



neofleX benchtop MALDI-TOF/TOF: Providing next-level MS/MS performance for more structural insight in life sciences applications

Latest technologies incorporated in neofleX benchtop MALDI-TOF/TOF enable a new level of MS/MS performance with 25x faster analysis speed, 100% increase in resolving power and 4x better sensitivity, thereby providing more structural detail in the analysis of complex biomolecules.

Abstract

- neofleX™ MALDI-TOF/TOF provides next-level MS/MS performance in a true benchtop format enhancing structural analysis in a wide range of life sciences applications.
- As a benefit from the latest TOF/TOF technologies incorporated, neofleX, when compared to previous generation instruments, offers up to 25x faster MS/MS acquisition speed, more than 100% increase in resolving power for fragment ions and 4x better MS/MS sensitivity.
- neofleX delivers high-quality MS/MS data showing isotopic fidelity across the entire fragment ion mass range irrespective of precursor mass. This provides particular benefit for the analysis of larger peptides beyond the size of tryptic peptides resulting in enhanced sequence coverage and, thus, increased confidence of sequencing results.
- neofleX is fully capable of MS/MS operation in both ion polarities ensuring optimum sensitivity for molecules ionizing best in either positive or negative ion mode.
- neofleX facilitates MS/MS experiments under high-energy CID conditions providing additional structural information required for discrimination of isobaric amino acids leucine and isoleucine in peptides and for linkage analysis in oligosaccharides.
- Outstanding MS/MS sensitivity is of great benefit to neofleX' unique capabilities in top-down protein analysis, allowing for verification of the terminal status in intact proteins by T³-Sequencing.

Keywords:
MALDI-TOF/TOF,
MS/MS technology,
LID, high-energy CID, *he*CID,
MALDI-MSD, Top-Down
Sequencing, T³-Sequencing,
peptides, proteins, glycans,
glycopeptides, modifications

neofleX MS/MS technology brief

neofleX MALDI-TOF/TOF incorporates latest innovative Bruker MALDI technologies in a lab-space efficient benchtop format. The instrument enables ultimate analysis speed, provides a level of analytical performance previously unseen on benchtop MALDI instruments and, thus, enhances lab productivity by leveraging simple and fast MALDI analysis workflows.

Besides other distinguishing technology features, such as 10 kHz smartbeam 3D laser, enhanced imaging detectors, and an ion source design enabling tool-free maintenance, neofleX's greatly enhanced MS/MS capabilities are particularly outstanding. When compared to previous generation TOF/TOF instruments such as autofleX maX, neofleX acquires MS/MS data 25x faster, and offers a more than 100% increase in MS/MS resolving power together with 4x better MS/MS sensitivity. The unmatched quality of neofleX MS/MS data provides more structural detail in the analysis of a wide range of complex biomolecules, such as peptides, proteins, carbohydrates and other modalities.

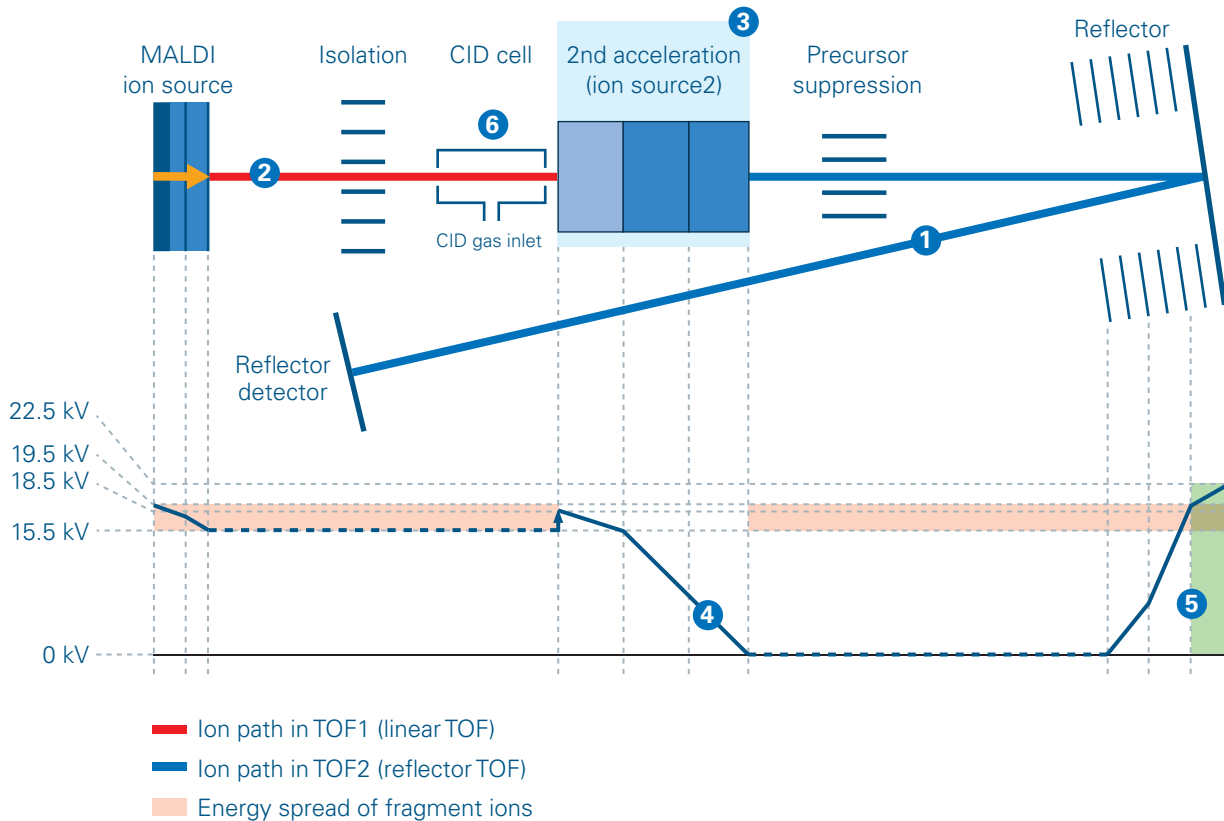


Figure 1. Principal scheme of the neofleX benchtop MALDI-TOF/TOF instrument when operated in TOF/TOF mode.

Bullet points indicate key technology features contributing to enhanced neofleX MS/MS performance as described in the text section below.

Various key technology features contribute to the greatly enhanced neofleX MS/MS performance (see also principal scheme shown in Figure 1):

- 1 An effective flight path length of 2.60 m (+0.45 m when compared to autoflex instruments) accommodated in a true benchtop format provides for enhanced resolving power.
- 2 Increased TOF1 fragmentation path length results in increased fragmentation yield and, thus, improved MS/MS sensitivity.
- 3 A newly designed ion source 2 allows for optimum ion focusing resulting in increased resolution and sensitivity.
- 4 Utilization of a static ion source 2 acceleration potential (replacing the LIFT pulser technology used previously in autoflex and ultrafleX series instruments) enables MS/MS experiments at 5 kHz laser repetition rate translating into 25x faster MS/MS acquisition speed when compared to autoflex maX.
- 5 A three-stage large-aperture reflector design enables optimum focusing of the ion beam, thus yielding enhanced resolution.
- 6 Larger physical dimensions of the CID cell, its positioning closer to ion source 2, and CID gas pressure tunable via the instrument control software allow for optimized fragmentation efficiency under high-energy CID (*he*CID) conditions.

To illustrate the tremendous leap in data quality provided by the new TOF/TOF technology, a side-by-side comparison of neofleX with previous generation instruments, autoflex maX and ultrafleXtreme, is shown in Figure 2. neofleX clearly outperforms previous instrument models with regard to MS/MS resolution by up to 100% as shown here for various fragment ions from Glu-1-Fibrinopeptide B (precursor m/z 1570.68). At the same time, neofleX offers superior MS/MS sensitivity as shown on the right-hand side in Figure 2. The zoomed MS/MS spectra originate from 250 amol Glu-Fib. For the most prominent fragment ion appearing at m/z 1056.5, neofleX achieves a signal-to-noise ratio 4x better than autoflex maX (S/N 40 vs. S/N 10).

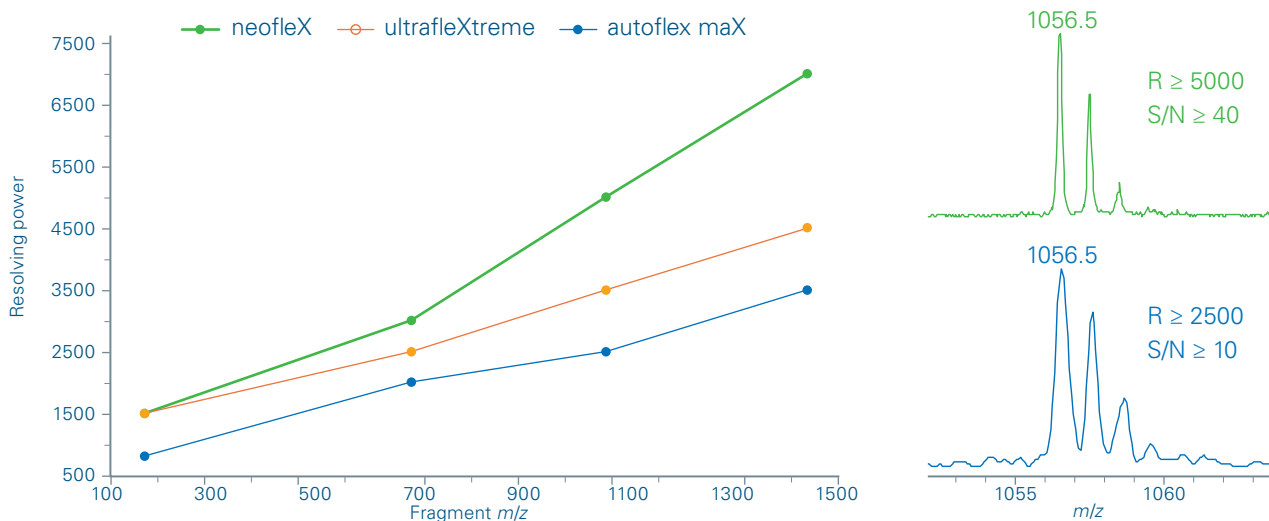


Figure 2. neofleX benchtop MALDI-TOF/TOF provides a major leap in MS/MS performance over previous generation TOF/TOF instruments and offers far superior MS/MS resolution and sensitivity

Benefits provided by superior neoflex TOF/TOF technology

Enhanced sequencing of large peptides with high-quality MS/MS data

neoflex allows for rapid MS/MS analysis of differently sized molecules. High-quality MS/MS spectra of parent ions within the m/z range 500-5000 are obtained using one single pre-installed default acquisition method without the need for further method tuning (see Figure 3). MS/MS acquisition can be performed in a fully automated sequence allowing for unattended recording of large numbers of MS/MS spectra from a prepared MALDI plate in either data-dependent or targeted workflows.

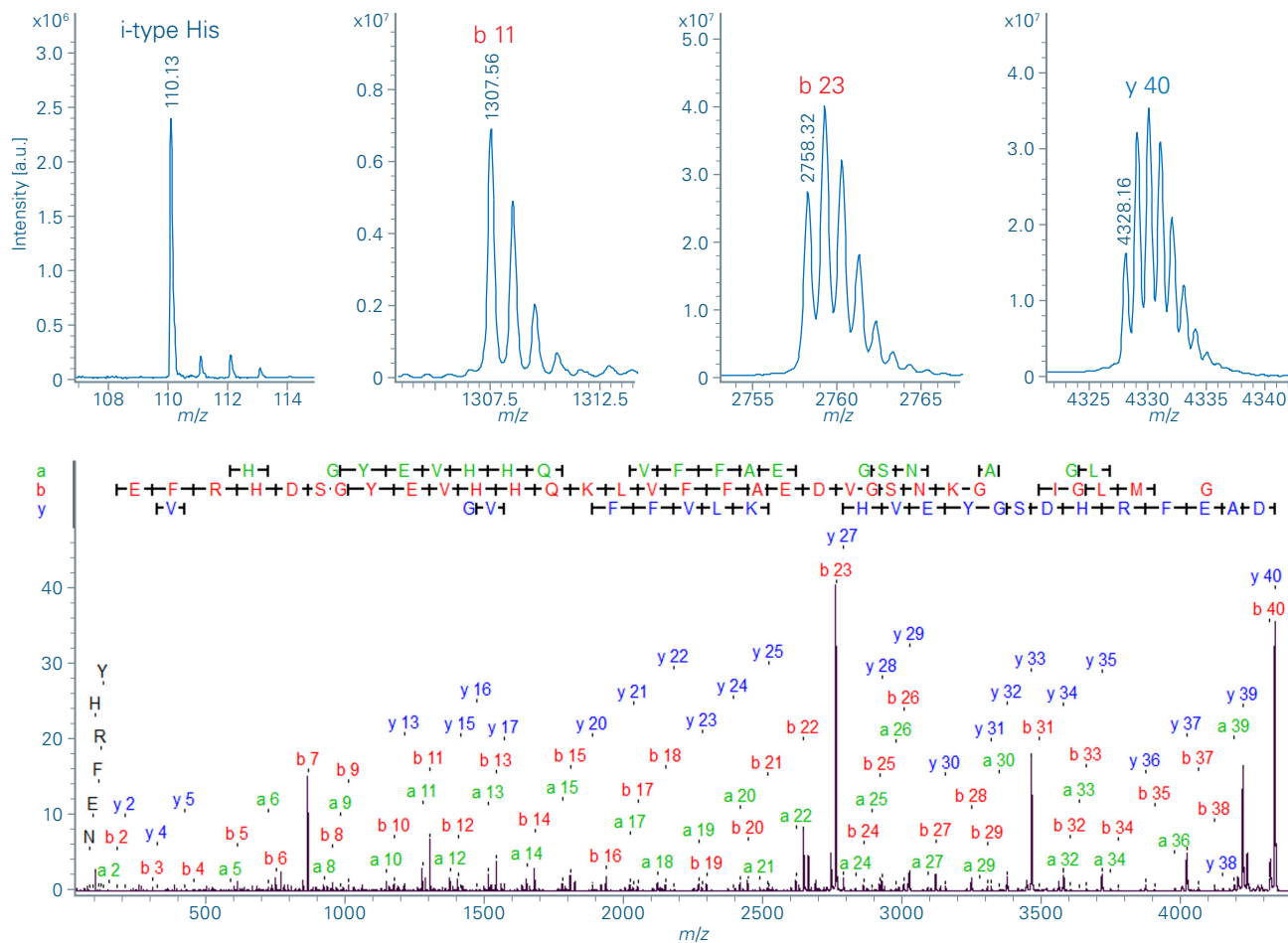


Figure 3. (+)MALDI-MS/MS spectrum of amyloid-beta peptide [1-40], precursor m/z 4328.16.

Even for large precursors beyond the size of tryptic peptides neoflex achieves isotopic resolution across the entire fragment mass range, from the largest b- or y-ions down to smallest immonium ions. Isotopic fidelity of neoflex MS/MS data goes along with enhanced mass accuracy (here: 0.05 Da, averaged over all matching fragments) and translates directly into higher sequence coverage and, thus, increased confidence of sequencing results. Spectrum annotation was performed in BioTools, part of Bruker BioPharma Compass® software.

Extended analyte space through positive and negative ion mode MS/MS

neoflex is fully capable of MS/MS operation in both ion polarities. This ensures optimum sensitivity in the structural analysis of compounds ionizing particularly well in either positive or negative ion mode.

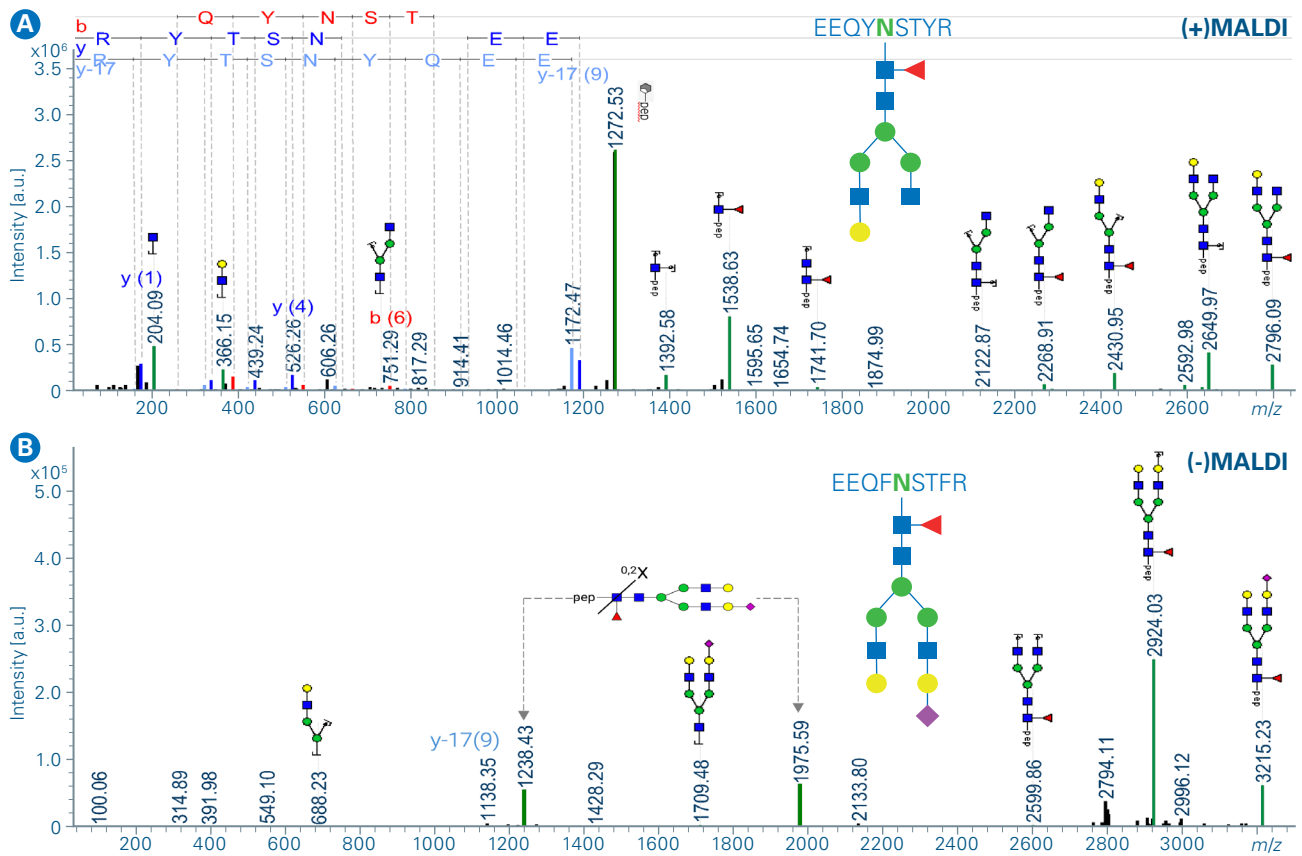


Figure 4. MALDI-MS/MS spectra of two IgG N-glycopeptides acquired in positive and negative ion mode, respectively.

A N-glycopeptide EEQYNSTYR carrying neutral glycan G1F was analyzed in positive ion mode and yielded an information-rich fragment ion spectrum confirming both peptide sequence and carbohydrate composition. **B** N-glycopeptide EEQFNSTFR carrying acidic glycan G2FS1, due to the additional negative charge brought in by the sialic acid residue, was analyzed in negative ion mode. The resulting MS/MS spectrum confirmed the presence of a terminal sialic acid residue by means of the respective highly abundant (-291 *m/z*) neutral loss fragment ion, and provided definite information regarding peptide and glycan moiety masses. Spectra annotation was performed in Bruker BioPharma Compass software.

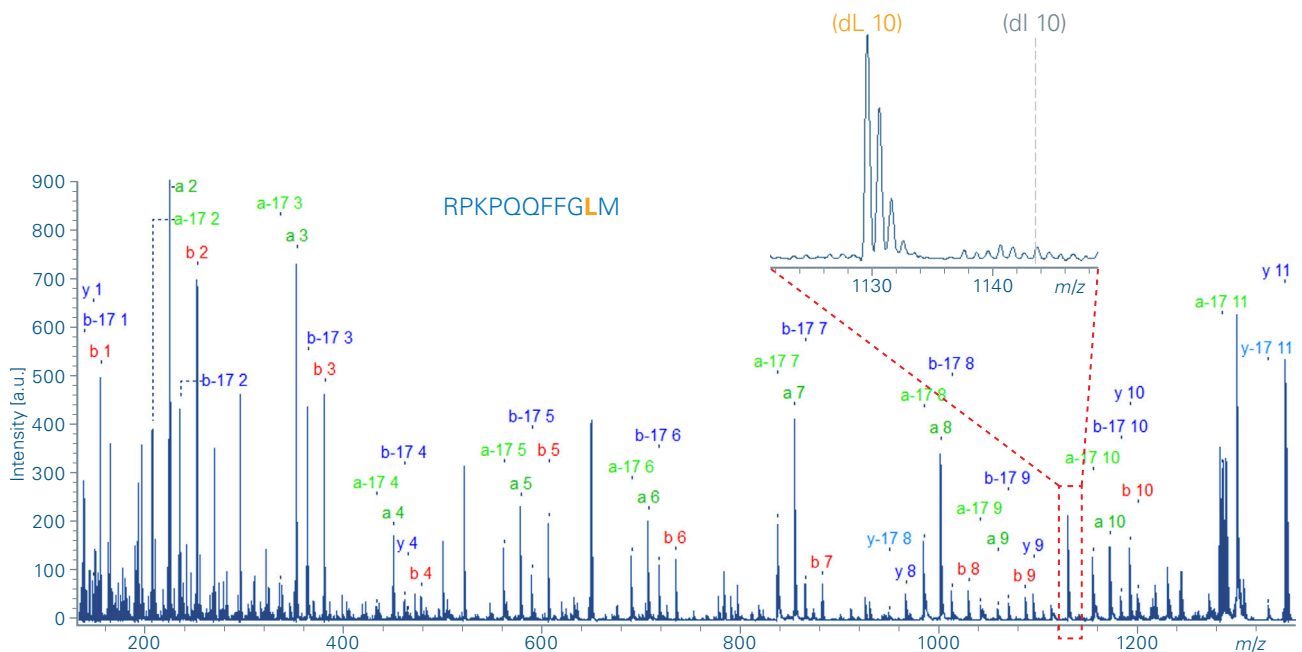


Figure 5. (+)MALDI-*he*CID-MS/MS spectrum of substance P peptide.

Nitrogen was used as a collision gas at a system pressure of approximately 1.5×10^{-6} mbar to provoke the formation of d-type side-chain fragment ions. Presence of fragment ion (dL10) together with the absence of the respective fragment (dl10) provided clear evidence for the presence of leucine at position 10 in the peptide sequence. Spectrum annotation was performed in Biotools, part of Bruker BioPharma Compass software.

More structural detail revealed with high-energy CID

neoflex allows for acquisition of MALDI-MS/MS spectra employing different fragmentation conditions:

- **Laser-induced dissociation (LID)**

MS/MS spectra acquired without supply of collision gas are dominated by fragments originating from laser-induced dissociation (LID). MALDI-LID-MS/MS spectra of peptides typically show very straightforward a,b,y-ion backbone fragmentation (see Figure 3) and are, therefore, highly suited for protein identification by means of protein sequence database searching using search engines such as MASCOT.

- **High-energy collision-induced dissociation (*he*CID)**

When increasing the instrument's system pressure to the low to medium 10^{-6} mbar region, high-energy collision induced dissociation occurs as an additional fragmentation pathway. MS/MS spectra acquired under *he*CID conditions feature further fragment ion types, f.e. originating from side-chain or cross-ring cleavages, and, thus, may provide additional structural information, such as discrimination between isobaric amino acids leucine and isoleucine in peptides (Figure 5) or structure of linkages in oligosaccharides (Figure 6).

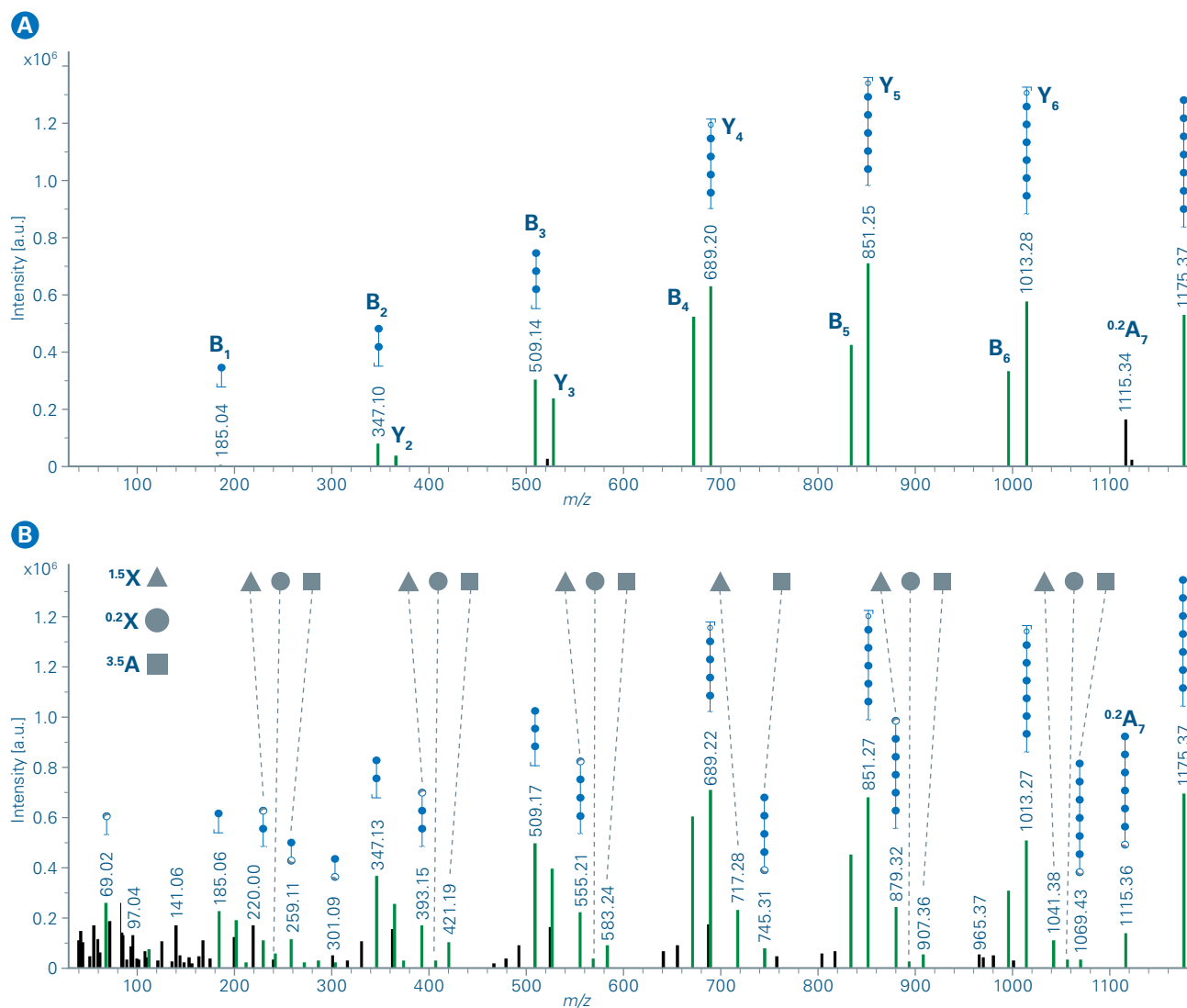


Figure 6. (+)MALDI-MS/MS spectra of maltoheptaose.

(A) The spectrum acquired under LID conditions (no supply of CID gas) is dominated by backbone fragments of B and Y type. (B) In the spectrum acquired under *he*CID conditions using nitrogen as a collision gas at a system pressure of approximately 2.5×10^{-6} mbar, cross-ring cleavages are observed in addition to B,Y backbone fragmentation allowing for detailed structural analysis of the linkages connecting the monosaccharide units. Spectrum annotation was performed in Bruker BioPharma Compass software.

Unique neoflex top-down MS/MS capabilities with T³-Sequencing

neoflex is highly capable of MALDI Top-Down Sequencing (MALDI-TDS) of intact proteins by MALDI In-Source Decay (MALDI-ISD) allowing for rapid-turnaround sequence verification of protein expression products. While MALDI-ISD spectra provide N- and C-terminal sequence readout at a depth of typically up to 100 amino acid residues each, access to the very near-terminal regions (typically the first 8-10 terminal amino acid residues) is hampered by the complex spectral background in MALDI-ISD-TOF spectra in the *m/z* region below 1000. However, by means of T³-Sequencing, i.e. isolation and fragmentation of selected N- or C-terminal MALDI-ISD fragments, the terminal status of a protein can be verified explicitly (Figure 7). T³-Sequencing as a pseudoMS³ method benefits a lot from the outstanding MS/MS sensitivity neoflex provides.

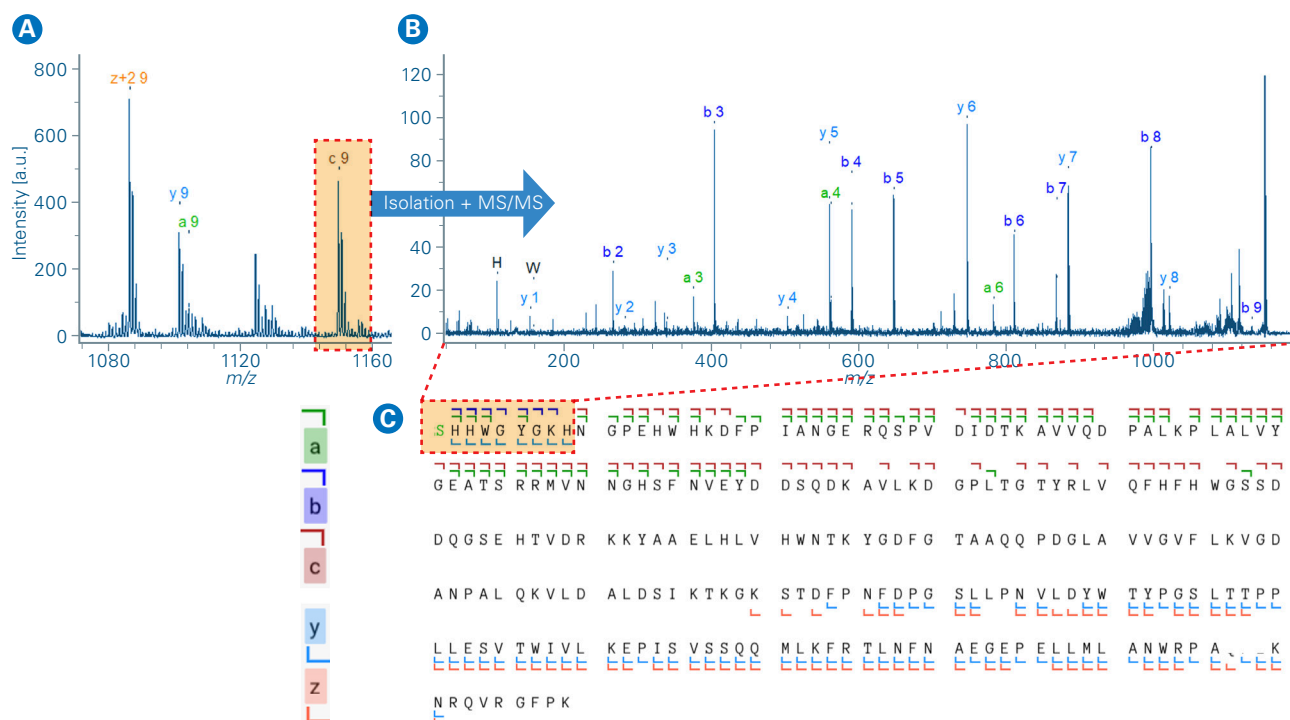


Figure 7. N-terminal T³-Sequencing of 29 kDa bovine carbonic anhydrase II (CAII).

(A) shows a zoomed view on the MALDI-ISD-TOF spectrum from which the CAII protein sequence could be verified as shown in the sequence map depicted in **(C)**. For explicit verification of the protein's N-terminal status, MALDI-ISD fragment c9 was isolated and subjected to further fragmentation in TOF/TOF mode. The resulting T³ spectrum shown in **(B)** confirmed the presence of an acetylated N-terminus and provided sequence readout for the near-terminal sequence region [1-9]. Data analysis was performed in Bruker OmniScope™ software.

Conclusions

- neoflex represents a milestone in MALDI-TOF/TOF development providing next-level MS/MS performance in a true benchtop format.
- When compared to previous-generation TOF/TOF instruments, neoflex offers up to 25x faster MS/MS acquisition speed, a more than 100% increase in resolving power for fragment ions, 4x better MS/MS sensitivity and greatly enhanced *h*eCID efficiency.
- With its unique MS/MS capabilities neoflex benchtop MALDI-TOF/TOF paves the way to more structural insight in life sciences applications by enhancing the analysis of a wide range of critically important biomolecules such as peptides, proteins, carbohydrates and other modalities.

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