LABERCA

Oniris VetAgroBio Nantes ÉCOLE NATIONALE

INRAE

TRACES

Ghent, 3-6 June 2025

INTEGRATIVE METABOLOMICS APPROACH

FOR

IMPROVED DETECTION OF GROWTH PROMOTER MISUSE IN LIVESTOCK

C Cloteau, J Boccard, M Letertre
B Le Bizec, G Dervilly

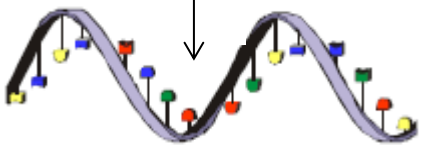
OMICS

INFORMATION STORAGE
« *what can happen?* »

PROCESSING
« *what makes it happen?* »

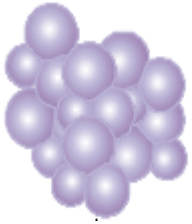
EXECUTION
« *what has happened / is happening?* »

DNA



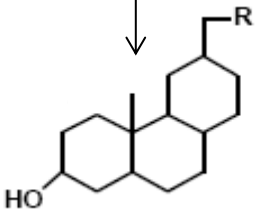
RNA

TRANSCRIPTOMICS



PROTEINS

PROTEOMICS



METABOLITES

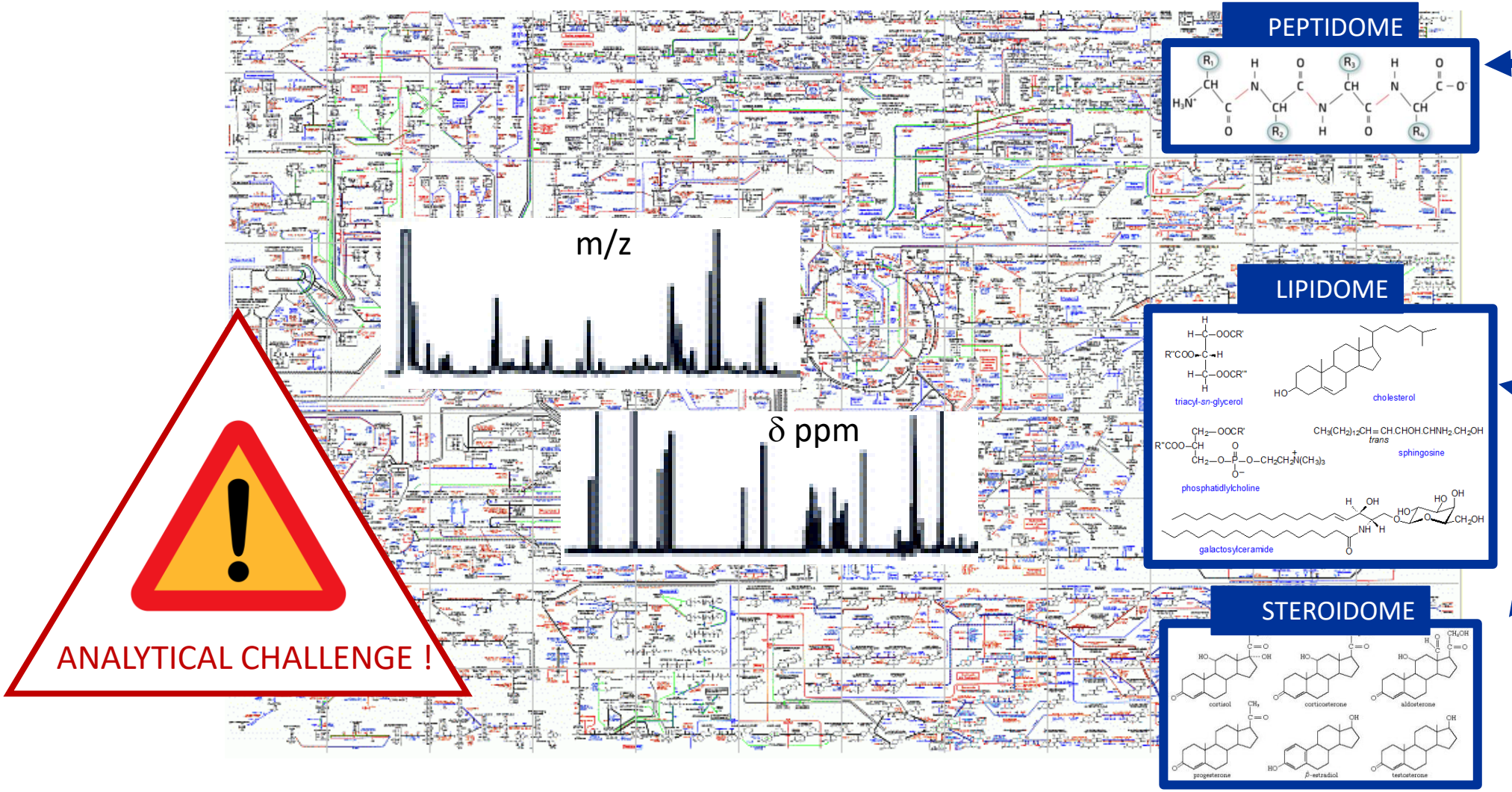
METABOLOMICS

BIOMARKERS OF EFFECT

METABOLOMICS

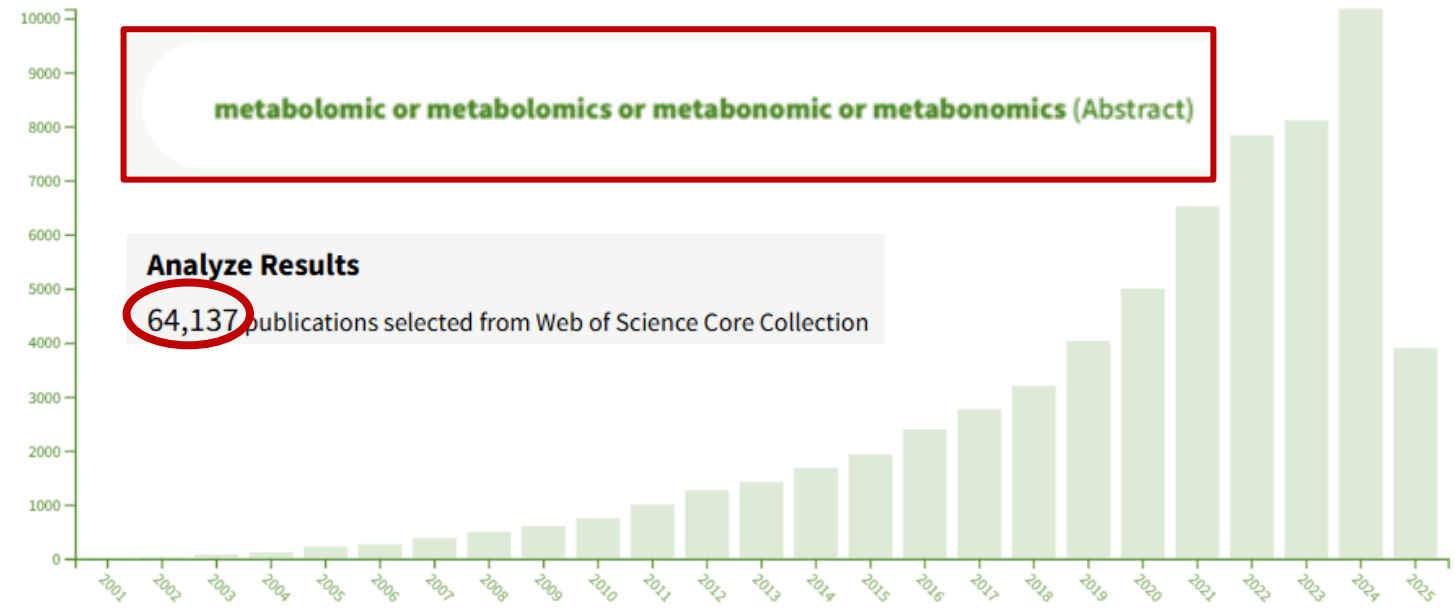
Metabolome = the total metabolite pool

All low molecular weight (MW < 1000 Da) organic molecules in a sample

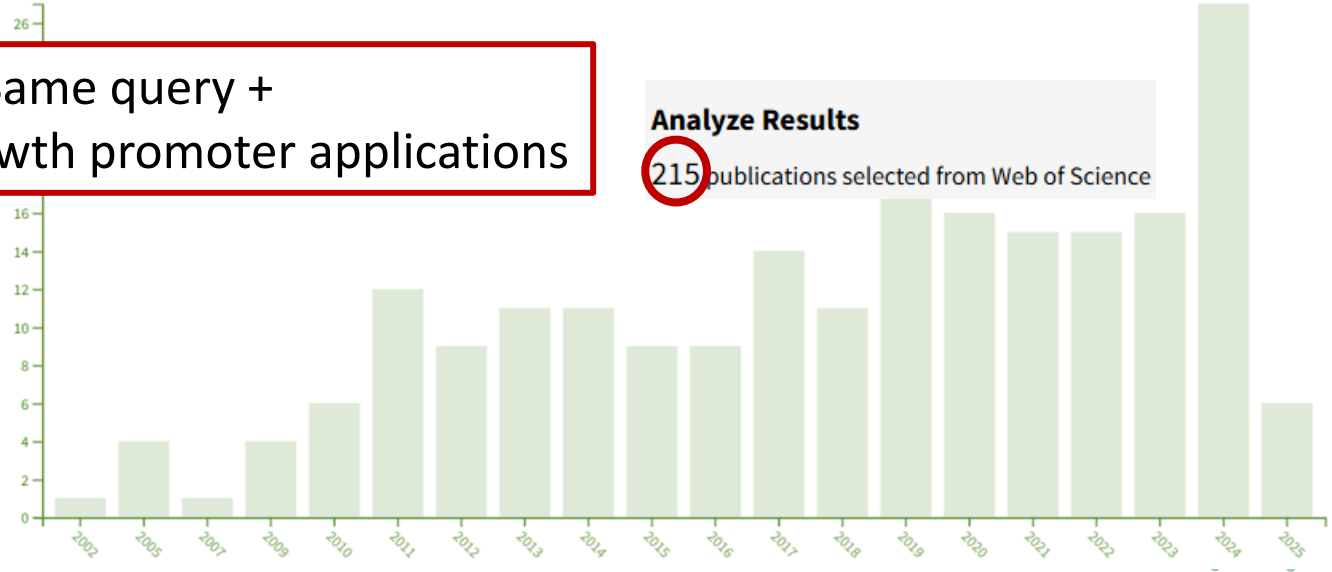


Peptides
Oligonucleotides
Sugars
Nucleosides
Organic acids
Ketones
Aldehydes
Amines
Amino acids
Lipids
Steroids
Alkaloids

METABOLOMICS: A Promising Tool?

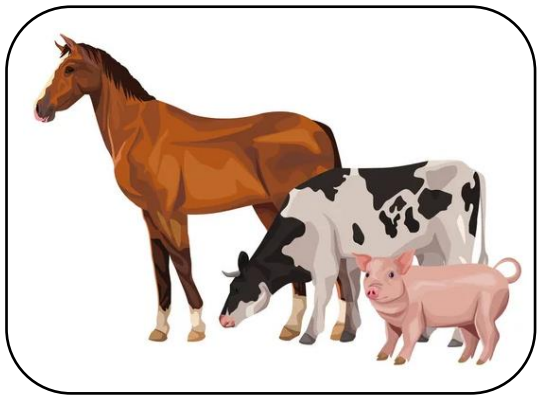


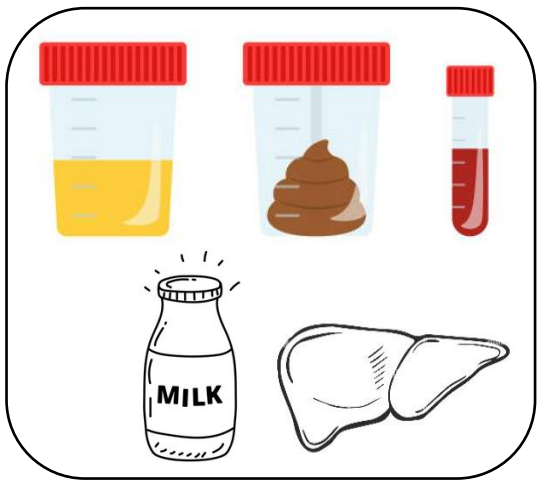
Same query +
anabolic / growth promoter applications



METABOLOMICS to screen for anabolic practices

2005





HRMS
RPLC / HILIC
ESI +/-

β-AGONISTS
Clenbuterol, ractopamine, cimaterol, zilpaterol, mabuterol

STEROIDS
Nandrolone, trenbolone, testosterone, estradiol

CORTICOSTEROIDS
Prednisolone, Dexamethasone

SARMS
Ostarine, Bicalutamide

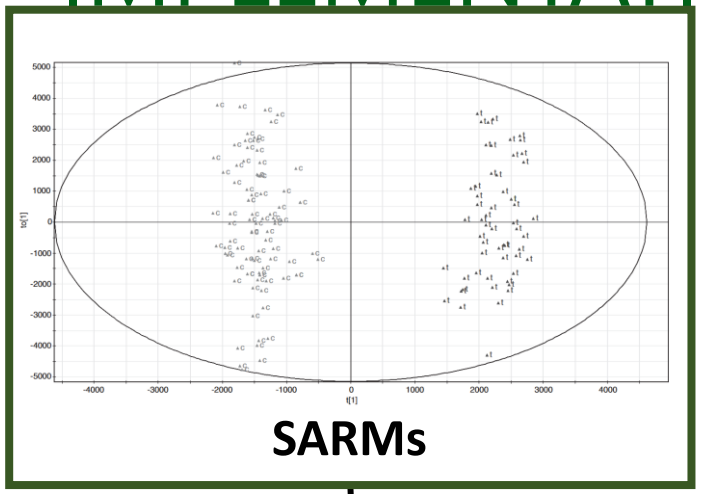
rGH
rbGH, reGH

2025

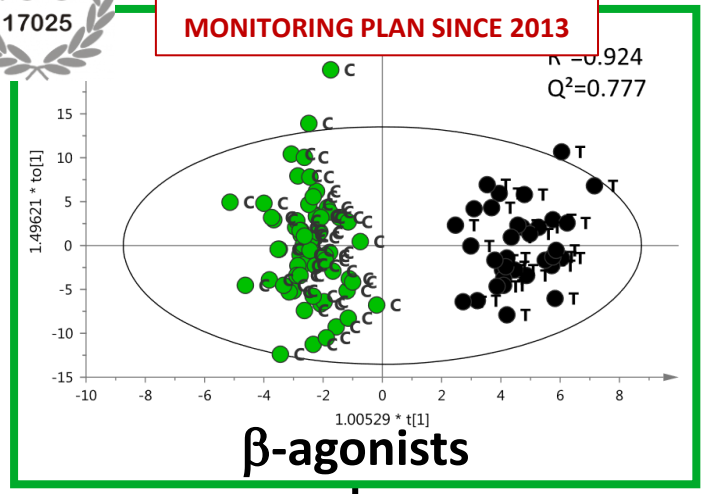
FROM PROOF OF CONCEPT TO IMPLEMENTATIC

ISO 17025

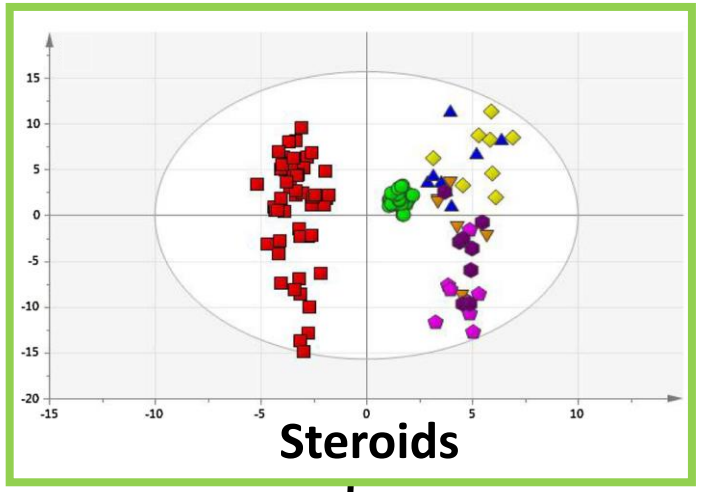
FRENCH NATIONAL MONITORING PLAN SINCE 2013



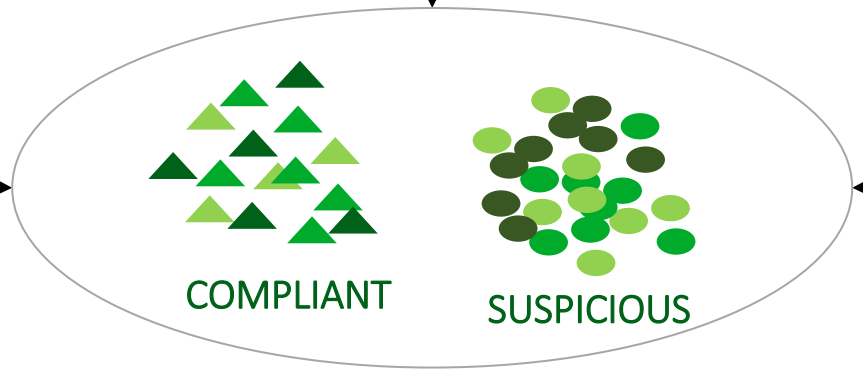
SARMs



β-agonists



Steroids



TOWARDS A UNIVERSAL MODEL FOR ANABOLIC AGENT DETECTION?

DIFFERENT METHODS

INCOMPATIBLE DATA

EXPERIMENTAL DESIGN

STEROIDS

- **Boldenone** — 1 mg/kg
- **Boldione** — 100 mg/jour, 4 days
- **Nandrolone Estradiol** — 150 mg every 2 weeks, 3x

β-AGONISTS

- **Clenbuterol** — 8 µg/day, 21 days
- **Clenbuterol** — 0.7 mg/day, 21 days

SARMS

- **Ostarine** — 200 mg or 2.4 mg/day, 21 days

6 EXPERIMENTS

3 ANABOLIC CLASSES (oral or IM)

60 ANIMALS (2 breeds, different regions)

>500 URINE SAMPLES (1/3 CONTROL, 2/3 TEST)

6 animals
Pre-ad (n=10)
Post-ad (n=54)
D35

D38

D33

animals
Post-ad (n=51)
Contrôles (n=26)

12 animals
Pre-ad (n=17)
Post-ad (n=76)
Contrôles (n=83)
D21

Frison Holstein

METABOLOMICS WORKFLOW

n=502

10 kDa filtration

Normalisation

RPLC & HILIC

Full scan
ESI+ & ESI-

Fingerprint 1
RPLC-ESI+

Fingerprint 2
RPLC-ESI-

Fingerprint 3
HILIC-ESI+

Fingerprint 4
HILIC-ESI-

DATA PROCESSING

Data Set 1
RPLC-ESI+

Data Set 2
RPLC-ESI-

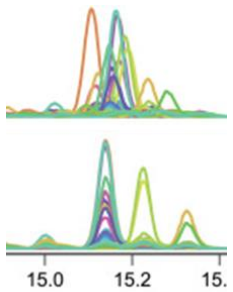
Data Set 3
HILIC-ESI+

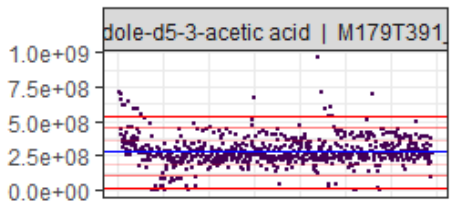
Data Set 4
HILIC-ESI-

PEAK PICKING

> 5700 features

- m/z dev: 5 ppm
- Pre Filter: 3, 3000
- Noise filter : 1000
- S/N: 4
- Peak Width: 3,30 s

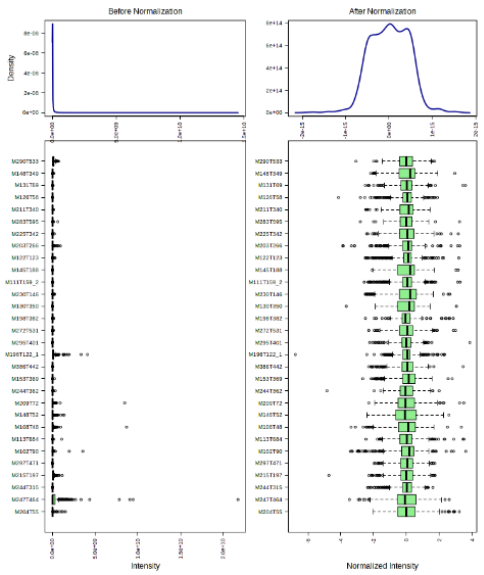




FILTERING

> 2900 features

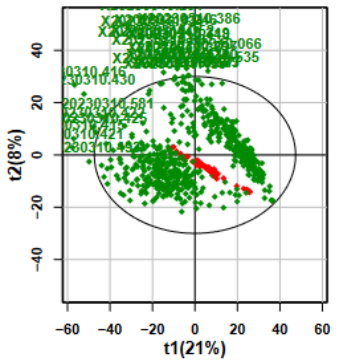
- NA < 20 %
- FoldChange > 2

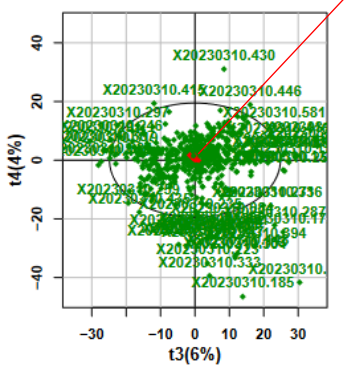


SIGNAL NORMALISATION

1700 features

- LOESS
- Log 10
- Pareto



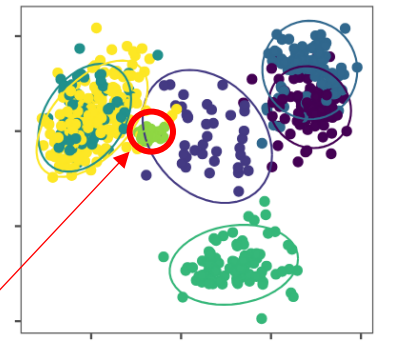


SAMPLE NORMALISATION

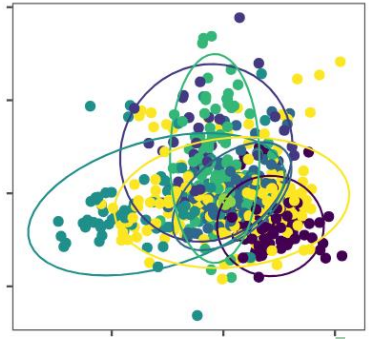
1060 features

- T0 normalisation

$$time\ 0\ centered = \frac{postT(x) - preT(\text{x reference})}{SD(\text{x} - \text{x reference})}$$



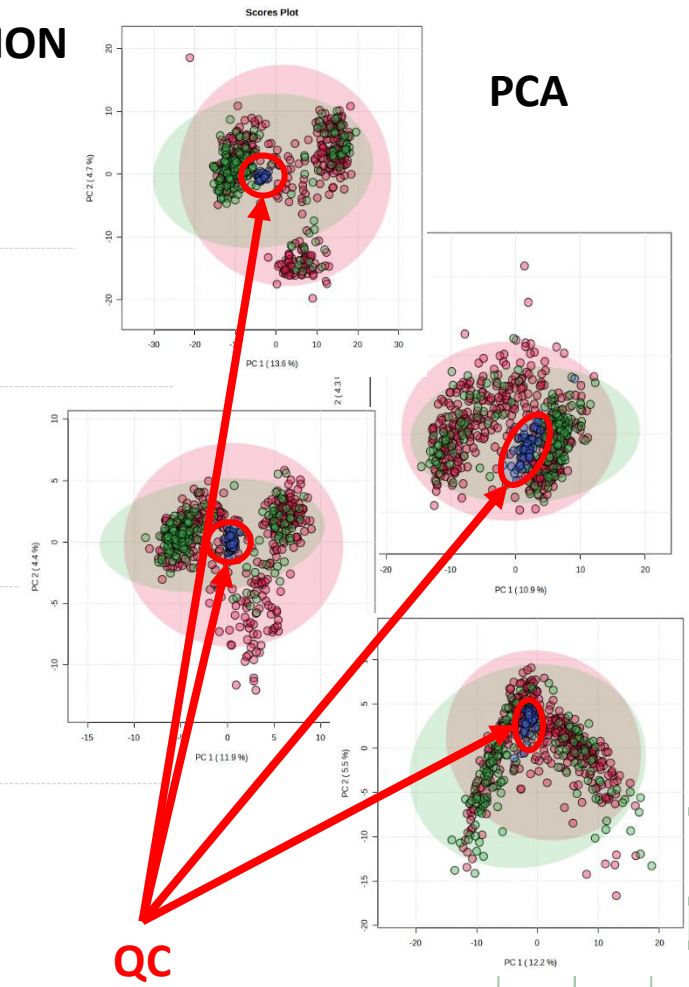
EXP.1
EXP.2
EXP.3
EXP.4
EXP.5
EXP.6



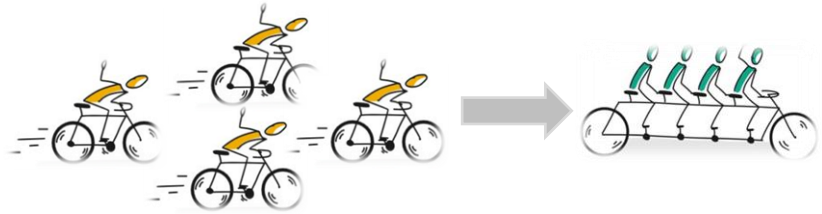
DATA PROCESSING

	PEAK PICKING	FILTERING	SIGNAL NORMALISATION	SAMPLE NORMALISATION
Data Set 1 RPLC-ESI+	> 5700 features	> 2900	1700	1060
Data Set 2 RPLC-ESI-	> 5100 features	> 1800	1198	980
Data Set 3 HILIC-ESI+	> 4800 features	> 1400	500	377
Data Set 4 HILIC-ESI-	> 3900 features	> 1400	533	398

PCA



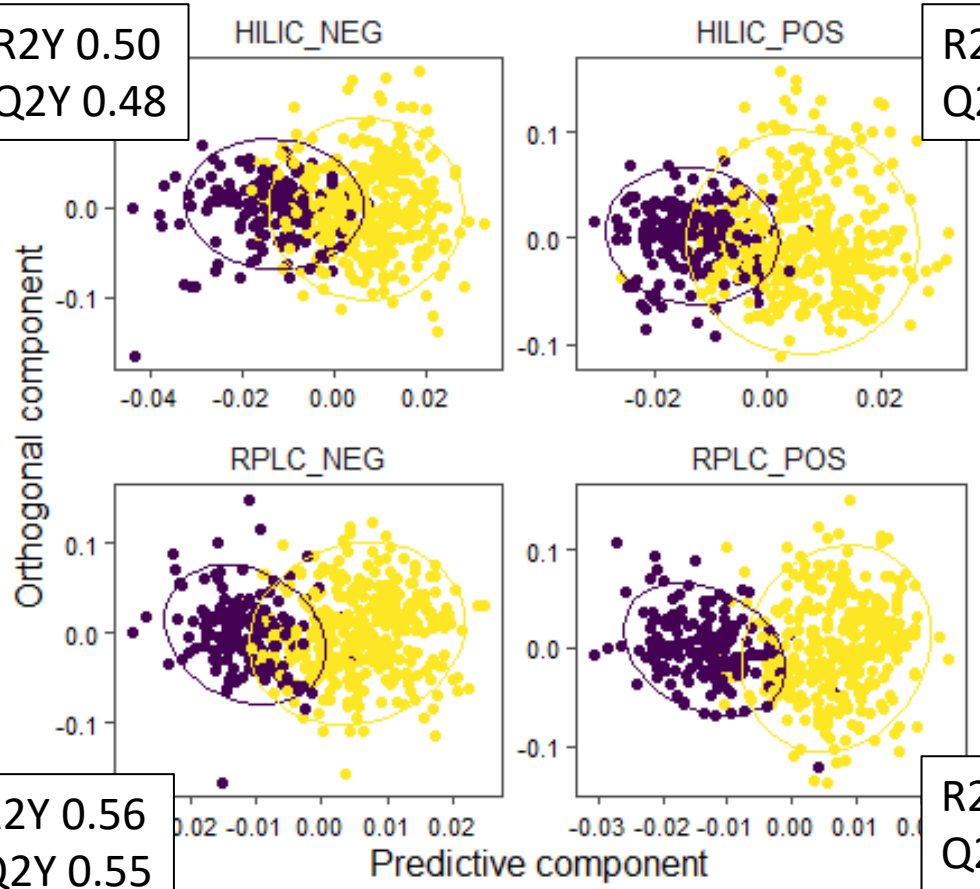
MULTI-BLOCK INTEGRATION



OPLS

R2Y 0.50
Q2Y 0.48

R2Y 0.53
Q2Y 0.43

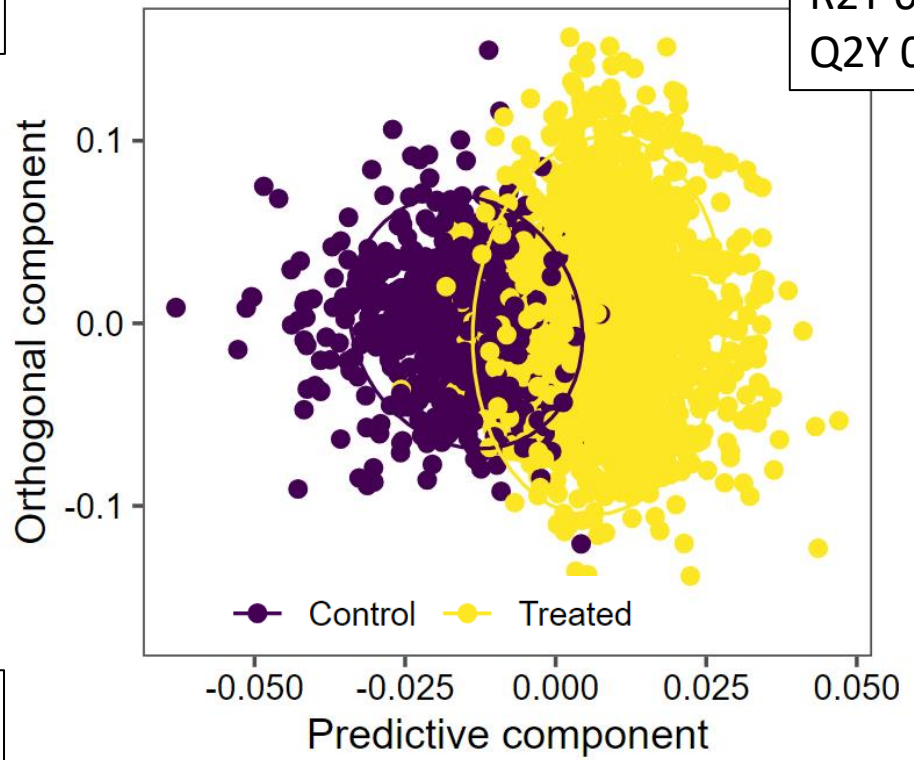


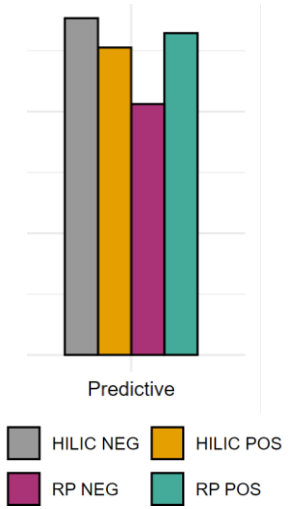
R2Y 0.56
Q2Y 0.55

R2Y 0.68
Q2Y 0.62

Consensus OPLS

R2Y 0.70
Q2Y 0.65



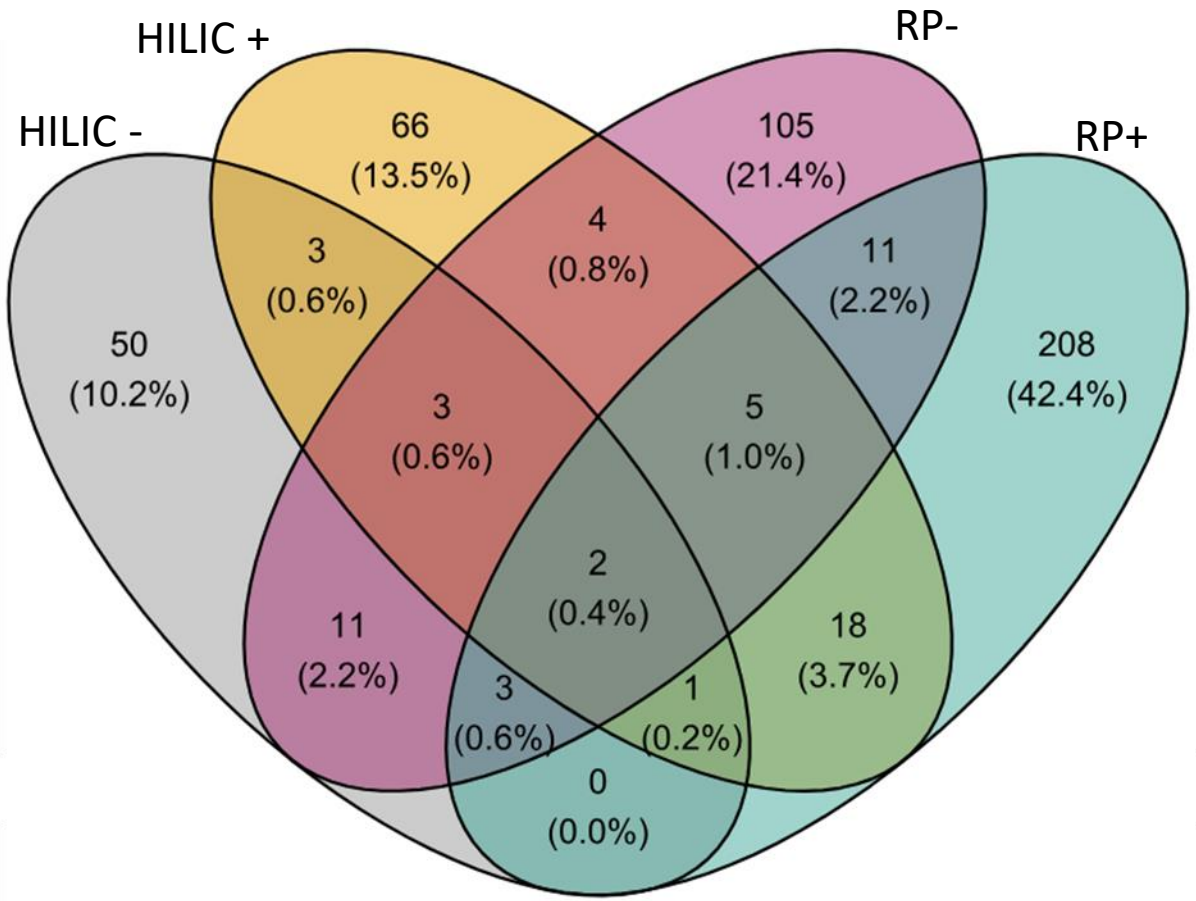


RStudio®

(Boccard *et al.* 2013)

BIOMARKERS INVESTIGATION

- MS/MS experiments
  (iDDA on QC samples, PRM on selected metabolites)
- Database search using W4M
- In silico annotation with SIRIUS



Schymanski scale	Level 5	Level 4	Level 3	Level 2	Level 1
Nb features	20	5	11	28	10

Level 5: m/z
Level 4: Molecular formula (adduct, isotope)
Level 3: Potential candidates with SIRIUS (class, structure)
Level 2: MS and MS/MS spectral match with databases
Level 1 : Standard match

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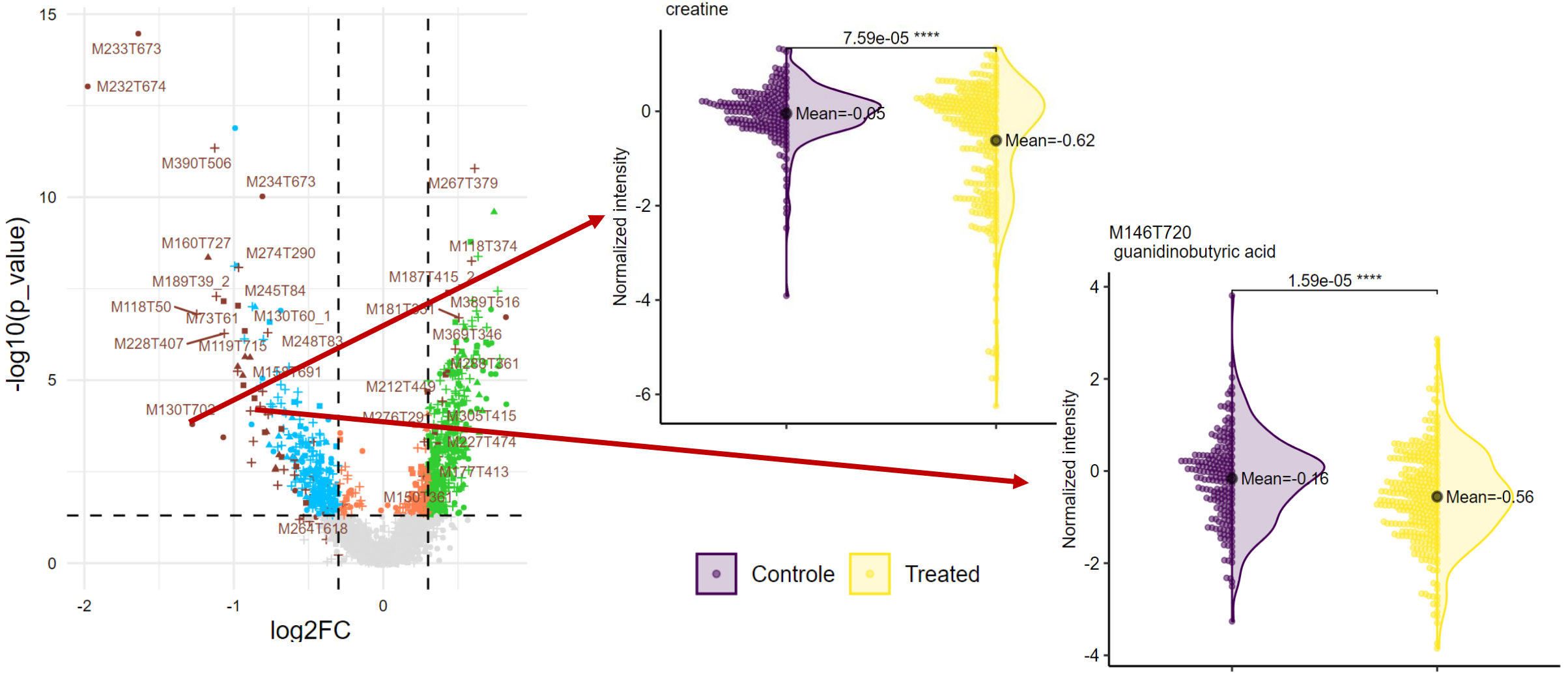
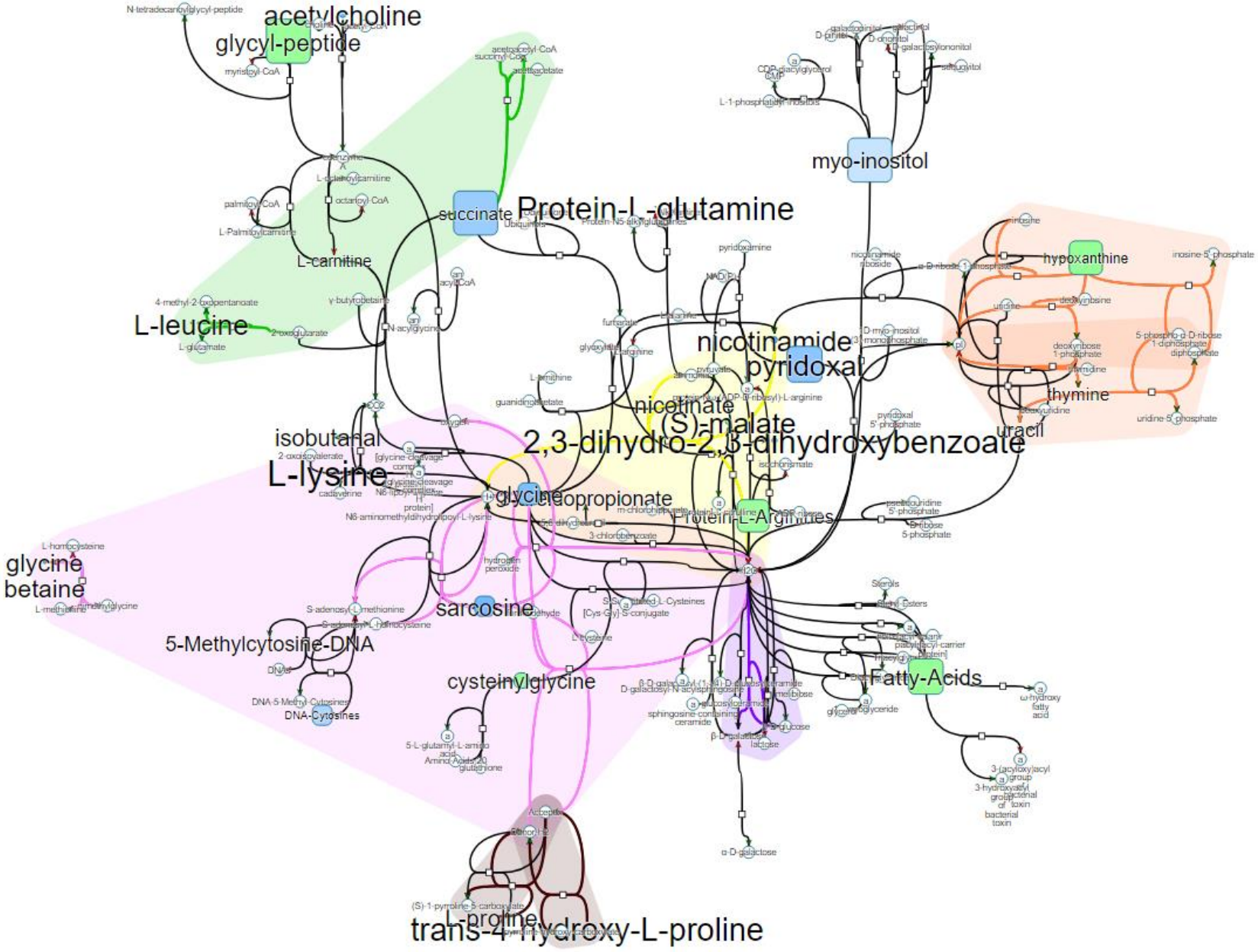
BIOMARKERS INVESTIGATION

Univariate analysis (fold change + Wilcoxon test)



PATHWAYS INVESTIGATION



Metabolic pathways

- Glycine
- Leucine
- Proline
- Lactose
- Nucleosides
- Pyridine

CONCLUSIONS

- **AMBITION**: Develop a unified metabolomics model to screen for multiple anabolic agents in calves

Thank you for your attention