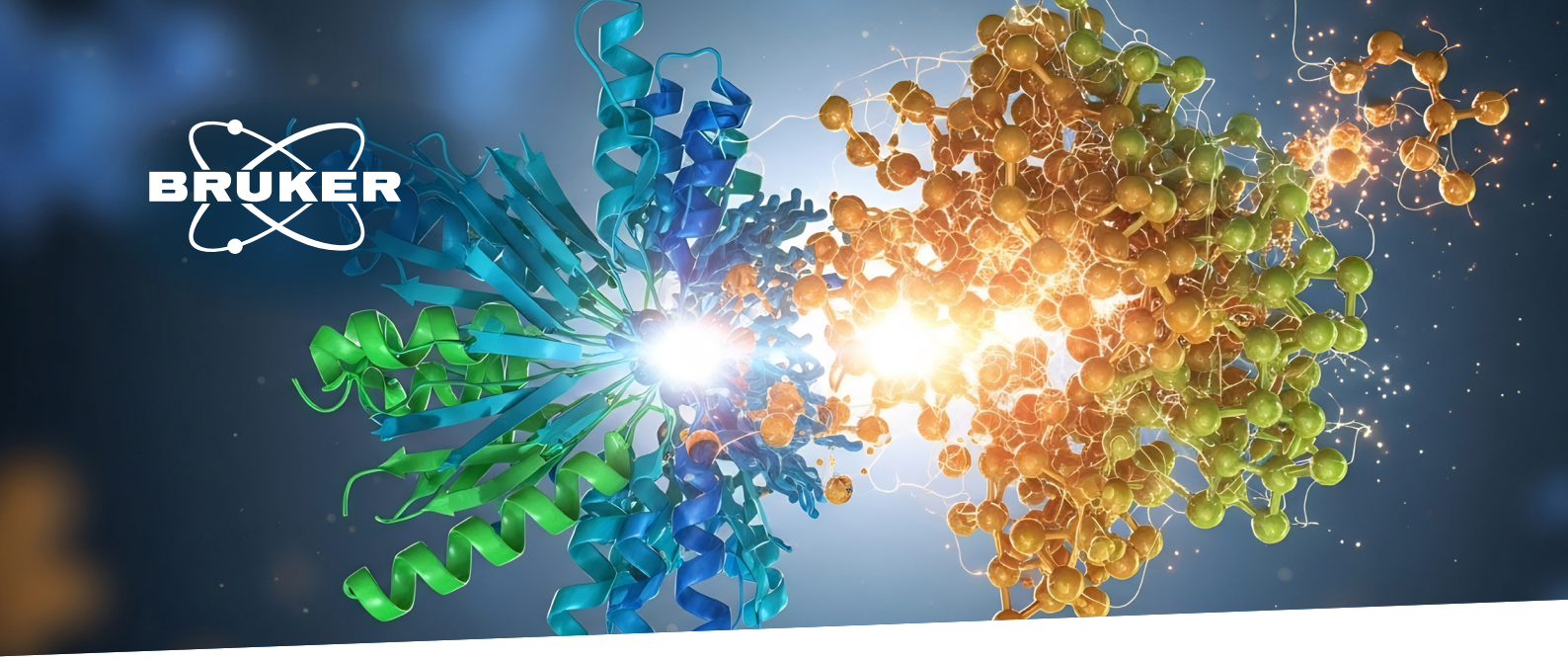

Native Top-Down Mass Spectrometry on the timsOmni Platform Enables Deep Structural Analysis of Protein Complexes

Deep sequencing of the native BamA-E complex on timsOmni with multistage top-down MS^n and ion-enrichment enables comprehensive analysis of protein subunits and lipid anchors. The platform's advanced capabilities facilitate efficient charge-state selection, subunit ejection, and multilevel fragmentation, providing high signal-to-noise ratios and revealing structural details under native conditions.

Abstract

Native mass spectrometry (nMS) provides a direct view of protein complexes, preserving oligomeric states, cofactors, lipidation, and ligand interactions. When combined with top-down MS, it can reveal proteoforms and structural details impossible to obtain from digested peptides alone. However, native top-down MS traditionally faces significant challenges: insufficient ion transmission of high mass complexes, extensive ion heating that disrupts non covalent interactions and restricted access to MS^n fragmentation of low abundance subunits or modifications.

Using the BamABCDE membrane protein complex as a representative system (Figures 1–4), the timsOmni™ demonstrates efficient subunit ejection, charge state selection, and multi level MS^n sequencing with ion enrichment to boost fragment S/N. These results illustrate the platform's ability to map subunits, define lipid anchors and characterize low abundance proteoforms under native conditions.

Keywords: timsOmni native, native top down, multimodal fragmentation, ion enrichment, proteoform analysis, Omnitrap activation, TIMS separation, electron fragmentation, PTM mapping, high mass transmission

Introduction

Native mass spectrometry preserves non covalent interactions and near solution conformations of protein complexes, making it well suited for studying oligomerization, proteoforms and ligand or lipid binding. However, applying top-down MS under native conditions remains difficult. High mass ions require gentle transmission to maintain structural integrity. Until now, insufficient access to controlled multi stage MS^n has restricted the ability to isolate subunits, probe low abundance species, or map modifications.

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The timsOmni platform addresses these limitations through precise control of ion activation, efficient ion handling, and multi modal fragmentation within the Omnitrap® Q2 and Q5 region. These capabilities enable selective subunit release, targeted ion enrichment, and deep MS^n sequencing from natively sprayed complexes, allowing native top-down workflows to access structural details such as subunit composition and lipid modifications that are difficult to reach with conventional instrumentation.

Methods

Native MS analysis of the BamABCDE complex was performed using the NEOS source with coated, open type nanospray tips (Cellomics) biased at 1.3 kV. Ions were sampled through a 1.0 mm i.d. glass inlet at 8 mbar pressure on the timsOmni platform. Efficient desolvation was achieved by accelerating ions to 120 eV in funnel 2, while higher values enabled the release of intact subunits. Individual subunit charge states were isolated using the quadrupole mass filter and subjected to MS^2 fragmentation using $_R$CID, ECD, and EID. Low abundance fragments of interest were further interrogated by MS^3 using an ion enrichment method to maximize signal to noise. Data processing and sequence confirmation were performed in OmniScape™.

Results and Discussion

The timsOmni native workflow (Figure 1) enabled systematic dissociation of the BamABCDE complex into individual subunits using in source collision induced dissociation (Figure 2), followed by MS^n analysis for deep sequencing and characterization. This approach provided detailed insight into N-terminal lipidation across all lipoprotein subunits. At the MS^3 level, resonance CID generated low abundance b type fragments carrying the lipid modification, which were selectively isolated and enriched to improve signal to noise and enable an additional stage of fragmentation (Figure 3). The strength of this strategy is illustrated by MS^4 resonance

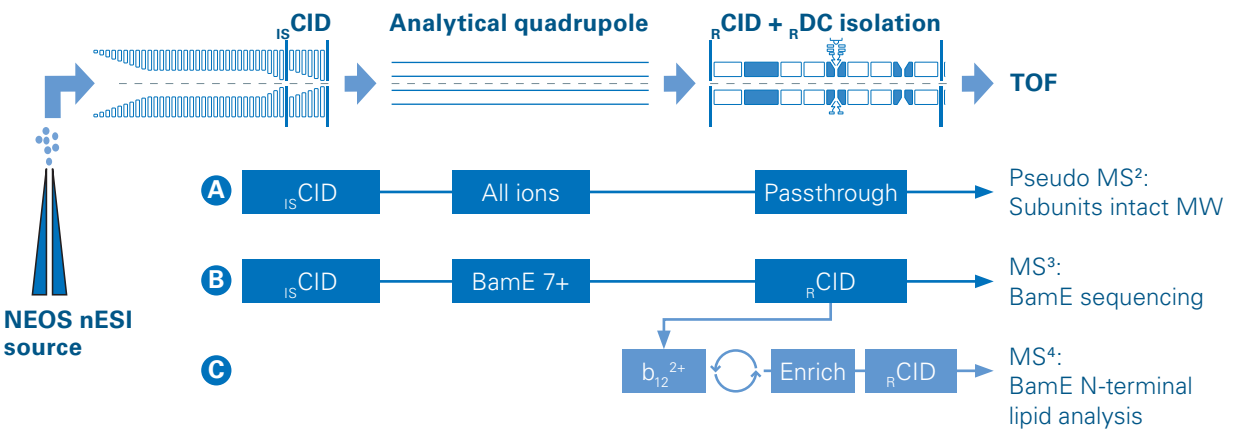

Figure 1. Native Top-Down Workflow Overview. (A) Complex activation, (B) Subunit sequencing, (C) Deep sequencing with enrichment

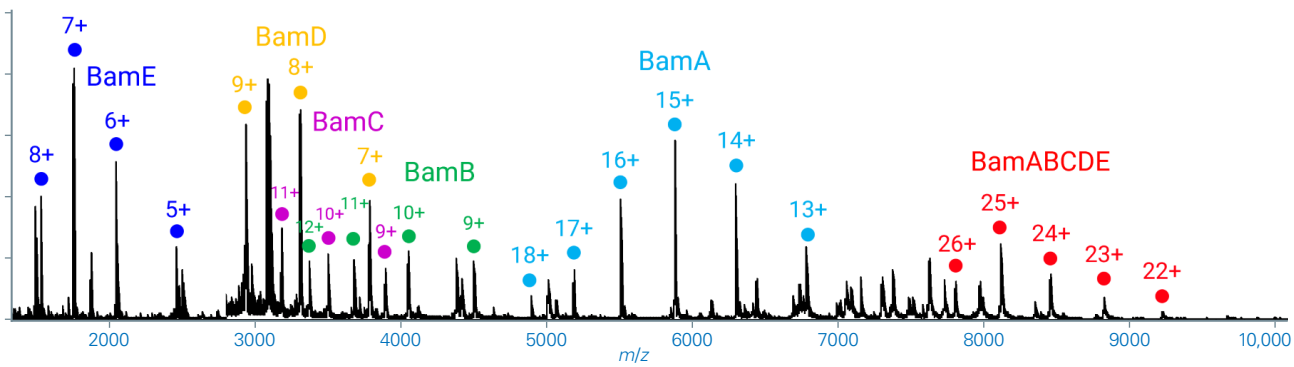

Figure 2. Wide *m/z* range pseudo-MS^2 spectrum with ejected subunits and complex.

CID of the b_{12}^{2+} product ion from the BamE subunit, which enabled direct structural elucidation of the lipid anchor (Figure 4). Extensive neutral loss series revealed the presence of palmitic acid, oleic acid, and the corresponding 1-oleoyl-2-palmitoyl glycerol species, while additional loss of an S-linked diacylglycerol unit indicated attachment of the glycerol moiety to the cysteine side chain. Further loss of a hexadecyl group confirmed N-terminal linkage of a second palmitic acid, allowing full structural characterization of the lipid modification. Application of electron induced dissociation at the MS^4 stage, although capable of generating fragments across lipid alkyl chains, produced predominantly backbone fragments localized near an aromatic residue (Y), limiting double bond localization. The MS^n workflow was subsequently extended to other subunits, with MS^3 CID of BamD yielding characteristic b-type ions with CH_2 and C_2H_4 losses and the same lipid-associated mass shift observed for BamE. Further MS^4 analysis of the most intense fragment enabled confident localization of the lipid modification at the N-terminal of the subunit.

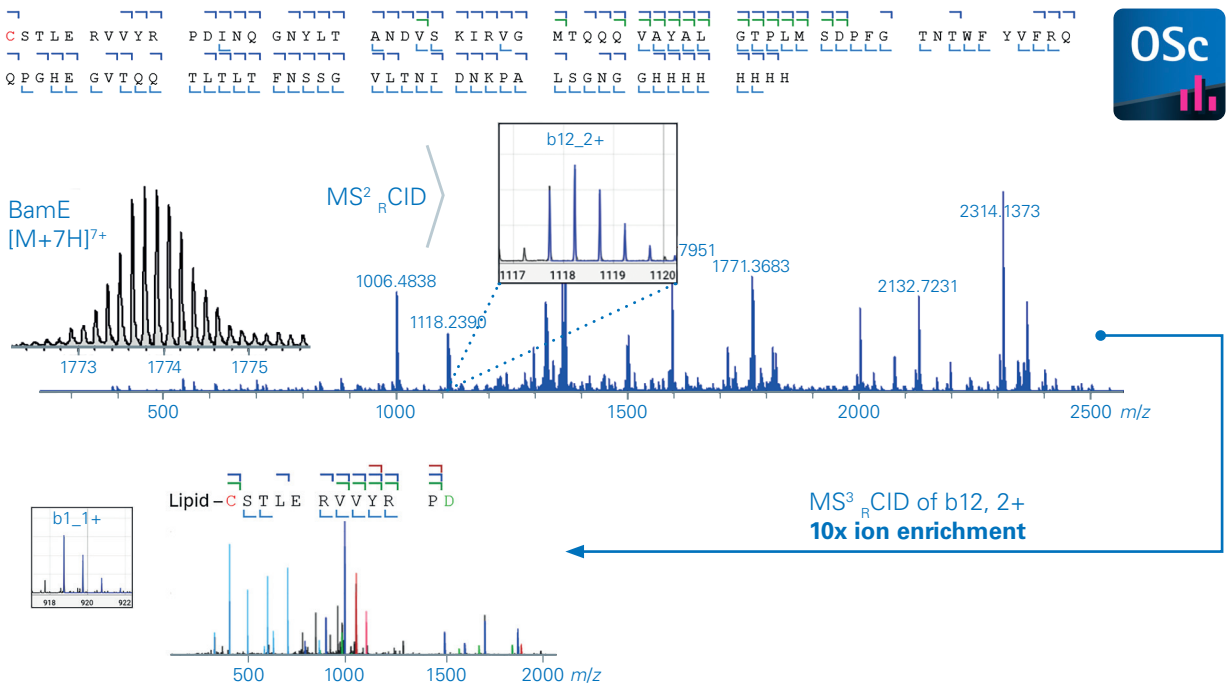

Figure 3. Subunit comprehensive characterization with pseudo-MS^4 ($_R$CID/$_R$CID) after enrichment of b12 ion.

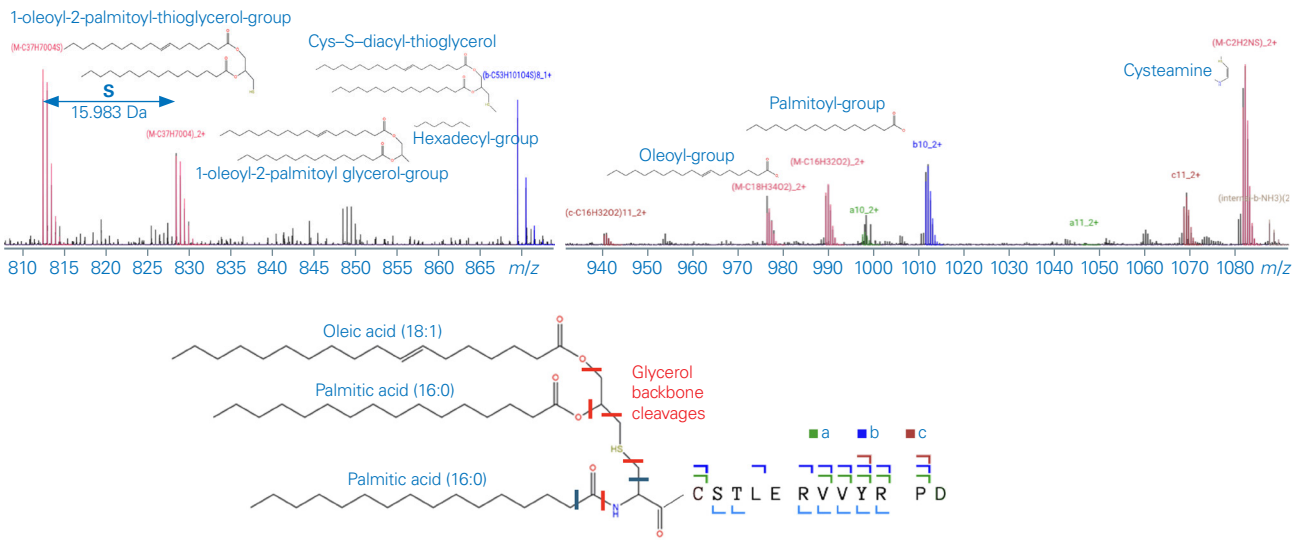

Figure 4. Assignment of lipid fragment for identification of the N-terminal lipid anchor.

Conclusion

- **Deep top-down sequencing:** Multistage MS^n across RCID, CCID, ECD, and EID yields enriched fragment ladders with high structural specificity.
- **Lipid localization and identification:** Ion enrichment loops allow full characterization of low abundance modified subunits.
- **High mass readiness:** The Omnitrap platform enables the selective dissociation and analysis of large-protein assemblies.
- **Integrated analysis:** OmniScape workflows support sequence confirmation and PTM assignment across multiple MS levels

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