Quantitation of Peptides by Miniature Mass Spectrometry

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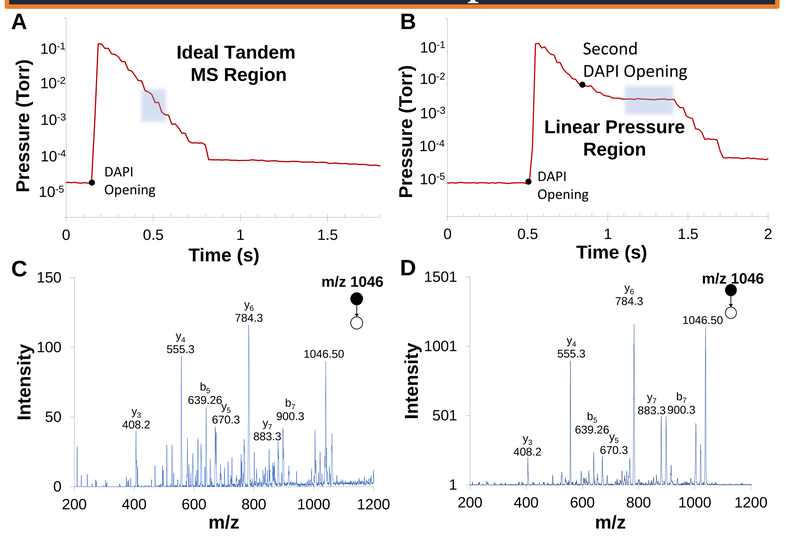
Introduction

Peptide analysis is an essential tool in quantifying disease-specific biomarkers and characterizing protein structure. Advancements in immunoassay peptide analysis have allowed for multiplexed peptide quantitation and sequencing. By pairing immunoassays to the high sensitivity of mass spectrometry, MS-based peptide analysis can provide vital quantitative information for accurate disease diagnosis.

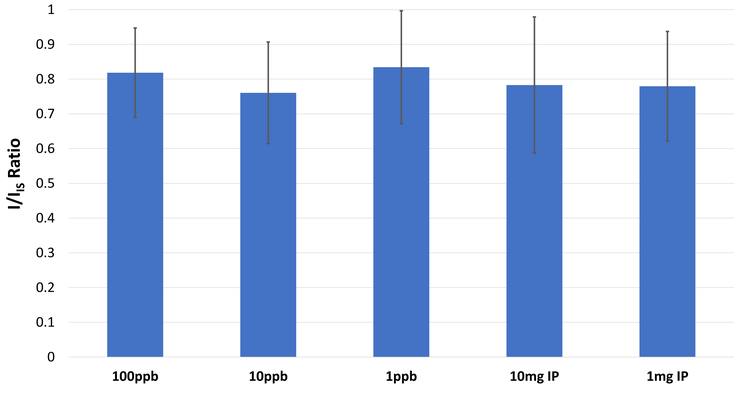
Gap: *Despite the potential for MS-based peptide analysis, identified biomarkers are under-utilized in real-world applications due to technological limitations in identification and quantification of targeted peptides outside of analytical laboratories.*

Pressure Condition Optimization



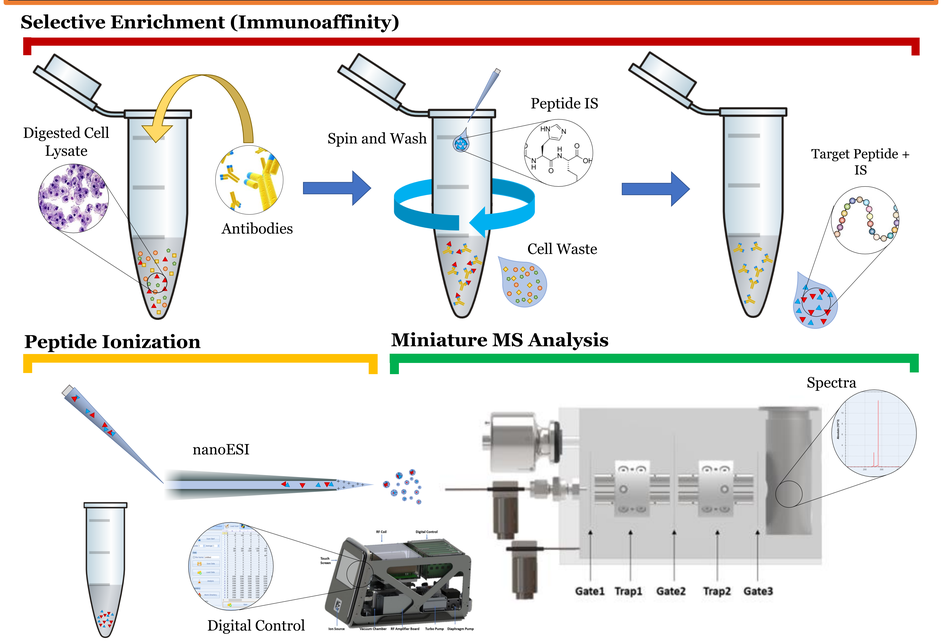
The single DAPI opening pressure curve (A) falls within the optimal tandem MS pressure region (blue square) for 200ms. The opening of the second DAPI for 300ms (B) creates an observable linear pressure region that can be altered by changing the inner diameter capillary of the auxiliary DAPI. (C) and (D) show the respective MS^2 spectra based upon the pressure curve used. The SNR is approximately 10 times higher using the curve in (B) compared to (A). Both spectra were obtained using synthetic *Met* peptide at a concentration of 25uM.

Ionization of *Met* in Background Peptides



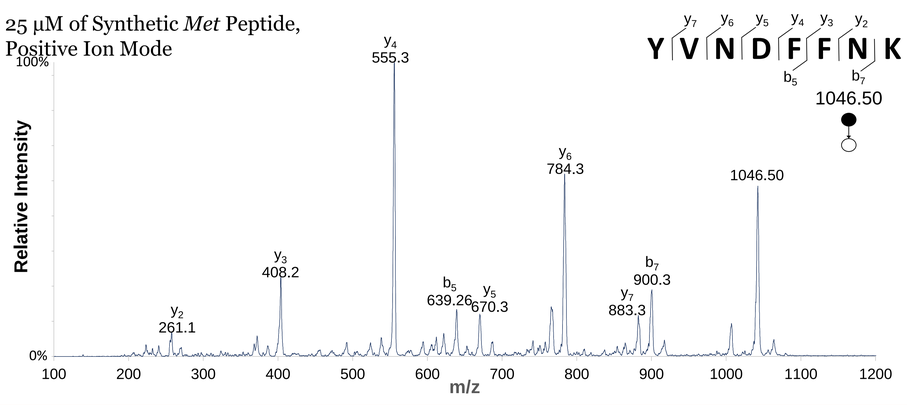
Both *Met* peptide and its internal standard were spiked into varying concentrations of mouse liver peptides from 1ppb to 100ppb. Analyte to internal standard ratios were compared to verify a non-significant effect of background peptides on analyte ionization. Several real immunoprecipitated (IP) samples with 10mg and 1mg starting protein with 1µM *Met* and IS spiked were also analyzed to verify the working range of background peptide in real IP samples and its effect on ionization before further analysis of *Met* using the miniature MS.

Overview



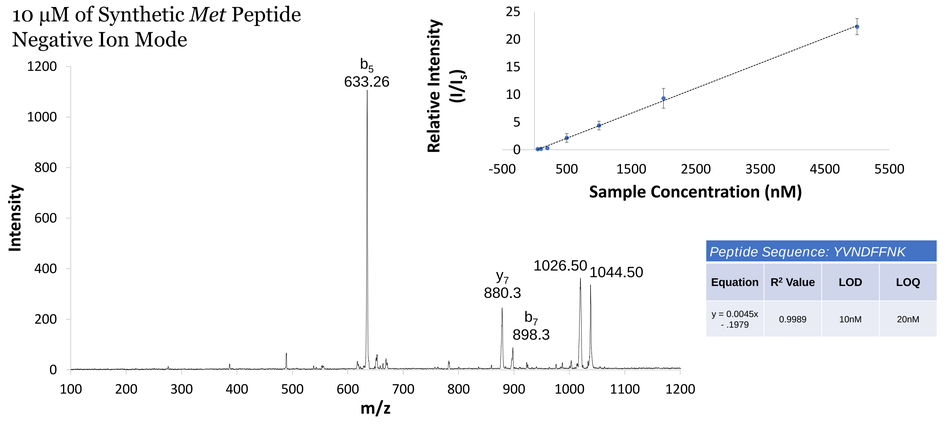
Peptide Fragment Identification

25 µM of Synthetic *Met* Peptide, Positive Ion Mode



Quantitation of Peptide by Mini Beta

10 µM of Synthetic *Met* Peptide Negative Ion Mode



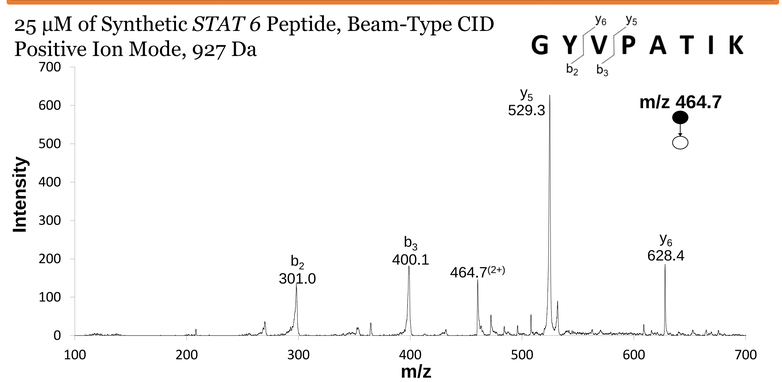
Peptide Sequence: YVNDFFNK			
Equation	**R^2 Value**	**LOD**	**LOQ**
y = 0.0045x - .1979	0.9989	10nM	20nM

Experimental

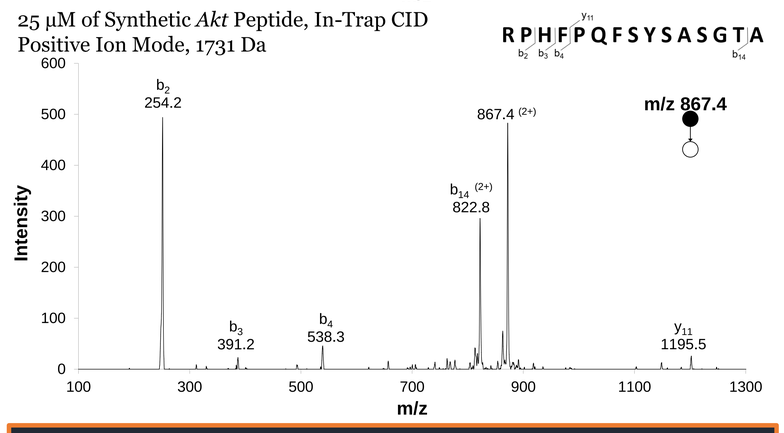
- Several synthetic peptides from *STAT6, Met,* and *Akt* proteins are analyzed using the Mini Beta.
- Manipulation of pressure curves within the chamber allows for optimization of tandem MS efficiencies such as collision-induced dissociation, ion transfer, and analyte isolation.
- Utilizing the dual-trap configuration of the Mini Beta, peptide tandem MS analysis is performed by using Q1 to isolate target ions and Q2 as a mass analyzer.

Low/High Mass Peptide Spectra

25 µM of Synthetic *STAT 6* Peptide, Beam-Type CID Positive Ion Mode, 927 Da



25 µM of Synthetic *Akt* Peptide, In-Trap CID Positive Ion Mode, 1731 Da



Conclusion

- Quantitation of several peptides of varying mass ranges was achieved by the Mini Beta.
- The Mini Beta achieves nanomolar-level sensitivity, with LOD and LOQ observed at 10nM and 20nM, respectively.
- This approach will also be applied to quantify the *Met* peptide in several cell lines with varying peptide expression levels.

Acknowledgements

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