Agenda

▸ Welcome from the Skyline team!

▸ **Jump start DIA analysis with DDA data in Skyline**

▸ Introduction with Brendan MacLean

  ▸ Workflow overview

  ▸ Method overview and considerations

  ▸ Alternative workflows

▸ Live tutorial with Jarrett Egertson – Contributor – DIA

▸ Audience Q&A – submit questions to Google Form:
  http://tinyurl.com/QA4Skyline (also in chat window)

▸

Chromatography-based Quantification

▸ Hypothesis testing (Verification)

▸ SRM

▸ MS1 chromatogram extraction

▸ Targeted MS/MS (PRM)

▸ **Data independent acquisition (DIA/SWATH)**

Skyline

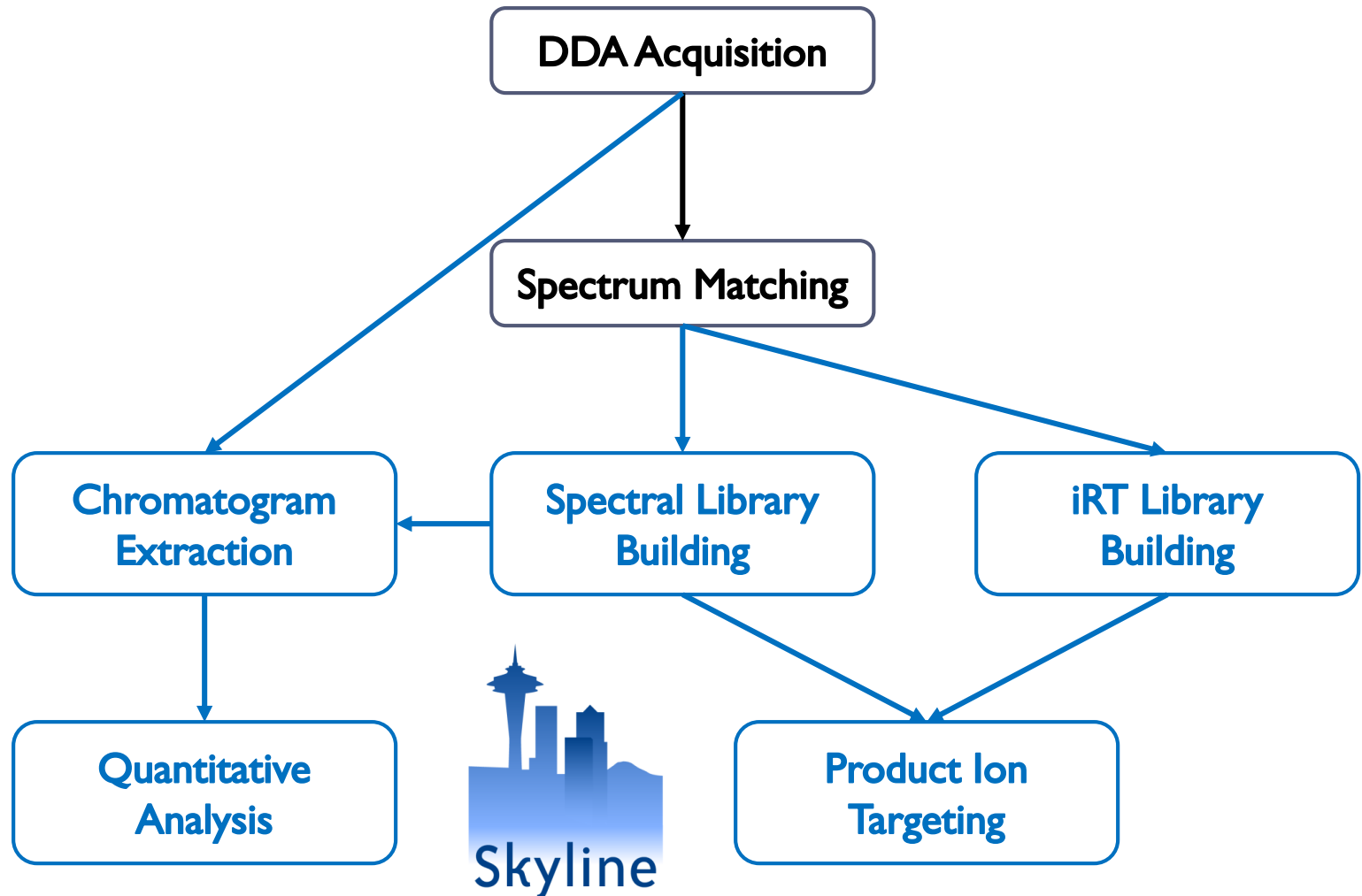| Acquisition	Targeted	Survey
More Selective	PRM	DIA
Less Selective	SRM	MS1

Got HYPOTHESIS??

Multiple Instrument Vendors

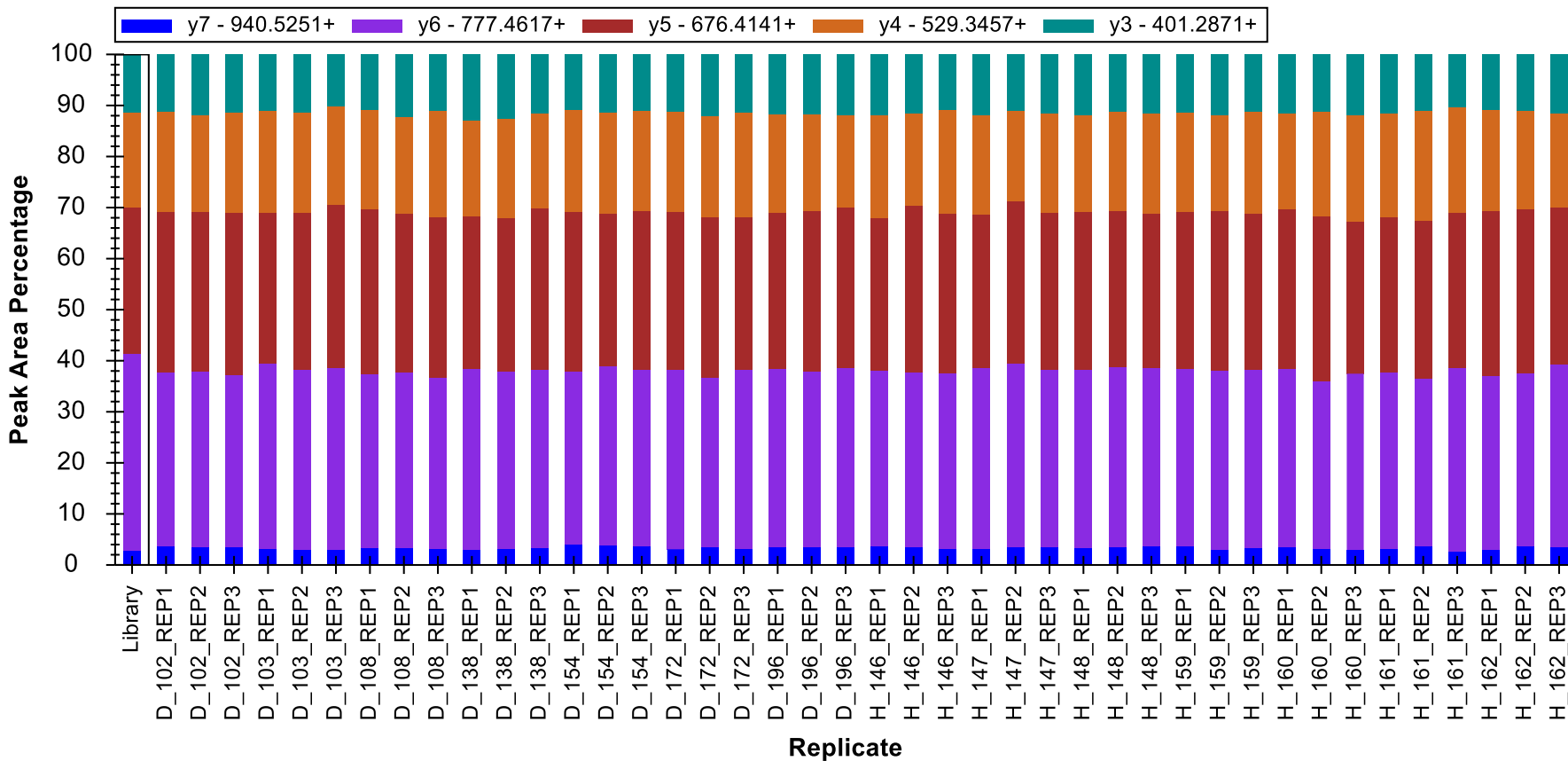# Webinar 1 Overview

Overview

DDA Acquisition
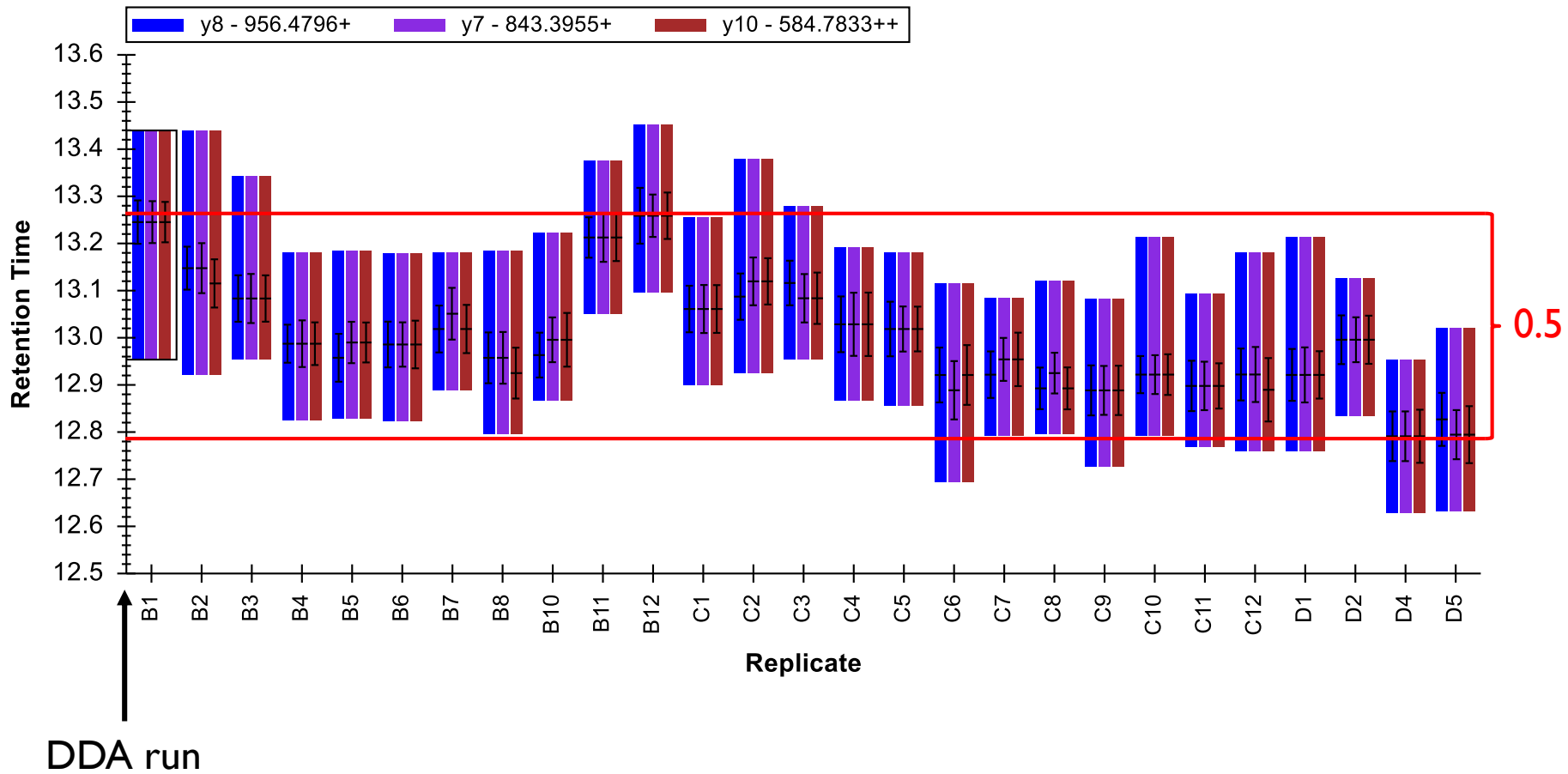
DIA Acquisition

Prior Knowledge and Consistency

▸ Relative product ion abundance

  ▸ Spectral libraries

▸ Retention time

  ▸ iRT libraries

▸ Powerful enough to be used cross-lab / cross experiment

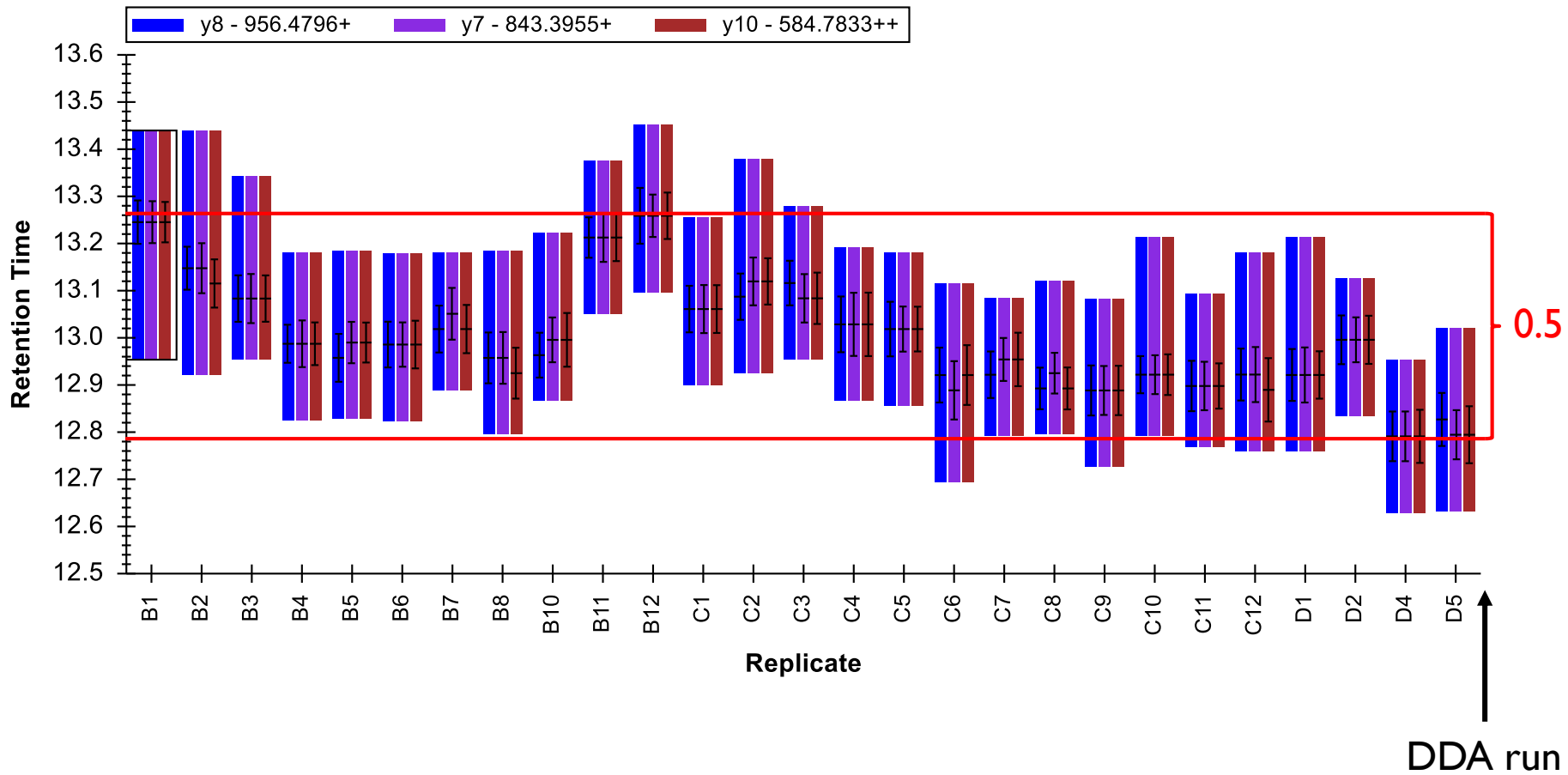 ▸ Measure, store, re-use

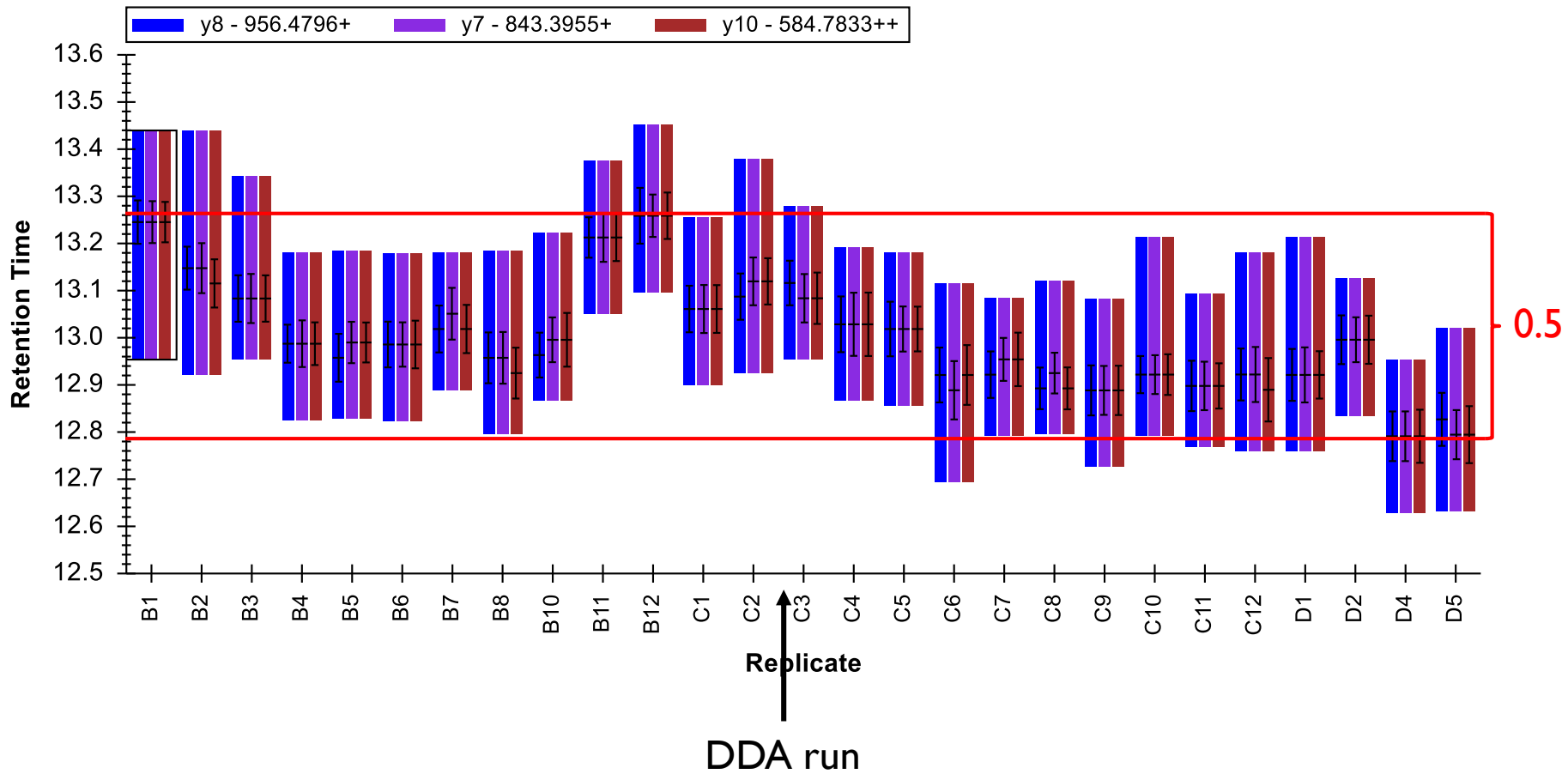▸ More powerful run-to-run
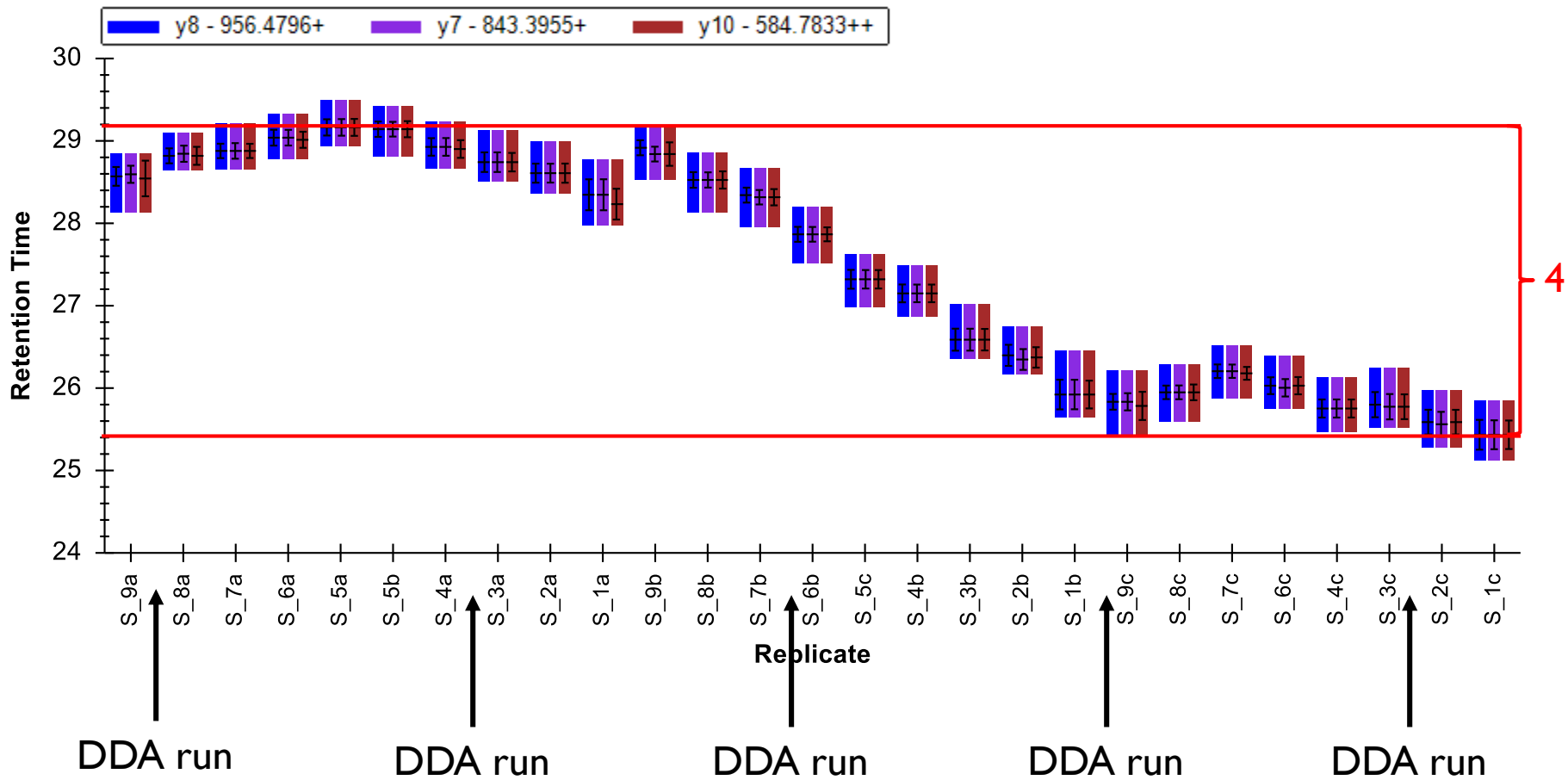
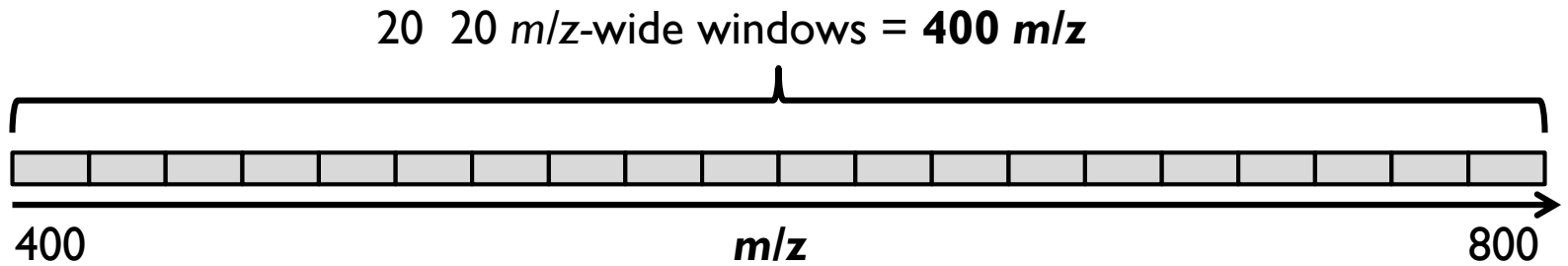# Relative Product Ion Abundance

Retention Time
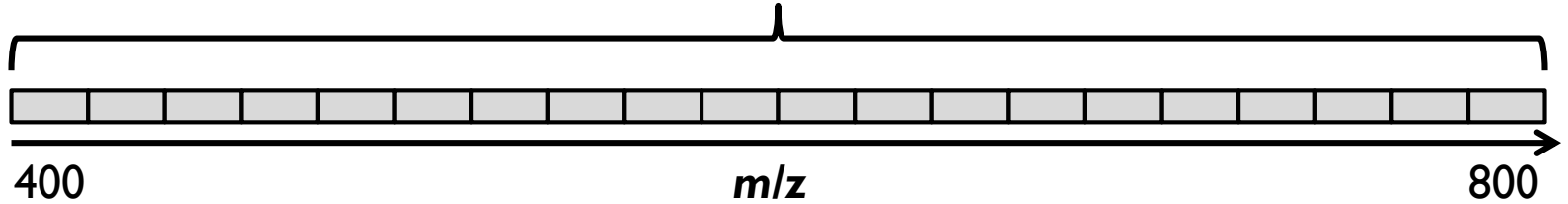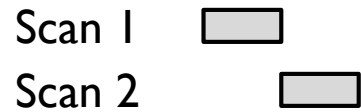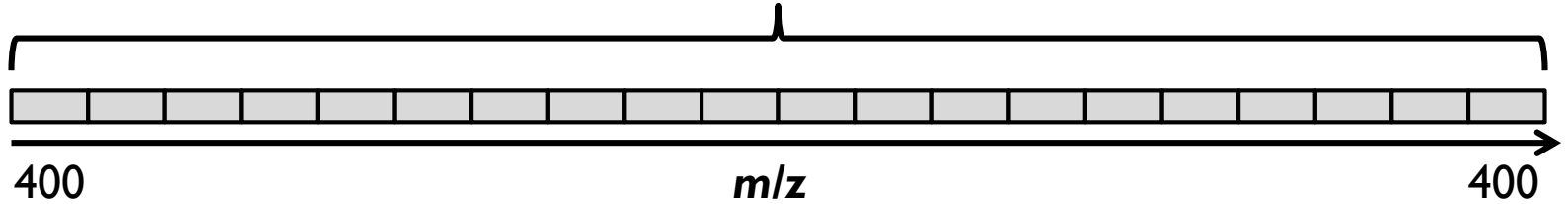
Retention Time

Retention Time

Retention Time

Data Independent Acquisition (DIA)

20 20 *m/z*-wide windows = **400 *m/z***

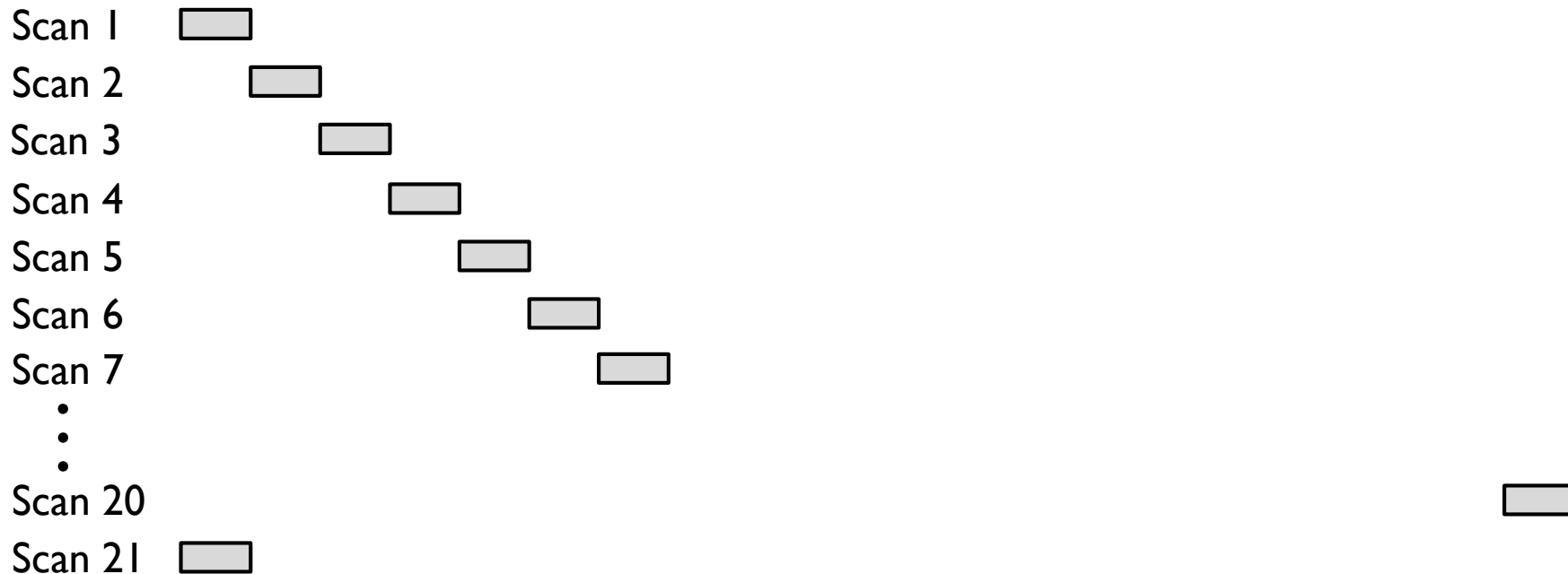400   *m/z*   800

Data Independent Acquisition (DIA)

20 20 *m/z*-wide windows = **400 *m/z***

400   ***m/z***   800

Scan 1

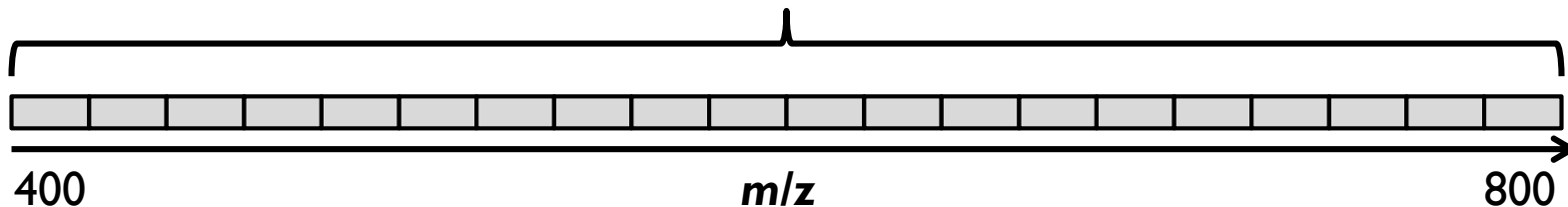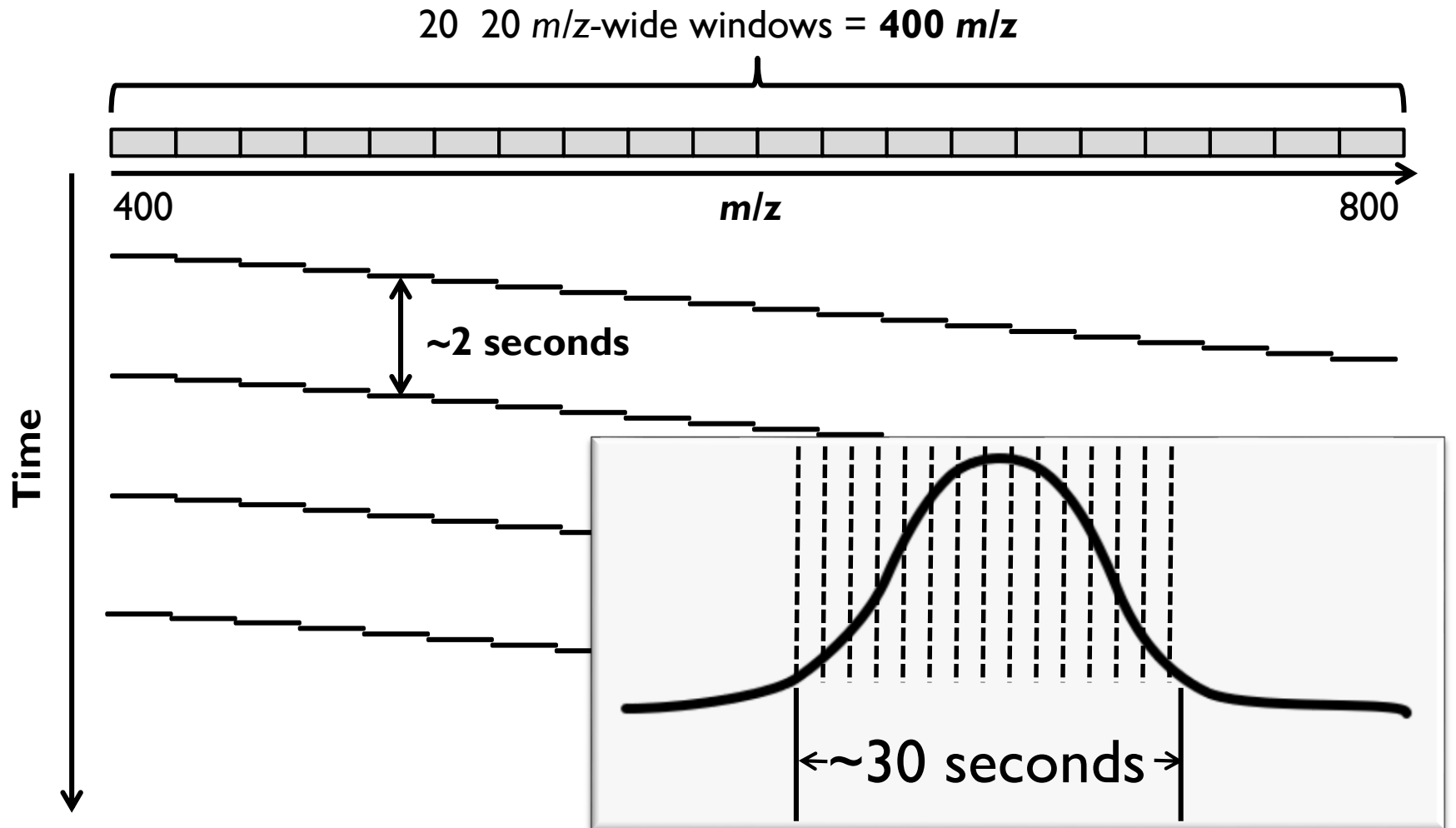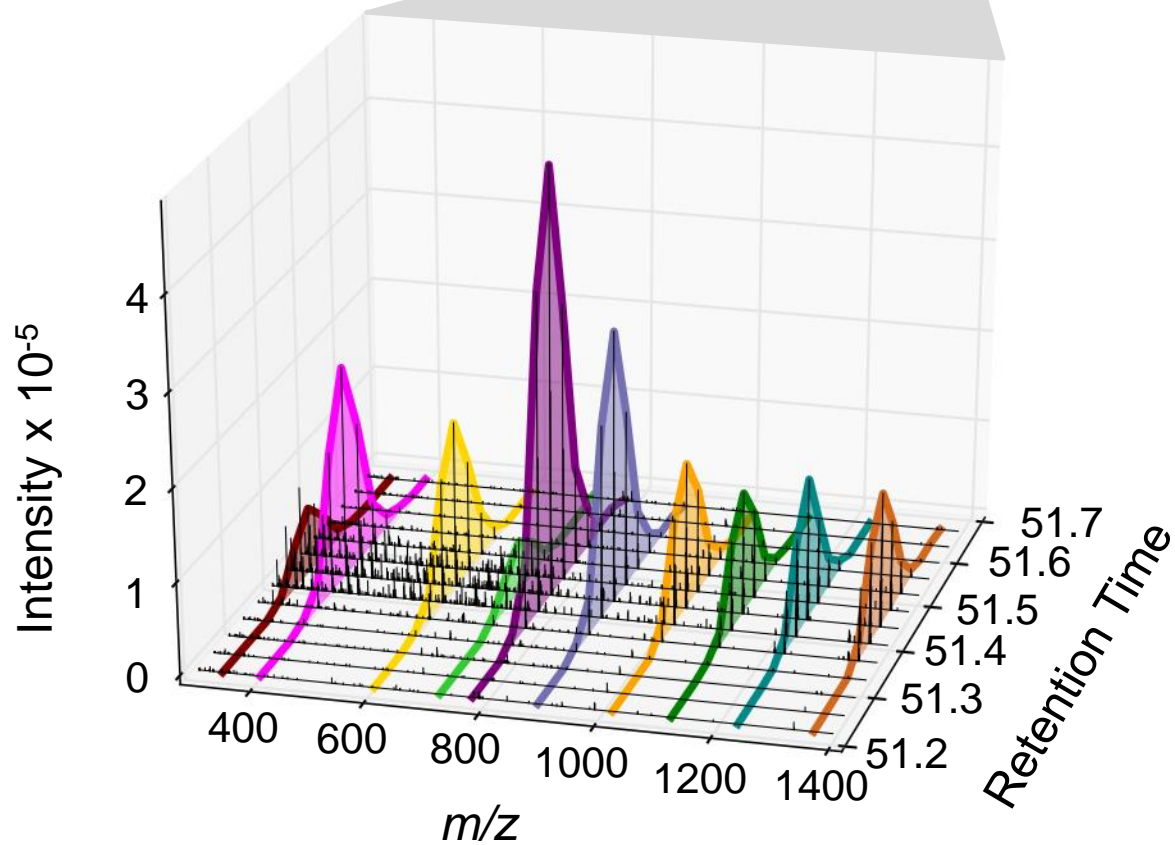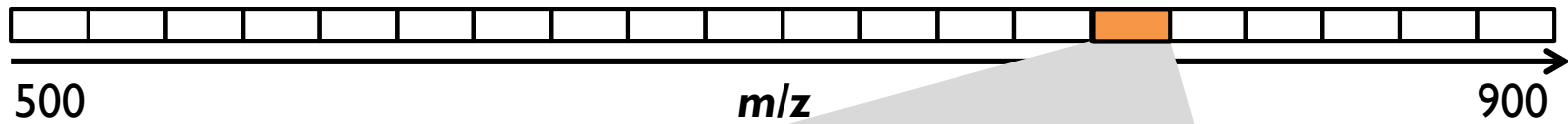# Data Independent Acquisition (DIA)

20 20 *m/z*-wide windows = **400 *m/z***

400   *m/z*   400

Scan 1

Scan 2

Data Independent Acquisition (DIA)

20 20 *m/z*-wide windows = **400 *m/z***

400   ***m/z***   800

Scan 1
Scan 2
Scan 3
Scan 4
Scan 5
Scan 6
Scan 7
•
•
•
Scan 20
Scan 21

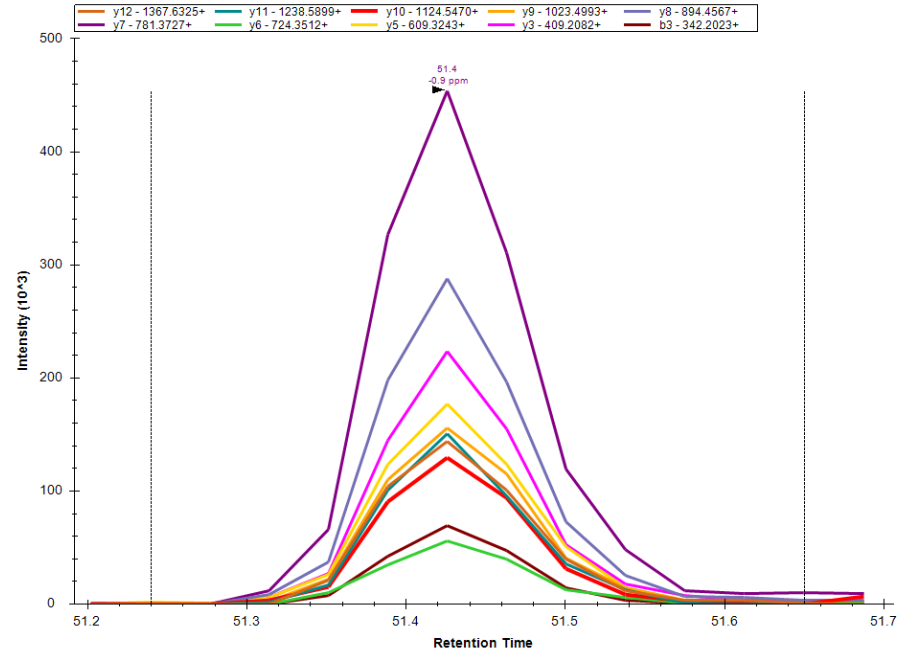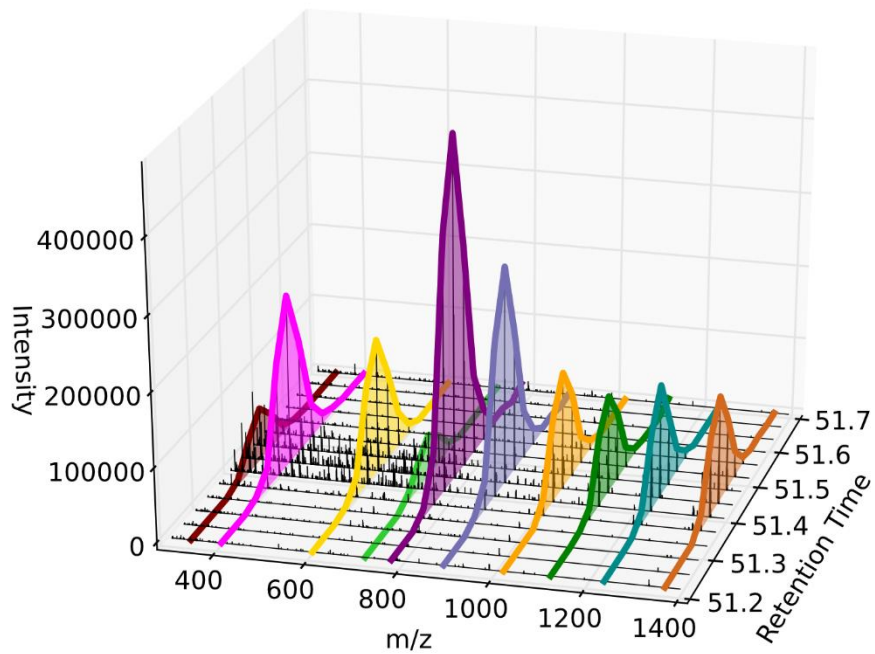# Data Independent Acquisition (DIA)

20 20 *m/z*-wide windows = **400 *m/z***

400   ***m/z***   800

Time

~2 seconds

←~30 seconds→

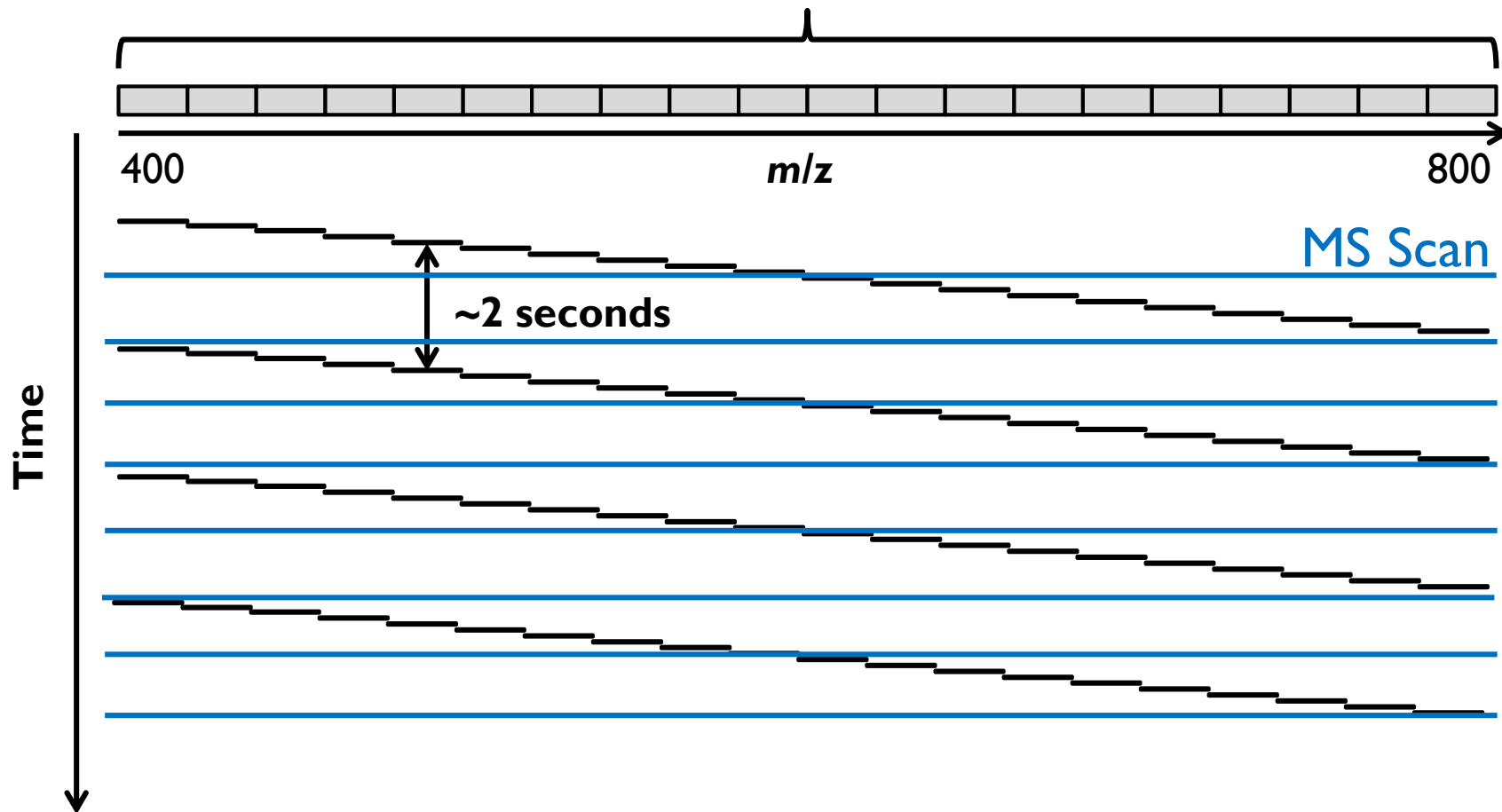Venable, J.D. et al. Nat. Meth. 2004. Gillet, L.C. et al. MCP 2012

Extraction of Fragment Ions
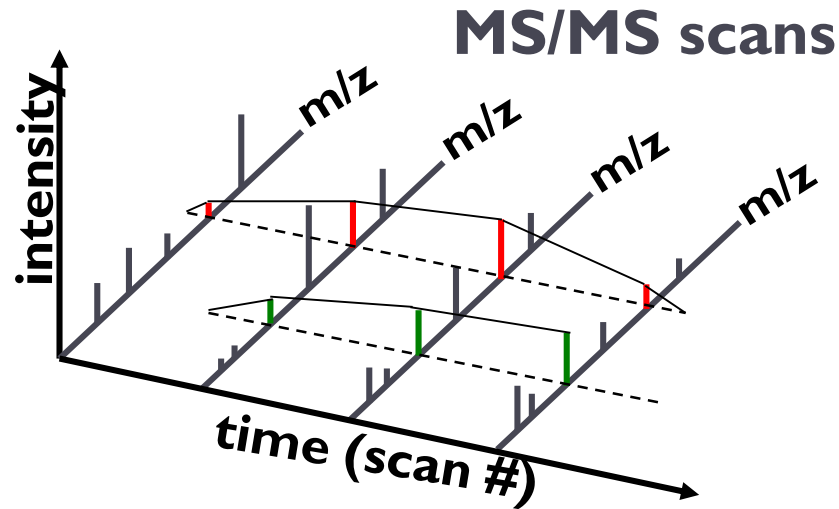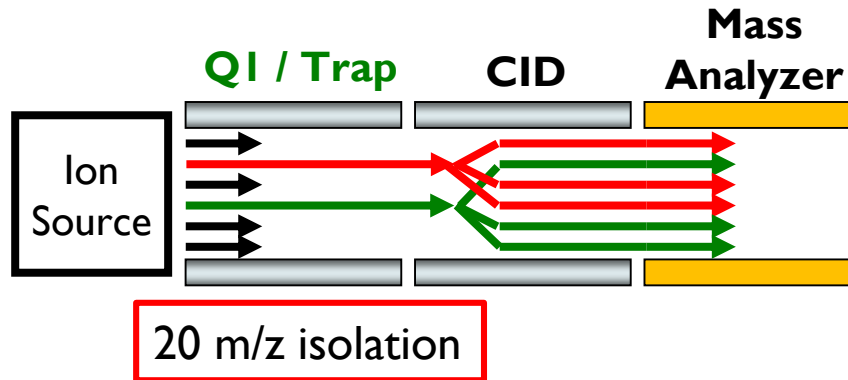
Extraction of Fragment Ions

Measuring Precursor Ions

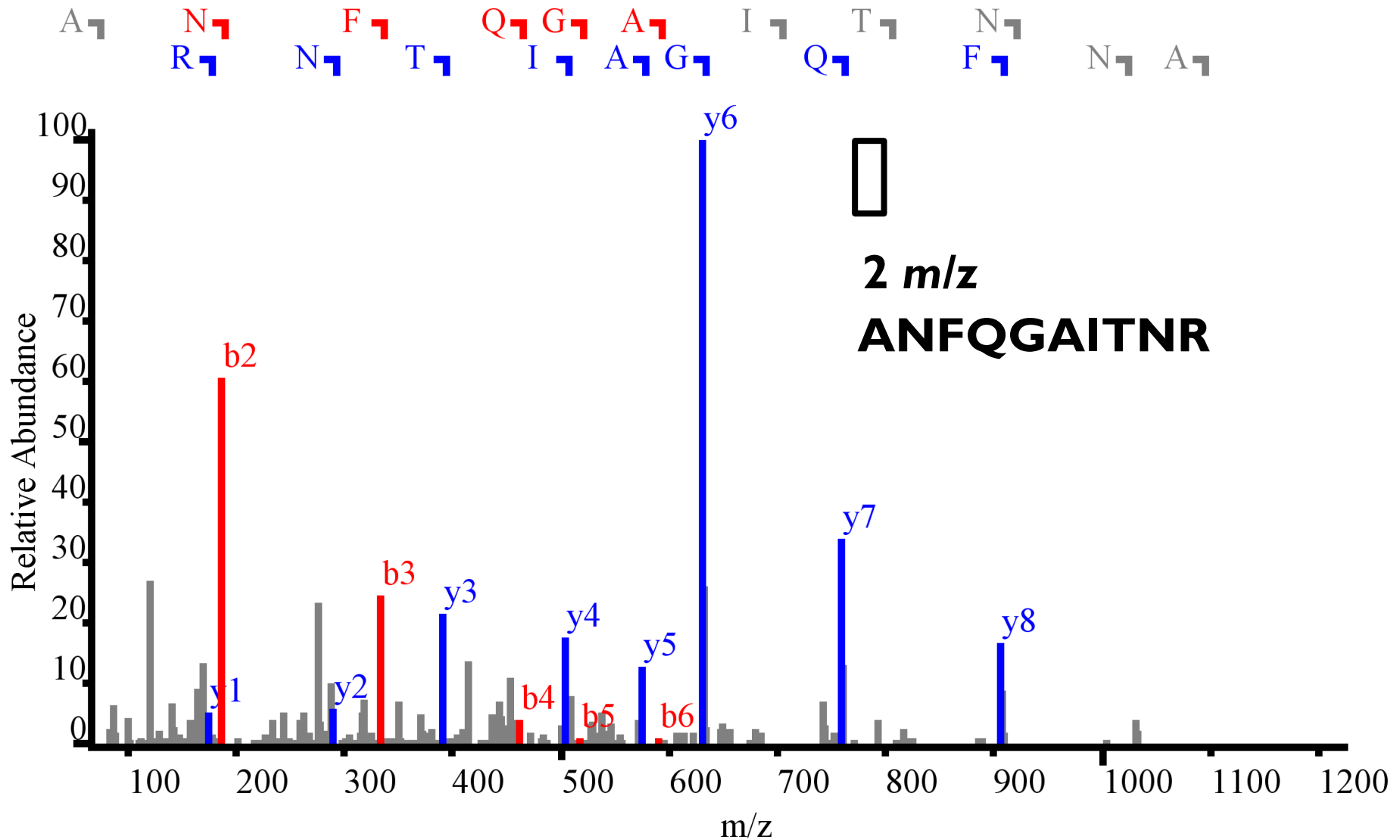# Precursor and Product Ion Information
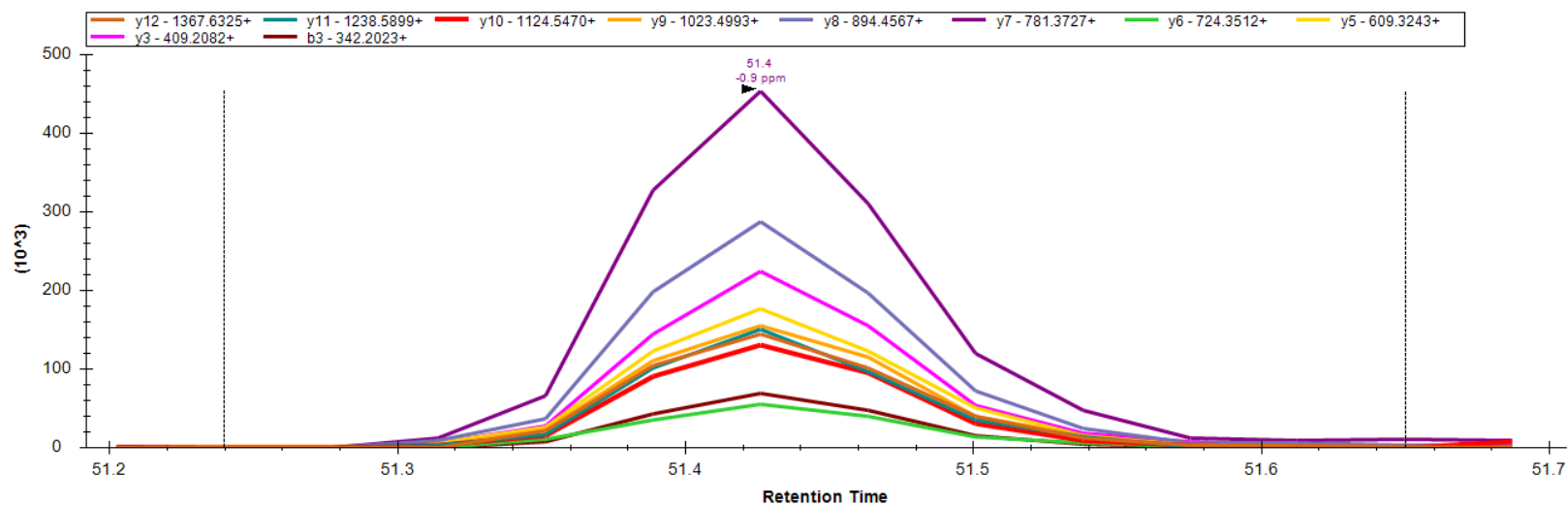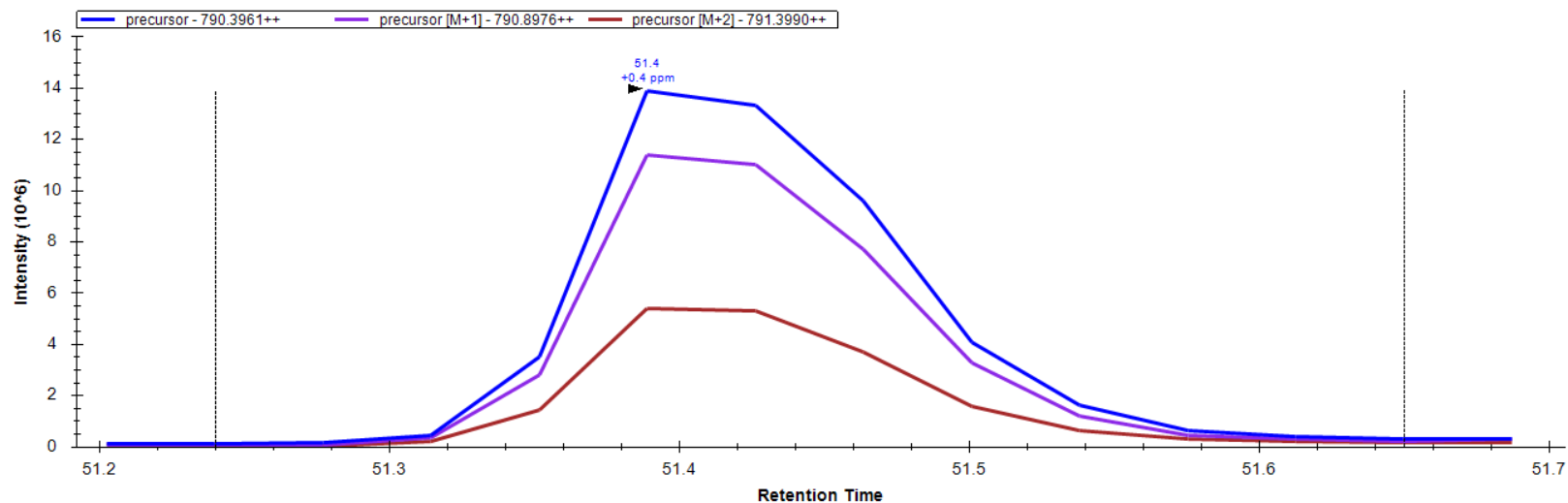
DIA Chromatogram Extraction

Product Ion Selectivity

Product Ion Selectivity (DIA)
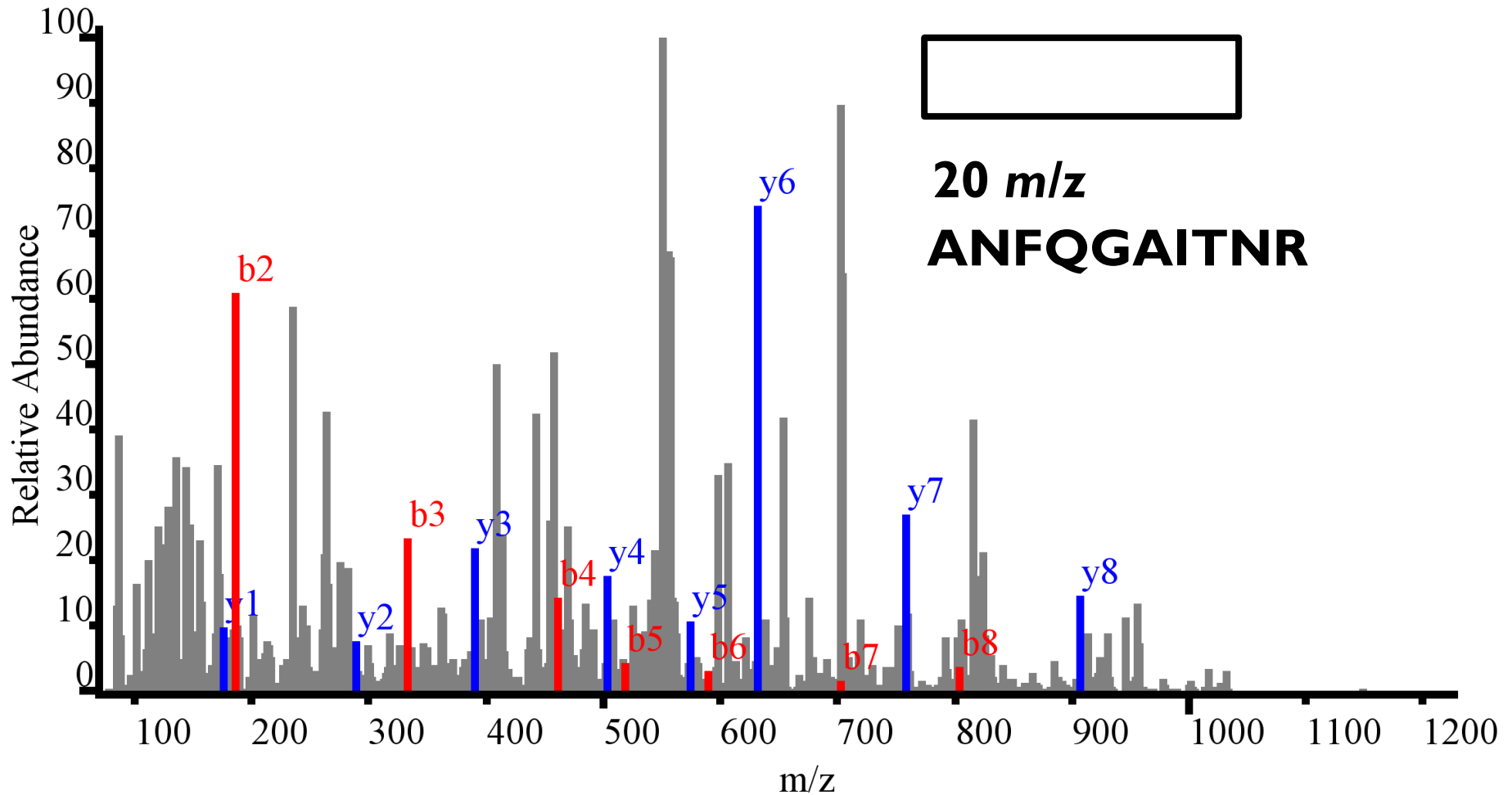
When Selectivity Becomes Sensitivity
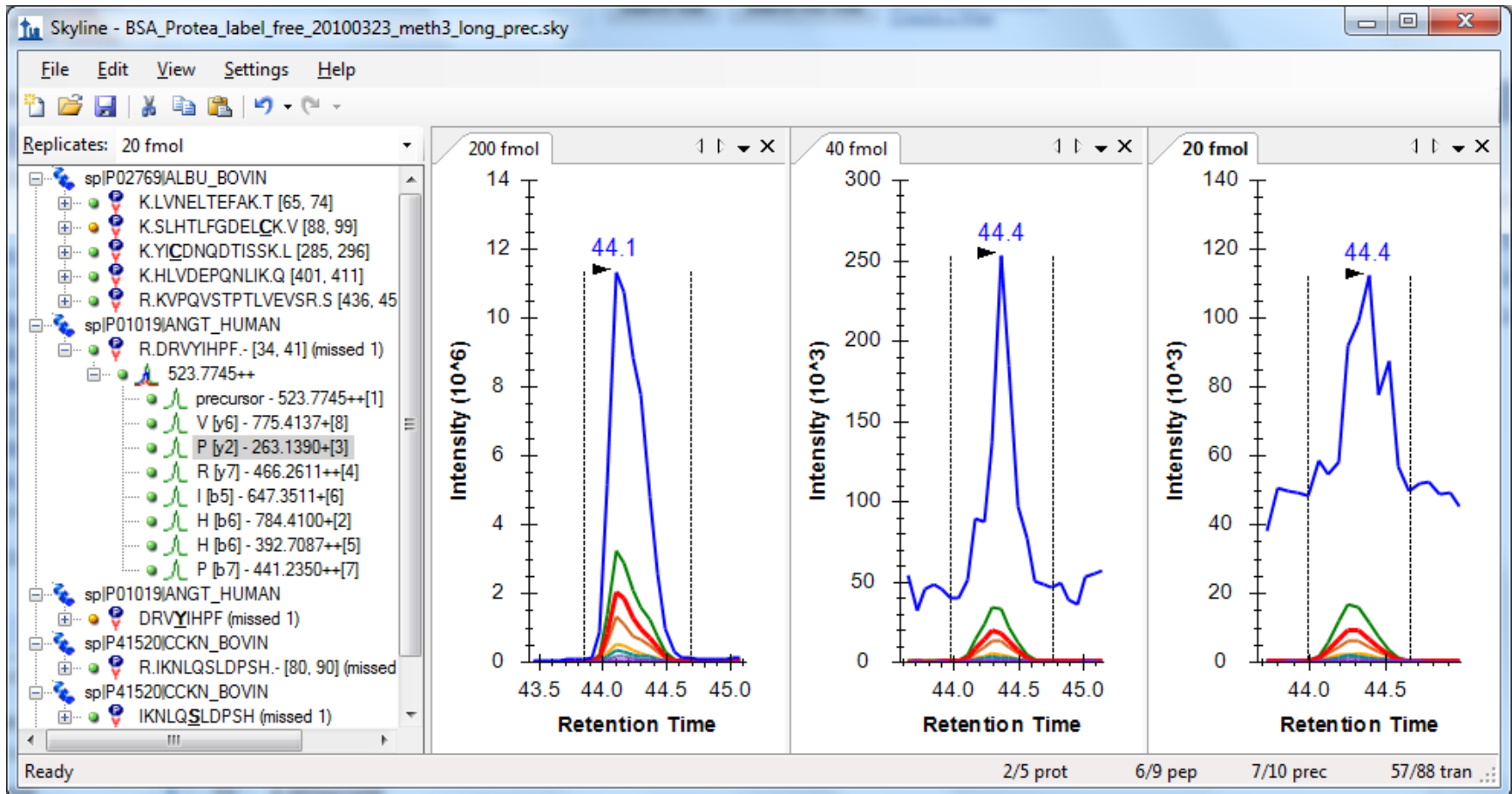
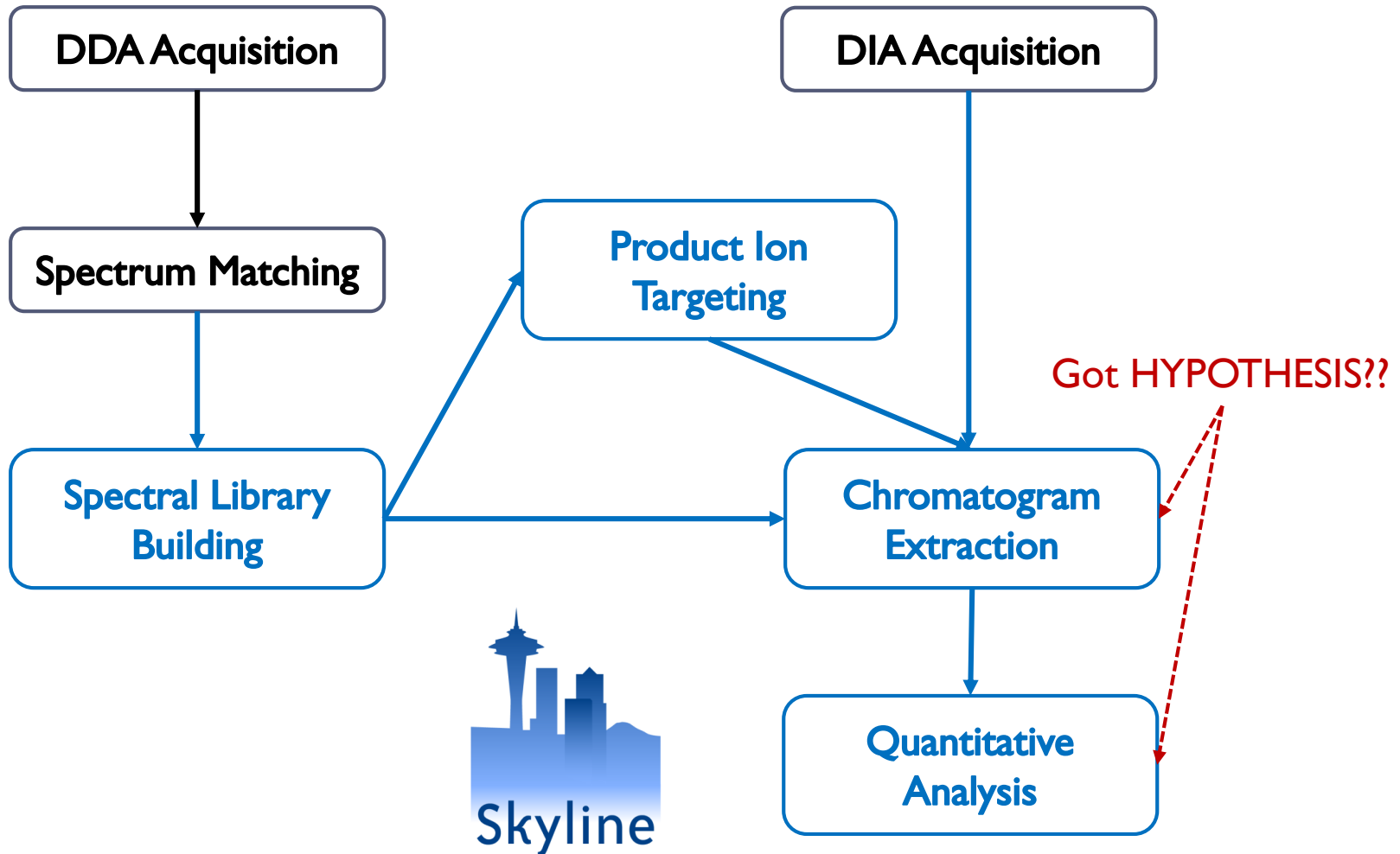
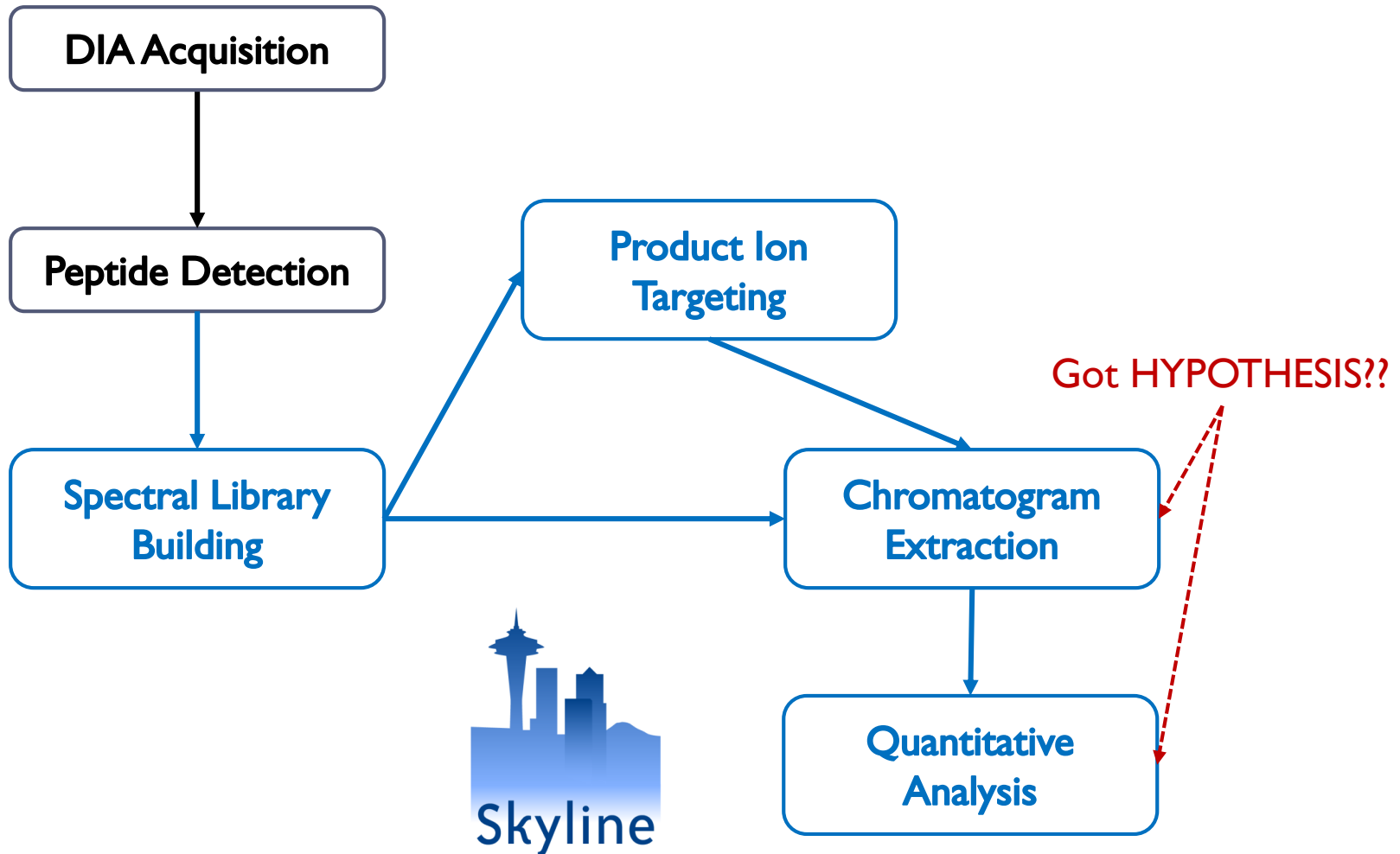Decreasing noise increases signal-to-noise

DIA Trade-Offs

▸ Selectivity

▸ Cycle time

▸ Measured range

▸ All ion digital record – image quality?

Overview

DIA-Centric Workflow

DIA-Centric Workflow
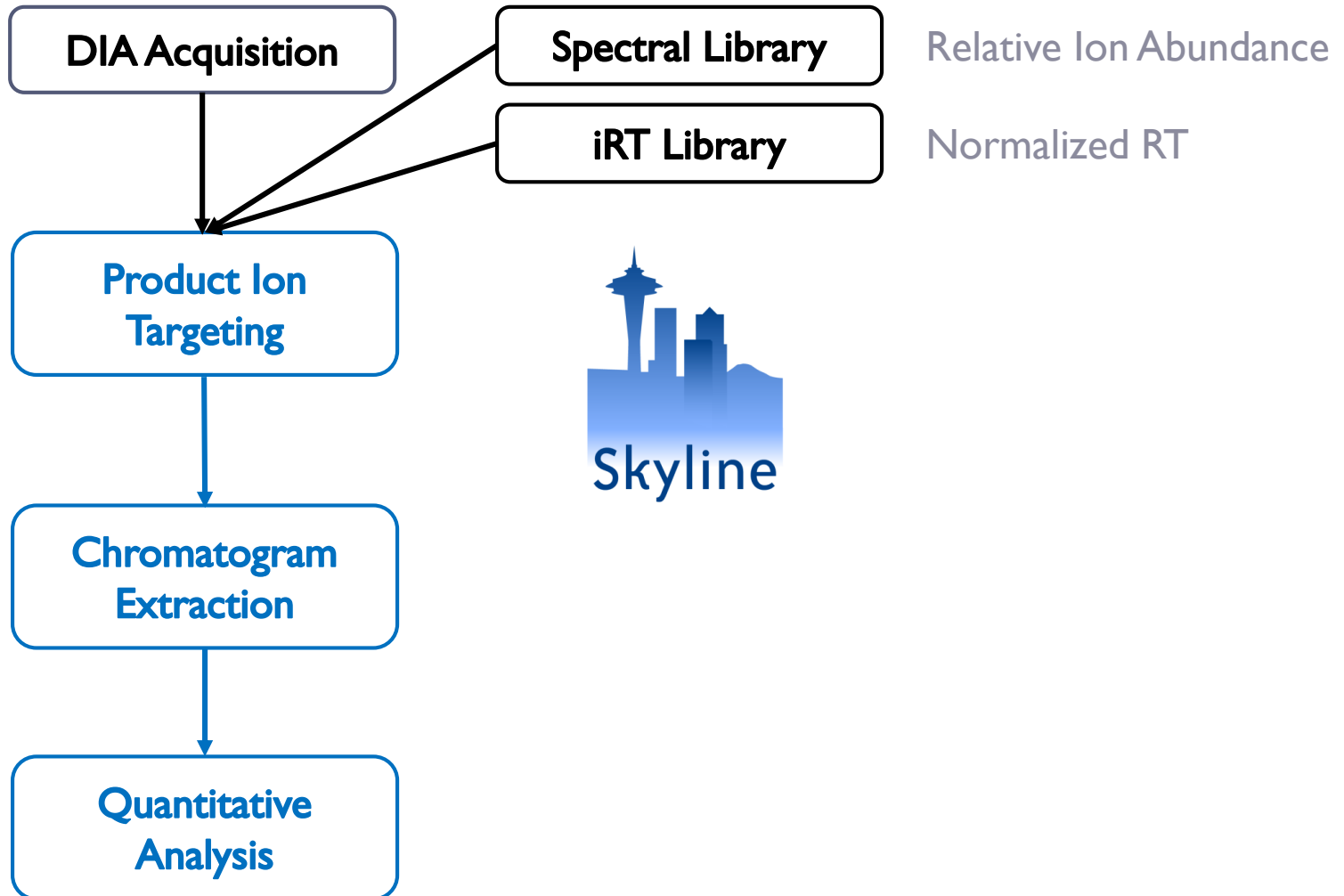
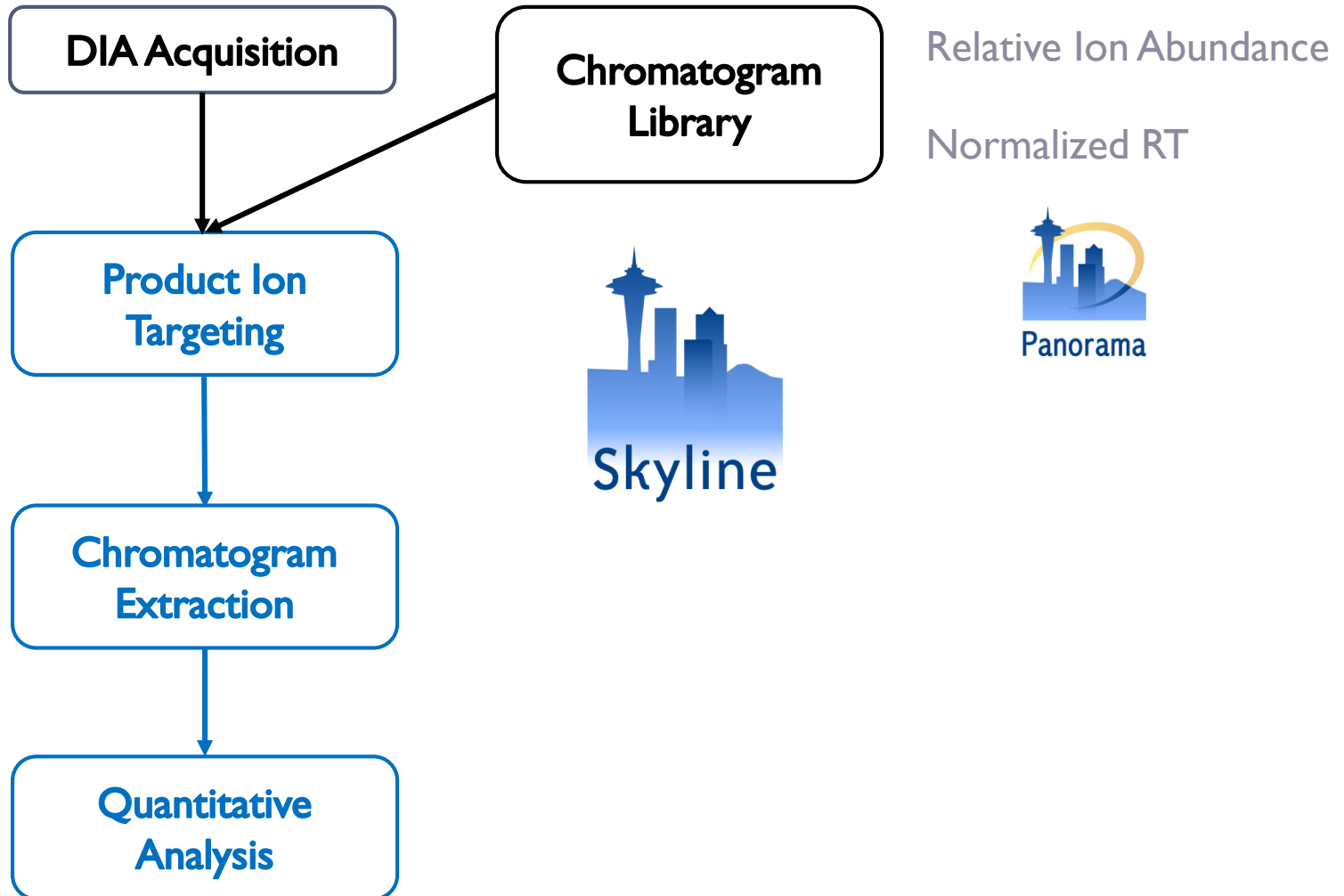**DIA Acquisition**

↓

Peptide Detection

- ▸ Pecan - MacCoss
- ▸ MS Pathfinder - Smith
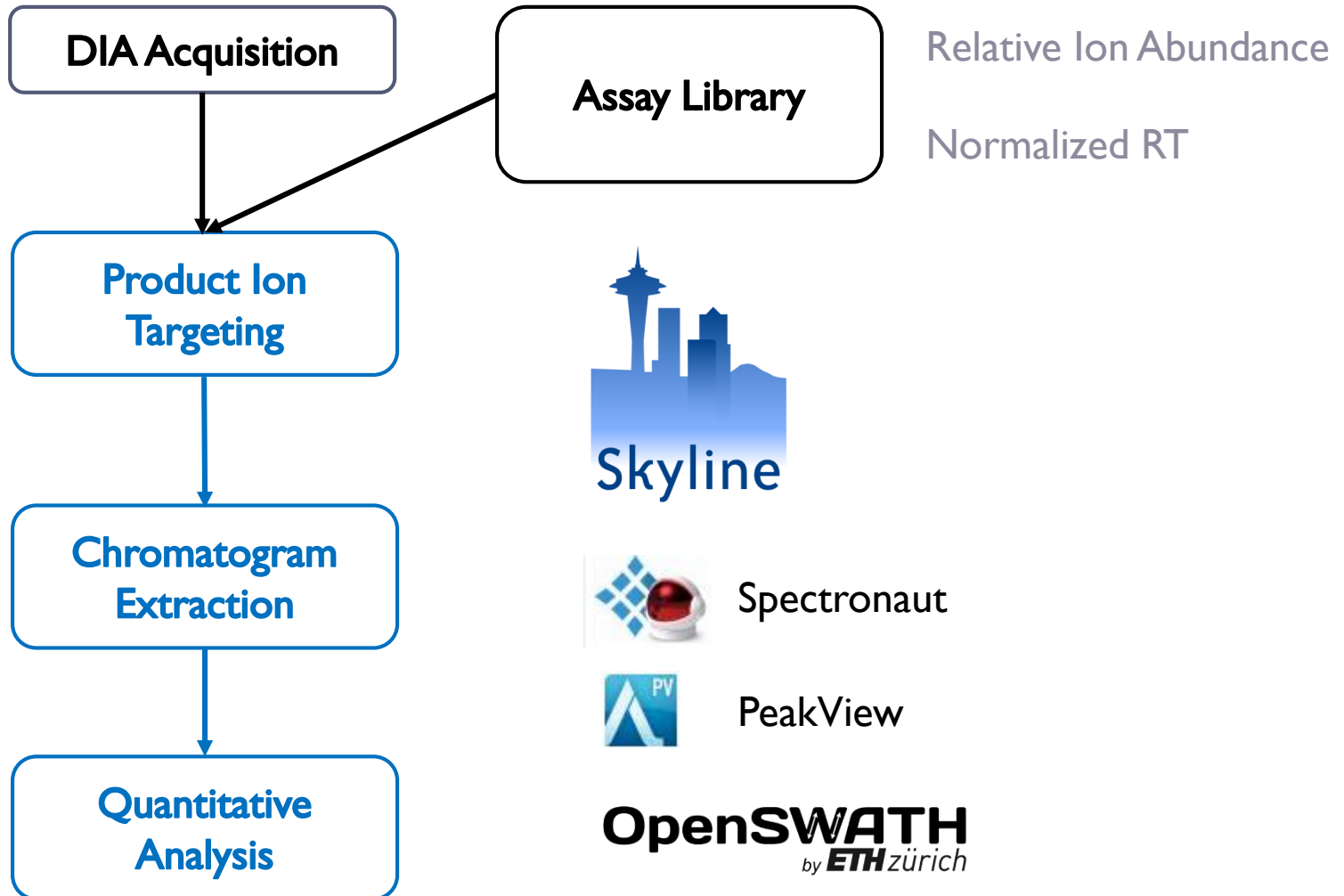- ▸ Umpire - Nesvizhskii
- ▸ Bandeira

Prior Knowledge Workflow

Prior Knowledge Workflow

Prior Knowledge Workflow

Tutorial

- DIA using interspersed DDA

Learn More

- Webinar #3: Tuesday, Jan. 13th – PRM

- Weeklong Targeted Proteomics Course at UW
  - March 23-27 (apply by Nov. 21)
- Workshops at US HUPO and ASMS
- Weeklong Course at ETH, Zurich
  - June 22-26
- Weeklong Course at PRBB, Barcelona
  - November 15-20
- Weeklong Course in Boston
  - TBA

Questions?

▸ Ask any questions you have on the topic of using DDA data in Skyline.

http://tinyurl.com/QA4Skyline

▸ Take the post-webinar survey:

http://tinyurl.com/Survey4Webinar