A Versatile Isobaric Tag Enables Proteome Quantification in Data-Dependent and Data-Independent Acquisition Modes

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Abstract We present a versatile acetyl-alanine-glycine (Ac-AG) tag that conceals quantitative information in isobarically labeled peptides and reveals it upon tandem MS in the form of peptide-coupled reporter-ions. Since the peptide-coupled reporter-ion is precursor-specific while fragment ions of the peptide backbone originating from different labeling channels are identical, the Ac-AG tag is compatible with both DDA and DIA. By isolating the monoisotopic peak of the precursor ion in DDA, intensities of the peptide-coupled reporter-ions represent the relative ratios between constituent samples, whereas in DIA, the ratio can be inferred after deconvoluting the peptide-coupled reporter-ion isotopes.

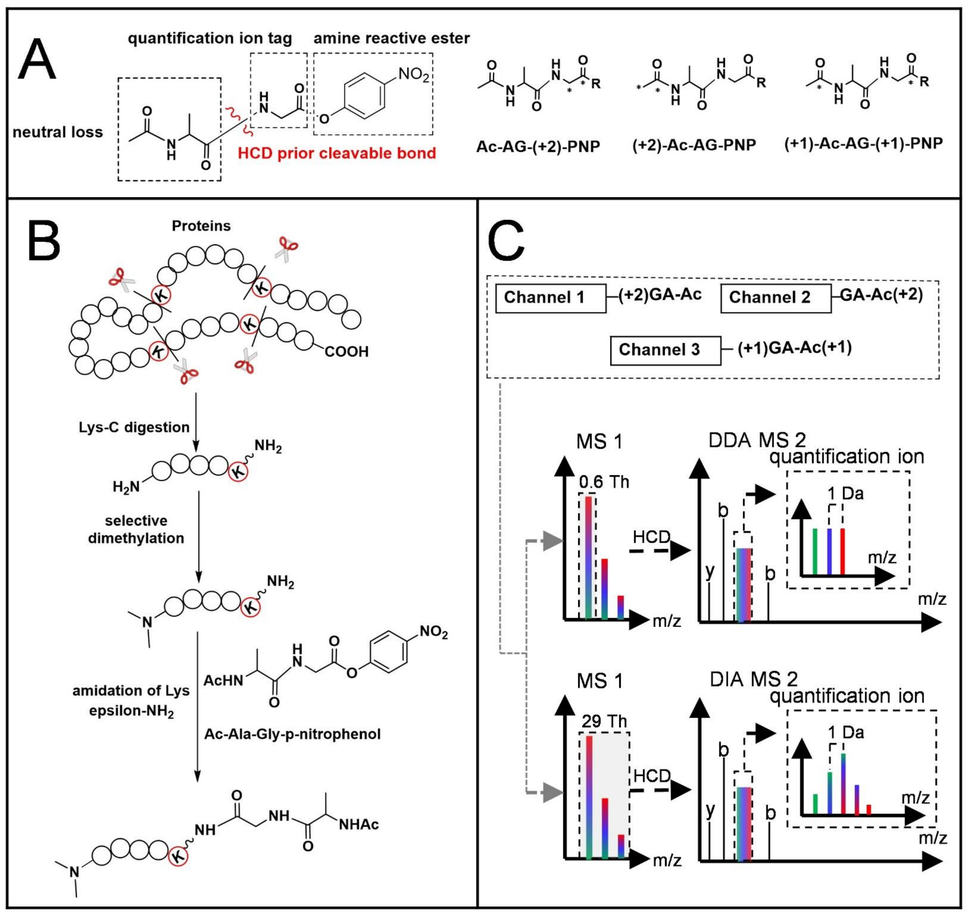


Figure 1. A. Molecular structure and isotopic distribution of the Ac-AG-PNP tags. **B**. Sample preparation and isobaric labeling steps. **C**. LC-MS/MS concept map for a mixture of triplex labeled samples.

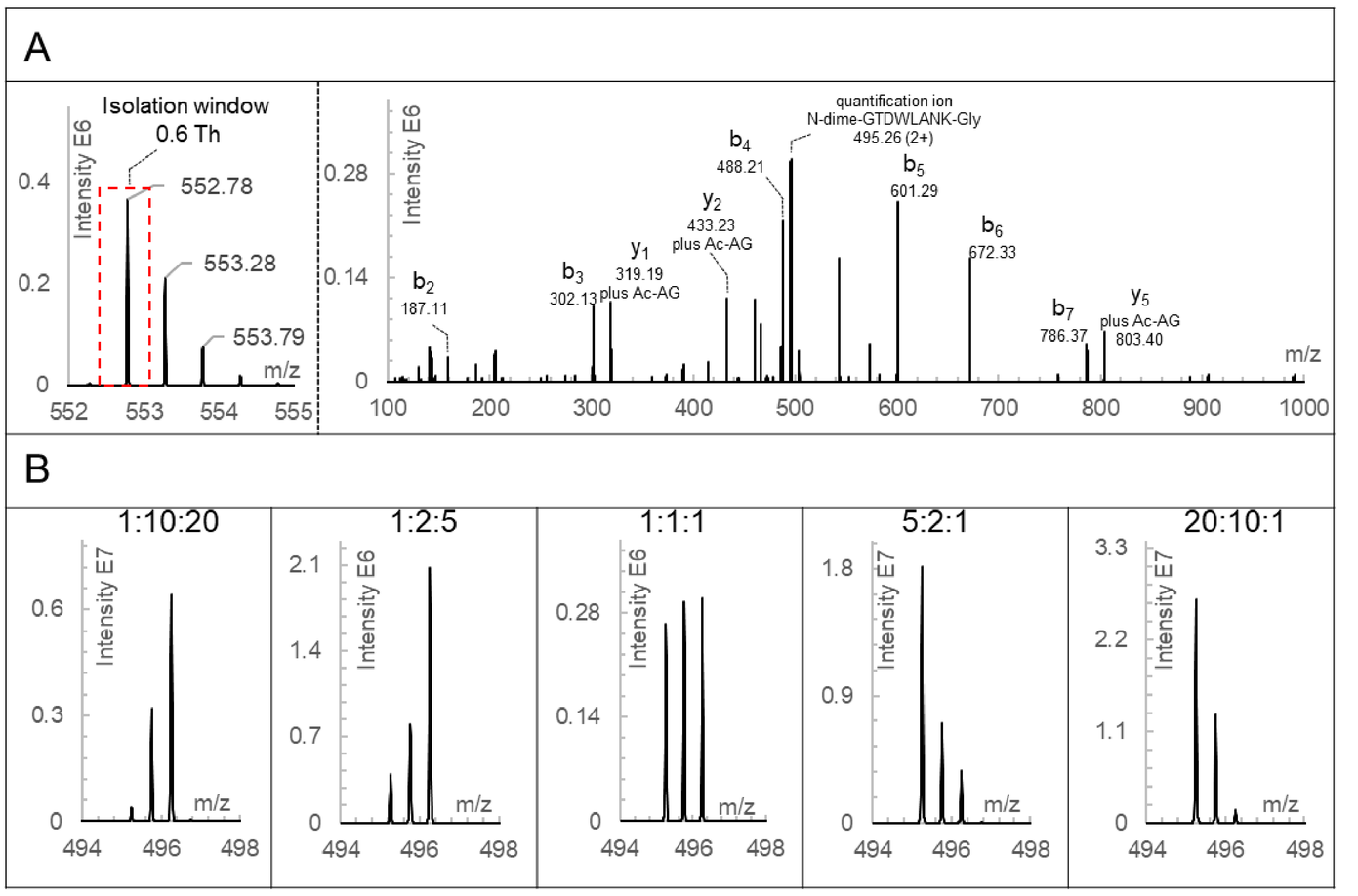


Figure 2. A. Precursor ion selection and MS2 spectrum of triplex-labeled peptide with a combined NCE of 18 and 28 acquired in DDA. **B**. Peptide-coupled reporter-ions in the DDA MS2 spectra at various ratios.

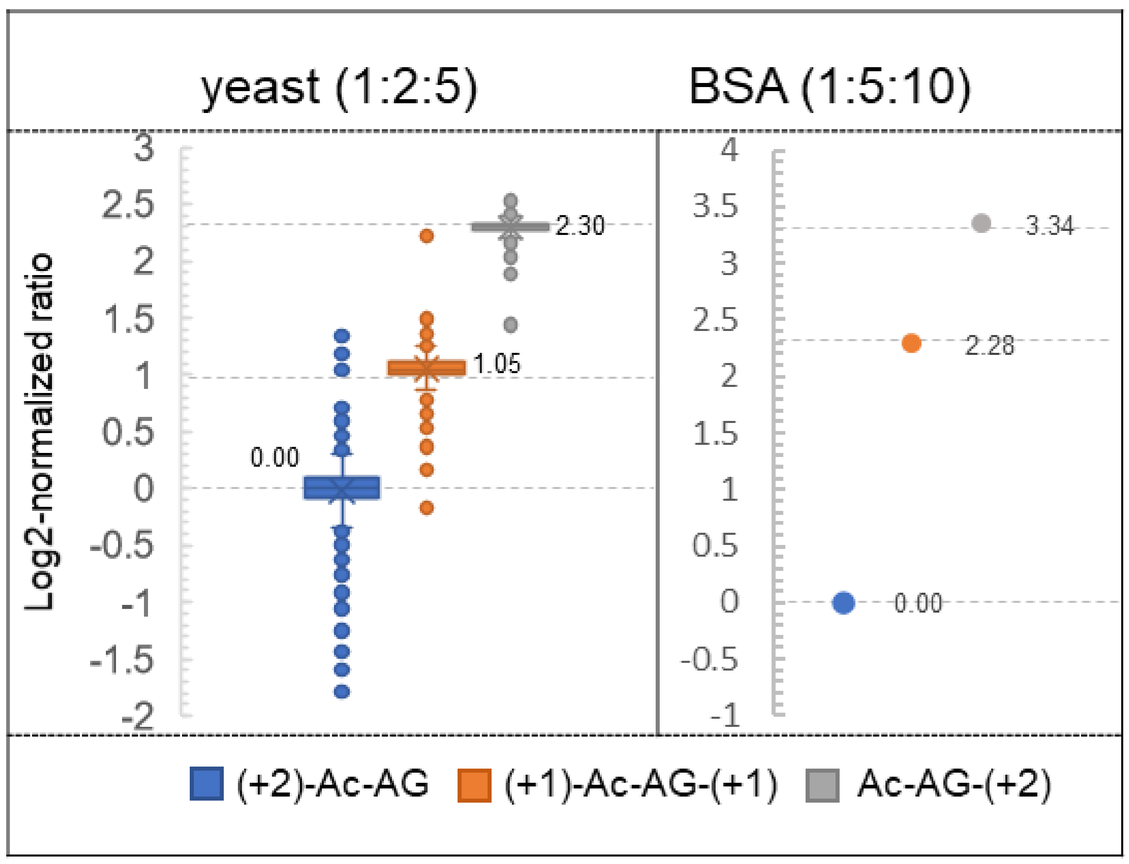
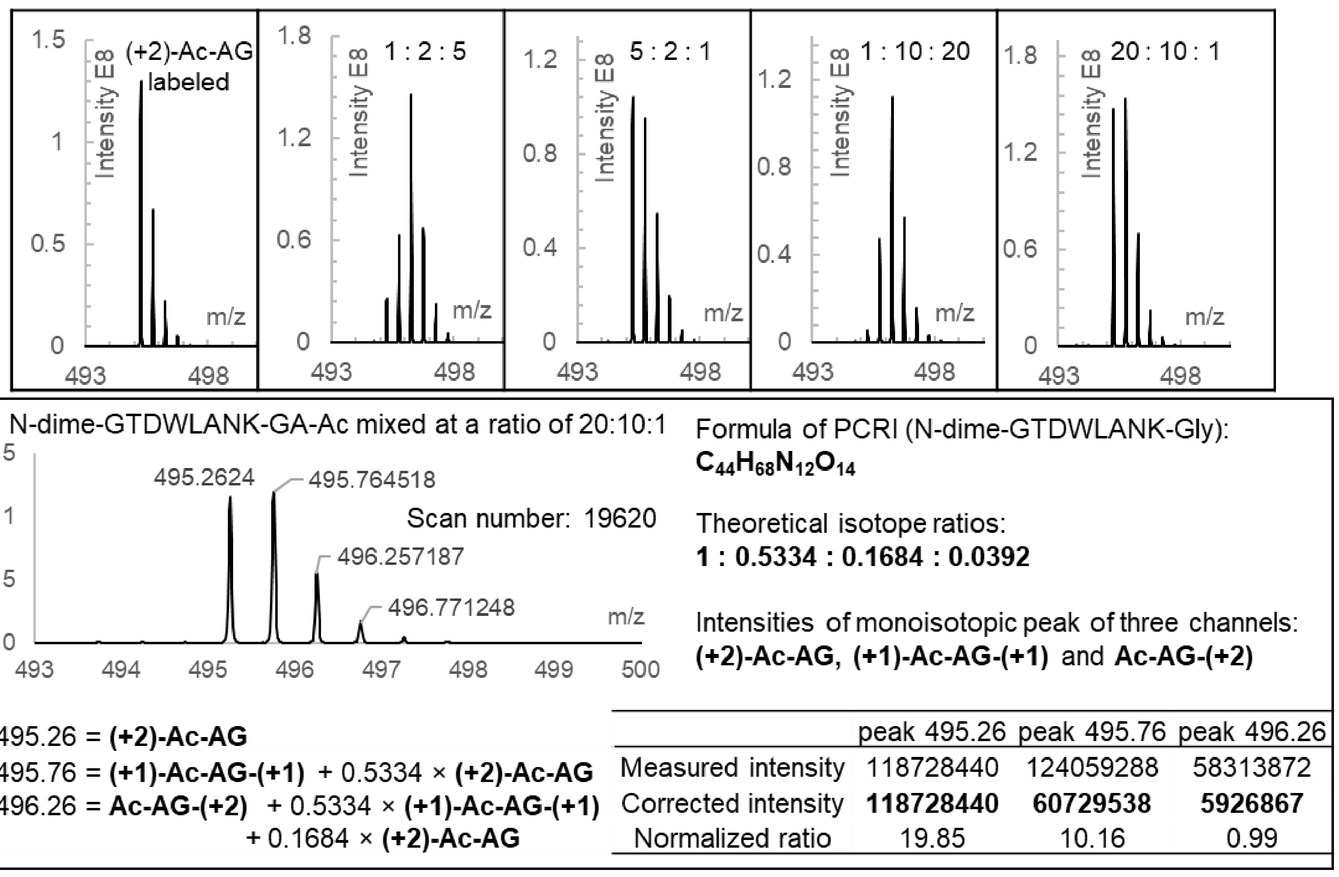


Figure 3. Quantification of a mixed yeast-BSA sample in DDA mode. Expected values for log2-normalized mixing ratios are shown as dotted lines.



	peak 495.26	peak 495.76	peak 496.26
Measured intensity	118728440	124059288	58313872
Corrected intensity	**118728440**	**60729538**	**5926867**
Normalized ratio	19.85	10.16	0.99

Figure 4. Different shapes of peptide-coupled reporter-ions of differentially mixed sample and deducing quantitative ratios from overlapped isotope envelope in DIA data.

Conclusions

The Ac-AG tag maintains the advantages of existing peptide-coupled reporter-ion-based quantification methods in DDA mode but also allows multiplexing in the DIA mode without sacrificing the rate of data acquisition or complicating MS2 spectra. The proteome quantification capabilities in DDA and DIA were demonstrated by triplex labeling of a yeast proteome spiked with BSA over a 10-fold dynamic range.

Acknowledgements

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