Understanding the Square-Wave Nature of Q1 Isolation for Data-Independent SWATH® Acquisition

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ABSTRACT

Data-Independent SWATH® Acquisition has become a popular method of analysis due primarily to its ability to robustly quantify large numbers of analytes in complex biological samples. In this work, signal intensity across the full width of the SWATH® Q1 window is investigated to determine whether there is a decrease in signal intensity near the boundaries. By staggering the m/z value at which the fixed-width SWATH® windows start across a set of replicate analyses, each peptide will be in different positions relative to the boundary in each experimental condition. The data shown here demonstrates that signals for individual peptides are consistent except within 0.5 Da of the SWATH® window boundary. These results reinforce the practice of acquiring data using a strategy that overlaps SWATH® windows by 1 Da to ensure data fidelity. Under these conditions, there should be no unintentional bias for peptides located near a SWATH® Q1 window boundary when migrating from fixed-window to variable-window SWATH® acquisition.

INTRODUCTION

Due to its ability to robustly quantify large numbers of analytes in complex biological samples, Data-Independent SWATH® Acquisition has become a popular method of analysis.[1] Initial execution of SWATH® acquisition used equal size "steps" across the m/z space to profile samples, covering the entire m/z range of interest on a chromatographic time scale (see Figure 1). Recent advances in SWATH® data acquisition allow for the Q1 isolation window size to vary. In this variable-window SWATH® approach,[2] smaller windows increase selectivity in precursor dense regions of m/z (see Figure 2). In going from fixed-window SWATH® to variable-window SWATH® acquisition, many precursor positions will vary relative to the boundaries. When this happens, a bias could be introduced due to the position of the precursor if quadrupole transmission is non-uniform.

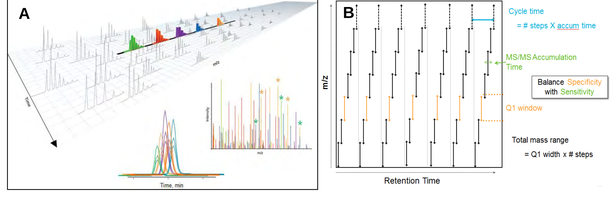

Figure 1. SWATH® Acquisition. The SWATH data acquisition strategy (A) utilizes a wider Q1 window to isolate multiple precursor ions which are simultaneously fragmented in Q2. Fragment ions are collected at high resolution in the TOF analyzer. After data collection, known fragment ions for each peptide (different colors in part A) are extracted as XIC's for quantitation. The Q1 window size in SWATH® acquisition (B) is a balance between specificity (Q1 window width in orange) and sensitivity (accumulation time in green) needed for a cycle time (blue) allowing multiple measurements across the chromatographic peak.

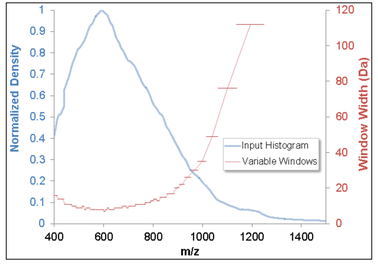

Figure 2. Variable Window SWATH® Acquisition. To achieve better specificity in complex matrices, smaller Q1 windows are desirable especially in the m/z dense regions where many peptide precursors are measured (around m/z 600). The m/z density histograms constructed from the TOF MS data for the proteome of interest (blue line) can be used to construct variable sized windows, where the density of precursors in each of the isolation windows is equalized across the m/z range. Note that the width of the window varies from less than 10 Da to over 100 Da.

MATERIALS AND METHODS

Data Acquisition: Trypsin digests of whole cell lysates from HeLa cells are analyzed in triplicate using five different SWATH® acquisition methods. Each method covers 200 Da of m/z space between approximately m/z 500 and 700 with ten 20-Da steps. The five methods start at m/z 500, 504, 508, 512, and 516, respectively and the usual 1 Da overlap was not used for this testing (built using the variable window import feature). In addition to the data-independent MS/MS scans, a TOFMS scan covering m/z 400-800 is included for each acquisition cycle. The full cycle will be acquired in 1.3 seconds (250 ms for TOFMS, and 100 ms for each of 10 SWATH® MS/MS scans) to ensure robust quantitation across each chromatographic peak. The nanoLC gradient of 60-minutes provides sufficient separation and 15- to 20-second wide peaks.

Data Processing: All SWATH® data was manually processed using SWATH® Software 2.0 and ratios were computed in Excel. Based on the way the data is acquired, each peptide precursor monoisotopic m/z will fall within 2 Da of a SWATH® window boundary in one of the five acquisition methods. In the other four, the same peptide will be at least 4 Da away from any boundary. The measurement from the "near boundary" method is compared to the other four "non-boundary" measurements. Since the TOFMS scan is acquired for each peptide along with the SWATH® data, it can be used to normalize the quantitative result within each result file (red points in Figure 5). Ninety-three peptides of modest to high precursor ion intensity with three, four, or five fragment ions measured in all 15 data files are chosen as representative peptides for this study to reduce interference introduced in the TOFMS data used for normalization.

RESULTS

Data acquired using SWATH® acquisition are interpreted based on prior knowledge of the fragmentation spectrum of the precursor. When a TOFMS spectrum is acquired along with the data-independent MS/MS data, the precursor and fragment ions will display correlated extracted ion chromatograms (XICs). An example of this correlation is shown in Figure 3. The specificity gained by utilizing MS/MS, rather than TOFMS, data for quantitation is shown in the cleaner baseline and elimination of the peak at 43.7 minutes in the TOFMS data (and has been reported in other work[1]). Figure 5A shows a bias when the precursor m/z is just above the low-mass limit for Q1. For non-boundary conditions (Figure 5B), this quantitative bias is not observed.

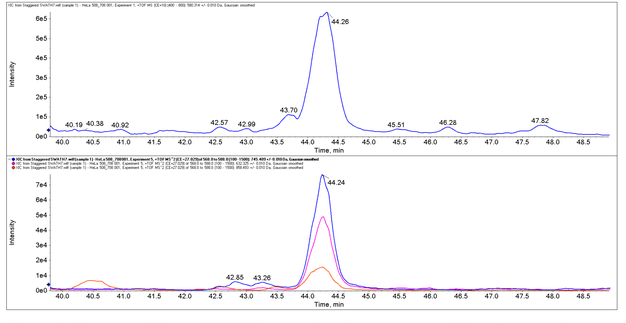

Figure 3. Comparing MS and MS/MS Extracted Ion Chromatograms (XICs). Precursor (top) and fragment ion (bottom) extracted ion chromatograms for peptide VDSLLENLEK were extracted from the SWATH acquisition data. Precursor m/z is 580.314 for the doubly-charged precursor (top), while m/z for the fragments (bottom) are 632.325 (y5, pink), 745.409 (y6, blue) and 858.493 (y7, red). Notice that the precursor and fragment ions overlap in LC time, confirming they belong to the same peptide.

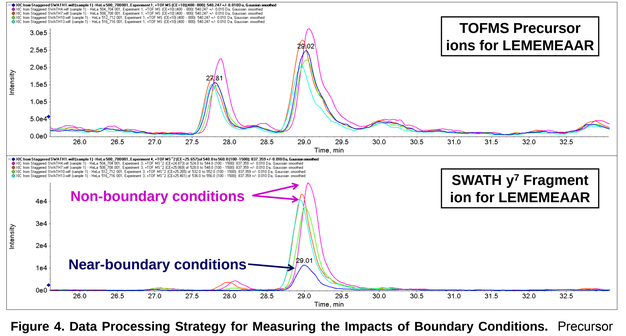

Figure 4. Data Processing Strategy for Measuring the Impacts of Boundary Conditions. Precursor (top, m/z 540.247) and fragment ion (bottom, m/z 837.359) extracted ion chromatograms for peptide LEMEMEAAR acquired using five different SWATH acquisition methods (5 separate LC injections). Note that for the precursor ions, all XIC profiles are approximately equivalent, while in the SWATH data, the near boundary condition (blue trace) shows a distinctly smaller peak area than the other four non-boundary conditions. The ratio of near-boundary to non-boundary conditions is plotted in Figure 5.

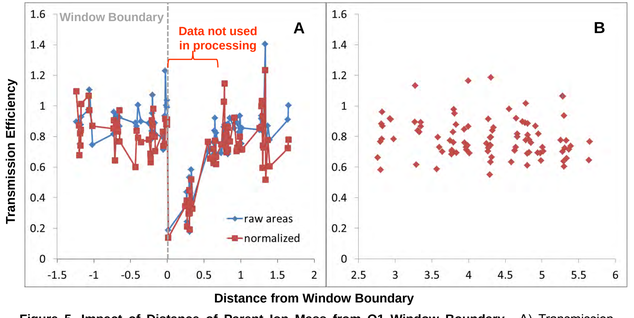

Figure 5. Impact of Distance of Parent Ion Mass from Q1 Window Boundary. A) Transmission efficiency is the fragment ion peak area ratio of near-boundary value divided by the average of the 4 other "non-boundary" conditions. This was computed for 93 representative peptides plotted against their distance in m/z from the edge of a Q1 isolation window (zero on the x-axis). B) Normalized peak area ratios for the same 93 peptides comparing one "non-boundary" condition to the average of three other "non-boundary" conditions. Note during data processing, when precursor mass lies in the 1Da overlap between Q1 windows, the quantitation is done from the end of the first window rather than the beginning of the second window (unless the precursor is right on the very edge of the first window and cut-off)

CONCLUSIONS

An underlying assumption in the data-independent SWATH® acquisition strategy is that a peptide's precursor m/z position relative to the boundaries of the Q1 isolation window has negligible impact on the quantitative measurement of that peptide. The results shown here indicate that employing the standard practice of a 1-Da overlap of SWATH windows ensures there is no quantitative bias in the resulting data. There is a small amount of reduced transmission at the very front of the Q1 transmission window but this is not used during data processing with SWATH® 2.0 and OneOmics™ processing tools.

REFERENCES

1. Gillet, LC; Navarro, P; Tate, S; Rost, H; Selevesek, N; Bonner, R; Aebersold, R. Targeted Data Extraction of the MS/MS Spectra Generated by Data Independent Acquisition: a New Concept for Consistent and Accurate Proteome Analysis. Molecular Cellular Proteomics (2012) 11, 1-17.
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TRADEMARKS/LICENSING

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