EFTMS2026.org 27-30 April 2026 - Rouen, France
European Fourier Transform Mass Spectrometry Workshop

# ADVANCING TOP-DOWN PROTEOMICS FOR COMPREHENSIVE CHARACTERIZATION OF PROTEIN COMPLEXES AND PROTEOFORMS

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Native top-down proteomics emerges as a powerful tool for structural biology to study proteins and their complexes in their native states, allowing the structural characterization of macromolecular complexes, protein-ligand interactions, together with comprehensive proteoform characterization. We have developed a hybrid top-down proteomics method that combines trapped ion mobility spectrometry (TIMS) with ultrahigh-resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS), enabling the analysis of protein conformers, gas-phase structural isomers, and proteoform heterogeneity. We reported the complete structural elucidation of intact O-glycan proteoforms in S protein regional-binding domain using FTICR-MS. Moreover, we **have developed a "native nanoproteomics" method to allow direct enrichment and native top-down FTICR-MS analysis of endogenous cardiac troponin complex from human heart tissue, providing insights into its structure and dynamics. Furthermore, we developed** a unified software package, MASH Native, for analysis of native top-down proteomics data to process complex datasets, providing a “one-stop shop” for characterizing both native protein complexes and proteoforms. To address the challenges in the native chromatography separation, we have developed online multi-dimensional chromatography approach incorporating multiple orthogonal chromatography separation modes to enable separation of endogenous proteins from complex mixtures under nondenaturing conditions, preserving noncovalent interactions for native top-down proteomics. We successfully detected identified endogenous protein complexes (>350 kDa) in human heart tissue. Using ultra-high resolution FTICR-MS, we have also uncovered the higher-order structural characteristics, protein-ligand binding, and proteoform heterogeneity of protein complexes. These top-down proteomics innovations could advance our understanding of protein structure-function relationships and provide new tools for probing disease mechanisms.

***Keywords***
Top-down proteomics, FTICR, proteoform, MDLC, diseases