Agenda

- Welcome from the Skyline team!
- Getting the most out of DDA Data with Brendan MacLean
  - Workflow overview
  - Keys to successful MS1 filtering in Skyline
  - Live tutorial

- A Scientist’s View: Using Skyline for MS1 Filtering with Birgit Schilling – Researcher, Author and Skyline user

Chromatography-based Quantification

- Hypothesis testing (Verification)

- SRM

- **MS1 chromatogram extraction**

- Targeted MS/MS (PRM)

- Data independent acquisition (DIA/SWATH)

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<th>Acquisition</th>
<th>Targeted</th>
<th>Survey</th>
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<td>More Selective</td>
<td>Targeted-MS/MS</td>
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<td>SRM</td>
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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

DDA Acquisition

Spectrum Matching
Overview

DDA Acquisition

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

DDA Acquisition

Spectrum Matching

Chromatogram Extraction

Spectral Library Building

Skyline
MS1 Chromatogram Extraction

ABRFSPRGPEPTIDESR
MS1 Chromatogram Extraction in Skyline

Multiple Instrument Vendors

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

*THE SCIENCE OF WHAT’S POSSIBLE.*
Overview

Got HYPOTHESIS??

Chromatogram Extraction

Spectral Library Building

Product Ion Targeting

iRT Library Building

Quantitative Analysis

Skyline

DDA Acquisition

Spectrum Matching
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. 18th – 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.
  

- Take the post-webinar survey:
  