NARROWBAND TWO-DIMENSIONAL MASS SPECTROMETRY AND LABEL-FREE RELATIVE QUANTIFICATION OF HISTONE PEPTIDES

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Introduction

- Two-dimensional mass spectrometry (2D MS) is a method for tandem mass spectrometry that does not require ion isolation.[1]
- 2D MS can be used to separate peptide and protein isoforms that cannot be easily separated through LC methods (e.g. methylations)

Goals

- Method development for high resolution 2D MS (precursor-fragment correlation)
- Application of 2D MS for label-free relative quantification of histone peptide isoforms.

Experimental Methods

- H3 histone peptide with no modifications, mono-, di-, and tri-methylation (K7) at 0.1 µM in methanol/water with 1% acetic acid each.
- FT-ICR mass spectrometer with positive ESI, ECD fragmentation, pulse sequence shown in Figure 1.

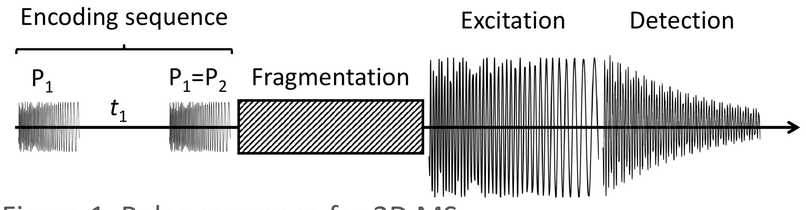

Figure 1. Pulse sequence for 2D MS.

- Ion radii in the ICR cell modulation during P_1-t_1-P_2.
- Fragment ion abundances modulation during fragmentation.
- Fourier transformation along detection and along t_1: correlations between precursor and fragment ion signal.
- Resolving power of second dimension: accuracy of precursor-fragment correlation.
- Broadband Nyquist frequency: 250 kHz,
- Narrowband Nyquist frequency: 62.5 kHz
- Data processing with SPIKE[2,3]
- Narrowband 2D MS: 125 kHz offset (see Figure 2)

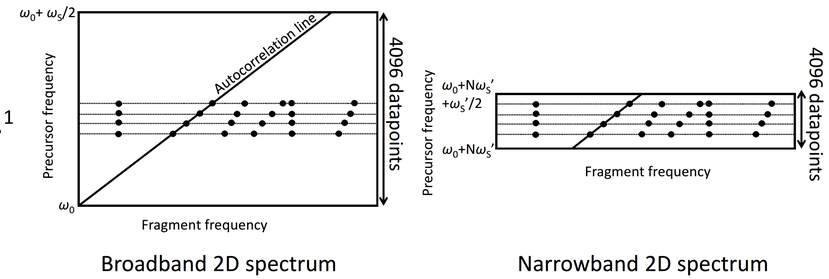

Figure 2. Comparison between a two-dimensional mass spectrum obtained by broadband method (left) and narrowband method (right).

Results and Discussion

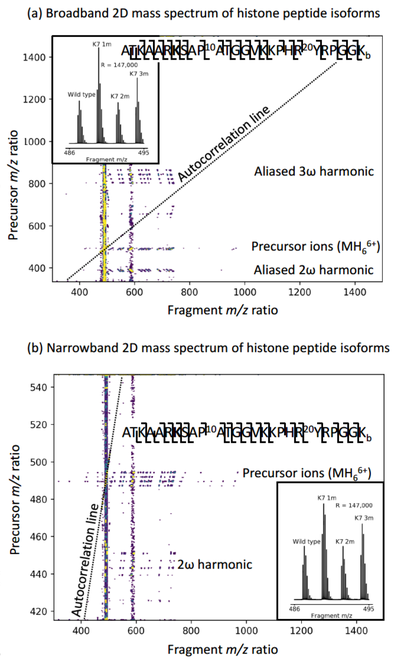

Figure 3. (a) Broadband and (b) narrowband 2D mass spectra of histone peptide isoforms with autocorrelation line and sequence coverage.

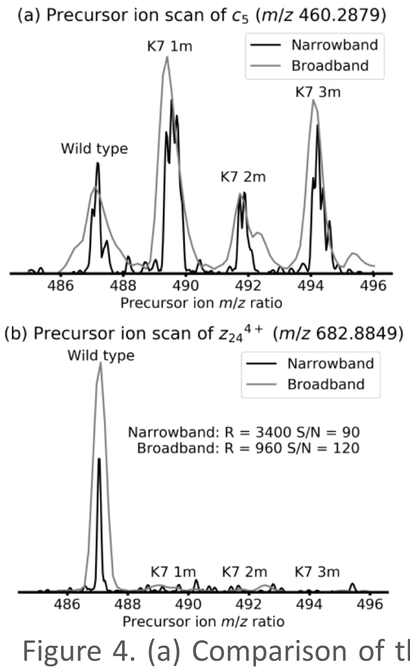

Figure 4. (a) Comparison of the precursor-fragment correlation for the c_5 fragment between broadband and narrowband. (b) Comparison for z_{24}^{4+} (single isotope).

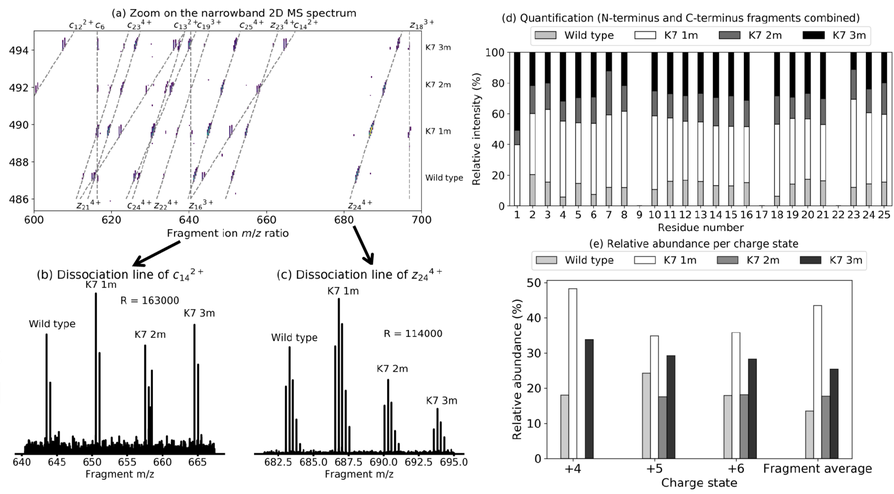

Figure 5. (a) Zoom on the narrowband 2D mass spectrum. (b-c) Extracted dissociation lines. (d) Label-free relative quantification with fragment abundances. (e) Label-free relative quantification of the four histone peptide isoforms for 6+ precursor ion, 5+ and 4+ charge-reduced states and average of fragment ions.

Conclusion

- Narrowband 2D MS offers more accurate precursor-fragment correlation by a factor of 4.
- Peptide modifications can be located by comparing vertical precursor ion scans (fragments without modification) and dissociation line (fragments with modification).[4]
- Label-free relative quantification results by 2D MS are consistent with sample preparation.

Acknowledgements

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References

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[4] M. van Agthoven, A.M. Lynch, T.E. Morgan, C.A. Wootton, Y.P.Y. Lam, L. Chiron, M. Barrow, M.-A. Delsuc, P. O'Connor, Anal. Chem. 90 (2018) 3496-3504.