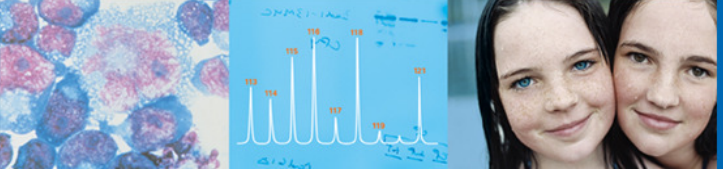

# Improved Depth of Coverage for Single Spot Analysis

## DynamicExit™ Algorithm on the AB SCIEX TOF/TOF™ 5800 System

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MALDI TOF and MALDI TOF/TOF mass spectrometry has been widely used for the identification of proteins separated either by 1- or 2-dimensional gel electrophoresis. Both separations allow the reduction in complexity of the complex mixtures by separating their components at the protein level. 1D gel electrophoresis partially reduces the complexity of samples, yielding bands of ~100 proteins. On the other hand, 2D gel electrophoresis gel separation uses an orthogonal strategy that produces less complexity per spot and makes the detection of multiple protein isoforms more feasible. In recent years, more efforts are focused on identifying the low level components separated in these gels in a more automated, and a higher throughput fashion.

There are a number of new acquisition features in the AB SCIEX TOF/TOF™ 5800 System which will provide improved protein identification results for gel spot analysis. Intelligent data acquisition software with the DynamicExit™ Algorithm maximizes spot utilization by only acquiring MSMS data until a user-defined data quality threshold is reached. A variable rate 1kHz laser increases the speed of each acquisition by collecting more shots per unit time. In addition, a continuous sample stage motion provides a further increase in acquisition efficiency (no longer moving and acquiring in single locations). These acquisition features combined with the new hardware improvements on the 5800 system provides high quality MS/MS data with minimized sample consumption, allowing deeper analysis into each sample and much faster acquisition for more gel spot analysis per unit time.

## Key Features for Gel Spot Protein Identification

- The variable rate 1000Hz laser provides ~5-7 fold increase in MS/MS acquisition speed[1].
- DynamicExit™ Algorithm acquisition further increases MS/MS acquisition speed (~2x) while maintaining high data quality by intelligently adjusting the MS/MS acquisition time per spectrum (total number of laser shots / spectrum) based on spectral quality[2].
- An EasyAccess™ Job Setup Wizard, ideal for multi-user environments and gel spot analysis.
- Programmable self-cleaning MALDI source enables regular source cleaning without breaking vacuum for increased instrument up-time.
- ProteinPilot™ software with the novel Paragon™ database searching algorithm identifies peptides from more MS/MS spectra with the ability to search for many peptide features simultaneously.

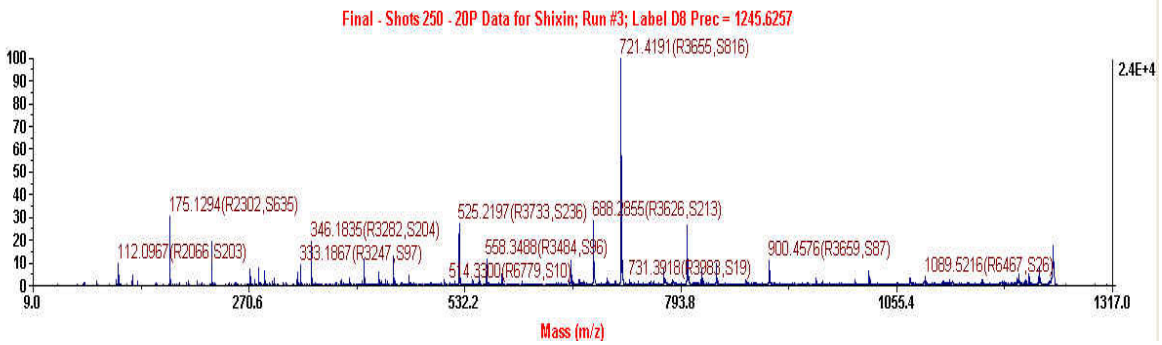


**Figure 1. High Quality MS/MS using DynamicExit™ Software.** High quality MS/MS spectrum of peptide SHGQDYLVGNR (m/z=1245.598) was acquired with DynamicExit™ Software. A good quality MS/MS spectrum was achieved after only 250 laser shots. Although a maximum of 4000 shots is set, the software stopped acquisition on this precursor after 250 shots and moved to acquire the next precursor.

## Materials and Methods

***Sample Preparation:*** A medium complexity protein mixture (digested with trypsin) was used as a model system to show the advantage of the new DynamicExit™ Algorithm. A dilution series of the peptide mixture was prepared and then mixed with α-cyano-4-hydroxycinnamic acid matrix (7.5 mg/mL, Protea Biosciences) in 1:1 ratio. Each sample (0.5µL) was then spotted onto a single spot on a standard stainless steel plate. The final amounts of total peptide in each spot were 25ng, 12.5ng, 6.25ng, 3.125ng and 1.56ng.

***Acquisition parameters:*** A survey scan was performed in MS reflector mode for a total of 1000 shots. Then a maximum of 200 precursors with signal/noise level above 10 were automatically selected for MS/MS. For the 4800 MALDI TOF/TOF™ Analyzer and AB SCIEX TOF/TOF™ 5800 system without DynamicExit Algorithm, a total of 4000 shots were acquired for each precursor in MS/MS mode. For 5800 with DynamicExit Algorithm enabled, the data quality was monitored in real time and the data acquisition was stopped once the spectral quality reached the pre-defined high quality threshold or a maximum of 4000 shots.

***Database searching:*** Each dataset was processed with ProteinPilot™ Software 3.0 using the Paragon™ Algorithm[1] and ProGroup™ Algorithm.

## Speed of Data Acquisition

The AB SCIEX TOF/TOF™ 5800 system is equipped with a new variable rate laser which operates at a maximum rate of 1 kHz. The frequency can be quickly and conveniently changed within the software. The stage is also programmed to run in continuous motion to improve the efficiency of acquisition. The total acquisition time on the 4800 for all spots was 92 minutes, but only 18 minutes on the 5800 system. The faster laser on the 5800 affords a 5-fold increase in acquisition speed for single spot analysis.

## Power of the DynamicExit™ Algorithm

Traditionally, MALDI spectra are acquired using a fixed number of laser shots for MS and MS/MS acquisition. This approach however wastes valuable acquisition time and sample by acquiring longer than necessary on high abundance peptides. This also diminishes the chances of getting good quality data on the lower abundance precursor masses. The new DynamicExit Algorithm directly addresses this by monitoring the MS/MS data quality in real time during acquisition and automatically terminating acquisition once a pre-defined MS/MS spectral quality has been reached (Figure 1). Acquisition then advances to the next precursor, minimizing sample consumption.

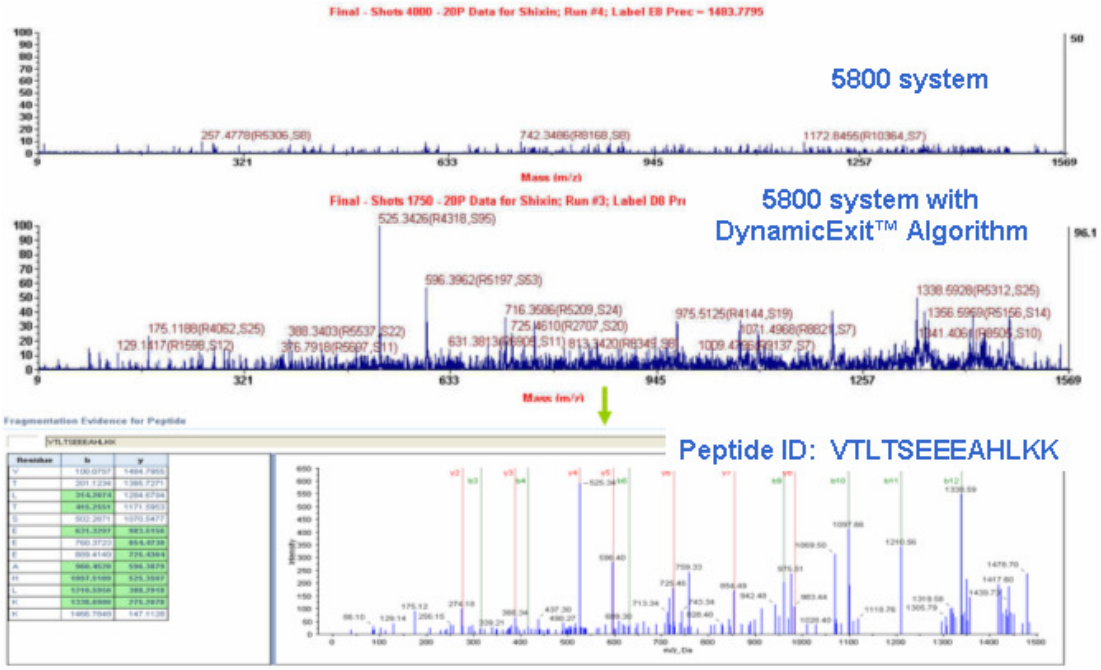


**Figure 2. Comparing MS/MS Quality of the 159th Precursor.** When intelligent data acquisition is used, higher quality MS/MS is obtained on a lower abundant precursor (middle pane) which produces a peptide identification (bottom). When a fixed MS/MS acquisition is used, the sample spot is consumed by the time the 159th precursor is reached and good MS/MS spectrum is not obtained (top). MSMS spectrum of Peptide VTLTSEEEAHLKK (m/z=1483.780).

Another way to assess the improvement in sample consumption is to investigate the spectral quality of MS/MS collected on a single spot after many MS/MS acquisitions. In Figure 2, the MS/MS of the 159$^{th}$ precursor from the 5800 system is shown for the acquisition without the DynamicExit Algorithm (top) and with the DynamicExit Algorithm (middle). The spectrum from this low abundant precursor acquired using this intelligent data acquisition strategy yielded a peptide identification (bottom) while the top spectrum did not. Sample consumption per spot is minimized with the DynamicExit Algorithm, allowing us to dig deeper into each sample spot and obtain “good enough” quality on the lower abundance peptides.

The effect can be further illustrated by plotting the total number of proteins and peptides identified from the 4800 system, or the 5800 system without and with the DynamicExit Algorithm enabled (Figure 3). As can be seen clearly from the plot, substantially more peptides and proteins were identified from TOF/TOF™ 5800 System with the DynamicExit Algorithm feature enabled. Finally, there is an additional increase in acquisition speed when using the real time spectral quality assessment (Figure 3, bottom). No time or sample is wasted with this acquisition strategy.

## Conclusions

- Coupling the faster repetition rate laser and the DynamicExit™ Algorithm, the AB SCIEX TOF/TOF™ 5800 System can obtain more protein identifications and higher sequence coverage within a shorter acquisition time.
- Reduced sample consumption means more information can be obtained from every MALDI spot, improving both gel spot and LC MALDI workflows.

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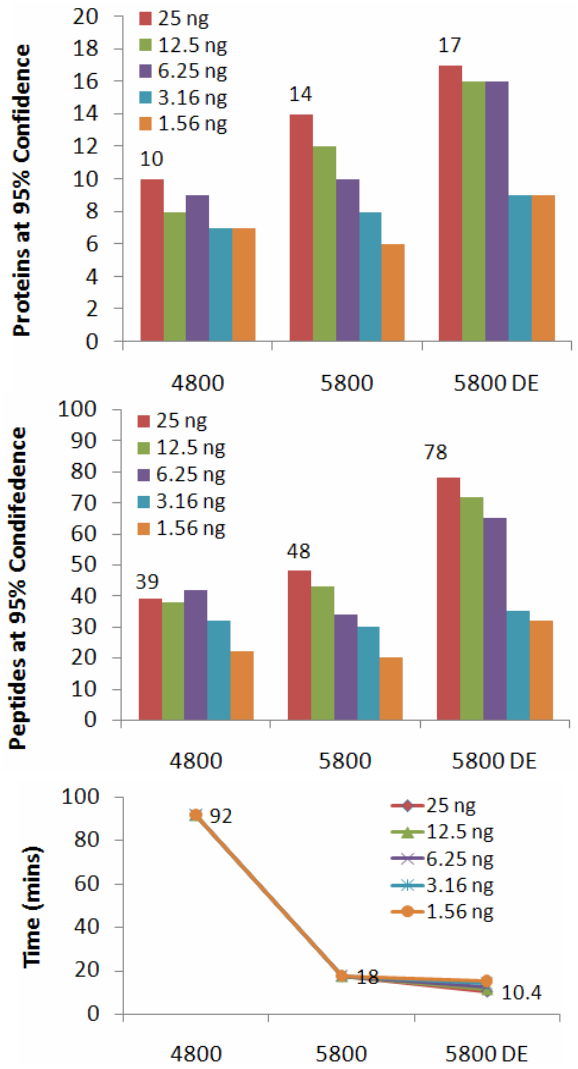


**Figure 3. Protein / Peptide IDs from a Single Spot.** DynamicExit™ Algorithm on the AB SCIEX TOF/TOF™ 5800 System provides more protein (top) and peptide IDs (middle) from a single spot in a much faster acquisition time (bottom).

## References


1. Effects of Intelligent Data Acquisition and Fast Laser Speed on Analysis of Complex Protein Digests. AB SCIEX Technical Note 2009.
2. Assessing the Complementarities of MALDI and ESI for Protein Identification in Complex Mixtures. AB SCIEX Technical Note 2009.
3. Shilov IV *et al.* (2007) *Mol. Cell Prot.* **6.9**, 1638.





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