Automated Creation and Refinement of Complex Scheduled SRM Methods for Targeted Proteomics

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Overview:
Selected Reaction Monitoring (SRM) is a technique widely used for the quantitative measurement of target compounds in complex mixtures. Increasingly it is being used for the hypothesis driven analysis of protein differences across large numbers of biological samples. One difficulty in making targeted proteomics routine is the complexity and labor involved in producing an optimized instrument method that measures many target peptides in a single analysis. Unfortunately the generation of these methods and their refinement is still largely a manual process. We have developed the software program Skyline that greatly shortens the path from hypothesis to a fully optimized instrument method.

Introduction:
Skyline presents an intuitive user interface for working with proteins, peptides and fragment ions, with rich support for in silico digestion and transition prediction. Using ProteoWizard[1] to fully support instruments from:
- Agilent
- Applied Biosystems
- Thermo Fisher
- Waters

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Methods:
Initial Complex Method:
- 312 Peptides with matching MS/MS library spectra
- 2688 Transitions (y3 – y N-1)
- 5S Sample injections

Monitoring 313 Peptides by SRM:
The resulting 55 Thermo RAW files were imported into the original Skyline document, beginning the process of refinement.

Figure 5: The unrefined method with transitions ordered by intensity or the matching MZTab2 [2] library spectra

Refining for measurable peptides:
The Skyline document editor proved effective for further refining the list. Within ½ hour, the list had been reduced to the three best transitions for 135 peptides with clear signal.

Figure 6: The editable tree view, combined with the chromatogram and MS/MS library spectrum views

Descriptive statistics
- 2908 Transitions (y3 – y N-1)
- 55 Sample injections

Results:
Peptide modifications
- Peptide modifications

Perfect Rank Correlation
- Perfect rank correlation

Scheduling:
- For a final scheduled method we chose the constraints:
  - 1.5 second cycle time
  - 70 concurrent transitions maximum at any time
  - >20ms dwell time

Figure 7: Skyline shows that the chosen constraints will present the form for creating the lists.

Further Refinement:
After running 2 sample injections unscheduled on a new column, the list was reduced to 121 peptides, but had to be further reduced to 105 peptides due to an unexpected decrease in signal. The Skyline method editor was used to order the list and add a 4 minute window, over 4 more replicates to test for stable chromatography.

Figure 8: Skyline radically streamlines the process of refining complex SRM methods for targeted proteomics experiments.

Conclusions:
- Skyline radically streamlines the process of refining complex SRM methods for targeted proteomics experiments.
- The streamlining allows optimized methods to be created by refining initial broad measurements in an actual biological matrix.
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References:

Preparation for a Quantitative Assay:
With 105 proven valid peptides, and stable chromatography that will allow us to further reduce the scheduling window to 2 minutes, we are ready to order peptides for a high-repetition quantitative assay.

Figure 2: The report editor helped create a comma separated value (CSV) report for further statistical analysis before choosing the final scheduling window.

Figure 3: The report editor helped create a comma separated value (CSV) report for further statistical analysis before choosing the final scheduling window.

Figure 4: The report editor helped create a comma separated value (CSV) report for further statistical analysis before choosing the final scheduling window.

Figure 5: The report editor helped create a comma separated value (CSV) report for further statistical analysis before choosing the final scheduling window.

Figure 6: The report editor helped create a comma separated value (CSV) report for further statistical analysis before choosing the final scheduling window.