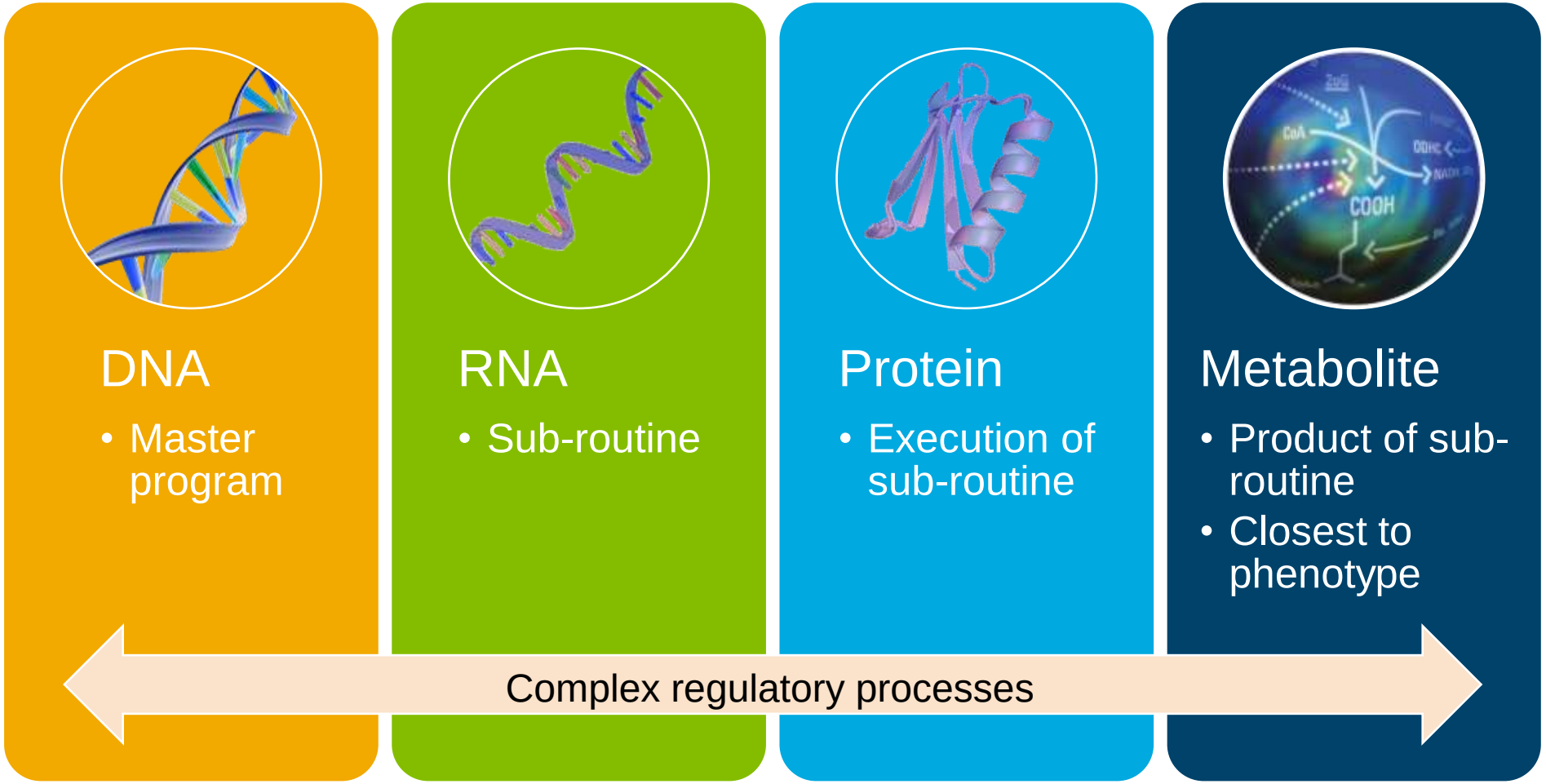
Accelerate Your Research with Advanced Omics Solutions

Christine Miller
Omics Market Manager
ASMS 2018

Agilent
Trusted Answers

Biology Is Integrated

Multi-omics increases biological understanding



Agilent Solutions for -Omics

The complexity of biology presents enormous challenges to understanding even simple systems.

Agilent is a trusted leader in developing the:

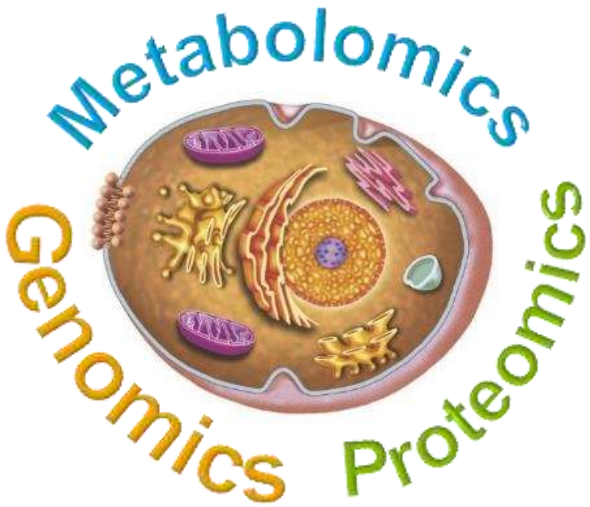
Instrumentation

Consumables

Analytical methods

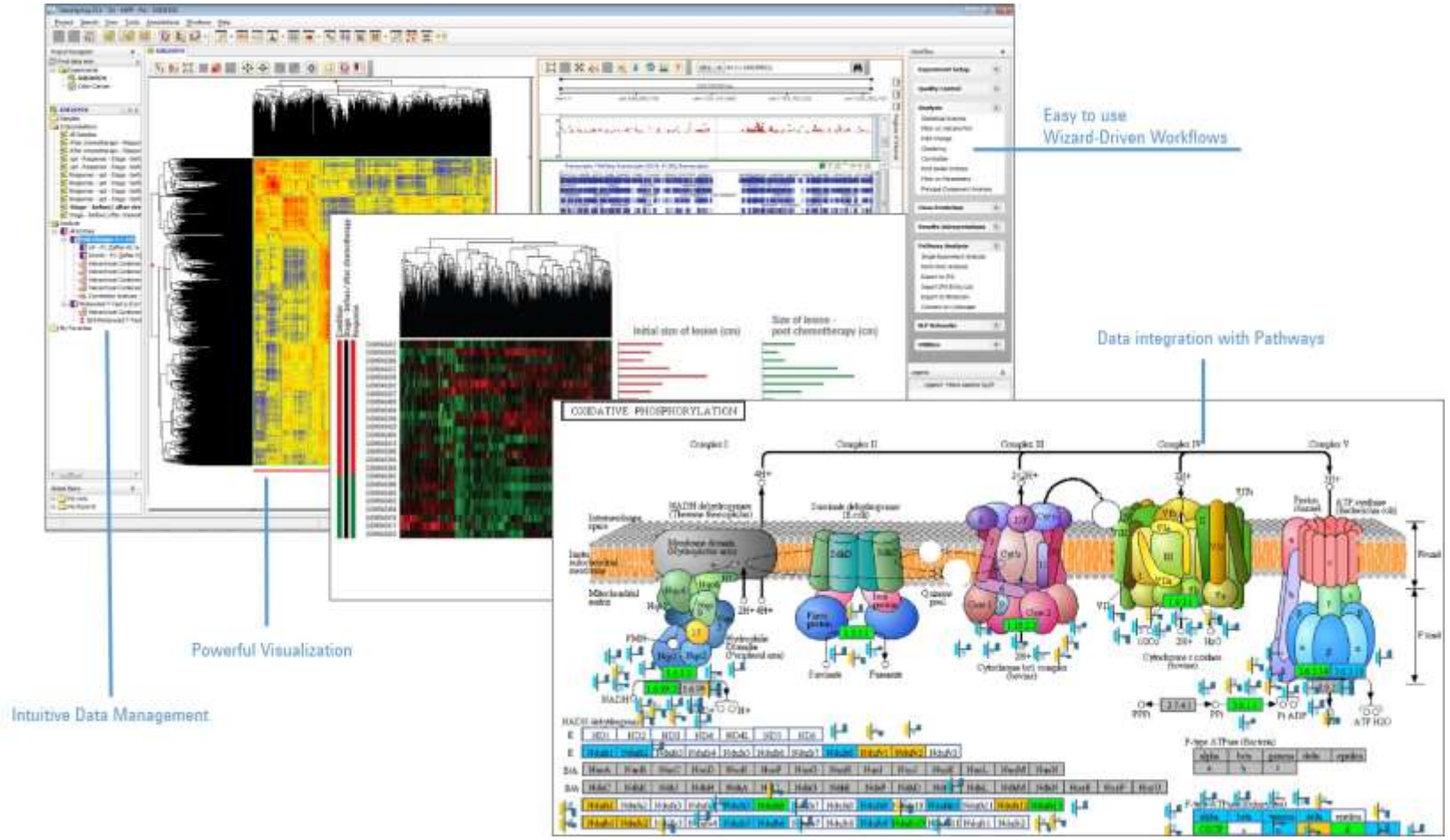
Software solutions

…needed to integrate multi-omics data.



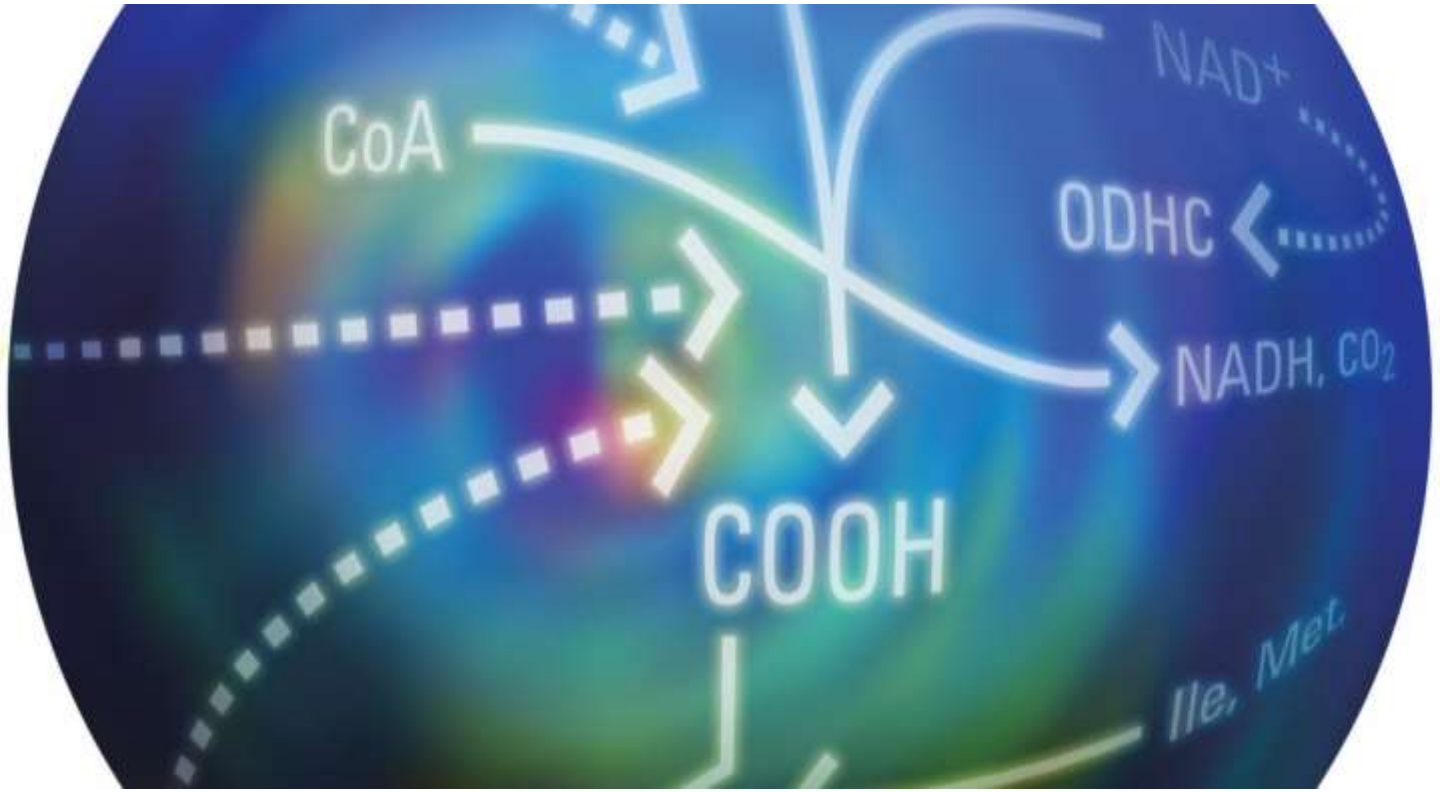
Mass Profiler Professional and Pathway Architect

Chemometric analysis and biological contextualization



- Supports multi-omics analysis
- Includes multivariate statistical analysis
- Offers correlation analysis for discovery of new biological relationships
- Connects meta data to biology
- Visualizes results directly on pathways
- Create pathway-directed experiments

Metabolomics Workflows

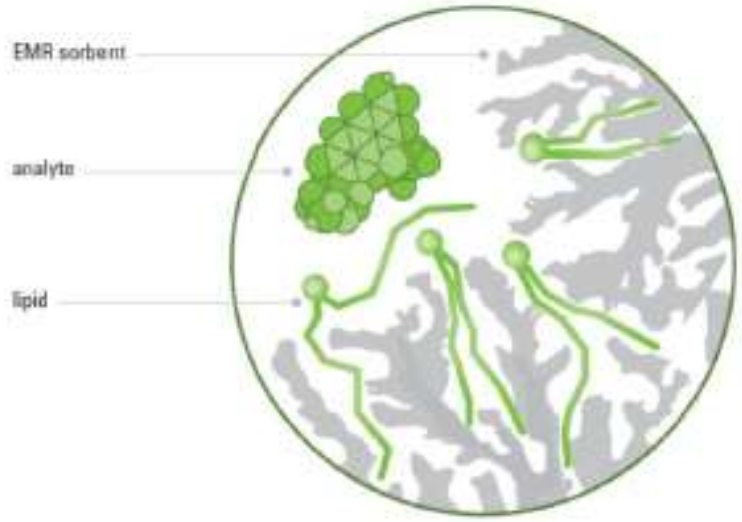


Captiva EMR-Lipid

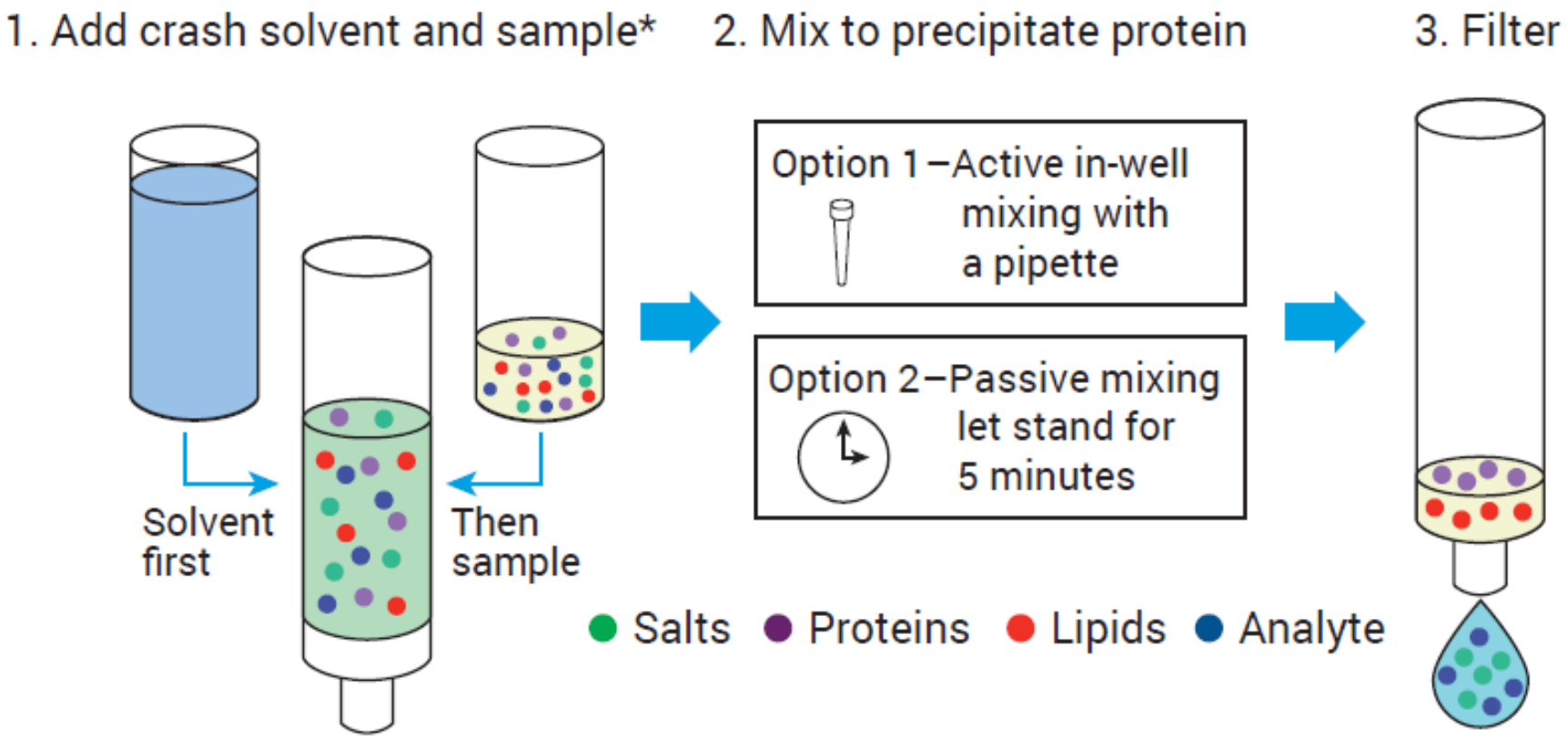
Improved efficiency: Unique EMR—Lipid mechanism combines size exclusion and hydrophobic interactions between the sorbent and the long aliphatic chain of the lipids

Better speed and precision: Solvent retention frit streamlines and automates your in-well protein precipitation workflow

An easier flow: An advanced filter design and construction technology ensure clog-free operation



Captiva EMR-Lipid Cleanup Procedure



* Alternatively, protein precipitation (Steps 1 and 2) can be performed off-line (Option 3), at which point the sample can be transferred to Step 3.

Efficiency of Biological Fluid Matrix Removal Using Agilent Captiva EMR-Lipid Cleanup (5991-8006EN)

Demonstrates phospholipids removal in a variety of common biological fluids based on in-well protein precipitation

- Serum and CSF
- Human plasma with various anti-coagulants (five type)
- Animal plasma with various anti-coagulants (four type)

Comparison with major competitors products

Ease-of-elution for in-well protein precipitation (PPT)

Efficiency of Biological Fluid Matrix Removal Using Agilent Captiva EMR—Lipid Cleanup

Application Note

Clinical Research

Authors

Limian Zhao and Derick Lucas
Agilent Technologies, Inc.

Abstract

The Agilent Captiva Enhanced Matrix Removal—Lipid (Captiva EMR—Lipid) is the next generation of EMR product, and is formatted in SPE cartridges or 96-well plates. Phospholipids are widely recognized as the prominent interferences in biological fluids. They not only affect the MS response of many analytes negatively, but are also difficult to remove from samples without analyte loss. This study demonstrates the application of Captiva EMR—Lipid cartridges and plates for phospholipid removal in various biological fluids. The phospholipid removal capabilities of Captiva EMR—Lipid were evaluated for many biological fluids from human and animal sources, with or without the addition of different anticoagulants. The procedure involves an *in situ* protein precipitation step followed by pass-through cleanup by Captiva EMR—Lipid. The efficiency of matrix removal was determined by the weight of residual matrix and the chromatographic profile of phospholipids through a precursor ion scan for product ion 184 *m/z*. A thorough comparison study of currently available products was evaluated for phospholipid removal based on the recommended product protocols. The results demonstrated that Captiva EMR—Lipid provides >99 % phospholipid removal, superior eluent clarity, easier flow, and substantially less clogging when compared to other products performance.

Impact of Phospholipid Removal

Demonstrates impact of phospholipids in biological fluids on LC/MS analysis

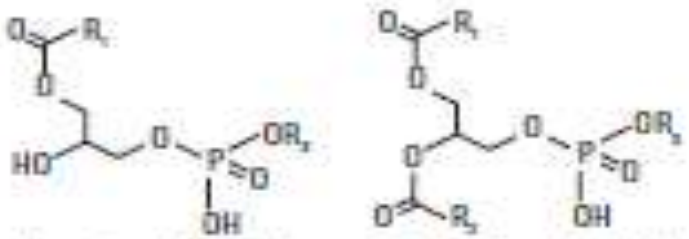


Figure 1. Chemical structures of the two most important groups of phospholipid.

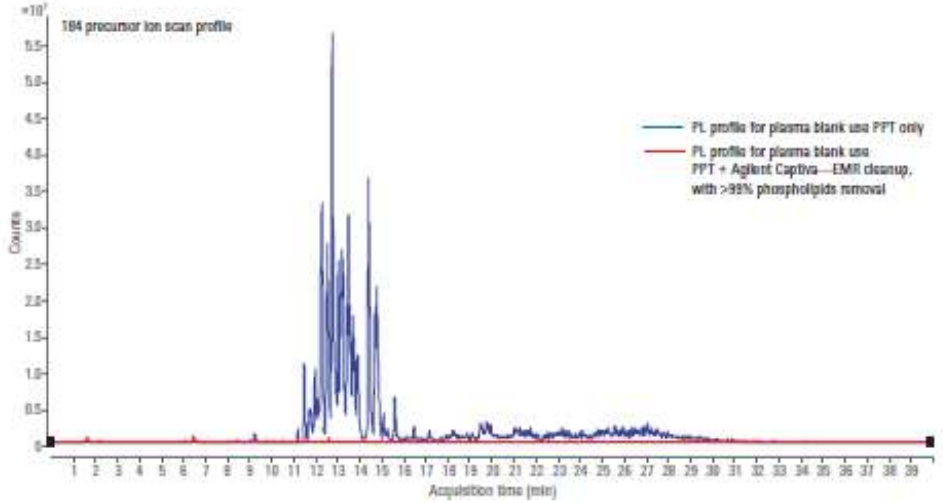
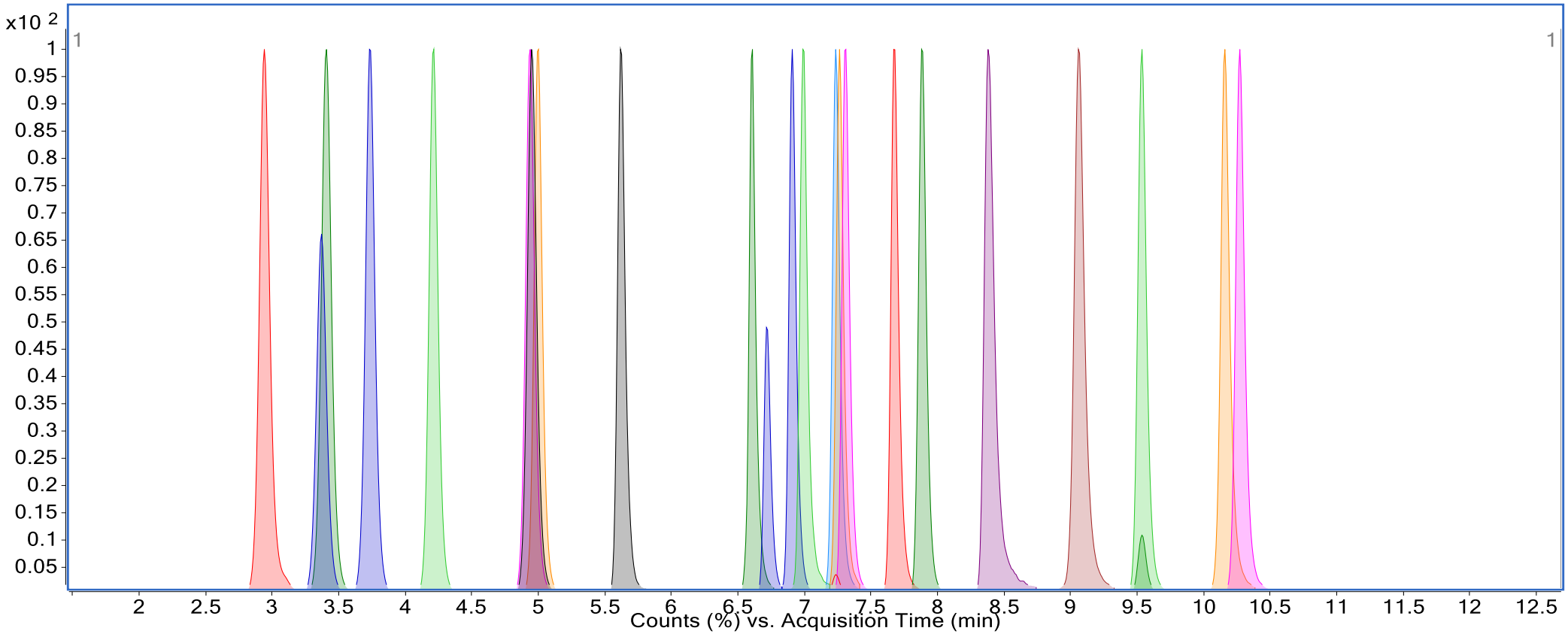


Figure 4. Overlapped chromatograms for phospholipids profile by monitoring a precursor ion scan for 184 m/z.

Captiva EMR-Lipid provides removal of >99% of phospholipids in various biological fluids.

Underivatized Amino Acid Analysis

Poroshell HILIC-Z, 2.7 µm, 2.1x100mm



Mobile Phase A = 20 mM ammonium formate in water, pH=3
Mobile Phase B = 20 mM ammonium formate in 90% acetonitrile in water, pH3
Flow Rate = 0.6 mL/min
Agilent Jet Stream source, positive ion mode

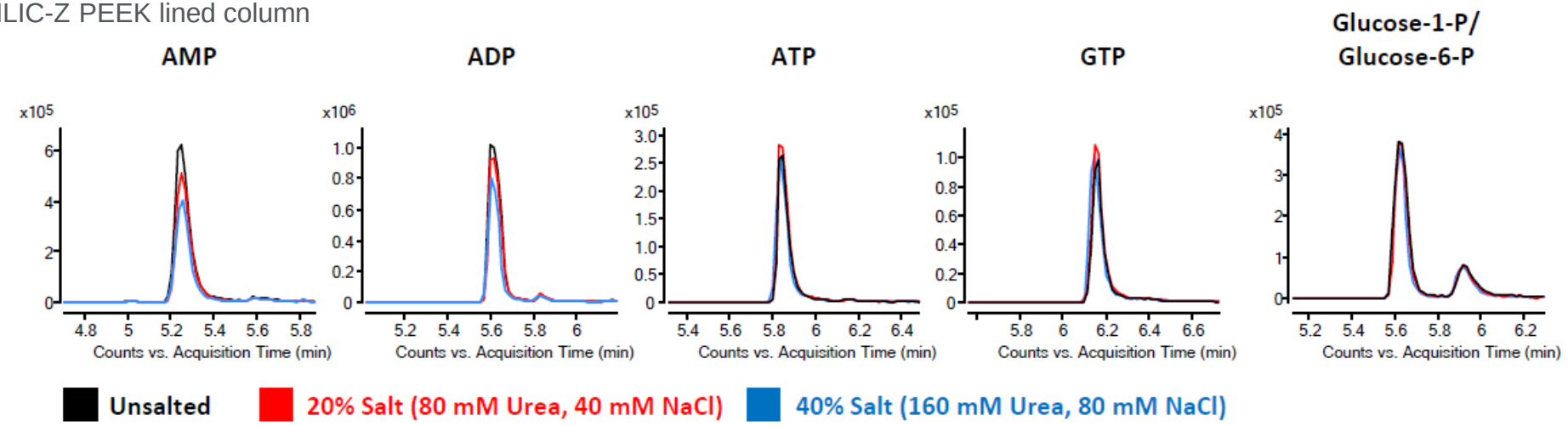
Robust Performance in Negative Mode

Mock Urine Sample		
Major Urine Components	**[Concentration]**	**[mM]**
Urea	23.3 g/L	390 mM
Chloride	8.4 g/L	236 mM
Sodium	4.4 g/L	191 mM
pH 6.3		

10-compound metabolomics test mix spiked with and without salt
Mobile Phase 10 mM ammonium formate pH=9

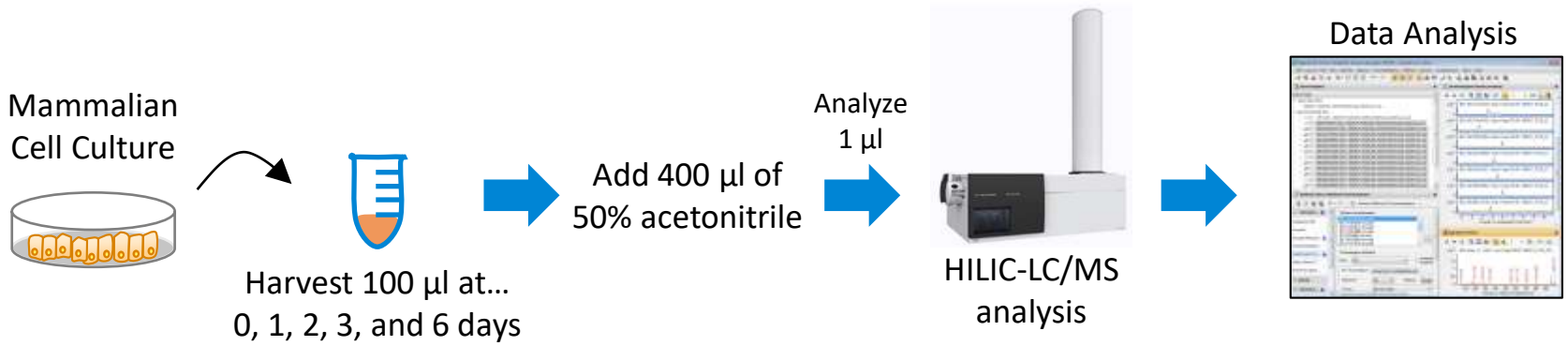
Made a 4M Urea, 2M NaCl stock solution for salt spike-in experiment.

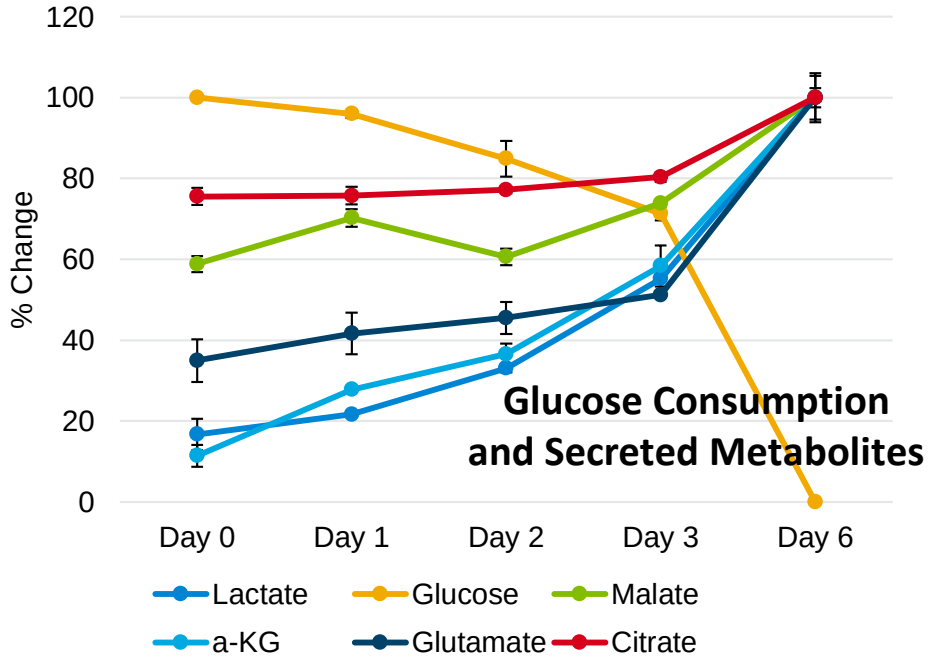
HILIC-Z PEEK lined column

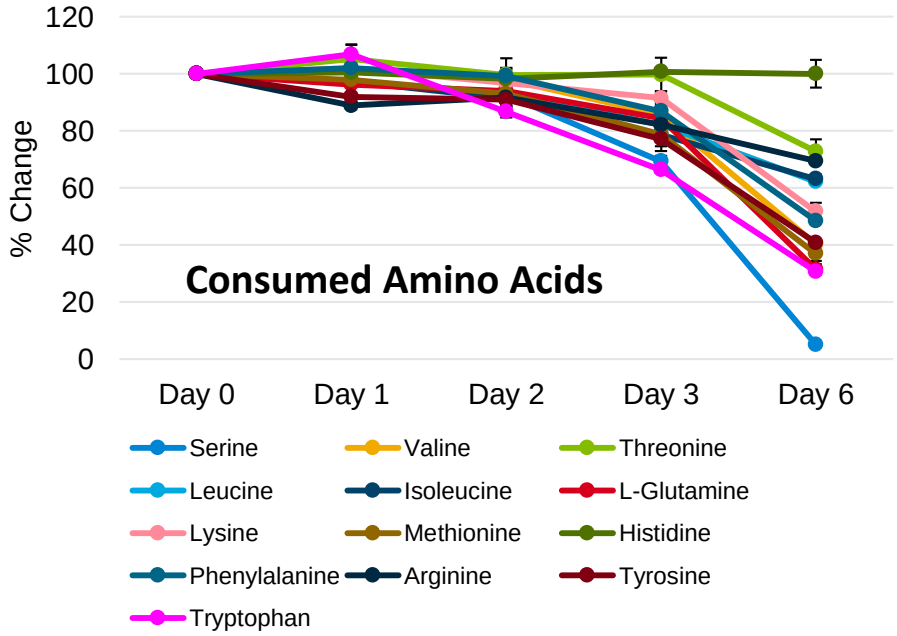


Metabolomics Analysis of Culture Media

Poroshell HILIC-Z, 2.7 µm, 2.1x100 mm



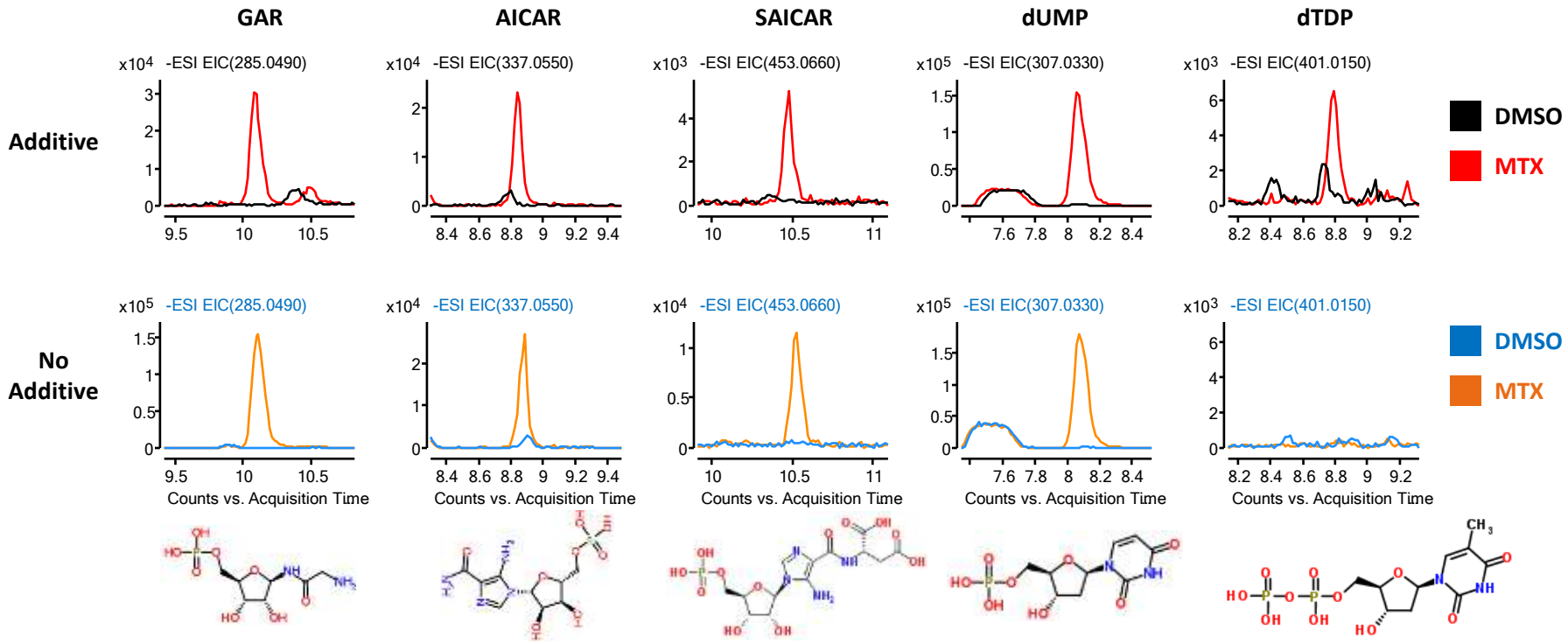




Purine Metabolism in MTX-treated Cells

Poroshell HILIC-Z, 2.7 µm, 2.1x100 mm

MTX = methotrexate-treated K562 leukemia cells



Mobile Phase A = 20 mM ammonium formate in water, pH=9
Mobile Phase B = 20 mM ammonium formate in 90% acetonitrile in water, pH9
Agilent Jet Stream source, negative ion mode

Agilent Instrumentation For Metabolomics

7000D GC/QQQ

5977B GC/MS

7100 CE

1260 SFC

7200B GC/Q-TOF

1290 Infinity II UHPLC

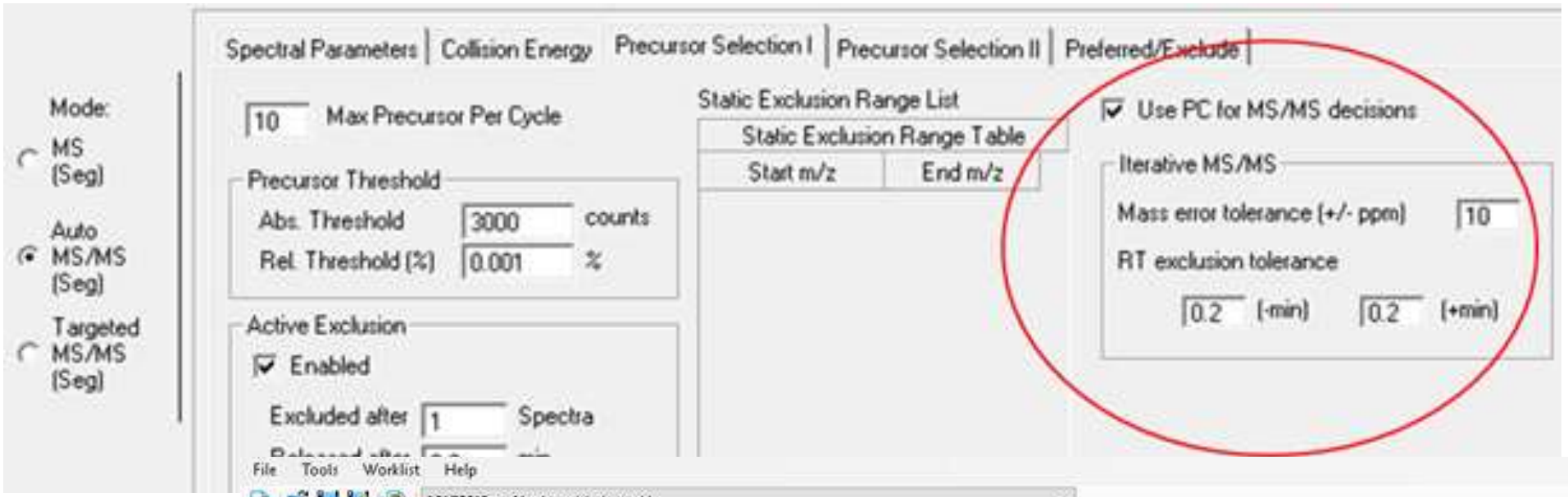
Hi-DEF Q-TOF 6500 series

Q-TOF 6500 series

QQQ 6400 Series

TOF 6200 series

New MS/MS Functionality on the LC/Q-TOF: Iterative Exclusion

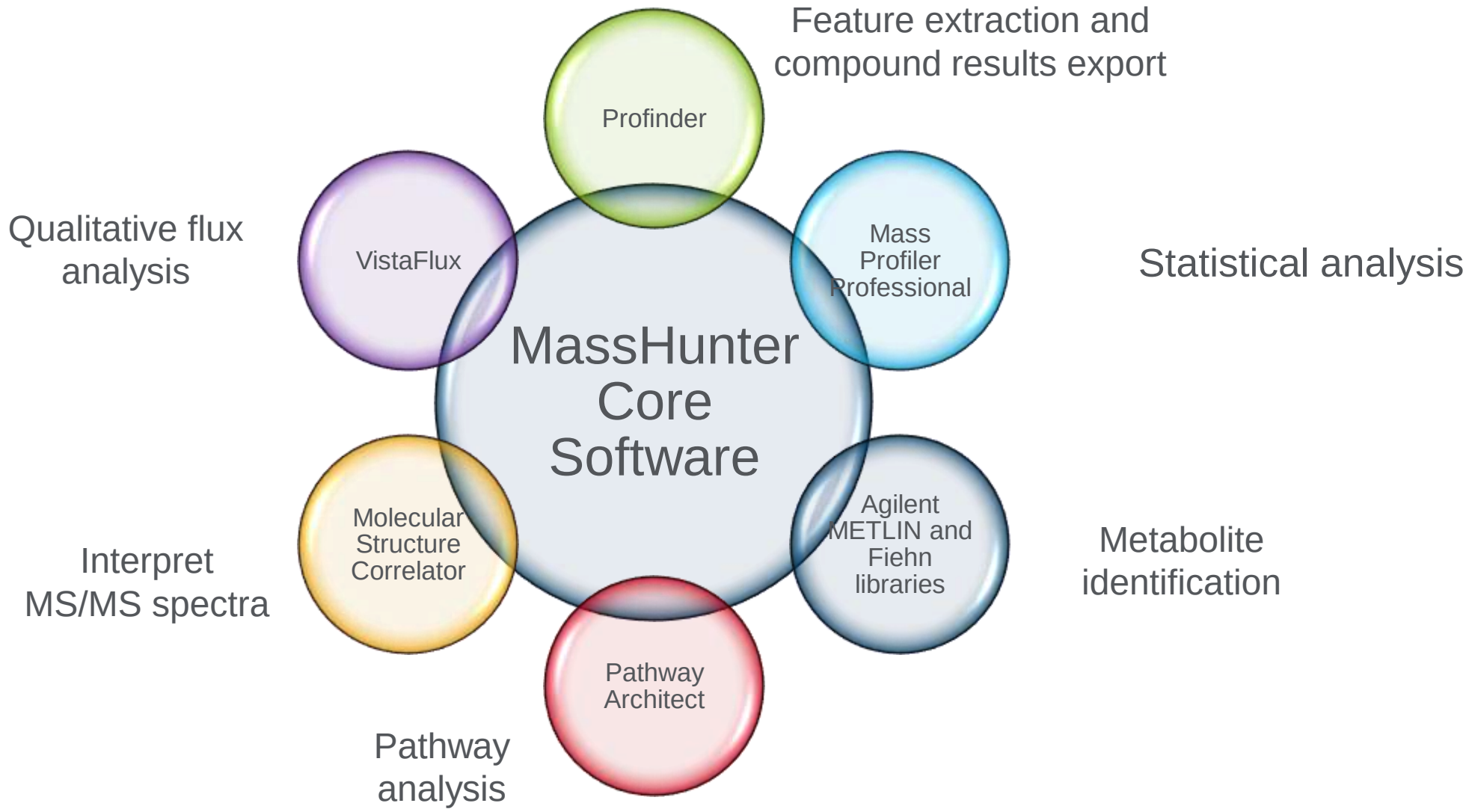


File Tools Worklist Help

20170913-mAb-deamidation.wkl

	Sample Name	Sample Position	Method	Data File	Sample Type	Inj Vol (µl	Iterative	
1	wash	Vial 1	peptide-mapping-15min-400uL-6s.m	wash01.d	Sample	20		wash
2	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r001.d	Sample	3		
3	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r002.d	Sample	3		
4	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r003.d	Sample	3		
5	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r004.d	Sample	3		
6	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-iterative-r001.d	Sample	3	start	
7	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-iterative-r002.d	Sample	3	iterative	
8	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-iterative-r003.d	Sample	3	iterative	
9	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-auto-r001.d	Sample	3		
10	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-auto-r002.d	Sample	3		
11	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-auto-r003.d	Sample	3		
12	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-iterative-r001.d	Sample	3	start	
13	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-iterative-r002.d	Sample	3	iterative	
14	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-iterative-r003.d	Sample	3	iterative	
15	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-2ug-auto-r001.d	Sample	10		
16	mAb untreat desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-2ug-auto-r002.d	Sample	10		

Agilent Metabolomics Application Software

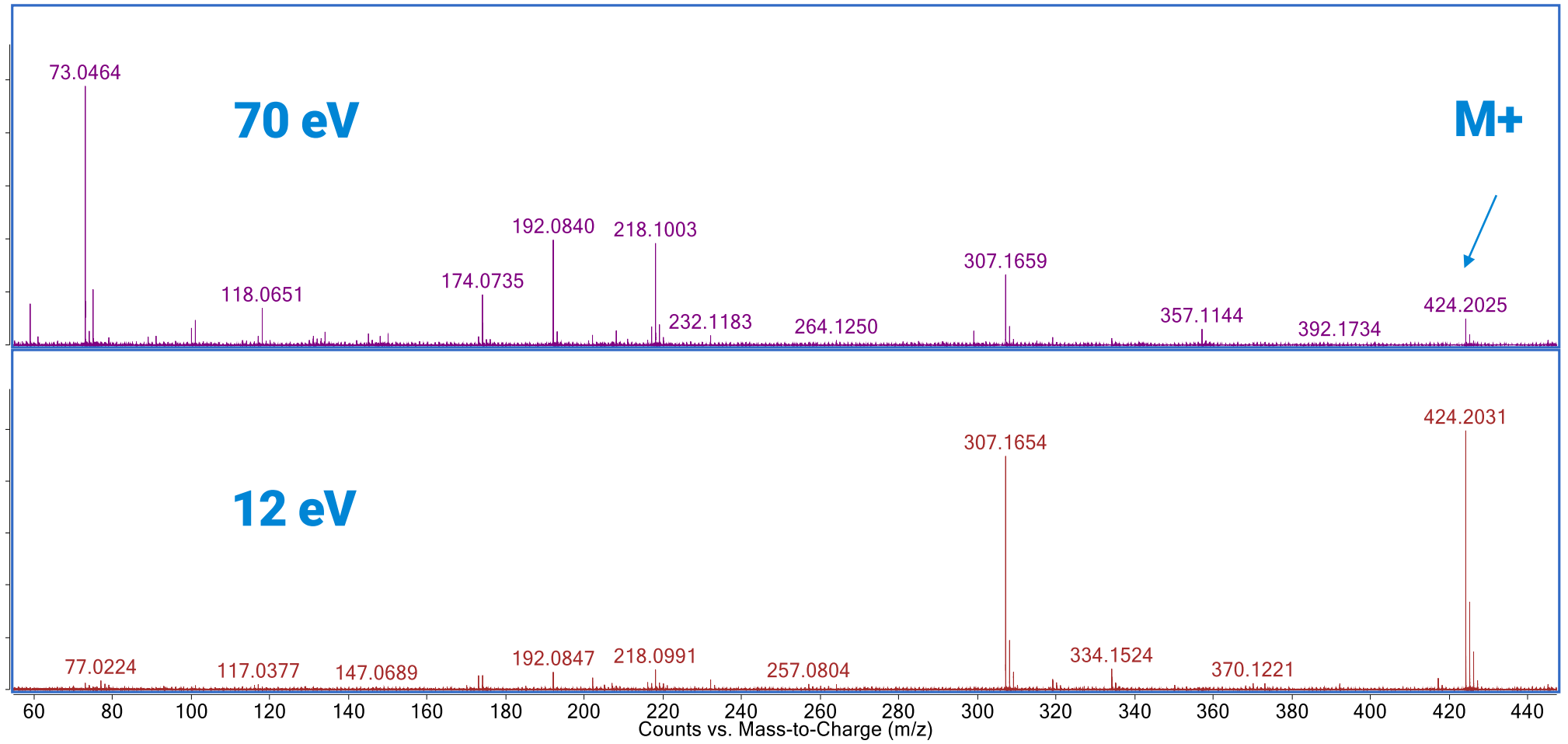


The Agilent 7250 GC/Q-TOF

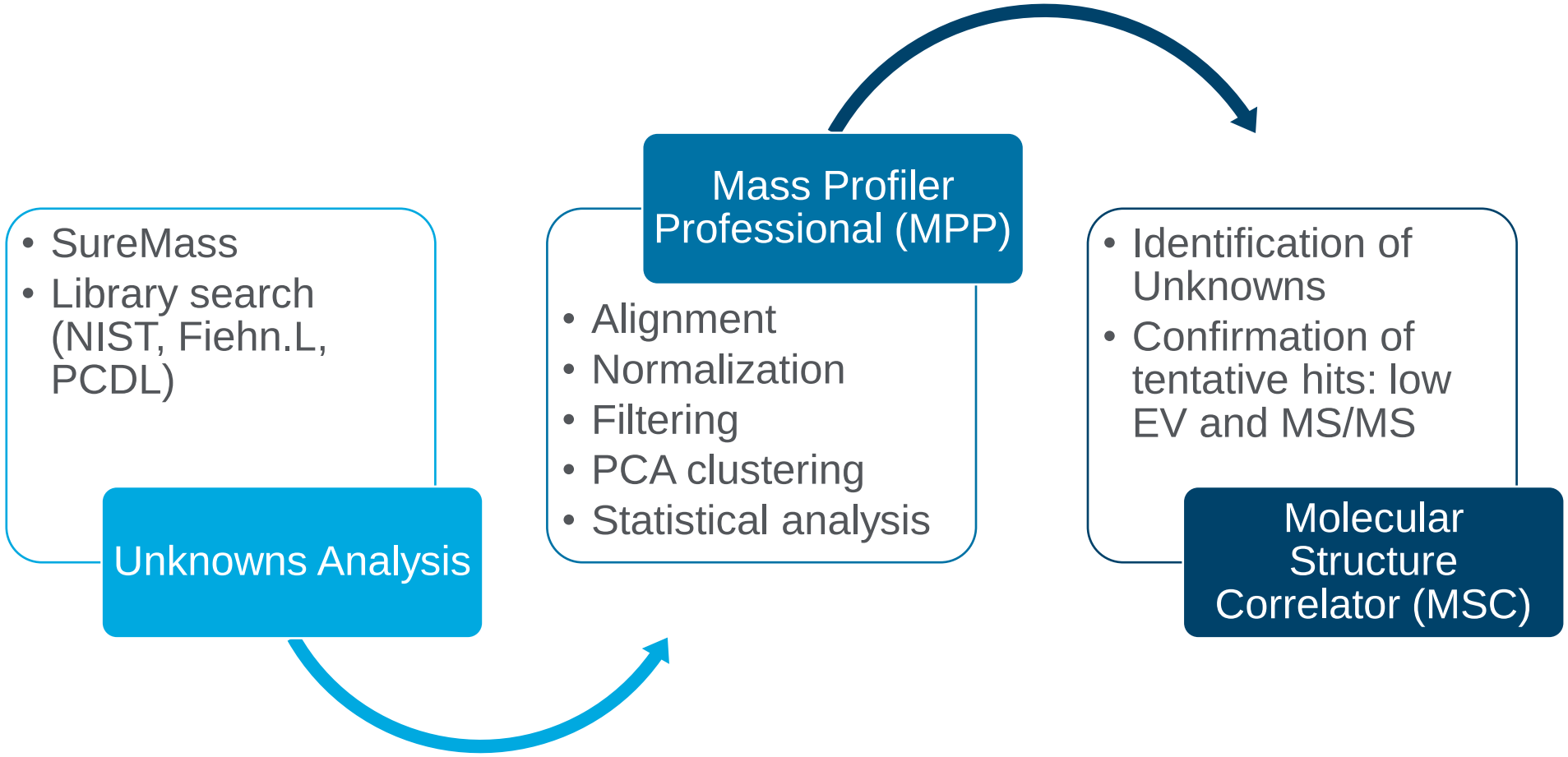
Using Low Electron Energy to Confirm Molecular Ion

Produces Less Complex EI Spectra

Kynurenine, 3TMS



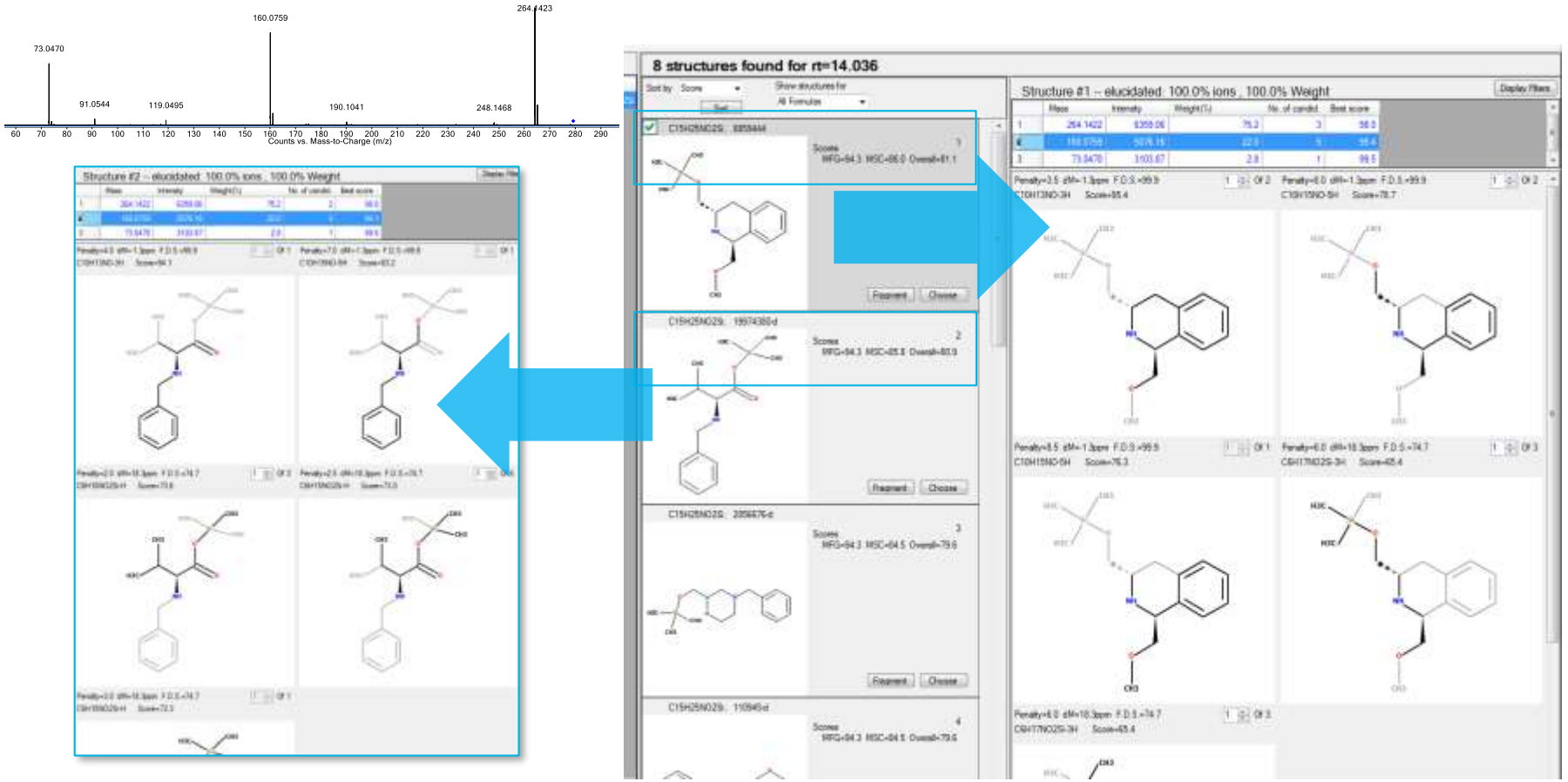
Untargeted GC/Q-TOF Workflow



Unknown Identification Using MSC

Correlate MS/MS Fragment Ions With Proposed Structures

MS/MS, *17 eV CE 20V*



MassHunter Profinder

The Power of 3D Batch Feature Finding

Create Batch

- Add all experimental data files to a project
- Optionally add group information

Untargeted MFE

- Extract features from each data file in batch
- Compile list of all features with composite spectra

Targeted MFE

- Use compiled list for targeted feature extraction of each data file in batch

Single software for untargeted and targeted feature extraction

Fast, multi-threaded batch processing

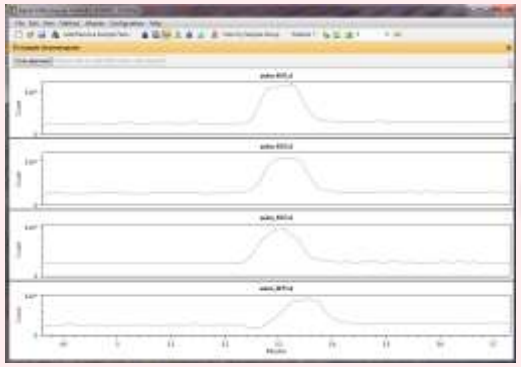
Recursive batch analysis minimizes false positives and negatives

Compound centric review and manual editing

MassHunter Profinder B.08.00 SP3

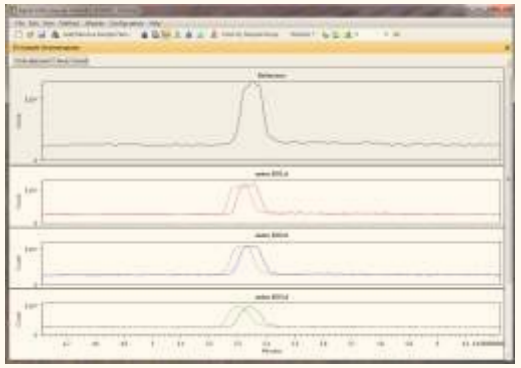
Chromatowarping for CE/MS & LC/MS

Chromatograms



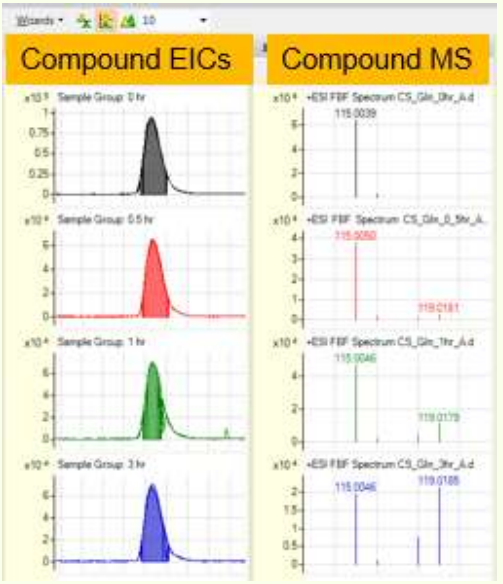
Original chromatograms with retention time variation

Aligned



Warped chromatograms with aligned retention times

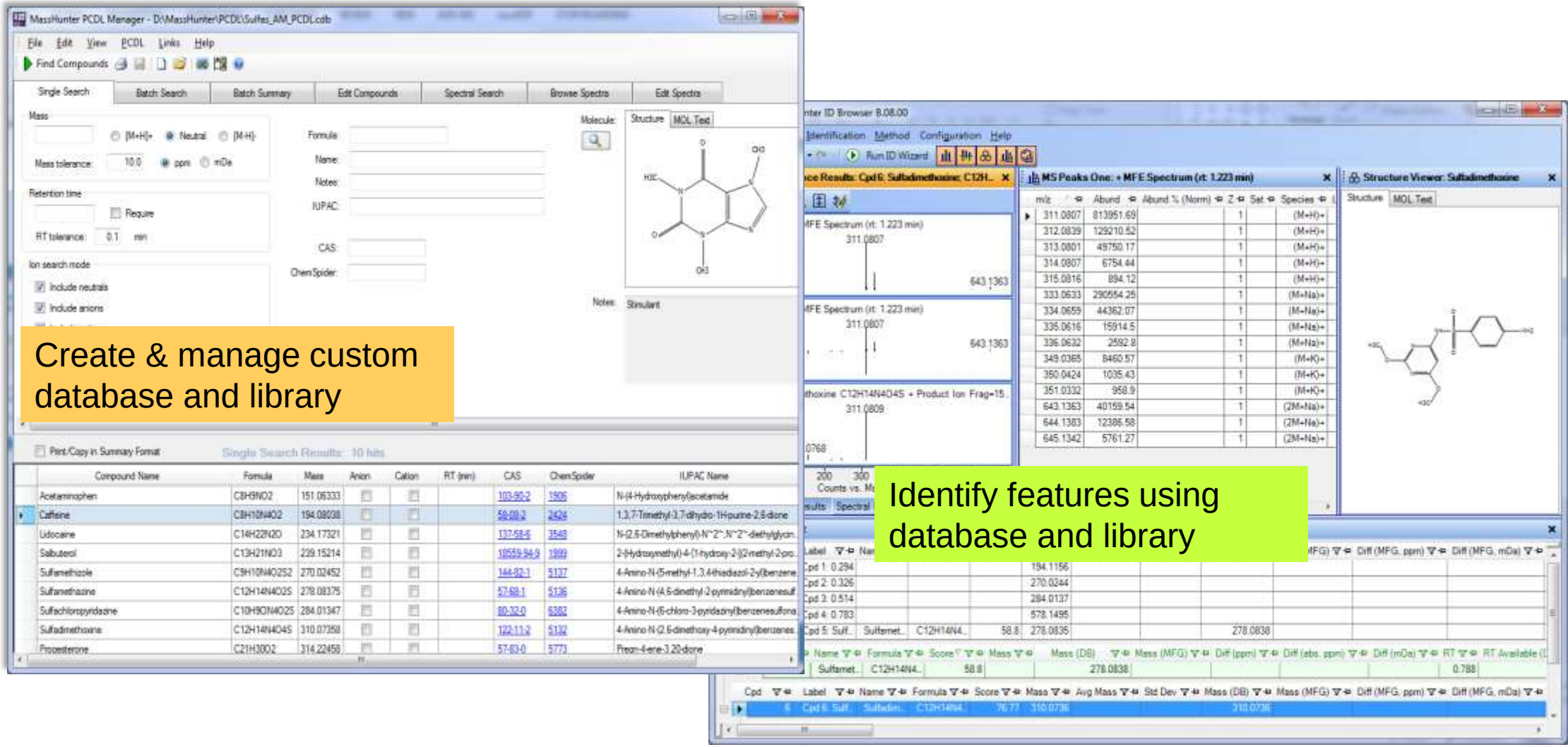
Feature Extraction



Extracted features with compound spectra

PCDL Manager and ID Browser 8.0

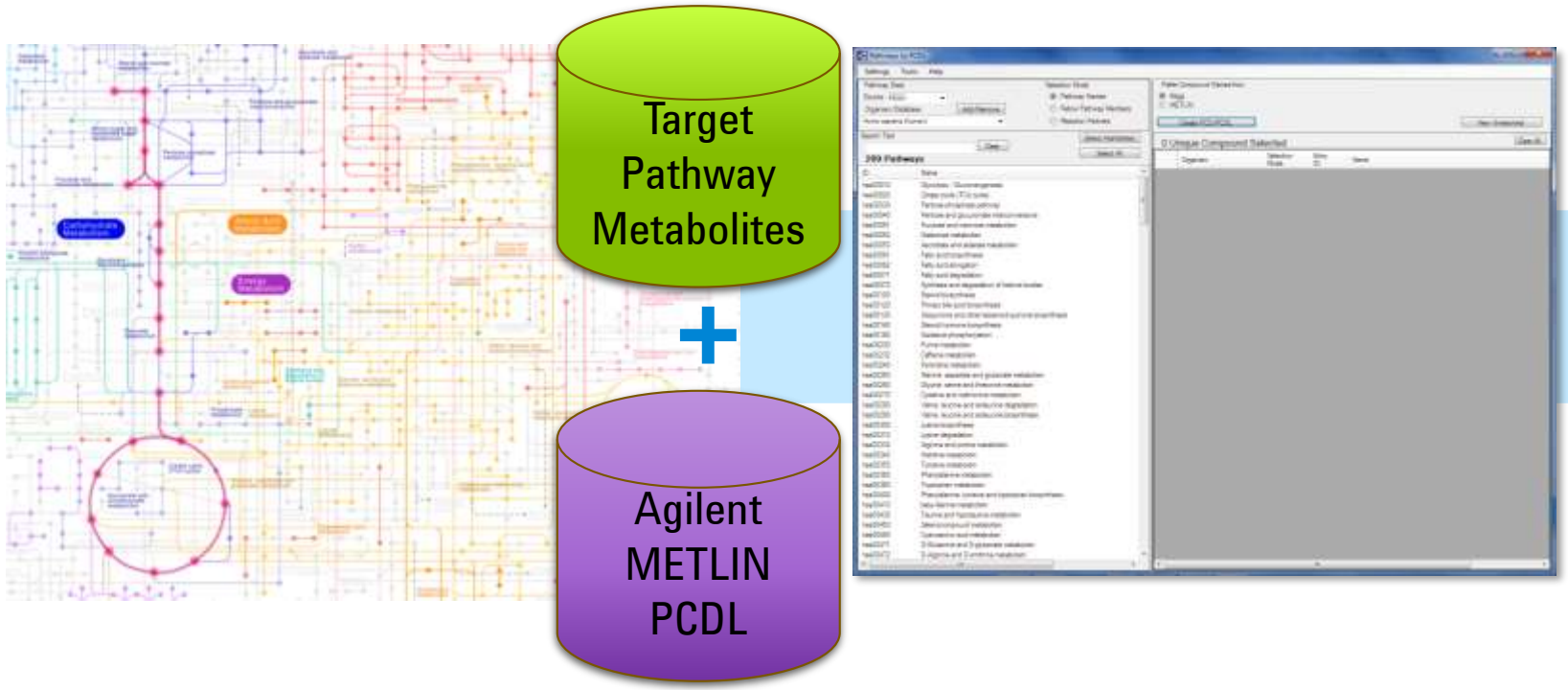
IM and MS/MS Library Support

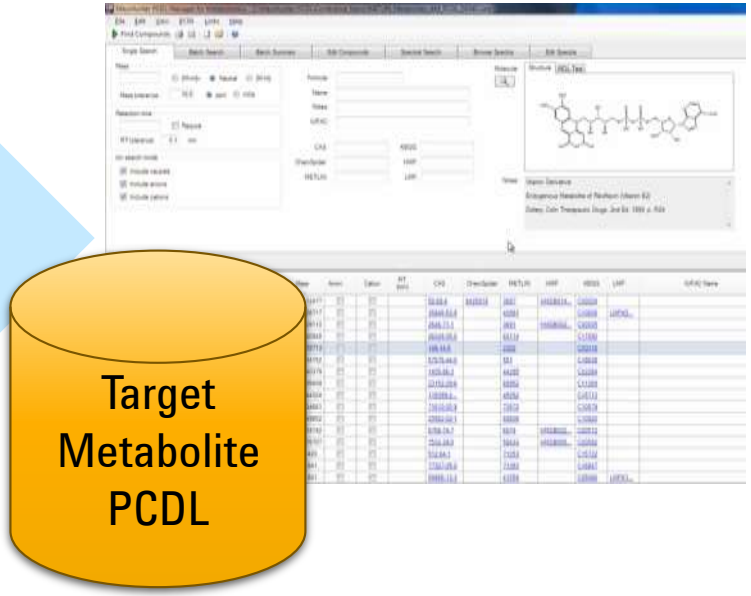


Pathway-directed Metabolomics: Create a Target Metabolite Database

Pathways to PCDL and PCDL Manager

Use Pathways to PCDL to specify pathway(s) for target database optionally including information from Agilent METLIN





Manage target database in PCDL Manager

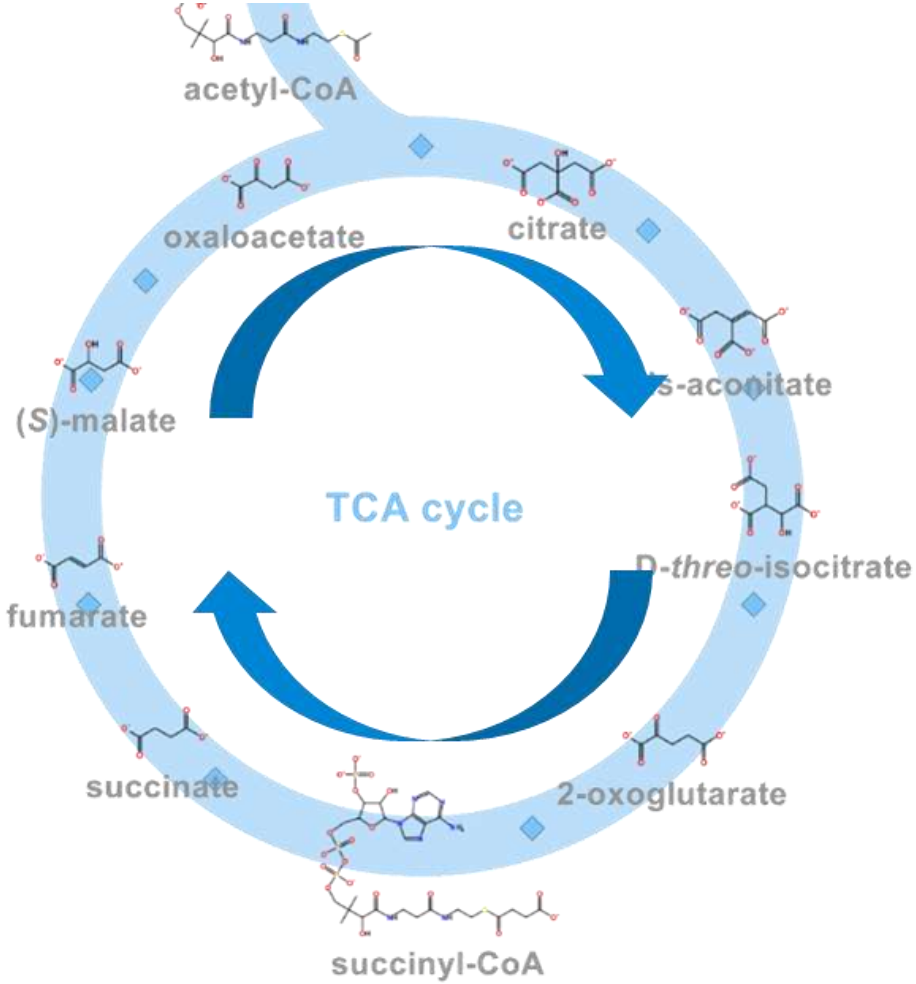
Stable Isotope Tracing

Qualitative flux analysis

Metabolomics provides static information on cellular molecular composition

Qualitative flux analysis reveals *in vivo* pathway activity

Qualitative flux analysis tracks the flow of metabolites through a pathway



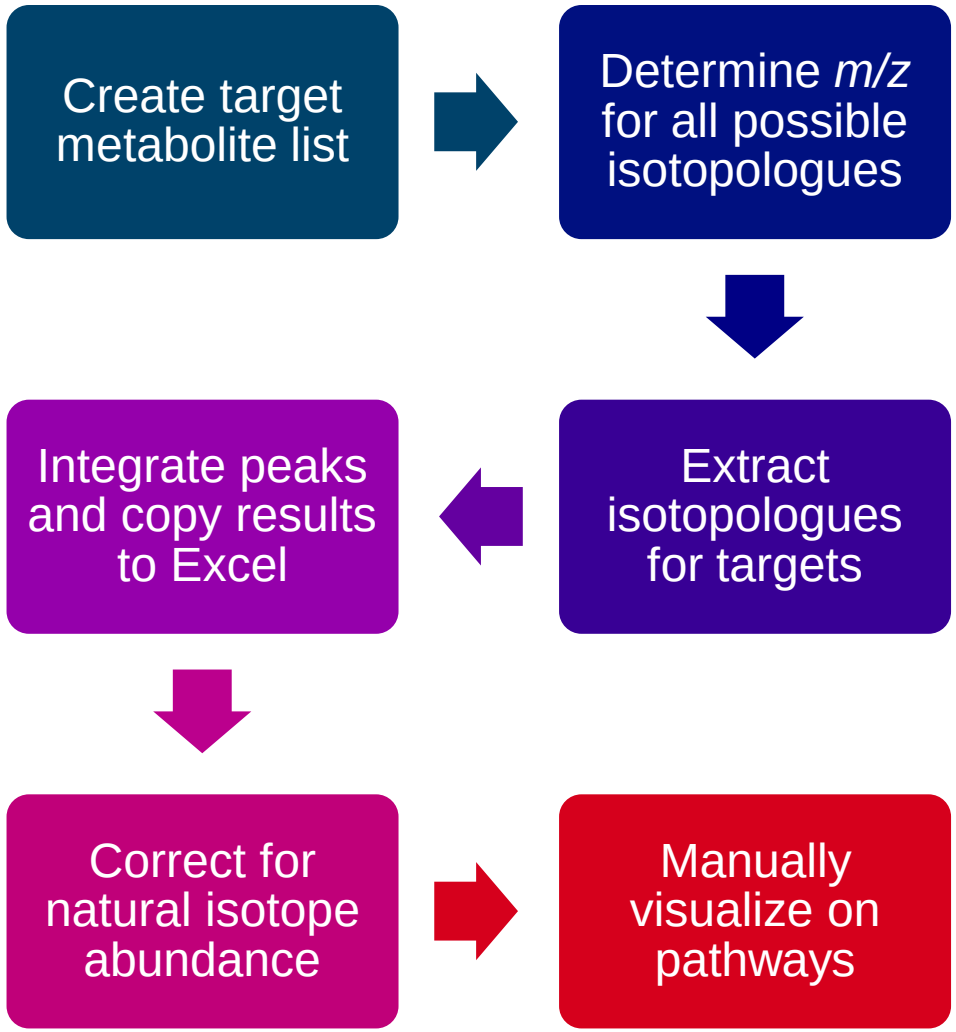
Stable Isotope Tracing for Qualitative Flux Analysis

Manual Process

Multi-step manual process is

- Tedious
- Error-prone
- Time-consuming

This limits the number of compounds analyzed!

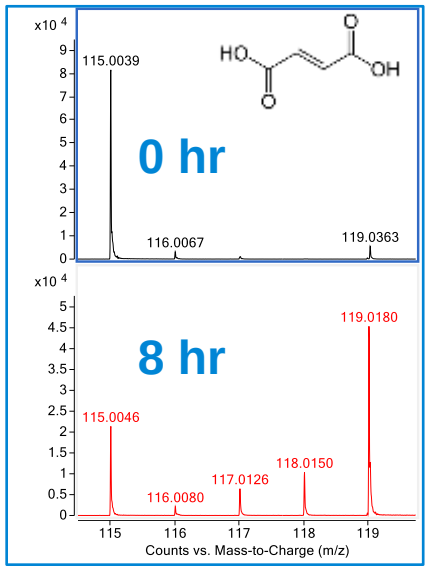


VistaFlux Stable Isotope Tracing

Isotopologue tracking

Use of stable isotope labels (^{13}C, ^{15}N, and ^{2}H)

Monitor stable isotope incorporation



Fumarate $C_4H_4O_4$

Isotopologues

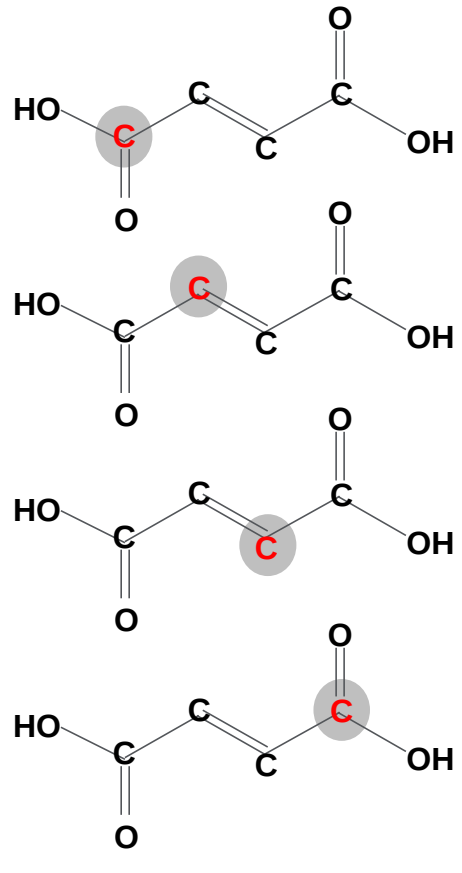
M+0: $^{13}C_0C_4H_4O_4$

M+1: $^{13}C_1C_3H_4O_4$

M+2: $^{13}C_2C_2H_4O_4$

M+3: $^{13}C_3C_1H_4O_4$

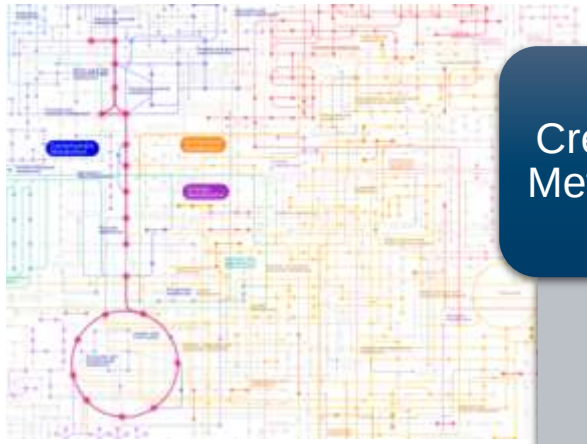
M+4: $^{13}C_4C_0H_4O_4$



4 M+1 Isotopomers

Stable Isotope Tracing Using MassHunter VistaFlux

Agilent VistaFlux workflow

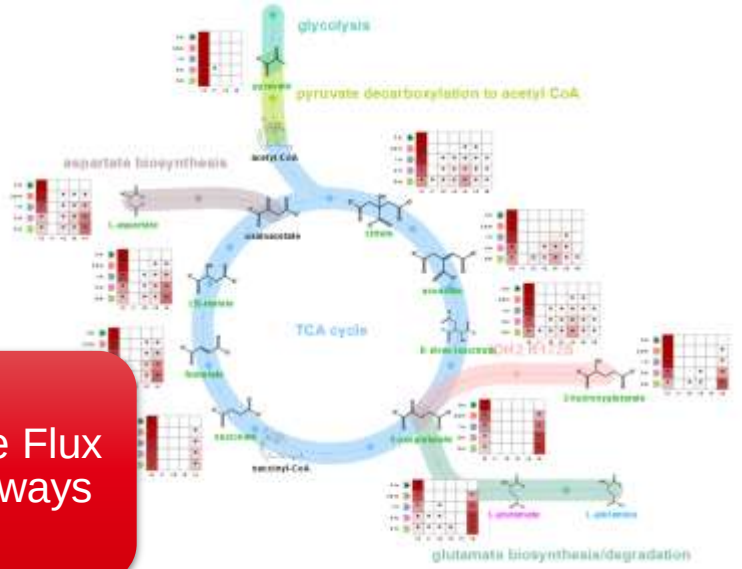


Create Target Metabolite List

Acquire TOF Data

Extract Isotopologues

Visualize Flux on Pathways



Easy creation of target metabolite list

High quality data collection

Efficient batch isotopologue extraction of targets

Flexible pathway visualization of metabolic fluxes

Metabolomics dMRM Database and Analytical Method

Routine analysis of central carbon pathway metabolites

What is it?

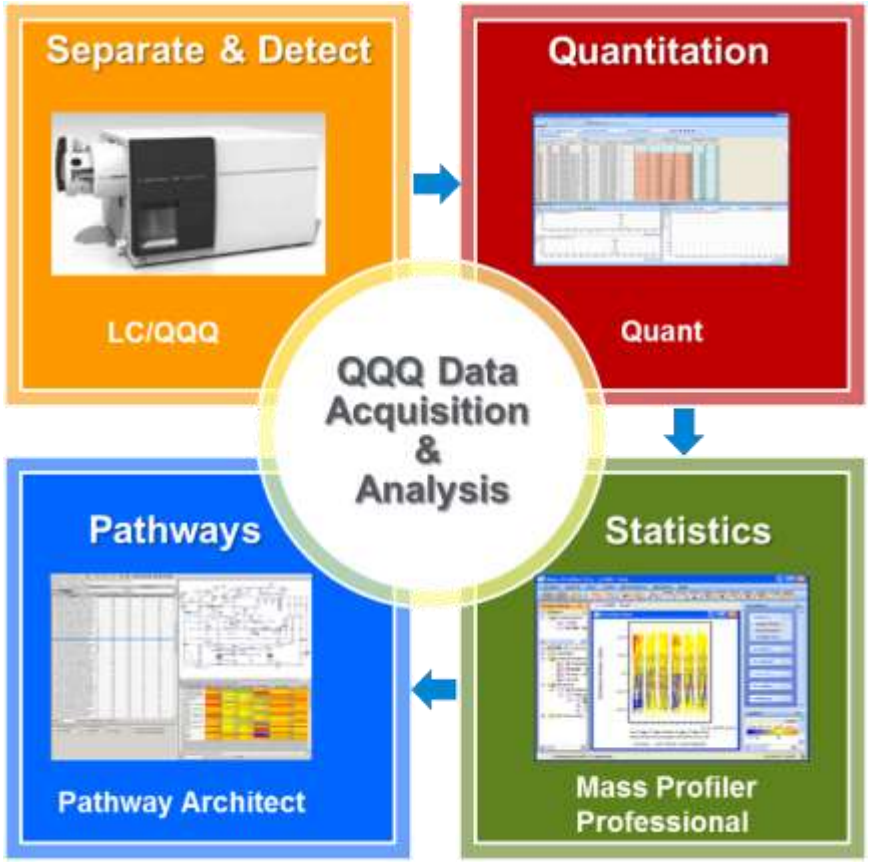
- An optimized LC/MS database and analytical method for 219 central carbon metabolites
- Designed for 1290 Flex pump and 6460/6470 QQQ LC/MS Systems
- Provides an optimized method and database with stable, robust chromatography

Why develop an analytical method for central carbon metabolism?

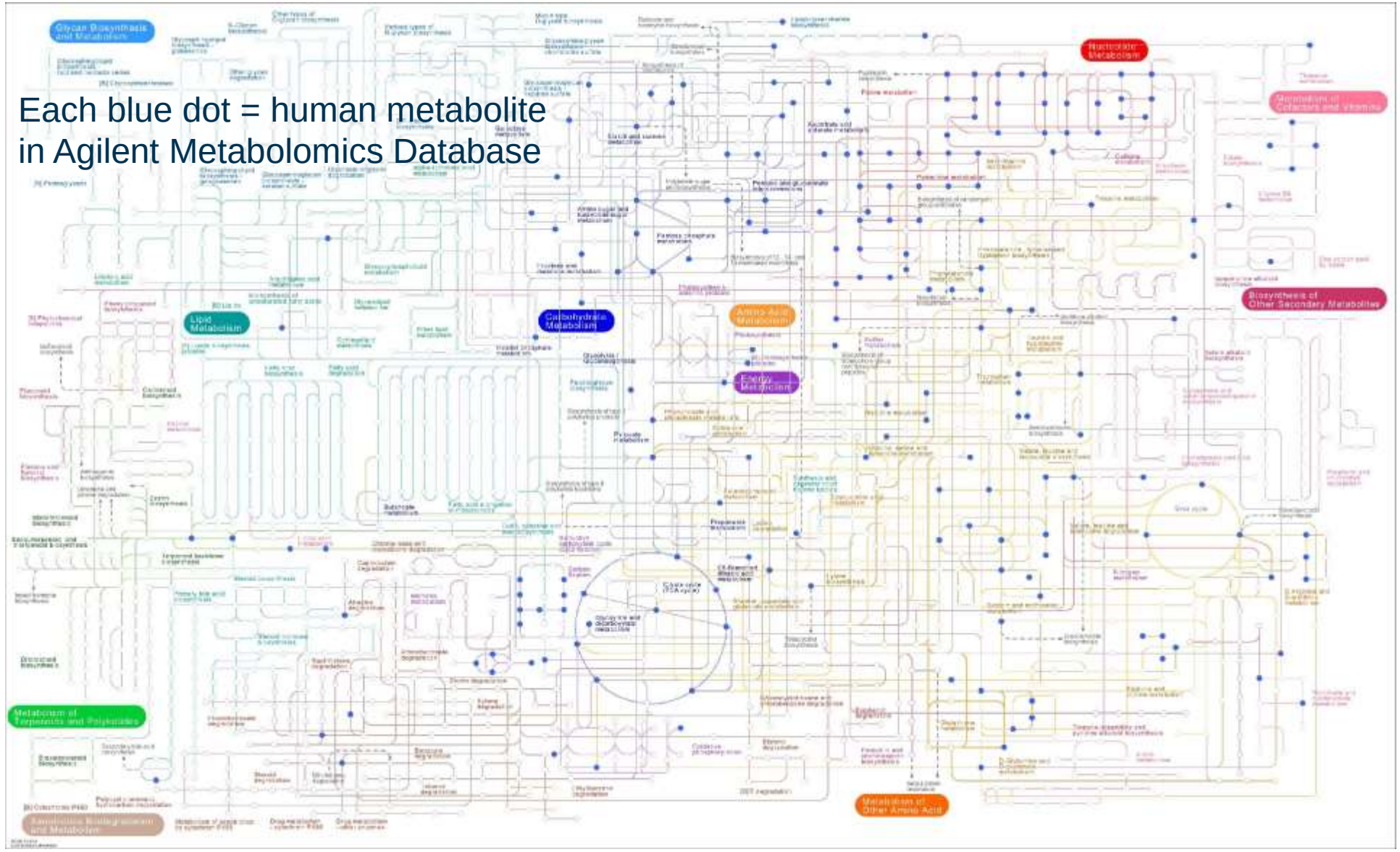
- Central carbon metabolism is associated with energy metabolism and synthesis of important metabolites

Why develop a targeted analytical method for LC/QQQ?

- Easy, sensitive, robust and routine analysis with simplified data analysis (compared to discovery metabolomics)
- Low cost of operation and low capital cost



KEGG Map of Human Metabolites in Database



XF Technology Empowers You to Answer Your Questions About Cellular Function in Real-time

Real-time kinetic measurements

Label-free multiparameter sensor
H^+ & O_2

Advanced software & analytics

Integrated injection ports
for real-time compound testing

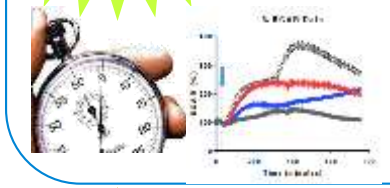
Live cell analysis
with 2D & 3D plate options

Ready-to-use validated kits & reagents

Seahorse XF Assays Go Beyond What Cells Are to Reveal What They **Are Doing**

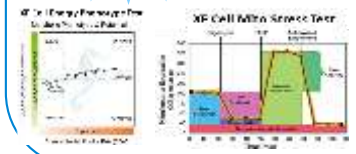
Real-Time Kinetics

- **Dynamic biology**
  Read-out functional changes as they occur
- **Highly sensitive**
  Never miss an unexpected response.



Multi-parameter

- **Complete picture**
  Simultaneously measure key metabolic pathways
- **Data rich**
  Automated compound injection enables real-time in situ modulation.



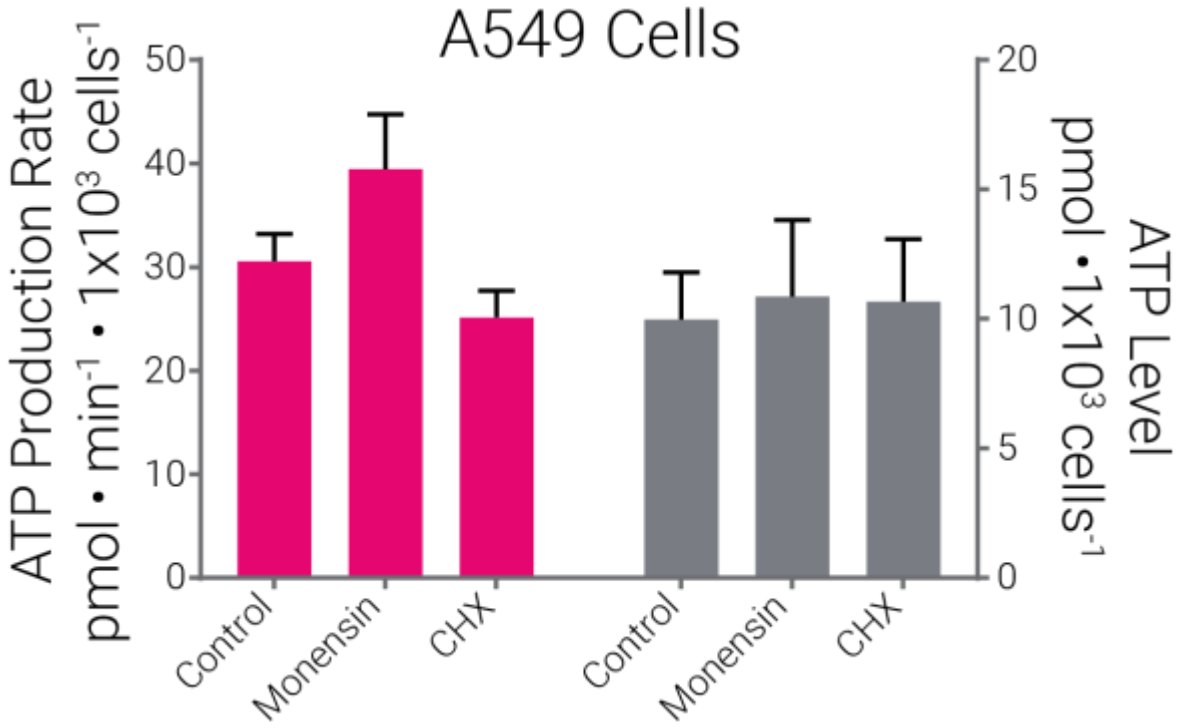
In situ

- **More relevant**
  Measure any cells, mitochondria, spheroids, islets, more.
- **Efficient**
  Requires <40-200K cells per well.

Label-free

- **Reduce Complexity**
  No need to select appropriate labels, arrays, panels, etc.
- **Non-invasive**
  Won't interfere with cell function

XF Real-Time ATP Rate Assay Uncovers Cellular ATP Demand that is missed by ATP Level Assay



Changes in ATP Production Rate correlate with changes in cellular activity.

More informative than measurements of intracellular ATP level for monitoring dynamics of cellular function.

Monensin: increases ATP demand due to increase in Na+ import and Na+/K+ ATPase activity

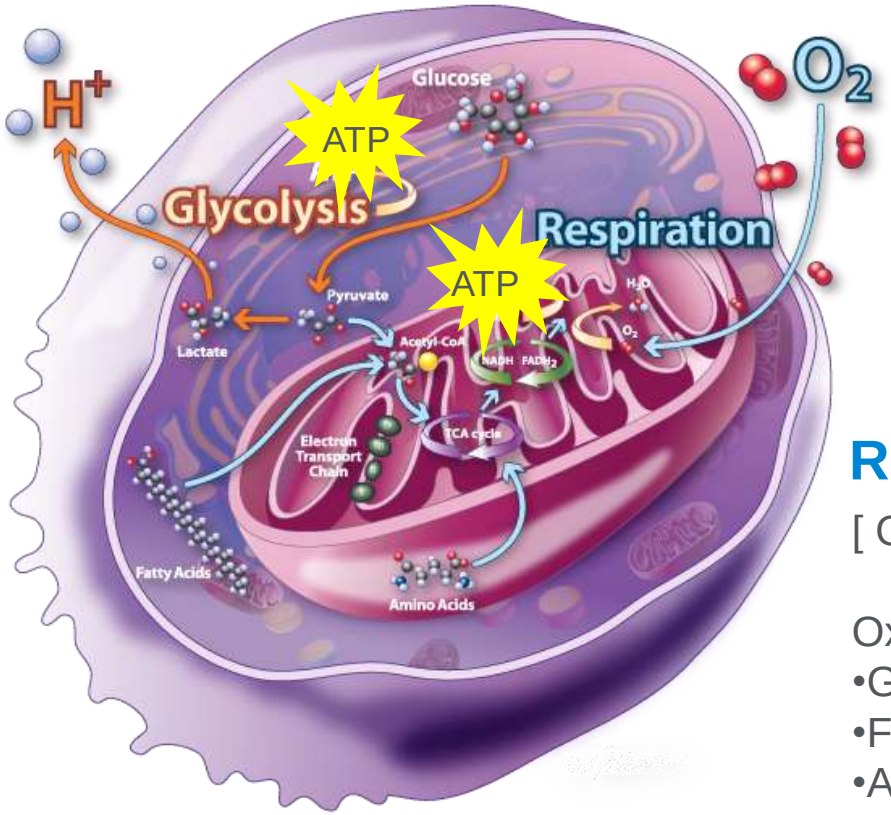
Cycloheximide: decreases ATP demand due to inhibition of protein synthesis

How is ATP Production Rate Measured?

Glycolysis

[pH]

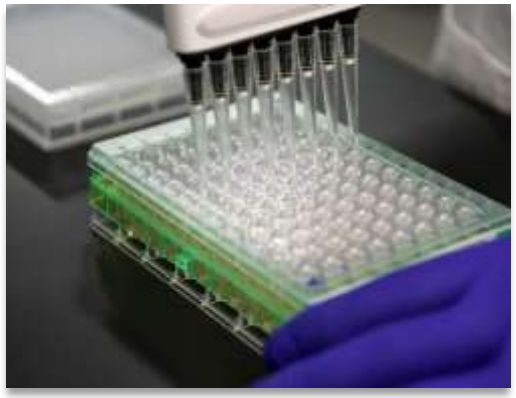
Fermentation of:
•Glucose



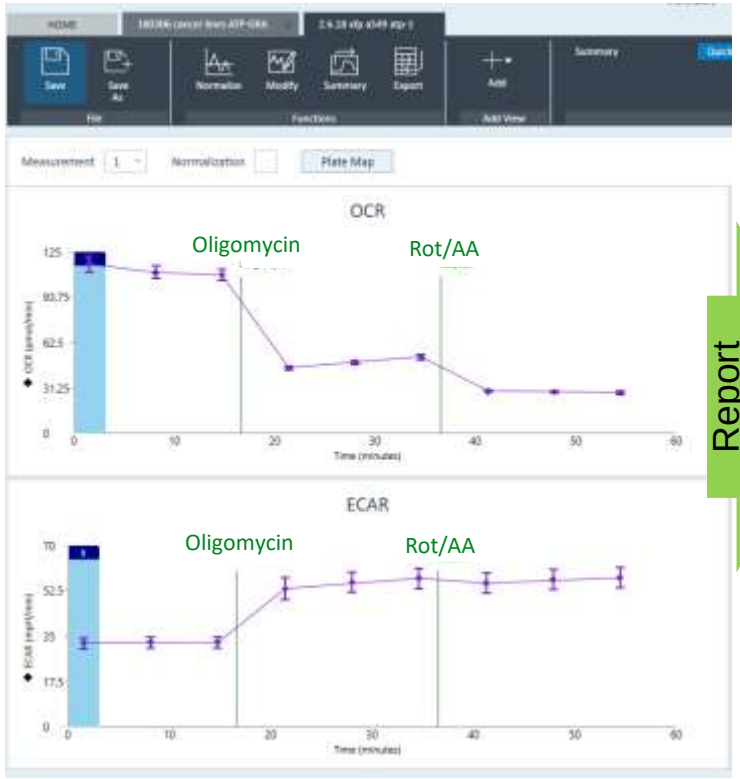
Respiration

[Oxygen]

Oxidation of:
•Glucose
•Fatty Acids
•Amino Acids



How is ATP Production Rate Measured?



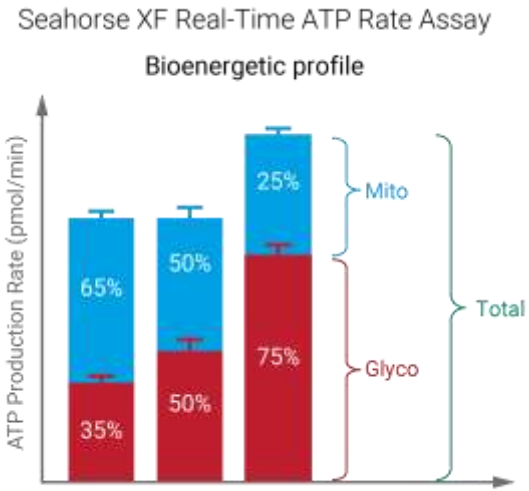
Report Generator

mitoATP Production Rate

+

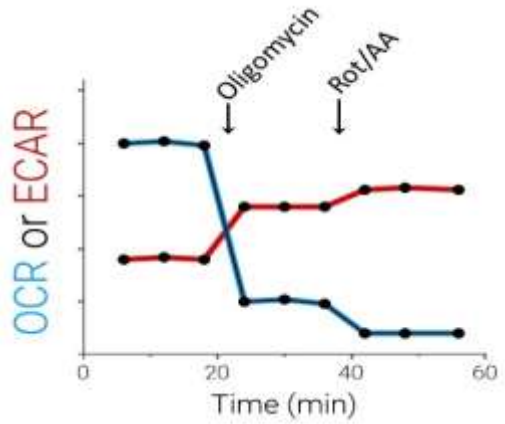
glycoATP Production Rate

Total ATP Production Rate



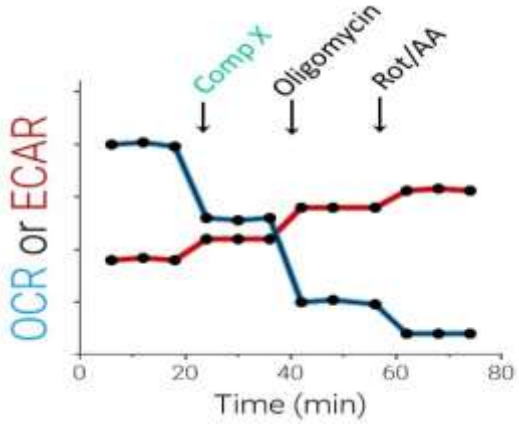
Assay Design

Standard XF Real-Time ATP Rate Assay



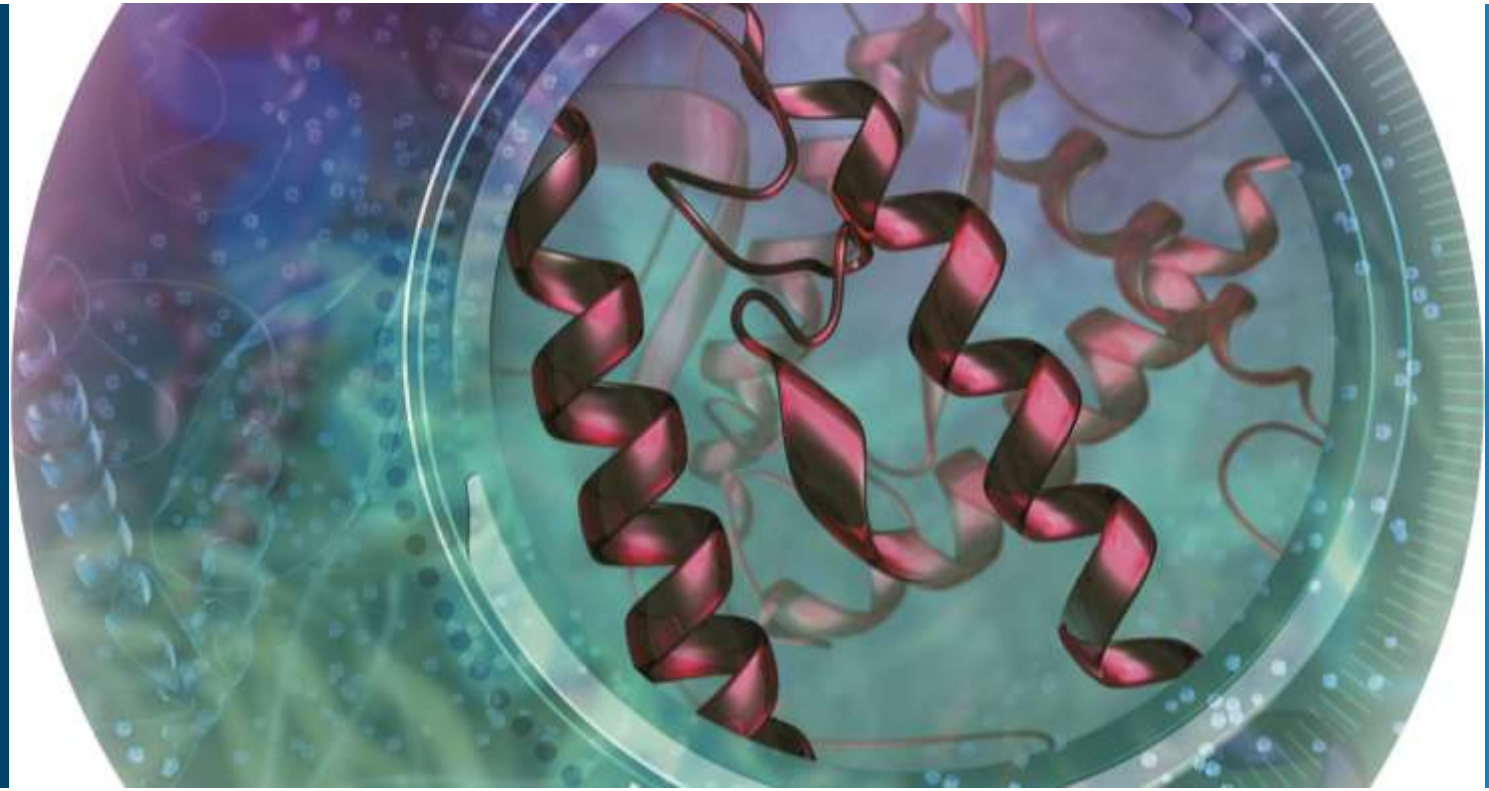
- To quantify metabolic phenotype of a cell type, to compare different cell types, genetic modifications, pre-treatments with compounds
- Outputs:
  - Basal mitoATP, glycoATP, total ATP rates
  - ATP Rate Index

Induced XF Real-Time ATP Rate Assay



- To study acute effect of compounds, compounds effect over time, mitotoxicity, metabolic switch or pathway liabilities induced by compounds
- Outputs:
  - Basal AND Induced (post-treatment) mitoATP, glycoATP, total ATP rates
  - Basal and Induced ATP Rate Index

Proteomics Workflows



Agilent Instrumentation For Proteomics

Agilent AssayMAP Bravo

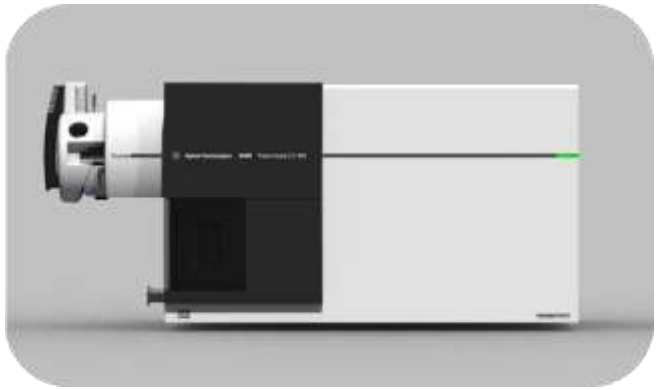
Agilent Jet Stream source

Nanodaptor

Nanospray source

1290 Infinity II UHPLC

QQQ 6400 Series



Q-TOF 6500 series

Agilent Jet Stream Ion Source

Superior to capillary LC

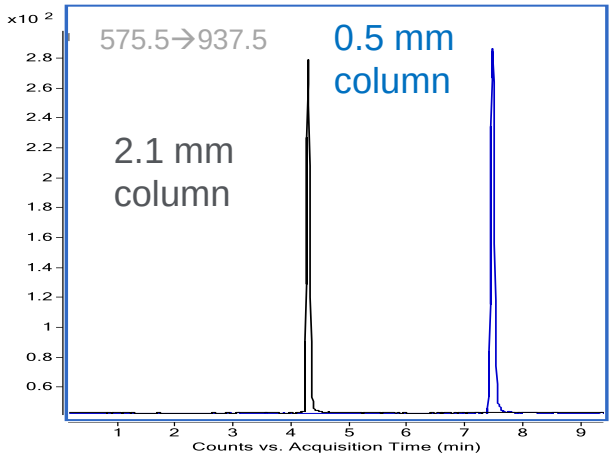
Jet Stream provides 3-5x signal increase compared to ESI

Agilent Jet Stream showed same signal (and LOD) for

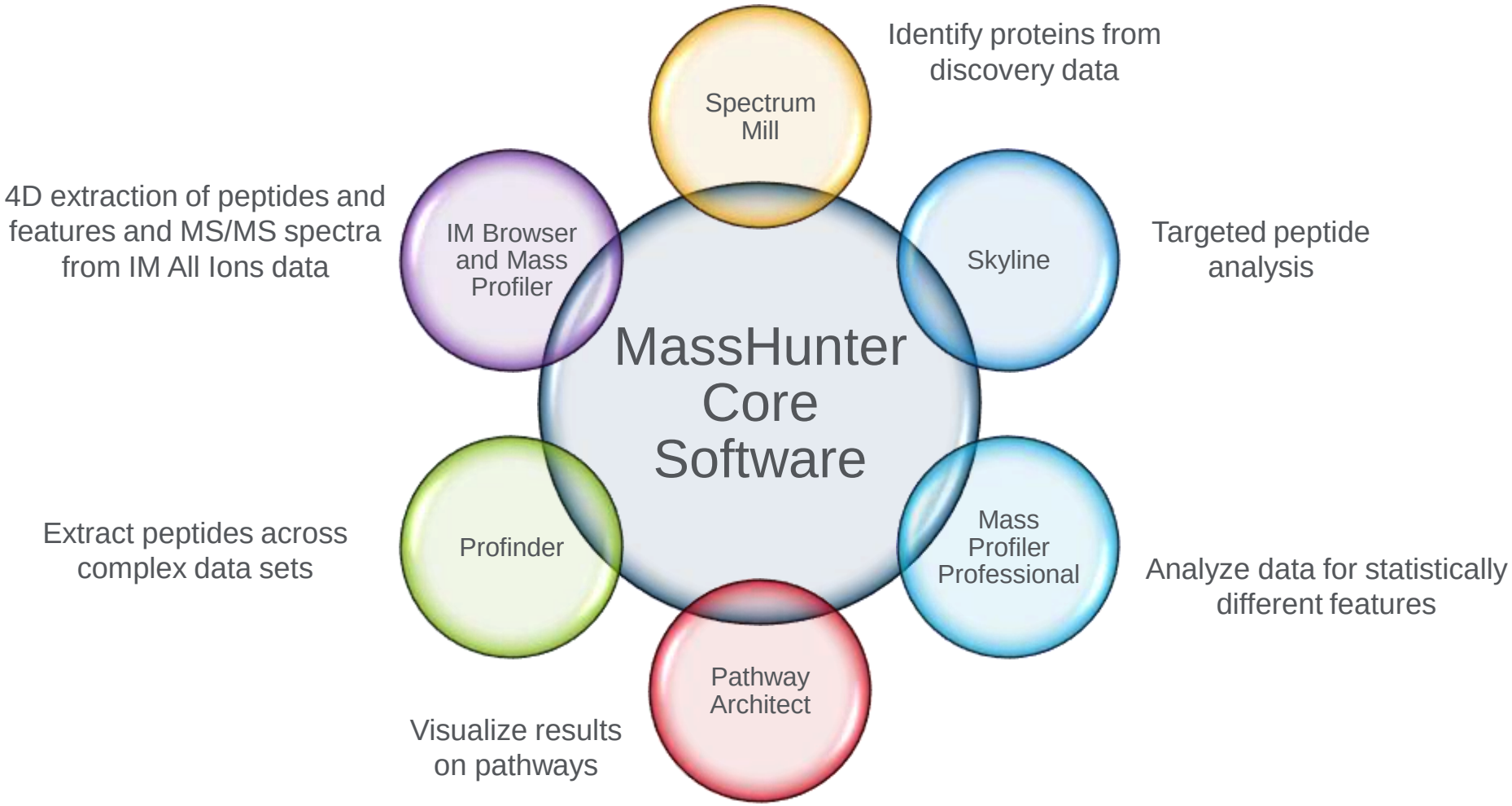
- 2.1 mm ID column at 400 µL/min
- 0.5 mm ID column at 17 µL/min

Agilent Jet Stream is not concentration dependent like ESI

- Analytical sensitivity depends on absolute amount (mass) of analyte in source not concentration of analyte in droplet
- Published results for small molecules: Buckenmaier S, Miller CA, van de Goor T, Dittmann MM. J Chromatogr. A 2015, 1377:64-74.

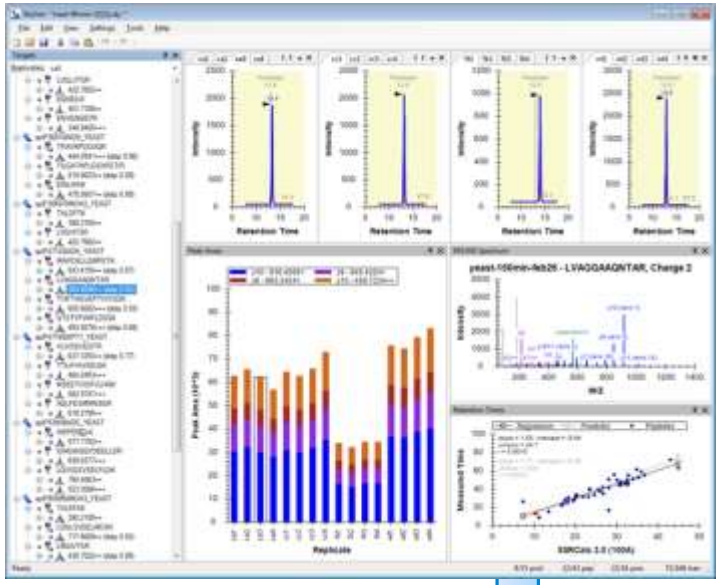


Agilent Proteomics Application Software

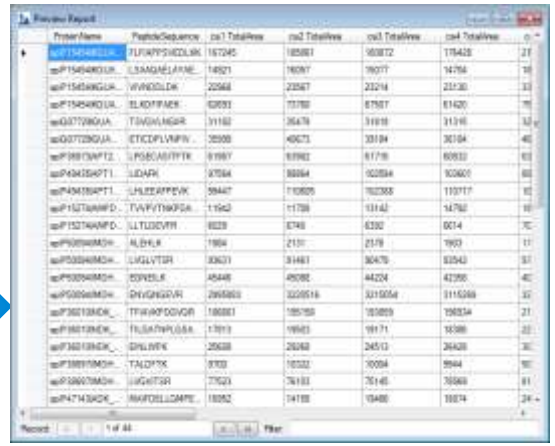


Targeted Proteomics Workflows

From Skyline to MPP

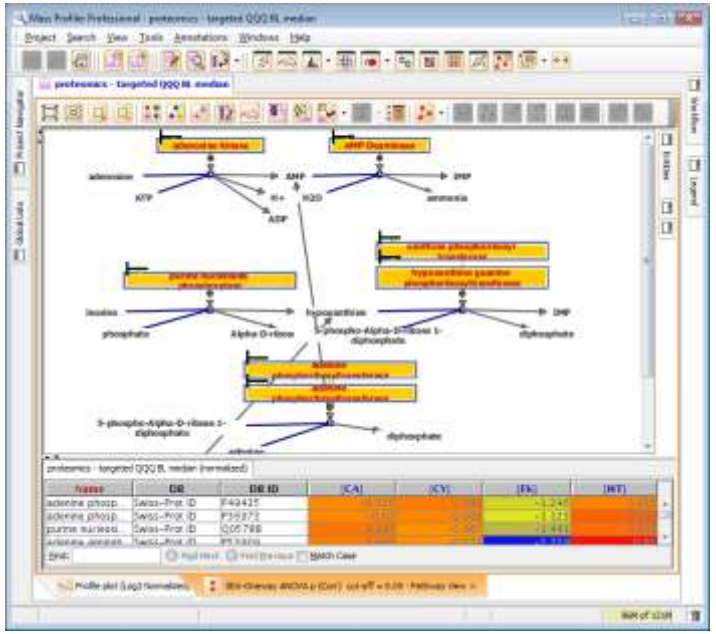


Review and process QQQ or Q-TOF results in Skyline

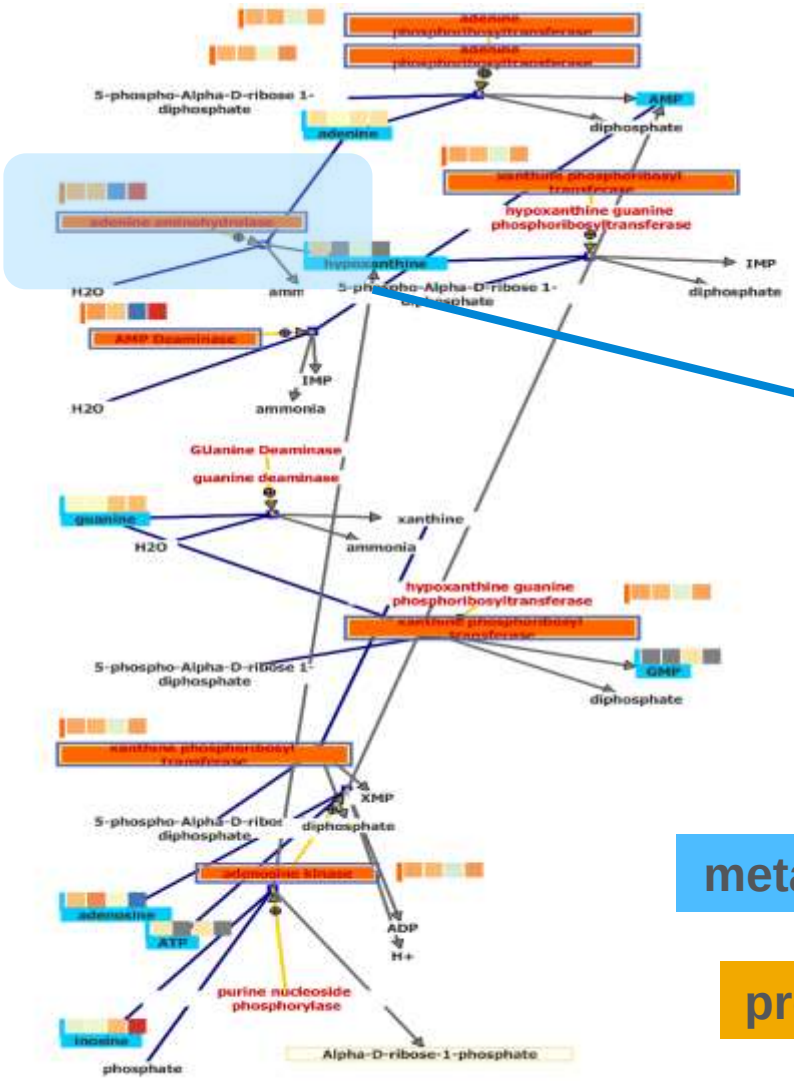


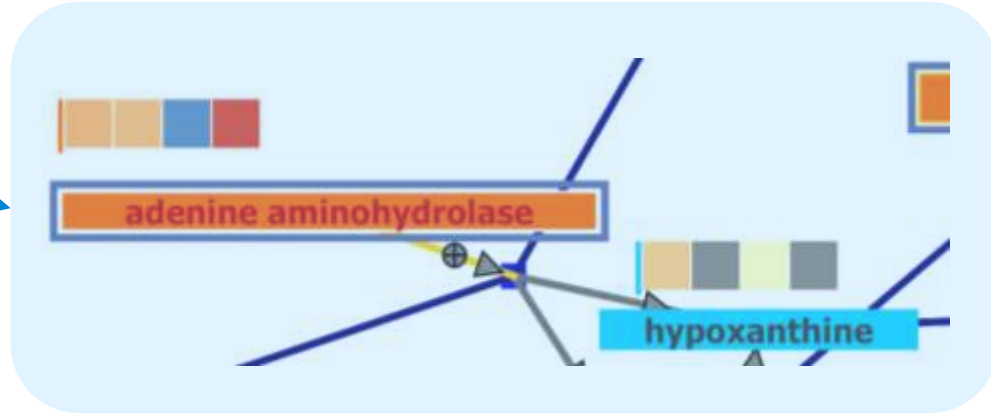
Export results to MPP

Pathway visualization in Pathway Architect



Multi-omics Pathway Visualization of Results





metabolites

proteins

Any 2 omics experiments can be combined

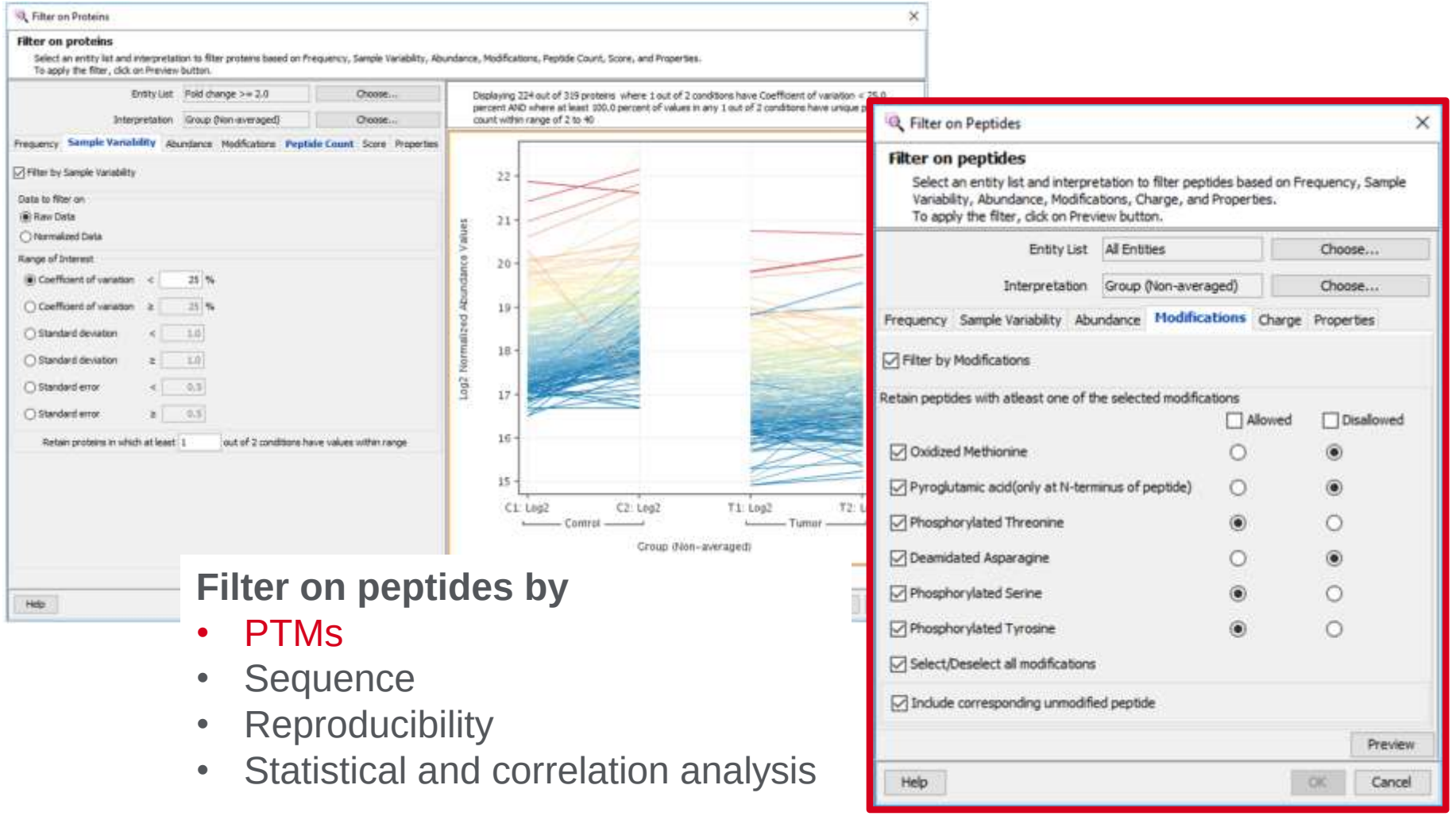
Results are mapped to the pathway entities and displayed as quilt plots

MPP Proteomics Analysis
Enabling Protein and Peptide Level Analysis

- Imports protein and peptide-level information from Spectrum Mill or Skyline
- Filters available for peptide-level and protein-level (abundance, frequency, PTMs)
- Protein/Peptide Entity Inspector for visualizing results

Enhanced Proteomics Workflow in MPP

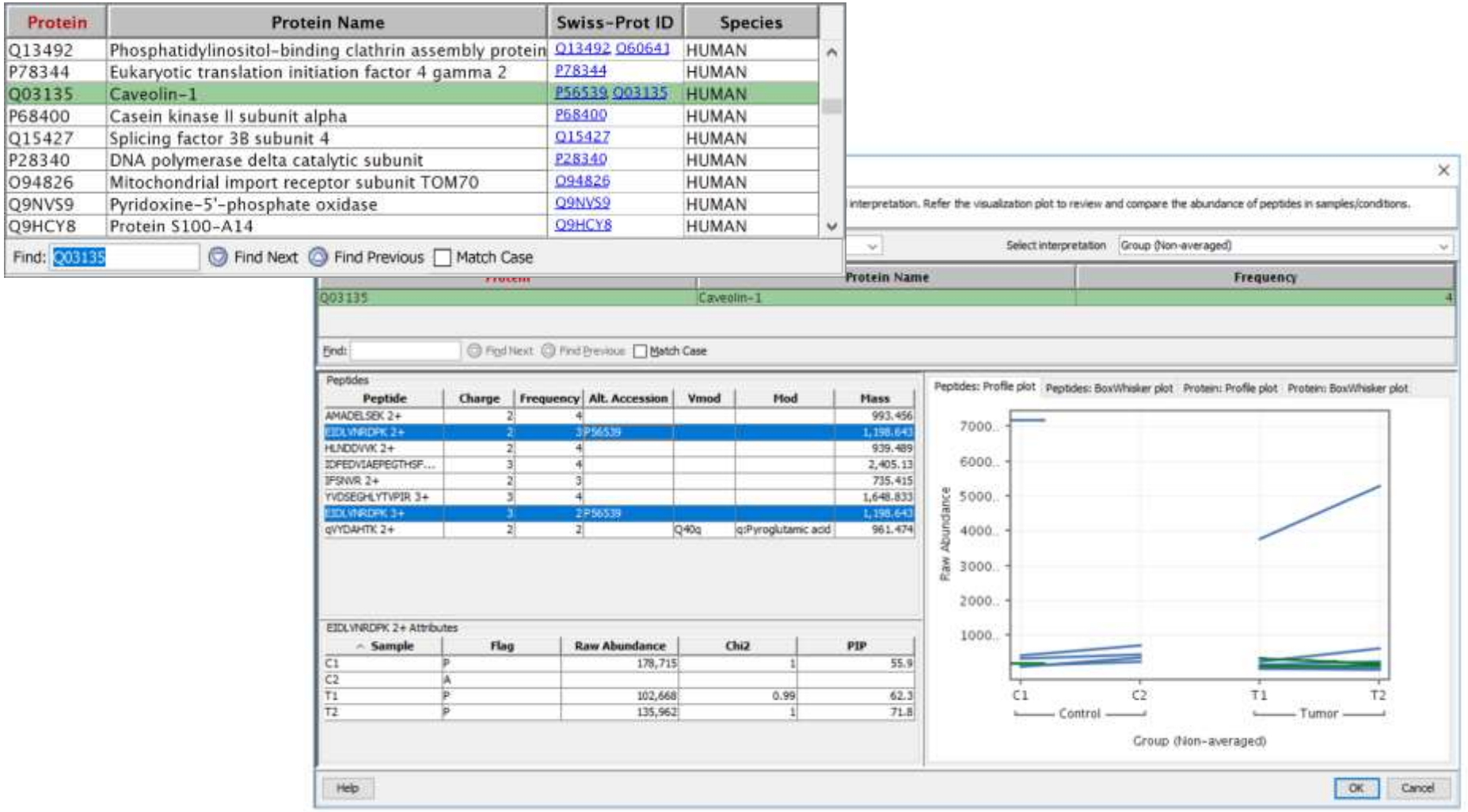
Filter, Analyze and Visualize at Protein and Peptide Levels



Filter on peptides by

- PTMs
- Sequence
- Reproducibility
- Statistical and correlation analysis

Proteomics Visualizations in MPP
Display Both Protein and Peptide Information

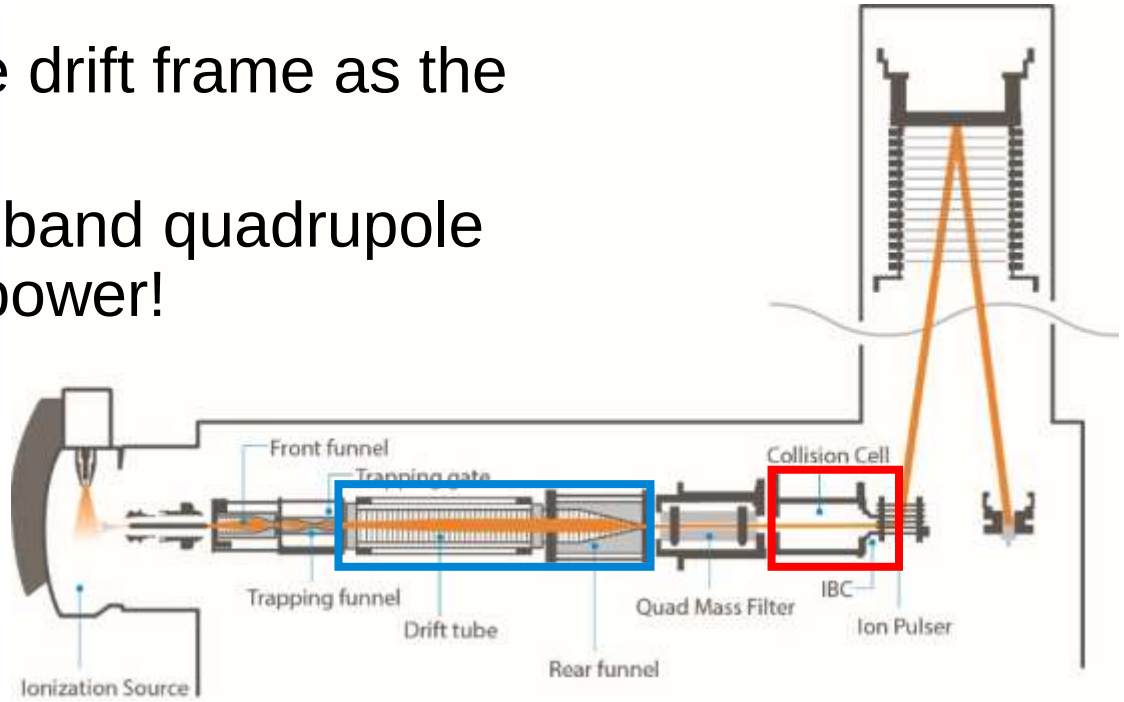


Agilent IM All Ions MS/MS for Proteomics

Drift separation instead of quadrupole isolation

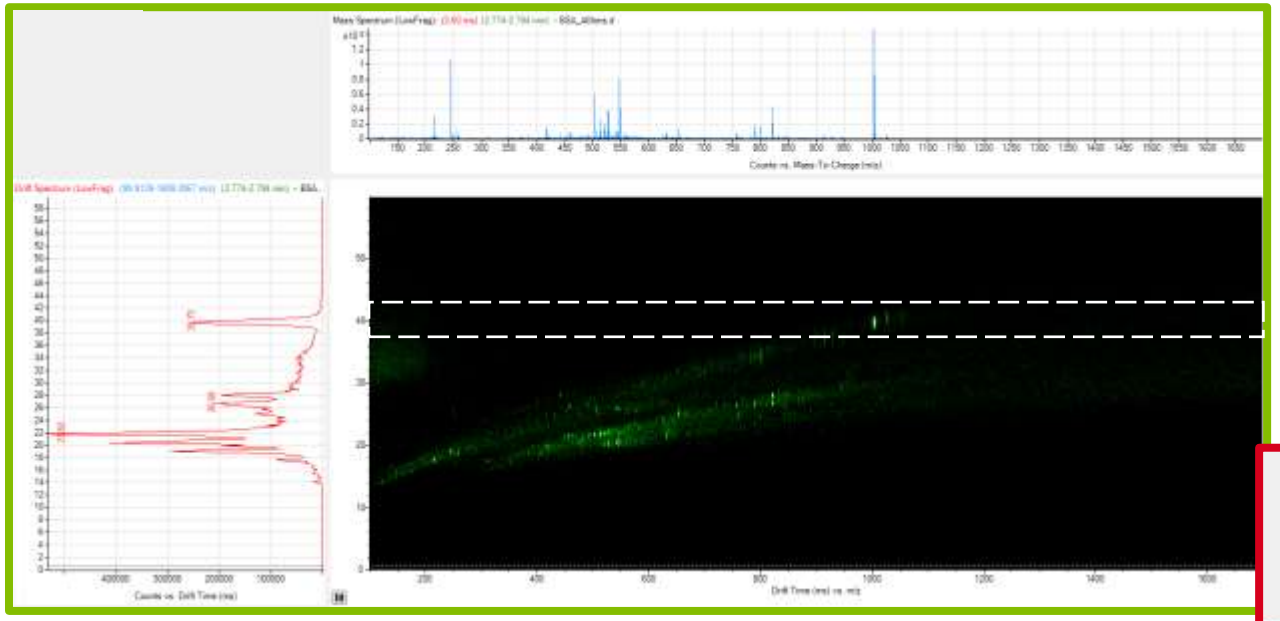
- All ions entering the collision cell are subjected to fragmentation voltage
- Fragment ions will be in the same drift frame as the precursor

Better duty cycle compared to wide-band quadrupole isolation with equivalent resolution power!



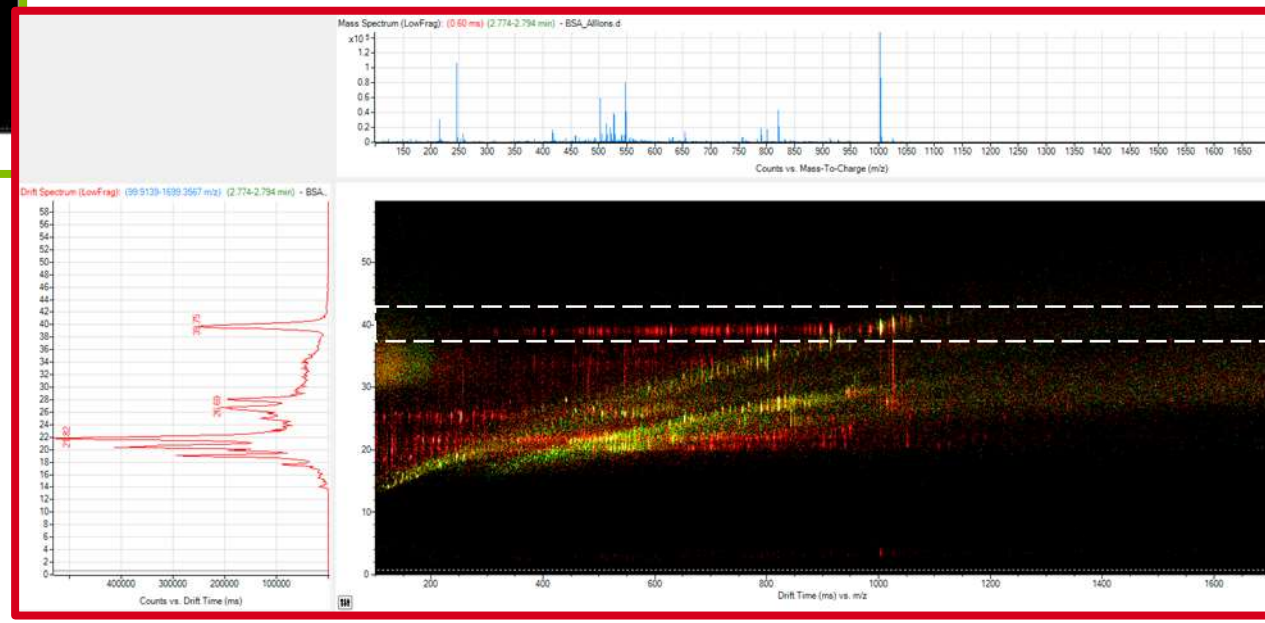
IM All Ions MS/MS Acquisition for Proteomics

Alternates between no collision energy and collision energy



No collision energy
(MS only)

With collision energy
(MS/MS)

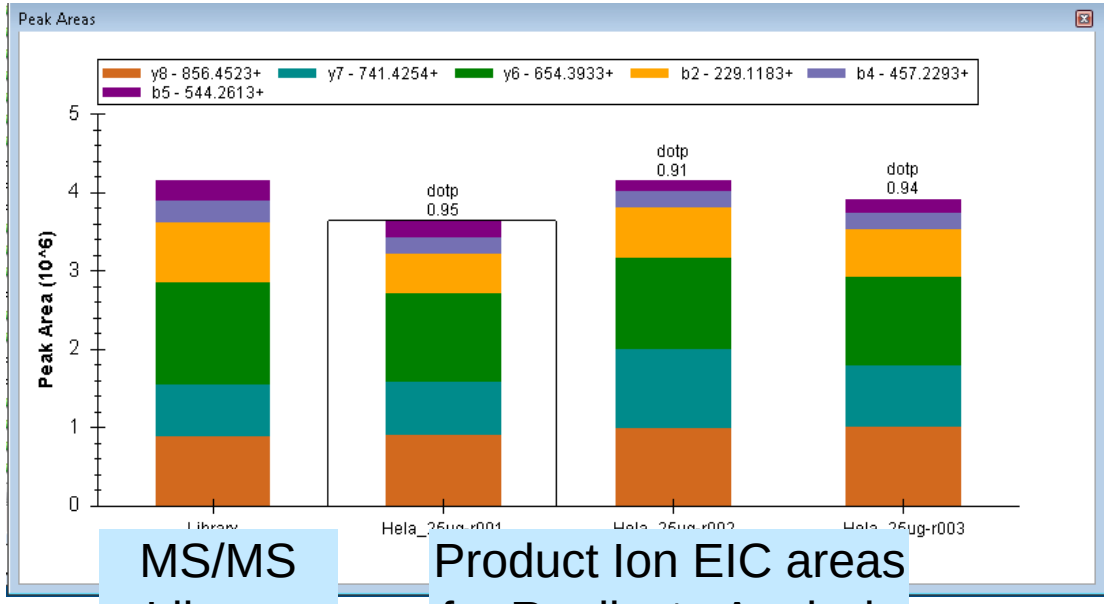


IM All Ions MS/MS for Proteomics
Reproducible MS/MS Results

No precursor isolation so product ions always produced

Fast cycle time compared to wide-band isolation

More points across chromatographic peak yields better reproducibility

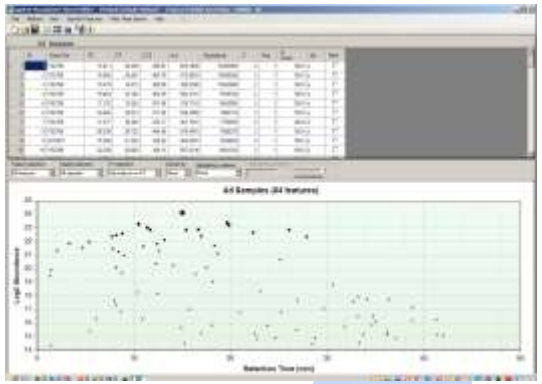


MS/MS Library

Product Ion EIC areas for Replicate Analysis

Ion Mobility Proteomics Workflows

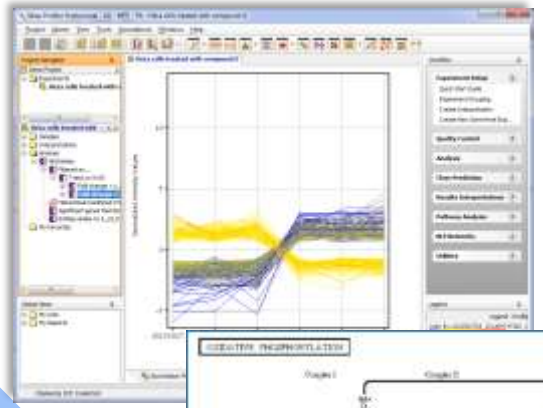
Discovery and targeted analysis

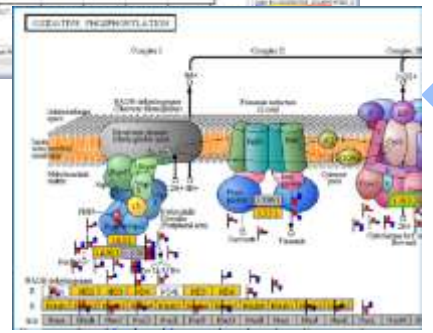


Mass Profiler

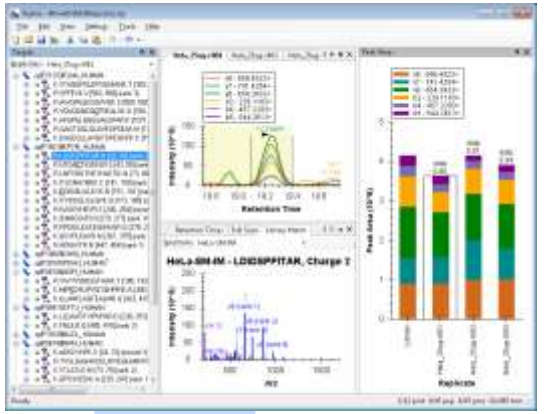
Spectrum Mill

MPP

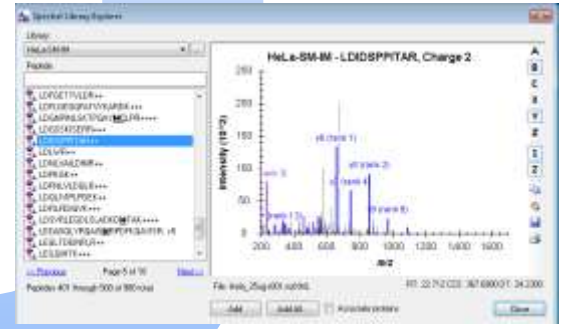




Pathway Architect



Skyline



MS/MS Library Viewer

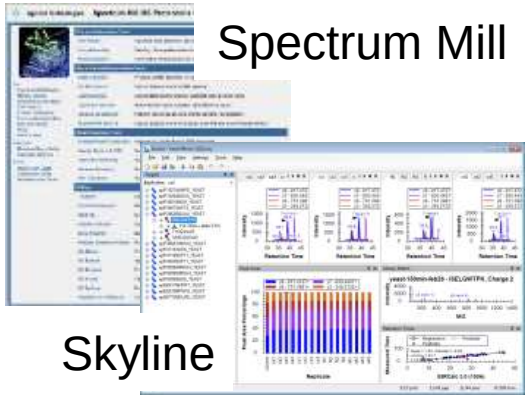
Discovery

Targeted

Accelerate Your Research With a Complete Pathway Centric Workflow

Acquire data → Perform data analysis → **Accelerate your research** → Statistical and correlation analysis → Map results to pathways

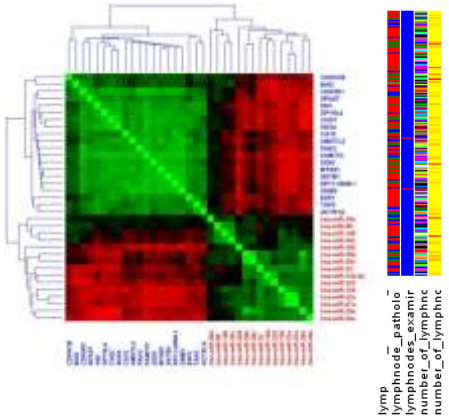
6500 Series Q-TOF



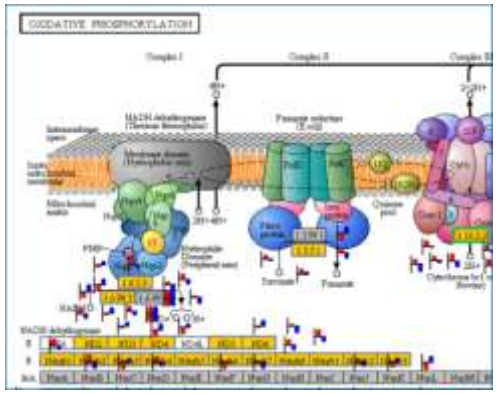
Spectrum Mill

Skyline

Profinder



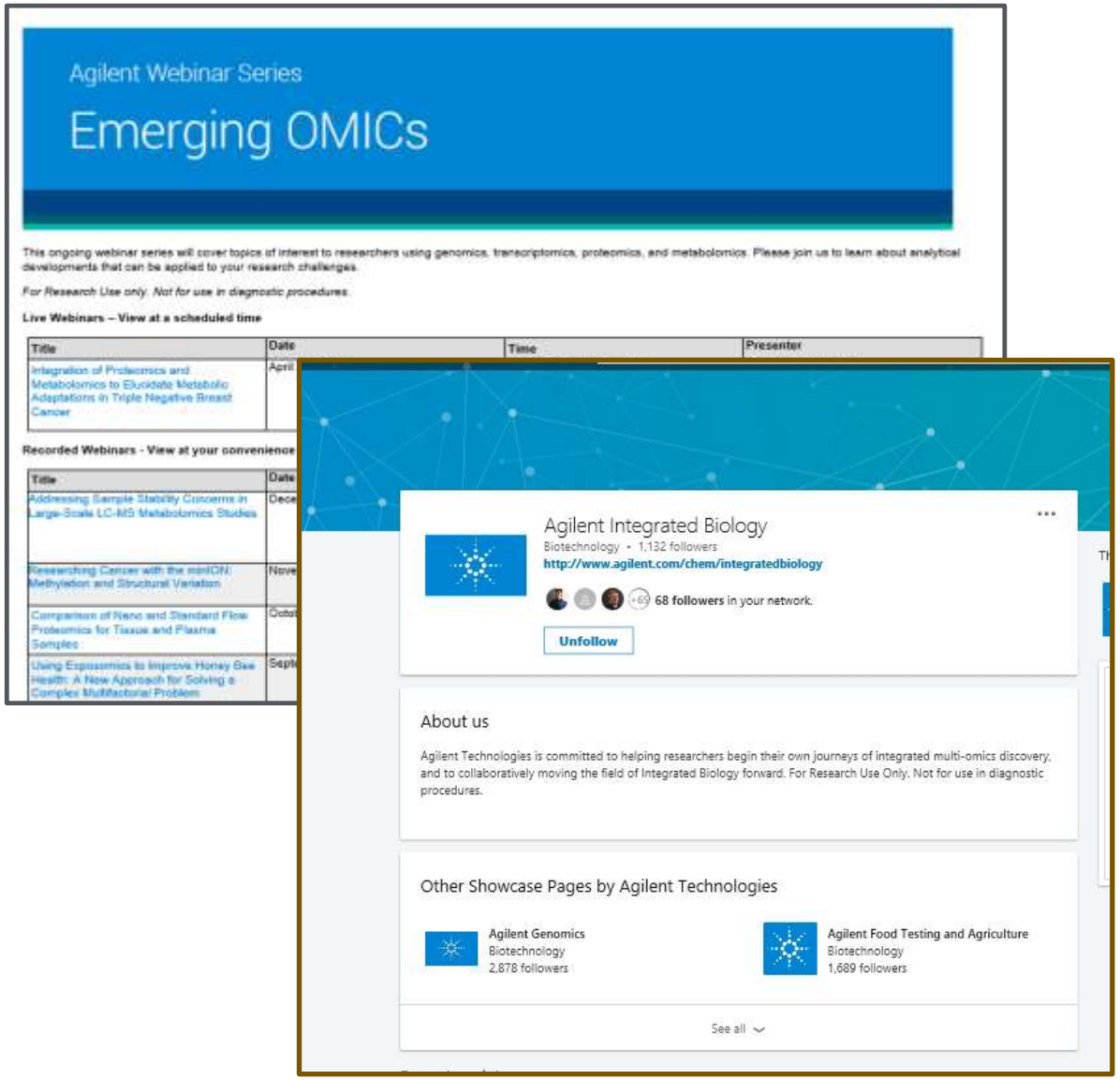
Mass Profiler Professional



Pathway Architect

Pathway-directed targeted omics experiments

Latest Information From Agilent



Quarterly Omics eSeminar Series

http://www.agilent.com/en-us/training-events/eseminars/emerging-omics

LinkedIn

Agilent Integrated Biology page

https://www.linkedin.com/company/agilent-integrated-biology?trk=rr_brands_carousel_logo

Questions