ASMS 2024 WP-685

Sequence Analysis of Proteins enhanced by TIMS-Enabled Next-Generation MALDI Top-Down Sequencing using a Dedicated Software Workflow

Arndt Asperger[1], Mariangela Kosmopoulou[2], Dodge Baluya[3], Detlev Suckau[1]

[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [2]Fasmatech, NCSR Demokritos, Athens, Greece; [3]Bruker Daltonics Inc., San Jose, CA, USA

Introduction

MALDI Top-Down Sequencing (MALDI-TDS) has proven itself a powerful method for characterization of intact proteins regarding primary sequence, terminal status and near-terminal modifications.

When using a trapped-ion mobility spectrometry (TIMS) enabled qTOF type MALDI instrument, MALDI-TDS benefits from accurate mass, high resolving power and TIMS separation of MALDI in-source-decay (ISD) fragment ions according charge state and terminal origin.

Here we introduce a new software-aided workflow for efficient analysis of TIMS-enabled MALDI-TDS datasets aiming for high-confidence Top-Down sequence confirmation of proteins at unparalleled sequencing depth.

Methods

Purified proteins (Carbonic Anhydrase II, MW 29 kDa; Nanobody_X, 15 kDa) were prepared on a AnchorChip MALDI target (20 pmol per spot) using SDHB matrix.

MALDI-TDS data were acquired on a Bruker timsTOF flex operated in positive ion polarity MALDI-ISD-MS, MALDI-ISD-TIMS-MS or MALDI-ISD-TIMS-MS/MS (T3-Sequencing) mode.

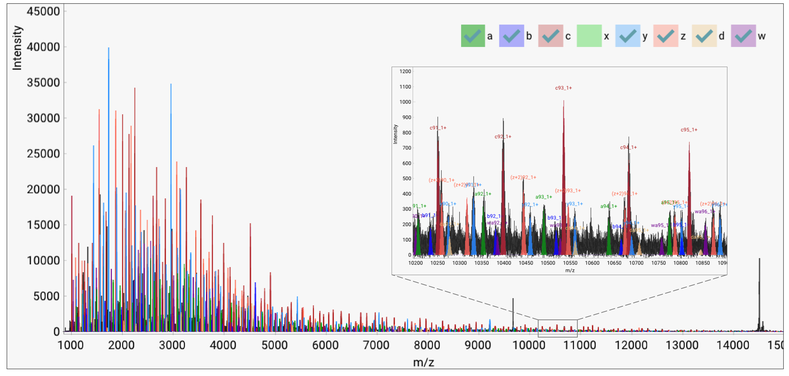
Pre-processing of raw data (i.e. spectra extraction from ion mobility regions, smoothing, baseline correction) was performed in Bruker DataAnalysis software

Processed spectra were exported as ASCII files and analyzed in Bruker OmniScape software performing m/z features detection and sequence matching against target protein sequences. Sequence matching results from individual MALDI-TDS analyses (i.e. with or without TIMS) were compiled using OmniScape´s Result Combination feature to yield an overall sequence coverage.

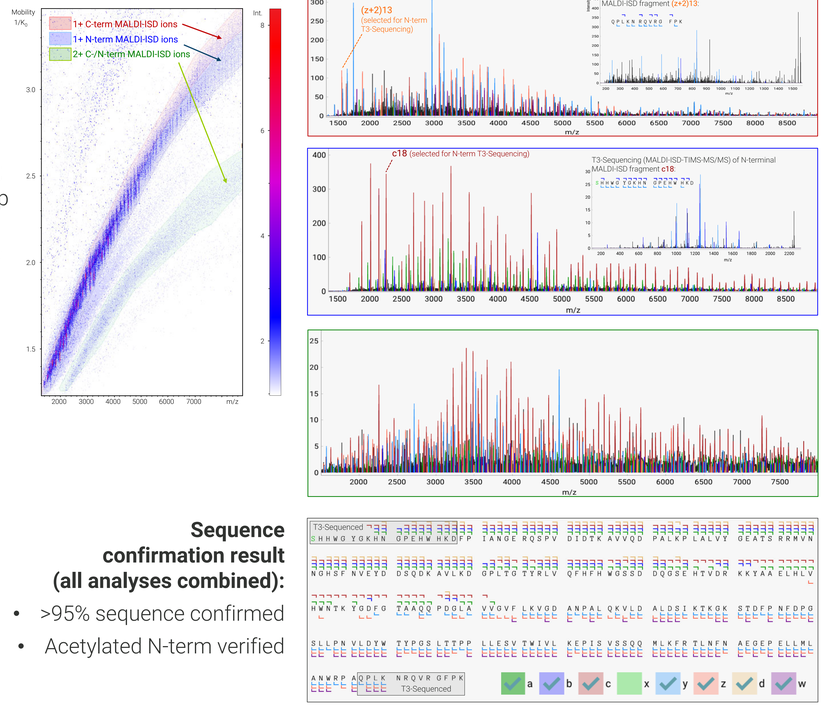
Results

Data processing workflow:
Bovine Carbonic Anhydrase II, MW 29 kDa

MALDI-ISD-MS:



MALDI-ISD-TIMS-MS(/MS):

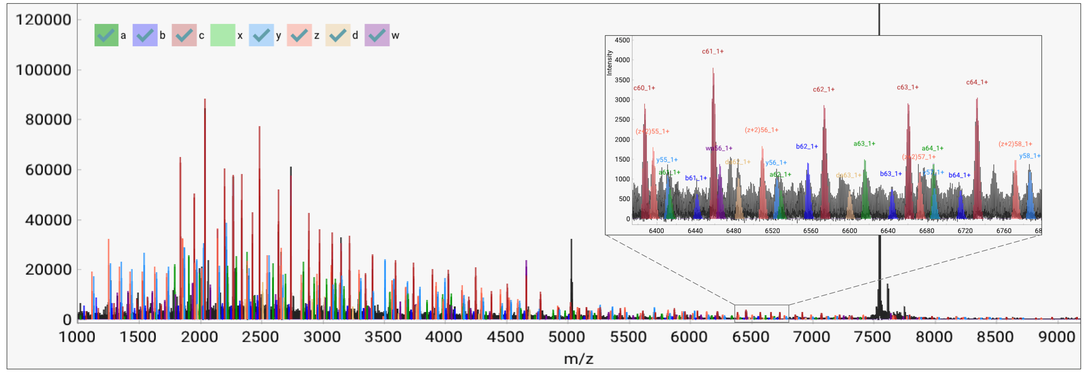


Sequence confirmation result (all analyses combined):

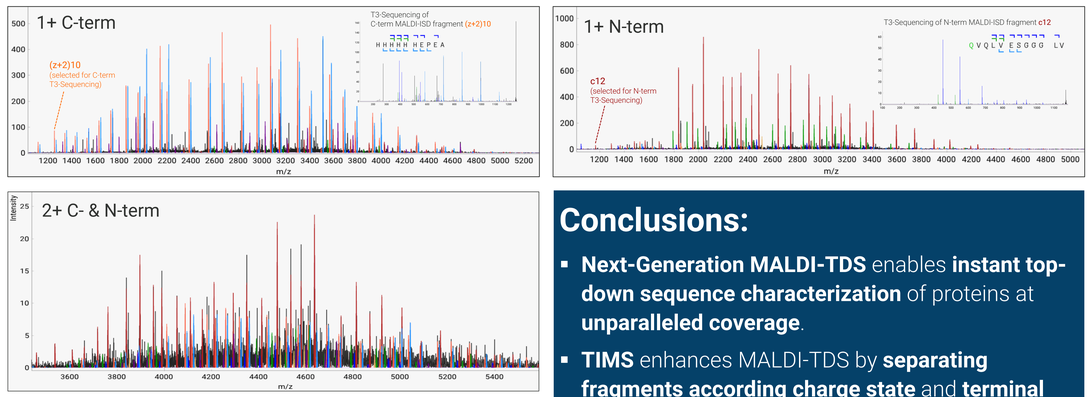
- >95% sequence confirmed
- Acetylated N-term verified

Application example:
Nanobody_X, MW 15 kDa (*sample courtesy: Prof. Jan Steyaert, Vrije Universiteit Brussel, Belgium*)

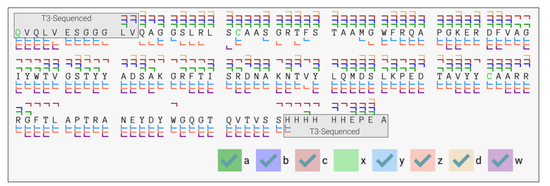
MALDI-ISD-MS:



MALDI-ISD-TIMS-MS(/MS) (spectra extracted from ion mobility regions):



Sequence confirmation result (all analyses combined):



- 100% sequence confirmed
- N-term pyroGlu verified as major proteoform (minor proteoform: unmodified N-term)

Conclusions:

- **Next-Generation MALDI-TDS** enables **instant top-down sequence characterization** of proteins at **unparalleled coverage**.
- **TIMS** enhances MALDI-TDS by **separating fragments according charge state** and **terminal origin**.
- **OmniScape software** provides an intuitive interface for **simplified top-down sequence confirmation**.
- OmniScape´s **Result Combination feature** provides **total sequence coverage** from multiple **compiled sequencing analyses**.
- OmniScape offers a dedicated **DeNovo workflow** (not shown here).

TIMS-enabled MALDI Top-Down Sequencing

The authors declare no competing financial interest