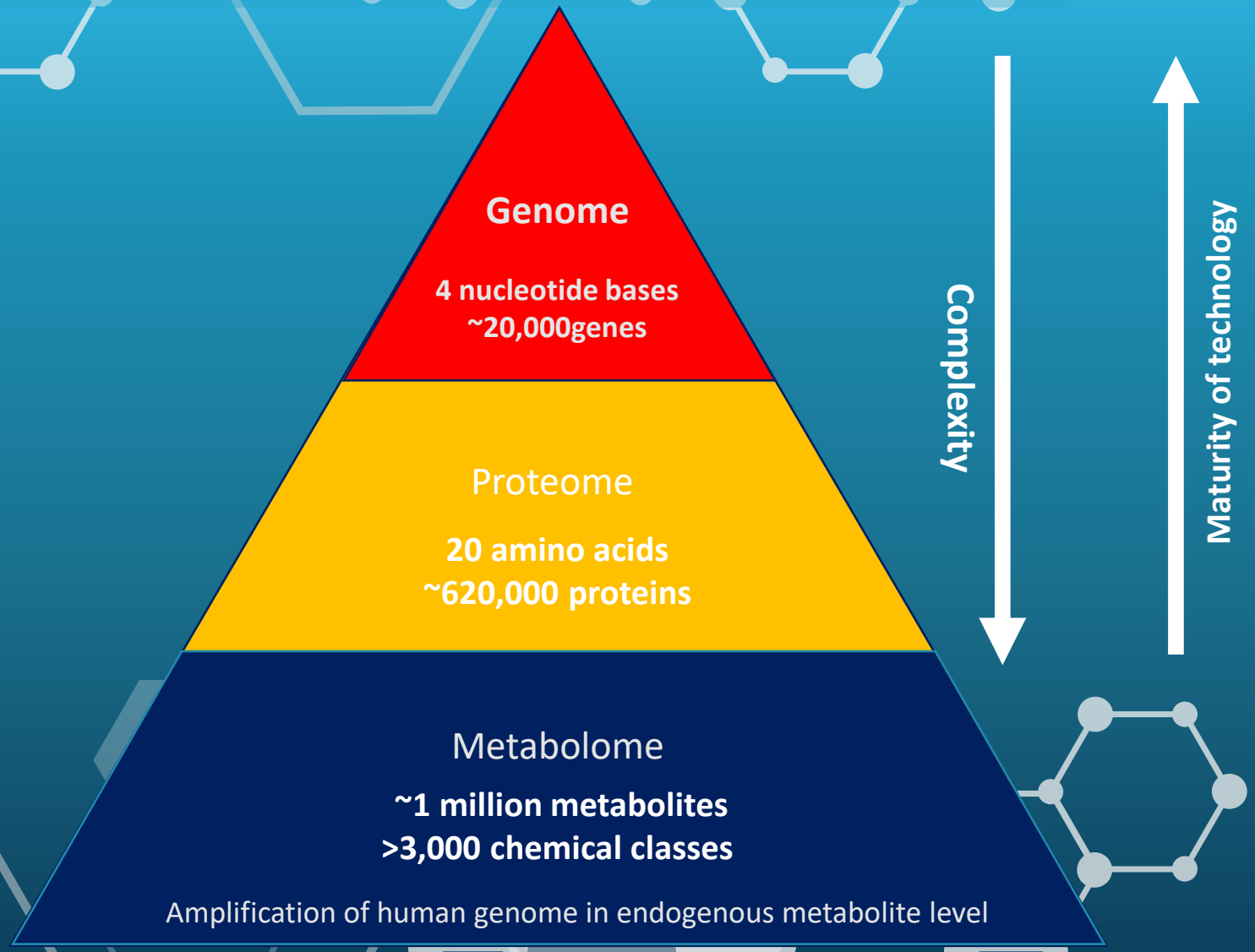
Mass Spectrometry Facility
Advanced Analysis Centre

UNIVERSITY of GUELPH

METABOLOMIC PROFILING

- Identify and Quantify

METABOLOME COMPLEXITY



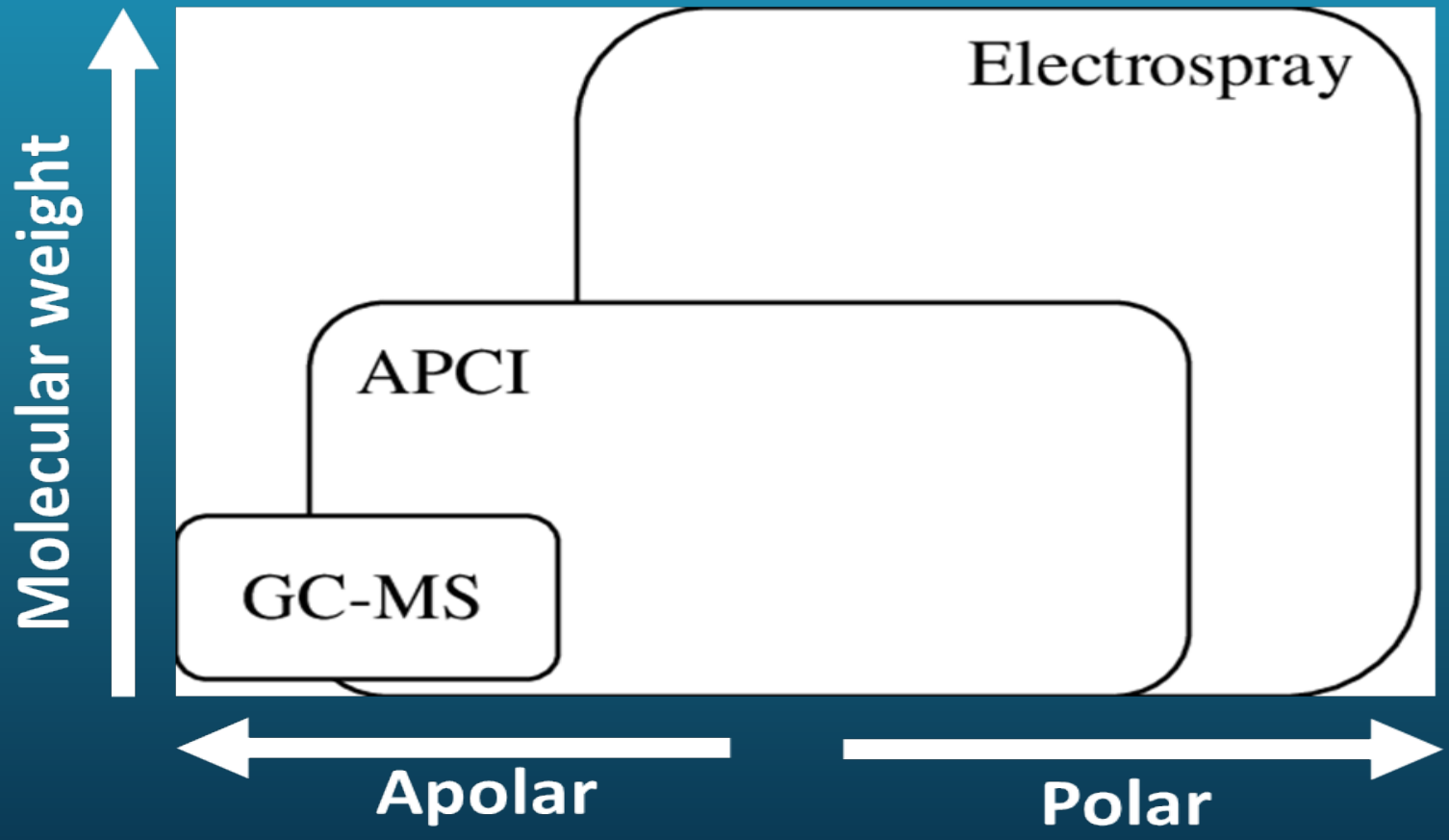
Adapted from Wishart DS. 2019

Mass Spectrometry Based Metabolite Profiling/Metabolomics Workflow

- Experiment design
- Method development
- Sample preparation
- Data acquisition
- Data processing & analysis

NO ION LEFT BEHIND!

Chromatography → Ionization → Mass Analyzer



Method development

Sample preparation

Chromatography → Ionization → Mass Analyzer

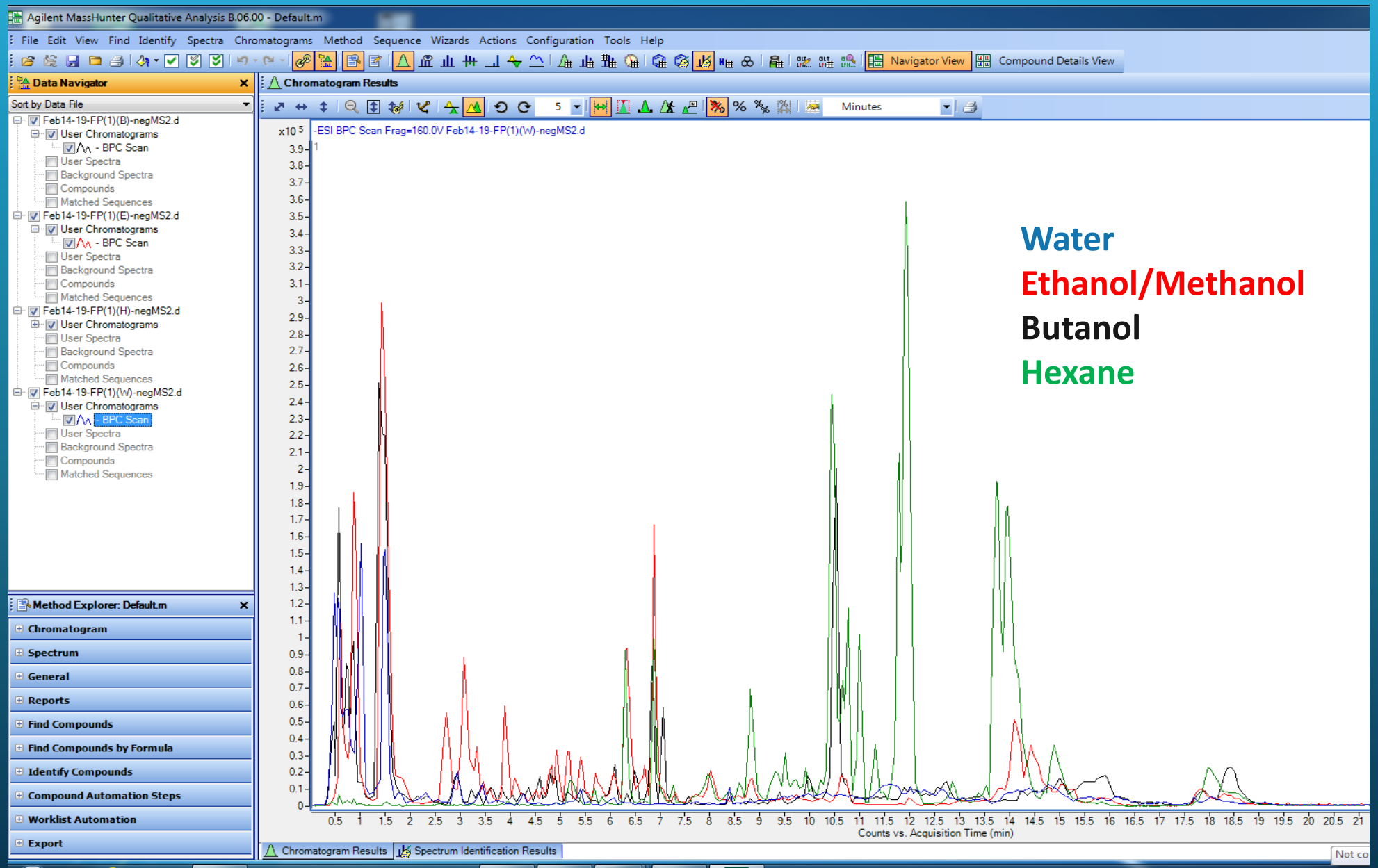
- Recovery rate
- Clean-up, SPE, filtering, centrifugation
- Ion suppression
- Sample handling
- Extraction solvents, vials, caps PTFE/silicon
- Eluents, chromatography column

Method development

Sample preparation

LC-MS

Comparing Different Extraction Solvents for LC-MS

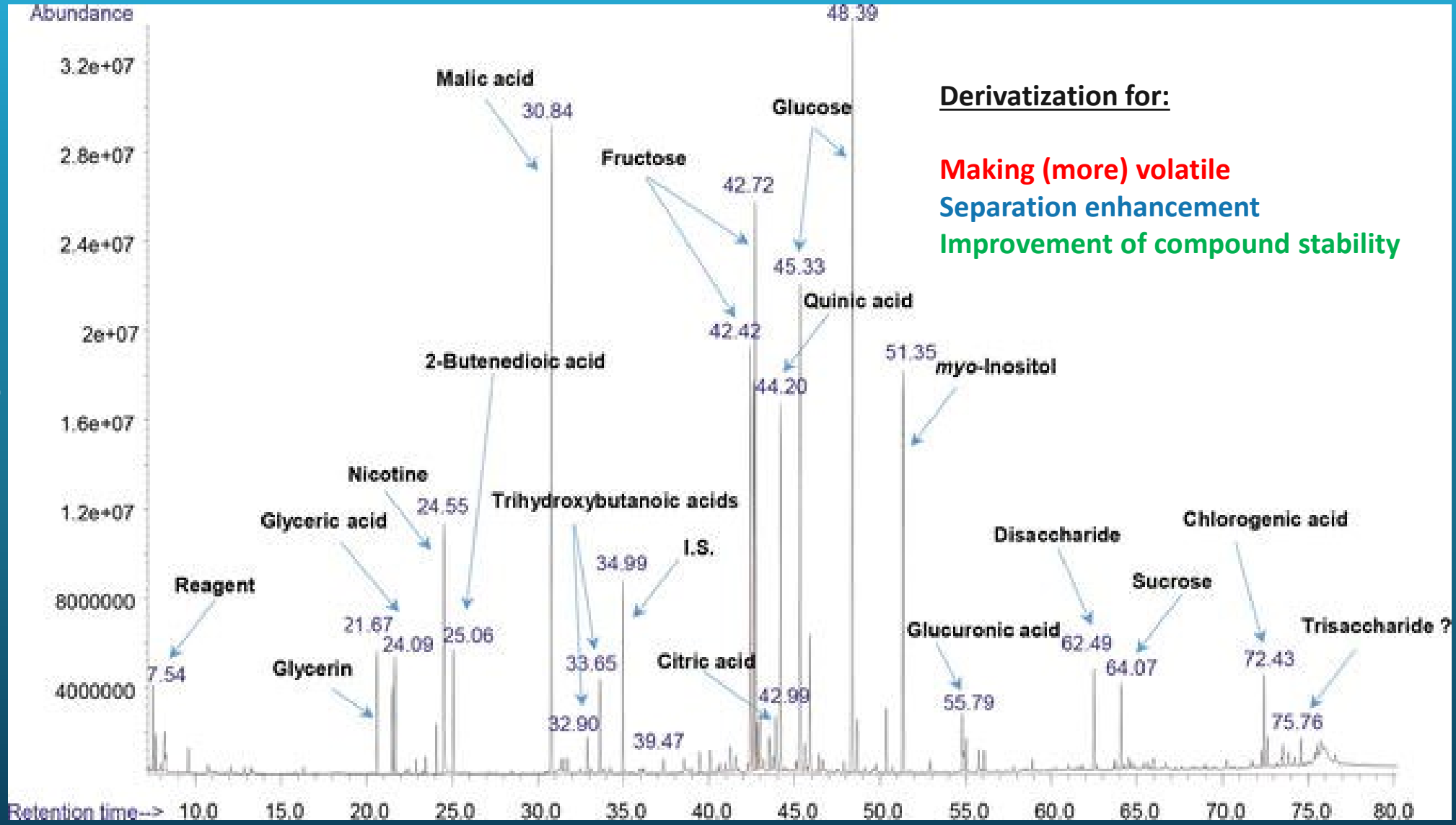


Method development

Sample preparation

GC-MS

GC/MS Chromatogram of silylated tobacco extract



Derivatization for:

Making (more) volatile

Separation enhancement

Improvement of compound stability

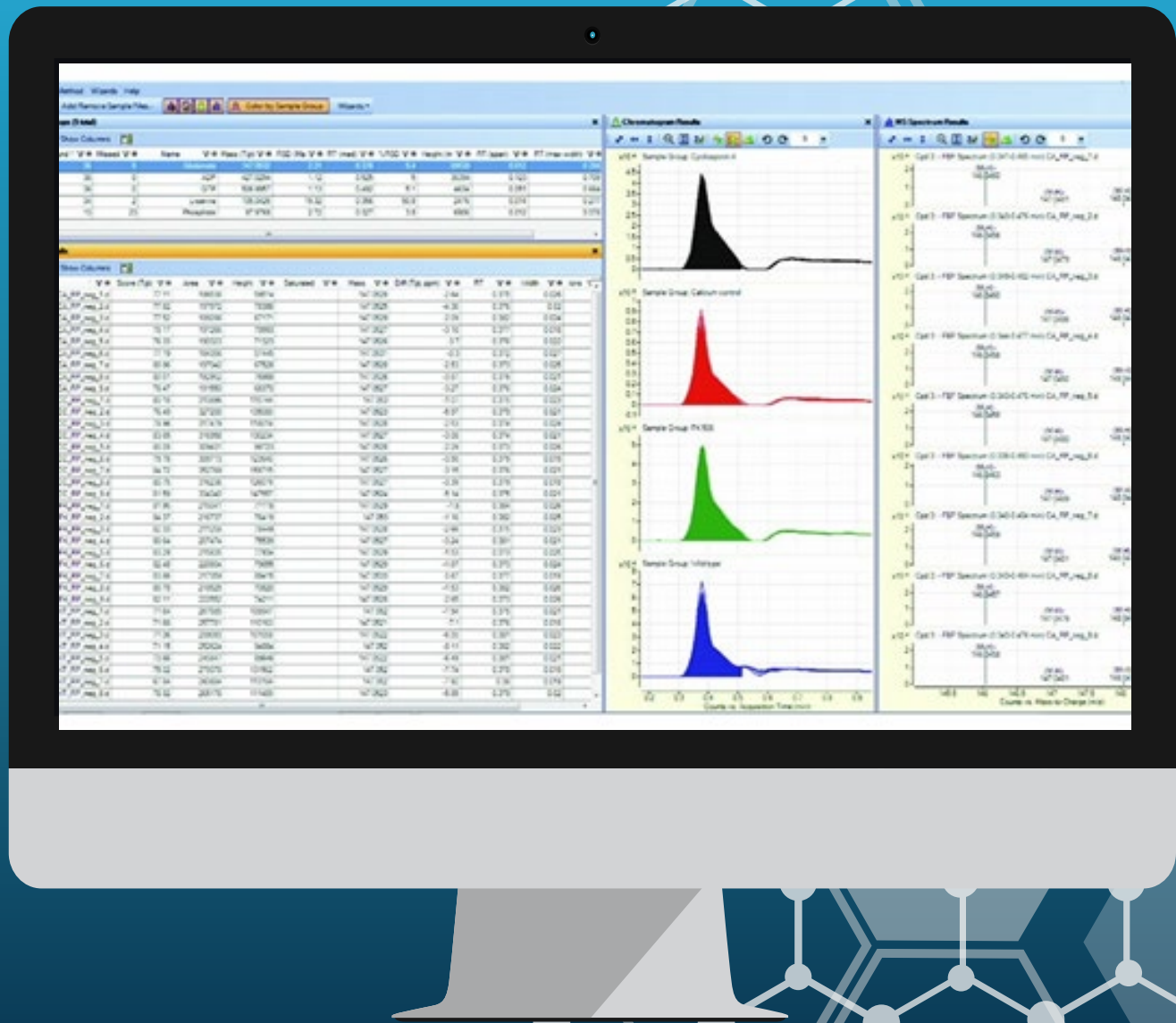
Moldoveanu & David, "Derivatization Methods in GC and GC/MS" IntechOpen, 2018

Data acquisition

- Randomized samples
- Precolumn
- Blank run, “Junk” run, Pooled QC
- Internal standard(s)
- Mass calibration, LockMass, LockSpray, Rt reference standards
- Data-dependent and data-independent acquisition (DDA & DIA)

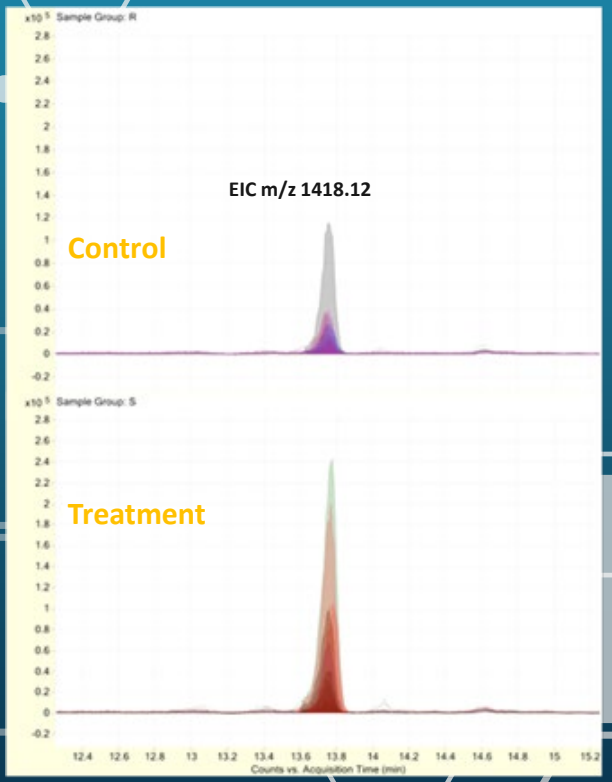
Data processing & analysis

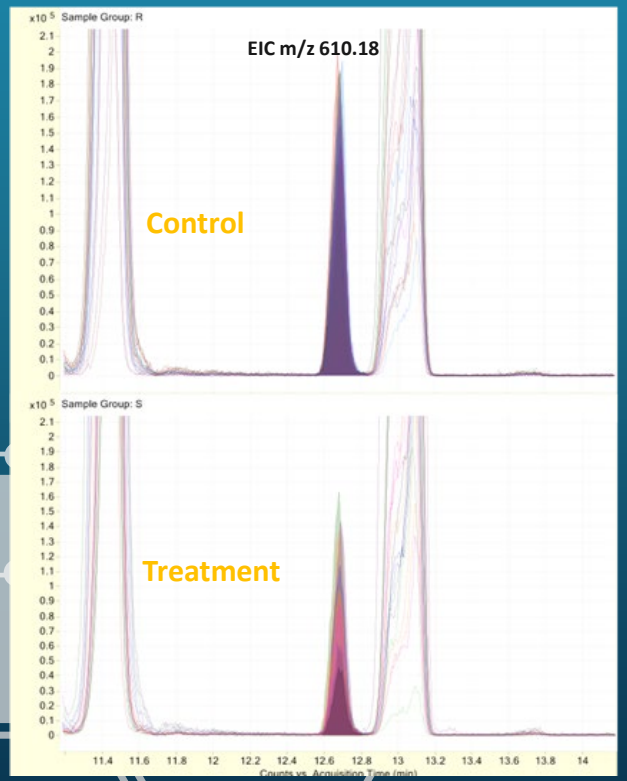
- PEAK-PICKING
- DECONVOLUTION, ADDUCTS
- ISOTOPIC PATTERN
- ALIGNMENT
- GAP FILLING
- NORMALIZATION
- MS2 ANALYSIS
- ANNOTATION, ID
- INTERPRETATION



Data processing & analysis

Potentially significant molecular features discriminate between two groups





MULTIPLE MODELS FOR CONSENSUS BUILDING

Univariate analysis

- volcano plots
- fold change analysis
- t-test

Multivariate analysis

- principal component analysis (PCA),
- partial least squares-discriminant analysis (PLS-DA)
- hierarchical clustering (HC)
- support vector machines (SVM)
- random forests (RF)

FROM MS DATA TO MOLECULAR INSIGHT

Metabolite identification remains a major challenge in untargeted MS-based metabolomics

MS1 Level

MOLECULAR FORMULA DETERMINATION

Golden Rules (Kind & Fiehn, Bioinformatics 2007)

MS2 Level

or

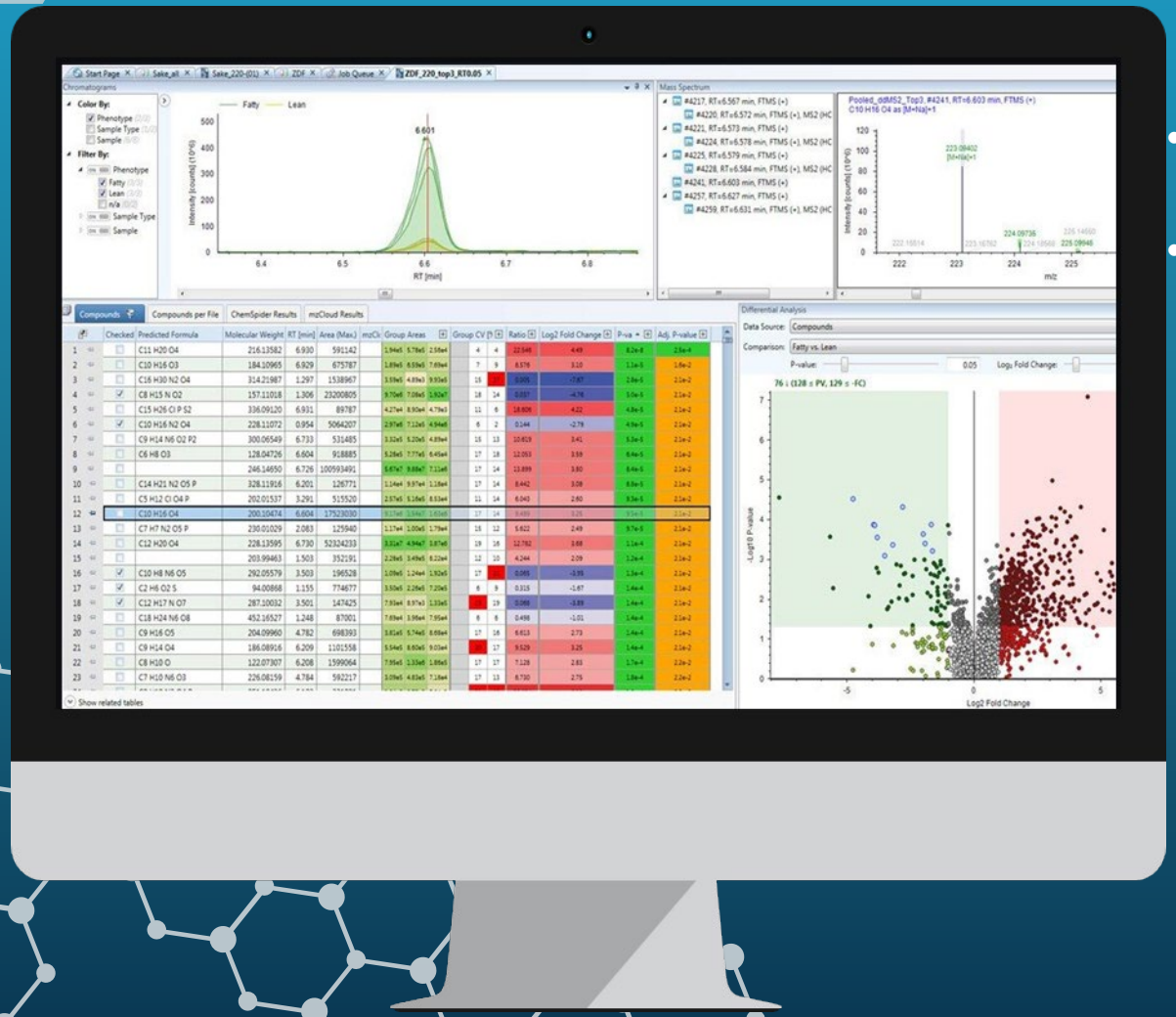
EI ionization at MS1

STRUCTURE ELUCIDATION

Shattering Pattern & Fragment Mass Measurement

FROM MS DATA TO MOLECULAR INSIGHT

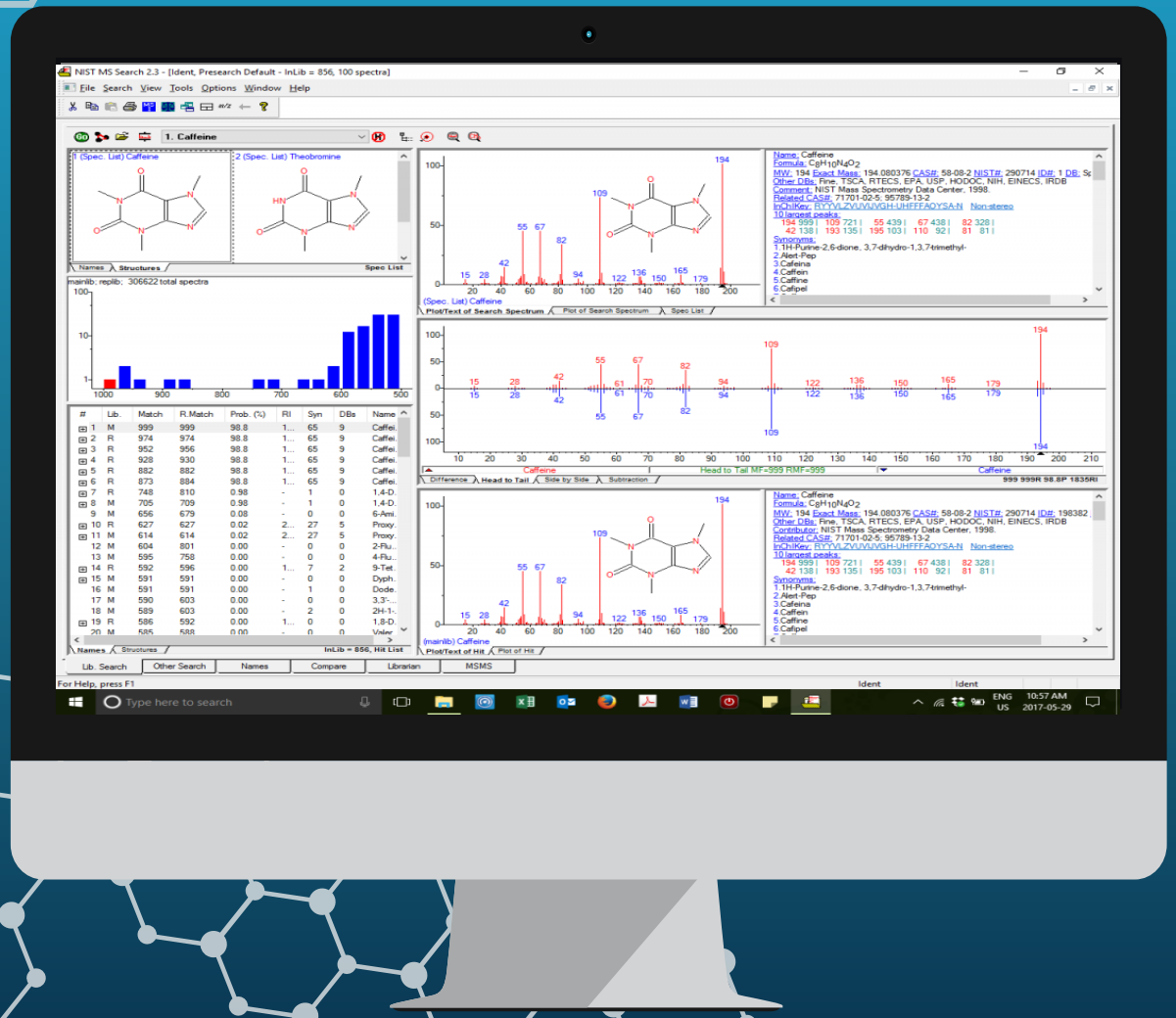
MPP
MassProfiler



- Differential analysis of multiple sample sets & statistical tools ANOVA, PCA, volcano plots, hierarchical trees, SOMs.
- METLIN Database

FROM MS DATA TO MOLECULAR INSIGHT

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE



350,704 electron ionization (EI) spectra
306,643 compounds, 43,774 replicate spectra
447,289 retention index (RI) values

SUGGESTED RESOURCES FOR LC/GC-MS DATA PROCESSING & ANALYSIS

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY U.S. DEPARTMENT OF COMMERCE

MZmine

MPP MassProfiler

MetaboAnalyst 5.0

Statistics

Meta-analysis

Multi-omics

Visualization

XCMS

LC/MS and GC/MS Data Analysis

Rdisop

Molecular formula calculations

CAMERA

Collection of annotation related methods for mass spectrometry data

MetShot

High throughput prioritized acquisition and processing of tandem mass spectra

MassBank

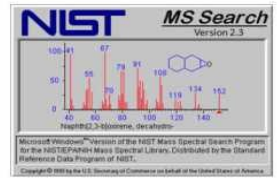
Tandem MS Reference Database

MetFrag

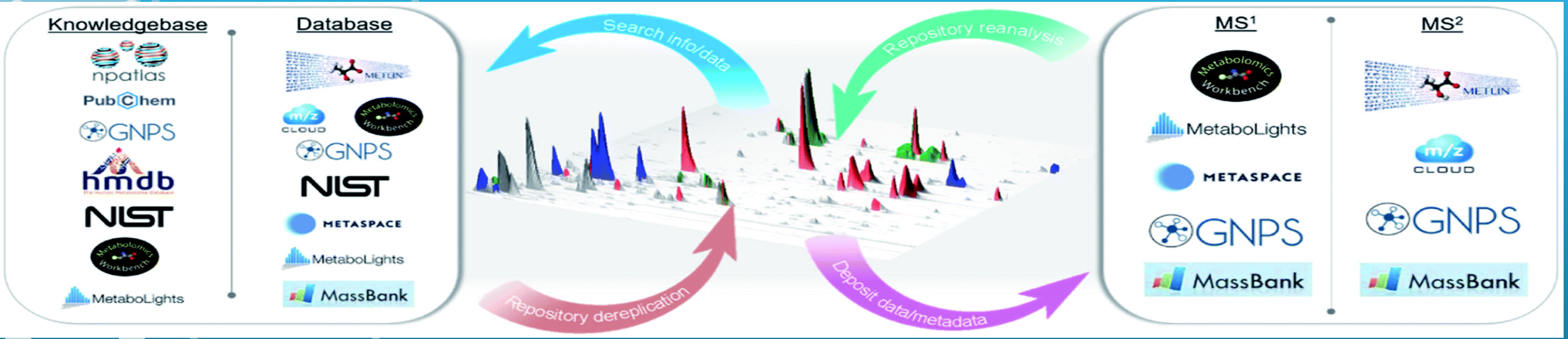
In-silico Metabolite identifikation

MetFamily

For the identification of regulated metabolite families.



RESOURCES FOR MASS SPECTROMETRY BASED MOLECULAR ID



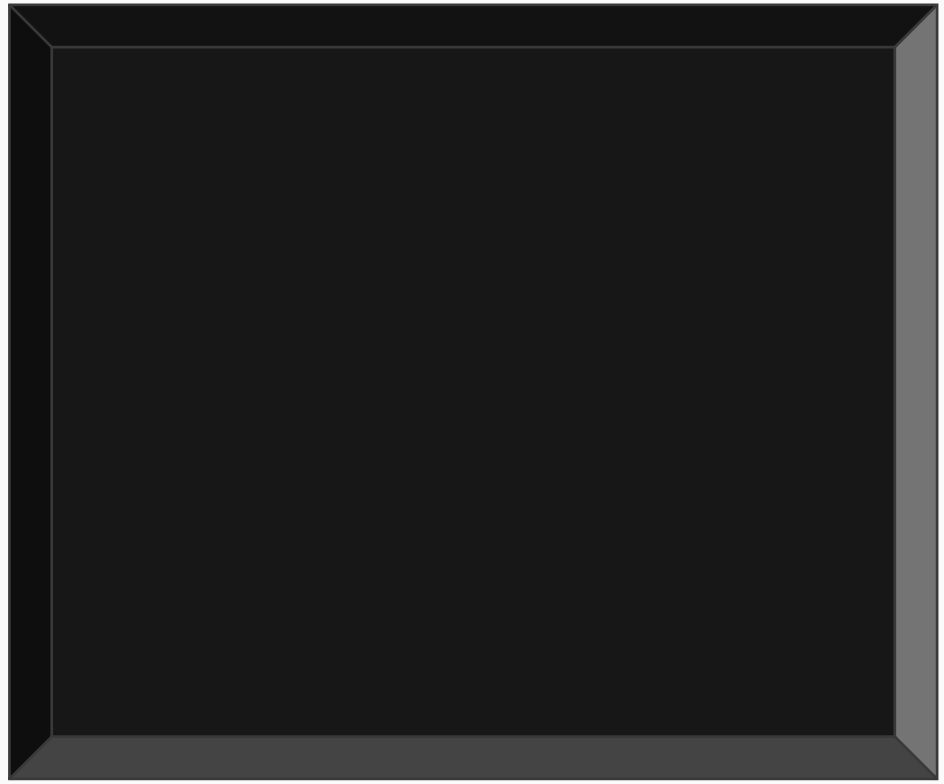
Picture credit: Jarmusch et al., Natural Product Reports, 2021

Knowledgebase and Database ID		
Known	Confirmation of presence	Matching obtained data
Unknown	Networking ID	*de novo*

What's in my sample?

"The hardest thing of all is to find a black cat in a dark room, especially if there is no cat"

Confucius



cat???