# Rapid-Turnaround Top-Down Sequence Verification and Characterization of Modified Proteins and Oligonucleotides Using a Benchtop MALDI-TOF/TOF Instrument

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## Introduction

MALDI Top-Down Sequencing (MALDI-TDS) is a proven method for rapid-turnaround verification and characterization of intact proteins, oligonucleotides, PEGylated peptides and other modalities. MALDI-TDS benefits from the formation of singly charged MALDI In-Source Decay (MALDI-ISD) fragments eliminating the need for demanding and potentially error-prone charge deconvolution routines and, thus, making interpretation of MALDI-TDS data simple and straightforward.

Here we highlight the unique MALDI-TDS capabilities of the neofleX™ benchtop MALDI-TOF/TOF for verification and characterization of modified proteins and oligonucleotides using the following samples for demonstration:

i) a recombinant protein expression product featuring a disulfide bridge and a minor, terminally truncated proteoform
ii) a heavily modified RNA 39-mer (2´O-methyl, 2´fluoro, phosphorothioate)
iii) a 5´-DBCO labeled DNA 21-mer.

## Methods

A 29.7 kDa recombinant protein of *Thermobifida alba estl* gene coding for cutinase F7IX06-9ACTN, expressed in L-arabinose inducible *E.coli* host cells (concentration 40 pmol/µl), was prepared on the MALDI plate using either 2,5-DHAP (for intact mass measurement) or SDHB (for MALDI-ISD top-down sequencing) as a matrix.

Two oligonucleotide samples (concentration 10 pmol/µl), a heavily modified RNA 39-mer (MW 12.5 kDa) and a 5´-DBCO-labeled DNA 21-mer (MW 7 kDa), were prepared on the MALDI plate using 3-HPA or 2,4,6-THAP as MALDI matrix.

All data were acquired on a Bruker neofleX benchtop MALDI-TOF/TOF instrument using default application methods in positive linear mode (intact mass analysis) and positive reflector mode (MALDI-ISD top-down sequencing).

Data analysis of MALDI-TDS spectra was performed using Bruker OmniScape™ and Biotools software.

## References

Bruker Application Note MT-142, www.bruker.com

## Acknowledgement

Sample courtesy of the recombinant protein sample by Prof. Bart Devreese and Dr. Isabel Vandenberghe, University of Gent, Belgium, is gratefully acknowledged.

## Results

### i) 29.7 kDa recombinant protein of *Thermobifida alba estl* gene coding for cutinase F7IX06-9ACTN, expressed in L-arabinose inducible *E.coli* host cells

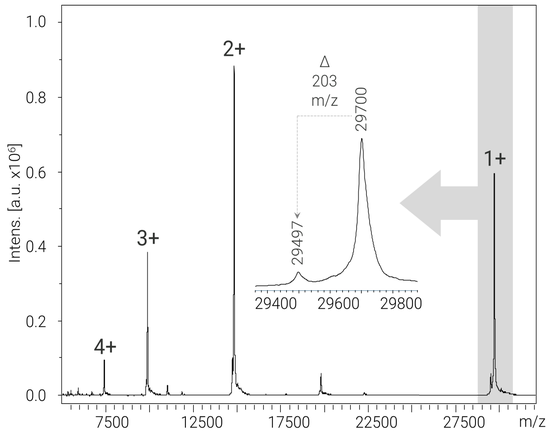


**Figure 1: Intact-mass MALDI-TOF spectrum**
Intact-mass analysis **confirmed the molecular weight** of the expected recombinant expression product, but **indicated a protein heterogeneity** in form of an **additional minor proteoform** appearing approx. 203 Da below the mass of the expected expression product. Based on its measured mass, this minor proteoform **could suggest a truncated sequence version lacking the N-terminal dipeptide MA**.

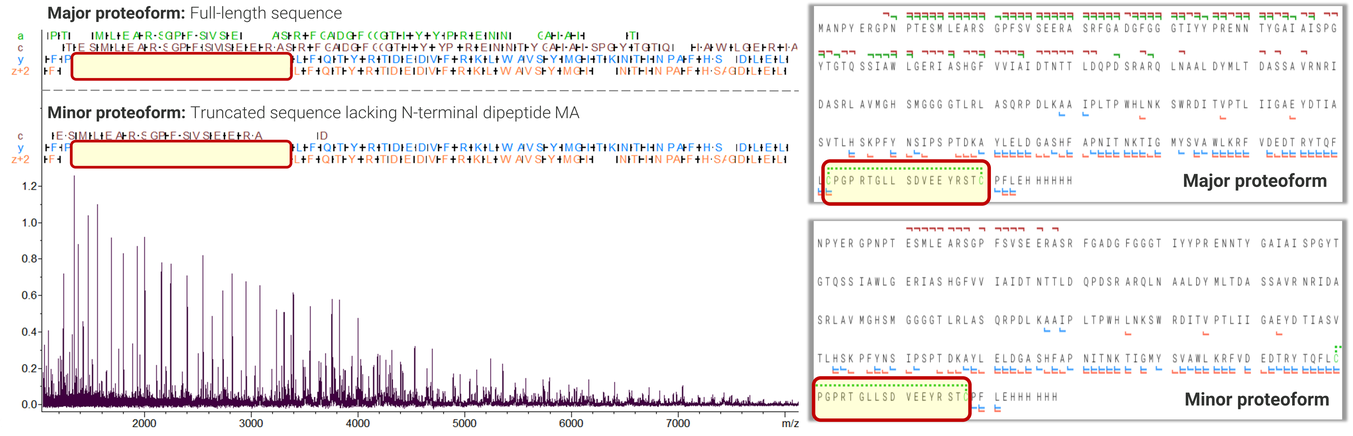


**Figure 2: MALDI-ISD top-down sequencing result**
The MALDI-ISD spectrum acquired from the protein sample (*Figure 2, left*) provided the following information:
i) The protein sequence of the **major proteoform was confirmed** by means of MALDI-ISD **sequence readout** at a depth of **80 amino acid residues from N- and C-terminus each**, resulting in a **MS/MS sequence confirmation percentage of >50% provided in a single spectrum** (*Figure 2, left*, sequence annotation above dashed line, *Figure 2, top right*, sequence map).
ii) A **minor proteoform** detected in the intact-mass spectrum at a distance of approx. [-203 Da] was elucidated as **truncated sequence version lacking the N-terminal dipeptide MA** based on the occurrence of a low abundant matching series of N-terminal c-type MALDI-ISD fragment ions (*Figure 2, left*, sequence annotation below dashed line, *Figure 2, bottom right*, sequence map).
iii) A gap observed in the series of matching C-terminal fragment ions (y, z+2) revealed the presence of a disulfide bridge between cysteine residues 242 and 260.

### ii) Modified RNA 39-mer (2'O-Me, 2'fluoro, phosphorothio.)

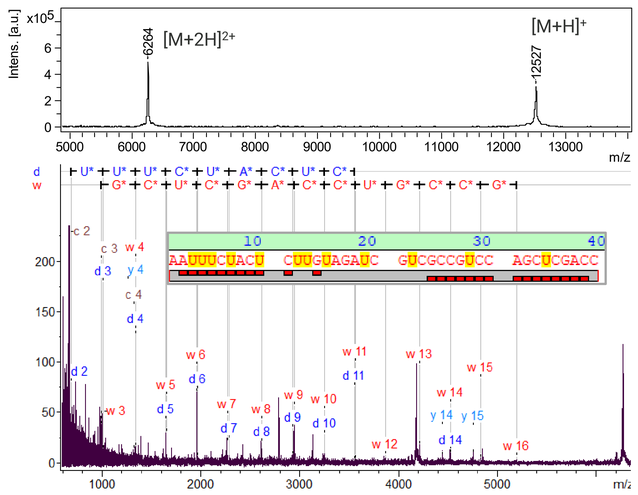


**Figure 3: Intact-mass MALDI-TOF spectrum (top) and MALDI-ISD spectrum (bottom)**
The sequence of the RNA 39-mer, including its **complex modification profile** (2´fluoro, 2´O-methyl, phosphorothioate) was **confirmed** by the MALDI-ISD data with **~67% MS/MS sequence coverage** based on matching d- and w-type fragment ions mainly.

### iii) 5'-DBCO labeled DNA 21-mer

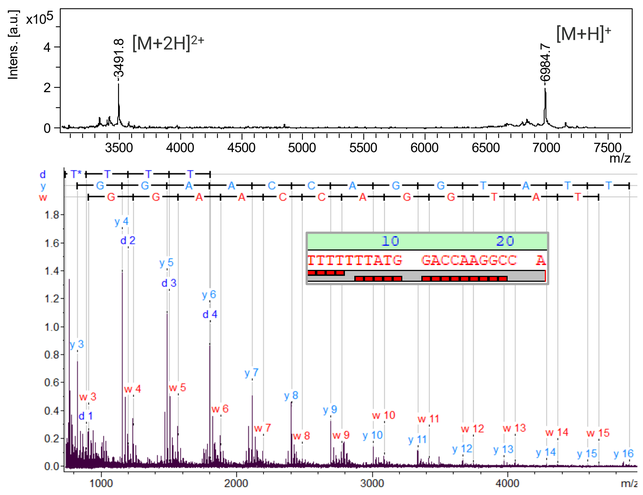


**Figure 4: Intact-mass MALDI-TOF spectrum (top) and MALDI-ISD spectrum (bottom)**
The **sequence** of the 5'-DBCO-labeled DNA 21-mer was **successfully verified**, including **localization** of the **DBCO-label at its 5´-end**, based on matching d-, y- and w-type fragment ions yielding **~85% MS/MS sequence coverage**.

## Conclusions

- MALDI-TDS performed on neofleX benchtop MALDI-TOF/TOF provides a simple but highly efficient LC-free tool for fast-turnaround top-down sequence verification and characterization of modified proteins, oligonucleotides and other biomolecular modalities.
- MALDI-TDS uses singly charged MALDI-ISD fragments making data analysis particularly easy and straightforward, without a need for charge deconvolution.
- MALDI-TDS allows for seamless switching between sequence analysis of proteins and oligonucleotides without any down-time as is typically the case in LCMS based workflows due to different eluent/modifier system requirements.
- Speed and flexibility of MALDI-TDS facilitate swift decision making in biopharma development as well as deeper insights in structural biology research.

**MALDI-TOF/TOF; MALDI-TDS**



**COI Disclosure:** S.V, S.S and A.A. are employees of Bruker Corporation. Bruker manufactures and sells instrumentation and software used in this study.