A RAPID APPROACH TO THE IDENTIFICATION AND CHARACTERISATION OF TRYPTIC PEPTIDES USING HIGH LINEAR VELOCITY NANOBORE UPLC SEPARATIONS COUPLED WITH ESI MS/MS

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OVERVIEW

- Here we present a rapid LC-MS/MS method, DDA^X (Accelerated DDA), for a Q-Tof mass spectrometer
- During the course of a UPLC experiment a single MS Survey acquisition and up to 8 MS/MS acquisitions can be acquired per second
- DDA^X, in combination with nano-scale Ultra Performance Liquid Chromatography, can significantly reduce the analysis time, without any loss in protein sequence coverage

INTRODUCTION

Mass spectrometry has now firmly established itself as the primary technique for identifying proteins due to its unparalleled speed, sensitivity and specificity. Strategies can involve either analysis of the intact protein, or more commonly digestion of the protein using a specific protease that cleaves at predictable residues along the peptide backbone. This provides smaller stretches of peptide sequence that are more amenable to analysis via mass spectrometry. When coupled with protein level pre-fractionation strategies, thus reducing the complexity of the protein mixture, this approach has proven highly successful in comprehensive protein identification and characterisation. A common approach to protein pre-fractionation is the use of 1 dimensional PAGE, coupled with LC-MS/MS. The downside of this approach is the number of gel samples, or fractions, to be analysed by the LC-MS/MS system. With typical analytical HPLC run times of 45 minutes to 1 hour the amount of time required to analyses one top level sample can be prohibitive.

DDAX (Accelerated Data Directed Analysis), is a data acquisition routine for the Q-Tof Premier instrument. Using modern, state of the art electronics combined with highly optimised code, the mass spectrometer decision making process has been minimised and DDAX can acquire a single MS survey acquisition and up to 8 MS/MS acquisitions in under 1 second – top pane Figure 1.

A unique feature of this acquisition method is ion-flux control. Depending upon the intensity of the precursor ion, either 80, 160 or 240 ms of MS/MS acquisition time will be performed on the ion of interest – bottom pane Figure 1. The interscan delay is omitted with 160 or 240 ms MS/MS acquisitions, thereby saving acquisition time, and improving duty cycle respectively.

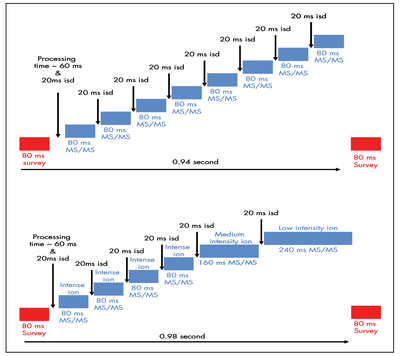

Figure 1. Schematic representation of DDA^X. The top pane illustrates a cycle with fixed MS/MS acquisition times and the lower pane a cycle with ion-flux control applied.

METHODS

nanoscale UPLC Conditions

Nanoscale UPLC separations were performed with a nanoACQUITY™ UPLC™ system (Waters Corporation), using a Symmetry® C18 5 µm, 20 mm x 180 µm trap column and a BEH C18 1.7 µm, 15 cm x 75 µm analytical reversed phase column (Waters Corporation, Milford, MA).
Samples were initially loaded & transferred with aqueous 0.1% formic acid solution to the pre-column at a flow rate of 15 µL/min for 1 min. Mobile phase A was water with 0.1% formic acid and B 0.1% formic acid in acetonitrile. The peptides were separated with a gradient of 10 to 65% B over 3 minutes at a flow rate of 900 nL/min, followed by a 1 minute rinse with 90% of mobile phase B. The column was re-equilibrated at initial conditions for 4 minutes. The total cycle time, injection to injection, was 10 minutes.

MS Conditions
DDA^X setup

The only input required is the intensity threshold of an ion in the MS mode to trigger an MS/MS acquisition, and the MS/MS acquisition time . During the course of an MS/MS acquisition, the collision energy is ramped, using a pre-defined collision energy profile, maximizing the data quality and information, whilst minimizing the time required. Deisotoping and database searching of the fragment ion spectra is automatically performed using ProteinLynx™ Global SERVER v2.2.5 software.

The Q-Tof Premier mass spectrometer was operated with a NanoLockSpray™ source. External calibration was initially performed and the data subsequently post-acquisition lock mass corrected using Glu-fibrinopeptibe B, to ensure high mass acuracy.

RESULTS

Operating the nanoACQUITY UPLC system at 900nL/min on a 15cm x 75µm column with a column temperature of 60ºC, results in a backpressure of ~9,300 psi. This allows the sample run time – including loading and re-equilibration – to be reduced to 10 min. Chromatographic peak widths at base can be 3 seconds or less, placing a significant demand on the acquisition system of the mass spectrometer . For example, a total of 27 MS and MS/MS spectra can be acquired using DDA^X, across a 3 second wide chromatographic peak.

PROTEIN STANDARDS

A typical chromatogram and MS/MS fragment ion spectrum from the separation and analysis of 20 fmol of a BSA tryptic digest are shown in Figures 2 and 3, respectively. Compared to a traditional DDA experiment, the amino acid sequence coverage typically improves by 20% and analysis time can be greatly reduced, from approximately 60 minutes down to 10 minutes.

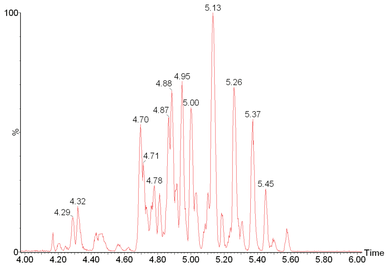

Figure 2. Nanoscale UPLC-DDA^X-MS/MS separation of 20 fmol Bovine Serum Albumin injected on column

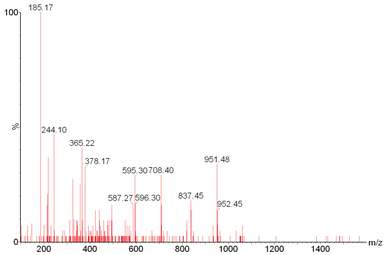

Figure 3. Single 140 msec DDA^X MS/MS spectrum of the doubly charged ion, m/z 595.82

SIMPLE PROTEIN MIXTURE

A second example is shown in Figure 4, illustrating the LC-DDA^X-MS/MS separation of 50 fmol of 4 tryptically digested protein standards in 6 min. An example spectrum from this experiment is shown in Figure 5.

Figure 6 shows the chromatograms obtained from a 2D gel spot, from the separation of a Human cell line. Figure 7 shows the protein identification of the same gel spot and the success rate across the whole 96 gel samples analysed

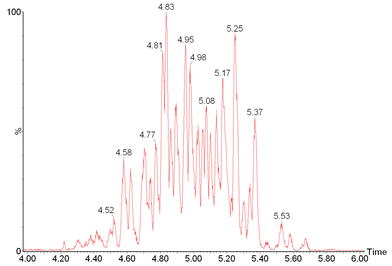

Figure 4. Nanoscale UPLC-DDA^X-MS separation of a four protein digest mixture (50fmoles per component). The protein mixture was made up of BSA, Enolase, Alcohol dehydrogenase and Phosphorylase B.

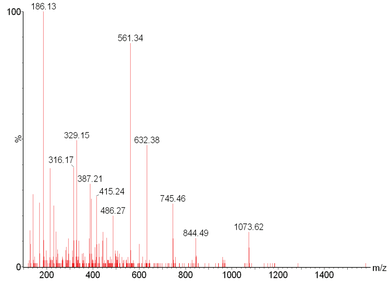

Figure 5. Single 140 msec DDA^X MS/MS spectrum of the doubly charged ion, m/z 643.84.

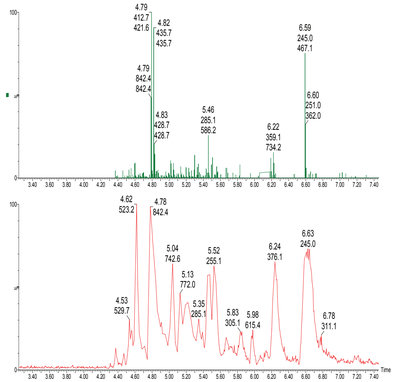

Figure 6. MS and MS/MS BPI chromatograms obtained from the analysis of a 2D gel isolated protein from a Human cell line.

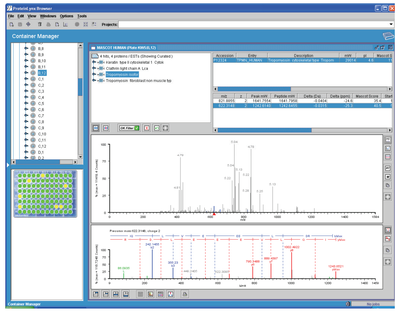

Figure 7. ProteinLynx Global SERVER identification from the LC-DDA^X-MS/MS analysis of the Human 2D gel spot shown above. The lower left pane shows the success rate across the 96 well pate. Total analysis time for 96 samples was 16 hours.

COMPLEX PROTEIN MIXTURES

An example of a complex biological mixture is shown below in Figure 8, illustrating the LC-DDA^X-MS/MS analysis of 0.1 µg of tryptically digested Yeast protein sample in 25 min. In total almost 2,000 MS/MS spectra were acquired over the 25 minute acquisition.

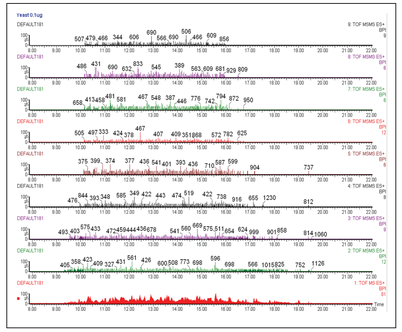

Figure 8. Nanoscale LC-DDA^X-MS/MS separation of 0.1 µg of a yeast tryptic digest

CONCLUSION

- **DDA^X allows the rapid acquisition of both MS and MS/MS data, with a single MS survey acquisition and up to 8 MS/MS acquisitions acquired in under 1second**
- **No compromise in mass spectral resolution is observed at the faster scan rates, which are required for UPLC separations over short gradients**
- **The combination of DDA^X with nanoscale UPLC enables simple protein mixtures, such as 1D and 2D gel spots, to be analysed by LC-MS/MS in under ten minutes.**

Acknowledgments
The authors would like to acknowledge Peter James (Lund University) for the supply of the Human 2D gel spots.

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