# ProMex: a New Feature Extraction Algorithm for Top-down Mass Spectrometry based on Summed Spectra

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## Overview

- ProMex: **Pr**otein **M**ass **Ex**tractor
- A new feature finding tool for extracting masses, charge states, elution times, and abundances of intact proteins in top-down mass spectra
- It clusters isotopic envelope peaks distributed across multiple charge states, scans and natural isotopes
- Extracted features are used for protein identification, characterization of post-translational modifications and quantification of proteoforms.

## Introduction

- Top-down mass spectra are complex and noisy
  - Low signal-to-noise ratio
  - Complex mixtures of hundreds or thousands proteoforms

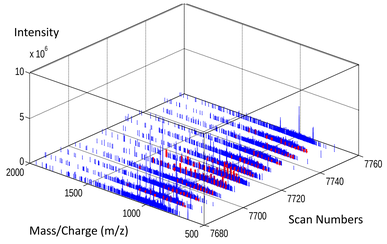



**Figure 1.** An example of top-down LC-MS spectra. Marked red are isotopic envelope peaks from a 28K Da intact protein. They are eluted over a period of time with multiple charge states.

## Methods

### Clustering Isotopic Envelopes

- ProMex clusters isotopic envelopes across multiple charge states and scans

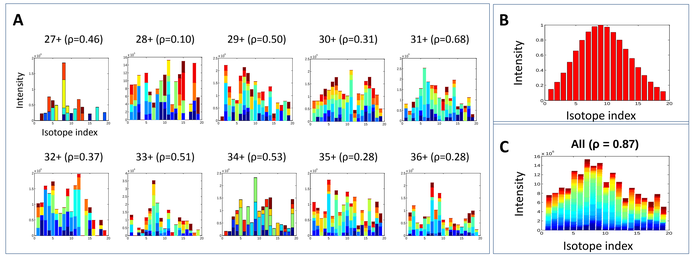



**Figure 2.** (A) Summed isotopic envelopes of the 28K protein in Figure 1 across multiple scans (12 spectra) at each charge state between 27+ and 36+. Numbers in parenthesis are Pearson's correlations to theoretical isotopic envelope, (B) Theoretical isotopic envelope, and (C) Sum of all isotope envelopes in (A).

- Summed envelopes becomes more similar to their theoretical envelopes

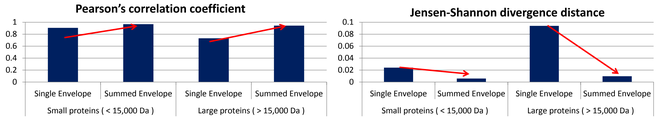



**Figure 3.** Summing envelopes increases similarity to theoretical isotopic envelopes, in particular for large proteins (e.g. >15K Da). Pearson's correlation coefficient and Jensen Shannon divergence distance are used for measuring the similarity between experimental and theoretical envelopes.

### Scoring

- Extracted features are evaluated and filtered out using various scoring metrics

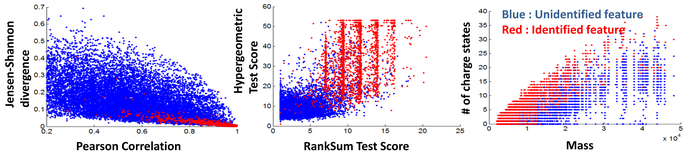



**Figure 4.** Scoring metrics such as Pearson's correlation, Jensen-Shannon divergence, Hypergeometric test score, Ranksum test score and differences in m/z values can separate good and bad features.

## Results

### Protein Identification

- Features extracted by ProMex are used for proteoform identification
- Increase in number of identifications

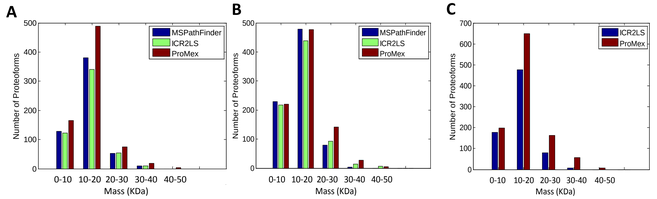



**Figure 5.** The number of unique proteoforms identified at 1% false discovery rate for three datasets from Shewanella oneidensis samples. (A) Short gradient C2 RPLC; Gradient Length = 180 min, (B) Long gradient C2 RPLC; Gradient Length = 850 min, and (C) Long gradient C2 RPLC; Gradient Length = 900 min

- ProMex extracts features of large intact proteins correctly

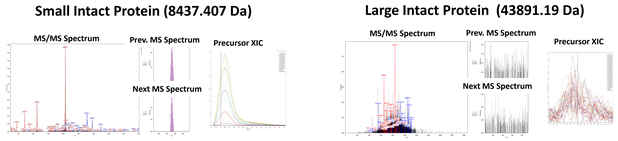



**Figure 6.** Examples of identified proteoforms: MS/MS spectra, adjacent MS spectra, and Precursor XICs of natural isotopes. Matched b ion, y ion and isotopic envelope peaks are marked blue, red, and purple, respectively.

### Label-free Quantification

- Discriminatory features are identified by selecting the features whose abundances are significantly different among multiple groups of samples

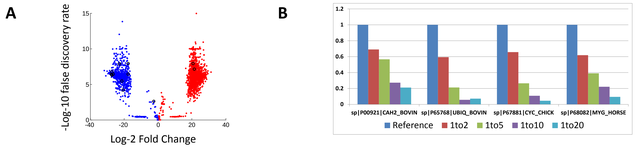
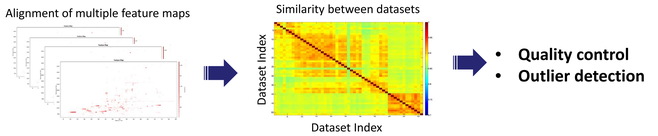



**Figure 7.** A known amount of a set of 4 intact protein standards were spiked into a ovarian tumor lysate background. A statistical analysis on extracted features identifies the spike-in proteins with their correct abundance levels. (A) volcano plot to compare spike-in samples. All spike-in proteins are detected above a 1% false positive threshold (B) The abundance of features corresponding to the spike-in proteins matches well to the spike-in levels

*(Note: Figure 7 will be updated with new data)*

## Measuring Reproducibility

- The similarity of aligned feature maps represents the overall difference between samples




## Discovering Unknown Modifications

- Infer unknown modifications from frequent mass differences between co-eluted features

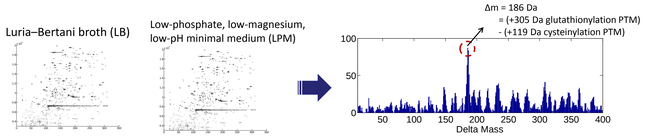



**Figure 8.** Protein S-thiolation switch in Salmonella Typhimurim in response to infection like (LPM) conditions versus basal (LB) conditions. The most frequent mass difference between aligned features is 186 Da, which corresponds to S-glutathionylation-to-Scysteinylation switch. Datasets used in Ansong et al., 2013

## Conclusions

- ProMex is a new feature finding tool which clusters ion peaks scattered over multiple charge states and spectra to better define and allow identification of isotopic envelopes.
- Compared to existing deconvolution algorithms, it increased the number of proteoform identifications by more than 10%.
- In particular, ProMex significantly increased proteoform identifications with masses over 25,000 Da, which were mostly missed by other tools.
- ProMex can be used for label-free quantification of intact proteins.

## Acknowledgements

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