

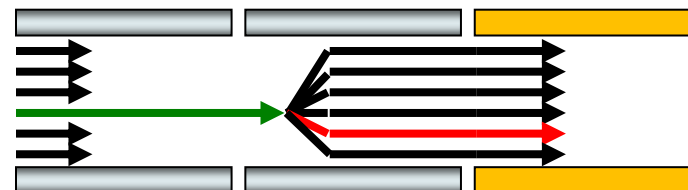


Skyline

Targeted Proteomics Environment

Quantitative Proteomics with Bruker Q-TOF
Instruments and Skyline

Brendan MacLean



Quantitative Proteomics

- ▶ **Spectrum-based**
 - ▶ Spectral counting
 - ▶ Isobaric tags

- ▶ **Chromatography-based**
 - ▶ SRM
 - ▶ MSI chromatogram extraction
 - ▶ Targeted MS/MS
 - ▶ Data independent acquisition (DIA)



Quantitative Proteomics

- ▶ Spectrum-based
 - ▶ Spectral counting
 - ▶ Isobaric tags
- ▶ Chromatography-based
 - ▶ SRM
 - ▶ **MSI chromatogram extraction**
 - ▶ **Targeted MS/MS**
 - ▶ **Data independent acquisition (DIA)**



micrOTOF-Q and maXis series

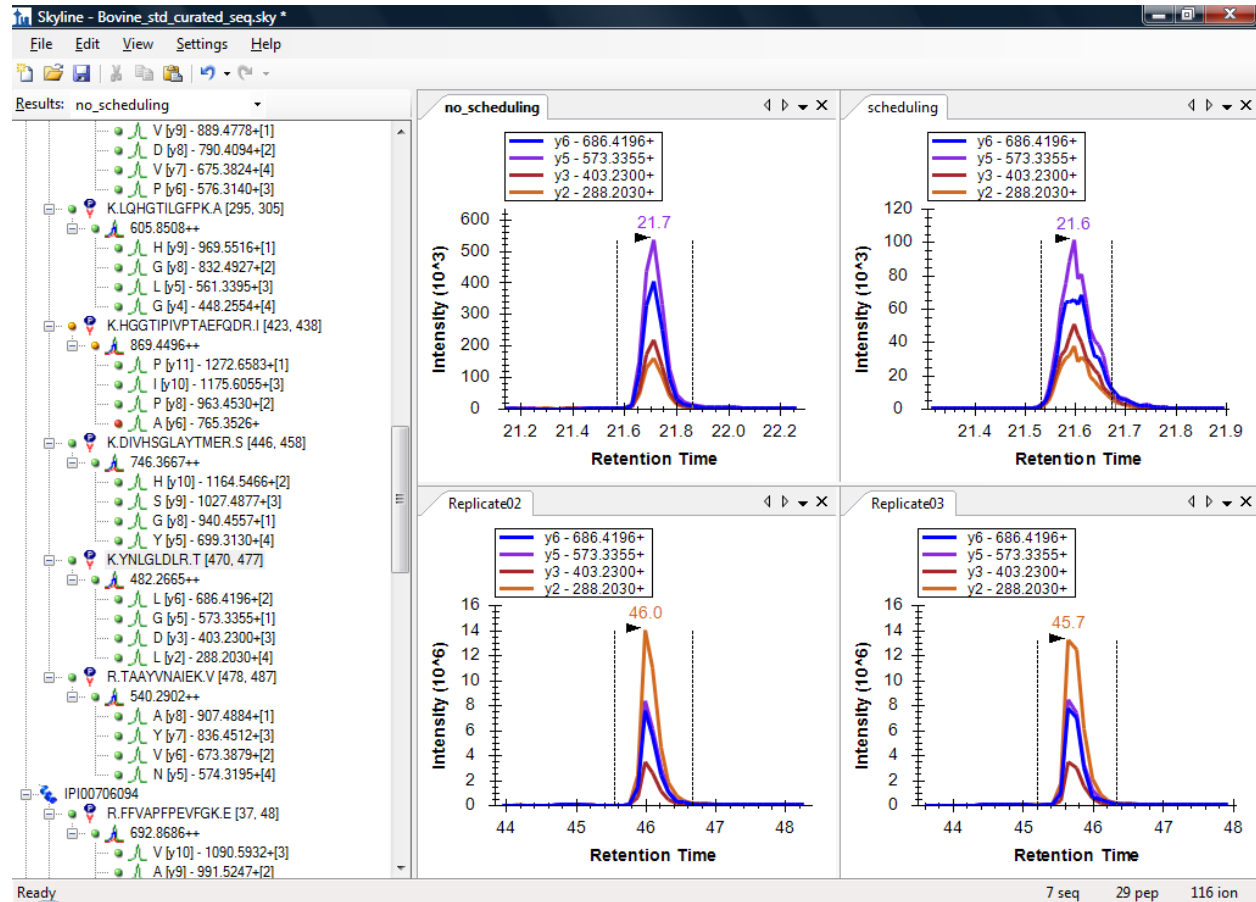


2010 Multi-Instrument SRM Tool for NCI CPTAC Verification Working Group



▶ AB SCIEX
4000 Q Trap

▶ Thermo-
Scientific
TSQ Ultra



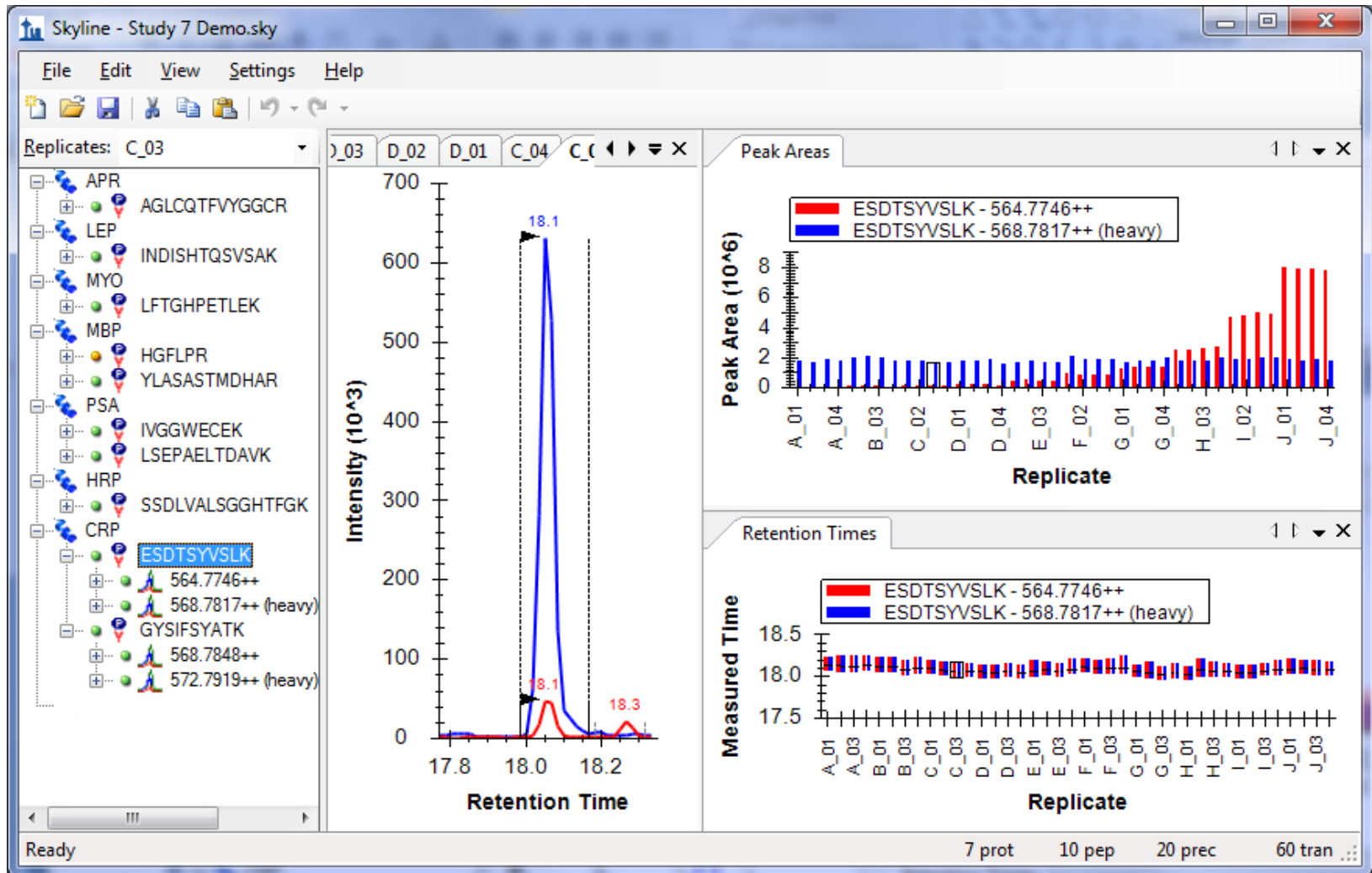
Support Multiple Instrument Vendors

- ▶ Selected Reaction Monitoring
- ▶ Exporting transition lists & native methods
- ▶ Importing **native** instrument output files

- ▶ AB Sciex
- ▶ Agilent Technologies
- ▶ Thermo-Scientific
- ▶ Waters



Graphic Display of Information



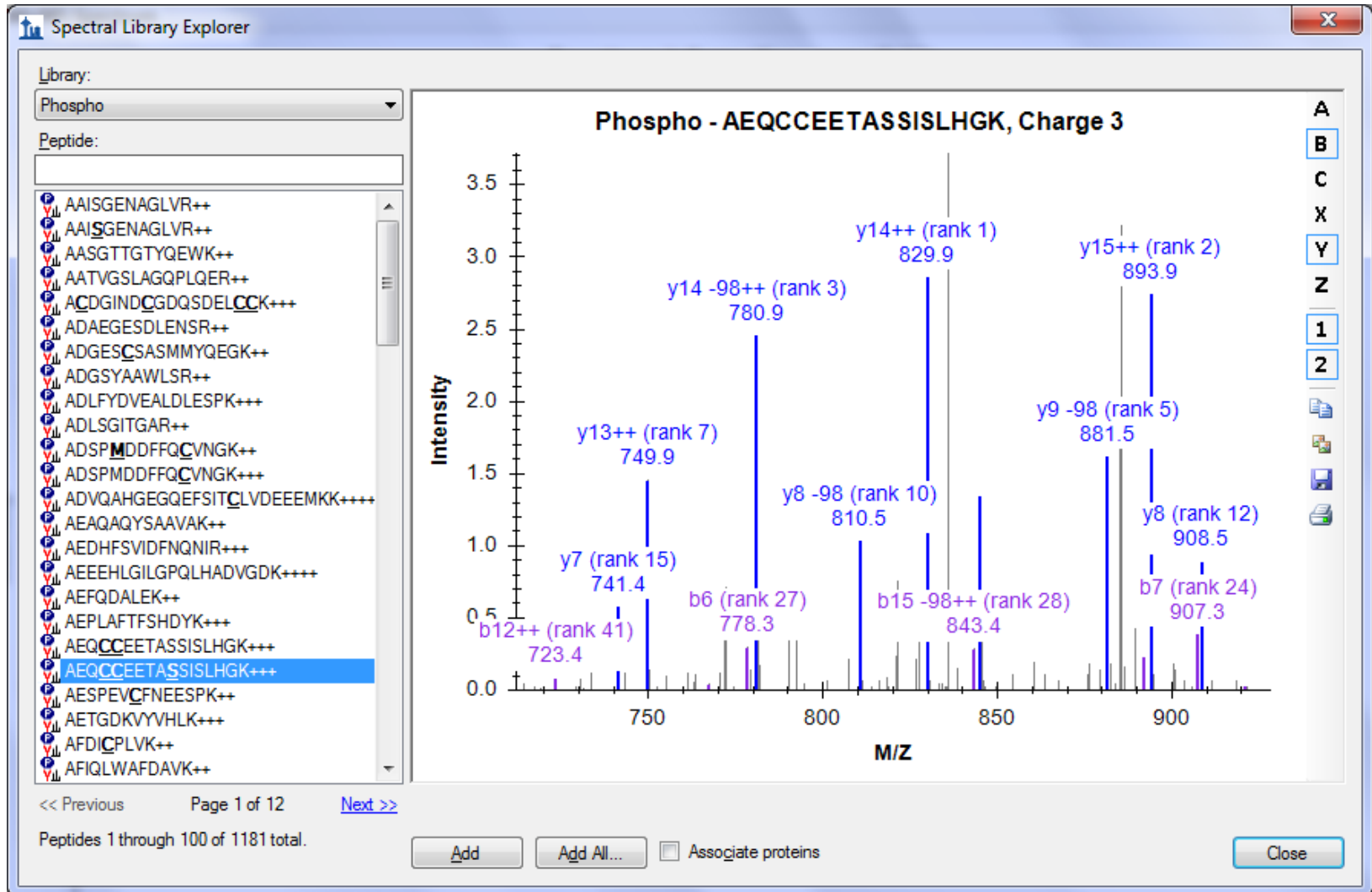
MS/MS Spectral Library Sources

- ▶ Global Proteome Machine
- ▶ MacCoss Lab
- ▶ NIST
- ▶ Peptide Atlas

- ▶ Build your own from peptide search results
 - ▶ Mascot
 - ▶ Myrimatch / IDPicker
 - ▶ OMSSA
 - ▶ Protein Pilot
 - ▶ Protein Prospector
 - ▶ Scaffold – mzIdentML / MGF
 - ▶ Spectrum Mill
 - ▶ TPP – pepXML / mzXML files – Peptide Atlas
 - ▶ X! Tandem
 - ▶ Waters MSe

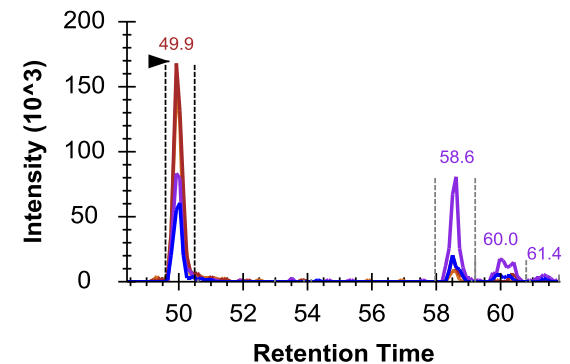
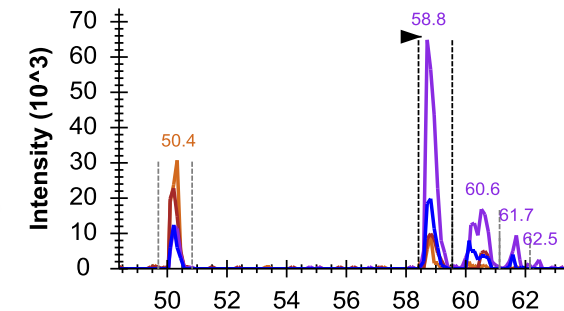
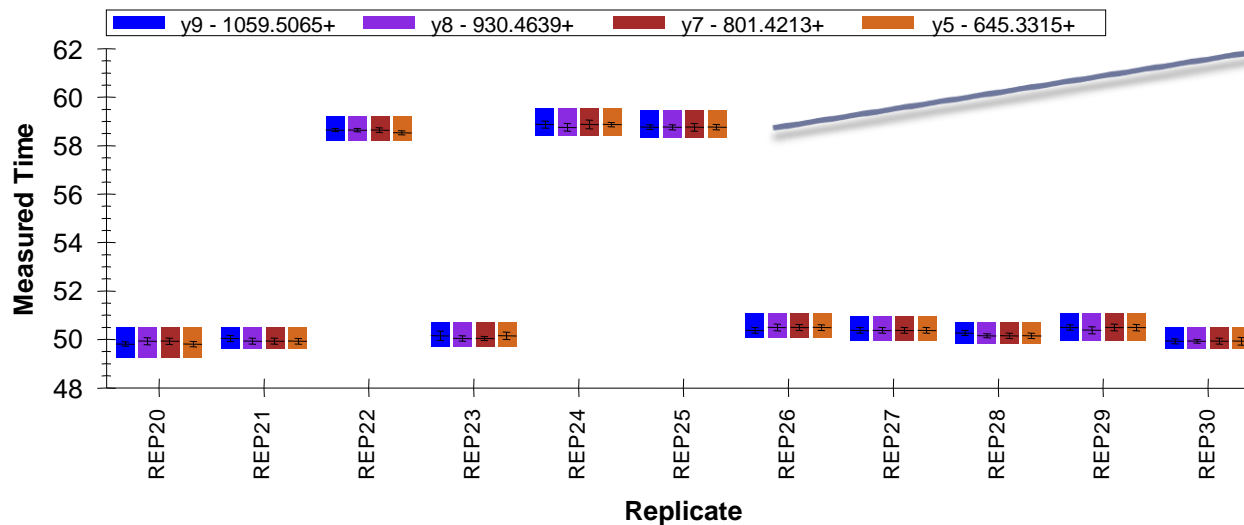
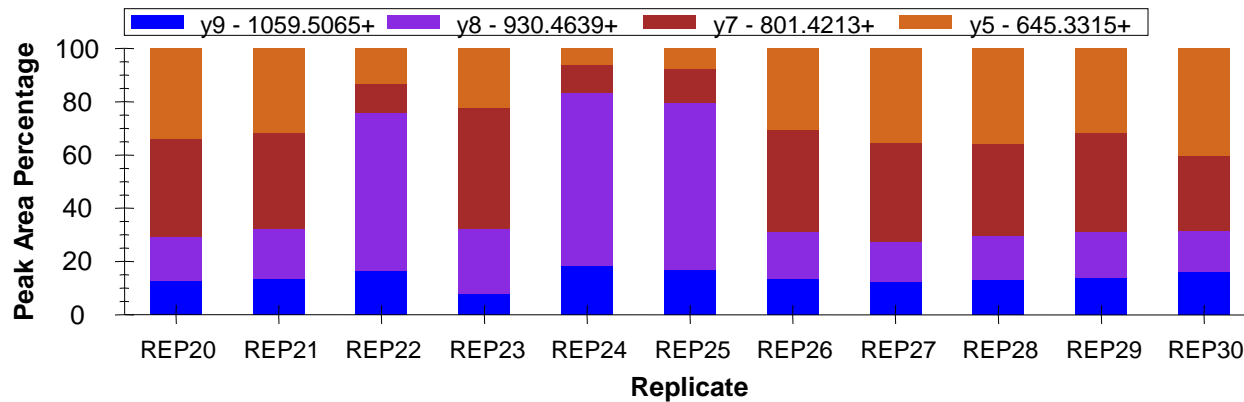


Spectral Library Explorer



Finding Issue Quickly (wrong peak)

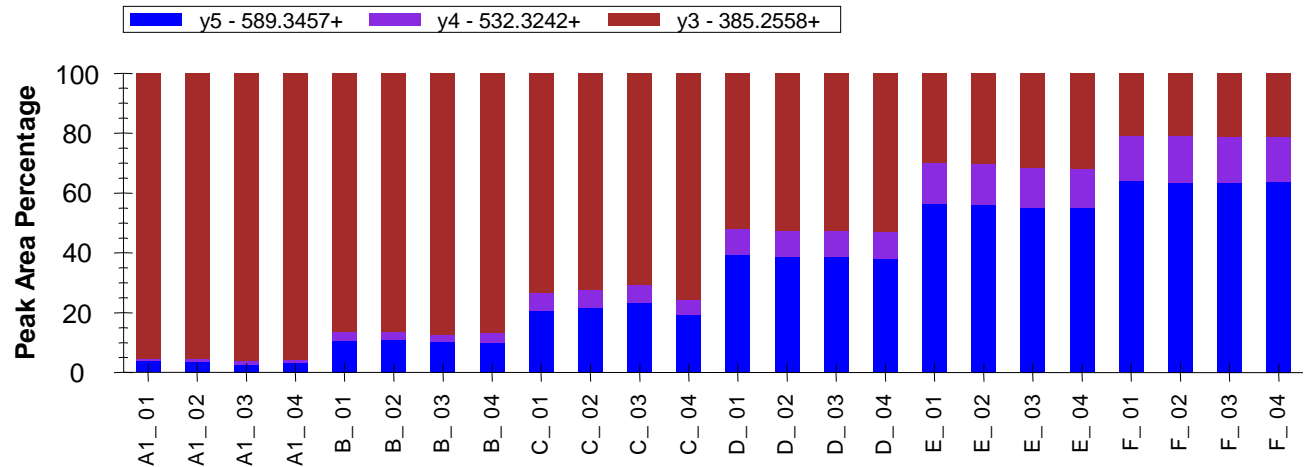
IVGYLDEEGVLDQNR



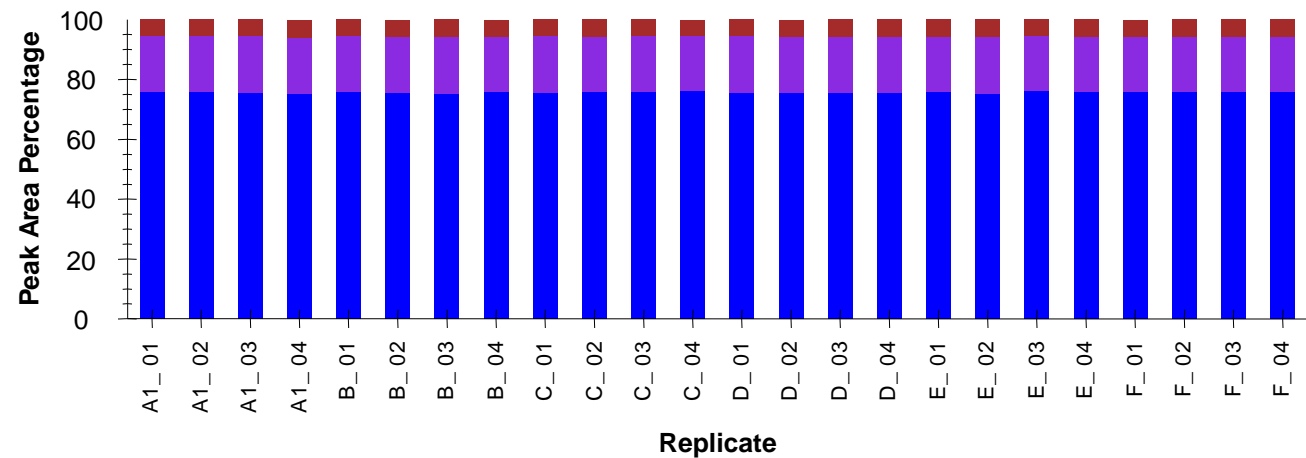
Finding Issues at a Glance (interference)

HGFLPR

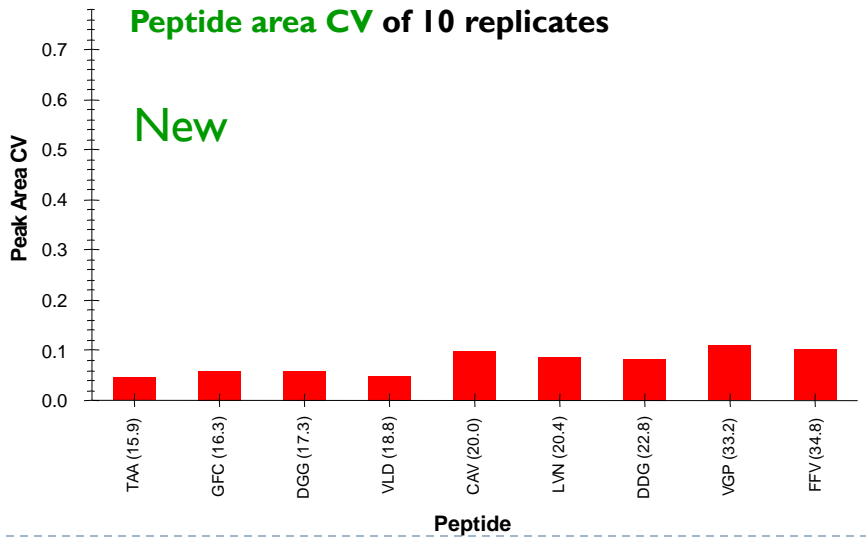
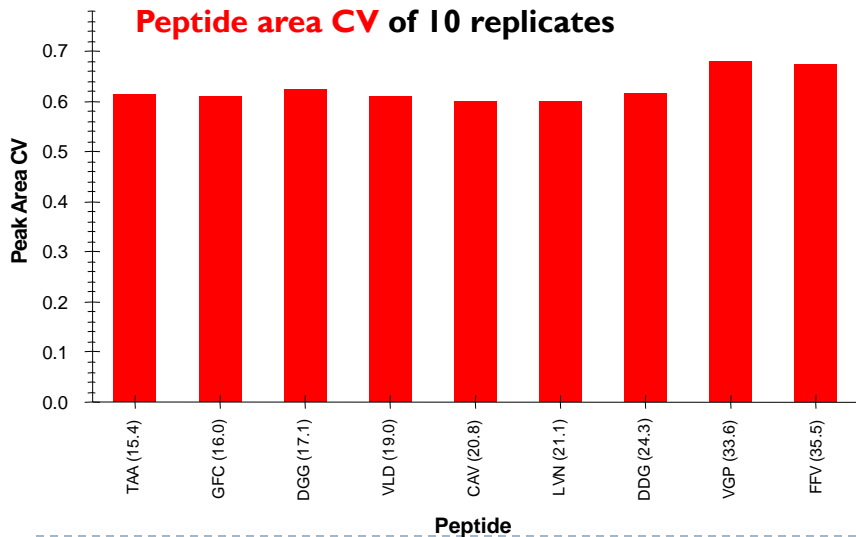
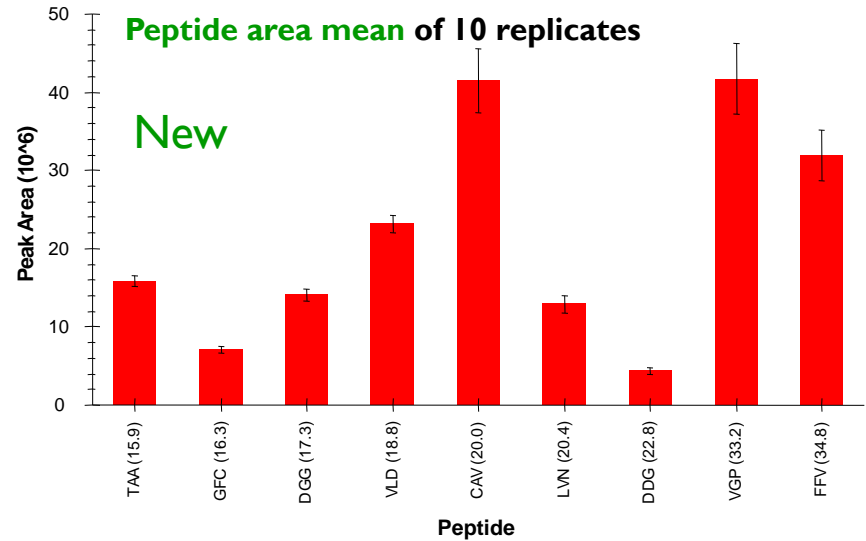
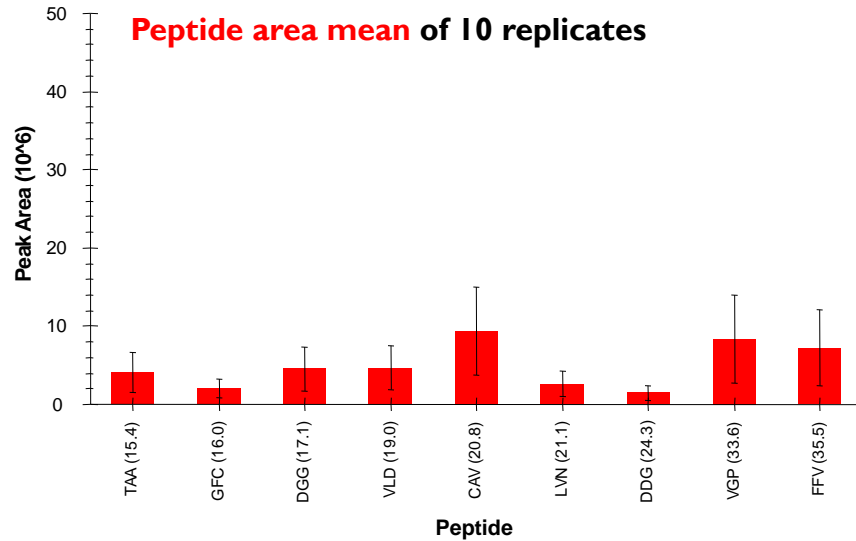
Unlabeled



Labeled
I3C R



Finding Issues Early (lack of precision)



Sharing Reports

PeptideSeq	Replicate	Pre	Fragr	AverageM	PeptideRt	PrecursorI	ProductM	Retention	Fwhm	Area
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.96	533.295	966.5142	24.99	0.13	273200
VLVLDTDYK	6ProtMix_	2	y8	24.62	25.05	533.295	966.5142	25.02	0.15	291000
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.91	533.295	966.5142	24.91	0.17	220600
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.79	533.295	966.5142	24.79	0.14	331650
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.73	533.295	966.5142	24.76	0.15	346800
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.7	533.295	966.5142	24.7	0.13	265800
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.65	533.295	966.5142	24.65	0.14	301200
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.36	533.295	966.5142	24.36	0.13	290800
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.1	533.295	966.5142	24.1	0.09	170600
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.33	533.295	966.5142	24.33	0.1	147700
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.47	533.295	966.5142	24.5	0.07	900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.5	533.295	966.5142	24.5	0.03	100
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.5	533.295	966.5142	24.45	0.03	100
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.96	533.295	853.4302	24.96	0.14	2528350
VLVLDTDYK	6ProtMix_	2	y8	24.62	25.05	533.295	853.4302	25.05	0.15	2816900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.91	533.295	853.4302	24.91	0.16	2215900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.79	533.295	853.4302	24.79	0.15	3202250
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.73	533.295	853.4302	24.73	0.15	3098300
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.7	533.295	853.4302	24.7	0.14	2618300
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.65	533.295	853.4302	24.65	0.13	2990900
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.36	533.295	853.4302	24.36	0.14	2794850
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.1	533.295	853.4302	24.1	0.1	1676000
VLVLDTDYK	6ProtMix_	2	y8	24.62	24.33	533.295	853.4302	24.33	0.13	1533550

Edit Report X

Report Name: Preview...

- Peptides
 - Precursors
 - Transitions
 - Results
 - PrecursorPeakFoundRatio
 - BestRetentionTime
 - MaxFwhm
 - MinStartTime
 - MaxEndTime
 - TotalArea
 - TotalBackground
 - TotalAreaRatio
 - TotalAreaNormalized
 - LibraryDotProduct
 - UserSetTotal
 - OptStep
 - OptCollisionEnergy
 - OptDeclusteringPotential
 - Note
 - Results Summary
 - Charge
 - IsotopeLabelType
 - NeutralMass
 - Mz
 - CollisionEnergy
 - DeclusteringPotential
 - ModifiedSequence

Add >

FileName
 SampleName
 ProteinName
 PeptideSequence
 ReplicateName
 PrecursorCharge
 FragmentIon
 AverageMeasuredRetentionTime
 PeptideRetentionTime
 RatioToStandard
 PeptideNote
 PrecursorMz
 ProductMz
 RetentionTime
 Fwhm
 Area
 Height
 IsotopeLabelType
 UserSetPeak
 TransitionReplicateNote
 PrecursorNote
 StartTime
 EndTime
 MinStartTime
 MaxFwhm
 MaxEndTime

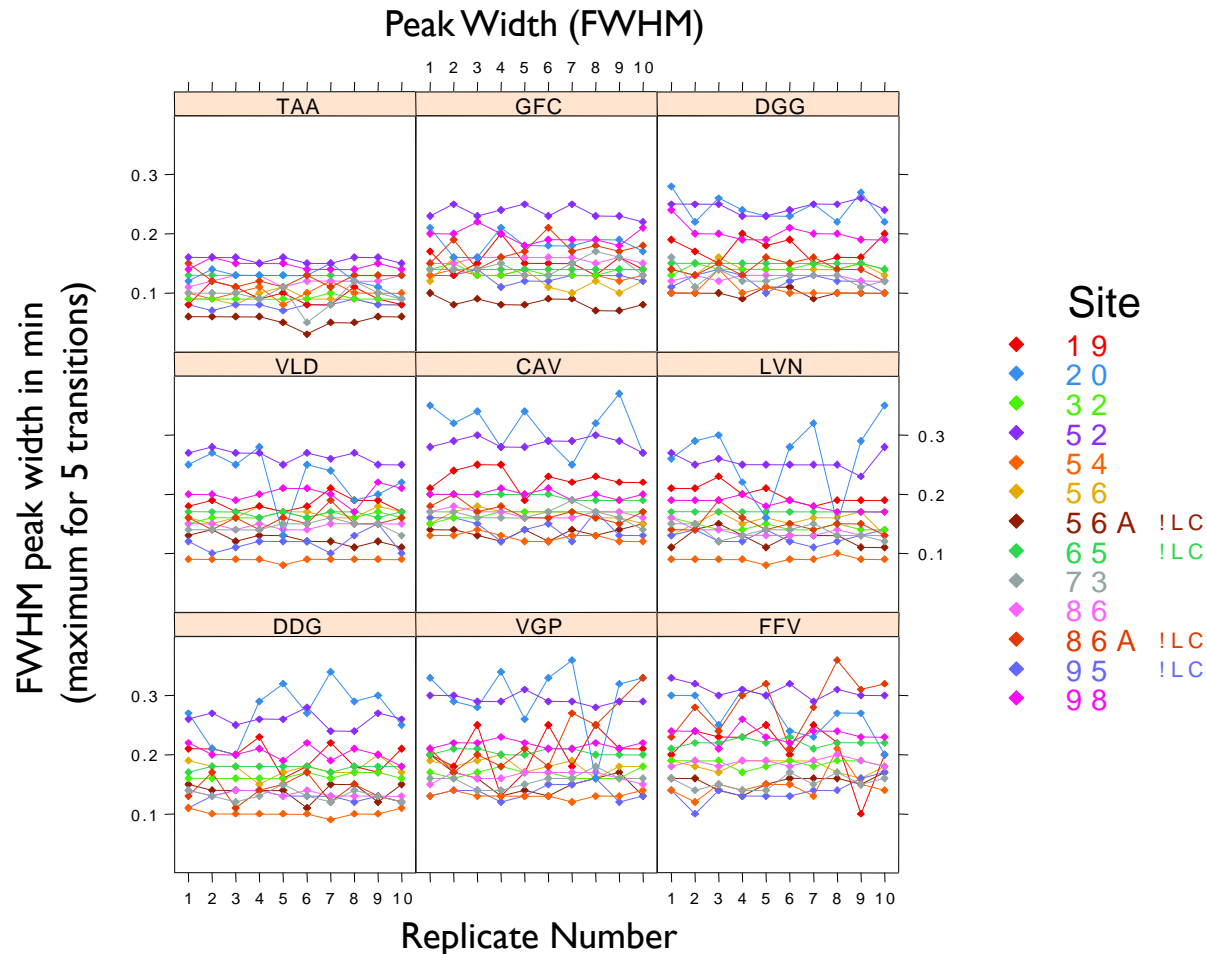
Pivot Replicate Name
 Pivot Isotope Label

OK
Cancel



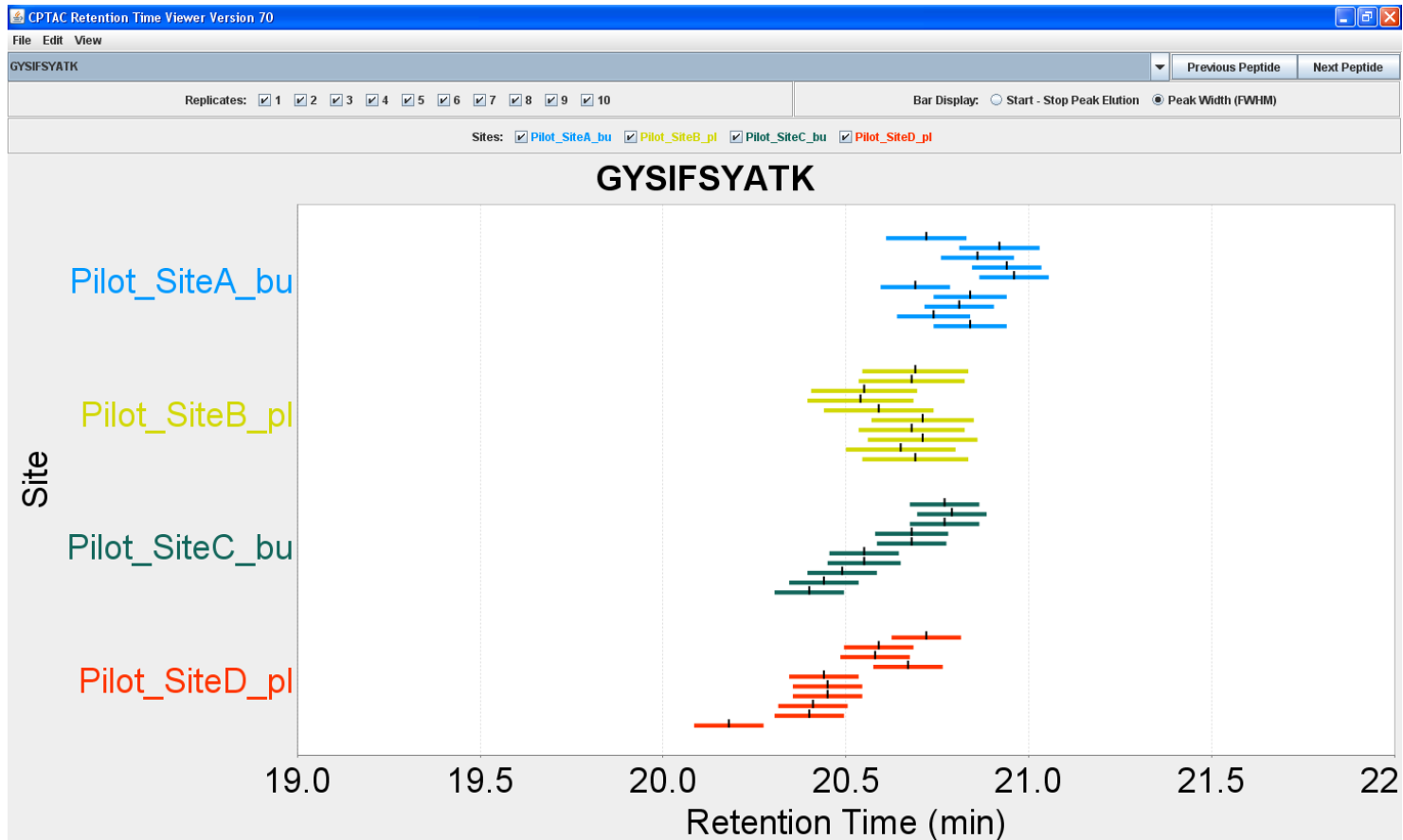
Downstream Analysis with Statistical Tools

► Analysis of Study 9S reports with R



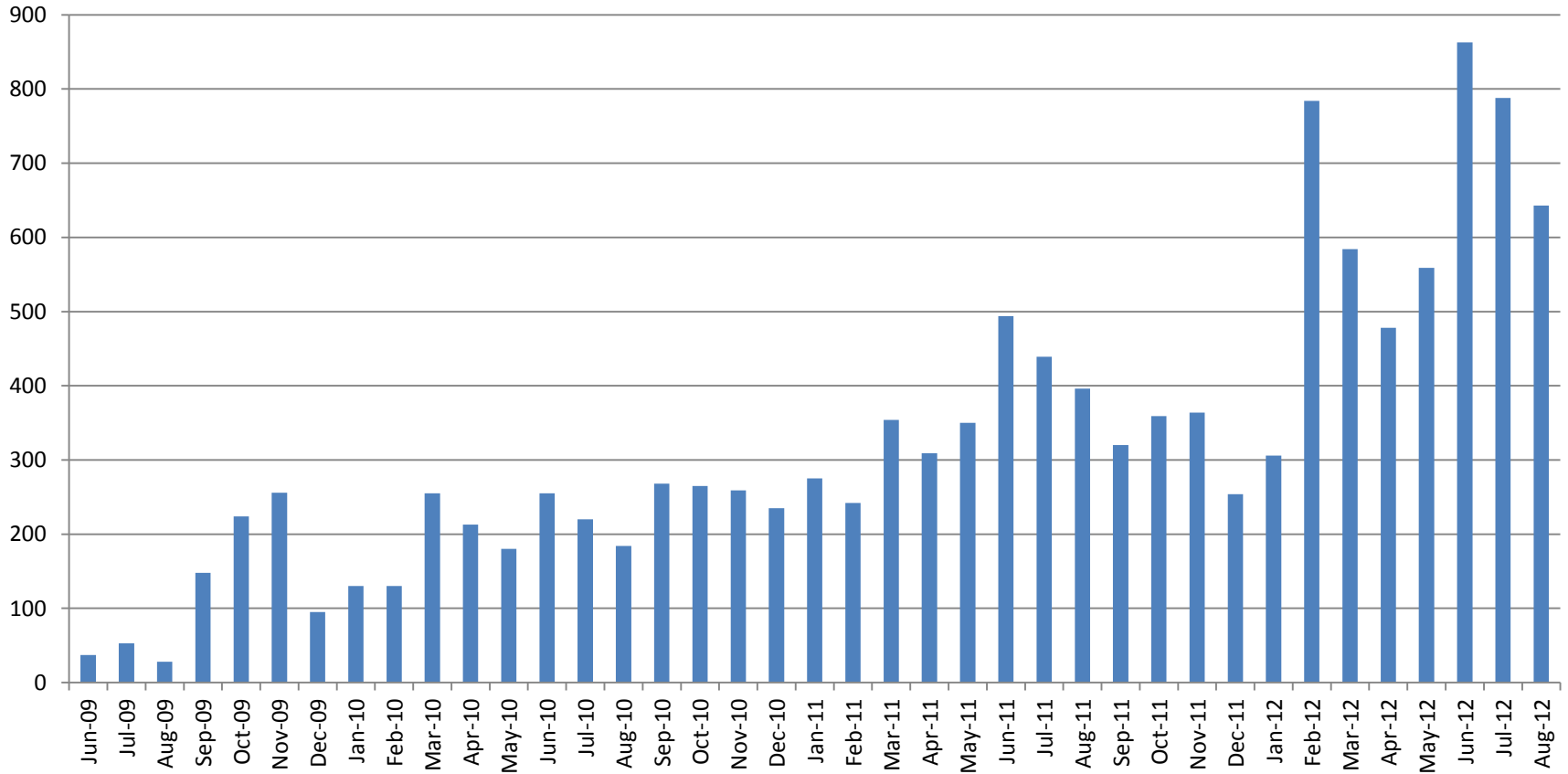
Deeper Analysis with Custom Tools

- ▶ Analysis of reports with Retention Time Viewer (Java program)



Skyline Adoption (>12,500 Installations)

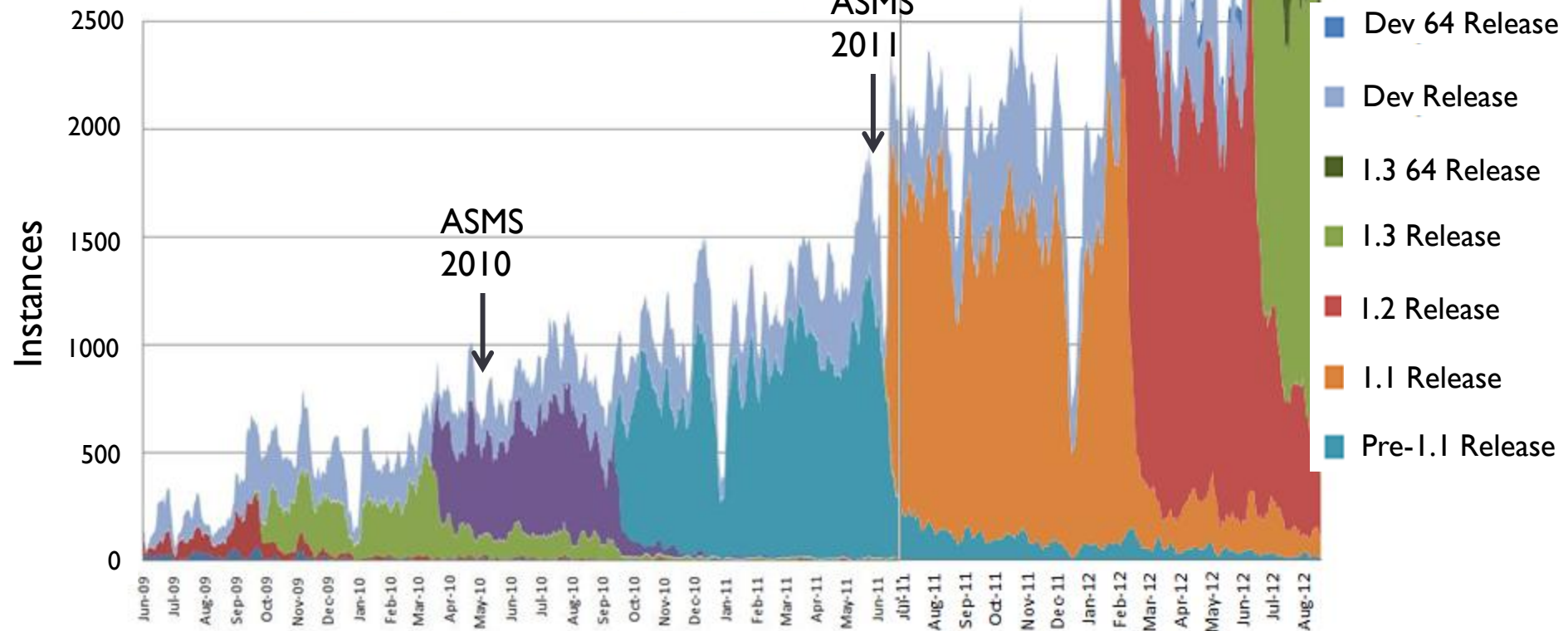
New Skyline Installations by Month



▶ 660 registered users

Skyline Use

Skyline Instances Started Trailing 7 Days



> 100 citations

2012 Support Multiple Instrument Vendors

- ▶ Full-Scan
- ▶ Exporting isolation lists & native methods
- ▶ Importing native instrument output files

- ▶ AB SCIEX SWATH™
- ▶ Agilent Technologies DIA
- ▶ Bruker DIA & All Ions DIA
- ▶ Thermo-Scientific DIA & Multiplexed DIA
- ▶ Waters MSe™



Skyline MS1 Full-Scan Settings

Transition Settings

Prediction | Filter | Library | Instrument | Full-Scan

MS1 filtering

Isotope peaks included: Count
Precursor mass analyzer: TOF

Peaks: 3
Resolving power: 10,000

Isotope labeling enrichment: Default

MS/MS filtering

Acquisition method: None
Product mass analyzer:

Isolation scheme:
Resolution: Th

Retention time filtering

Include all matching scans
 Use only scans in retention time scheduling windows
 Use only scans within 5 minutes of MS/MS IDs

OK Cancel

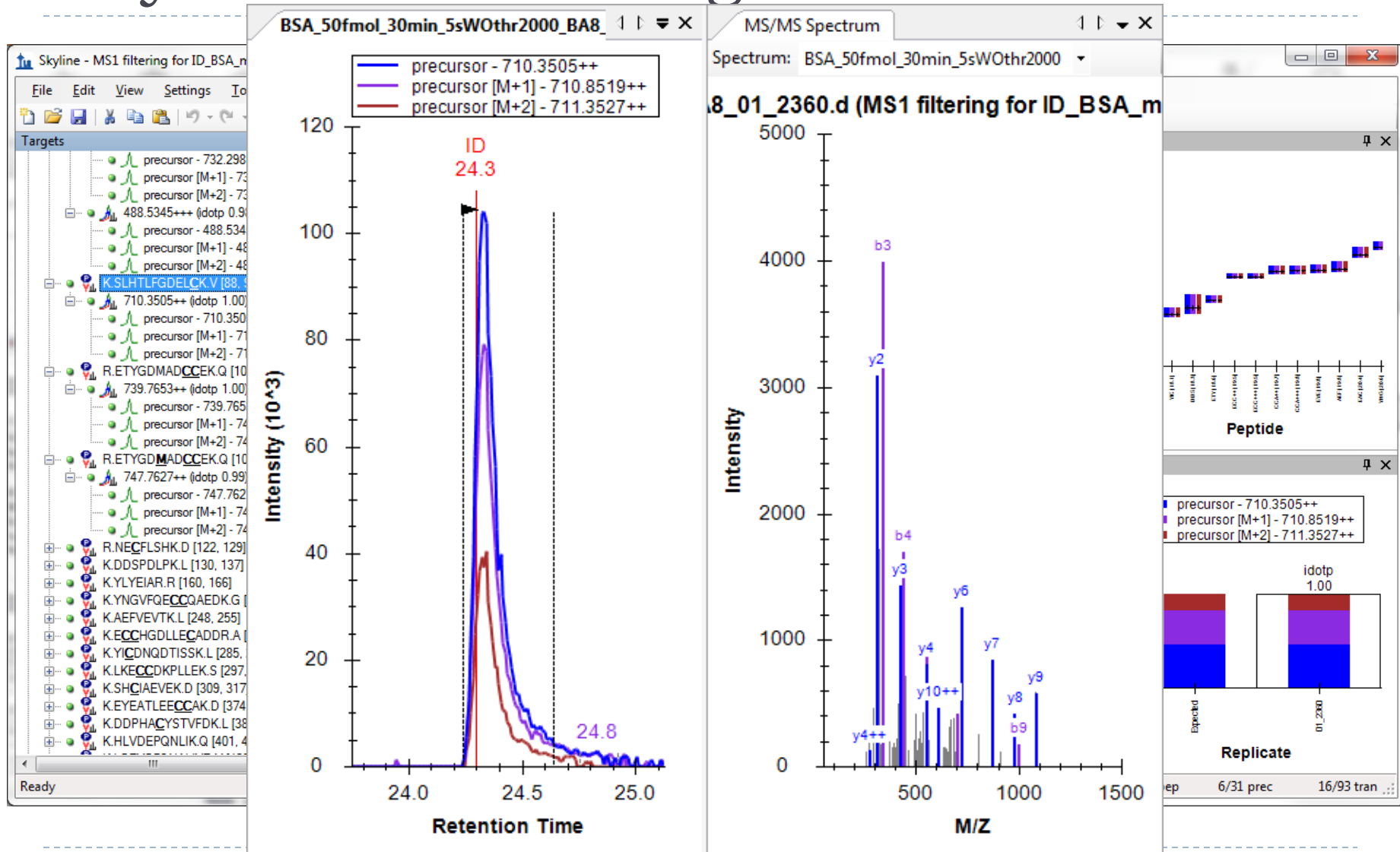
MS1 filtering

Isotope peaks included: Count
Precursor mass analyzer: TOF

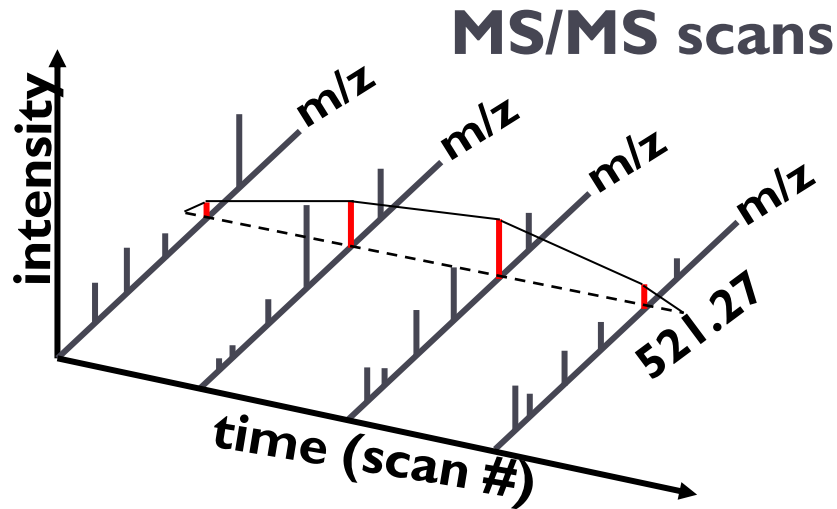
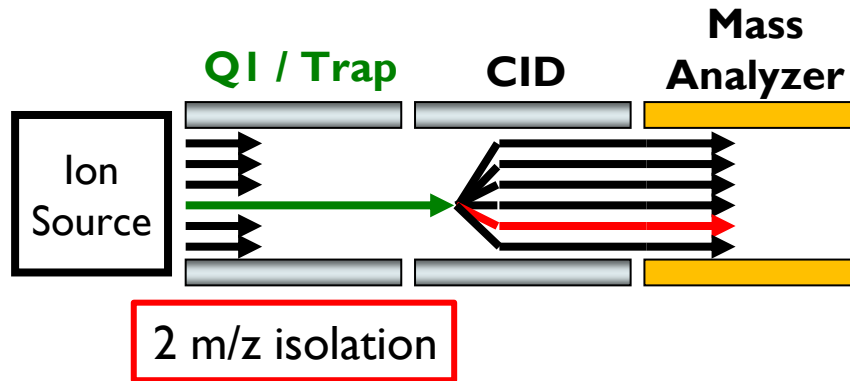
Peaks: 3
Resolving power: 10,000

Isotope labeling enrichment: Default

Skyline MS1 Filtering Data



Targeted MS/MS



Skyline Targeted MS/MS Settings

Transition Settings

Prediction Filter Library Instrument Full-Scan

MS1 filtering

Isotope peaks included: Count
Precursor mass analyzer: QIT

Peaks: 1
Resolution: 0.7 Th

Isotope labeling enrichment:

MS/MS filtering

Acquisition method: Targeted
Product mass analyzer: QIT

Isolation scheme:
Resolution: 0.7 Th

Filter only retention time scheduling windows

OK Cancel

MS1 filtering

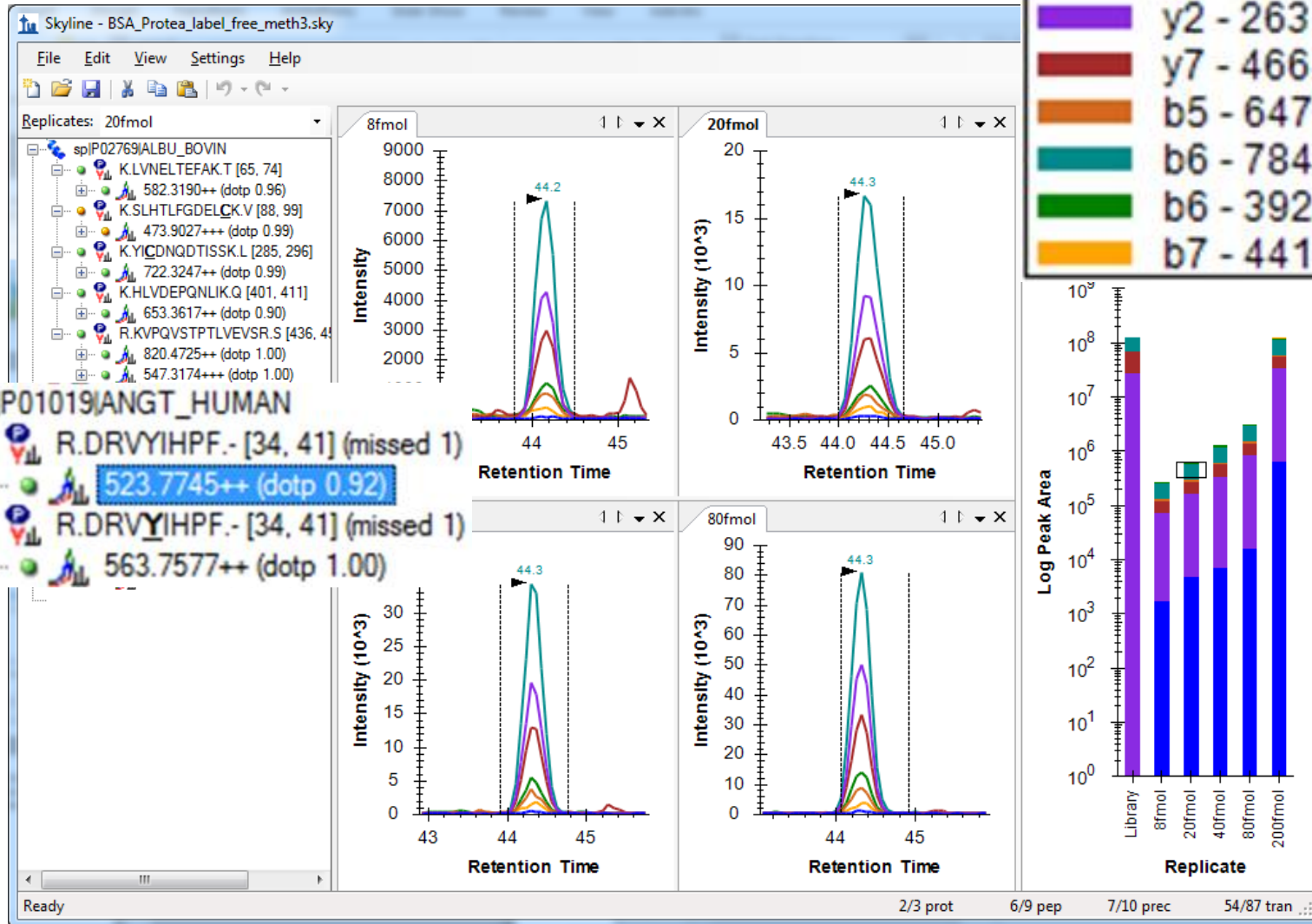
MS/MS filtering

Acquisition method: Targeted
Product mass analyzer: QIT

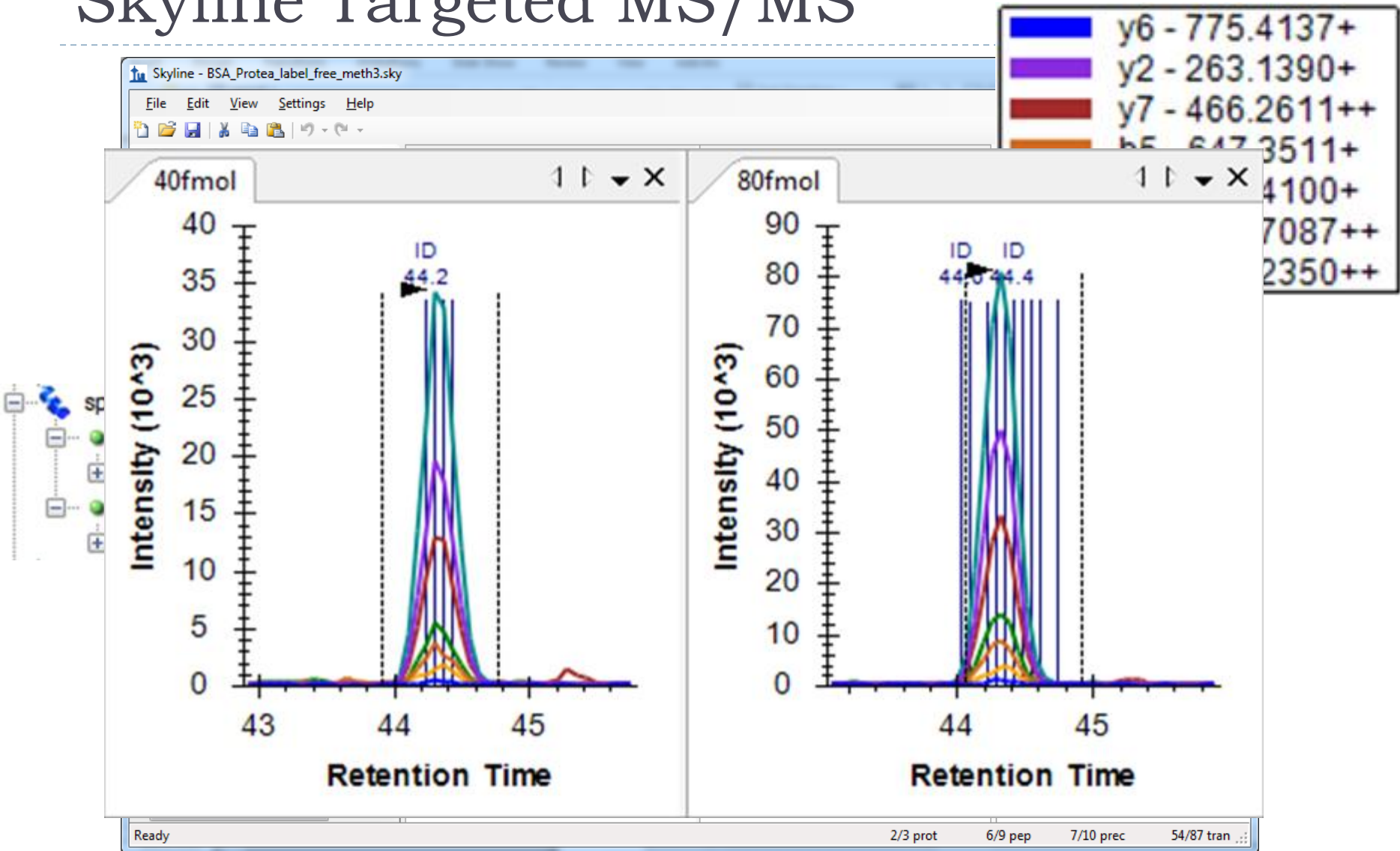
Isolation scheme:
Resolution: 0.7 Th



Skyline Targeted MS/MS



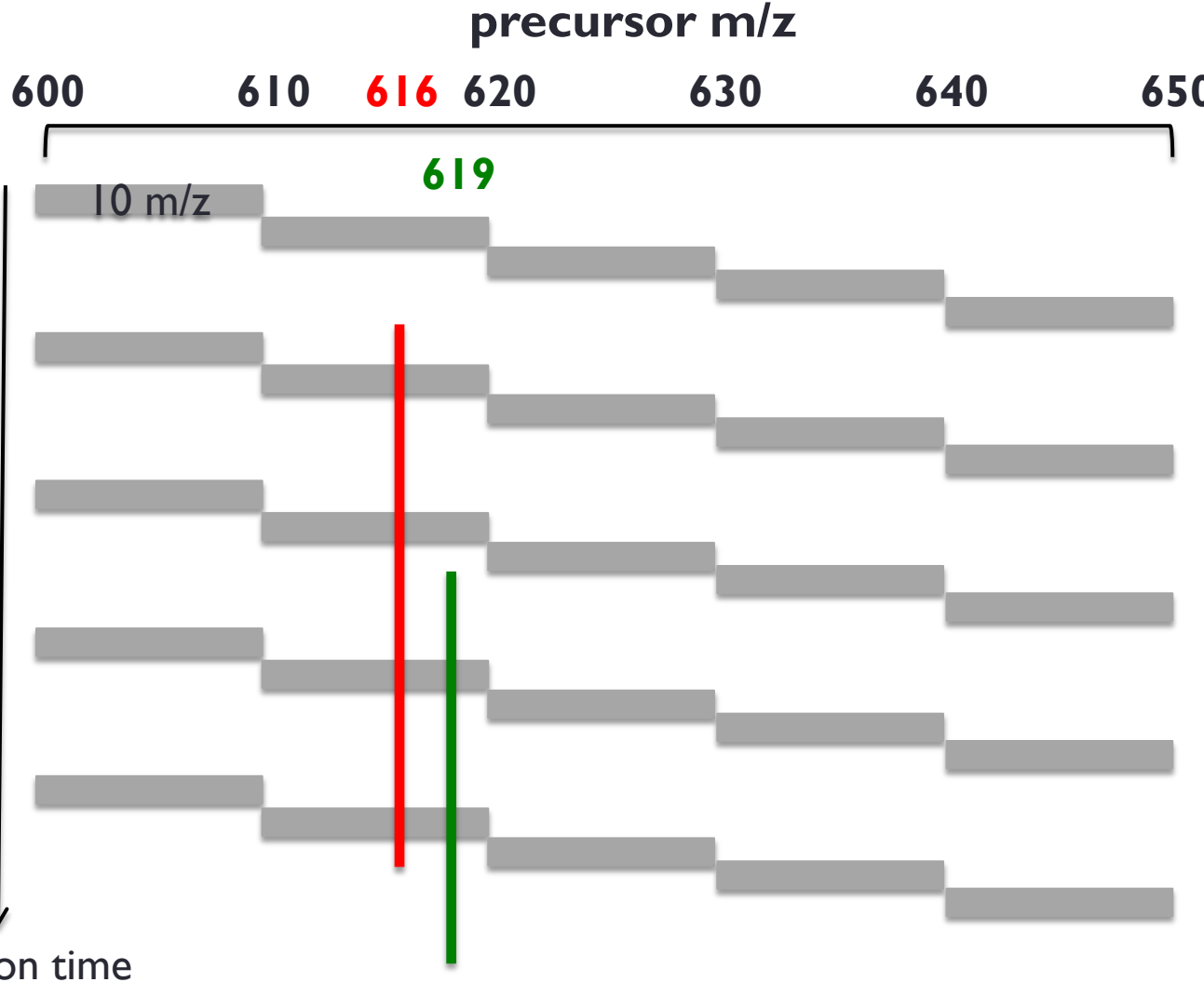
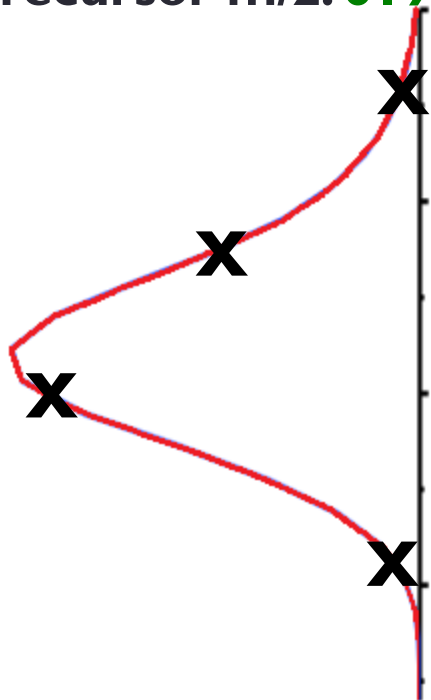
Skyline Targeted MS/MS



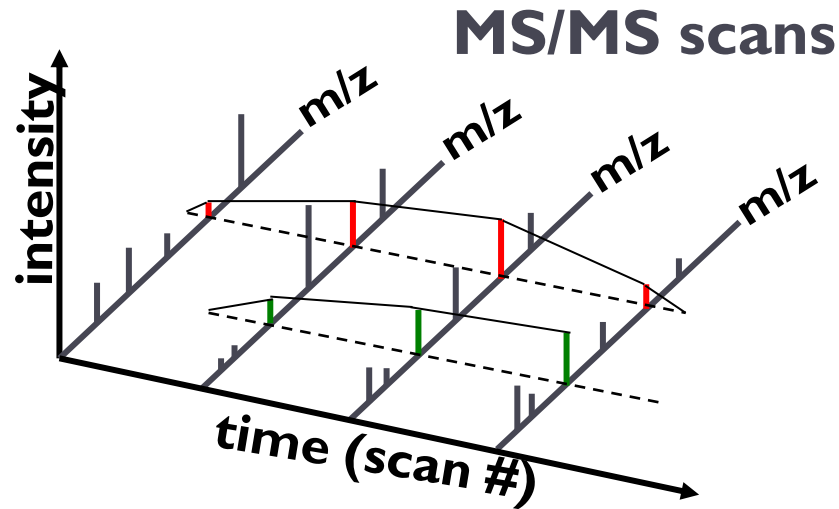
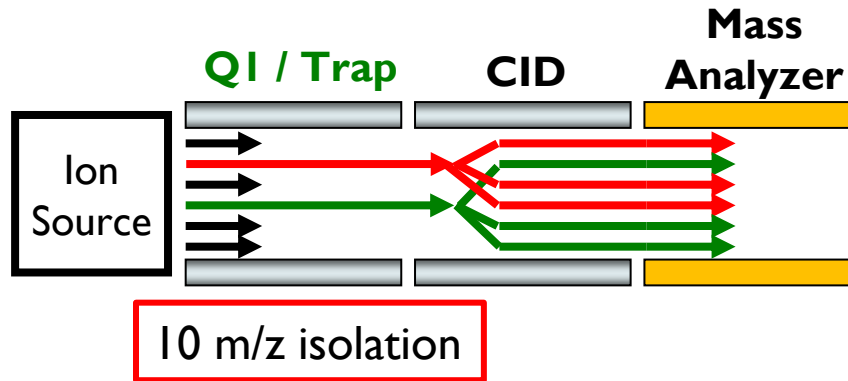
Traditional DIA Isolation Scheme

SVEDFMAAMQR
Precursor m/z: **616**

VGGNGADYALATK
Precursor m/z: **619**

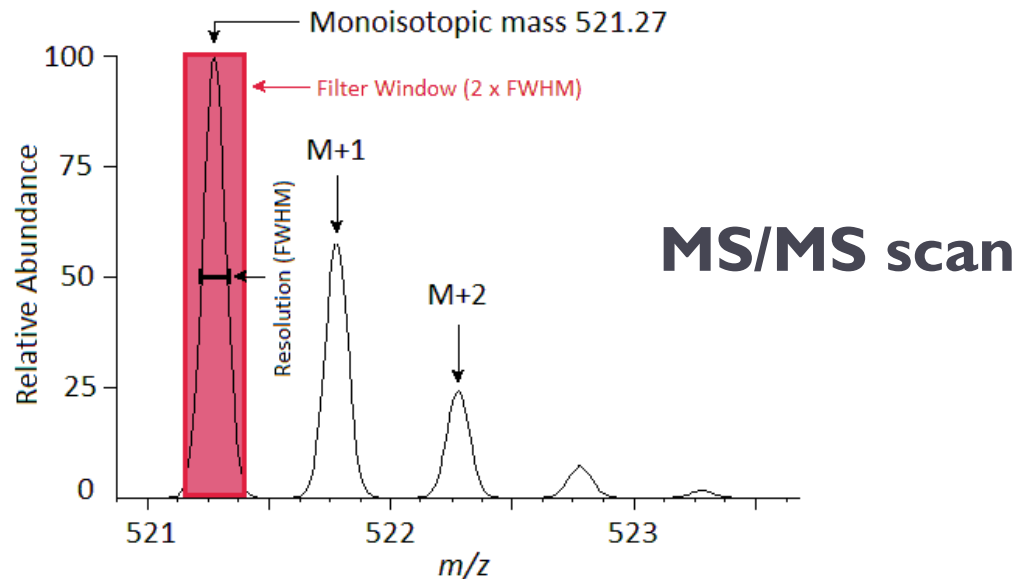


DIA Chromatogram Extraction



DIA Chromatogram Extraction Trade-Offs

- ▶ **Sensitivity**
 - ▶ Dwell / Accumulation time
 - ▶ Cycle time
- ▶ Selectivity lost by wide precursor isolation
- ▶ Selectivity gained vs. SRM by narrow product extraction



Skyline Bruker DIA Settings (25 m/z Extraction Windows)

The image shows two overlapping software windows from Skyline Bruker. The background window is the 'Transition Settings' dialog, and the foreground window is the 'Edit Isolation Scheme' dialog.

Transition Settings (Background Window):

- MS1 filtering: []
- MS/MS filtering: []
- Acquisition method: **DIA**
- Product mass analyzer: **TOF**
- Isolation scheme: **SWATH** (highlighted with a red box)
- Resolving power: **10,000**
- Default: []
- Use only scans within **5** minutes of MS/MS IDs

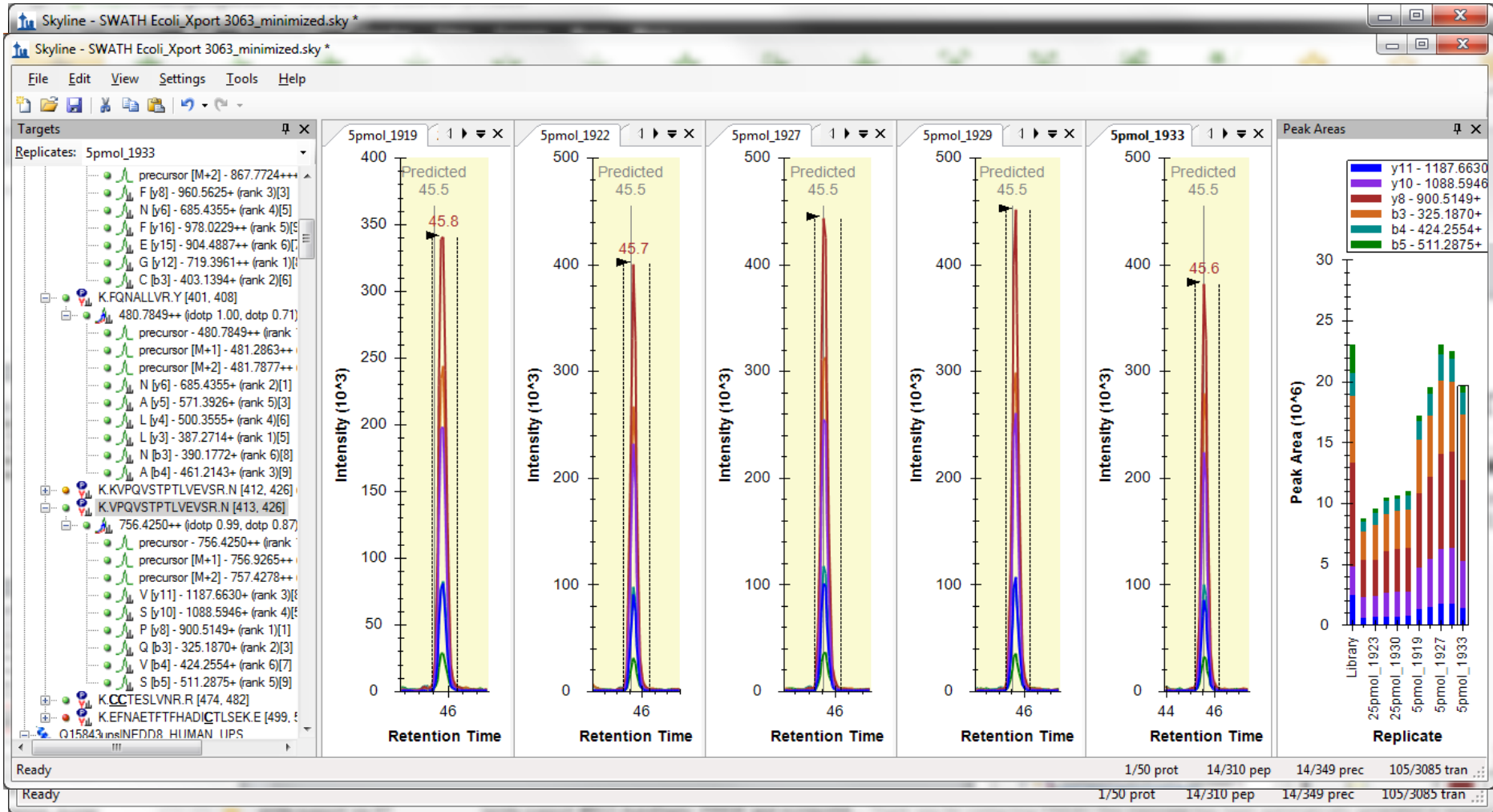
Edit Isolation Scheme (Foreground Window):

- Name: **SWATH**
- Use results data isolation targets: []
- Isolation width: [] Th
- Asymmetric: []
- Prespecified isolation windows: [] **Calculate...**
- Table of isolation windows:

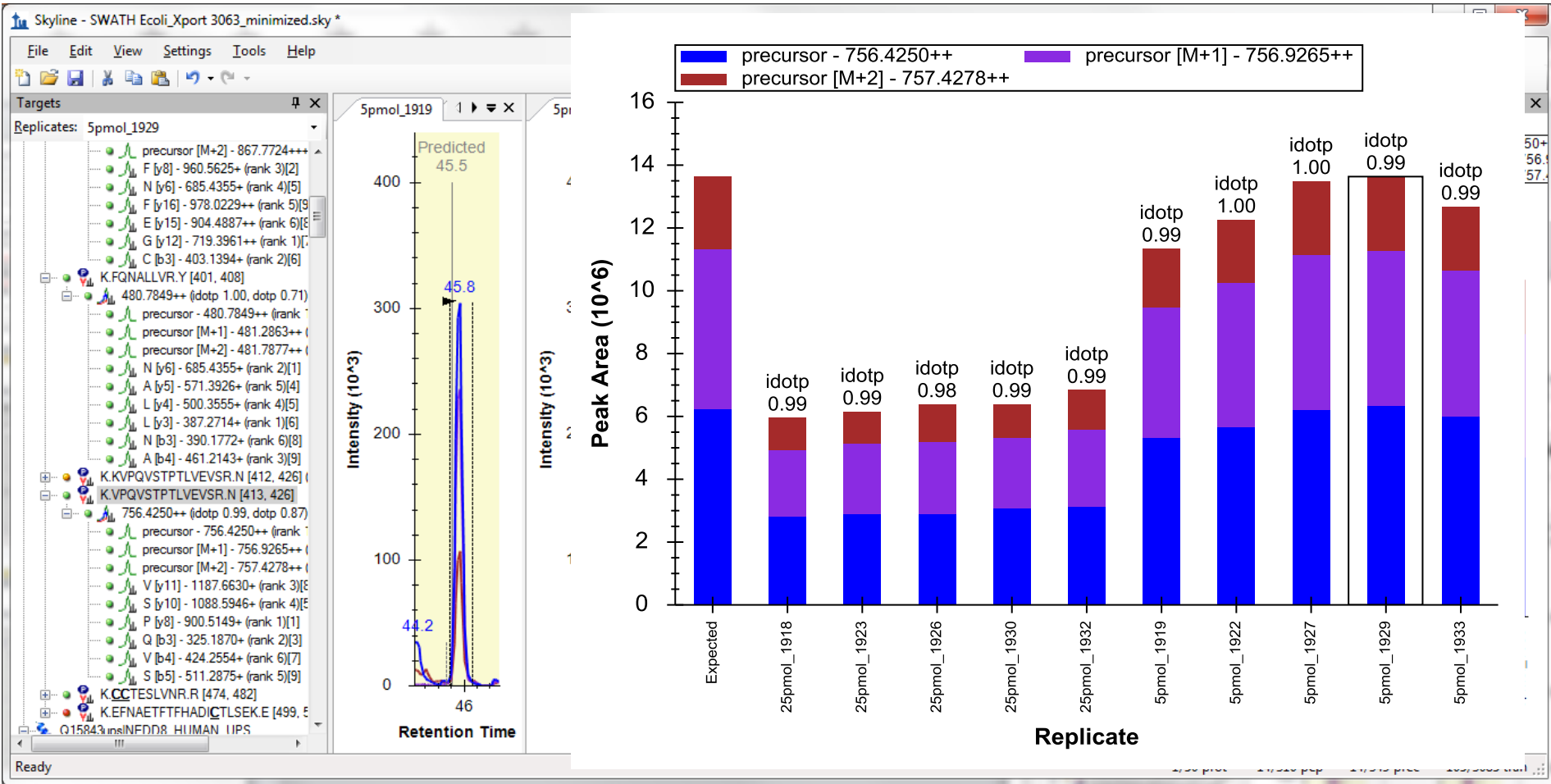
	Start	End
▶	400	425
	425	450
	450	475
	475	500
	500	525
	525	550
	550	575

- Multiplexed acquisition: []
- Windows per scan: []
- Margins: **None**
- Specify target: []

Bruker DIA Data (Product Ions)



Bruker DIA Data (Precursor Ions)



Bruker All Ions DIA Settings (50 – 1500 m/z Range)

The image displays two overlapping software dialog boxes for configuring mass spectrometry settings. The left dialog, titled "Transition Settings", shows the "Full-Scan" tab with "MS1 filtering" options: "Isotope peaks included" set to "Count" and "Precursor mass analyzer" set to "TOF". Below this, the "MS/MS filtering" section includes "Acquisition method" set to "DIA", "Product mass analyzer" set to "TOF", "Isolation scheme" set to "All ions" (highlighted with a red box), and "Resolving power" set to "10,000". The "Retention time filtering" section at the bottom has "Include all matching scans" selected. The right dialog, also titled "Transition Settings", shows the "Full-Scan" tab with "Min m/z" set to "50" and "Max m/z" set to "1500", both with "Th" (threshold) units. It also includes a "Dynamic min product m/z" checkbox (unchecked) and "Match tolerance m/z" field. Both dialog boxes have "OK" and "Cancel" buttons at the bottom.

MS/MS filtering

Acquisition method: **DIA**

Product mass analyzer: **TOF**

Isolation scheme: **All ions**

Resolving power: **10,000**

Transition Settings (Right):

Min m/z: **50** Th

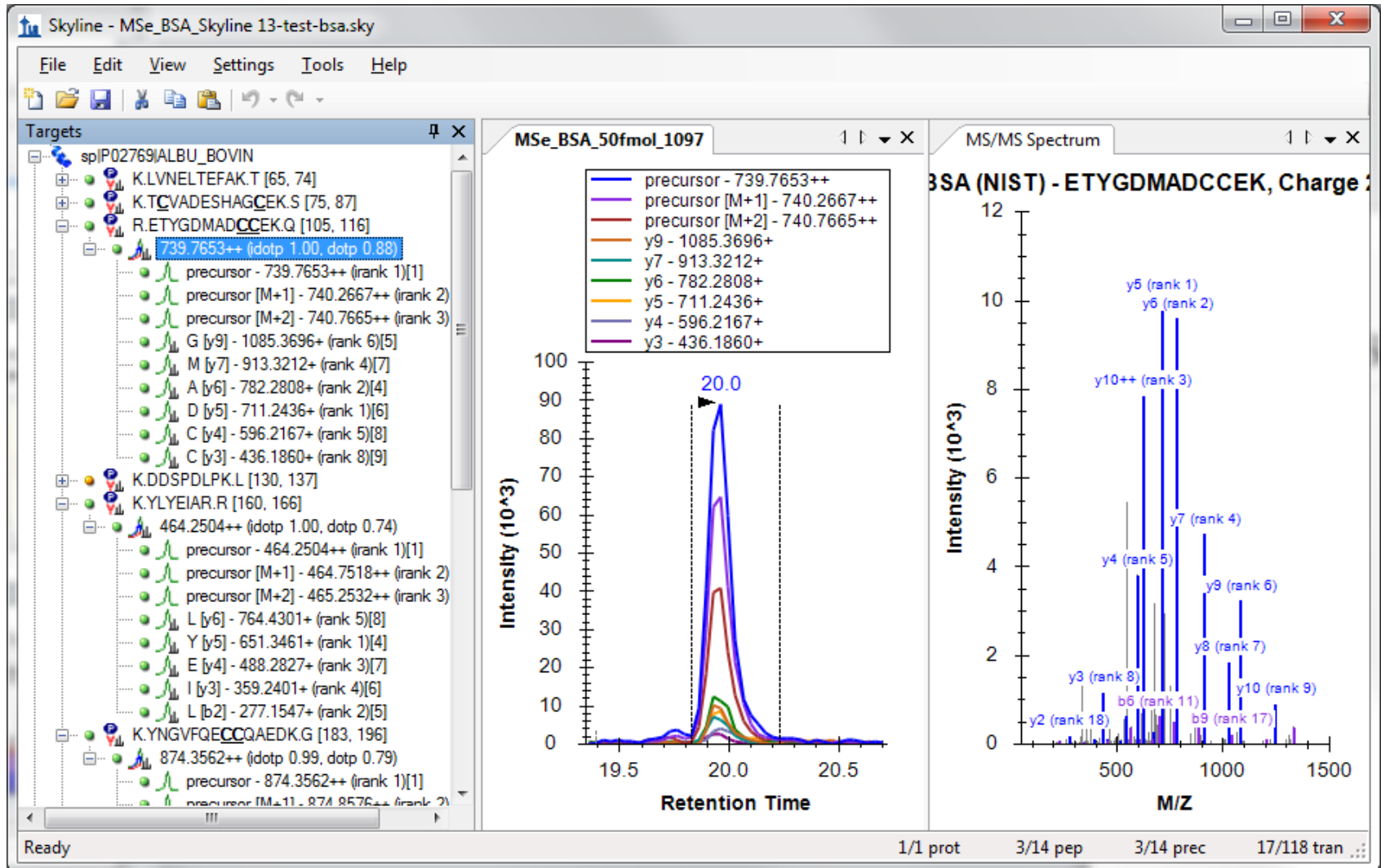
Max m/z: **1500** Th

Match tolerance m/z: []

Min time: **10** min

Max time: **40** min

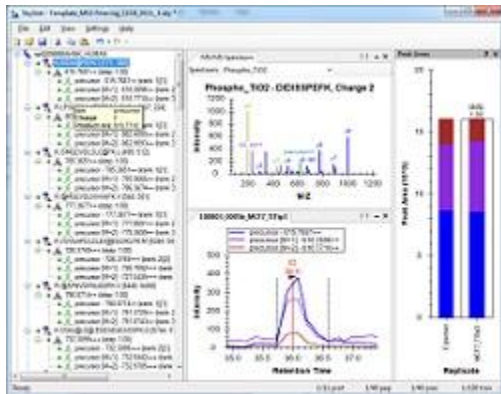
Bruker All Ions DIA Data



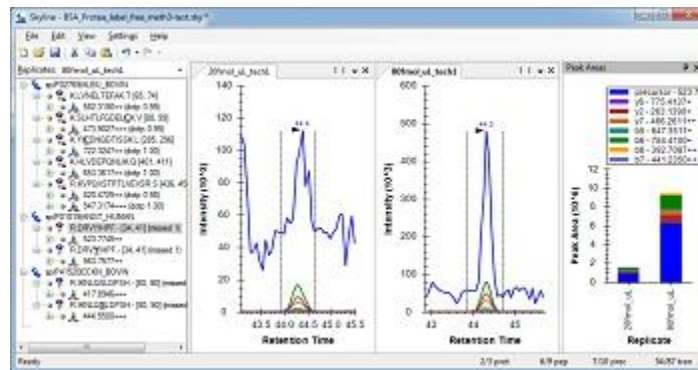
Getting Started

- ▶ Freely available & Open Source
<http://proteome.gs.washington.edu/software/skyline>
- ▶ Self-updating web installation (v1.4 coming soon)
- ▶ 2 full-scan filtering tutorials (DIA coming soon)

MSI Full-Scan Filtering



Targeted MS/MS



Data Independent Acquisition



- ▶ Support board and issues list
- ▶ 8 other tutorials & 3 instructional videos (full-scan video soon)