A NOVEL MALDI ORTHOGONAL TIME OF FLIGHT MASS SPECTROMETER

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OVERVIEW

- An experimental tandem MALDI TOF is described and preliminary data from peptide digest standards are presented.
- The instrument utilizes an orthogonal TOF for mass acquisitions. This is preceded by a TOF for precursor ion selection in the MS/MS mode.
- The instrument does not utilise collisional damping gas and RF confinement.
- In MS mode, time dependant ion bunching optics cause ions over the mass range of interest to arrive simultaneously into the oa-TOF pusher region.
- In MS/MS mode, the time dependant optics are disabled and the oa-TOF is energised at the moment the precursor ion of interest and it's associated PSD fragments arrive in the pusher assembly.
- For MS or MS/MS, each laser shot provides a full mass spectrum requiring only one calibration.

INTRODUCTION

The use of an oa-TOF analyser with MALDI for MS and MS/MS, is known to have a number of advantages. Only one calibration function is required, and good mass measurement is easily obtained since flight times are not affected by ion source conditions (unlike axial MALDI TOF-TOF systems).
Furthermore, oa-TOF has a particular advantage when recording MS/MS spectra since the whole spectrum is recorded for each laser shot.
Usually, in MALDI oa-TOF systems, a RF collisional damping interface is used. This can give rise to matrix clustering effects that limit the detection of low level analytes. Sample consumption rates are also relatively high enabling only a limited number of laser shots per sample.

METHODS

The layout of the experimental system is shown in figure 1. Samples are irradiated by a UV laser (Spectra Physics, 337si) at 10Hz. Initially, ions are accelerated along the axis shown as TOF1. They pass through a "mass buncher", (the operation of which is described below) and a CID gas cell. In both MS and MS/MS, ions with kinetic ion energies ranging from 10ev to 500eV arrive at the oa-TOF pusher assembly simultaneously where they are accelerated orthogonally into TOF2, the oa-TOF mass analyser. The accelerating voltage of the oa-TOF is 10kV and the effective flight path is 0.8m. TOF2 incorporates a linear reflectron and microchannel plate (MCP) ion detector. The detector is 140mm long in order to accommodate the axial energy spread of up to 500eV.

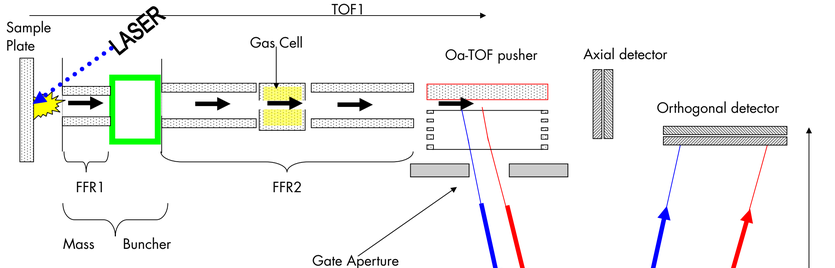

Figure 1. Schematic of instrument

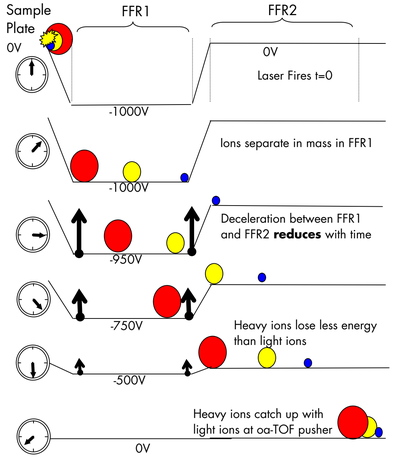

Figure 2. Potential energy diagram of "mass buncher" ion optics

MS Mode—Mass Bunching

To record an MS spectrum with the oa-TOF, ions over the mass range of interest are arranged to arrive at the pusher electrode of the oa-TOF simultaneously. This is achieved by the application of a time dependant deceleration field to the axial beam of TOF1 as shown in figure 2. Initially, ions are accelerated through 1000V into field free region 1 (FFR1).

At this point their kinetic energies are 1000eV and they arrive at the deceleration region between FFR1 and FFR2 in order of mass. The deceleration field is changed so that heavier ions emerging into FFR2 have slightly higher velocities than lighter ions. This causes ions of different masses to arrive at the oa-TOF together. The waveform of the electric field applied to the deceleration stage is sinusoidal.

MS/MS Mode

In MS/MS mode, the buncher is disabled and ions arrive at the oa-TOF pusher in order of mass. Ions that fragment travel at approximately the same velocity as their precursor. Fragment ions are formed by the process of post source decay (PSD) or by collision induced decomposition after allowing gas into the CID gas cell. By controlling the delay time between the laser pulse and the oa-TOF pusher, it is possible to select just the precursor ion of interest and its associated fragment ions. The precursor ion selectivity may be controlled by changing the width of the Gate Aperture.

RESULTS

All data are from standard "dried droplet" loadings of 1ul of matrix (5mg/ml HCCA in 50:50 Water:ACN) and 1ul of analyte. Calibration is multipoint external followed by single point internal correction. All MS/MS data is post source decay data.

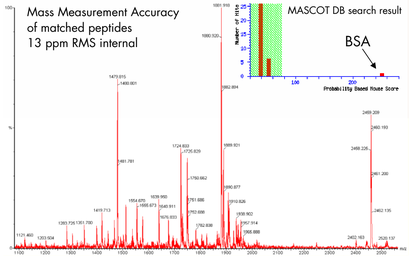

Figure 3. MS Mode BSA protein digest (100fm)

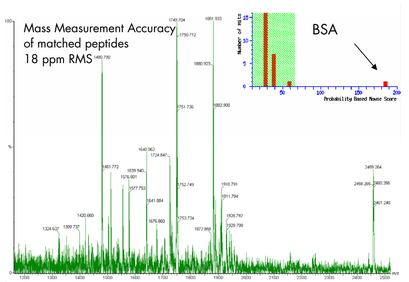

Figure 4. MS Mode BSA protein digest (1fm)
Figures 3 and 4: MS spectra for 100fm and 1fm loadings of bovine serum albumin (BSA) tryptic digest.

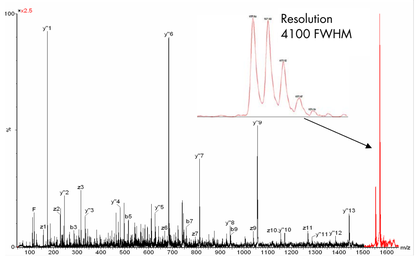

Figure 5 MS/MS GluFibrinopeptide (100fm) MH+ 1570.6

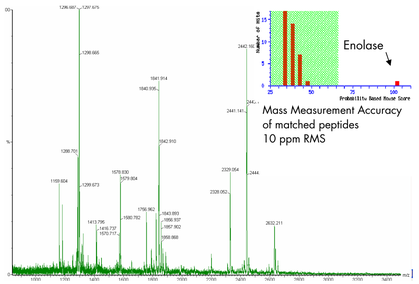

Figure 6. MS Mode Enolase protein digest (100fm)

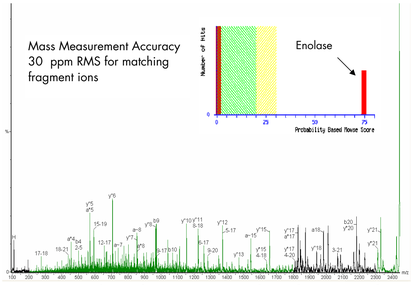

Figure 7. MS/MS of MH+ 2441 Enolase digest (100fm)

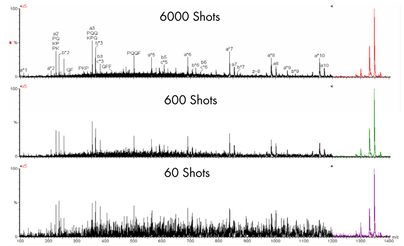

Figure 8. MS/MS of Substance-P (4fm) illustrating low sample consumption and enhancement in signal to noise with increasing number of laser shots.

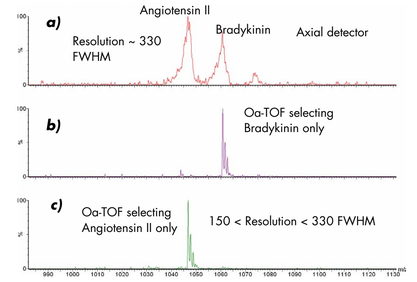

Figure 9. Precursor Ion Selection - mixture of Angiotensin II MH+1046.6 (100fm) and Bradykinin (100fm) MH+1060.6. Spectrum a) recorded on the axial detector indicates the maximum resolution attainable with a FWHM of 3Da (i.e. approximately 330). With the pusher activated at 28.0 us after the laser fired, oa-TOF spectrum b) was recorded. Similarly, at 28.2us, spectrum c) was recorded. With a Gate Aperture of 1mm, both species are fully resolved indicating a minimum resolution of 150 FWHM.

DISCUSSION

Despite the modest resolving power of 4000 FWHM, the mass measurement accuracy shown is 15ppm and 30 ppm (RMS) in MS and MS/MS modes respectively. For MS/MS, this performance is comparable with other TOF/TOF configurations.
Since the oa-TOF is decoupled from the ion source, the system is more amenable to automated data acquisition as the system is more forgiving to variations in laser energy, matrix thickness and sample plate position. Even for a low concentration sample (Substance-P 4fm loading) MS/MS spectra of many thousands of laser shots were acquired (figure 8).

CONCLUSIONS

- A new concept of MALDI-TOF-TOF benefiting from an oa-TOF mass analyser has been demonstrated.
- Sample consumption rate is relatively low allowing many thousands of laser shots per sample.
- No collisional damping gas with RF confinement optics are required.
- Oa-TOF allows the same calibration for both MS as MS/MS.

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