
Chimeric Spectra and Data-Driven DIA analysis

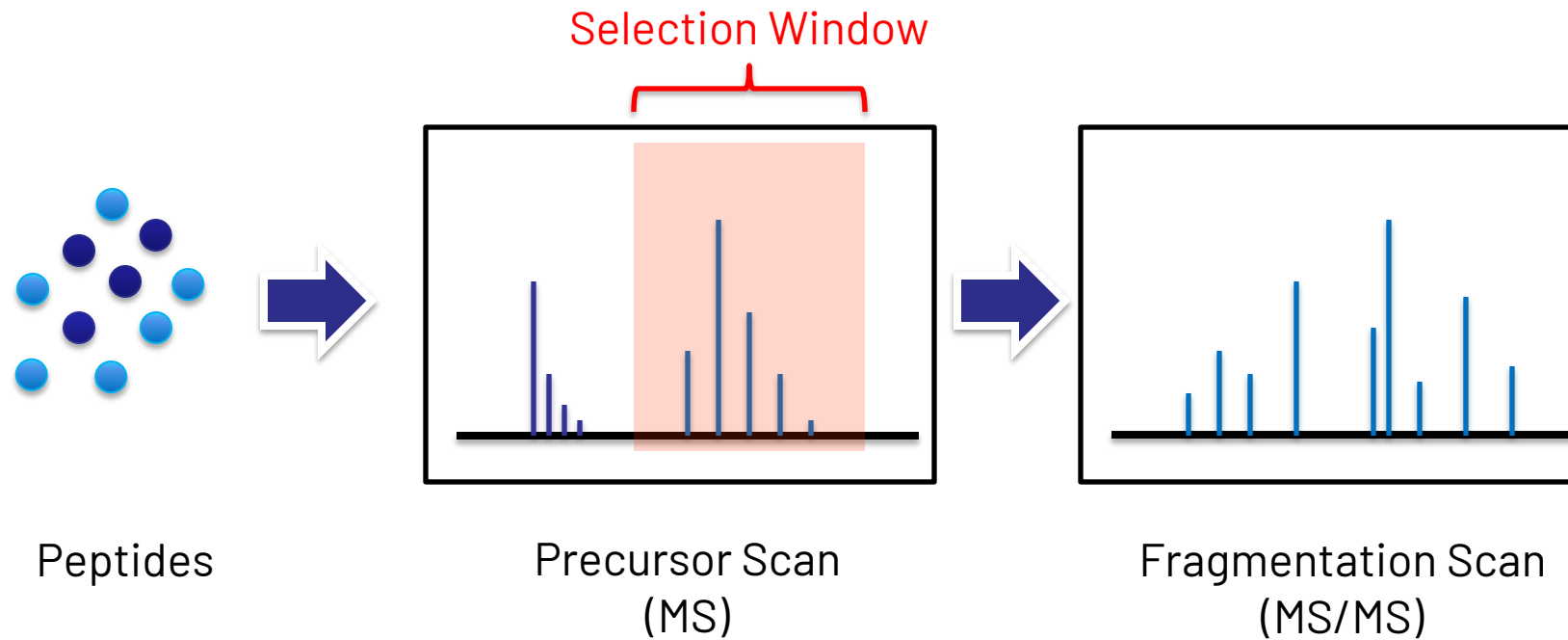
reSpect, DISCo, Quantic

reSpect

**Finding chimeric peptides from
high resolution MS/MS spectra**

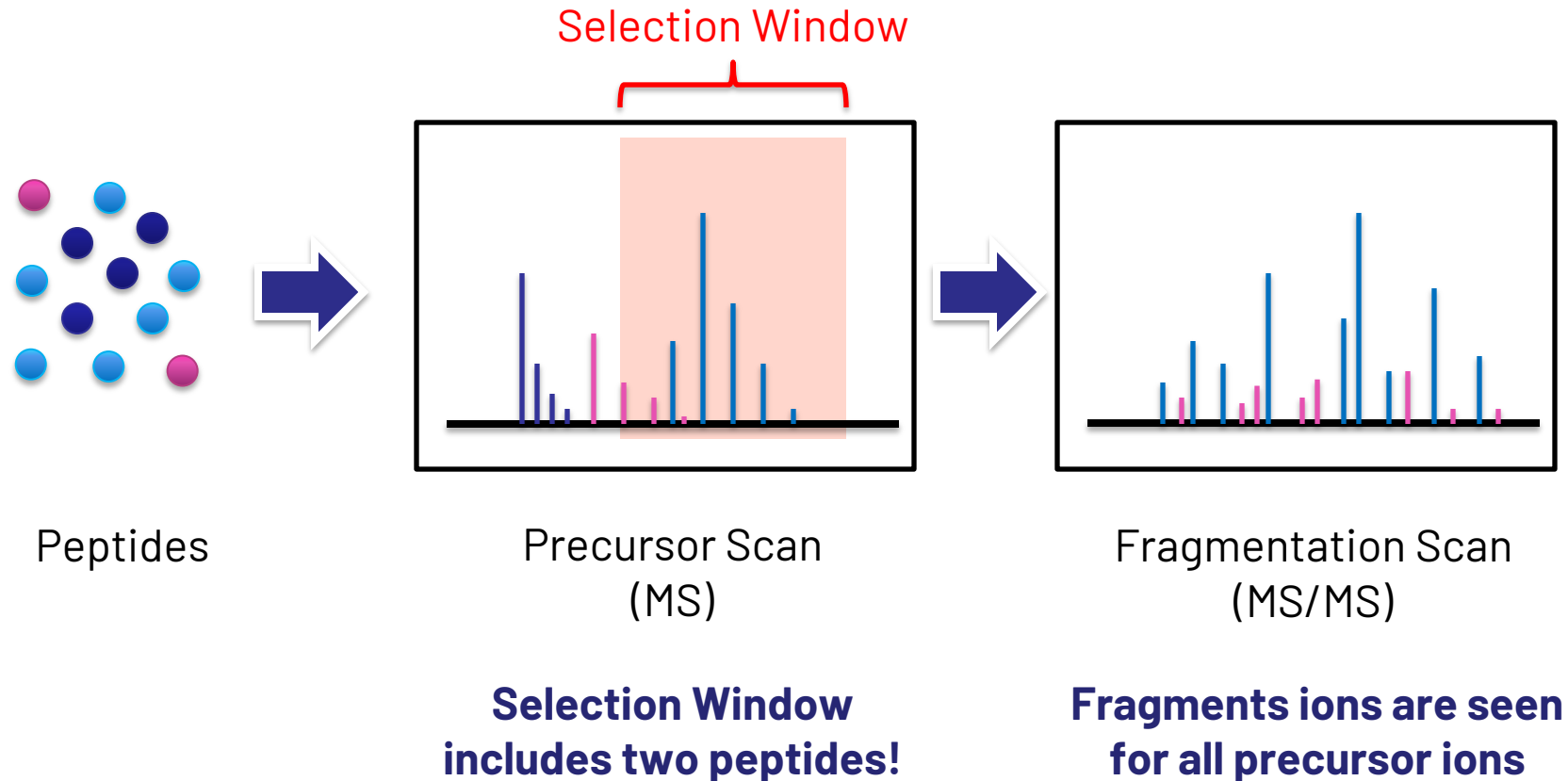
MS/MS

- **Standard Shotgun (MS/MS) Workflow**



Chimeric MS/MS

- **Chimeric spectra contain multiple precursor ions**



Factors Contributing To Chimeric Spectra

- **Chimeric spectra are a common occurrence in LC-MS/MS**
 - ***proportional*** to sample complexity
 - ***proportional*** to selection window size
 - influenced by chromatography conditions

**Database search algorithms
only report one peptide per
spectrum!**

Search Algorithms Do Not Find Chimeras

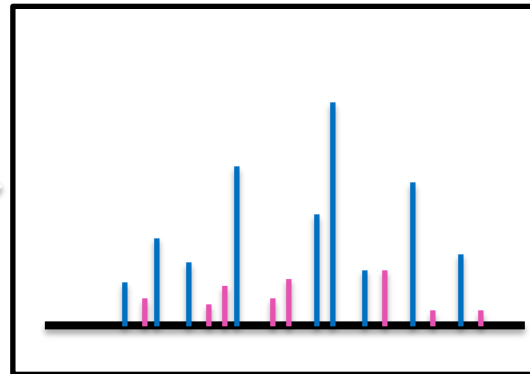
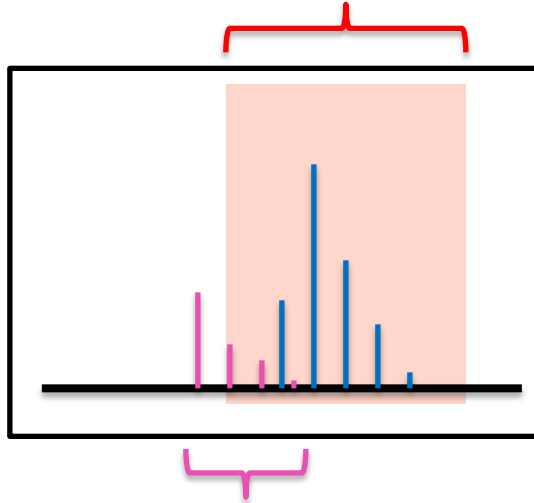
- **Selection window of the instrument is *much wider* than the peak resolution**
 - Search algorithms use only the measured precursor mass
 - For example: ± 25 ppm
- **Selection window may include *peptides outside the search mass***
 - Those peptides never considered during search
 - For example: different charge leads to mass outside ± 25 ppm tolerance

Chimeric MS/MS

Selected Ion (A):
Mass = 615.74
Charge = 3+

Database Search:
Peptides ~ 1844.22 Da

**Peptide (A)
Found!!**



Chimeric Ion (B):
Mass = 614.85
Charge = 2+

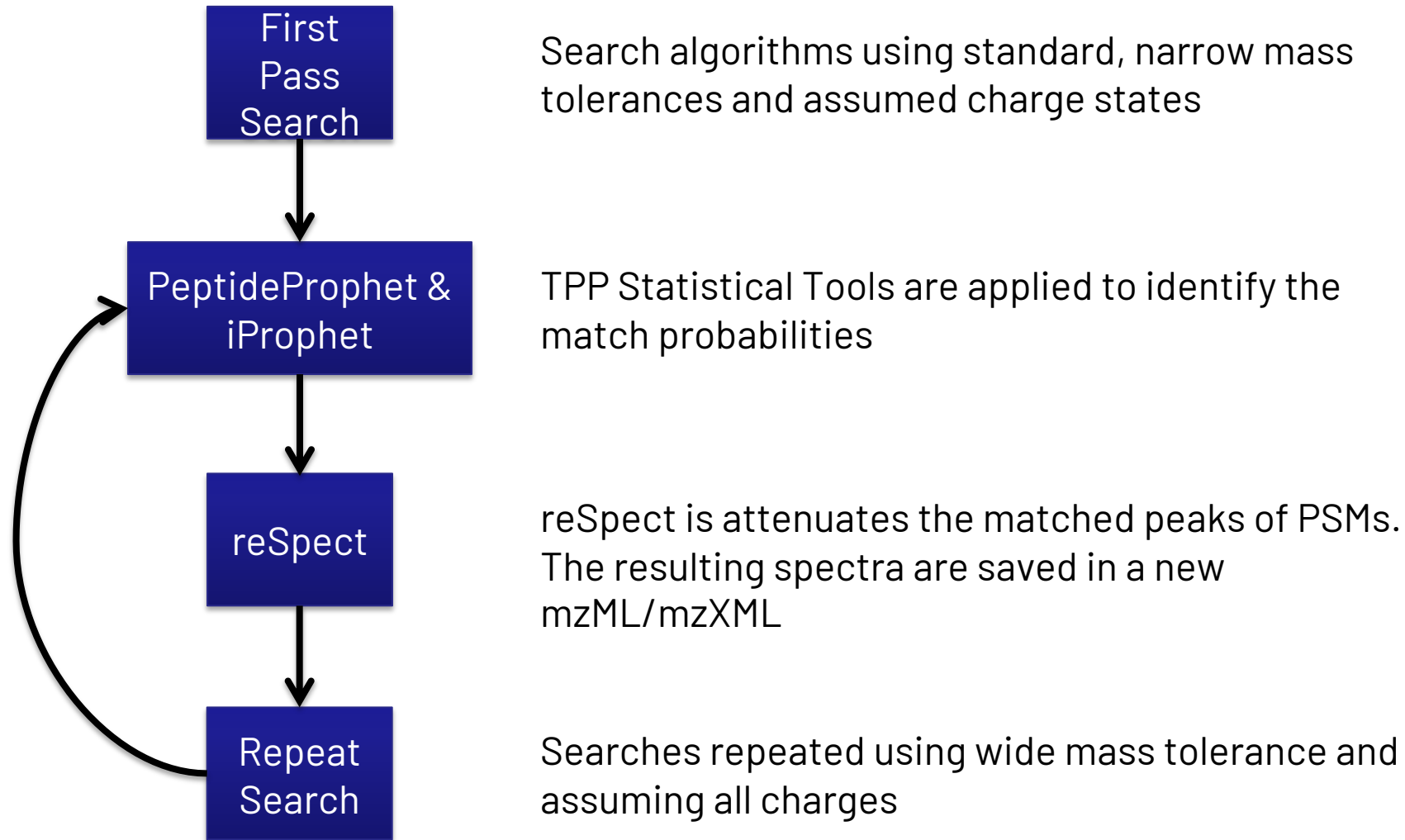
Not Searched:
Peptides ~ 1227.70 Da

**Peptide (B) NOT
Found!!**

reSpect

- **reSpect**
 - Allows identification of chimeric peptides from spectra.
 - Works with existing search algorithms.

reSpect: The Approach



reSpect: Attenuation Original $P = 0.999$

COMET/Lorikeet Spectrum Viewer
 (TPP v4.7 POLAR VORTEX rev 0, Build 201403171616 (linux))

FTQDTQPHYIYSPR, MH+ 1981.9970, m/z 661.3372
 File: F11.08453.08453.3, Scan: 8453, Exp. m/z: 661.3377, Charge: 3

ions:
 a 1* 2* 3*
 b 1* 2* 3*
 c 1* 2* 3*
 x 1* 2* 3*
 y 1* 2* 3*
 z 1* 2* 3*
 [Deselect All]

Neutral Loss:
 NH₃ (*)
 H₂O (o)
 Immonium ions
 Reporter ions

Mass Type:
 Mono Avg

Mass Tol: 0.1
 Update

Peak Assignment:
 Most Intense
 Nearest Match
 Peak Detect

Peak Labels:
 Ion m/z
 None

Width: 800
 Height: 400

Click and drag in the plot to zoom X: Y: Zoom Out Print Enable tooltip

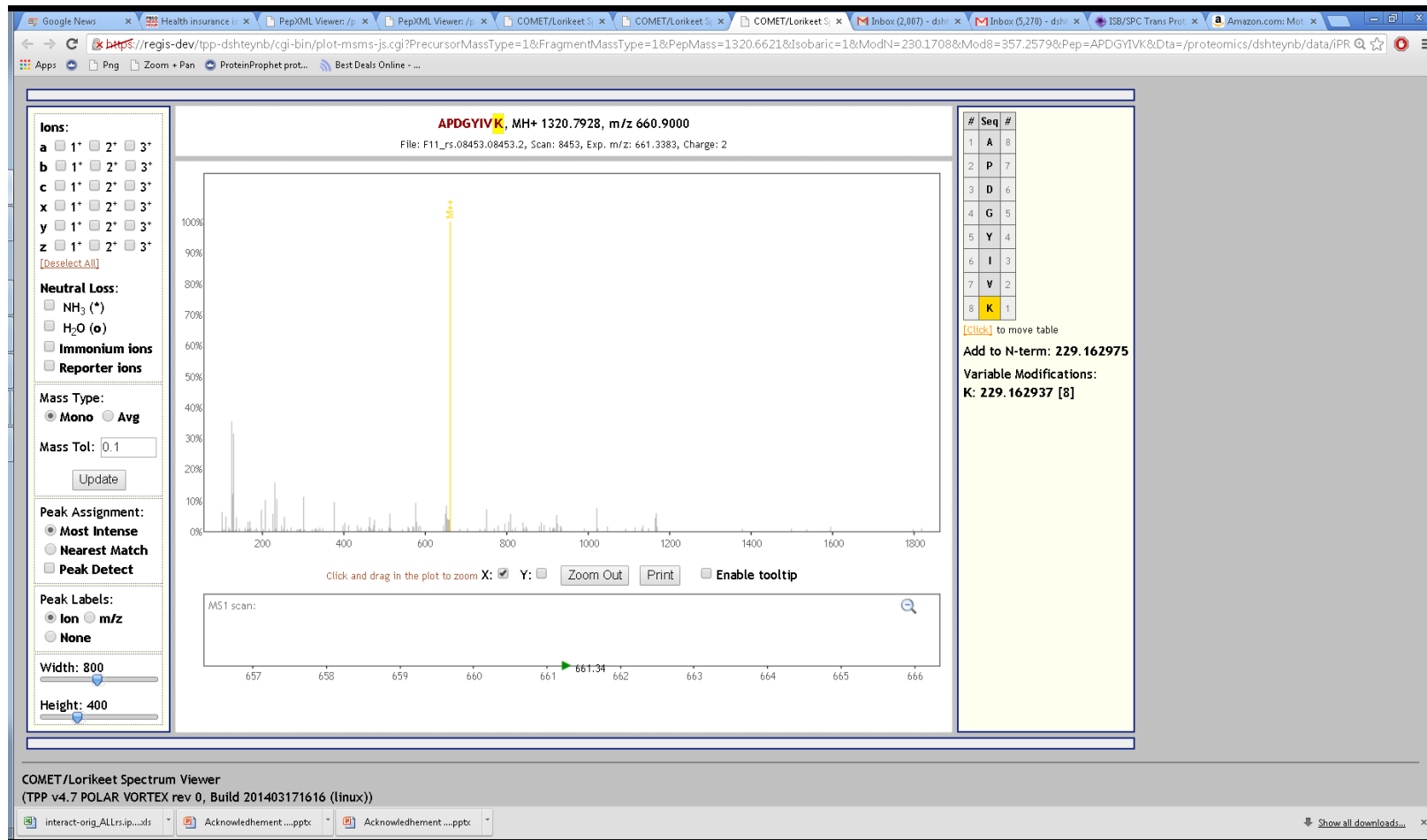
MS1 scan: 8446, RT 5094.20

b+	b2+	b3+	#	Seq	#	y+	y2+	y3+
377.2387	189.1230	126.4177	1	F	14			
478.2863	239.6468	160.1003	2	T	13	1605.7656	803.3864	535.9267
606.3449	303.6761	202.7865	3	Q	12	1504.7179	752.8626	502.2442
721.3719	361.1896	241.1288	4	D	11	1376.6593	688.8333	459.5580
822.4195	411.7134	274.8114	5	T	10	1261.6324	631.3198	421.2156
950.4781	475.7427	317.4976	6	Q	9	1160.5847	580.7960	387.5331
1047.5309	524.2691	349.8485	7	P	8	1032.5261	516.7667	344.8469
1184.5898	592.7985	395.5348	8	H	7	935.4734	468.2403	312.4960
1347.6531	674.3302	449.8892	9	Y	6	798.4145	399.7109	266.8097
1460.7372	730.8722	487.5839	10	I	5	635.3511	318.1792	212.4552
1623.8005	812.4039	541.9384	11	Y	4	522.2671	261.6372	174.7605
1710.8325	855.9199	570.9490	12	S	3	359.2037	180.1055	120.4061
1807.8853	904.4463	603.3000	13	P	2	272.1717	136.5895	91.3954
			14	R	1	175.1190	88.0631	59.0445

[Click] to move table
 Add to N-term: 229.162975

reSpect: Attenuation

$$I^{rs} = (1-P) * I^{orig}$$



reSpect: Second Match $P = 0.995$

COMET/Lorikeet Spectrum Viewer
(TPP v4.7 POLAR VORTEX rev 0, Build 201403171616 (linux))

APDGYIVK, MH+ 1320.7928, m/z 660.9000
File: F11_rs.08453.08453.2, Scan: 8453, Exp. m/z: 661.3383, Charge: 2

ions:
a 1* 2* 3*
b 1* 2* 3*
c 1* 2* 3*
x 1* 2* 3*
y 1* 2* 3*
z 1* 2* 3*
[\[Deselect All\]](#)

Neutral Loss:
 NH₃ (*)
 H₂O (o)
 Immonium ions
 Reporter ions

Mass Type:
 Mono Avg

Mass Tol: 0.1

Peak Assignment:
 Most Intense
 Nearest Match
 Peak Detect

Peak Labels:
 Ion m/z
 None

Width: 800
Height: 400

Click and drag in the plot to zoom X: Y: Enable tooltip

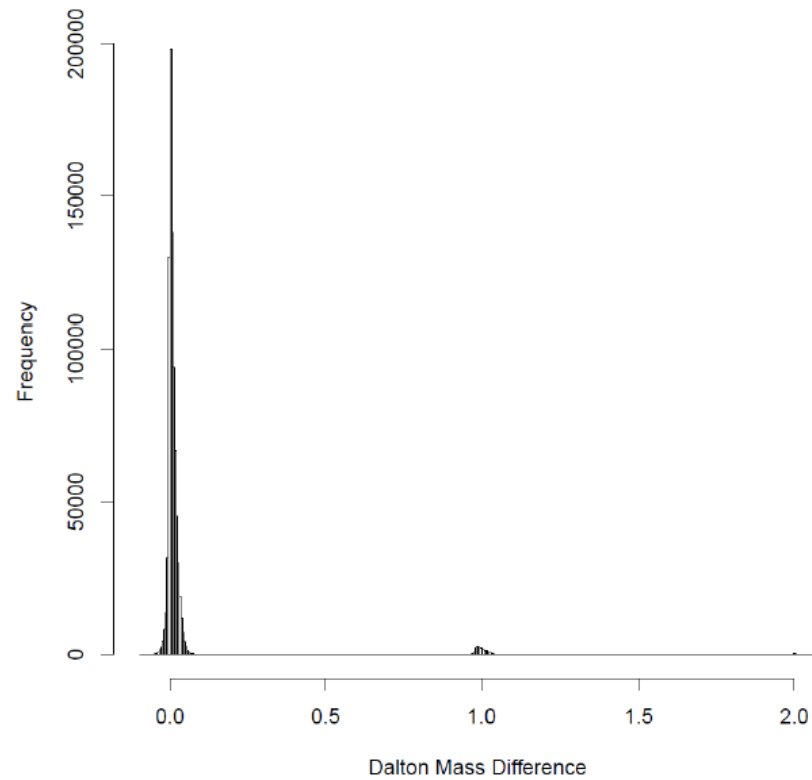
MS1 scan:

b+	b2+	b3+	#	Seq	#	y+	y2+	y3+
301.2074	151.1073	101.0740	1	A	8			
398.2601	199.6337	133.4249	2	P	7	1020.5927	510.8000	340.8691
513.2871	257.1472	171.7672	3	D	6	923.5400	462.2736	308.5182
570.3085	285.6579	190.7744	4	G	5	808.5130	404.7601	270.1759
733.3719	367.1896	245.1288	5	Y	4	751.4915	376.2494	251.1687
846.4559	423.7316	282.8235	6	I	3	588.4282	294.7177	196.8143
945.5243	473.2658	315.8463	7	V	2	475.3442	238.1757	159.1196
			8	K	1	376.2757	188.6415	126.0968

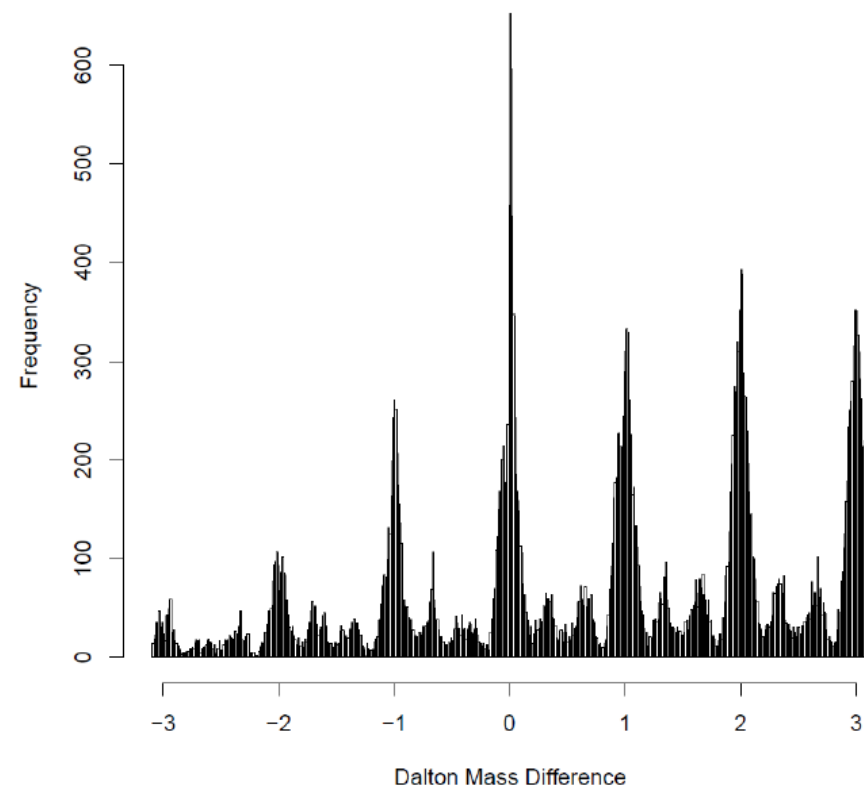
[\[Click\]](#) to move table
Add to N-term: 229.162975
Variable Modifications:
K: 229.162937 [8]

reSpect: Mass Differences

First Pass Mass Differences



reSpect Mass Differences



Chimeric Depth

- **Chimeric spectra may contain more than two precursors**
- **reSpect can be iteratively applied until novel PSMs are exhausted**

reSpect: The Original

PepXML Viewer /proteom... comet-pep.xml COMET/Lorikeet Spectrum... COMET/Lorikeet Spectrum... COMET/Lorikeet Spectrum... COMET/Lorikeet Spectrum... PepXML Viewer /proteom...

<https://regis-dev/tp-dshsteynb/cgi-bin/plot-msms-js.cgi?PrecursorMassType=1&FragmentMassType=1&PepMass=1659.9082&Isobaric=1&Pep=SKVVVFEDAPAGIAAGK&Dt=/proteomics/dshsteynb/data/mhooopmann/HiRes/semitryp>

Ions:

a 1* 2* 3*

b 1* 2* 3*

c 1* 2* 3*

x 1* 2* 3*

y 1* 2* 3*

z 1* 2* 3*

[\[Deselect All\]](#)

Neutral Loss:

NH₃ (*)

H₂O (o)

Immonium ions

Mass Type:

Mono Avg

Mass Tol:

Peak Assignment:

Most Intense

Nearest Match

Peak Detect

Peak Labels:

Ion m/z

None

Width:

Height:

SKVVVFEDAPAGIAAGK, MH+ 1658.9112, m/z 553.6419

File: 120913-Yeast-02.42285.42285.3, Scan: 42285, Exp. m/z: 554.31, Charge: 3

Click and drag in the plot to zoom X: Y: Enable tooltip

MS1 scan:

b+	b2+	b3+	#	Seq	#	y+	y2+	y3+
88.0393	44.5233	30.0180	1	S	17			
216.1343	108.5708	72.7163	2	K	16	1571.8792	786.4432	524.6312
315.2027	158.1050	105.7391	3	V	15	1443.7842	722.3957	481.9329
414.2711	207.6392	138.7619	4	V	14	1344.7158	672.8615	448.9101
513.3395	257.1734	171.7847	5	V	13	1245.6474	623.3273	415.8873
660.4079	330.7076	220.8075	6	F	12	1146.5790	573.7931	382.8645
789.4505	395.2289	263.8217	7	E	11	999.5106	500.2589	333.8417
904.4775	452.7424	302.1640	8	D	10	870.4680	435.7376	290.8275
975.5146	488.2609	325.8430	9	A	9	755.4410	378.2241	252.4852
1072.5673	536.7873	358.1940	10	P	8	684.4039	342.7056	228.8062
1143.6045	572.3059	381.8730	11	A	7	587.3511	294.1792	196.4552
1200.6259	600.8166	400.8802	12	G	6	516.3140	258.6606	172.7762
1313.7100	657.3586	438.5748	13	I	5	459.2926	230.1499	153.7690
1384.7471	692.8772	462.2539	14	A	4	346.2085	173.6079	116.0743
1455.7842	728.3957	485.9329	15	A	3	275.1714	138.0893	92.3953
1512.8057	756.9065	504.9401	16	G	2	204.1343	102.5708	68.7163
			17	K	1	147.1128	74.0600	49.7091

[\[Click\]](#) to move table

COMET/Lorikeet Spectrum Viewer
 (TPP v0.0 Development trunk rev 0, Build 201309231229 (linux))

reSpect: The First Iteration

COMET/Lorikeet Spectrum Viewer
(TPP v4.7 POLAR VORTEX rev 0, Build 201403171616 (linux))

File: 120913-Yeast-02_rs.42285.2, Scan: 42285, Exp. m/z: 554.3106, Charge: 2

GLILVGGYGTR, MH+ 1105.6364, m/z 553.3218

ions:
a 1* 2* 3*
b 1* 2* 3*
c 1* 2* 3*
x 1* 2* 3*
y 1* 2* 3*
z 1* 2* 3*
[\[Deselect All\]](#)

Neutral Loss:
 NH₃ (*)
 H₂O (o)
 Immonium ions
 Reporter ions

Mass Type:
 Mono Avg

Mass Tol: 0.1

Peak Assignment:
 Most Intense
 Nearest Match
 Peak Detect

Peak Labels:
 Ion m/z
 None

Width: 650
Height: 400

Click and drag in the plot to zoom X: Y: Enable tooltip

b+	b2+	#	Seq	#	y+	y2+
58.0287	29.5180	1	G	11		
171.1128	86.0600	2	L	10	1048.6150	524.8111
284.1969	142.6021	3	I	9	935.5309	468.2691
397.2809	199.1441	4	L	8	822.4468	411.7271
496.3493	248.6783	5	V	7	709.3628	355.1850
553.3708	277.1890	6	G	6	610.2944	305.6508
610.3923	305.6998	7	G	5	553.2729	277.1401
773.4556	387.2314	8	Y	4	496.2514	248.6293
830.4771	415.7422	9	G	3	333.1881	167.0977
931.5247	466.2660	10	T	2	276.1666	138.5870
		11	R	1	175.1190	88.0631

[\[Click\]](#) to move table

reSpect: The Second Iteration

COMET/Lorikeet Spectrum Viewer

File: 120913-Yeast-02_rs.42285.42285.2, Scan: 42285, Exp. m/z: 554.3112, Charge: 2

ELYEVDVLK, MH+ 1107.5932, m/z 554.3002

ProteinProphet prot... Index of /proteomic... Best Deals Online - ...

Zoom + Pan

Update

MS1 scan: 550 551 552 553 554 555 556 557 558 559

Width: 650

Height: 400

COMET/Lorikeet Spectrum Viewer
(TPP v0.0 Development trunk rev 0, Build 201309231229 (linux))

1⁺ 2⁺ 3⁺
 1⁺ 2⁺ 3⁺
 1⁺ 2⁺ 3⁺
 1⁺ 2⁺ 3⁺
 1⁺ 2⁺ 3⁺
 1⁺ 2⁺ 3⁺
[\[Deselect All\]](#)

Neutral Loss:
 NH₃ (*)
 H₂O (o)
 Immonium ions

Mono Avg
 Mass Tol: 0.5

Most Intense
 Nearest Match
 Peak Detect

Ion m/z
 None

X: Y: Enable tooltip

b+	b2+	#	Seq	#	y+	y2+
130.0499	65.5286	1	E	9		
243.1339	122.0706	2	L	8	978.5506	489.7790
406.1973	203.6023	3	Y	7	865.4666	433.2369
535.2399	268.1236	4	E	6	702.4032	351.7053
634.3083	317.6578	5	V	5	573.3606	287.1840
749.3352	375.1712	6	D	4	474.2922	237.6498
848.4036	424.7055	7	V	3	359.2653	180.1363
961.4877	481.2475	8	L	2	260.1969	130.6021
		9	K	1	147.1128	74.0600

[\[click\]](#) to move table

reSpect: The Third Iteration

PepXML Viewer /proteom... comet-pep.xml COMET/Lorikeet Spectrum... COMET/Lorikeet Spectrum... COMET/Lorikeet Spectrum... COMET/Lorikeet Spectrum... PepXML Viewer /proteom...

<https://regis-dev/tp-dshtheynb/cgi-bin/plot-msms-js.cgi?PrecursorMassType=1&FragmentMassType=1&PepMass=1659.9137&Isobaric=1&Pep=STEQIRPFATAAVLR&Dta=/proteomics/dshtheynb/data/mhoopmann/HiRes/semityrp/12>

STEQIRPFATAAVLR, MH+ 1659.9177, m/z 553.9774
 File: 120913-Yeast-02_rs_rs.42285.42285.3, Scan: 42285, Exp. m/z: 554.3118, Charge: 3

Ions:

a 1* 2* 3*

b 1* 2* 3*

c 1* 2* 3*

x 1* 2* 3*

y 1* 2* 3*

z 1* 2* 3*

[\[Deselect All\]](#)

Neutral Loss:

NH₃ (*)

H₂O (o)

Immonium ions

Mass Type:

Mono Avg

Mass Tol:

Peak Assignment:

Most Intense

Nearest Match

Peak Detect

Peak Labels:

Ion m/z

None

Width:

Height:

Click and drag in the plot to zoom X: Y: Enable tooltip

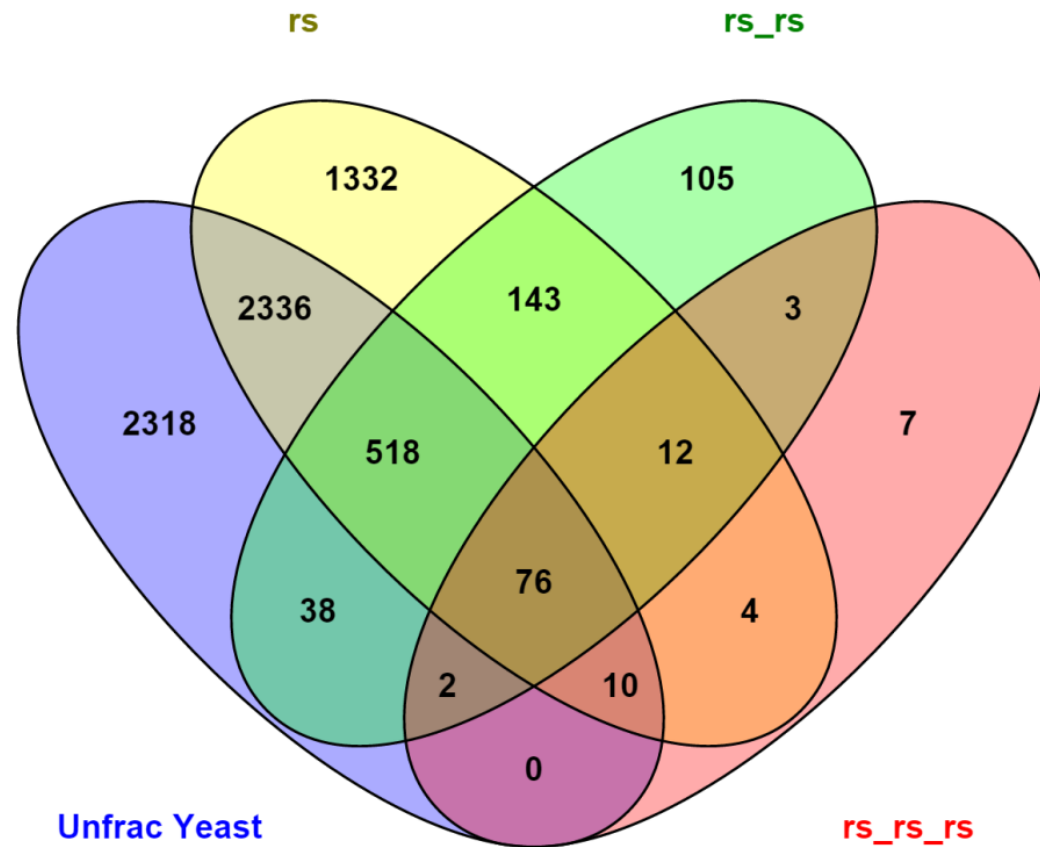
MS1 scan:

b+	b2+	b3+	#	Seq	#	y+	y2+	y3+
88.0393	44.5233	30.0180	1	S	15			
189.0870	95.0471	63.7005	2	T	14	1572.8857	786.9465	524.9667
318.1296	159.5684	106.7147	3	E	13	1471.8380	736.4226	491.2842
446.1882	223.5977	149.4009	4	Q	12	1342.7954	671.9013	448.2700
559.2722	280.1397	187.0956	5	I	11	1214.7368	607.8720	405.5838
715.3733	358.1903	239.1293	6	R	10	1101.6527	551.3300	367.8891
812.4261	406.7167	271.4802	7	P	9	945.5516	473.2795	315.8554
959.4945	480.2509	320.5030	8	F	8	848.4989	424.7531	283.5045
1030.5316	515.7694	344.1821	9	A	7	701.4304	351.2189	234.4817
1131.5793	566.2933	377.8646	10	T	6	630.3933	315.7003	210.8026
1202.6164	601.8118	401.5437	11	A	5	529.3457	265.1765	177.1201
1273.6535	637.3304	425.2227	12	A	4	458.3085	229.6579	153.4410
1372.7219	686.8646	458.2455	13	V	3	387.2714	194.1394	129.7620
1485.8060	743.4066	495.9402	14	L	2	288.2030	144.6051	96.7392
			15	R	1	175.1190	88.0631	59.0445

[\[Click\]](#) to move table

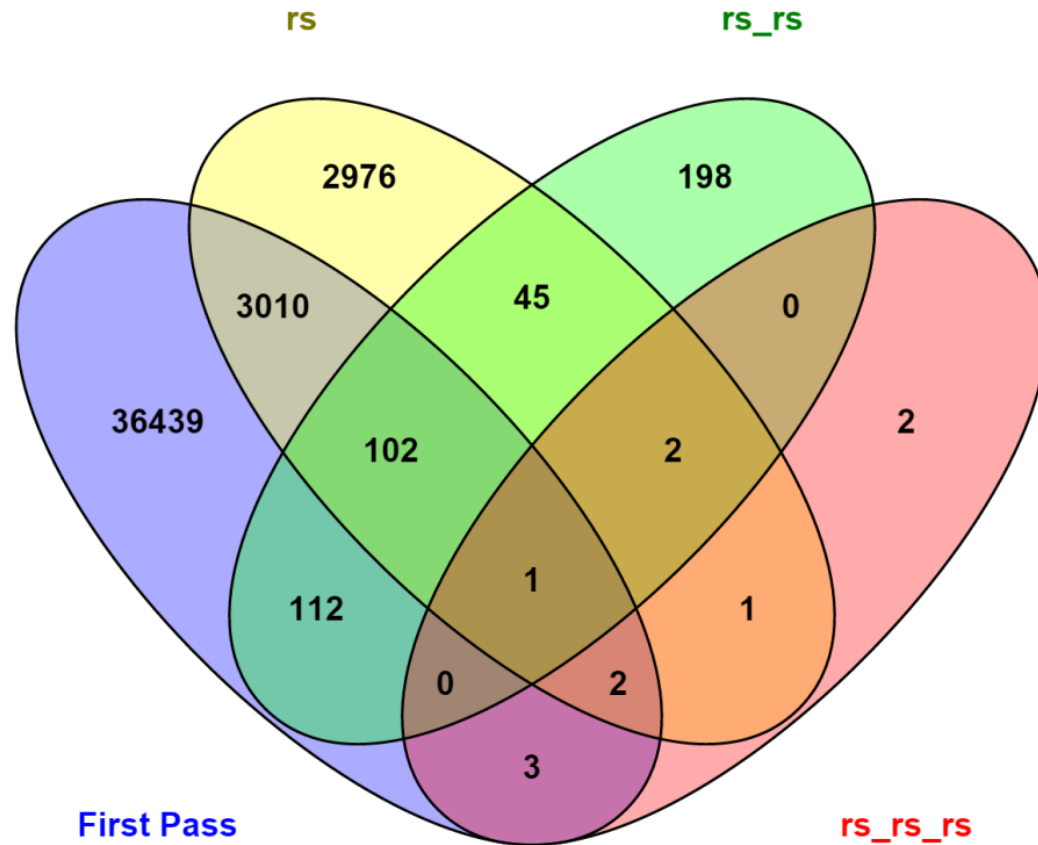
COMET/Lorikeet Spectrum Viewer
 (TPP v0.0 Development trunk rev 0, Build 201309231229 (linux))

reSpect: No Fractionation



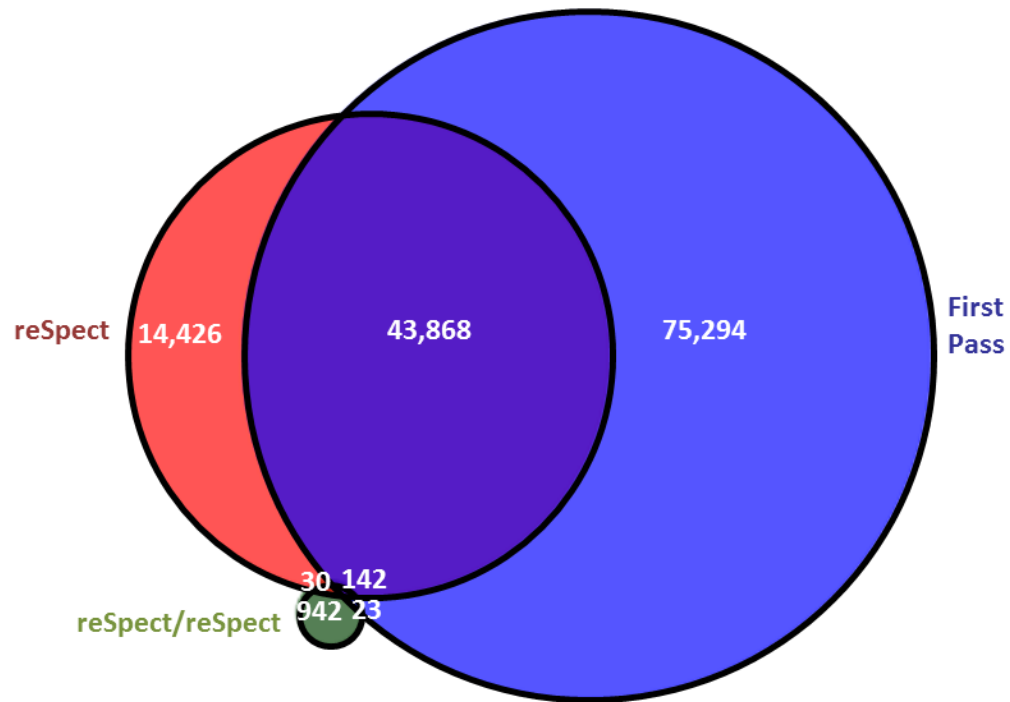
- **4 Replicate Yeast 0-Exactive Runs**
- **347,000 spectra**
- **30.3% Boost in Distinct Peptide Identification**

reSpect: Medium Fractionation



- **iPRG2013 study**
- **118,000 spectra**
- **Orbitrap Velos, 14 RP fractions of whole cell lysate of human peripheral blood mononuclear cells**
- **8.1% Boost in Distinct Peptide identifications**

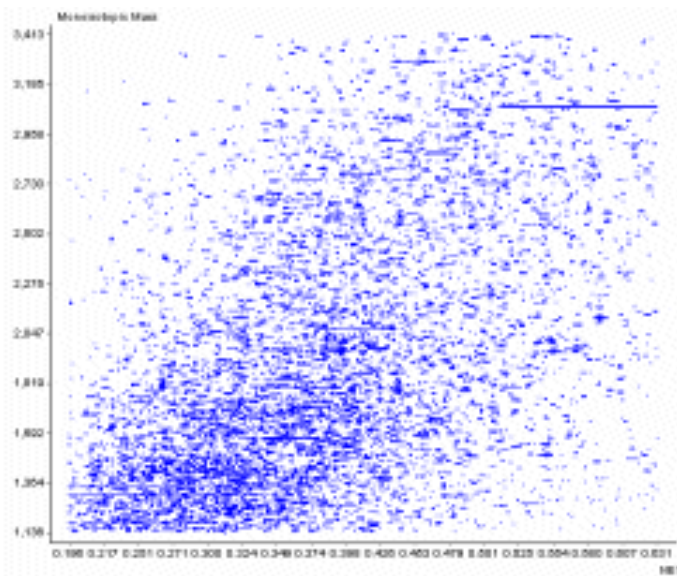
reSpect: High Fractionation



- **Human Cancer Cell Line**
- **Orbitrap Velos, 48 fractions**
- **12.9% Boost in Distinct Peptide Identifications**

Different Datasets, Different Chimeras

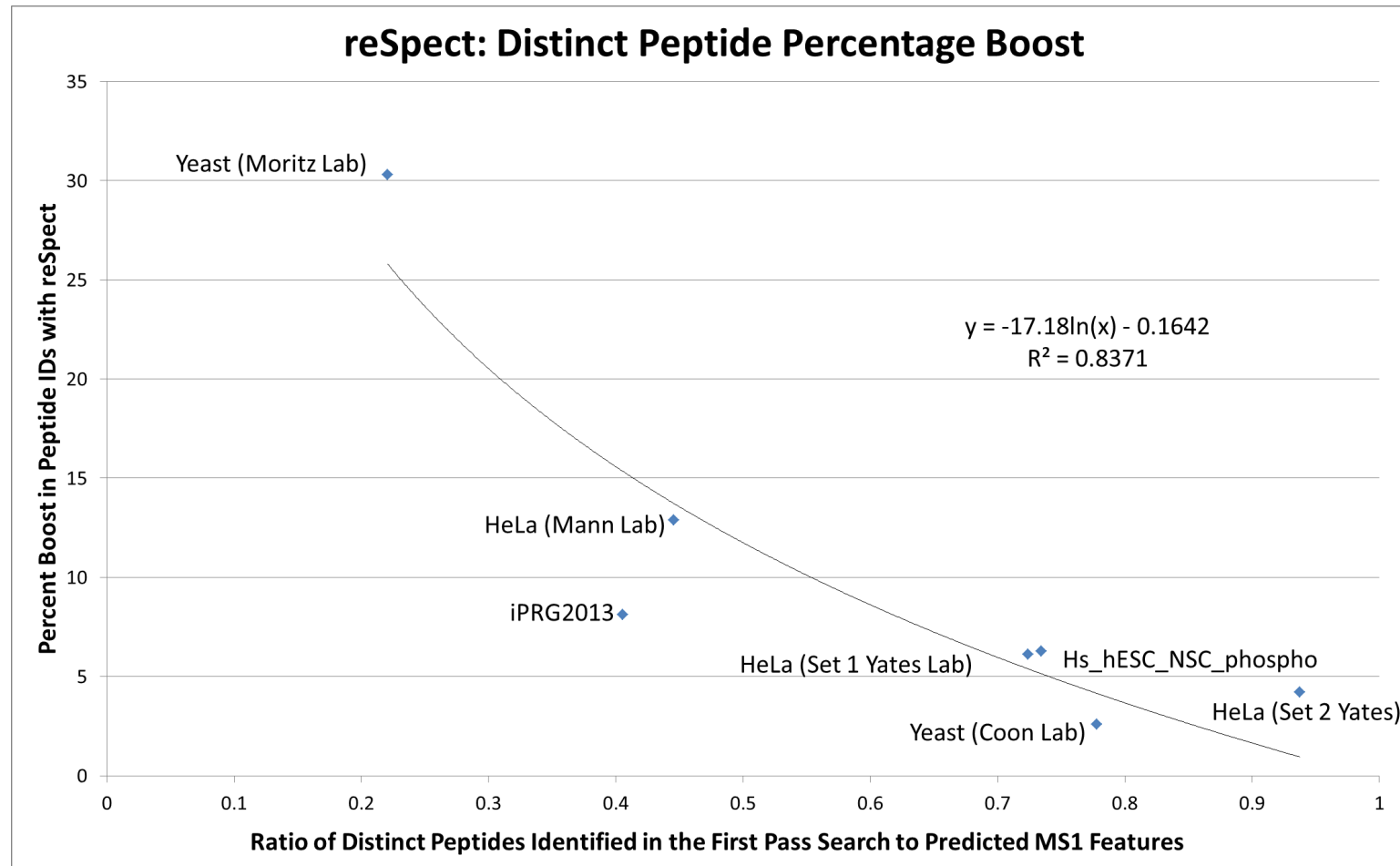
- **Sample complexity and instrument settings have largest impact on chimeric spectra**



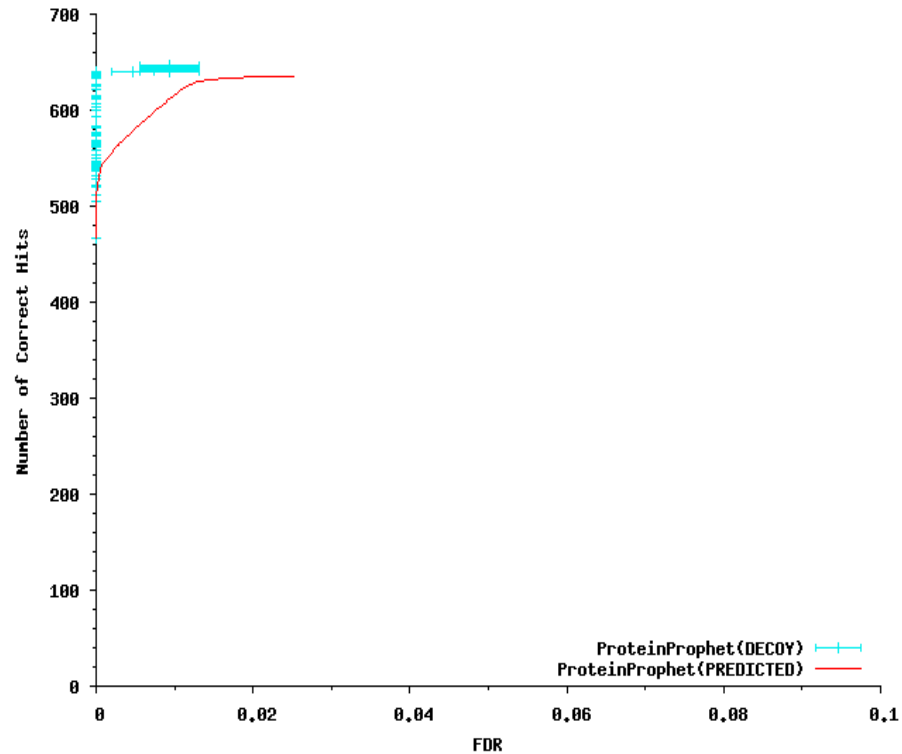
Precursor analysis algorithms can estimate number of precursors to solve.

These numbers correlate with PSMs identified.

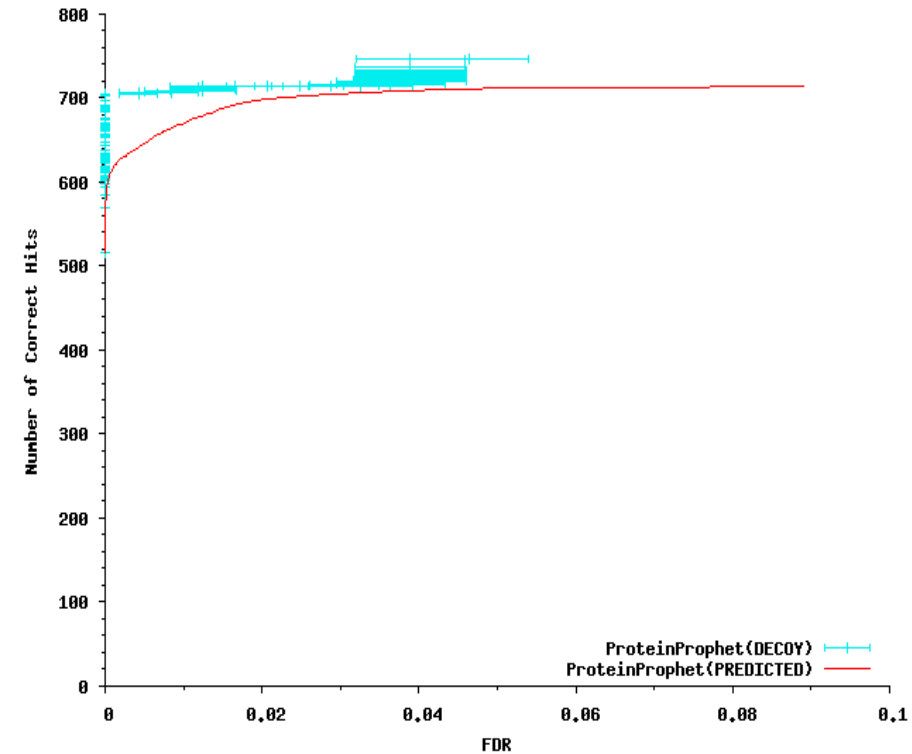
reSpect: Different Datasets



reSpect: Identifies More Proteins



Protein level performance on the Moritz Lab yeast dataset **BEFORE reSpect analysis.**



Protein level performance on the Moritz Lab yeast dataset **AFTER reSpect analysis.**

reSpect in the TPP

The screenshot shows a web browser window with the following elements:

- Browser Tab:** ISB/SPC Trans Proteomic P... x +
- Address Bar:** localhost/tpp-bin/tpp_gui.pl
- Page Title:** ISB/SPC Trans Proteomic Pipeline - home
- Navigation Bar:** Home | Files | Account | Pre-Process | mzXML Utils | Analysis Pipeline (Comet) | Decoy | Utilities | SpectraST Tools | Jobs 3 27
- Menu:** Home | FILES | ACCOUNT | PRE-PROCESS | mzXML UTILS
- Messages:** [Show / Hide]
 - Welcome, guest.
- Dropdown Menu (highlighted):**
 - reSpect
 - Qual Score
 - Compare Proteins Extract potentially c
 - Libra Conditions
 - DNA to AA
 - RT Prediction
 - RT Training
- Footer:** Welcome

reSpect in the TPP

The screenshot shows a web browser window with the address bar displaying `localhost/tpp-bin/tpp_gui.pl`. The page title is "ISB/SPC Trans Proteomic Pipeline - respect". The navigation menu includes: Home, reSpect, QualScore, Compare Proteins, Libra Conditions, DNA to AA, RT Prediction, and RT Training. The user is logged in as a guest, with a "Log Out" button. A "Messages" section is visible with a "Show [+]" link. The main content area is divided into three sections:

- 1. Specify pepXML file with probabilities** [Show / Hide]
 - File list:
 - `c:/inetpub/wwwroot/ISB/data/ETDOutput.xml`
 - `c:/inetpub/wwwroot/ISB/data/pepxml.xml`
 - Buttons: "Select/Unselect All" and "Remove".
 - Form: "Add Files" button and "Or choose from list: c:/inetpub/wwwroot/ISB/data/ [.xml]" dropdown.
- 2. Options** [Show / Hide]
 - Minimum probability:
 - m/z tolerance (ions): Daltons
 - Enter additional options to pass directly to the command-line (expert use only!)
- 3. Look for Chimeric Spectra!**
 - Button: "Run reSpect"

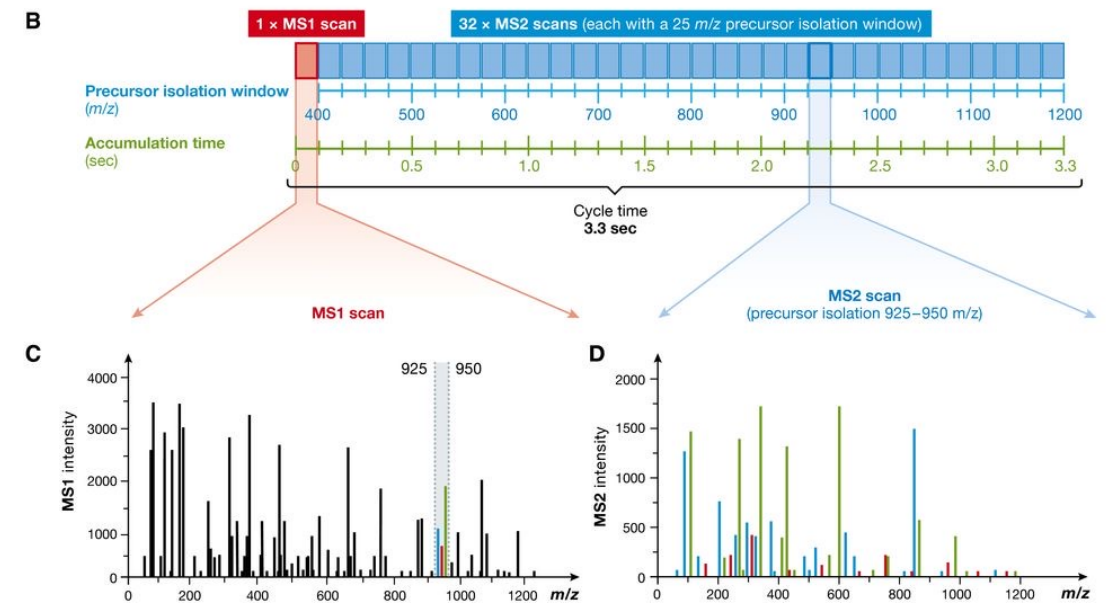
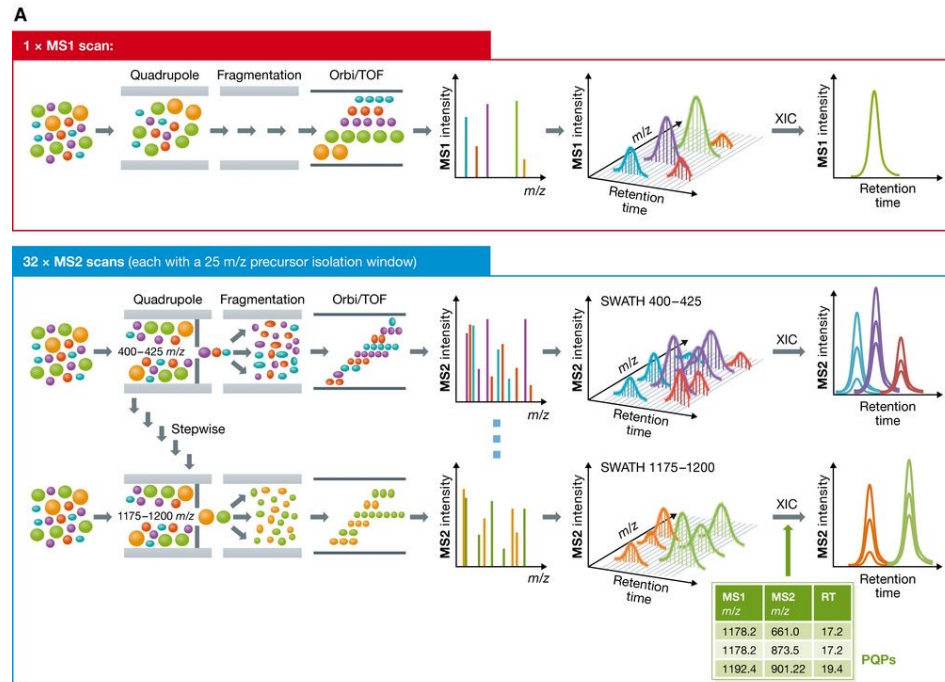
reSpect: Summary

- **Explained peaks are *attenuated* according to the quality of the match amongst medium and high scoring spectra**
- **Attenuated spectra are *researched* with wide mass window and assuming all charges**
- **Uses *same validation* with PeptideProphet and iProphet to estimate error rates**
- ***Automated* within the TPP framework**
- **Identifies new peptide sequences even in highly fractionated data**
- **Works best on *high mass accuracy MS²* data**
 - Q-Exactive
 - Orbitrap Velos
 - QTOF
- **Also works nicely on *standard mass accuracy MS²* data**
 - LTQ

Principles of Data Independent Acquisition

Slides courtesy of Dr. Mukul Midha

Principle of sequentially windowed acquisition in DIA/SWATH-MS



Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial, Ludwig, C. et al Molecular Systems Biology 2018

SWATH-MS/DIA experiment setup.....So much to choose from!!

Instruments.....

**Nano flow or
Micro flow?**

**LC separation
considerations?**

**Sample
datasets...**

**Library free
approach**

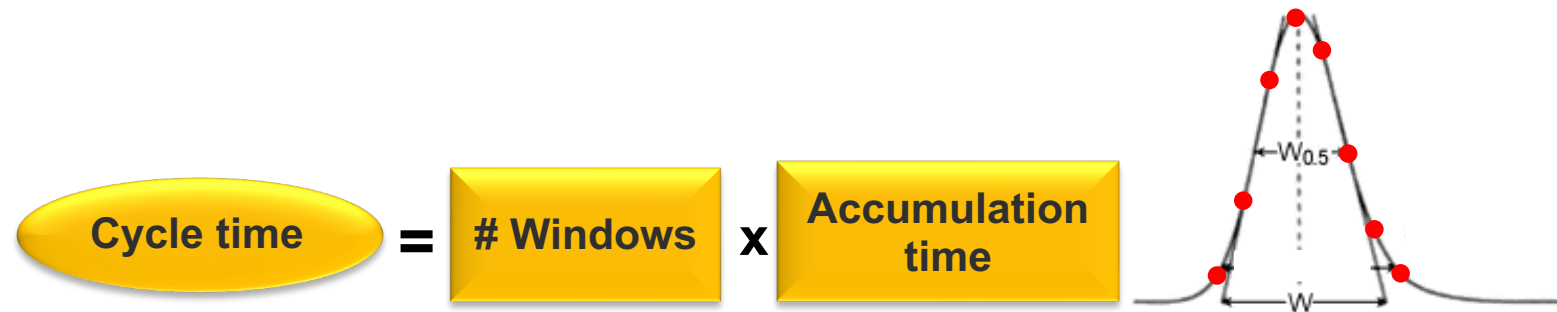
**Spectral ion
Libraries**

**Fixed or Variable
windows?**

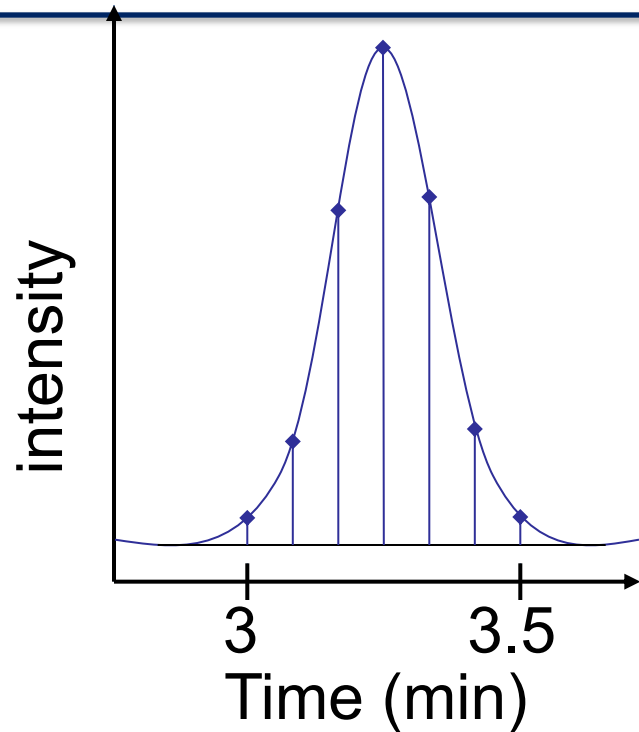
**Software, software,
software.....**

What are the critical acquisition attributes for DIA/SWATH-MS?

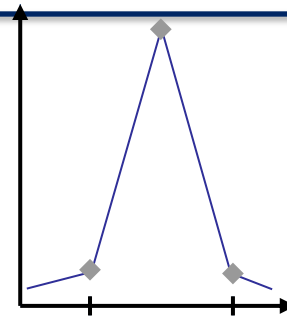
- ✓ High resolution MS/MS
- ✓ Cycle Time
- ✓ Q1 Isolation Windows
- ✓ Dynamic Range



DIA Acquisition- Quantitation

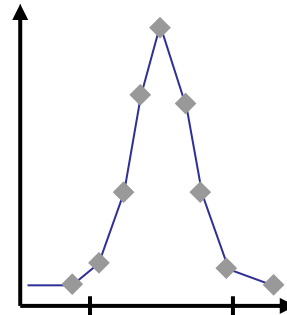


ex. 30 s peak width at base
 3 s cycle will collect 10 DPPP
 <3 s will over sample
 >3 s will under sample

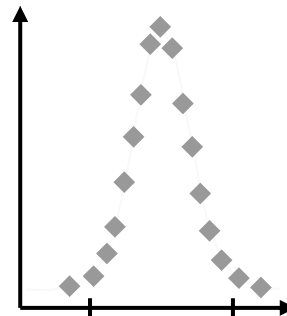


Data Points Per Peak
(DPPP)

<7 DPPP = under sampling

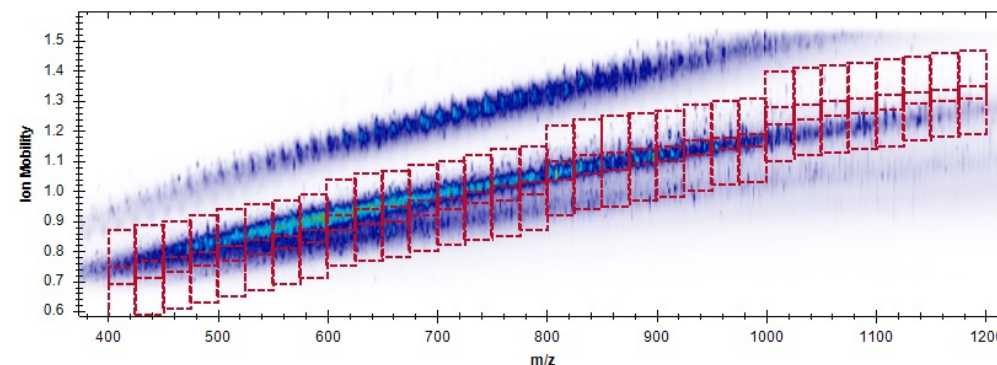
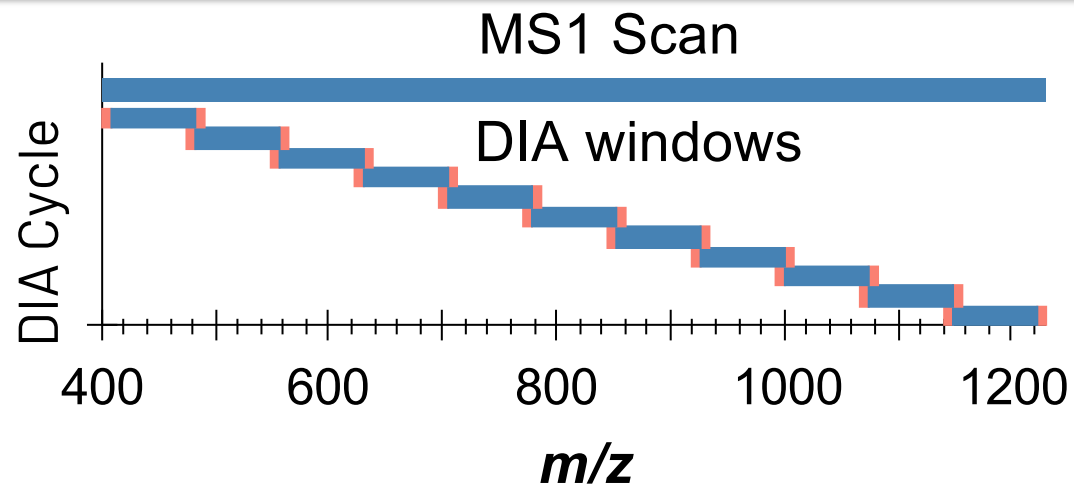


7-10 DPPP = optimal
 sampling



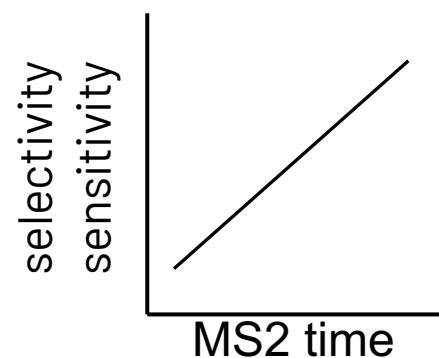
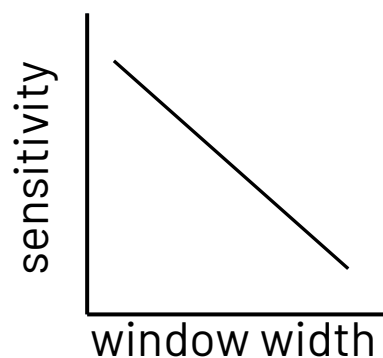
>10 DPPP = over sampling

DIA/SWATH-MS precursor isolation windows



windows x MS2 acquisition time = cycle time

42 (20 m/z width, 400-1200 m/z , 1 Da overlap) x 60 ms = 3.6 s

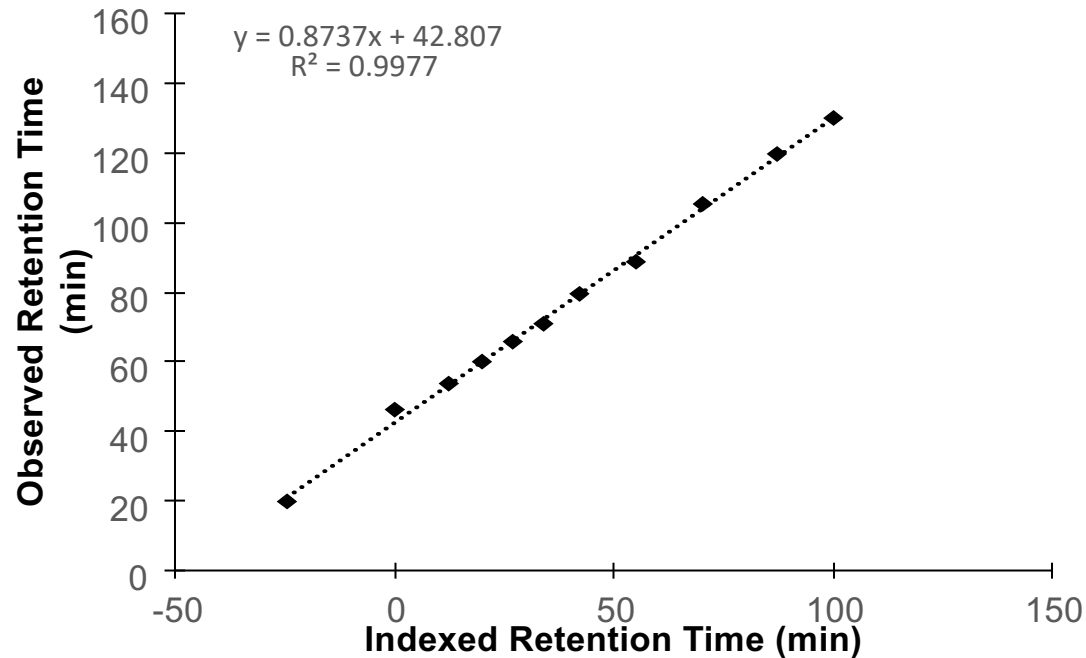


Choices are dependent on chromatography, application and platform.

DIA data analysis – retention time normalization

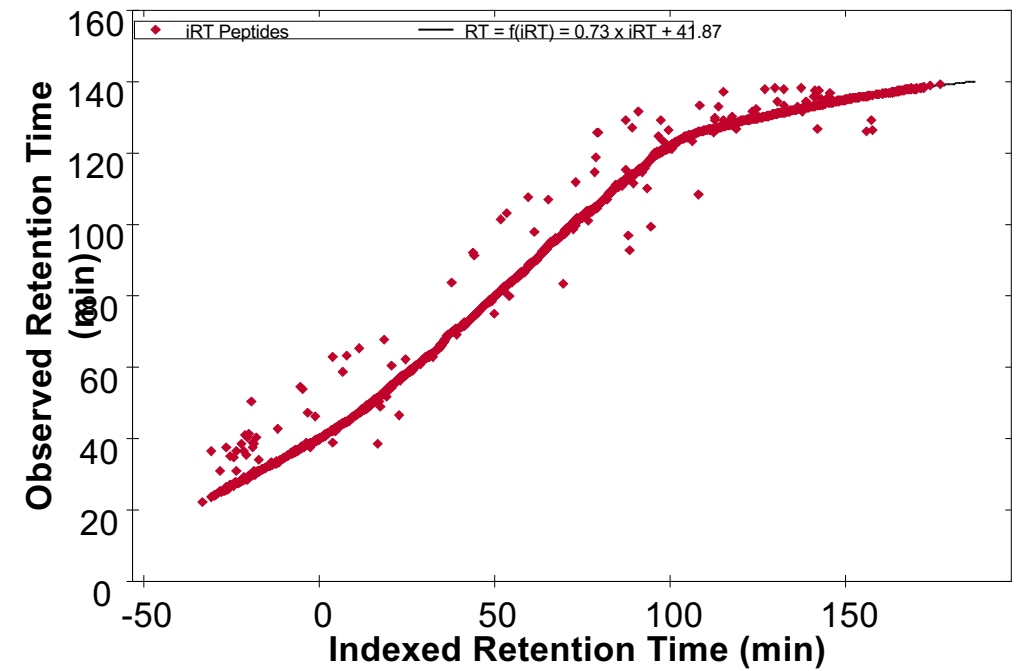
■ iRT spike

- set of non-endogenous peptides
- Used to convert to iRT scale (linear)

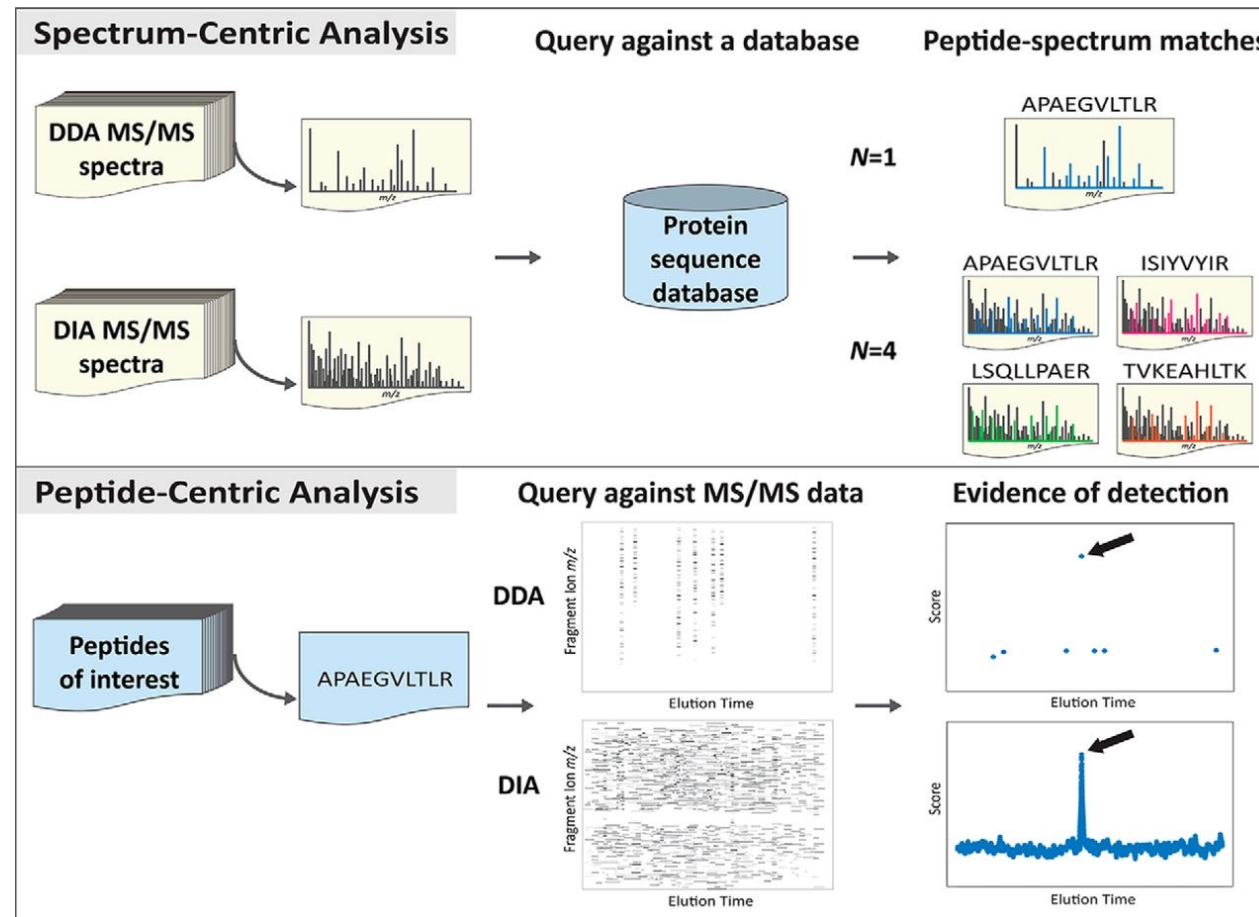


■ Observed peptides/features

- High-precision iRT (non-linear)
- Anchor points

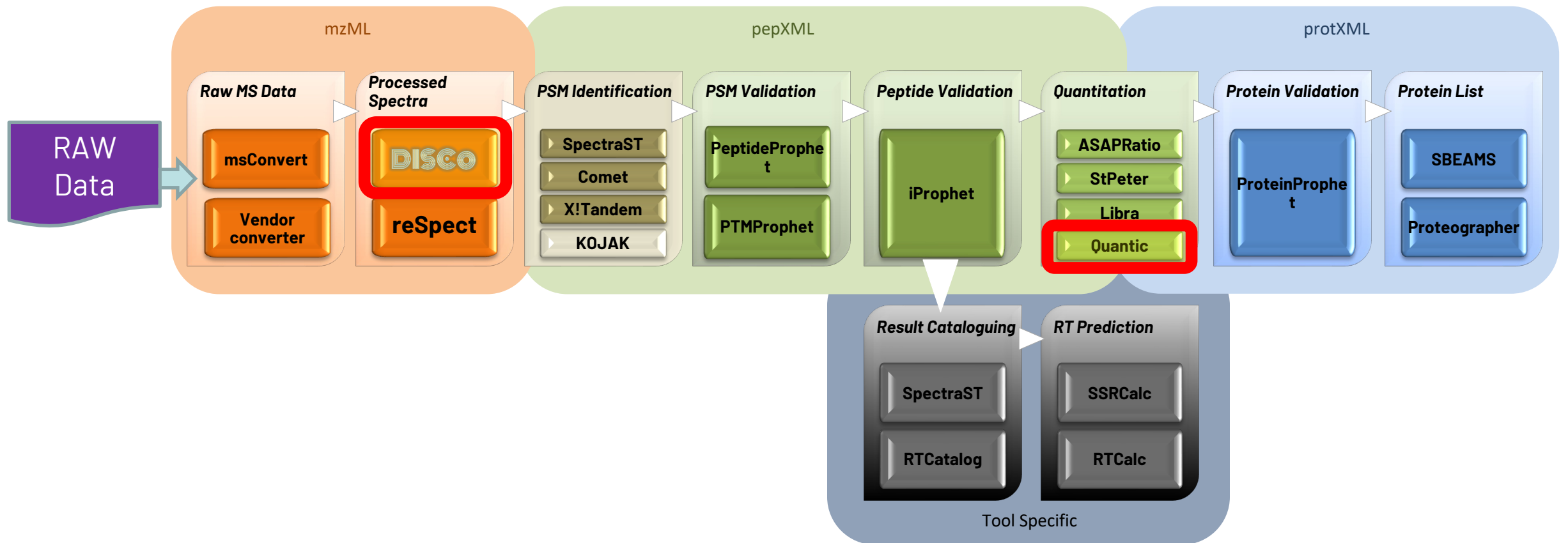


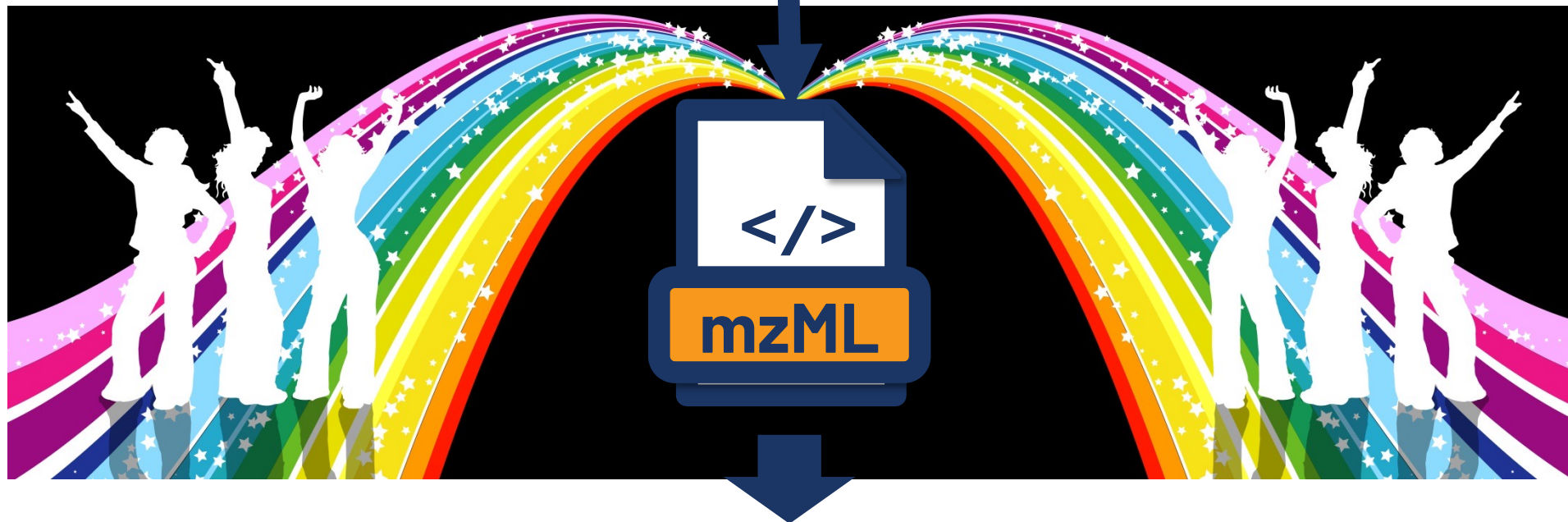
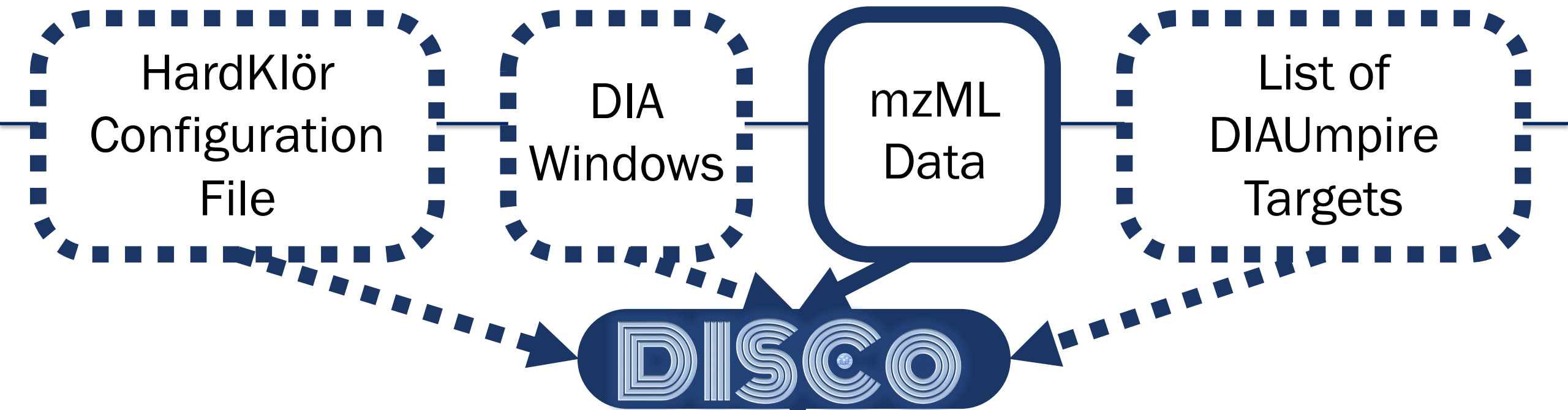
DIA/SWATH-MS: Library based and library free approach



**DISCO:TPP Software for Data-Driven DIA Analysis
and Quantitation**

Trans-Proteomic Pipeline a.k.a. TPP





- **Works on timsTOF PASEF DIA data**
- **DIA Windows is not required if isolation_window info available**
- **Multiple scan windows sizes for data point extraction**
- **Minimum amount of correlation required to select fragments depends on the scan window size (number of data points)**
- **Kernel Density smoothing of raw signals**
- **Better utilization of multi-threading resources**
- **Fail-safe mzML file reading**

USAGE: DiscoFilter <OPTIONS> <mzML/mzXML_input_file>

OPTIONS:

TARGETS=<targets_file> Use specified <target_file> to look for MS1 features (default: use HardKlör internally)

UMPTARGETS=<targets_file> Use specified <target_file> to look for MS1 features (default: use HardKlör internally)

HKCONFIG=<config_file> Use specified <config_file> for Hardklör (default: use Hardklor.conf)

WINDOWS=<DIA_windows_file>

MAXSCANWINDOW=<number>: Use specified maximum number of scans over which to track (default=17). This option applies only when the UMPTARGETS are not specified, when UMPTARGETS are specified the SCANWINDOW is set to the maximum peak width defined in the UMPTARGETS file.

MINSCANWINDOW=<number>: Use specified minimum number of scans over which to track (default=7). This option applies only when the UMPTARGETS are not specified, when UMPTARGETS are specified the SCANWINDOW is set to the maximum peak width defined in the UMPTARGETS file.

AVGSCANHALF=<number>: Use specified number of +- scans over which to average MS1 scans prior to feature detection, set to 0 for no averaging. (default=0)

MZPREC=<number>: Set mz precision in 'points after the decimal', used for binning and averaging. Applies in combination with AVGSCANHALF= greater than 0. (default=2.1)

MININTENS=<number>: Filter out peaks below minimum intensity factor in each spectrum, set as a non-negative number (default=off)

MAXPPM=<number>: Maximum Allowed PPM signal offset best defined in powers of 2 (e.g. 2, 4, 8, 16, etc. default=32)

PPMFWHM=<number>: Full Width at Half Maximum expected for Mass PPM profile of peaks (default=16)

IMFWHM=<number>: Full Width at Half Maximum expected for Inverse Reduced Ion Mobility profile of peaks (default=0.05)

IONMOBBINS=<number>: Set number of bins to partition Ion Mobility. (default=10)

SUFFIX=<string>: Set suffix for output file (default='_ds')

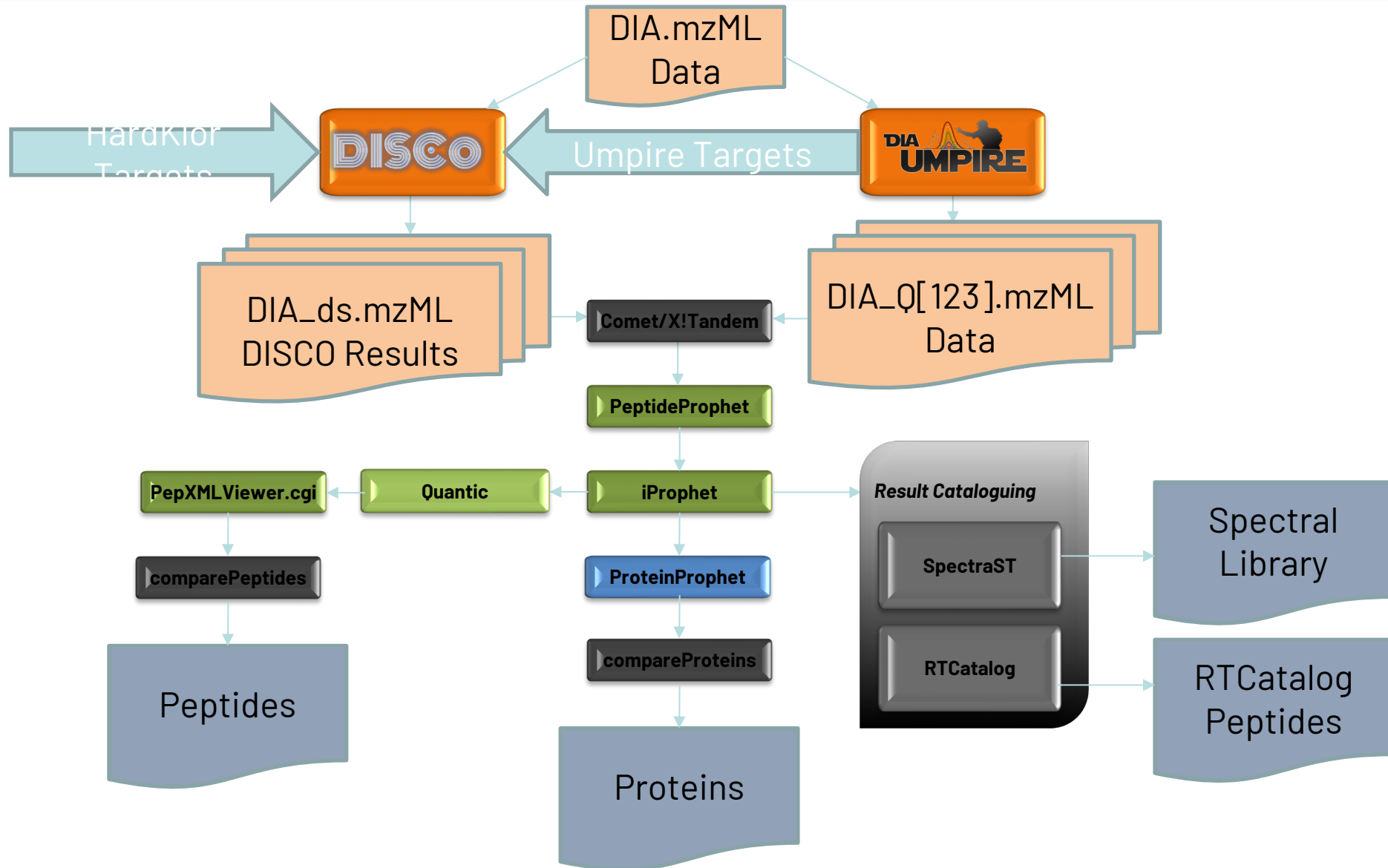
THREADS=<number>: Use specified number of threads (default=1).

STARTSCAN=<number>: Starting scan to process (default=1).

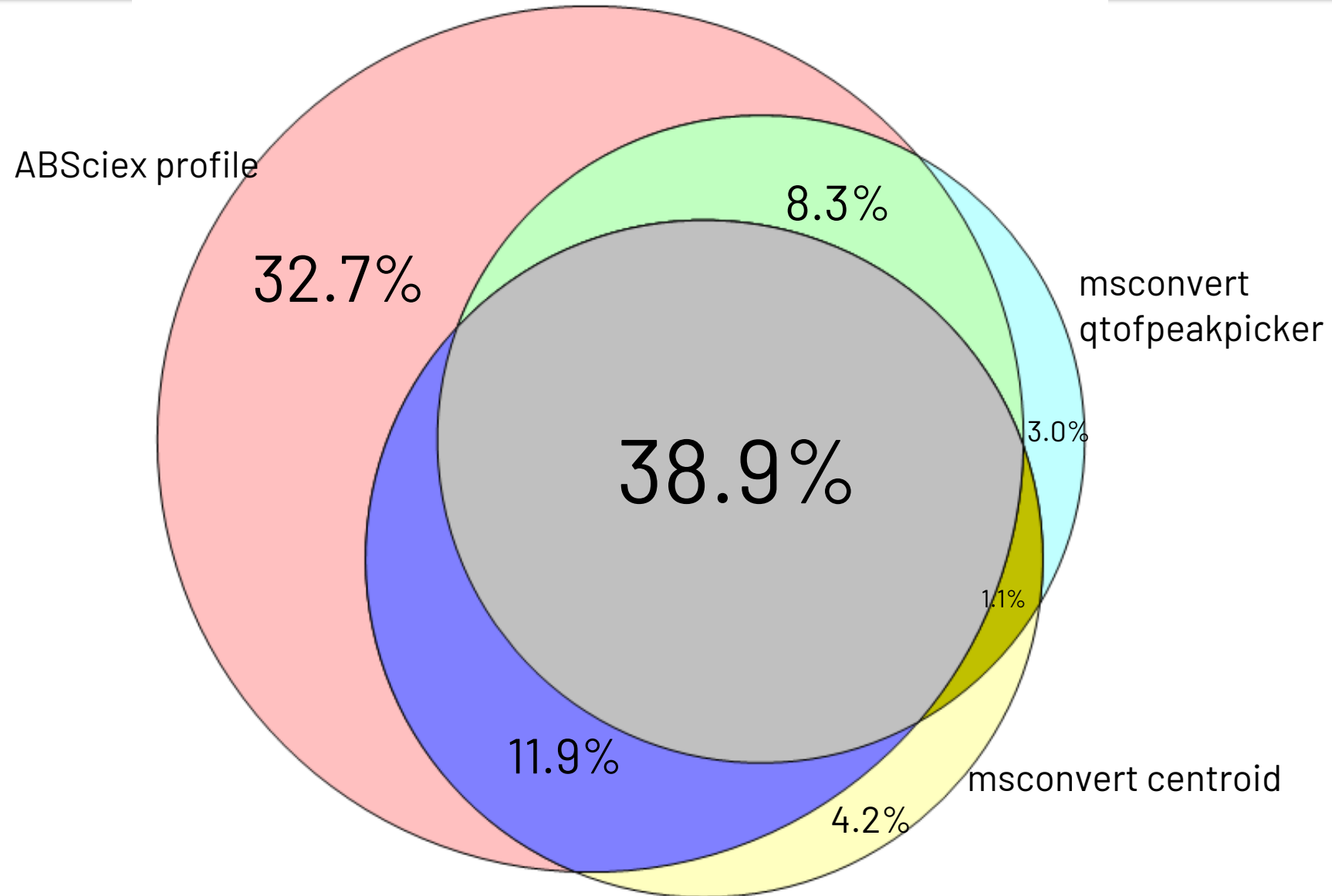
ENDSCAN=<number>: Ending scan to process (default: process until the end of the run).

- DISCO matches identified **precursor** signals to fragment signals by looking for those that have a similar shape in Retention Time Space
- Fragment signals are sorted according to the following criteria, in order:
entropy → *distance* → *correlation* → *intensity*
- Type I Error Rate α and Type II Error Rate β , N is sample size (points across peak)
- For each MS¹ target the following MS² peaks are selected:
 - Up to 1000 peaks with correlation $\geq rMax$ ($\alpha = 0.01, \beta = 0.05$)
 - Up to 500 peaks with correlation $\geq rMid1$ ($\alpha = 0.03, \beta = 0.1$)
 - Up to 200 peaks with correlation $\geq rMid2$ ($\alpha = 0.05, \beta = 0.2$)
 - Up to 100 peaks with correlation $\geq rMin$ ($\alpha = 0.1, \beta = 0.333$)
 - Peak intensities are scaled by the correlation

Data-Driven DIA Workflow



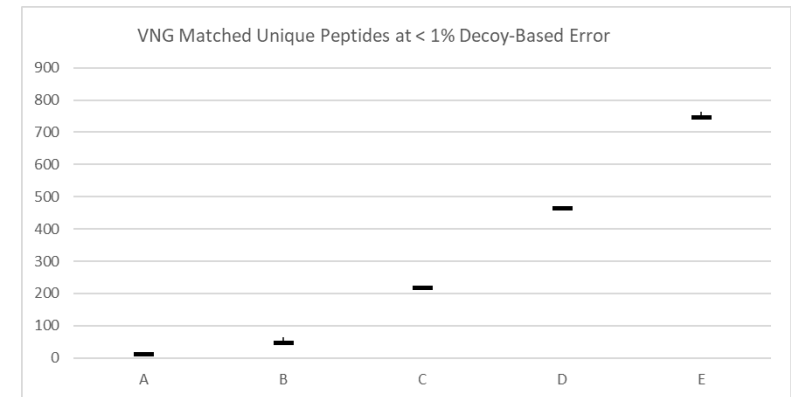
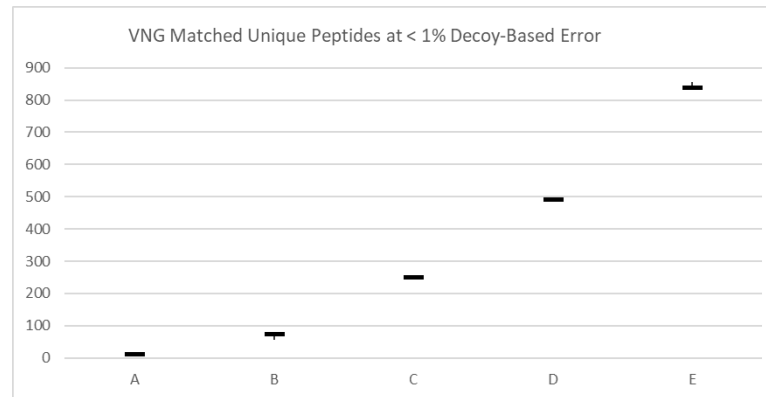
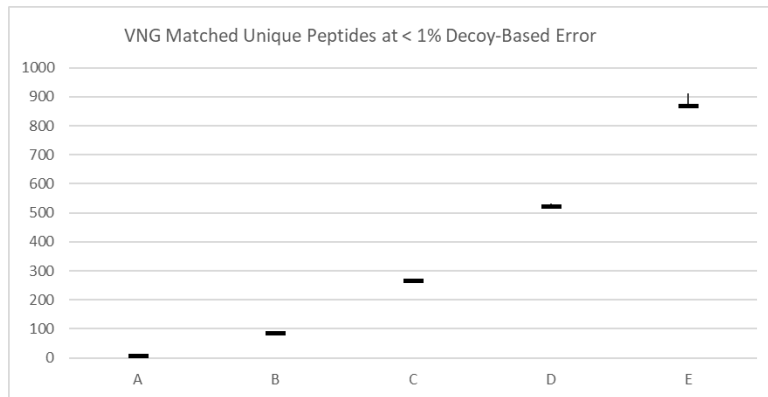
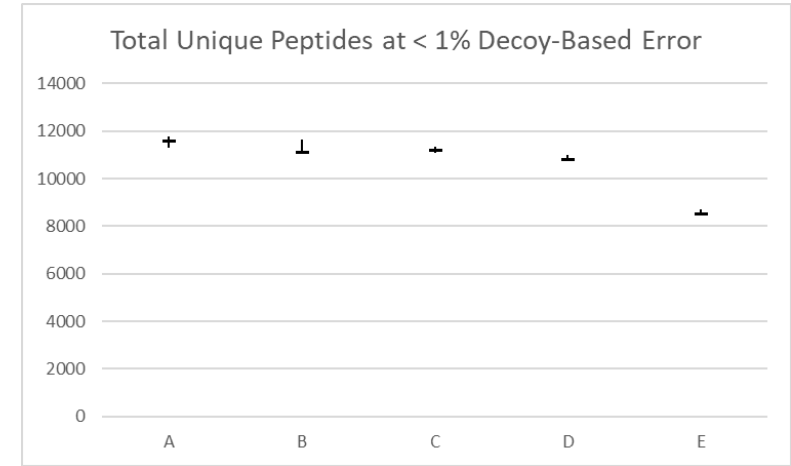
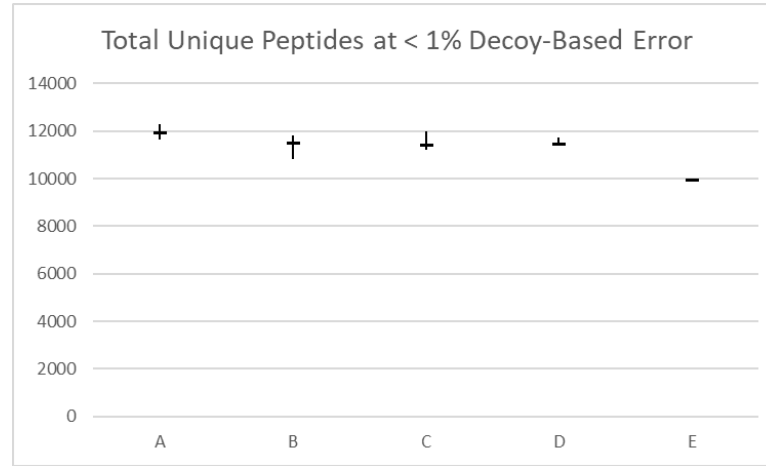
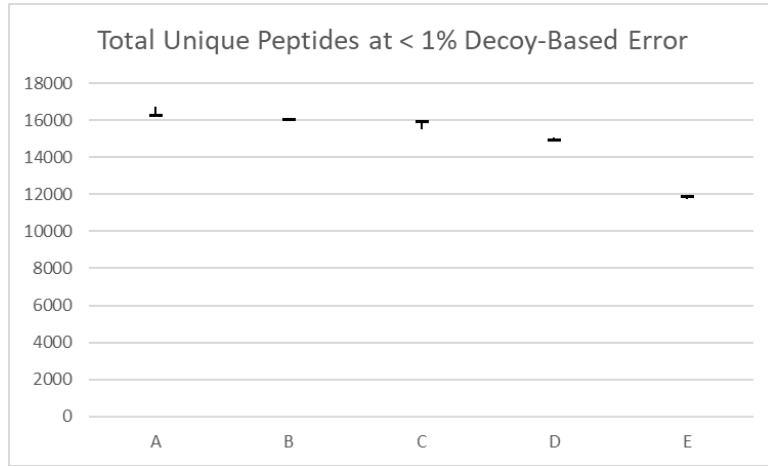
For TTOF 6600 Converter Choices Matter!



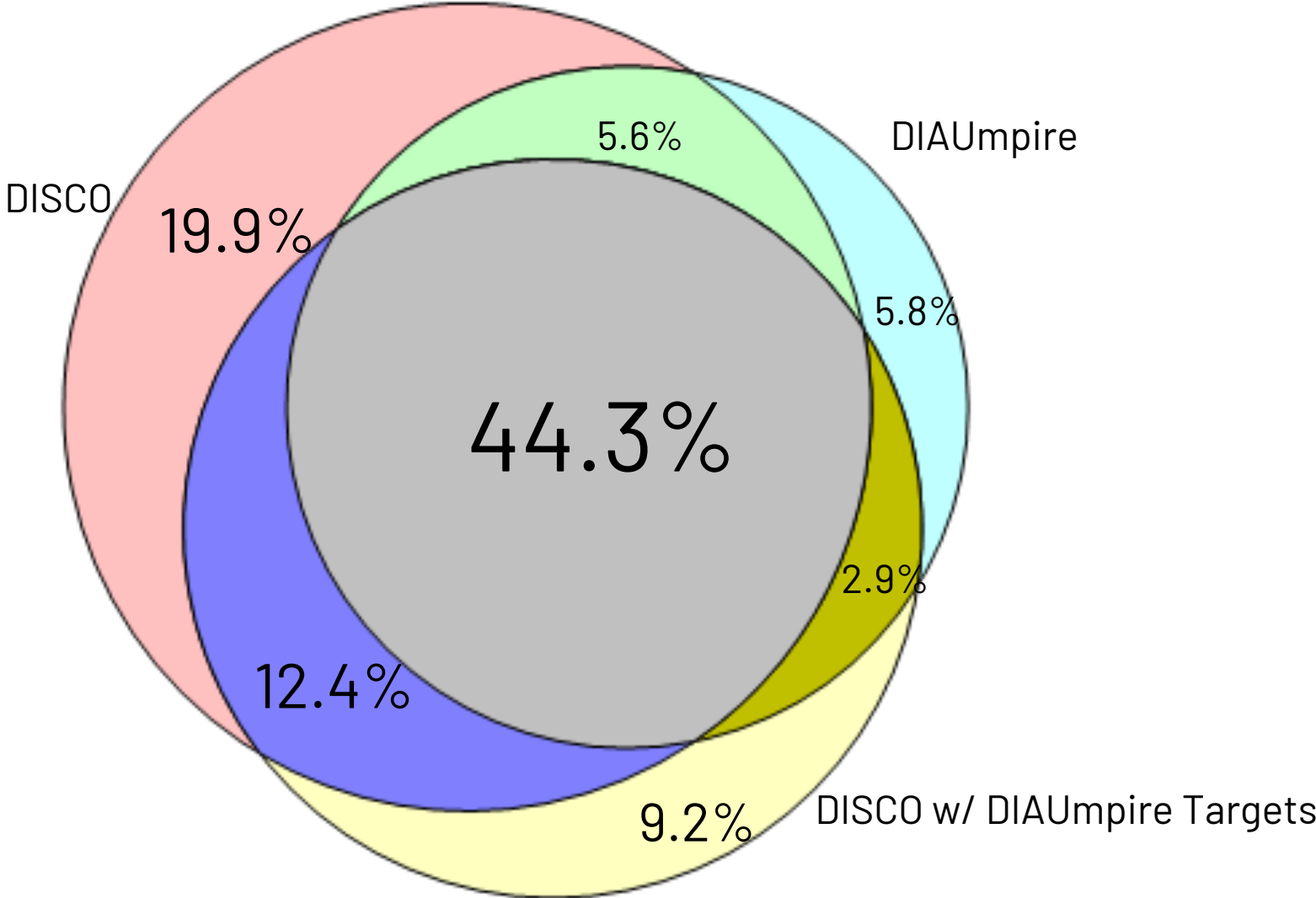
Benchmarking HeLa/Halo Dataset

Sample	HeLa Background (ug)	Halo Variable (fmol)	Halo E/x Ratio	Halo log2 (E/x)
A	3.52	1.56	256	E/A= 8
B	3.52	6.25	64	E/B= 6
C	3.52	25	16	E/C= 4
D	3.52	100	4	E/D= 2
E	3.52	400	1	E/E= 0

Hela/Halo TTOF6600 : DISCO – DIAUmpire – DISCO w/ Umpire Targets



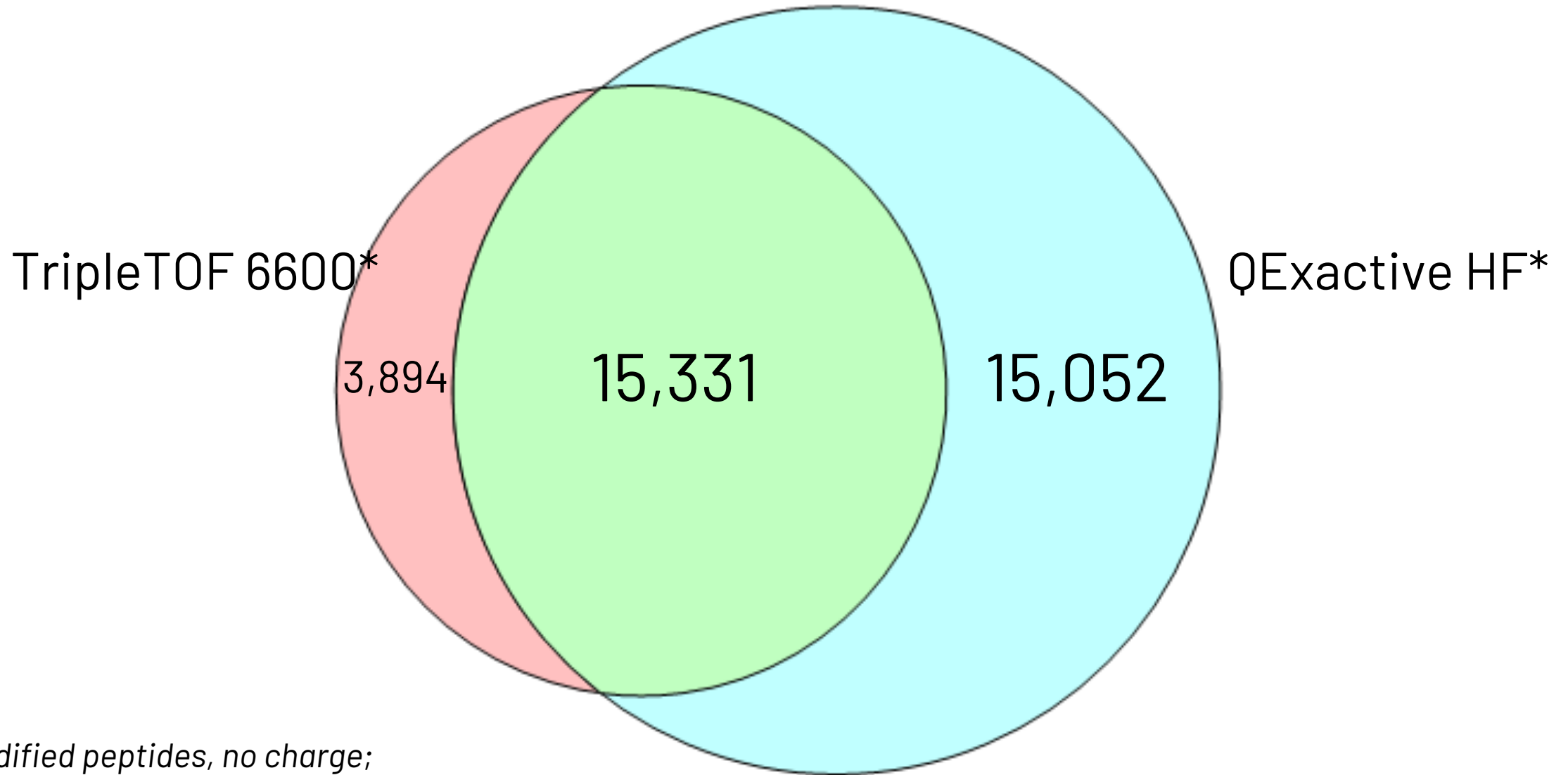
DISCO - DIAUmpire - DISCO w/ DIAUmpire Targets



Mass-Spectrometry Setups

	Triple TOF 6600	Q Exactive HF
Sample Type	HeLa Halo	HeLa Halo
Sample Load (µg)	3.5	0.88
Column Details	500µm X 150mm X 2.4µm	75µm X 500mm X 1.9µm
Flow (nl/min)	5000	300
Gradient length (min)	98	98
Median Peak Width (sec)	22.2	15.6
Acquisition Mode	SWATH	DIA
# Windows	100 variable	100 fixed (6m/z)
MS1 m/z range	400-1250	400-1000
MS1 scans- Accumulation time/Max fill time	250ms	200ms
MS2 scans- Accumulation time/Max fill time	25ms	10ms
Cycle Time (sec)	2.92	4.45

DISCO Peptide Identification: TTOF 6600 vs QE HF



**modified peptides, no charge;
combining 2 search engines (Comet and X!Tandem)*

DISCO Quantitation Options

- **MS1**
 - For each spectrum coming from an MS1 feature, DISCO stores the integrated precursor peak intensity stored as precursor_intensity in mzML
- **MS2 using QuanticParser**
 - For each PSM, Quantic computes the **Annotated Ion Current** (antic_score) by summing the intensities of annotated fragments in the spectrum
- **Hybrid – Combined MS1 and MS2 Quantitation**
 - Average of MS1 and MS2 quantitation results

DISCO Peptide Quantitation from PSMs

MS1

- For each run r , for each peptide ion s compute
 - **maximum MS1 precursor_intensity** over all PSMs in run r matching peptide ion s

$$I_s^r = \max(I_x: \forall(\text{PSMs } x \text{ in } r \text{ where } x \text{ matches } s))$$

- **probability weighted MS1 intensity** over all PSMs in r matching s ,

$$W_s^r = \frac{\sum_{\forall(\text{PSMs } x \text{ in } r \text{ matching } s)} p_p(x)p_i(x)I_x}{\sum_{\forall(\text{PSMs } x \text{ in } r \text{ matching } s)} p_p(x)p_i(x)}$$

where:

I_x is the precursor_intensity of PSM x

$p_p(x)$ is PeptideProphet probability and $p_i(x)$ is iProphet probability, of PSM x

DISCO Peptide Quantitation from PSMs

MS2

- For each run r , for each peptide ion s compute
 - **max MS1 antic_score** over all PSMs in run r matching peptide ion s

$$A_s^r = \max(A_x: \forall(\text{PSMs } x \text{ in } r \text{ where } x \text{ matches } s))$$

- **probability weighted antic_score** over all PSMs in r matching s ,

$$a_s^r = \frac{\sum_{\forall(\text{PSMs } x \text{ in } r \text{ matching } s)} p_p(x)p_i(x)A_x}{\sum_{\forall(\text{PSMs } x \text{ in } r \text{ matching } s)} p_p(x)p_i(x)}$$

where:

A_x is the antic_score of PSM x

$p_p(x)$ is PeptideProphet probability and $p_i(x)$ is iProphet probability, of PSM x

DISCO Peptide Quantitation from PSMs

Hybrid

- For each run r , for each peptide ion s compute
 - Compute Hybrid intensity

$$H_s^r = \frac{1}{2}(I_s^r + a_s^r)$$

where:

I_s^r is the maximum precursor_intensity of peptide ion s in run r

a_s^r is the weighted antic_score of peptide ion s in run r

DISCO Peptide Quantitation Sample Means

- For each peptide ion s over n replicates r_1, \dots, r_n of classification c , compute sample means:

- MS1

$$\overline{W}_s^c = \frac{1}{n} \sum_{i=1}^n W_s^{r_i}$$

- MS2

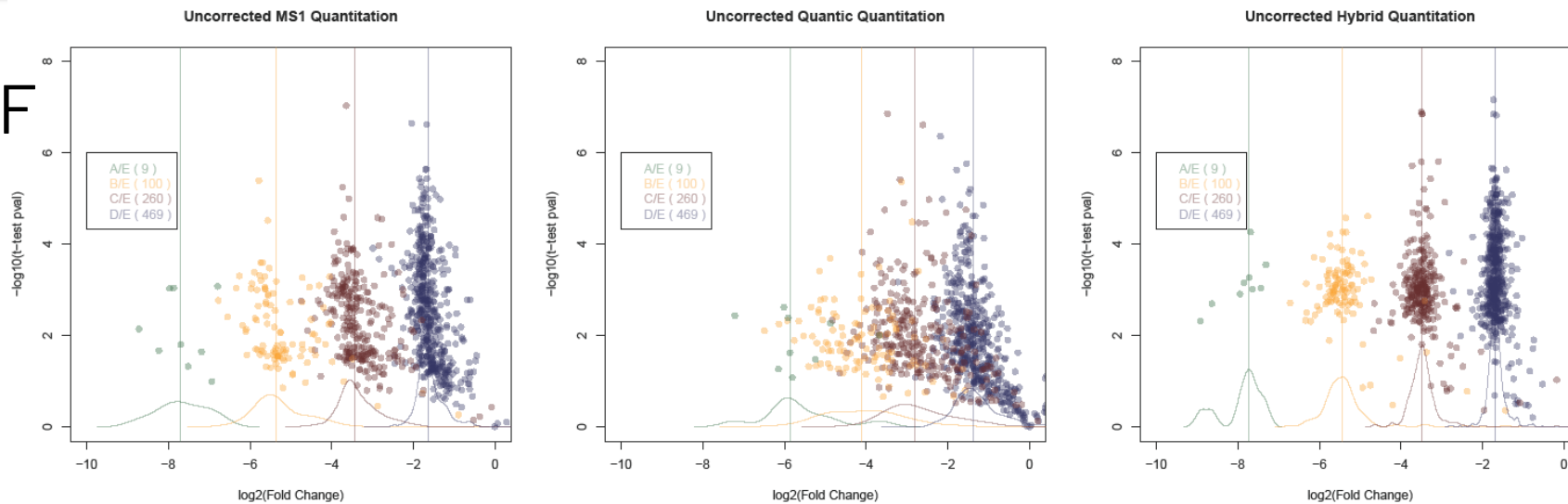
$$\overline{a}_s^c = \frac{1}{n} \sum_{i=1}^n a_s^{r_i}$$

- Hybrid

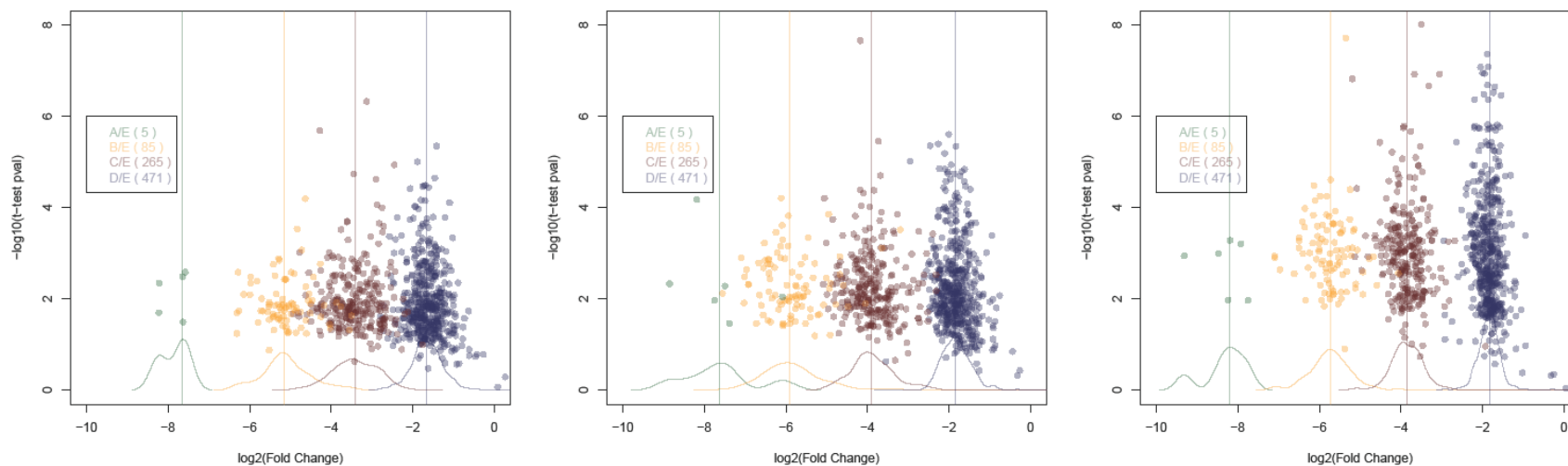
$$\overline{H}_s^c = \frac{1}{n} \sum_{i=1}^n H_s^{r_i}$$

DISCO Halo Quantitation (not corrected for background)

QExactive HF

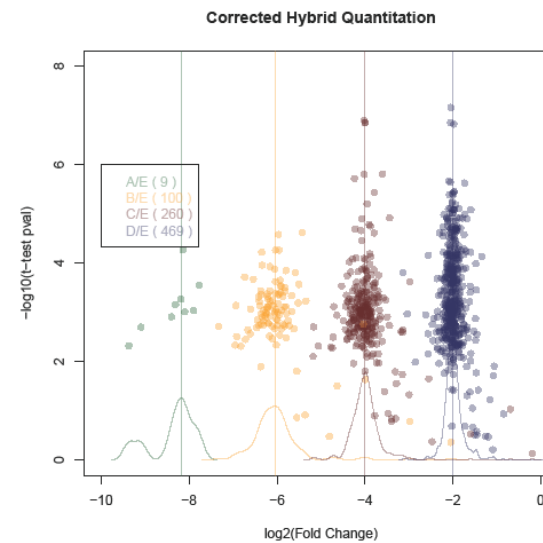
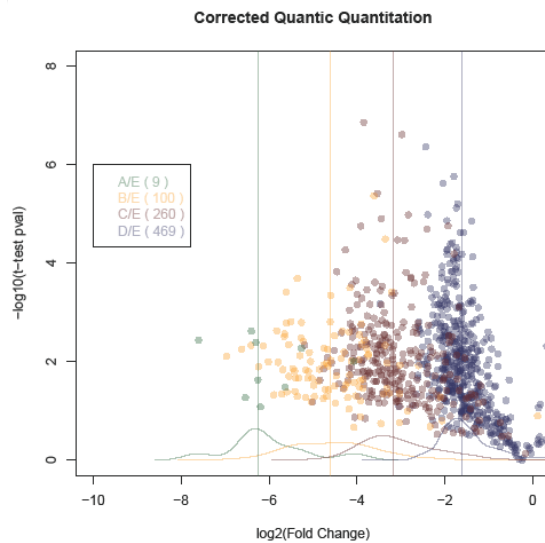
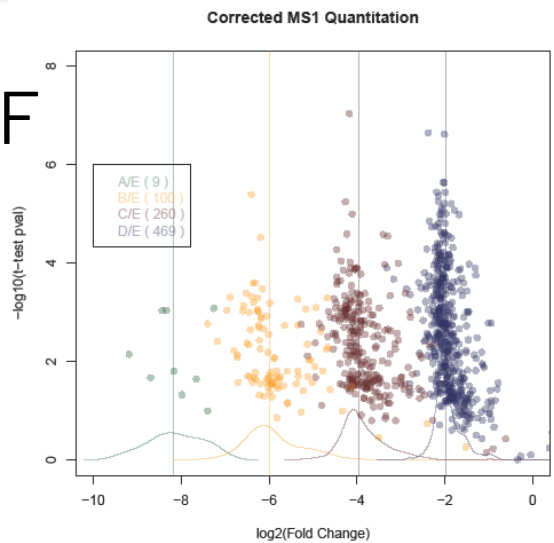


TTOF 6600

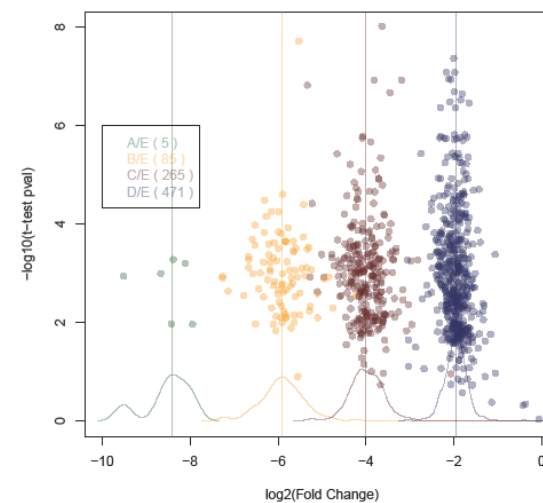
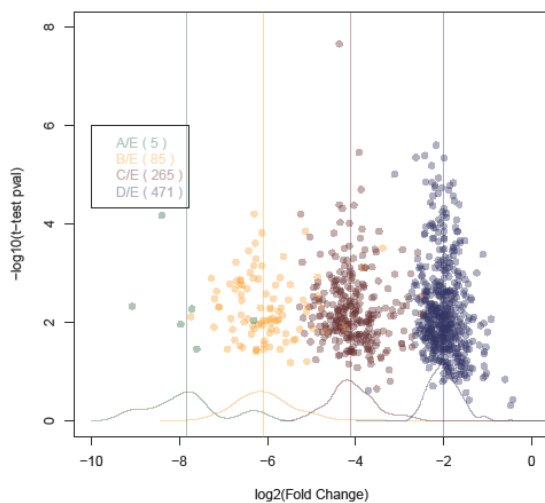
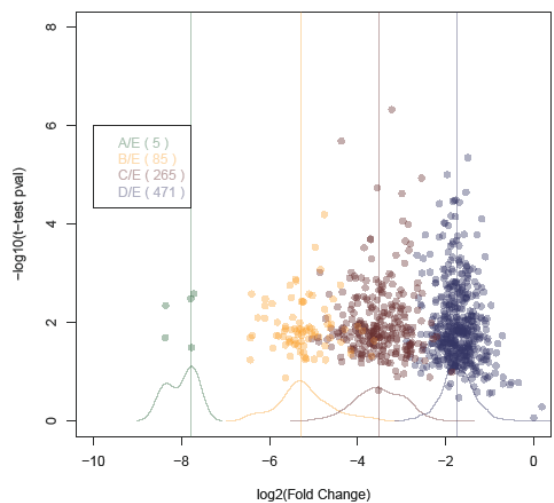


DISCO Halo Quantitation (corrected for background)

QExactive HF

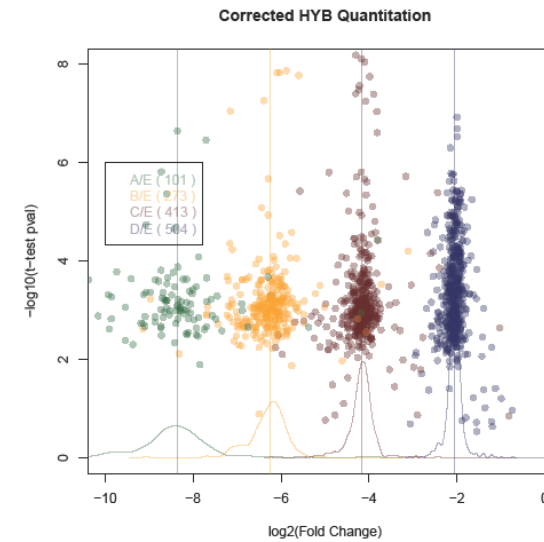
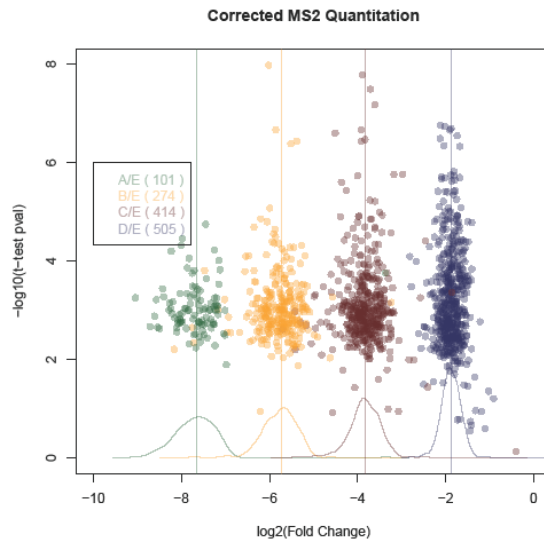
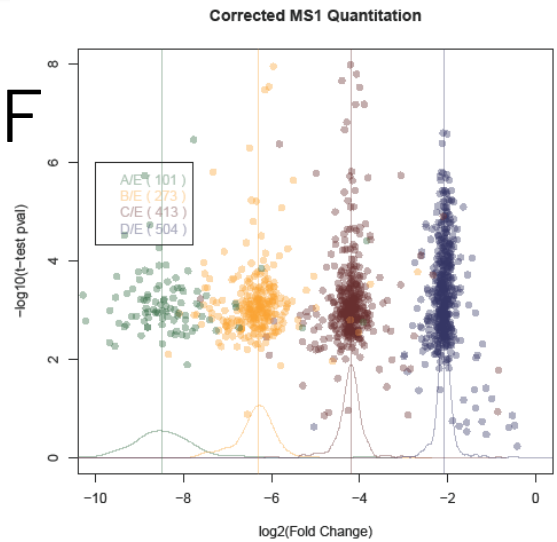


TTOF 6600

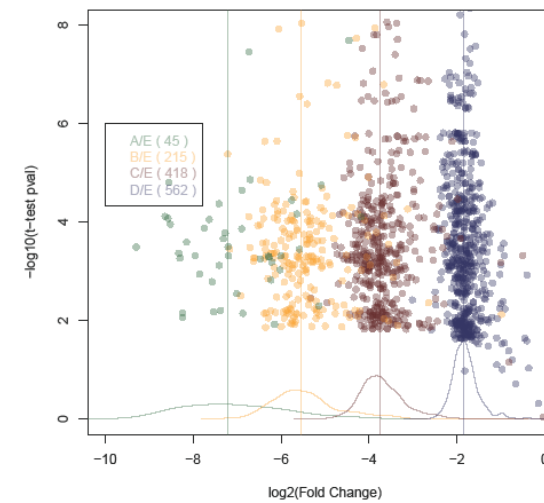
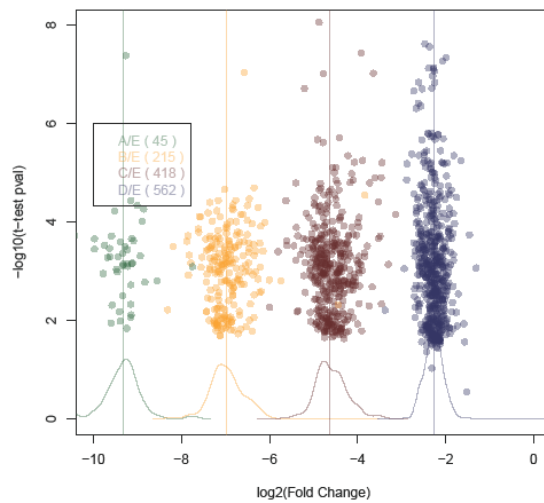
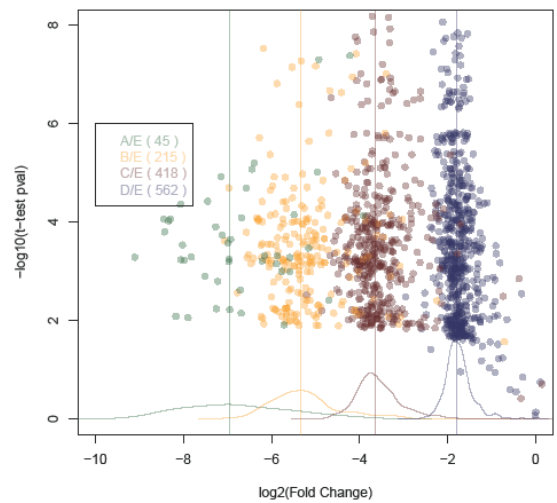


Spectronaut Halo Quantitation (corrected for background)

QExactive HF



TTOF 6600



Conclusions

- **Choice of data converter and centroiding options are important to ensure identifications are not lost due to the file converter**
- **DISCO identification rate improves upon DIAUmpire**
 - Combining approaches maximizes data-driven identifications
- **DISCO quantitation computes accurate ratios, reflecting the ground truth of the dataset**
 - Hybrid quantitation reduces variance in ratios
 - Correcting ratios using the median background ratio is important for accurate ratio estimation

QuanTic

**Applied to CID Cleavable Isobaric Tag
Quantitation**

A Collision-Induced Dissociation Cleavable Isobaric Tag for Peptide Fragment Ion-Based Quantification in Proteomics

Xiaobo Tian, Marcel P. de Vries, Hjalmar P. Permentier, and Rainer Bischoff*



Cite This: <https://dx.doi.org/10.1021/acs.jproteome.0c00371>



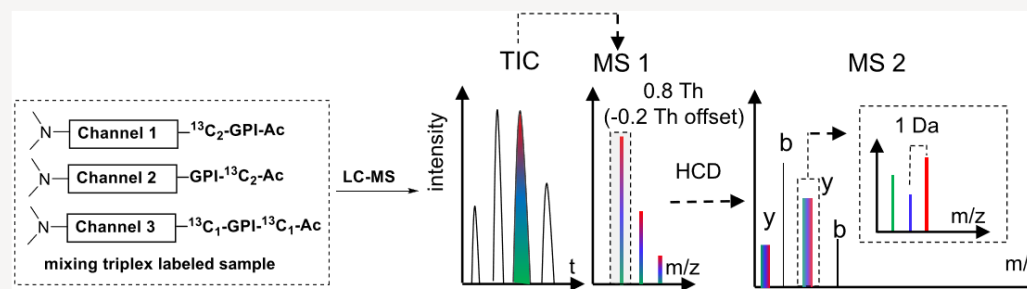
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Supporting Information



ABSTRACT: Quantifying peptides based on unique peptide fragment ions avoids the issue of ratio distortion that is commonly observed for reporter ion-based quantification approaches. Herein, we present a collision-induced dissociation-cleavable, isobaric acetyl-isoleucine-proline-glycine (Ac-IPG) tag, which conserves the merits of quantifying peptides based on unique fragments while reducing the complexity of the b-ion series compared to conventional fragment ion-based quantification methods thus facilitating data processing. Multiplex labeling is based on selective N-terminal dimethylation followed by derivatization of the ϵ -amino group of the C-terminal Lys residue of LysC peptides with isobaric Ac-IPG tags having complementary isotope distributions on Pro-Gly and Ac-Ile. Upon fragmentation between Ile and Pro, the resulting y ions, with the neutral loss of Ac-Ile, can be distinguished between the different labeling channels based on different numbers of isotope labels on the Pro-Gly part and thus contain the information for relative quantification, while b ions of different labeling channels have the same m/z values. The proteome quantification capability of this method was demonstrated by triplex labeling of a yeast proteome spiked with bovine serum albumin (BSA) over a 10-fold dynamic range. With the yeast proteins as the background, BSA was detected at ratios of 1.14:5.06:9.78 when spiked at 1:5:10 ratios. The raw mass data is available on the ProteomeXchange with the identifier PXD 018790.

KEYWORDS: isobaric labeling, tandem mass spectrometry, fragment ion, quantitative proteomics, stable isotope

Concept of the Ac-AG-tag

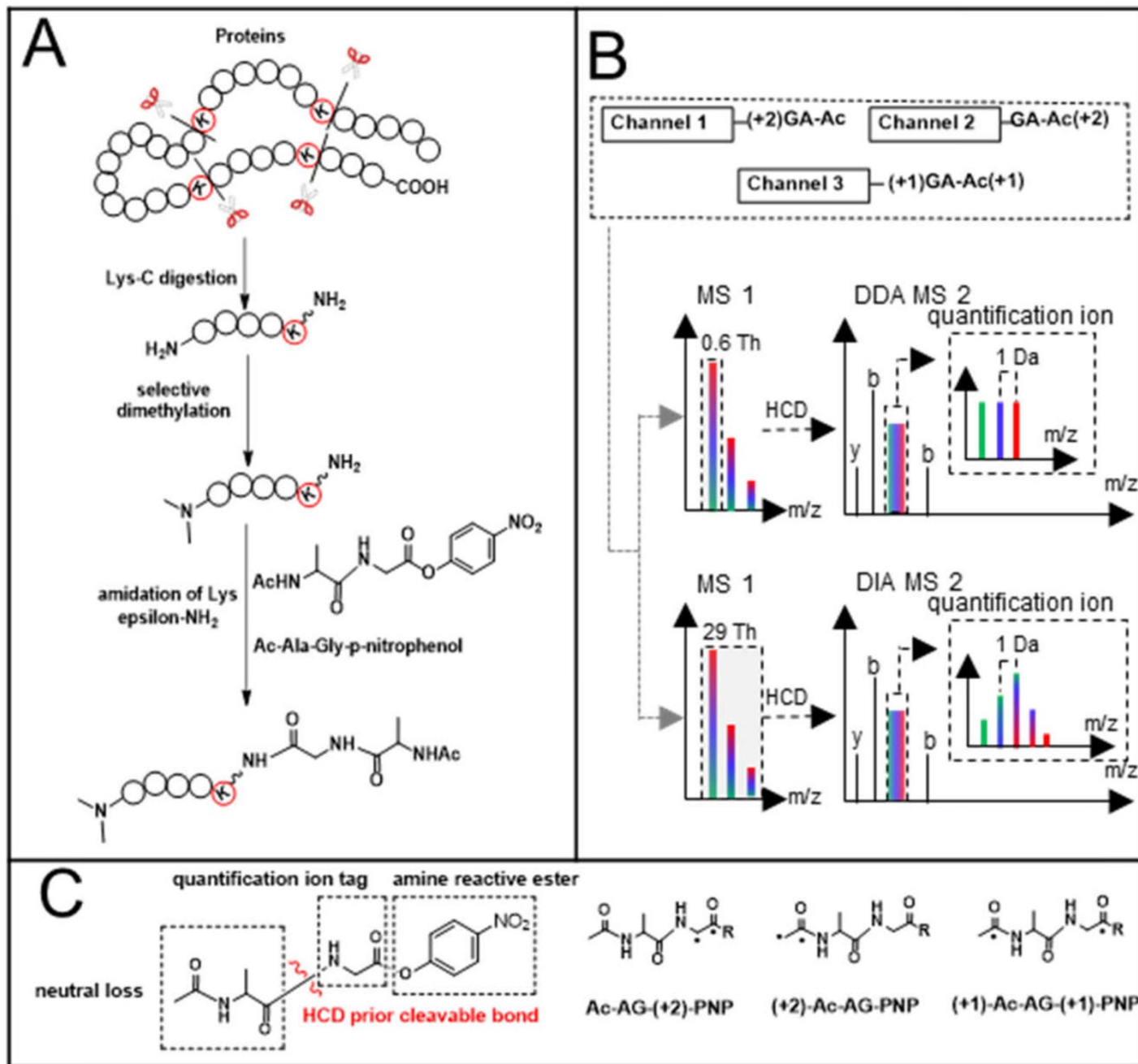


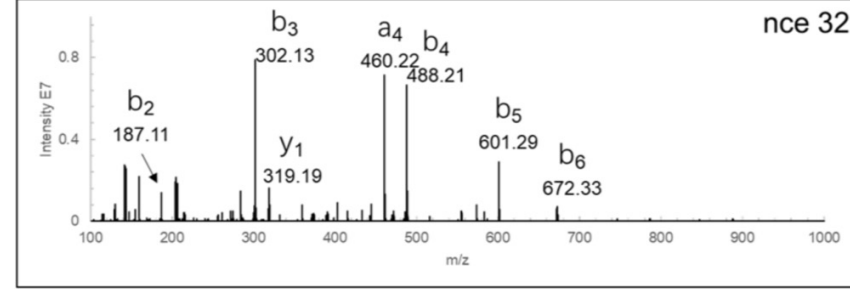
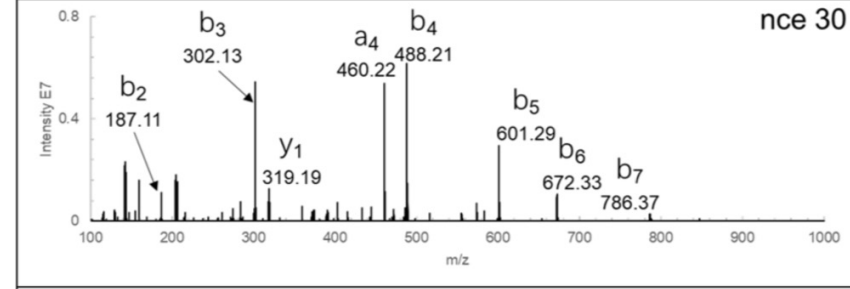
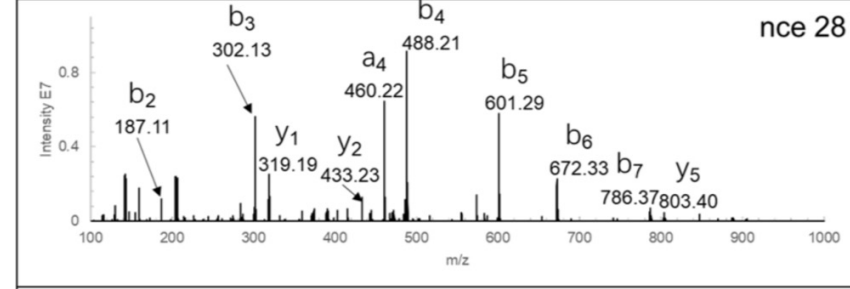
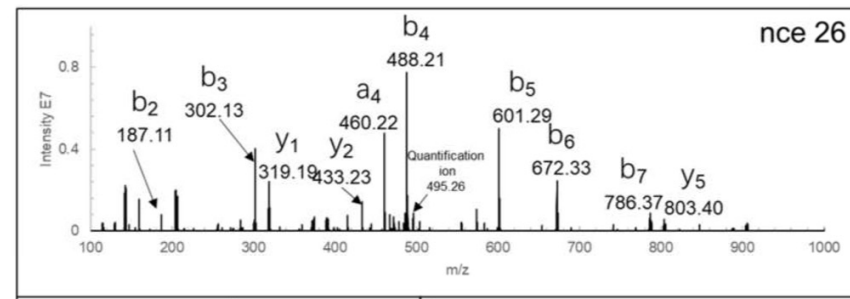
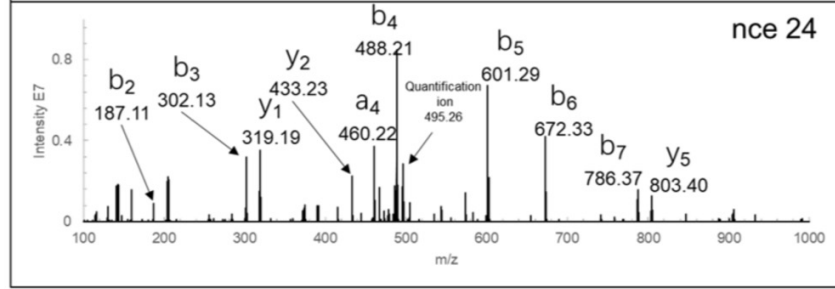
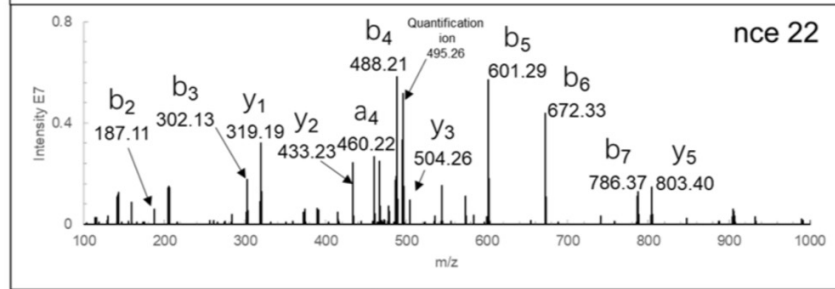
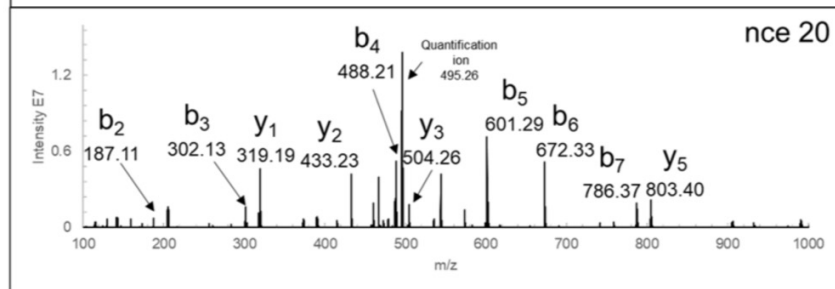
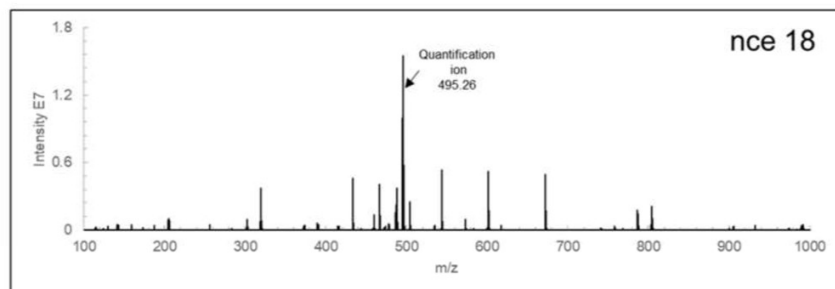
Fig. 1. Schematic view of the Ac-AG approach.

A) Isobaric labeling steps;

B) LC-MS/MS concept map for a mixture of triplex labeled samples;

C) Functional design of the Ac-AG-PNP tag (¹³C isotope locations are marked with “*”).

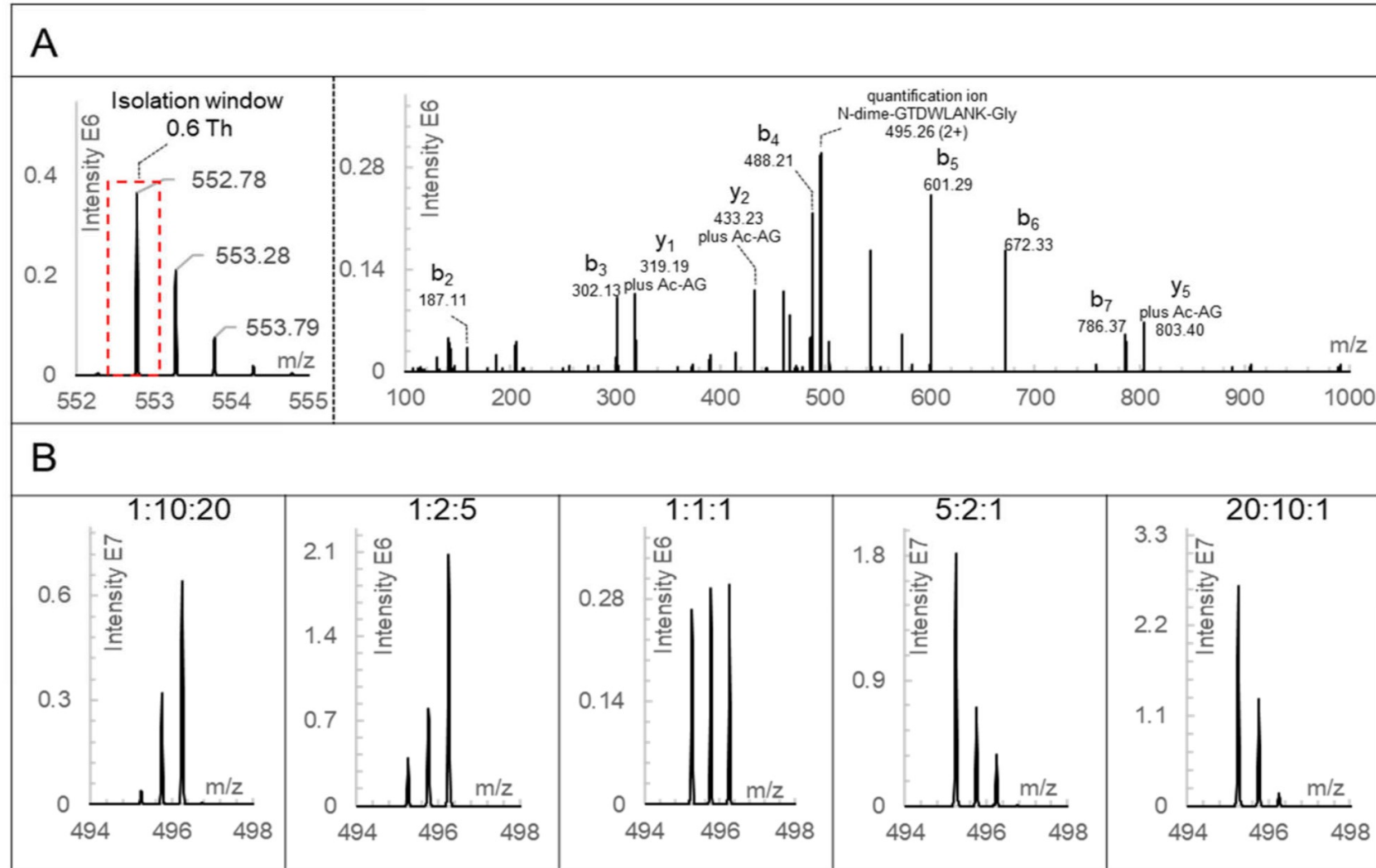
Studying peptide fragmentation with various NCEs



NCE 18 for producing peptide-coupled reporter-ion

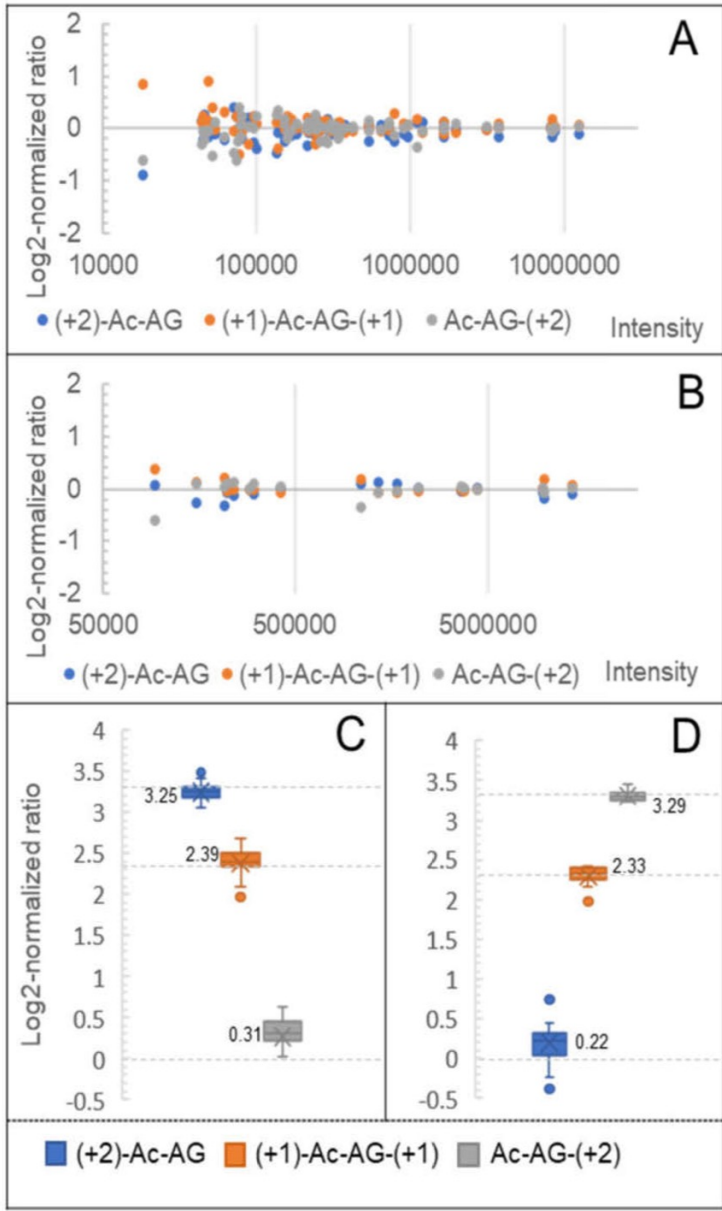
NCE 28 for formation of fragments of the peptide backbone

Quantification at the peptide level



A) precursor isolation with a window of 0.6 Th and MS2 spectrum of triplex-labeled N-dime-GTDWLANK-GA-Ac with a combined NCE of 18 and 28. **B)** Peptide-coupled reporter-ions in the DDA MS2 spectra at ratios of 1:10:20, 1:2:5, 1:1:1, 5:2:1 and 20:10:1.

Quantification of triplex labeled LysC BSA



Calculating the ratio for PSM 6134 (AEFVEVTK) from triplex labeled BSA mixed at a ratio of 5:2:1

scan_number	intensity (+0)	intensity (+1)	intensity(+2)	total_intensity (3 channels)	sequence
6134	72202240	28389638	13828063	114419941	AEFVEVTK

Normalizer (weighted average) = $(72202240 + 28389638 + 13828063)/(1+2+5) = 14302492.63$
 Ratio of 'spectrum of 6134' = $72202240 / 14302492.63 : 28389638 / 14302492.63 : 13828063 / 14302492.63$
 = 5.05 : 1.98 : 0.97

Calculating peptide ratios based on top 3 most intense PSMs.

Calculating protein ratios based on top 3 most intense peptides.

A) Log2-normalized ratio distribution at the PSM level at a mixing ratio of 1:1:1; **B)** Log2-normalized ratio distribution at the peptide level at a mixing ratio of 1:1:1; **C)** Log2-normalized ratio at the peptide level at a mixing ratio of 10:5:1; **D)** Log2-normalized ratio at the peptide level at a mixing ratio of 1:5:10. Expected values for log2-normalized mixing ratios are shown as dotted lines.

QuanTic **New Option for Quantitation**

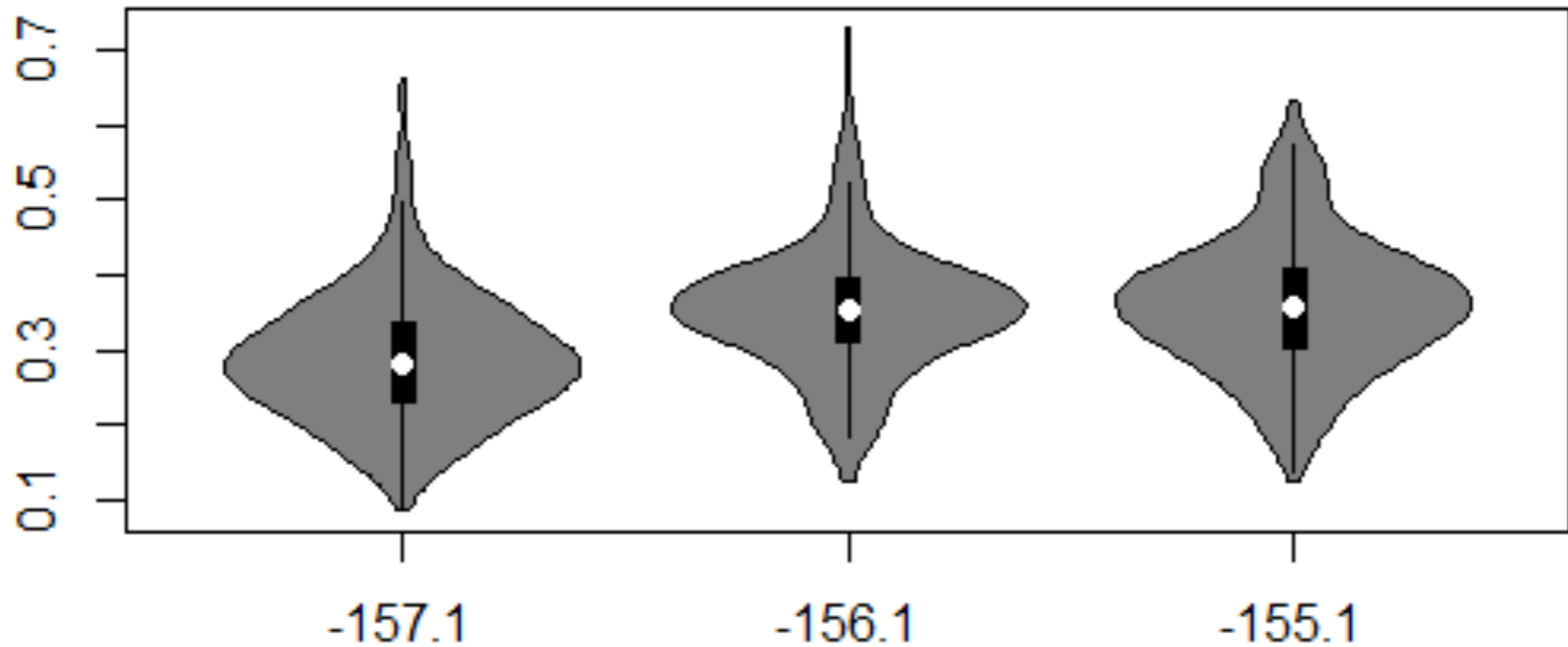
K:311.17:-155.1:-156.1:-157.1

**<amino acids, n, or
c>:<mass_shift>:<neut_loss1>:...:<neut_lossN> Specify
modifications with neutral losses for quantitation (default: off)**

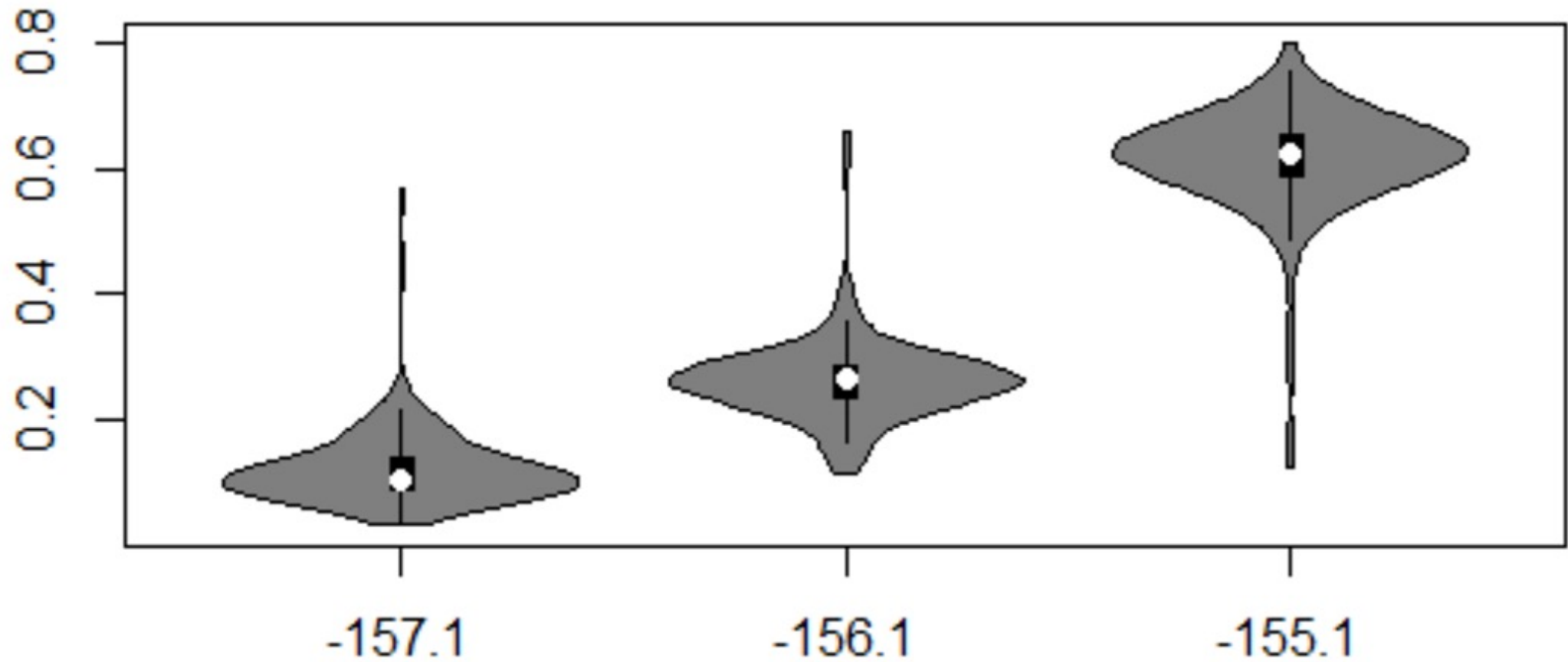
***Quantify the intensity of neutral loss peaks associated with a modification for all fragments retaining the modification, when all are available for a given fragment ion, skip y1**

PROB	SPECTRUM	EXPECT	IONS	PEPTIDE	PROTEIN	NPROTS	CALC_MASS	QUANTIC	QUANTIC_TIC	QUANTIC_QUANT	QUANTIC_STRING
1.0000	Ac-IPG-yeast-1-1-1.06237.06237.3 SR	0.00011	10/52	K, n29.04AVYAGENFHHGDK439.26.L, - RA	sp P00925 ENO2_YEAST	1	1895.9385	3.89711e+06	4.34866e+07	1347818.1,1269763.4,735574.9	M(-155.1),0,581.291,0
1.0000	Ac-IPG-yeast-1-1-1.09376.09376.3 SR	0.000245	11/64	K, n29.04TFEAAMRIGSEVYHNLK439.26.S RA	sp P00925 ENO2_YEAST	1	2304.1791	1.30834e+06	1.07953e+07	524884.3,472688.3,315971.1	
1.0000	Ac-IPG-yeast-1-1-1.08330.08330.3 SR	1.2e-07	17/72	R, n29.04SVYDSRGNPTVEVELTTEK439.26.G RA	sp P00924 ENO1_YEAST +1	2	2462.2395	1.95827e+06	1.42429e+07	417081.5,289362.8,194558.9	
1.0000	Ac-IPG-yeast-1-1-1.07870.07870.3 SR	0.000231	13/52	K, n29.04LSRAIQTANIALEK439.26.A RA	sp P00950 PMG1_YEAST	1	1866.0793	1.70548e+06	1.16365e+07	300725.9,277894.0,107346.1	
1.0000	Ac-IPG-yeast-1-1-1.07563.07563.3 SR	0.000203	12/48	K, n29.04DVTREYIINLHK439.26.R RA	sp P0C2H8 RL31A_YEAST +1	2	1926.0430	702968	1.24334e+07	251100.6,227770.3,126984.0	
0.9988	Ac-IPG-yeast-1-1-1.06329.06329.3 SR	0.696	11/52	K, n29.04LVLVRHGQSEWNEK439.26.N RA	sp P00950 PMG1_YEAST	1	2033.0913	681717	4.77765e+06	247265.2,229580.6,123972.0	
0.9994	Ac-IPG-yeast-1-1-1.07926.07926.3 SR	0.00217	10/72	R, n29.04SIAPAYGIPVVLHSDHC160.03AK439.26.K RA	sp P14540 ALF_YEAST	1	2373.2370	1.62851e+06	8.71115e+06	243413.1,221734.3,165115.4	M(-155.1),0,740.385,-0.001,6254.6;M(-156.1),0,740.051,-0.0
0.9988	Ac-IPG-yeast-1-1-1.08861.08861.3 SR	0.00522	11/68	K, n29.04IGGIGTVPVGRVETGVIK439.26.P RA	sp P02994 EF1A_YEAST	1	2090.2318	633286	6.6591e+06	225457.1,206643.7,131845.3	
0.9999	Ac-IPG-yeast-1-1-1.06736.06736.3 SR	0.00335	9/56	K, n29.04IYPGRGTLFVRGDSK439.26.L RA	sp P04449 RL24A_YEAST +1	2	2004.1011	575172	2.72473e+06	222247.7,199969.1,143789.9	
0.9023	Ac-IPG-yeast-1-1-1.11233.11233.2 SR	0.0726	4/34	R, n29.04SFDVPPPPIDASSPFQK439.26.G RA	sp P00950 PMG1_YEAST	1	2254.1376	639804	1.99186e+06	189855.8,148777.9,103828.8	
1.0000	Ac-IPG-yeast-1-1-1.10847.10847.3 SR	0.000426	15/64	K, n29.04ALENTPRFLAILGGAK439.26.V RA	sp P00560 PGK_YEAST	1	2106.2056	482013	3.15874e+06	172527.2,154608.7,110860.0	
1.0000	Ac-IPG-yeast-1-1-1.09442.09442.3 SR	5.7e-07	15/64	K, n29.04IVSNASC160.03TTNC160.03LAPLAK439.26.V RA	sp P00358 G3P2_YEAST +3	4	2158.0981	2.00572e+06	1.93312e+07	169416.0,167327.3,118868.3	
1.0000	Ac-IPG-yeast-1-1-1.09266.09266.3 SR	2.14e-06	11/76	K, n29.04TSAVAALTEVRAEFAALAK439.26.L RA	sp P17076 RL8A_YEAST +1	2	2354.2548	815505	7.16469e+06	158161.9,161845.1,89759.4	
0.9975	Ac-IPG-yeast-1-1-1.08020.08020.3 SR	0.385	8/60	K, n29.04YAQDGAGIERELARIK439.26.K RA	sp P14126 RL3_YEAST	1	2128.1495	824834	2.78199e+06	132469.4,109744.6,89193.2	
1.0000	Ac-IPG-yeast-1-1-1.07359.07359.3 SR	0.00275	11/60	K, n29.04TVMIAAHGNSLRGLVK439.26.H RA	sp P00950 PMG1_YEAST	1	2005.1361	349374	2.41765e+06	108782.1,94074.6,71460.5	
0.9223	Ac-IPG-yeast-1-1-1.06350.06350.3 SR	1.6	3/40	K, n29.04NIVEFHSDHMK439.26.L RA	sp P06169 PDC1_YEAST	1	1694.8305	160852	3.35394e+06	104704.5,86711.9,84888.3	
1.0000	Ac-IPG-yeast-1-1-1.05512.05512.3 SR	0.000841	3/40	M, n29.04NFSHGSEYEHK439.26.S RA	sp P00549 KPYK1_YEAST	1	1706.7908	208583	4.87777e+06	103630.2,105907.0,71181.5	
1.0000	Ac-IPG-yeast-1-1-1.05694.05694.3 SR	0.0145	9/44	K, n29.04ALNEEAFAARLLK439.26.N RA	sp P0CX82 RL19A_YEAST +1	2	1737.9592	377602	882798	102408.1,91357.6,76992.9	
0.9999	Ac-IPG-yeast-1-1-1.09738.09738.3 SR	0.0738	7/68	K, n29.04SFLESVIRDSVYTYTEHAK439.26.R RA	sp P02309 H4_YEAST	1	2420.2442	309493	1.58101e+06	97209.5,78974.3,64076.4	
0.9953	Ac-IPG-yeast-1-1-1.07541.07541.2 SR	0.0568	6/14	R, n29.04EVLGEQGK439.26.D RA	sp P00549 KPYK1_YEAST	1	1197.6460	364756	2.79228e+06	88068.0,78618.8,77815.7	
0.9792	Ac-IPG-yeast-1-1-1.08567.08567.3 SR	0.183	5/56	R, n29.04LVIVTDPRSDAQAIK439.26.E RA	sp P32905 RSSA1_YEAST	1	1964.1161	280769	1.64864e+06	86562.6,102413.0,56210.7	
1.0000	Ac-IPG-yeast-1-1-1.07839.07839.3 SR	3.3e-05	13/56	F, n29.04AEALRIGSEVYHNLK439.26.S RA	sp P00924 ENO1_YEAST	1	2038.1066	280726	2.43851e+06	83421.3,39456.8,23738.8	
0.9892	Ac-IPG-yeast-1-1-1.05824.05824.3 SR	0.958	6/48	K, n29.04LVERAVSEDPRIK439.26.M RA	sp P32527 ZUO1_YEAST	1	1850.0480	618037	1.97821e+06	79985.4,76044.3,66386.6	
1.0000	Ac-IPG-yeast-1-1-1.09271.09271.3 SR	3.18e-05	13/68	K, n29.04YVDEQVELADAAAPPEAK439.26.L RA	sp P16387 ODPA_YEAST	1	2280.1380	421155	1.87633e+06	77323.9,86621.9,47663.7	
1.0000	Ac-IPG-yeast-1-1-1.04614.04614.3 SR	0.0229	9/48	K, n29.04TAEQVAERAARK439.26.A RA	sp P05737 RL7A_YEAST	1	1738.9545	447613	1.1847e+06	71672.2,71510.2,64837.1	

QuanTic Normalized Results 1:1:1



QuanTic Normalized Results 1:2:5



A Versatile Isobaric Tag Enables Proteome Quantification in Data-Dependent and Data-Independent Acquisition Modes

Xiaobo Tian, Marcel P. de Vries, Hjalmar P. Permentier, and Rainer Bischoff*



Cite This: *Anal. Chem.* 2020, 92, 16149–16157



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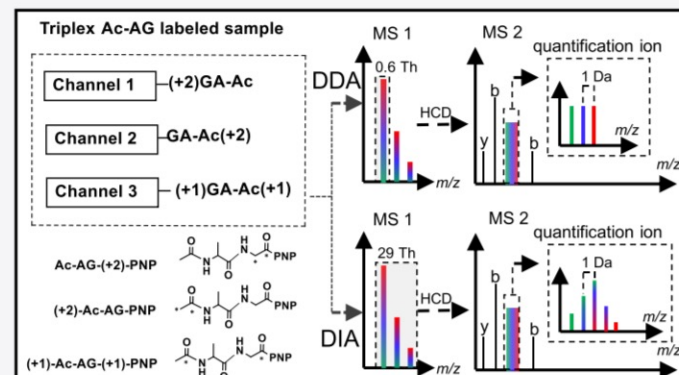
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ABSTRACT: Quantifying proteins based on peptide-coupled reporter ions is a multiplexed quantitative strategy in proteomics that alleviates the problem of ratio distortion caused by peptide cofragmentation, as commonly observed in other reporter-ion-based approaches, such as TMT and iTRAQ. Data-independent acquisition (DIA) is an attractive alternative to data-dependent acquisition (DDA) due to its better reproducibility. While multiplexed labeling is widely used in DDA, it is rarely used in DIA, presumably because current approaches lead to more complex MS2 spectra, severe ratio distortion, or to a reduction in quantification accuracy and precision. Herein, we present a versatile acetyl-alanine-glycine (Ac-AG) tag that conceals quantitative information in isobarically labeled peptides and reveals it upon tandem MS in the form of peptide-coupled reporter ions. Since the peptide-coupled reporter ion is precursor-specific while fragment ions of the peptide backbone originating from different labeling channels are identical, the Ac-AG tag is compatible with both DDA and DIA. By isolating the monoisotopic peak of the precursor ion in DDA, intensities of the peptide-coupled reporter ions represent the relative ratios between constituent samples, whereas in DIA, the ratio can be inferred after deconvoluting the peptide-coupled reporter ion isotopes. The proteome quantification capability of the Ac-AG tag was demonstrated by triplex labeling of a yeast proteome spiked with bovine serum albumin (BSA) over a 10-fold dynamic range. Within this complex proteomics background, BSA spiked at 1:5:10 ratios was detected at ratios of 1.00:4.87:10.13 in DDA and 1.16:5.20:9.64 in DIA.



K:172.075845:-113.0477:-114.0511:-115.0545

COUPLED

- Option that restricts the quantitation to using only the unfragmented precursor-coupled neutral losses for QuanTic

DIAMODE

- Option that allows deconvolves quantitative values for multiple isotope, problem common to DIA data

QuanTic **QuanticProteinParser**

- **Combine Quantic PSM quantitation to compute protein level quantities using PSM stats weighted by intensity**
- **Fully integrated with ProteinProphet**
 - Input and output are the same protXML file
- **Requires no user input other than the input file**
- **Visualized by ProtXMLViewer.cgi**

QuanTic ProtXML Protein View

ProtXML Viewer: [interact-Ac-AG-yeast-125_DDA.NOSTATIC.quantALLions.prot.xml](#)

protXML

#	Main Entry Accession	NTT	Probability	# Tot PSMs	% Coverage Weight	% Spectrum ids NSP	Quantic: n	weight
1	gi 21961605 gb AAH34697.1 (584 aa) (CONTAMINANT) Keratin 10 [Homo sapiens]	1.0000		3	6.3%	0.07%	3	
2	sp O13516 RS9A_YEAST (197 aa) 40S ribosomal protein S9-A OS=Saccharomyces cere...	1.0000		9	29.9%	0.11%	6	
3	sp O14467 MBF1_YEAST (151 aa) Multiprotein-bridging factor 1 OS=Saccharomyces cere...	1.0000		7	44.4%	0.17%	6	
4	sp P00330 ADH1_YEAST (348 aa) Alcohol dehydrogenase 1 OS=Saccharomyces cerevisi...	1.0000		44	56.9%	0.90%	25	
5	sp P00331 ADH2_YEAST (348 aa) Alcohol dehydrogenase 2 OS=Saccharomyces cerevisi...	1.0000		2	25.3%	0.23%	10	
6	sp P00431 CCPR_YEAST (361 aa) Cytochrome c peroxidase, mitochondrial OS=Sacchar...	1.0000		2	4.4%	0.05%	2	
7	sp P00445 SODC_YEAST (154 aa) Superoxide dismutase [Cu-Zn] OS=Saccharomyces c...	1.0000		9	50.0%	0.21%	6	
8	sp P00498 HIS1_YEAST (297 aa) ATP phosphoribosyltransferase OS=Saccharomyces cer...	1.0000		3	9.4%	0.07%	3	
9	sp P00560 PGK_YEAST (416 aa) Phosphoglycerate kinase OS=Saccharomyces cerevisia...	1.0000		82	74.8%	1.94%	40	
10	sp P00815 HIS2_YEAST (799 aa) Histidine biosynthesis trifunctional protein OS=Saccharo...	1.0000		9	12.4%	0.22%	8	
11	sp P00817 IPYR_YEAST (287 aa) Inorganic pyrophosphatase OS=Saccharomyces cerevis...	1.0000		16	44.9%	0.39%	11	
12	sp P00830 ATPB_YEAST (511 aa) ATP synthase subunit beta, mitochondrial OS=Sacchar...	1.0000		4	9.4%	0.10%	4	
13	sp P00931 TRP_YEAST (707 aa) Tryptophan synthase OS=Saccharomyces cerevisiae (str...	1.0000		4	5.9%	0.09%	3	
14	sp P00942 TPIS_YEAST (248 aa) Triosephosphate isomerase OS=Saccharomyces cerevis...	1.0000		21	51.2%	0.46%	12	
15	sp P00950 PMG1_YEAST (247 aa) Phosphoglycerate mutase 1 OS=Saccharomyces cere...	1.0000		30	58.7%	0.70%	17	
16	sp P00958 SYMC_YEAST (751 aa) Methionine-tRNA ligase, cytoplasmic OS=Saccharom...	1.0000		5	6.8%	0.12%	3	
17	sp P02293 H2B1_YEAST (131 aa) +1	1.0000		5	31.3%	0.12%	5	
18	sp P02309 H4_YEAST (103 aa) Histone H4 OS=Saccharomyces cerevisiae (strain ATCC 20...	1.0000		3	36.9%	0.07%	2	
19	sp P02400 RLA4_YEAST (110 aa) 60S acidic ribosomal protein P2-beta OS=Saccharomy...	1.0000		3	31.8%	0.07%	2	
20	sp P02406 RL28_YEAST (149 aa) 60S ribosomal protein L28 OS=Saccharomyces cerevisi...	1.0000		11	43.0%	0.25%	7	
21	sp P02407 RS17A_YEAST (136 aa) +1	1.0000		13	58.1%	0.31%	8	
22	sp P02829 HSP82_YEAST (709 aa) ATP-dependent molecular chaperone HSP82 OS=Sa...	1.0000		3	46.3%	0.55%	31	
23	sp P02994 EF1A_YEAST (458 aa) Elongation factor 1-alpha OS=Saccharomyces cerevisi...	1.0000		33	58.5%	0.80%	23	
24	sp P04147 PABP_YEAST (577 aa) Polyadenylate-binding protein, cytoplasmic and nuclea...	1.0000		16	30.0%	0.38%	14	
25	sp P04449 RL24A_YEAST (155 aa) 60S ribosomal protein L24-A OS=Saccharomyces ce...	1.0000		10	33.5%	0.13%	9	
26	sp P04456 RL25_YEAST (142 aa) 60S ribosomal protein L25 OS=Saccharomyces cerevisi...	1.0000		11	49.3%	0.26%	8	
27	sp P04801 SYTC_YEAST (734 aa) Threonine-tRNA ligase, cytoplasmic OS=Saccharomy...	1.0000		10	10.9%	0.24%	9	
28	sp P04802 SYDC_YEAST (557 aa) Aspartate-tRNA ligase, cytoplasmic OS=Saccharomy...	1.0000		7	14.0%	0.17%	6	
29	sp P04806 HXKA_YEAST (485 aa) Hexokinase-1 OS=Saccharomyces cerevisiae (strain A...	1.0000		24	37.3%	0.48%	16	
30	sp P04807 HXKB_YEAST (486 aa) Hexokinase-2 OS=Saccharomyces cerevisiae (strain A...	1.0000		18	29.2%	0.34%	13	
31	sp P04840 VDAC1_YEAST (283 aa) Mitochondrial outer membrane protein porin 1 OS=S...	1.0000		5	15.2%	0.12%	5	

QuanTic ProXML Peptide View

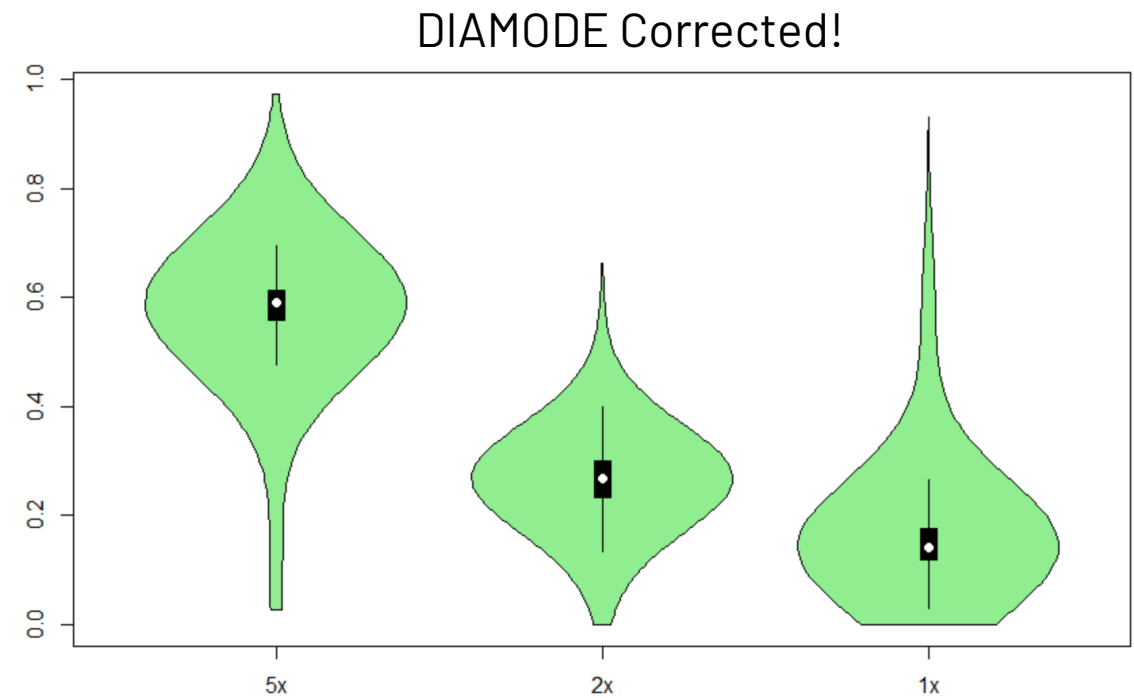
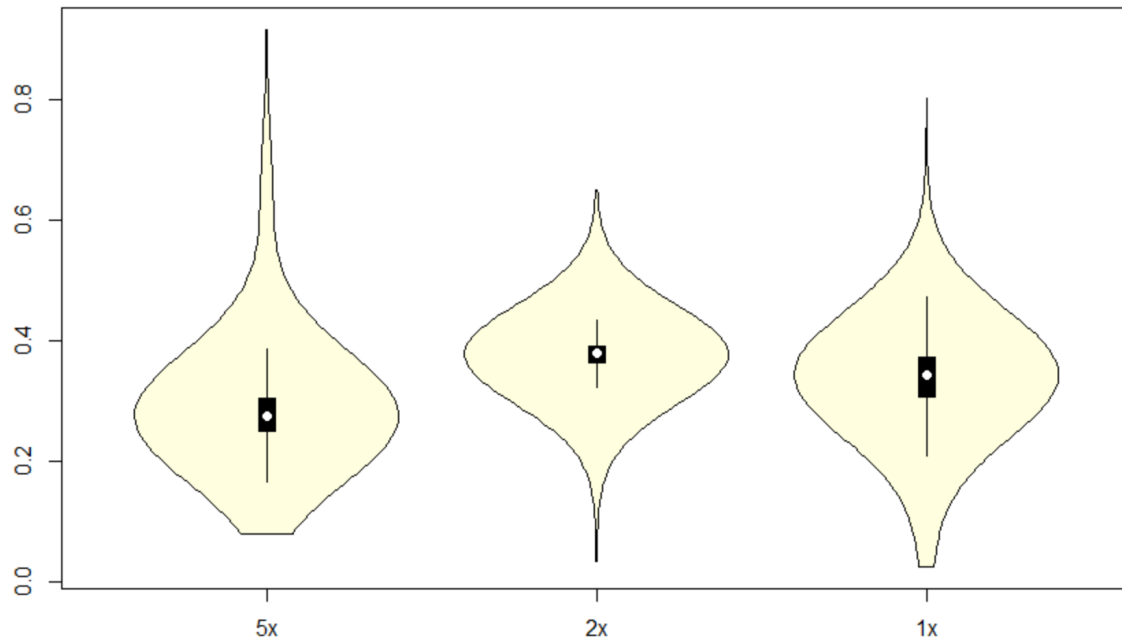
ProtXML Viewer: [interact-Ac-AG-yeast-125_DDA.NOSTATIC.quantALLions.prot.xml](#)

protXML

#	Main Entry Accession	NTT	Probability	# Tot PSMs	% Coverage Weight	% Spectrum ids NSP	Quantic: n	weight
4	sp P00330 ADH1_YEAST (348 aa) Alcohol dehydrogenase 1 OS=Saccharomyces cerevisi...		1.0000	44	56.9% 0.90%		25	
+2	n ₂₉ AM ₁₄₇ GYRVL	0.9987		1	0.26	24.50		
+2	n ₂₉ AMGYRVLGIDGGEGK ₃₀₀	0.9997		1	1.00	23.77	1	0.89
+2	n ₂₉ AM ₁₄₇ GYRVLGIDGGEGK ₃₀₀	0.9997		1	1.00	23.77	1	1.18
+3	n ₂₉ AM ₁₄₇ GYRVLGIDGGEGK ₃₀₀	0.9991		1	1.00	23.77	1	15.63
+2	n ₂₉ ANELLINVK ₃₀₀	0.9997		3	1.00	23.77	3	7.41
+2	n ₂₉ ATDGGAGVINV	0.9997		1	1.00	23.77		
+2	n ₂₉ ATDGGAGVINVSVS	0.9995		1	1.00	23.77		
+2	n ₂₉ CCSDVFNQVVK ₃₀₀	0.9996		1	1.00	23.77		
+2	n ₂₉ DIPVPK ₃₀₀ PK ₃₀₀	0.9997		6	0.50	24.26	6	24.95
+2	n ₂₉ DIVGAVLK ₃₀₀	0.9973		2	1.00	23.77	2	1.38
+2	n ₂₉ EAAIEASTR	0.9565		1	0.36	24.46		
+3	n ₂₉ EELFRSIGGEVFIDFTK ₃₀₀ EK ₃₀₀	0.7543		1	1.00	24.29		
+2	n ₂₉ EK ₃₀₀ DIVGAVLK ₃₀₀	0.4201		1	1.00	24.34		
+2	n ₂₉ GAAAGGLGSLAVQYAK ₃₀₀	0.9997		1	0.50	24.26	1	0.46
+3	n ₂₉ GAAAGGLGSLAVQYAK ₃₀₀	0.9997		1	0.50	24.26	1	0.58
+2	n ₂₉ GQIVGRYVVDTSK ₃₀₀	0.9997		2	1.00	23.77	2	2.44
+3	n ₂₉ GQIVGRYVVDTSK ₃₀₀	0.9996		1	1.00	23.77	1	4.84
+2	n ₂₉ GVIFYESHGK ₃₀₀	0.9997		3	1.00	23.77	3	61.56
+3	n ₂₉ GVIFYESHGK ₃₀₀	0.9997		1	1.00	23.77	1	2.38
+2	n ₂₉ GYRVLGIDGGEGK ₃₀₀	0.9997		1	1.00	23.77	1	2.20
+2	n ₂₉ IGDYAGIK ₃₀₀	0.9992		2	0.50	24.27	2	16.80
+2	n ₂₉ LAVQYAK ₃₀₀	0.9777		1	0.50	24.30	1	1.78
+3	n ₂₉ LPLVGGHEGAGVVGMGENVK ₃₀₀	0.9997		1	0.50	24.26	1	0.31
+3	n ₂₉ LPLVGGHEGAGVVGM ₁₄₇ GENVK ₃₀₀	0.9996		1	0.50	24.27	1	0.44
+2	n ₂₉ RVLGIDGGEGK ₃₀₀	0.9997		1	1.00	23.77	1	1.60
+2	n ₂₉ SANLM ₁₄₇ AGHWVAIS	0.9994		1	1.00	23.77		
+2	n ₂₉ SDVFNQVVK ₃₀₀	0.9994		1	0.55	24.21	1	1.44
+2	n ₂₉ TVLVGMPAGAK ₃₀₀	0.9997		1	1.00	23.77	1	1.61
+2	n ₂₉ VLGIDGGEGK ₃₀₀	0.9993		1	1.00	23.77	1	5.20
+2	n ₂₉ VVGLSTLPEIYEK ₃₀₀	0.9997		3	1.00	23.77	3	0.78
+3	n ₂₉ VVGLSTLPEIYEK ₃₀₀	0.9997		2	1.00	23.77	2	0.80
+2	n ₂₉ YVVDTSK ₃₀₀	0.9935		1	0.33	24.44	1	4.32

QuanTic **DIAMODE**

- **Deconvolves overlapping isotopes of DIA data to extract quantitative signals, uses Mike's Mercury8 code to predict theoretical isotopic distributions of identified peptides**

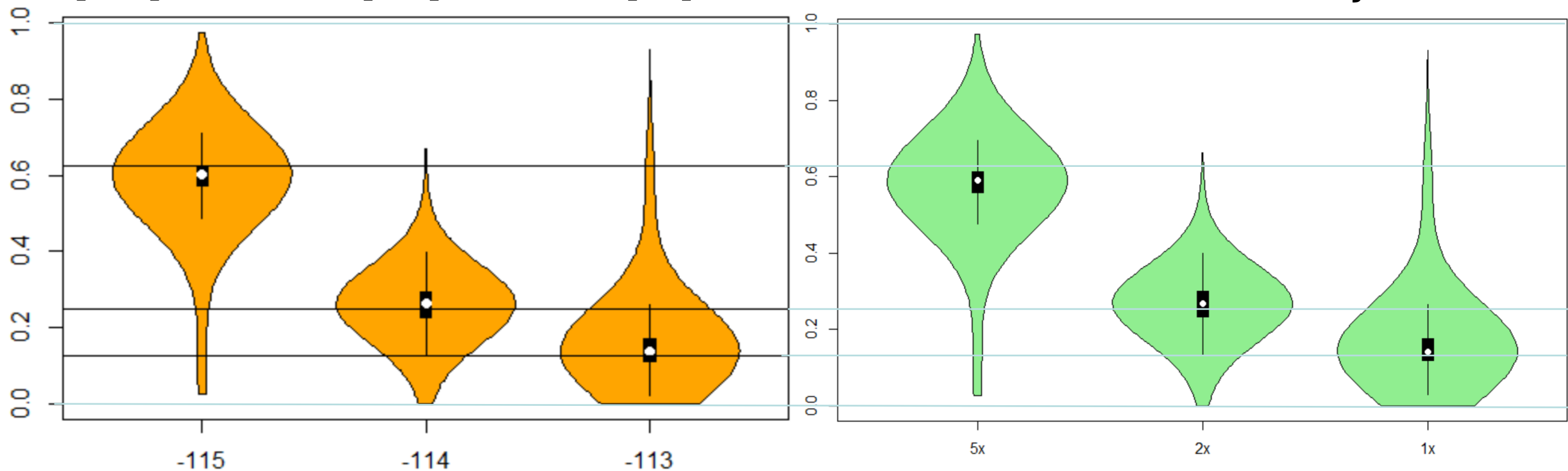


QuanTic : PTM Molecular Formula

- QuanTic allows the user to specify PTM molecular formulas for better isotopic distribution estimation with Mercury8

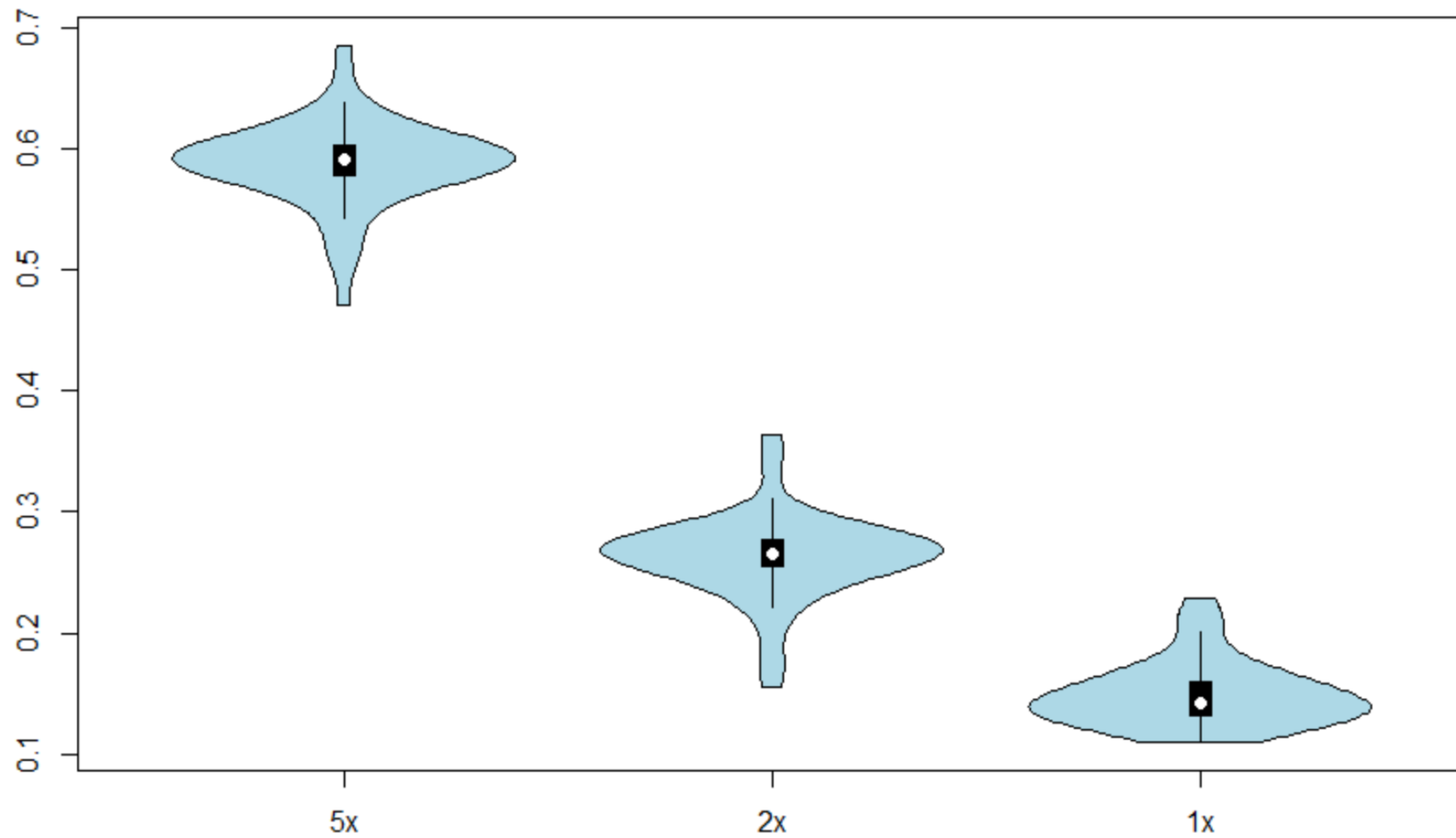
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vs No PTM Molecular Formula Adjustments



QuanTic

Protein Level DIAMODE



Acknowledgements

- **Mukul Midha**
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- **Zhi Sun**
- **Mike Hoopmann**
- **Kristian Swearingen**
- **Luis Mendoza**
- **Eric Deutsch**
- **Rob Moritz**

- **Thank you for your attention!**