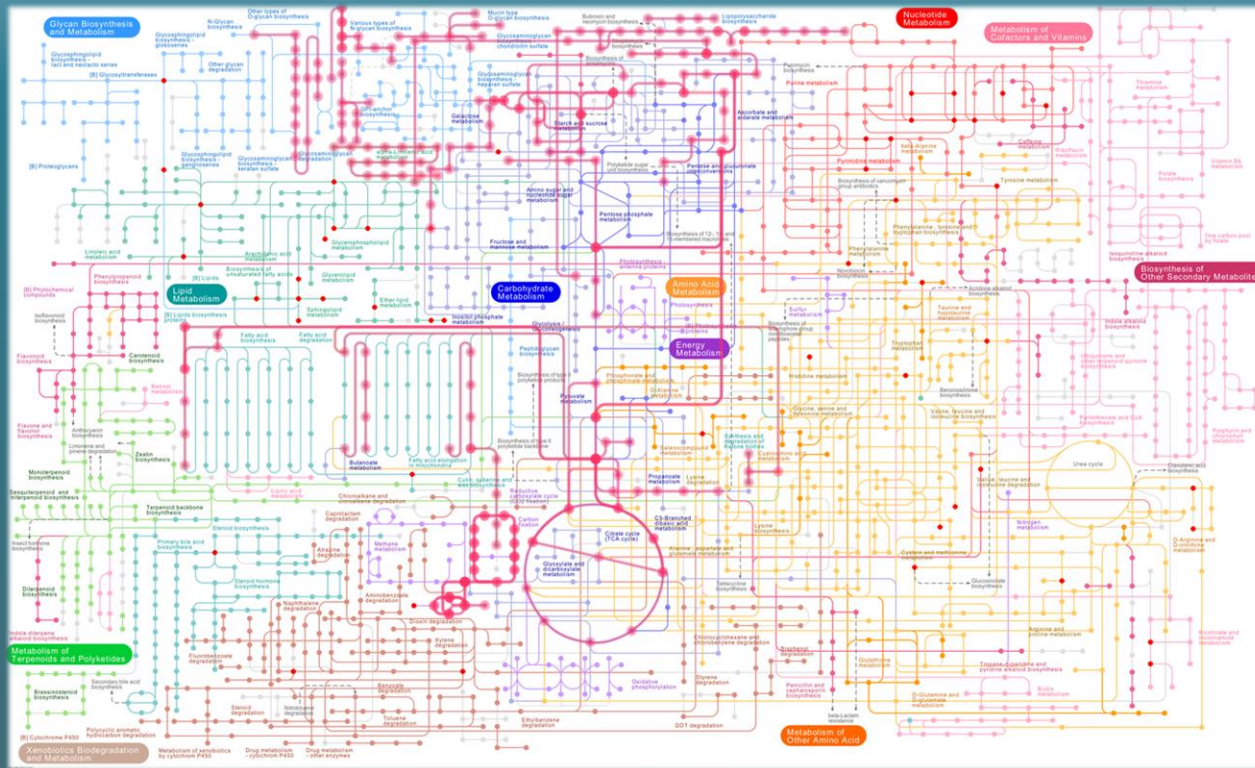


Untargeted/Semitargeted LC-MS metabolomics

Dr Alexandros Pechlivanis
Dr Panagiotis A Vorkas

Imperial College
London

Expanding Metabolome Coverage



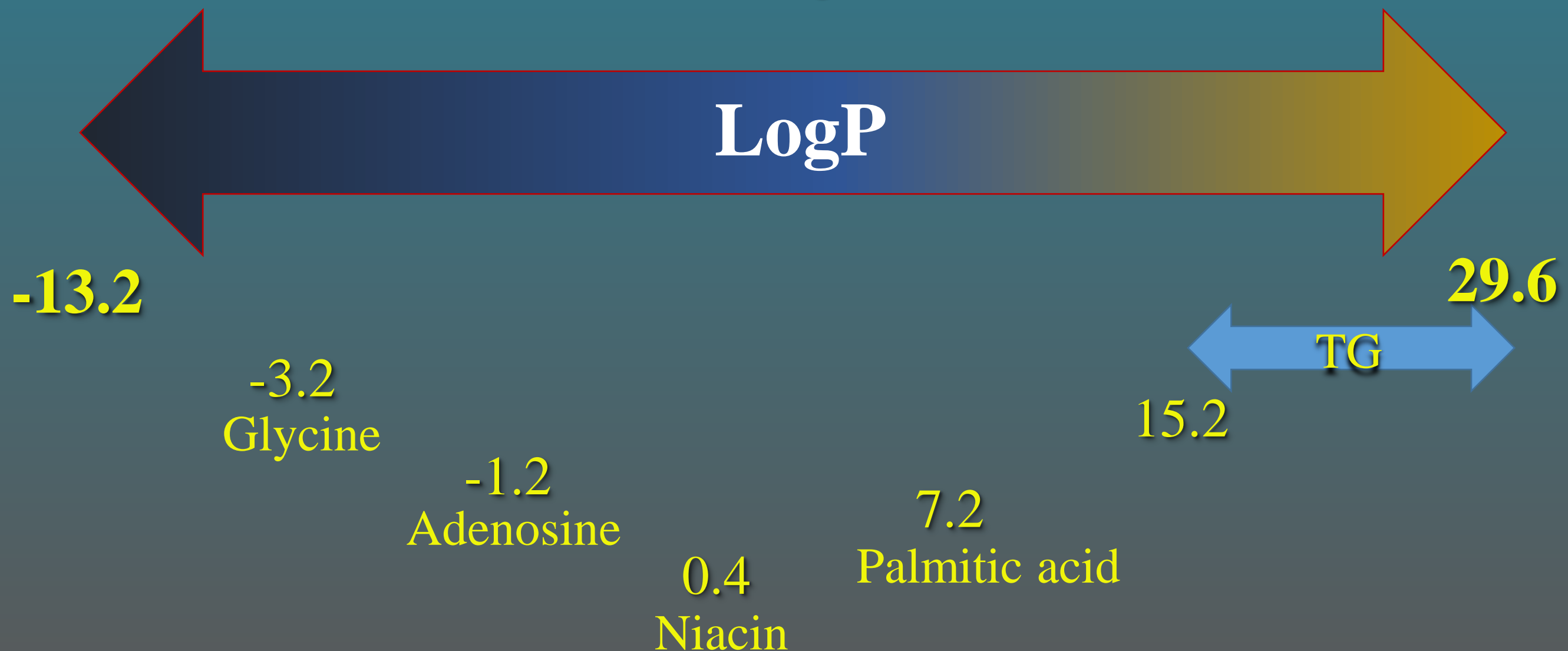
➤ **Maximise the possibility of detecting disease dysregulated compounds**

➤ **Capture the most relevant pathway/reaction**

The vast physicochemical diversity of the metabolome

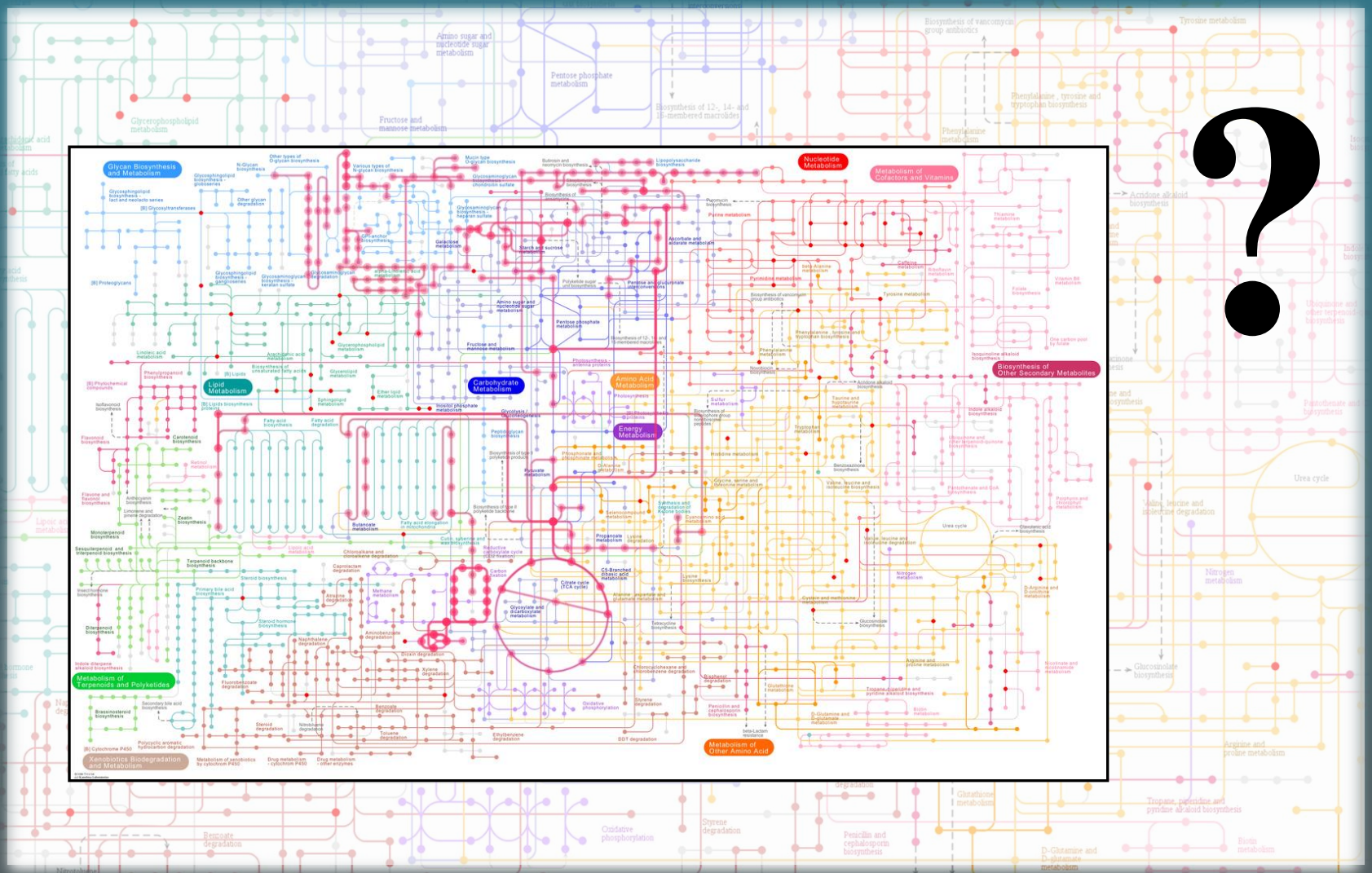
- Wide range of lipophilicity
- Wide range of functional groups

Humans: 30,000 endogenous metabolites



Untargeted Metabolic Profiling

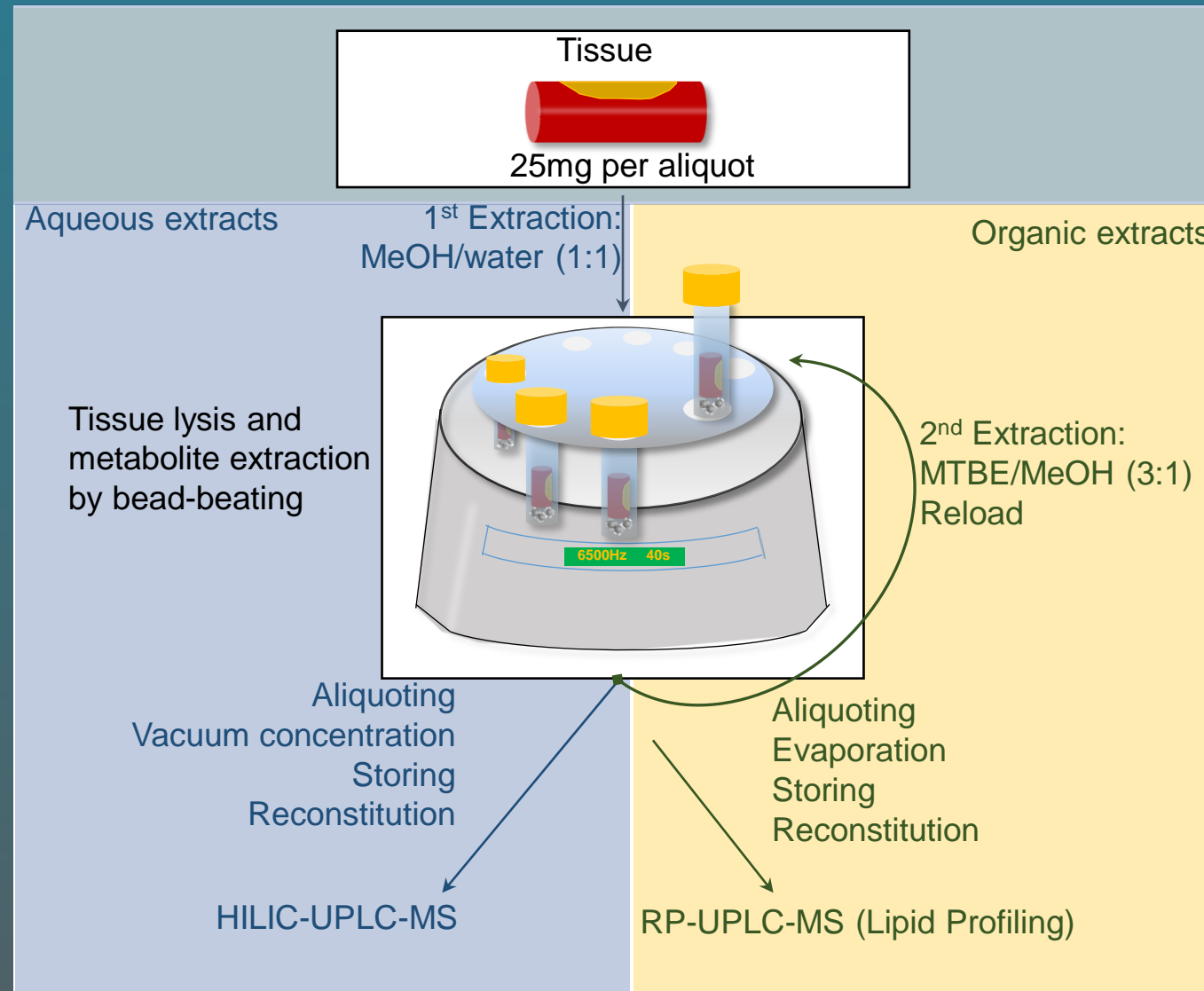
- Semi-Targeted
- Targeted



“Untargeted Does *not* Mean Unplanned” Patti G

Sample Preparation

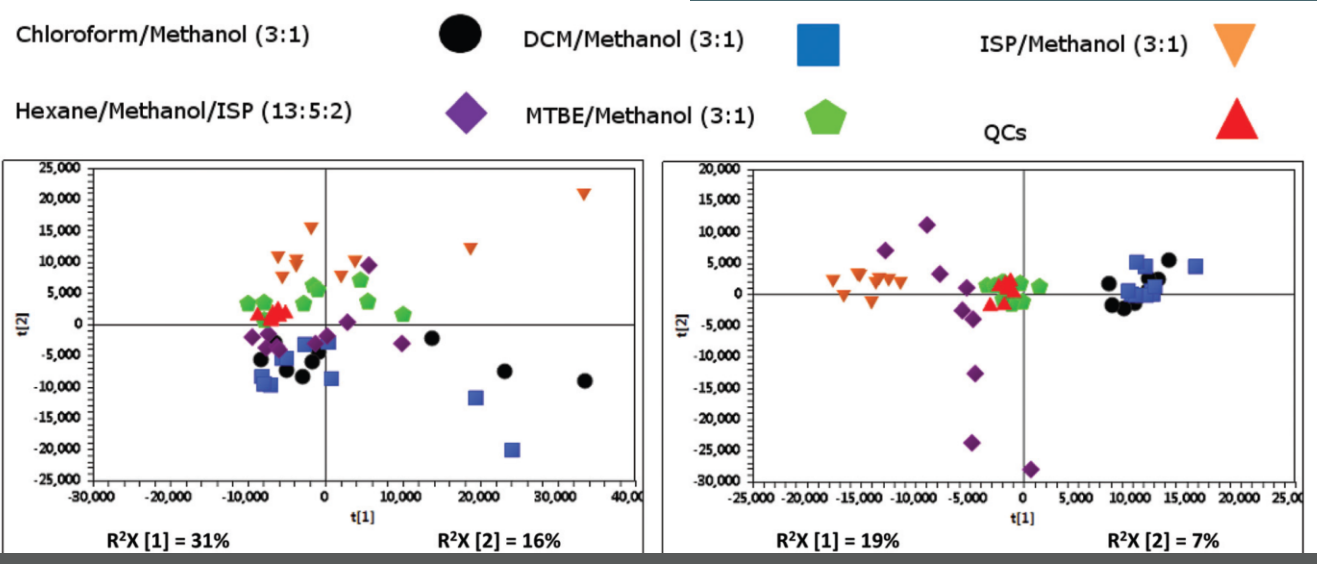
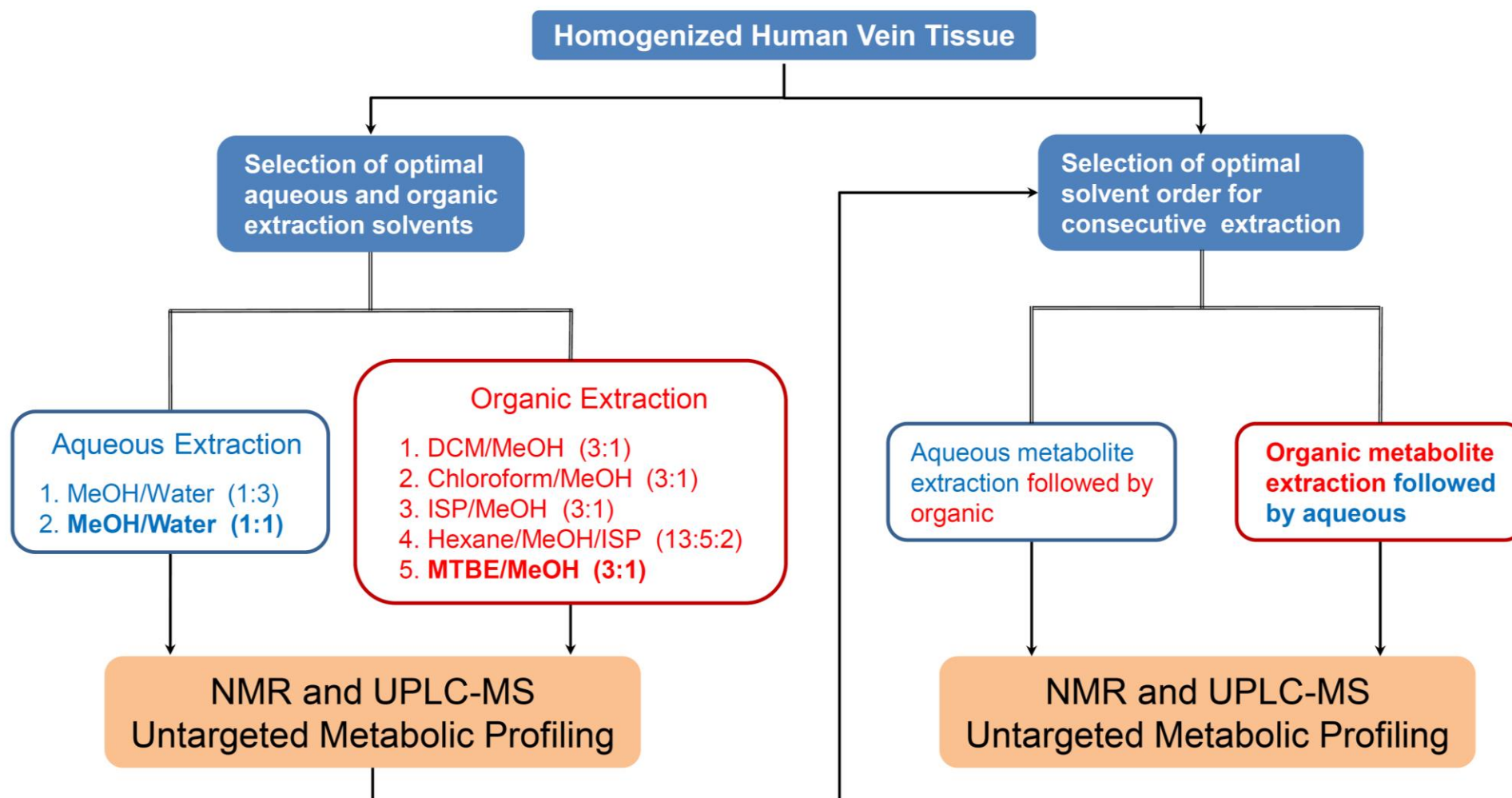
Tissue Extraction



Sample Preparation

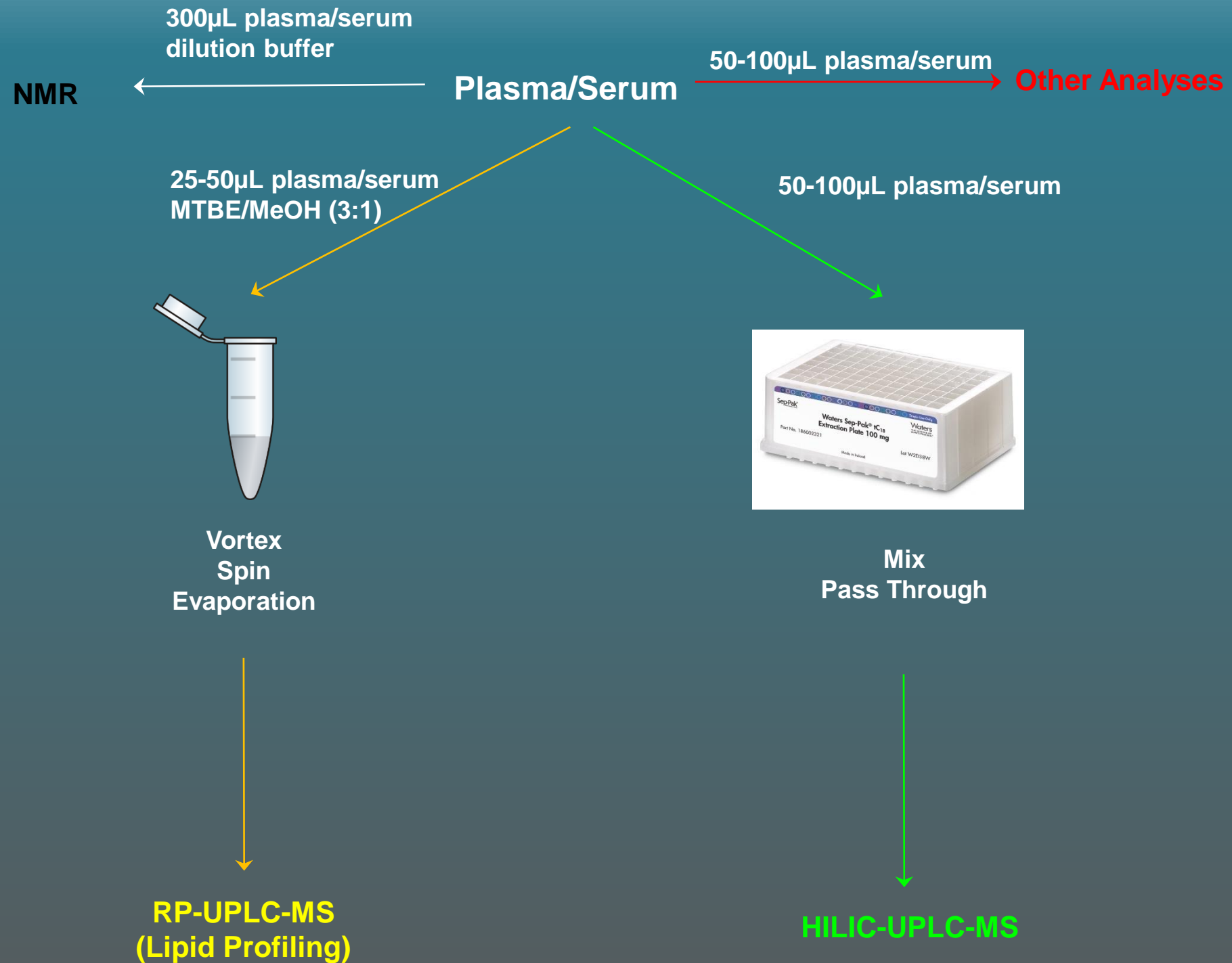
Tissue Extraction Optimisation

Tissue Extraction Optimization for Untargeted Metabolic Profiling



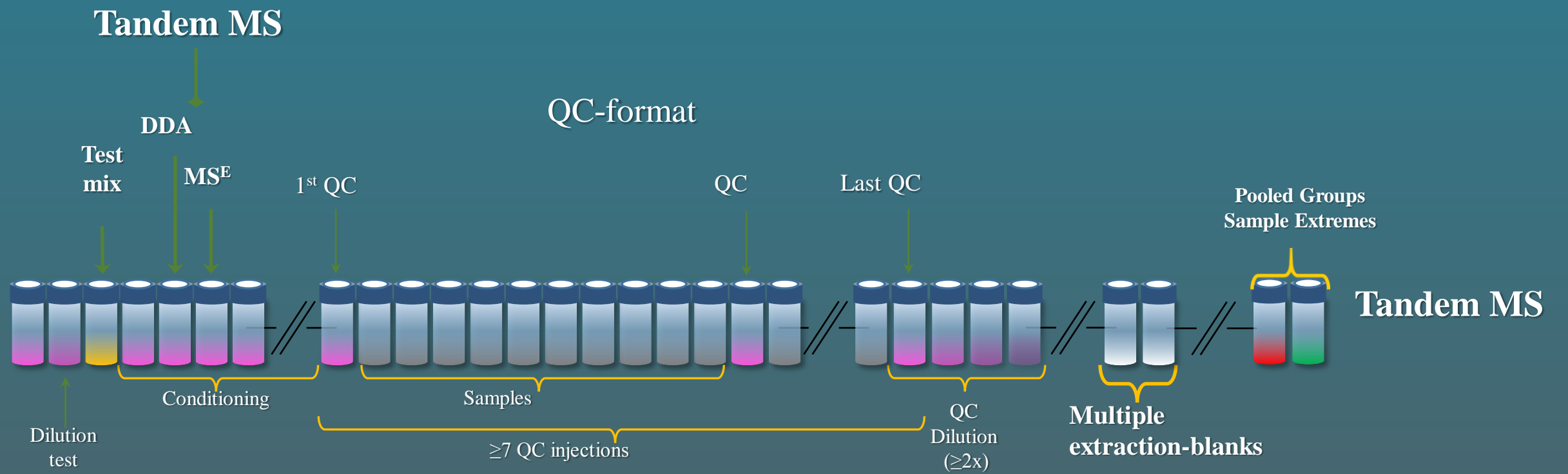
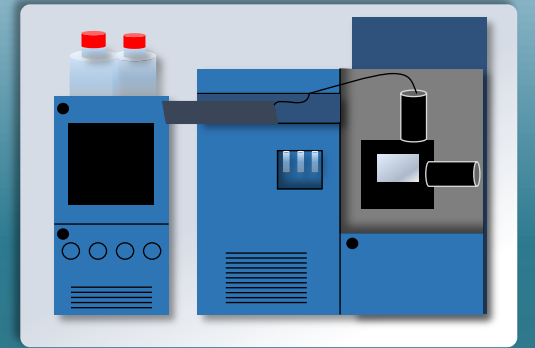
Sample Preparation

Plasma/Serum



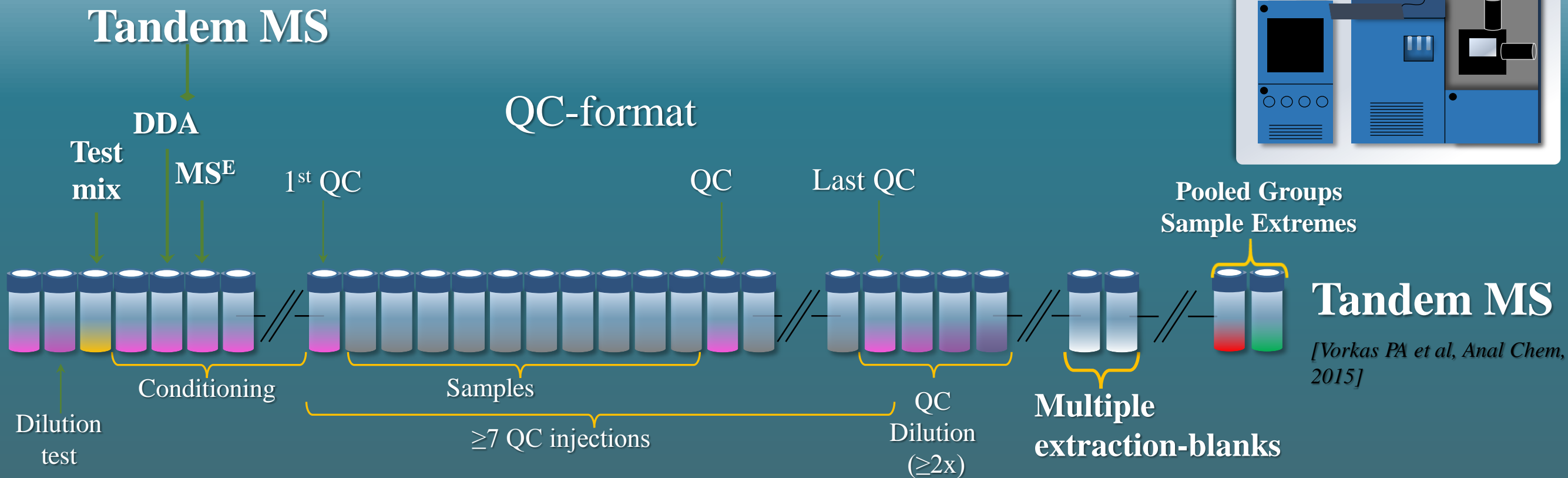
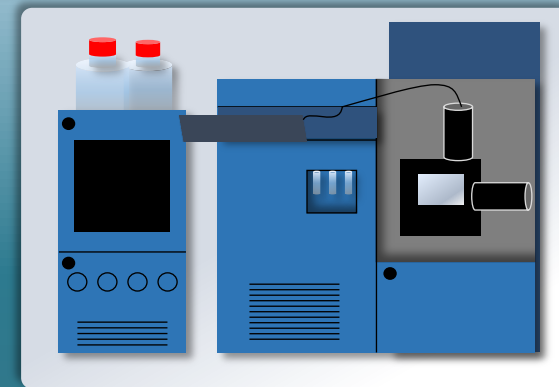
Sample Analysis

UPLC-MS



Sample Analysis

UPLC-MS



- **Know your method**
 - Use methods from literature
 - Run authentic standards

- **Run a test mix**
 - Check RT
 - Accurate mass
 - Frequent adducts

- **Unbiased tandem MS acquisition**
 - Run Data Dependent Acquisition (DDA)
 - MSE^E

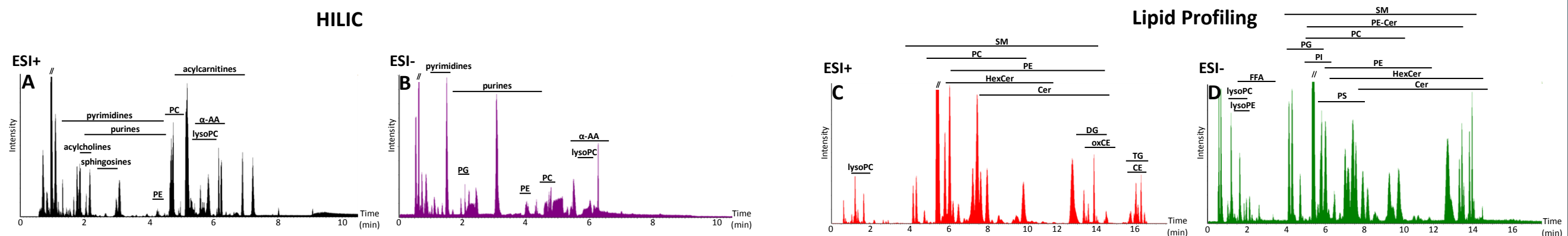
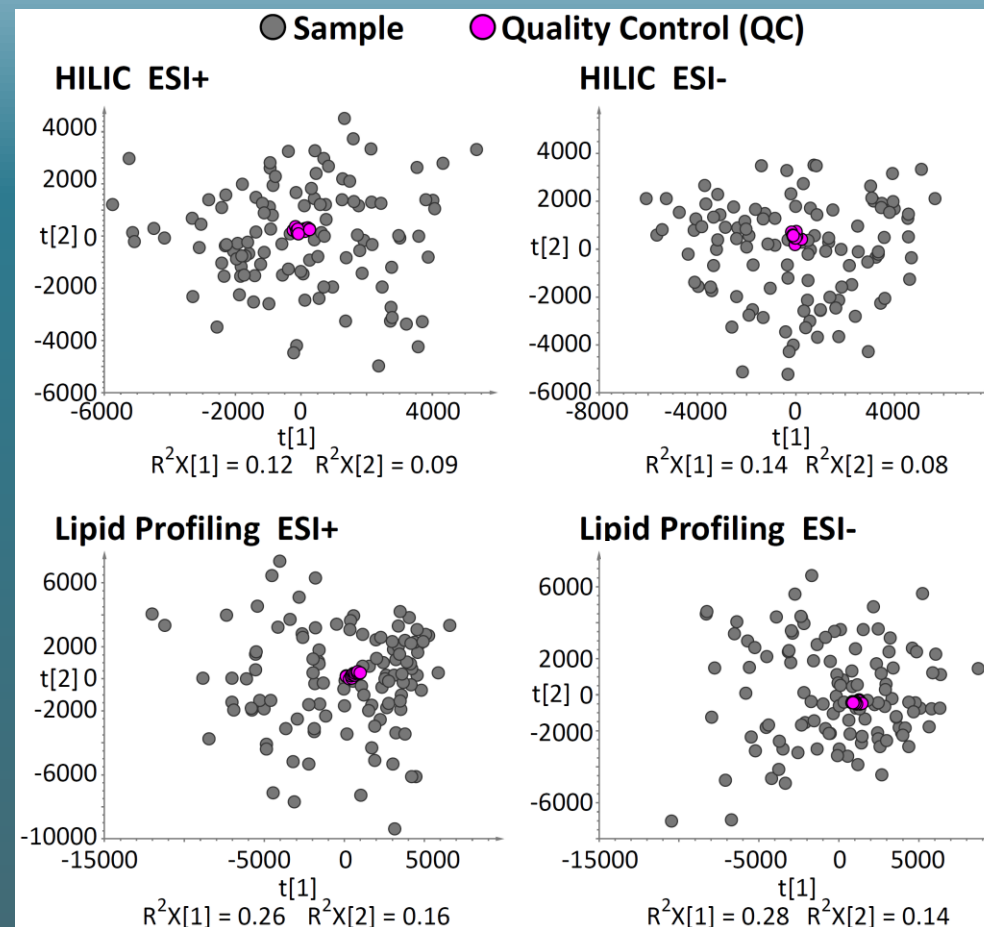
(Use pooled/QC samples – pooled groups/extremes)

- **Run sample preparation blanks**

- **Run Pos and Neg modes with the same gradient**

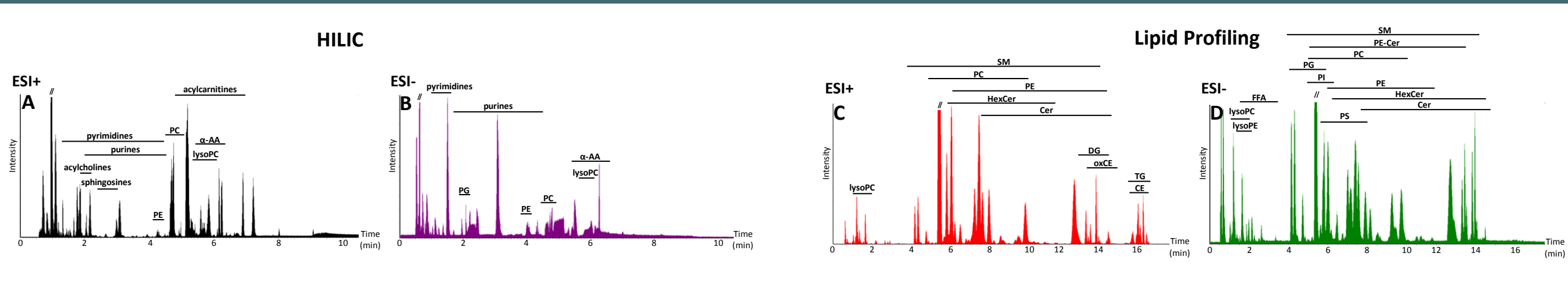
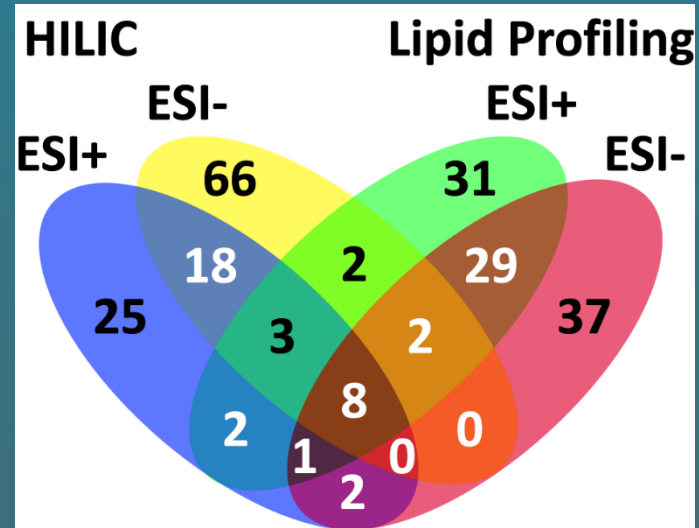
Sample Analysis

UPLC-MS



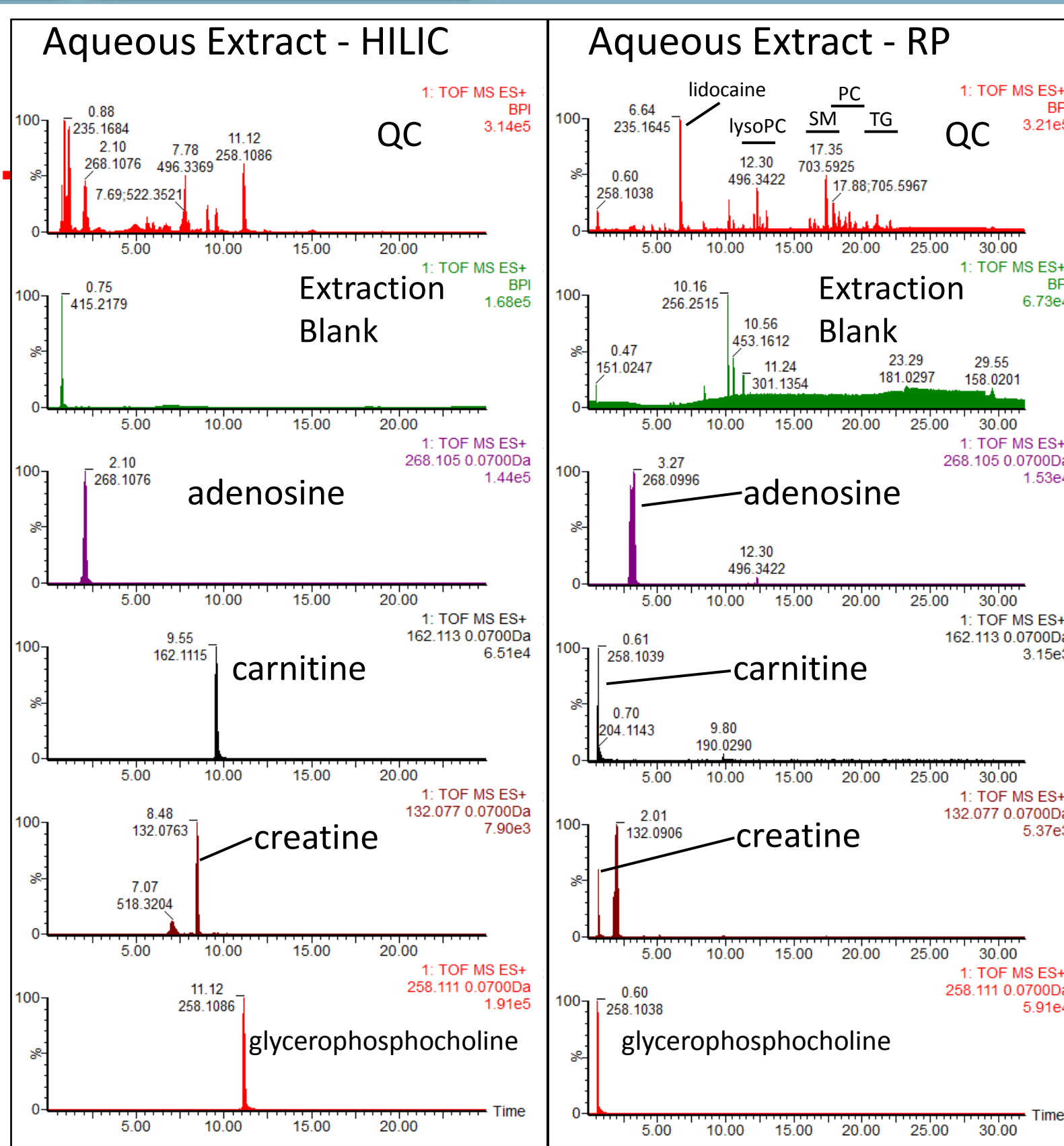
Sample Analysis

UPLC-MS

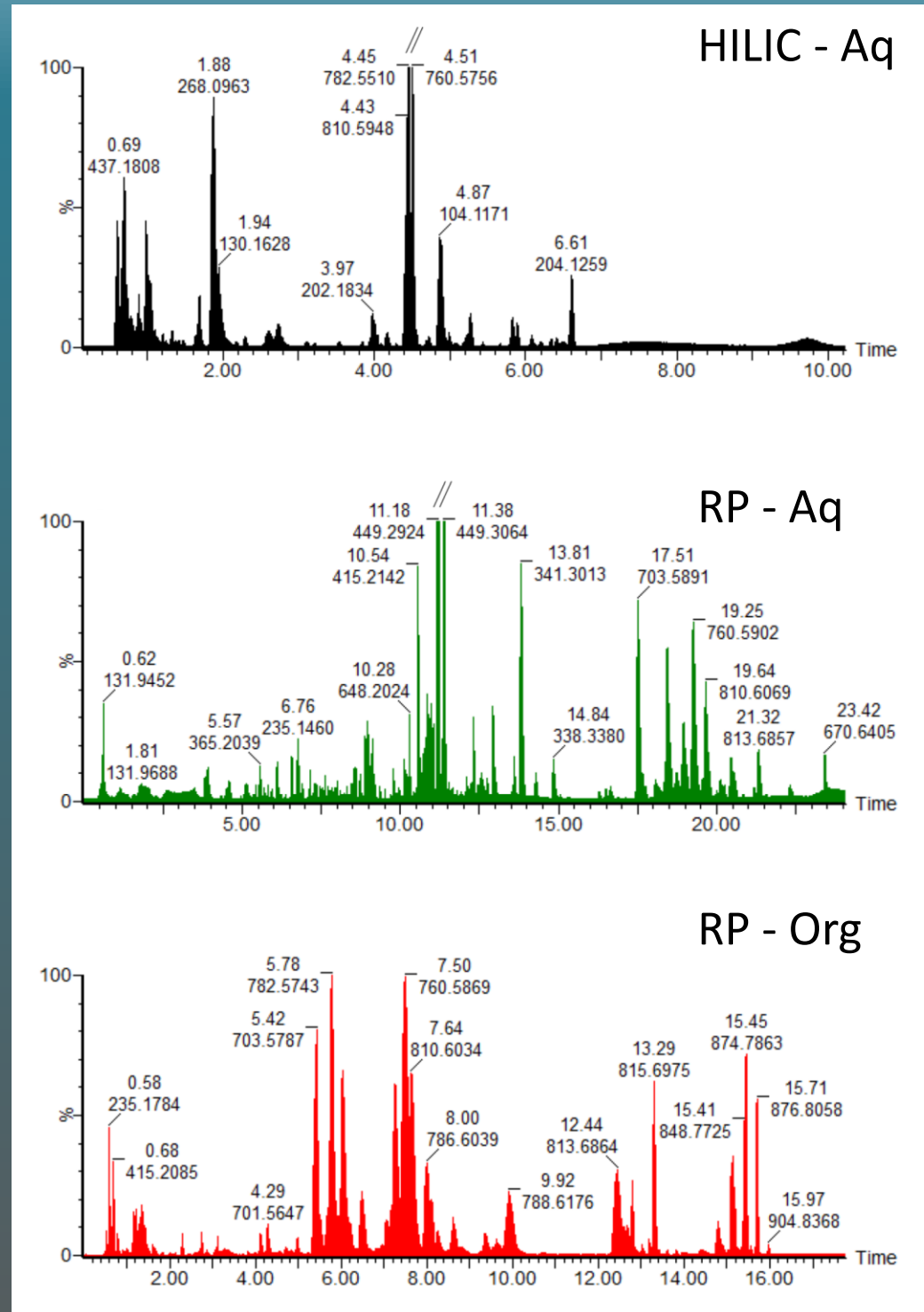


Sample Analysis

UPLC-MS

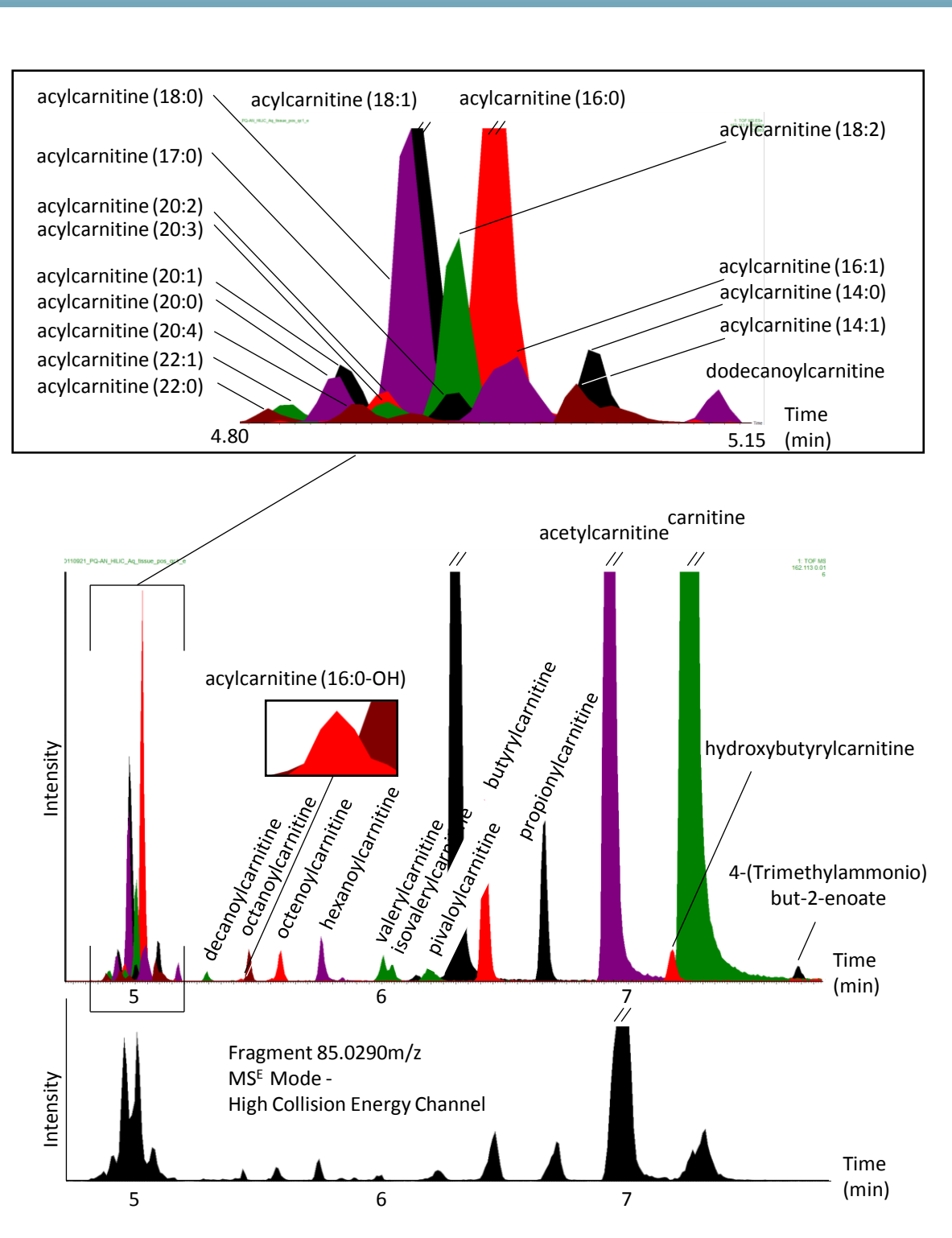


UPLC-MS



Sample Analysis

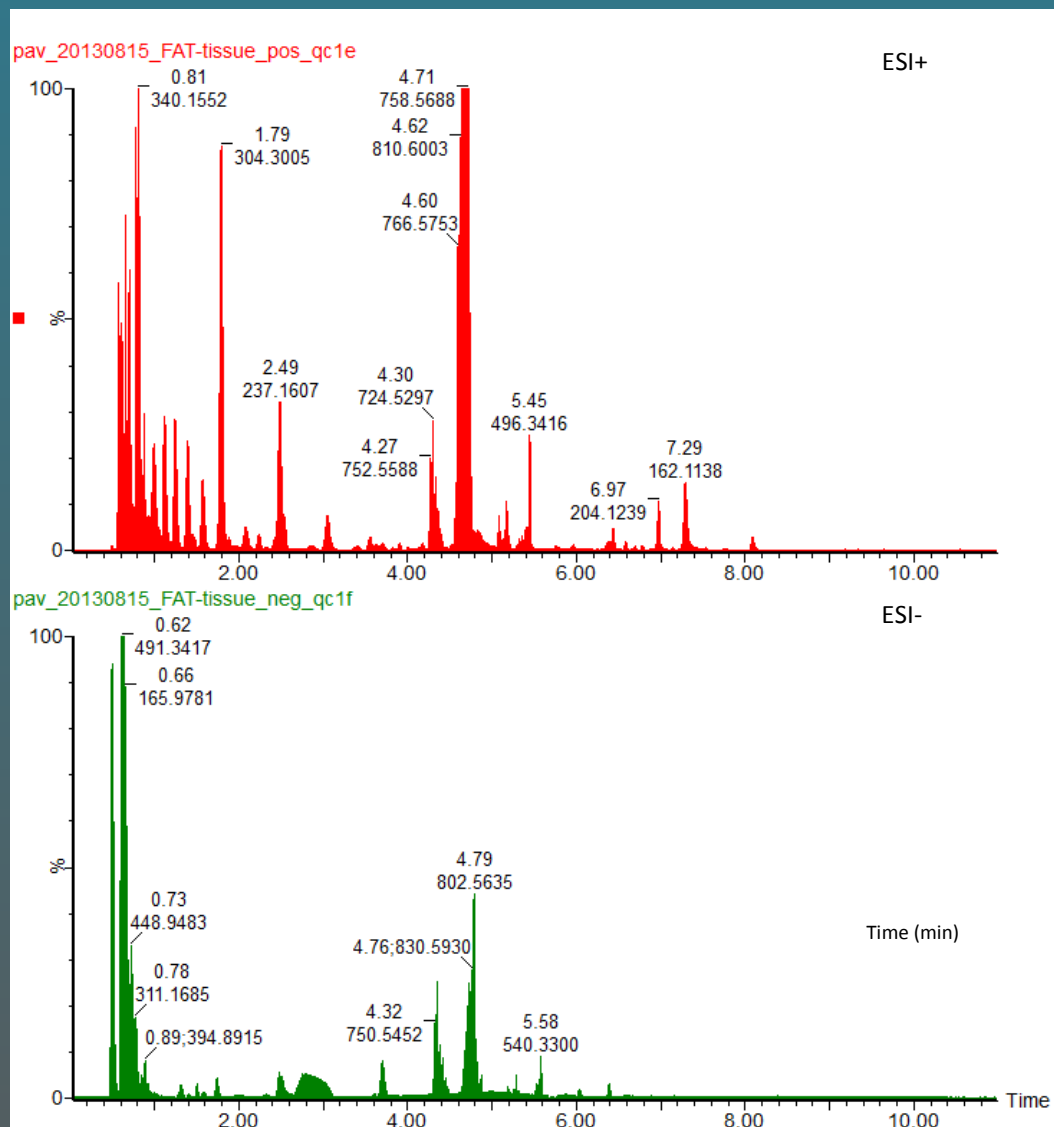
UPLC-MS



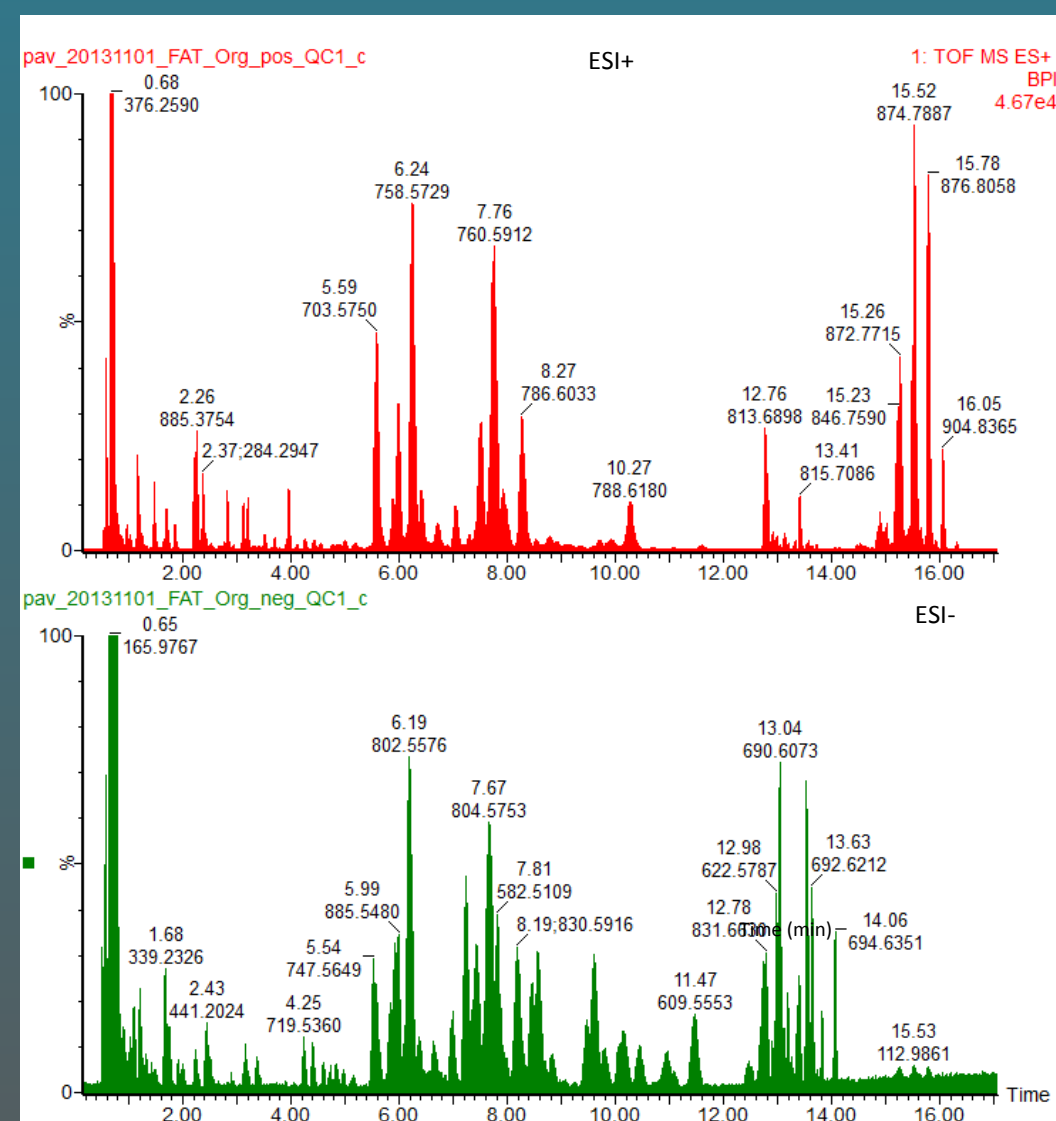
Application

Human adipose tissue

HILIC – Human Adipose Tissue Aqueous Extract



RP - Lipid Profiling – Human Adipose Tissue Organic Extract



Applications

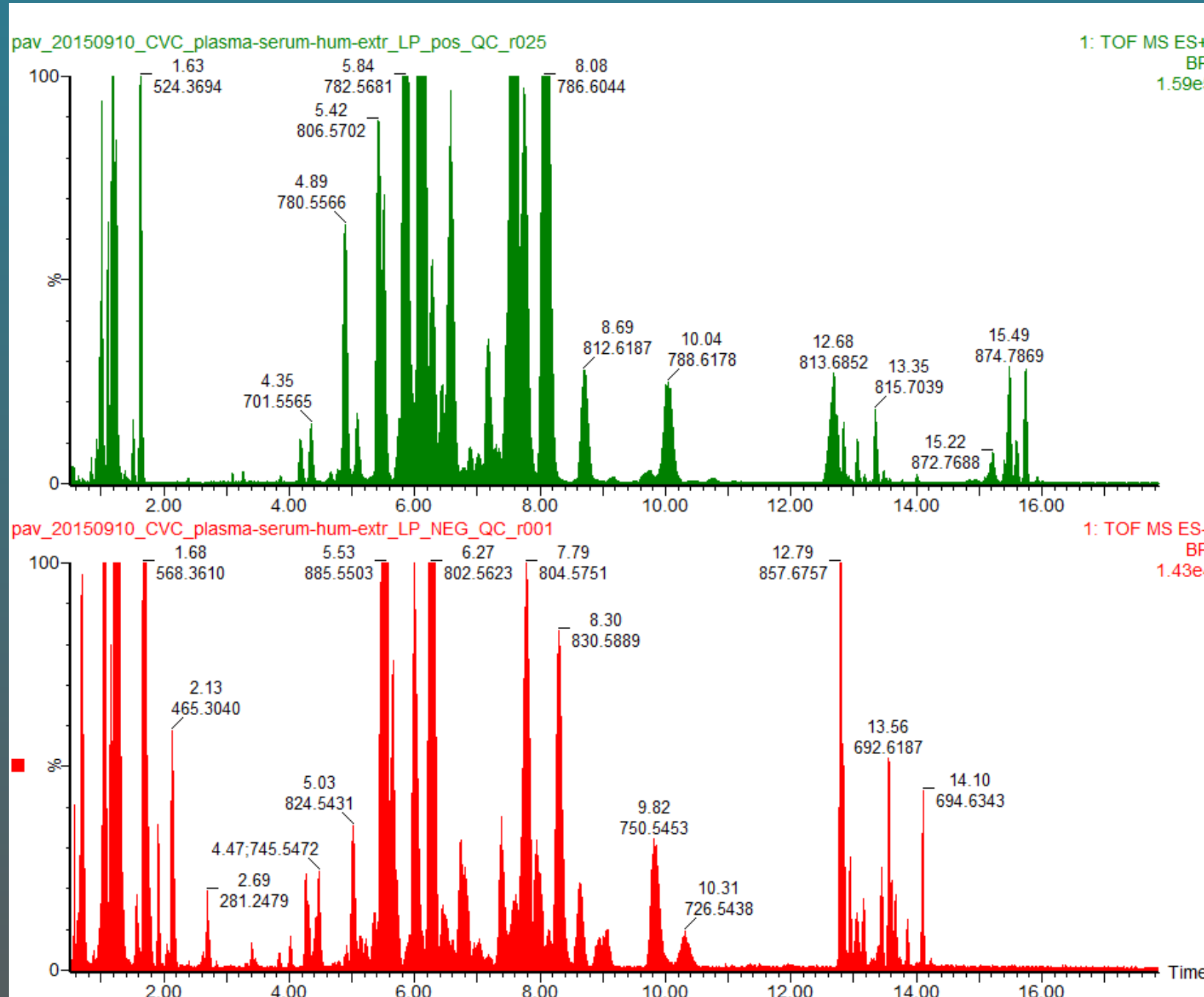
Other tissue types

- **Vascular (Plaque)**
- **Adipose**
- **Liver**
- **Heart**
- **Breast (Cancer)**
- **Brain**
- **Colon**
- **Cecum**
- **Jejunum**
- **Duodenum**
- **Pancreas**
- **Plasma/Serum**
- **Urine**
- **Cells**

Application

Human Serum

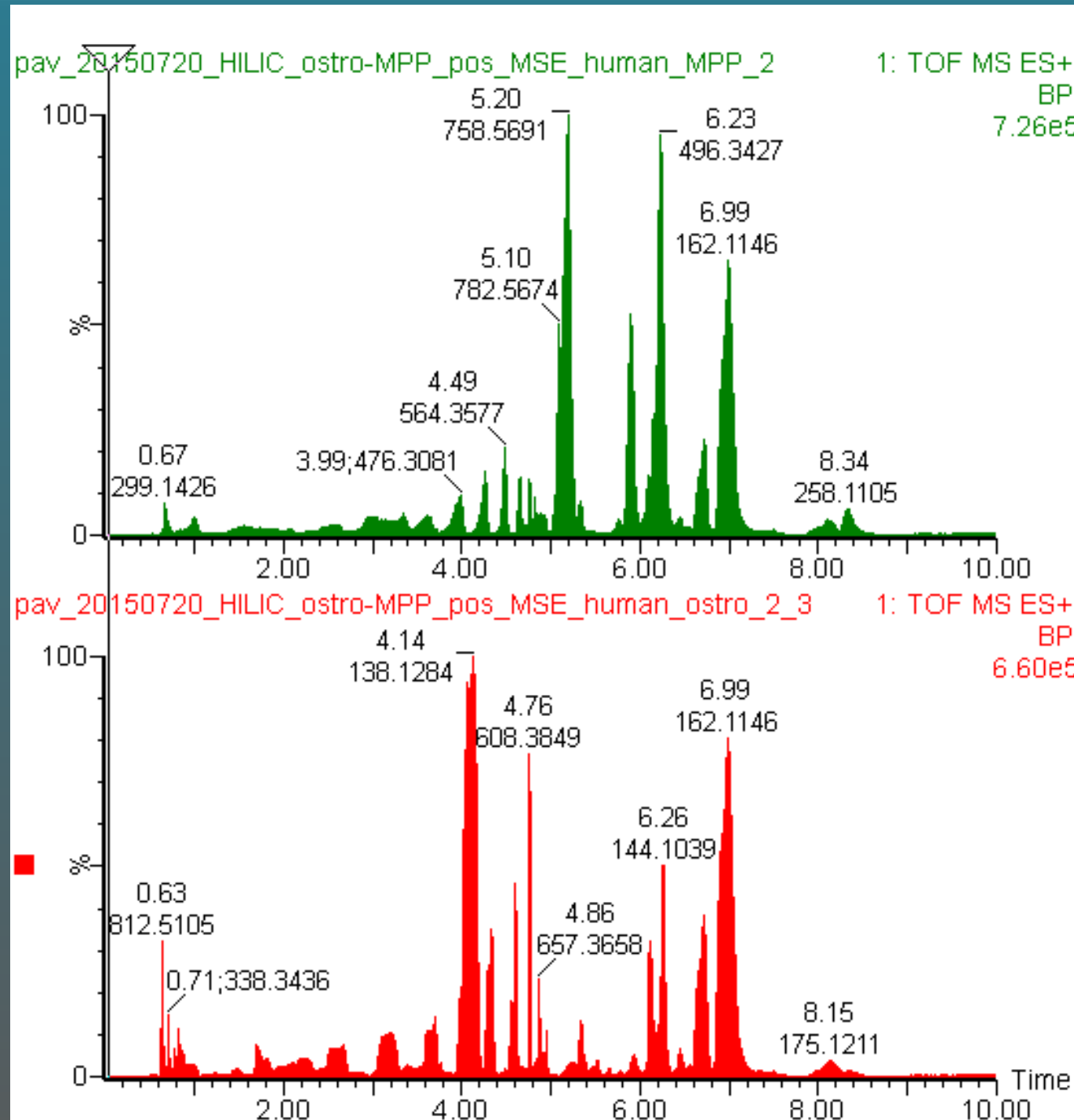
Lipid Profiling



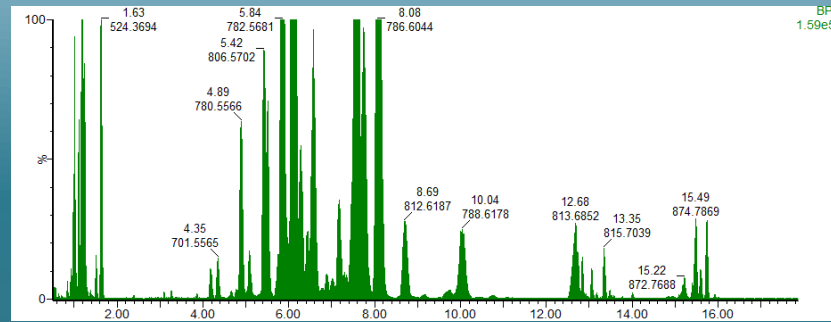
Application

Human Serum

HILIC



Data Deconvolution



Peak-picking



Grouping



RT-correction

Using Correlation – CAMERA

Preprocessing with
XCMS

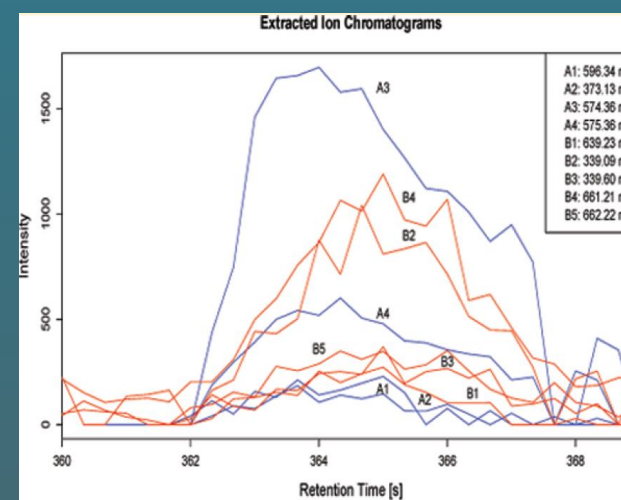


Annotation Workflow



peak grouping after retention
time (groupFWHM)

peak group verification with
peakshape correlation (groupCorr)



id	mz	rt	isotopes	adduct	pc
65	176.04	280.09			4
76	136.05	280.43	[14][M+1]1+		5
77	135.05	280.43	[14][M]1+		5
74	153.06	280.43		[M+H] ⁺ 152.05437	5
75	175.04	280.43		[M+Na] ⁺ 152.05437	5
73	197.02	280.76		[M+2Na-H] ⁺ 152.05437	5
78	377.74	286.15			6
79	732.5	286.49			6
83	488.32	286.82		[M+Na] ⁺ 465.33205	7
82	466.34	286.82		[M+H] ⁺ 465.33205	7
...					

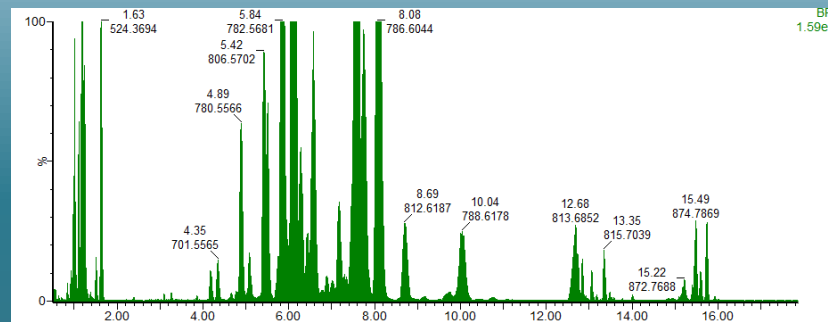
pc: result of the peak correlation based
annotation

adduct: annotation hypothesis for the
adduct species. The value after the brackets
is the estimated molecular mass

isotopes: annotation and the number after
the brackets is the charge of the isotope.

CAMERA: Collection of Algorithms for METabolite pROfile ANnotation

Data Deconvolution



Peak-picking



Grouping



RT-correction



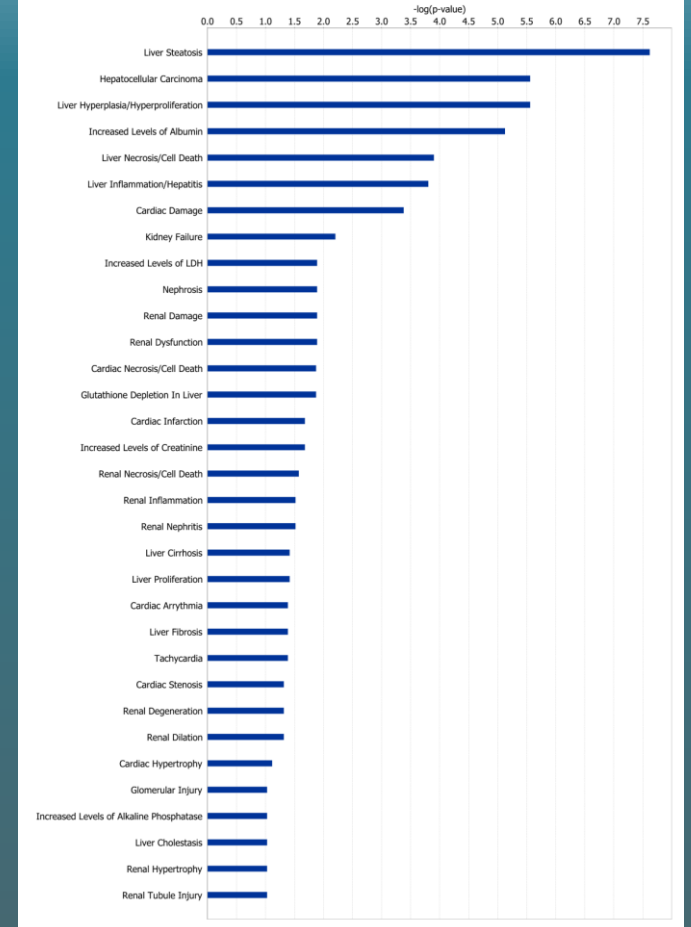
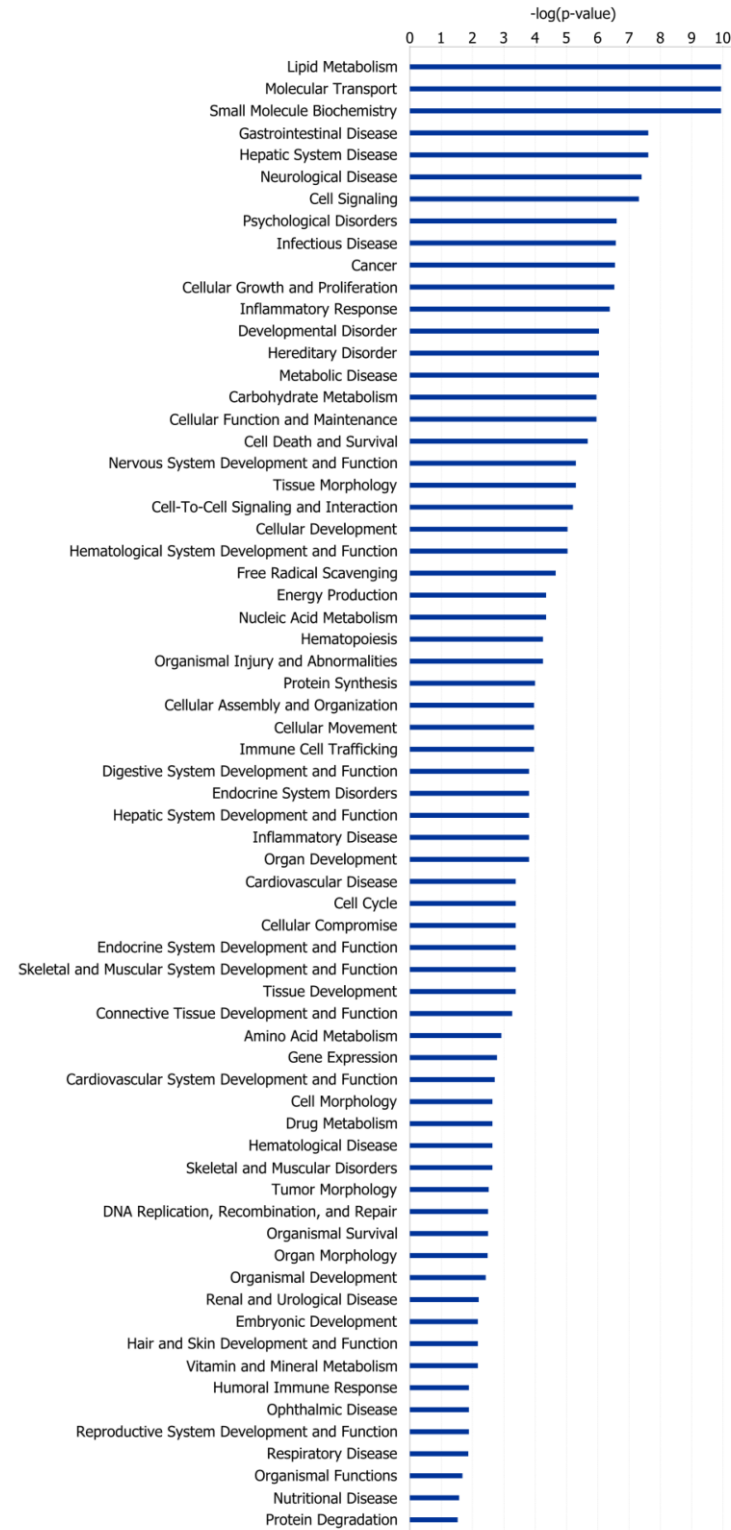
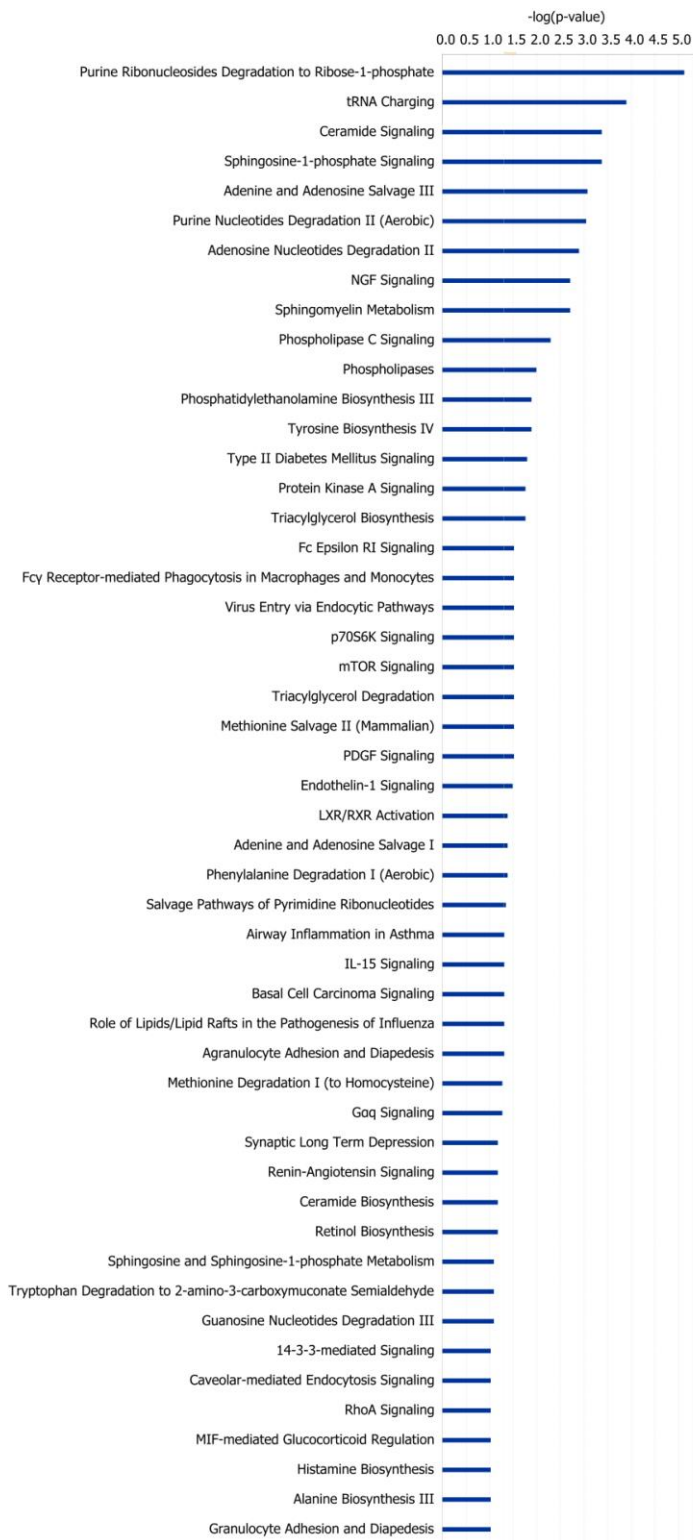
Statistics



Metabolite Structure Elucidation



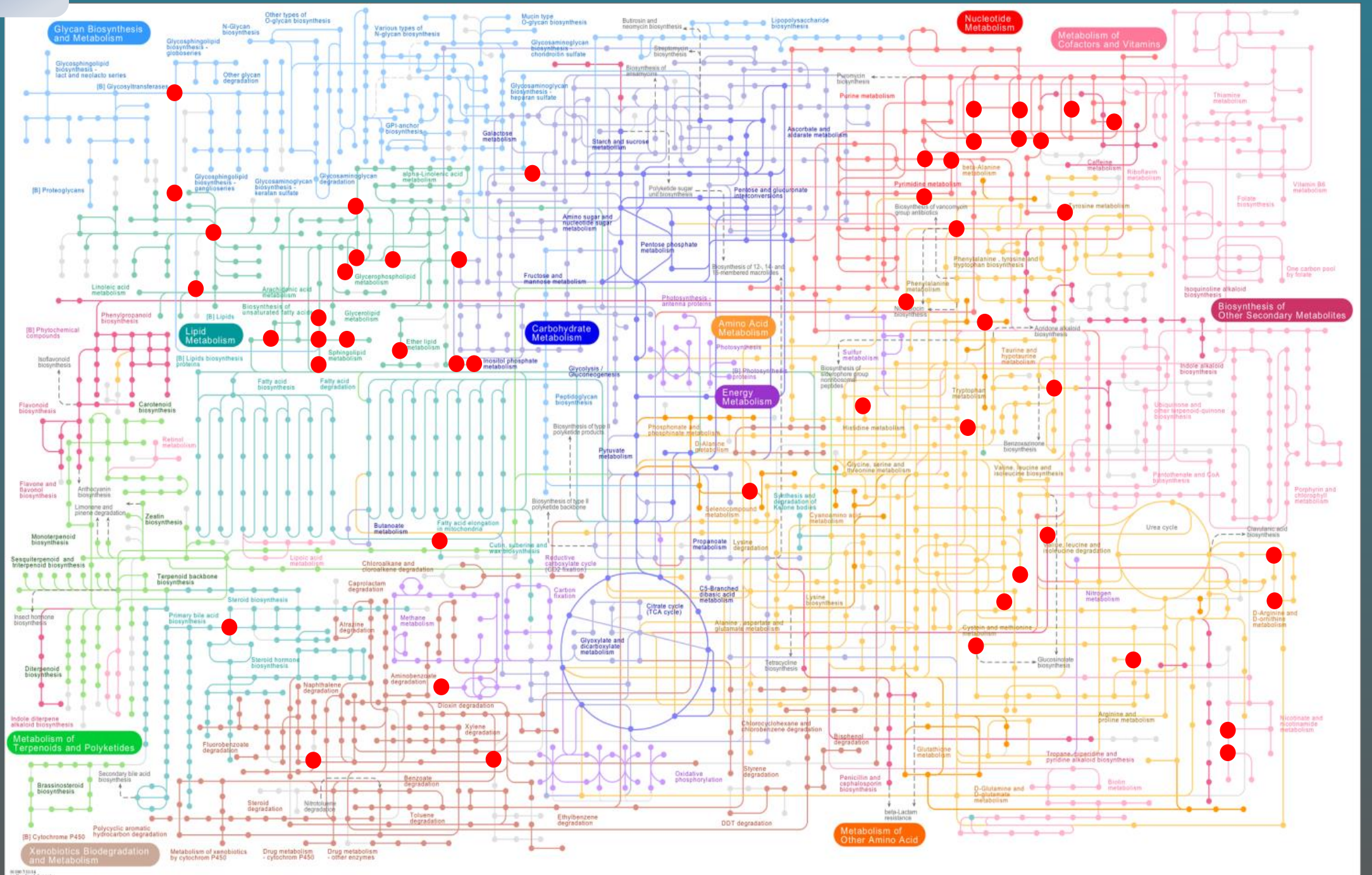
Metabolite Coverage Pathway Mapping



- 89 /100 KEGG IDs recognised by IPA
- Mapped 182 canonical pathways
- 60 mapped to 1041 diseases and biological functions
- 36 to 99 toxicity functions

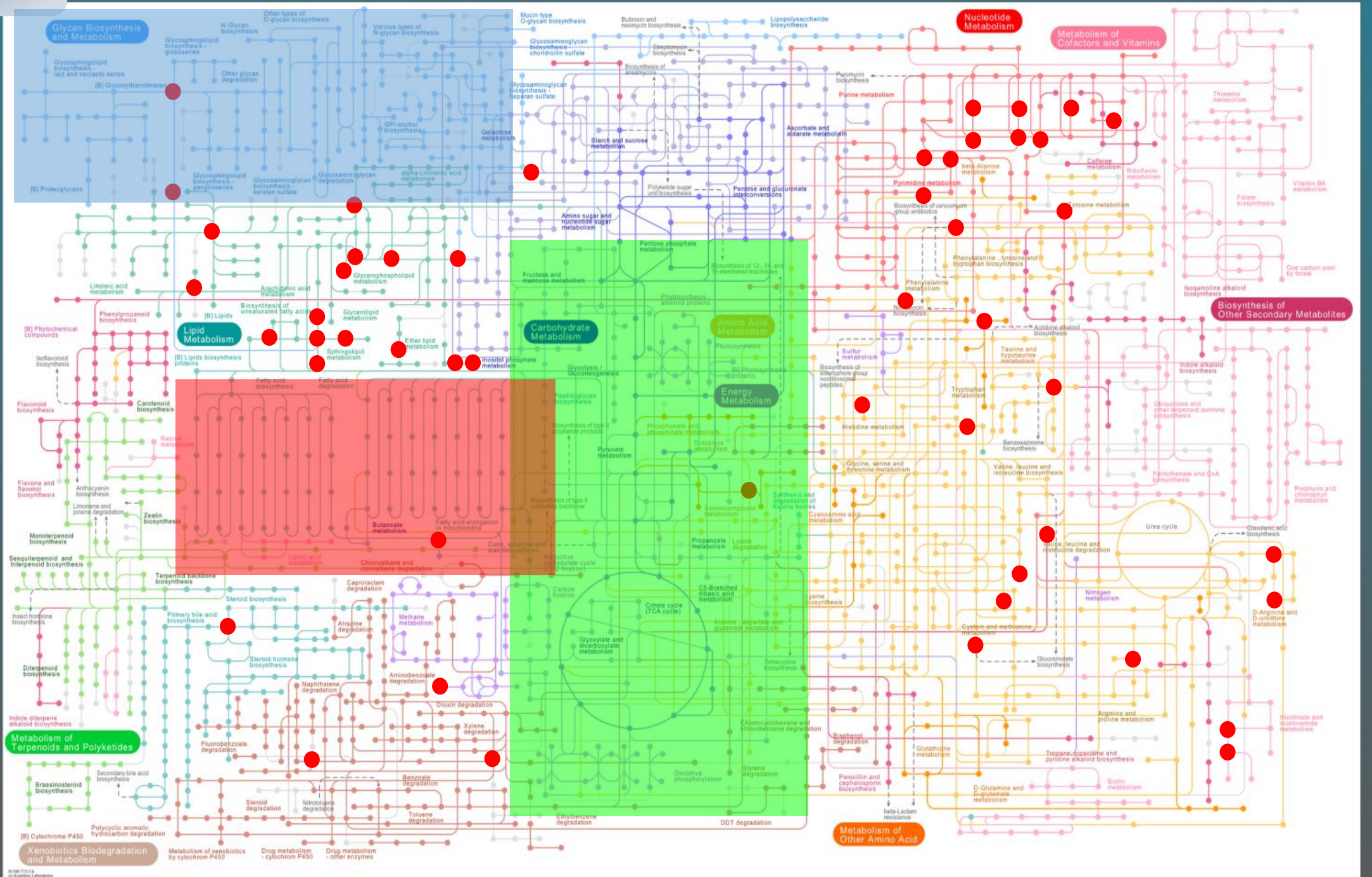
Metabolite Coverage Pathway Mapping

187/229 assigned
a KEGG ID
100 Unique



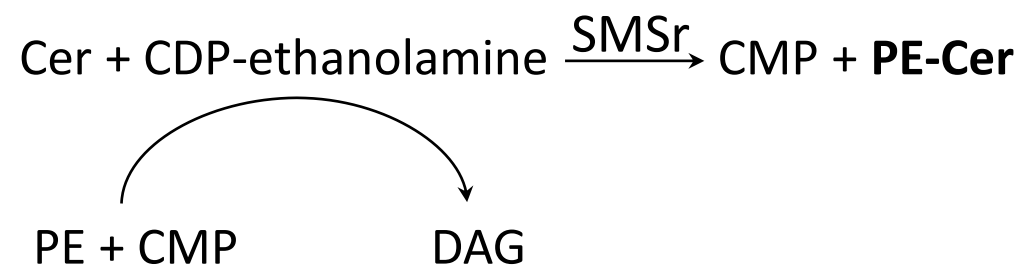
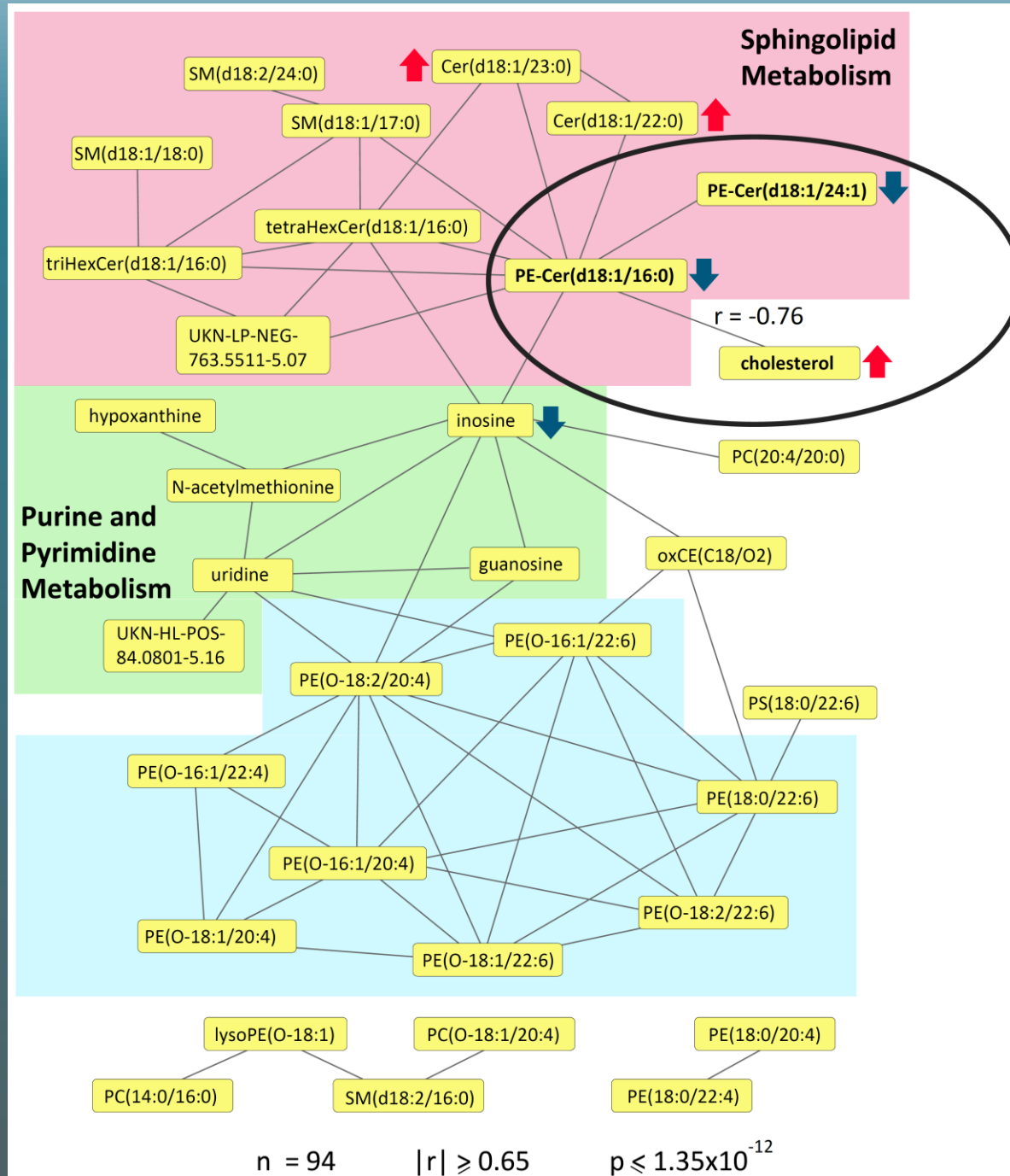
Metabolite Coverage Pathway Mapping

187/229 assigned
a KEGG ID
100 Unique

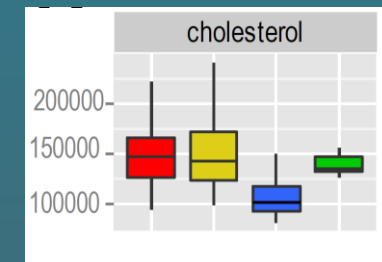
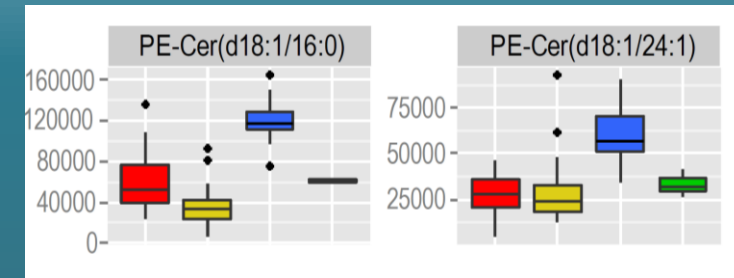


Applications

Atherosclerosis

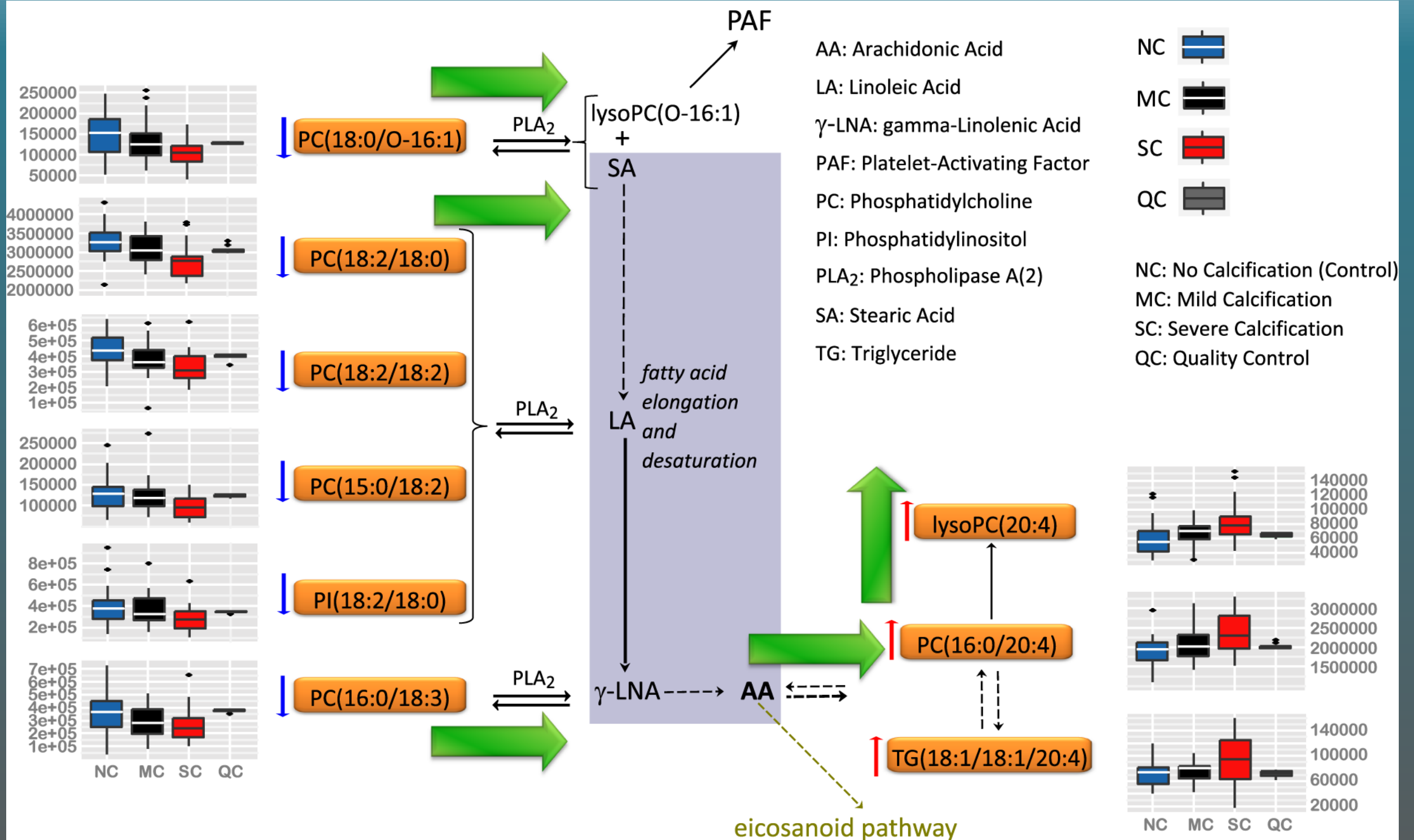


$p = 9 \times 10^{-12}$



Applications

Coronary Artery Calcification (Serum)



Many thanks to:

CSM:

Prof Elaine Holmes

Dr Elizabeth Want

Prof Jeremy Nicholson

Dr Matt Lewis

Dr Dina Spagou

Dr Maria Gomez-Romero

Dr Paul Benton

Dr Claire Boulange

Dr Renaud Mestdagh

Mr Gabriel Valbuena

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Dr Giorgis Isaac

Dr John P Shockcor

ASVS (ICL):

Prof Alun Davies

Mr Muzaffar Anwar

Mr Joseph Shalhoub

Umea Heart Centre

Prof Michael Henein

Thank you

Dr Panagiotis A Vorkas

**Imperial College
London**