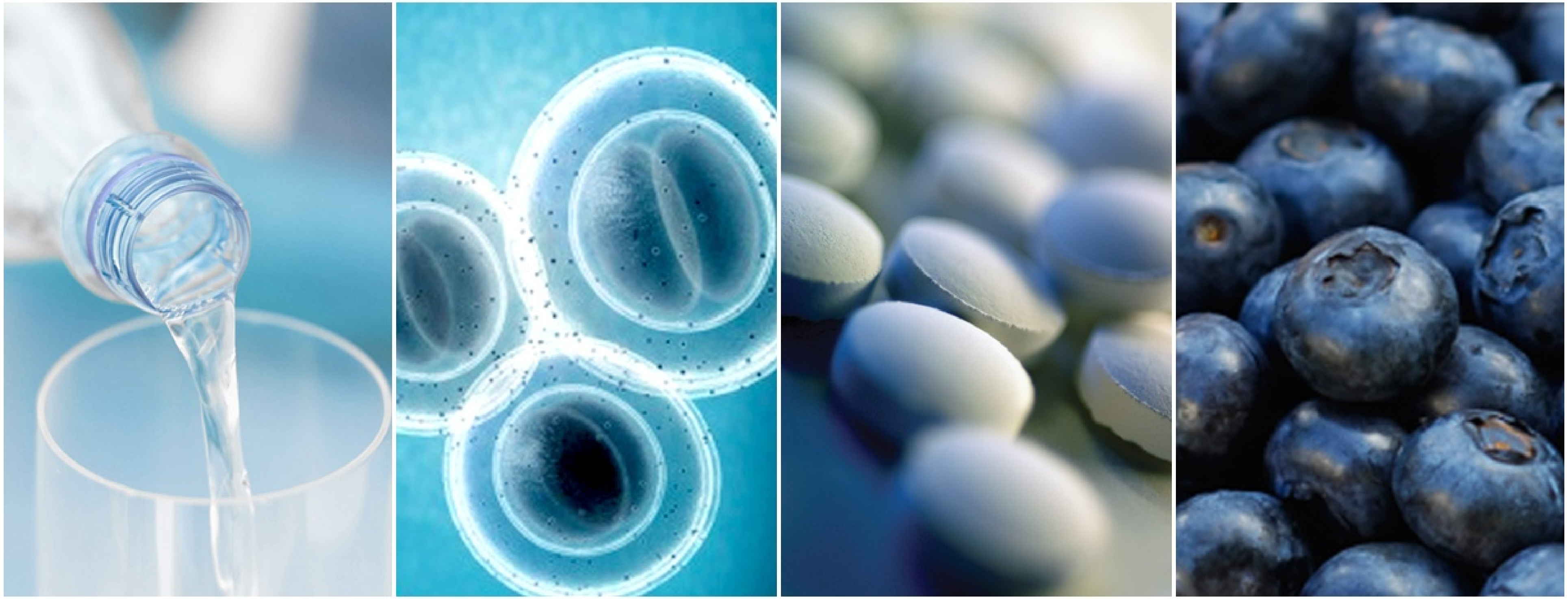


AB SCIEX TripleTOF™ 5600
Selected applications in proteomics

Tomáš Korba, AB SCIEX, Praha

Agenda

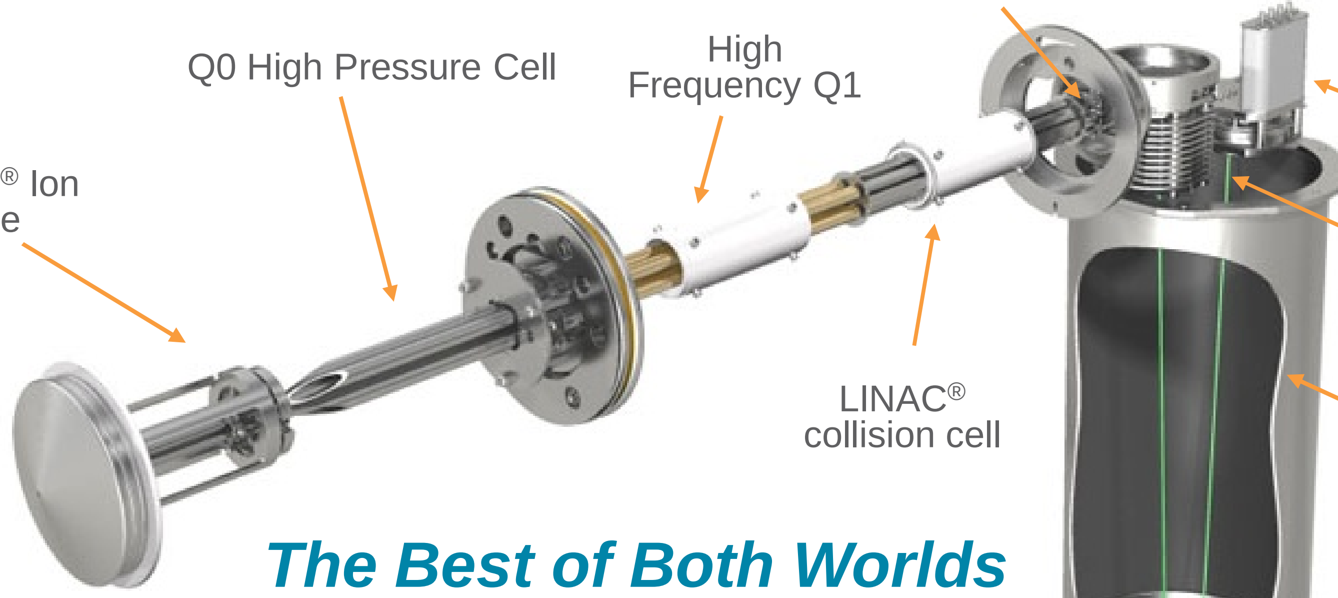
- The AB SCIEX TripleTOF™ 5600
  - introduction to the reasoning and technology
- Qualitative & Quantitative Workflows
  - Protein identification
  - Relative quantitation – label and labelfree workflows
  - Absolute quantitation

The AB SCIEX TripleTOF™ 5600 System

- Highest Sensitivity
  - In MS and MS/MS mode
- SmartSpeed™ 100 Hz Acquisition
  - 10 ms accumulation times
- High Resolution
  - ~30 000 at all speeds
- EasyMass™ Accuracy
  - ~1 ppm RMS
- Linear Dynamic Range
  - ~ 4 orders
- Reliability
  - 3-year standard warranty

AB SCIEX TripleTOF™ 5600 System

Ion Path Innovations



Accelerator TOF™ Analyzer

Ion compression optics

High Frequency Q1

Q0 High Pressure Cell

QJet® Ion Guide

LINAC® collision cell

40 GHz Multichannel TDC Detector

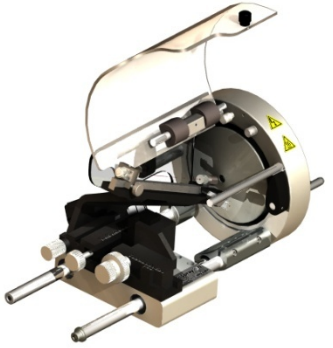
30kHz Accelerator

15 kV Acceleration voltage

Two-stage reflectron

The Best of Both Worlds

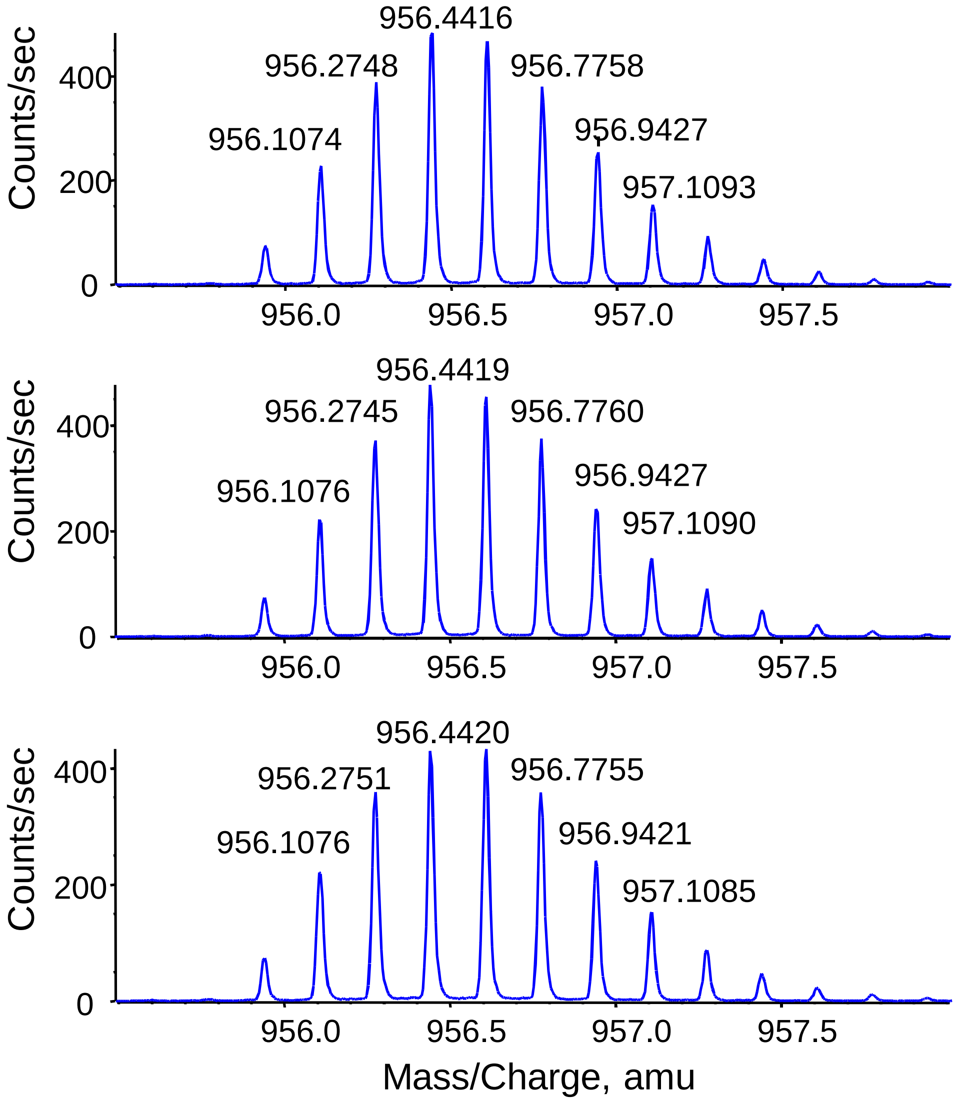
Industry Leading TurboV™ Ion Sources



NanoSpray® III Source

High Resolution at the Highest Speed

SmartSpeed™ Acquisition - Bovine Insulin, 6+ Charge State



1 s

R=43,750

100 ms

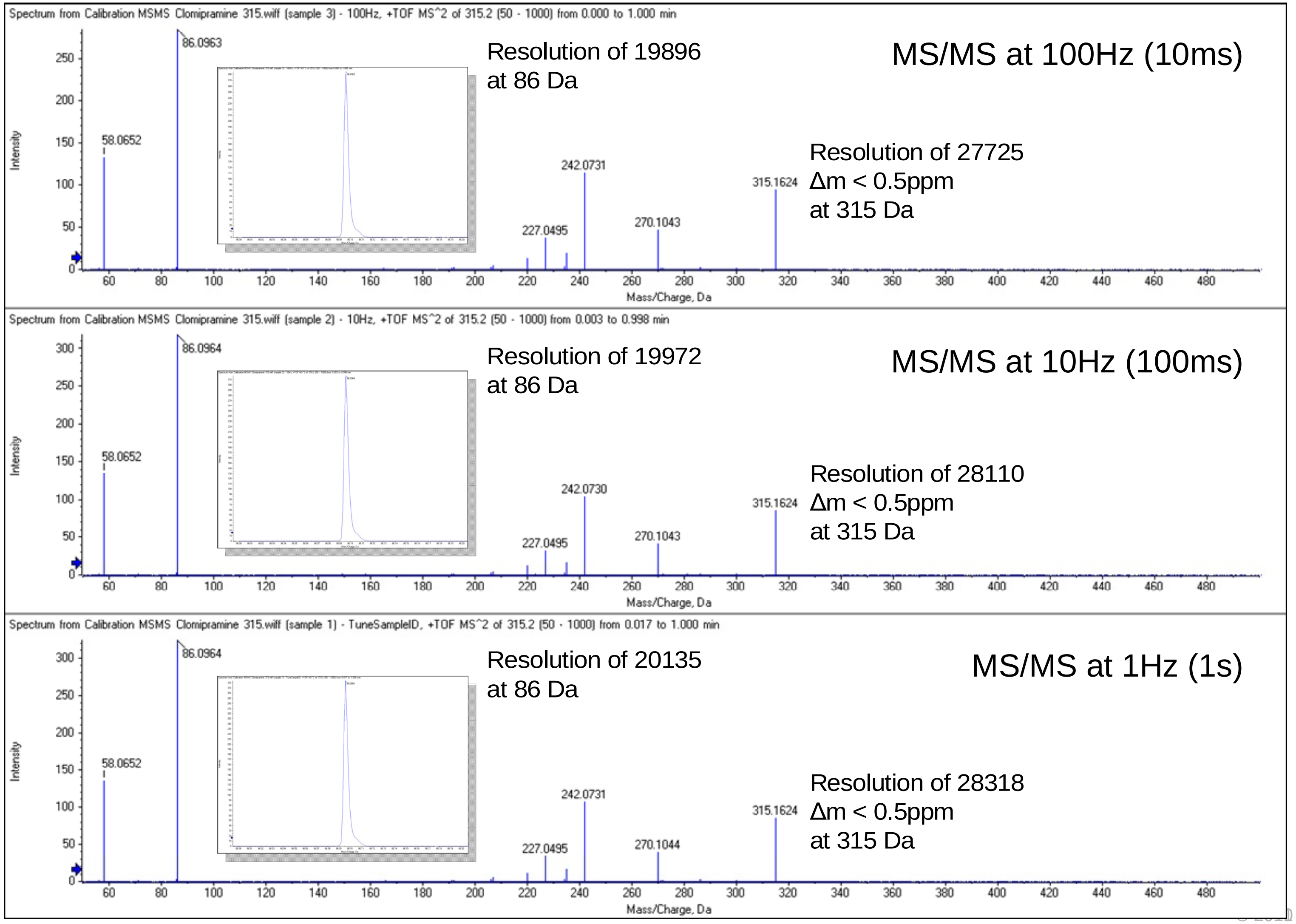
R=44,020

10 ms

R=44,000

High Resolution at the Highest Speed

MS/MS of Clomipramine using Different Accumulation Times



Spectrum from Calibration MSMS Clomipramine 315.wiff (sample 3) - 100Hz, +TOF MS^2 of 315.2 (50 - 1000) from 0.000 to 1.000 min

MS/MS at 100Hz (10ms)

Resolution of 19896
at 86 Da

Resolution of 27725
Δm < 0.5ppm
at 315 Da

Spectrum from Calibration MSMS Clomipramine 315.wiff (sample 2) - 10Hz, +TOF MS^2 of 315.2 (50 - 1000) from 0.003 to 0.998 min

MS/MS at 10Hz (100ms)

Resolution of 19972
at 86 Da

Resolution of 28110
Δm < 0.5ppm
at 315 Da

Spectrum from Calibration MSMS Clomipramine 315.wiff (sample 1) - TuneSampleID, +TOF MS^2 of 315.2 (50 - 1000) from 0.017 to 1.000 min

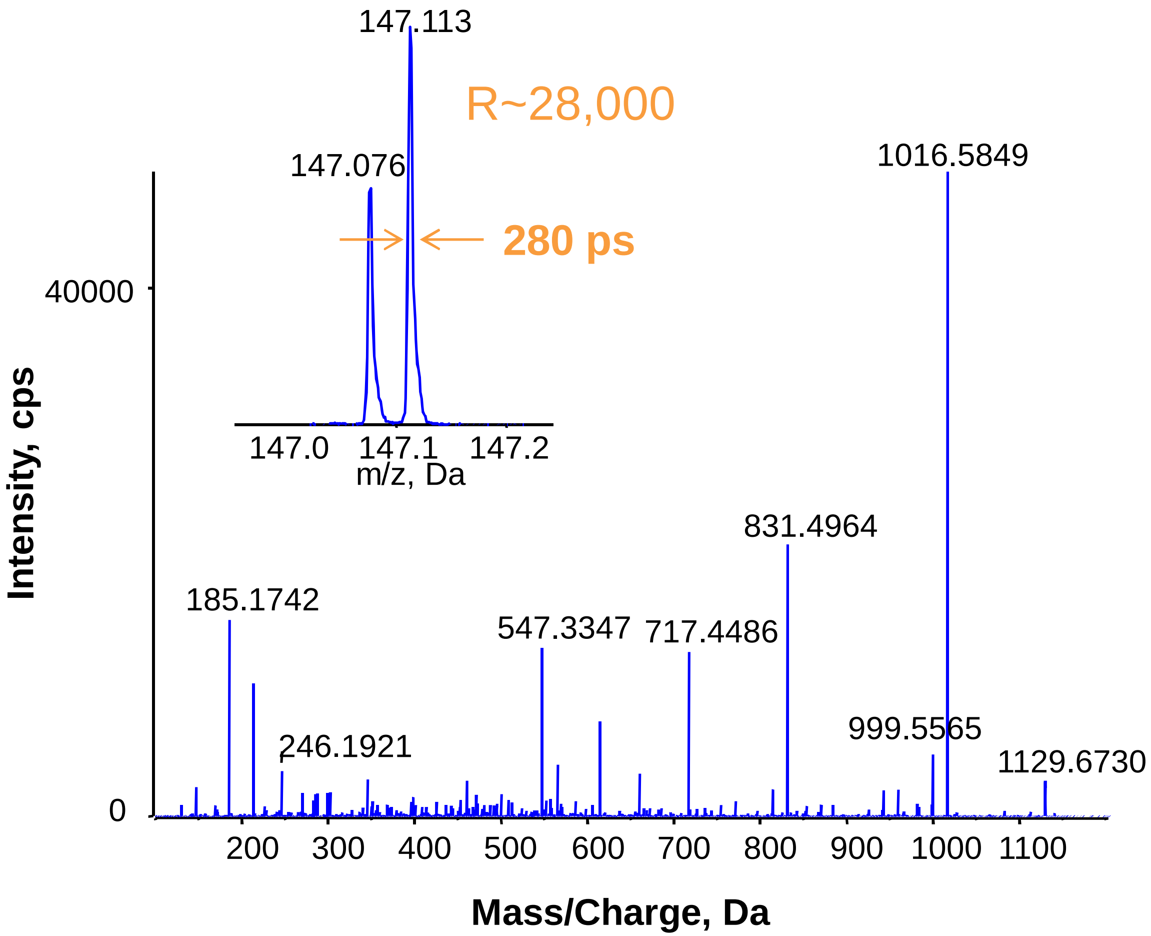
MS/MS at 1Hz (1s)

Resolution of 20135
at 86 Da

Resolution of 28318
Δm < 0.5ppm
at 315 Da

High Resolution at Low m/z

10x More Data Points Across a Peak than 4 GHz ADC



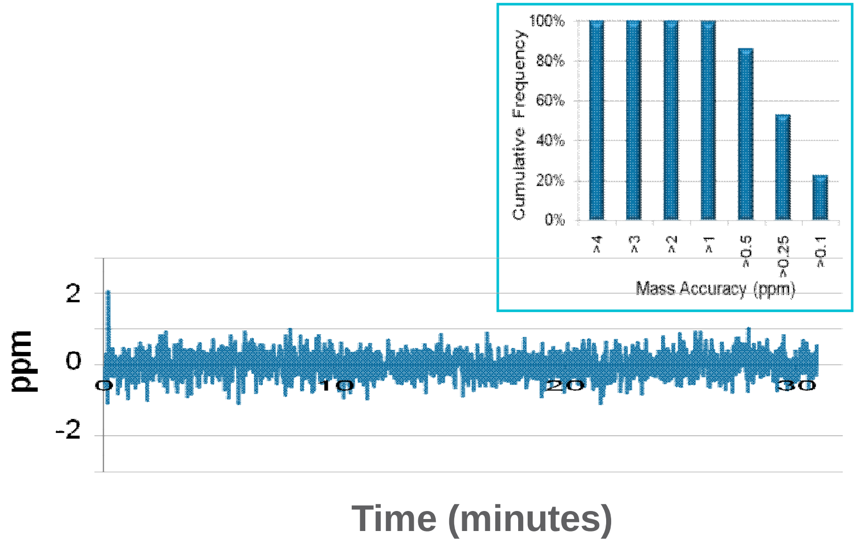
R~28,000

280 ps

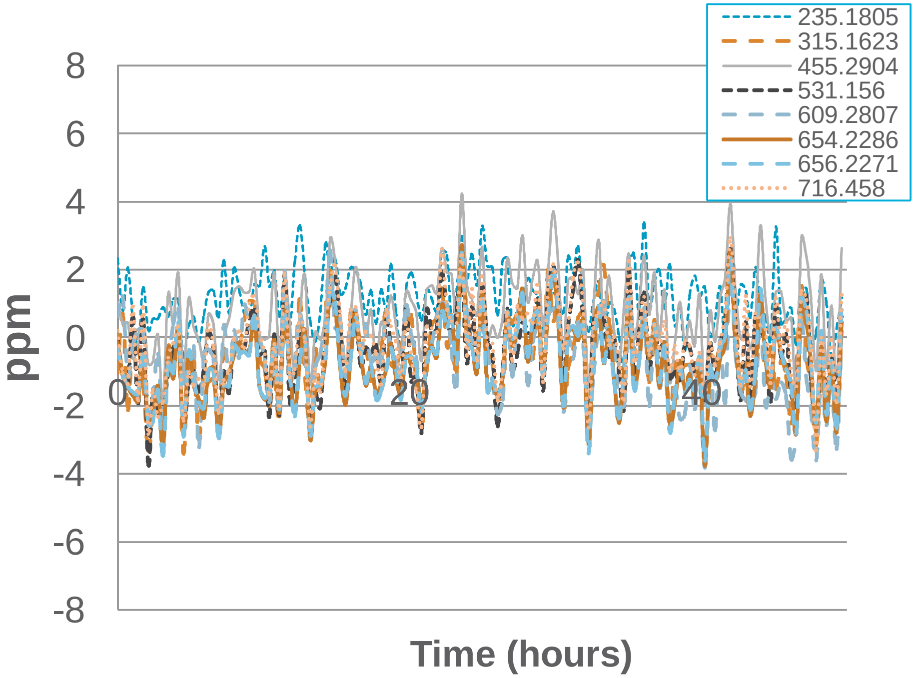
- Ultra fast 40 GHz Time to Digital Converter (TDC) with 25 ps per bin
- Resolution across the mass range provides more information content
- Y1 ions of Lys and Gln can be resolved!

Mass Accuracy and Stability

EasyMass™ Accuracy with External Calibration



- Infusion for 30 minutes in ESI mode
- Mass accuracy <1 ppm



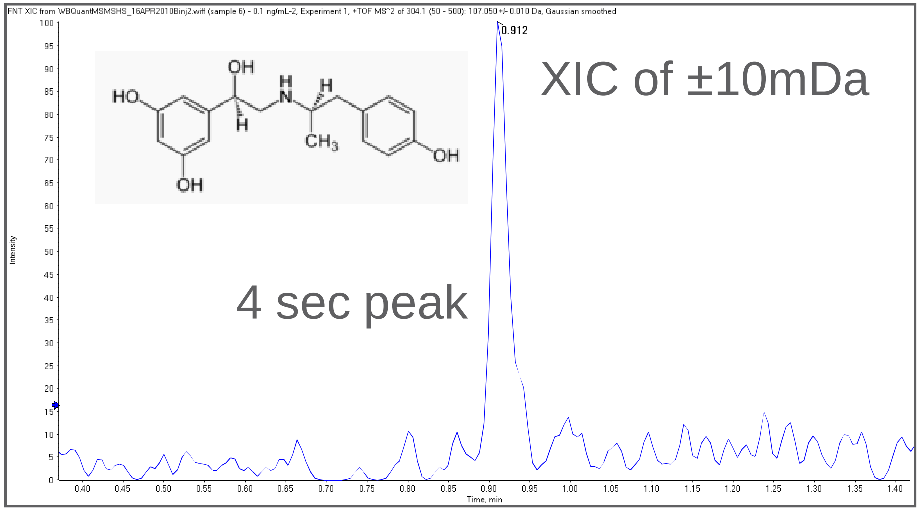
- 144 LC/MS runs over 50 hours
- Mass accuracy RMS = 1.37

- Novel calibration methodology maintains calibration without recalibration or internal calibration – easy to use

High Sensitivity and Wide Linear Range

Quantitation of Fenoterol in Whole Blood using TOF-MS/MS

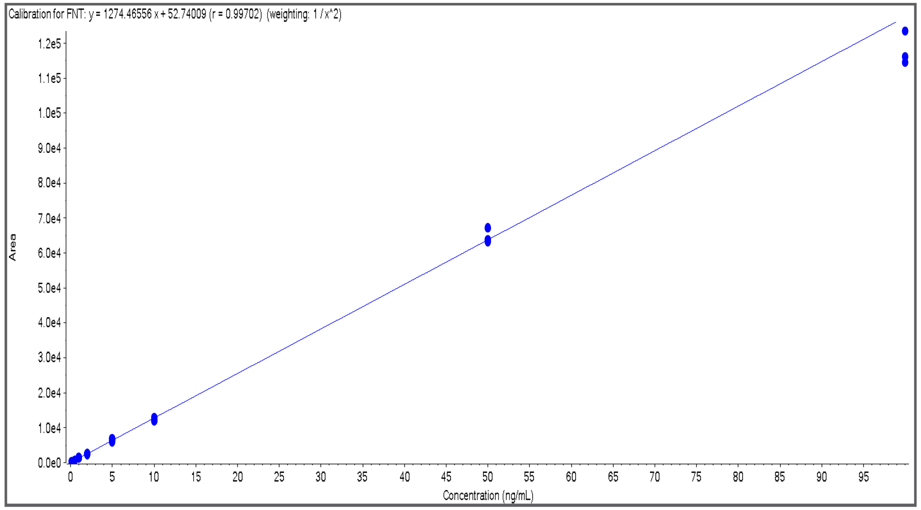
62.5 fg on column (9.5% CV)



XIC of ±10mDa

4 sec peak

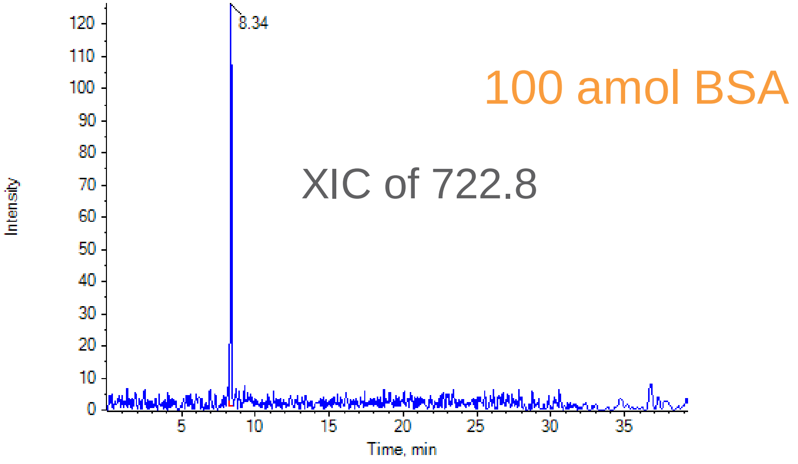
0.1 – 100ng/mL

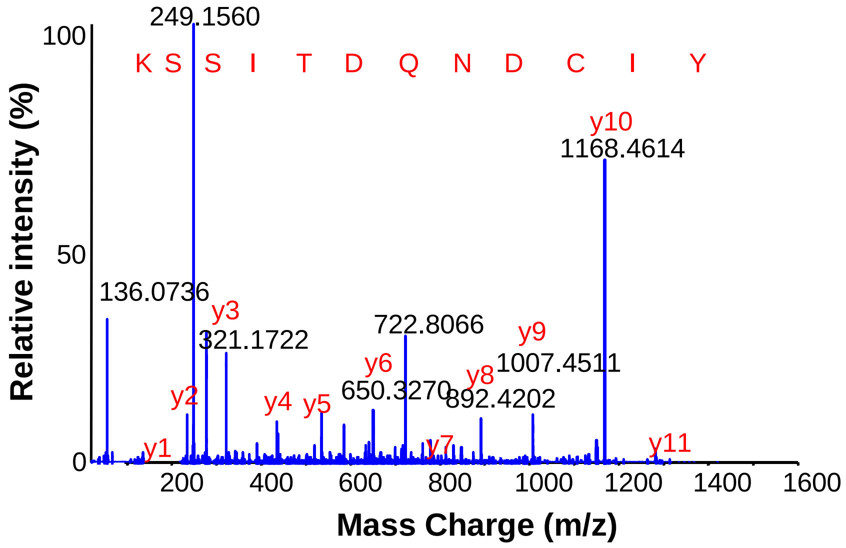


Actual Concentration	Num. Values	Mean	Standard Deviation	% CV	Accuracy
0.1	3 of 3	9.93E-02	9.42E-03	9.48	99.3
0.25	3 of 3	2.42E-01	1.44E-02	5.95	96.76
0.5	3 of 3	5.28E-01	6.83E-03	1.29	105.66
1	3 of 3	1.10E+00	4.48E-02	4.07	110.23
2	3 of 3	1.96E+00	7.56E-02	3.87	97.79
5	3 of 3	5.00E+00	3.06E-01	6.12	100.04
10	3 of 3	9.59E+00	3.29E-01	3.43	95.92
50	3 of 3	5.09E+01	1.65E+00	3.24	101.73
100	3 of 3	9.26E+01	3.76E+00	4.06	92.57

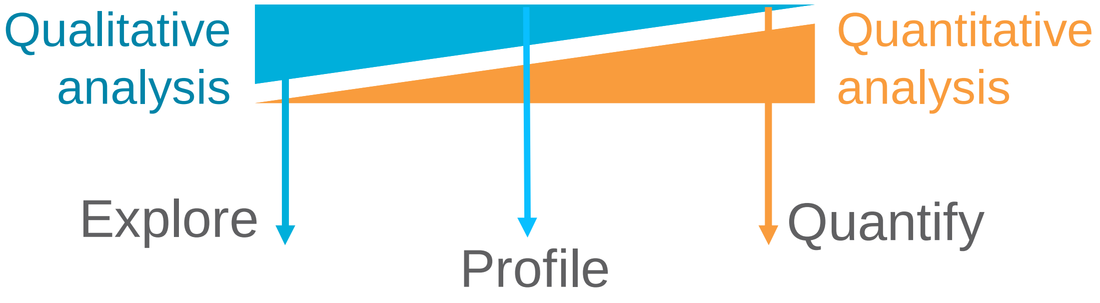
High Sensitivity = High Speed Acquisition

- Combined high mass accuracy and resolution with high sensitivity allows for very high acquisition speed with good S/N
- More MS/MS per unit time
  - Up to 100 scans per second
  - 20,000 resolution (high sensitivity mode)
  - 30,000 resolution (high resolution mode)
  - 50 ms TOF MS/MS accumulation time on 100 amol with excellent S/N





Powerful Workflows Enable Qualitative and Quantitative Data Acquisition



Qualitative analysis

Quantitative analysis

Explore

Profile

Quantify

Agenda

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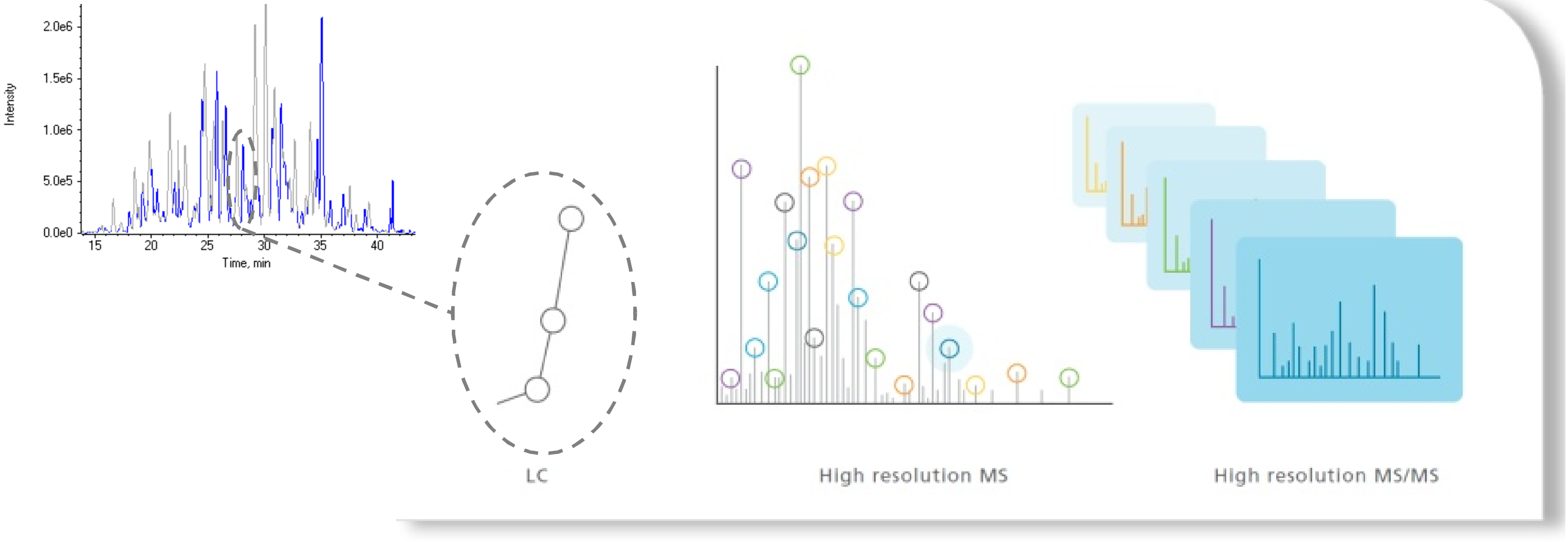
Ÿ Qualitative & Quantitative Workflows

- Protein identification

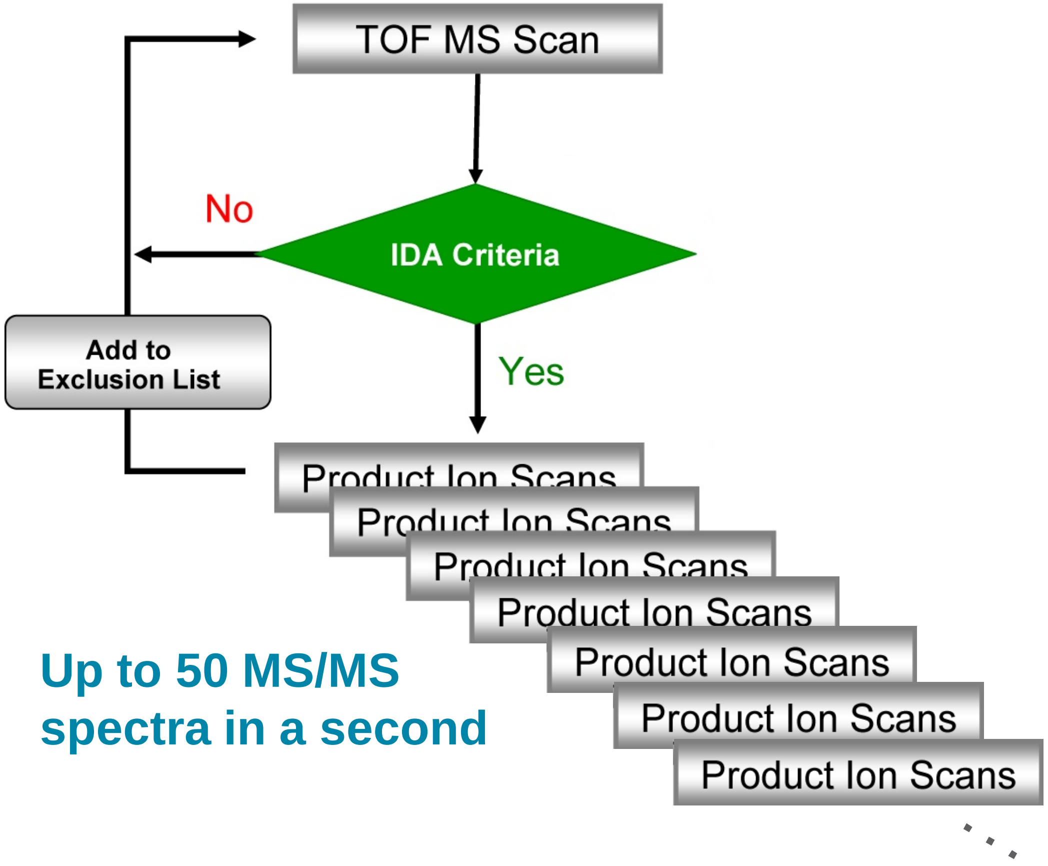
-

EXPLORE Workflow for Protein ID



- The TripleTOF™ 5600 System has high sensitivity and speed for in depth exploration of complex samples
- Up to 50 precursors in a second can be selected and high resolution, accurate-mass MS/MS is acquired

EXPLORE Workflow for Protein ID



TOF MS Scan

IDA Criteria

No

Add to Exclusion List

Yes

Product Ion Scans

Product Ion Scans

Product Ion Scans

Product Ion Scans

Product Ion Scans

Product Ion Scans

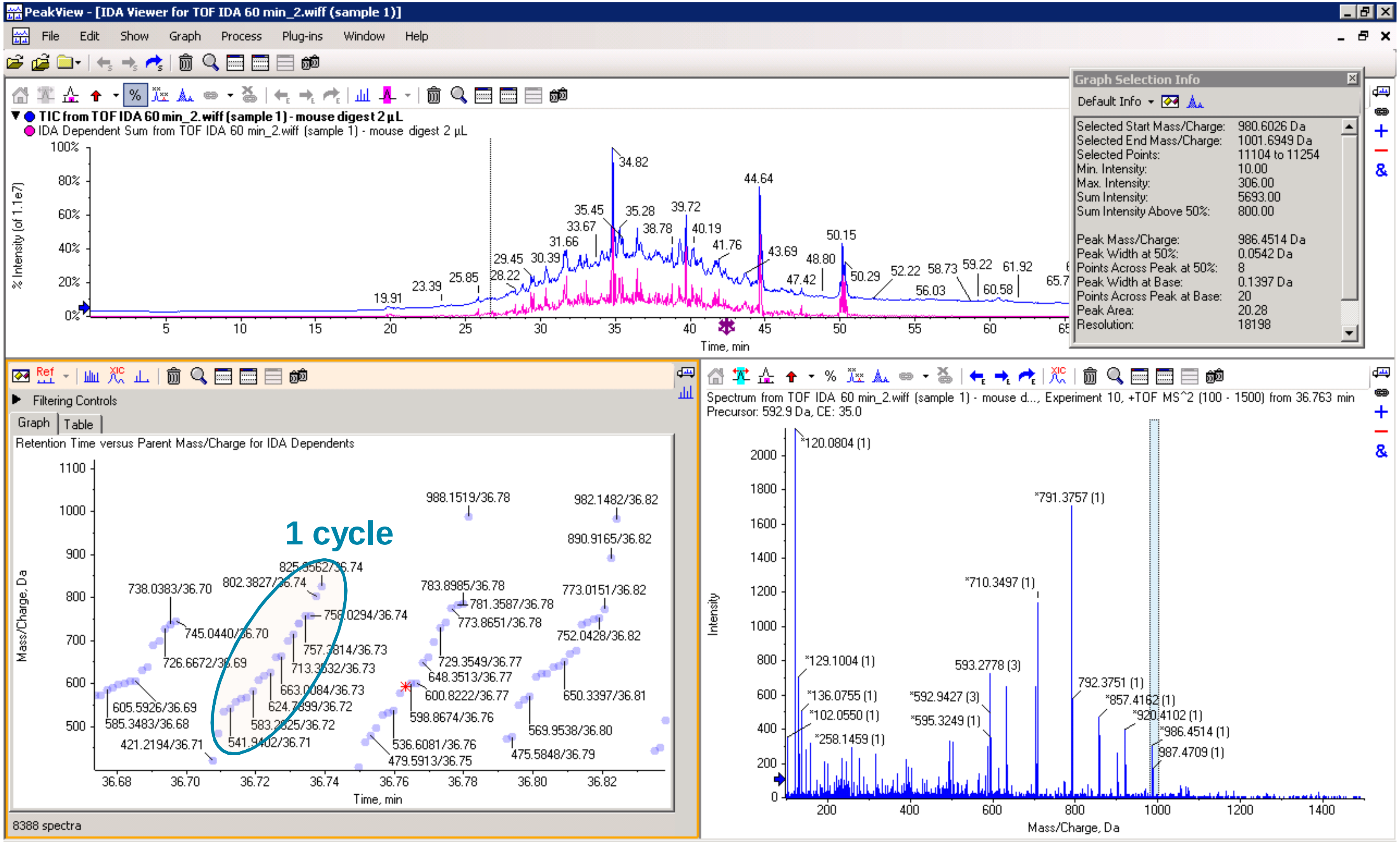
Product Ion Scans

Up to 50 MS/MS spectra in a second

- IDA Workflow
- Object is to spend as much time in MS/MS mode as possible
  - Cycle time is fixed
    - User specifies the maximum number of precursors and minimum accumulation time
    - Accumulation time will be dynamically distributed across available precursors
- Methods are turnkey – protein ID template methods provided with the software
  - Prot simple IDA
  - Prot complex IDA

High Resolution MS and MS/MS Spectra

PeakView™ Software



TIC from TOF IDA 60 min_2.wiff (sample 1) - mouse digest 2 µL

IDA Dependent Sum from TOF IDA 60 min_2.wiff (sample 1) - mouse digest 2 µL

Graph Selection Info

Default Info

Selected Start Mass/Charge:	980.6026 Da
Selected End Mass/Charge:	1001.6949 Da
Selected Points:	11104 to 11254
Min. Intensity:	10.00
Max. Intensity:	306.00
Sum Intensity:	5693.00
Sum Intensity Above 50%:	800.00
Peak Mass/Charge:	986.4514 Da
Peak Width at 50%:	0.0542 Da
Points Across Peak at 50%:	8
Peak Width at Base:	0.1397 Da
Points Across Peak at Base:	20
Peak Area:	20.28
Resolution:	18198

Filtering Controls

Graph | Table

Retention Time versus Parent Mass/Charge for IDA Dependents

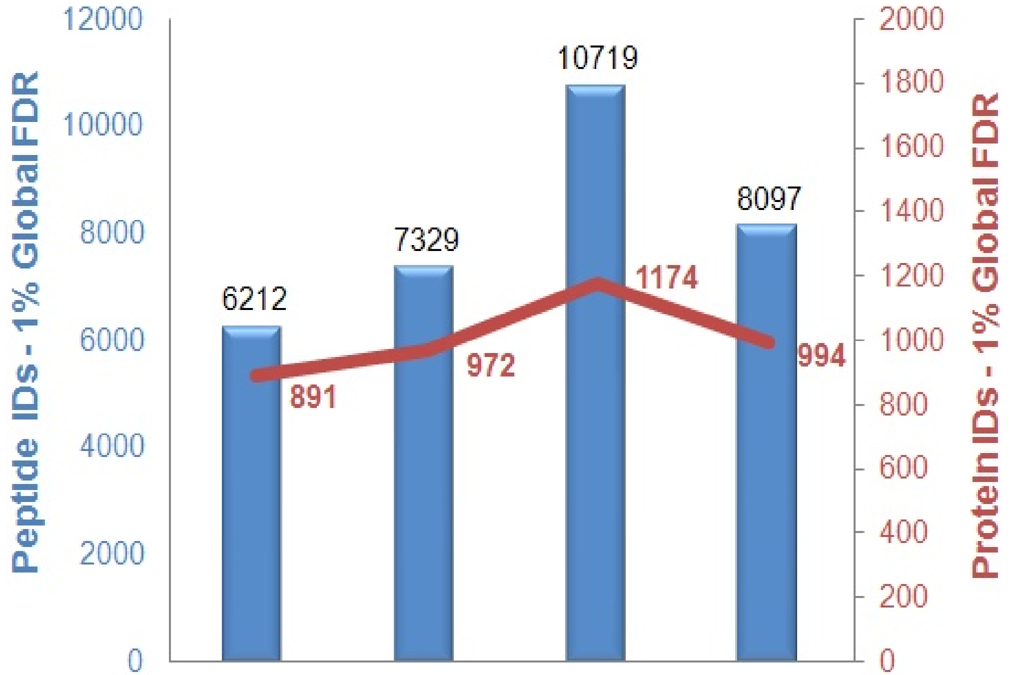
8388 spectra

Spectrum from TOF IDA 60 min_2.wiff (sample 1) - mouse d..., Experiment 10, +TOF MS^2 (100 - 1500) from 36.763 min

Precursor: 592.9 Da, CE: 35.0

Protein and Peptide Identifications

Yeast Cell Lysate

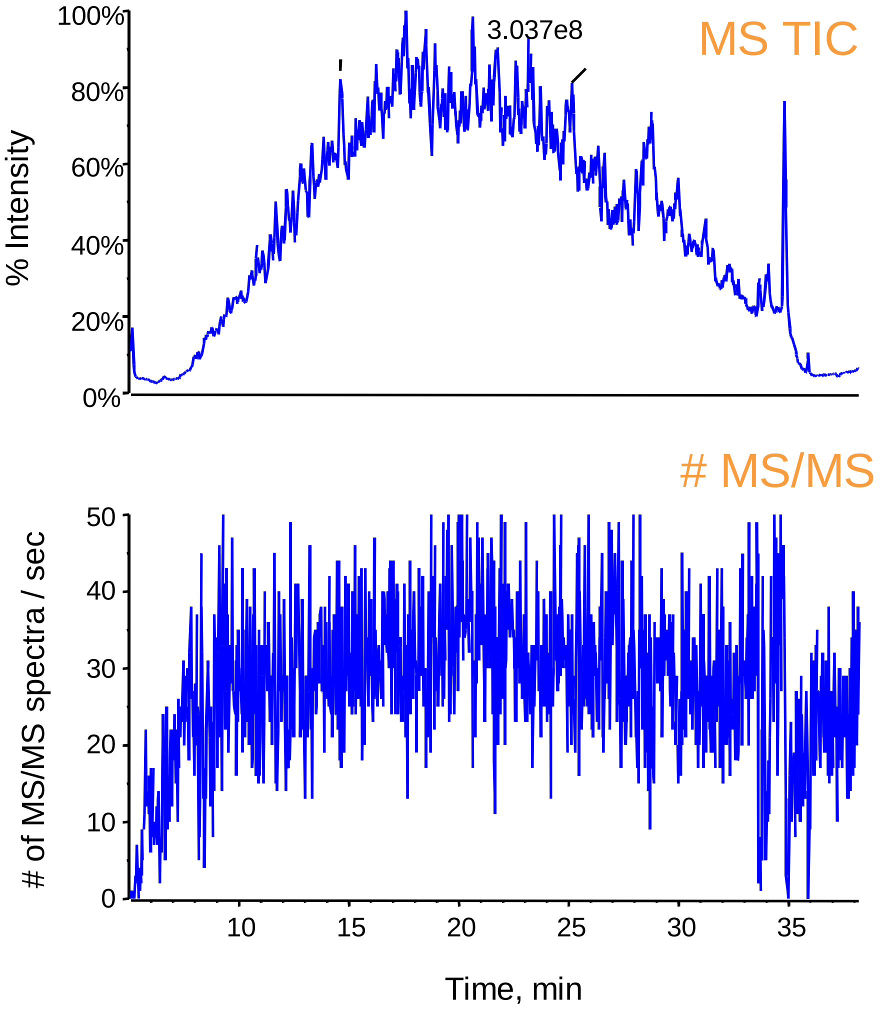


Experiment #	1	2	3	4
Column Loading (ng)	500	1000	2000	2000
Gradient Length (min)	60	60	60	30
Avg MS/MS per sec	14	16	28	34

- Single injections of yeast were run using varied gradient lengths and varied loading amounts
- As sample amount is increased and gradient is shortened, the system acquires more MS/MS data in order to keep up with sample complexity

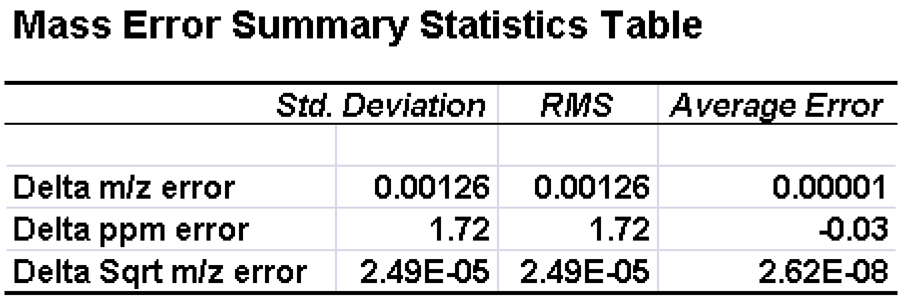
Fast Acquisition of MS/MS Spectra

- TIC of Experiment 4 (2000 ng sample with 30 min gradient) shows how peptide rich the chromatogram is
- The number of MS/MS obtained in each cycle is computed across the gradient
- In this experimental case, the average number of MS/MS is 34 spectra per sec with some cycles hitting the maximum of 50 MS/MS per sec



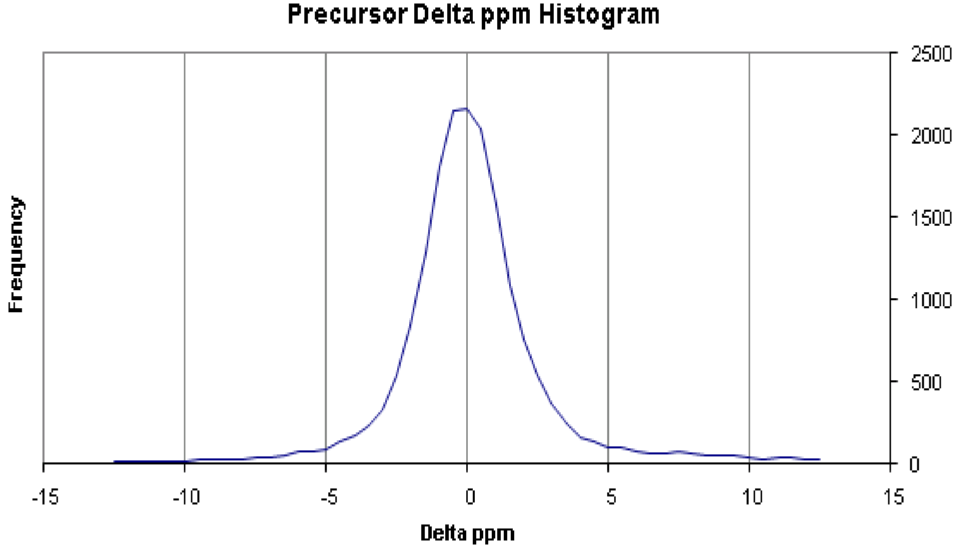
High Mass Accuracy at High Acquisition Rates

- Yeast whole cell lysate run with a 60 min gradient, 2000 ng on column
- Processed with ProteinPilot™ Software 4.0
- RMS error is 1.7 ppm across all the peptides detected, including the low abundant species



Mass Error Summary Statistics Table

	Std. Deviation	*RMS*	*Average Error*
Delta m/z error	0.00126	0.00126	0.00001
Delta ppm error	1.72	1.72	-0.03
Delta Sqrt m/z error	2.49E-05	2.49E-05	2.62E-08



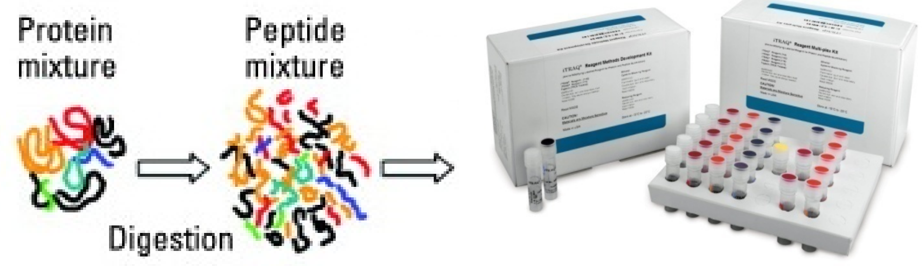
Agenda

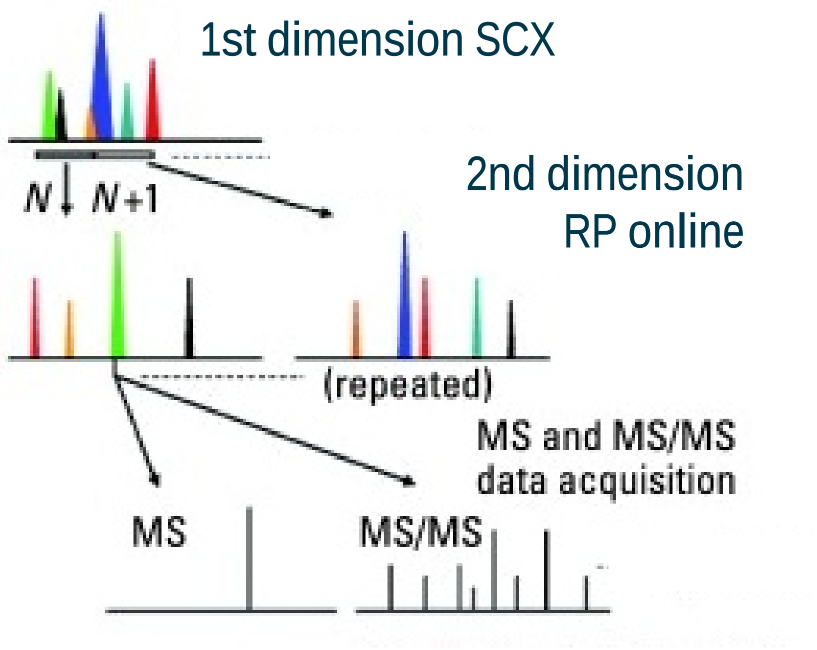
- Qualitative & Quantitative Workflows

  - Relative quantitation – label and labelfree workflows

Protein Quantitation in Arabidopsis Leaf Extracts

Quantify the Proteomics Effects of a Gene Mutation Study in Plants



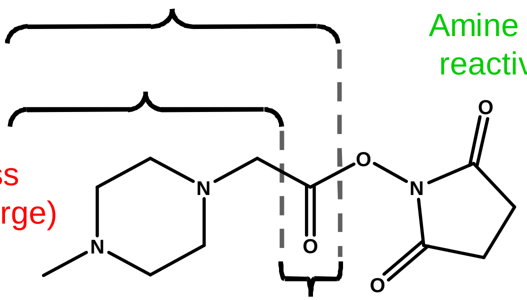


- ~100 µg of total protein from each plant extract was digested and labeled with an amine tagging reagent
- 10 SCX peptide fractions were collected then subjected to online RP-nanoLC-MS/MS
- ~ 200 – 500 ng total protein/injection for each nanoLC-MS/MS acquisition

***Collaboration with Chen et al, University of Florida**

iTRAQ Workflow

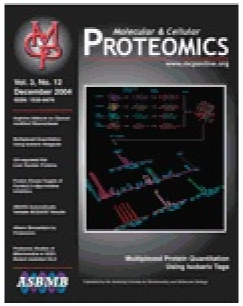
Isobaric Tag
Total mass = **145**



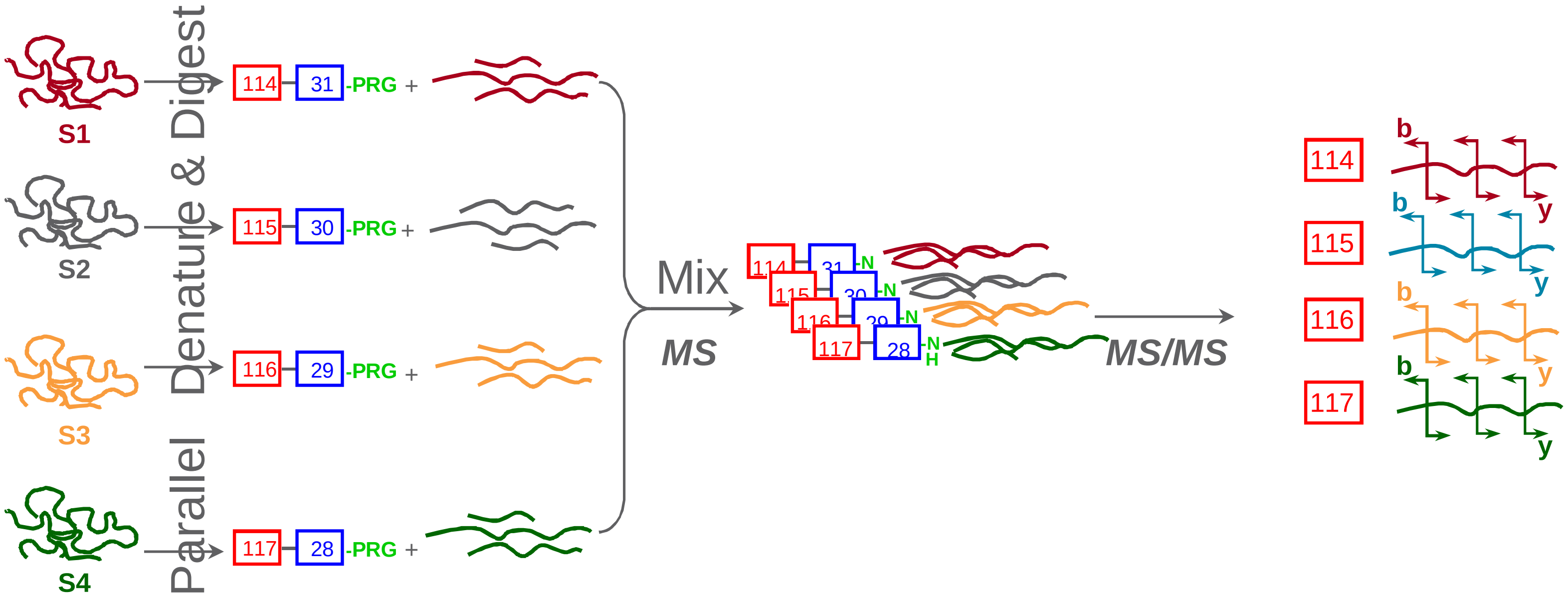
Amine specific peptide reactive group (NHS)

Reporter Group mass
114 –117 (Retains Charge)

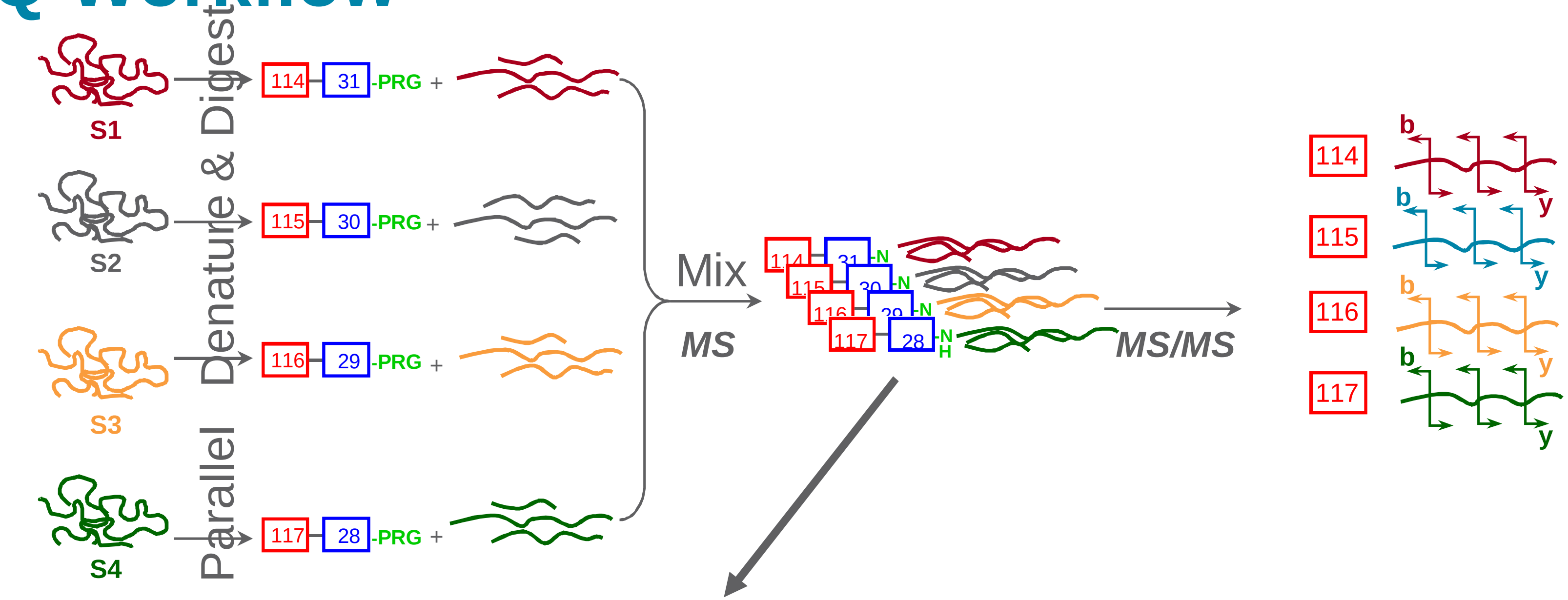
Balance Group
Mass **31-28** (Neutral loss)

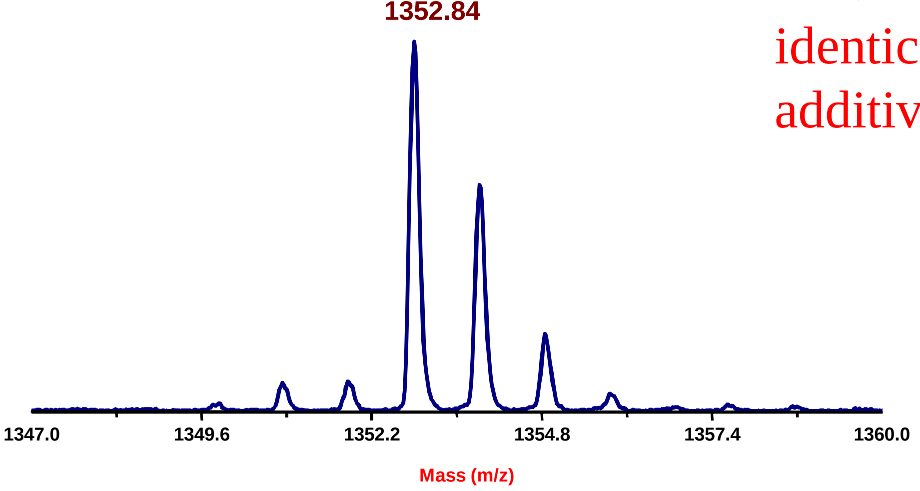


(Ross et. al, *MCP 3*, 1154-1169, 2004)

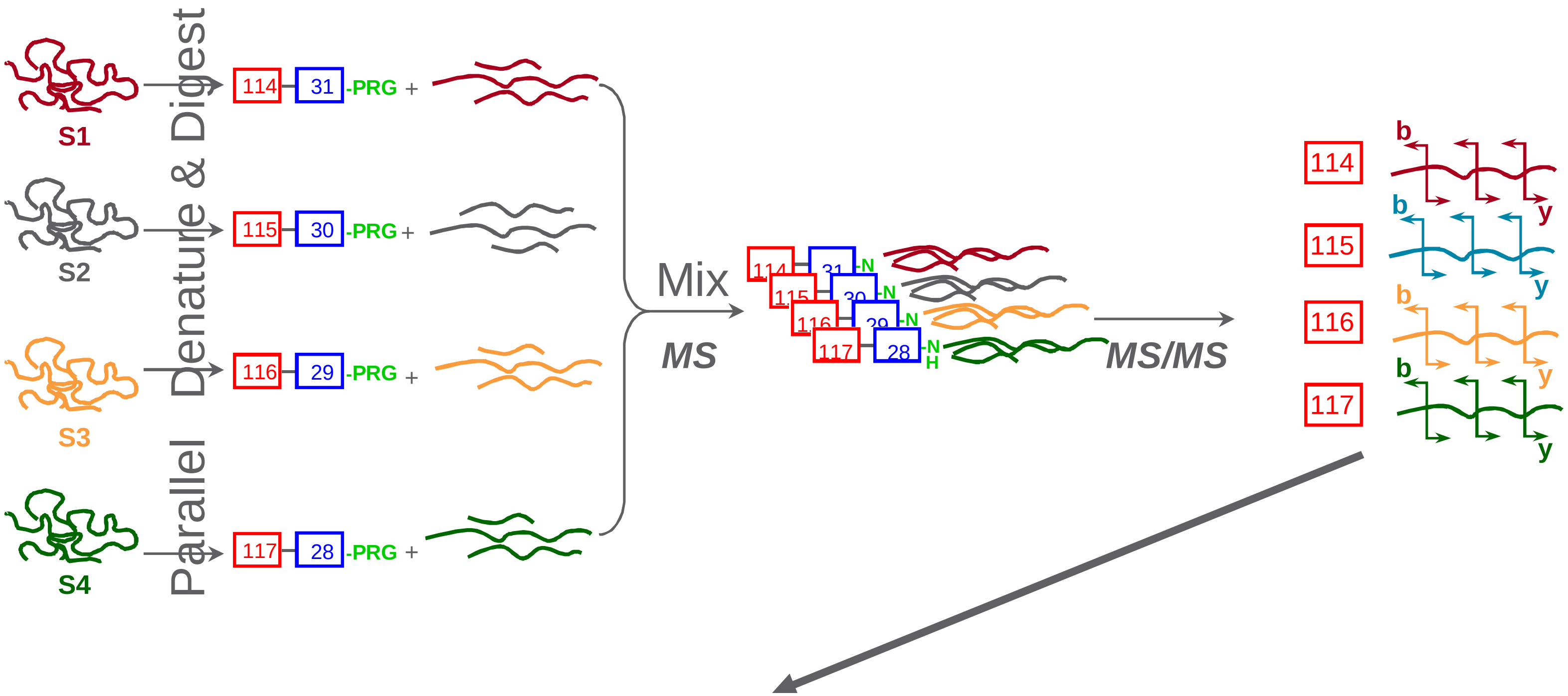


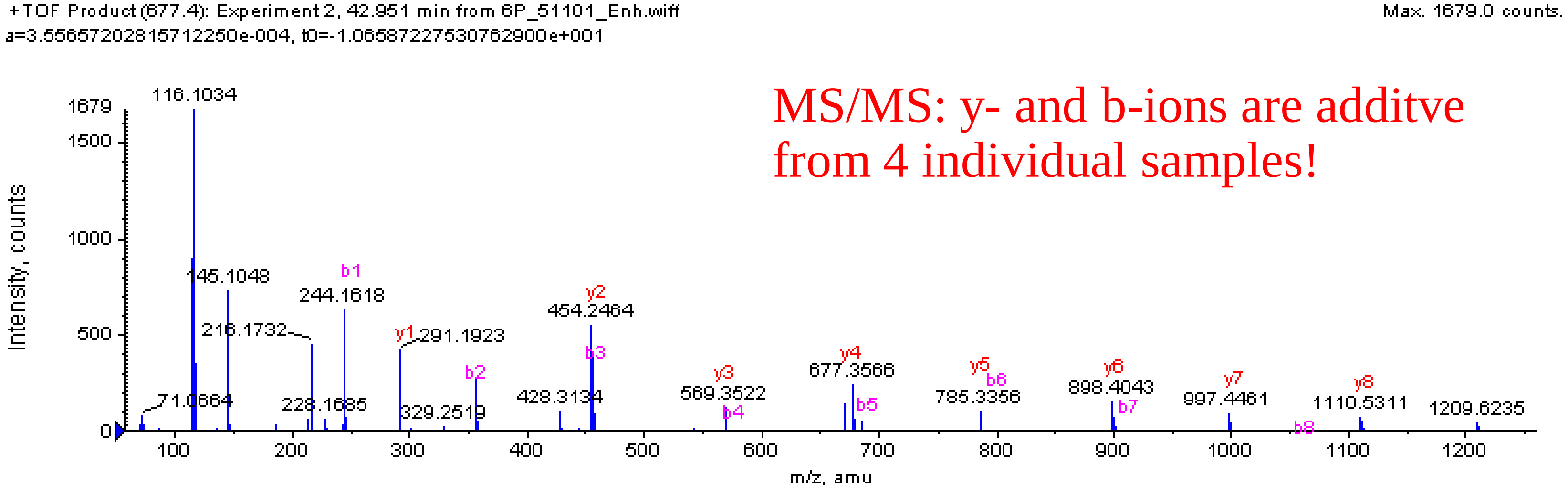
iTRAQ Workflow





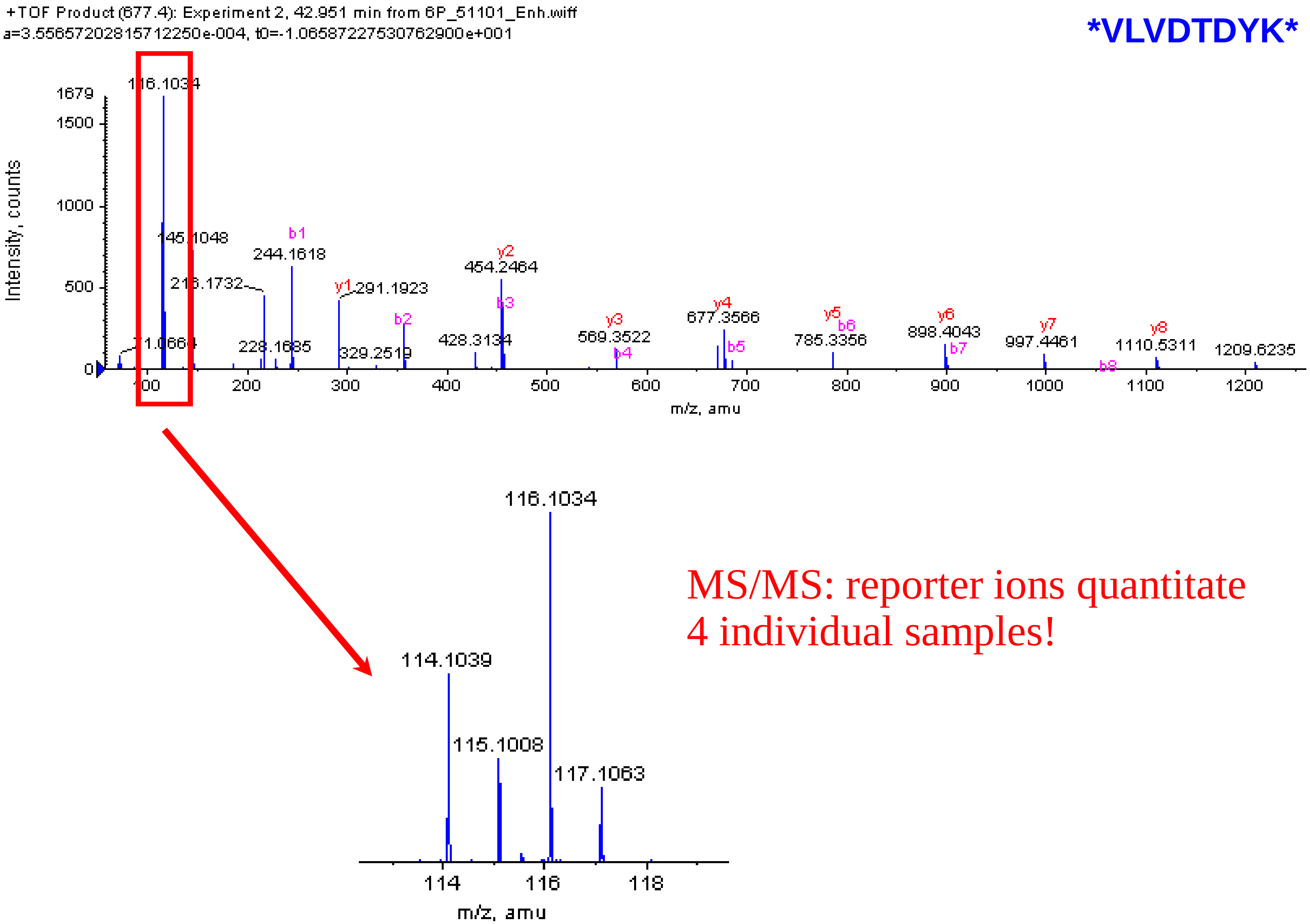
MS: intact labelled peptides are identical, signal intensities are additive!





MS/MS: y- and b-ions are additve from 4 individual samples!

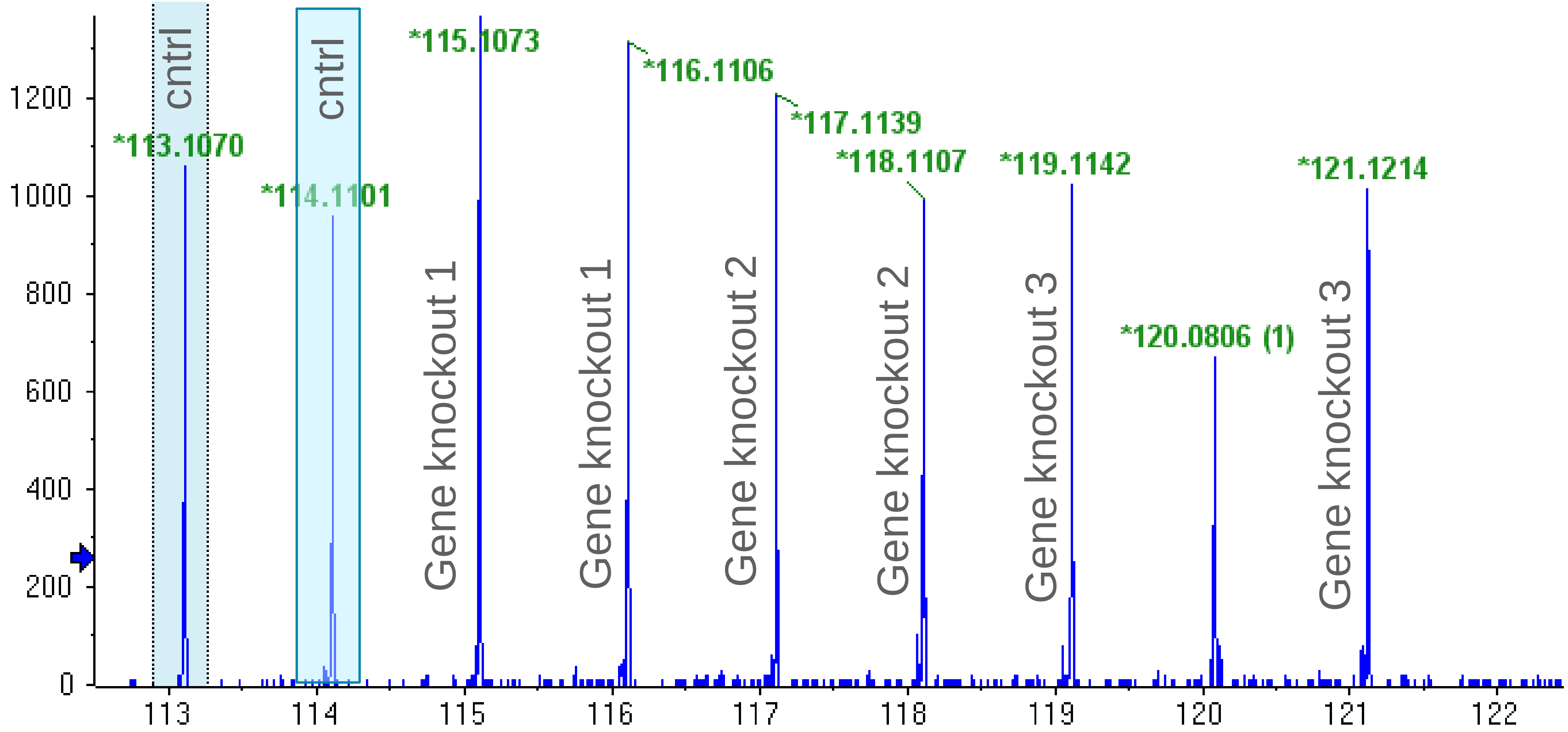
VLVDTDYK



MS/MS: reporter ions quantitate 4 individual samples!

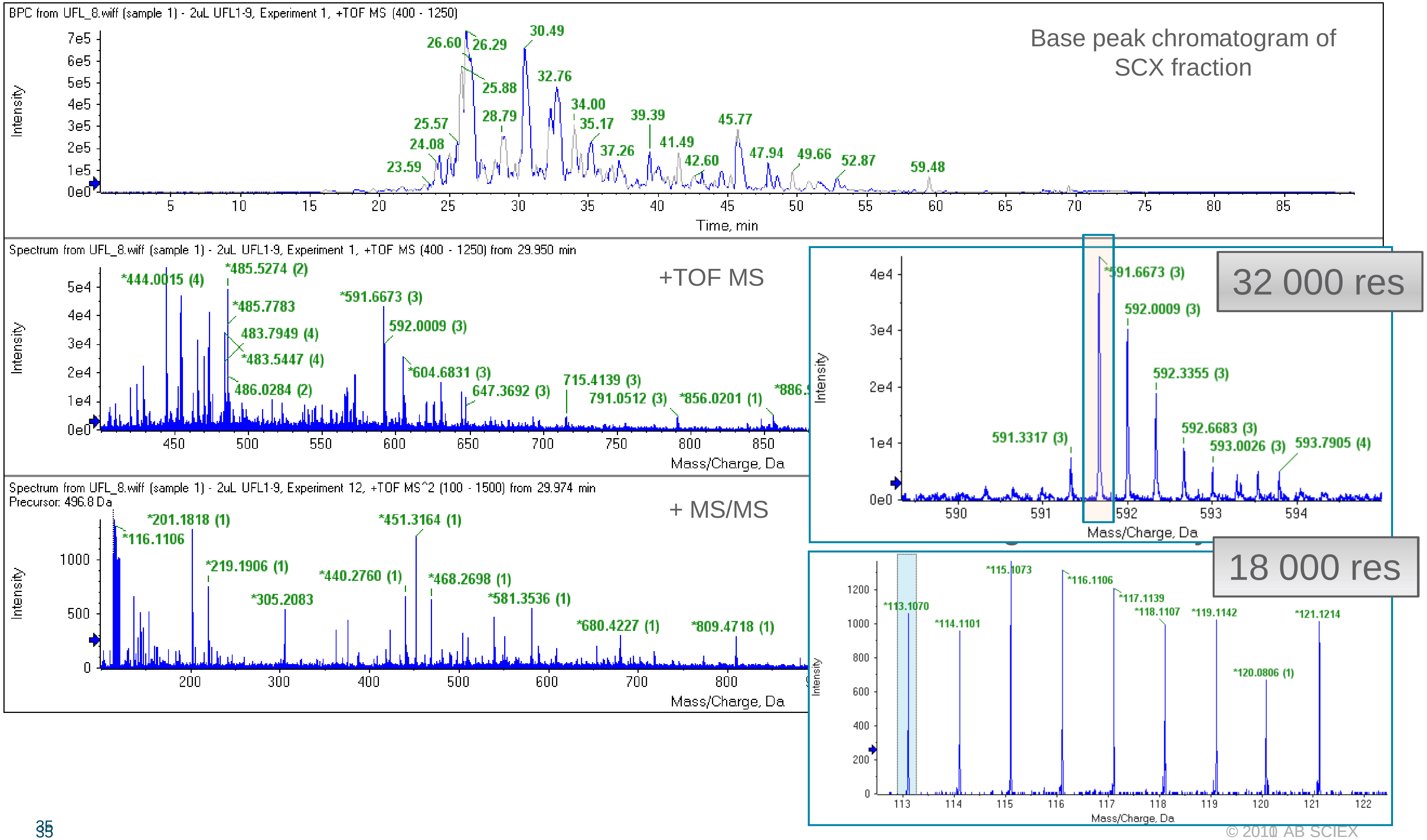
Protein Quantitation in Arabidopsis Leaf Extracts

Quantify the Proteomics Effects of a Gene Mutation Study in Plants



- 10 SCX fractions, 10 – 150 mM KCl, 2 x biological replicates [20 samples]
- 60 min nanoLC seperation

High Resolution TOF MS & MS/MS Data


Base peak chromatogram of SCX fraction

+TOF MS

+ MS/MS

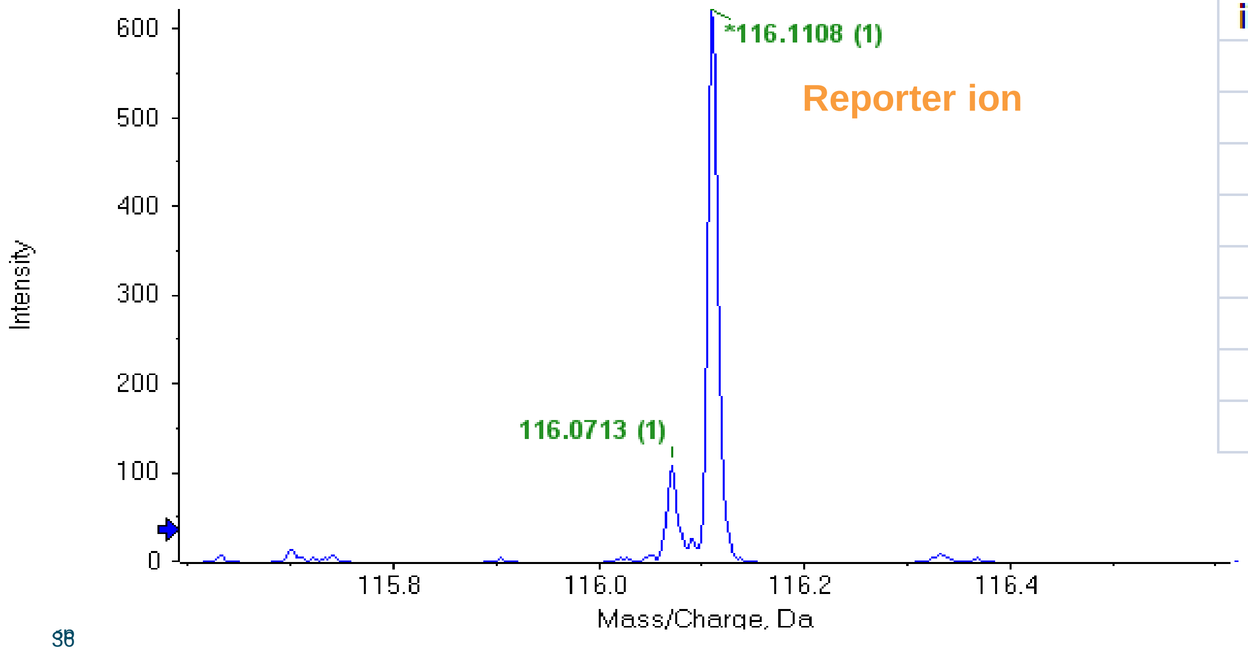
32 000 res

18 000 res

High Resolution MS/MS Data for Quant

Amine Labeling Reagents for Protein Expression Analysis

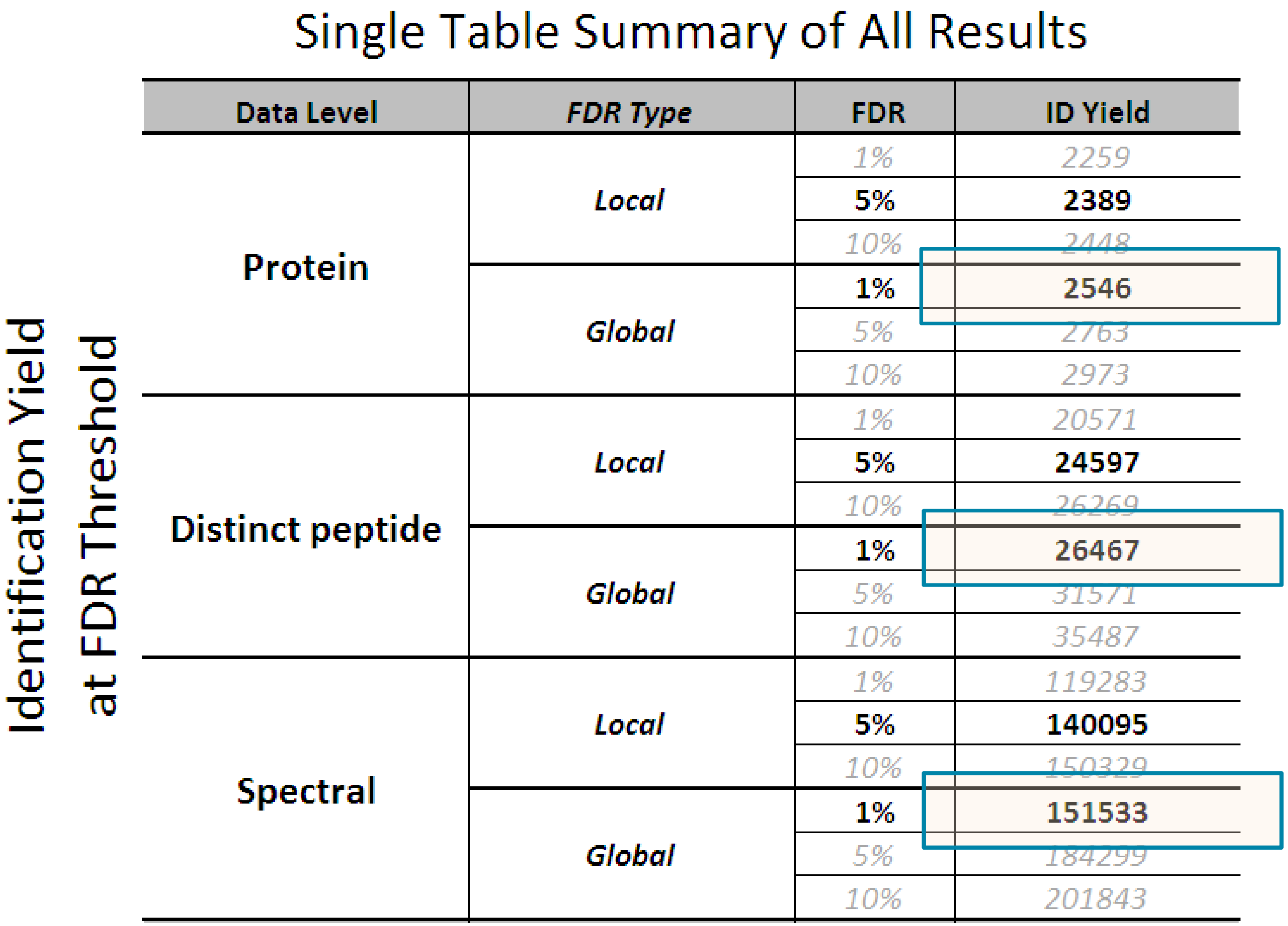
- 40 GHz Multichannel TDC detector collects ion counts at 25 ps bin-sizes delivering enough points across the reporter ions peaks for good resolution
- Benefiting from high resolution at low mass, the reporter ions are well resolved from fragments close in mass



iTRAQ reporter ions 8-plex
113.1073
114.1107
115.1077
116.1111
117.1144
118.1115
119.1148
121.1215

High Speed Acquistion of MS/MS

For Greatest Depth of Coverage



Single Table Summary of All Results

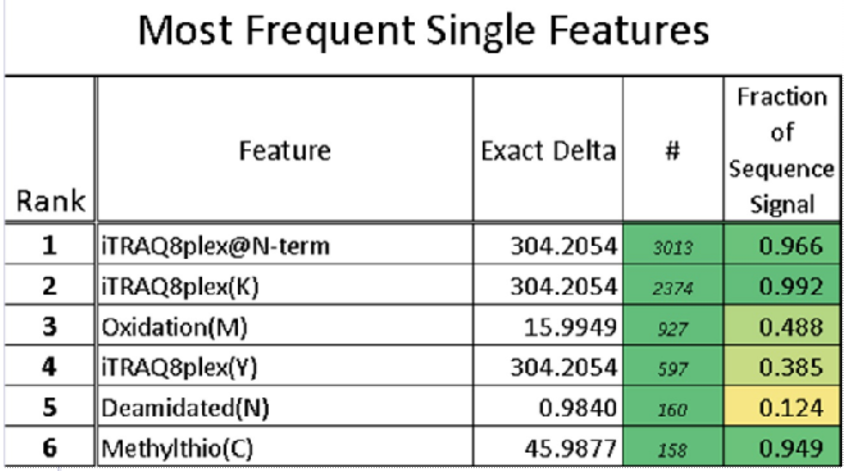
Identification Yield at FDR Threshold

Data Level	FDR Type	FDR	ID Yield
Protein	***Local***	*1%*	*2259*
		5%	**2389**
		10%	*2448*
	Global	**1%**	**2546**
		5%	*2763*
		10%	*2973*
Distinct peptide	***Local***	*1%*	*20571*
		5%	**24597**
		10%	*26269*
	Global	**1%**	**26467**
		5%	*31571*
		10%	*35487*
Spectral	***Local***	*1%*	*119283*
		5%	**140095**
		10%	*150329*
	Global	**1%**	**151533**
		5%	*184299*
		10%	*201843*

- 15 MS/MS per second
- Integrated FDR report
- More MS/MS and powerful search engine yields deep proteome coverage

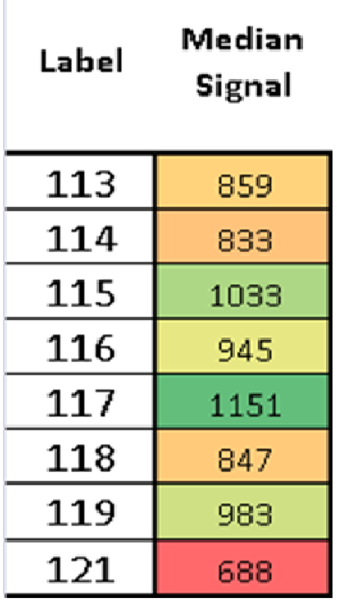
High Confidence in Quantitation

- High labeling efficiency
  - 96.6% on N-term sites
  - 99.2% on Lys residues
- High sensitivity in low mass region for good ion statistics
- Over 90% of confidently identified peptides have valid & quantifiable ratios

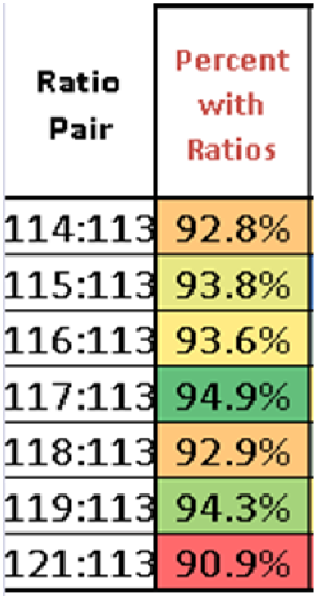


Most Frequent Single Features

Rank	Feature	Exact Delta	#	Fraction of Sequence Signal
1	iTRAQ8plex@N-term	304.2054	3013	0.966
2	iTRAQ8plex(K)	304.2054	2374	0.992
3	Oxidation(M)	15.9949	927	0.488
4	iTRAQ8plex(Y)	304.2054	597	0.385
5	Deamidated(N)	0.9840	160	0.124
6	Methylthio(C)	45.9877	158	0.949



Label	Median Signal
113	859
114	833
115	1033
116	945
117	1151
118	847
119	983
121	688

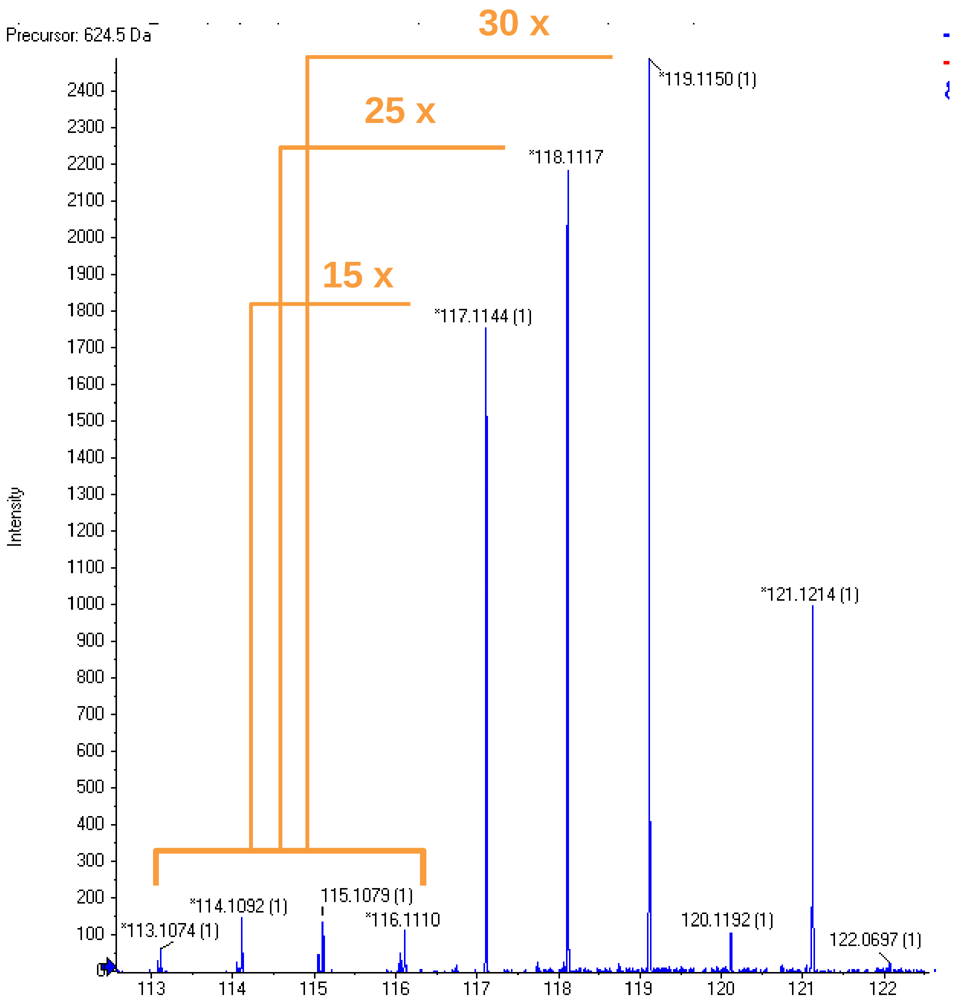


Ratio Pair	Percent with Ratios
114:113	92.8%
115:113	93.8%
116:113	93.6%
117:113	94.9%
118:113	92.9%
119:113	94.3%
121:113	90.9%

Dynamic Ranges of Isobaric-Tagging Quantitation

Extending Measurable Differences Between Reporter Ions

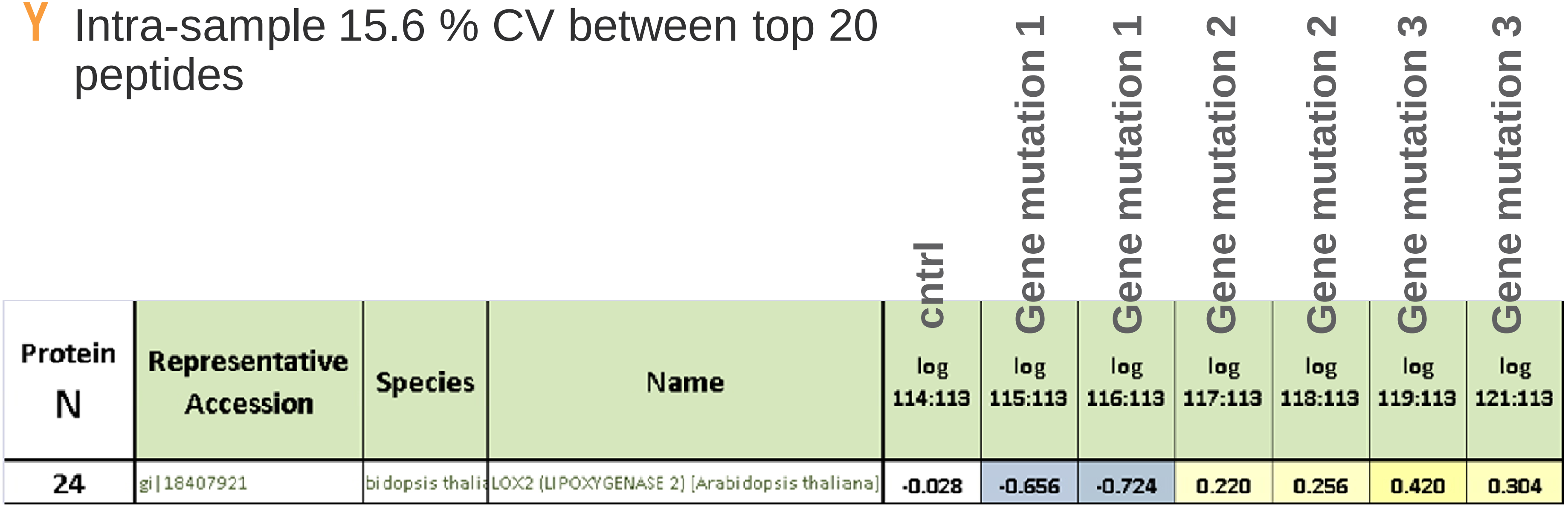
- Expanded view of reporter ions for peptide VHSGNK from *serine carboxy-peptidase*
- Here shown is a 30 x difference in reporter ion measurement



High Analytical Reproducibility

Quantitation of LOX2 Protein

- 32 peptides quantified for LOX2
- Intra-sample 15.6 % CV between top 20 peptides



Protein N	Representative Accession	Species	Name	cntrl log 114:113	Gene mutation 1 log 115:113	Gene mutation 1 log 116:113	Gene mutation 2 log 117:113	Gene mutation 2 log 118:113	Gene mutation 3 log 119:113	Gene mutation 3 log 121:113
24	gi|18407921	bidopsis thali	LOX2 (LIPOXYGENASE 2) [Arabidopsis thaliana]	-0.028	-0.656	-0.724	0.220	0.256	0.420	0.304

23.6% CV
cntrl

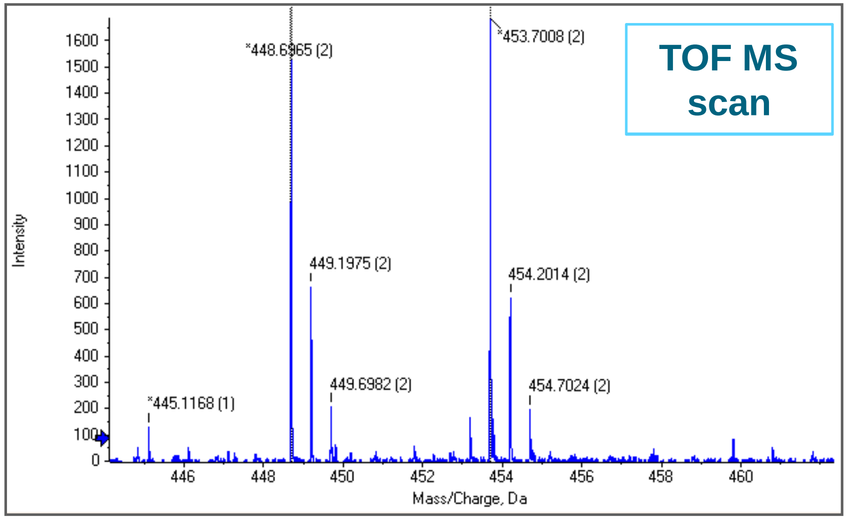
14.3% CV
Mutant 1

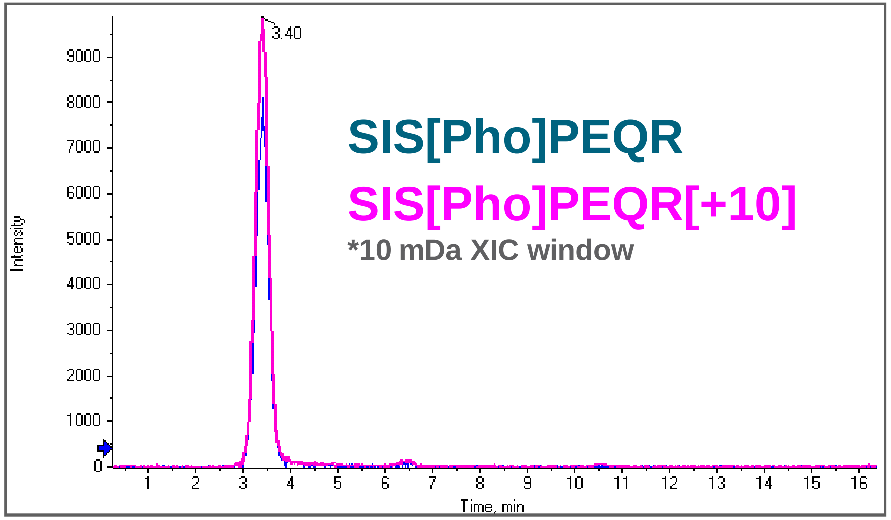
11.6% CV
Mutant 2

15.4% CV
Mutant 3

Phosphopeptide Identification & Quantitation

Protein Kinase Phosphopeptide Characterization using SILAC

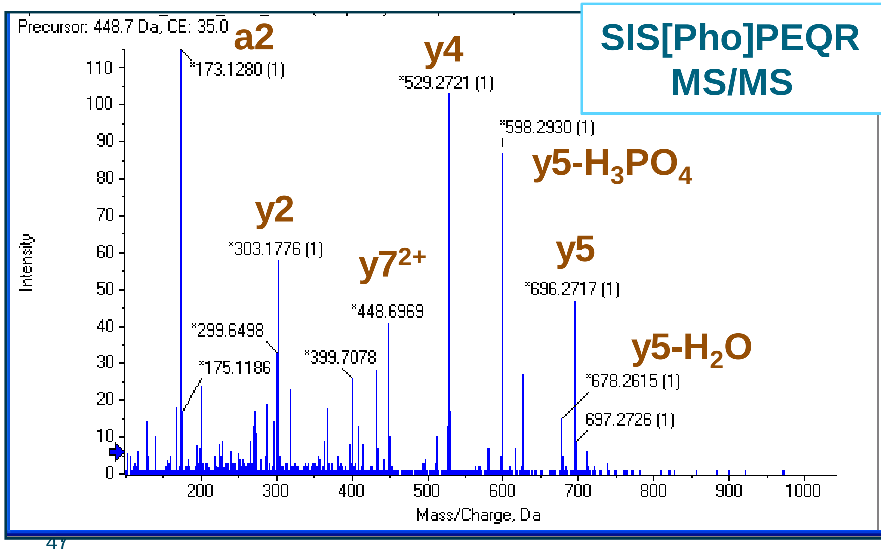




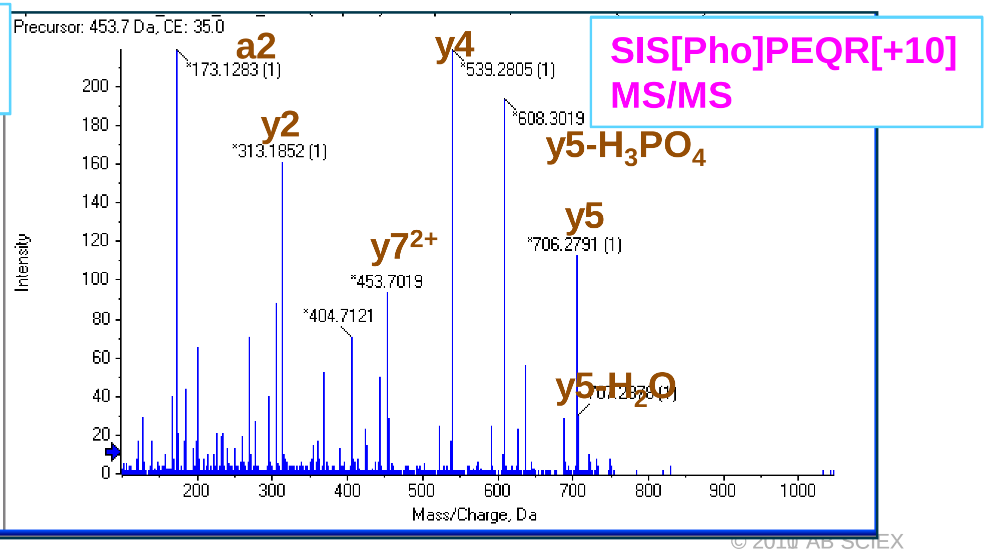
SIS[Pho]PEQR

SIS[Pho]PEQR[+10]

***10 mDa XIC window**



SIS[Pho]PEQR MS/MS



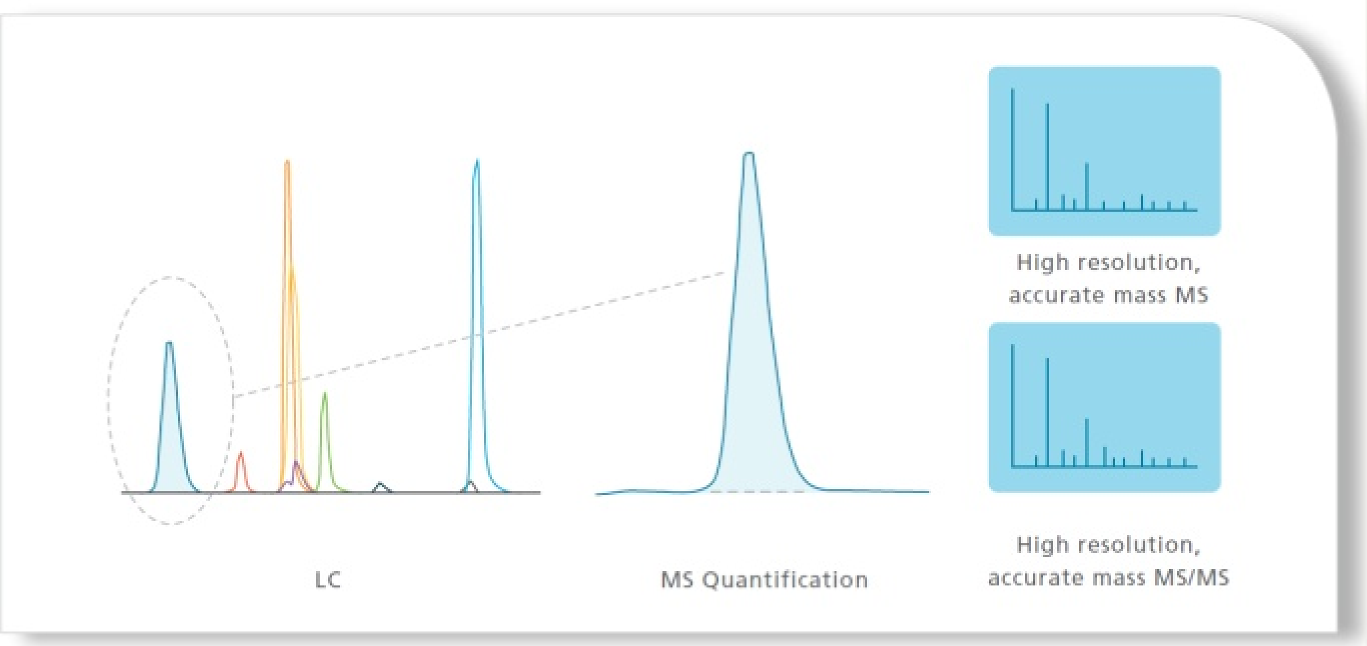
SIS[Pho]PEQR[+10] MS/MS

Agenda

- Qualitative & Quantitative Workflows

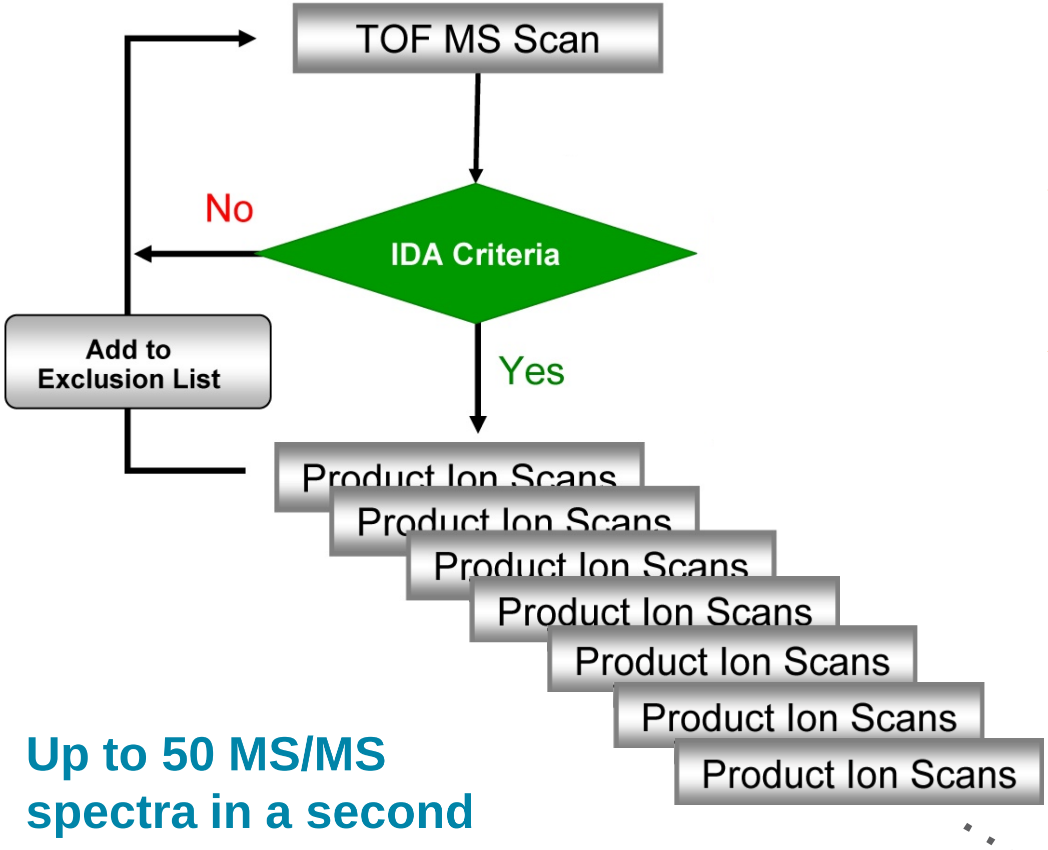
- Relative quantitation – label and labelfree workflows

Profile Workflow



- In “Profiling” mode the AB SCIEX TripleTOF™ 5600 System delivers high-resolution MS quantification of virtually every compound in the sample, especially when fast chromatography is used
- Because choosing Quant **OR** Qual is a thing of the past, high-resolution, accurate-mass MS/MS data confirms the identity of the compounds
- The data can be mined retrospectively to continue to yield information way into the future

EXPLORE / PROFILE Workflows



Up to 50 MS/MS spectra in a second (Explore)

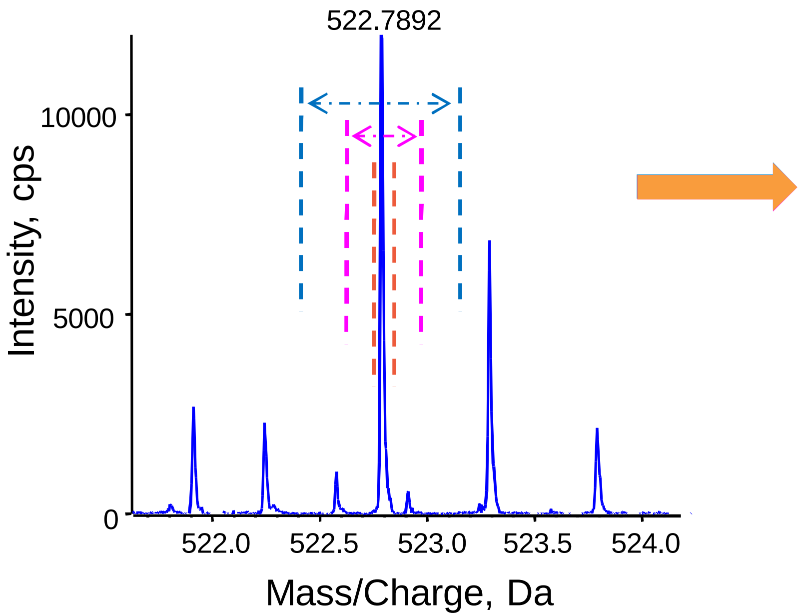
10 – 20 MS/MS spectra in a second (Profile)

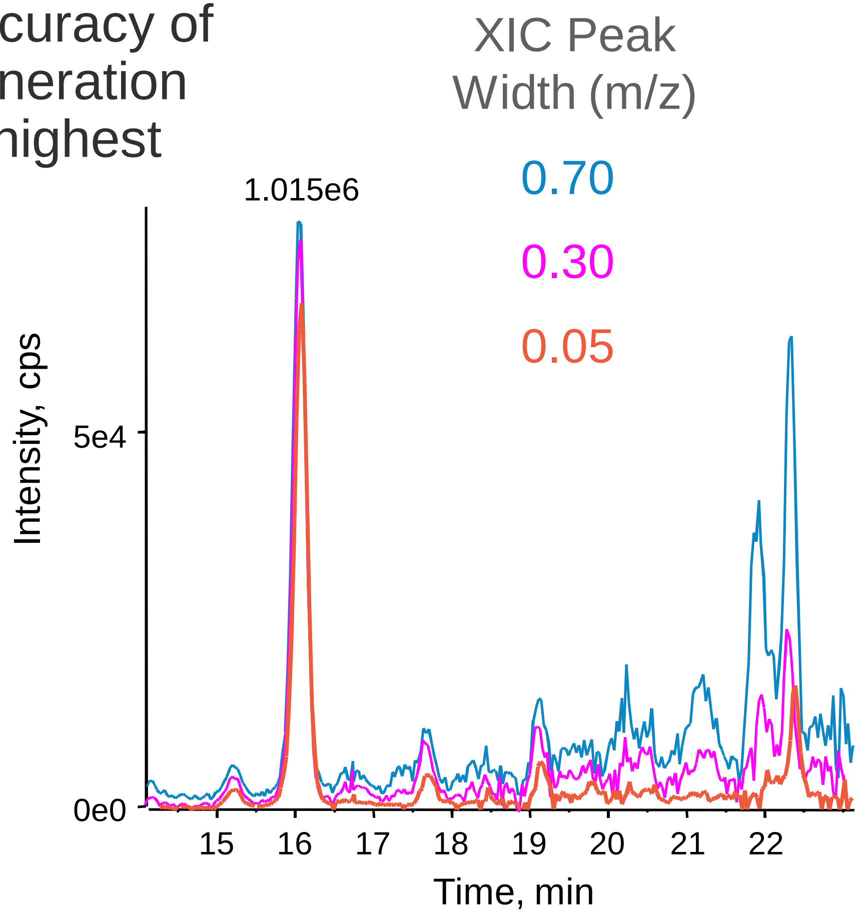
- IDA Workflow
- Cycle time is fixed
  - User specifies the maximum number of precursors and minimum accumulation time
  - Accumulation time will be dynamically distributed across available precursors

High Resolution MS Quantitation

Accurate Mass and High Resolution XIC Increase Specificity

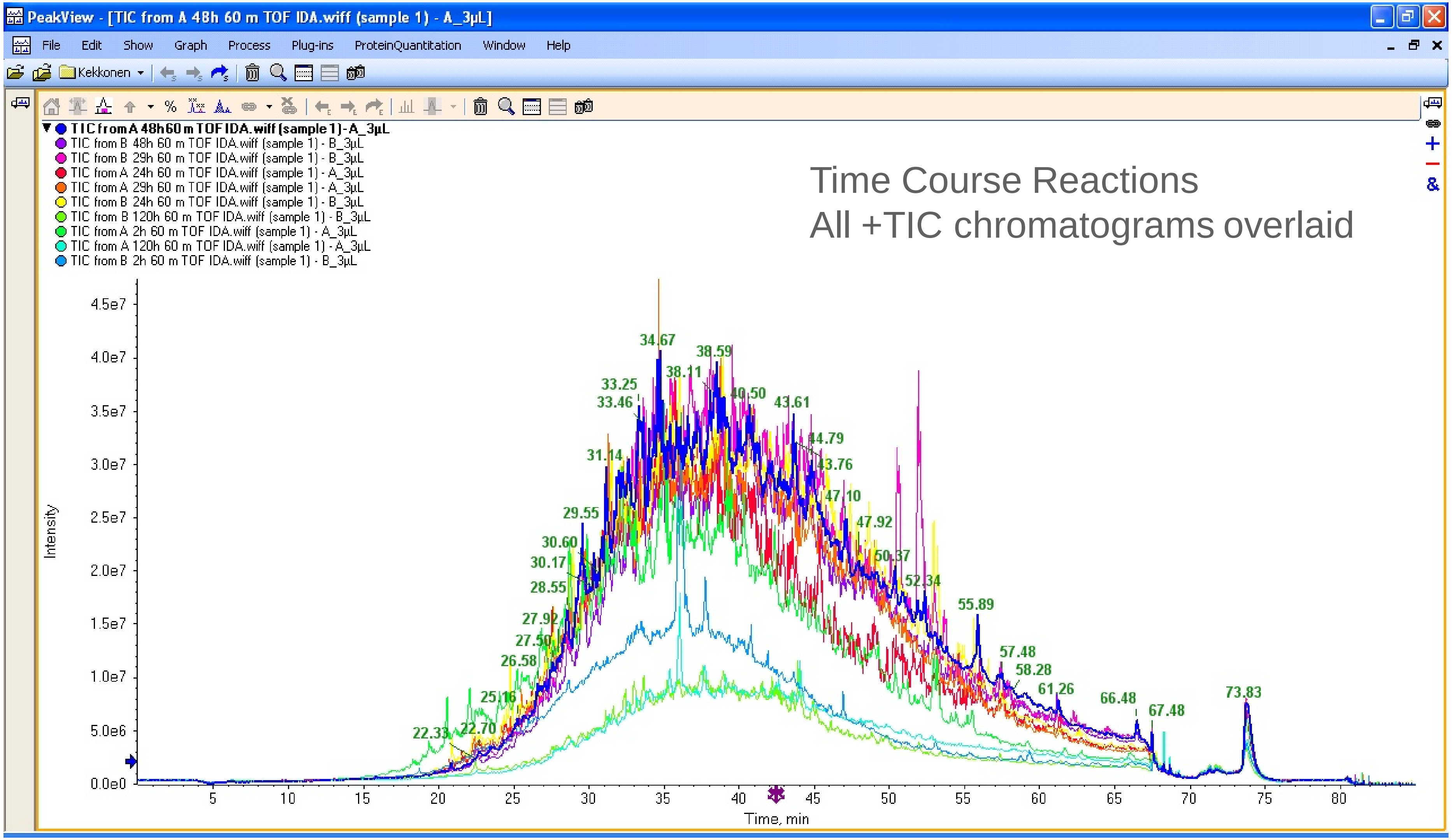
- High resolution and high mass accuracy of the TOF MS data enables the generation of MS XICs at high resolution for highest specificity.





Protein/Peptide Profiling in PeakView™ Software

Label-free Approach to Peptide Quantitation and MS/MS Sequencing

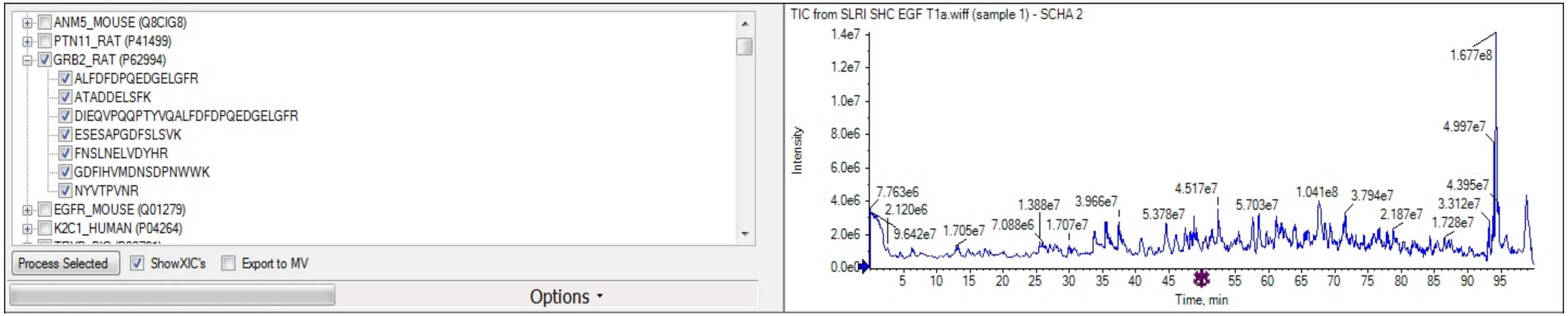


Time Course Reactions

All +TIC chromatograms overlaid

TOF-MS Quantitation of Grb2

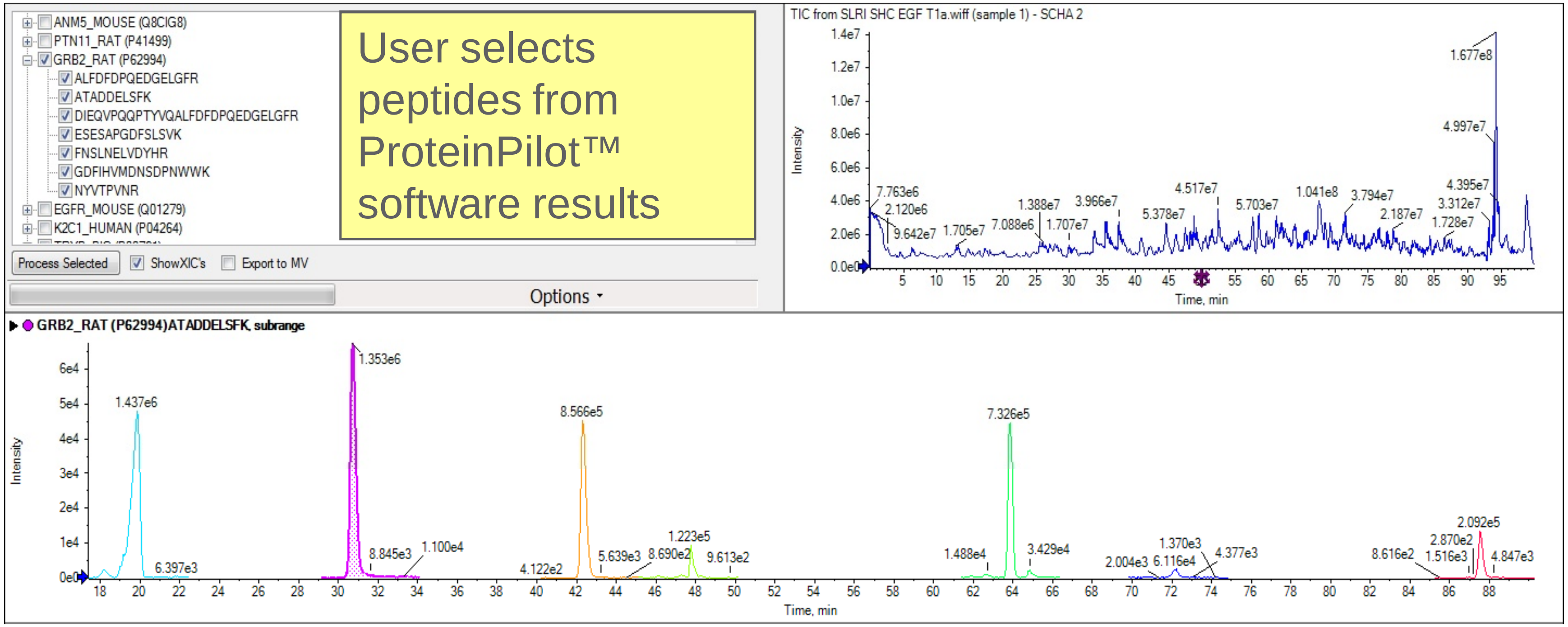
PeakView™ Software



☐ ANM5_MOUSE (Q8CIG8)
☐ PTN11_RAT (P41499)
☑ GRB2_RAT (P62994)
  ☑ ALFDFDPQEDGELGFR
  ☑ ATADDELSFK
  ☑ DIEQVPQQPTYVQALFDFDPQEDGELGFR
  ☑ ESESAPGDFSLSVK
  ☑ FNSLNELVDYHR
  ☑ GDFIHVMDNSDPNWWK
  ☑ NYVTPVNR
☐ EGFR_MOUSE (Q01279)
☐ K2C1_HUMAN (P04264)

Process Selected ☑ ShowXIC's ☐ Export to MV

XIC's for all Grb2 Peptides with High Quality MS/MS Identifications



⊞ ☐ ANM5_MOUSE (Q8CIG8)
⊞ ☐ PTN11_RAT (P41499)
⊟ ☑ GRB2_RAT (P62994)
  ☑ ALFDFDPQEDGELGFR
  ☑ ATADDELSFK
  ☑ DIEQVPQQPTYVQALFDFDPQEDGELGFR
  ☑ ESESAPGDFSLSVK
  ☑ FNSLNELVDYHR
  ☑ GDFIHVMDNSDPNWWK
  ☑ NYVTPVNR
⊞ ☐ EGFR_MOUSE (Q01279)
⊞ ☐ K2C1_HUMAN (P04264)

Process Selected ☑ ShowXIC's ☐ Export to MV

Options ▾

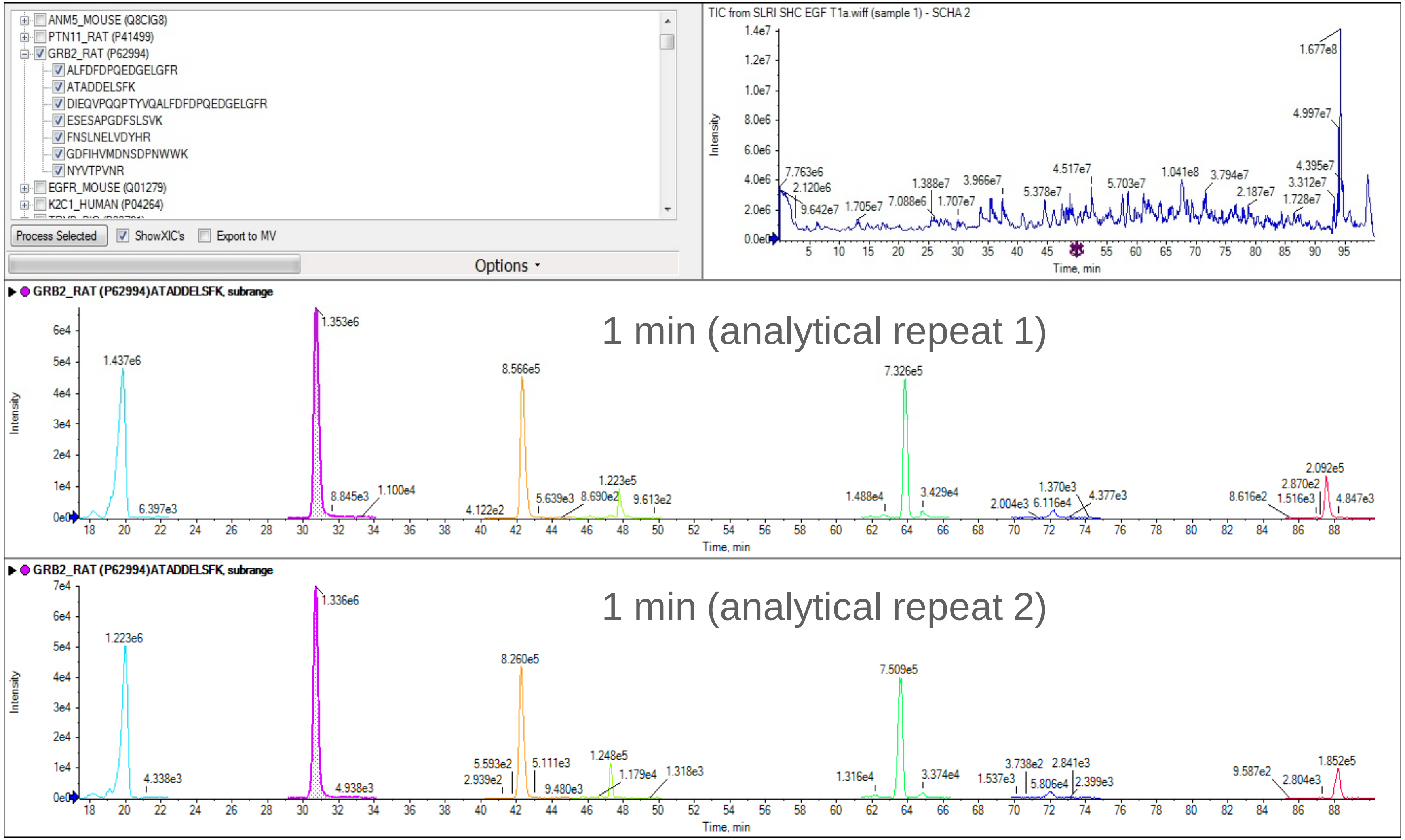
User selects peptides from ProteinPilot™ software results

TIC from SLRI SHC EGF T1a.wiff (sample 1) - SCHA 2

▶ ● GRB2_RAT (P62994)ATADDELSFK, subrange

XIC’s for all Grb2 Peptides with High Quality MS/MS Identifications

Analytical Replicates



- ⊞ ☐ ANM5_MOUSE (Q8CIG8)
- ⊞ ☐ PTN11_RAT (P41499)
- ⊟ ☑ GRB2_RAT (P62994)
  - ☑ ALFDFDPQEDGELGFR
  - ☑ ATADDELSFK
  - ☑ DIEQVPQQPTYVQALFDFDPQEDGELGFR
  - ☑ ESESAPGDFSLSVK
  - ☑ FNSLNELVDYHR
  - ☑ GDFIHVMDNSDPNWWK
  - ☑ NYVTPVNR
- ⊞ ☐ EGFR_MOUSE (Q01279)
- ⊞ ☐ K2C1_HUMAN (P04264)

Process Selected ☑ ShowXIC's ☐ Export to MV

TIC from SLRI SHC EGF T1a.wiff (sample 1) - SCHA 2

GRB2_RAT (P62994)ATADDELSFK, subrange

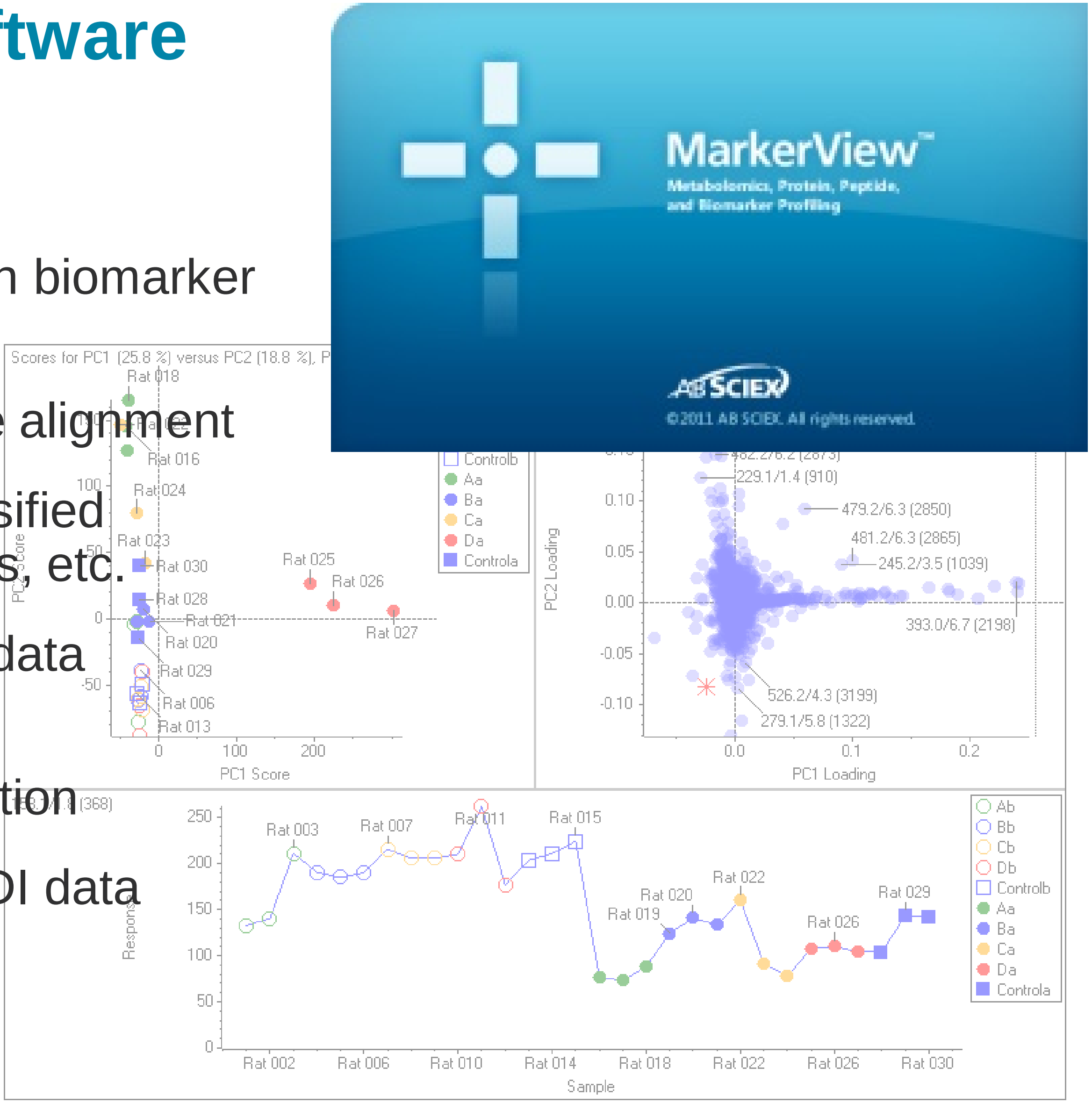
1 min (analytical repeat 1)

GRB2_RAT (P62994)ATADDELSFK, subrange

1 min (analytical repeat 2)

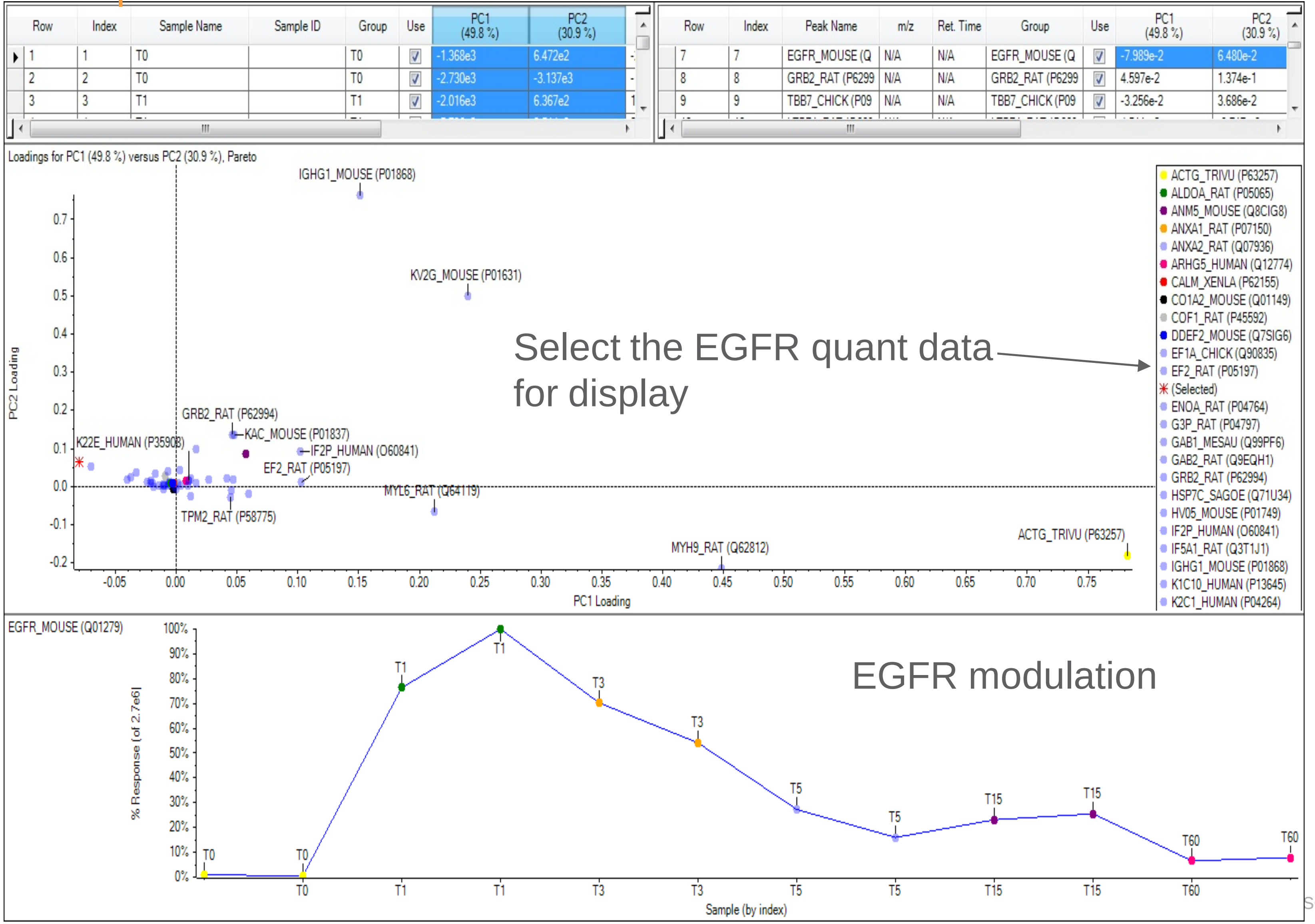
MarkerView™ Software

- Metabolomic and protein biomarker profiling workflows
- Mass and retention time alignment
- Classified and non-classified workflows - PCA, T-tests, etc.
- Informative, interactive data visualization
- Automate report generation
- Supports ESI and MALDI data



Principle Component Analysis; What are the Ds

Direct Imports into MarkerView™ Software



Row	Index	Sample Name	Sample ID	Group	Use	PC1 (49.8 %)	PC2 (30.9 %)
1	1	T0		T0	☑	-1.368e3	6.472e2
2	2	T0		T0	☑	-2.730e3	-3.137e3
3	3	T1		T1	☑	-2.016e3	6.367e2

Row	Index	Peak Name	m/z	Ret. Time	Group	Use	PC1 (49.8 %)	PC2 (30.9 %)
7	7	EGFR_MOUSE (Q	N/A	N/A	EGFR_MOUSE (Q	☑	-7.989e-2	6.480e-2
8	8	GRB2_RAT (P6299	N/A	N/A	GRB2_RAT (P6299	☑	4.597e-2	1.374e-1
9	9	TBB7_CHICK (P09	N/A	N/A	TBB7_CHICK (P09	☑	-3.256e-2	3.686e-2

Loadings for PC1 (49.8 %) versus PC2 (30.9 %), Pareto

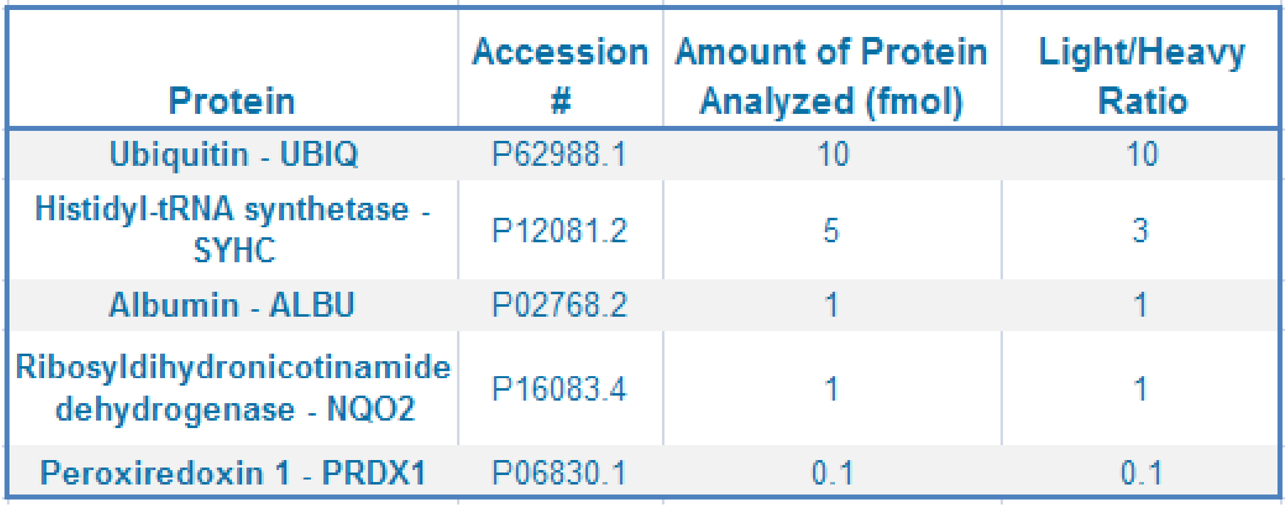
Select the EGFR quant data for display

EGFR modulation

Standard Protein Description

sPRG 2009 Sample

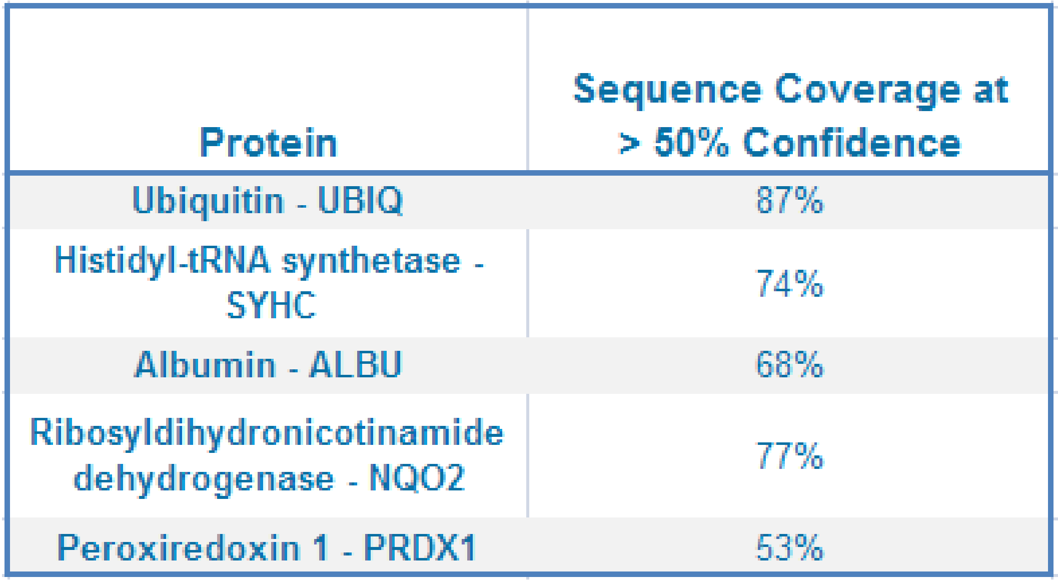
- ABRF sPRG2009 proteomic standard mixture contains 5 target and a set of heavy labeled peptide analogues in medium complexity matrix
- Developed to assess quantitative reproducibility across multiple instruments and labs.



Protein	Accession #	Amount of Protein Analyzed (fmol)	Light/Heavy Ratio
Ubiquitin - UBIQ	P62988.1	10	10
Histidyl-tRNA synthetase - SYHC	P12081.2	5	3
Albumin - ALBU	P02768.2	1	1
Ribosyldihydronicotinamide dehydrogenase - NQO2	P16083.4	1	1
Peroxiredoxin 1 - PRDX1	P06830.1	0.1	0.1

Qualitative Analysis of Standard Protein Sample

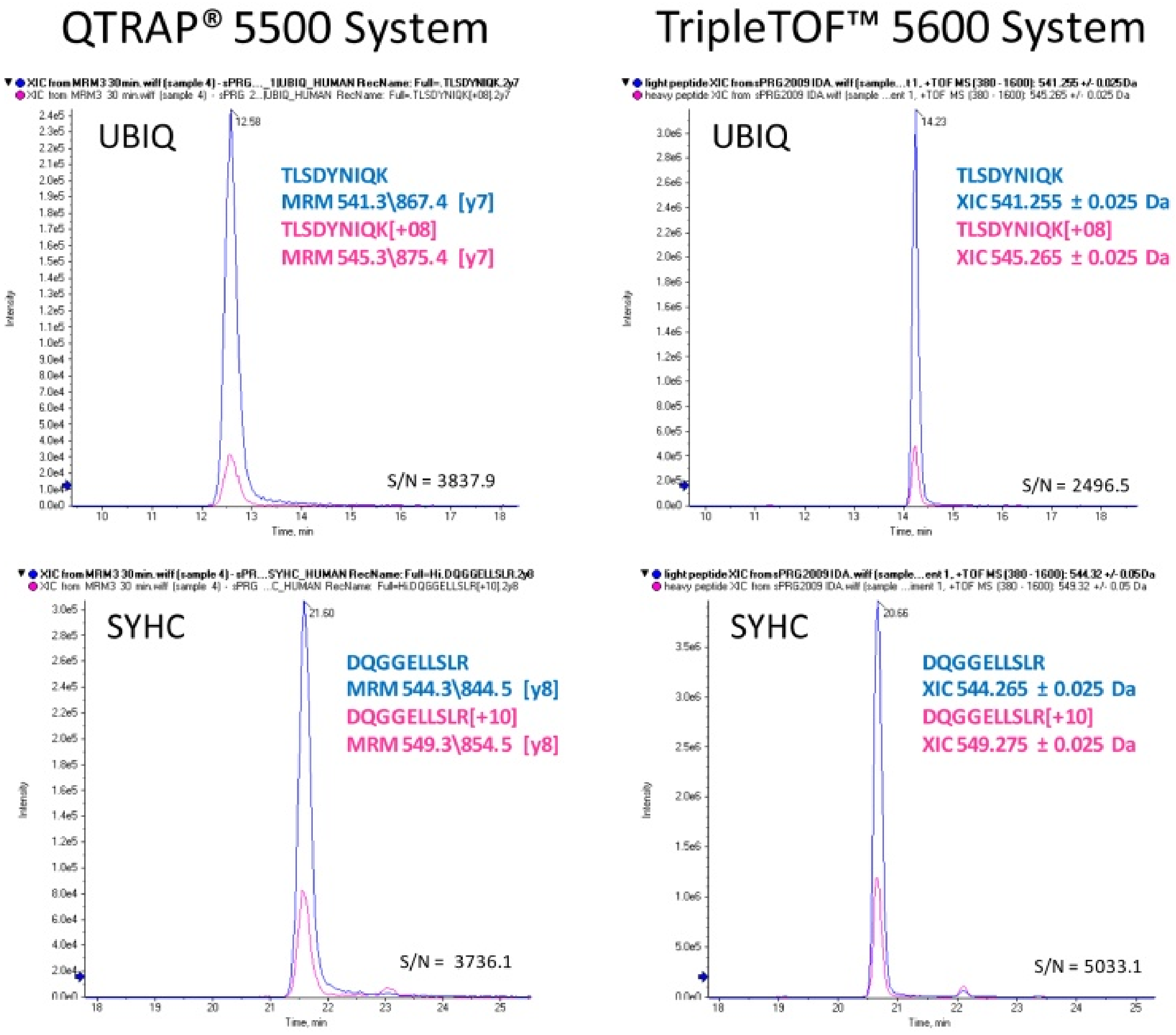
- The MS/MS data from the TripleTOF™ 5600 system was searched using the ProteinPilot™ Software
- 32 proteins and 501 unique peptides were identified at a 1% global false discovery rate
- High sequence coverage was obtained for the protein targets



Protein	Sequence Coverage at > 50% Confidence
Ubiquitin - UBIQ	87%
Histidyl-tRNA synthetase - SYHC	74%
Albumin - ALBU	68%
Ribosyldihydronicotinamide dehydrogenase - NQO2	77%
Peroxiredoxin 1 - PRDX1	53%

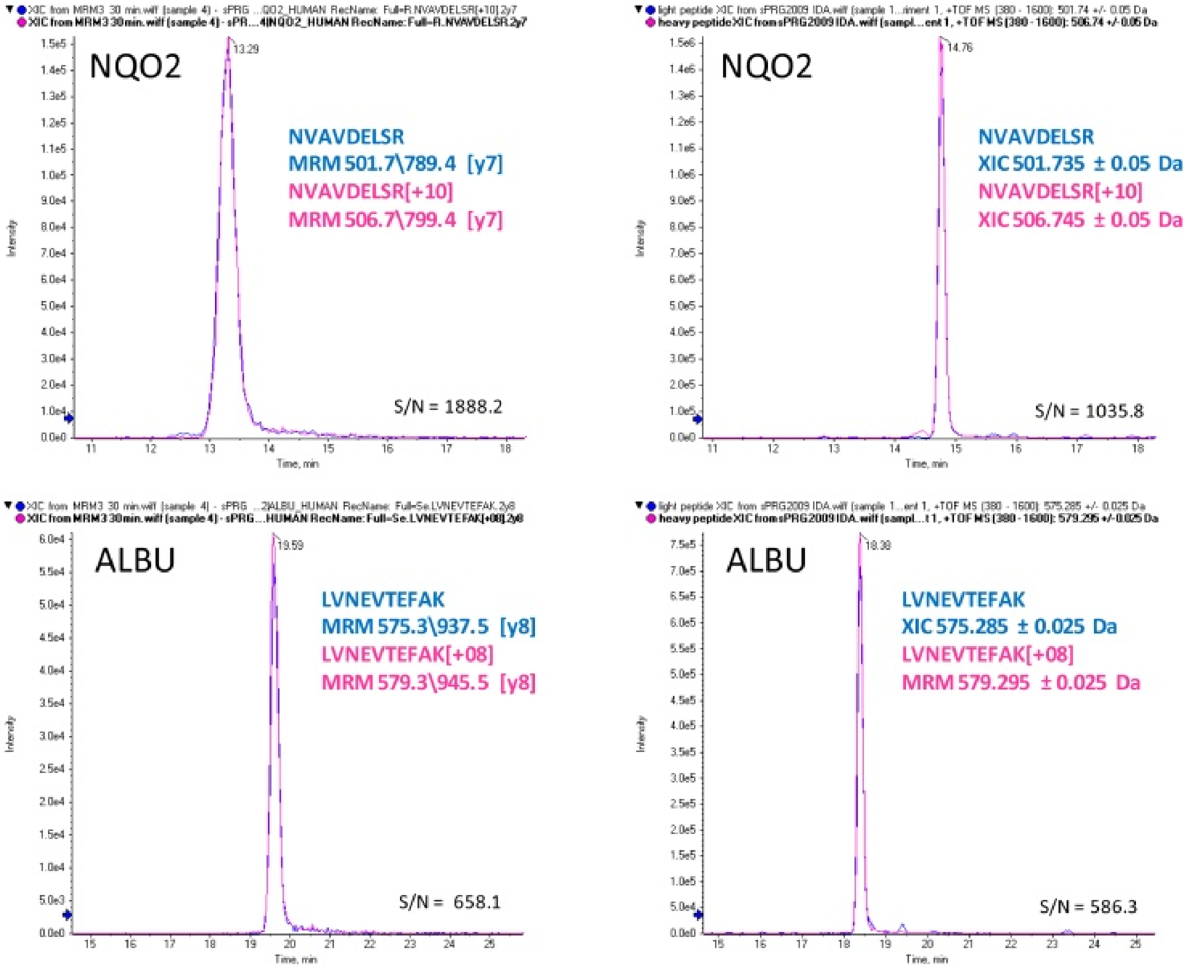
Comparing Quantitative Quality

- A comparison between the quantitation obtained from extracted ion chromatograms (XICs) from TOF MS on the TripleTOF™ System and the best MRM transition acquired on the QTRAP® 5500 System for the light (blue trace) and heavy (pink trace) labeled peptide set for each target protein.



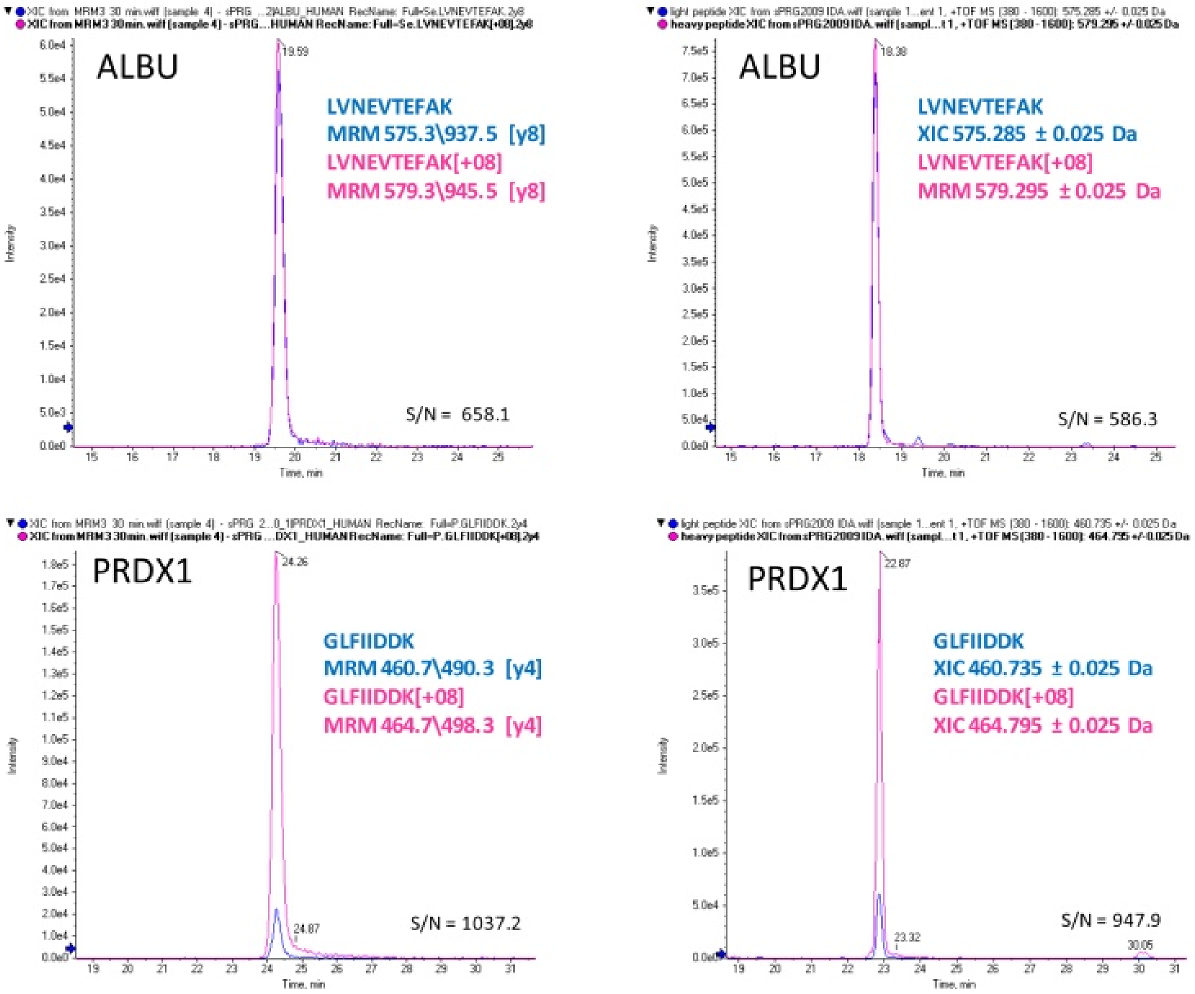
Comparing Quantitative Quality

- A comparison between the quantitation obtained from extracted ion chromatograms (XICs) from TOF MS on the TripleTOF™ System and the best MRM transition acquired on the QTRAP® 5500 System for the light (blue trace) and heavy (pink trace) labeled peptide set for each target protein.



Comparing Quantitative Quality

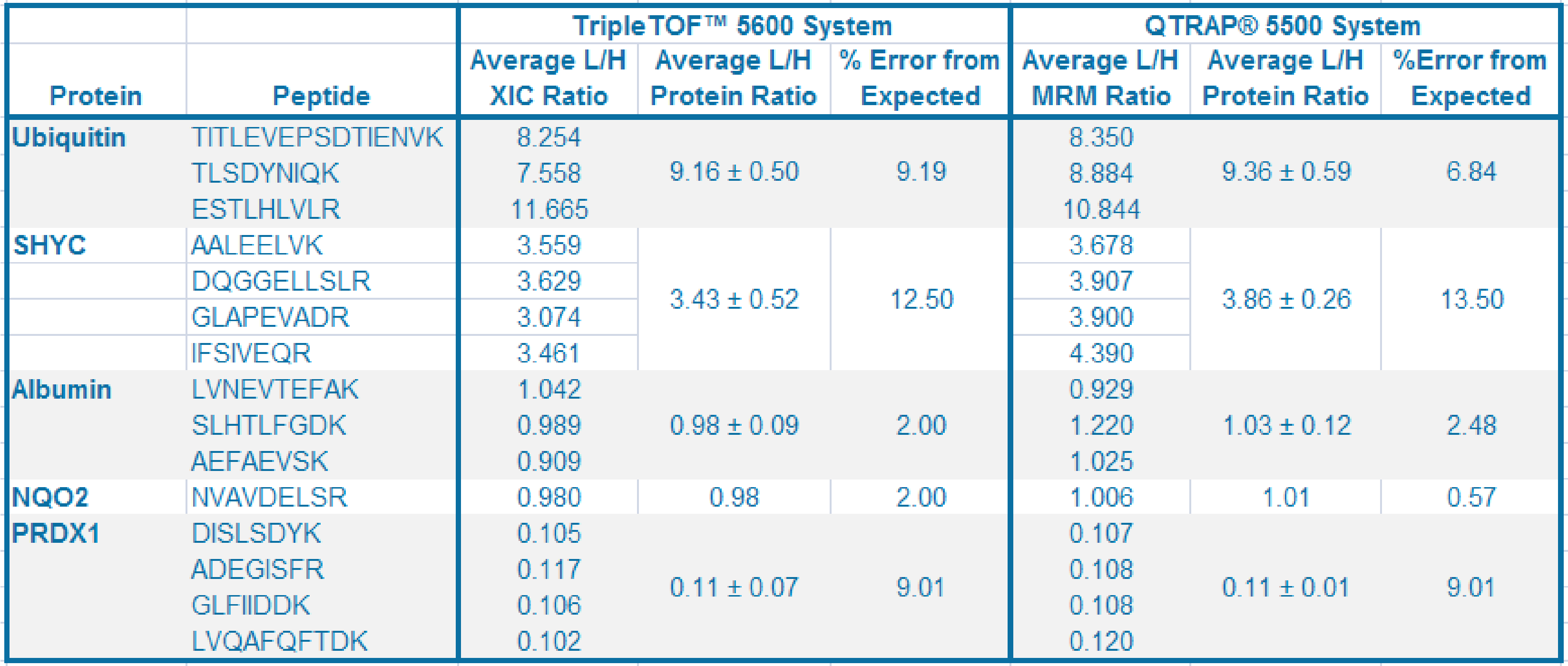
- A comparison between the quantitation obtained from extracted ion chromatograms (XICs) from TOF MS on the TripleTOF™ System and the best MRM transition acquired on the QTRAP® 5500 System for the light (blue trace) and heavy (pink trace) labeled peptide set for each target protein.



Assessing Quantitative Accuracy

TripleTOF™ 5600 System and QTRAP® 5500 System

- Measured light / heavy protein ratios for each protein agreed very well with the expected ratio
- Relative % experimental error from expected as below 12.5% for all measurements with an average of 7%



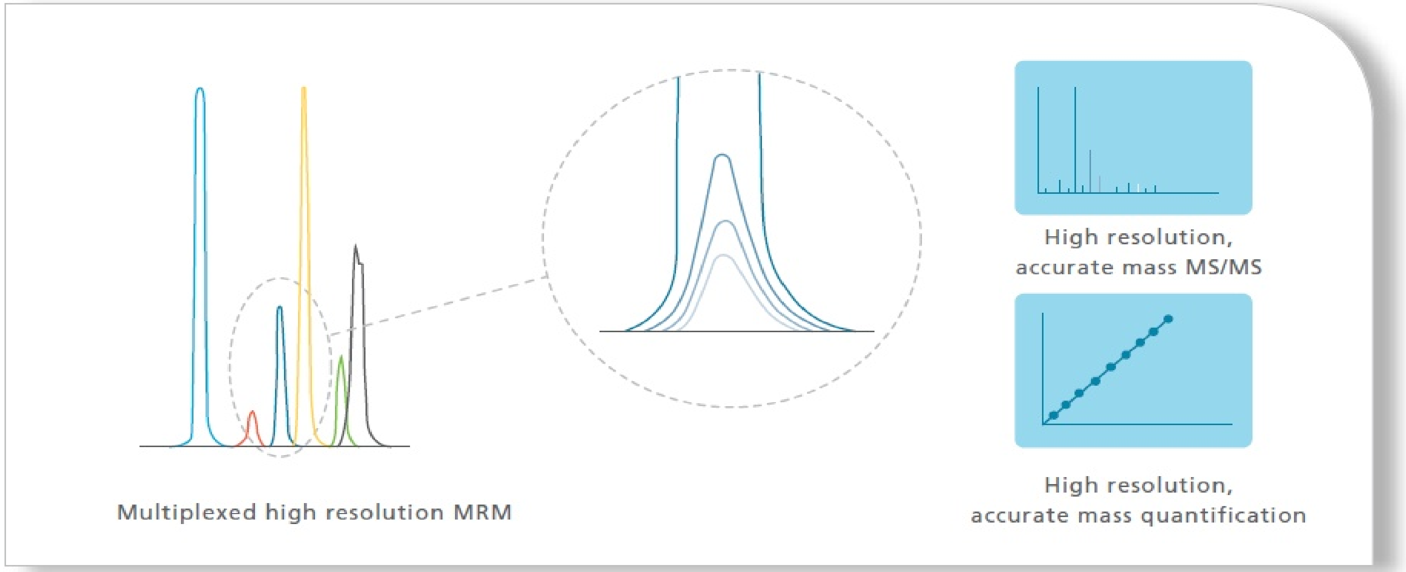
		TripleTOF™ 5600 System			QTRAP® 5500 System		
Protein	**Peptide**	**Average L/H XIC Ratio**	**Average L/H Protein Ratio**	**% Error from Expected**	**Average L/H MRM Ratio**	**Average L/H Protein Ratio**	**%Error from Expected**
Ubiquitin	TITLEVEPSDTIENVK	8.254	9.16 ± 0.50	9.19	8.350	9.36 ± 0.59	6.84
	TLSDYNIQK	7.558			8.884		
	ESTLHLVLR	11.665			10.844		
SHYC	AALEELVK	3.559	3.43 ± 0.52	12.50	3.678	3.86 ± 0.26	13.50
	DQGGELLSLR	3.629			3.907		
	GLAPEVADR	3.074			3.900		
	IFSIVEQR	3.461			4.390		
Albumin	LVNEVTEFAK	1.042	0.98 ± 0.09	2.00	0.929	1.03 ± 0.12	2.48
	SLHTLFGDK	0.989			1.220		
	AEFAEVSK	0.909			1.025		
NQO2	NVAVDELSR	0.980	0.98	2.00	1.006	1.01	0.57
PRDX1	DISLSDYK	0.105	0.11 ± 0.07	9.01	0.107	0.11 ± 0.01	9.01
	ADEGISFR	0.117			0.108		
	GLFIIDDK	0.106			0.108		
	LVQAFQFTDK	0.102			0.120		

Agenda

-
- Qualitative & Quantitative Workflows

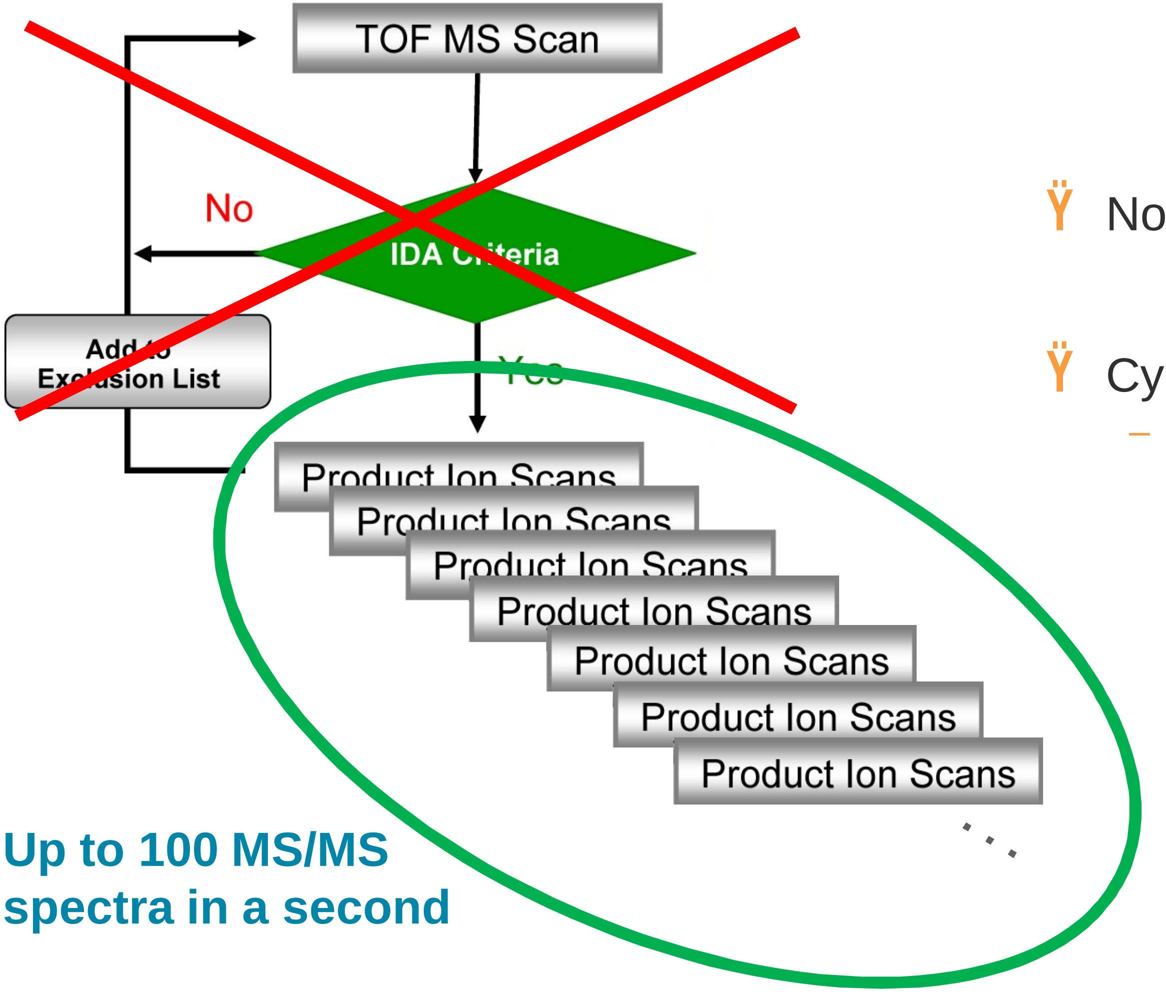
  - Absolute and relative quantitation in high resolution MS/MS

QUANTIFY Workflow



- High-resolution MRM-like quantification using the TripleTOF™ 5600 system
- LOQ’s and dynamic range equivalent to high performance QQQ
- Cycle times as fast as 10 milliseconds – compatible with fast separations
- Minimal method development required

QUANTIFY Workflow



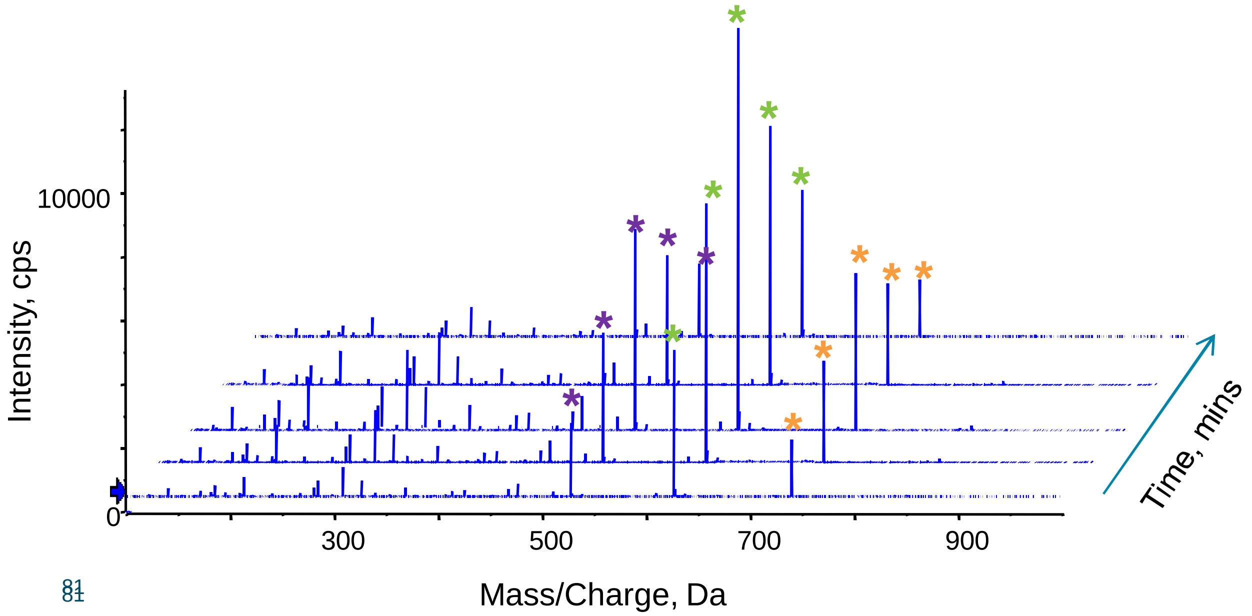
Up to 100 MS/MS spectra in a second

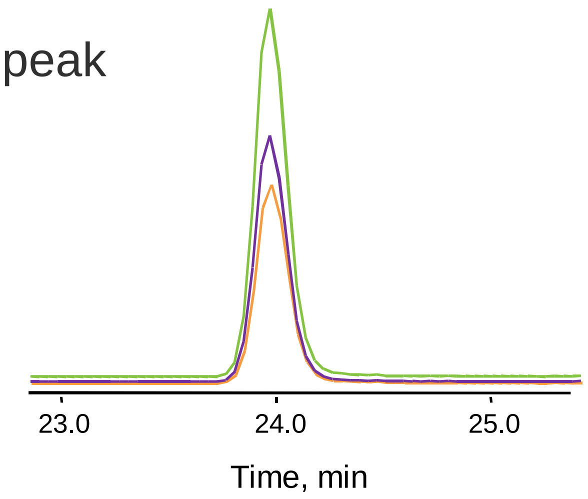
- Non-IDA Workflow
- Cycle time is fixed
  - User specifies precursors and accumulation time

Looped TOF MS/MS

Post Acquisition Data Extraction

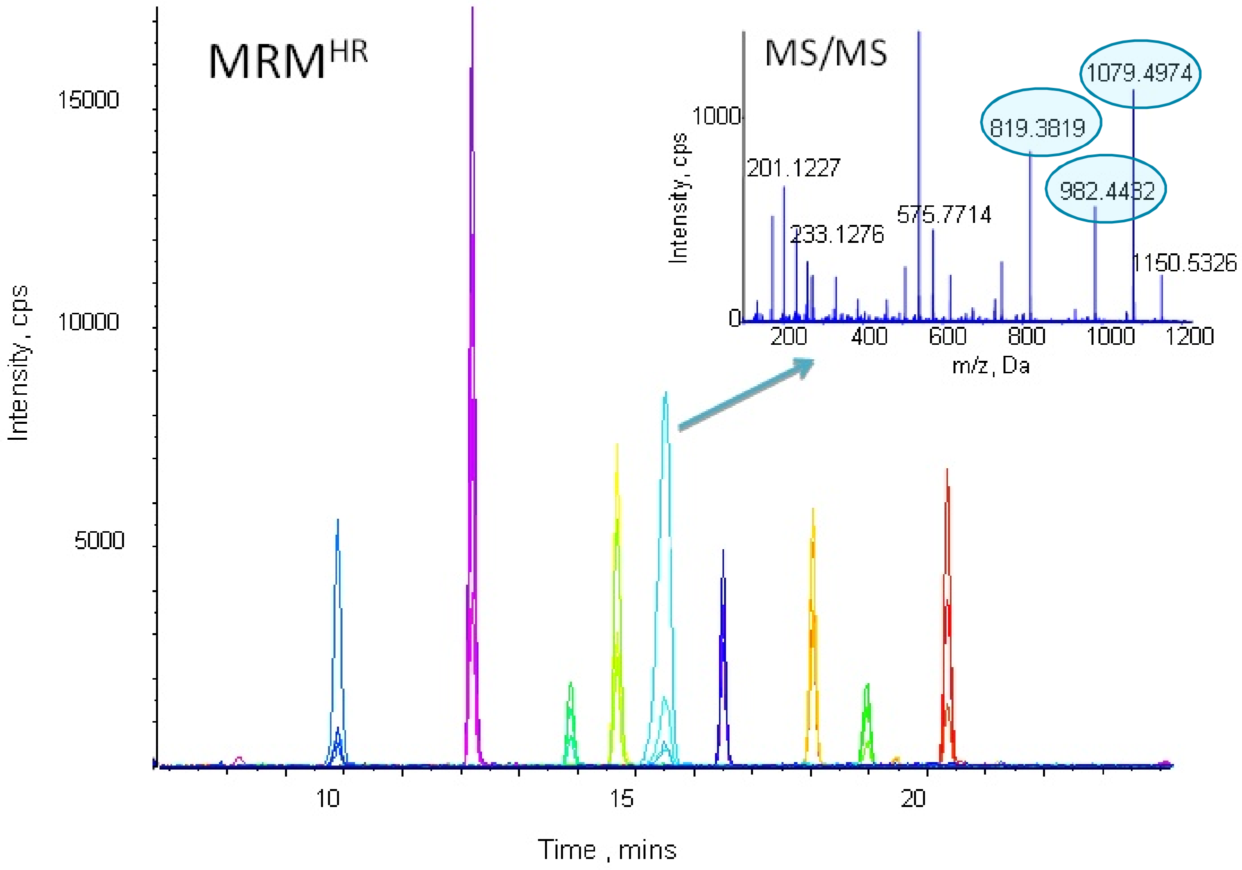
- Looped full scan MS/MS is acquired across the LC peak at high resolution, >15,000 or >30,000
- High resolution XIC are generated post-acquisition, single or multiple
- Quantitation processing then is similar to MRM
- Fast acquisition ensures sufficient points across the peak





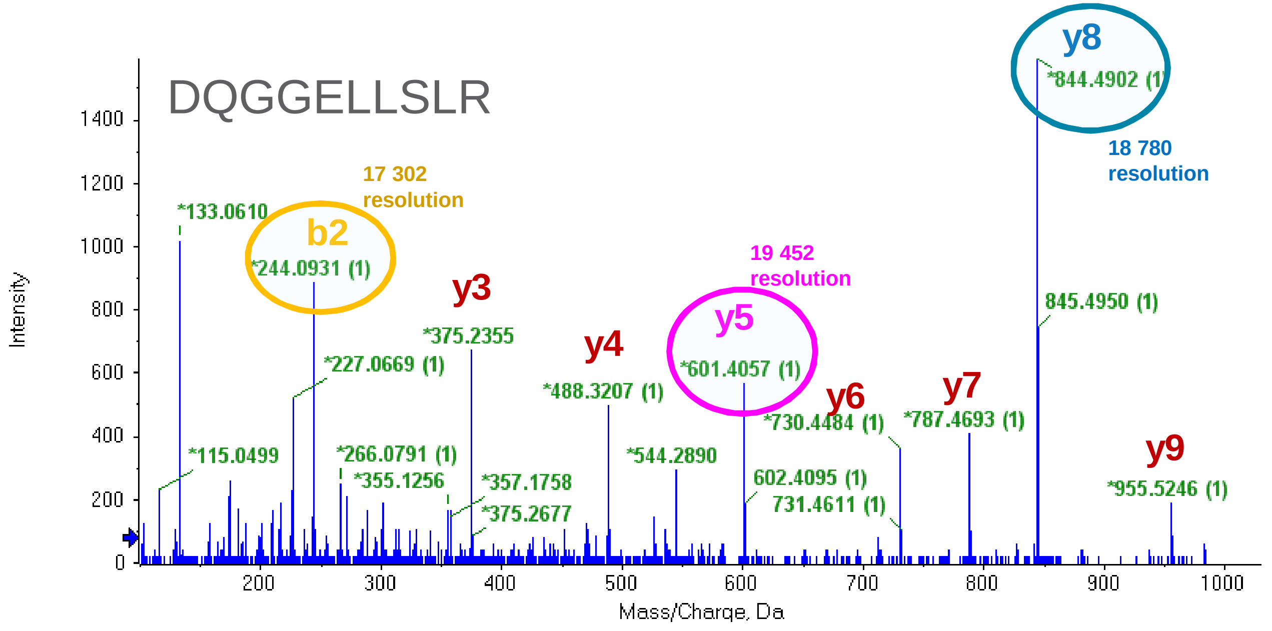
Getting So Much More From MRM^{HR}

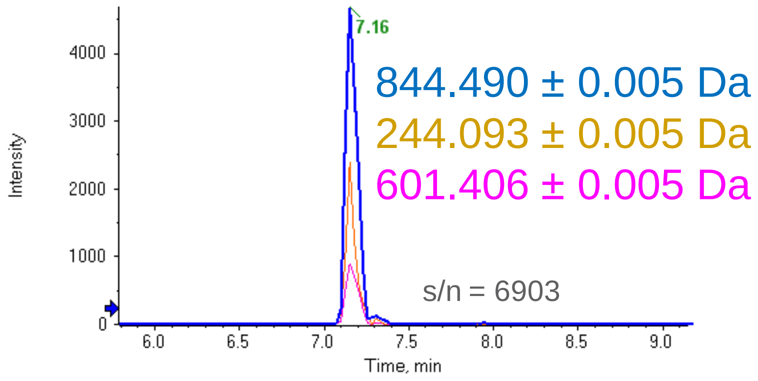
High Sensitivity Peptide Quantitation

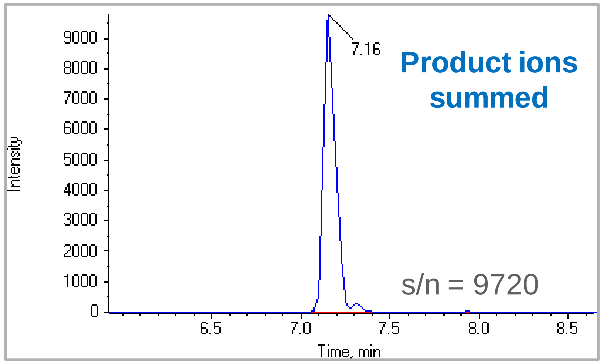


Looped Full Scan TOF MS/MS for Peptide Quant

High Resolution MRM-like Quantitation - MRM^{HR}





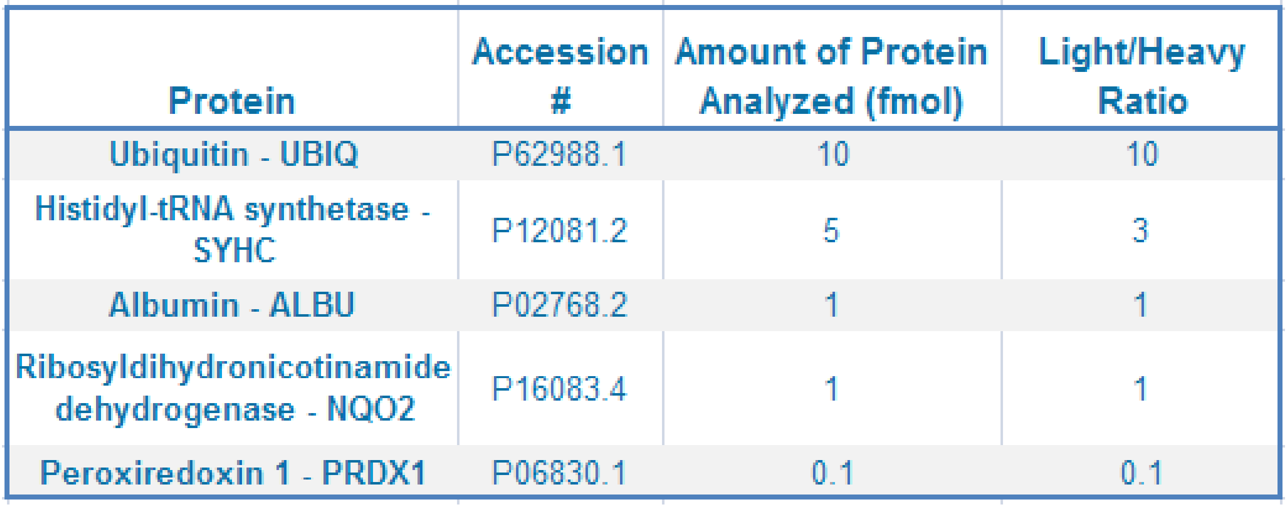


- Looped full scan MS/MS is acquired across the LC peak at high resolution, >15000
- High resolution XIC are generated post-acquisition in 5 mDa windows
- Multiple fragment ions can be chosen and/or summed

Quantitative Standard Protein Mix

sPRG 2009 Sample Description

- ABRF sPRG2009 proteomic standard mixture contains 5 target and a set of heavy labeled peptide analogues in medium complexity matrix
- Developed to assess quantitative reproducibility across multiple instruments and labs.



Protein	Accession #	Amount of Protein Analyzed (fmol)	Light/Heavy Ratio
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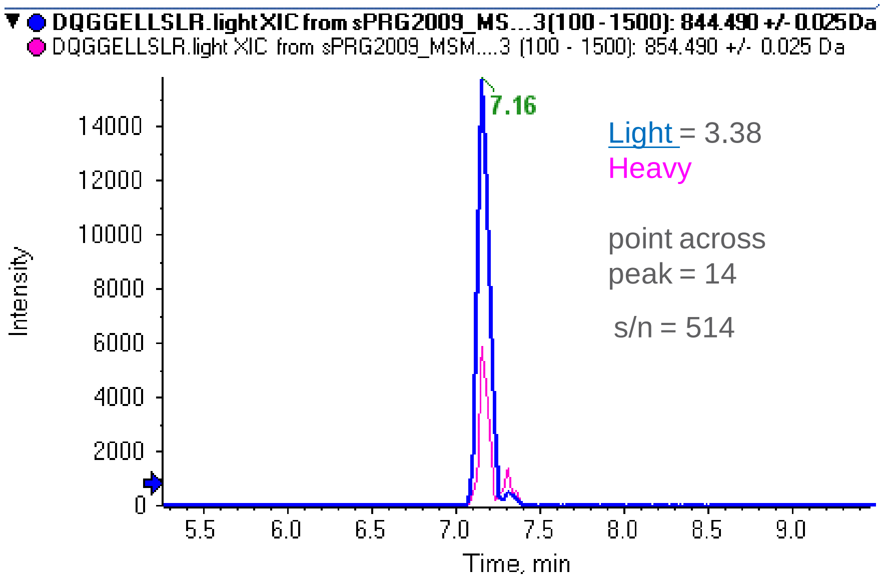
Ribosyldihydronicotinamide dehydrogenase - NQO2	P16083.4	1	1
Peroxiredoxin 1 - PRDX1	P06830.1	0.1	0.1

Peptide Quantitation at HPLC Flow

Sensitivity & Selectivity using the MRM^{HR} Approach

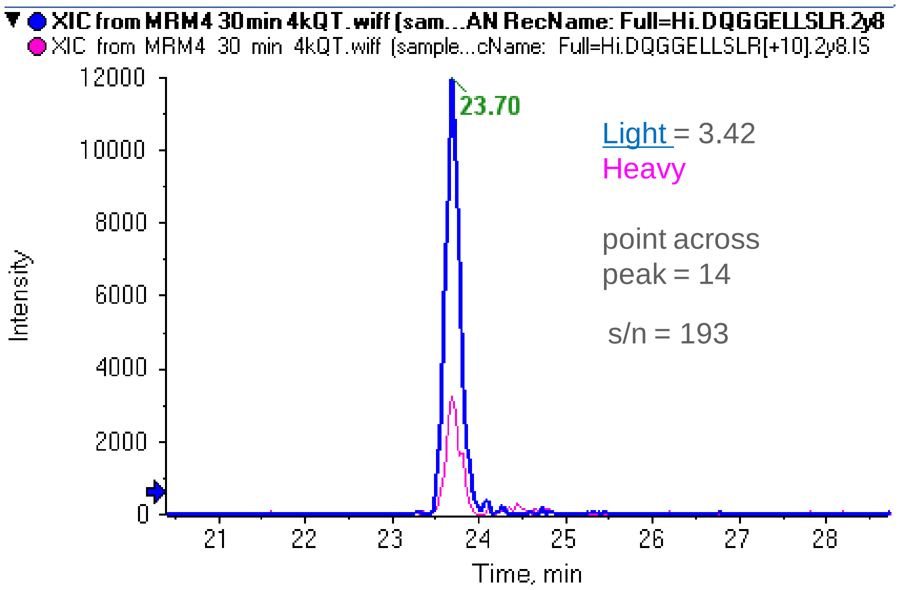
TripleTOF™ 5600 System



XIC [y8] DQGGELLSLR

XIC [y8] DQGGELLSLR[+10]

4000 QTRAP® System



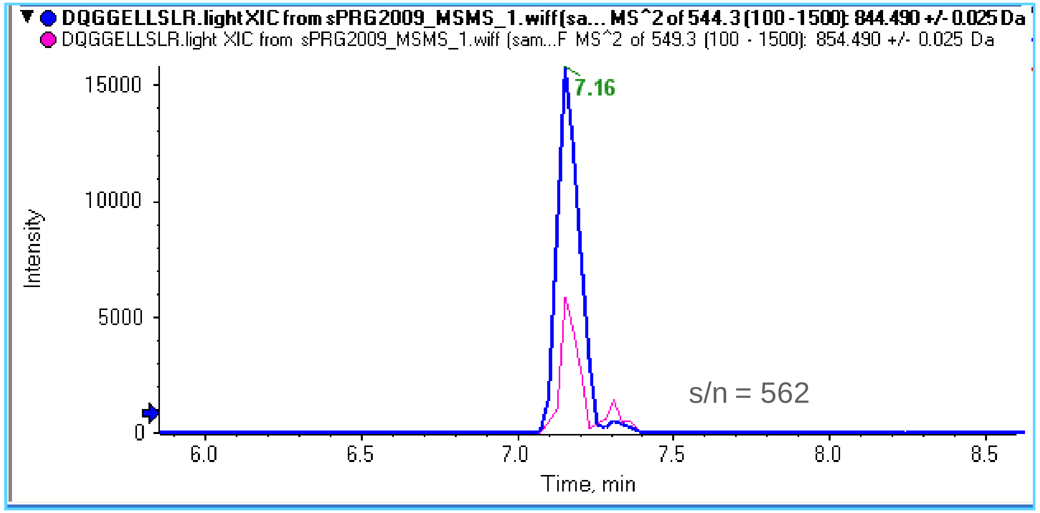
MRM [y8] DQGGELLSLR

MRM [y8] DQGGELLSLR[+10]

- Accurate Peptide Quantitation with 4000 QTRAP-level sensitivity at high flow chromatography [200 µL/min]

Two Modes of High Resolution Peptide Quant

High Res TOF MS vs MRM^{HR} Quantitation

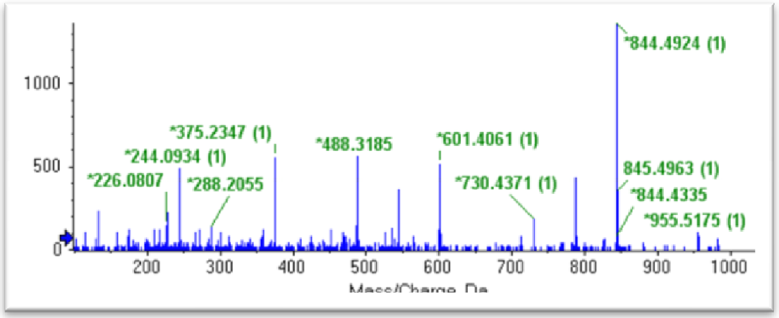


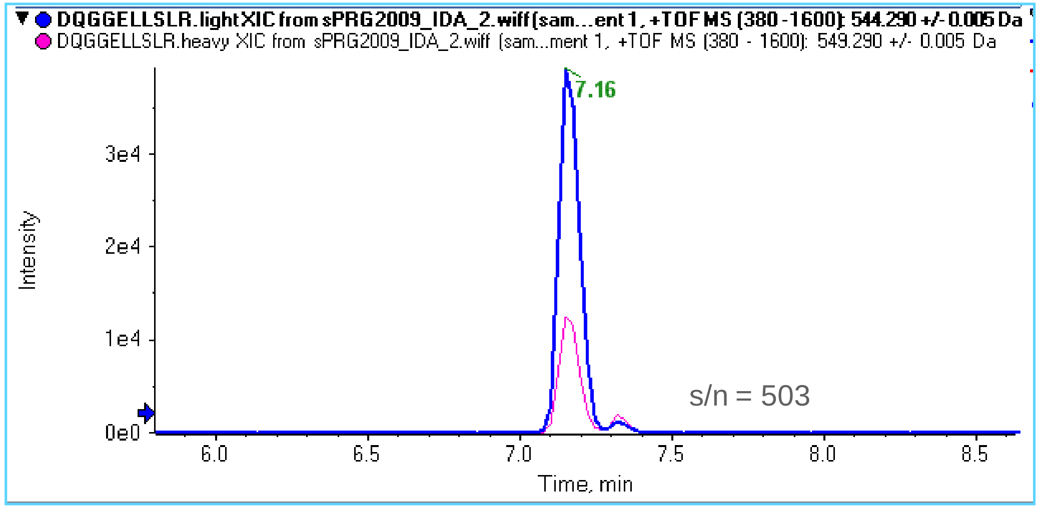
s/n = 562

MRM^{HR}

XIC [y8] DQGGELLSLR

XIC [y8] DQGGELLSLR[+10]



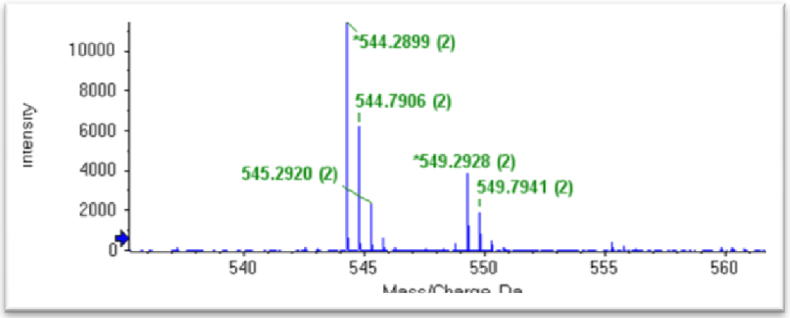


s/n = 503

High Res TOF MS

DQGGELLSLR ± 5 mDa

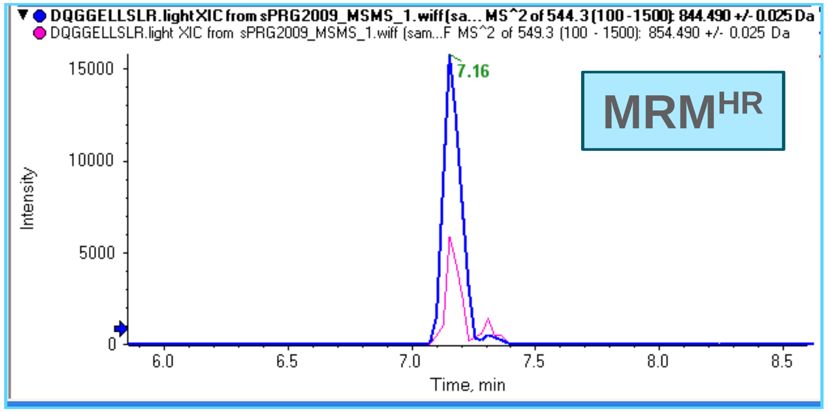
DQGGELLSLR[+10] ± 5 mDa

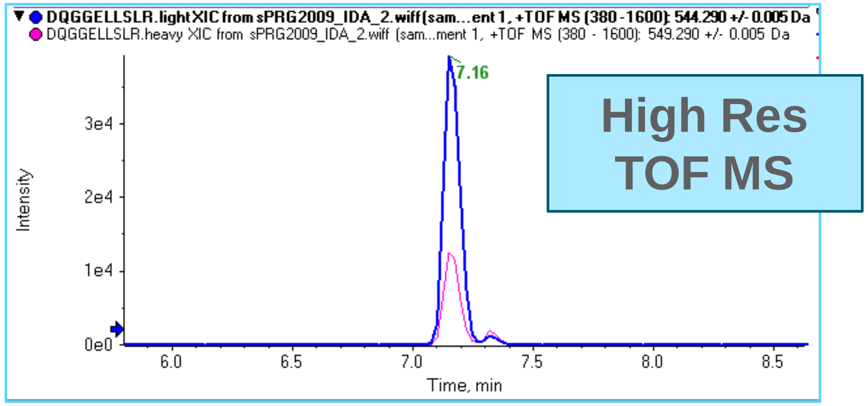


Two Modes of High Resolution Peptide Quant

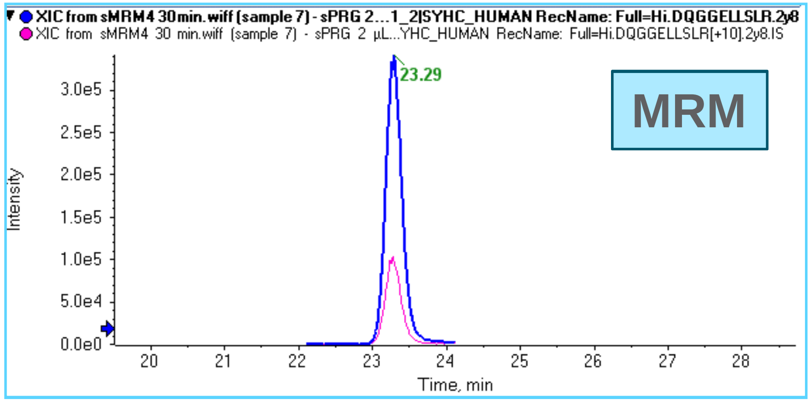
High Res TOF MS vs MRM^{HR} Quantitation of DQGGELLSLR light|heavy

TripleTOF™ 5600 System

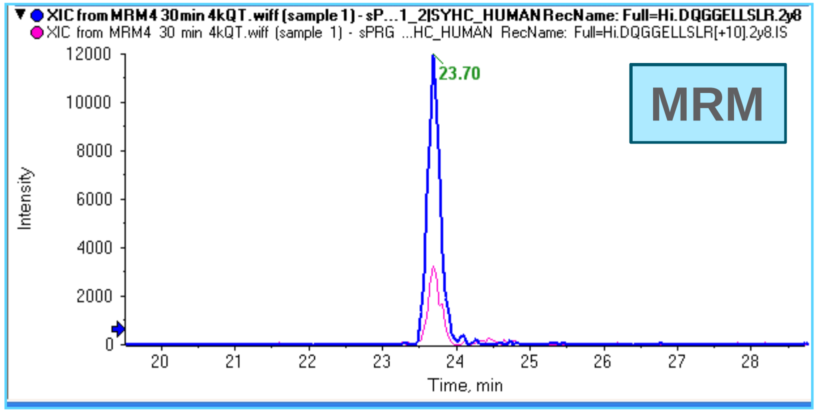




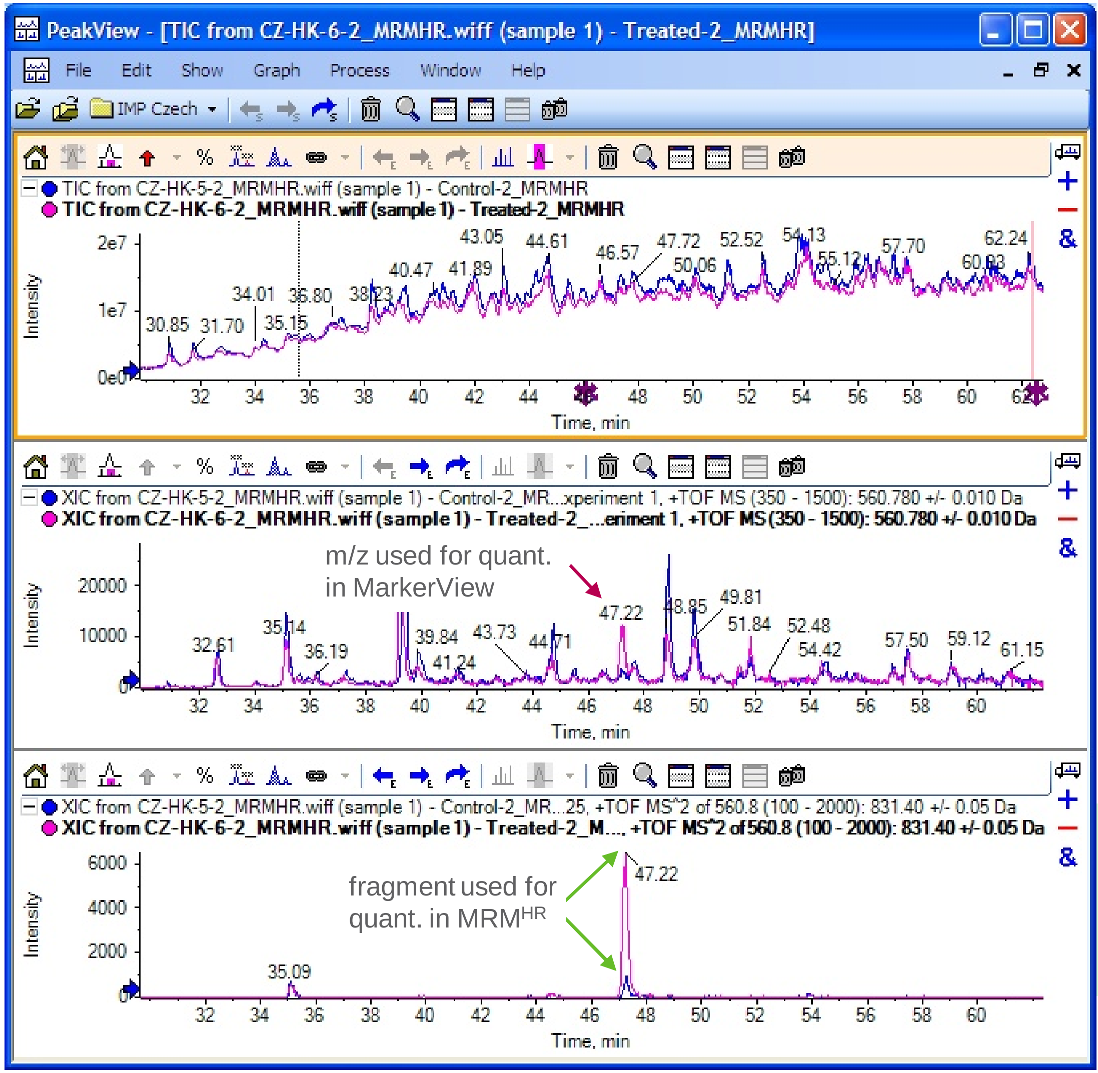
QTRAP® 5500 System



4000 QTRAP® System



MS vs. MS/MS based quantitation


PeakView - [TIC from CZ-HK-6-2_MRMHR.wiff (sample 1) - Treated-2_MRMHR]

TIC from CZ-HK-5-2_MRMHR.wiff (sample 1) - Control-2_MRMHR
TIC from CZ-HK-6-2_MRMHR.wiff (sample 1) - Treated-2_MRMHR

XIC from CZ-HK-5-2_MRMHR.wiff (sample 1) - Control-2_MR...xperiment 1, +TOF MS (350 - 1500): 560.780 +/- 0.010 Da
XIC from CZ-HK-6-2_MRMHR.wiff (sample 1) - Treated-2_...eriment 1, +TOF MS (350 - 1500): 560.780 +/- 0.010 Da

m/z used for quant.
in MarkerView

XIC from CZ-HK-5-2_MRMHR.wiff (sample 1) - Control-2_MR...25, +TOF MS^2 of 560.8 (100 - 2000): 831.40 +/- 0.05 Da
XIC from CZ-HK-6-2_MRMHR.wiff (sample 1) - Treated-2_M..., +TOF MS^2 of 560.8 (100 - 2000): 831.40 +/- 0.05 Da

fragment used for
quant. in MRM^{HR}

MS based XIC of peptide m/z
the lower amount is close to the overall background
à low S/N à weak quant. accuracy
(this holds true esp. for very complex samples)

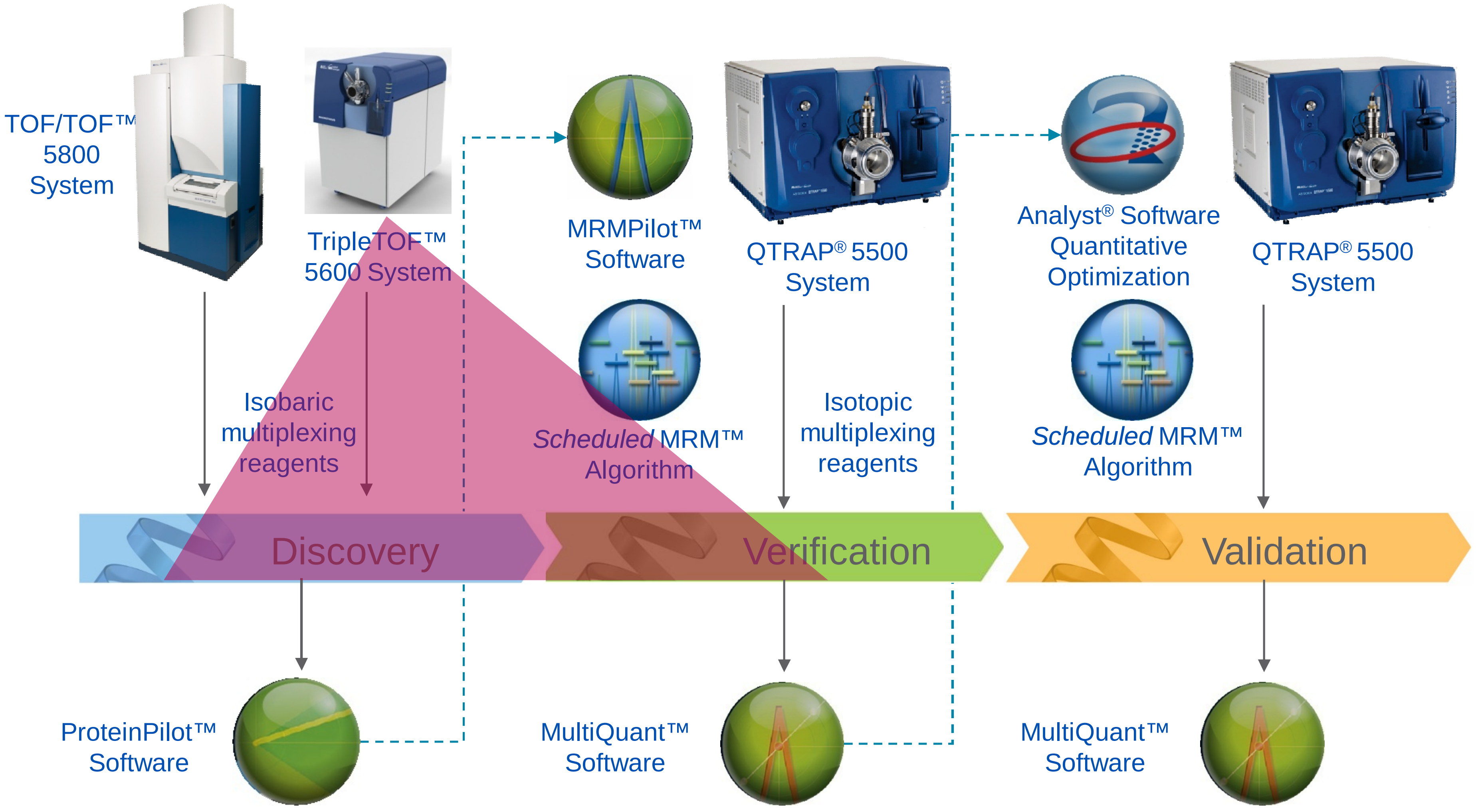
MS/MS based XIC of a specific fragment of the peptide
background is very low à high S/N à excellent quant. accuracy

Summary

- TripleTOF 5600
  - High resolution, speed, sensitivity at the same time

- Protein ID
  - Explore workflow
- Protein Quant
  - Relative (Discovery) from MS/MS
    - iTRAQ
      - Explore Workfow
  - Relative (Discovery) from MS
    - SILAC, label free
      - Profile Workfow
  - Targeted (Verification)
    - Quantify Workflow (MRM^{HR})

Protein and Biomarker Research Pipeline



TOF/TOF™ 5800 System

TripleTOF™ 5600 System

MRMPilot™ Software

QTRAP® 5500 System

Analyst® Software Quantitative Optimization

QTRAP® 5500 System

Isobaric multiplexing reagents

Scheduled MRM™ Algorithm

Isotopic multiplexing reagents

Scheduled MRM™ Algorithm

Discovery

Verification

Validation

ProteinPilot™ Software

MultiQuant™ Software

MultiQuant™ Software

Thank you for your attention

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