A Novel Benchtop MALDI-TOF/TOF Platform for Top-Down Protein Characterization

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Introduction

MALDI top-down sequencing (TDS) is a powerful approach for the analysis of intact proteins or protein subunits, providing sequence readout of up to 100 amino acid residues from each terminus and detailed information on modifications. This approach combines MALDI in-source decay (ISD) analysis with T^3-sequencing (pseudo-MS^3) of ISD fragment ions. Over the years it has been successfully applied to monoclonal antibodies, nanobodies, PEGylated proteins, bacterial glycoconjugates, and small membrane proteins. This study aims to evaluate the efficacy of a novel benchtop MALDI-TOF/TOF platform in performing comprehensive TDS analysis of NISTmAb and other proteins.

Methods

NISTmAb was cleaved with IdeS protease, deglycosylated with EndoS endoglycosidase, reduced with DTT, and fractionated by LC on a C_4 column using a short 10-minute gradient (Figure 1). NISTmAb subunits, fragments and TNF-α were prepared with SDHB matrix for MALDI-TDS analyses. All MALDI measurements were performed on a Bruker neofleX benchtop MALDI-TOF/TOF platform. MALDI-ISD spectra were acquired in reflector mode, and T^3-sequencing spectra were acquired in MS/MS mode. MALDI-ISD and T^3 spectra were analyzed in OmniScape software.

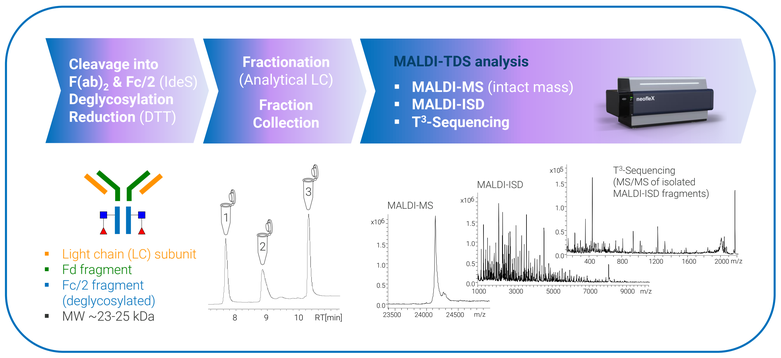

Figure 1. MALDI-TDS workflow for sequence verification of therapeutic mAbs

Table 1. Sequence coverage and terminal status of NISTmAb subunits and fragments determined by MALDI-TDS

	MALDI-TDS Seq. Coverage [%]	Terminal status as confirmed by MALDI-ISD/T3-Sequencing N-terminus	C-terminus
Light Chain	92.5	Unmodified	Unmodified
Heavy Chain	40.0	Gln->pGlu	Lys deletion
Fd fragment	83.6	Gln->pGlu	n.a.
Fc/2 fragment	84.2	n.a.	Lys deletion

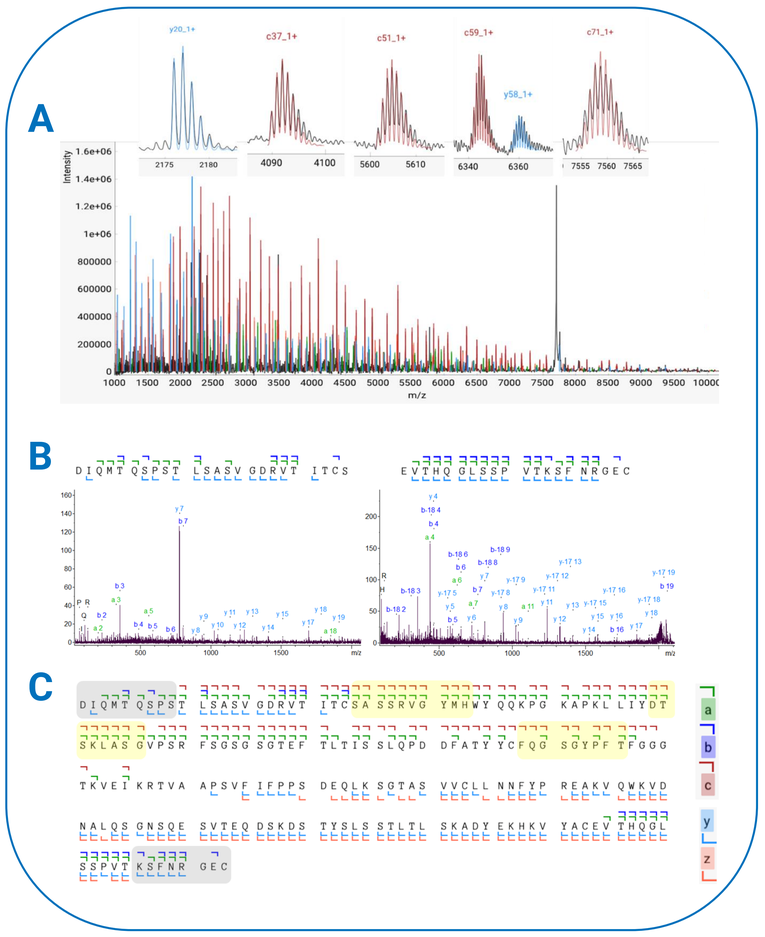

Figure 2. MALDI-TDS analysis of NISTmAb light chain subunit showing MALDI-ISD spectrum annotated in OmniScape software with zoomed-in view of individual fragment ions across the whole mass range (A), T^3 spectra acquired from fragment ions c24 (left) and y20 (right) confirming that both termini are not modified (B), and color-coded sequence coverage map with CDR regions highlighted in yellow and T^3 sequence coverage in gray (C)

Results

- Comprehensive MALDI-TDS analysis of the NISTmAb light chain subunit achieved 92.5% sequence coverage (Table 1). Isotopic resolution of ISD fragment ions was achieved across the mass range of the MALDI-ISD spectrum (Figure 2A).
- The set of two T^3 spectra confirmed that the light chain´s N- and C-termini are not modified (Figure 2B). All three complementarity-determining regions (CDRs) were verified (Figure 2C).
- MALDI-TDS analysis of the Fd fragment attained 83.6% sequence coverage (Table 1). T^3 spectrum of the N-terminal c12 fragment ion confirmed pGlu modification at the N-terminus (Figure 3).
- Two out of three CDRs were confirmed in the Fd fragment sequence. The total of five out of six CDRs were verified in the NISTmAb sequence (Figures 2C and 3C).

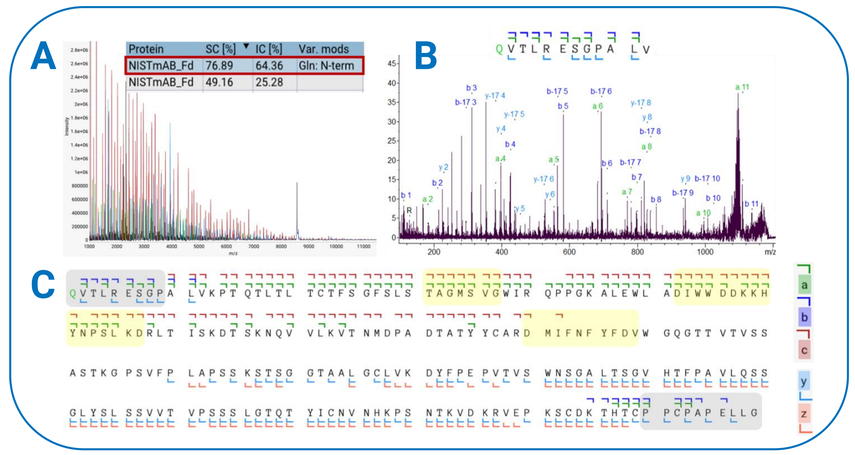

Figure 3. MALDI-TDS analysis of NISTmAb fragment Fd. MALDI-ISD spectrum showing match for the pGlu-modified N-terminus (A), T^3 spectrum of ISD fragment ion c12 confirming pGlu modification at the N-terminus (B), and color-coded sequence coverage map (C)

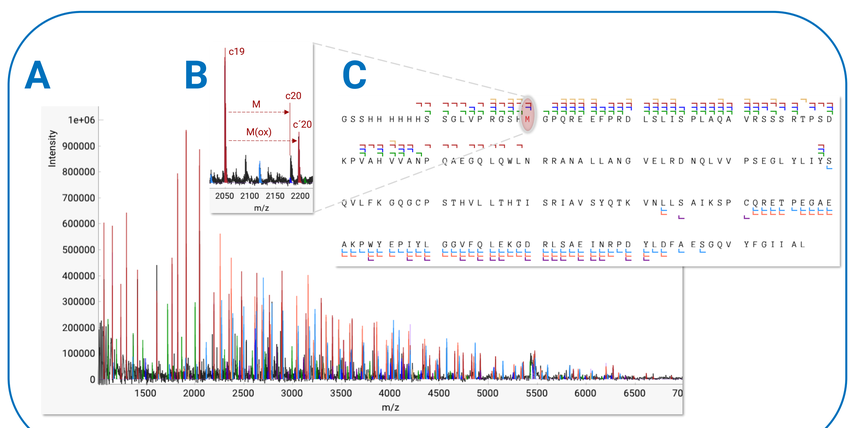

Figure 4. MALDI-TDS analysis of TNF-α. MALDI-ISD spectrum (A) with zoomed view of c20 ISD fragment ion confirming Met20 oxidation (B) and color-coded sequence coverage map highlighting the oxidized Met20 residue (C)

- MALDI-TDS of TNF-α revealed oxidation of Met20 residue (Figure 4). Additional work is being performed to confirm galactosylation of the N-terminus.

Conclusions

- MALDI-TDS workflow was successfully adapted to the novel neofleX platform, which allowed rapid sequence verification, localization of PTMs, deletions, and preparation artifacts.
- High sequence coverage of NISTmAb facilitated verification of 5 out of 6 CDRs.
- The T^3-sequencing part of the MALDI-TDS workflow confirmed the presence of a pGlu modification at the N-terminus of the heavy chain (Fd fragment) and the deletion of Lys from the C-terminus of the heavy chain (Fc/2 fragment).

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