

Agenda

- ▶ Welcome from the Skyline team!
- ▶ **PRM Targeted Proteomics Using Full-Scan MS2**
- ▶ Introduction with Brendan MacLean
- ▶ PRM Introduced by Bruno Domon – PRM Researcher
- ▶ Tutorial with Brendan MacLean
 - ▶ Workflow overview
 - ▶ Data processing tutorial
- ▶ Audience Q&A – submit questions to Google Form:
<http://tinyurl.com/QA4Skyline> (also in chat window)



Parallel Reaction Monitoring Synonyms

- ▶ MRM-HR™
 - ▶ Targeted MS/MS (MacLean, ASMS 2011) - tMSMS
 - ▶ Psuedo-SRM (Sherrod, JPR 2012)
 - ▶ PRM (Peterson, MCP 2012)
-
- ▶ More mass spectrometry synonyms
<http://tinyurl.com/ms-synonyms>



Chromatography-based Quantification

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

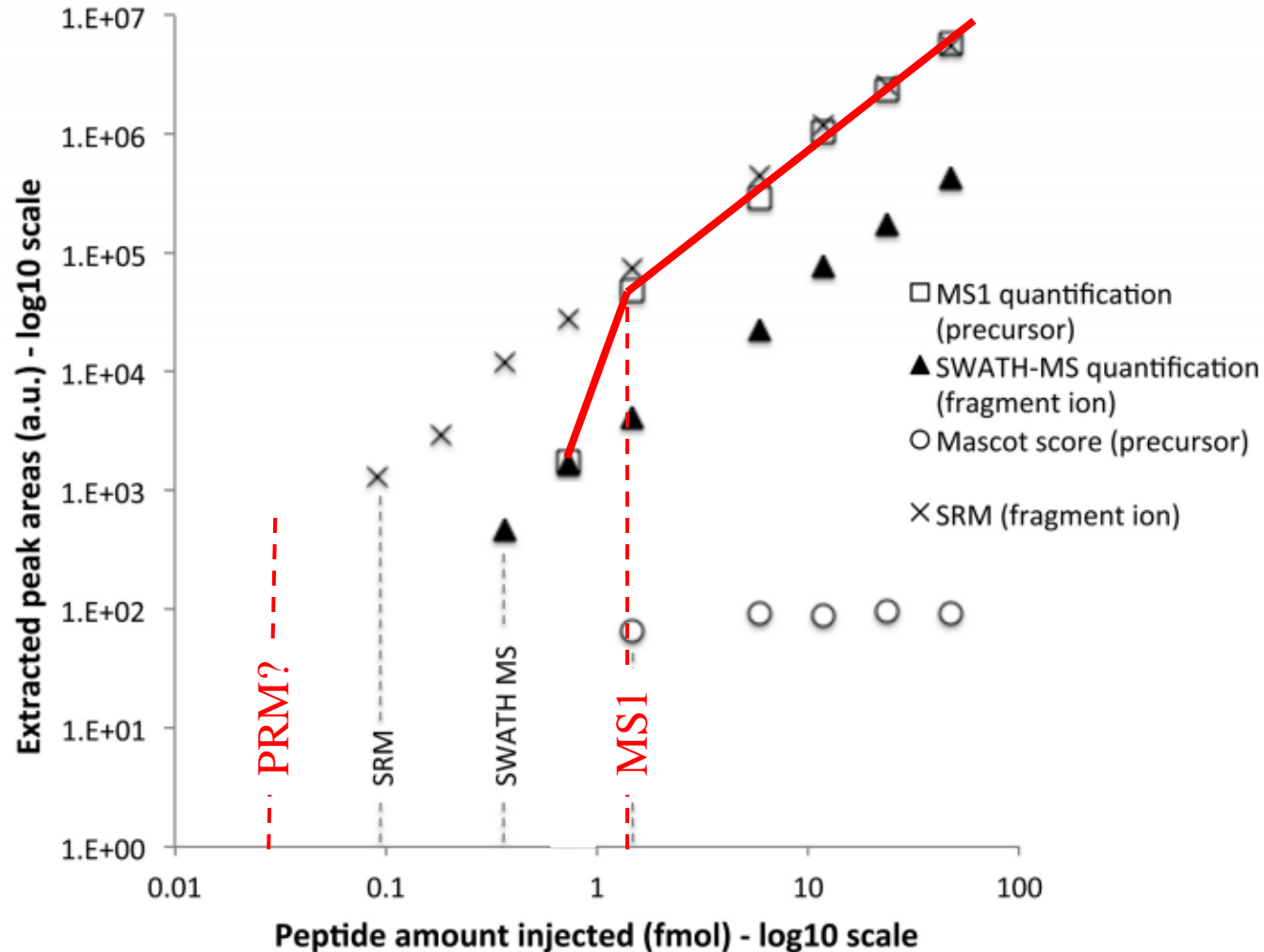


Acquisition	Targeted	Survey
More Selective	PRM	DIA
Less Selective	SRM	MSI

Got HYPOTHESIS



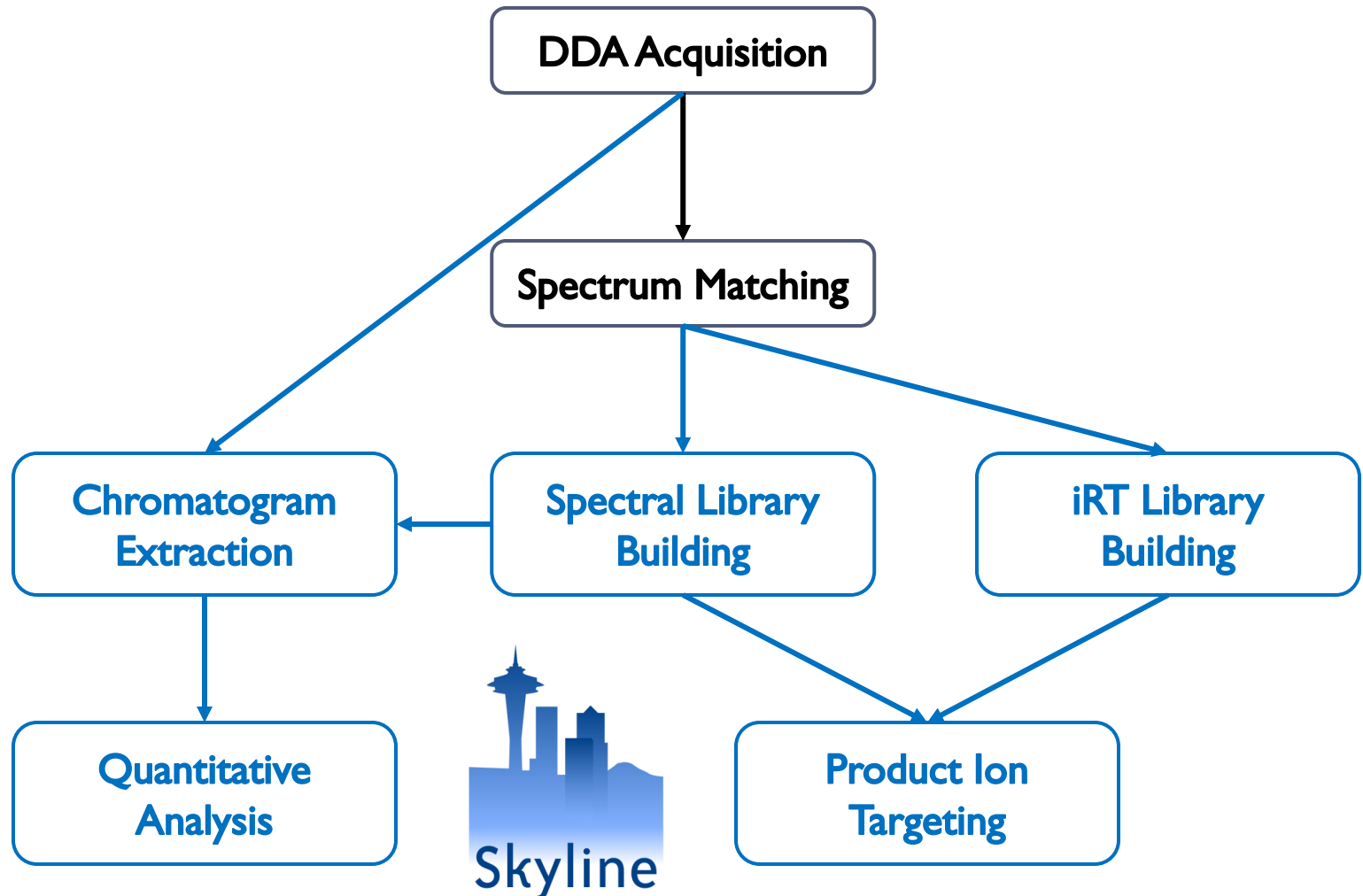
Dynamic Range for PRM?



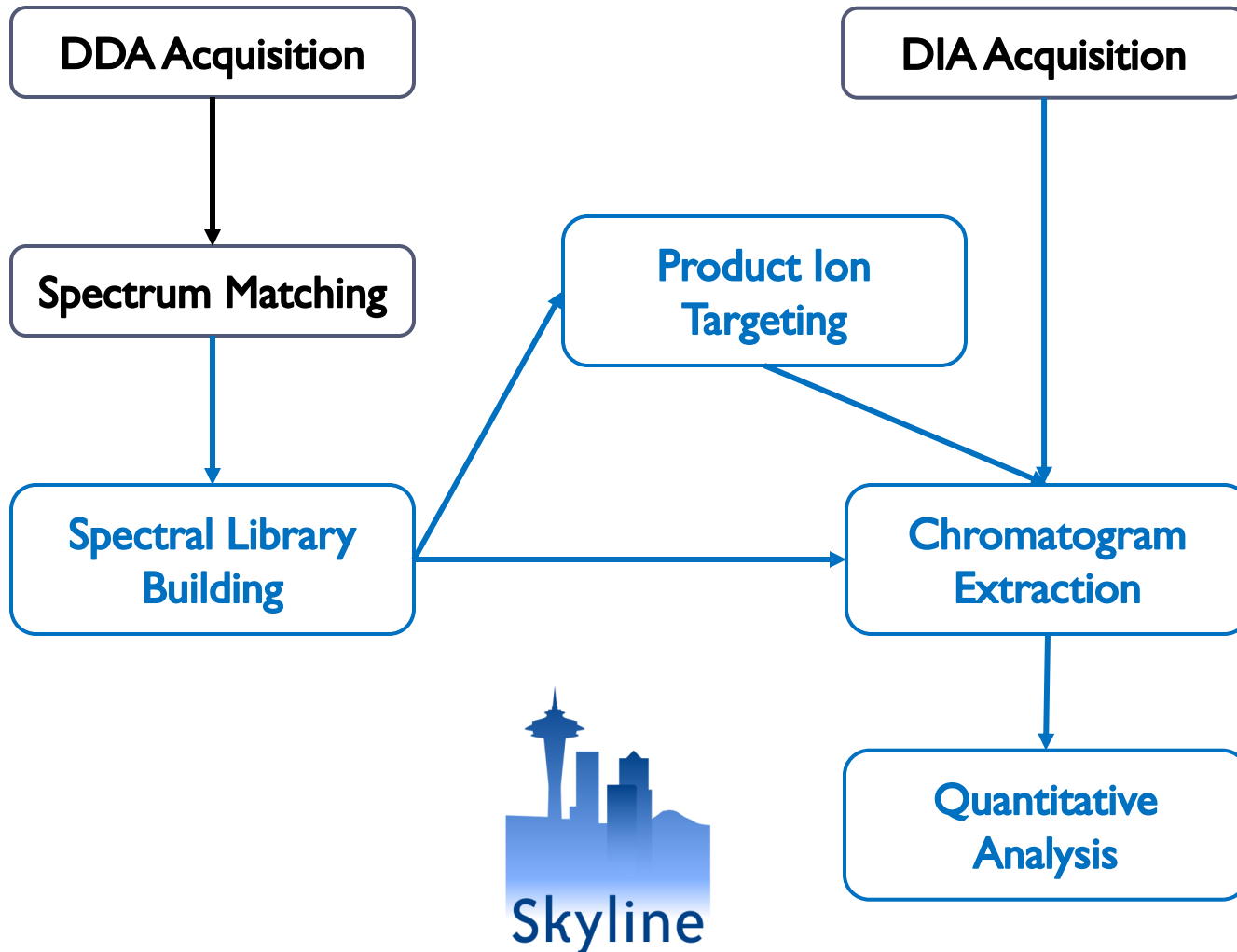
Multiple Instrument Vendors



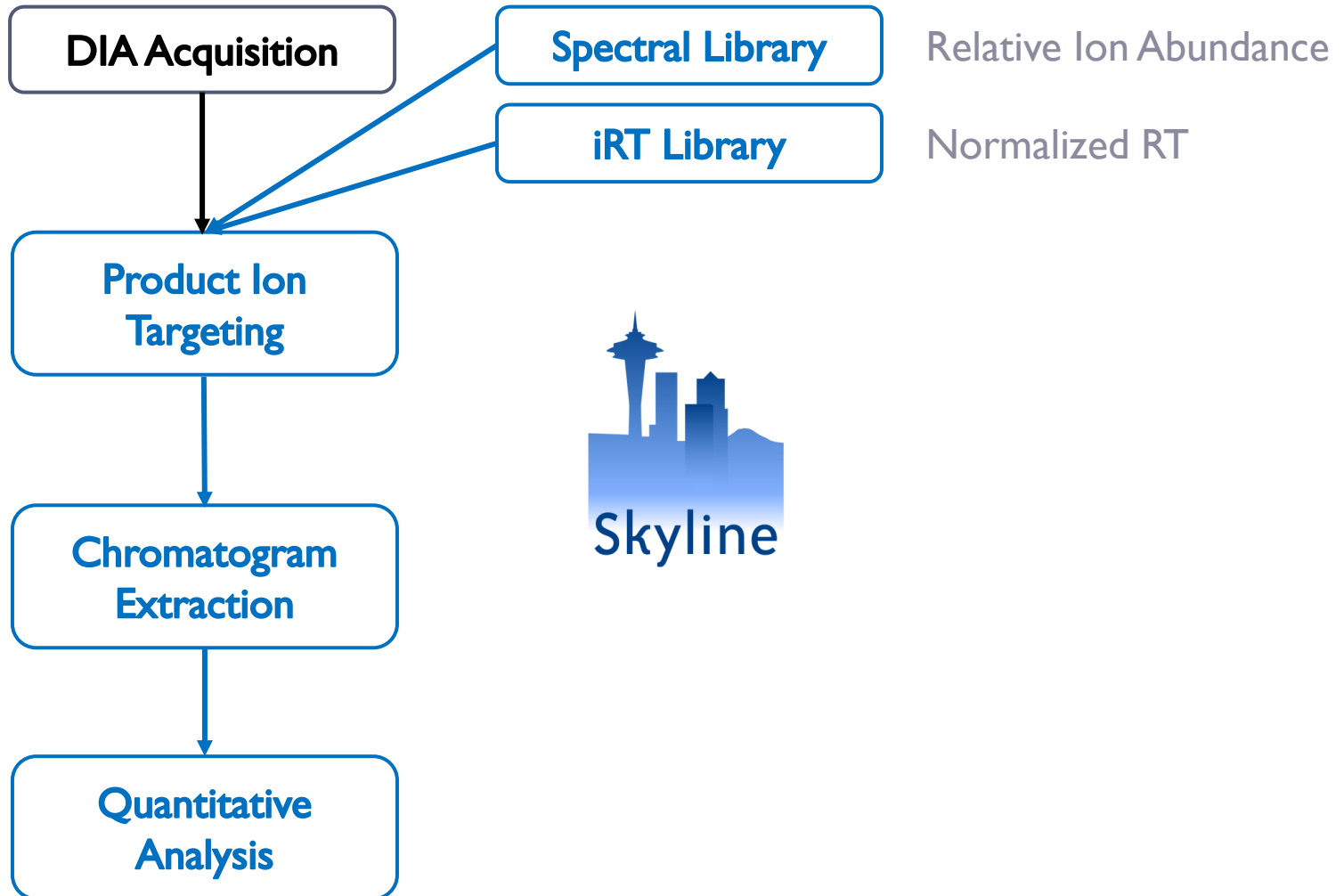
Webinar 1: DDA Workflow Overview



Webinar 2: DIA Workflow Overview



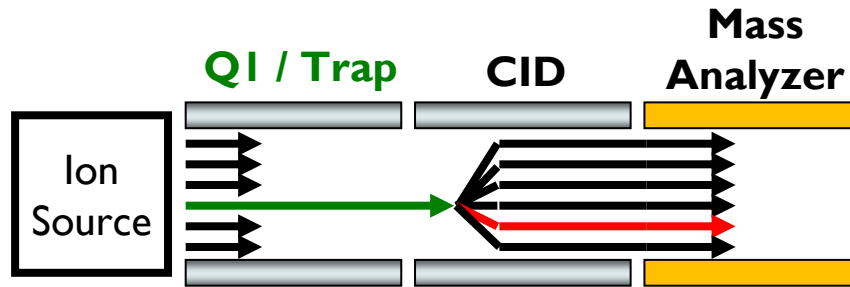
DIA Prior Knowledge Workflow



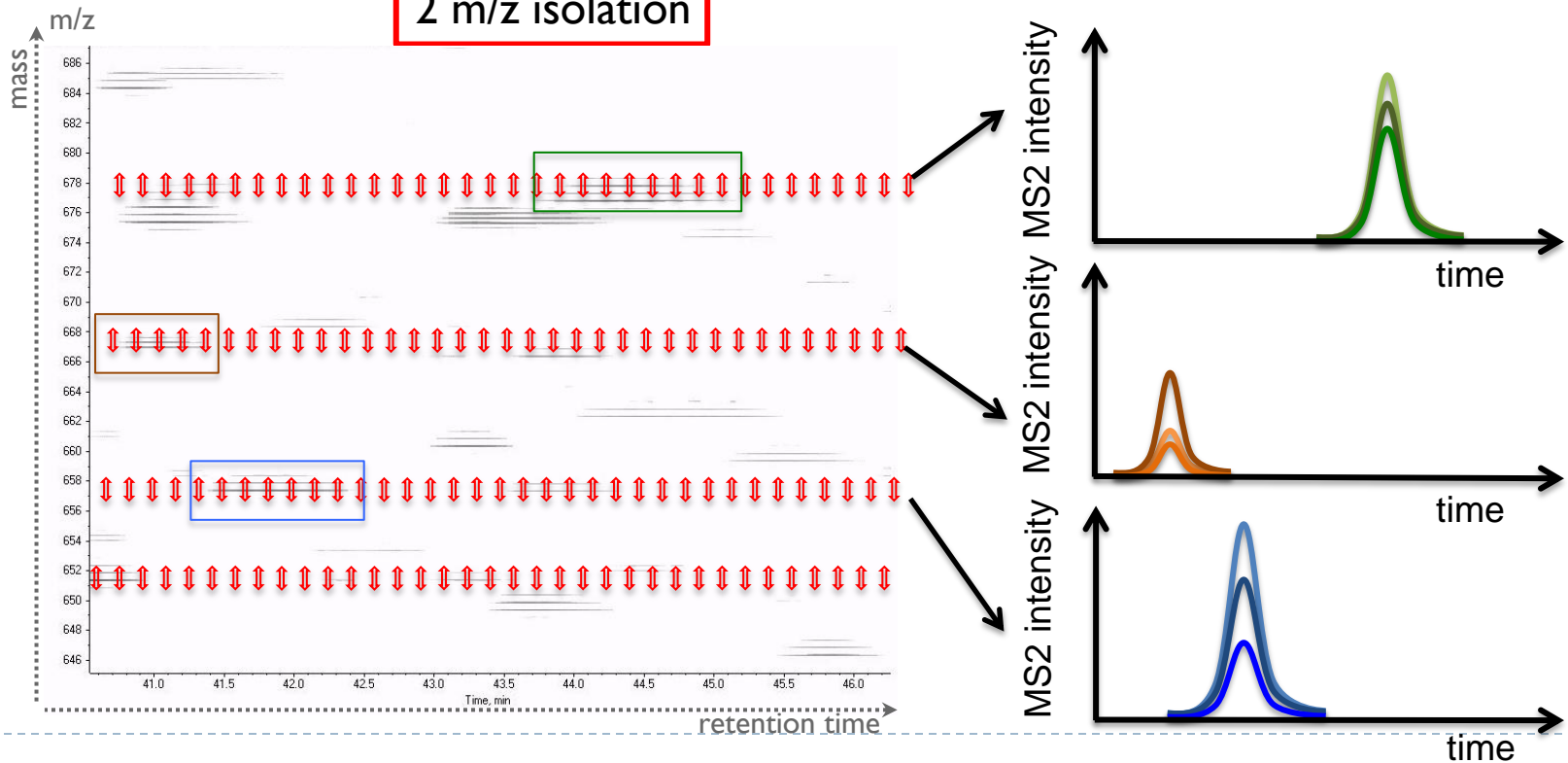
Label-Free PRM Workflow Overview



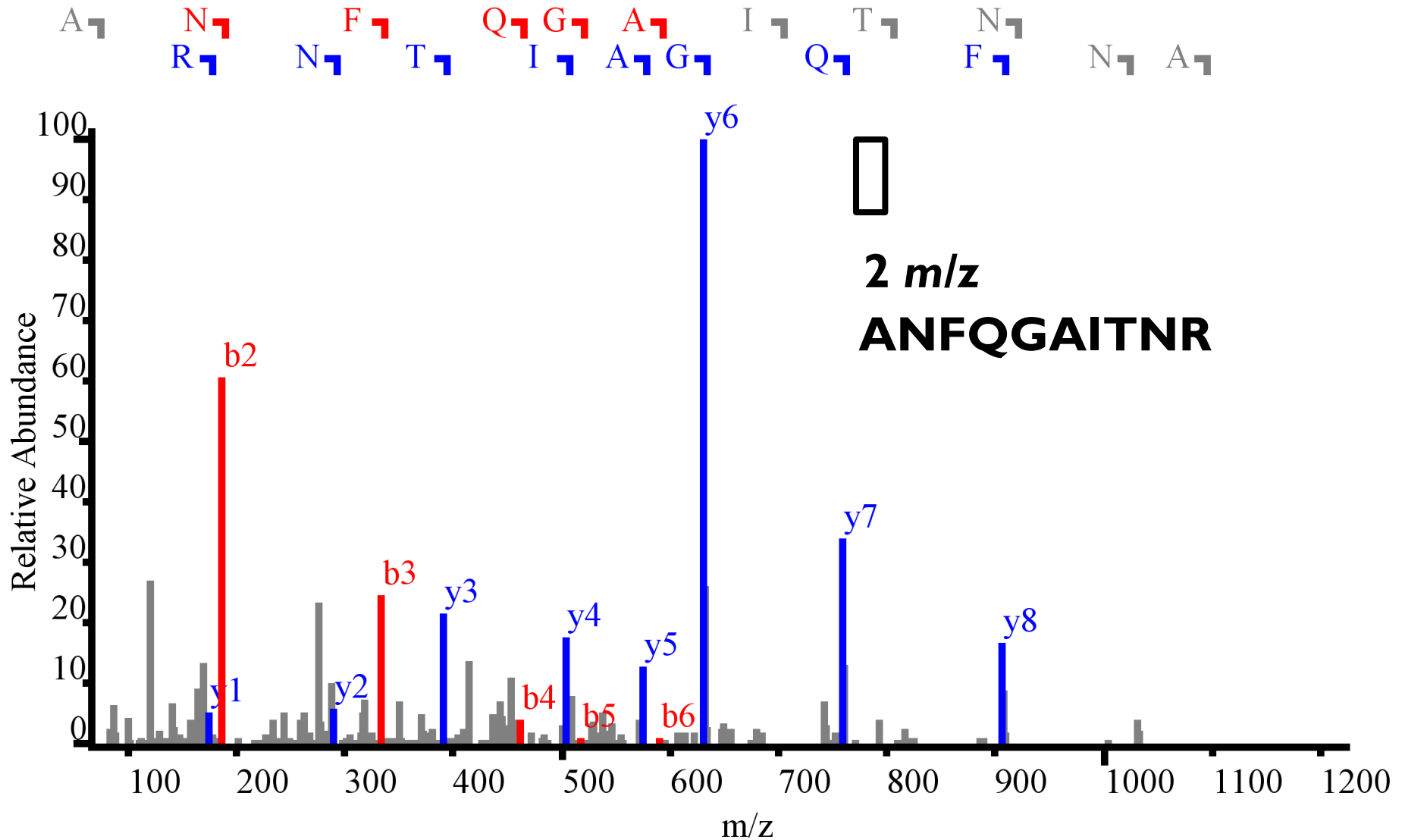
PRM Acquisition



2 m/z isolation

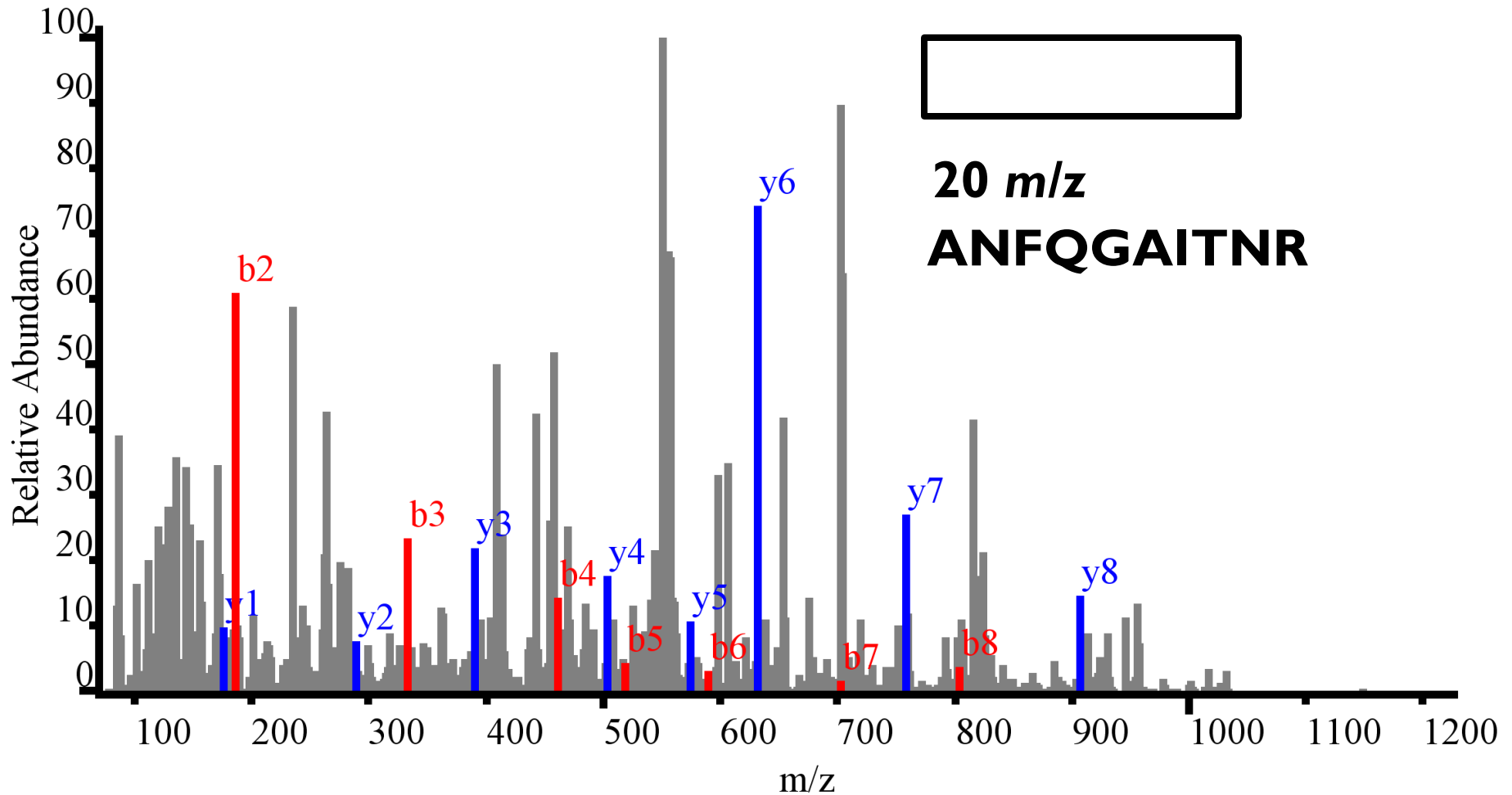


Product Ion Selectivity (PRM and DDA)

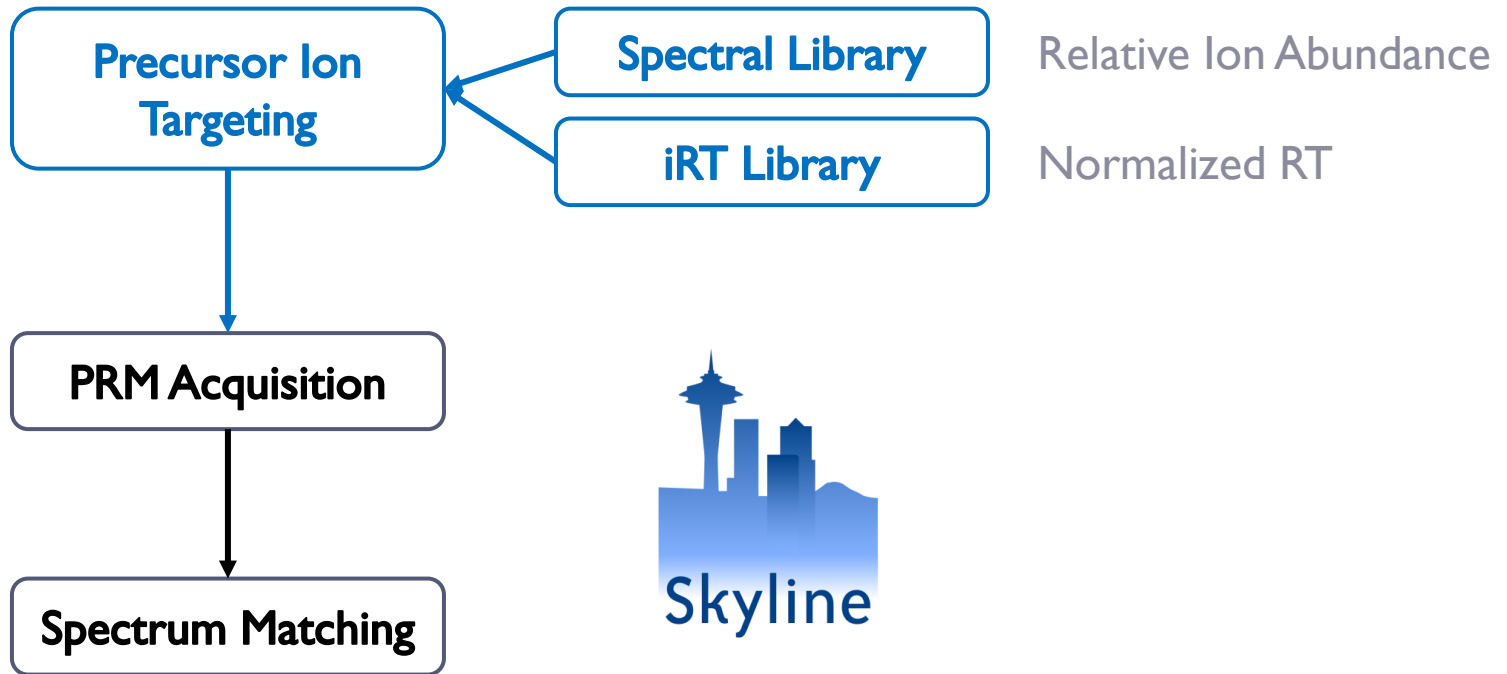


Product Ion Selectivity (DIA)

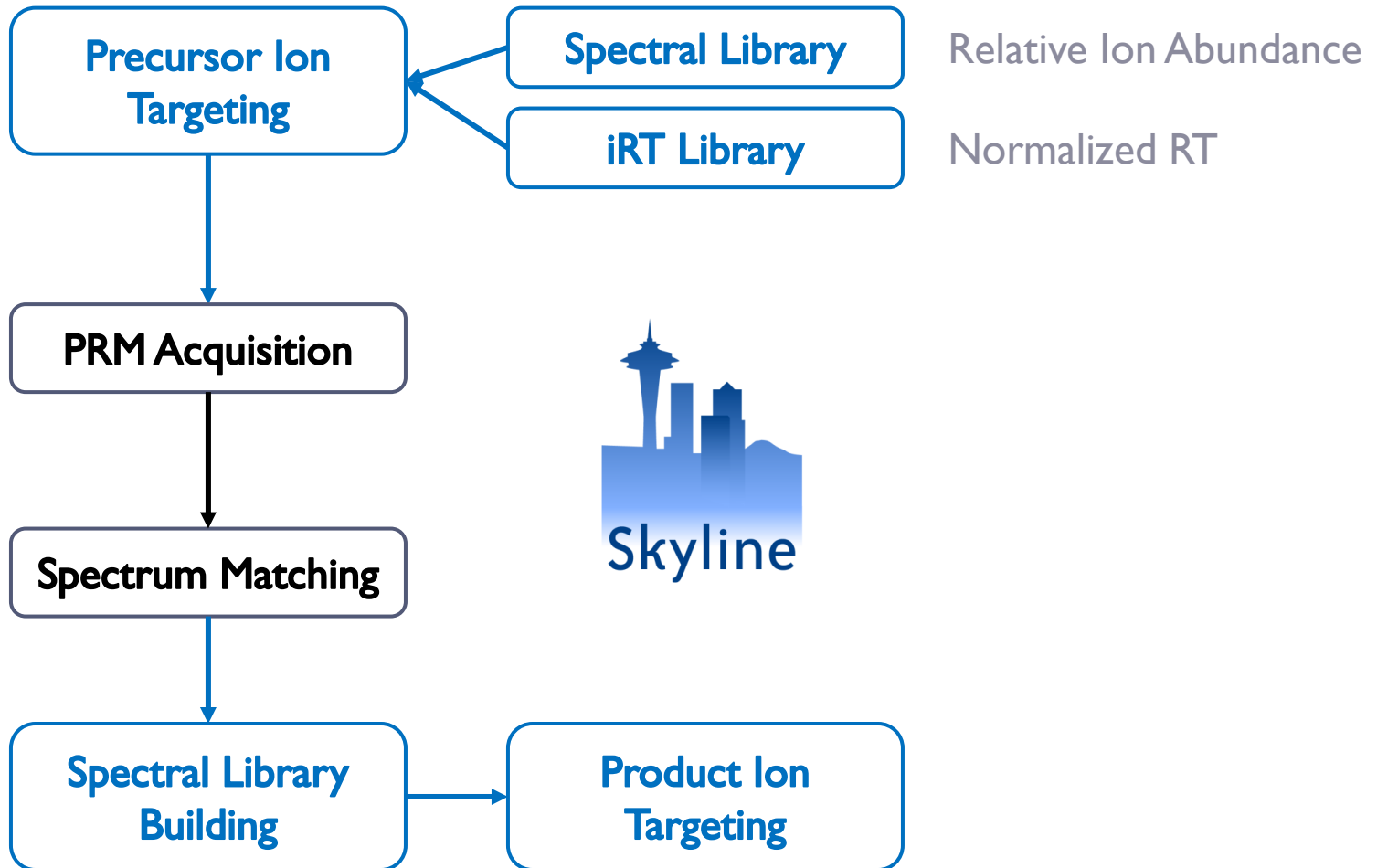
N₁ R₁ N₂ F₁ T₁ Q₁ G₁ A₁ I₁ T₁ N₁
R₁ N₁ T₁ I₁ A₁ G₁ Q₁ F₁ N₁ A₁



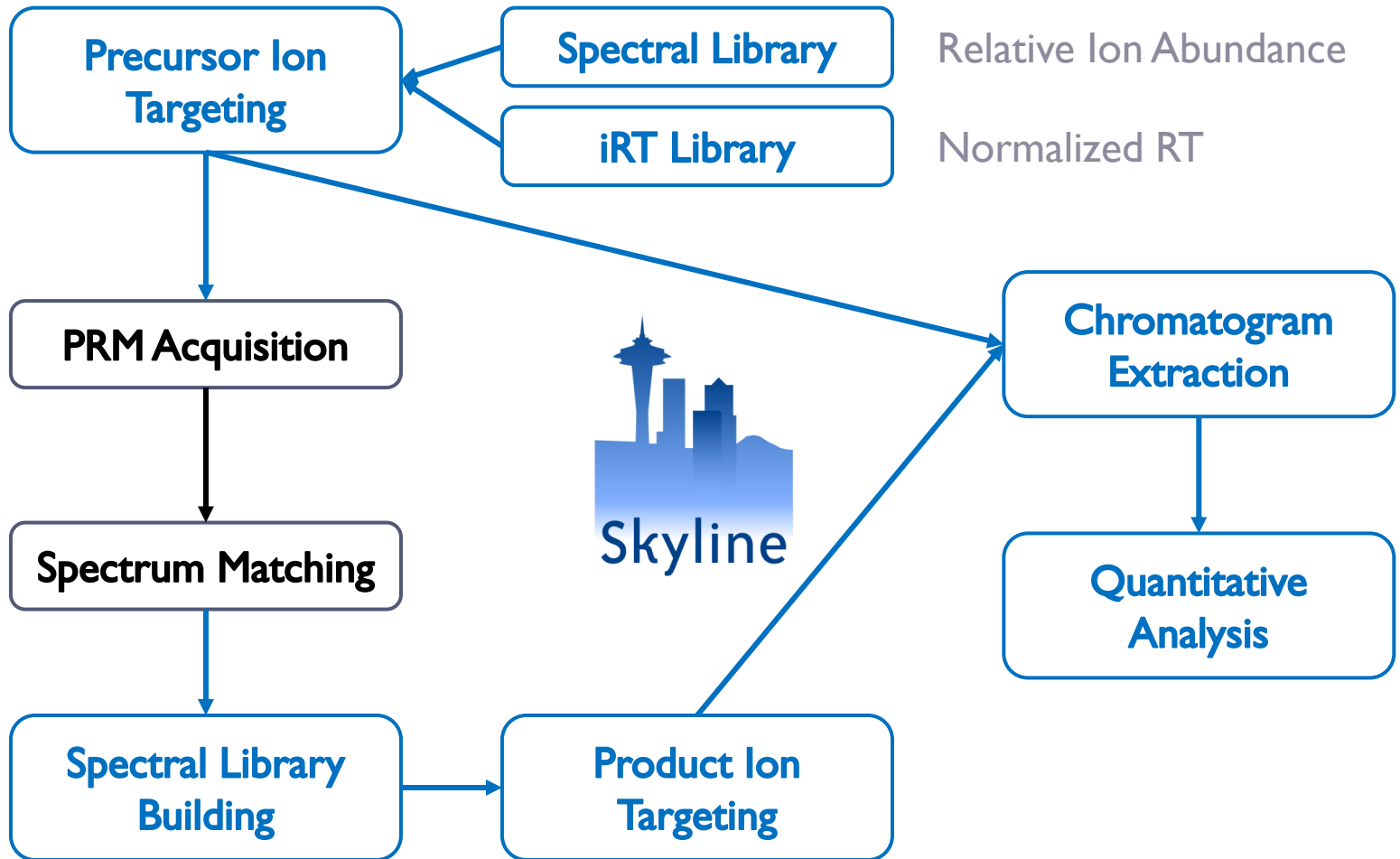
Label-Free PRM Workflow Overview



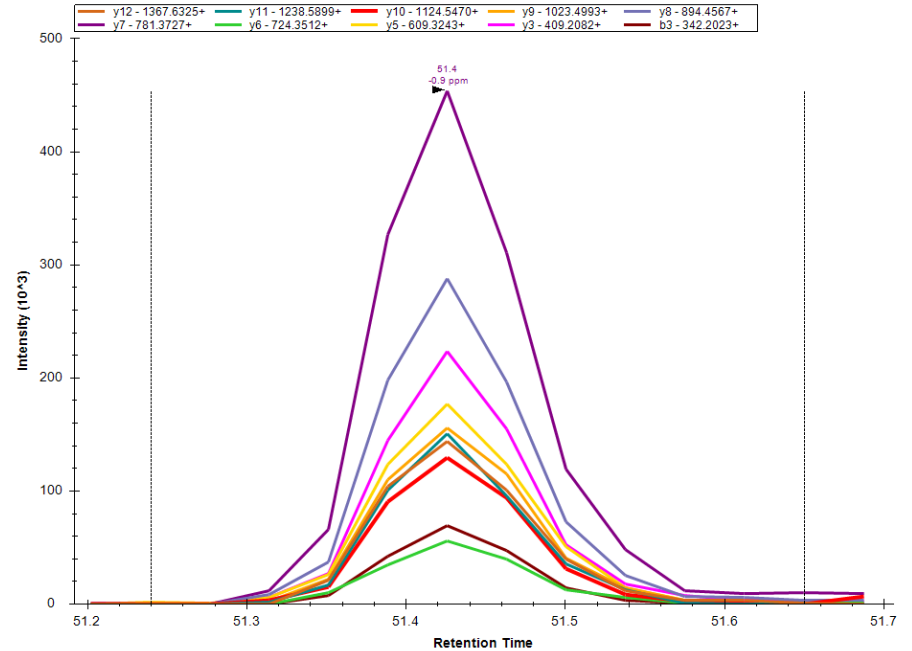
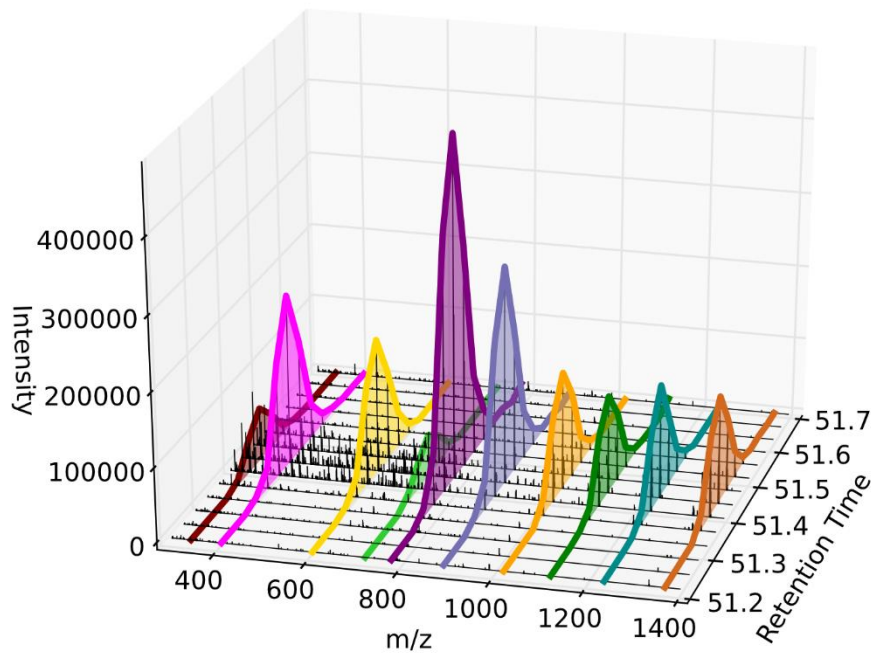
Label-Free PRM Workflow Overview



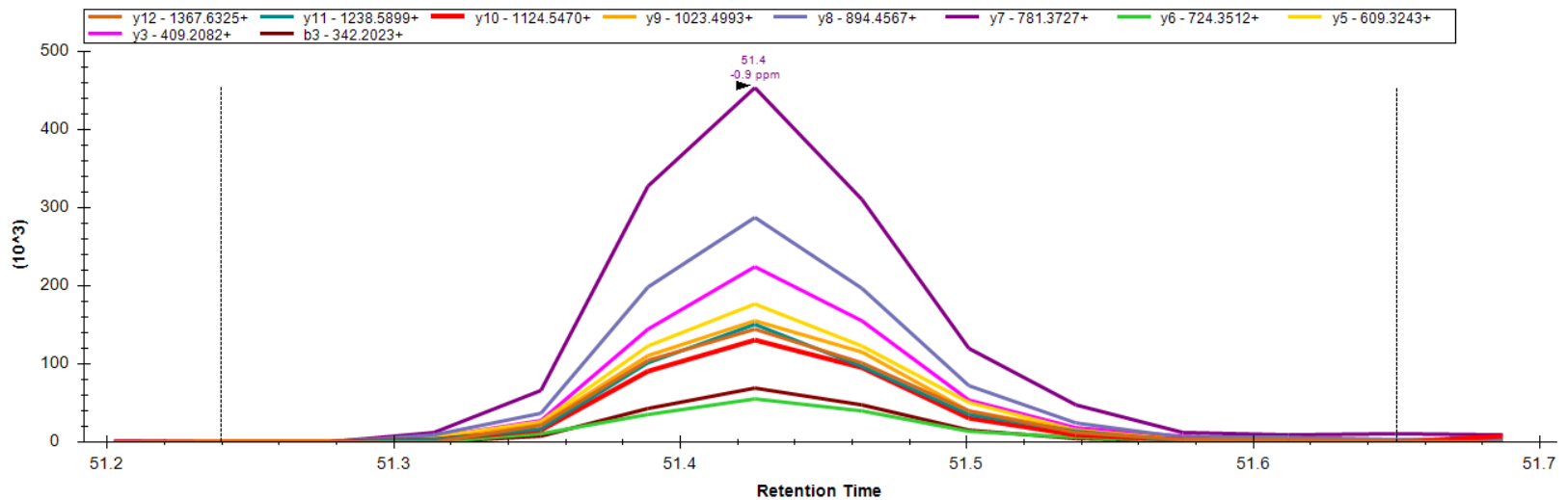
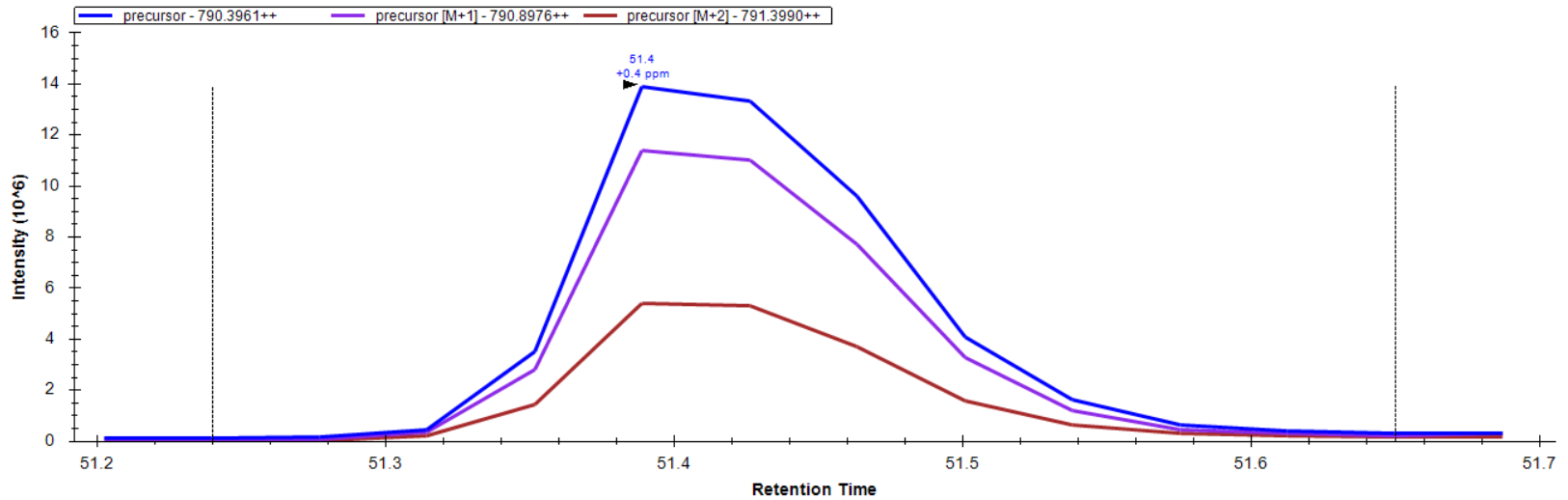
Label-Free PRM Workflow Overview



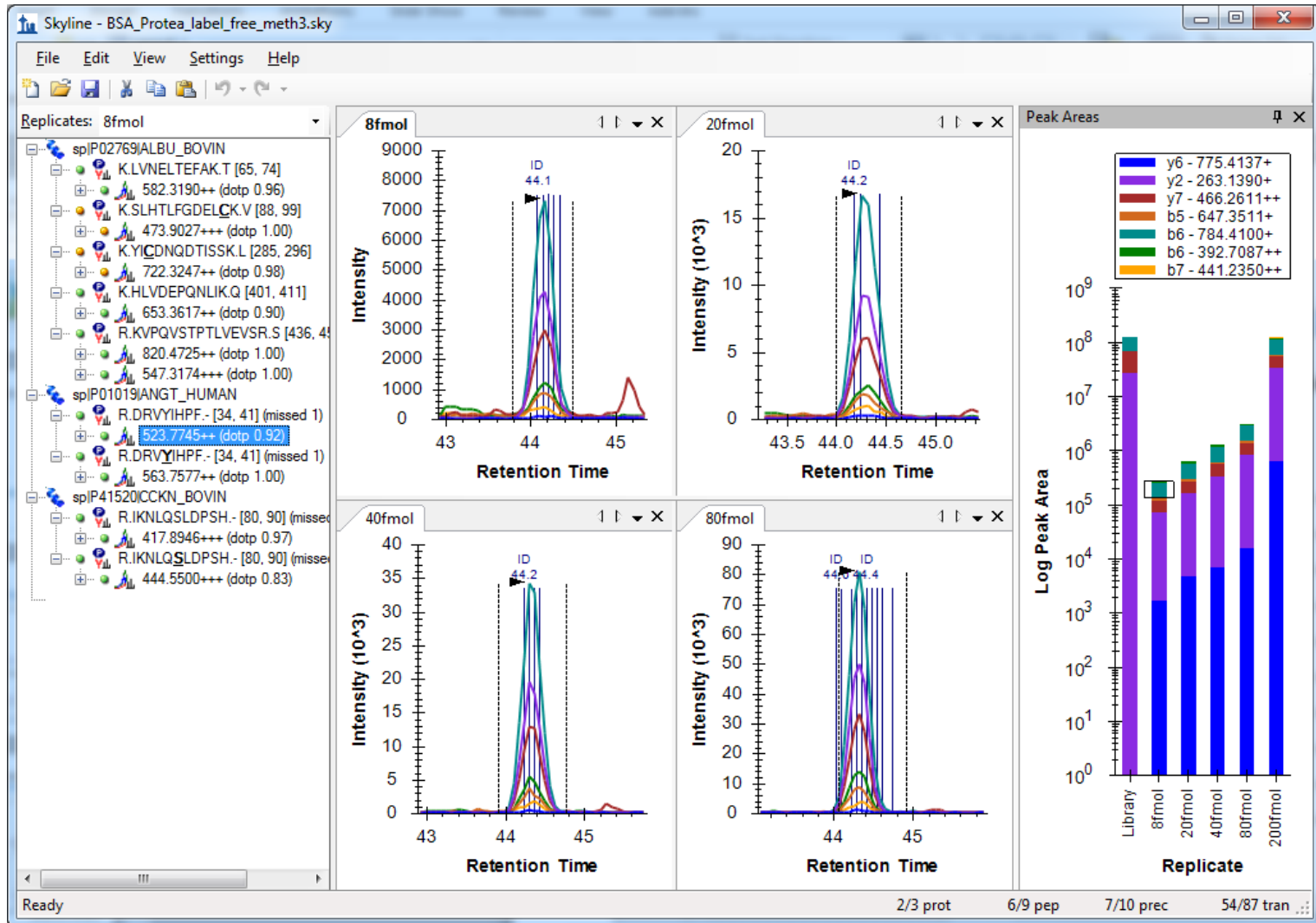
Extraction of Fragment Ions



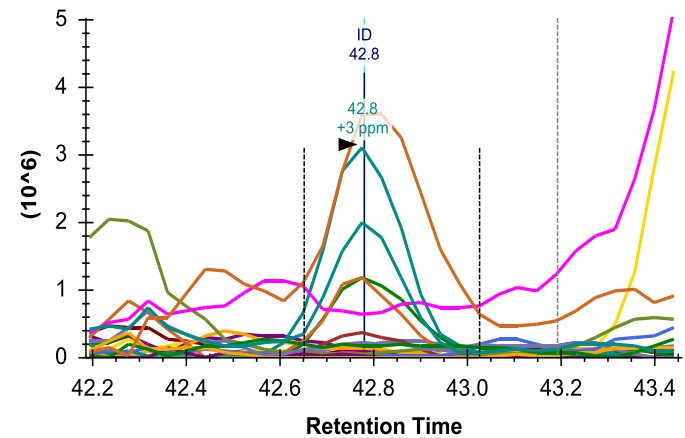
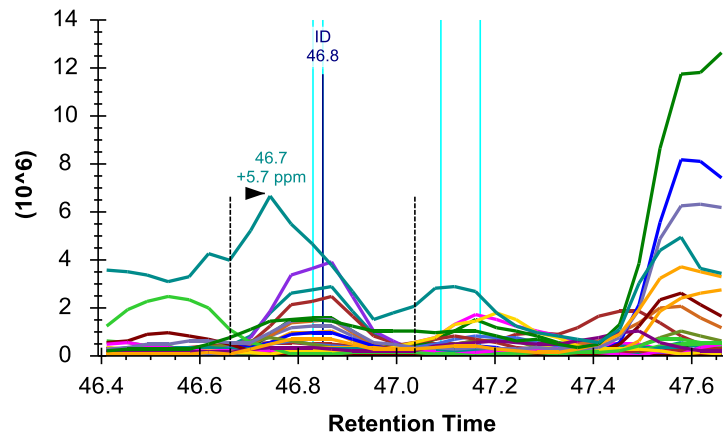
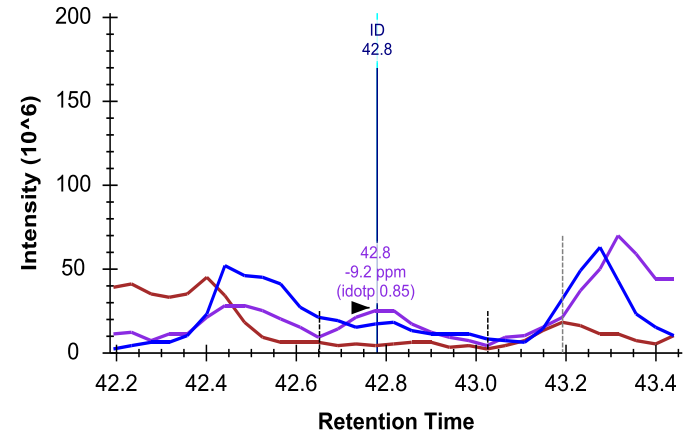
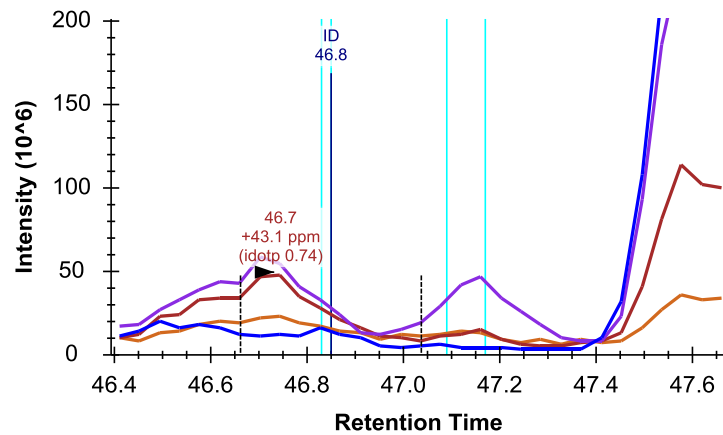
Precursor and Product Ion Information



Peptide Search MS/MS from PRM

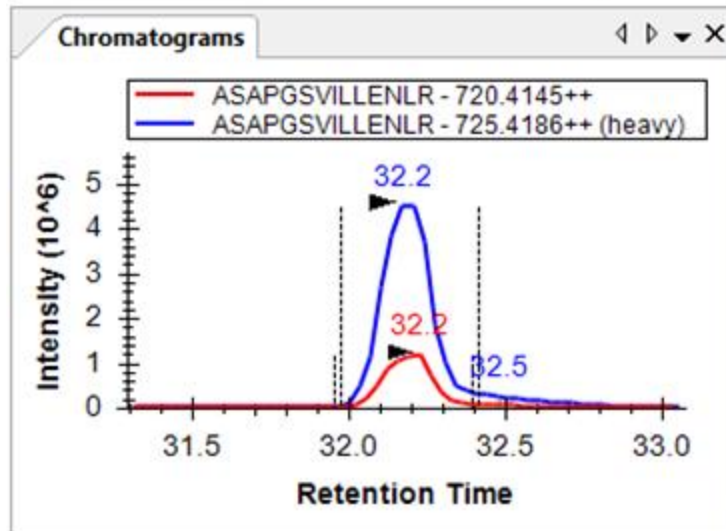
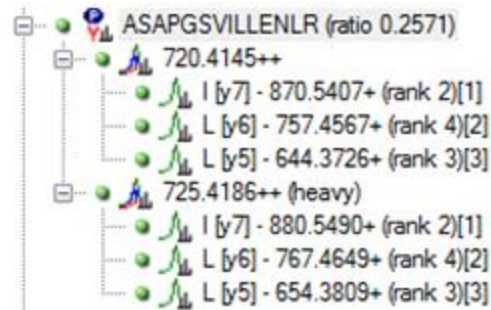


Proving Peak ID in Label-Free PRM



PRM with Labeled Internal Standards

► Extensive support in Skyline



Edit Heavy Modification

Name: Heavy R

Amino acid: R

Terminus:

Chemical formula

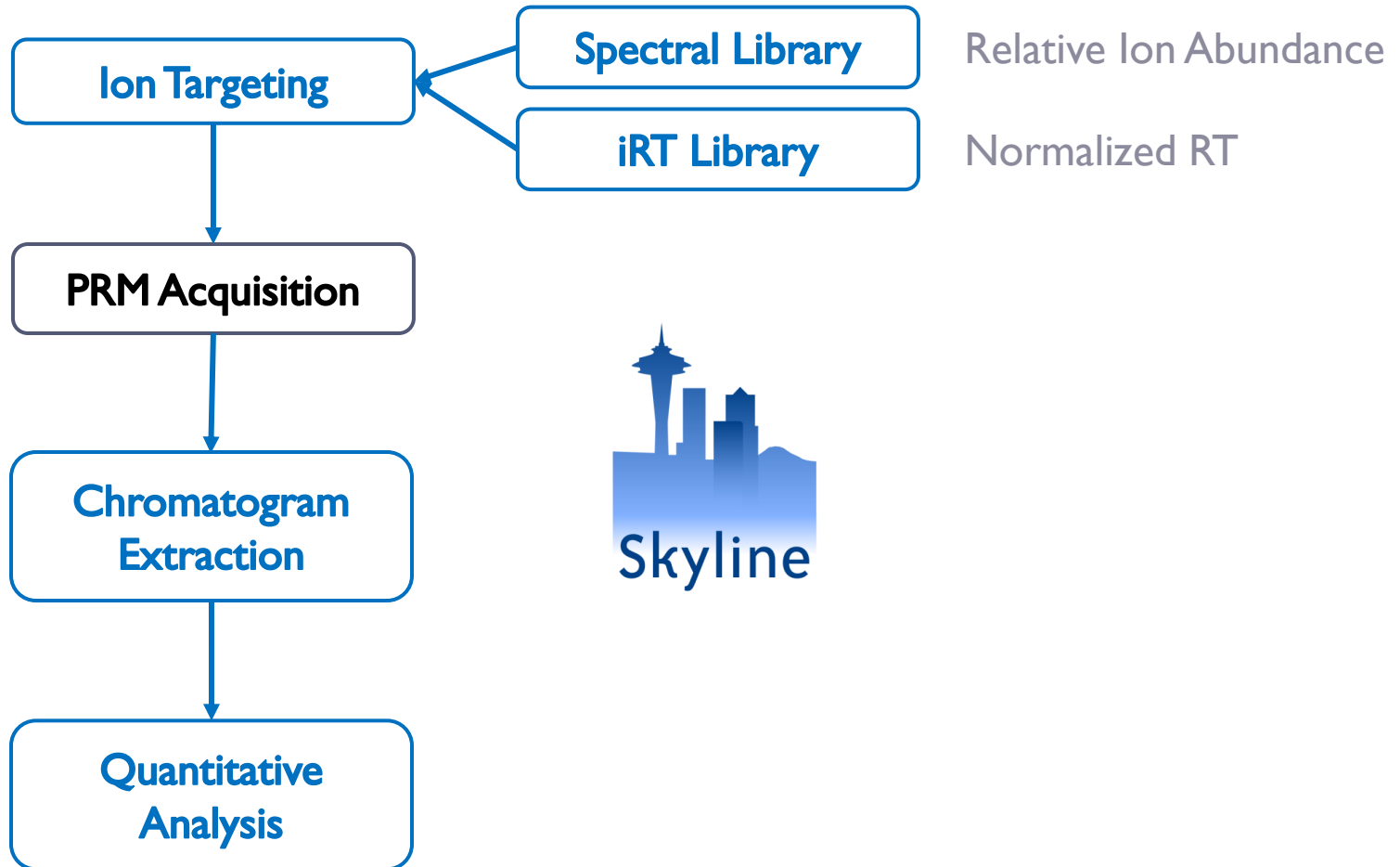
13C 15N 18O 2H

Monoisotopic Mass: 10.008269

Average Mass: 9.928665

OK Cancel

Labeled PRM Workflow Overview



Tutorial

- ▶ **Basic PRM Data Analysis**



Learn More

- ▶ Webinar #4: Tuesday, Feb. 10th – Targeted Method Design
- ▶ Workshops at US HUPO and ASMS
- ▶ Weeklong Course in Northeastern University, Boston
 - ▶ May 18-22
- ▶ Weeklong Course at ETH, Zurich
 - ▶ June 22-26
- ▶ Weeklong Course at PRBB, Barcelona
 - ▶ November 15-20
- ▶ More... TBA



Questions?

- ▶ Ask any questions you have on the topic of PRM experiments with Skyline.

<http://tinyurl.com/QA4Skyline>

- ▶ Take the post-webinar survey:

<http://tinyurl.com/Survey4Webinar>





Skyline Tutorial Webinar #3

This ends this Skyline Tutorial Webinar.

Please give us feedback on the webinar at the following survey:

<http://tinyurl.com/ocbnzfv>

A recording of today's meeting will be available shortly at the Skyline website.

We look forward to seeing you at a future Skyline Tutorial Webinar.

