

## PERFORMANCE EVALUATION AND APPLICATIONS OF IMPROVED MALDI-TOF MS-MS SYSTEM

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The prototype TOF-TOF system was recently modified by adding an electrically isolated collision cell, a timed gate for suppressing unimolecular fragments formed after the MS-2 ion source. Additional focusing elements have been included so that both precursor and fragment ions are efficiently detected by a small (18 mm) fast, dual channel plate detector. High-speed electronics and a kHz laser have also been added to the system.

Measured resolving power for the new system is greater than 15,000 over the range typically covered for protein digests (ca. 600-4000 m/z) and mass error with internal calibration is less than 10 ppm for all peaks greater than 1% of the base peak. Resolution varies between 2000 and 5000 for fragment ions in MS-MS mode and mass error is typically less than 0.03 da for all detected fragment ions. Absolute efficiency is greater than 1 ion/100 molecules desorbed, and 1 femtomole of peptide loaded is generally sufficient to yield a high quality MS-MS spectrum with a total acquisition time of a few seconds using a 200kHz laser.

This new instrument has been applied to protein identification by conventional trypsin digestion, and with other non-specific enzymes such as pepsin. Results obtained from tryptic molecular weight maps can be less definitive than is required in some cases. In the absence of MS/MS experiments, no convenient means is available to acquire additional information from the same sample to verify or confirm the results once an accurate mass list is generated. Furthermore, not all proteins are amenable to convenient digestion with trypsin or the other proteases that reliably cleave at specific residues. Nearly every tryptic digest contains one or more "unexpected" peptides arising from cleavage at a residue other than Lys or Arg, due to chymotryptic activity or other adventitious cleavage. These peptides are normally ignored in searches, but represent available information that is not being used. We present here results showing that MS/MS data produced in a MALDI TOF-TOF instrument can provide extremely reliable protein identifications from a database with the use of almost any proteolytic enzyme. No sequence data interpretation is required, and all of the operations involved are easily automatable.

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