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Application News

MALDI-7090

No.MO424

# Top-Down In-Source Decay (ISD) Fragmentation and ISD$^n$ Sequencing for Protein Characterization

## Introduction

Matrix-assisted laser desorption/ionization (MALDI) in-source decay (ISD) refers to fragmentation occurring in the MALDI plume prior to ion extraction from the source region during the desorption/ionization steps. The metastable decay process is triggered when the laser fluence is increased 5–20% above the ionization threshold. In contrast to a bottom-up approach, where the protein of interest is subjected to enzymatic digestion followed by MS/MS sequencing, MALDI-ISD is referred to as a top-down approach. Such top-down methods have been previously used for extensive characterization of post-translational modifications. This pseudo-MS/MS technique uses hydrogen radical transfer from the matrix to the analyte molecules to provide fragmentation and sequencing of the N- and C-termini of intact proteins.

A limitation to MALDI-ISD is the overlap of lower mass fragment signals with chemical noise and matrix-related ions. This can be circumvented using ISD$^n$ : MS/MS fragmentation of precursor ions created by ISD. However, using conventional MALDI TOF-TOF instrumentation, ISD and particularly ISD$^n$ approaches had a limited resolution. The introduction of patented technology, Axial Spatial Distribution Focussing (ASDF™)[1], in the MALDI-7090 TOF-TOF instrument (figure 1) significantly improves the fragment ion resolution during MS/MS acquisitions. Through correction of the axial spatial distribution of the ions generated, the MS/MS mass resolution and consequently the ISD$^n$ resolution is significantly increased and becomes essentially independent of the laser power used to ionize the sample.

This application note describes the combination of ISD and ISD$^n$ with high resolution MS/MS using the MALDI-7090 TOF-TOF and demonstrates the unparalleled resolution delivered by the instrument using CID and ASDF.

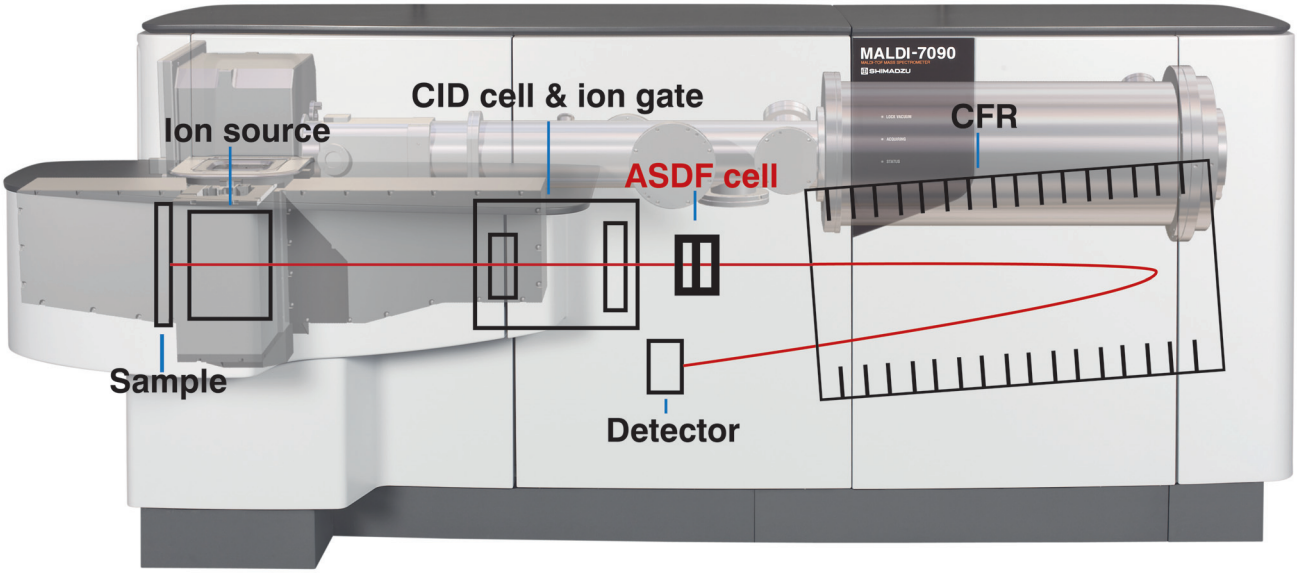


**Figure 1: Schematic of the MALDI-7090 MALDI TOF-TOF mass spectrometer.**

## Experimental

BSA (bovine serum albumin, 66 kDa) and Avastin (bevacizumab monoclonal antibody, 150 kDa) were provided by Aix-Marseille University (France). The proteins were purified using dialysis filtration. Samples were prepared for MALDI analysis using 1,5-diaminonaphthalene matrix (saturated in 50:50 acetonitrile:$H_2O$ 0.1% TFA). Samples were analyzed using a MALDI-7090 TOF-TOF mass spectrometer equipped with UV laser, CID cell and ASDF. For CID, helium gas (rather than heavier gases such as air or argon) was introduced into the collision cell allowing selected precursors to undergo high-energy collisions (HE-CID, 20 keV lab collision-energy) during MS/MS analysis. This instrument geometry demonstrated $ISD^n$ fragment ion resolutions in excess of 8000 (FWHM), essentially independent of the laser power. The resulting fragment ions were measured in the TOF analyzer and resolved using a curved-field reflectron.

## Results

During ISD on a conventional MALDI TOF-TOF instrument, depending on the matrix used, fragmentation proceeds via a radical or thermal pathway, resulting in the formation of primarily $c_n$ and $(z_n+2)$ ions or $b_n$ and $y_n$ ions, respectively. The advantages of using the MALDI-7090 TOF-TOF for ISD analysis of proteins were demonstrated using BSA and Avastin. This monoclonal antibody is a good *in vitro* model to demonstrate the potential of the described technique for the characterization of very large proteins. ISD of these proteins was induced by increasing the laser power by around 30% above the ionization threshold value. In the MALDI-7090 TOF-TOF instrument, this increase led to the detection of an extensive c-ion fragment series.

The MALDI-7090 TOF-TOF in-source decay spectra obtained for BSA and Avastin proteins using linear and reflectron modes are illustrated in figures 2 and 3.

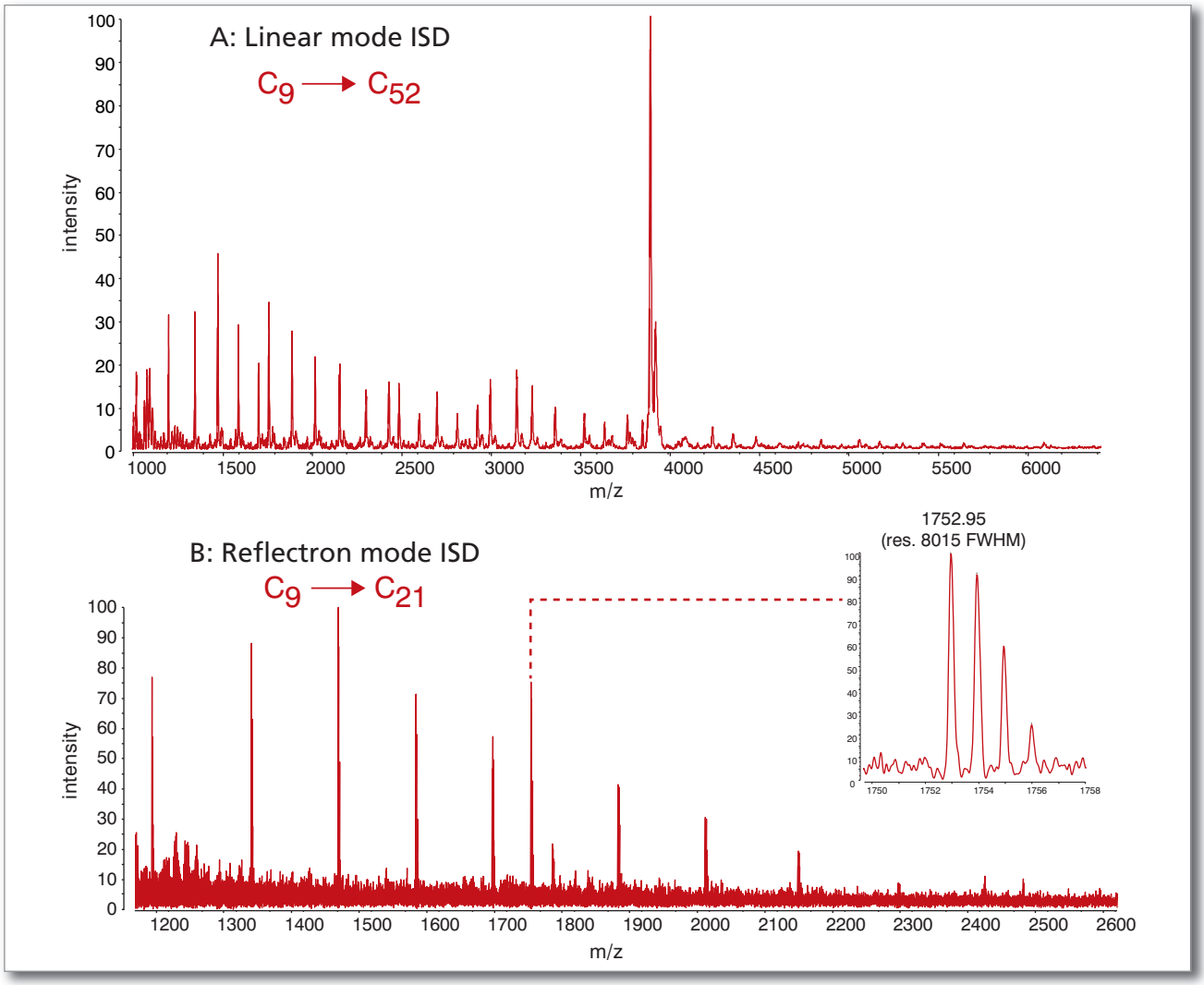


**Figure 2: ISD spectra of BSA with 1,5-DAN matrix in linear mode (A), reflectron mode (B). Inset (panel (B)): Expanded m/z region showing the $c_{15}$ ion (m/z 1753) of BSA.**

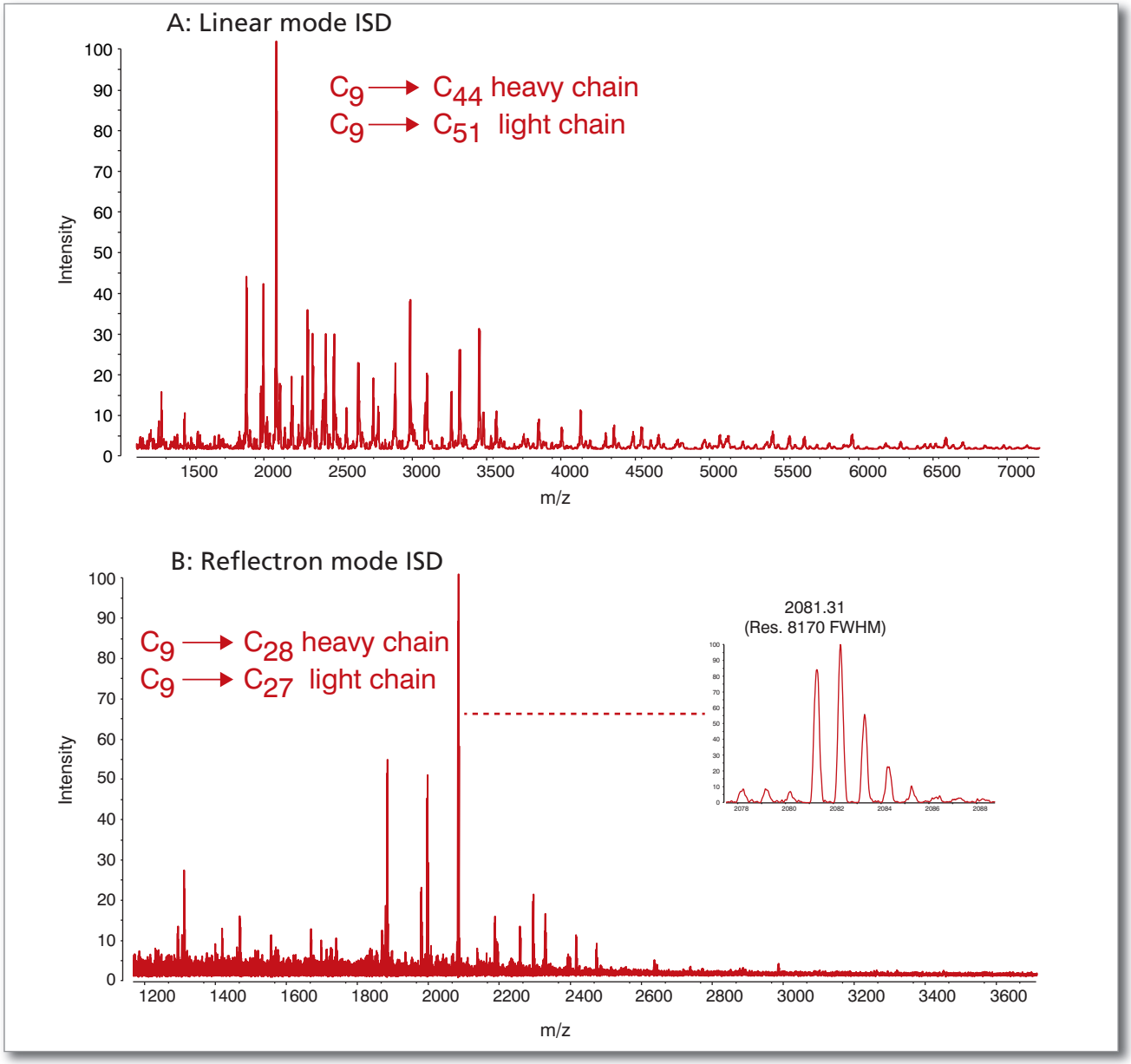


**Figure 3: ISD spectra of Avastin with 1,5-DAN matrix in linear mode (A), reflectron mode (B). Inset (panel (B)): Expanded m/z region showing the $c_{21}$ ion (m/z 2081) of Avastin heavy chain.**

Significant c-ion series from the N-termini of the proteins were detected:

$c_9$ to $c_{52}$ for BSA in linear mode

$c_9$ to $c_{21}$ for BSA in reflectron mode

$c_9$ to $c_{44}$ and $c_9$ to $c_{51}$ in linear mode for Avastin heavy and light chains respectively

$c_9$ to $c_{28}$ and $c_9$ to $c_{27}$ in reflectron mode for Avastin heavy and light chains respectively.

$ISD^n$ sequencing of the ion at m/z 2081 was performed using a combination of CID and ASDF. Figure 4 shows the resulting spectrum and fragment ion interpretation, confirming the correct sequence for ISD fragment ion $c_{21}$ from Avastin heavy chain.

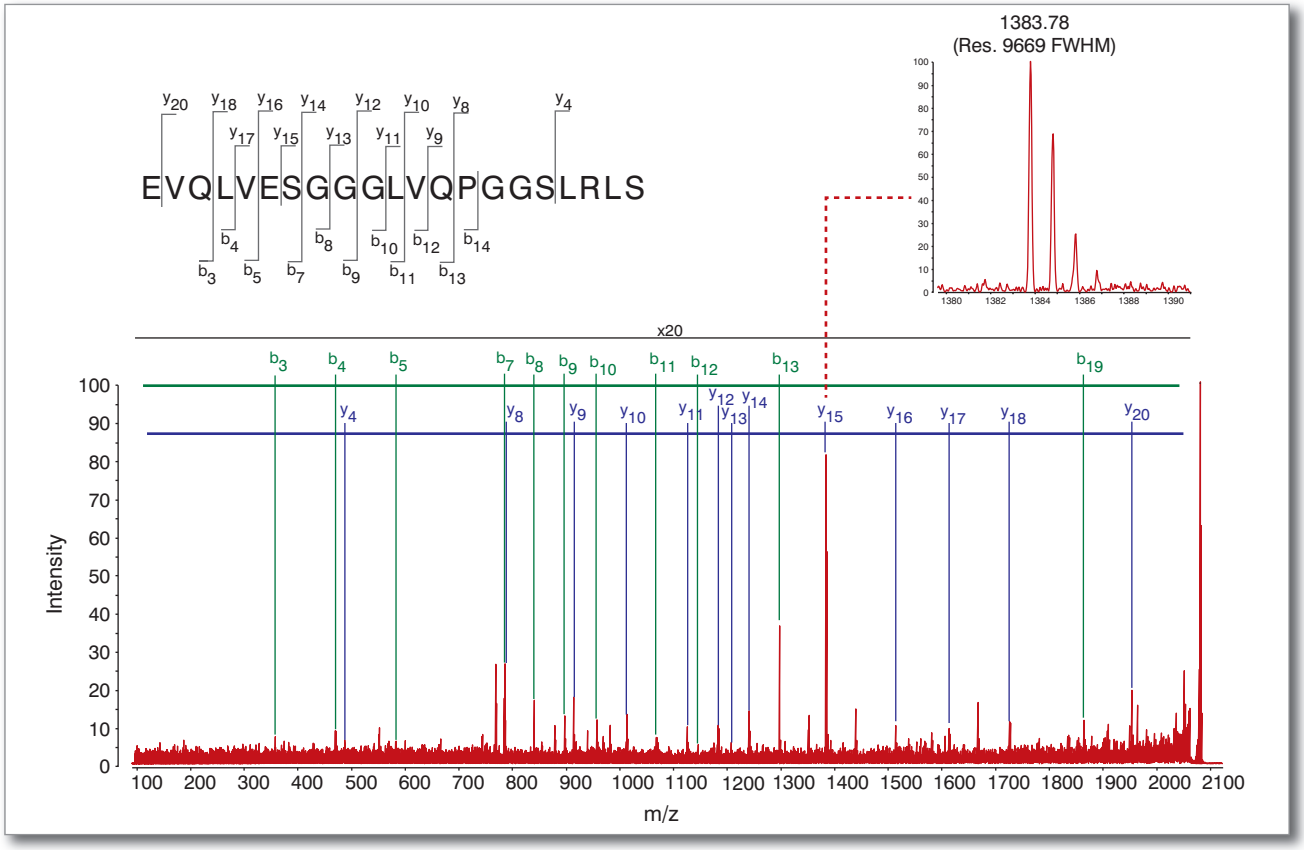


**Figure 4: ISD$^n$ sequencing of the $c_{21}$ ion from Avastin heavy chain (m/z 2081) using CID and ASDF. Inset: Expanded m/z region showing the $y_{15}$ ISD$^n$ fragment ion illustrating high resolution MS/MS using ASDF.**

## Conclusions

ISD and ISD$^n$ performed on the ultra-fast MALDI-7090 TOF-TOF mass spectrometer offers several advantages for detailed characterization of biomolecules compared with ISD performed on conventional MALDI TOF-TOF instruments.

The high performance of the MALDI-7090 affords excellent resolution and good mass accuracy. The unique ASDF technology in the MALDI-7090 minimizes the adverse effect of increasing the laser power on mass resolution that is typically observed in conventional MALDI TOF-TOF MS/MS, effectively decoupling resolution from laser fluence.

## Acknowledgements

The samples were kindly provided by Dr. Daniel Lafitte and his team at the Proteomic and Technological Innovation Platforme Timone PIT2, Aix Marseille University, France.

References

(1) Axial Spatial Distribution Focussing, patent: WO-2010010333-A1.

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