

# AXIMA-QIT™

Analysis of high mass peptides using a novel  
Matrix-Assisted Laser Desorption Ionization  
Quadrupole Ion Trap Time-of-Flight  
(MALDI QIT TOF) mass spectrometer

- MS, MS/MS and MS/MS/MS with high resolution and accuracy enables identification of fragments differing by only 1 Da
- MS/MS/MS provides additional fragment and sequence information not achieved by MS/MS alone
- MS/MS sensitivity at very low fmol levels

# Analysis of high mass peptides using a novel MALDI QIT TOF mass spectrometer

## Introduction

Matrix assisted laser/desorption ionization (MALDI) has become a widely accepted analytical technique in the structural determination of biological molecules<sup>1,2</sup>. Its sensitivity at the femtomole level, the ability to generate singly-charged ion species and the limited susceptibility towards contaminants render MALDI the preferred ionization method for immediate and rapid identification of proteins isolated via sub-fractionation of the entire proteome. In a typical experiment, the protein of interest is hydrolyzed by a specific proteolytic enzyme generating peptide mixtures that are analyzed by MALDI. The masses of the observed proteolytic peptide ions are subsequently compared to those values obtained by performing *in silico* digestion of all protein entries in a database (also referred to as *peptide mass fingerprinting* (PMF))<sup>3</sup>.

Such strategy has become routine in many laboratories. In order to confirm the identity of a putative database hit, additional data derived from tandem mass spectrometry (MS/MS) of the peptide ion are often necessary.

An innovative configuration for a MALDI hybrid mass spectrometer was reported recently by different groups. The three-dimensional device has two functions: firstly, it traps externally generated MALDI ions by quickly ramping the RF potential applied to the ring electrode. Secondly, during MS/MS, the ion of interest is isolated by ejecting the unwanted ions. After collisionally activated decomposition (CAD) within the ion trap, the fragment ions are analyzed in the time-of-flight analyzer.

Here, we report the analysis of large peptide ions both in MS and MS/MS modes using a novel hybrid MALDI QIT TOF (AXIMA-QIT™) in which ions are formed in a high-vacuum region<sup>4</sup>. The ability of the instrument to isolate high mass peptide ions in the trap is demonstrated using human adrenocorticotrophic hormone (fragment 18-39) and oxidized bovine insulin chain B. Subsequent tandem mass analysis of these peptide precursor ions generates product ion spectra containing fragmentation patterns from which the peptide sequence can be partially deduced.

## Methods

High resolution mass spectra were acquired on a AXIMA-QIT™ instrument. The design of the mass spectrometer is depicted schematically in Figure 1. Acquisition and data processing are controlled by LAUNCHPAD™ software. The instrument consists of four main sections: the ion source, the introduction region, the ion trap and the reflectron time-of-flight mass analyzer. The target is positioned in the ion source using an XY sample stage. The pressure in the ion source is measured by an ion gauge and it is typically at  $6 \times 10^{-6}$  Torr. Matrix assisted laser desorption of peptides is produced by pulses of light (337nm, 3ns pulse width) generated by a nitrogen laser with a maximum pulse rate of 10 Hz.

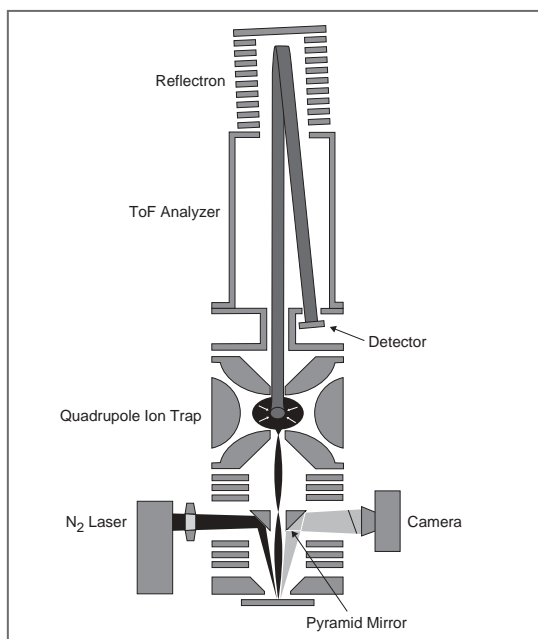


Figure 1. Schematic illustration of the MALDI QIT TOF mass spectrometer

Spatial focussing is provided by two Einzel lenses separated by a pyramid mirror and ions are injected axially into the trap. During ion introduction, no RF potential is applied to the ion trap. To trap ions, a retarding potential is applied to the end-cap adjacent to the time-of-flight and an RF with a frequency of 500 kHz is applied to the ring electrode (rapid RF startup). Amplitudes and the corresponding bias voltage are chosen according to peptide mass under investigation. The rapid “RF-startup” method provides trapping efficiency close to 100%<sup>4</sup>. Once trapped, the ions are cooled using argon. The pressure in the trap is held at  $6 \times 10^{-3}$  Torr. The TOF mass analyzer is employed to acquire the data in both MS and MS/MS modes of operation. Prior to MS/MS analysis, precursor ions can be isolated using the filtered noise field (FNF) method which generates a notched broadband signal composed of frequency components. The window used for precursor ion isolation is defined by using the concept of resolution ( $m/\Delta m$ ) where  $m$  corresponds to 1000. Two resolutions are shown here: 70 and 250, ie  $\pm 7$  m/z and  $\pm 2$  m/z at 1000 m/z, respectively. In order to induce fragmentation of the precursor ion, resonant excitation is induced by the application of a supplementary AC potential to the endcap electrodes. For CAD, argon is used as collision gas. Following decomposition, the product ions are extracted into the TOF for mass analysis by applying a potential between the two end-caps. In both MS and MS/MS modes, ions are pulsed into the TOF with an accelerating voltage of 10 kV. The TOF is externally calibrated using fullerite deposited directly onto the sample stage. All spectra were obtained with the standard instrument settings.

Peptides (Sigma-Aldrich) were prepared as 1 pmol/ $\mu$ l stock solutions and serially diluted prior to analysis. Peptide solution (0.5  $\mu$ l) was mixed on the target with a fresh saturated solution of 2,5 dihydroxybenzoic acid (DHB) in 0.1%(v/v) TFA/50%(v/v) acetonitrile.

## Results

The efficiency of searching a completely sequenced genome with a single peptide mass increases greatly by using peptides bigger than 2000 Da. It follows that the larger the peptide, the fewer the number of protein matches to database. This is extremely important when protein mixtures, containing more than one component, are studied. In such context, certainty of identification can only be achieved by providing sequence information derived from MS/MS data. Since MALDI generates predominantly singly charged ions, higher confidence in protein identification employing a database searching strategy can be achieved when peptide ions exceeding 2000 amu are selected for MS/MS analysis. To utilise this approach, the mass analyzer which isolates the precursor ion (quadrupole or ion trap) has to be capable of selecting these ions.

To demonstrate that AXIMA-QIT™ can isolate and fragment ions in the range 2000-3500 Da, mass spectra of peptides were generated. Figure 2 illustrates an example of spectra obtained from mixtures containing [Glu<sup>1</sup>]-Fibrinopeptide B and oxidized bovine insulin chain B. MALDI analysis was performed by loading 5 fmol of each component on-target. In the case of [Glu<sup>1</sup>]-Fibrinopeptide B (Figure 2a), the spectrum is dominated by the ion signal corresponding to the singly-charged peptide ion, indicated with [M+H]<sup>+</sup> at m/z 1570.7. A lower intensity ion signal at m/z 1552.7 is observed 18 Da below the [M+H]<sup>+</sup> which derives from the loss of water from the protonated peptide. The inset shows that the isotopic cluster is fully resolved and a resolution ~7000 (FWHM) is observed. Figure 2b displays a mass spectrum of oxidized bovine insulin B chain with a resolution of ~9000 (FWHM). The high resolving power makes possible identification of all ions which compose the entire isotopic envelope.

Figure 3 illustrates the spectra of ACTH 18-39 and oxidized bovine insulin chain B following precursor ion isolation. Depletion of the isotopic distribution of a specific peptide can be obtained by narrowing or enlarging the frequency range, in which the secular frequency of the precursor ion lies. An increase of the resolution for precursor ion selection leads to an isotopic cluster with fewer components. In such experiments, ion isolation is performed by selecting only the monoisotopic ions (m/z 2465.18 ACTH and m/z 3494.70 insulin B chain). For the peptide ions under investigation, the whole isotopic envelope can be isolated by applying a wide window for precursor ion selection (resolution= 70) (Figures 3a and 3c). When the interval of masses isolated decreases (resolution= 250), more ions are ejected and the isotopic distribution of the envelope changes. Isolation of the precursor ion of ACTH 18-39 with m/z 2465.18 produces an isotopic cluster in which the monoisotopic species is the most abundant ion in the spectrum (Figure 3b). Similarly, a decrease of the window, during isolation of oxidized insulin B chain ion at m/z 3494.70 generates a spectrum in which a different isotopic distribution is observed. In fact the isotopic envelope is dominated mostly by the monoisotopic ion and by the ion incorporating <sup>13</sup>C at m/z 3495.7 (Figure 3d).

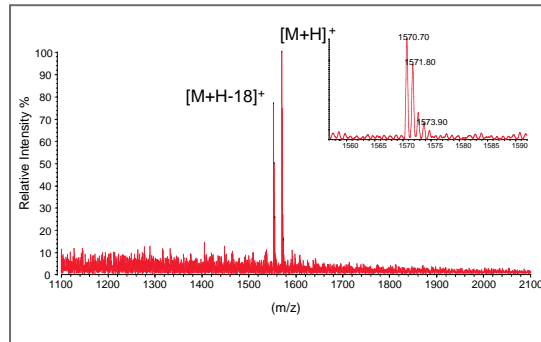


Figure 2. (a) AXIMA-QIT™ mass spectrum of 5 fmol of [Glu<sup>1</sup>]-Fibrinopeptide B

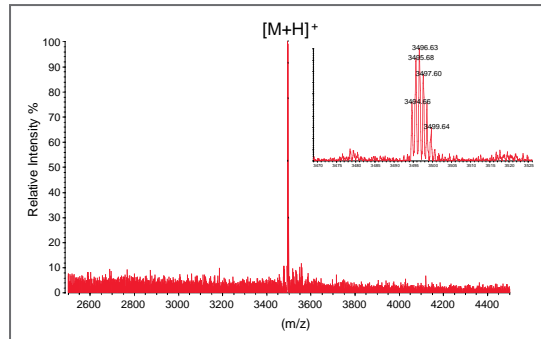


Figure 2. (b) AXIMA-QIT™ mass spectrum of 5 fmol of insulin B chain

The ability to isolate high mass peptide ions with high resolution is an important feature with regards to the analysis of complex mixtures. However, improvements in sensitivity during MS/MS can be observed when the entire isotopic envelope of the precursor ion is isolated in the ion trap. Figure 4 illustrates the product ion spectrum of 5 femtomole of oxidized bovine insulin B chain with m/z 3494. The entire isotopic envelope was selected using a resolution of 70 for precursor ion isolation. Fragmentation is extensive and b/y type fragments dominate the spectrum. An almost complete series of  $\gamma$  ions from  $\gamma_8$  to  $\gamma_{24}$  is clearly identifiable in the mass spectrum and a large proportion of the primary sequence can be easily deduced.

Ion trap devices offer the possibility to perform multiple-stage mass spectrometry (MS<sup>n</sup>). Repeated selection of ions and subsequent CAD can provide identification of new portions within the sequence of oxidized insulin chain B. Figure 5 shows the second generation product ion spectrum (MS/MS/MS) of  $\gamma_9$  corresponding to the protonated peptide RGFYTPKA with m/z 1085. Although the fragment ion distribution is dominated by b<sub>9</sub> corresponding to the peptide bond cleavage C-terminal to the lysine residue, new b-type ions are observed which can be used for peptide sequencing.

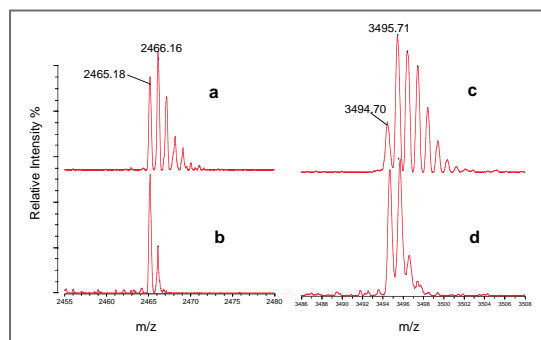


Figure 3. (a) Precursor ion isolation of the monoisotopic ion of ACTH 18-39 at m/z 2465.18 using a resolution of 70.

(b) ACTH 18-39 mass spectrum produced by isolation of the monoisotopic ion at 2465.18 with a resolution of 250

(c) Precursor ion isolation of the monoisotopic ion of insulin chain b (m/z 3494.70) by employing a resolution of 70

(d) Insulin chain b spectrum displaying the isolation of the monoisotopic ion at 3494.70. The resolution used was 250.

Figure 4. Product ion spectrum of 5 fmol of insulin chain b. The precursor ion  $[M+H]^+$  at 3494.5 was selected by using a resolution of 70

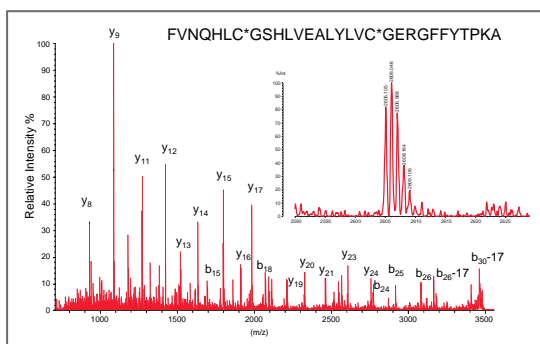


Figure 5. MS/MS/MS of  $y_9$  generated by dissociation of  $[M+H]^+$  at 3494.5

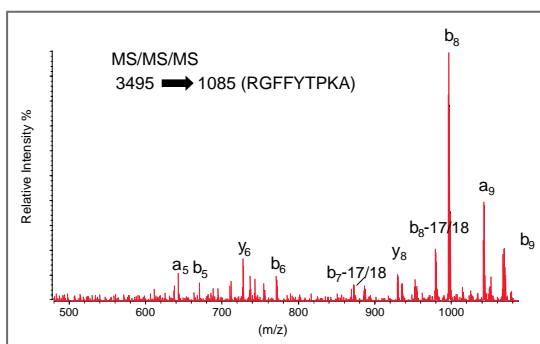
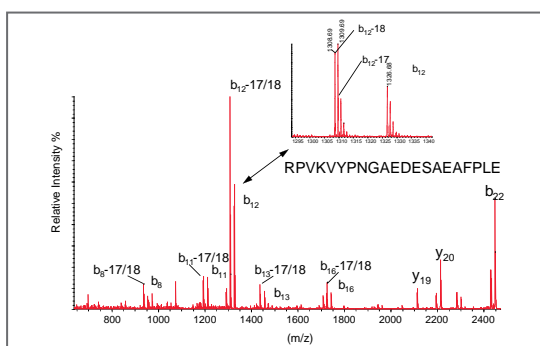


Figure 6. MS/MS spectrum of 5 fmol of ACTH 18-39



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The fragmentation behaviour of the  $y_9$  fragment ion in MS/MS/MS is attributable to the co-presence of an arginine at N-terminus and a lysine located in proximity of the C-terminus.

Figure 6 displays the product ion spectrum of  $[M+H]^+$  of ACTH 18-39 (RPVKVYPNGAEDESAEAFPLEF) at m/z 2465. The MS/MS spectrum shows a predominant ion cluster at m/z 1308.6 resulting from the loss of  $H_2O/NH_3$  from  $b_{12}$  at m/z 1326.7. As previously described, promotion of selective cleavage adjacent to Asp is attributable to the free carboxylic group on the side chain of the aspartate residue. This can initiate selective cleavage on the peptide backbone producing  $b_{12}$ . Furthermore, the high resolution obtained on a MALDI QIT TOF instrument allows precise determination of the type of product ions resulting from the dissociation of  $b_{12}$ . The anomalous isotopic distribution of the ion at m/z 1308 reveals that the isotopic cluster is generated by a combination of two ions differing by 1 Da. N-terminal product ions resulting from the loss of  $NH_3$  ( $b_{12}-NH_3$ ) and from the loss of  $H_2O$  ( $b_{12}-H_2O$ ) overlap generating the ion signal depicted in the inset of Figure 6. Overlapping of the product ions resulting from the loss of  $NH_3/H_2O$  is also observed for the other b-type ions present in the spectrum.

## Conclusion

- novel hybrid quadrupole ion trap time of flight mass spectrometer with high vacuum MALDI source
- ability to isolate MALDI ions > 3000 Da with high resolution from low femtomole peptides
- MS/MS analysis of oxidized bovine B chain insulin shows extensive fragmentation
- MS<sup>3</sup> spectrum identifies new stretches of amino acid sequence, not achieved by MS/MS alone
- valuable for sequence analysis of complex mixtures

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