

Hybrid MALDI MSⁿ System with Wide Dynamic Range and Low PPM Mass Accuracy for High Throughput Identification of Proteins

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Overview

Purpose: To demonstrate the MALDI Duo source performance attached to an LTQ Orbitrap XL™ system

Methods: Reliability of mass calibration, Full scan and MS/MS performance, Peptide Mass Fingerprinting with accurate mass.

Results: The MALDI Duo source coupled to the LTQ Orbitrap hybrid MS provides reliable high resolution, accurate mass spectral data.

Introduction

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules such as proteins, peptides, oligonucleotides and oligosaccharides, which tend to be fragile and fragment when ionized by other ionization methods. It is most similar in character to electrospray ionization both in relative softness and with the ions produced (although MALDI causes much fewer multiply charged ions).

The ionization is triggered by a pulsed laser beam (normally a nitrogen laser of few nanoseconds pulse duration). A matrix, which is a small organic molecule absorbing at the laser wavelength, is used to isolate the analyte molecules from each other and to absorb the laser light resonantly. The matrix initiates the soft vaporization ("desorption") and ionization of analyte molecules.

This presentation shows first results of the new MALDI source coupled to a high resolution, accurate mass detector; ions produced in a Thermo Scientific MALDI Duo ion source are detected in an LTQ Orbitrap XL instrument. This allows the detection of (singly charged) MALDI ions with mass resolution up to 100,000 (@ m/z 400) with accurate mass (3 ppm or better).

Materials & Methods

The MALDI Duo is equipped with a Nitrogen Laser (LTB, Berlin, Germany) which operates at 337.1 nm wavelength, 3 ns pulse duration and 60 Hz repetition rate. The beam diameter is about 80 x 100 μm on the sample plate.

MALDI preparations were performed according to the protocol for MS-Cal4 purchased from SIGMA™ (in which a variety of different peptides as well as matrix molecules are used for calibration of all devices in the hybrid mass spectrometer. All LTQ as well as all Orbitrap devices can be calibrated straight forward with MS-Cal4.

Enzymatic digests were purchased from Michrom Bioresources Inc. USA. MALDI preparations contained 5 fmol of the enzymatically digested protein on the sample plate. The phosphopeptide analyzed is part of the peptide standard from Invitrogen (P33357), Molecular Probes Inc., USA. HCCA matrix was used for all experiments shown here.

Figure 4: 1 fmol Angiotensin DRVYIHPF MH+ = 1046.541791 u.

HCCA matrix in full scan mode m/z 800 – 2000. Insets of the molecular ion at m/z 1046 and of the background noise. Signal to noise is > 50 : 1. Average of 5 single scans, 5 LS each.

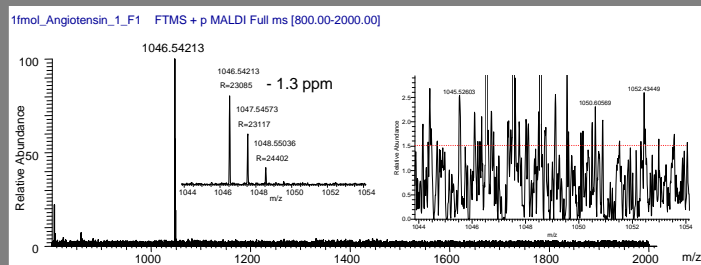


Figure 5. Analysis of a phosphopeptide with the sequence VPIGRFDRRVPtVE, in which T is phosphorylated. HCCA matrix.

A: FT-MS full scan plus inset into m/z 1720, single scan
B: FT-MS/MS, CID @ m/z 1720, single scan
C: FT-MS/MS, HCD @ 1720, Av of 5 scans

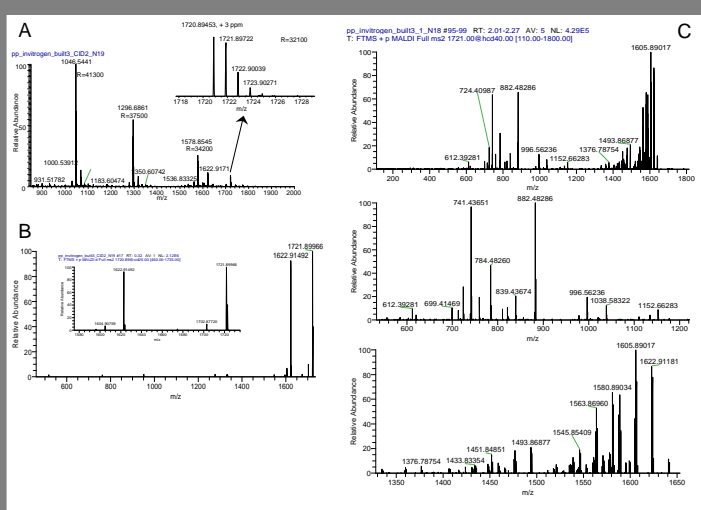
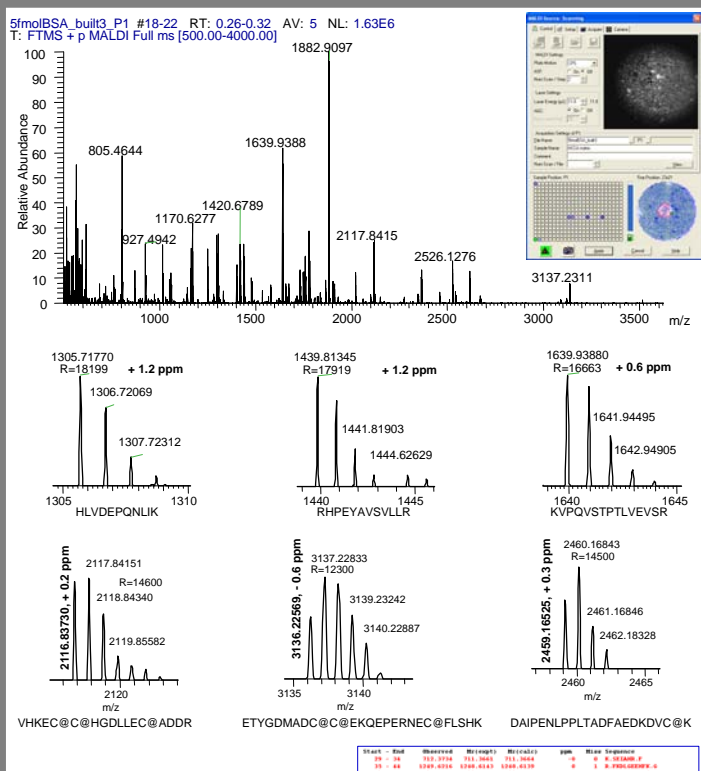


FIGURE 6. PMF, Peptide Mass Fingerprinting for a 5 fmol load of an enzymatic digest of Bovine Serum Albumin, HCCA matrix.

5 scans, 20 laser shots each. Insets from full scan of some proteolytic peptides are shown. Monoisotopic MH+ masses are submitted to Mascot for protein ID. Protein is unambiguously identified as Bovine Serum Albumin. C@ is the carboxymethylated Cysteine.



Mascot Search Results

Search title: 10fmolBSA
 Database: SwissProt 34.1 (377883 sequences; 101973233 residues)
 Taxonomy: Mammalia (mammals) (34895 sequences)
 Timestamp: 5 Sep 2007 at 12:31:19 GMT
 Top Score: 137 seq. ALBU_BOVIN, Serum albumin precursor - Bos t...

Rank	Seq. ID	Accession	Length	Score	ppm	Missed Sequence
1	1	Q5UQ58	715	137.000	0.0	ALBU_BOVIN
2	2	Q5UQ58	715	136.999	0.0	ALBU_BOVIN
3	3	Q5UQ58	715	136.998	0.0	ALBU_BOVIN
4	4	Q5UQ58	715	136.997	0.0	ALBU_BOVIN
5	5	Q5UQ58	715	136.996	0.0	ALBU_BOVIN

Probability Based Mowse Score

Protein score is $10^{\text{Log}P}$, where P is the probability that the observed match is a random event. Protein scores greater than 60 are significant ($p < 0.05$).

Conclusions

- MALDI ions analyzed with LTQ Orbitrap technology are detected with accurate mass.
- MS/MS data obtained with Higher Energy Collision Dissociation (HCD) are accessible and are also detected with accurate mass.
- HCD is able to provide fragment ions over a significantly larger mass range than CID (in the LTQ).
- Low sample amounts - 1 fmol Angiotensin - loaded onto the plate are detected with signal to noise ratios > 10 : 1.
- Phosphopeptide analysis can be performed using an HCD scan triggered by an accurately detected neutral loss by CID.
- PMF printing is easily accessible on a low femtomole load on target.

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FIGURE 1. Instrument schematics of the MALDI DUO LTQ Orbitrap XL system (for a better view, the ion source is enlarged relative to the mass spectrometer).

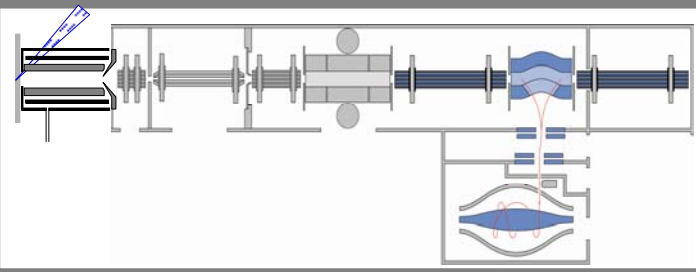


FIGURE 2. Accuracy and Precision of Mass Determination

A: Normal Mass Range m/z 150 – 2000. Check mass calibration for a duration of 25 min for a 1 day old mass calibration
B: High Mass Range m/z 350 – 4000. Check mass calibration for a duration of 5 min for a 0.5 day old mass calibration

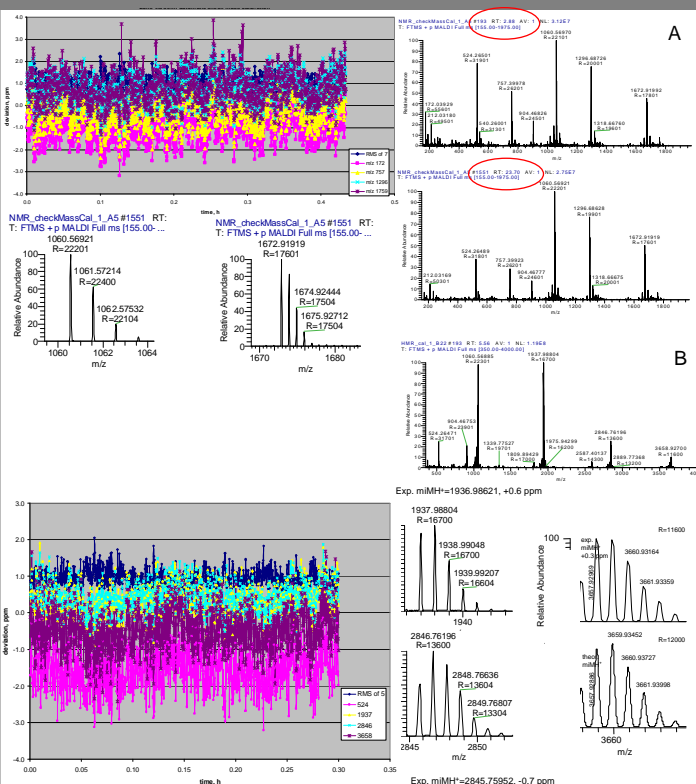


FIGURE 3. MS/MS spectrum on m/z 2845, Melittin from honey bee. Higher Energy Collision Dissociation (HCD)

