Skyline: Targeted Proteomics with Extracted Ion Chromatograms from Full-Scan Mass Spectra

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http://proteome.gs.washington.edu/software/skyline

Overview:

The freely-available Skyline Targeted Proteomics Environment is becoming the standard tool for targeted proteomics investigation, with its support for instruments from all major triple quadrupole vendors and its broad support for spectral libraries. Until this year, however, its use for quantitative experiments has been limited to selected reaction monitoring (SRM) on triple quadrupole mass spectrometers. More recently, Skyline has been extended in version 1.1 to support bulk extraction of ion chromatograms from both MS1 and MS/MS full-scan spectra into the now familiar protein- and peptide-centric Skyline user interface. A wide range of new experimental applications are enabled, from targeted full-scan MS/MS acquisition to post-peptide search analysis of data independent acquisition MS/MS or even standard MS1 scans from shotgun experiments.

Introduction:

Until now, targeted proteomics applications have focused almost exclusively on triple quadrupole instruments, because of their high sensitivity and quantitative accuracy in measuring a small number of product ions per peptide precursor. While quantitative tools exist for the analysis of ion trap (IT) and time of flight (TOF) data, few are as comprehensive in their feature sets or as easy to use as Skyline. Barriers remain in the transition from shot-gun, discovery experiments performed with one set of instruments and tools to targeted experiments performed with different instruments and tools.

To address these issues and give proteomics investigators powerful new options for targeted experiments and targeted analysis of shotgun data, we have implemented new features for extracting time, intensity chromatograms from MS1 and MS/MS data from the IT and Q-TOF instruments of:

- AB SCIEX
- Waters
- Thermo Fisher

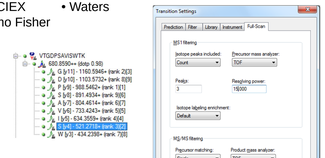


Figure 1: A targeted peptide (above) and the full-scan settings form (right) in Skyline, configured to filter both MS1 and MS/MS scans from Q-TOF data

Methods:

The method implemented in Skyline version 1.1 extracts time, intensity chromatograms, as shown in Figure 2a, b and c, from the full mass spectra of TOF and IT instruments.

This has been tested on a range of instruments using calibration curves. We have also gathered a complete set of calibration curve data from both triple quadrupole and full-scan instruments, which we intend to analyze fully in the coming months.

Figure 2a: Schematic of a Q-TOF instrument, depicting all product ions of a specific precursor being measured in the TOF mass analyzer.

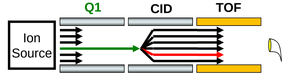


Figure 2b: Time-intensity chromatogram extraction of a single product ion is performed in-situ, from collected full-scan data.

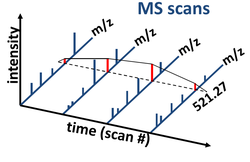
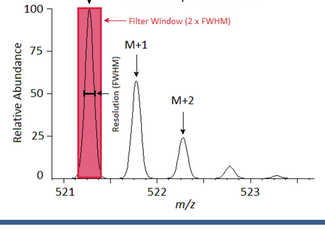


Figure 2c: High-resolution data allow more specific filtering of ions, and separation of individual peaks in the isotope distribution. Skyline sums intensities within a single resolution to either side of the predicted mass-to-charge ratio.



Results:

Figure 3: Skyline full-scan settings and imported data from a targeted MS/MS calibration curve experiment run on a Thermo Fisher LTQ, low resolution instrument. The precursor ion, in blue, is filtered from MS1 scans taken at the end of each cycle. At low intensity, interference becomes an issue for the precursor in the MS1 scans at this low resolution, while the product ions filtered from MS/MS remain adequately selective.

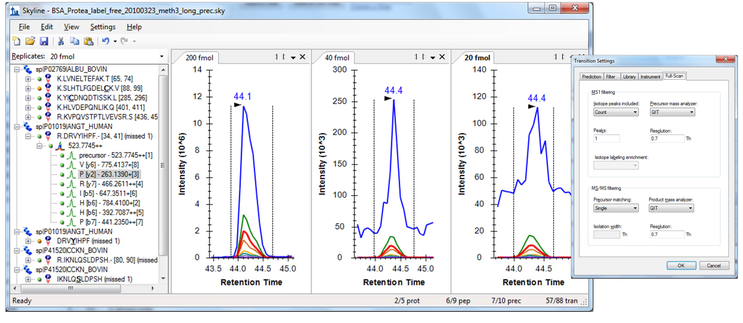
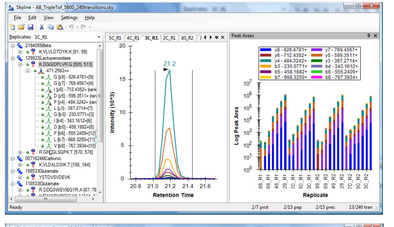


Figure 4: Skyline full-scan settings and imported data from a multiplatform calibration curve experiment exploring targeted MS/MS versus SRM. Data shown here are from AB SCIEX 5600 Triple TOF (top) and Waters Synapt G2 (bottom).



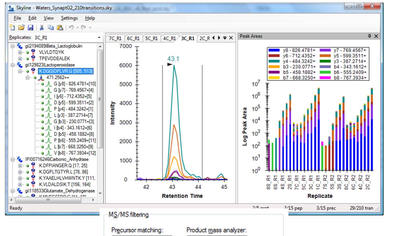
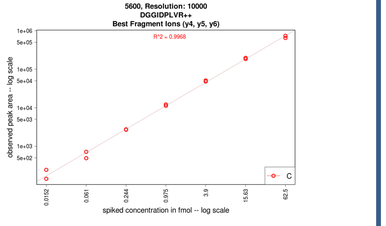


Figure 6a & b: Plots showing linearity of response (top) and peak area consistency (bottom) in targeted MS/MS on the AB SCIEX 5600 Triple TOF.



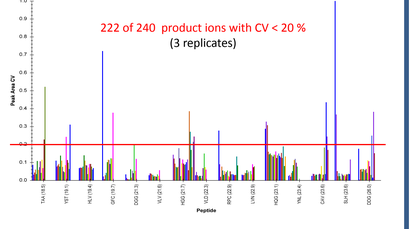


Figure 4: Skyline full-scan settings and imported data from the MS1 scans of a calibration curve experiment run on a Thermo Fisher LTQ-FT at 50,000 resolving power, using data dependent acquisition of MS/MS scans. In this high resolution data, peaks of the precursor isotope distribution are filtered into separate chromatograms for greater selectivity and peak identity confirmation without product ion chromatograms.

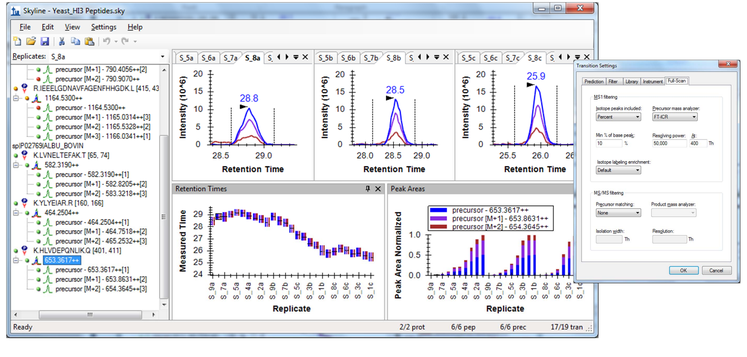
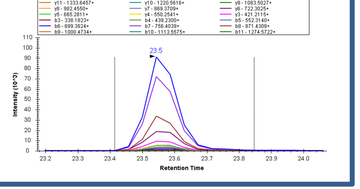


Figure 5: Adding precursor isotope peaks from MS1 can add information. Here the monoisotopic, M+1 and M+2 peaks for the peptide SLHTLFGDELCK coelute with product ion peaks.



Conclusions:

- New chromatogram extraction features in Skyline support a variety of new approaches to targeted and quantitative proteomics analysis using TOF and IT instruments.
- In depth statistical analysis of acquired calibration data is required to determine trade-offs in instrumentation choices.

Future work includes:

- Statistical analysis of calibration curves between triple quadrupole and full-scan instruments
- Method export for Waters and AB SCIEX
- Support overlapping isotope envelopes in MS1
- Integrate MS/MS peptide identification results

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