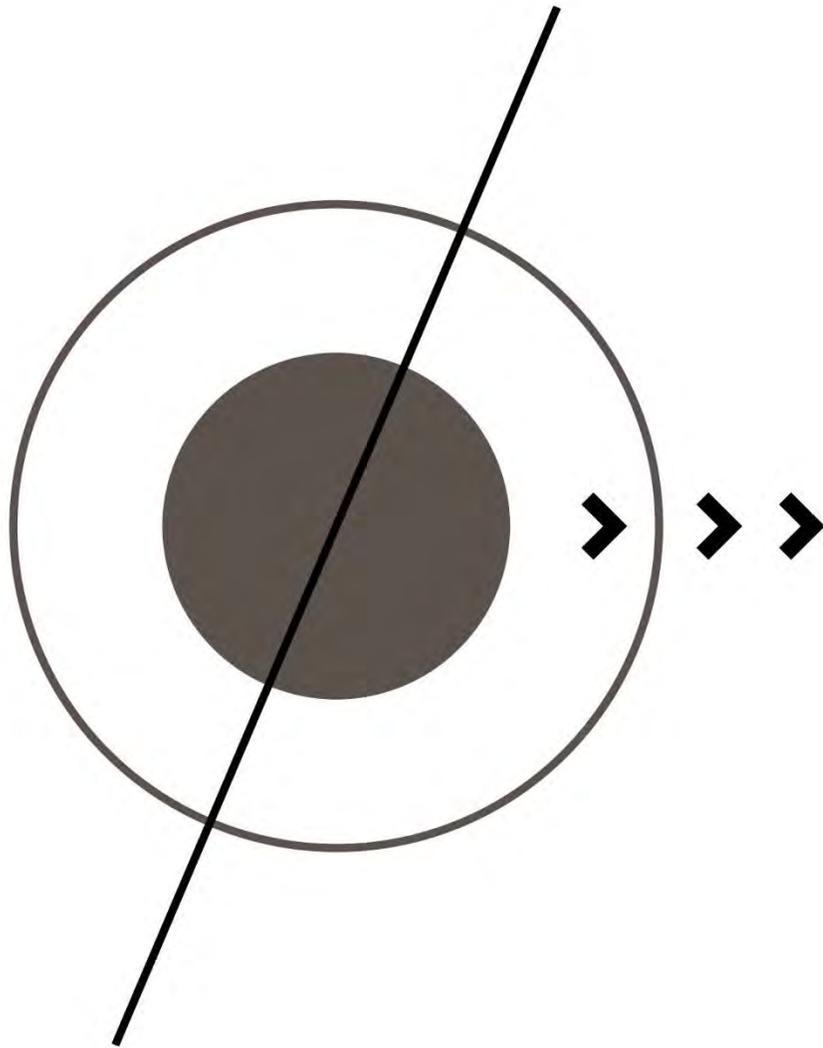
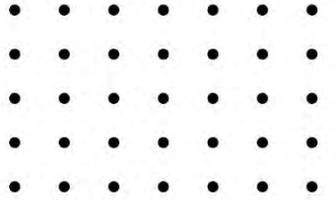
> >>
Contact us :

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Fanny.aprahamian@gustaveroussy.fr



Annexes : targeted methods

Validated metabolites: generic profiling (P1)

Hundreds of metabolites entries in the GR compound database
Schymanski's level annotation 1/2/3

Ascorbic acid
NAD

Niacinamide
Nicotinic acid
Pantothenic acid
Retinol
Riboflavin

S-adenosylhomocysteine
S-adenosylmethionine
S-lactoylglutathione

Succinyladenosine
Taurine
Trimethyl-lysine

N2-acetyllysine
N6-acetyllysine
NAAG
N-acetylaspartic acid
N-acetylglutamic acid
N-acetylglutamine
N-acetylputrescine
N-acetylspemidine

Butyrylglycine
Choline phosphate
Glycerol-3-phosphate
Glycerophosphorylcholine
Hexanoylglycine
O-phosphoethanolamine

PCae(14:0)
PCae(15:0)
PCae(16:0)
PCae(16:1)
PCae(17:0)
PCae(18:0)
PCae(18:1)
PCae(18:2)
PCae(20:1)
PCae(20:3)
PCae(20:4)
PCae(20:5)
PCae(22:6)
Phosphocreatine
Sphingosine
Sphingosine-1-phosphate

2-hydroxyglutaric acid
3-hydroxybutyric acid
cis-Aconitic acid
Malonic acid
Phosphoenolpyruvic acid
Succinic acid
trans-Aconitic acid

Aspartic acid
Cysteine
Glutamic acid
Glutamine
Histidine
Isoleucine
Leucine
Methionine
Ornithine
Phenylalanine
Proline
Threonine
Tryptophan
Tyrosine

Arachidonic acid
Capric acid
Caproic acid
Caprylic acid
Eicosadienoic acid
Eicosenoic acid
Isovaleric acid
Linoleic acid
Margaric acid
Myristic acid
Oleic acid
Sebacic acid
Stearic acid
32 FAs

Carnitine
34 ACs

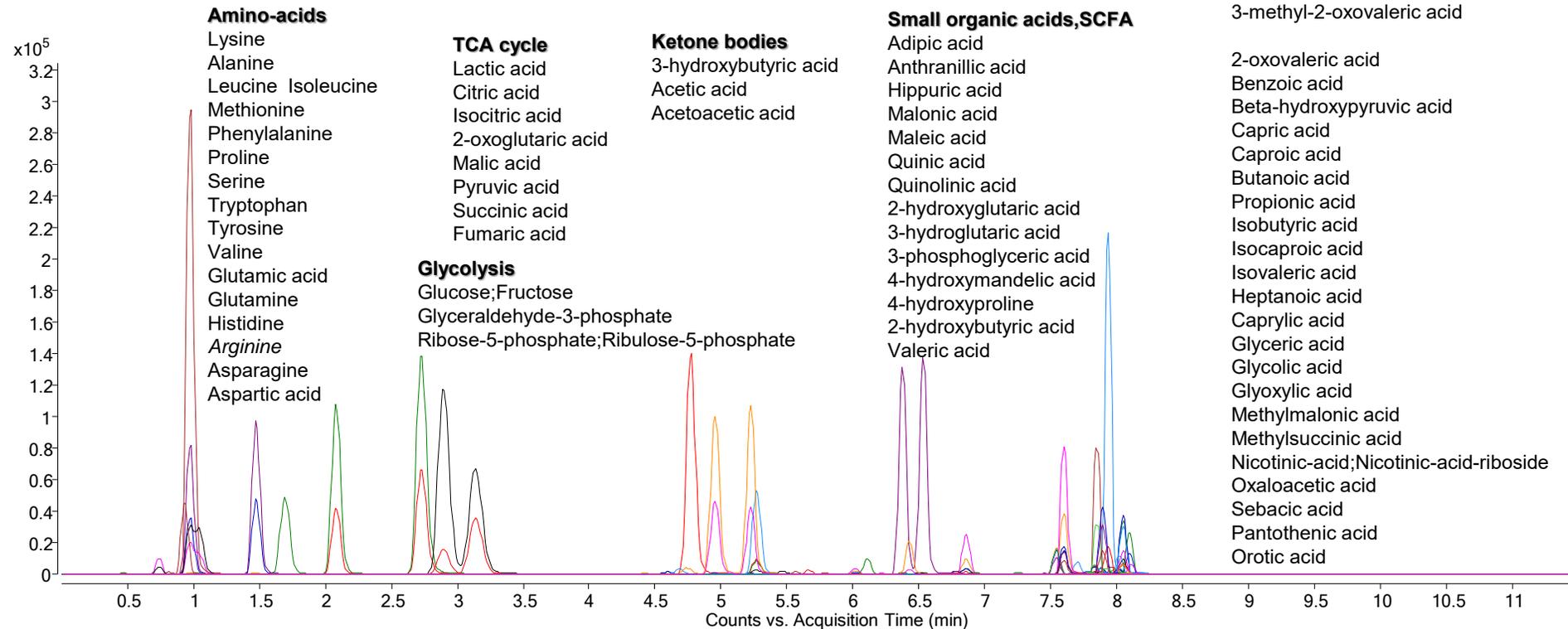
2-Phenylglycine
Anacardic acid
Benzoic acid
Caffeine
Cholic acid
Corticosterone
Cortisol
Coumaric acid
Cystamine
Cysteinylglycine
Dimethyl-oxoglutaric acid
Erythritol
Estradiol
Gamma-glutamylleucine
Gamma-glutamylphenylalanine
Gamma-glutamyltyrosine
Gentisic acid
Glycolic acid
Glycylglycine
Hippuric acid
Hydrocinnamic acid
Imidazole
Leucylproline
Lithocholic acid
N-glycolylneuraminic acid
Phenylalanylphenylalanine
Phenylbutyric acid
Proline betaine
Pyrogallol
Quinic acid
Taurocholic acid
Taurodeoxycholic acid
Ursodeoxycholic acid

2-deoxyadenosine
2-deoxycytidine
2-deoxyguanosine
5-methyluridine
Adenine
Adenosine
ADP
ADP-ribose
AMP
cAMP
CDP
CMP
CMP-N-acetylneuraminatone
CTP
cUMP
Cytidine
Cytosine
GDP
GMP
Guanosine
Hypoxanthine
IMP
Inosine
N1-methyladenosine
N1-methylguanosine
Thymidine
Thymine
UDP
UMP
Uracil
Ureidosuccinic acid
Uric acid
Uridine
Xanthine
Xanthosine

2-hydroxybutyric acid
3-indoxyl sulfic acid
3-methylhistidine
4-hydroxyproline
5-aminovaleric acid
5-methylthioadenosine
Aminocaproic acid
Anthranilic acid
Dimethylarginine
Dimethylglycine
GABA
Glutathione
Guanidinoacetic acid
Homovanillic acid
Hydroxyphenyllactic acid
Hypotaurine
Indole-3-butyric acid
Indole-3-lactic acid
Indole-3-propionic acid
Ketoisovaleric acid
Kynurenic acid
Kynurenine
Methionine sulfoxide
Methylindole-3-acetic acid
Ox. glutathione
Phenylacetylglutamine
Phosphoserine
Pipelicolic acid
Quinolinic acid
...

SCFA / KB / TCA / Aas / Glycolysis (T1)

- Short chain fatty acids, Ketone Bodies, Tricarboxylic acids cycle, amino acids, glycolysis
- Application : fecal contents, tissues, plasma/serum
- HPLC/MS-MS with derivatization, alternative to T1
- **72 metabolites** detected with sensitivity & discrimination



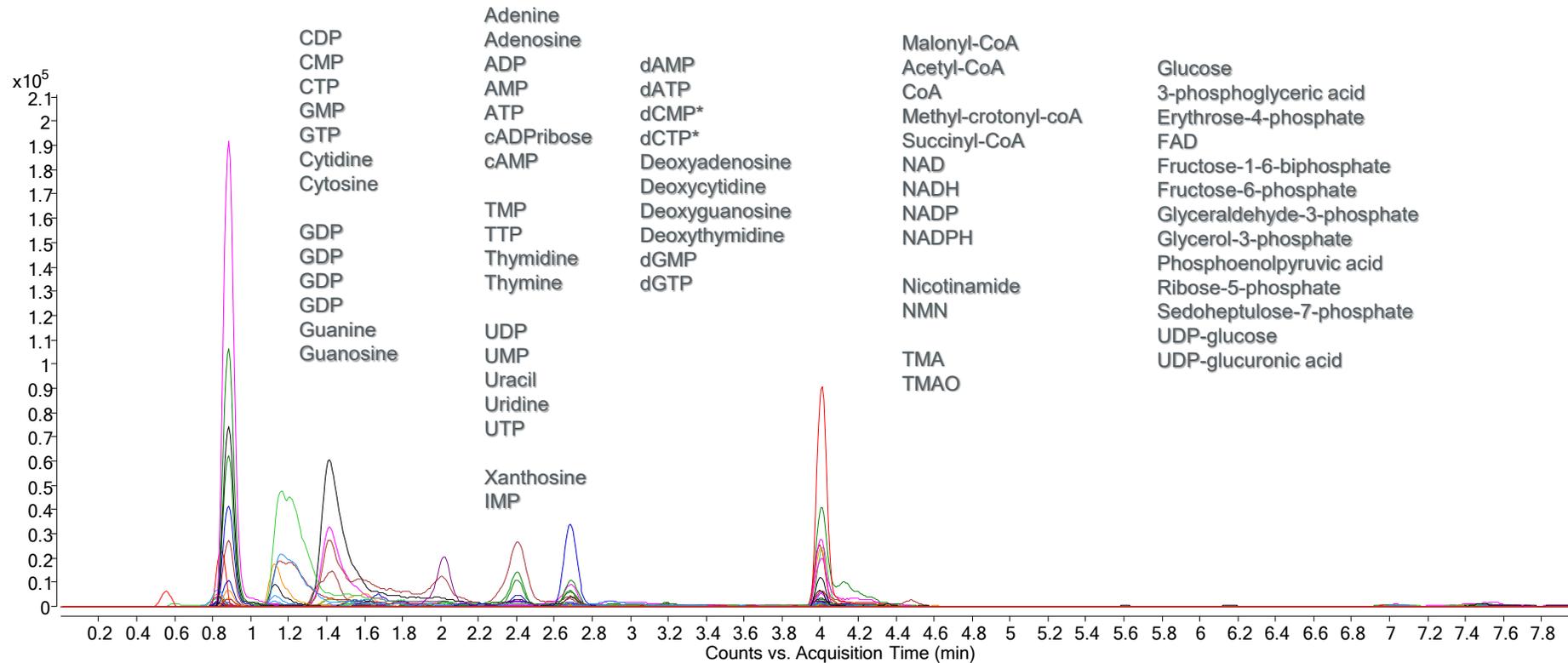
Currency metabolites (T2)

67 metabolites measured by UHPLC/MSMS

Applications: cultured cells, tissues and fecal content (dNTPs)

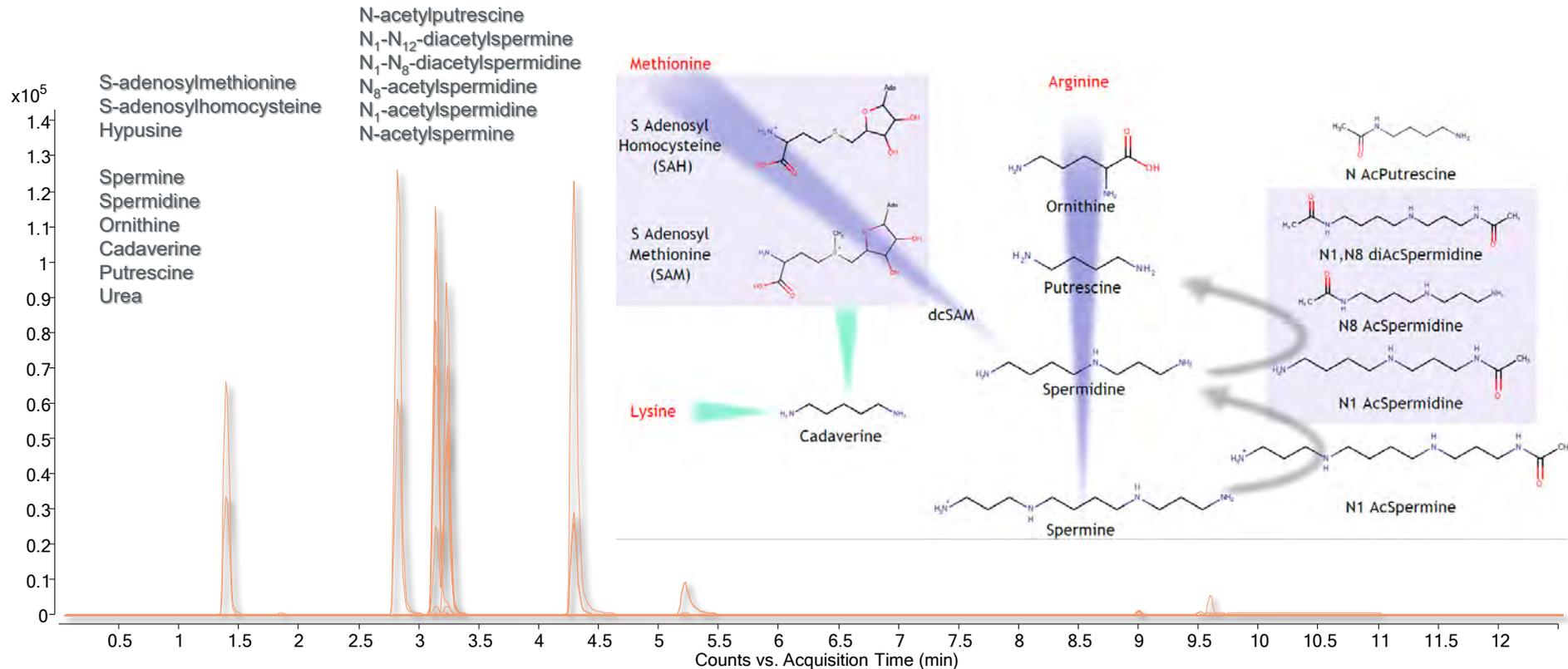
Accurate quantification of adenylate energy charge ratio

Glycolysis pathway



Polyamines (T3)

15 metabolites polyamines and derivated measured by UHPLC/MSMS + **13 Aa, TMA/TMAO**
 Applications: tissues, plasma, gut and cultured cells

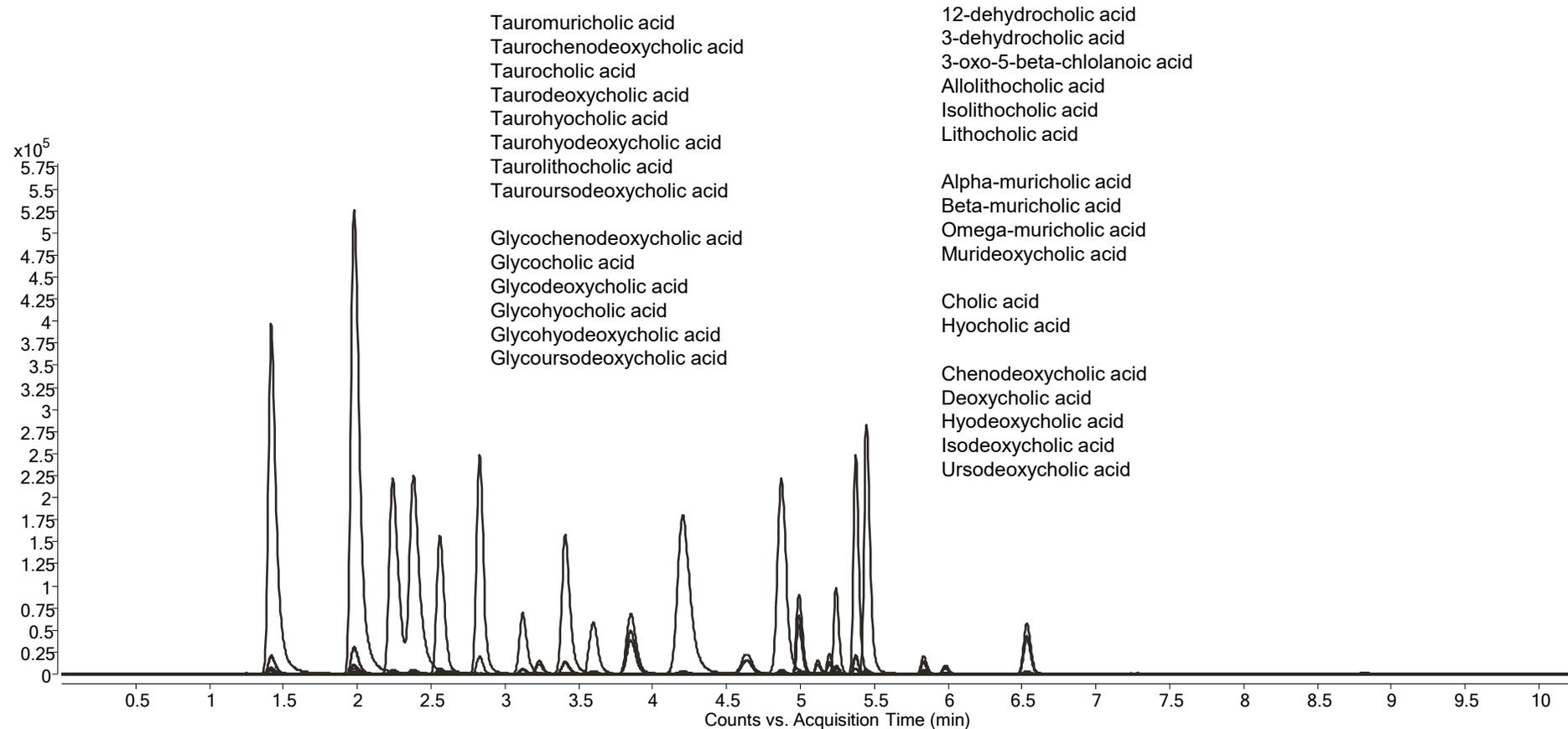


Alanine
 Arginine
 Beta-Alanine
 Betaine
 Citrulline
 Cysteine
 Isoleucine
 Leucine
 Lysine
 Sarcosine
 Tyrosine
 Valine

TMA
 TMAO

Bile acids (T4)

Applications : tissues, plasma and gut microbiota
 Biles Acids, unconjugated and Tauro/Glyco conjugated



>> NFPA (T5)

Ion pairing LC/QQQ, diversity, polar metabolites,

Glycolysis/PPP

Sedoheptulose-7-phosphate
Glycerol-3-phosphate
Glyceraldehyde-3-phosphate
Phosphoenolpyruvic acid
Fructose-6-phosphate
Fructose-1-6-biphosphate
Erythrose-4-phosphate
Ribose-5-phosphate

Oses

Sucrose
Sorbitol; Mannitol
Ribitol; Xylitol
Raffinose
UDP glucose
Glucose
Erythritol

Aminoacids

Tyrosine
Tryptophan
Taurine
Serine
Proline
Phenylalanine
Methionine
Lysine
Isoleucine
Leucine
Homoserine
Histidine
Histamine
Glycine
Glutamic acid
Beta-alanine
Aspartic acid
Arginine

Alanine
Cystine
Cysteine
Citrulline
S-adenosylhomocysteine
S-adenosylmethionine
Dimethylglycine
Hypusine
Hypotaurine
Kynurenine
Sarcosine
Ornithine

Nucleo(t/s)ides, bases

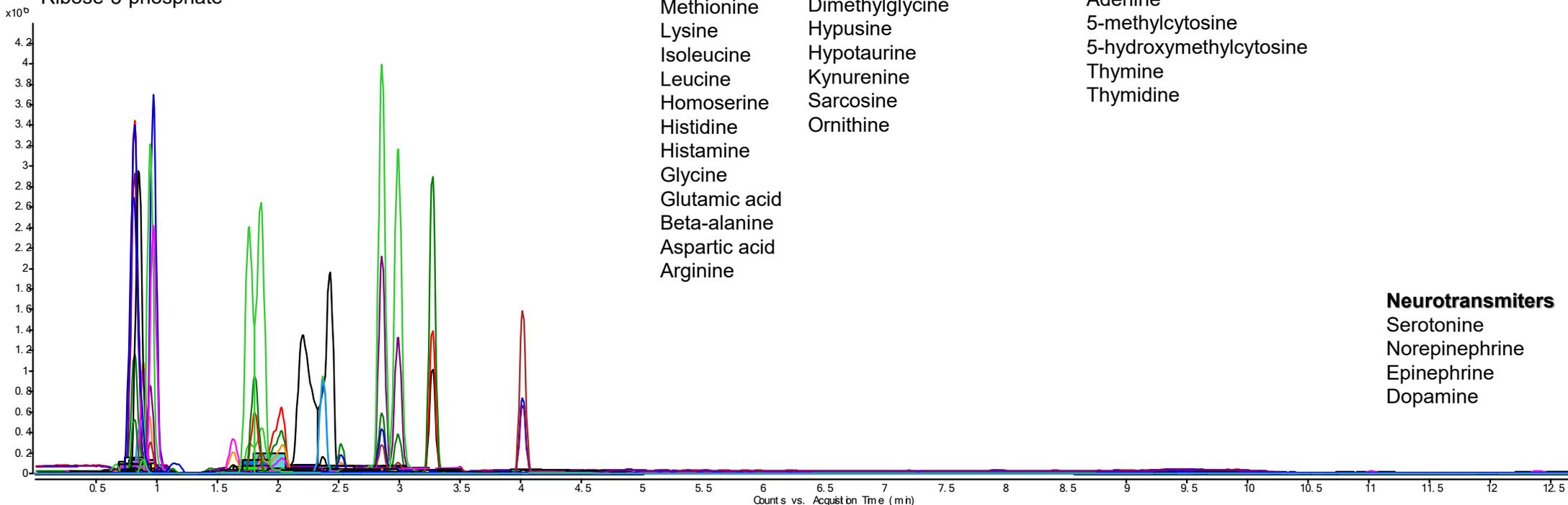
Nicotinamide
NAD
FAD
AMP
Guanosine
Guanine
Cytosine
Cytidine
Adenosine
Adenine
5-methylcytosine
5-hydroxymethylcytosine
Thymine
Thymidine

Small Organic acids

GABA
Betaine
Acetylcholine
4-hydroxyproline
3-methylhistamine
TMAO
TMA

Neurotransmitters

Serotonin
Norepinephrine
Epinephrine
Dopamine



>>> Lipidomic (T6)

Several classes covered

CE / DimethylCE / MethylCE

Ceramides

Cholesterol

MG / DG / TG

PC / PI / PG / PS

LPC / LPE / LPI

SM / Sphingosine-1-phosphate

Ubiquinone

