

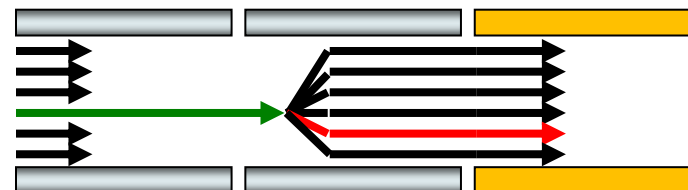


Skyline

Targeted Proteomics Environment

Targeted Proteomics Quantitative Analysis of Data
Independent Acquisition MS/MS in Skyline

Brendan MacLean; Jarrett Egertson; Donald M. Marsh;
Michael J. MacCoss

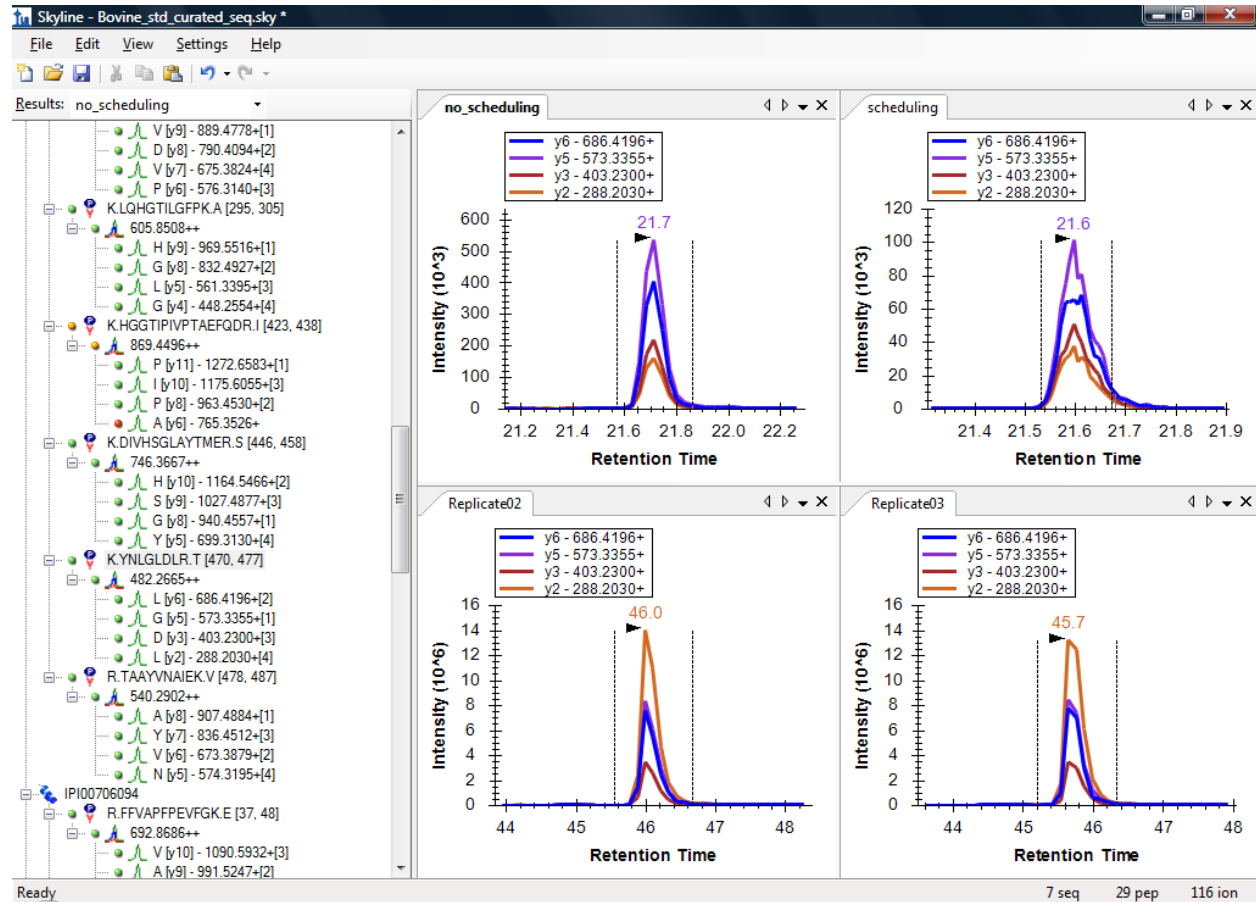


2010 Multi-Instrument SRM Tool for NCI CPTAC Verification Working Group

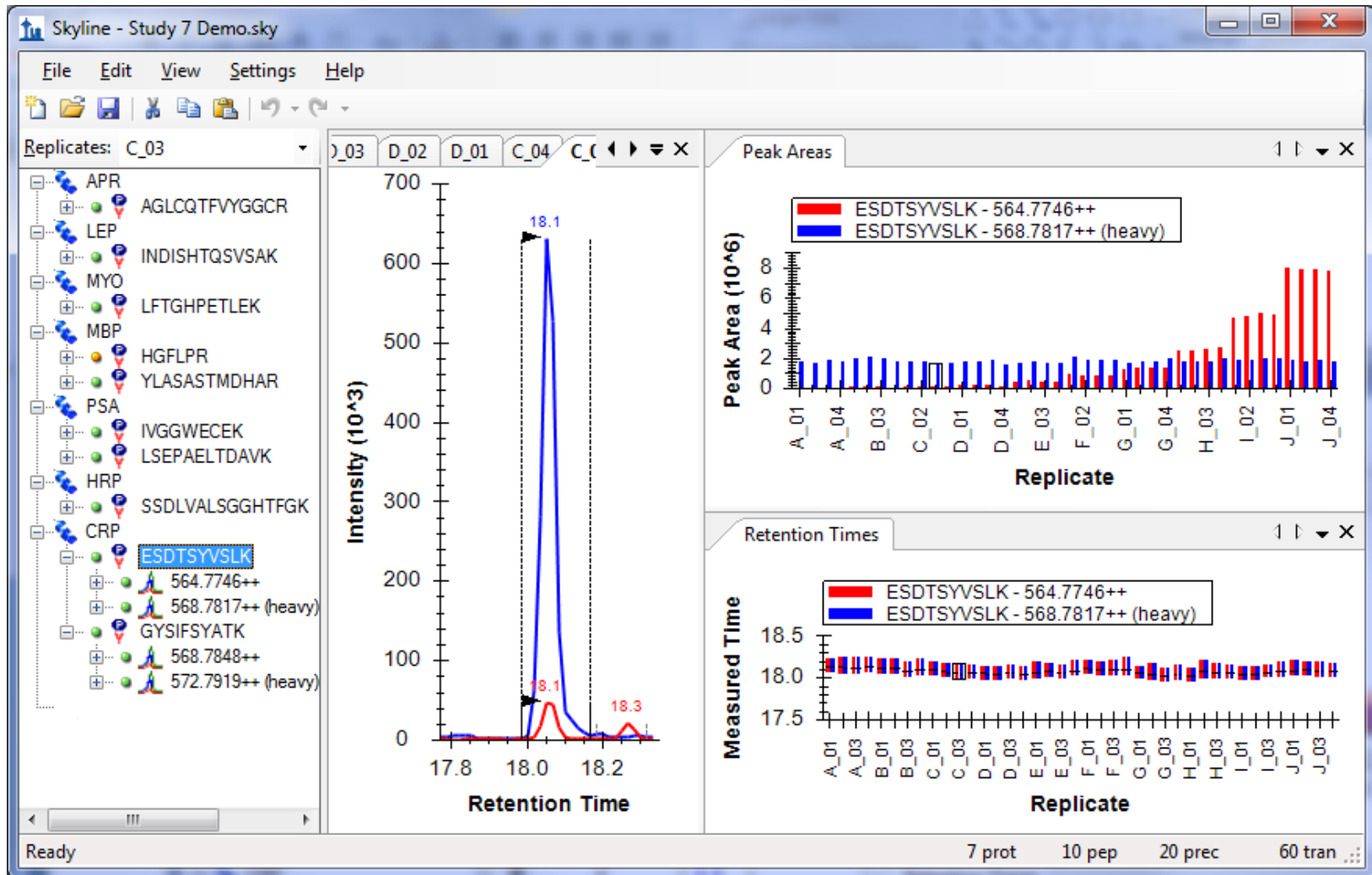


▶ AB SCIEX
4000 Q Trap

▶ Thermo-
Scientific
TSQ Ultra

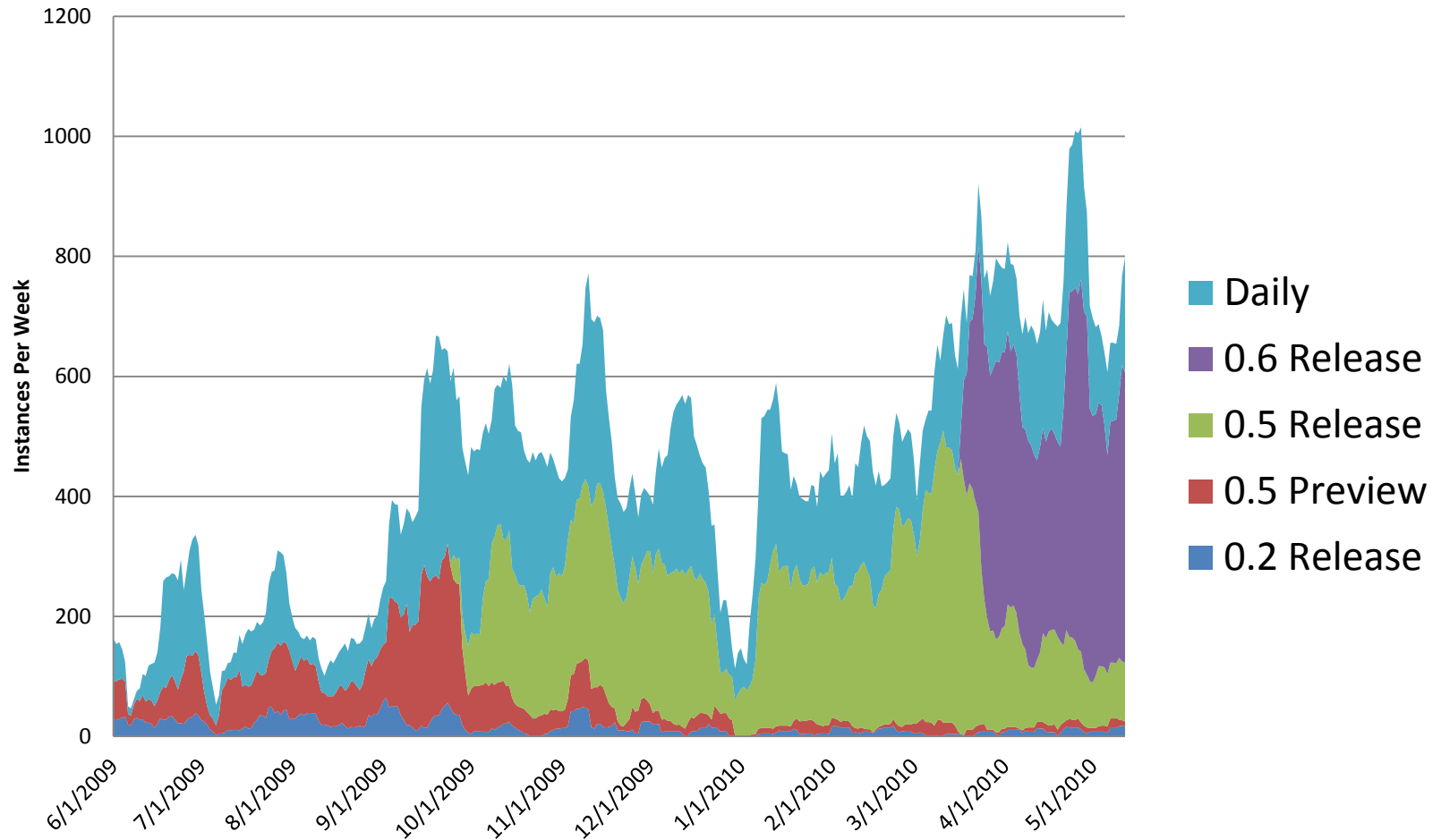


2010 Graphic Display of Information



2010 Skyline Adoption (>1500 installations)

Skyline Average Usage Per Week



Quantitative Proteomics

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

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



Skyline MS1 Full-Scan Settings

Transition Settings

Prediction Filter Library Instrument Full-Scan

MS1 filtering

Isotope peaks included: Percent
Precursor mass analyzer: FT-ICR

Min % of base peak: 20 %
Resolving power: 50,000 At: 400 Th

Isotope labeling enrichment: Default

MS/MS filtering

Acquisition method: None
Product mass analyzer:

Isolation scheme:
Resolution:

Filter only retention time scheduling windows

OK Cancel

MS1 filtering

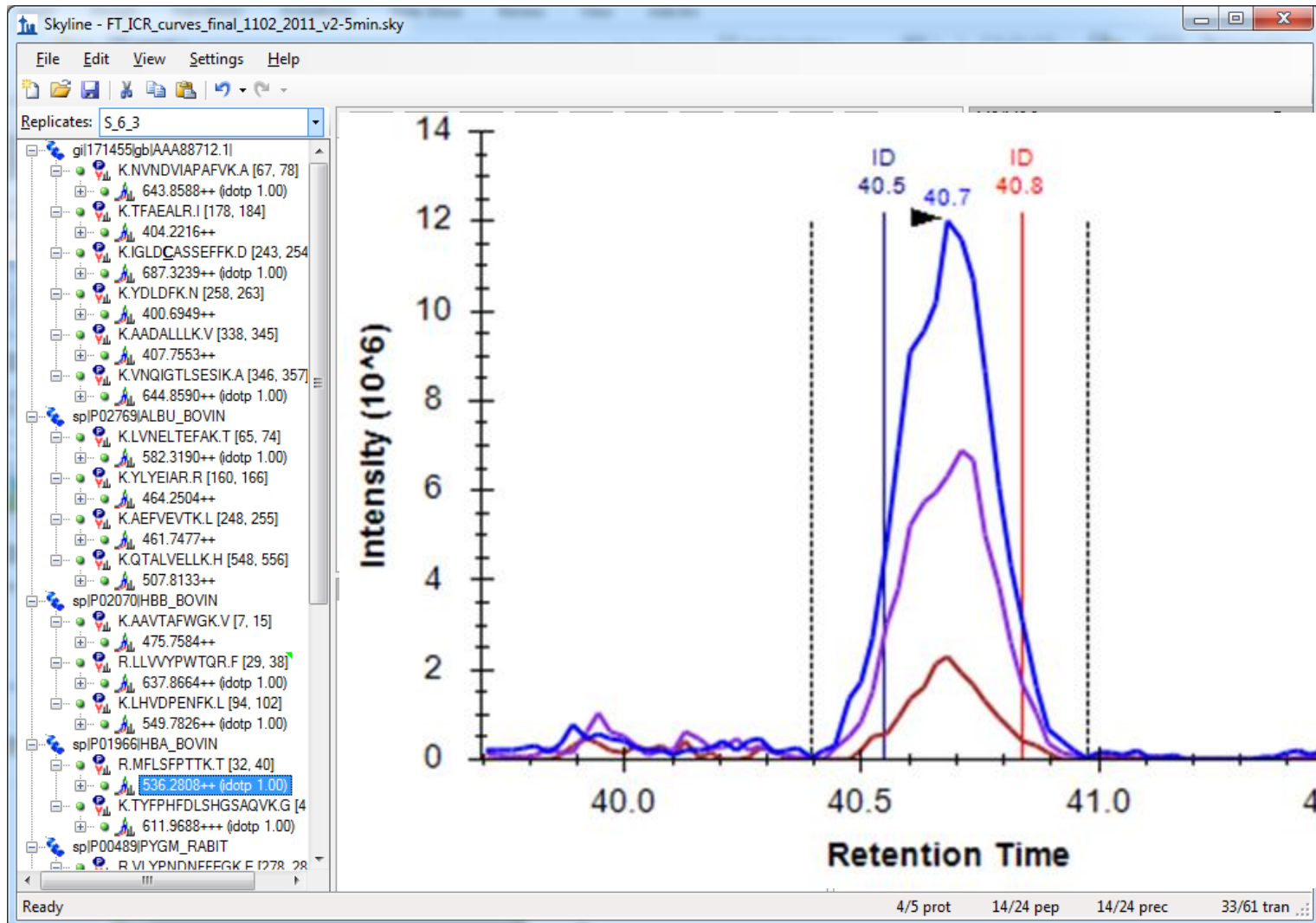
Isotope peaks included: Percent
Precursor mass analyzer: FT-ICR

Min % of base peak: 20 %
Resolving power: 50,000 At: 400 Th

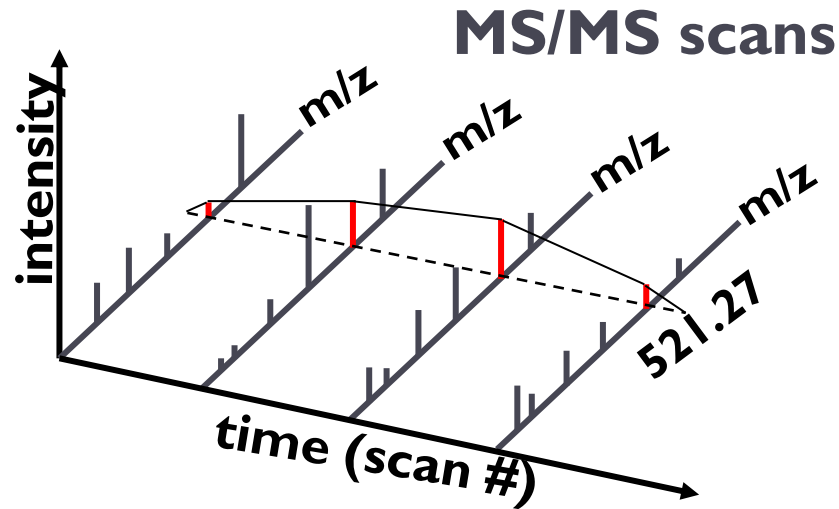
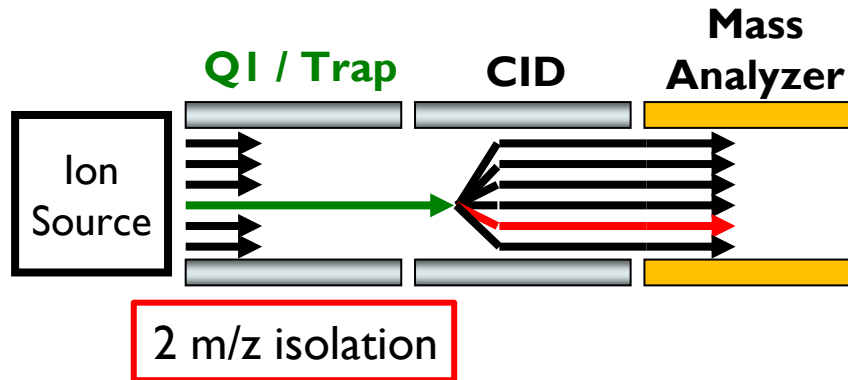
Isotope labeling enrichment: Default



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

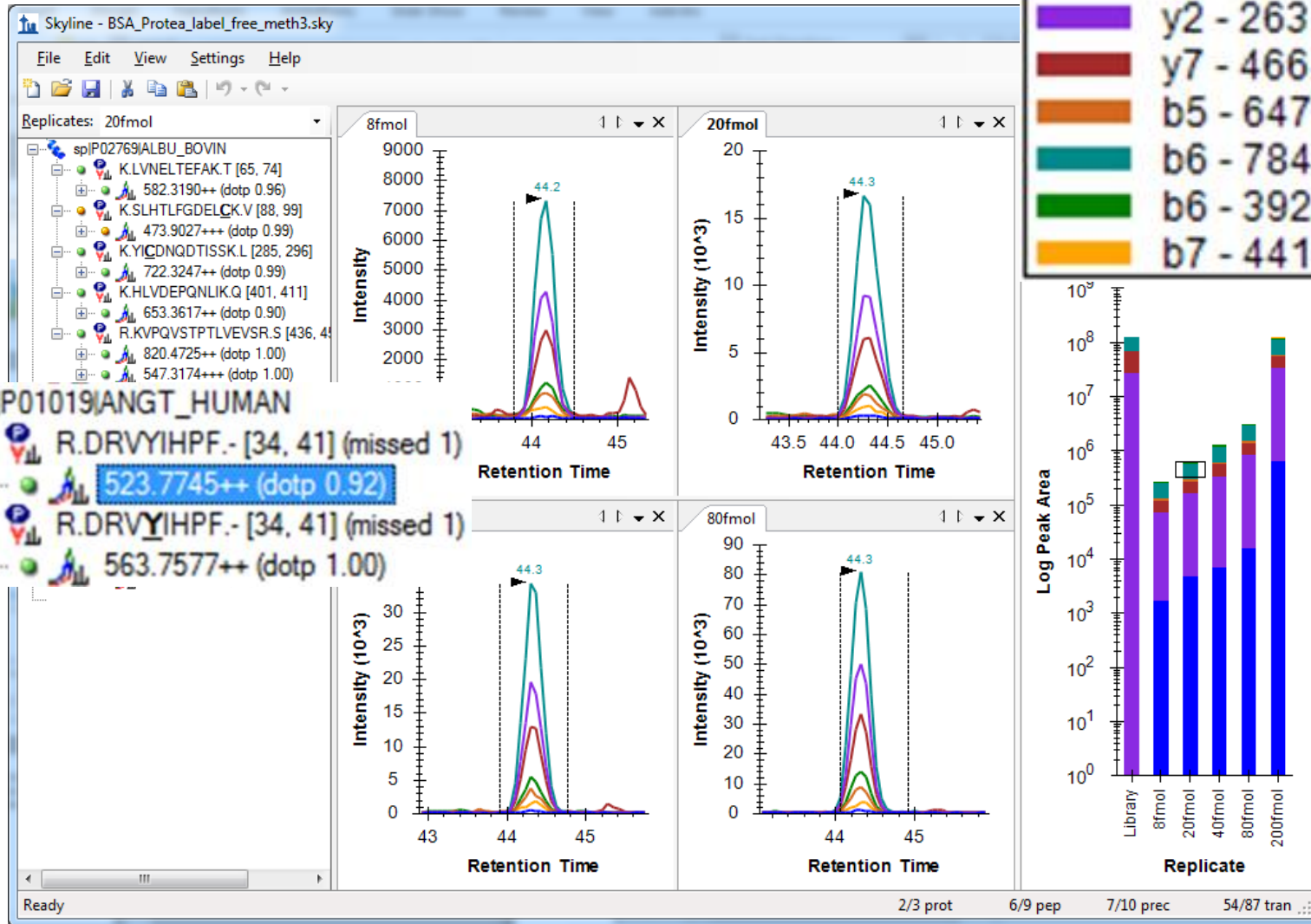
MS1 filtering

MS/MS filtering

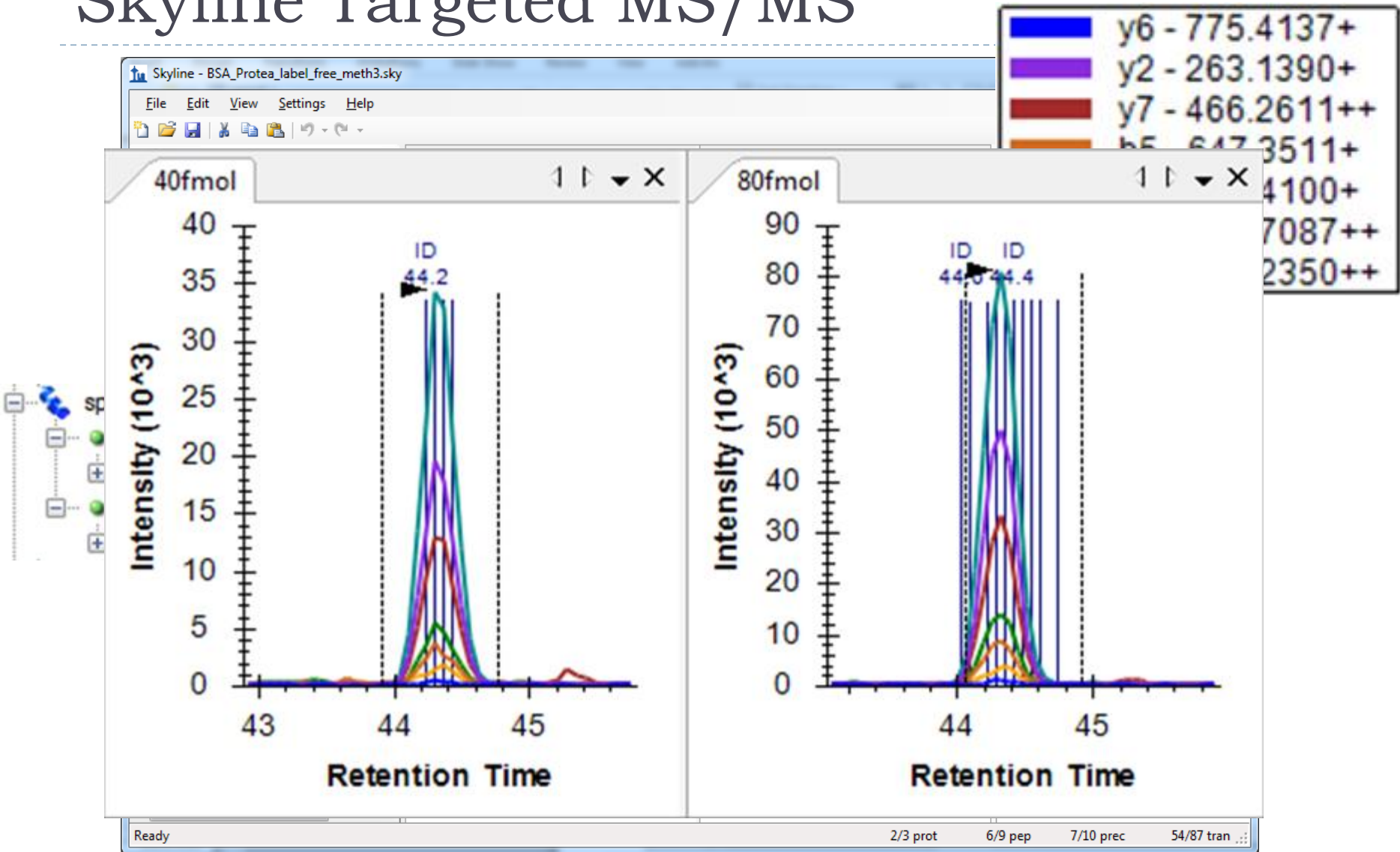
Acquisition method: Targeted
Product mass analyzer: QIT

Isolation scheme:
Resolution: 0.7 Th

Skyline Targeted MS/MS



Skyline Targeted MS/MS



2010 Support Multiple Instrument Vendors

- ▶ SRM
- ▶ Exporting transition lists & native methods
- ▶ Importing native instrument output files

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



2012 Support Multiple Instrument Vendors

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

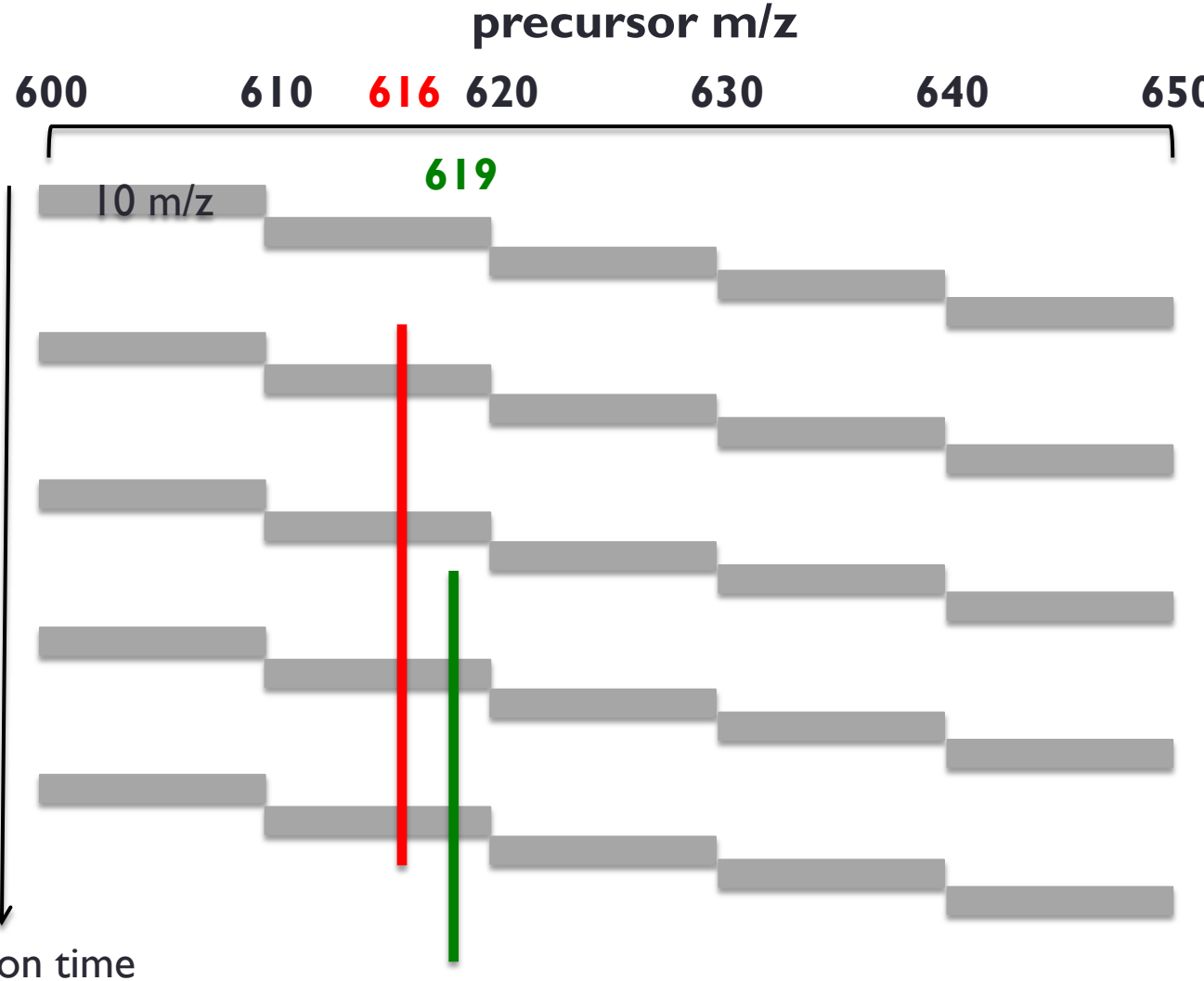
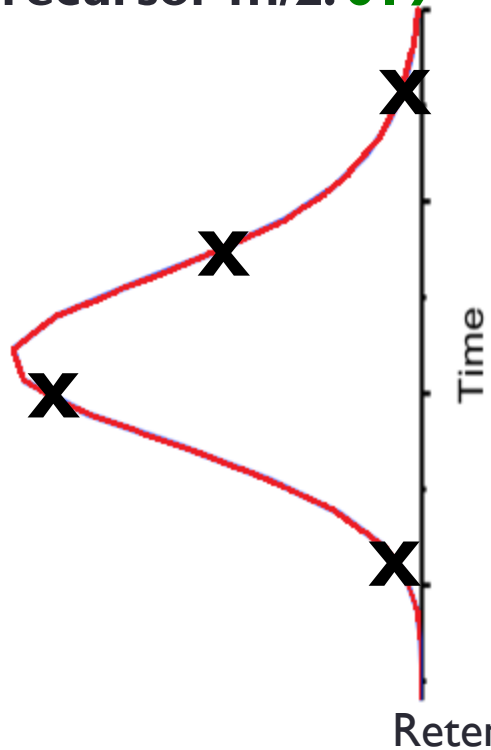
- ▶ AB SCIEX SWATH™
- ▶ Agilent Technologies DIA
- ▶ Thermo-Scientific DIA & Multiplexed DIA
- ▶ Waters MSe™



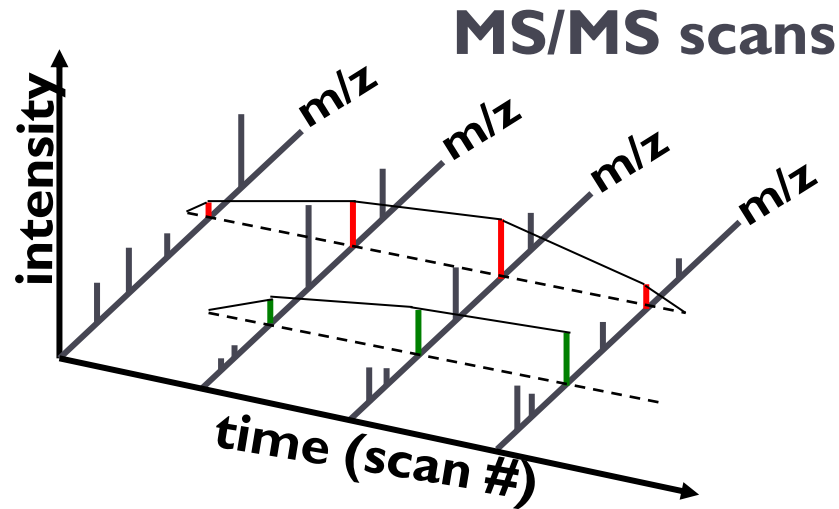
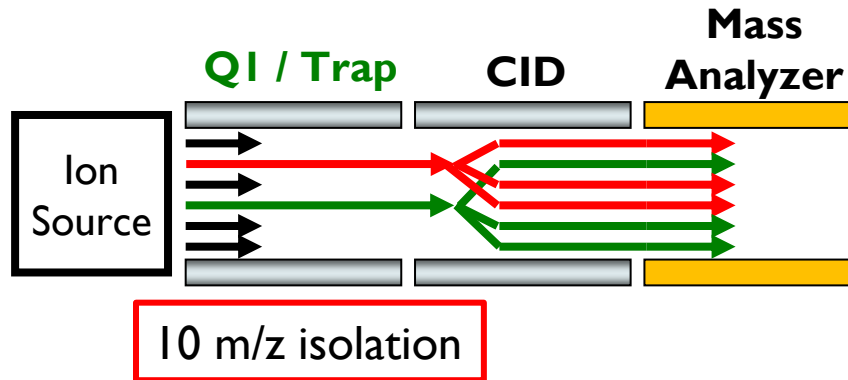
Traditional DIA Isolation Scheme

SVEDFMAAMQR
Precursor m/z: **616**

VGGNGADYALATK
Precursor m/z: **619**

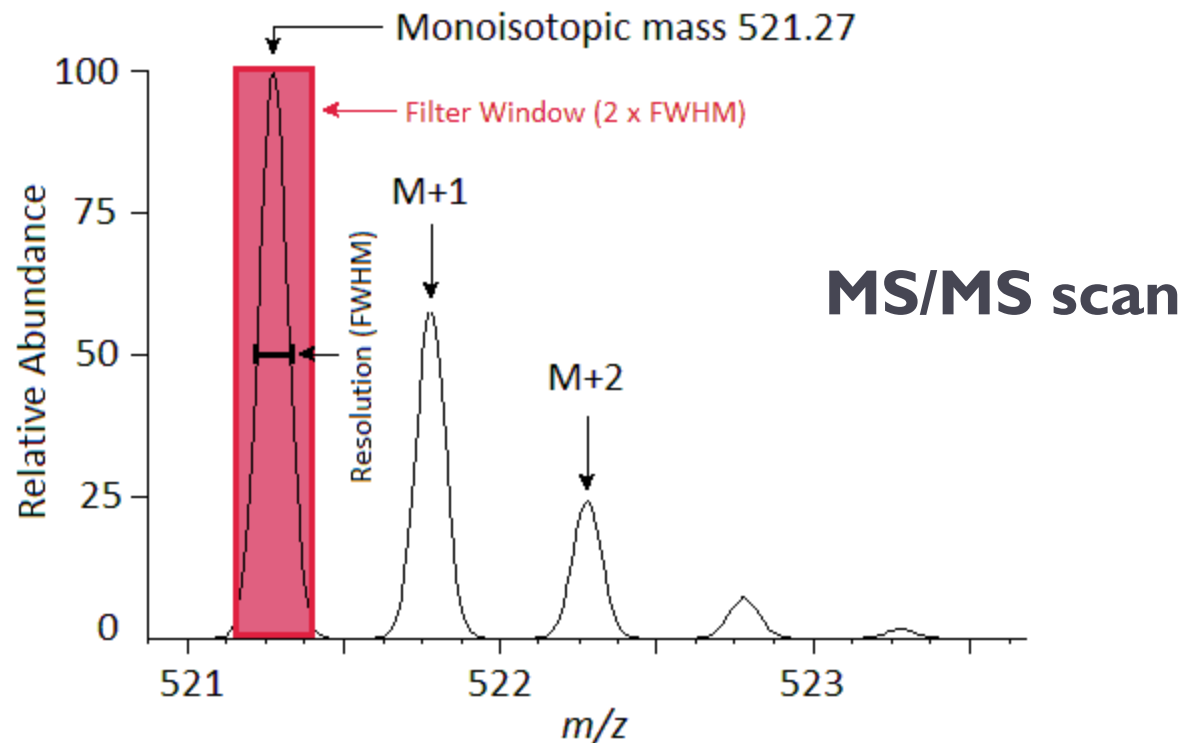


DIA Chromatogram Extraction



DIA High-Res. Chromatogram Extraction

- ▶ Selectivity lost by wide precursor isolation
- ▶ Selectivity gained vs. SRM by narrow product extraction



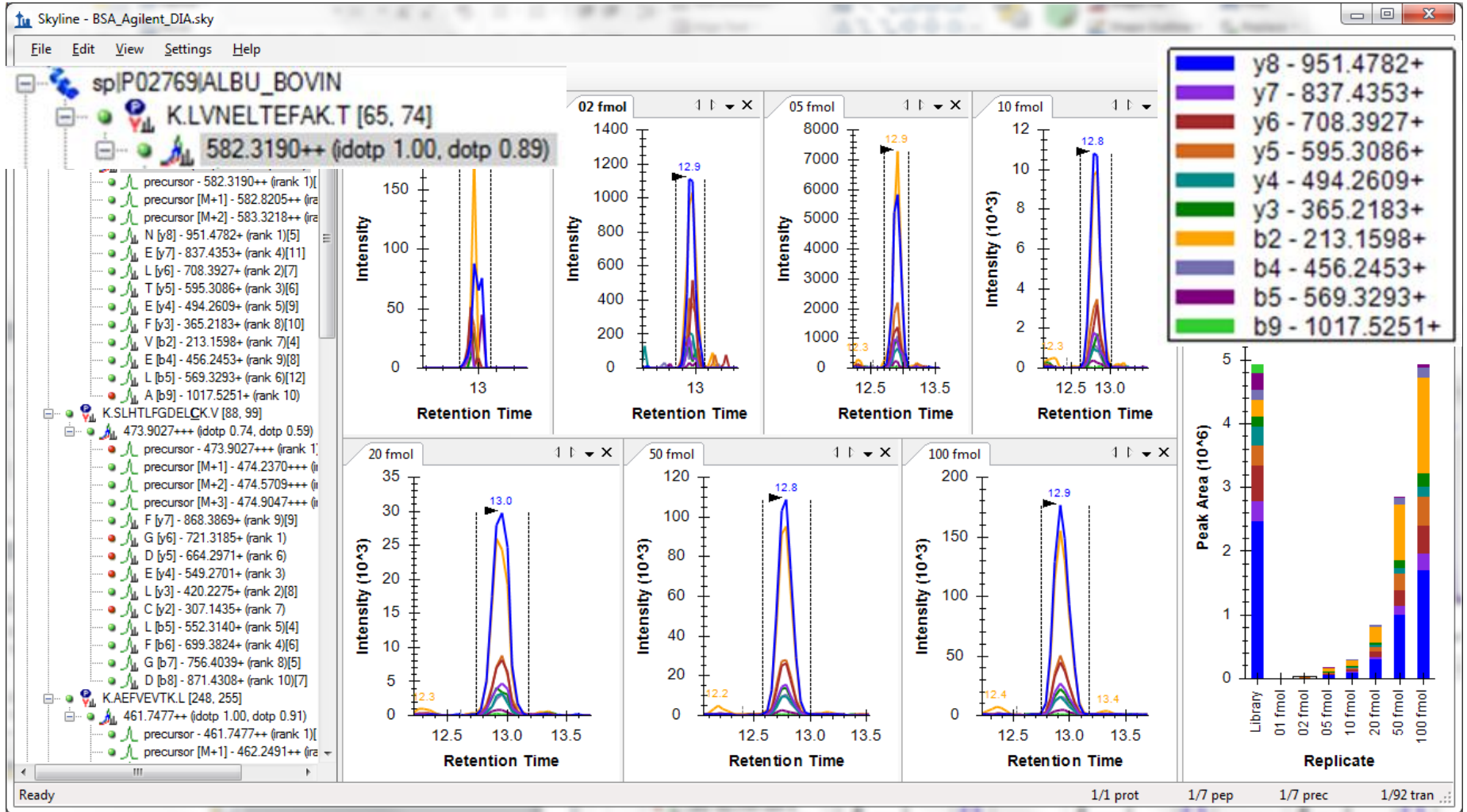
Skyline Agilent DIA Settings (11.5 m/z Extraction Windows)

The image displays two windows from the Skyline software interface. The left window, titled "Transition Settings", shows the "MS1 filtering" section. In this section, the "Acquisition method" is set to "DIA" and the "Product mass analyzer" is set to "TOF". The "Isolation scheme" dropdown menu is highlighted with a red box and contains the text "Results 11.5 Th". The "Resolving power" is set to "10,000".

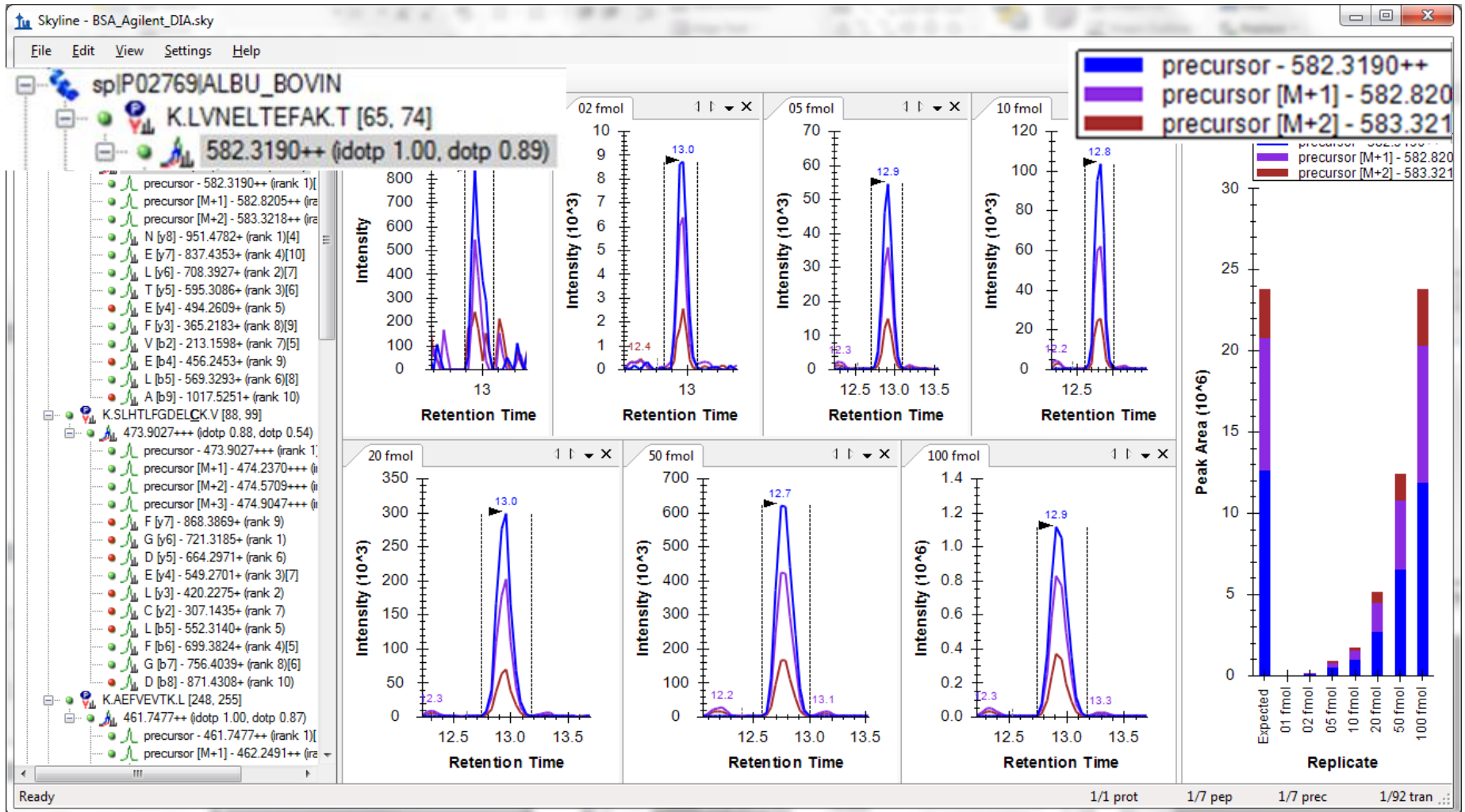
The right window, titled "Edit Isolation Scheme", is open. It shows the "Name" field set to "Results 11.5 Th". The "Use results data isolation targets" radio button is selected. The "Isolation width" is set to "11.5 Th". The "Asymmetric" checkbox is unchecked. The "Margins" dropdown is set to "None". The "Multiplexed acquisition" checkbox is unchecked. The "Windows per scan" field is empty. The "Specify target" checkbox is unchecked.

A red arrow points from the "Results 11.5 Th" dropdown in the "Transition Settings" window to the "Results 11.5 Th" text in the "Edit Isolation Scheme" window.

Agilent DIA Data (Product Ions)



Agilent DIA Data (Precursor Ions)



Skyline AB SCIEX SWATH™ Settings in v1.3 (32 x 25 m/z Extraction Windows)

Transition Settings

Prediction Filter Library Instrument Full-Scan

MS1 filtering

MS/MS filtering

Acquisition method: DIA

Product mass analyzer: TOF

Isolation scheme: **SWATH**

Resolving power: 10,000

Filter only retention time scheduling windows

OK Cancel

Edit Isolation Scheme

Name: SWATH

Use results data isolation targets

Isolation width: 2 Th

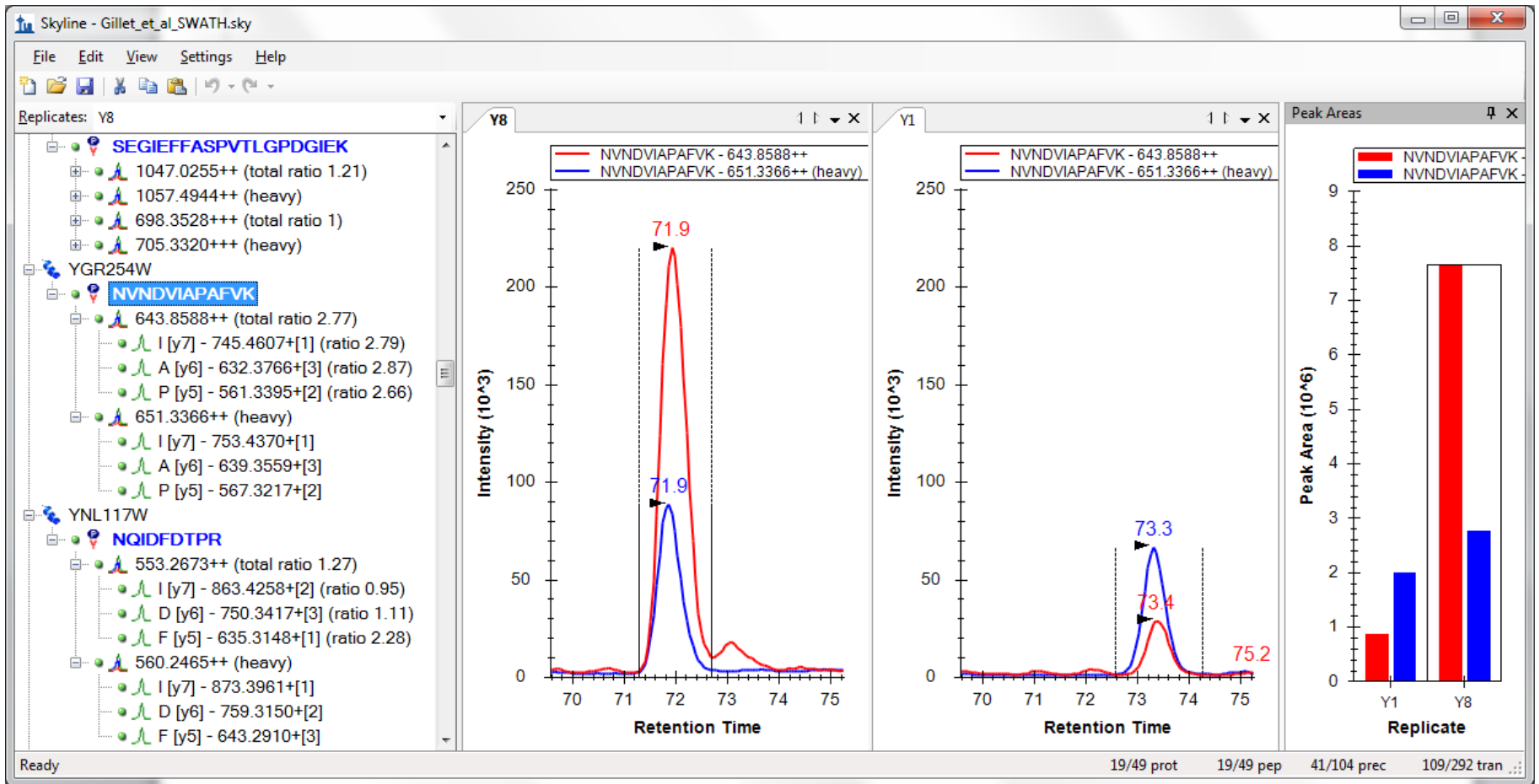
Prespecified isolation windows

	Start	End	Margin
▶	400.5	425.5	0.5
	425.5	450.5	0.5
	450.5	475.5	0.5
	475.5	500.5	0.5
	500.5	525.5	0.5
	525.5	550.5	0.5
	550.5	575.5	0.5

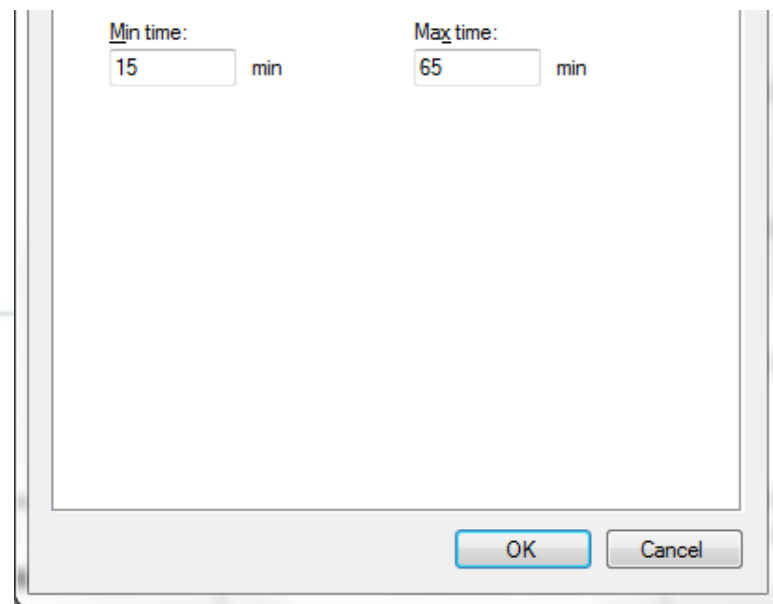
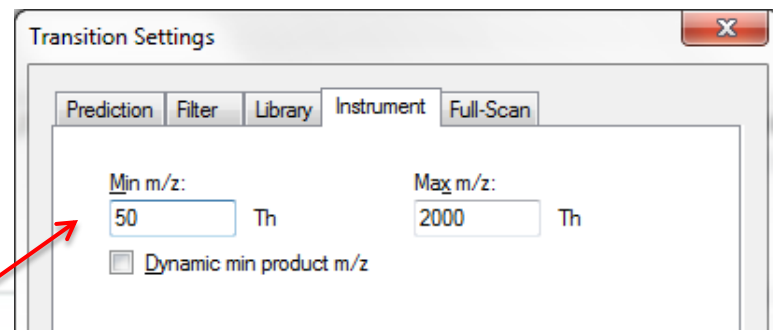
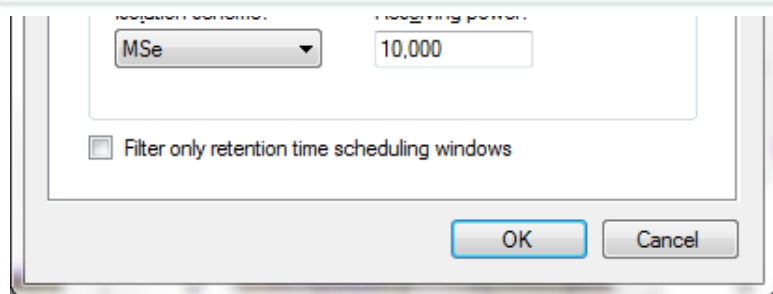
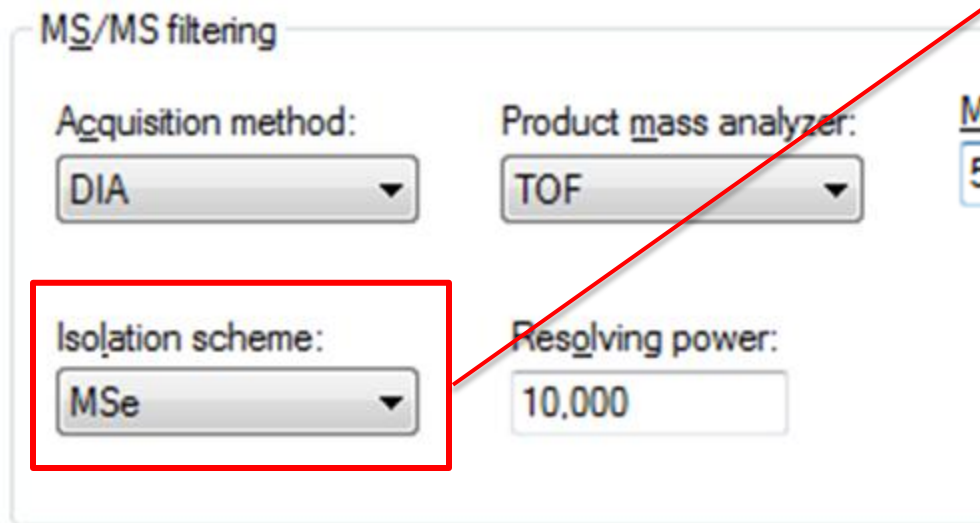
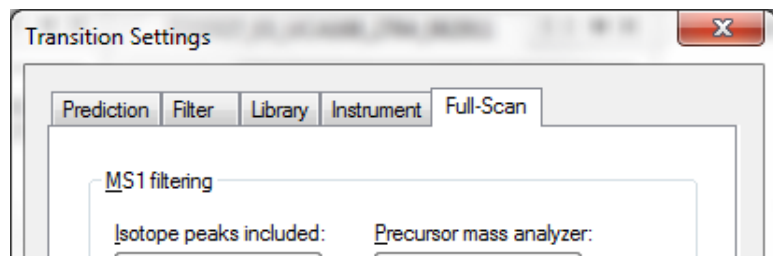
Margins: Symmetric

OK Cancel

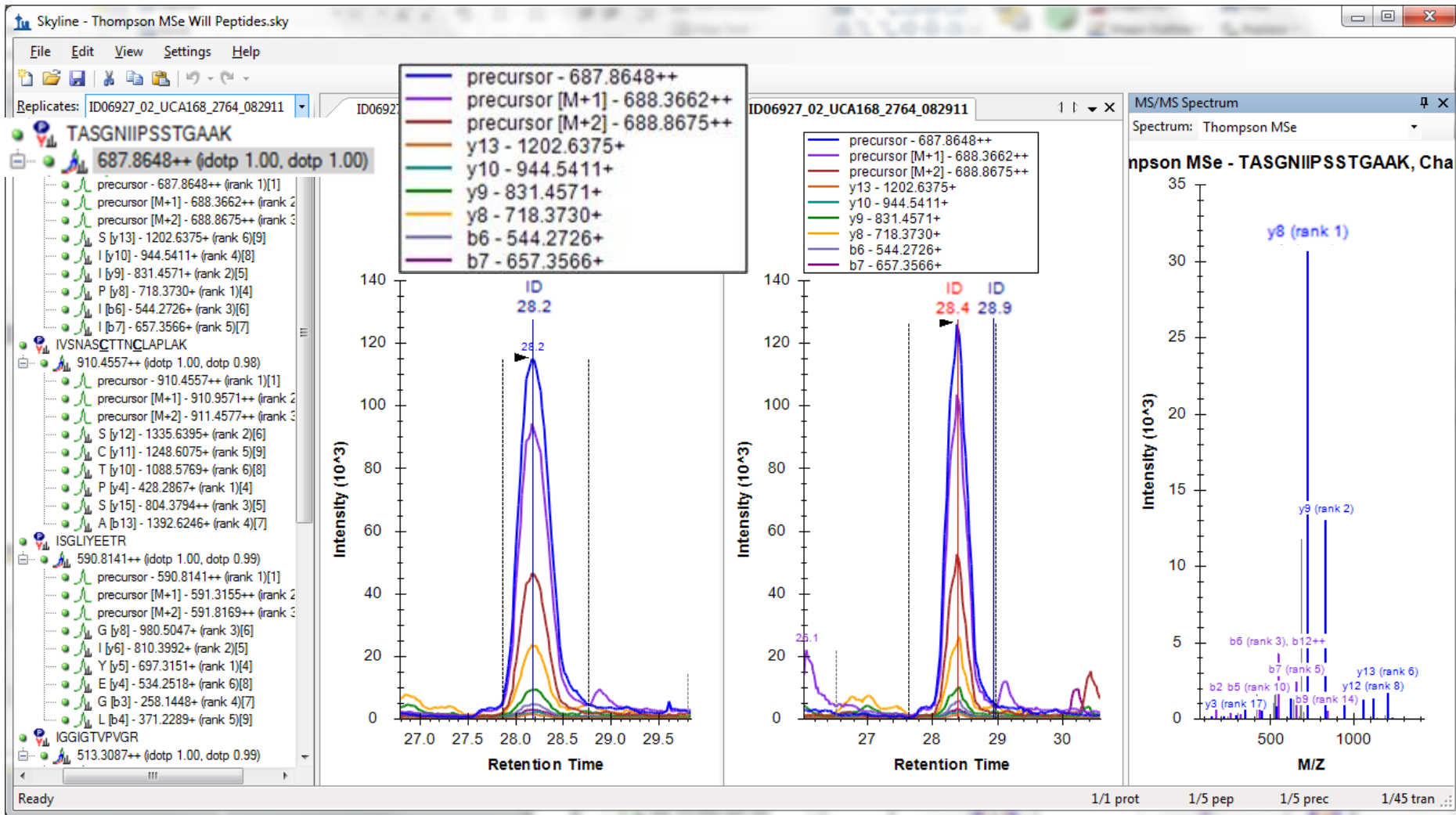
AB SCIEX SWATH™ Data



Skyline Waters MSe™ Settings in v1.3 (50 – 2000 m/z Range)



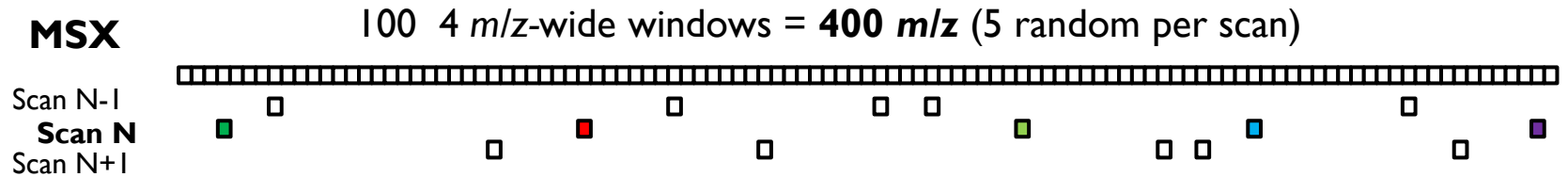
Waters MSe™ Data



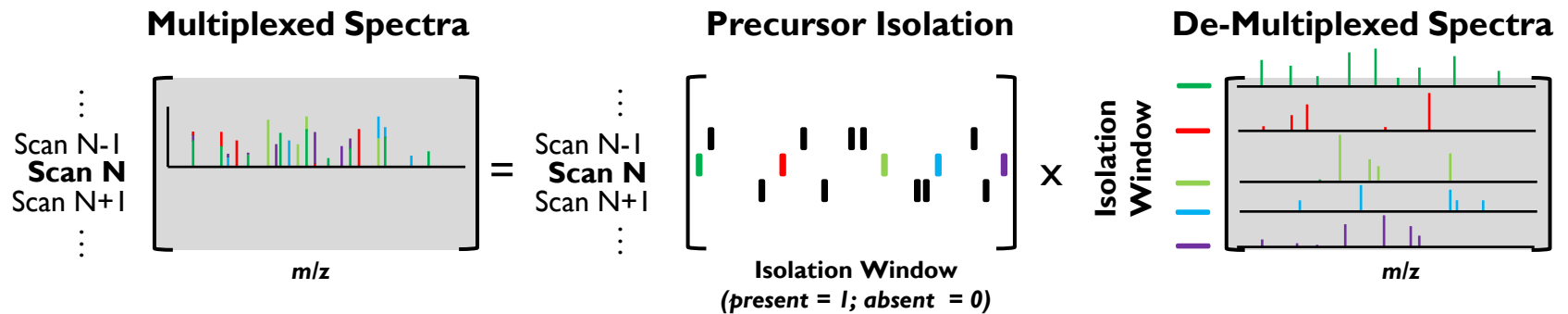
Multiplexed DIA (Q Exactive) in v1.3

▶ Jarrett Egertson – Thursday AM – ThP 571

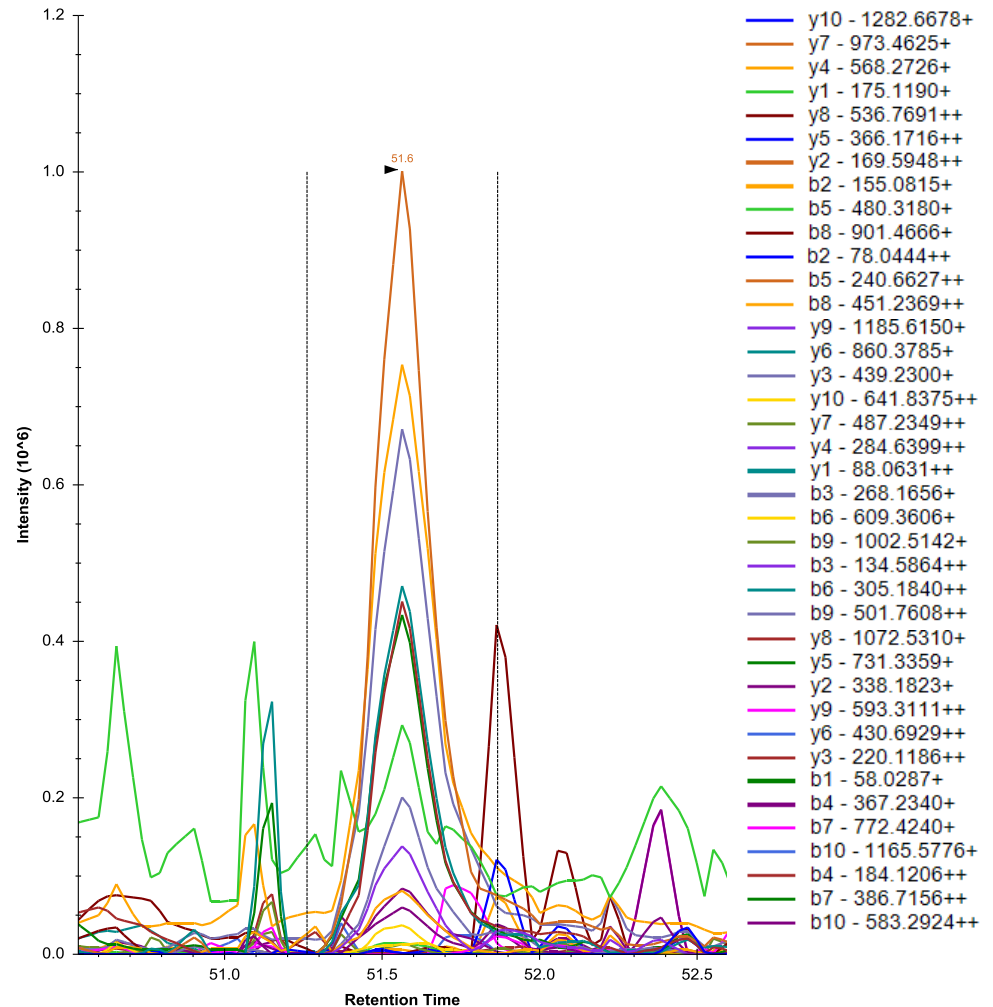
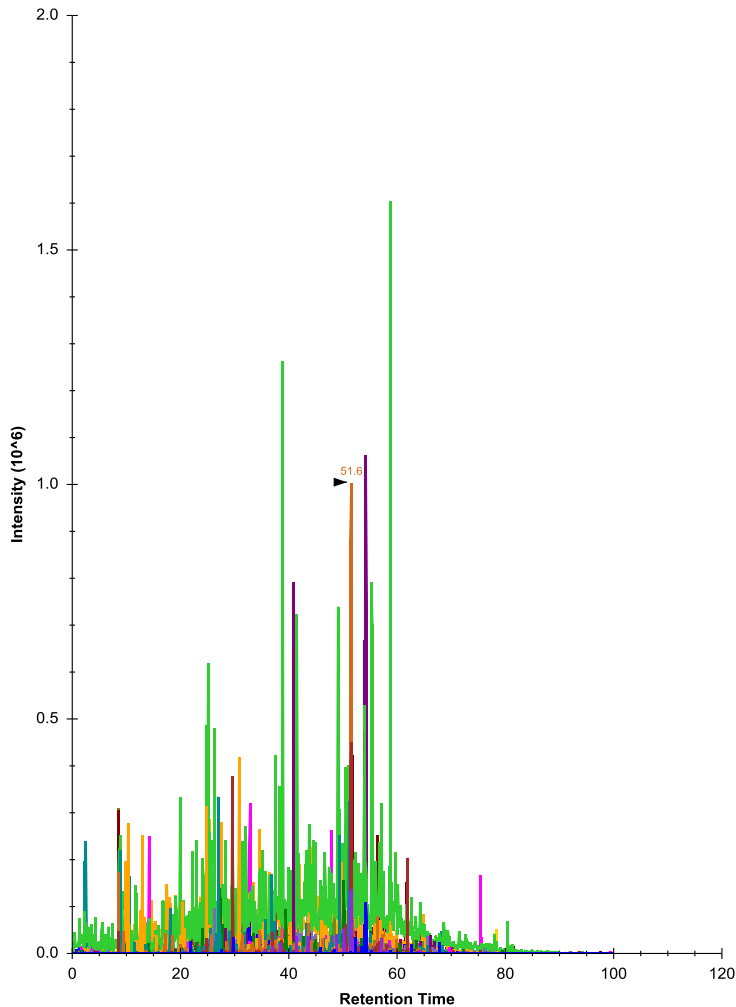
Acquisition



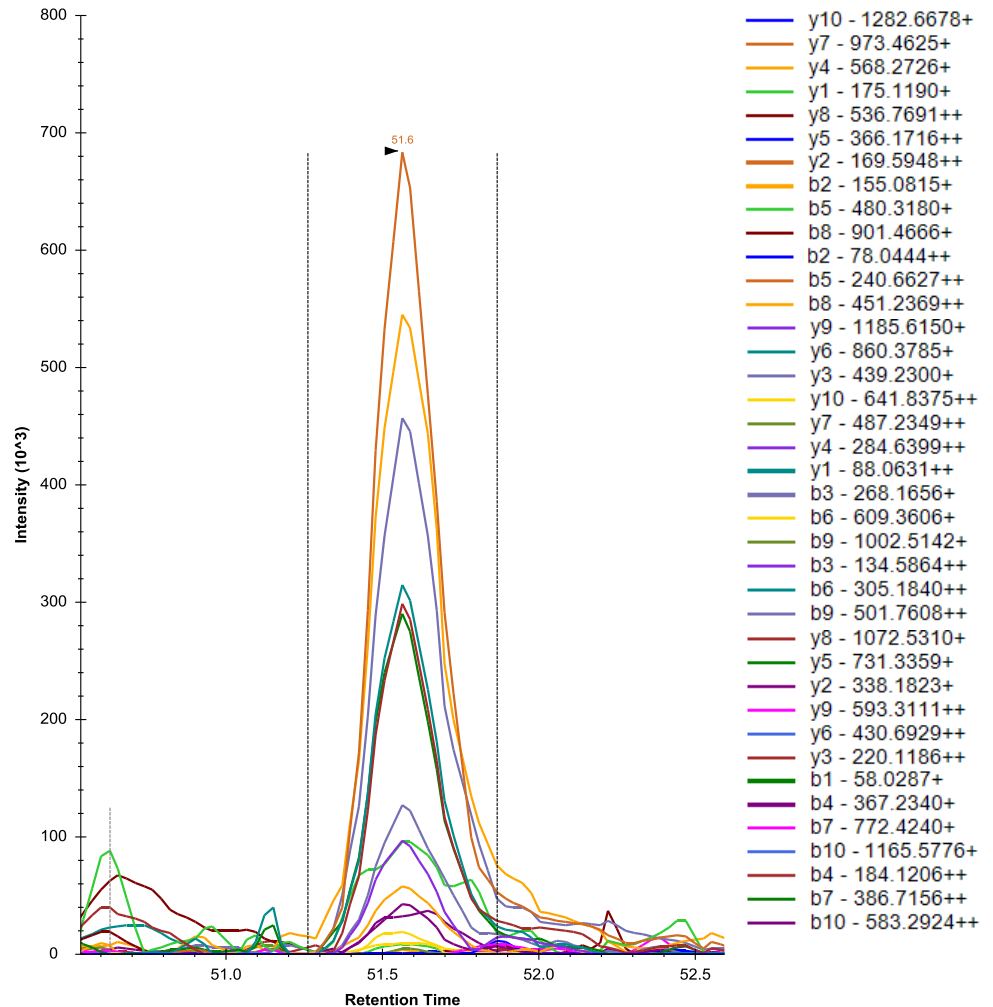
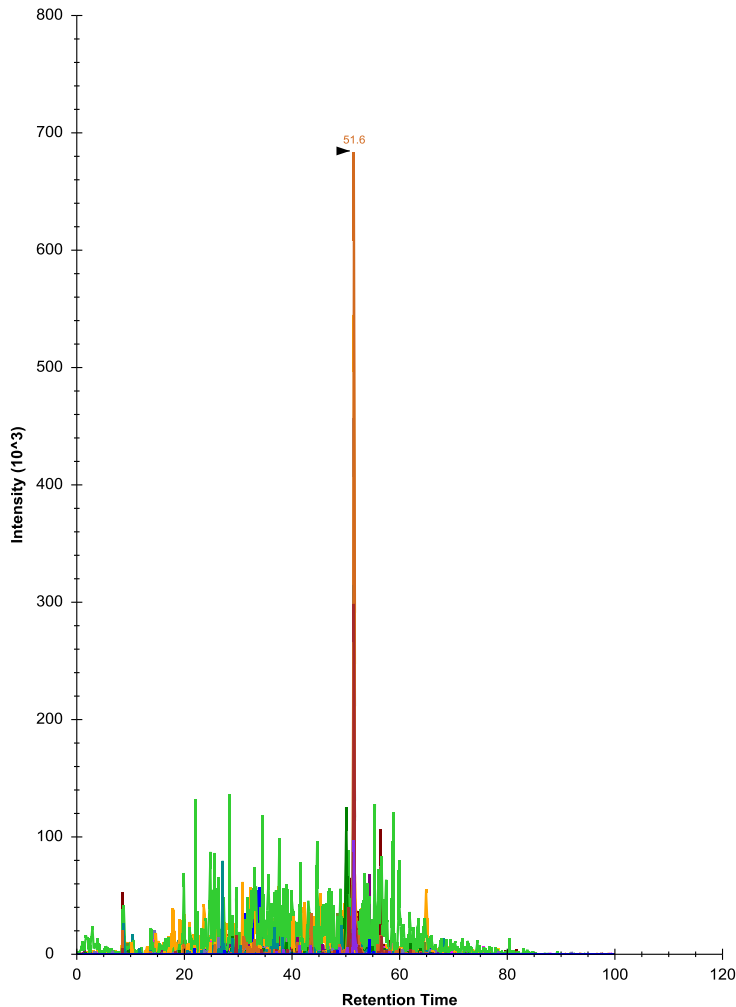
De-Multiplexing



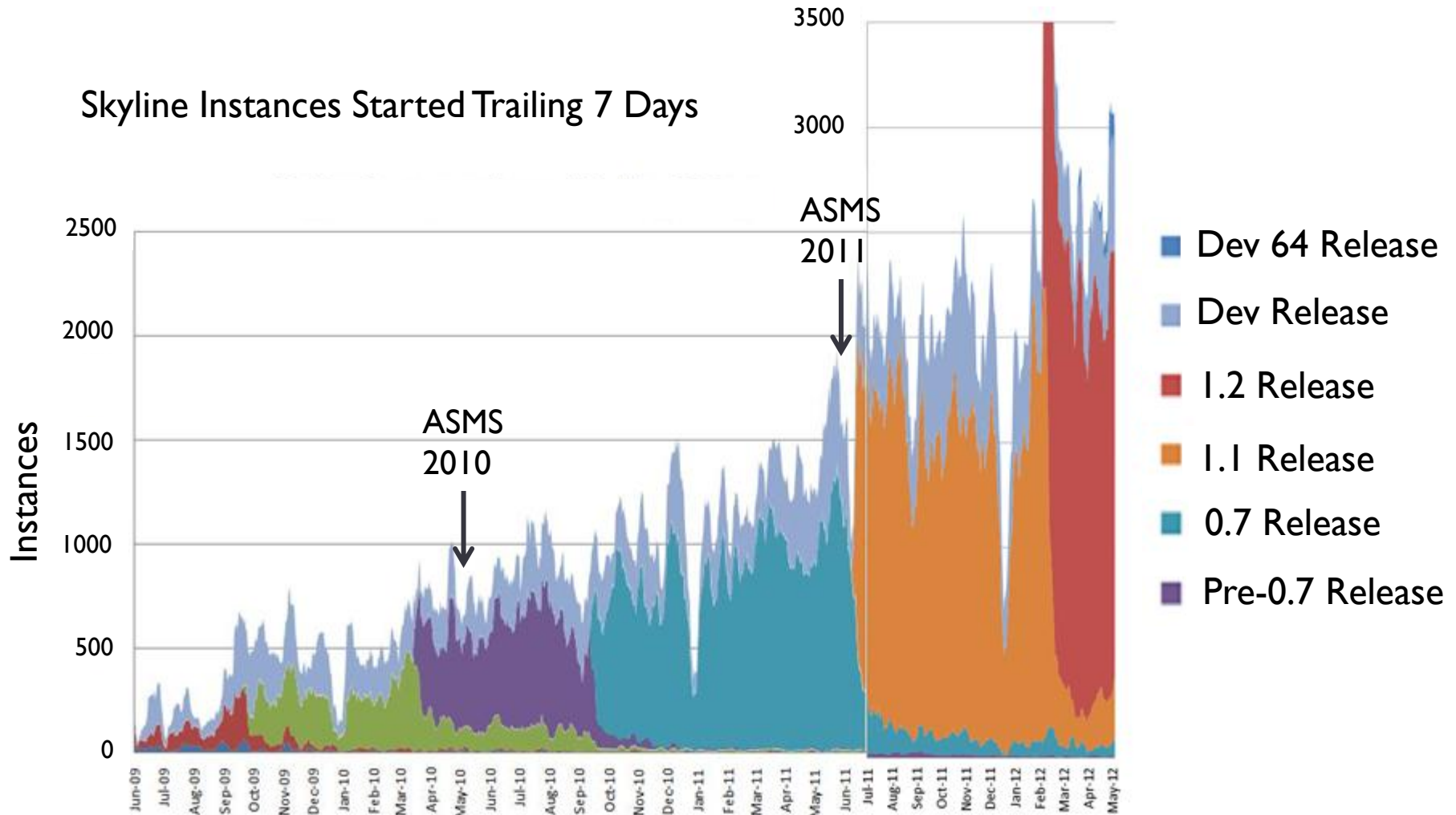
Multiplexed DIA Data (before)



Multiplexed DIA Data (after)



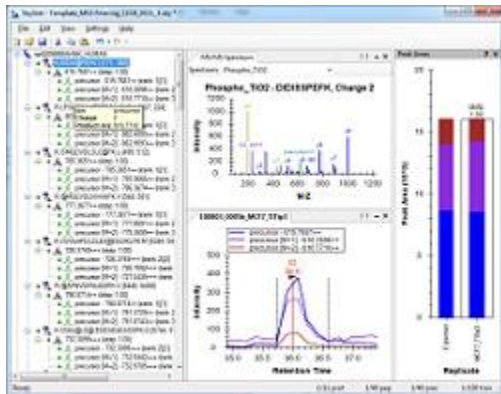
2012 Skyline Adoption (>10,000 installations)



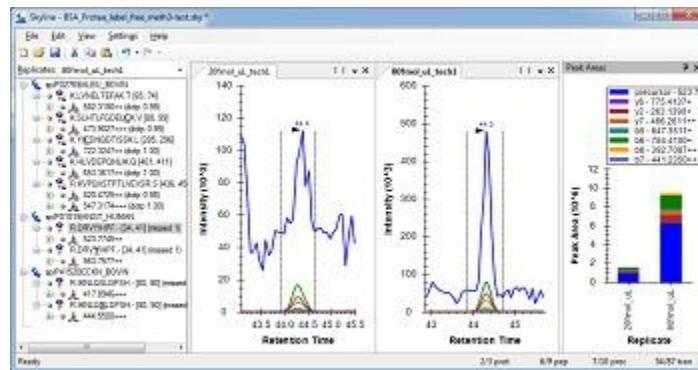
Getting Started

- ▶ Freely available & Open Source
<http://proteome.gs.washington.edu/software/skyline>
- ▶ Self-updating web installation (v1.3 coming soon)
- ▶ 2 full-scan filtering tutorials (DIA coming soon)

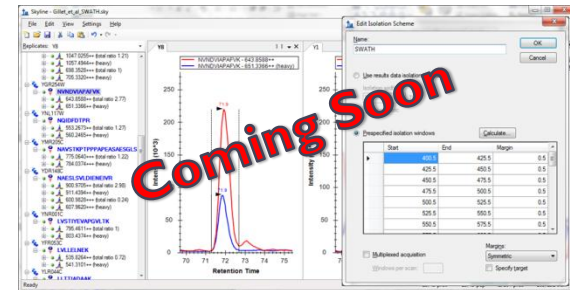
MSI Full-Scan Filtering



Targeted MS/MS



Data Independent Acquisition



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

Acknowledgments:

▶ Skyline Team

- ▶ Nick Shulman
- ▶ Vagisha Sharma
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- ▶ Michael Bereman

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- ▶ Christina Ludwig

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- ▶ Joe Roark
- ▶ Pat Perkins

▶ Thermo-Scientific

- ▶ Markus Kellmann
- ▶ Andreas Kuehn
- ▶ Vlad Zabrouskov

▶ Waters

- ▶ Laurence Firth
- ▶ James Langridge
- ▶ Roy Martin
- ▶ Kieran Neeson
- ▶ Keith Richards



A private repository of targeted proteomics assays for Skyline

WP 407 - Vagisha Sharma

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