Quantitation of Peptides by Miniature Mass Spectrometer

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Novel Aspect (limit 20 words):

Quantifying peptides through miniature mass spectrometry enables point-of-care peptide biomarker analysis.

Introduction (limit 120 words):

Miniature mass spectrometry (MS) systems with ambient ionization capability have been shown to have a great potential for biological and clinical applications such as therapeutic drug monitoring and lipid profiling. Analysis of protein/peptide biomarkers using miniature MS systems remains to be explored. Rapid affinity enrichment procedures such as immuno-enrichment are widely used for capturing and quantifying peptides digested from proteins. In this study, we investigated the development of an analytical platform integrating immuno-enrichment methods for capturing specific peptides and quantitation by the novel Mini β system.

Methods (Limit 120 words):

A novel miniature mass spectrometer with dual-trap configuration, Mini β, was used for peptide analysis. Several synthetic peptides, *Met* (Seq: TVNDFFNK), *Stat 6* (Seq: GYVPATIK) and *Akt (*Seq: RPHFPQFSYSASGTA) provided by Cell Signaling Technology, were ionized by electrospray and analyzed by miniature mass spectrometer. Utilizing the dual-trap configuration, peptides were isolated in the first linear ion trap and transferred to the second trap for either in-trap collision induced dissociation (CID) or beam-type CID. Optimization of the Mini β scan function included CID energy, isolation frequency, and manifold pressure. Further optimization studies like solvent type were conducted to develop the quantitation workflow. The resulting peptide quantitation method was verified through the addition of internal standard and generation of a calibration curve for *Met* peptide.

Preliminary results (Limit 300 words):

Several synthetic peptides were quantified using Mini β. *Stat 6, Akt,* and *Met* peptides were ionized by electrospray and identified with several significant CID fragments. The optimization of the Mini β scan function focused on CID type (in-trap or beam-type), isolation frequency, and

chamber pressure, while maintaining a short duty cycle. The pressure of vacuum manifold was maintained in the millitorr range where CID and ion mass transfer was optimal, improving the resulting signal-to-noise ratio by approximately 10 times. MS spectra were obtained using positive and negative ion modes. Due to lowered sensitivity of positive ion mode in nM concentrations, negative ion mode was used for MS/MS quantitation. *Met* peptide (1046 m/z) was chosen as the target peptide for MS/MS quantitation (m/z 1046 → m/z 633) and the solvent used was MeOH and water (v/v 3:7). The addition of acid or ammonia to the solvent led to decreases in overall sensitivity. The *Met* peptide internal standard (m/z 1048 → m/z 635) was used to determine the absolute concentration and generate a calibration curve to verify analytical performance. A calibration curve was established between 50 nM and 5 µM and identified a R^2 of 0.99 with RSD better than 20%. Limit of detection of 10nM and limit of quantitation of 20 nM were obtained. Studies on the effect of background peptide levels are being carried on identifying any loss of sensitivity in comparison to the standard quantitation curve. We are applying this method to quantify *Met* peptide in cell lines with varying expression levels.

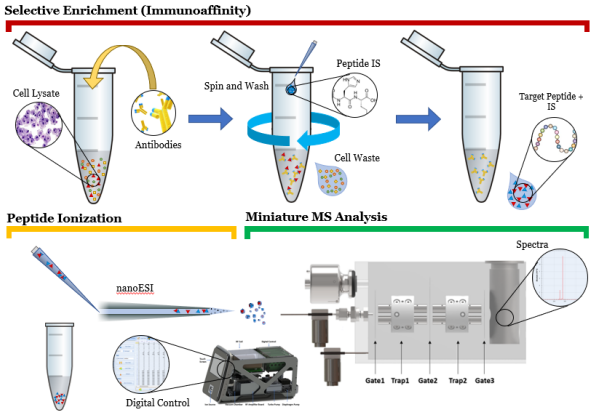

Figure 1: Overall Workflow for the quantitation of peptides by miniature mass spectrometry. Initial trypsin digests of cell lysates are completed, and Met peptide isolated for introduction to miniature mass spectrometer.

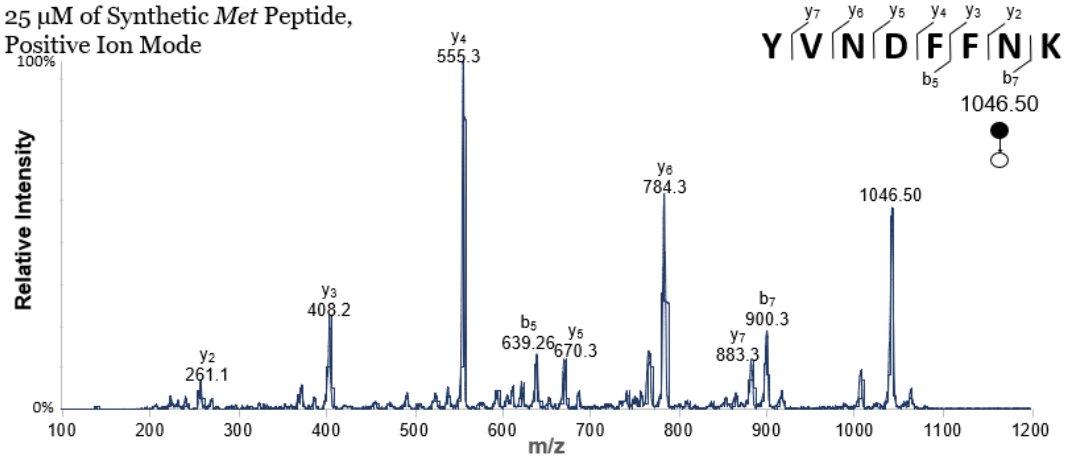

Figure 2: Standard Spectra of Synthetic Met peptide in Positive Ion Mode. In-Trap CID was done and several significant fragments were identified from the precursor Met peptide.

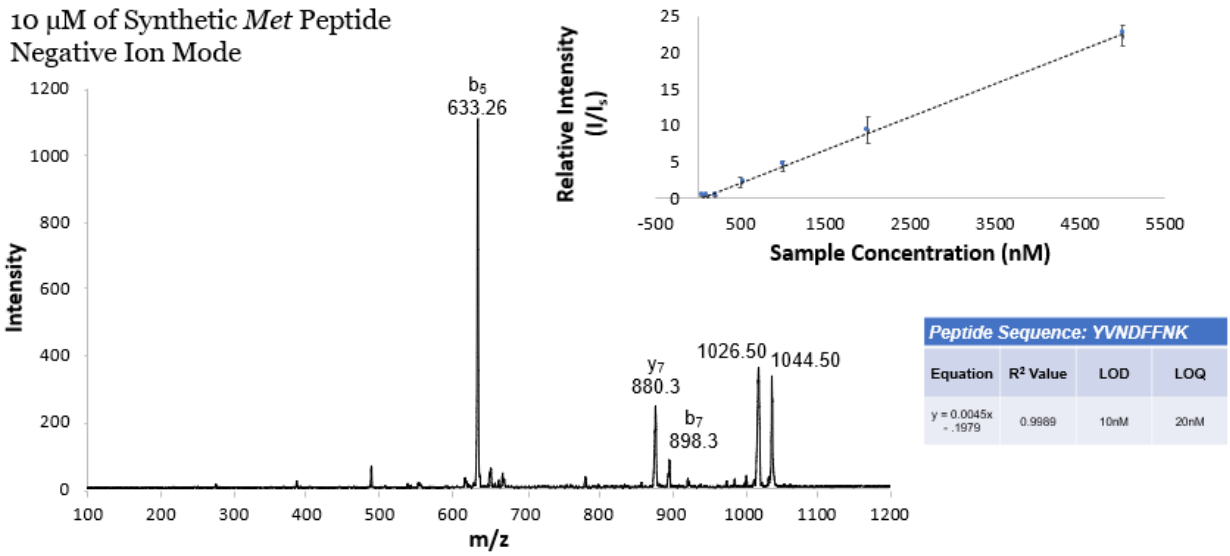

Figure 3: Generated Calibration Curve of Met Peptide using standards. The linear region of the curve ranges from 50nM to 5uM while identifying a LOD of 10nM using the miniature mass spectrometer.