Application Note: 30224

Biological Applications of AP MALDI with Thermo Scientific Exactive Orbitrap MS

Kerstin Strupat, Olaf Scheibner, Tabiwang Arrey, Maciej Bromirski, Thermo Fisher Scientific, Bremen, Germany

Key Words

- Exactive
- AP MALDI
- High Resolution/ Accurate Mass Performance
- In Source Decay
- Orbitrap™ Technology

Overview

The use of an AP MALDI ion source coupled to the Thermo Scientific Exactive mass spectrometer enables for a fast and sensitive analysis of:

- A proteolytic digest of a protein
- Mixtures of small molecules
- N- and C-terminal sequences of intact proteins via MALDI In Source Decay
- Straight forward exchange with ion sources such as HESI or APCI on a daily basis

Introduction

Atmospheric Pressure Matrix-Assisted Laser Desorption/ Ionization (AP MALDI) is a similar ion source technique to that of vacuum MALDI, utilizing UV or IR laser for ion formation. However, with AP MALDI, ions are created at atmospheric pressure instead of under vacuum. This ionization method has been shown to be an even "softer" ionization technique than vacuum MALDI due to fast thermalization/cooling of the ions' internal energy at atmospheric conditions. This feature is particularly important in the analysis of labile bio-molecules. AP MALDI is often utilized in many different applications including conventional analysis of bacteria, lipids, proteomics, small molecule and polymers but also in recent times in applications such as analysis of antioxidants and paint (pigments).

An additional benefit of using an AP source is the easy source interchange without venting the system and consequent long pumping time. AP MALDI allows the use of liquid matrices and matrices that are not vacuum compatible, without inhibiting vacuum conditions of the mass spectrometer.

In the herein described geometry, ion source and mass analysis are decoupled; i.e. MALDI ion production and mass analysis in the Orbitrap-based instrumentation is decoupled. As a consequence, sample morphology – such as thickness of matrix crystals – does neither sacrifice mass resolution nor accurate mass measurement.

In the past, the main disadvantage of AP MALDI technique compared to the conventional vacuum MALDI has been its limited sensitivity. With the introduction of AP MALDI Pulsed Dynamic Focusing (PDF) by MassTech, Inc. (Columbia, MD, USA) in 2004, ions are transferred into the mass spectrometer with high efficiency and attomole quantities of peptides have been reported in the peer-reviewed literature.[1] PDF removes the electric field that exists between sample plate and the ion inlet, thought to be responsible for the loss of ions during ion transfer to the ion inlet.

Figure 1: AP MALDI *PDF+* ion source coupled on Exactive MS.

This application note focuses on experimental results from coupling the AP MALDI *PDF+* ion source with the Exactive™ mass spectrometer, a high resolution accurate mass (HRAM) mass spectrometer. A brief description of the AP MALDI *PDF+* ion source is also provided. AP MALDI ions are resolved up to 100,000 (at *m/z* 200) at a rate of 1 spectrum per second with mass accuracy of 2 ppm or better.

Methods

Sample Preparation

Analytes are dissolved in HPLC grade water and mixed with alpha-cyano-4-hydroxy cinnamic acid (CHCA) or 1,5 diamino naphthalene (1,5-DAN) matrix according to published procedures. 0.5 µl of matrix-analyte mixtures are spotted on a stainless steel plate and air dried. MS and MS/MS are acquired at resolution setting of RP 100,000 at *m/z* 200. Plate, capillary, tube lens, skimmer voltages, capillary temperature, and the distance "plate – capillary" are varied and optimized for best conditions to liberate ions. Intact proteins are prepared using 1,5-DAN matrix for the detection of sequence specific In Source Decay (ISD) fragment ions with HRAM performance.

Mass Spectrometry

All experiments were performed on a Thermo Scientific Exactive benchtop mass spectrometer powered by Orbitrap™ technology using an AP MALDI *PDF+* source. A Nd:YAG laser beam at 355 nm was coupled into a 400 µm core diameter fiber forming a spot size of 500 x 650 µm². The mass spectrometer was operated in either negative or positive ion mode with Orbitrap full scan setting using resolving power of 50,000 or 100,000 at *m/z* 200 (FWHM).

Results

Despite MALDI ion production under atmospheric pressure, sensitivity of the herein described instrumentation can nicely compete with previously published, non AP MALDI work.

Figure 2 displays the FTMS full scan information obtained from 1 fmol deposits of Angiotensin II on plate. Each spectrum results from single FTMS full scan MS. MH^+ ion of Angiotensin is measured with accurate mass; signal-to-noise ratios of single FTMS scans are reported to be well above 50/1.

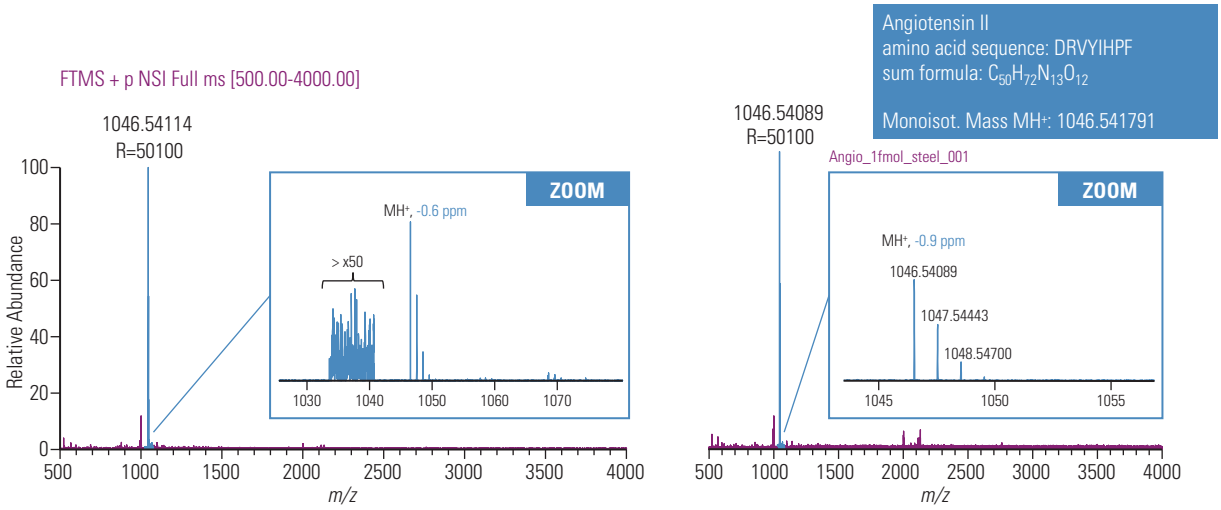


Figure 2: Two examples of an FTMS full scan acquired form a 1 femtomol deposit of Angiotensin II on plate, two different individual sample preparations. CHCA matrix. Single FTMS full scan, *m/z* 500 - 4000. S/N ratio > 50 / 1 in single scans can be obtained. Sample is not depleted, some hundreds of scans can be done from such 1 femtomole deposits.

A 10 fmol proteolytic digest of Human Serum Albumin was deposited with CHCA matrix. A single FTMS full scan onto such a sample is shown in Figure 3. Peak lists of monoisotopic masses of proteolytic fragments are submitted to Mascot for protein ID in a Peptide Mass Fingerprint (PMF) approach.

The zoomed spectrum in Figure 3 shows the assigned peptide with the sequence VFDEFKPLVEEPQNLIK measured with 33,000 resolution (at *m/z* 2,045). Isotope pattern of this peptide agrees nicely with its theoretical distribution.

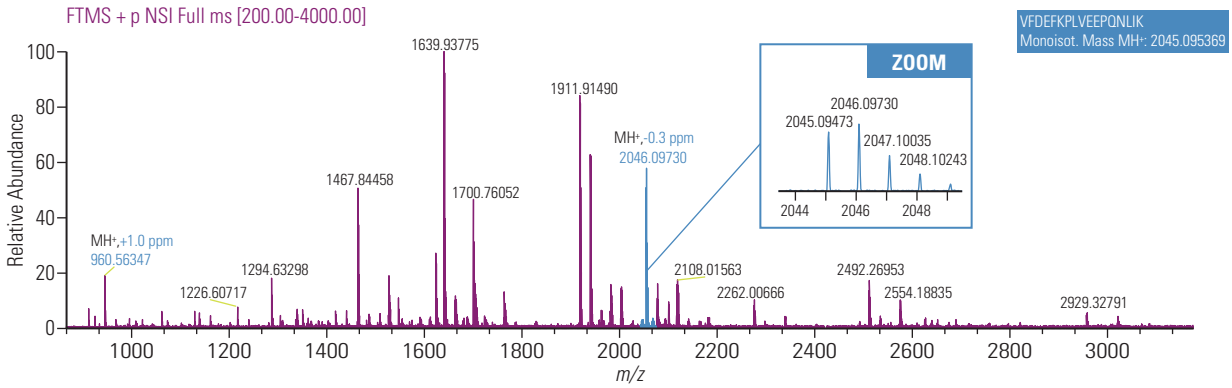


Figure 3: FTMS full scan mass spectrum of a 10 fmol proteolytic digest of Human Serum Albumin. Data displayed derives from a single scan (1 second). Mass accuracy is < 1 ppm (internal calibration).

Using 1.5-diamino naphthalene (1,5-DAN) as matrix, sequence-specific fragments such as b-, c-, y- and z-type ions can be generated simultaneously from intact proteins; fragments derive from N- and C-terminal ends of the protein.

This is exemplified by Lysozyme in Figure 6. Fragment ions are detected without any precursor selection or induced dissociation; fragment ions observed are intrinsically created upon the MALDI process with this particular matrix. For further reading see Reference [3].

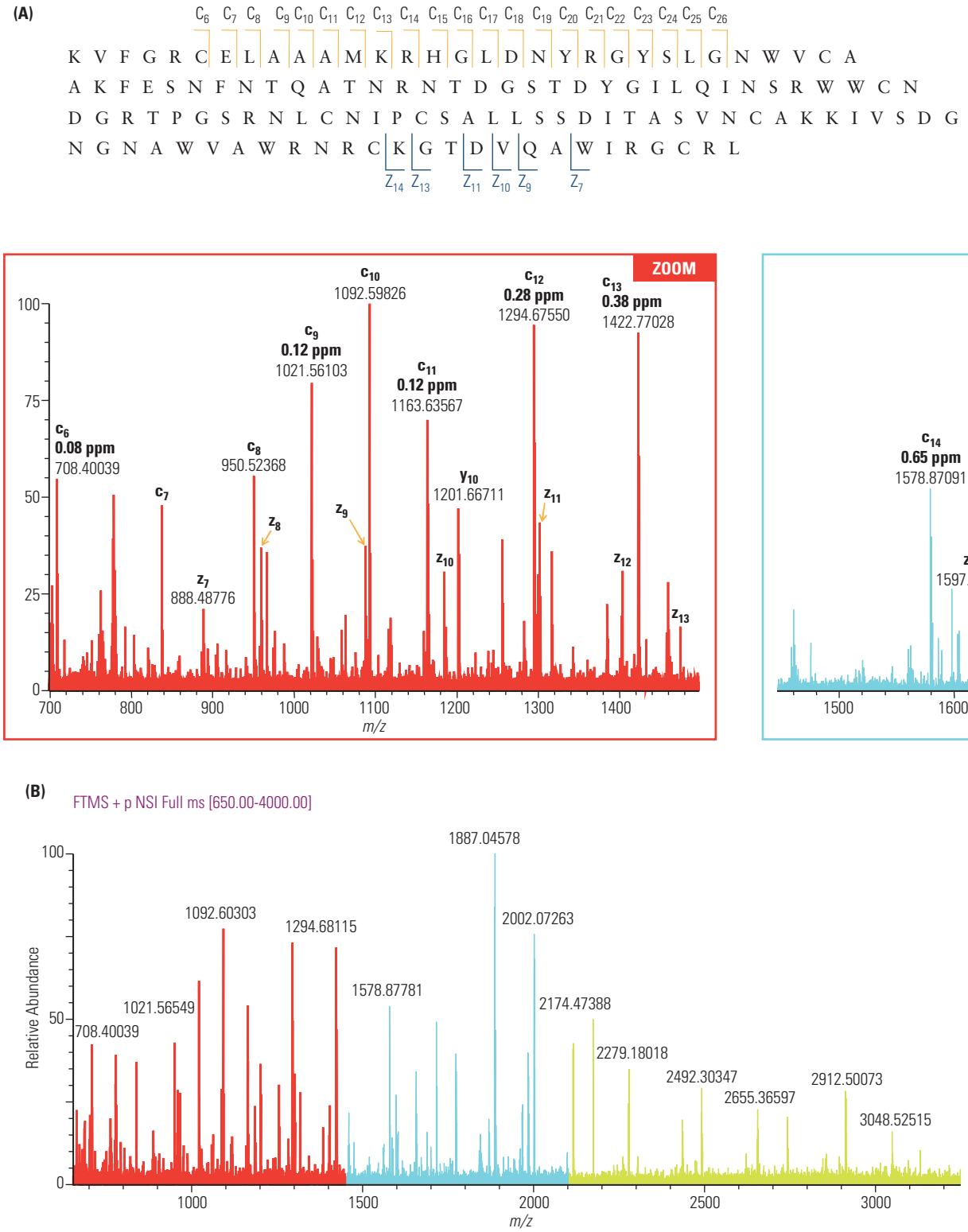
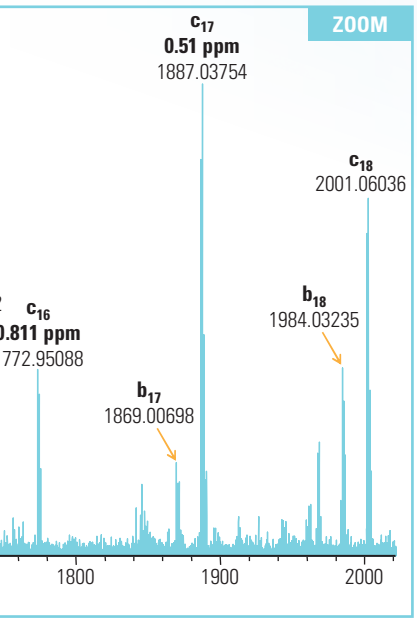
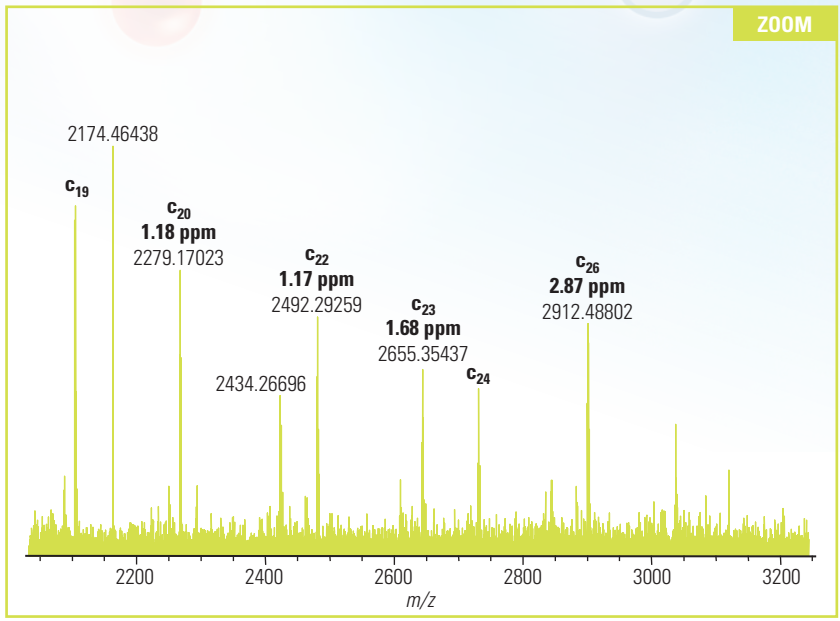


Figure 6: (A) amino acid sequence of Lysozyme and the alocated fragment ions. (B) FTMS full scan, intact Lysozyme analyzed with 1,5-DAN matrix. In Source Decay creates sequence specific N- and C-terminal fragment ions of proteins deposited as intact entities. Insets - displayed in red (*m/z* 700 - *m/z* 1500), blue (*m/z* 1500 - *m/z* 2100) and green (*m/z* 2100 - *m/z* 3200) colored spectra - provide further insight into sequence specific fragment ions observed. Refer to the bottom spectrum to see the entire FTMS full scan information. It is an average of 30 single FTMS full scan spectra.





Conclusions

AP MALDI combined with the HRAM performance of the Exactive MS enables:

- The analysis of small molecules at low ng concentrations without sacrificing the baseline or a loss of accurate mass.
- Identification of peptides from proteolytic digests of proteins at low concentrations upon a PMF approach using accurate $M_{monoisotopic}H^+$ masses.
- The analysis of N- and C-terminal sequences of proteins – deposited as intact species – upon MALDI In Source Decay specifically enabled using 1,5-DAN matrix. Sequence specific fragment ions can be assigned unambiguously.
- Further benefit of an AP MALDI ion source is the straightforward exchange with ion sources such as H-ESI, APCI, APPI, DART and other ionization techniques.
- Ease of use and no down time due to source exchange.

References

1 Trimpin et al.; *Anal Chem.* (**2010**) *82* 4998, (e.g.), and other papers by Trimpin et al. on "Laserspray Ionization"

2 Strupat et al.; *J. Am. Soc. Mass Spectrom.* (**2009**) *20* 1451

3 Thermo Scientific Application Note: 30218

Due to the sensitivity and high resolution of the Exactive mass spectrometer, a mixture of small molecules can be analyzed at low concentration and in presence of a MALDI matrix. Figure 4 displays a mixture of Atropine, Buspirone and Loperamide in CHCA matrix. Achieved mass resolution and mass accuracy for each compound are displayed in the figure.

Note that, even though there is a prominent CHCA matrix signal (CHCA dimer at *m/z* 379.09297), it does not suppress or affect the accuracy of mass determination of the Buspirone peak.

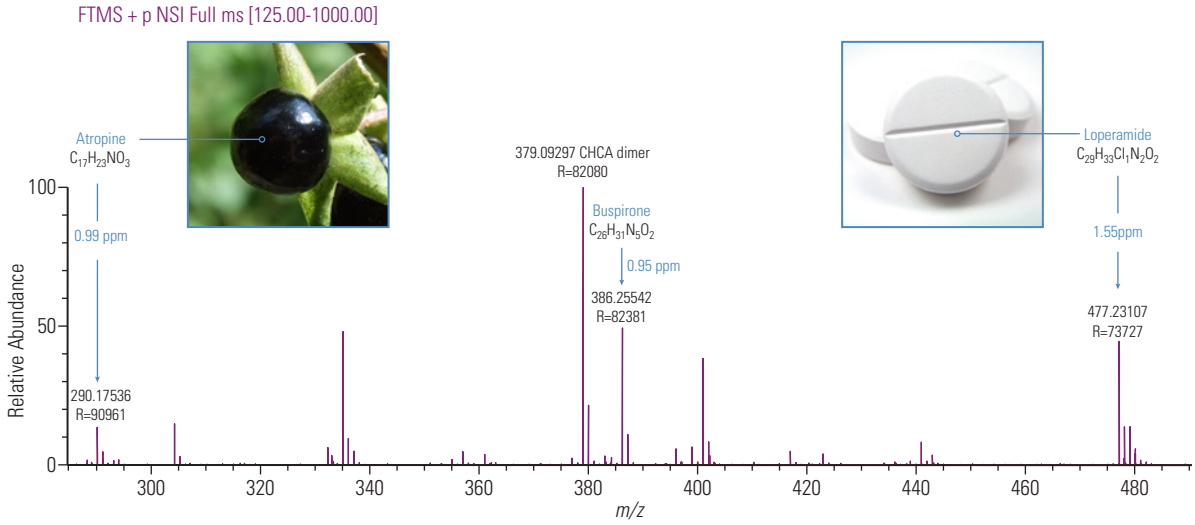


Figure 4: Inset into an FTMS full scan (*m/z* 125 – *m/z* 1000) of three small molecules, Atropine, Buspirone and Loperamide; CHCA matrix. A total of 1 ng of each molecule was present on the sample plate.

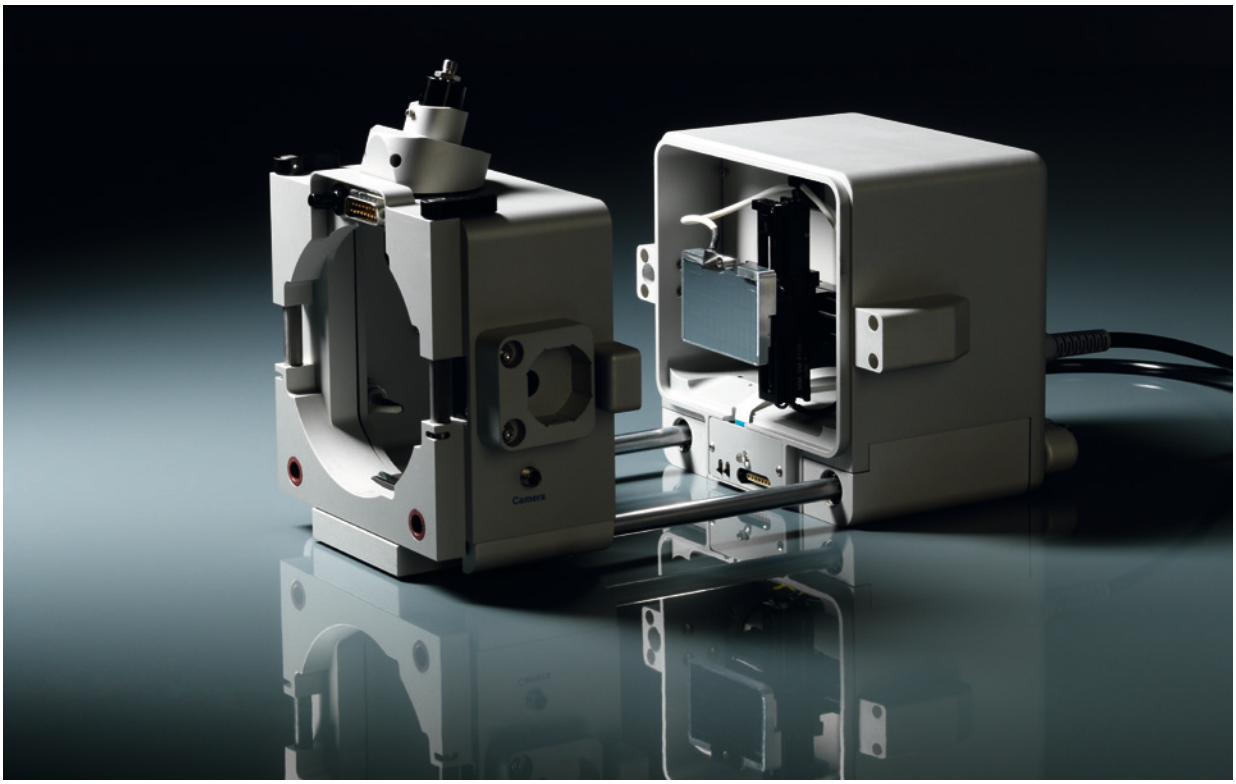


Figure 5: AP MALDI *PDF+* ion source, source opened to see the MALDI sample plate positioned on an xy stage located on the right hand side. Left hand side of the ion source allows source connection to Thermo Scientific ion trap and Orbitrap based instrumentations.

Laboratory Solutions Backed by Worldwide Service and Support

Tap our expertise throughout the life of your instrument. Thermo Scientific Services extends its support throughout our worldwide network of highly trained and certified engineers who are experts in laboratory technologies and applications. Put our team of experts to work for you in a range of disciplines – from system installation, training and technical support, to complete asset management and regulatory compliance consulting. Improve your productivity and lower the cost of instrument ownership through our product support services. Maximize uptime while eliminating the uncontrollable cost of unplanned maintenance and repairs. When it's time to enhance your system, we also offer certified parts and a range of accessories and consumables suited to your application.

To learn more about our products and comprehensive service offerings, visit us at www.thermoscientific.com.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa-Other
+27 11 570 1840
Australia
+61 3 9757 4300
Austria
+43 1 333 50 34 0
Belgium
+32 53 73 42 41
Canada
+1 800 530 8447
China
+86 10 8419 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 50 34 0
Finland/Norway/ Sweden
+46 8 556 468 00
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 561 688 8700
Middle East
+43 1 333 50 34 0
Netherlands
+31 76 579 55 55
New Zealand
+64 9 980 6700
Russia/CIS
+43 1 333 50 34 0
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Switzerland
+41 61 716 77 00
UK
+44 1442 233555
USA
+1 800 532 4752

Thermo Fisher Scientific (Bremen) GmbH Management System Registered to ISO 9001:2008

AN30224_E 06/11G

www.thermoscientific.com