

# Agenda

---

- ▶ Welcome from the Skyline team!
- ▶ Getting the most out of DDA Data with Brendan MacLean
  - ▶ Workflow overview
  - ▶ Keys to successful MSI filtering in Skyline
  - ▶ Live tutorial
- ▶ A Scientist's View: Using Skyline for MSI Filtering with Birgit Schilling – Researcher, Author and Skyline user
- ▶ Audience Q&A – submit questions to Google Form:  
<http://bit.ly/ZGDQ9K>



# Chromatography-based Quantification

---

- ▶ Hypothesis testing (Verification)
- ▶ SRM
- ▶ **MSI chromatogram extraction**
- ▶ Targeted MS/MS (PRM)
- ▶ Data independent acquisition (DIA/SWATH)



Acquisition	Targeted	Survey
More Selective	Targeted-MS/MS	DIA
Less Selective	SRM	MSI

Got **HYPOTHESIS??**

---



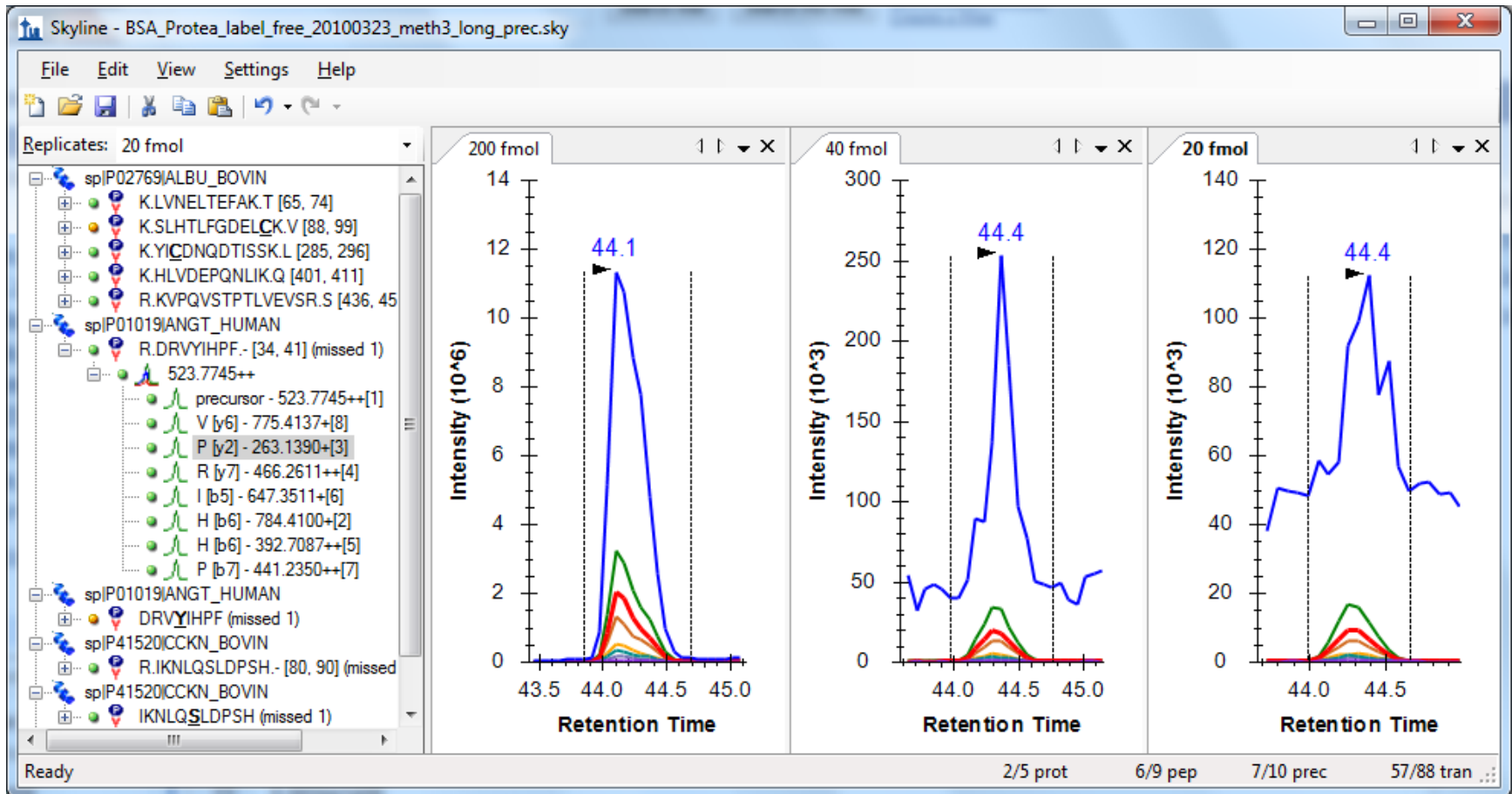
# Hypothesis Generation

---

- ▶ Tuning integration for 10,000+ peptides in Skyline?
- ▶ Use discovery tools to narrow the field
- ▶ Use Skyline without correction
  - ▶ Narrow the field with statistical analysis
  - ▶ Use Edit > Refine > Accept Peptides
  - ▶ Then tune integration
  - ▶ We will improve this workflow



# When Selectivity Becomes Sensitivity



Decreasing noise increases signal-to-noise

# Overview

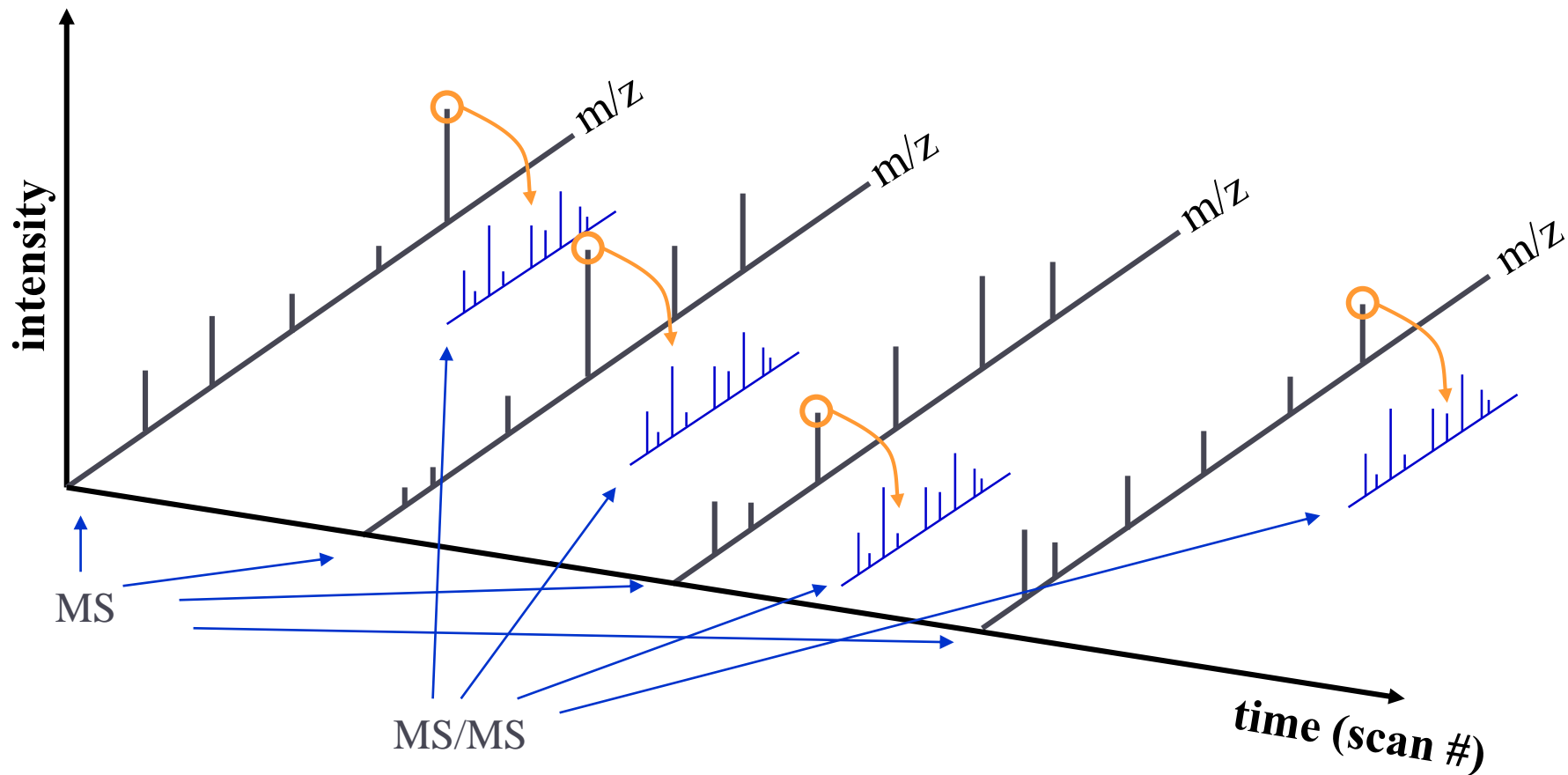
---

**DDA Acquisition**



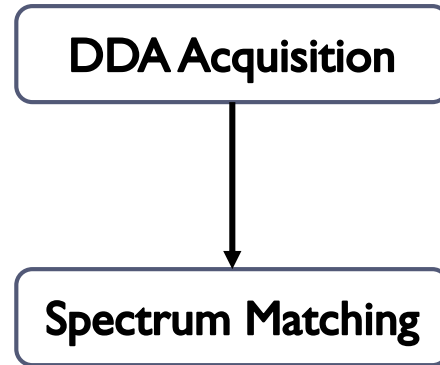
# DDA Acquisition

---



# Overview

---



# Overview

---

## DDA Acquisition

```
graph TD; A[DDA Acquisition] --> B[Spectrum Matching];
```

### Spectrum Matching

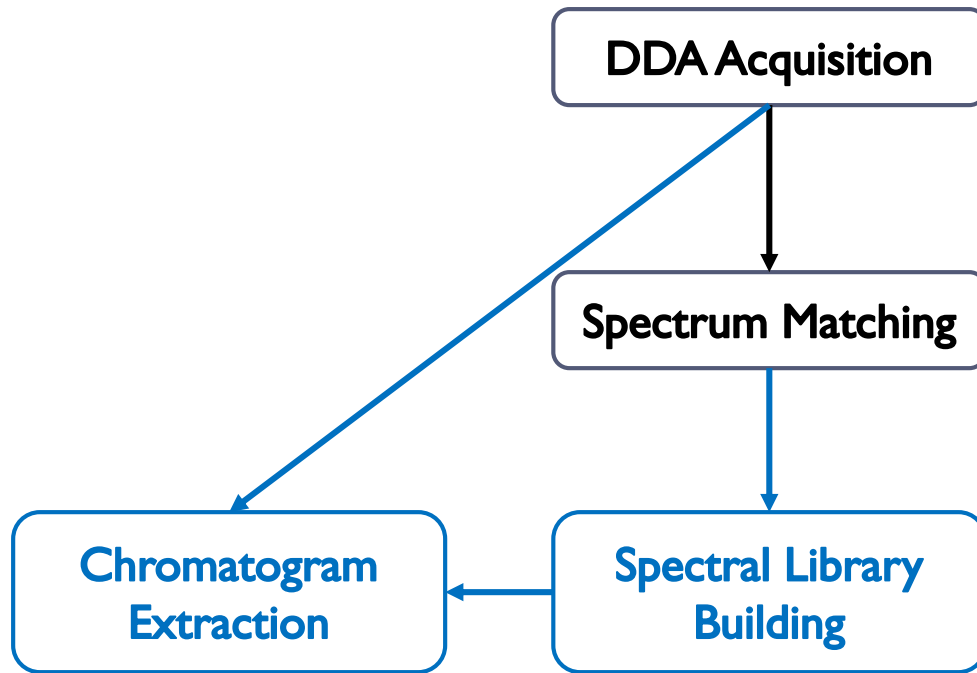
- ▶ *Mascot*
- ▶ ByOnic
- ▶ Myrimatch / IDPicker
- ▶ OMSSA
- ▶ MaxQuant Andromeda
- ▶ Morpheus
- ▶ MSGF+
- ▶ PRIDE XML
- ▶ Protein Pilot
- ▶ Protein Prospector
- ▶ Proteome Discoverer (MSF)
- ▶ Scaffold – mzIdentML / MGF
- ▶ Spectrum Mill
- ▶ TPP – pepXML / mzXML files – Peptide Atlas
- ▶ X! Tandem
- ▶ PLGS - Waters MSe





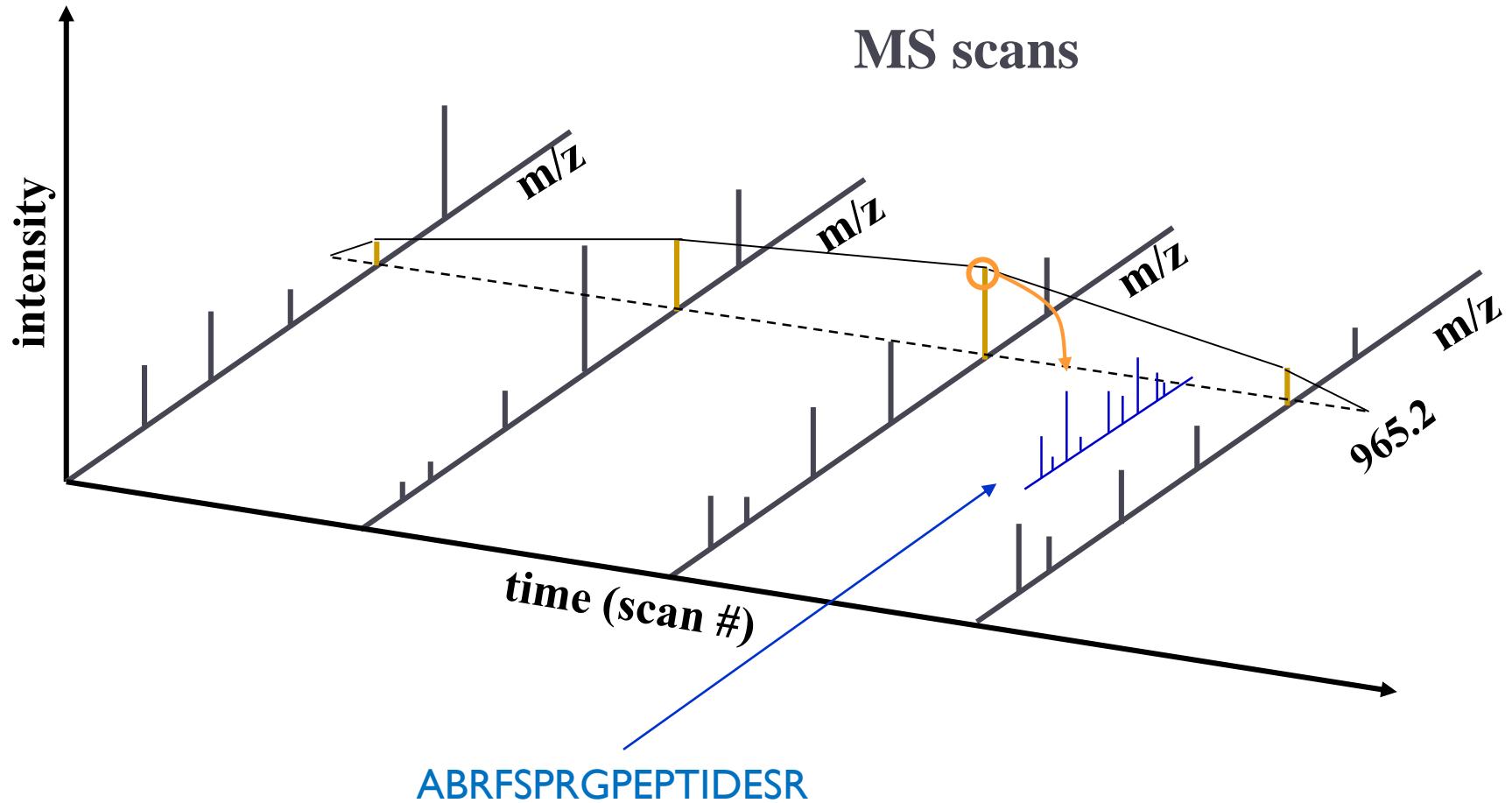
# Overview

---

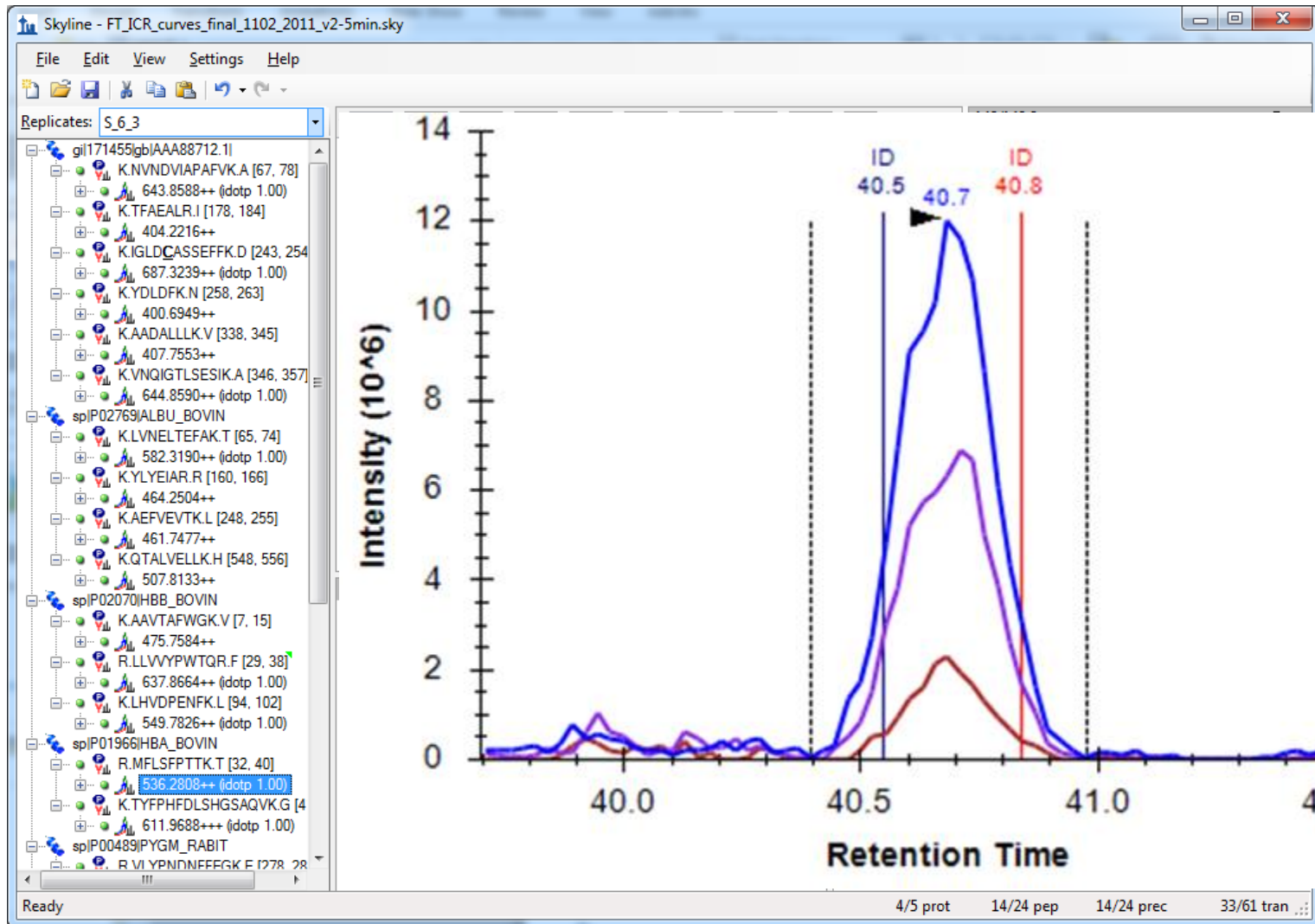


# MS1 Chromatogram Extraction

---



# MS1 Chromatogram Extraction in Skyline



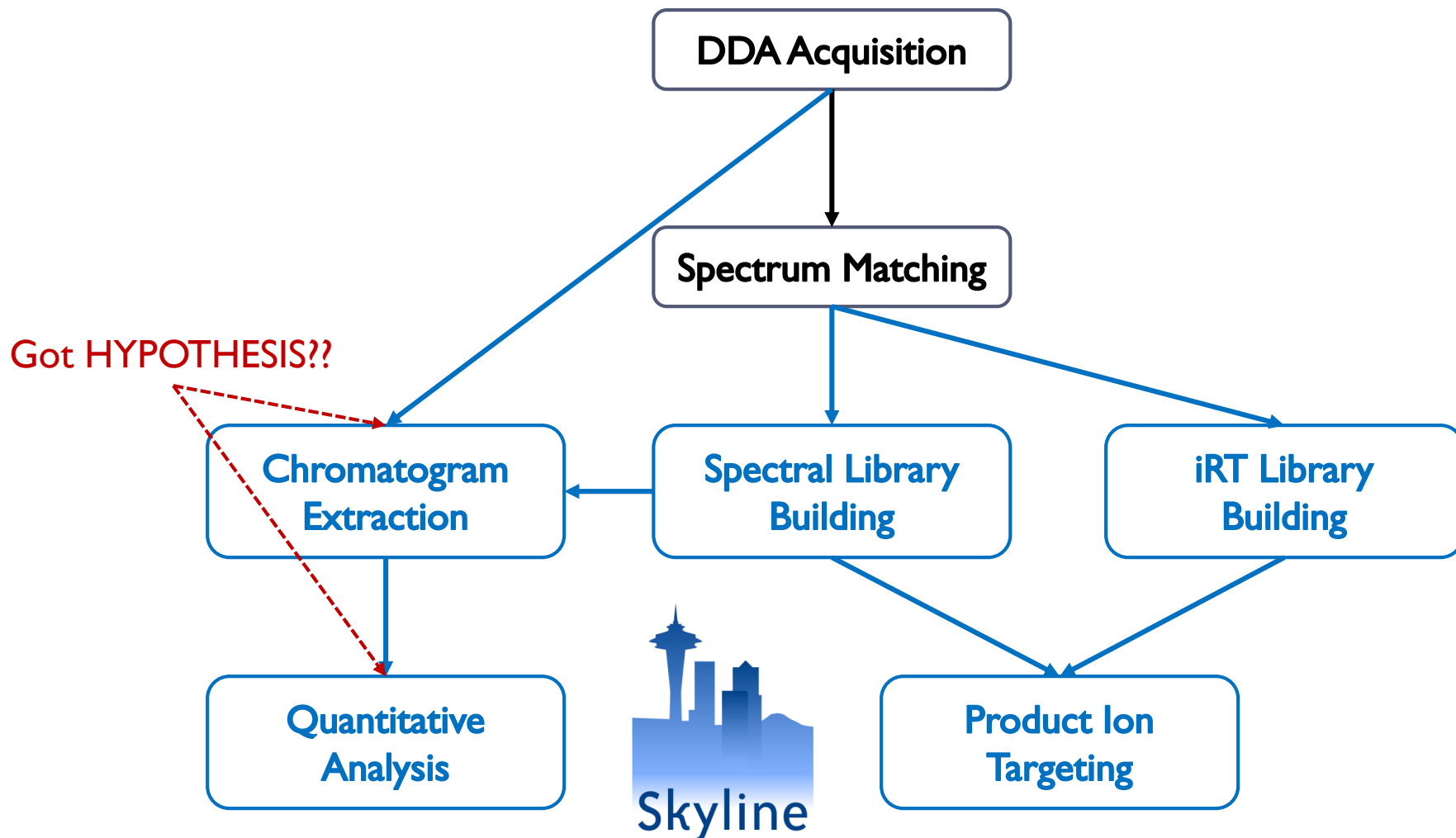
# Multiple Instrument Vendors

---



# Overview

---



# Tutorial

---

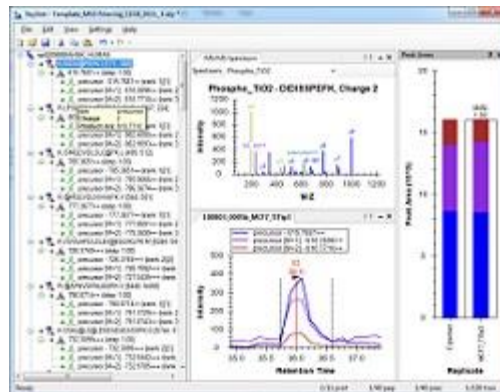
- ▶ MSI Filtering with Skyline



# Keys to Success with MS1 in Skyline

- ▶ Make sure you have ID annotations
  - ▶ Diagnose with Spectral Library Explorer
  - ▶ <http://tinyurl.com/Skyline-missing-ids>
  - ▶ Use File > Import > Peptide Search
- ▶ Review RT alignment in alignment viewer
- ▶ **Got HYPOTHESIS??**
- ▶ Review and manually adjust <5% of peaks

- ▶ Do the tutorial



# Learn More

---

- ▶ Webinar #2: Tuesday, Nov. 18<sup>th</sup> – Topic TBA
- ▶ 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://bit.ly/ZGDQ9K>

- ▶ Take the post-webinar survey:

<http://bit.ly/IsLpjoE>

