

Accelerate Your Research with Advanced Omics Solutions

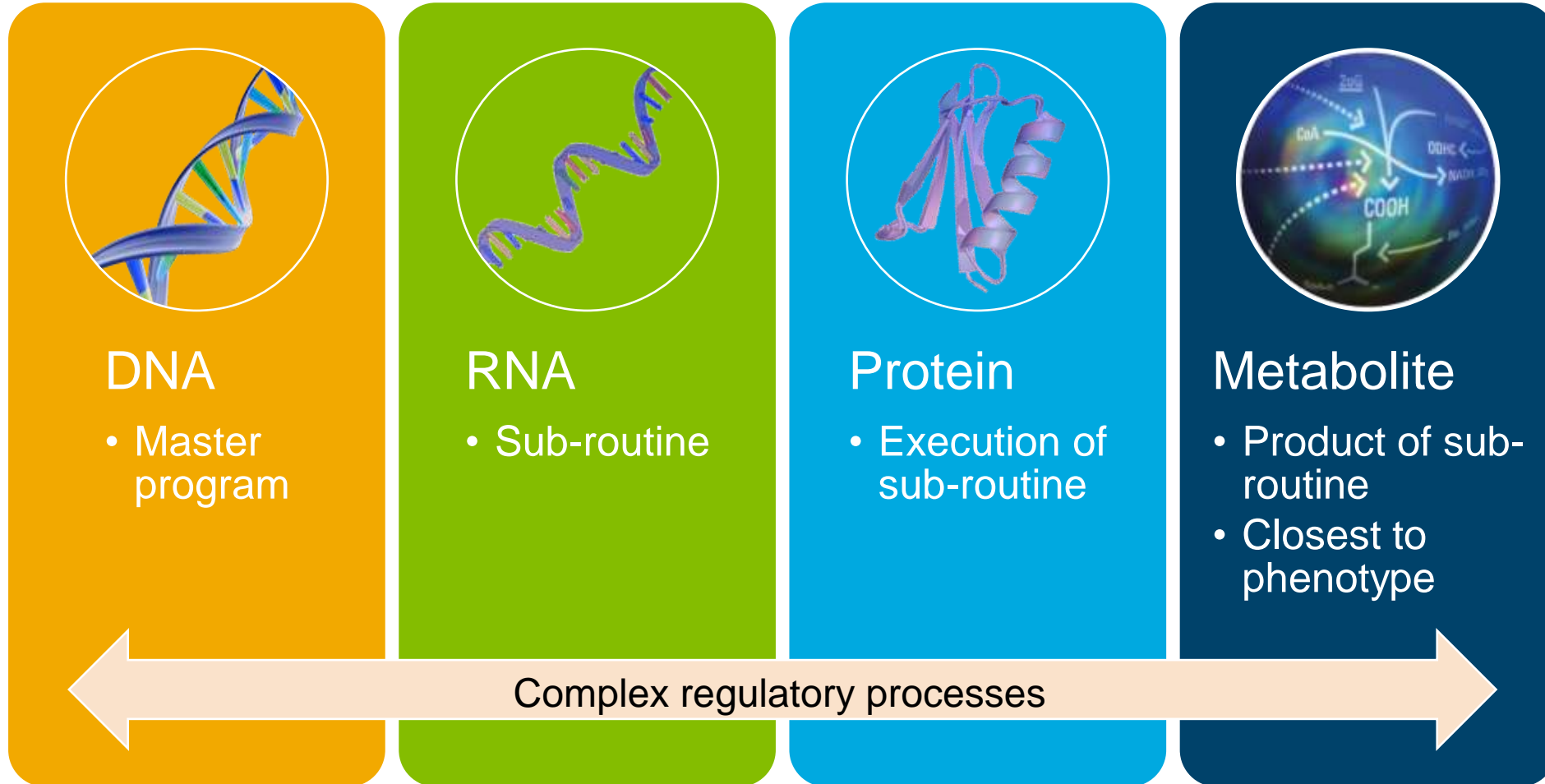
Christine Miller
Omics Market Manager
ASMS 2018

ASMS 2018 For Research Use Only. Not for use
in diagnostic procedures.



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:

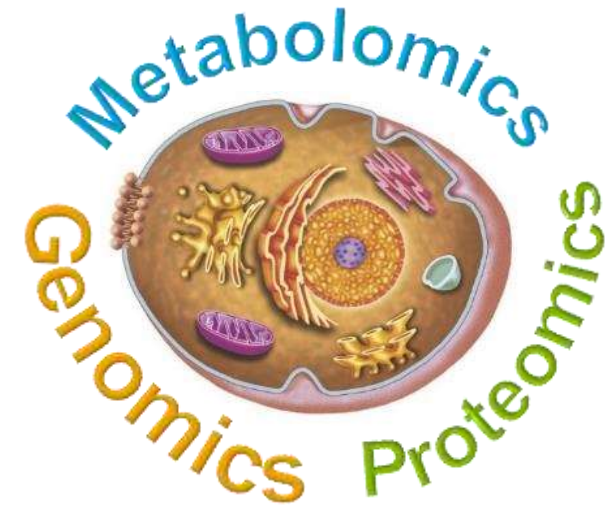
Instrumentation

Consumables

Analytical methods

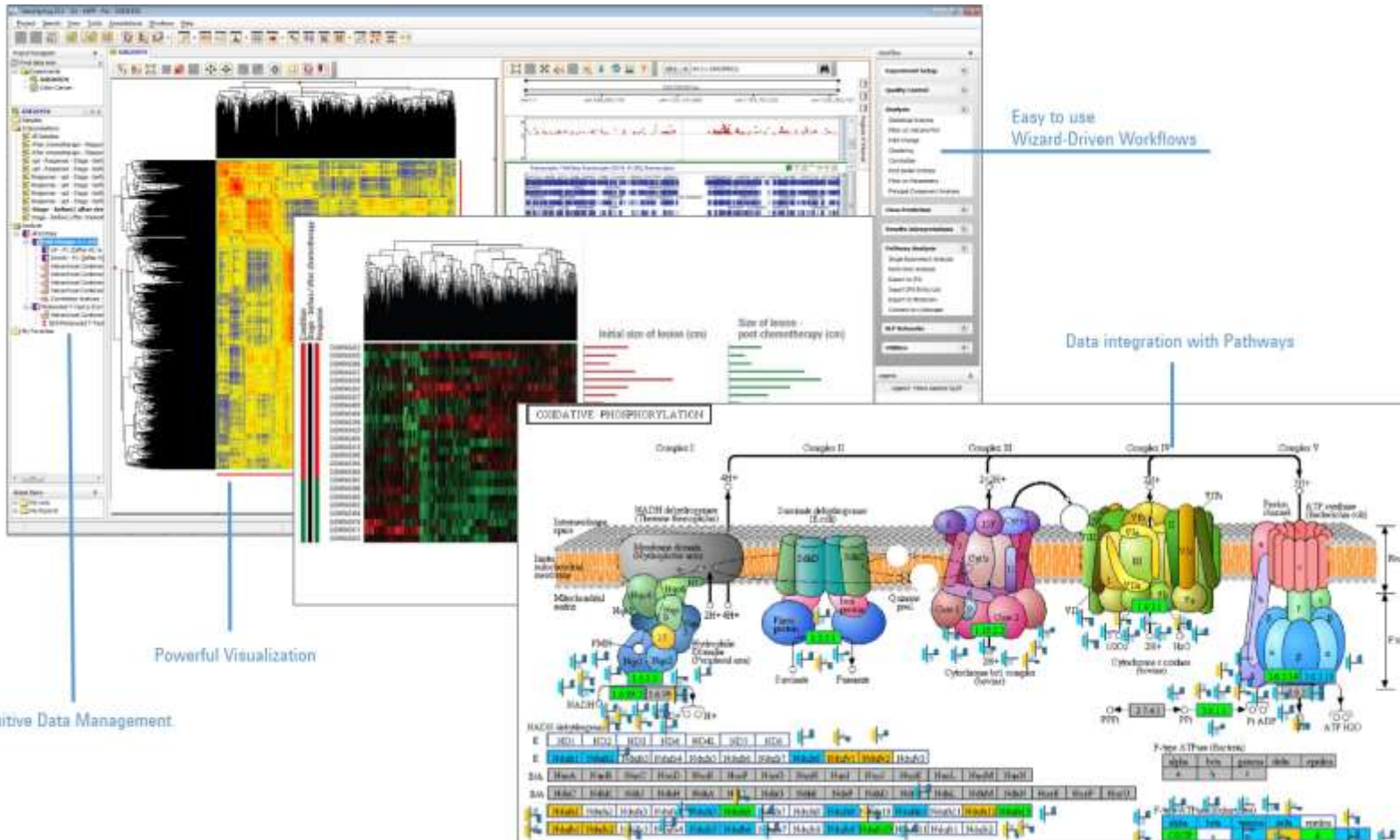
Software solutions

...needed to integrate multi-omics data.



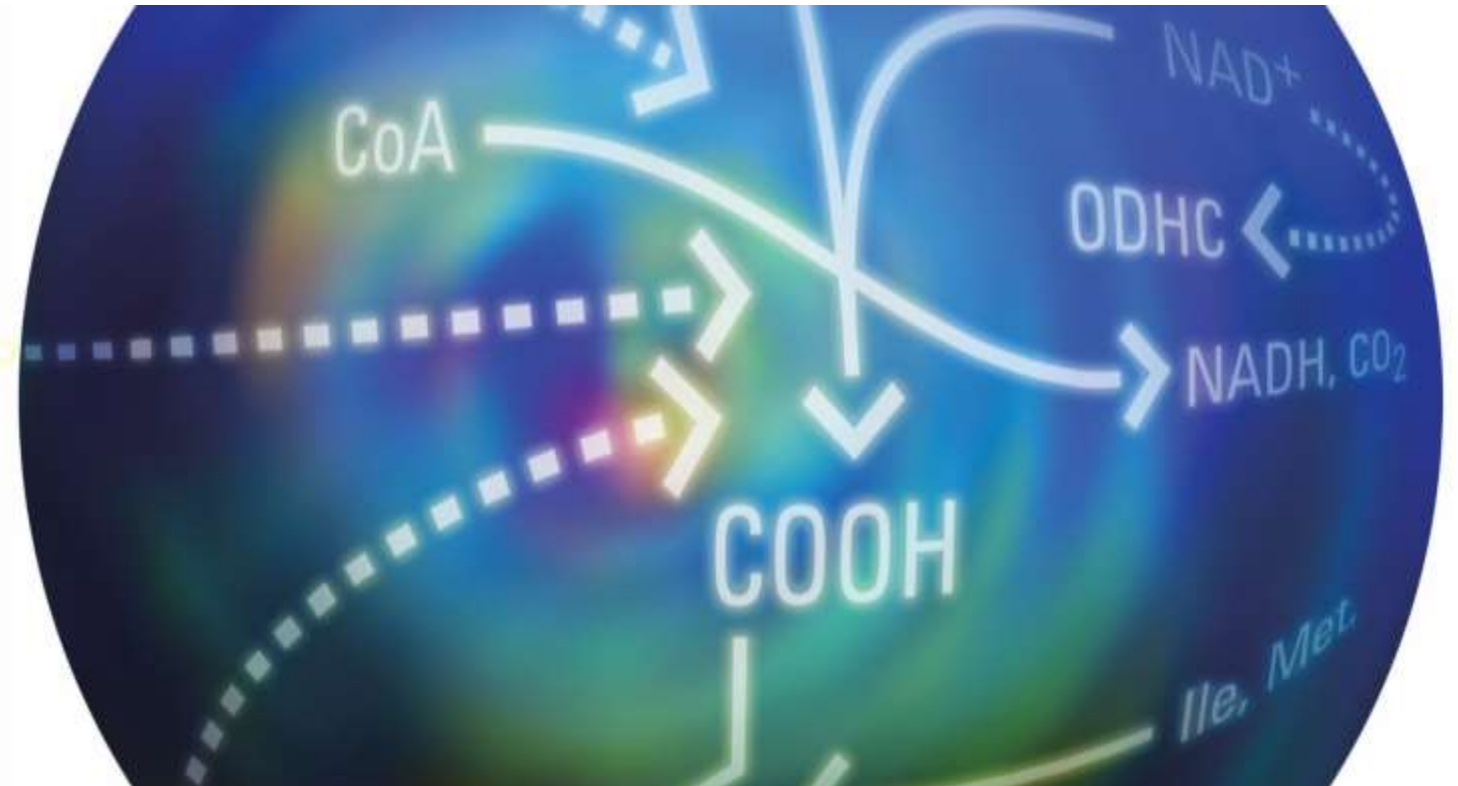
Mass Profiler Professional and Pathway Architect

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

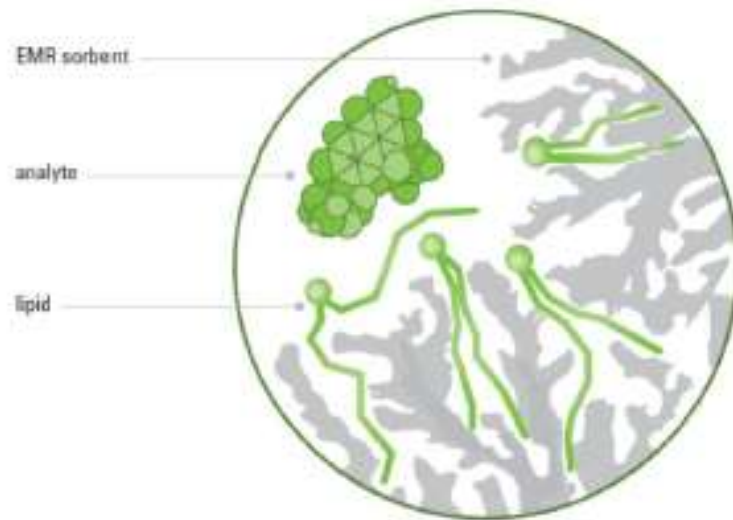


Captiva EMR-Lipid

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

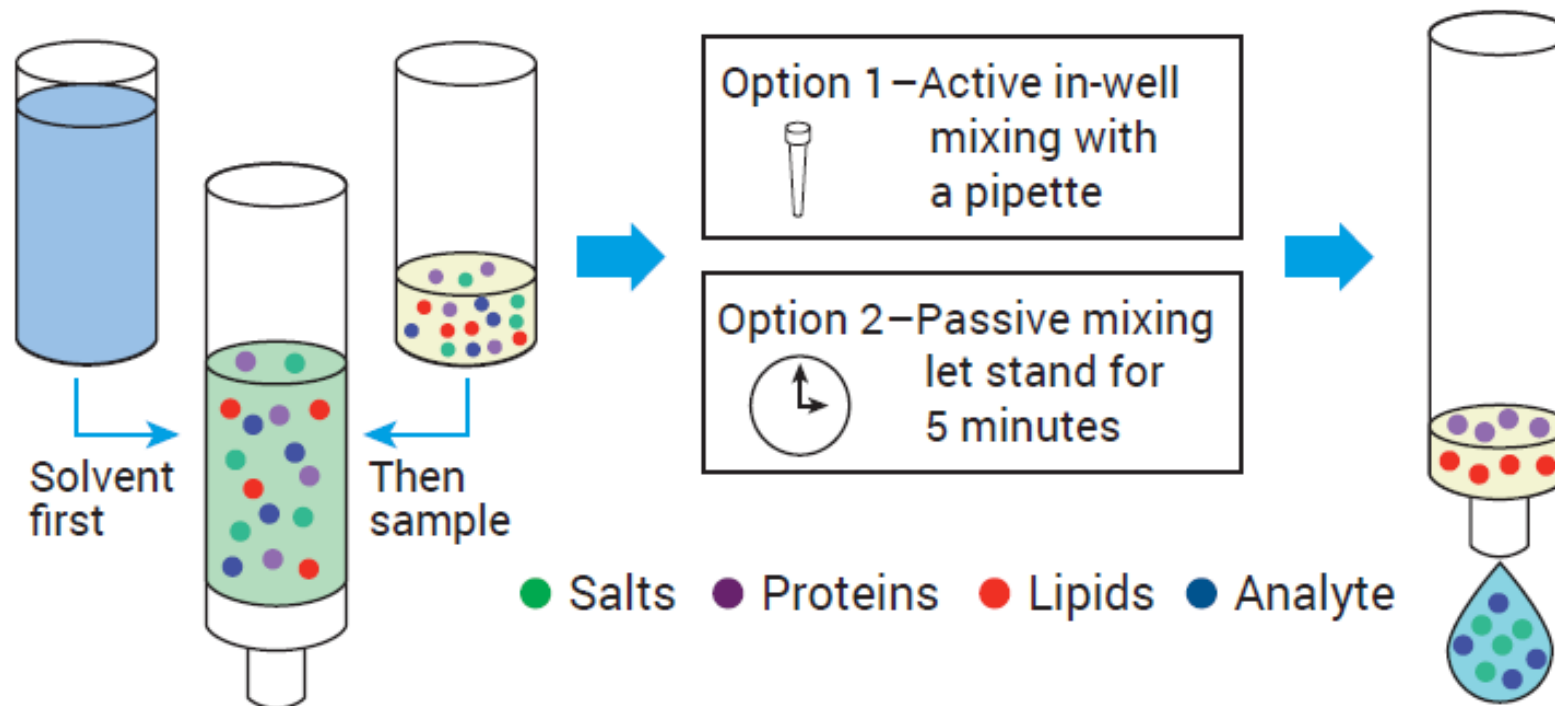
Better speed and precision: Solvent retention frit streamlines and automates your in-well protein precipitation workflow

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



Captiva EMR-Lipid Cleanup Procedure

1. Add crash solvent and sample*
2. Mix to precipitate protein
3. Filter



* 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

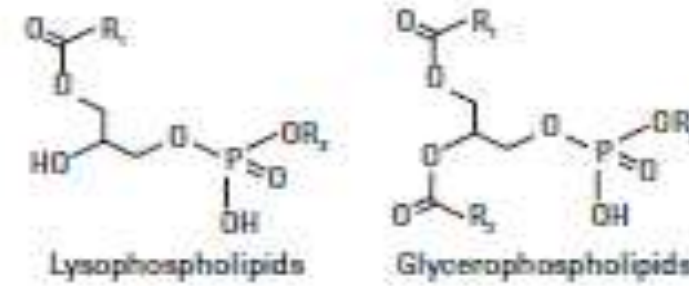


Figure 1. Chemical structures of the two most important groups of phospholipid.

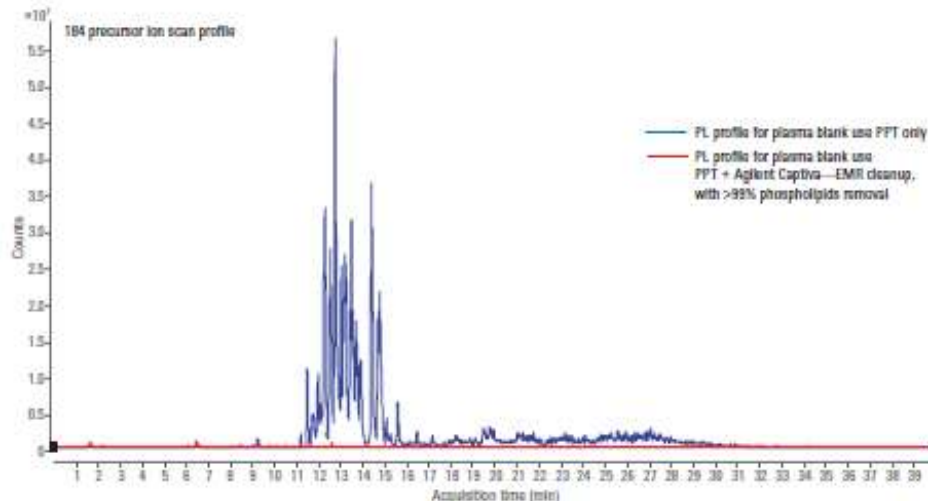
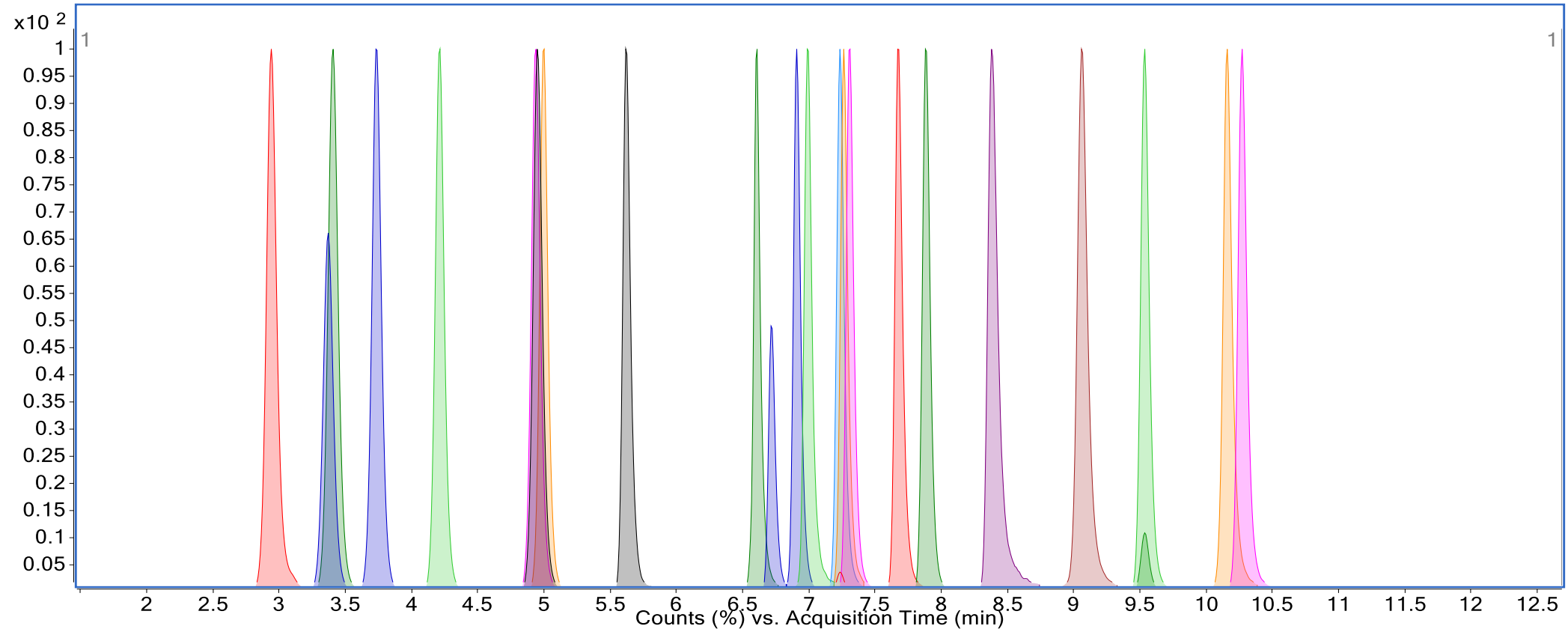


Figure 4. Overlapped chromatograms for phospholipids profile by monitoring a precursor ion scan for 184 m/z.

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

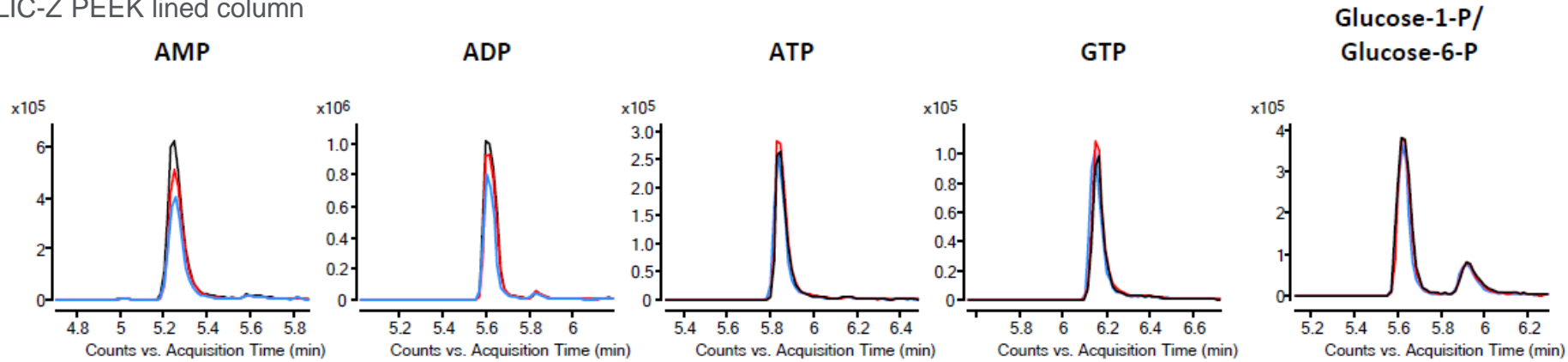
Mock Urine Sample		
Major Urine Components	[Concentration]	[mM]
Urea	23.3 g/L	390 mM
Chloride	8.4 g/L	236 mM
Sodium	4.4 g/L	191 mM
pH 6.3		

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.**

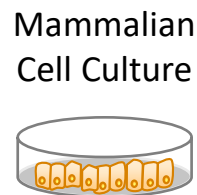
HILIC-Z PEEK lined column



■ Unsalted ■ 20% Salt (80 mM Urea, 40 mM NaCl) ■ 40% Salt (160 mM Urea, 80 mM NaCl)

Metabolomics Analysis of Culture Media

Poroshell HILIC-Z, 2.7 μm , 2.1x100 mm



Mammalian Cell Culture



Harvest 100 μl at...
0, 1, 2, 3, and 6 days



Add 400 μl of
50% acetonitrile

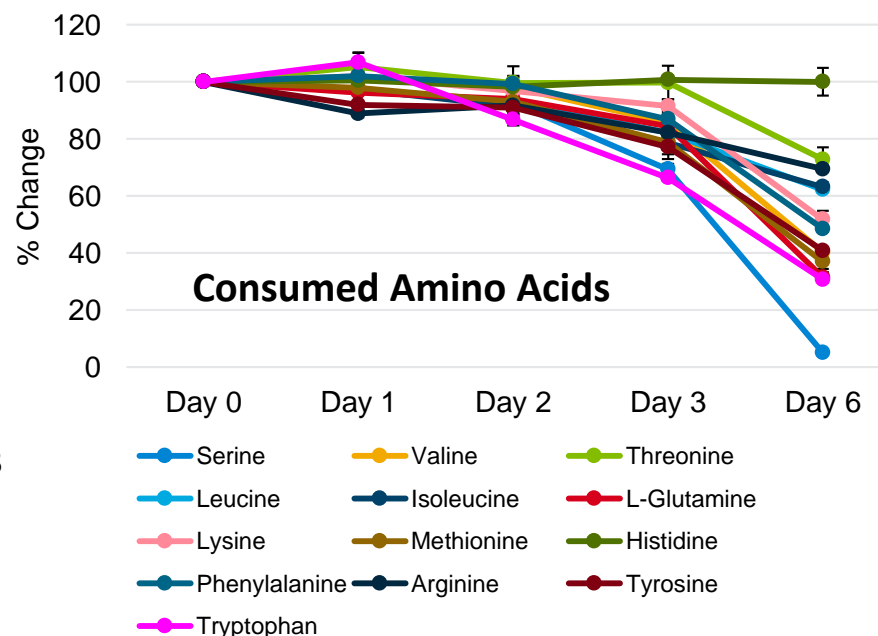
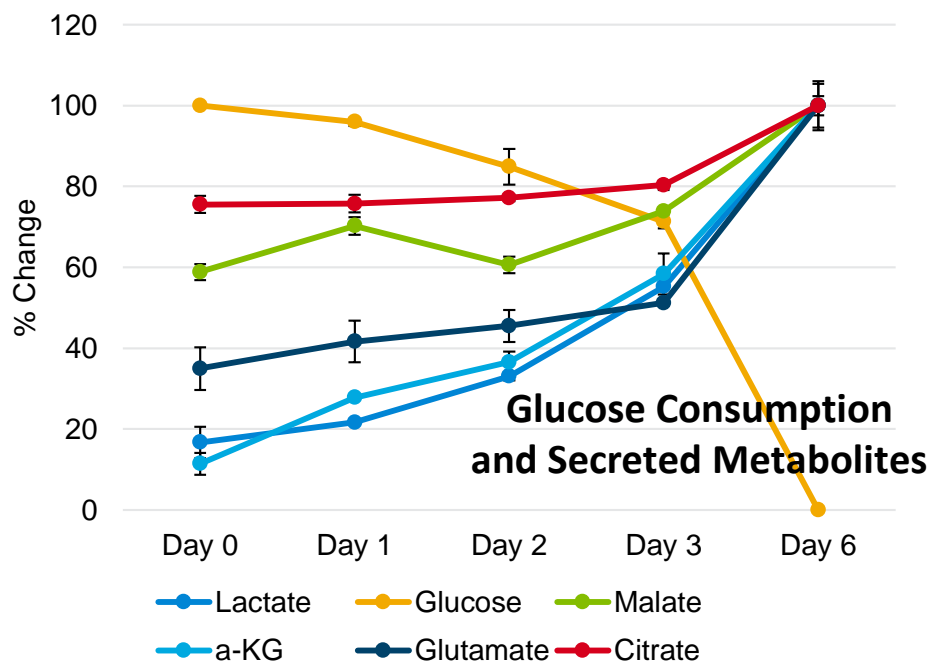
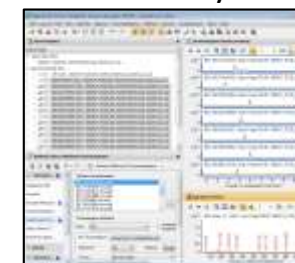
Analyze
1 μl



HILIC-LC/MS
analysis



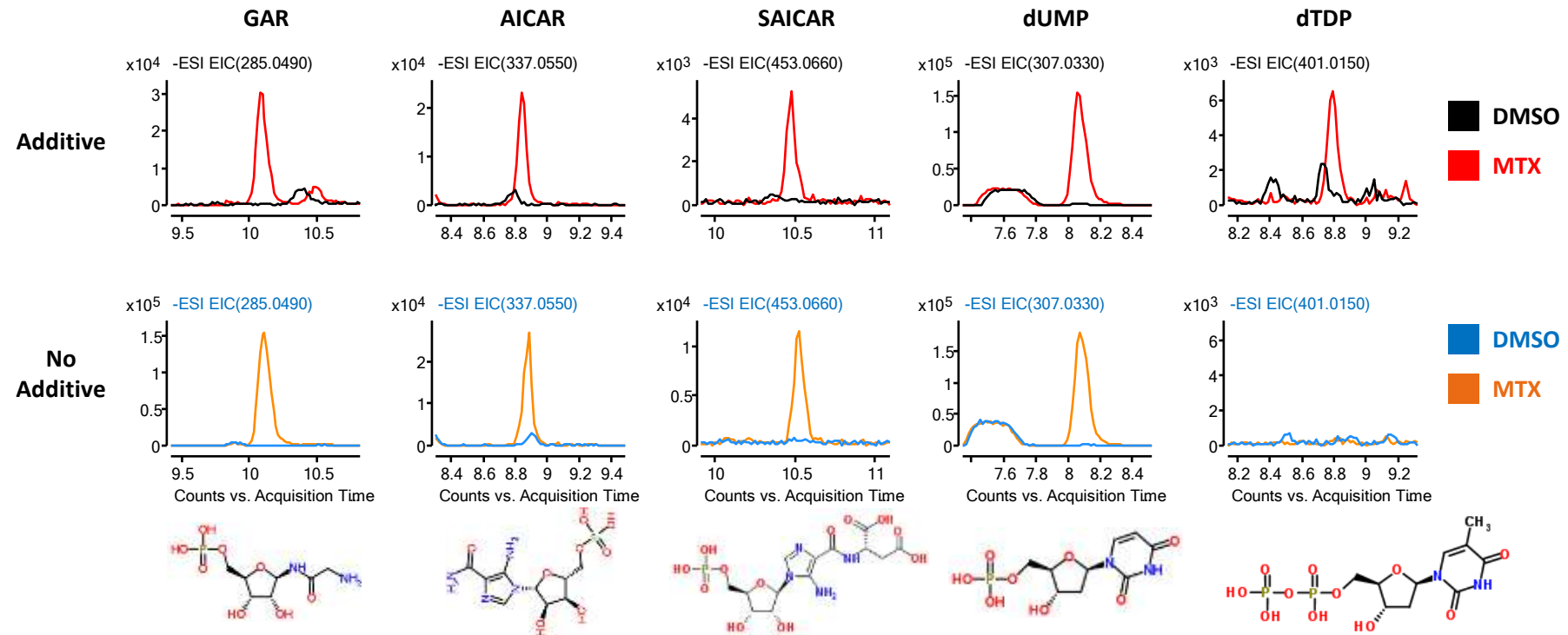
Data Analysis



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

Spectral Parameters | Collision Energy | Precursor Selection I | Precursor Selection II | Preferred/Exclude

Mode: MS (Seg) | Auto MS/MS (Seg) | Targeted MS/MS (Seg)

Max Precursor Per Cycle: 10

Precursor Threshold: Abs. Threshold 3000 counts, Rel. Threshold (%) 0.001 %

Active Exclusion: Enabled, Excluded after 1 Spectra

Static Exclusion Range List

Start m/z	End m/z

Use PC for MS/MS decisions

Iterative MS/MS

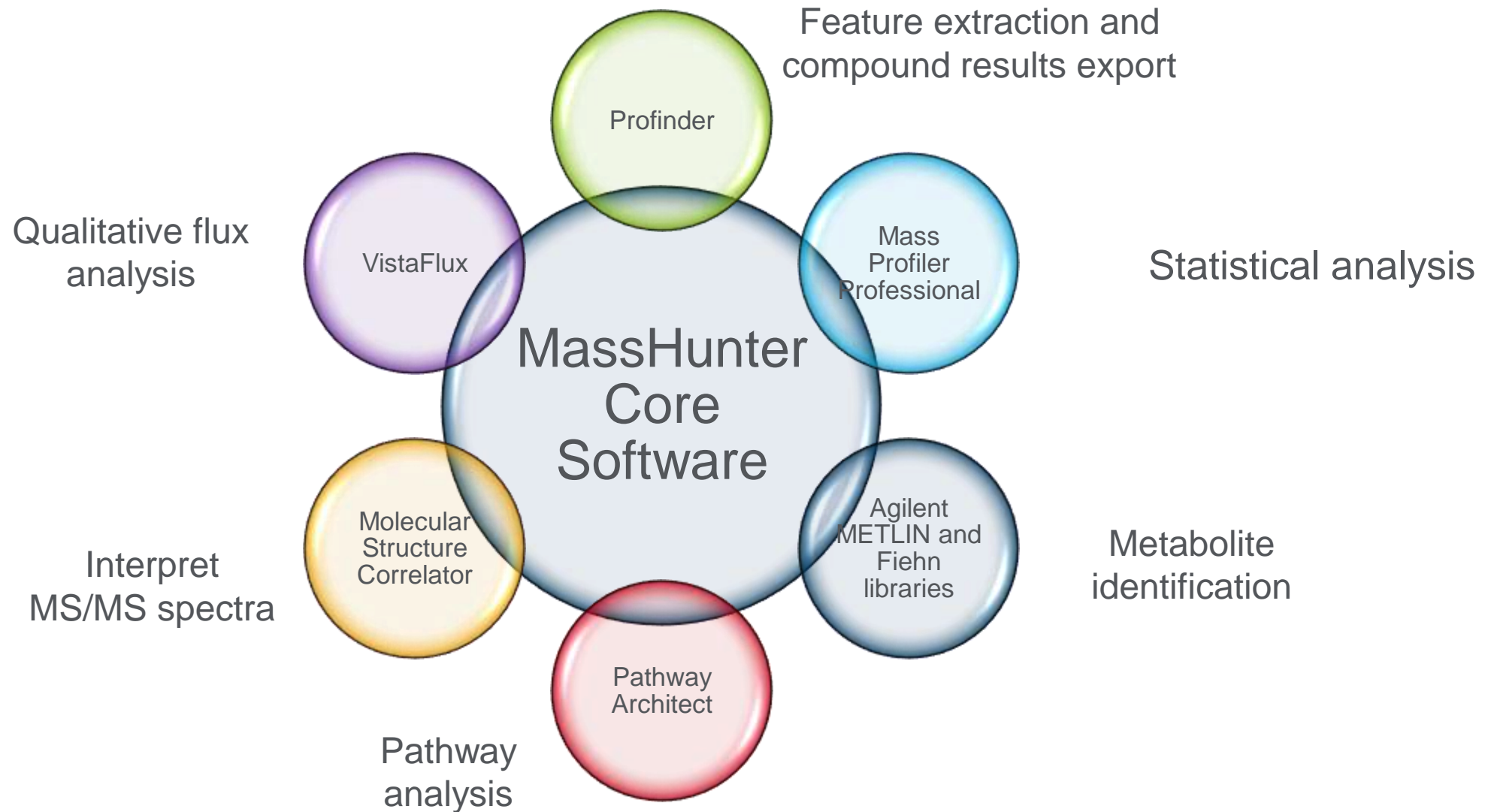
Mass error tolerance (+/- ppm): 10

RT exclusion tolerance: 0.2 (-min) 0.2 (+min)

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	
2	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r001.d	Sample	3	
3	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r002.d	Sample	3	
4	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r003.d	Sample	3	
5	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-auto-r004.d	Sample	3	
6	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-iterative-r001.d	Sample	3	start
7	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-iterative-r002.d	Sample	3	iterative
8	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-15min-400uL-6s.m	20170913-mAb-untreated-15min-iterative-r003.d	Sample	3	iterative
9	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-auto-r001.d	Sample	3	
10	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-auto-r002.d	Sample	3	
11	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-auto-r003.d	Sample	3	
12	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-iterative-r001.d	Sample	3	start
13	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-iterative-r002.d	Sample	3	iterative
14	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-iterative-r003.d	Sample	3	iterative
15	mAb untreated desalt 0.2ug/uL	P1-F4	peptide-mapping-30min-400uL-6s.m	20170913-mAb-untreated-30min-2ug-auto-r001.d	Sample	10	
16	mAb untreated 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



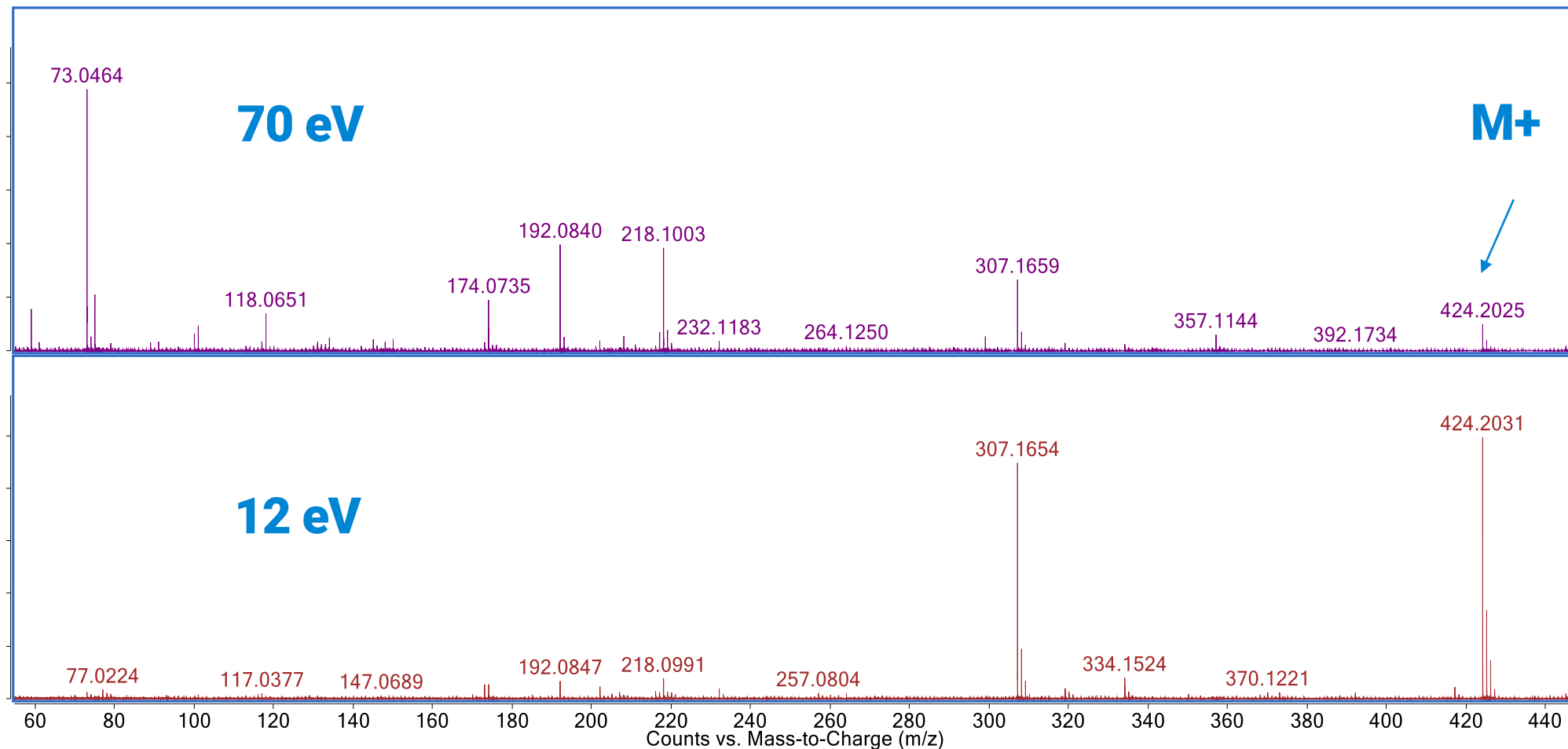
The Agilent 7250 GC/Q-TOF



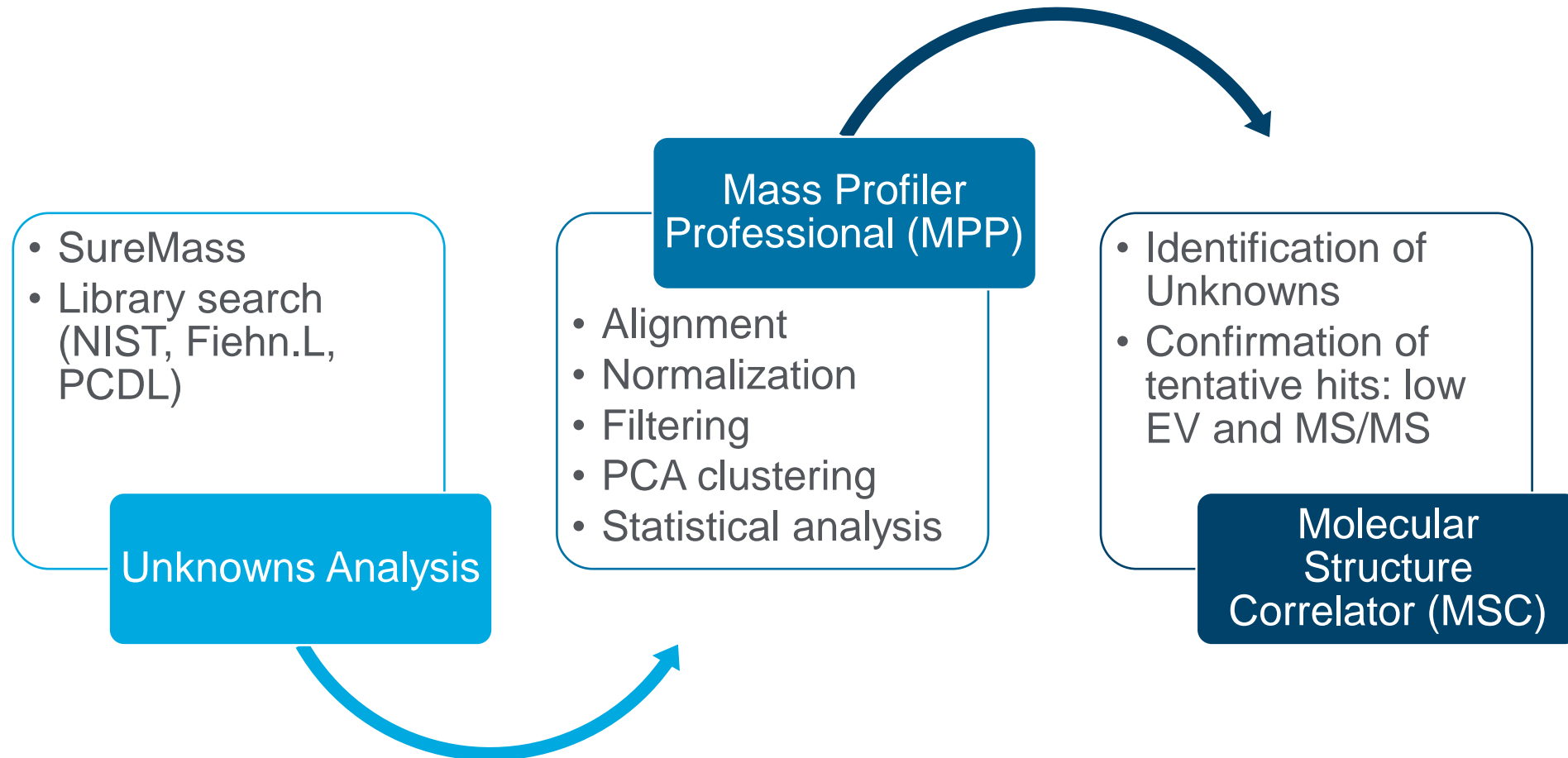
Using Low Electron Energy to Confirm Molecular Ion

Produces Less Complex EI Spectra

Kynurenine, 3TMS



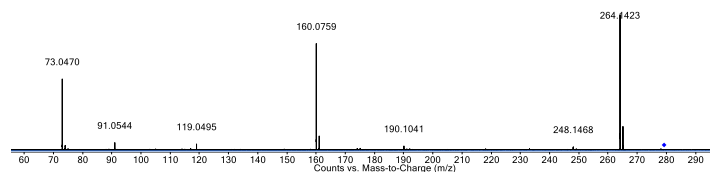
Untargeted GC/Q-TOF Workflow



Unknown Identification Using MSC

Correlate MS/MS Fragment Ions With Proposed Structures

MS/MS, 17 eV CE 20V



8 structures found for rt=14.036

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #1 -- elucidated: 100.0% ions, 100.0% Weight

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #2 -- elucidated: 100.0% ions, 100.0% Weight

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #3 -- elucidated: 100.0% ions, 100.0% Weight

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #4 -- elucidated: 100.0% ions, 100.0% Weight

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #5 -- elucidated: 100.0% ions, 100.0% Weight

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #6 -- elucidated: 100.0% ions, 100.0% Weight

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #7 -- elucidated: 100.0% ions, 100.0% Weight

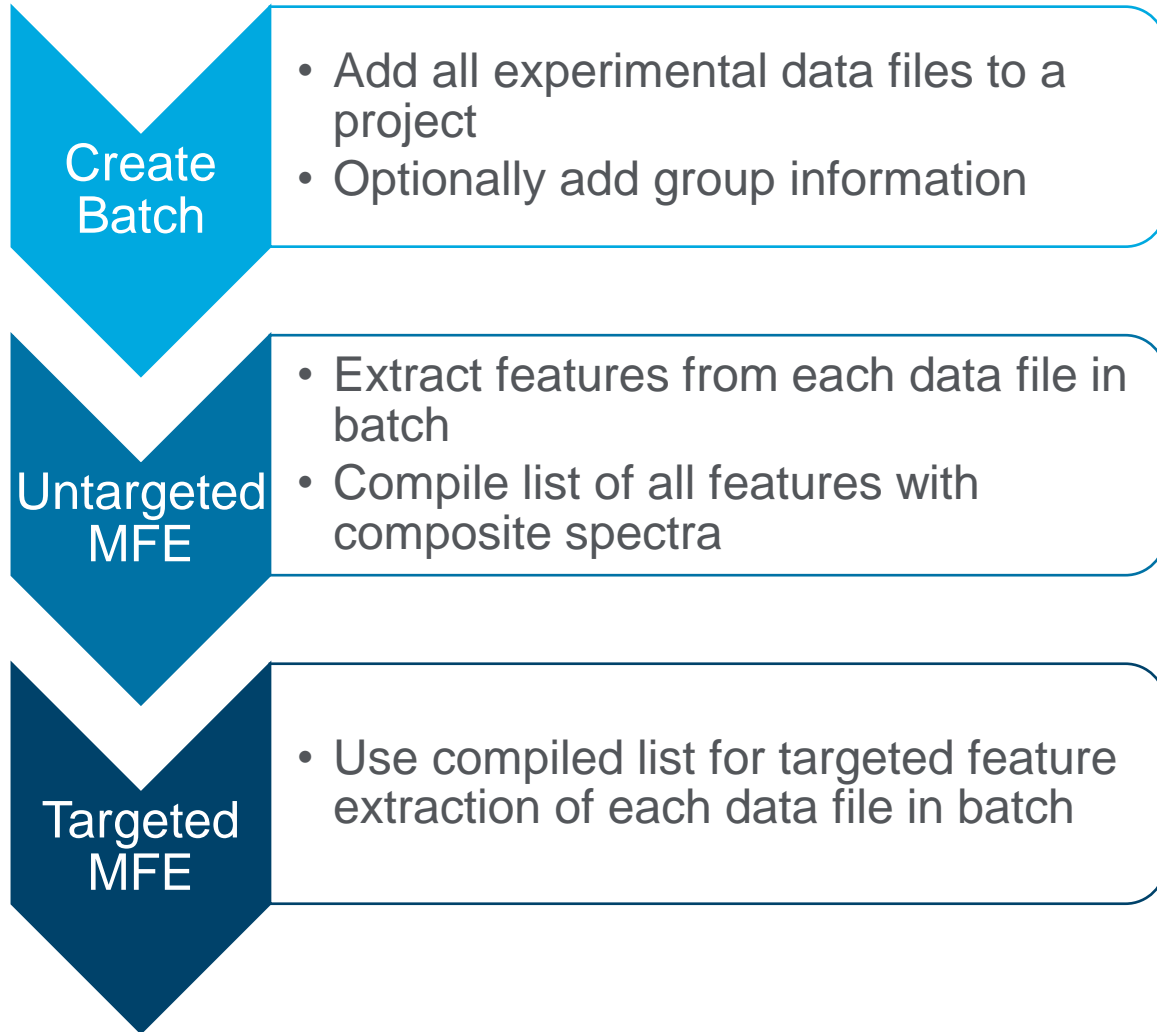
Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

Structure #8 -- elucidated: 100.0% ions, 100.0% Weight

Rank	Mass	Intensity	Weight (%)	No. of candidates	Best score
1	264.422	8293.06	75.3	3	98.9
2	160.0759	5076.18	22.9	1	98.8
3	73.0470	1103.87	2.8	1	98.5

MassHunter Profinder

The Power of 3D Batch Feature Finding



Single software for untargeted and targeted feature extraction

Fast, multi-threaded batch processing

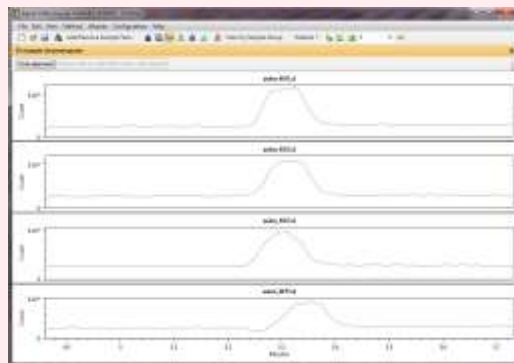
Recursive batch analysis minimizes false positives and negatives

Compound centric review and manual editing

MassHunter Profinder B.08.00 SP3

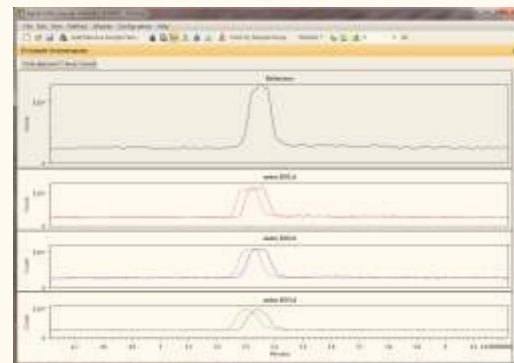
Chromatowarping for CE/MS & LC/MS

Chromatograms



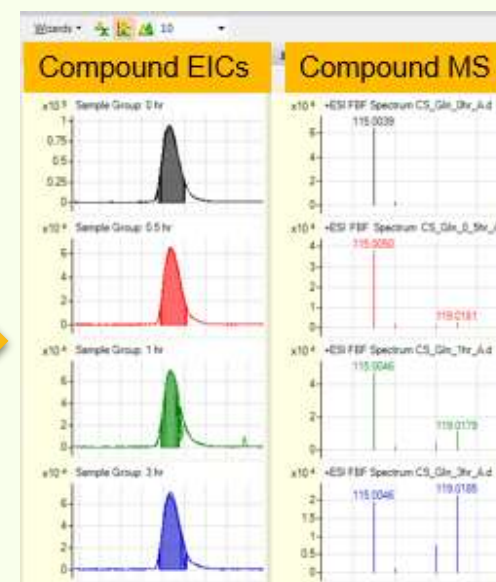
Original chromatograms with retention time variation

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

MassHunter PCDL Manager - D:\MassHunter\PCDL\Sulfas_AM_PCDL.cob

File Edit View PCDL Links Help

Find Compounds

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra Edit Spectra

Mass: [] [M-H]⁺ Neutral [M-H]⁻ Formula: [] Name: [] Notes: [] IUPAC: [] CAS: [] ChemSpider: []

Mass tolerance: 10.0 ppm mDa

Retention time: [] Require [] RT tolerance: 0.1 min

Ion search mode: [x] Include neutrals [x] Include anions

Molecule: Structure MOL Test

Notes: Sulfadiazine

Print/Copy in Summary Format Single Search Results: 10 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name
Acetaminophen	C8H9NO2	151.06333			103.902	1806		N-(4-Hydroxyphenyl)acetamide
Caffeine	C8H10N4O2	194.08038			58.082	2424		1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione
Lidocaine	C14H22N2O	234.17321			137.584	3548		N-(2,6-Dimethylphenyl)-N',N'-diethylglycine
Sabutamol	C13H21NO3	239.15214			185.599	1899		2-(Hydroxymethyl)-4-(1-hydroxy-2-(2-methyl-2-propyl)-2-oxoethyl)pyridine
Sulfamethazole	C9H10N4O2S2	270.02452			144.921	5127		4-Amino-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide
Sulfamethazine	C12H14N4O2S	278.08375			57.681	5136		4-Amino-N-(4,6-dimethyl-2-pyridinyl)benzenesulfonamide
Sulfachloropyridazine	C10H9ClN4O2S	284.01347			80.320	5382		4-Amino-N-(6-chloro-3-pyridazinyl)benzenesulfonamide
Sulfadimethoxine	C12H14N4O4S	310.07358			122.112	5132		4-Amino-N-(2,6-dimethoxy-4-pyridinyl)benzenesulfonamide
Progesterone	C21H30O2	314.22458			57.630	5773		Pregn-4-ene-3,20-dione

Create & manage custom database and library

MassHunter ID Browser 8.08.00

Identification Method Configuration Help

Run ID Wizard

Search Results: Cpd 6: Sulfadimethoxine; C12H14N4O4S

MS Peaks One: + MFE Spectrum (rt: 1.223 min)

m/z	Abund	Abund % (Norm)	Z	Set	Species
311.0807	813951.69		1		(M+H) ⁺
312.0839	129210.82		1		(M+H) ⁺
313.0801	49750.17		1		(M+H) ⁺
314.0807	6754.44		1		(M+H) ⁺
315.0816	894.12		1		(M+H) ⁺
333.0633	280554.25		1		(M+Na) ⁺
334.0655	44362.07		1		(M+Na) ⁺
335.0616	15914.5		1		(M+Na) ⁺
336.0632	2592.8		1		(M+Na) ⁺
349.0365	8460.57		1		(M+K) ⁺
350.0424	1035.43		1		(M+K) ⁺
351.0332	958.9		1		(M+K) ⁺
643.1363	40159.54		1		(2M+Na) ⁺
644.1383	12385.58		1		(2M+Na) ⁺
645.1342	5761.27		1		(2M+Na) ⁺

Structure Viewer: Sulfadimethoxine

Structure MOL Test

Label Name Formula Score Mass Avg Mass Std Dev Mass (DB) Mass (MFG) Diff (MFG, ppm) Diff (MFG, mDa)

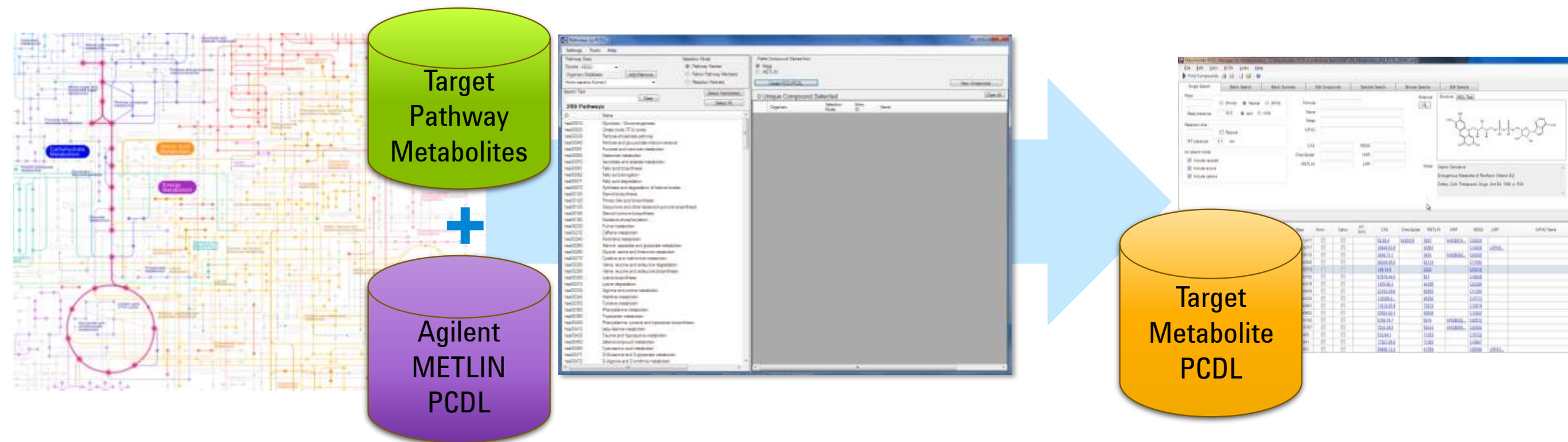
Cpd	Label	Name	Formula	Score	Mass	Avg Mass	Std Dev	Mass (DB)	Mass (MFG)	Diff (MFG, ppm)	Diff (MFG, mDa)
1	0.294			194.1156							
2	0.326			270.0244							
3	0.514			284.0137							
4	0.783			578.1495							
5	Sulf.	Sulfamet.	C12H14N4.	58.8	278.0838						
6	Sulf.	Sulfadim.	C12H14N4O4.	76.77	310.0736						

Identify features using database and library

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

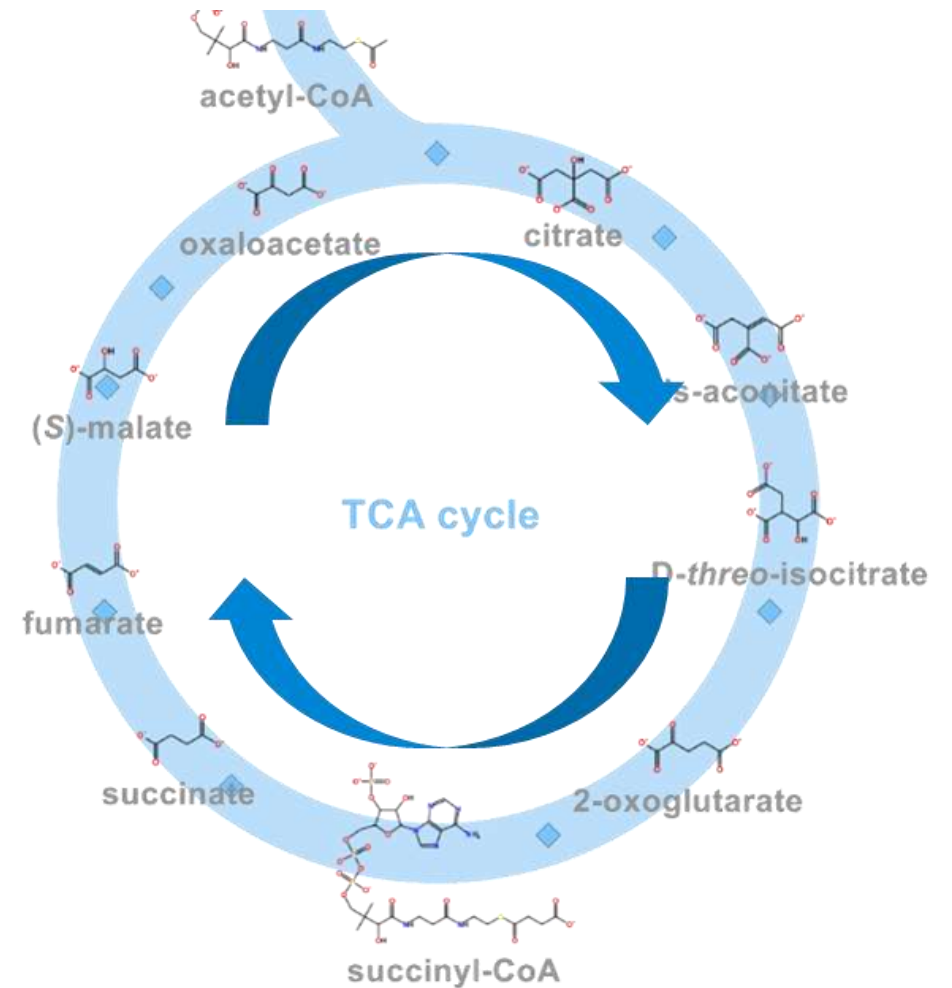
Stable Isotope Tracing

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



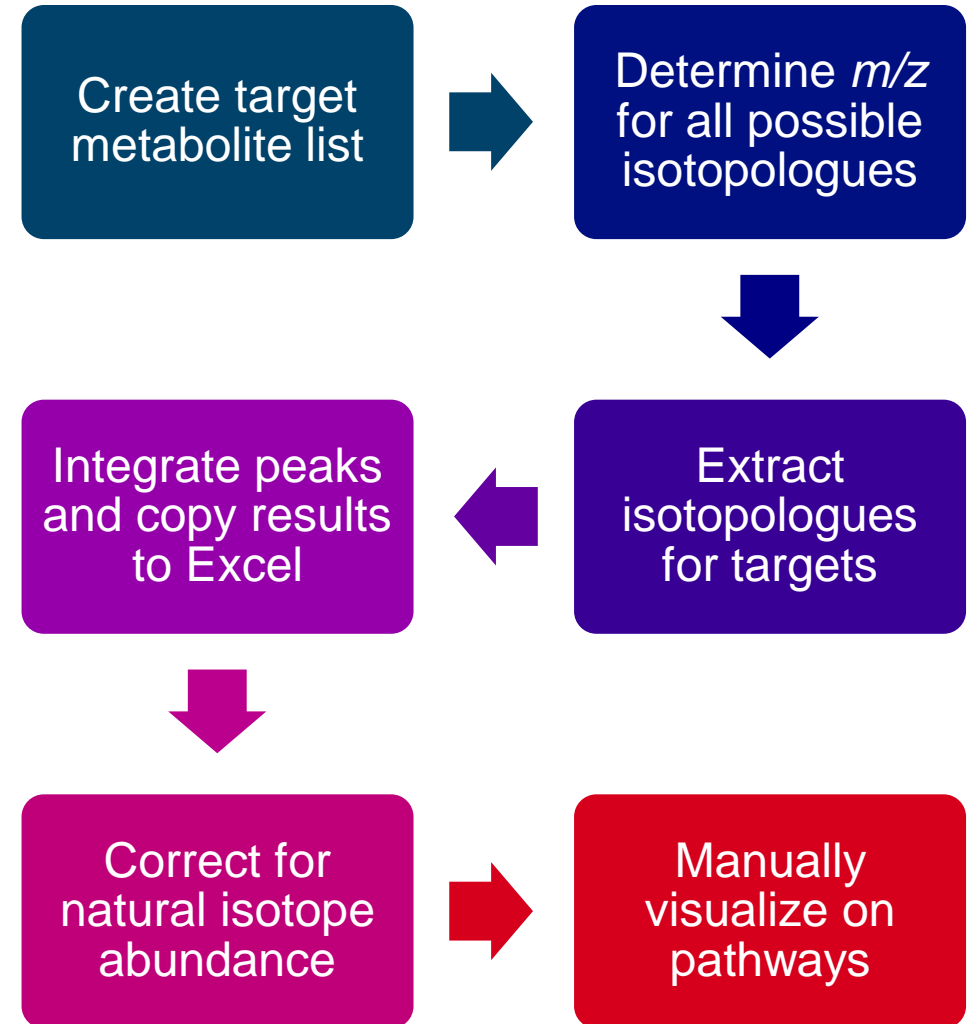
Stable Isotope Tracing for Qualitative Flux Analysis

Manual Process

Multi-step **manual** process is

- Tedious
- Error-prone
- Time-consuming

This limits the number of compounds analyzed!

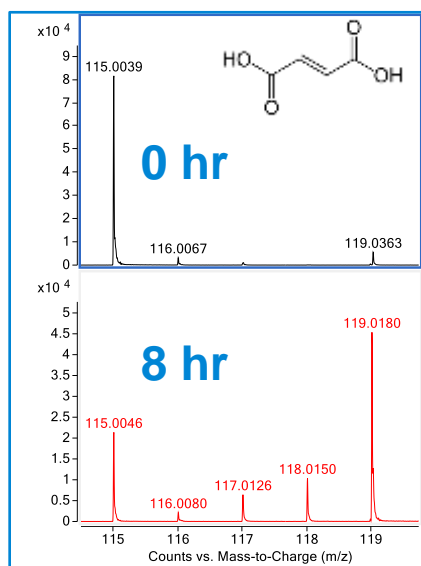


VistaFlux Stable Isotope Tracing

Isotopologue tracking

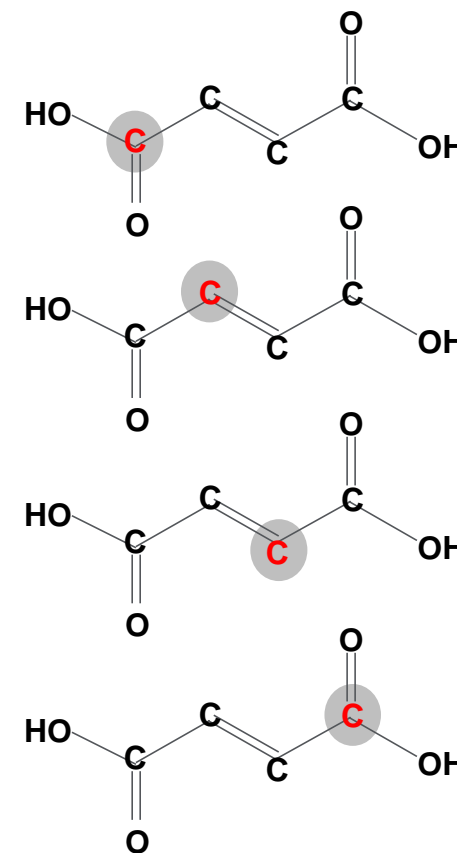
Use of stable isotope labels (^{13}C , ^{15}N , and ^2H)

Monitor stable isotope incorporation



Fumarate $\text{C}_4\text{H}_4\text{O}_4$

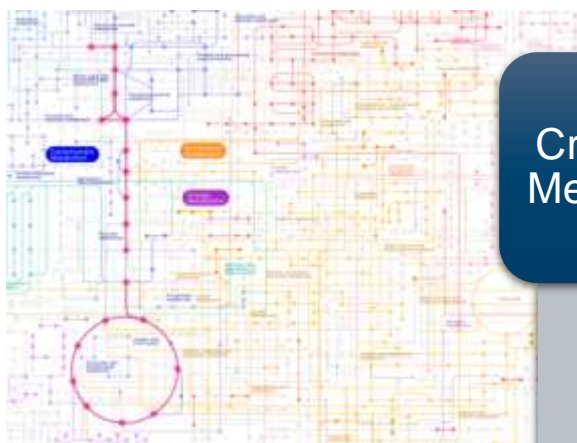
Isotopologues



4 M+1 Isotopomers

Stable Isotope Tracing Using MassHunter VistaFlux

Agilent VistaFlux workflow



Create Target Metabolite List

Acquire TOF Data

Extract Isotopologues

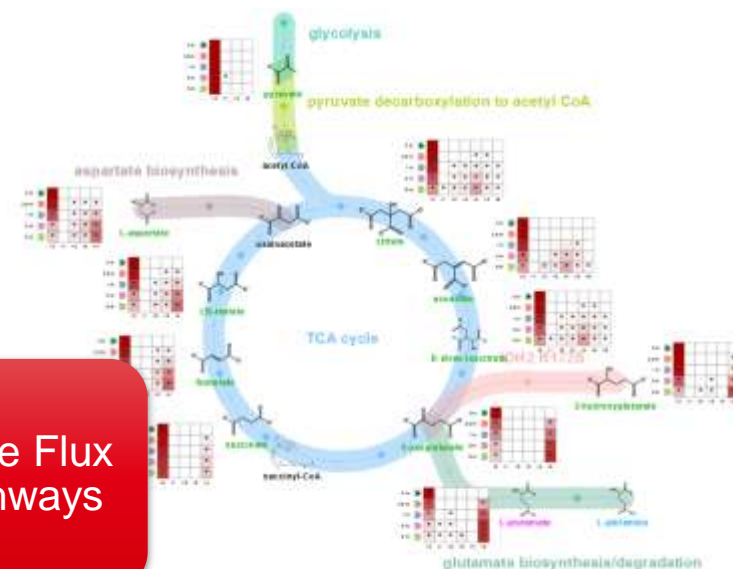
Visualize Flux on Pathways

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?

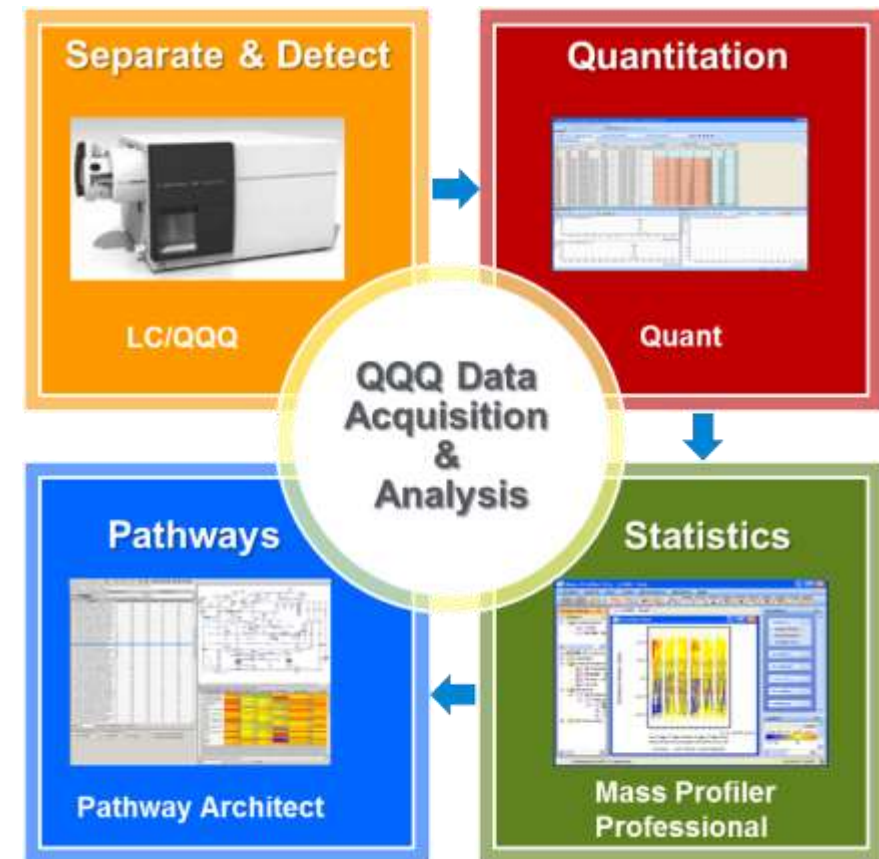
- An optimized LC/MS database and analytical method for 219 central carbon metabolites
- Designed for 1290 Flex pump and 6460/6470 QQQ LC/MS Systems
- Provides an optimized method and database with stable, robust chromatography

Why develop an analytical method for central carbon metabolism?

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

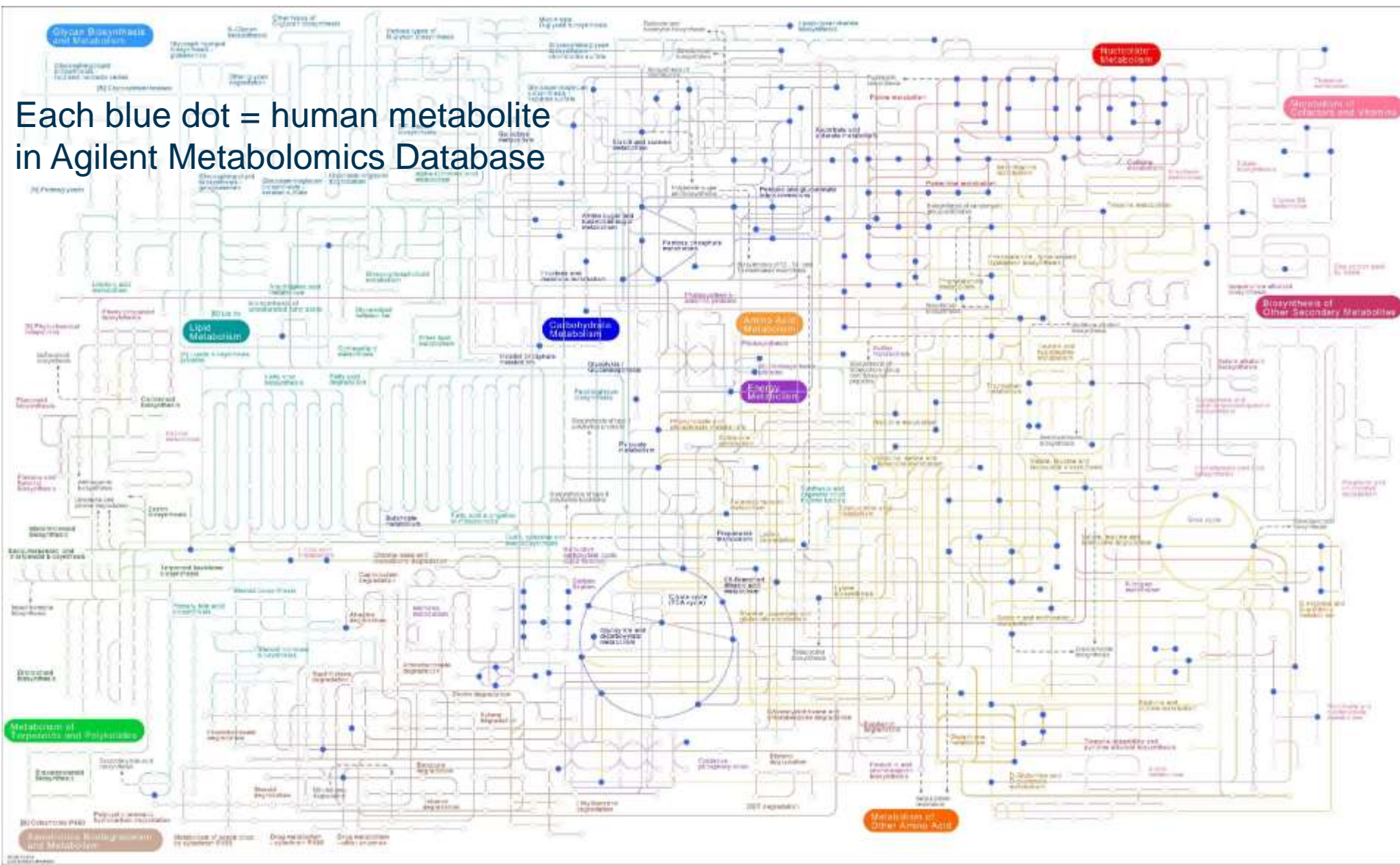
Why develop a targeted analytical method for LC/QQQ?

- 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

Each blue dot = human metabolite in Agilent Metabolomics Database

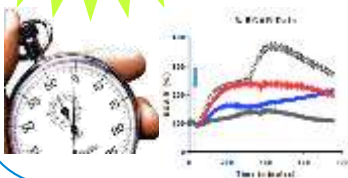


XF Technology Empowers You to Answer Your Questions About Cellular Function in Real-time



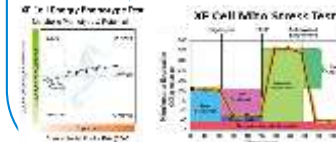
Seahorse XF Assays Go Beyond What Cells Are to Reveal What They Are Doing

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



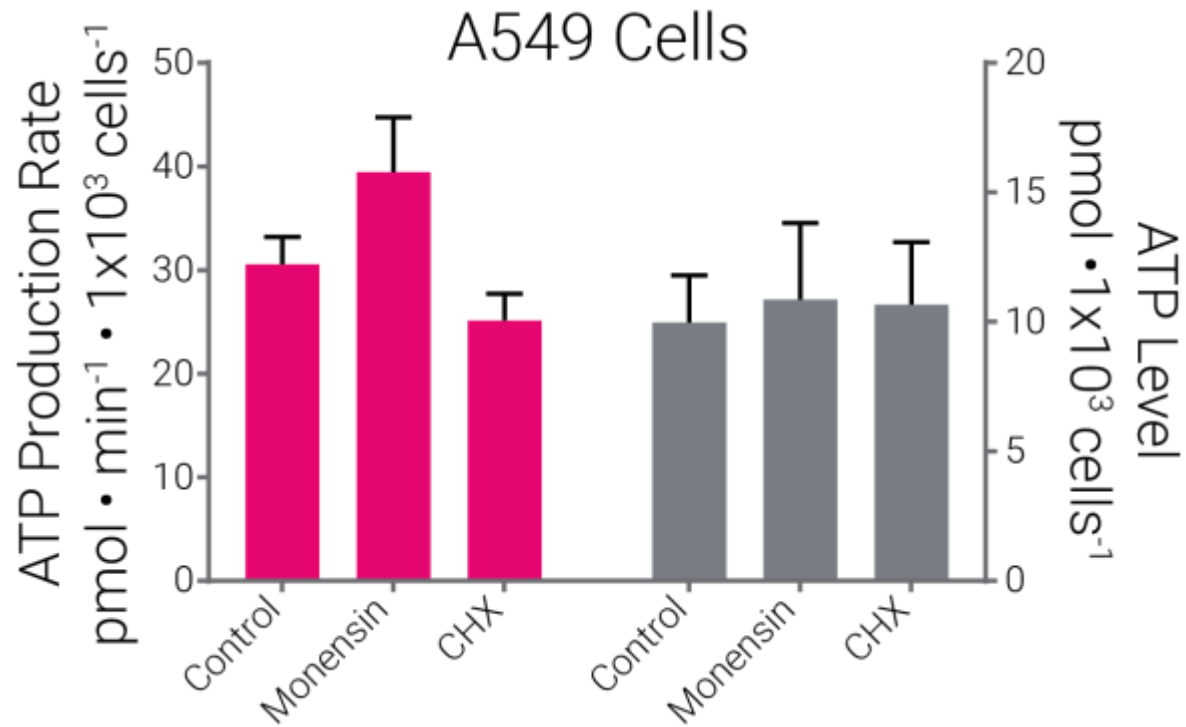
- **More relevant**
Measure any cells, mitochondria, spheroids, islets, more.
- **Efficient**
Requires <40-200K cells per well.

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

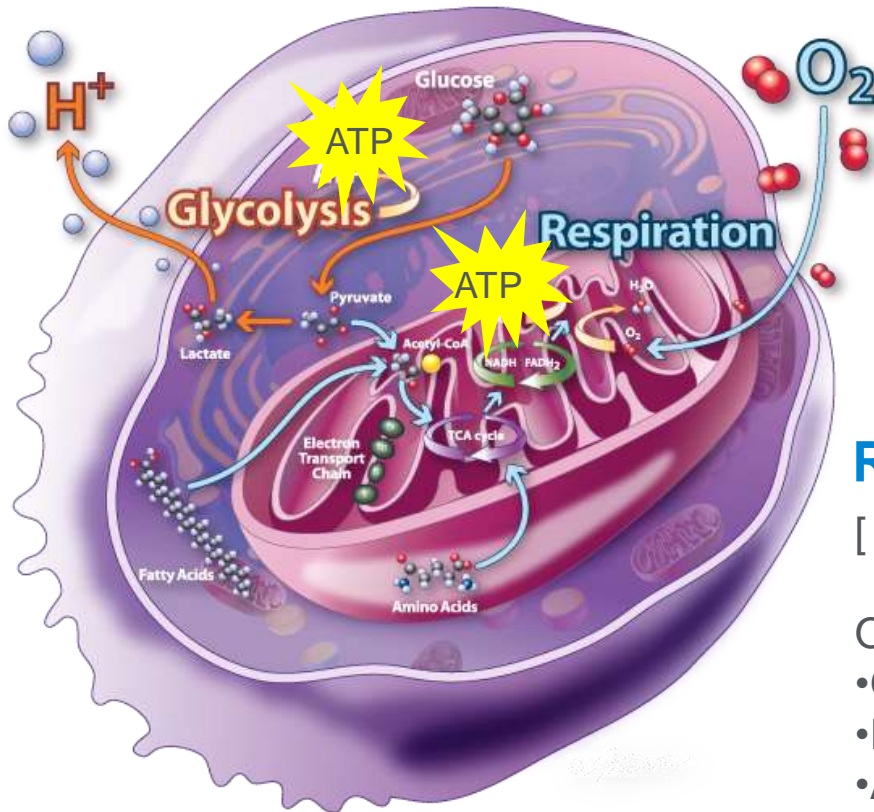
Cycloheximide: decreases ATP demand due to inhibition of protein synthesis

How is ATP Production Rate Measured?

Glycolysis

[pH]

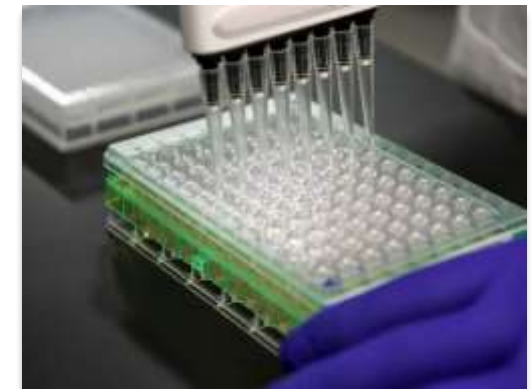
Fermentation of:
•Glucose



Respiration

[Oxygen]

Oxidation of:
•Glucose
•Fatty Acids
•Amino Acids



How is ATP Production Rate Measured?



Report Generator

mitoATP Production Rate

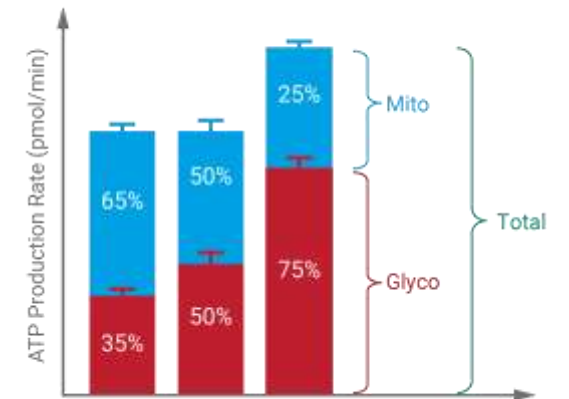
+

glycoATP Production Rate

Total ATP Production Rate

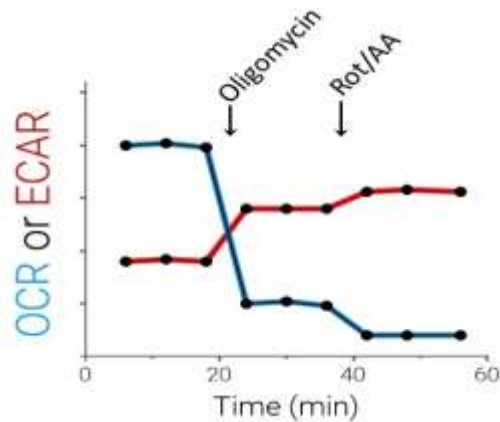
Seahorse XF Real-Time ATP Rate Assay

Bioenergetic profile



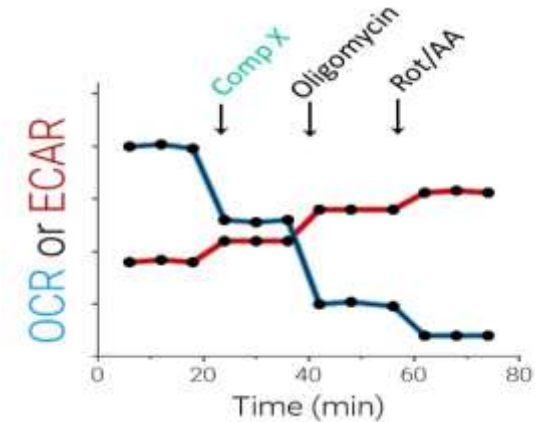
Assay Design

Standard XF Real-Time ATP Rate Assay



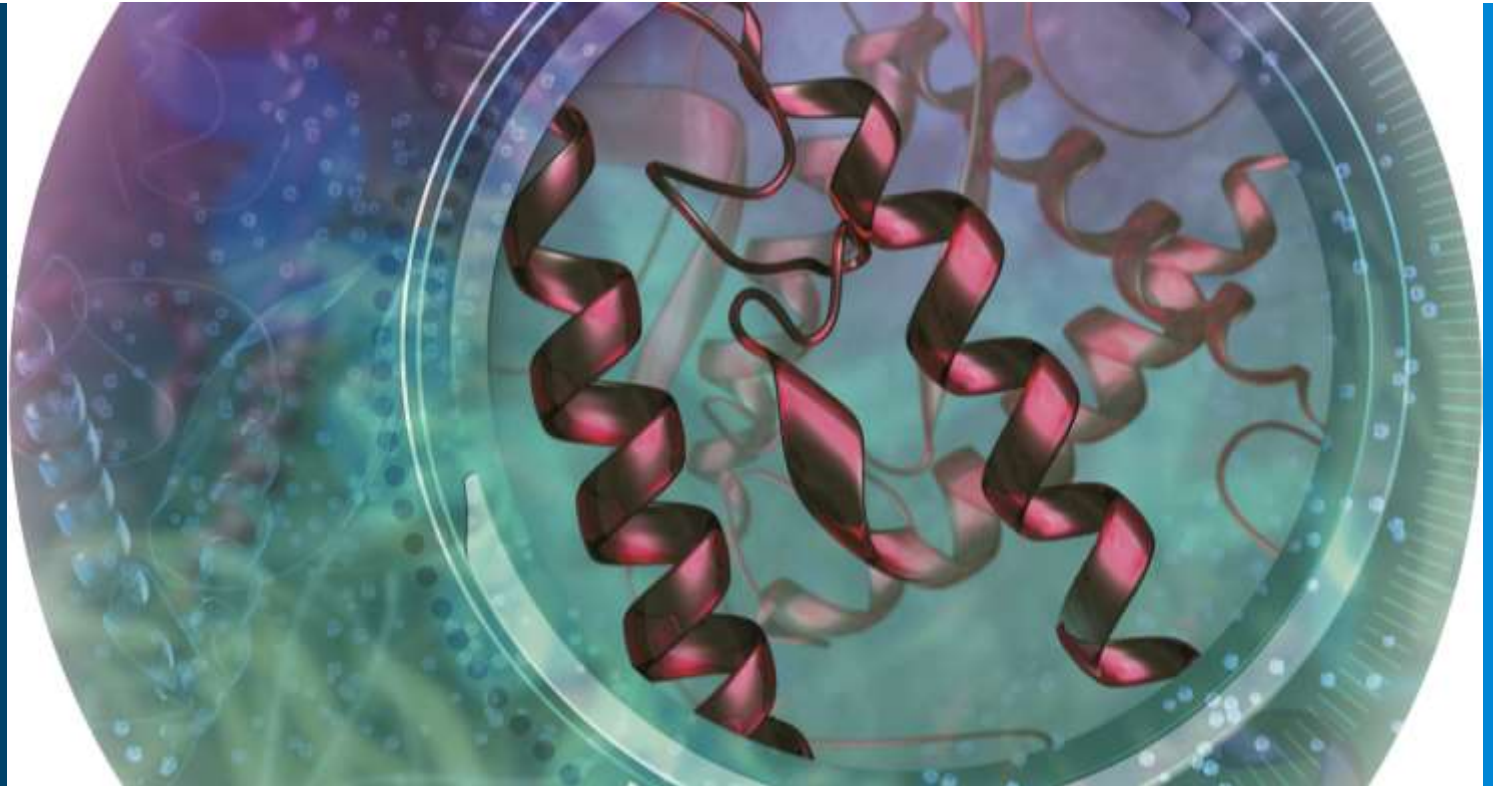
- To quantify metabolic phenotype of a cell type, to compare different cell types, genetic modifications, pre-treatments with compounds
- Outputs:
 - Basal mitoATP, glycoATP, total ATP rates
 - ATP Rate Index

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

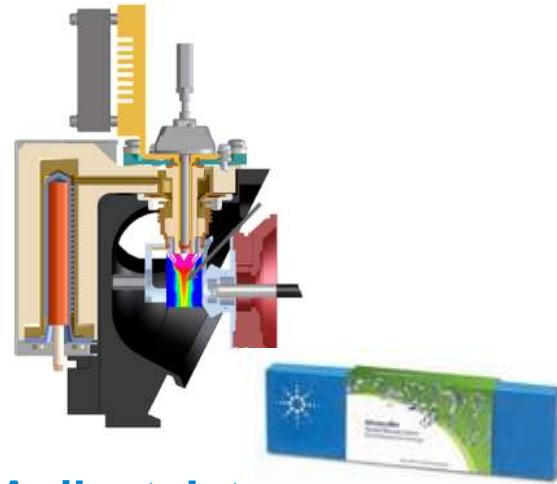
Proteomics Workflows



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

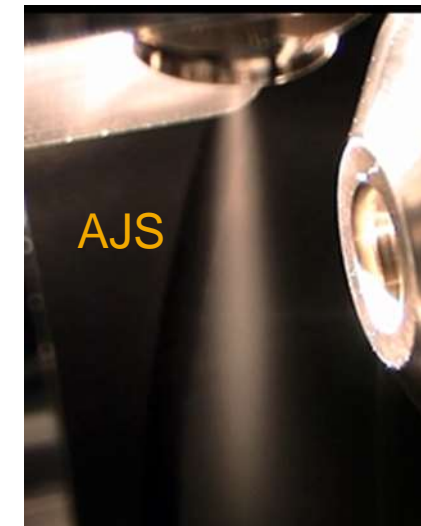
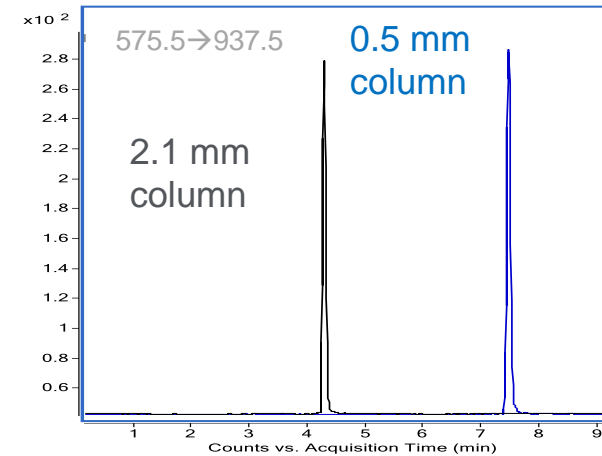
Jet Stream provides 3-5x signal increase compared to ESI

Agilent Jet Stream showed same signal (and LOD) for

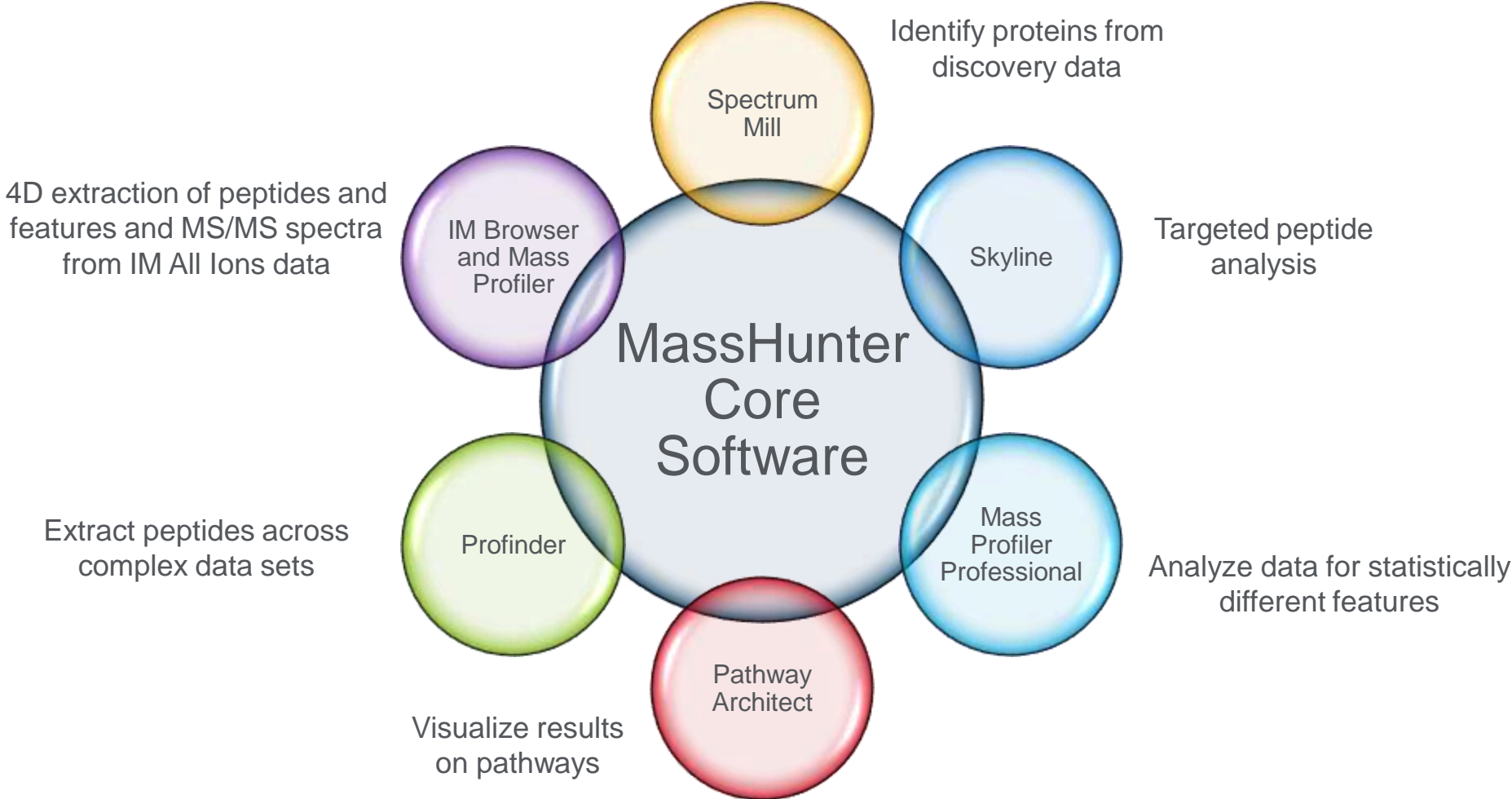
- 2.1 mm ID column at 400 $\mu\text{L}/\text{min}$
- 0.5 mm ID column at 17 $\mu\text{L}/\text{min}$

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.

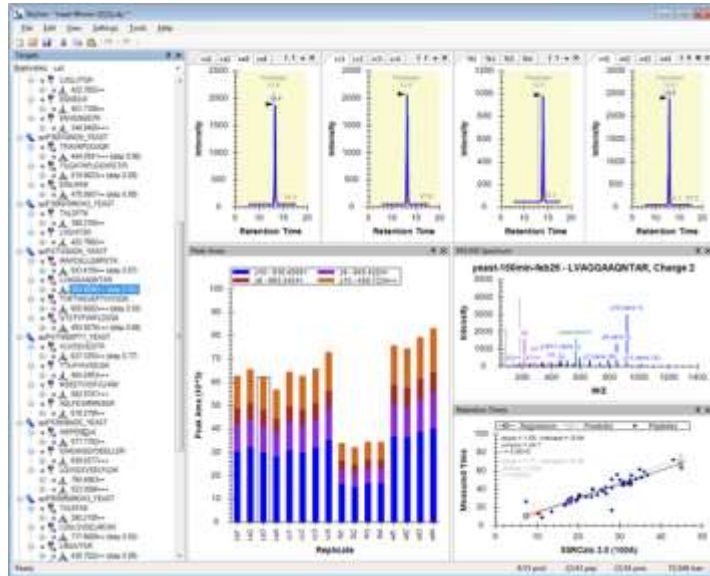


Agilent Proteomics Application Software



Targeted Proteomics Workflows

From Skyline to MPP

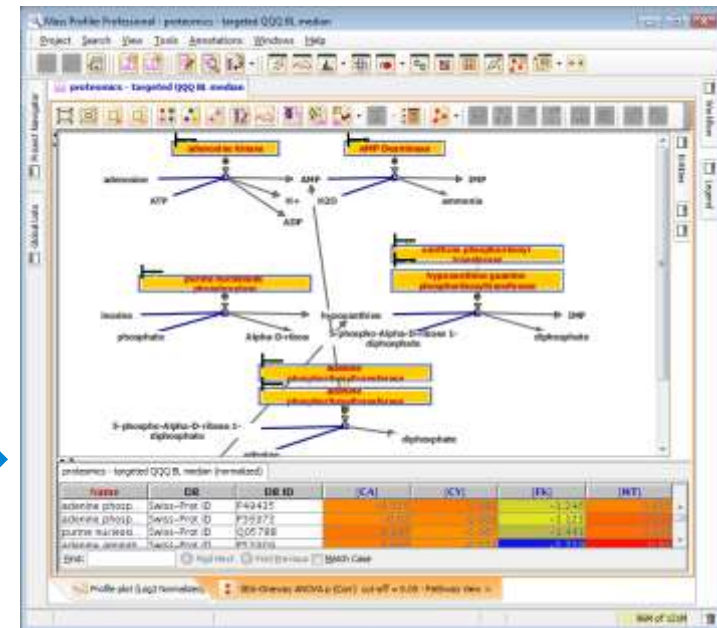


Review and process QQQ or Q-TOF results in Skyline

Protein Name	Peptide Sequence	ms1 TotalIons	ms2 TotalIons	ms3 TotalIons	ms4 TotalIons
pp17548QGLK	TLKRPYSKDLK	15245	18583	16582	17428
pp17548KGLK	LSMAGSLKLNK	14921	16097	19077	14754
pp17548KGLK	WINDSLK	22548	22887	23274	23130
pp17548KGLK	ELKDFPKK	8393	7370	8787	8142
pp37728QAL	TSVGNLGR	31160	36476	31919	31316
pp37728QAL	ETICDPLKPKV	32508	40673	38184	38184
pp38324PTL	LSFGKSLPKK	91987	87982	81716	80933
pp38324PTL	LDLAK	97084	88884	92884	90600
pp38324PTL	LLKELPKPKK	58487	71825	78285	110717
pp11244WFD	LVVPTKPKK	11562	11798	13142	14780
pp11244WFD	LLTLDVFR	8828	8749	8280	8074
pp39344MDL	ALDLK	1884	2131	2178	1993
pp39344MDL	LSGLVFR	30621	31483	30476	33243
pp39344MDL	EDVSLK	45448	45082	44224	42390
pp39344MDL	DNVGGVFR	295883	320518	321508	311628
pp39344MDL	TFVAKDQGR	18881	18078	18329	18824
pp39344MDL	TLNATPLGK	13919	19983	19171	18388
pp39344MDL	LDLNEK	35628	26762	24510	26428
pp39344MDL	TALDVK	8783	10322	10084	8944
pp39344MDL	LSGLVFR	27623	26183	26148	28868
pp39344MDL	MAKDELGKTE	18082	14188	13488	18874

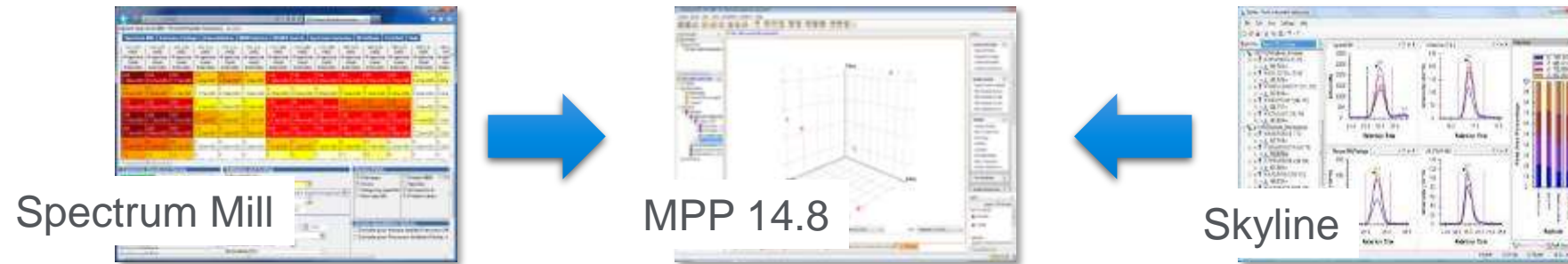
Export results to MPP

Pathway visualization in Pathway Architect



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

The image displays two overlapping software windows from the MPP (Mass Profiling Profiler) application. The background window is titled "Filter on Proteins" and shows options for filtering based on Frequency, Sample Variability, Abundance, Modifications, Peptide Count, Score, and Properties. The foreground window is titled "Filter on Peptides" and shows options for filtering based on Frequency, Sample Variability, Abundance, Modifications, Charge, and Properties. The "Filter on Peptides" window is highlighted with a red border. Below the windows is a list of filtering criteria for peptides, and a "Preview" button is visible at the bottom right of the foreground window.

Filter on peptides by

- PTMs
- Sequence
- Reproducibility
- Statistical and correlation analysis

Proteomics Visualizations in MPP

Display Both Protein and Peptide Information

Protein	Protein Name	Swiss-Prot ID	Species
Q13492	Phosphatidylinositol-binding clathrin assembly protein	Q13492 Q60643	HUMAN
P78344	Eukaryotic translation initiation factor 4 gamma 2	P78344	HUMAN
Q03135	Caveolin-1	P56539 Q03135	HUMAN
P68400	Casein kinase II subunit alpha	P68400	HUMAN
Q15427	Splicing factor 3B subunit 4	Q15427	HUMAN
P28340	DNA polymerase delta catalytic subunit	P28340	HUMAN
O94826	Mitochondrial import receptor subunit TOM70	O94826	HUMAN
Q9NVS9	Pyridoxine-5'-phosphate oxidase	Q9NVS9	HUMAN
Q9HCY8	Protein S100-A14	Q9HCY8	HUMAN

Find: Find Next Find Previous Match Case

Protein Name	Frequency
Q03135 Caveolin-1	4

Find: Find Next Find Previous Match Case

Peptide	Charge	Frequency	Alt. Accession	Vmod	Mod	Mass
AMAEISEK 2+	2	4				993.456
EIDLVRDPK 2+	2	3	P56539			1,198.643
HINDDIVK 2+	2	4				939.489
IDFEDVIAEPEGTHSF...	3	4				2,405.13
IFSNVR 2+	2	3				735.415
YDSEIHLTYVPIR 3+	3	4				1,648.833
EIDLVRDPK 3+	3	2	P56539			1,198.643
QYDAHTK 2+	2	2		Q40q	q:Pyroglutamic acid	961.474

EIDLVRDPK 2+ Attributes					
Sample	Flag	Raw Abundance	Chi2	PIP	
C1	P	178,715		1	55.9
C2	A				
T1	P	102,668	0.99		62.3
T2	P	135,962		1	71.8

Peptides: Profile plot Peptides: BoxWhisker plot Protein: Profile plot Protein: BoxWhisker plot

Group (Non-averaged)

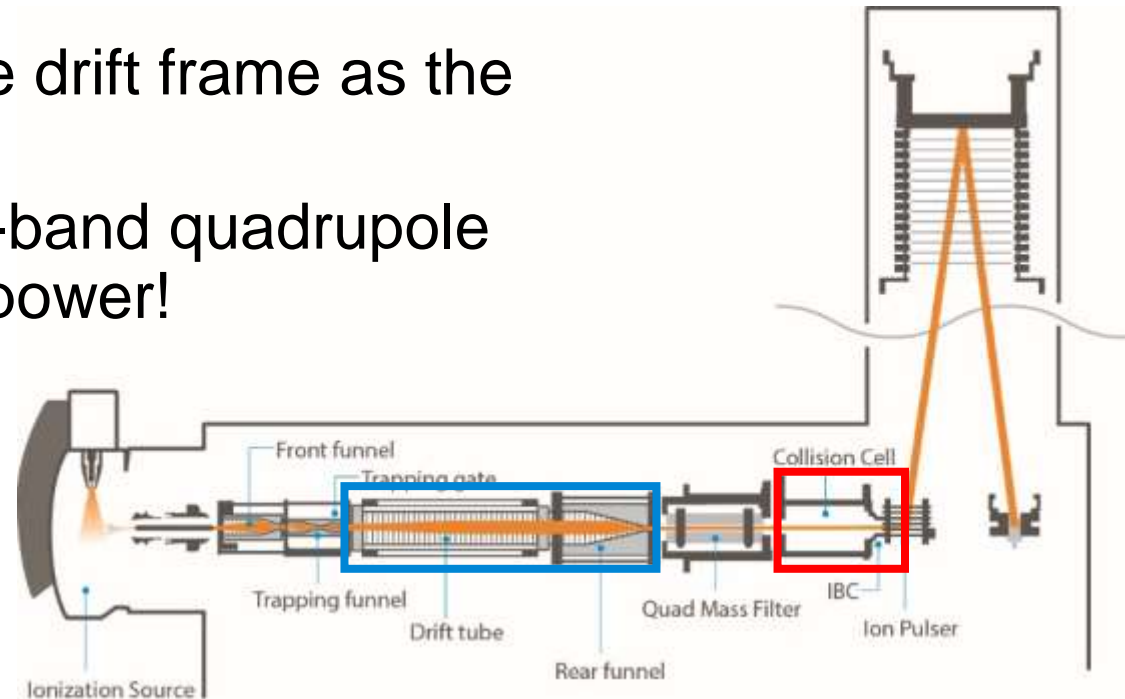
OK Cancel

Agilent IM All Ions MS/MS for Proteomics

Drift separation instead of quadrupole isolation

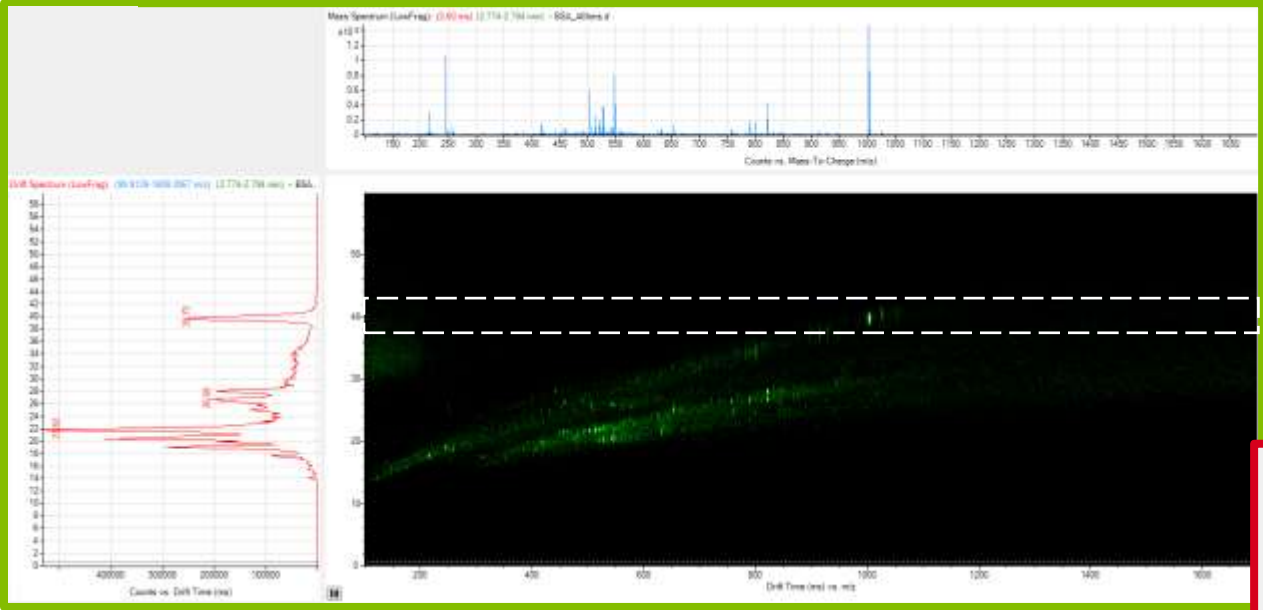
- All ions entering the **collision cell** are subjected to fragmentation voltage
- Fragment ions will be in the same drift frame as the precursor

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



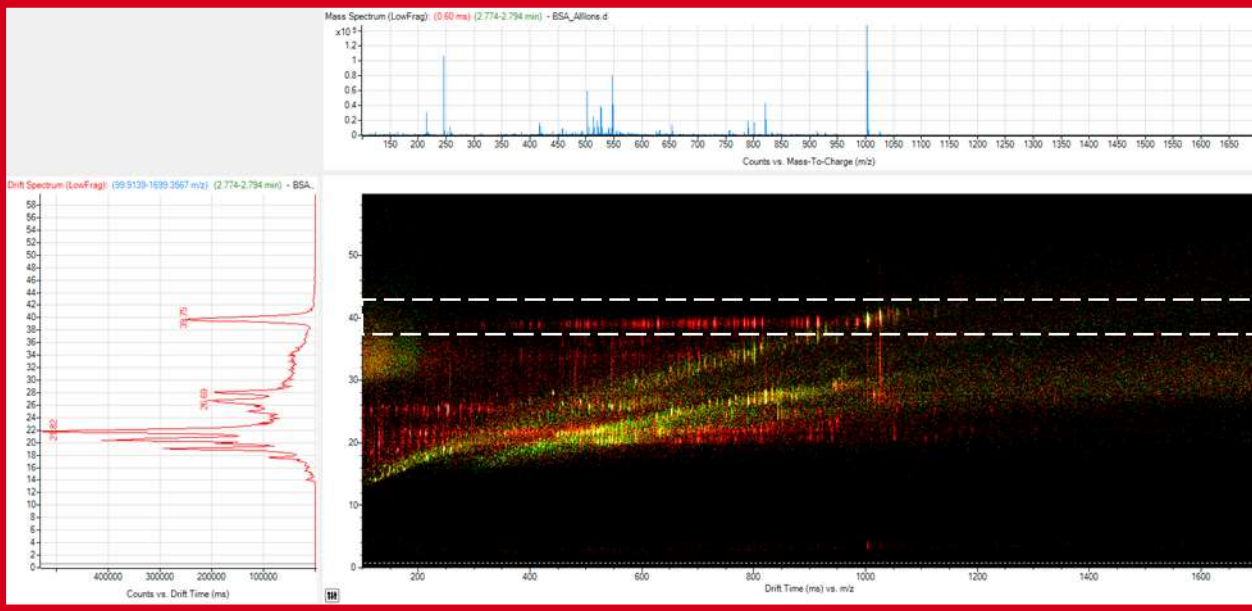
IM All Ions MS/MS Acquisition for Proteomics

Alternates between no collision energy and collision energy



No collision energy
(MS only)

With collision energy
(MS/MS)



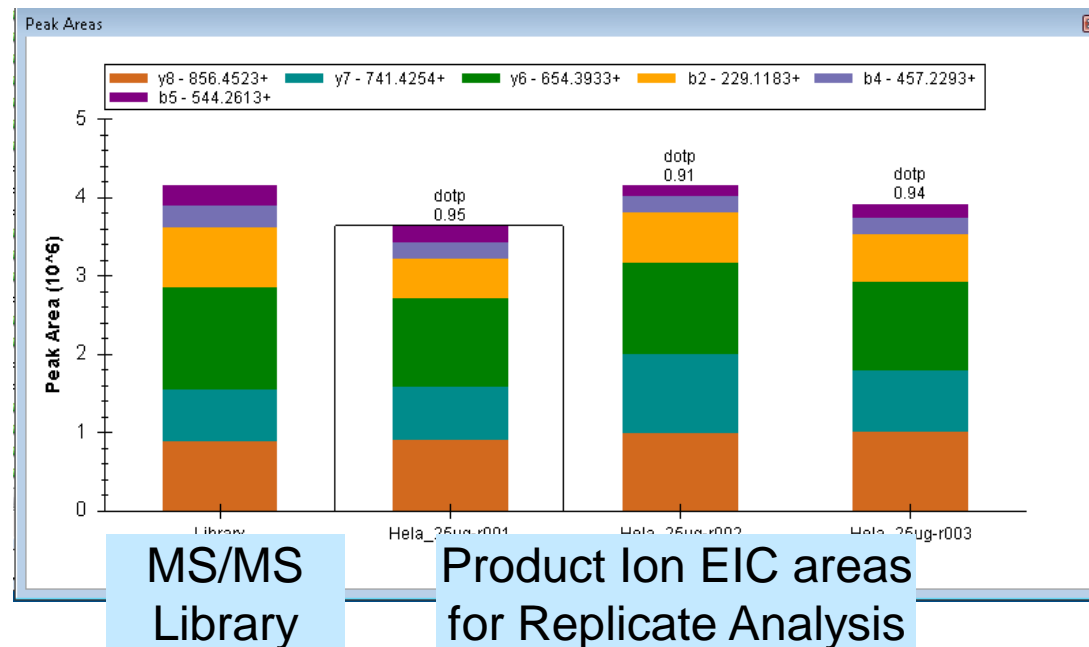
IM All Ions MS/MS for Proteomics

Reproducible MS/MS Results

No precursor isolation so product ions always produced

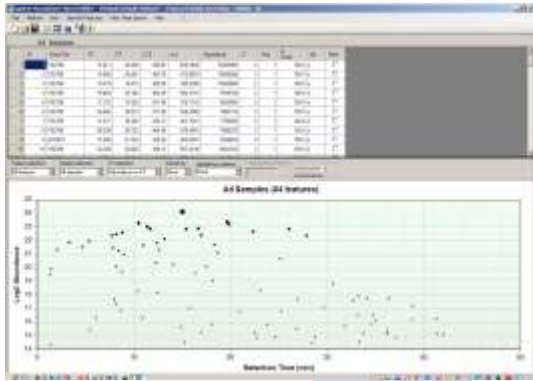
Fast cycle time compared to wide-band isolation

More points across chromatographic peak yields better reproducibility

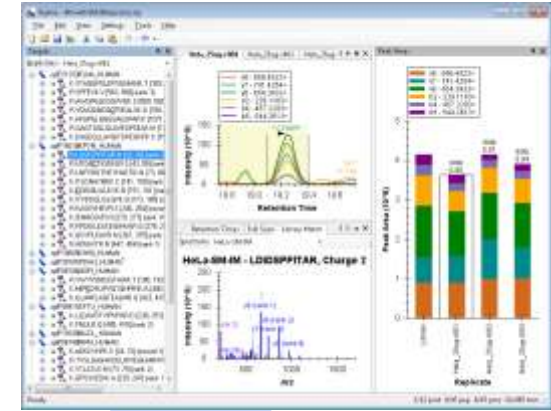


Ion Mobility Proteomics Workflows

Discovery and targeted analysis



Mass Profiler

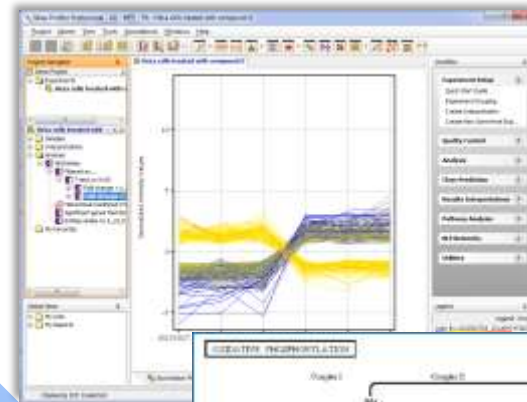


Skyline

MPP

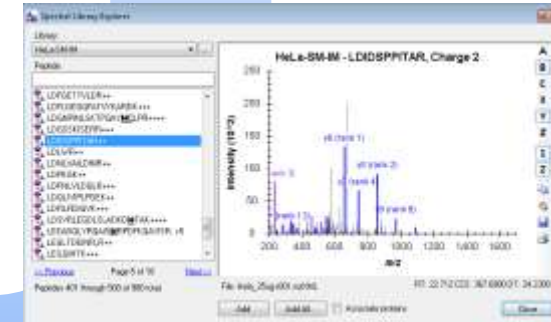


Spectrum Mill



Pathway Architect

MS/MS Library Viewer



Targeted

Discovery

Accelerate Your Research With a Complete Pathway Centric Workflow

Acquire data

Perform data analysis

Accelerate
your
research

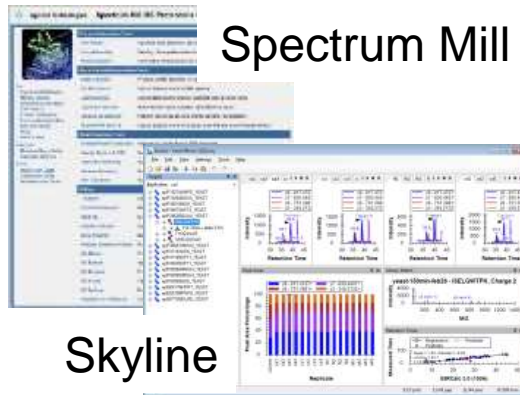
Statistical and
correlation
analysis

Map results to
pathways

6500 Series
Q-TOF



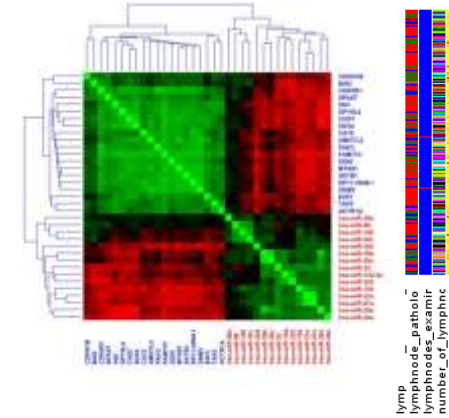
Spectrum Mill



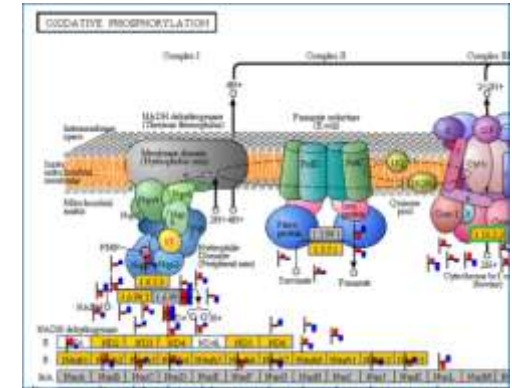
Skyline



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

Agilent Webinar Series

Emerging OMICs

This ongoing webinar series will cover topics of interest to researchers using genomics, transcriptomics, proteomics, and metabolomics. Please join us to learn about analytical developments that can be applied to your research challenges.

For Research Use only. Not for use in diagnostic procedures.

Live Webinars – View at a scheduled time

Title	Date	Time	Presenter
Integration of Proteomics and Metabolomics to Elucidate Metabolic Adaptations in Triple Negative Breast Cancer	April		

Recorded Webinars - View at your convenience

Title	Date
Addressing Sample Stability Concerns in Large-Scale LC-MS Metabolomics Studies	December
Researching Cancer with the next-Gen Methylation and Structural Variations	November
Comparison of Nano and Standard Flow Proteomics for Tissue and Plasma Samples	October
Using Exposomics to Improve Honey Bee Health: A New Approach for Solving a Complex Multifactorial Problem	September

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