

Characterization of protein isoforms using tandem MS of intact and on-line digested proteins from a single intact protein HPLC separation

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Overview

- Integrated top-down bottom-up proteomics with parallel on-line MS analysis and digestion of intact proteins for high throughput applications
- Tandem MS of intact and on-line digested proteins for characterization of protein isoforms and posttranslational modifications (PTMs).
- Current integrated platform continues the trend of
 - Increased confidence in protein identifications and isoform characterization
 - Higher throughput
 - Reduced sample requirements for analysis

Introduction

The integrated top-down bottom-up proteomics approach[1,2] seeks to maximize the advantages from each methodology

- Top-down proteomics
 - Identify combinatorial PTMs
 - Characterize protein isoform distributions, e.g. modification site occupancy
- Bottom-up proteomics
 - Confident protein identification
 - Modification site localization

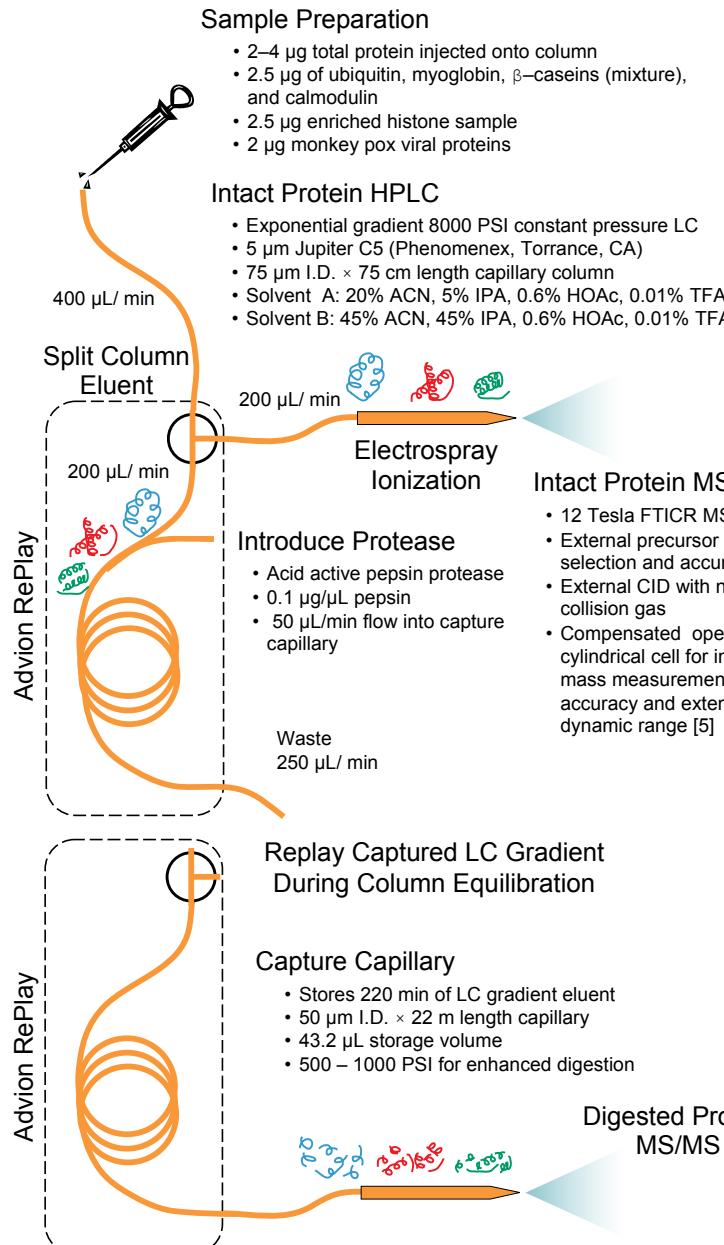
Previous integrated approaches have relied on fraction collection during LC-MS analysis of intact proteins [1,2]

Improved platform incorporates on-line digestion [3] and the RePlay system (Adion Biosciences, Ithaca, NY) [4] for analysis of intact and digested proteins from a single intact LC separation

Platform maintains correlation between intact and digested proteins for increased confidence in protein identifications [1,2,4]

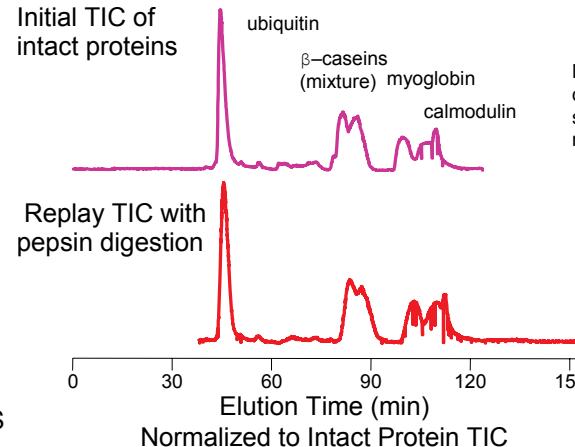
Utilization of collisional induced dissociation (CID) on both the intact and digested proteins for improved characterization of protein isoforms [2].

Methods

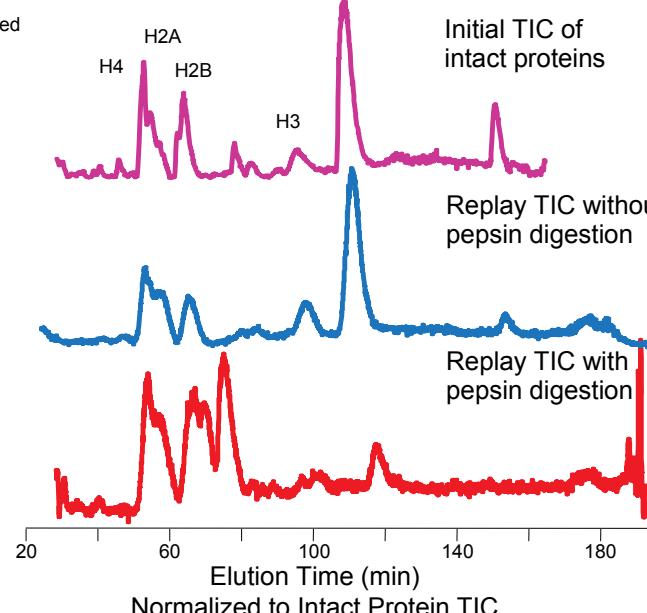


Results

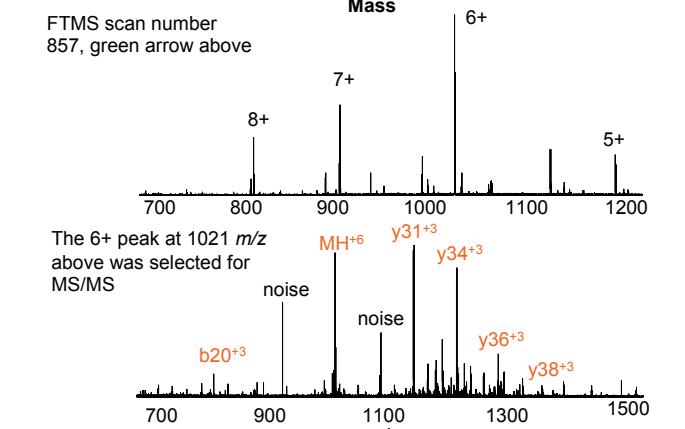
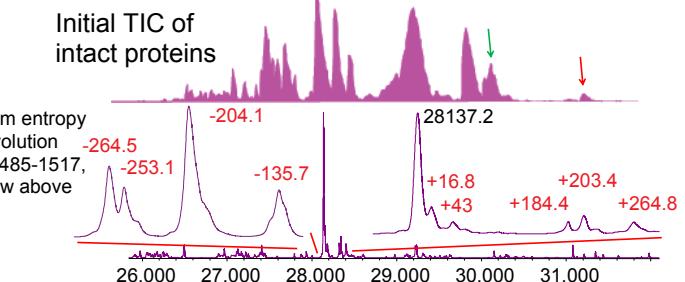
LC-MS/MS of standard protein mixture with replay pepsin digestion



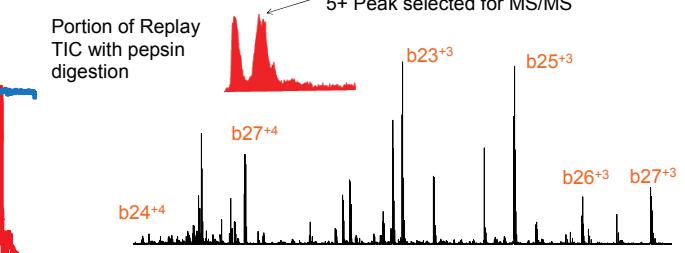
LC-MS/MS of enriched histone sample replay with and without pepsin digestion



LC-MS/MS of isolated Monkeypox viral proteins with replay pepsin digestion MS/MS



De novo MS/MS analysis using in-house tools identified GFTNKNKLEKLSTNKELEYSSSPQEPQLRNLDFLGLLECVKKN IPLTDIPTKD from Monkeypox structural protein VP8 based on the observed fragment peptides



Similar de novo analysis based on the observed fragment peptides identified the sequence GFTNKNKLEKLSTNKELEYSSSPQEPQLRNLDFLGLLECVKKN as a digestion product of the original larger protein fragment

Conclusions

- High correlation of LC elution features between top-down and bottom-up MS/MS analysis
- Enabled by effective on-line digestion of intact proteins
- Reduced sample requirements through the use of Advion RePlay technology

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