

## **MALDI TOF-MS System with High-Repetition Rate Laser for Fast Analysis of Multiplexed Separated Peptide Mixtures**

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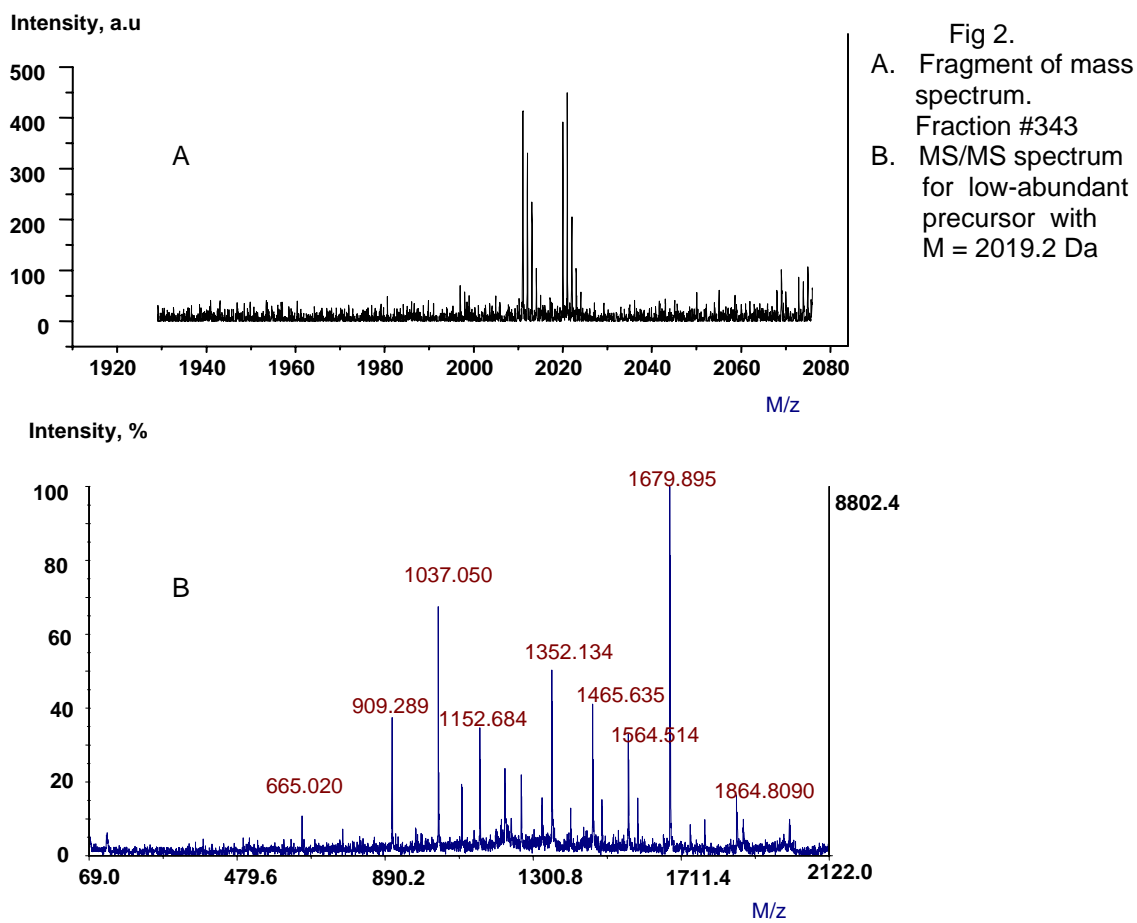
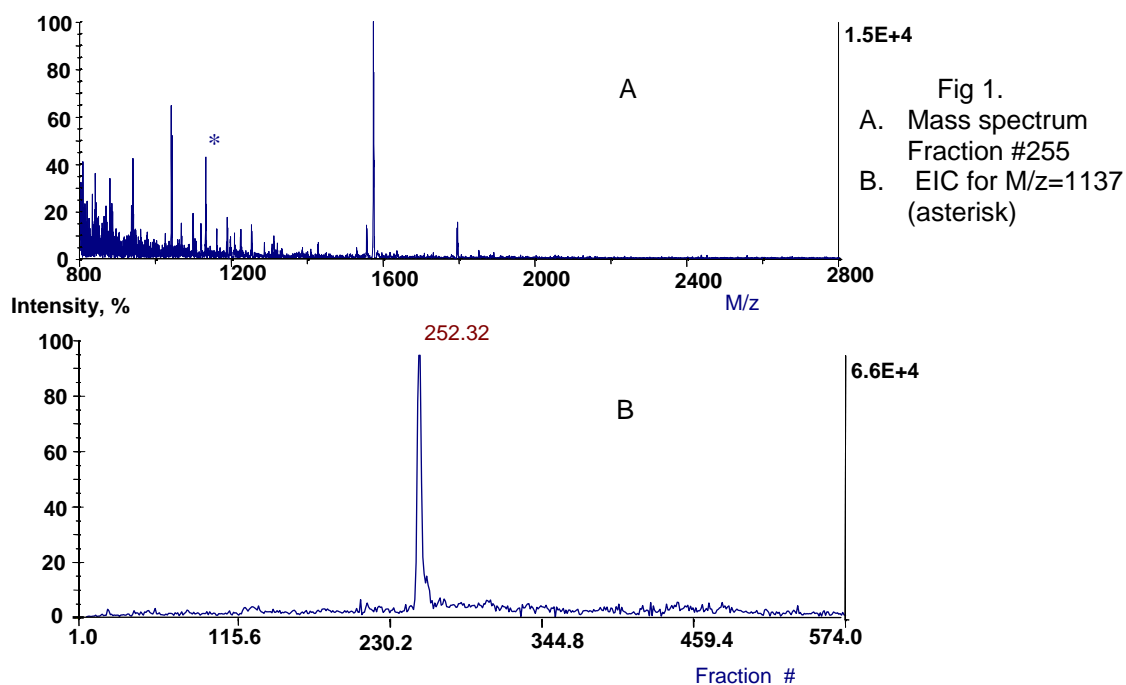
The high-throughput search for proteins determined by successive MS and MS/MS scans is currently attracting much interest. High chromatographic resolution in 2-D chromatography coupled to high mass accuracy in MALDI MS and MS/MS analyses can substantially increase the sample information content by increasing protein sequence coverage and by identifying lower abundance proteins. High-resolution separation of complex peptide mixtures in 2-D chromatography results in a very large number of fractions. Off-line analysis of these fractions using conventional MALDI TOF and TOF/TOF mass spectrometers (MS) equipped with 10 - 200 Hz lasers may require several days of continuous operations. Thus, the development of high-throughput TOF MS is essential to utilize full analytical capacity of MALDI method.

To enhance throughput of off-line MALDI analysis by a factor of 10 compared to conventional axial TOF instruments, a new MALDI TOF mass spectrometer has been developed in our laboratory. This instrument with 0.6 m ion mirror of 0.6 m and the total flight-time length of TOF mass spectrometer of 4.2 m provided high mass resolution over 1-4 kDa mass range. It utilized a 2-kHz laser, automatically scanned the MALDI plate, and stored the data on a hard disk. Initial MS analysis (500-1000 laser shots on a spot of 1.2 mm) used only a small fraction of the sample in the spot, allowing for several MS/MS scans of the same spot, based on results of peak picking, database searching, or the desire for high sensitivity. Our approach includes a fast MS scan of several standard (2"x2") MALDI plates containing samples simultaneously deposited from four or more high-resolution multiplexed LC separations. The stored MS spectra were then analyzed by the software program MEND<sup>1</sup> utilizing the information on the elution profiles of peptides in LC separations. This program provided m/z's of candidates and positioned these candidates on the plate for the subsequent MS/MS scan in 4700 TOF/TOF MS (Applied Biosystems, Framingham). Finally, the MS/MS spectra of precursors were obtained from chosen positions.

The samples were deposited using a sample deposition interface<sup>2</sup> on several standard 2 "x 2" MALDI plates as 1mm spots separated by a distance of 1.7 mm. Each spot contained 5-sec fractions of multiplexed LC separations.

The sample plate moved with the average speed of 4 mm/s allowing mass analysis of up to 2 spots/sec. Ion signals from MCP detector were digitized with 2-GHz acquisition board, averaged in real time, and recorded to the hard drive. High-stability power supplies and pulsers used for delayed extraction provided < 20 ppm/15 min temporal stability for ion flight times from a single spot. The laser beam (~200 micron in diameter) was steadily moving across the spot in a zigzag motion. This allowed illumination of fresh areas in the sample spot and resulted in reproducible ion flow from the sample. The averaged MS spectrum from a single spot was transferred to a hard disk in less than 50 ms time when the stage was moving between the spots. Monitoring of the high-voltage, the motion of the XY stage, generation of synch pulses for delayed extraction and data acquisition were controlled by several high-precision DAC and ADC boards.

A typical scanning time of each target containing 800 of 1mm spots with 1.7 mm center-to-center distances was 8 minutes (1000 laser shots per spot). For the same number of spots, analysis on the 4700 TOF/TOF mass spectrometer required 1 hour 50 minutes. Performance results are shown below.



**References:**

1. Rejtar, T.; Hu, P.; Juhasz, P.; Campbell, J.M.; Vestal, M.L.; Preisler, J.; Karger, B.L., *J. Proteome Res.*, **1**, 171-179 (2002).
2. Andreev, V.; Rejtar, T.; Moskovets, E.; Ivanov, A.; Karger, B. L., *Anal. Chem.* **75**(22), 6314 (2003)