Exploring Impact of Dynamic Accumulation for Improving MS/MS Quality of QqTOF Data

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INTRODUCTION

Recent innovations in QqTOF instrumentation has resulted in a large increase in MS and MS/MS acquisition speed providing deeper coverage of complex proteomes. In data dependent acquisition, selection and timing of MS/MS acquisition is triggered when a precursor mass surpasses a minimum threshold. At constant speed, this can create a dataset with a range of MS/MS spectral quality. Some workflows such as SCIEX iTRAQ® reagent quantitation or PTM characterization can benefit from higher spectral quality. Here an acquisition strategy that uses precursor intensity to adapt the MS/MS accumulation time was explored for its utility in improving these proteomic datasets.

To understand and optimize the dynamic accumulation (DA) workflow, a range of acquisition rates and precursor intensity combinations were explored using standard proteome samples. The optimized workflow was then applied to a range of proteomic samples to characterize impact.

MATERIALS AND METHODS

Sample Preparation: A E.*coli* tryptic digest was reduced, alkylated and digested for MS analysis. A portion of the digest was then labeled with iTRAQ® reagents 8-plex (SCIEX) and then mixed either a 1:1 ratios or with a mixed set of ratios.

Chromatography: Separation of a trypsin digest of complex proteomes was performed on an nanoLC System (Eksigent) using either a cHiPLC® system (Eksigent) in trap-elute mode or a capillary column in direct inject mode.

Mass Spectrometry: The MS analysis was performed on a TripleTOF® 5600+ and 6600 system (AB SCIEX) equipped with a NanoSpray® Source. Data collection was performed with Analyst® TF Software 1.7 beta, using a data dependent acquisition and a variety of dynamic accumulation acquisition strategies.

Data Processing: Proteins were identified using ProteinPilot™ Software and results were assessed using Excel tools.

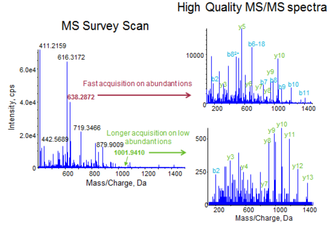


Figure 1. Dynamic Accumulation of MS/MS Spectra on a QqTOF Instrument. Biological samples have a broad dynamic range of analytes, therefore dynamically adjusting the acquisition time for MS/MS on these targets can provide improved data quality. The TOF MS intensity of the selected precursor is measured and used to scale the accumulation time for the MS/MS, low level precursors will be allocated more accumulation time vs. higher level precursors. The number of precursors per cycle will by dynamically adjusted such that the cycle time stays constant, to maintain quantitative quality of the experiment. This function can be used in combination with the other data dependent features such as rolling collision energy, dynamic background subtraction, multiple mass defect scanning, etc.

Improving MS/MS Spectral Quality

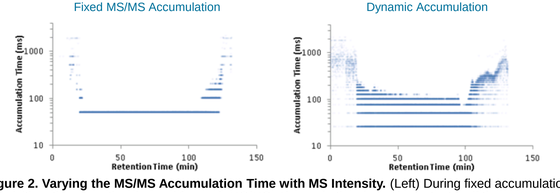


Figure 2. Varying the MS/MS Accumulation Time with MS Intensity. (Left) During fixed accumulation acquisition, the same time is used for each MS/MS. The cycle time is held constant so if there are not enough precursor per cycle as seen on the edges of the elution profile, fewer precursors are measured with longer accumulation time. (Right) During Dynamic Accumulation, the time of every MS/MS is adjusted according to the precursor intensity, so throughout the run there is a variety of accumulation times used.

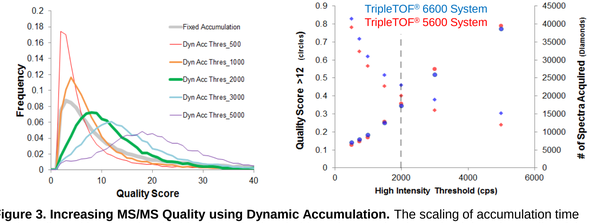
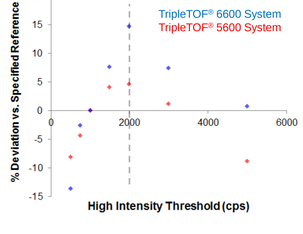


Figure 3. Increasing MS/MS Quality using Dynamic Accumulation. The scaling of accumulation time can be adjusted by changing the high intensity threshold. As that number is made higher, the quality of the spectra of the population of MS/MS increases (as more time is spent per MS/MS, Left). On the right, the median of the quality distribution was plotted vs. threshold and a steady increase in quality was observed (Circles). Also as more time is spent on the low abundant precursors as the threshold is increased, the total spectra collected per file decreases (Diamonds).

Figure 4. Tuning High Intensity Threshold for Optimal Results. Here, the high intensity threshold (above which the fastest accumulation time is performed) was adjusted and the impact on protein ID rates was assessed. On the TripleTOF® 6600 system loading 300 ng (blue), the best ID rates were obtained using the 2000 threshold (with minimum accumulation time of 25msec). In a similar experiment on the TripleTOF 5600 system loading 200 ng, a similar threshold optimum was obtained (red).



Improving iTRAQ® Reagent Results

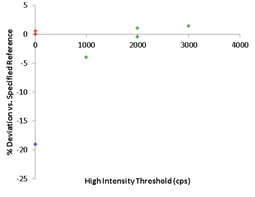


Figure 5. Dynamic Accumulation for Analysis of iTRAQ® Reagent Labeled Samples. A range of acquisition settings on the TripleTOF 5600 system were explored for the analysis of iTRAQ labeled samples using a 1:1 mixed sample and a load of 500 ng (30 min gradient). In this case, the standard IDA workflow (30 MS/MS x 50 msec per cycle, red) yielded similar ID rates to the dynamic accumulation methods (green). Sometimes for increased quantitation quality, fixed acquisition rates are slowed (15 MS/MS x 100 msec per cycle, blue), however this led to a 20% decrease in peptide IDs. For the quantitative comparison below, the standard fixed IDA was compared to the Dynamic accumulation acquisition with 3000 threshold.

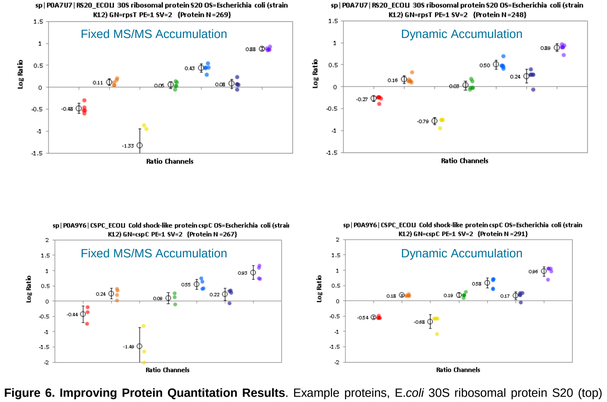
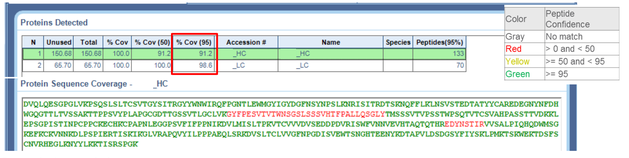


Figure 6. Improving Protein Quantitation Results. Example proteins, E.*coli* 30S ribosomal protein S20 (top) and cold shock-like protein cspC (bottom) were selected from the mixed ratio sample to assess the impact on protein level quantitation. (Left) For the fixed accumulation experiment, there was some variance observed between the peptide ratios measured for the protein, especially on the down-regulated channels. (Right) In the Dynamic Accumulation acquisition experiment, the quantitative variance was significantly reduced, showing much tighter clustering of the peptide ratios around the protein ratio.

Fixed MS/MS Accumulation



Dynamic Accumulation

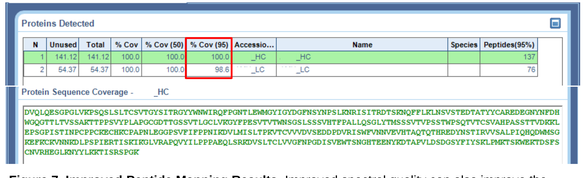


Figure 7. Improved Peptide Mapping Results. Improved spectral quality can also improve the peptide matching scores observed in a peptide mapping or database search results. Here a digest of a monoclonal antibody was analyzed with and without dynamic accumulation. The sequence coverage as for 95% confident peptides increased from 91% to 100% for heavy chain, and remained roughly the same for light chain.

CONCLUSIONS

- Biological samples have a broad dynamic range of analytes, therefore dynamically adjusting the acquisition time for MS/MS on these targets can provide improved MS/MS data quality throughout the data dependent acquisition experiment.
- Higher spectral quality can improve:
  - Quantitation in MS/MS for SCIEX iTRAQ® Reagent workflows
  - Peptide mapping sequence coverage
  - More confident ID of lower abundant species
  - SWATH™ Acquisition library generation
  - PTM characterization / localization

TRADEMARKS/LICENSING