Performance Characteristics of an AP MALDI Ion Source Applied to Exactive Plus Mass Spectrometer

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Introduction
Powerful Screening and Quantitation

The importance of Atmospheric Pressure Matrix Assisted Laser Desorption/Ionization (AP MALDI) technique for analyzing larger biological or synthetic samples is investigated on the novel single stage Orbitrap analyzer, the Thermo Scientific Exactive Plus MS. The Exactive Plus instrument allows access to mass range up to m/z 6000. The performance of this device is presented by detecting MALDI-ions produced under atmospheric pressure. Larger endogenous peptides are investigated with regard to mass resolution, isotope pattern and signal-to-noise ratio. Intact proteins are prepared with 1,5 DAN matrix for the application of In Source Decay (ISD) fragmentation of proteins; ISD fragments of medium-size proteins are interpreted.

Key Words
Exactive Plus, AP MALDI, High Resolution Accurate Mass Performance, In Source Decay, Orbitrap Technology

Overview
Ambient Mass Spectrometry: Fast Screening of Large Molecules

Novel Aspect: Mass range up to m/z 6000 demonstrated for AP MALDI MS and Orbitrap™-based technology

Purpose: Compatibility of Thermo Scientific™ Exactive™ Plus MS Instrumentation with focus on singly charged AP MALDI-produced ions at high mass range

Methods: AP MALDI PDF™ ion source attached to Exactive Plus MS

Results: Detection of bovine insulin at m/z 5734
In Source Decay fragmentation of Cytochrome C
Detection of 1 femtomole Angiotensin II with S/N > 50/1
Measurement of synthetic polymer PEG 3350

Samples are prepared using published preparation protocols for MALDI MS. Synthetic polymers such as PEGs are investigated, specifically the performance of the entire polymer distribution and the adduct ion formation are explored.
Methods

**Exactive Plus MS Instrumentation**

All experiments are performed on the Exactive Plus mass spectrometer, see Figure 1, using an AP MALDI PDF+ Ion source. The ion source is coupled in front of the heated capillary which is integrated in the S-lens. The peak-shaped heated capillary is held at approximately 400-450 °C for all experiments shown in this presentation. The peak front end of the heated capillary facilitates laser beam adjustment onto the sample plate. The Exactive Plus mass spectrometer is operated in Full Scan MS and AIF mode settings using resolving power of up to 140,000 at m/z 200 (FWHM) and with mass range up to m/z 6000.

**MassTech’s AP MALDI Source**

The AP MALDI PDF+ ion source (MassTech, Inc., USA), see Figure 2, is used for the herein presented experiments and results. Distance between sample plate and heated capillary in the MS-interface is adapted with suitable distance pieces (spacers) to a distance of about 1 mm.

A Nd:YAG laser beam at 355 nm (pulse duration 3-5 ns) is coupled into a 400 μm core diameter fiber forming a spot size of 500 x 650 μm². A spiral motion across the selected spot on the plate while scanning is checked in MassTech’s Target Software (Instrument Control Software of AP MALDI source). The laser repetition rate can be varied between 1 and 200 Hz in MassTech’s Target software. For most of the experiments repetition rate is set to 50 or 200 Hz.

**Mass Calibration with AP MALDI-Produced Ions in the Exactive Plus Instrument**

Mass calibration of the Orbitrap detector can be performed in MALDI mode of operation. User interface is shown in Figure 3 (bottom).

It shows the newly integrated mass calibration editor for user-defined mass lists. Specific m/z ratios can be activated or deactivated for the individual mass calibration. The mass calibration is performed for the selected m/z ratios after an automated quick ion flux stability check. Mass stability and mass accuracy are reported after mass calibration. Calibration procedure using the user-defined list lasts less than 1 minute. Insets into some m/z ratios of MALDI Calibration mixture MSCAL4 are given, see Figure 3 (top).

**Samples and Sample Preparation**

Sigma-Aldrich’s ProteoMass™ MALDI Calibration Kit for LTQ XL and LTQ Hybrids “MSCAL4” (MALDI Calibration mixtures) is prepared according to the protocol provided with the kit. Alpha-cyano-4-hydroxy cinnamic acid (CHCA) matrix is used to prepare the MALDI calibration solution (see Figure 3 (top)). If not otherwise mentioned in the figure captions, analyte molecules are dissolved in HPLC grade water and mixed with alpha-cyano-4-hydroxy cinnamic acid (CHCA) or 2,5-dihydroxy benzoic acid (2,5-DHB), respectively. For the analysis of In Source Decay fragment ions of intact, medium-size protein, 1,5 diaminonaphthalene (1,5-DAN) matrix is used.2 Volumes of 0.5-1 μL of matrix-analyte mixtures are spotted on a stainless steel plate and air dried. Polyethylene Glycol (PEG) is purchased from Sigma-Aldrich (P-3640), for sample preparation see results.
Conclusion
Fast and broad screening for confident compound ID

- For the first time, intact bovine insulin at m/z 5734 is detected on an Exactive series mass spectrometer.
- In Source Decay fragment ions of Cytochrome C are detected with 1,5 DAN matrix and N- and C-terminal ions are observed up to m/z 5000.
- S/N > 50/1 of a 1 femtomole Angiotensin deposited on plate is detected.
- For PEG 3350, a synthetic polymer, its polymer distribution is easily displayed and adduct formation is unambiguously elucidated with 2,5-DHB matrix and LiTFA addition.
- All Ion Fragmentation allows detection of HCD fragment ions over a wide mass range, from immonium-related ions to intact medium-size peptides.
- It is recommended to use high capillary temperatures (about 400 °C) and select the spiral motion for ease of data acquisition.

Acknowledgements
We would like to thank Dr. Andrew Hoteling, Bausch & Lomb, USA, for sharing the 2,5-DHB/ LiTFA matrix sample preparation protocol with us. Help in data interpretation of In Source Decay data obtained from our colleague Kai Scheffler is greatly appreciated.

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References
2. Thermo Scientific Application Note AN 30218, MALDI In Source Decay Fragment Ions of Peptides and Proteins Generated in a Collisional Cooling Interface and Detected with an Ion Trap – Orbitrap Hybrid Mass Analyzer
Results

Endogenous Peptide and Sequence-Specific Fragments of Medium-Size Protein

Bovine Insulin and fragment ions of intact horse heart Cytochrome C are analyzed as singly charged ions with the Exactive Plus MS.

In the first experiment, the performance with the extended mass range is shown and intact MH+ signal of bovine Insulin is detected using CHCA matrix. Figure 4 shows Orbitrap analyzer full scan spectrum from m/z 1500-6000 and insets into singly, doubly and triply charged ions of bovine insulin. Corresponding mass accuracy and resolving power are given in the insets. The same performance is obtained using 2,5 DHB matrix, data not shown.

In Source Decay of Intact Protein Cytochrome C

In the second experiment, 1,5-diaminonapthalene (1,5-DAN) serves as MALDI matrix, because it produces significant amount of specific N- and C-terminal sequence information from the In Source Decay process. Figure 5. Series of a-, b-, c-, y- and z-type ions are observed. Here, the z-type ion series is labeled in blue. Resolution and mass accuracy is given. Results are in agreement with those obtained using other MALDI interfaces. Method setup applies three mass ranges and 75 eV In Source CID energy. An inset from m/z 1200 to m/z 5000 into Orbitrap analyzer full scan information, and an inset into mass range (same spectrum) from m/z 2600 to m/z 3100 with signal assignments are shown.

1 femtomol Angiotensin II Amount on the Plate

The signal-to-noise ratio (S/N) of the mono isotopic peak of Angiotensin II MH+ (m/z 1046.5418) is evaluated. Sample preparation with CHCA matrix is performed according to the protocol in MASCAL/4. The S/N of a 1 femtomole Angiotensin II deposited on the sample plate is investigated. The laser repetition rate of 200 Hz, 50 ms injection time and Source CID were used for data acquisition. A total of 5 full scans were averaged per spectrum (Figure 6). The S/N reflects the general performance of low analyte loads on plate in MALDI and AP MALDI.

Figure 4. AP MALDI mass spectrum of bovine insulin at m/z 5734

Figure 5. MALDI In Source Decay of protein Cytochrome C using 1,5 DAN matrix

Figure 6. Inset into FTMS mass spectrum of a 1 femtomole load of Angiotensin II (m/z 1046.5418, C50 H72 N13 O12) on plate generated by AP MALDI
Results

All Ion Fragmentation of Proline_{14R} peptide

To show the ability of the “All Ion Fragmentation” (AIF) technique in the Exactive Plus mass spectrometer, a peptide with the sequence Proline_{14R} (Sigma Aldrich’s ProteoMass™ P_{14R} MALDI-MS Standard) and MH+ of m/z 1533.85765 (C_{76}H_{113}N_{18}O_{16}) is injected into the Higher Energy Collisional Dissociation (HCD) cell. AIF takes place in the collision cell attached to the C-trap (see Figure 1). Fragment ions are subsequently injected into the Orbitrap detector; results are shown in Figure 7. The spectrum is acquired using CHCA matrix, 200 Hz laser repetition rate, and applies Source CID ‘on’.

Synthetic Polymer (PEG) Analyzed with 2,5-DHB Matrix and LiTFA

This experiment involves (2,5-DHB matrix, the protocol is given as follows: a) concentration of polymer sample is 2 g/L in methanol (stock solution), b) 0.25 g/L of LiTFA, and c) 15 g/L of 2,5-DHB, respectively, are prepared in methanol. 70 μL of matrix solution is mixed with 10 μL of sample and 10 μL of LiTFA solution. 1 μL of the final mixture is deposited on the sample plate.

Addition of e.g. LiTFA in such synthetic polymer samples can be beneficial as it facilitates data interpretation in comparison to the default occurrence of Na+- and K+-ions. The Li+-adduct ion formation can compete successfully against Na+- or K+-adduct ion formation as shown in the mass spectrum for the PEG sample. Li+-adduct ions can be assigned easily and distinguished clearly from Na+- or K+-adduct ions. The expected mass increment of Δm = C_{12}H_{24}O = 44.02621 u between subsequent polymerization grades (D_n and D_{n+1}) is obvious in the mass spectra.

Figure 8 represents 10 scans averaged of the PEG sample prepared with 2,5-DHB matrix and addition of LiTFA. Orbitrap analyzer full scan data (m/z 400–m/z 6000) (bottom) and an inset into mass range around m/z 2600 with peak interpretation are shown (top); all masses are reflected with mass accuracy better than 3 ppm. The inset is compared to simulated data of PEG polymerization grade D_{60} (middle).
Performance Characteristics of Exactive Plus Instrumentation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Mass range</td>
<td>m/z 50-6,000</td>
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<tr>
<td>Resolving Power</td>
<td>Up to 140,000 (FWHM) @ m/z 200</td>
</tr>
<tr>
<td>Max scan rate</td>
<td>Up to 12 Hz at mass resolution setting of 17,500 @ m/z 200</td>
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| Mass accuracy                        | Internal: < 1 ppm RMS  
                                External: < 3 ppm RMS  
                                Under defined conditions |
| Sensitivity in Full MS mode          | 500 fg Buspirone on column S/N 100:1 acquired with H-ESI II probe    |
| Dynamic range                        | > 5000:1 in single scan, intra-scan dynamic range                    |
| Polarity switching                   | One full cycle in < 1 sec (one full-scan positive ion mode and negative ion mode each at mass resolution setting of 35,000 @ m/z 200) |
| Analog inputs                        | One analog input (0-1 V)  
                                One analog input (0-10 V)                                           |

Options
- Axial Higher energy Collision-induced Dissociation (HCD) cell
- NanoSpray Flex Ion source – single set-up for all online nanoflow applications
- ESI probe compatible with liquid flow rates of < 1 μL/min to 1 mL/min without splitting
- APCI source compatible with liquid flow rates of 0.5 μL/min to 2 mL/min without splitting
- APCI/APPI source compatible with liquid flow rates of 0.5 μL/min to 2 mL/min without splitting
- Metal needle kits for high and low flow analyses
- The Exactive Plus MS is upgradable to Q Exactive MS onsite

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