# MALDI In-Source Decay Ions Generated in a Collisional Cooling Interface and Detected With an Ion Trap – Orbitrap Hybrid Mass Spectrometer

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## Overview

**Purpose:** Evaluation of 1,5 diaminonaphthalene as MALDI matrix for peptide and protein sequencing.

## Introduction

MALDI-produced fragment ions can be generated by using 1,5-diamino-naphthalene (1,5-DAN) matrix. This matrix was previously applied to enable rapid reduction of disulfide bonds[1] and N-terminal and C-terminal sequencing[2] of peptides and small proteins. 1,5-DAN matrix was developed to specifically produce a significant amount of in-source decay fragments on axial time-of-flight (ToF) systems.[2] MALDI in-source decay (MALDI ISD) fragment ion production was initially described in the mid 1990s in studies employing delayed extraction in axial TOF systems by Brown et al.[3,4,5] A model explaining the underlying mechanism in MALDI was proposed by Takayama (N-$C_{\alpha}$ bond cleavage of peptide backbone via hydrogen abstraction) and its parallels to ECD were given.[6] Using 1,5-DAN Demeure *et al.* observed entire c- and z-type sequence ladders of sequence-specific fragment ions simultaneously in an axial TOF geometry.[2]

In contrast to previously applied axial TOF with relatively low pressure in front of the sample plate, the MALDI ion source used in this study liberates the MALDI-produced fragment ions into a region of significantly higher pressure (75 mtorr)[7], *i.e.* a collisional cooling regime. Ions (ISD fragment ions) are detected in a linear ion trap or an Orbitrap detector. In this report, we show 1,5-DAN matrix applied for the first time to a collisional cooling interface with Orbitrap detection. ISD fragment ions of peptides or proteins are observed upon Orbitrap detection. In addition, entire sequence tags can be observed. a-, b-, c-, y-, and z- type fragment ions are also observed simultaneously in a spectrum.

## Methods

**Reagents:** 1,5-diaminonaphthalene (1,5-DAN), ACTH (18-39), Insulin B chain (oxidized), and chicken lysozmye were purchased from Sigma-Aldrich (Steinheim, Germany).

**Matrix and Analyte Solutions:** 1,5-DAN was used as matrix and prepared as a saturated solution in water/acetonitrile 50/50 v/v with 0.1% TFA and centrifuged before usage.[2] The 1,5-DAN solutions were always prepared shortly before MS experiments because of the instability of 1,5-DAN in acetonitrile. Peptides and proteins were dissolved in HPLC grade water at concentrations of 5 pmol/µL and mixed in a ratio 1:1 with 1,5-DAN. 0.5 µL of each analyte/matrix mixture was spotted on a stainless steel target and air dried at ambient temperature.

**Mass Spectrometry**: All experiments were performed on a Thermo Scientific MALDI LTQ Orbitrap XL hybrid mass spectrometer, operating at intermediate pressure (75 mTorr).[7] Spectra were acquired from single scans or as averages from some (up to 3) µscans. Averages of some (up to 10 to 25) scans are displayed; a single µscan can easily meet a "number of charges" request of 1e6 in the Orbitrap™ detector with 50 laser shots.

**Data Processing:** Raw data were processed with Thermo Scientific Xtract software to obtain the monoisoptic mass lists that were submitted to ProSightPTM, https://prosightptm.northwestern.edu/ for fragment ion matching of known sequences.

## Results

Figure 1 displays the mass spectrum obtained from 1,5-DAN. Besides monomeric radical and protonated species, ions representing dimeric and trimeric structures are detected. The capabilities of this matrix to produce a mixture of the four most common low-energy sequence ions (c-, z-, b- and y-type ions) are demonstrated by three examples: a 14.3 kDa small-size protein (Figure 2, chicken lysozyme), a mid-size peptide at mass 2464 (Figure 3, ACTH fragment), and a larger peptide at mass 3495 (Figure 4, oxidized bovine Insulin b chain). All fragments are detected in the MALDI ISD full MS spectrum next to the intact precursor (Figures 3 and 4).

**FIGURE 1. MALDI ISD full MS spectrum of 1,5-DAN (M=158.08385) and proposed peak and structure assignments of the major species detected: the radical cation monomer, dimer, and trimer in a mixed population with their protonated species and isotope patterns.**

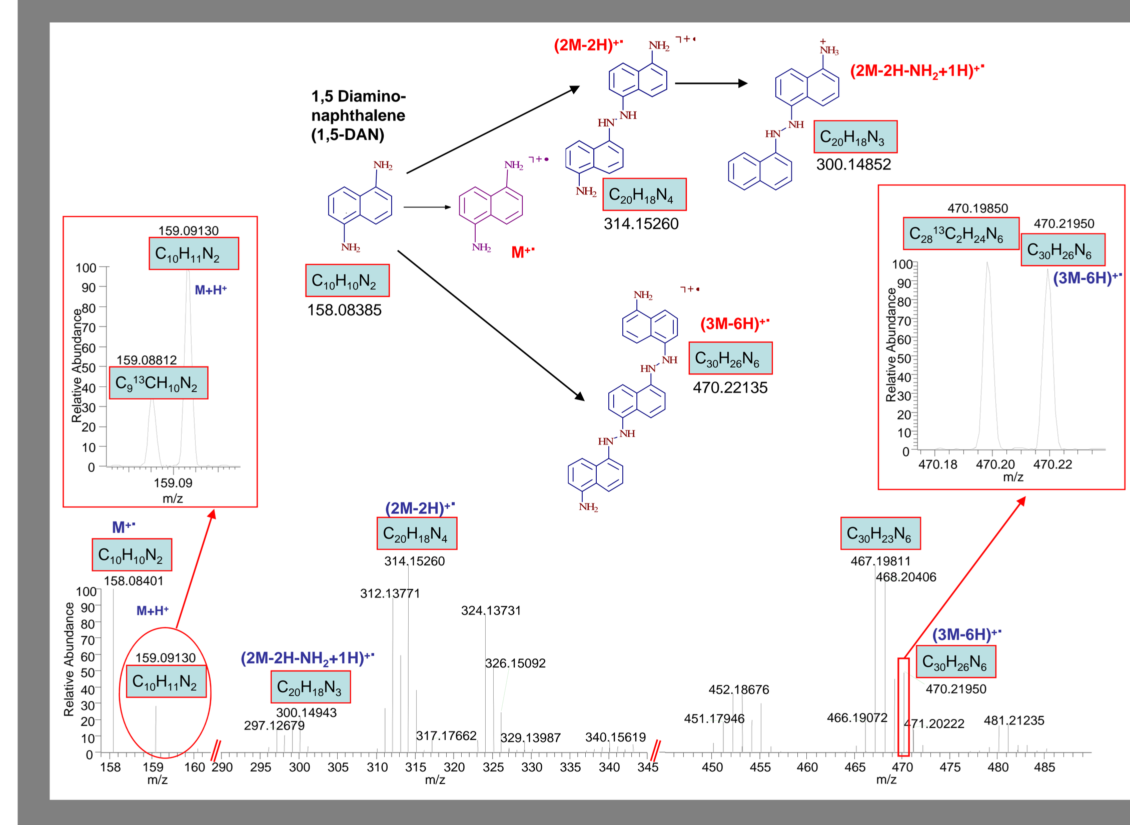
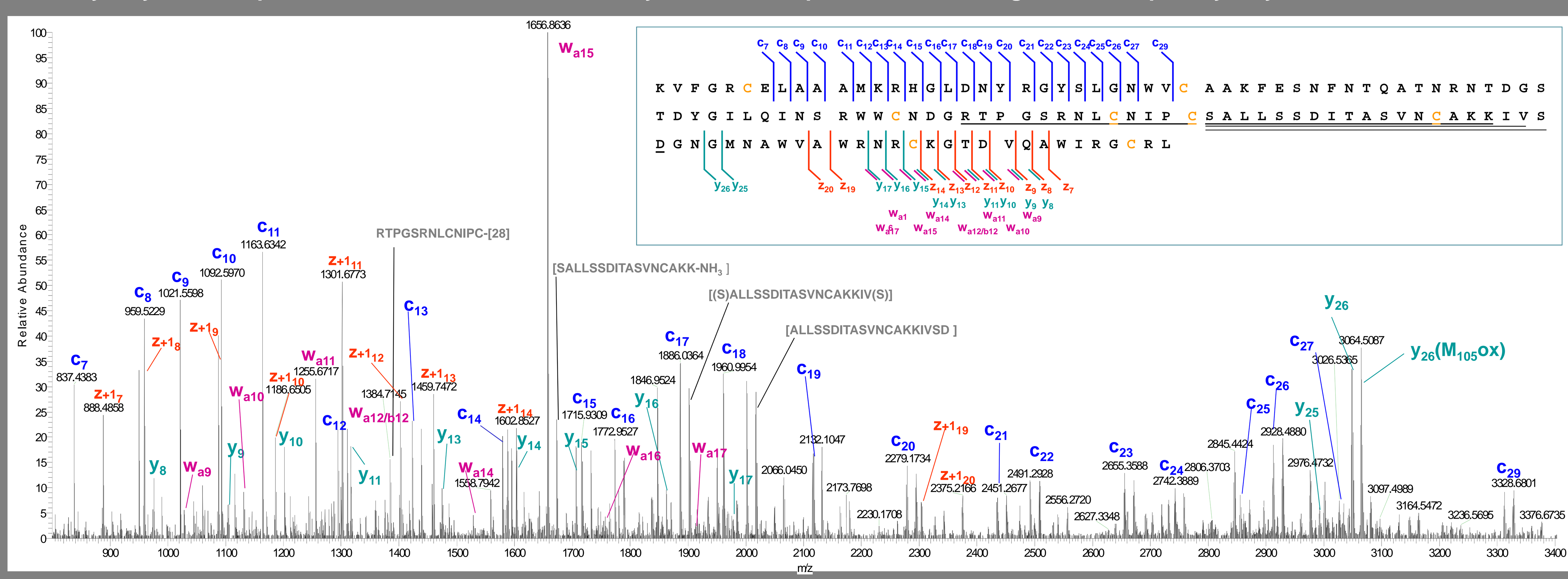


**FIGURE 2. MALDI ISD full MS spectrum of intact lysozyme (M=14.3 kDa) with fragment ion annotation of c-, z-, b-, and y-ions. A large portion towards the N-terminus is covered by a complete c-ion series. The C-terminus is covered by a smaller series of y- and z-ions. The middle section of the sequence is represented by 3 internal fragments. The c- and y- ions series demonstrates the reducing nature of 1,5-DAN since native lysozyme was spotted. Nevertheless, the reduced cysteines are represented in all fragments except for y/z7-y/z14.**



The spectrum of oxidized insulin B chain (Figure 4) demonstrates that fragmentation is rich and spontaneous, especially because no further energies and fragmentation reactions are involved. Fragmentation is intrinsically involved in the MALDI event, in particular, with this specific matrix 1,5-DAN. Series of a-, b-, c-, and z-ions next to w- and d-ions are detected simultaneously in a single full MS scan, covering the entire sequence of the peptide.

**FIGURE 3. MALDI ISD full MS spectrum of ACTH (18-39) (M=2464). Dominant series of b- and c-type ions are detected, complemented by a few a- ions and y-ions.**

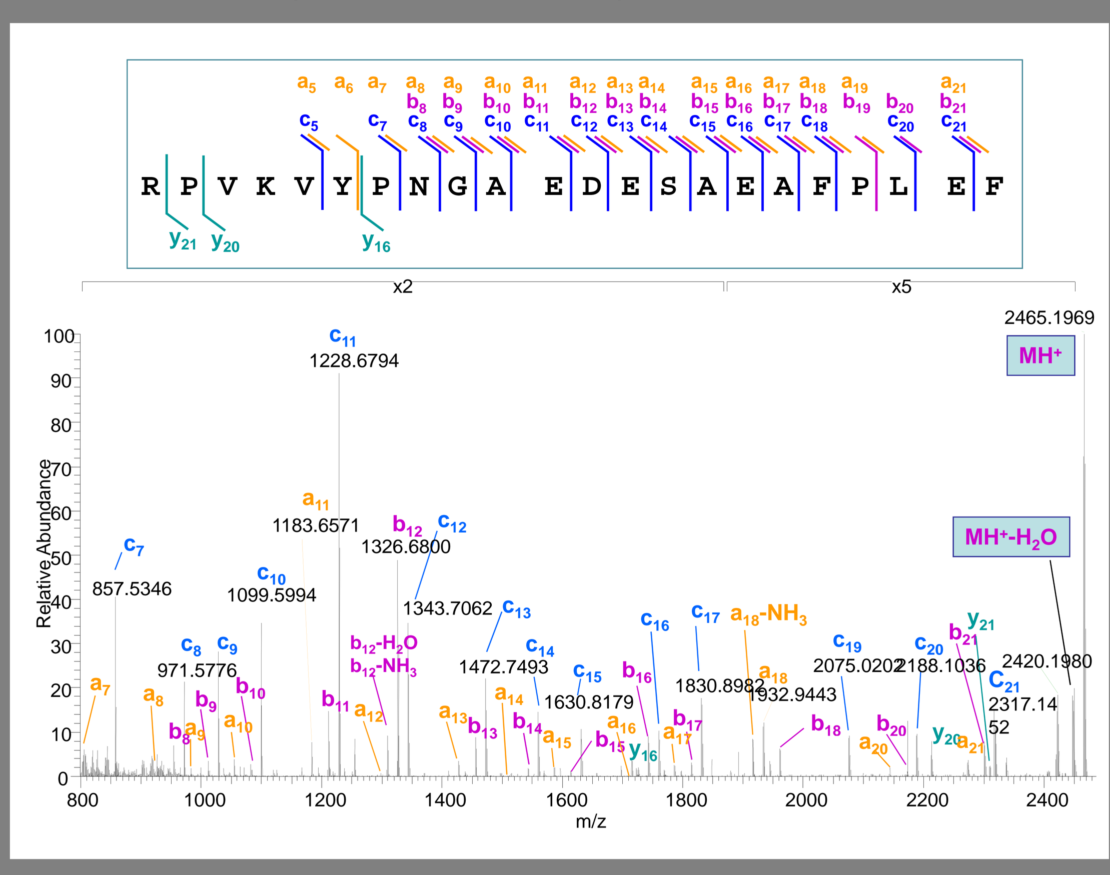
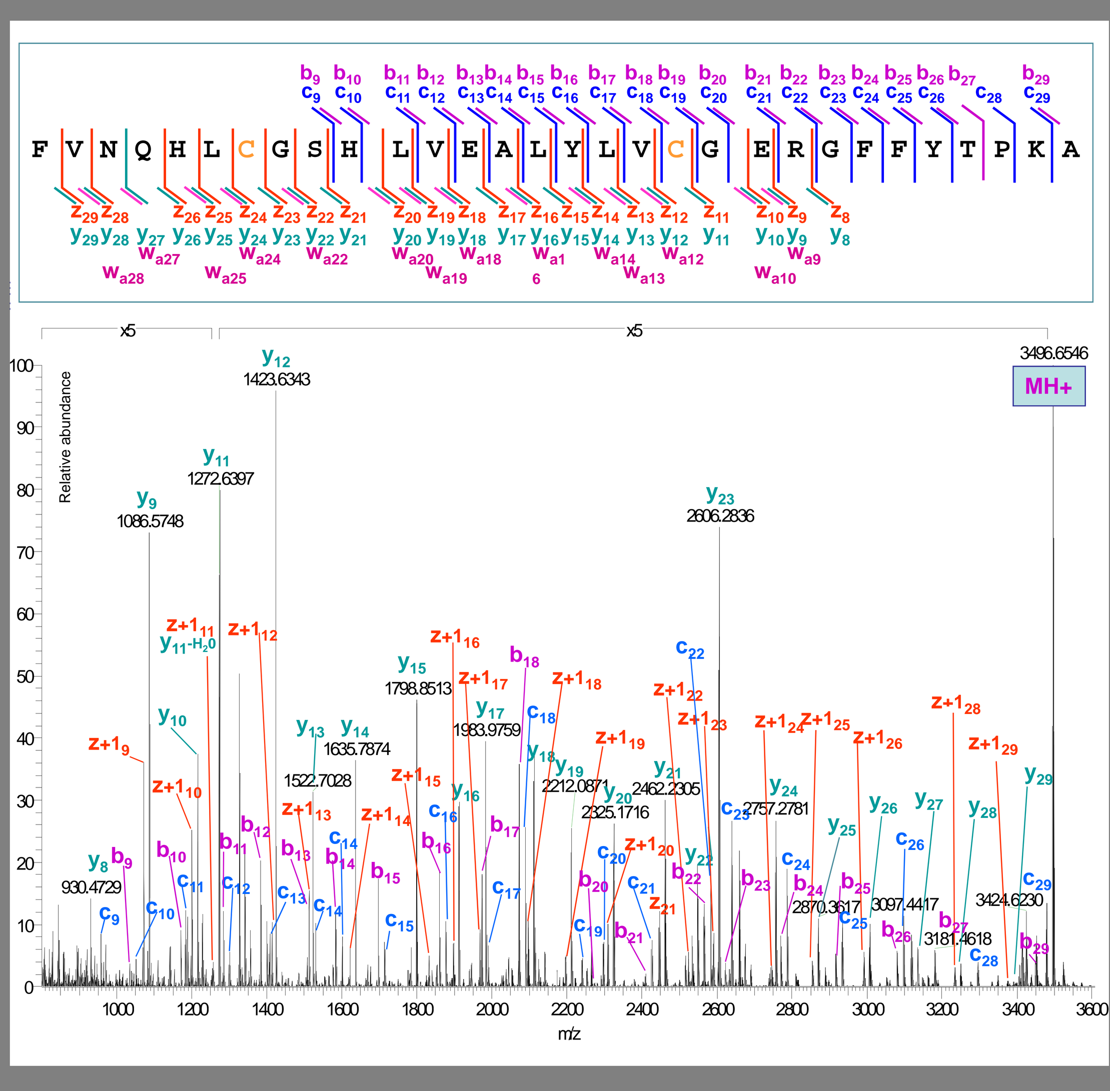


**FIGURE 4. MALDI ISD full MS spectrum of insulin B chain (oxidized) (M = 3495). All four series of b-, c-, y- and z-type ions are nearly completely detected, thus covering the full sequence of the peptide.**



Another characteristic of 1,5-DAN is its reducing nature that was observed upon the analysis of lysozyme in its native form (Figure 2). Lysozyme contains four disulfide bonds (6-127, 30-115, 64-80, 76-94) in the protein sequence without signal peptide (as shown in Figure 4) that are apparently reduced by the matrix. This allows the detection of cysteine-containing fragment ions. Analysis of the spectrum obtained from native lysozyme confirms the presence of six out the eight possible free cysteine residues in the sequence. The two cysteines, Cys30 and Cys64, not present in any of the observed fragment ions, are not connected via a disulfide bond in the native form, confirming that all four disulfide bridges of lysozyme are reduced by 1,5-DAN.

## Conclusion

- MALDI spectra acquired using 1,5-DAN matrix provide sequence-specific, fragment ion-rich, full MS spectra of peptides and proteins.
- Collisional cooling combined with accurate mass detection of sequence-specfic fragment ions in the Orbitrap detector allows the simultaneous detection of the series of c- and z-type ions, b-, and y-type ions, a-, d- and w-type ions, and also internal fragment ions.
- Fragments are detected in the MALDI ISD full MS spectrum without any precursor selection or resonance excitation.
- The fragmentation process intrinsic to the MALDI process is particularly pronounced with this matrix in comparison to other matrices such as 2,5 DHB or CHCA.
- This approach enables sequening of entire peptides, N- or C-terminal sequencing, and sequence confirmation of proteins.
- In a middle-down or top-down approach, sequences of larger peptides (< 4kD) and N-terminal or C-terminal sequence tags of intact proteins above 50 kDa can be obtained and assigned.
- A wealth of information about a peptide and/or protein can be obtained in a very short time in this top-down approach, and it represents an appealing alternative to top-down sequencing by ESI.

## References

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