

# High Sensitivity Peptide Sequence Identification using the 4800 MALDI TOF/TOF™ Analyzer



## Introduction

High sensitivity peptide sequencing of peptides in complex biological mixtures is essential to enable identification of low concentration components in proteomics experiments. The 4800 MALDI TOF/TOF™ Analyzer has been designed to feature laser irradiation on-axis to the ion beam path as well as improved ion optics for maximum ion transmission efficiency. The result is an instrument that delivers MS/MS data routinely at the sub-femtomole levels of peptides for definitive protein identification.

## Key Benefits

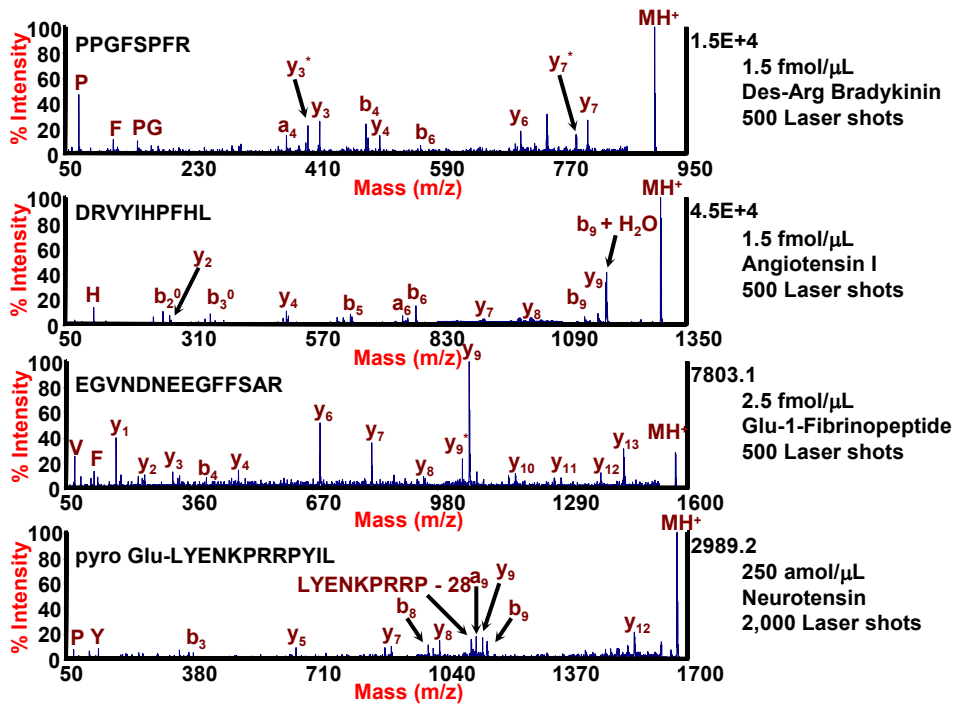
- Routine sub-femtomole MS/MS sequence information without the use of elaborate and expensive sample plate technology
- Highest sensitivity MALDI MS/MS instrument for 1D and 2D gel protein identification
- Combined with LC-MALDI and sophisticated result dependant workflows such as PTM discovery and non-redundant RDA, the 4800 MALDI TOF/TOF™ Analyzer provides the ultimate solution for protein identification and quantitation by LC-MS

## Discussion

High sensitivity MS/MS was demonstrated by analysis of two mixtures of peptides in interactive and batch modes. The first mixture comprised four synthetically pure peptides, with one of these peptides (Neurotensin) at a concentration of 250 amol/μL. The second mixture was a trypsin digest of beta-galactosidase. A serial dilution of this digest was prepared, and multiple beta-galactosidase tryptic peptides were identified by database searching of MS/MS spectra using GPS Explorer™ V3.5 Software.

A mixture of synthetically pure peptides was prepared and deposited onto a MALDI plate using a matrix of alpha-cyano-4-hydroxycinnamic acid (3.6 mg/mL) that also contained 10 mM ammonium citrate.

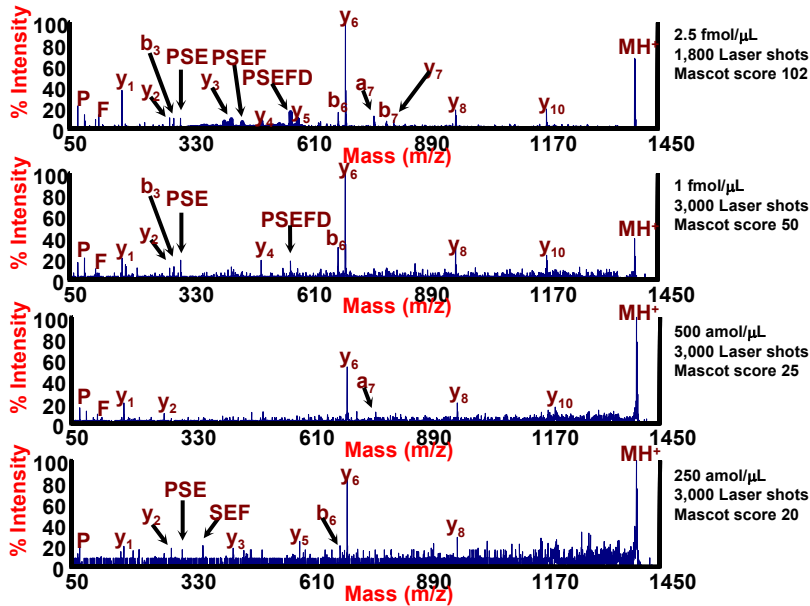
Samples were prepared using a standard dry droplet method that included diluting the peptide mixture with the matrix solution, spotting 0.6 μL of this sample onto multiple wells of a 192-well Opti-TOF™ single use plate, air drying of samples, and measurement of reflector mode spectra before collection of MS/MS spectra for each of the peptides of this mixture by a 4800 MALDI TOF/TOF™ Analyzer. The MS/MS experiments collected during these experiments are shown in Figure 1. In this example, the spectra collected for Des-Arg Bradykinin, Angiotensin I, and Glu-1-Fibrinopeptide resulted from 500 laser shots (~2.5s acquisition time), and the MS/MS spectrum collected for the Neurotensin (which was present at 250 amol/μL) was an average of only 2,000 laser shots. All of these spectra exhibit extremely high S/N ratios as well as a high coverage of y- and b-ions. All peptides were easily identified from the data shown in Figure 1 and demonstrate high quality MS/MS data collection at low peptide concentrations by the 4800 MALDI TOF/TOF™ Analyzer.



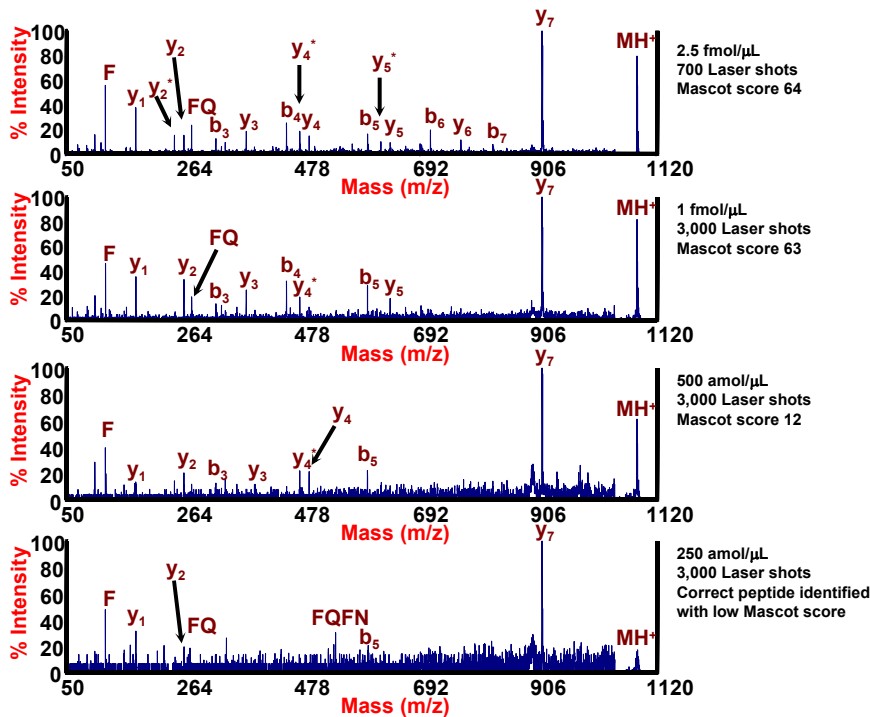
**Figure 1** MS/MS analysis of components of a mixture of 4 synthetically pure peptides (des-Arg Bradykinin, m/z 904.4; Angiotensin I, m/z 1296.5; Glu-1-Fibrinopeptide, m/z 1570.6; and Neurotensin, m/z 1672.7). All spectra were collected with the CID on using air as the collision gas.

A serial dilution of a trypsin digest of beta-galactosidase was prepared by diluting the solution digest in a matrix solution consisting of 3.6 mg/mL alpha-cyano-4-hydroxycinnamic acid and 10 mM ammonium citrate. The maximum concentration of the beta-galactosidase digest in this experiment was 2.5 fmol/μL and the minimum concentration was 50 amol/μL (final concentrations of the digest in matrix solution). A standard dry droplet method, that included spotting 0.6 μL of each sample onto a 192-well Opti-TOF™ single use plate, air drying samples, and collection of reflector spectra to identify precursor ions for MS/MS analysis by a 4800 MALDI TOF/TOF™ Analyzer. MS/MS spectra were collected in both interactive and batch modes, with equally high quality data collected by each of these modes of operation. The stop conditions feature of the 4000 Explorer v3.5 was used to preserve sample and minimize acquisition time. In this example the acquisition was stopped after four ions in the spectrum reached a S/N of 50:1 or 3000 laser shots were accumulated (see laser shot count in each spectrum). A number of precursor ions including m/z 1083.5 (GDFQFNISR), m/z 1394.7 (LSPEFDLSAFLR) and m/z 1787.7 (WSDGSYLEDDQDMWR) were selected for MS/MS analysis. A timed ion selector (TIS) resolution of 200 was used throughout these experiments and GPS Explorer™ V3.5 Software was used for peptide sequence identification (searching the NCBI protein database with an Ecoli filter). CID MS/MS spectra for the precursor ion m/z 1787.7 at the various concentration of the trypsin digest are shown in Figure 2. These data represent the high quality of MS/MS spectra that can be collected from low peptide concentrations by the 4800 MALDI TOF/TOF™ Analyzer. GPS Explorer™ was able to identify the correct sequence of this peptide from the database search with Mascot scores ranging from 21 at the 250 amol/μL level of the digest to approximately 70 at the 1 to 2.5 fmol/μL levels. Indeed, the GPS Explorer V3.5 software was able to correctly identify the peptide at both 50 and 100 amol/μL with lower Mascot scores.

In order to verify the high sensitivity obtainable two further precursor ions were selected from the beta-galactosidase digest. These data are shown in Figures 3 and 4. In all cases GPS Explorer™ was able to identify the correct peptide from a database search, and only one the spectra (m/z 1083.5 at the 250 amol/μL) did not have a significant Mascot score. These data confirm the levels of detection and sequence identification of the first example, and demonstrate the high sensitivity of the MS/MS mode of the 4800 MALDI TOF/TOF™ Analyzer.



**Figure 3** MS/MS spectra collected for m/z 1394.7 (LSPEFDLSAFLR) isolated from each sample of a serial dilution of a trypsin solution digest of beta-galactosidase. CID and optimized precursor modes were used to collect all spectra.



**Figure 4** MS/MS spectra collected for m/z 1083.5 (GDFQFNISR) isolated from each sample of a serial dilution of a trypsin solution digest of beta-galactosidase. CID and optimized precursor modes were used to collect all spectra.

## Summary

The high sensitivity in MS/MS mode of the 4800 MALDI TOF/TOF™ Analyzer was demonstrated by the analysis of a synthetically pure mixture of peptides and a serial dilution of an enzyme digest of beta-galactosidase. In both experiments a sub-femtomole concentration of a peptide was shown to be sufficient to produce high quality MS/MS data that identified the specific peptide sequence. Indeed, for the trypsin digest of beta-galactosidase, the GPS Explorer™ V3.5 Software correctly identified the sequence of two out of the three peptides (with significant Mascot scores) at the 250 amol/μL level of this digest, and demonstrate the phenomenal MS/MS performance of the 4800 MALDI TOF/TOF™ Analyzer without the use of complex sample plate technology. Experimental efficiency and throughput can be further maximized using the intelligent acquisition software allowing the MS/MS acquisition to stop when the user-specified data quality is reached.

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