

# *Use of RapidFire/MS for Metabolomics*

*- from untargeted to targeted and Pathway driven analysis*

RapidFire/MS

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Agilent Technologies

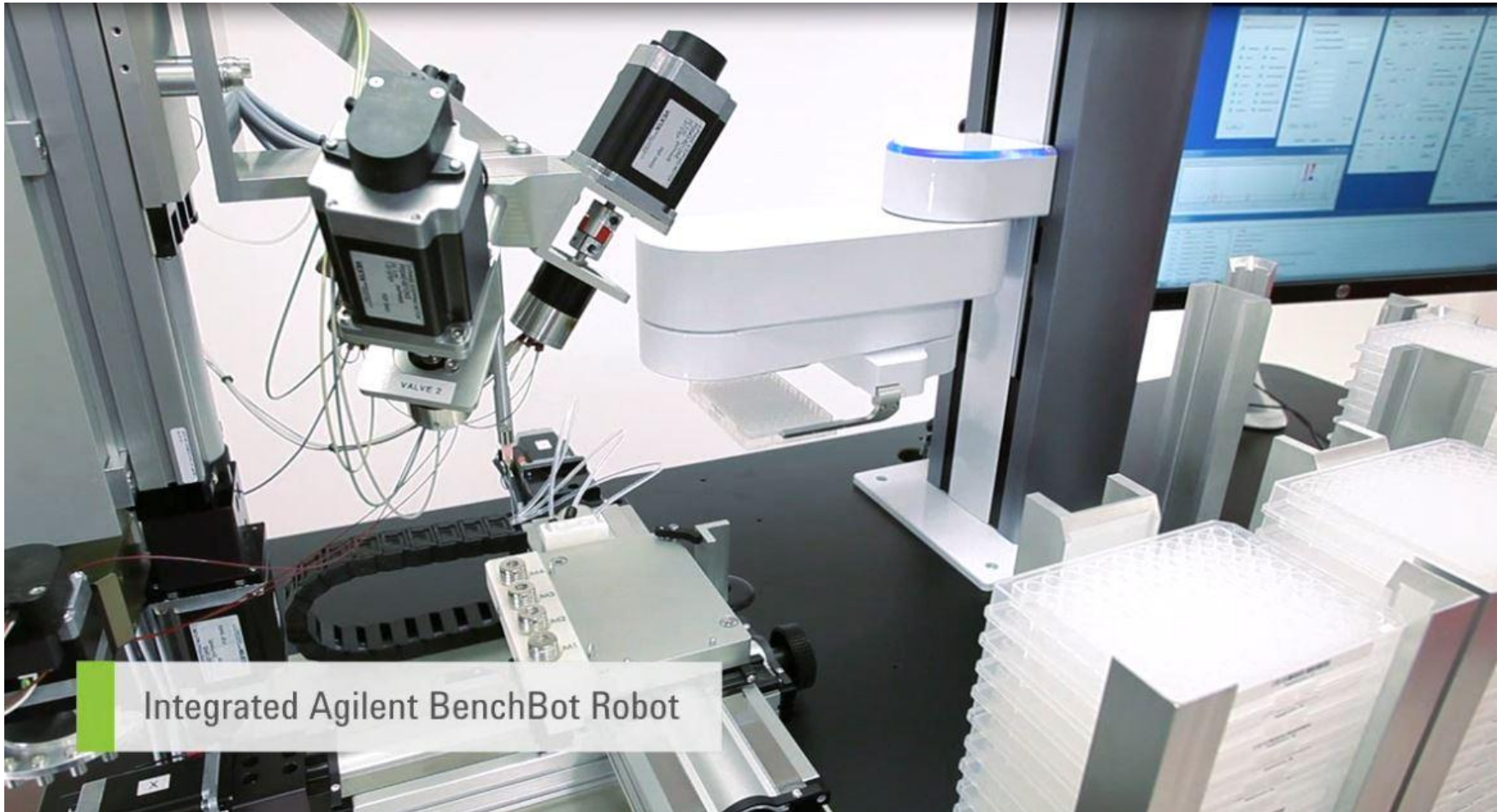
# Agilent RapidFire™ High-throughput Mass Spectrometry System



## Fast autosampler, SPE and Sample Quantitation

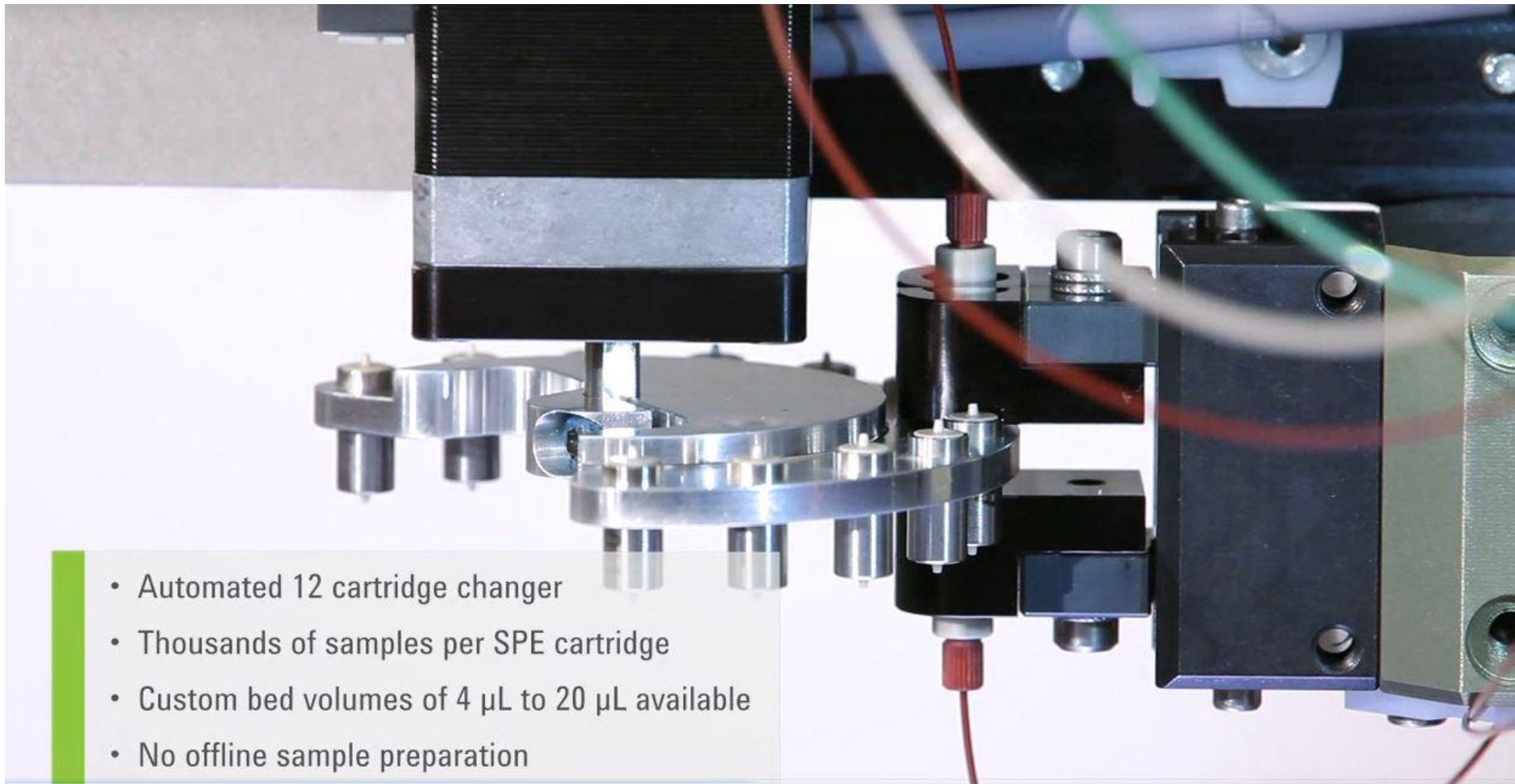
- Complementary to LC-MS
- Integrated, automated, micro-scale solid-phase extraction
- Integrates with standard ESI MS instruments
- Compatible with most biological matrices
- **cycle time: 5–10 seconds / sample**

# RapidFire 365 – plate handling



Capacity 63 plates → 20000 samples over 60 hours

# RapidFire 365 – cartridge changer



- Automated 12 cartridge changer
- Thousands of samples per SPE cartridge
- Custom bed volumes of 4  $\mu\text{L}$  to 20  $\mu\text{L}$  available
- No offline sample preparation

Capacity 12 cartridges → matching the Benchbot

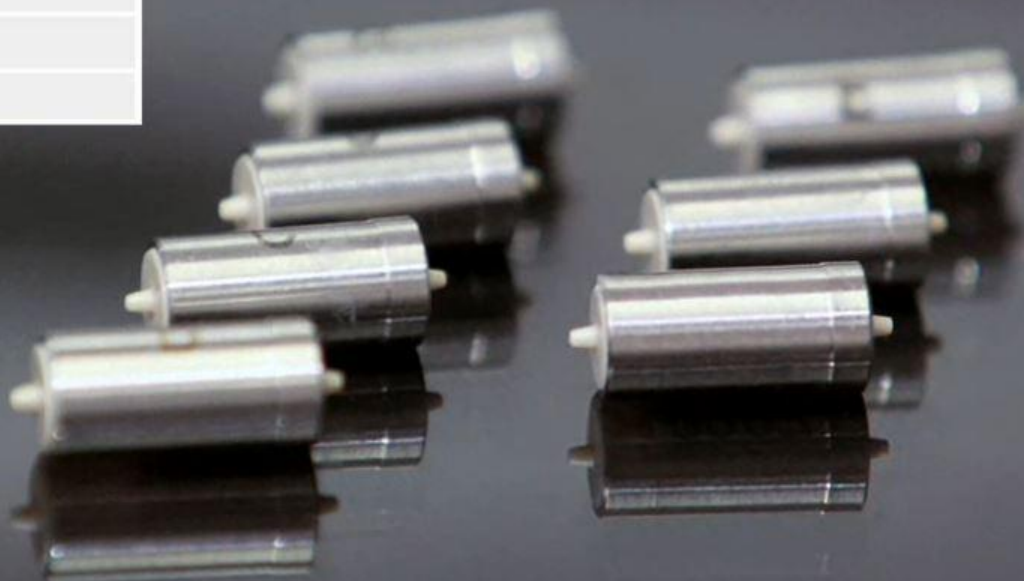
# RapidFire 365 - cartridges

Packing Material	Typical Applications
C4	Small molecules, peptides, oligos
Cyano	Hydrophobic compounds
C18	Proteins
Graphitic Carbon	Hydrophilic compounds, small molecules
C8	Proteins
Phenyl	Aromatic compounds
HILIC	Small polar molecules
Custom	Custom application

Up to 12 re-usable SPE cartridges

Same type or mix

2000+ injections per cartridge



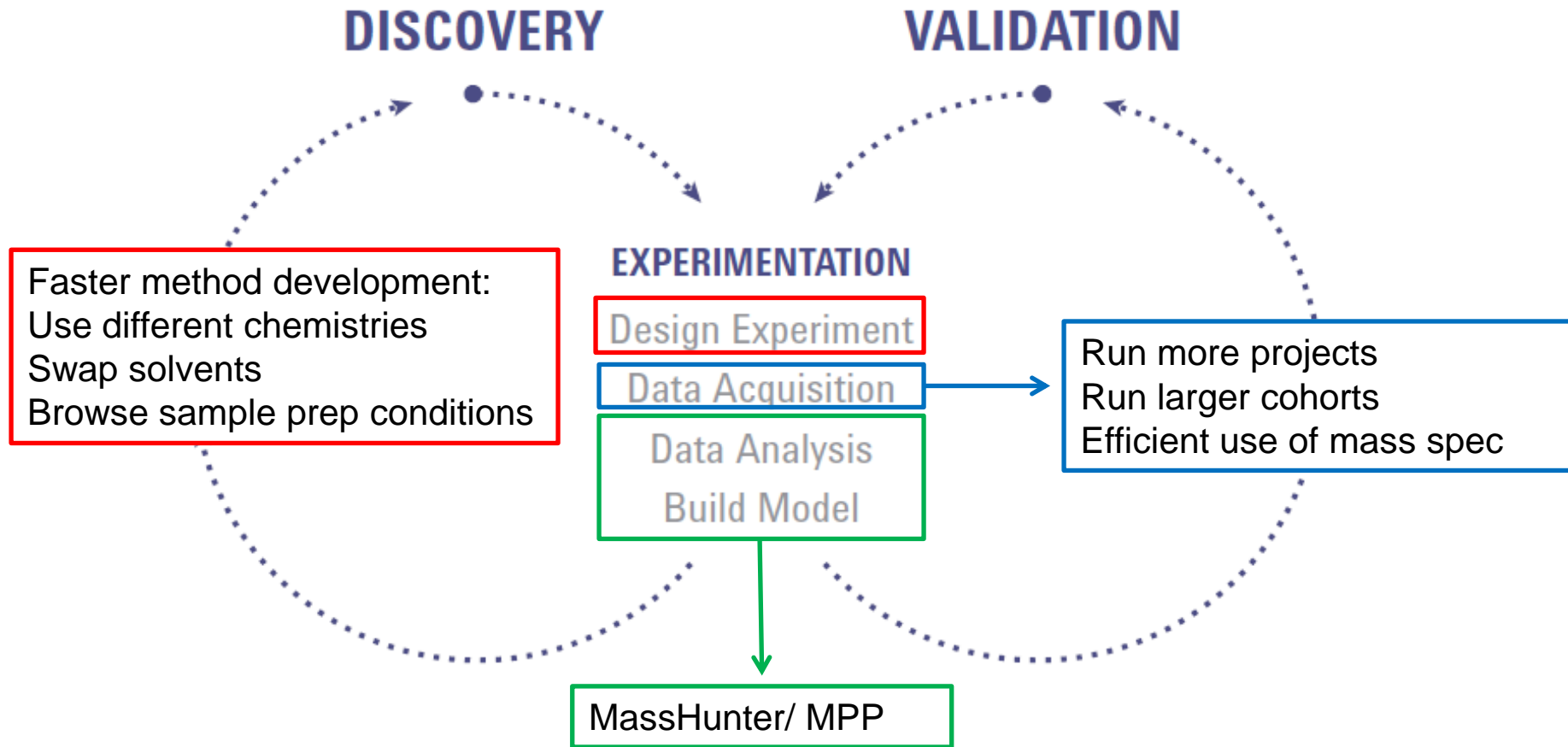
# RapidFire 365 – solvent delivery



3 pumps with 4 channels each → up to 12 different solvents

# Bottlenecks in a typical core facility/academia lab?

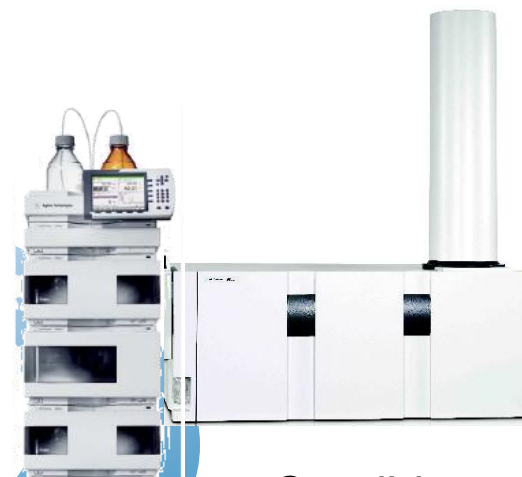
Accelerate all steps from method development to data processing



# Where to apply RapidFire in an Omics lab?

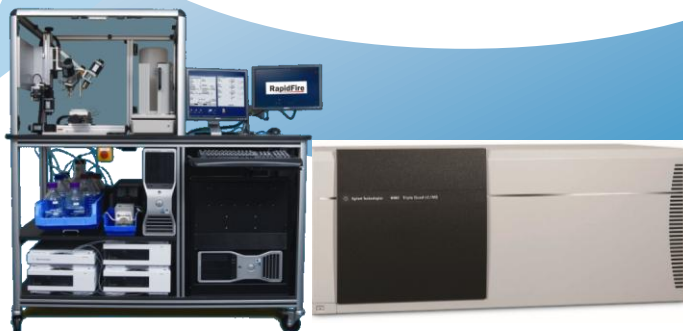


Fast method development/  
early discovery studies



Candidate  
biomarker discovery  
+ validation

**Software tools**



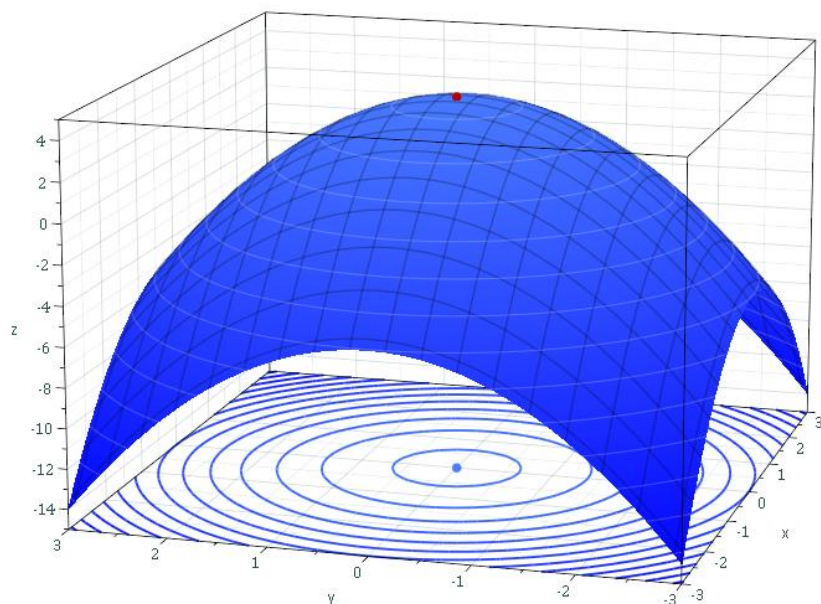
Fast validation



# Finding the sweet spot(s) of your samples

**Optimization - an act, process, or methodology of making something (as a design, system, or decision) as fully perfect, functional, or effective as possible**

**Simple View – 1 parameter**



**“Real” View – many parameters**



# „Screening“ of methods and parameters



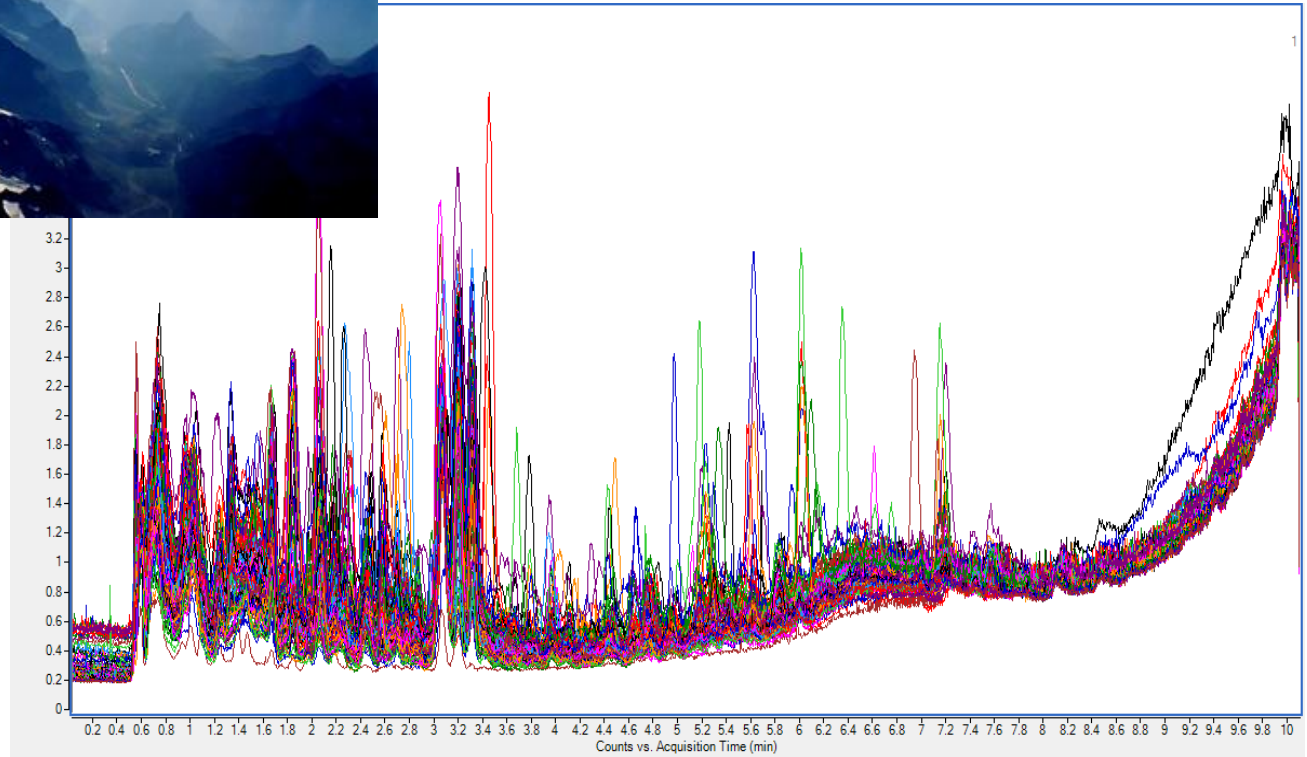
Typical „landscape“ of heterogenously distributed metabolites  
Ideally, each compound or at least compound class should be analyzed using a dedicated method. Finding the best parameter set can be cumbersome

# Untargeted profiling

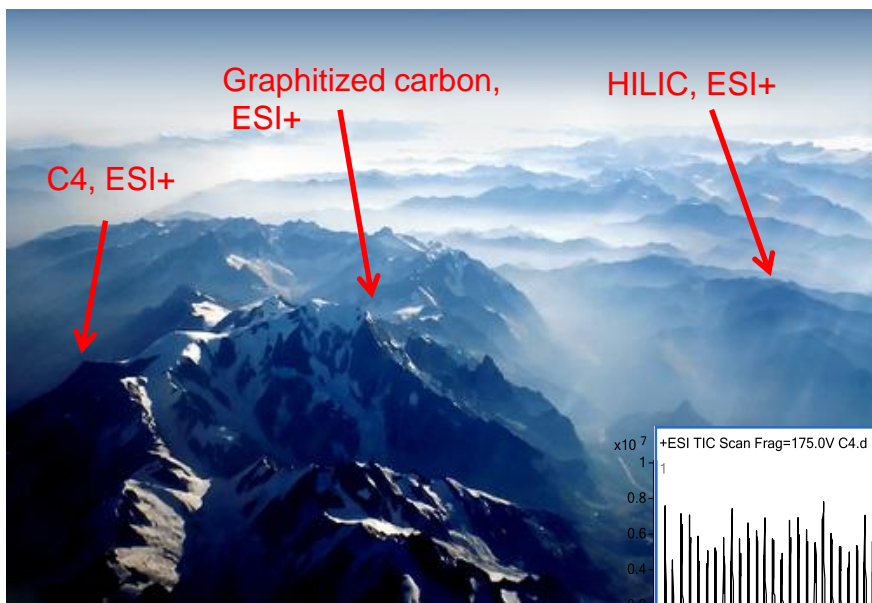


Case study: 58 kidney stone formers  
vs. 38 healthy individuals  
Analyzed using UHPLC/QTOF-MS  
C18 and ESI+ only

→ 20 hours run time

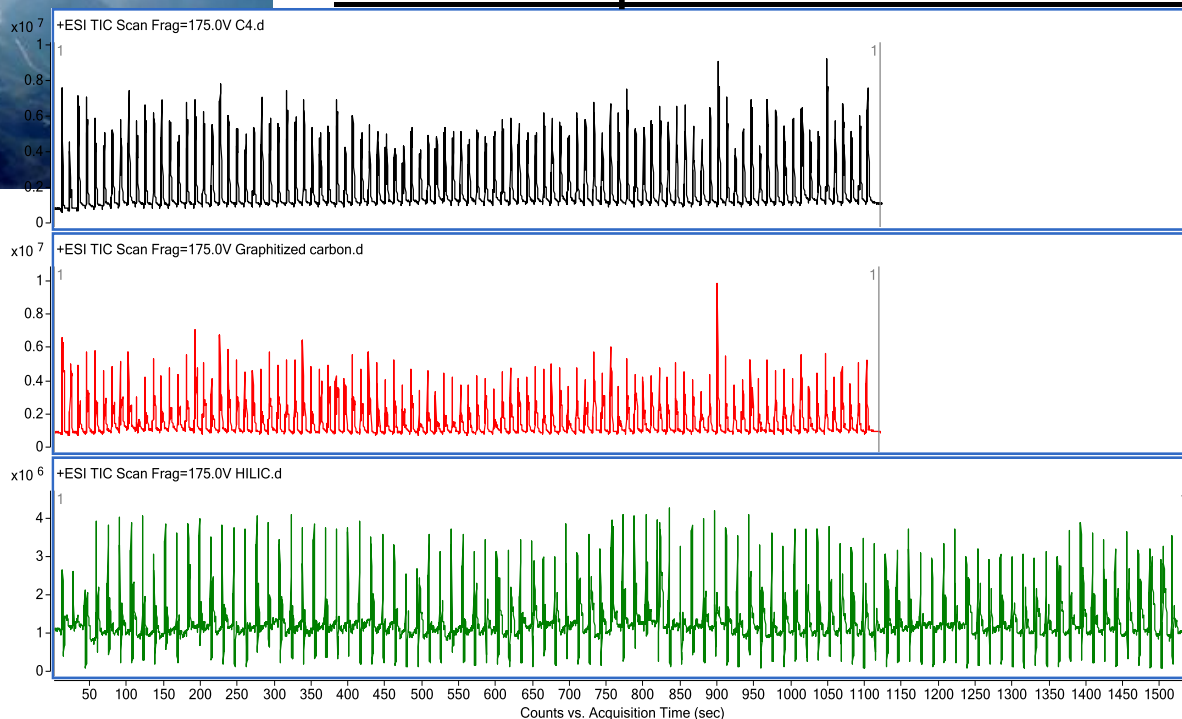


# Untargeted profiling

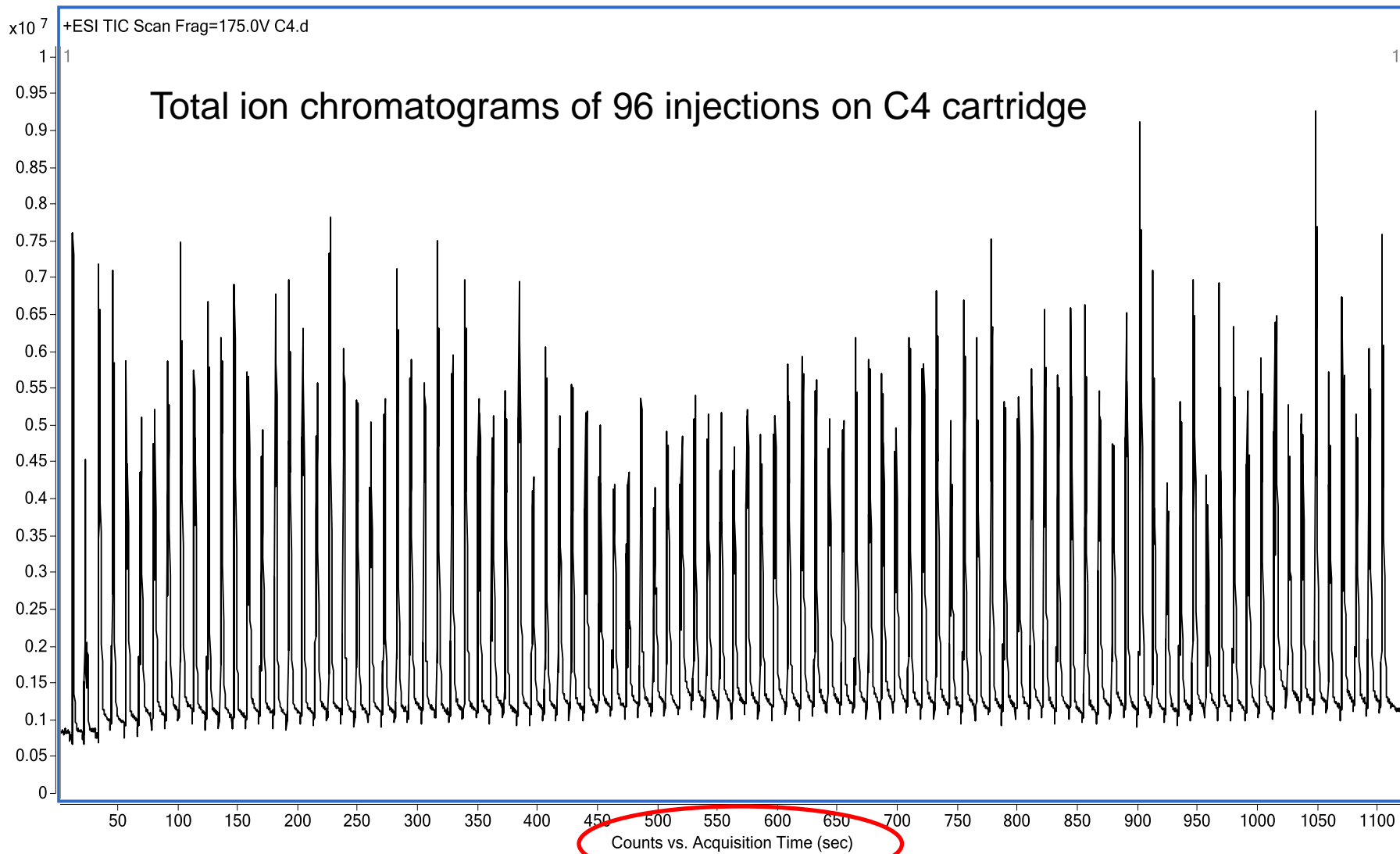


Case study: 58 kidney stone formers vs. 38 healthy individuals  
Analyzed using 3x using different chemistries

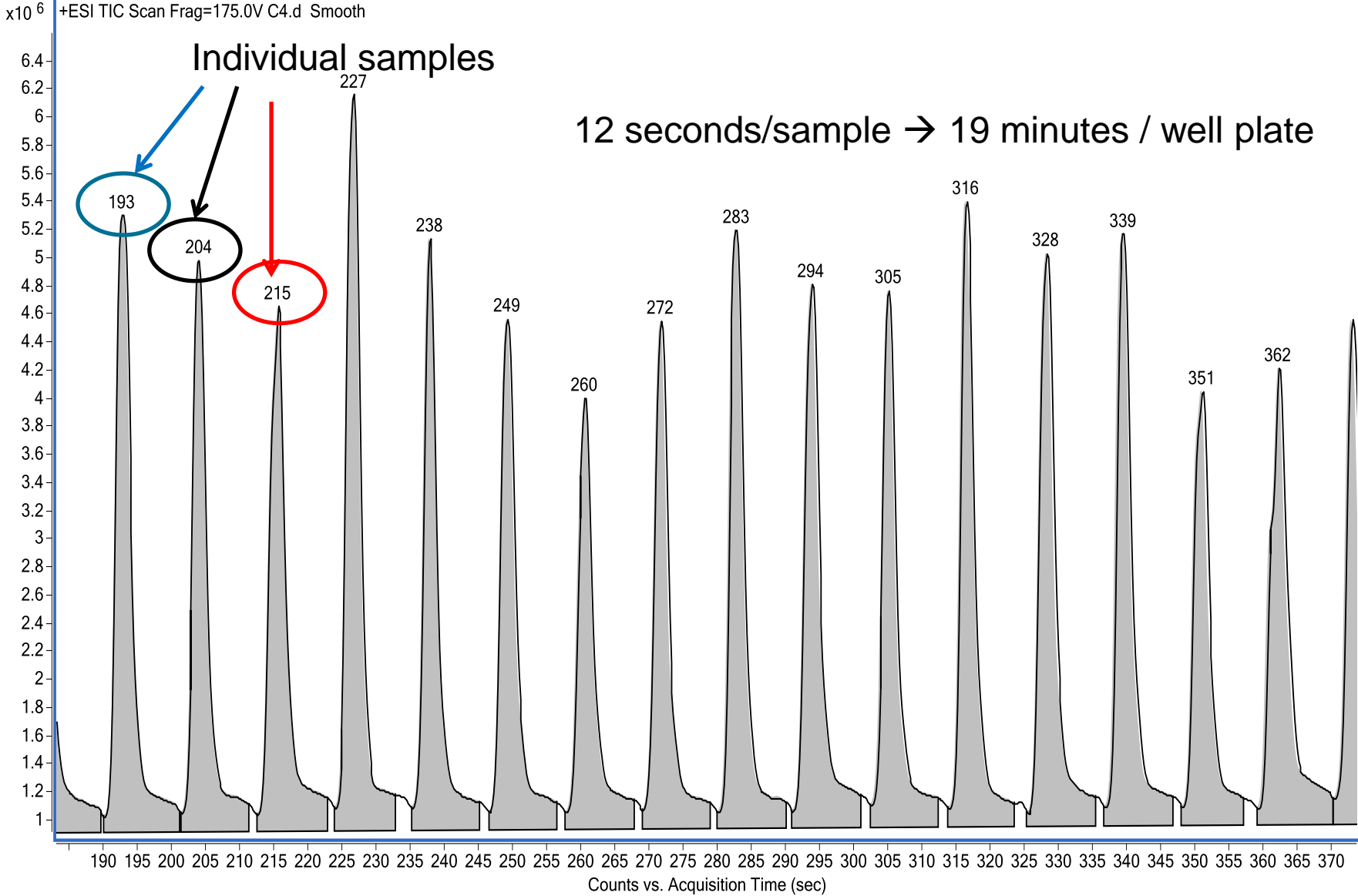
→ 1 hour run time,  
60-fold improvement vs LCMS



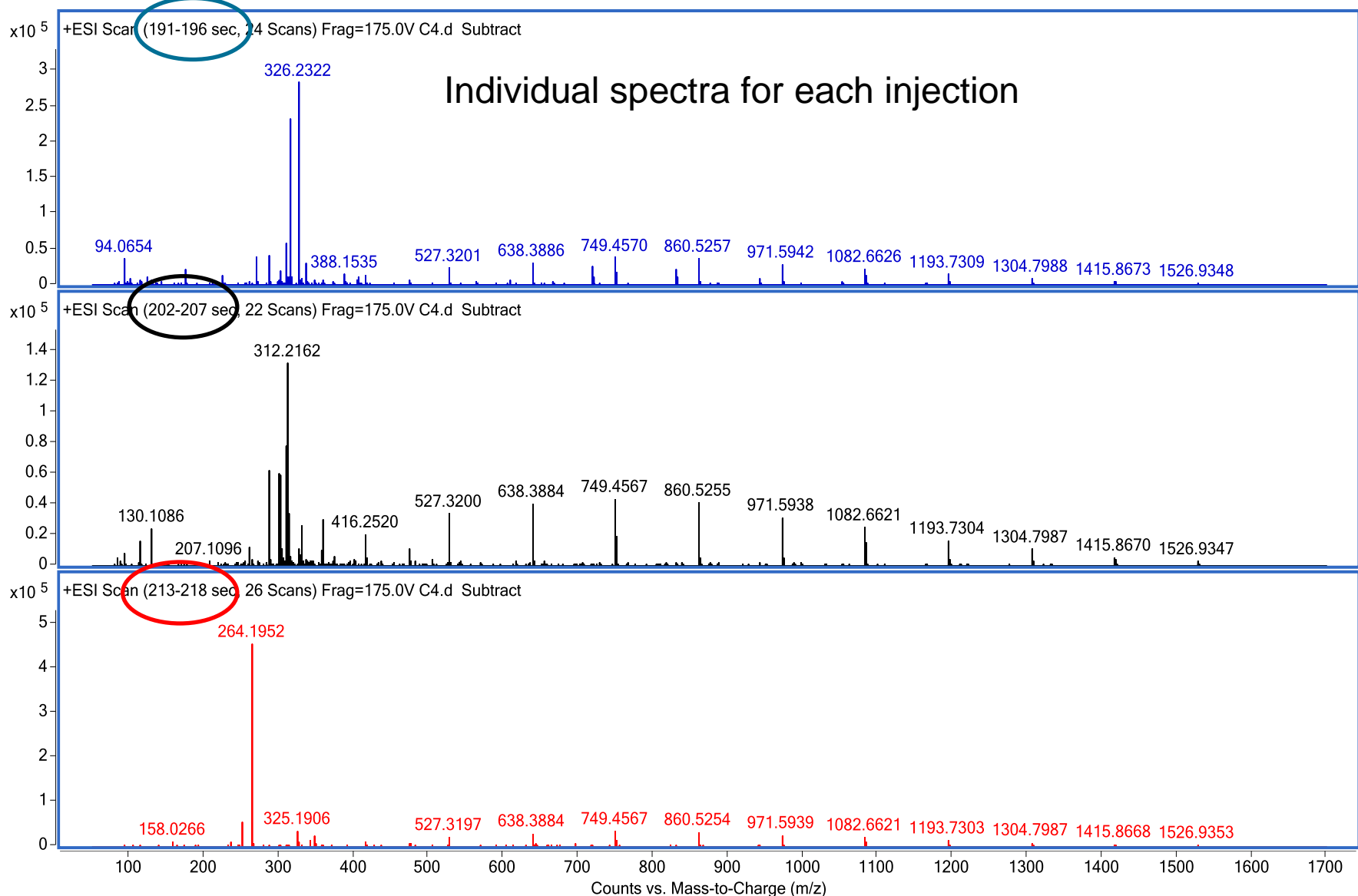
# Untargeted profiling



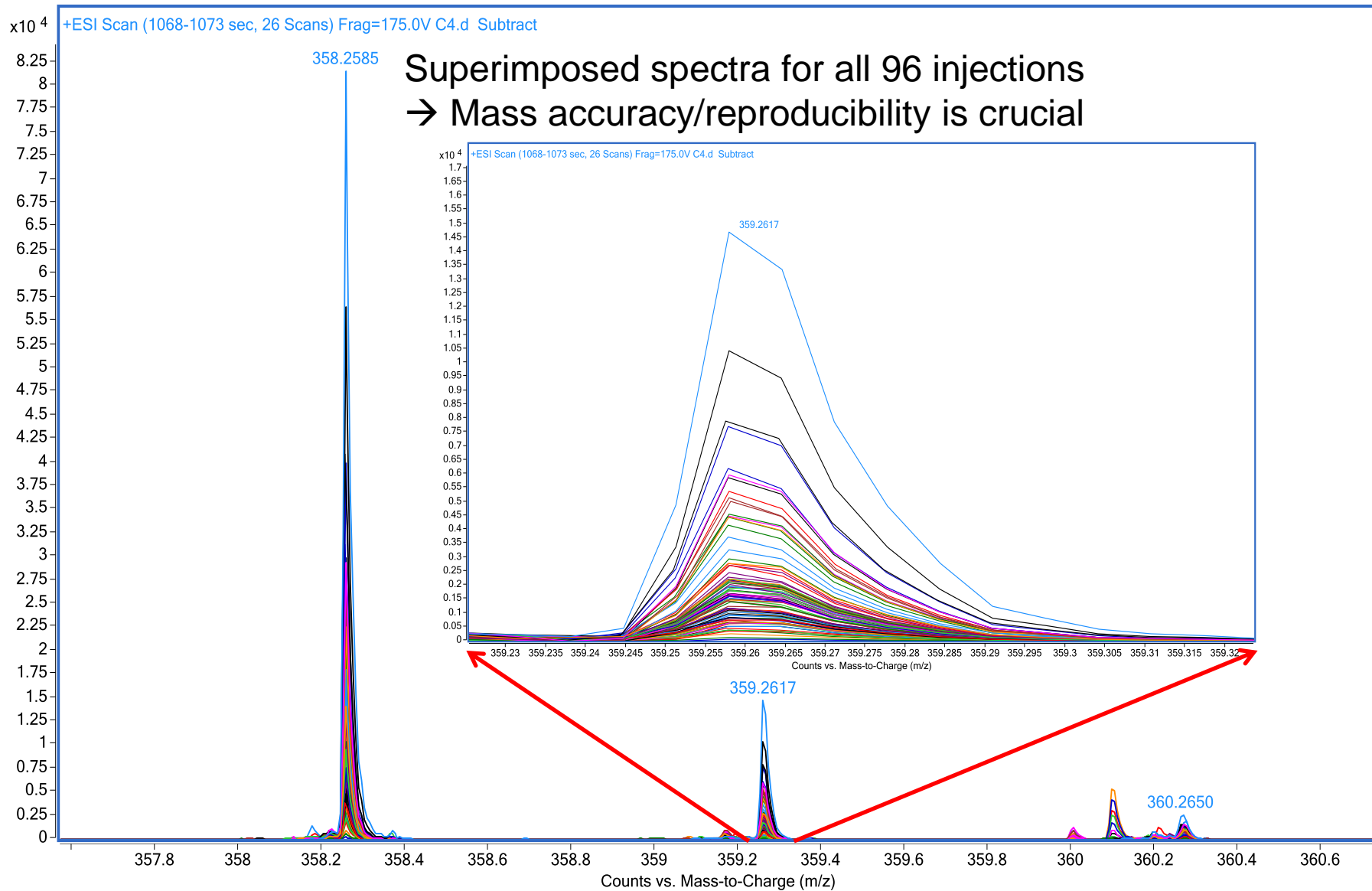
# Untargeted profiling



# Untargeted profiling



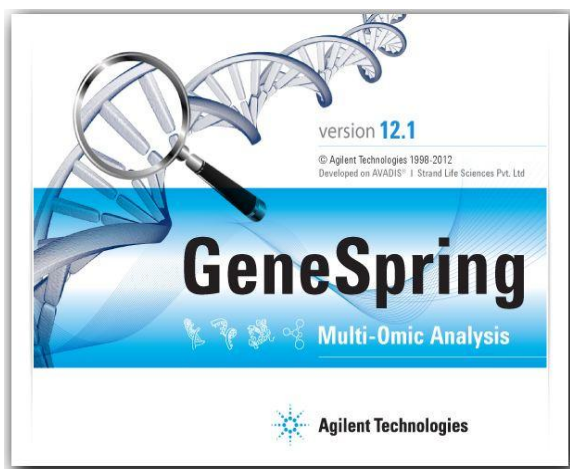
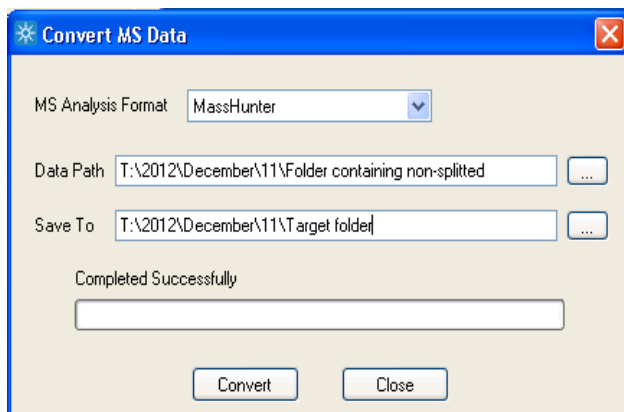
# Untargeted profiling





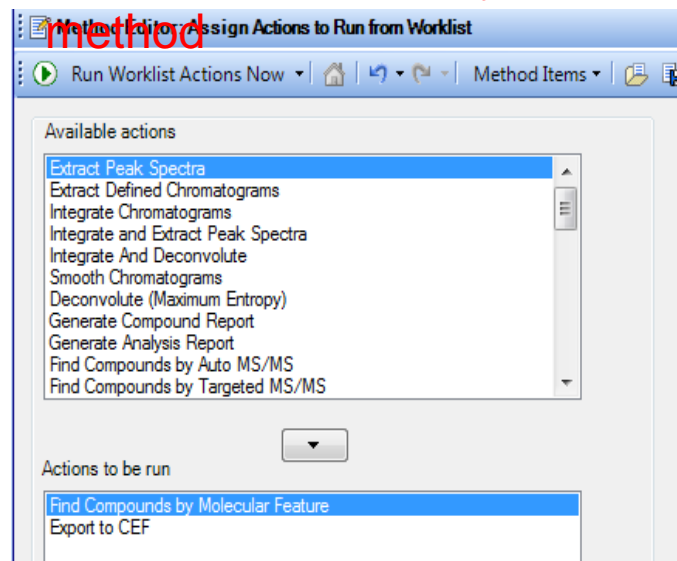
# Untargeted profiling

## 1. Convert/split data



## 3. Statistical analysis

## 2a. Create data analysis method

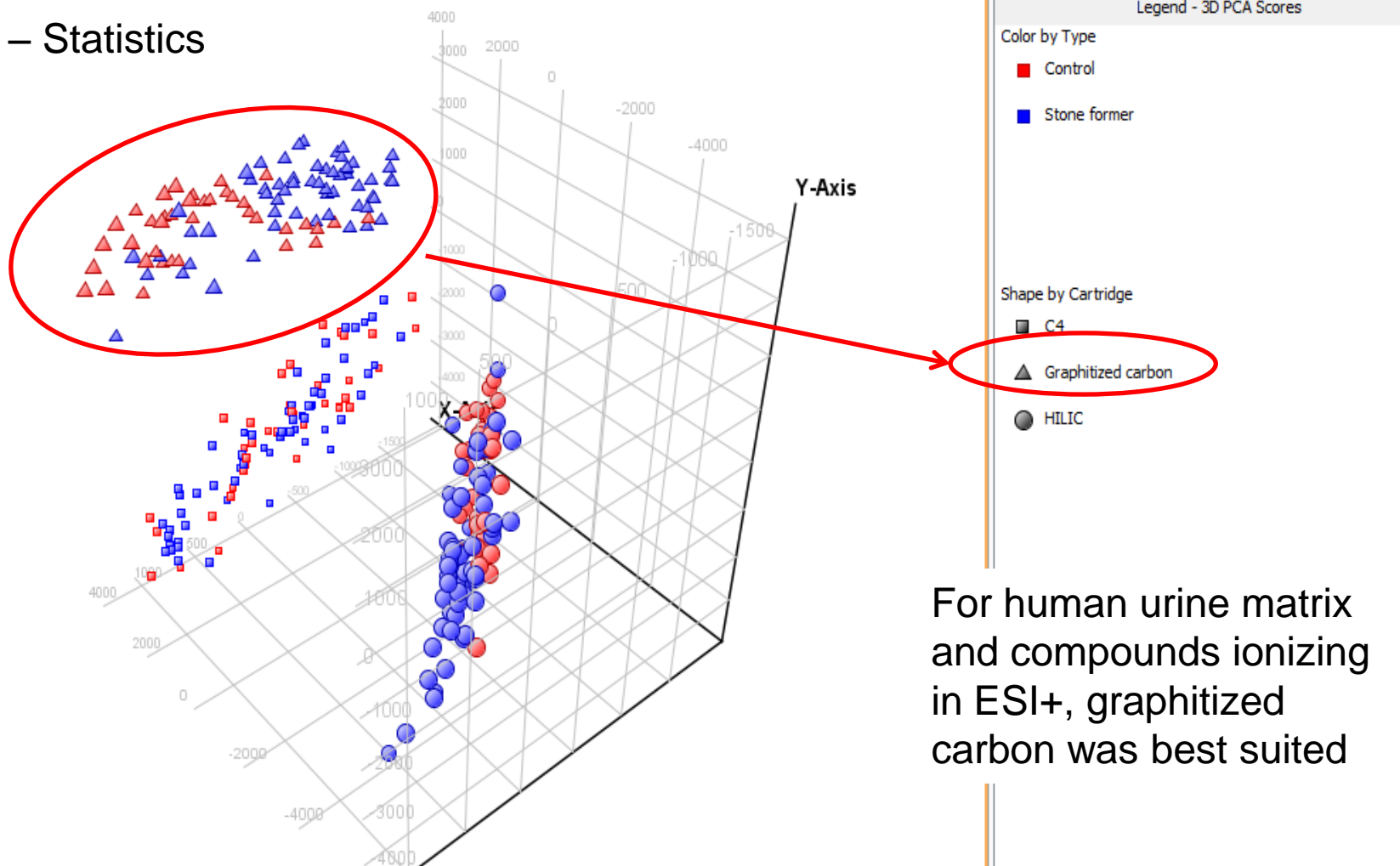


	<input type="checkbox"/>	Sample Name	Sample Position	Method	
1	<input type="checkbox"/>	Sample3	P1-A1	D:\MassHunter\damethods\MPP_RF.m	Inj00003-C4-A1.d
2	<input type="checkbox"/>	Sample4	P1-A2	D:\MassHunter\damethods\MPP_RF.m	Inj00004-C4-A2.d
3	<input type="checkbox"/>	Sample5	P1-A3	D:\MassHunter\damethods\MPP_RF.m	Inj00005-C4-A3.d
4	<input type="checkbox"/>	Sample6	P1-A4	D:\MassHunter\damethods\MPP_RF.m	Inj00006-C4-A4.d
5	<input type="checkbox"/>	Sample7	P1-A5	D:\MassHunter\damethods\MPP_RF.m	Inj00007-C4-A5.d
6	<input type="checkbox"/>	Sample8	P1-A6	D:\MassHunter\damethods\MPP_RF.m	Inj00008-C4-A6.d
7>	<input checked="" type="checkbox"/>	Sample9	P1-A7	D:\MassHunter\damethods\MPP_RF.m	Inj00009-C4-A7.d
8	<input checked="" type="checkbox"/>	Sample10	P1-A8	D:\MassHunter\damethods\MPP_RF.m	Inj00010-C4-A8.d
9	<input checked="" type="checkbox"/>	Sample11	P1-A9	D:\MassHunter\damethods\MPP_RF.m	Inj00011-C4-A9.d
10	<input checked="" type="checkbox"/>	Sample12	P1-A10	D:\MassHunter\damethods\MPP_RF.m	Inj00012-C4-A10.d

## 2b. Run worklist/sequence for data processing

# Untargeted profiling

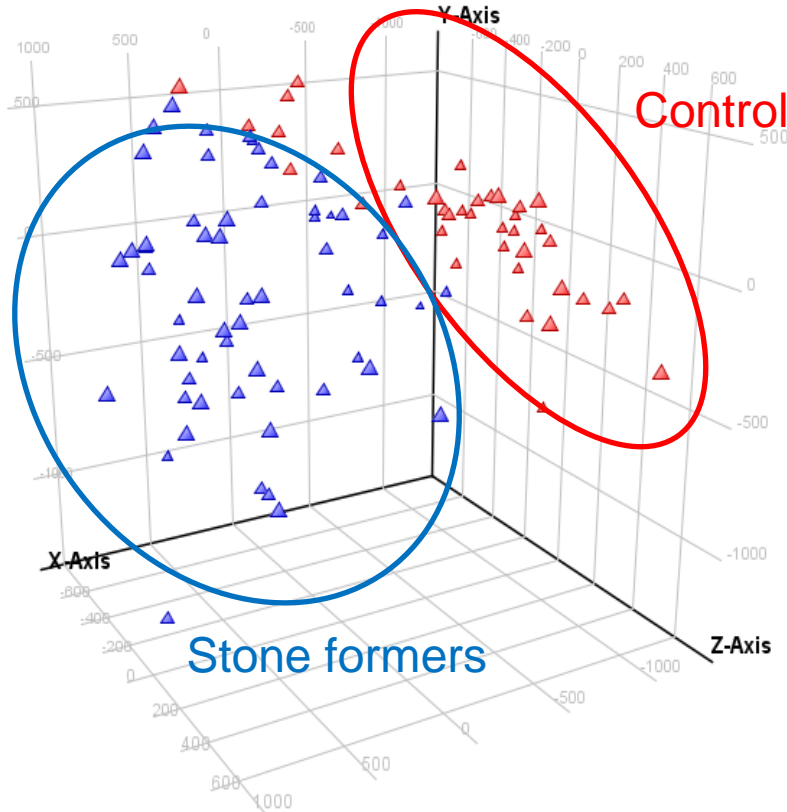
## Step 3 – Statistics



Hundreds and thousands of data points can be quickly browsed and evaluated

# Untargeted profiling

## Step 3 – Statistics



Legend - 3D PCA Score

Color by Type

■ Control

■ Stone former

Shape by Cartridge

▲ Graphitized carbon

Description

Algorithm: Principal Components A

Parameters:

Column indices = [1-96]

Pruning option = [numPrincipalCo

Mean centered = true

Scale = true

3-D scores = true

PCA on = Columns

X-Axis Component 1 (15.28%)

Y-Axis Component 2 (7.99%)

Z-Axis Component 3 (6.7%)

<< Back

Next >>

Finish

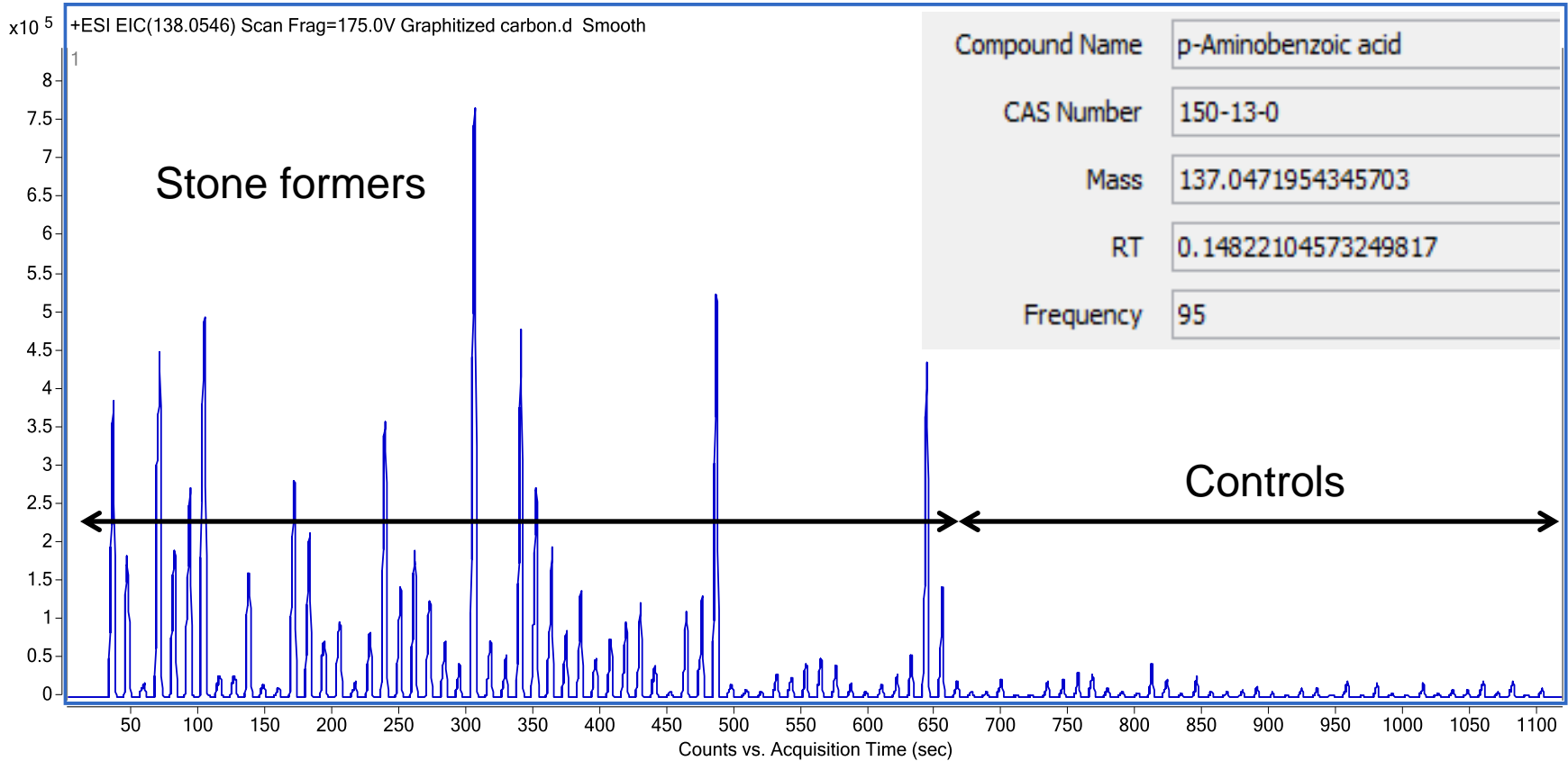
Cancel

2nd pass PCA showing only graphitized carbon data

# Untargeted profiling

## Step 3 – Statistics

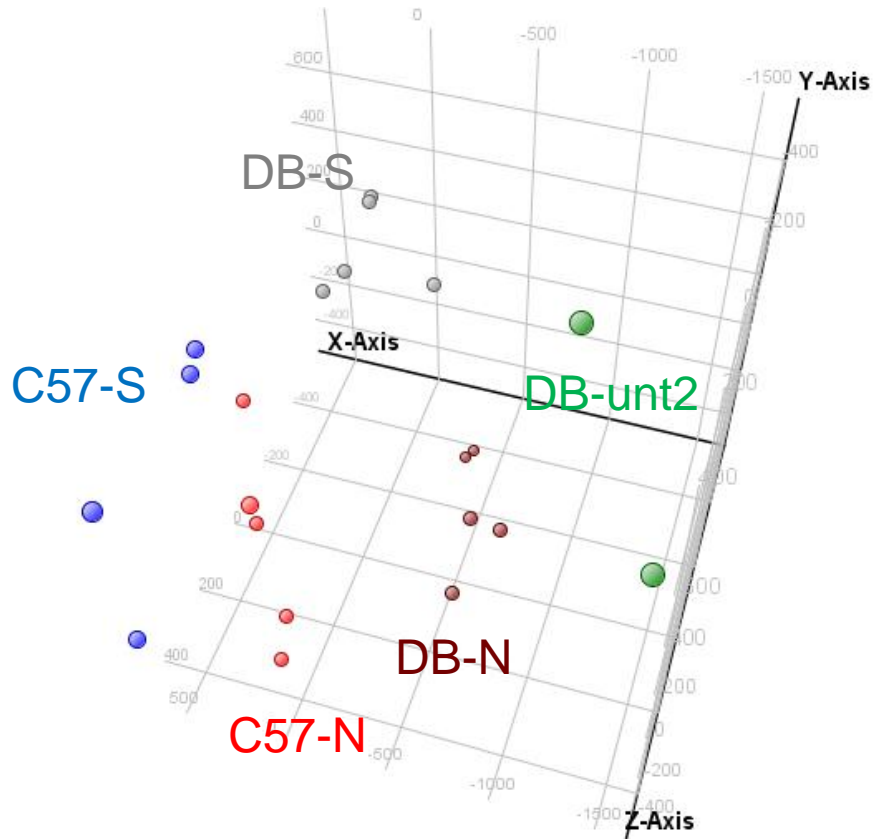
Extracted ion chromatogram for one statistically marked compound



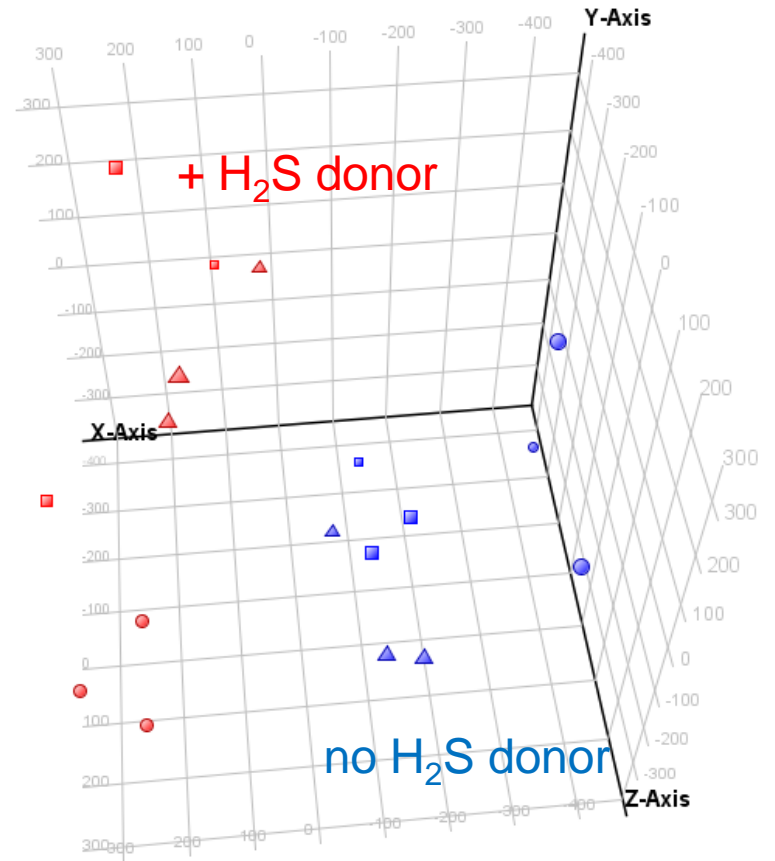
→ Validation by isotope pattern, MS/MS and retention time for isomers needs LC/MS

# Untargeted profiling

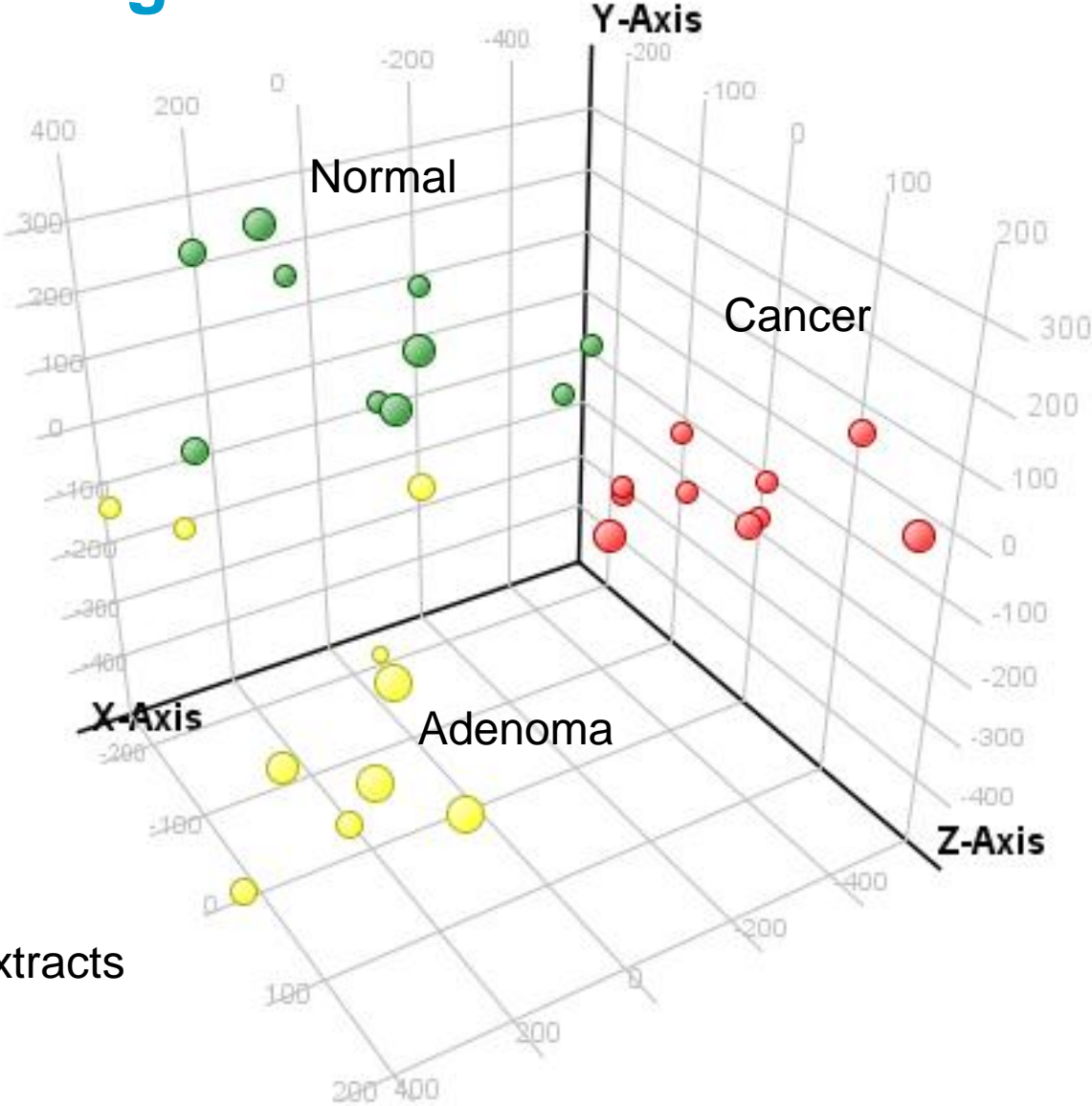
Mouse plasma extracts



3 different cell lines  
(n = 3 each, visualized by shape)



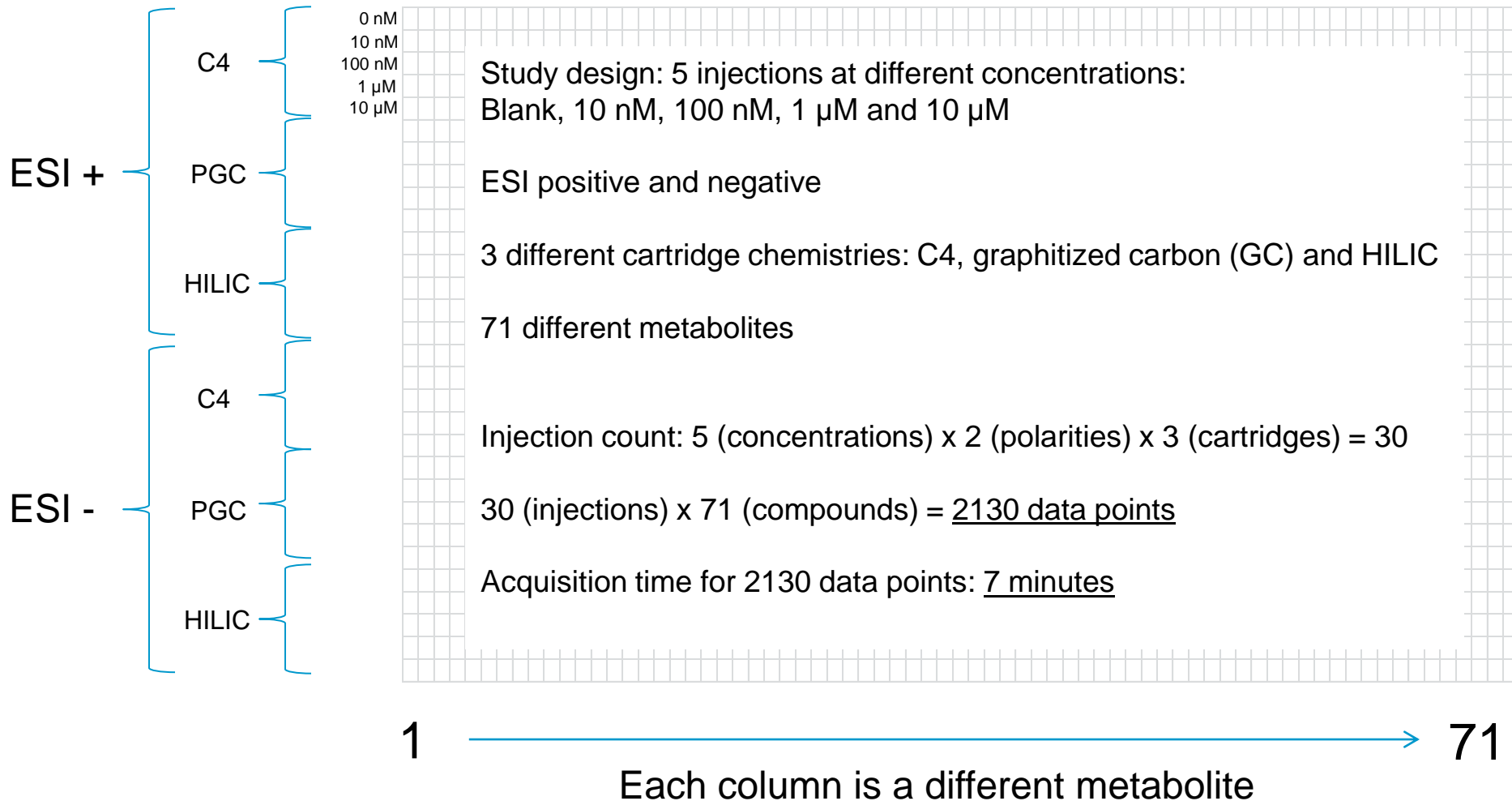
# Untargeted profiling



PCA plot of fecal matter extracts

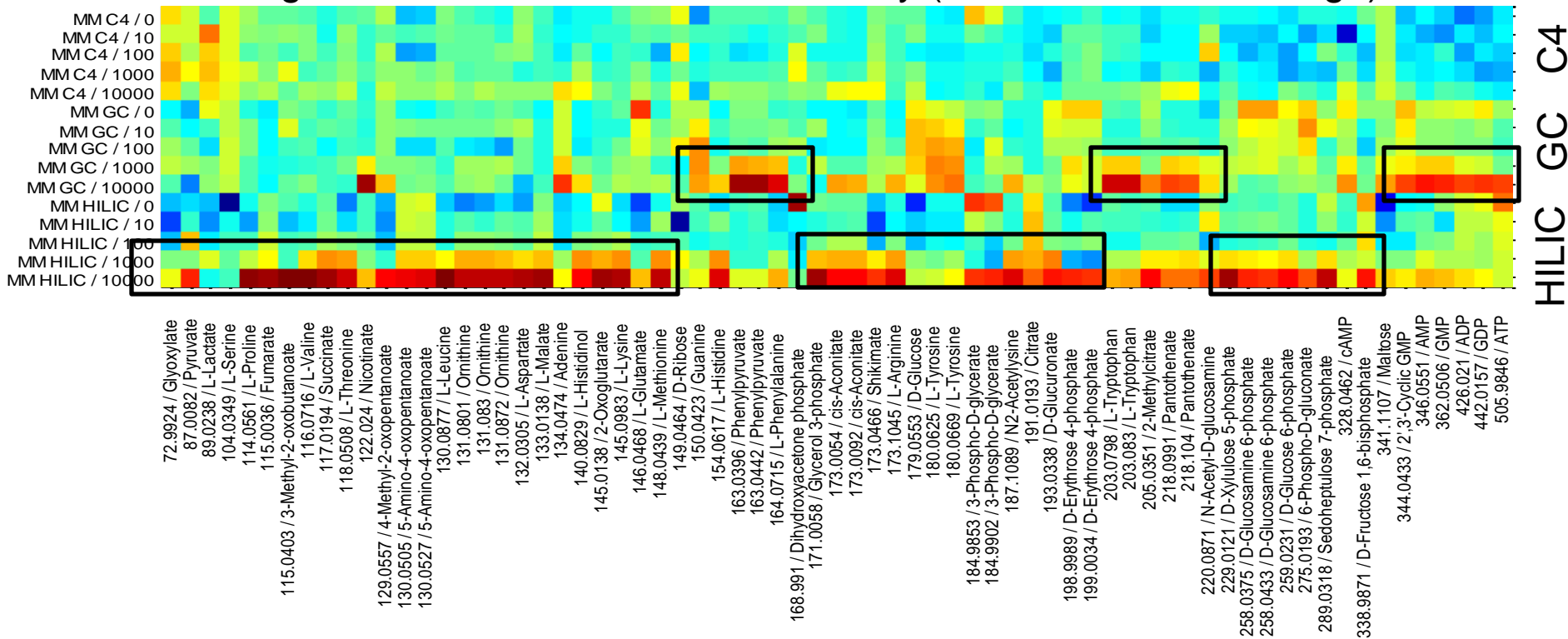
3 groups (n = 10 each)

# Targeted profiling and quantitation



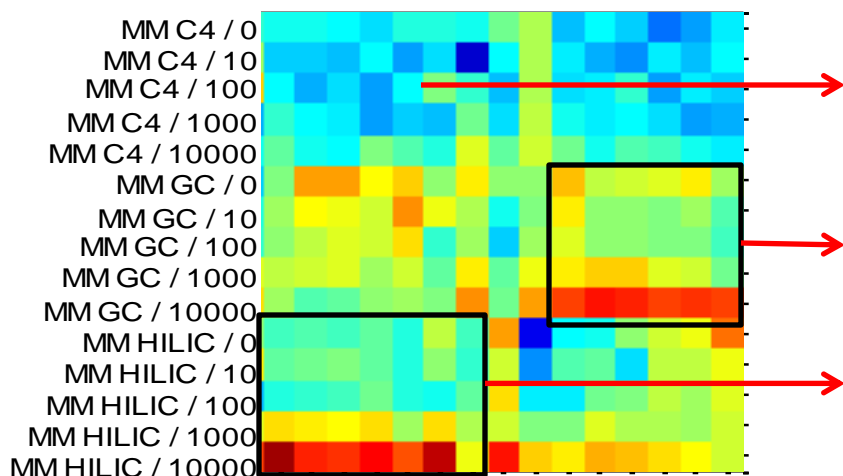
# Targeted profiling and quantitation

ESI negative data, color codes for intensity (blue: low → brown: high)





# Targeted profiling and quantitation



Low intensities for all compounds on C4

Good intensities and concentration based correlation for nucleotides using GC

Good intensities and concentration based correlation for sugar phosphates using HILIC

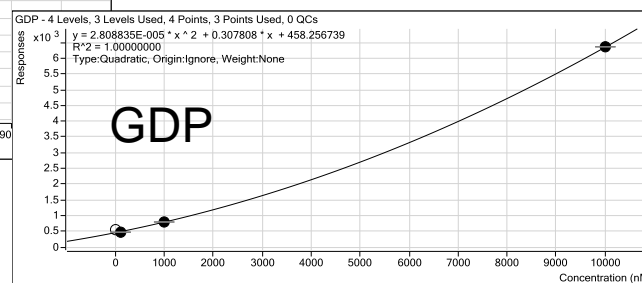
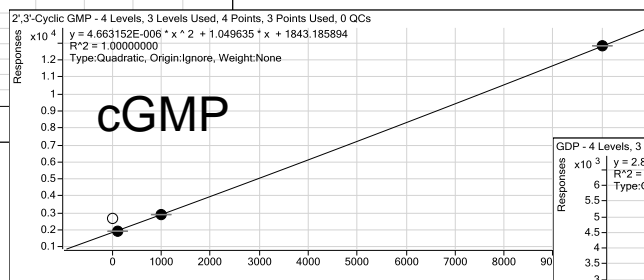
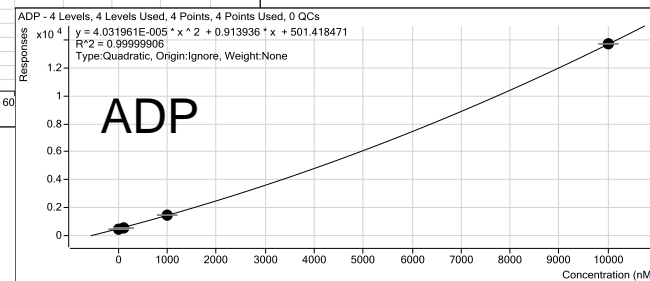
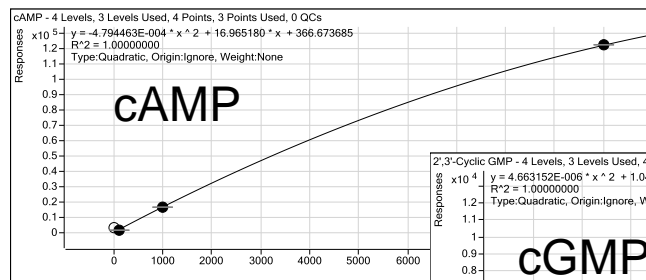
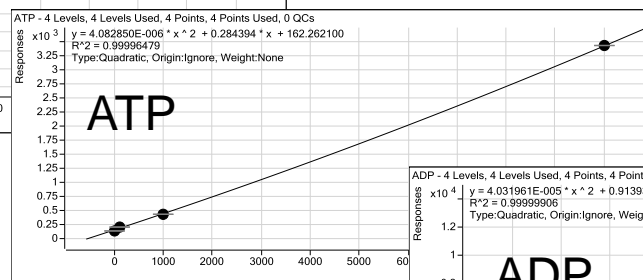
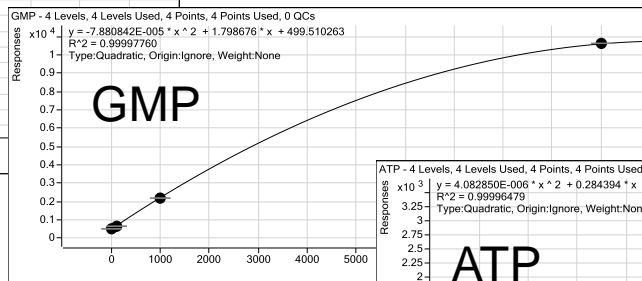
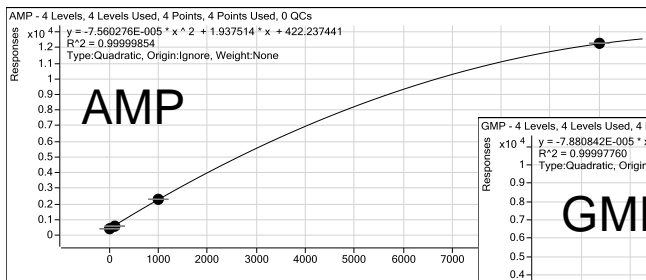
229.0121 / D-Xylulose 5-phosphate  
 258.0375 / D-Glucosamine 6-phosphate  
 258.0433 / D-Glucosamine 6-phosphate  
 259.0231 / D-Glucose 6-phosphate  
 275.0193 / 6-Phospho-D-gluconate  
 289.0318 / Sedoheptulose 7-phosphate  
 328.0462 / cAMP  
 338.9871 / D-Fructose 1,6-bisphosphate  
 341.1107 / Maltose  
 344.0433 / 2',3'-Cyclic GMP  
 346.0551 / AMP  
 362.0506 / GMP  
 426.021 / ADP  
 442.0157 / GDP  
 505.9846 / ATP

Details visualized on a compound class base

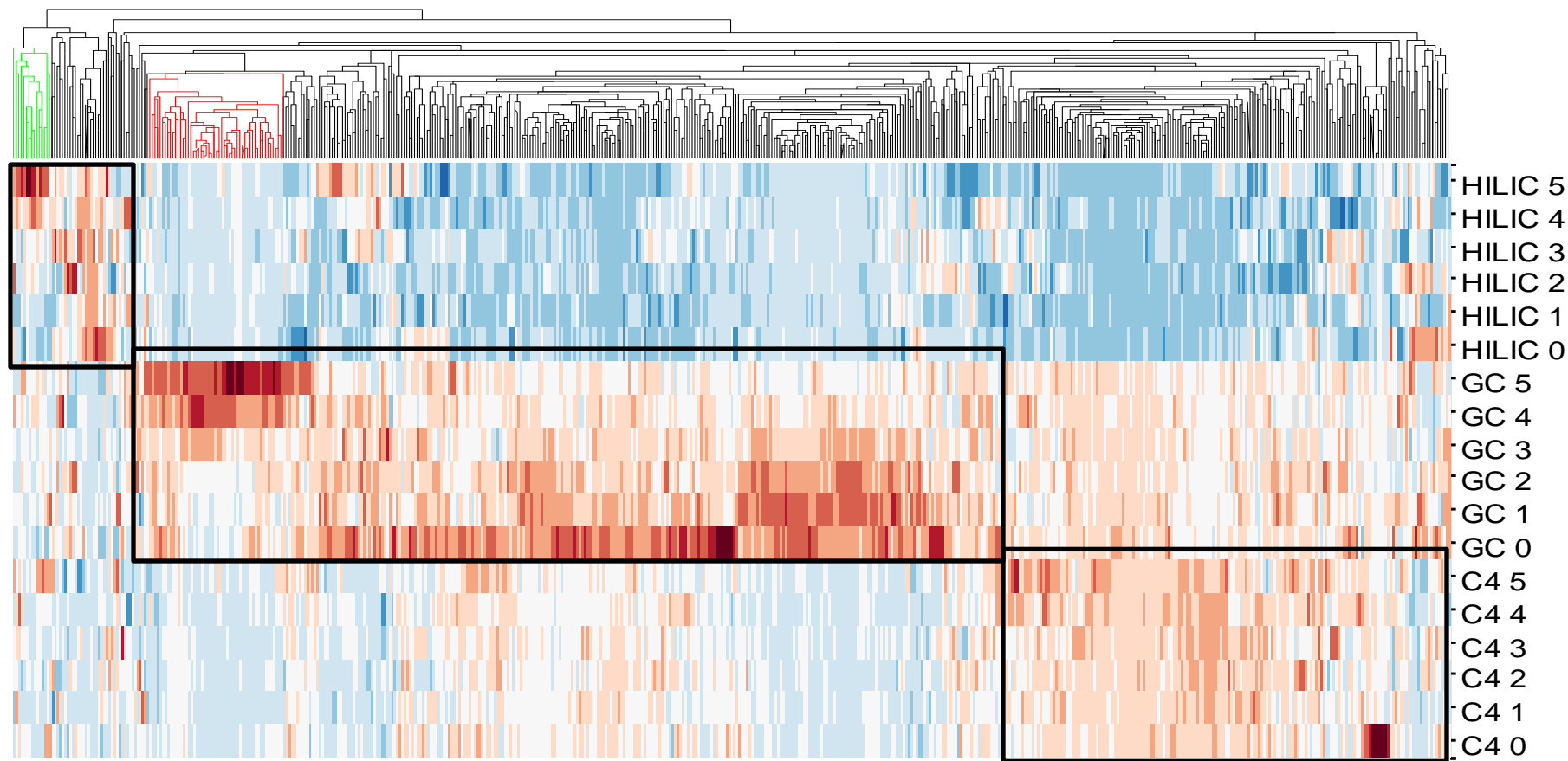
# Targeted profiling and quantitation

Calibration for nucleotides using GC  
10 nM – 10 μM (AMP,GMP,ADP,ATP)  
100 nM – 10 μM (cAMP,cGMP,GDP)

All  $R^2 > 0.999$ , quadratic fit



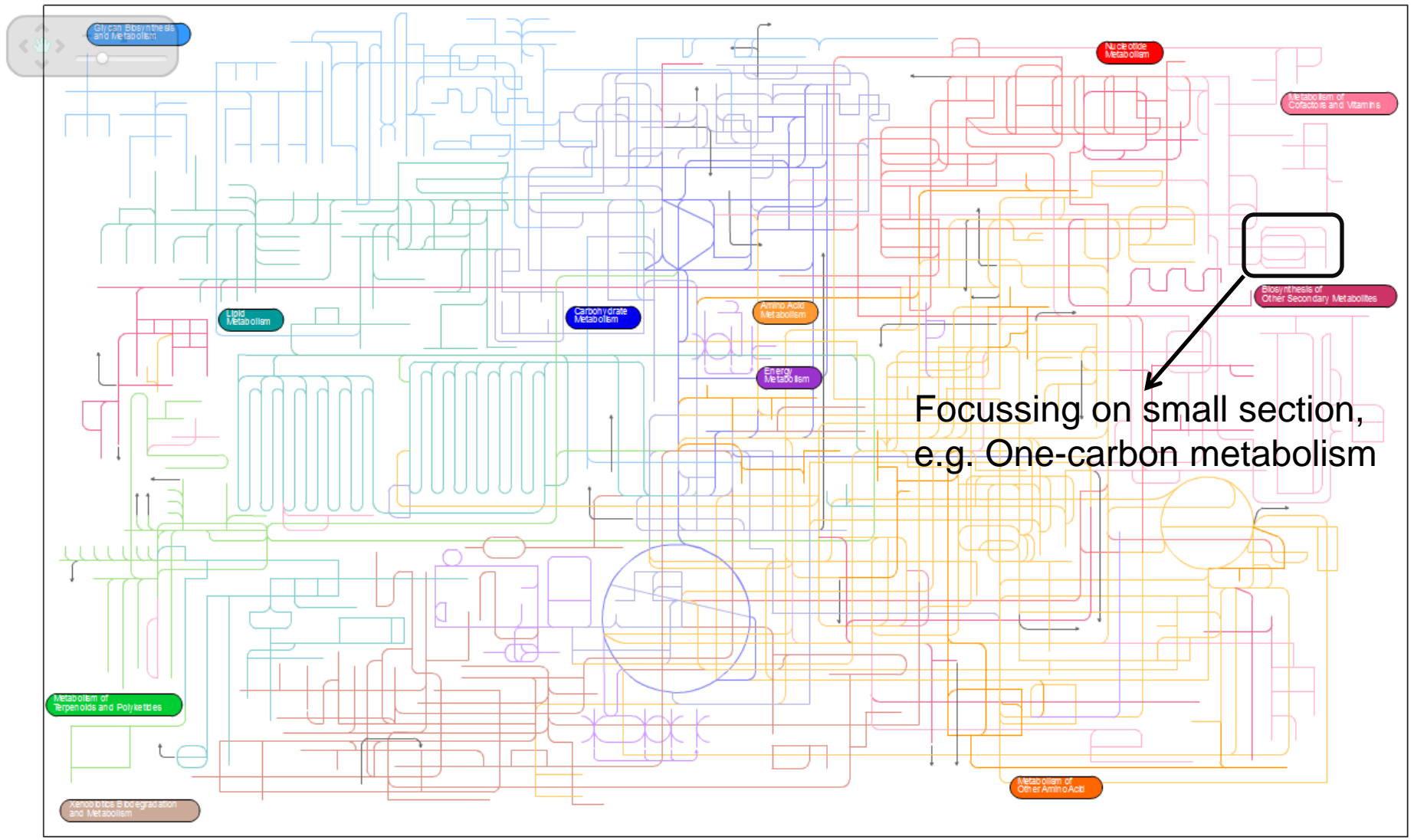
# Targeted profiling and quantitation



Enlarged hierarchical cluster analysis of all metabolites in E.coli extract using METLIN database search

→ Combine targeted and untargeted data for Pathway based analysis

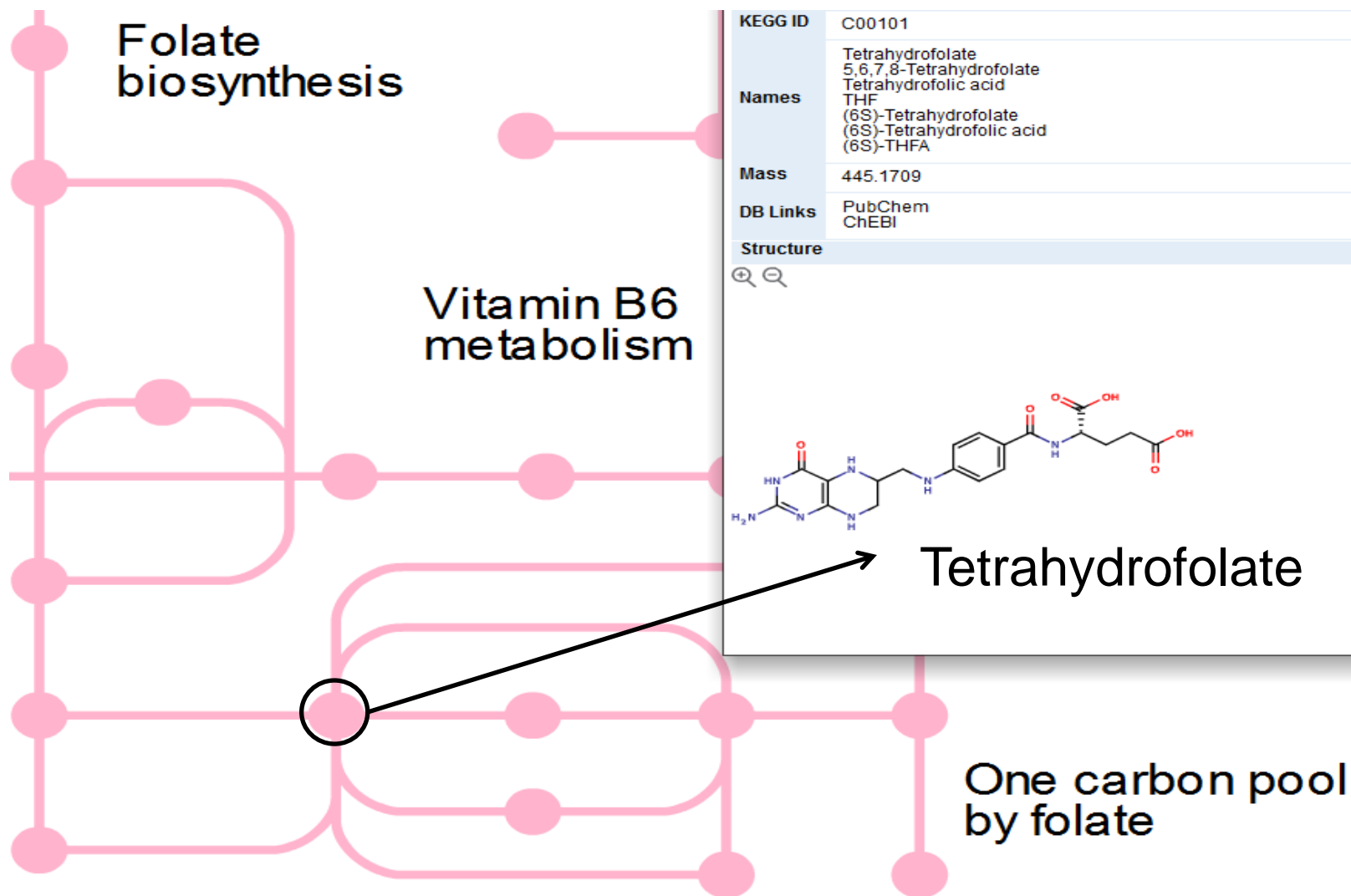
# Pathway driven analysis



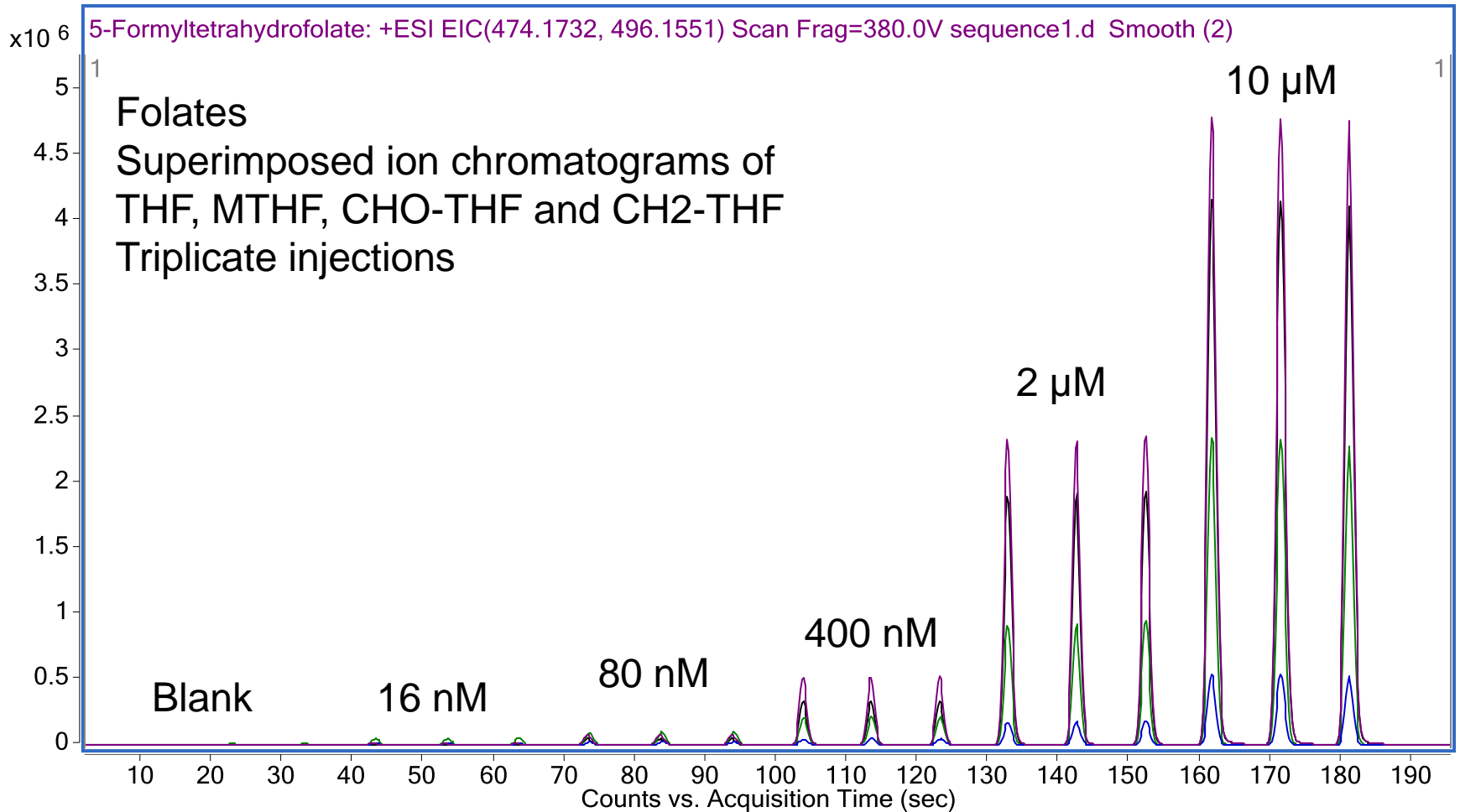
Focussing on small section,  
e.g. One-carbon metabolism

Human pathway map from: <http://pathways.embl.de/>

# Pathway driven analysis



# Pathway driven analysis



# Conclusions

- RF/MS is very well suited for all Metabolomics stages
- Time-to-answer is greatly reduced compared to LCMS, injection times are typically between 10-20 seconds per sample
- Semi-automated workflow with existing and proven tools including MPP workflow

# Contributions

Thank you to:

- Rebecca Konietzny, CCMP Oxford
- Nicola Zamboni, ETH Zürich
- Qiuying Chen and Steven Gross,  
Weill Cornell Medical College, NY