A New Data Dependent Acquisition Algorithm (Fast DDA) for the Rapid Characterization of Complex Mixtures

GOAL

To characterize complex protein digest samples using a new data dependent acquisition algorithm.

BACKGROUND

LC/MS/MS using data dependent acquisition has been widely employed to qualitatively characterize tryptic digests and subsequently identify the constituent proteins. While many studies have been conducted and a large number of instruments have been employed using this approach, it is well recognized to have a number of serious limitations, including under-sampling, irreproducibility, lack of in-sample dynamic range, and an inability to deal with chimericy. This has resulted in the development of alternative data independent strategies, such as LC/MS^{E}, to address these issues. However, data dependent acquisition is important for the analysis of samples labeled with isobaric quantitation reagents (*i.e.* iTRAQ and TMT), which cannot be currently addressed by data independent methods. In this technology brief, we present an improved DDA method for the characterization of such proteomic samples.

Obtain consistent results between injections and more confident results for DDA acquisitions

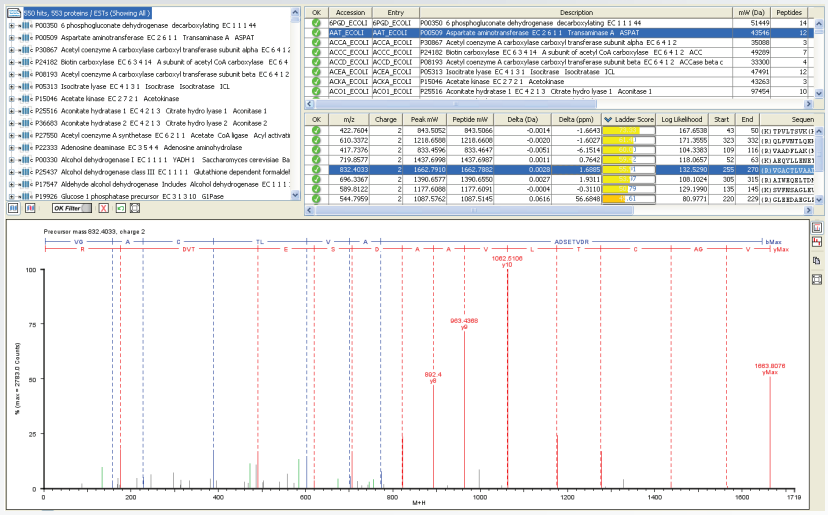

Figure 1. ProteinLynx Global SERVER™ results from the DDA analysis of 400 ng of a tryptic digest from an E.coli cytosolic fraction.

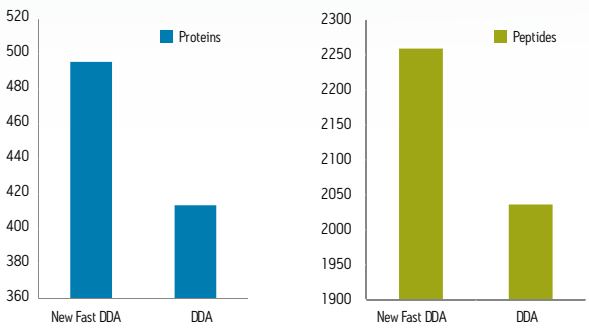

Figure 2. Summary of results at the protein and peptide levels for 400 ng of E.coli lysate run by the new Fast DDA algorithm, compared to the previous acquisition code.

THE SOLUTION

This new FastDDA algorithm includes the following features:

- Acquisition rates up to 30 MS/MS spectra per second
- Precursor selection (and exclusion) by charge state
- Rapid decision making, made during inter-scan delay
- New iTRAQ MS/MS function
- Inclusion list trigger based upon accurate mass of precursor ions

To test the performance of the new algorithm, a complex tryptic digest from the cytosolic fraction of *E.coli* was separated on a nanoACQUITY UPLC® System with an ACQUITY UPLC® BEH 1.7 µM, 75 µM x 100 mm Column. A gradient from 1% to 40% acetonitrile + 0.1% formic acid over 90 minutes was used at a flow rate of 300 nL/min. The UPLC® eluent was passed directly into the NanoFlow™ ion source of a Xevo® G2 QTof Mass Spectrometer. For all experiments, 400 ng of total protein digest were injected on column, and the FastDDA parameters that were used in these experiments are shown in Table 1. The results in terms of peptides and proteins identified for the Fast DDA algorithm are shown in Figure 1. The results obtained from the FastDDA code were then directly compared to the previous MassLynx™ Software DDA acquisition code, as shown in Figure 2.

It can clearly be seen that both the number of peptides and proteins identified by the new FastDDA algorithm is superior.

MS/MS spectra can be acquired at rates of up to 30 spectra per second, depending on the abundance of the ion. An example of this is demonstrated in Figure 3, which shows a 36 msec integration from a BSA tryptic digest. Despite the short integration time, the resolution and mass accuracy in both MS and MS/MS modes were maintained, providing highly-specific information for identification purposes.

Fast DDA parameters	
MS survey	*m/z* 330 to 1600, 0.50 s
MS survey	*m/z* 50 to 2000, 0.15 s
Number of components	Maximum of five components
Switch out	160 k counts on total ion chromatogram

Table 1. Fast DDA parameters used in this experiment.

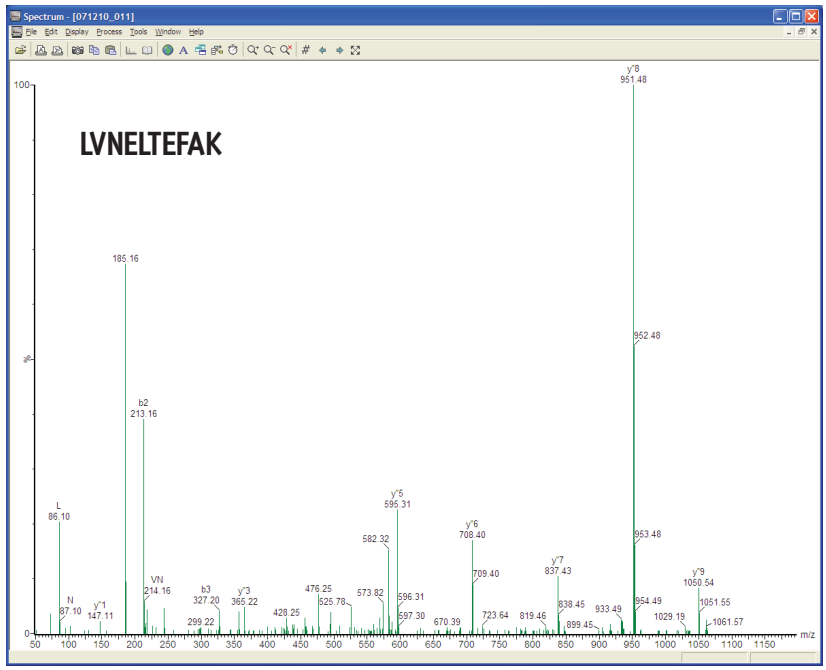

Figure 3. An example 36 msec MS/MS spectrum acquired from the FastDDA analysis of a BSA tryptic digest.

SUMMARY

Here we have described new FastDDA acquisition code. The benefits of the FastDDA acquisition code are that it delivers more consistent results between injections and it provides greater coverage of the peptides and proteins present. Together, these enable more confident results to be obtained for DDA acquisitions in proteomics laboratories.

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