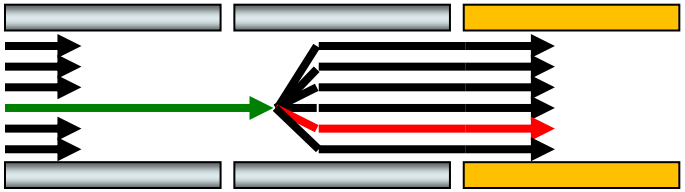
Skyline

Targeted Proteomics Environment

Quantitative Proteomics with Bruker Q-TOF Instruments and Skyline

Brendan MacLean

UNIVERSITY of WASHINGTON



Quantitative Proteomics

- Spectrum-based
  - Spectral counting
  - Isobaric tags
- Chromatography-based
  - SRM
  - MS1 chromatogram extraction
  - Targeted MS/MS
  - Data independent acquisition (DIA)

Quantitative Proteomics

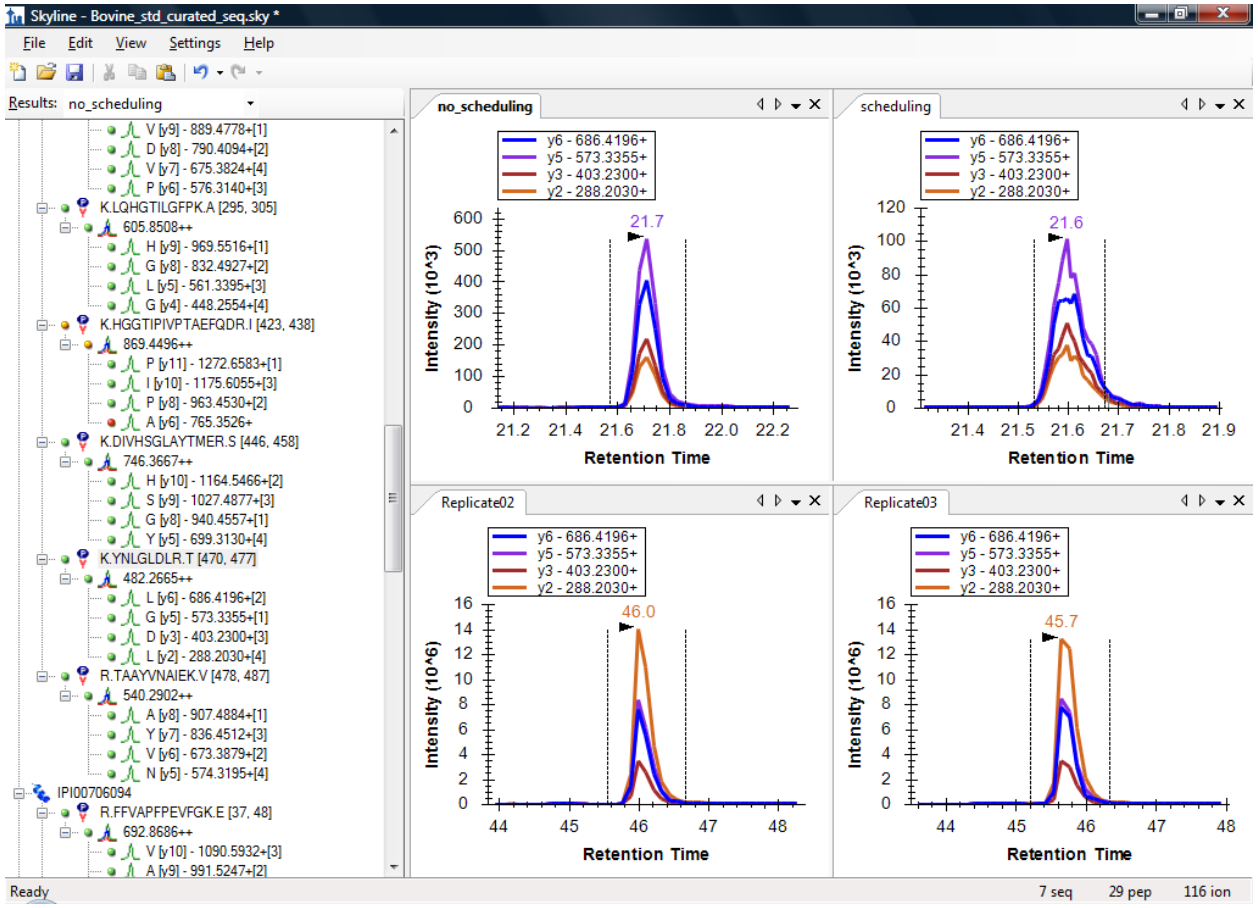
- Spectrum-based
  - Spectral counting
  - Isobaric tags

- Chromatography-based
  - SRM
  - **MS1 chromatogram extraction**
  - **Targeted MS/MS**
  - **Data independent acquisition (DIA)**

BRUKER **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



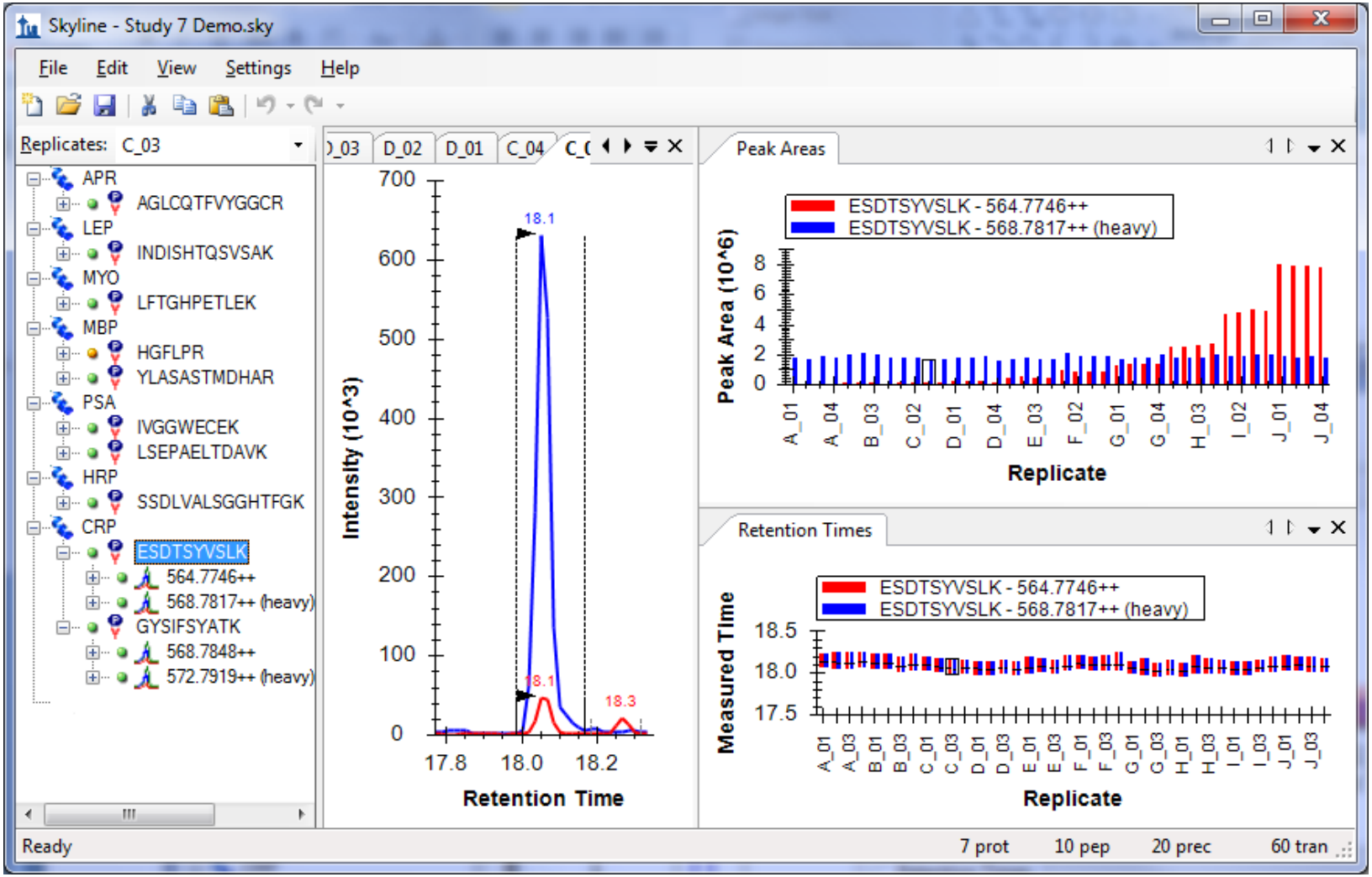
Kessner, D. et al. Bioinformatics. 2008.

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

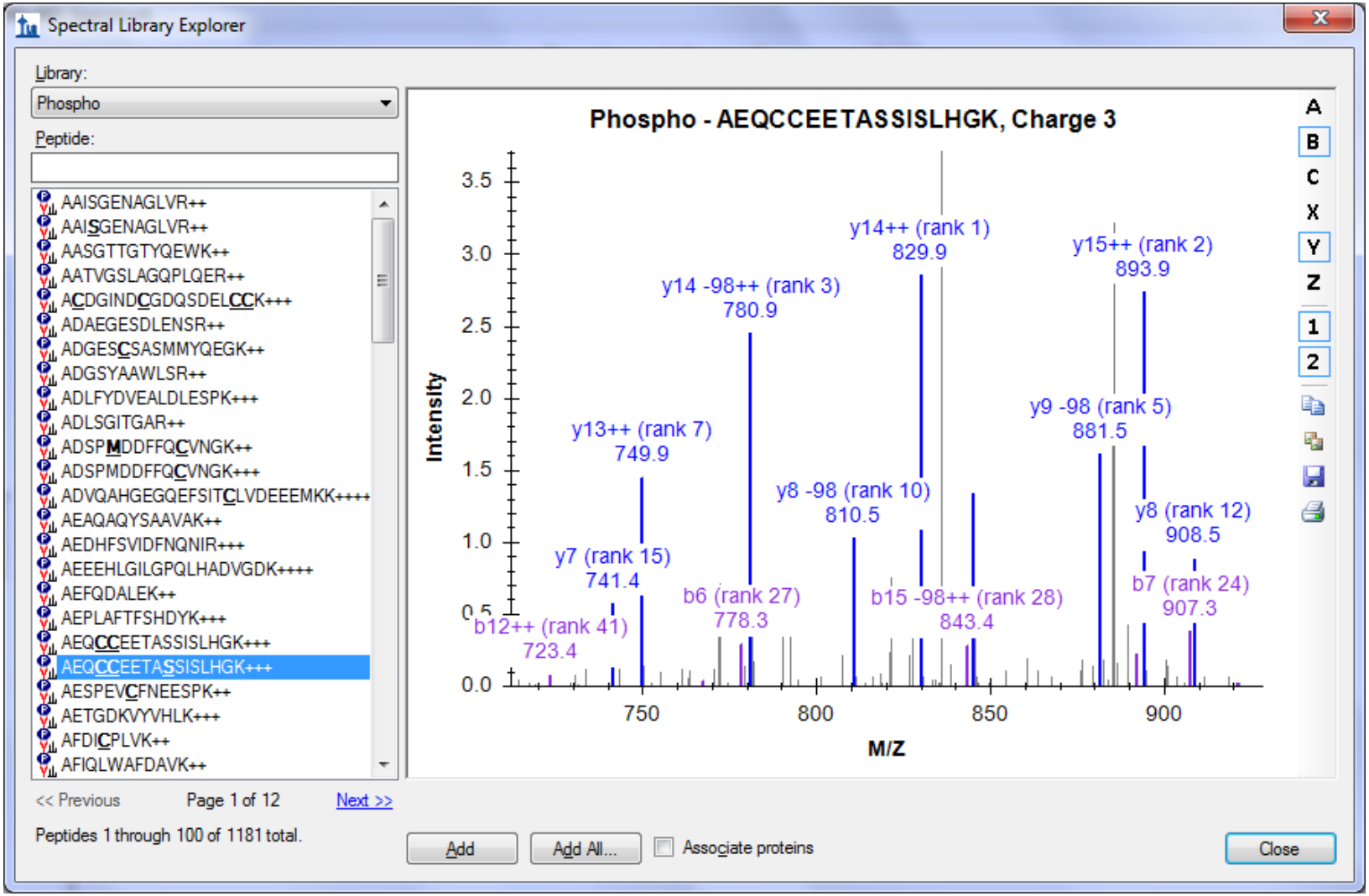


MS/MS Spectral Library Sources

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

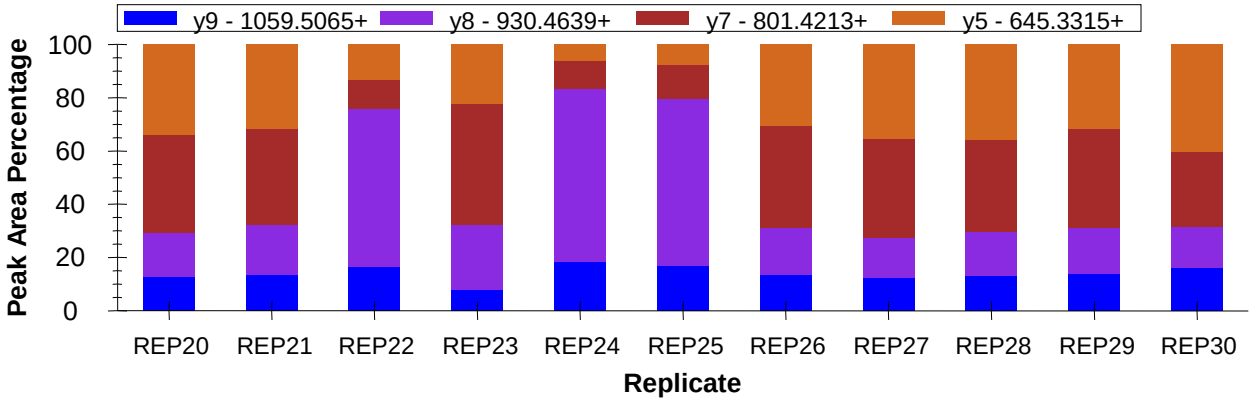
- Build your own from peptide search results
  - Mascot
  - Myrimatch / IDPicker
  - OMSSA
  - Protein Pilot
  - Protein Prospector
  - Scaffold – mzIdentML / MGF
  - Spectrum Mill
  - TPP – pepXML / mzXML files – Peptide Atlas
  - X! Tandem
  - Waters MSe

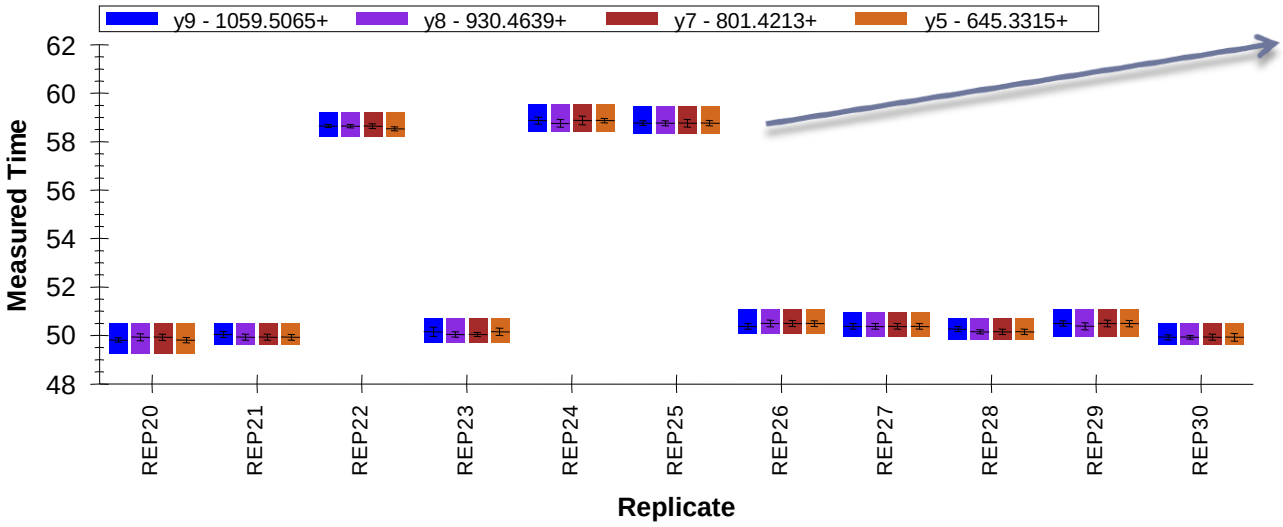
Spectral Library Explorer

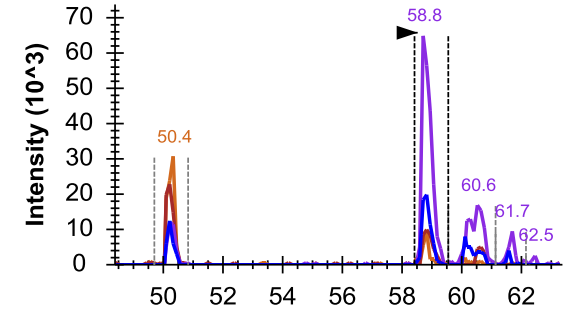


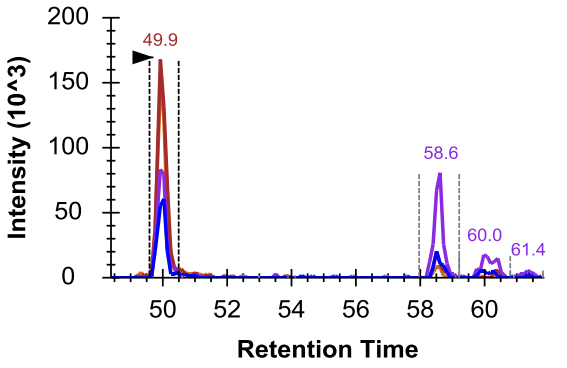
Finding Issue Quickly (wrong peak)

IVGYLDEEGVLDQNR







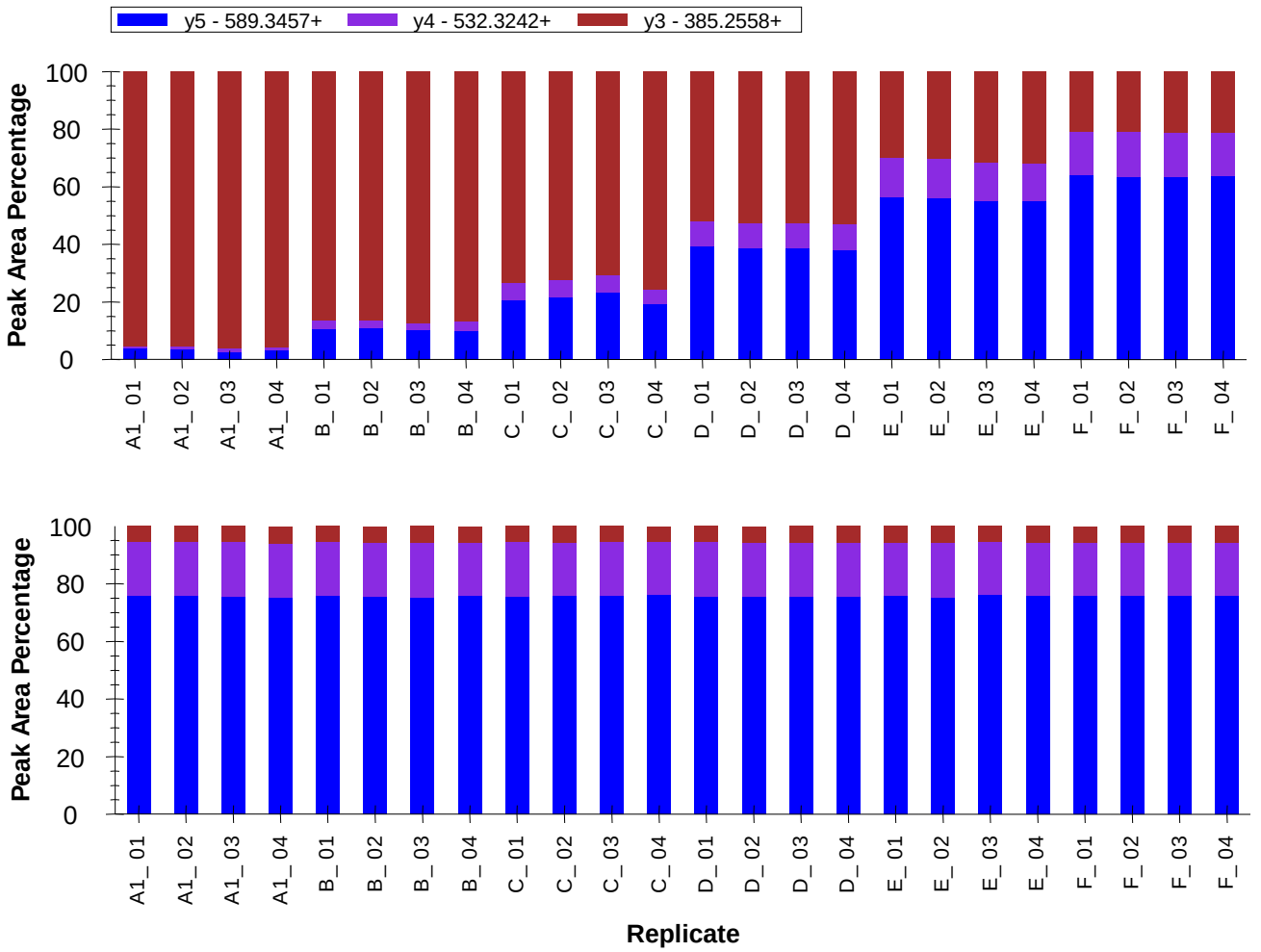


Finding Issues at a Glance (interference)

HGFLPR

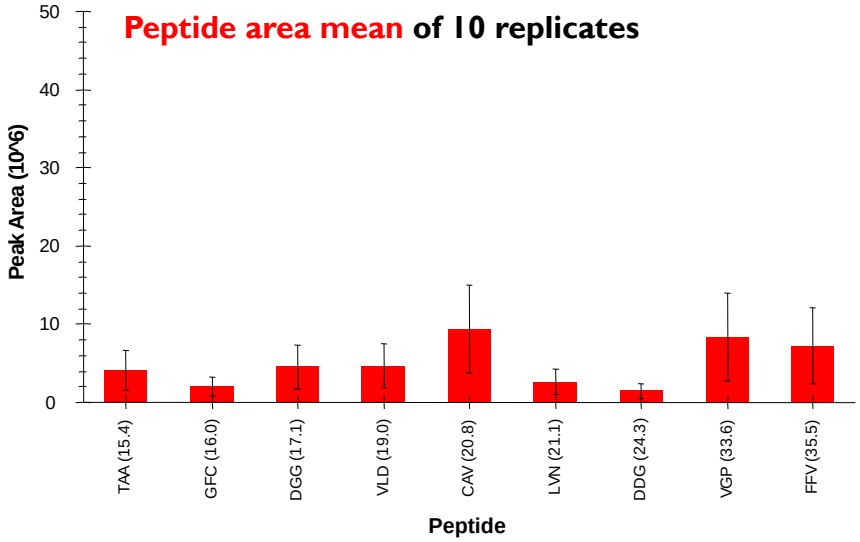
Unlabeled

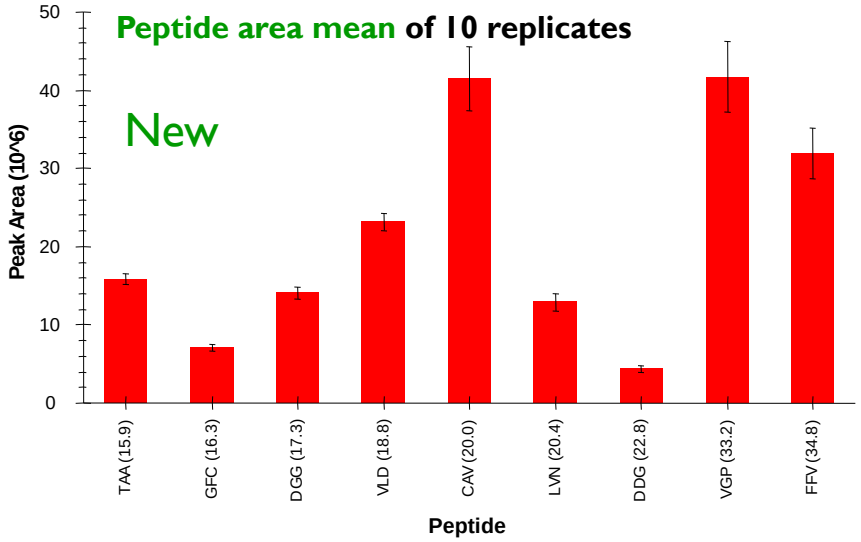
Labeled
13C R

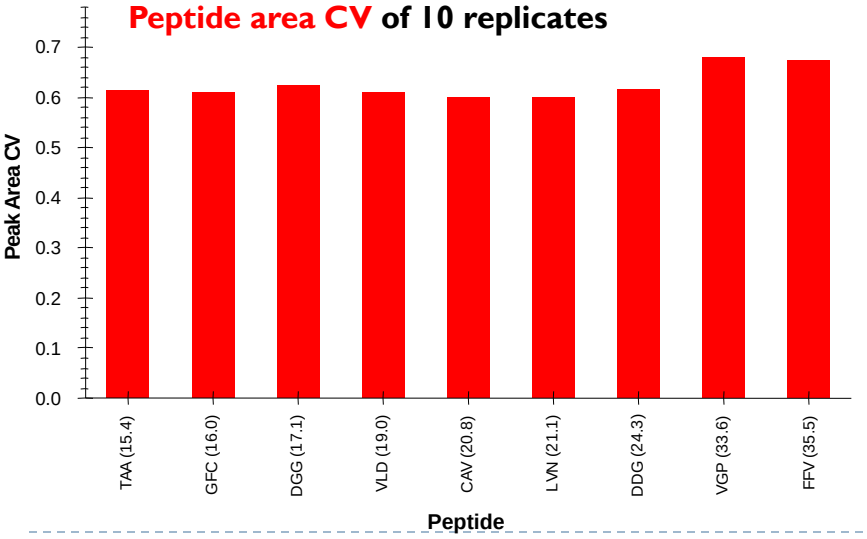


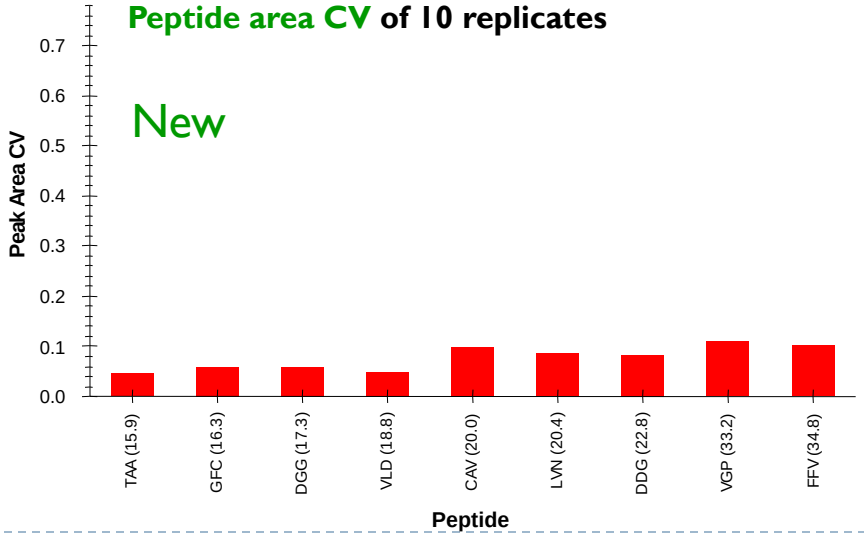
Abbatiello, S.E. et al. Clin. Chem. 2010.

Finding Issues Early (lack of precision)



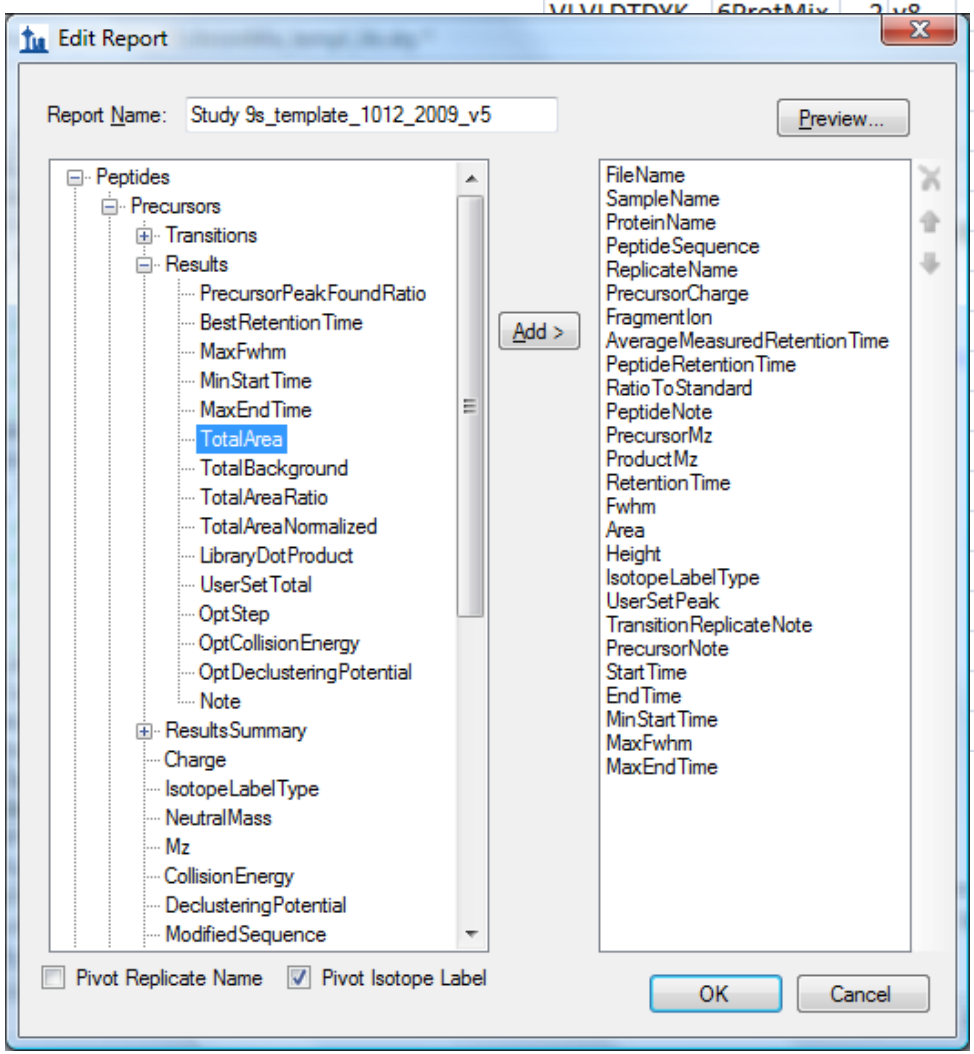






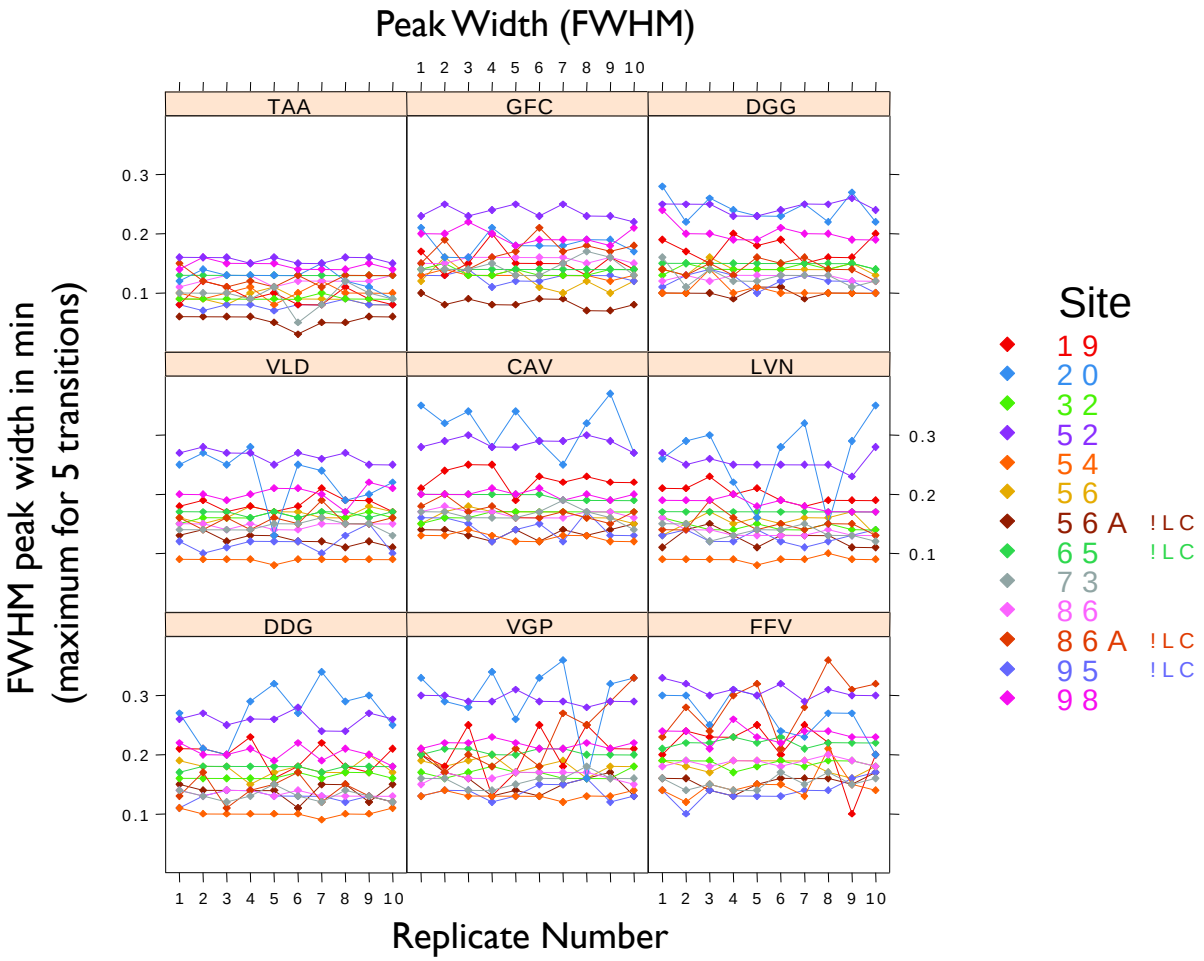
Sharing Reports

PeptideSequ	ReplicateI	Pre	Fragr	AverageM	PeptideRe	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
				24.62	24.73	533.295	966.5142	24.76	0.15	346800
				24.62	24.7	533.295	966.5142	24.7	0.13	265800
				24.62	24.65	533.295	966.5142	24.65	0.14	301200
				24.62	24.36	533.295	966.5142	24.36	0.13	290800
				24.62	24.1	533.295	966.5142	24.1	0.09	170600
				24.62	24.33	533.295	966.5142	24.33	0.1	147700
				24.62	24.47	533.295	966.5142	24.5	0.07	900
				24.62	24.5	533.295	966.5142	24.5	0.03	100
				24.62	24.5	533.295	966.5142	24.45	0.03	100
				24.62	24.96	533.295	853.4302	24.96	0.14	2528350
				24.62	25.05	533.295	853.4302	25.05	0.15	2816900
				24.62	24.91	533.295	853.4302	24.91	0.16	2215900
				24.62	24.79	533.295	853.4302	24.79	0.15	3202250
				24.62	24.73	533.295	853.4302	24.73	0.15	3098300
				24.62	24.7	533.295	853.4302	24.7	0.14	2618300
				24.62	24.65	533.295	853.4302	24.65	0.13	2990900
				24.62	24.36	533.295	853.4302	24.36	0.14	2794850
				24.62	24.1	533.295	853.4302	24.1	0.1	1676000
				24.62	24.33	533.295	853.4302	24.33	0.13	1533550



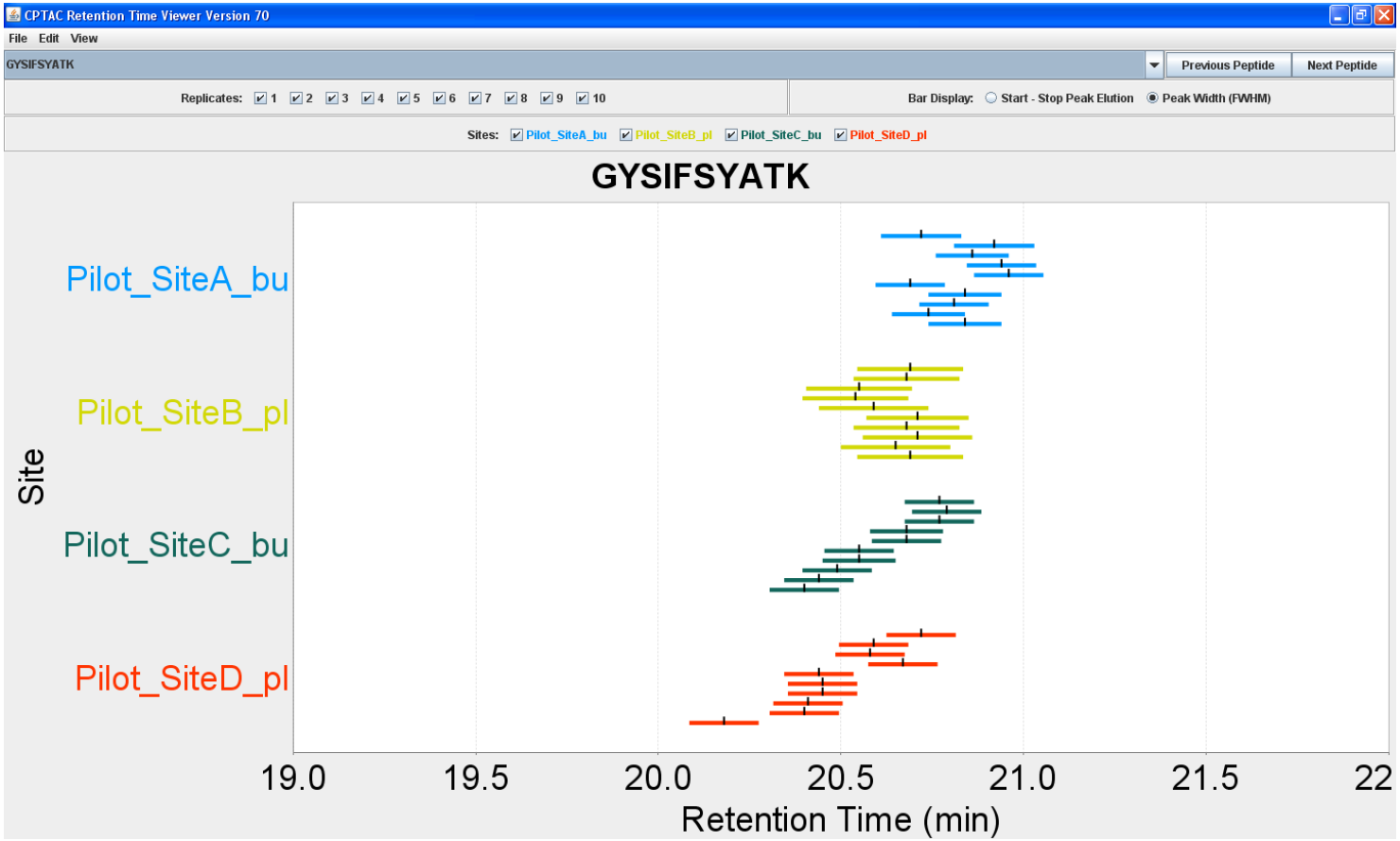
Downstream Analysis with Statistical Tools

- Analysis of Study 9S reports with R



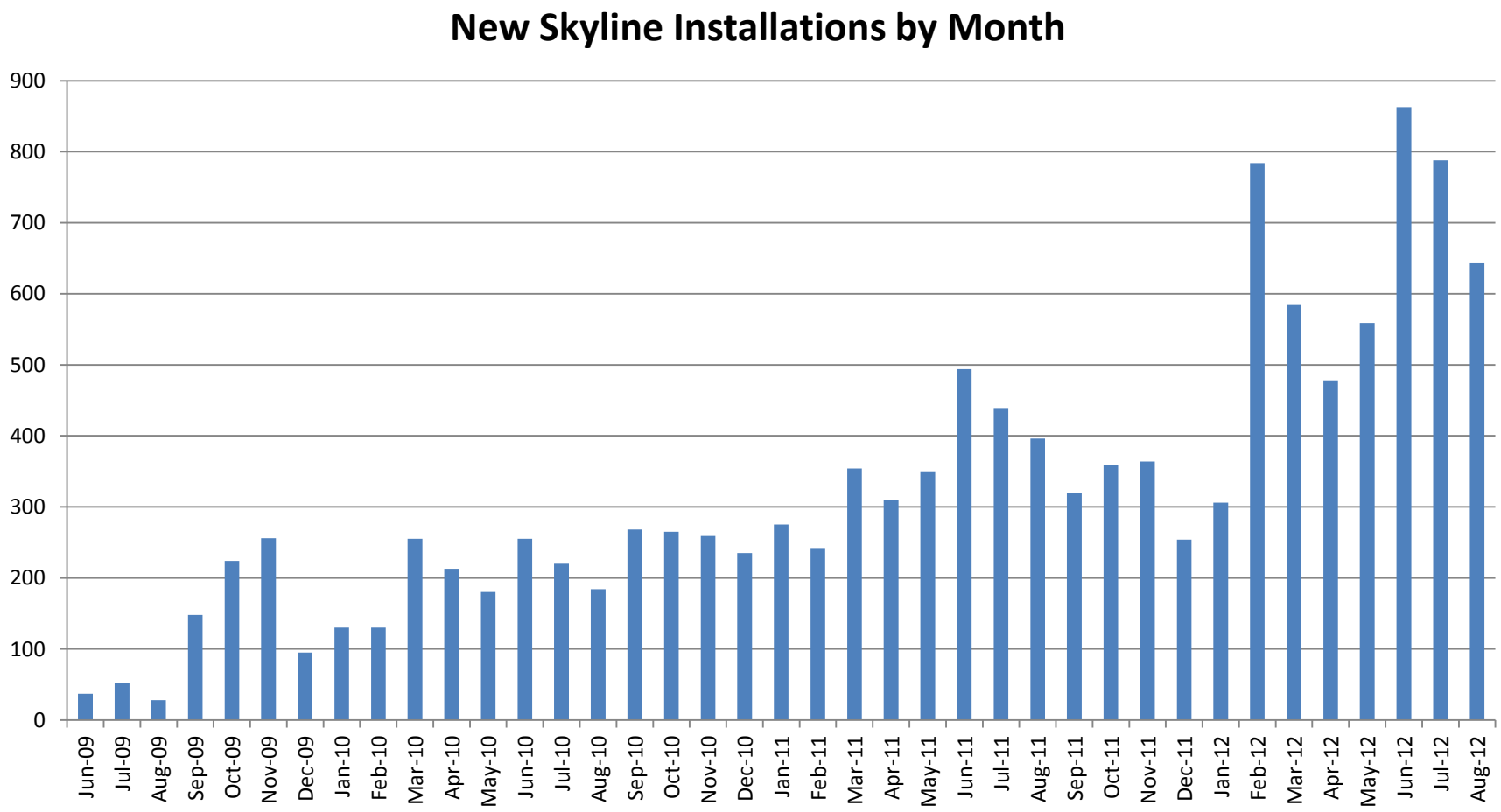
Deeper Analysis with Custom Tools

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



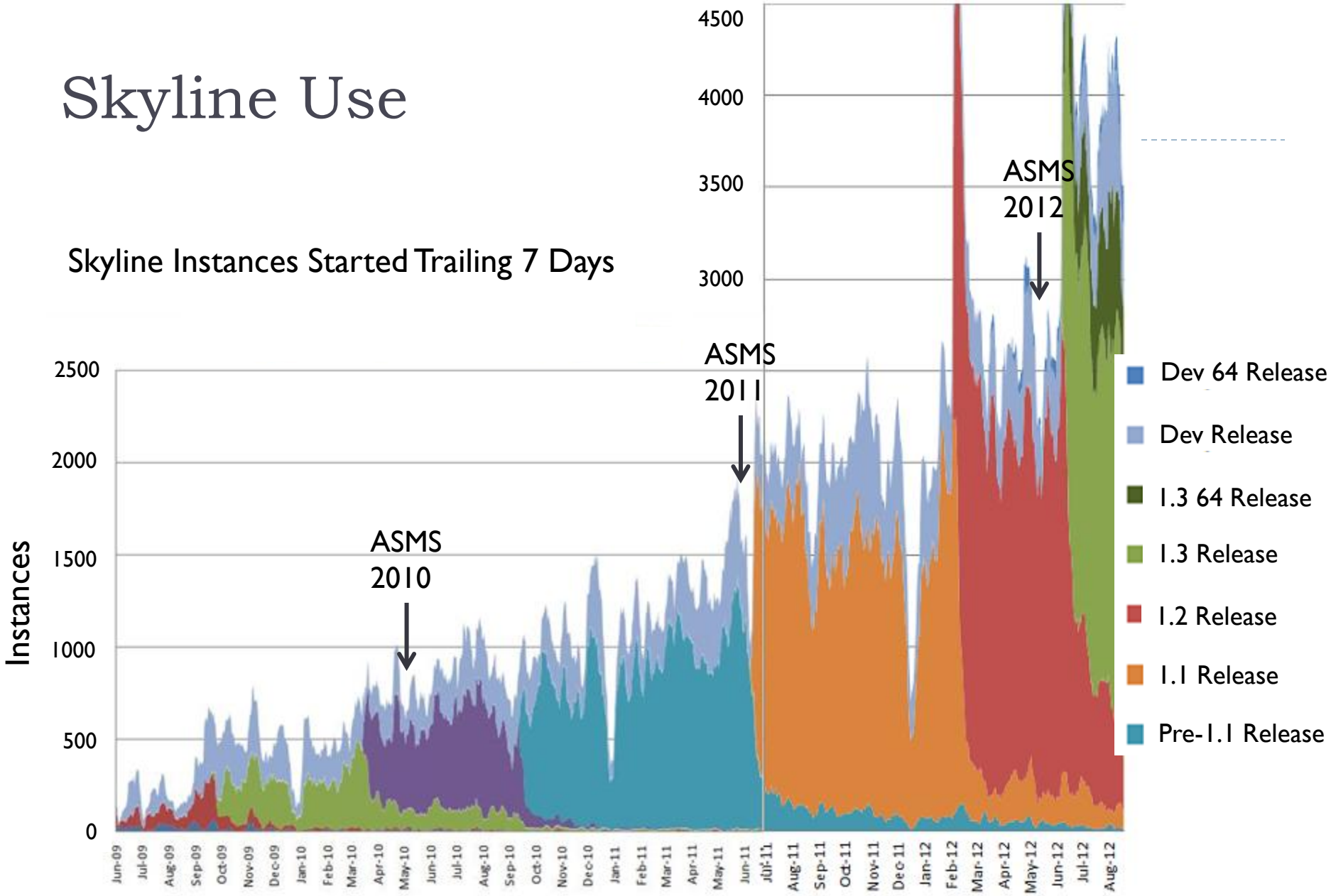
http://www.buckinstitute.org/Labs/thegibson/resources.asp

Skyline Adoption (>12,500 Installations)



- 660 registered users

Skyline Use



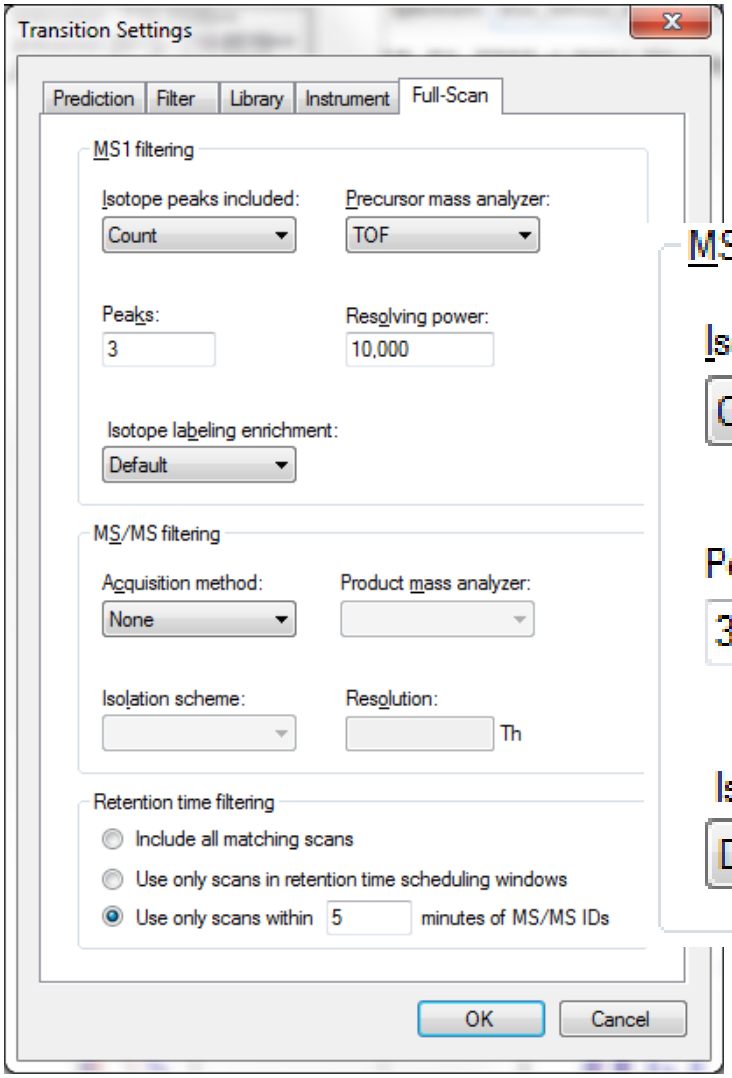
>100 citations

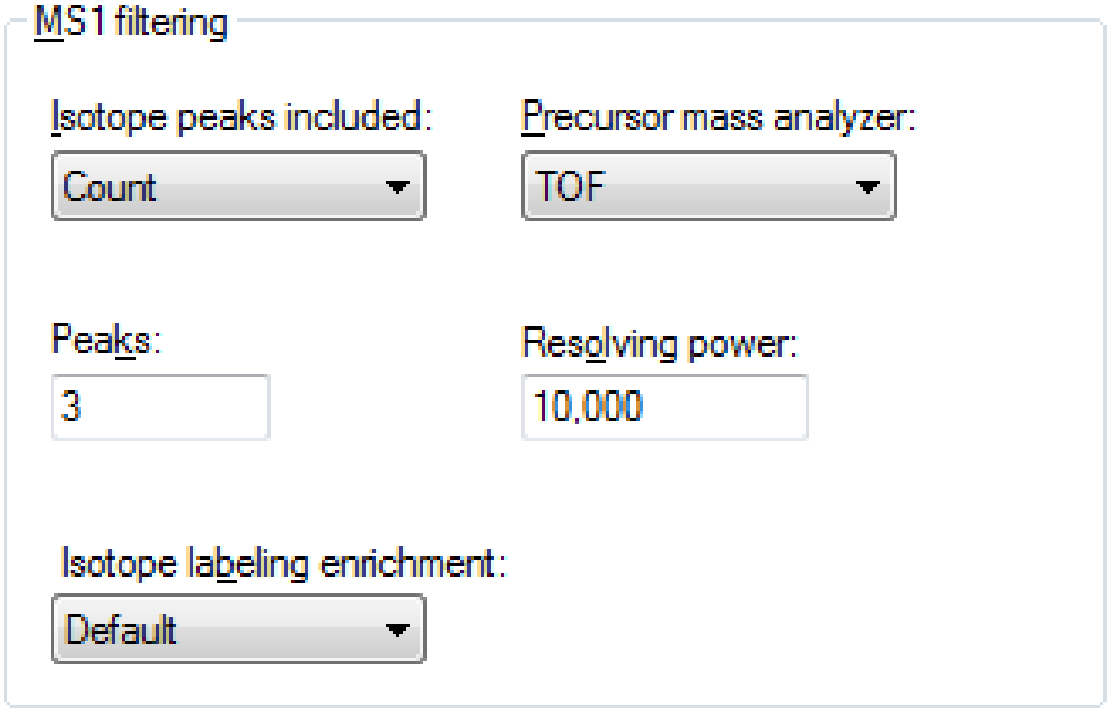
2012 Support Multiple Instrument Vendors

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

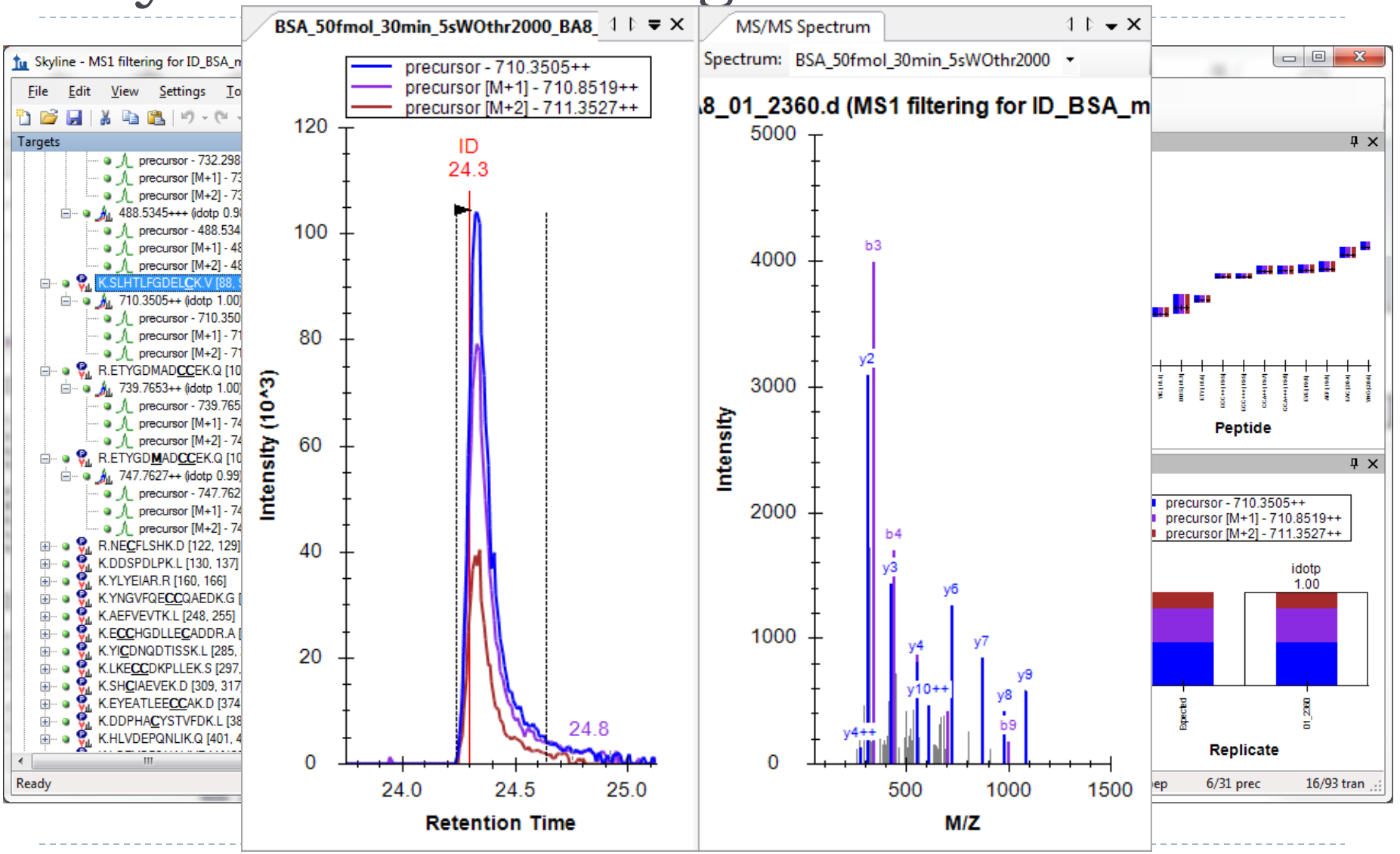
AB SCIEX	SWATH™
Agilent Technologies	DIA
Bruker	DIA & All Ions DIA
Thermo-Scientific	DIA & Multiplexed DIA
Waters	MSe™

Skyline MS1 Full-Scan Settings



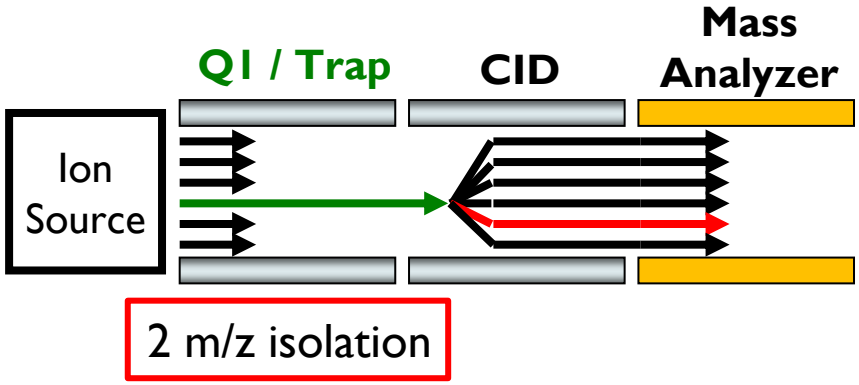


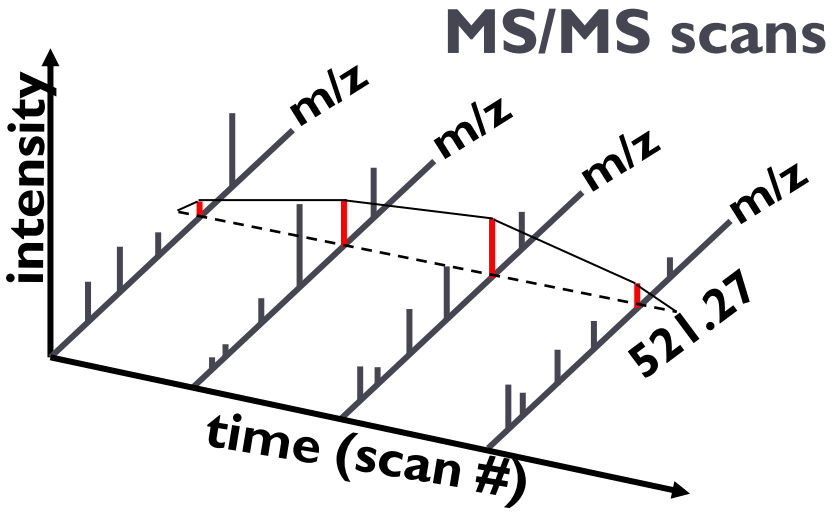
Skyline MS1 Filtering Data



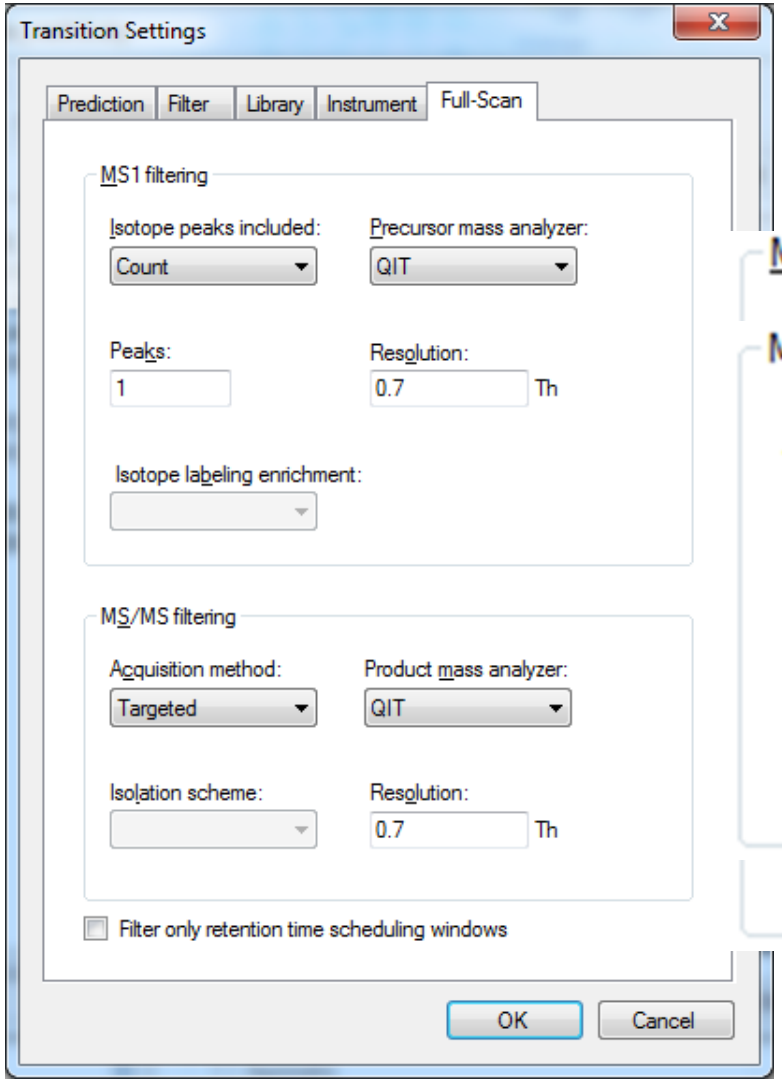
Schilling, B. et al. Mol. Cell. Prot. 2012.

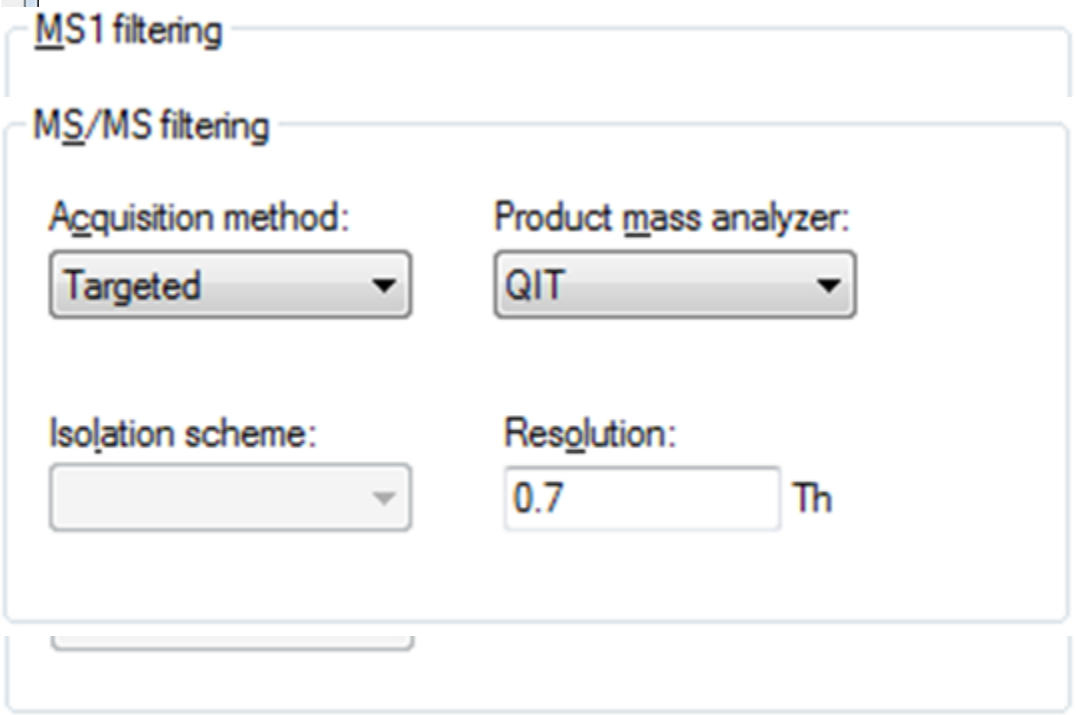
Targeted MS/MS





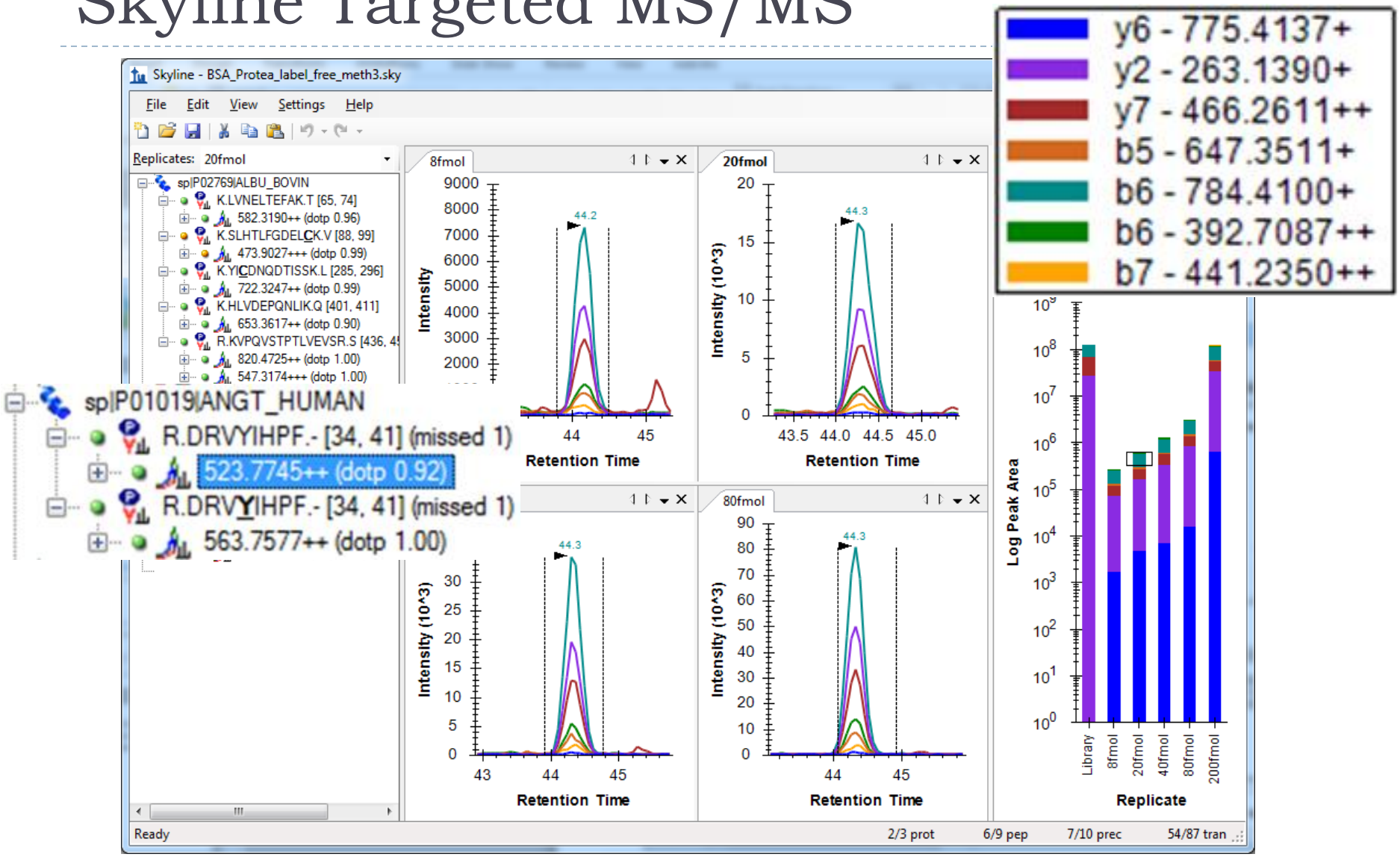
Skyline Targeted MS/MS Settings





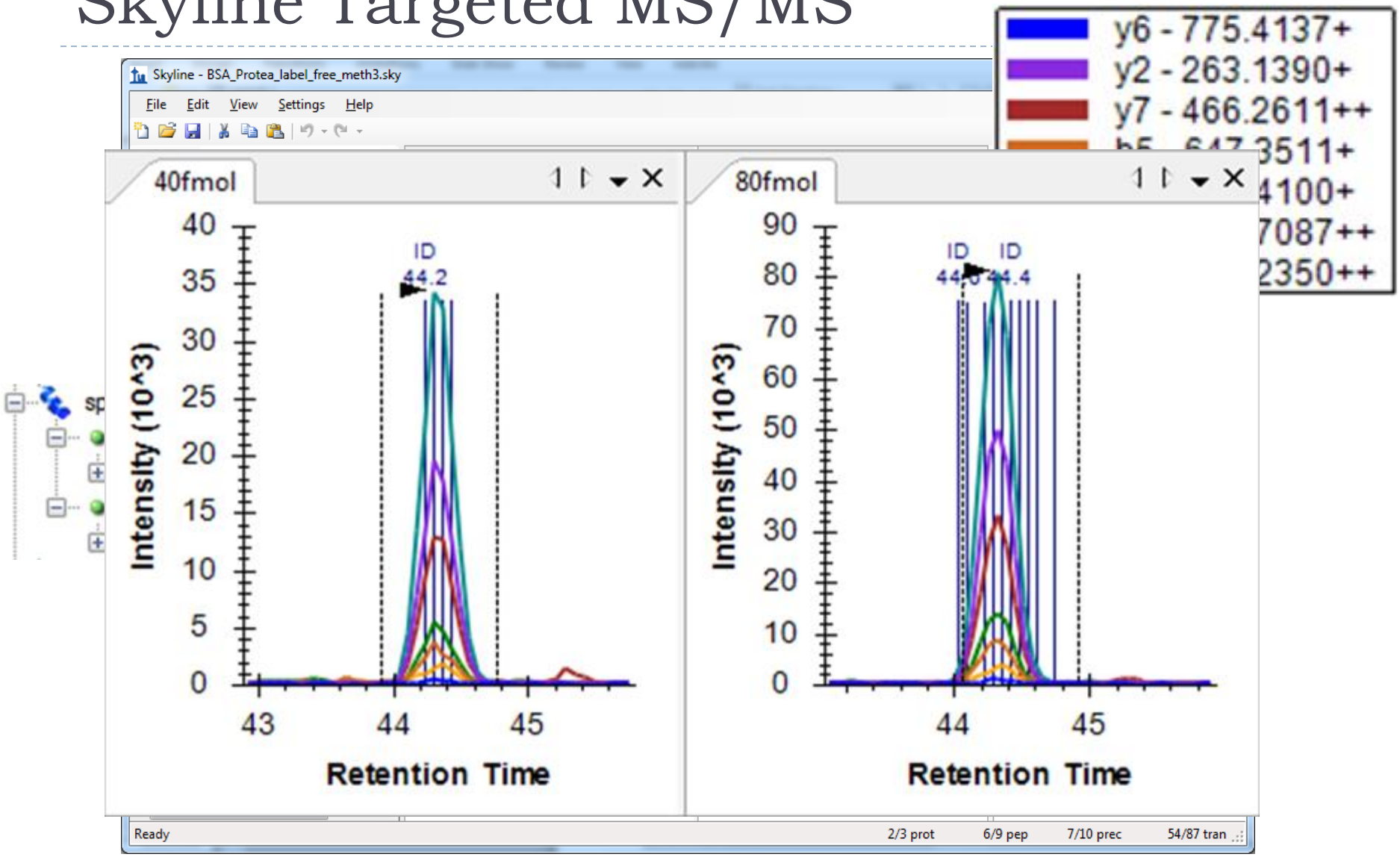
Tutorial

Skyline Targeted MS/MS



Sherrod, S. et al. J. Prot. Res. 2012.

Skyline Targeted MS/MS

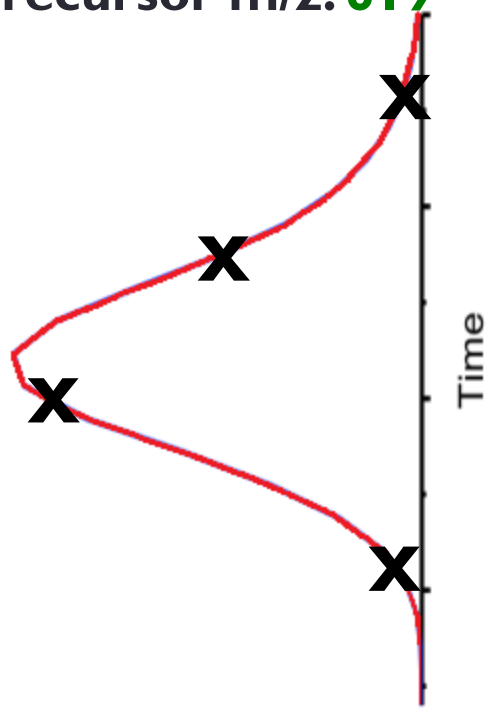


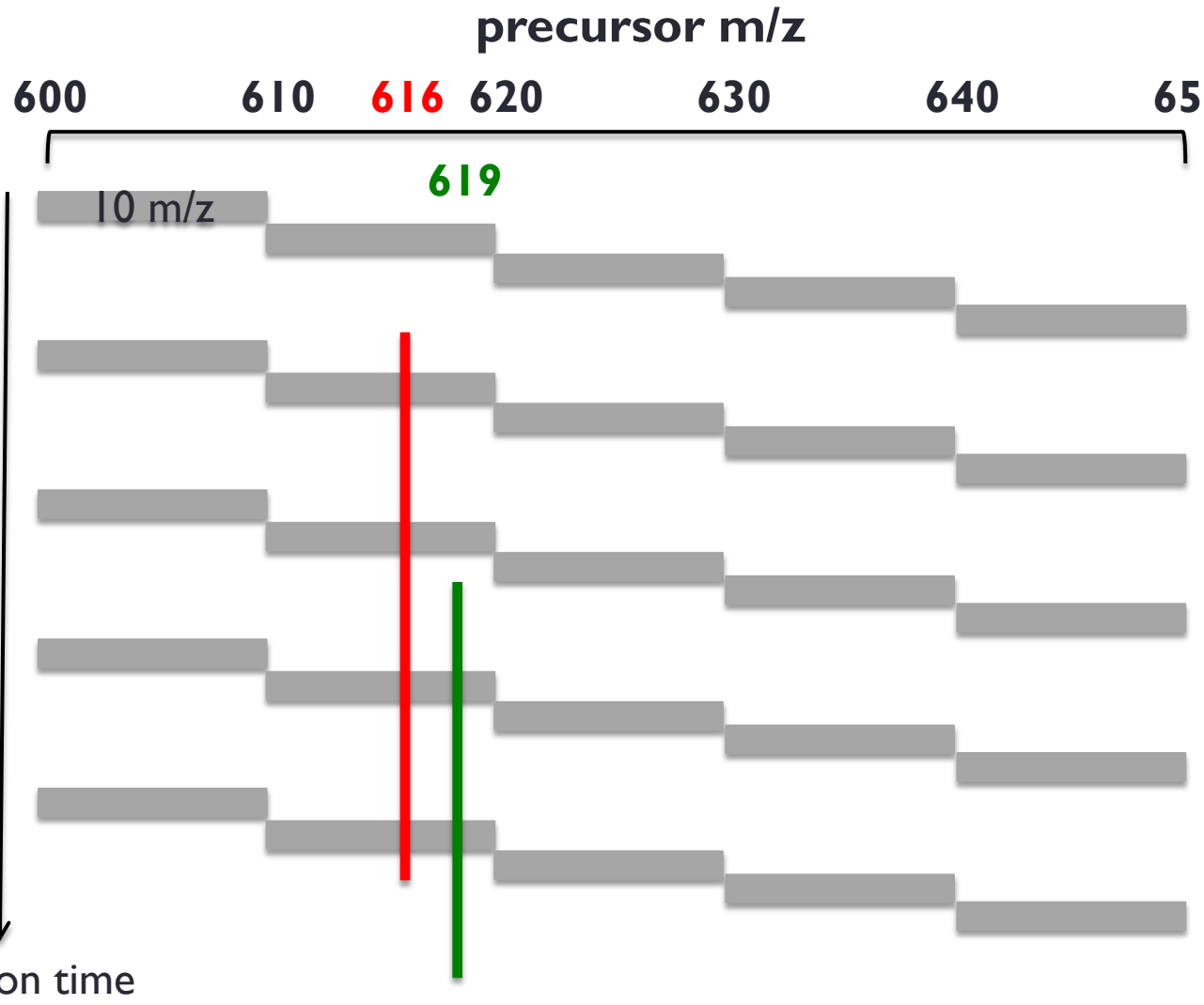
Sherrod, S. et al. J. Prot. Res. 2012.

Traditional DIA Isolation Scheme

SVEDFMAAMQR
Precursor m/z: 616

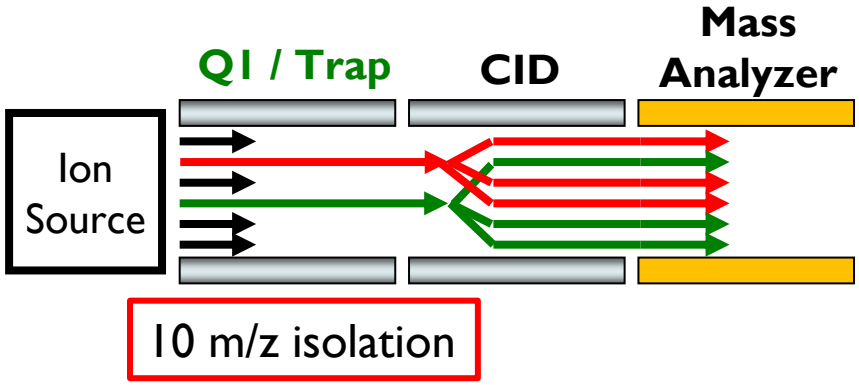
VGGNGADYALATK
Precursor m/z: 619

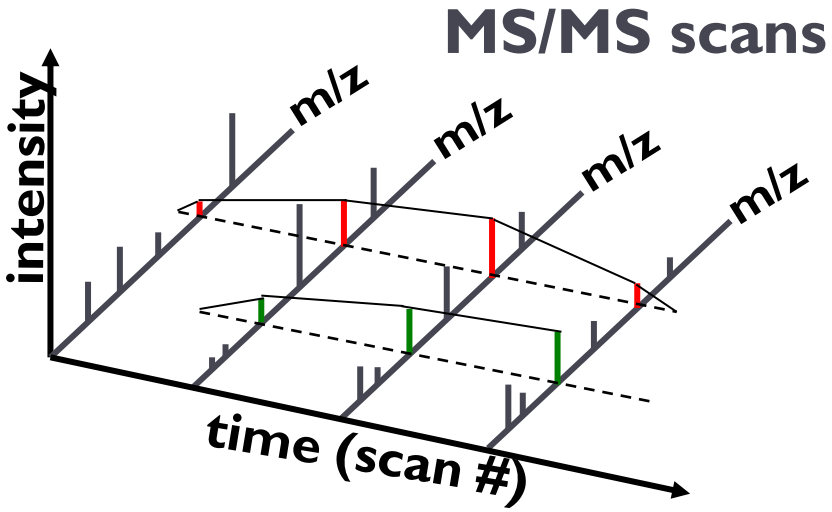




Venable, J.D. et al. Nat. Meth. 2004.

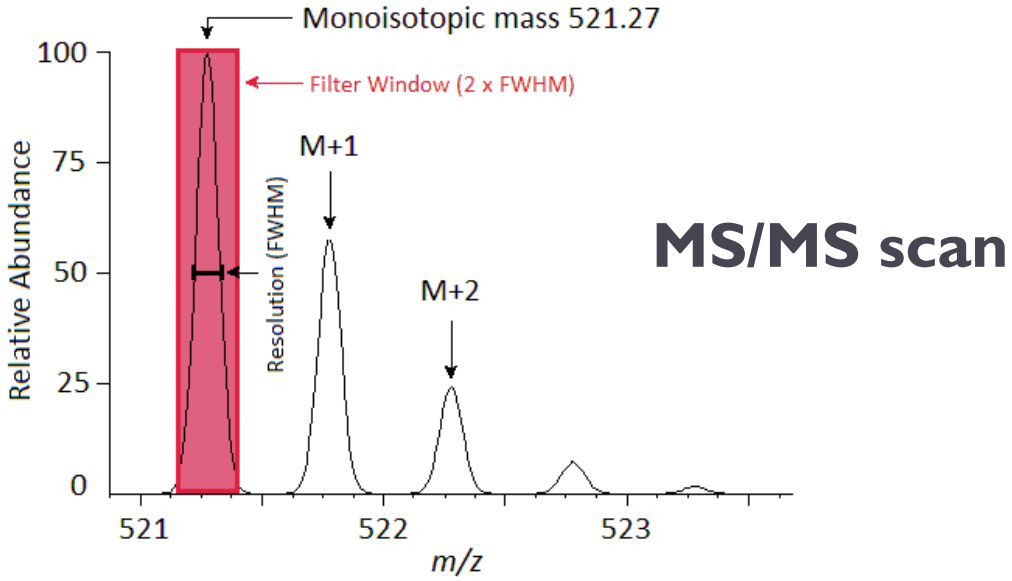
DIA Chromatogram Extraction





DIA Chromatogram Extraction Trade-Offs

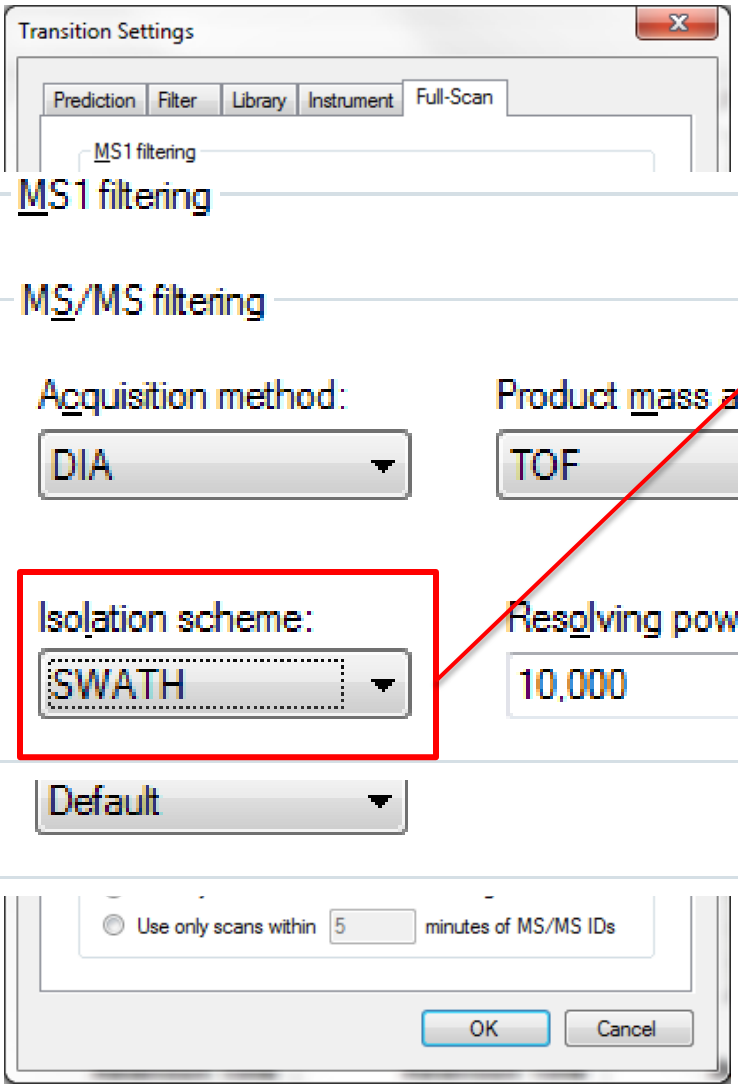
- Sensitivity
  - Dwell / Accumulation time
  - Cycle time
- Selectivity lost by wide precursor isolation
- Selectivity gained vs. SRM by narrow product extraction

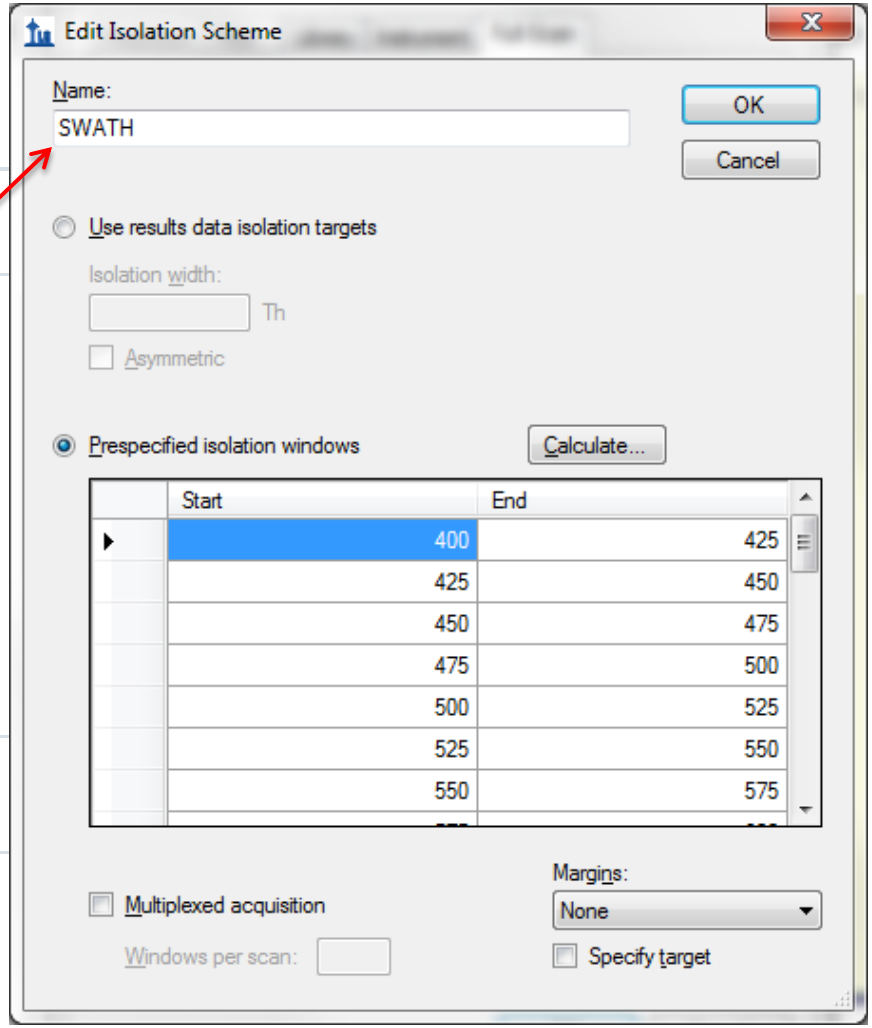


MS/MS scan

Gillet, L.C. et al. Mol. Cell. Prot. 2012.

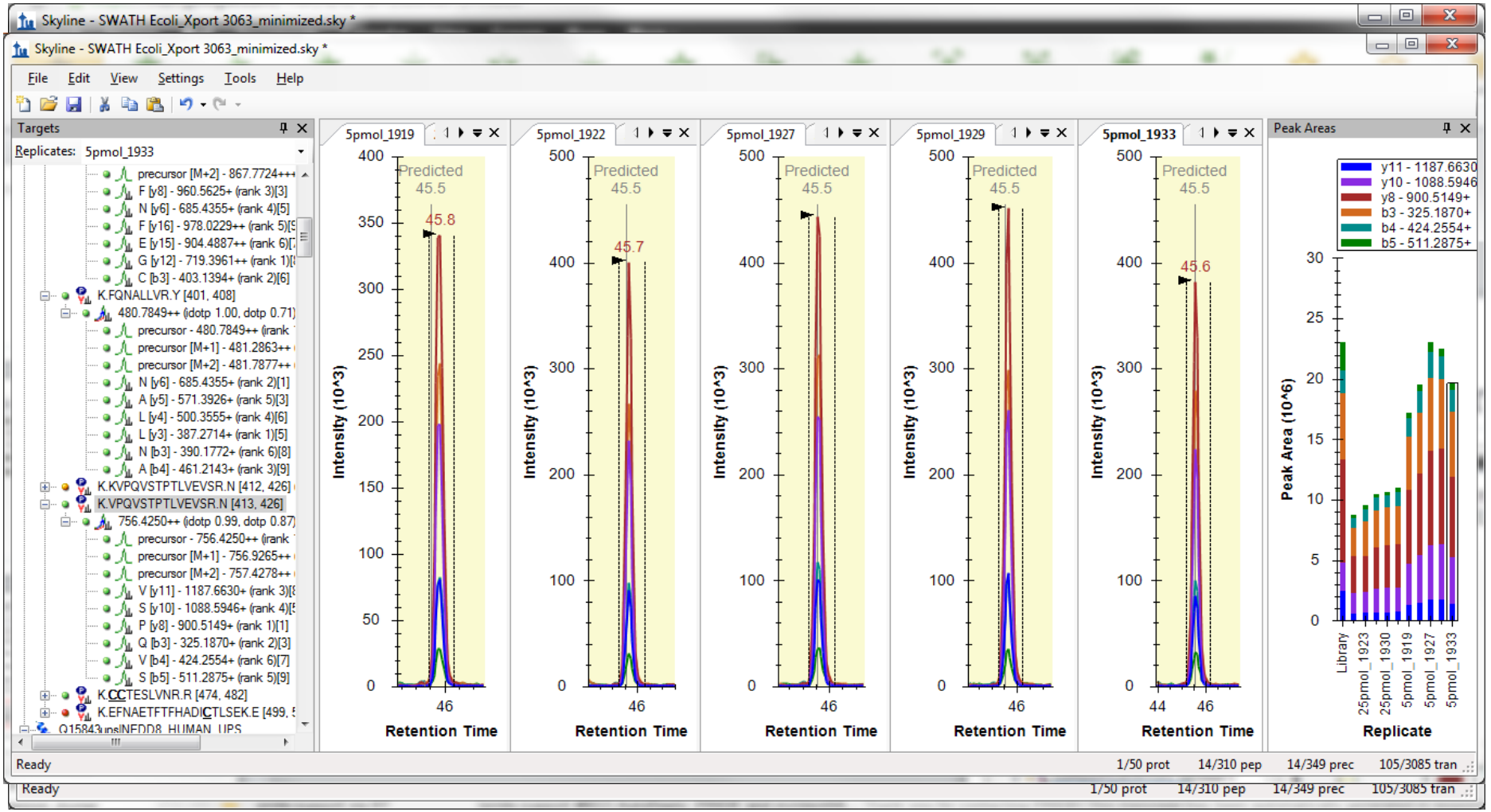
Skyline Bruker DIA Settings (25 *m/z* Extraction Windows)



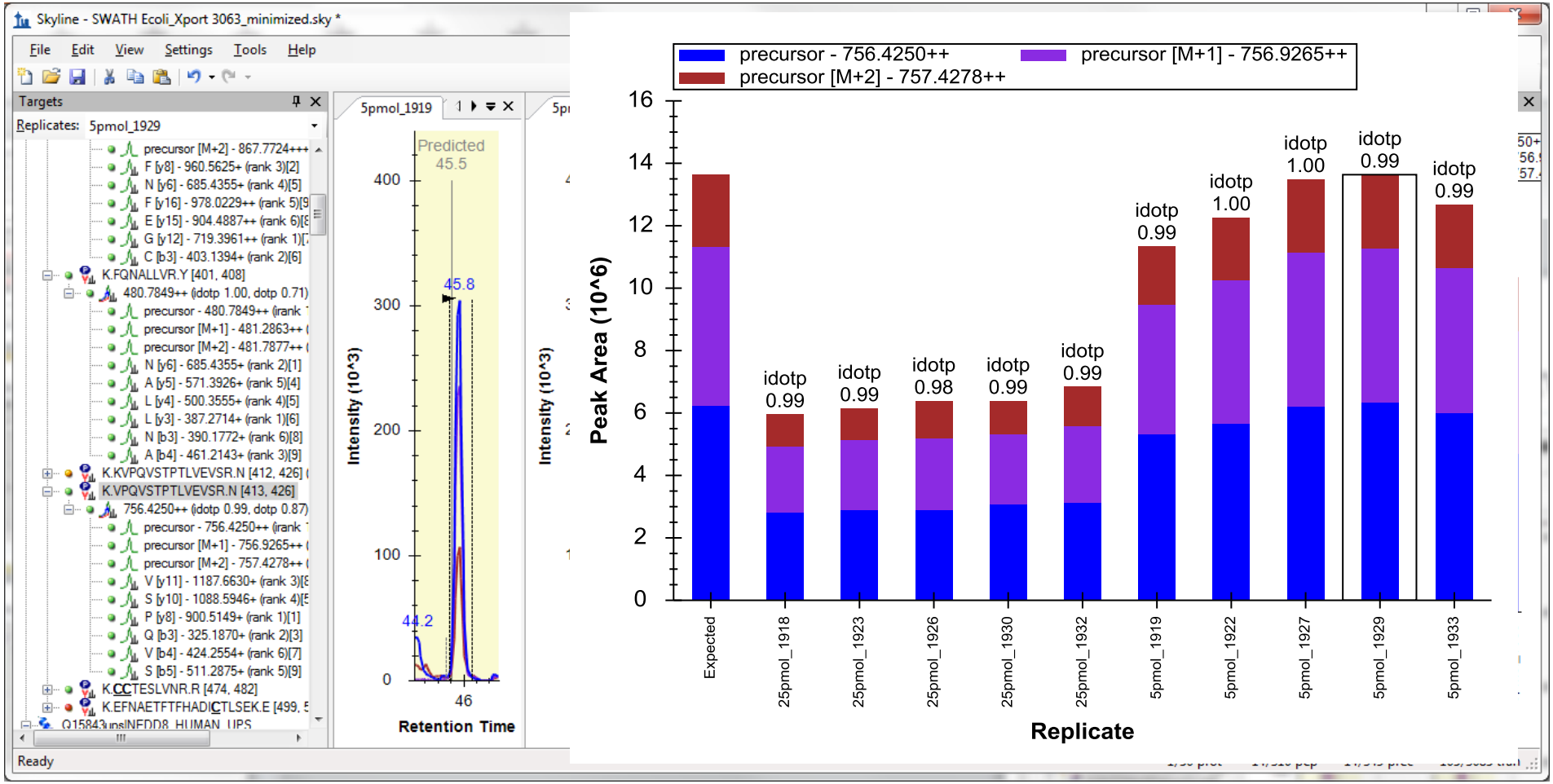


Gillet, L.C. et al. Mol. Cell. Prot. 2012.

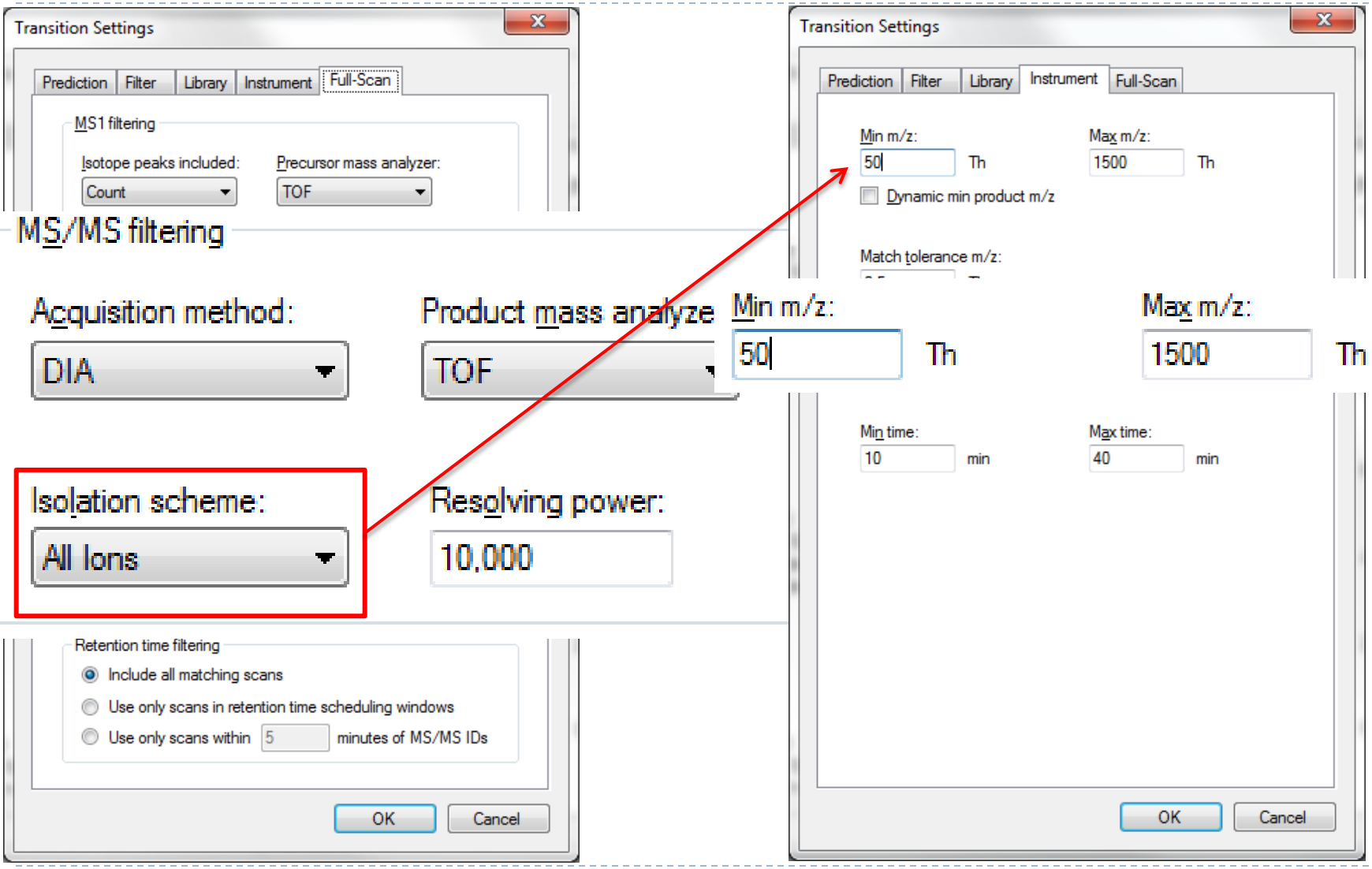
Bruker DIA Data (Product Ions)



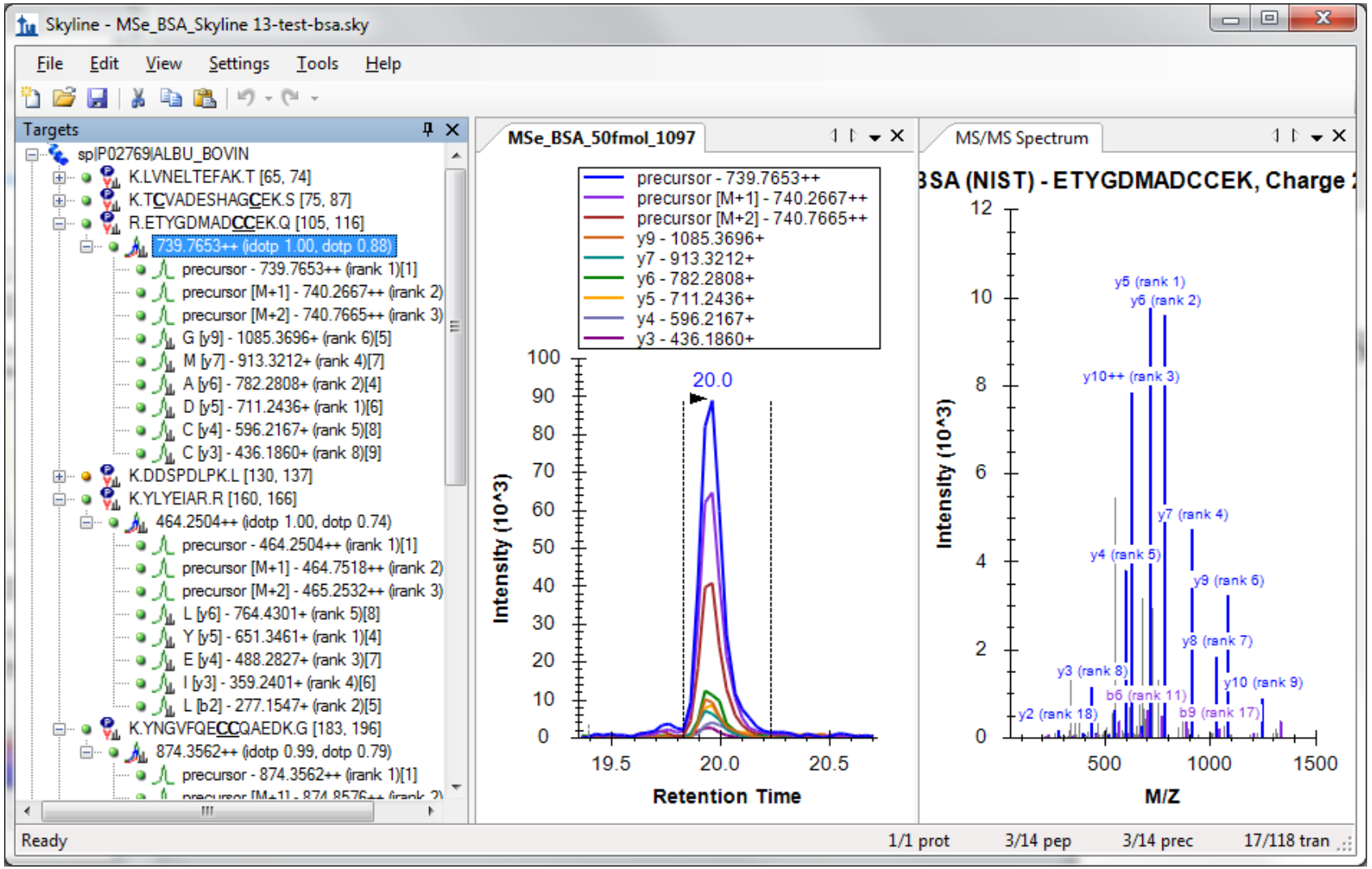
Bruker DIA Data (Precursor Ions)



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



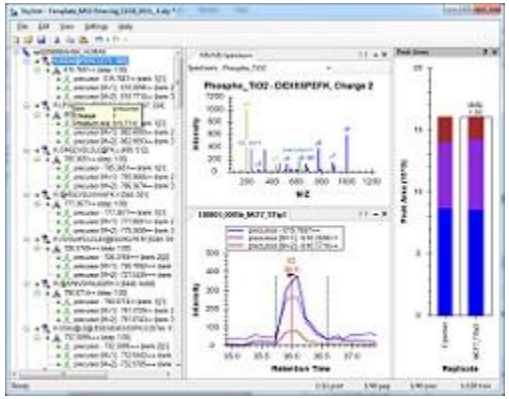
Bruker All Ions DIA Data



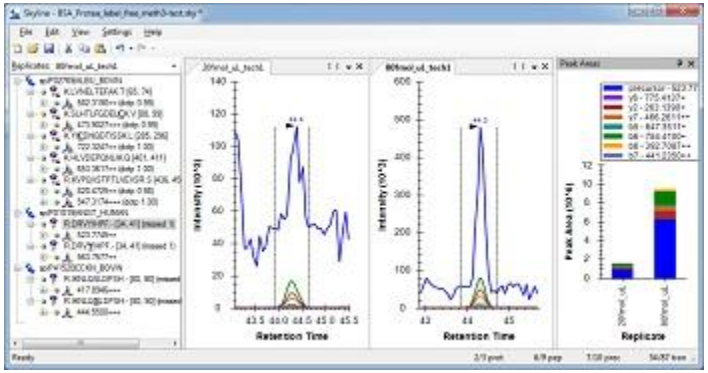
Getting Started

- 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)

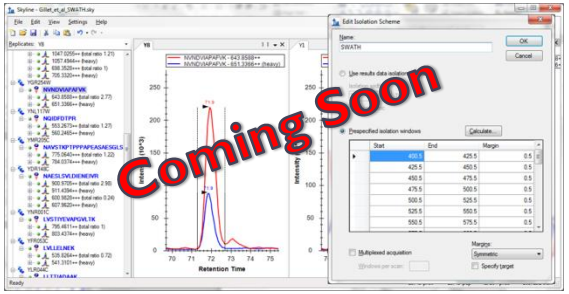
MS1 Full-Scan Filtering



Targeted MS/MS



Data Independent Acquisition



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