Ultrahigh-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry and Tandem Mass Spectrometry for Peptide *de Novo* Amino Acid Sequencing for a Seven-Protein Mixture by Paired Single-Residue Tranasposed Lys-N and Lys-C Digestion

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Introduction

Bottom-up tandem mass spectrometry (MS/MS) is regularly used in proteomics to identify proteins from an amino acid sequence database. De novo sequencing is also available for sequencing peptides with relatively short sequence lengths. We recently showed that paired Lys-C and Lys-N proteases produce peptides of identical mass and similar retention time, but different tandem mass spectra. Such parallel experiments provide complementary information, and allow for up to 100% MS/MS sequence coverage.

Experimental

Here, we report digestion by paired Lys-C and Lys-N proteases of a seven-protein mixture: human hemoglobin alpha, bovine carbonic anhydrase 2, horse skeletal muscle myoglobin, hen egg white lysozyme, bovine pancreatic ribonuclease, bovine rhodanese, and bovine serum albumin, followed by reversed-phase nanoflow liquid chromatography, collision-induced dissociation, and NHMFL's 14.5 T Fourier transform ion cyclotron resonance mass spectrometry.

Results and Discussion

Matched pairs of product peptide ions of equal precursor mass and similar retention times from each digestion are compared, leveraging single-residue transposed information with independent interferences to confidently identify fragment ion types, residues, and peptides. Pairs of the transposed product ions as well as complementary information from the parallel experiments allow for both high MS/MS coverage for long peptide sequences and high confidence in the amino acid identification. (**Fig. 1**) Moreover, the parallel experiments in the *de novo* sequencing reduce false-positive matches of product ions from the single-residue transposed peptides from the same segment, and thereby further improve the confidence in protein identification.

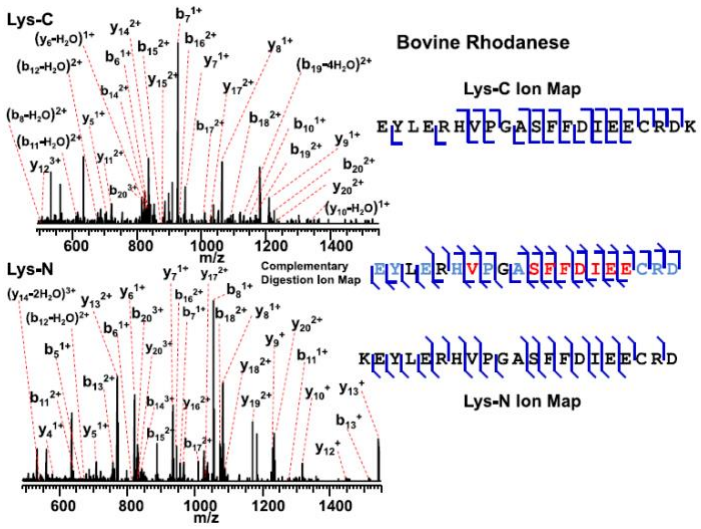

Fig. 1. Paired annotated CID positive product ion mass spectra, as for Fig. 1, for the same peptide from bovine rhodanese. The complementary transposed ions lead to an MS/MSsequence coverage of 100%.

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Reference

[1] Guan, X., *et al.*, Rapid Commun. Mass Spectrom. **31**, 207–217 (2017).