Advanced Native and Top-Down MS^n Workflows on the timsOmni Platform Enable Deep Structural Analysis of Membrane Protein Complexes

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Introduction

- Membrane proteins are essential for cellular function and major therapeutic targets, yet their structural and functional characterization remains challenging due to diverse oligomeric states, proteoforms, post-translational modifications, and interactions with lipids, cofactors, and partner proteins.
- Native mass spectrometry (MS), combined with top-down (TD) analysis, enables detailed characterization of membrane protein assemblies and subtle modifications such as lipidation.
- Here, we present advanced MS^n workflows on the timsOmni™ platform for comprehensive analysis of membrane proteins, their modifications, and functional complexes.

Methods

- **Sample introduction**: Coated, open type nanospray emitters (biased at 1.3kV), 1 mm i.d. glass inlet at 8 mbar
- **Desolvation/isCID**: Ion acceleration at 120 eV to 200 eV, 0.5 mbar
- **Mass selection**: Quadrupole mass filter for charge states of m/z<4500 Th; Q2 segment of the Omnitrap™ platform used to isolate larger ions, which can be accumulated to enhance signal intensity
- **Protein dissociation and fragmentation**: **Fig. 1**.
- **Data analysis**: OmniScape™ for MS^n data.

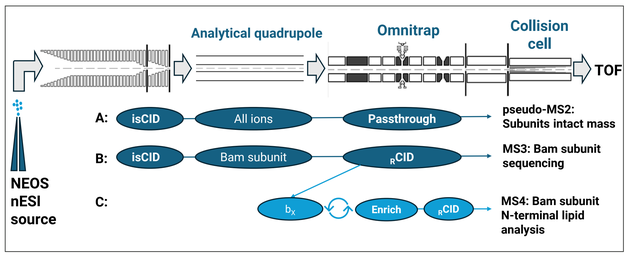

Fig. 1 Native Top-Down MS^n Workflow Overview.

Results

For the heteropentameric β-barrel assembly machinery BamABCDE complex, we achieved systematic dissociation of the intact complex into individual subunits using in-source collision-induced dissociation (isCID) followed by MS^n analysis. This approach enabled deep sequencing and detailed characterization of N-terminal lipidation on each lipoprotein subunit (**Fig. 2**).

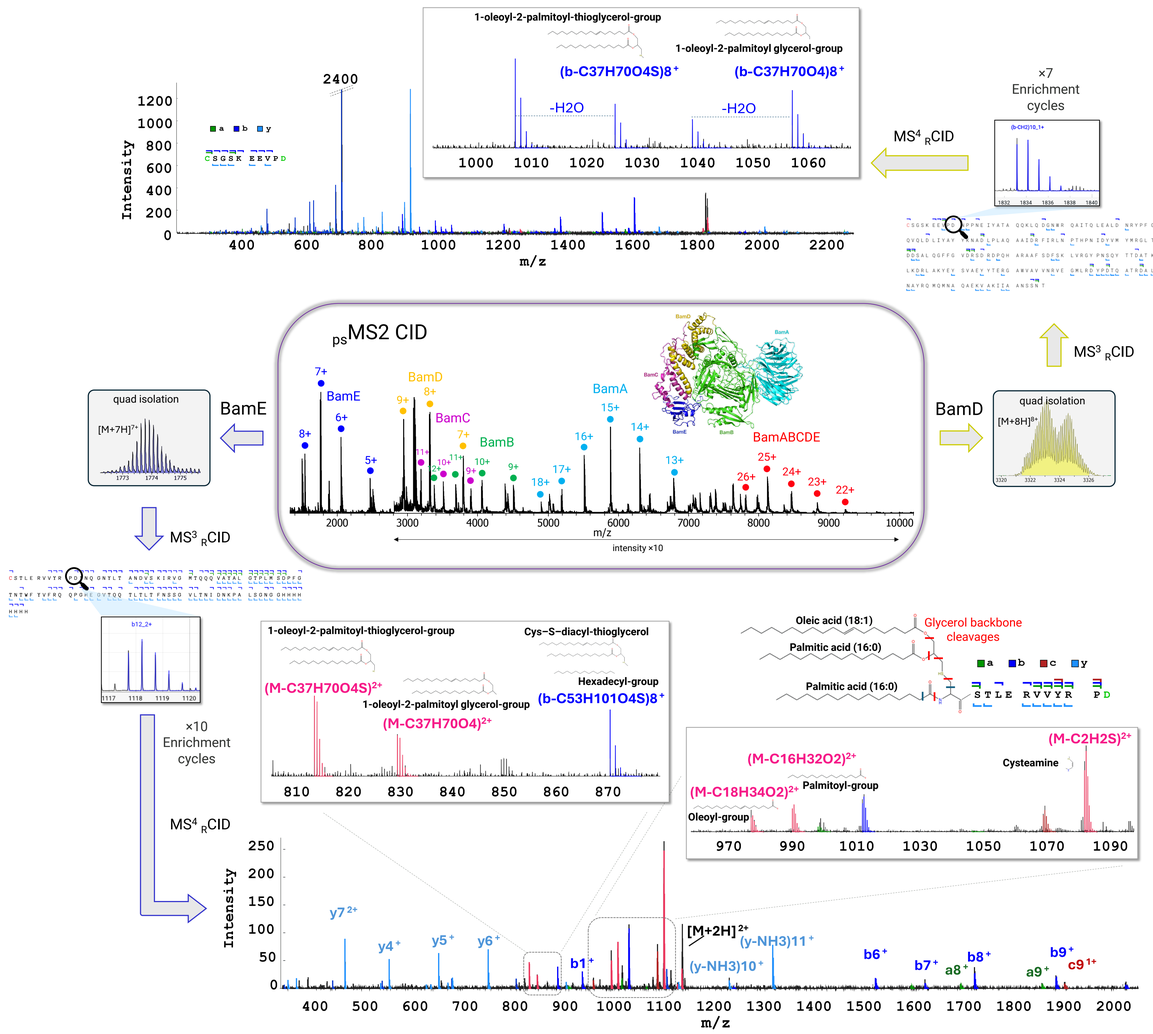

Fig. 2 The timsOmni platform enables the disassembly of BamABCDE intact membrane-protein complex, using psMS2 CID (isCID), for targeted subunit characterization. Two subunits were interrogated on the MS^3 level: BamE (workflow in blue arrows) and BamD (workflow in yellow arrows).
Ion enrichment of low-abundance lipidated fragment ions significantly improves signal-to-noise, enabling MS^4 fragmentation. Comprehensive localization and structural elucidation of N-terminal lipidation was achieved through MS^4 RCID, revealing the lipid-anchor architecture directly from protein-derived fragment ions.

Low-abundance b-type fragments carrying the lipid modification, generated by MS^3 resonance CID (RCID) in Q2, were selectively isolated and enriched, substantially improving signal-to-noise and enabling an additional stage of fragmentation.

The analytical strength of this ion-enrichment strategy is demonstrated by MS^4 RCID of the b_{12}^{2+} product ion from the BamE subunit, which allowed direct structural elucidation of the N-terminal lipidation. MS^4 RCID produced an extensive series of neutral-loss fragments, including palmitic acid (C16:0), oleic acid (C18:1), and the corresponding 1-oleoyl-2-palmitoyl-glycerol species. Additional neutral loss of an S-linked diacylglycerol unit (oleoyl plus palmitoyl) indicated attachment of the glycerol moiety to the cysteine side chain. Loss of a hexadecyl group further confirmed N-terminal linkage of a second palmitic acid to the cysteine residue, enabling full structural characterization of the lipid anchor.

The MS^n workflow was subsequently extended to the remaining lipoprotein subunits. In particular, MS^3 CID of BamD yielded a characteristic series of b-type ions exhibiting CH_2 and C_2H_4 losses and the same lipid-associated mass shift observed for BamE. The most intense species, $[b–CH_2]_{10}^+$, was further interrogated by MS^n and enabled the confident localization of the lipid on the N-terminal Cysteine. Additionally, lipid-related losses from both the precursor and the fragments are in agreement with the corresponding findings in the MS^n workflow for BamE.

Conclusion

- Native top-down MS^n workflows on the timsOmni platform, exploiting ion enrichment, enable localization and detailed structural elucidation of lipidation on a multimeric protein complex.

Proteomics: Intact Proteins and Top-Down Analysis

Conflicts of Interest
JN, MK, DB, IB, AS and DP are employees of Bruker, which develops and manufactures advanced mass spectrometry platforms for life science applications. This presentation includes data and findings related to Bruker's new timsOmni product.