

Combining Microfluidic HPLC Chip Technology with a Hybrid Triple Quadrupole FT-ICR Mass Spectrometer for Novel “Bottom-up” and “Top-Down” Protein Analyses

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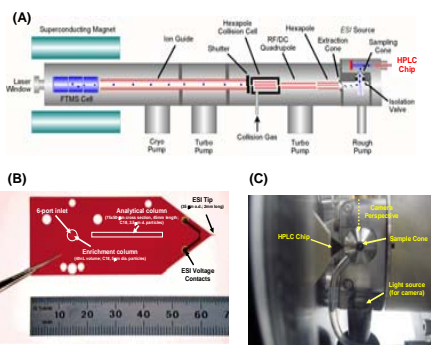
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INTRODUCTION

- The microfluidic HPLC chip integrates an enrichment column, a separation column, and a nanospray tip into a single polyimide structure. This avoids many of the pitfalls (i.e., peak broadening, dead volumes) encountered in traditional nanoLC systems. These HPLC chips have been demonstrated to provide excellent sensitivity and retention time reproducibility (Yin et al., 2005; Fortier et al., 2005).
- Combined with a 12T QFT FT-ICR mass spectrometer, the HPLC chip provides high performance chromatographic separations that complement the high resolving power and mass accuracy of the FT-ICR.
- With these tools in hand, we are developing new software that employs both high precision retention times and accurate mass measurements for the peptide and protein identification.

Figure 1. (A) Schematic diagram of the QFT-12 FT-ICR mass spectrometer. (B) Close-up image detailing the organization of the components of the HPLC chip. (C) Photograph showing the positioning of the HPLC chip in the source region of the QFT-12



METHODS

Mass Spectrometry

Experiments were performed using a QFT-12 triple-quadrupole FT-ICR mass spectrometer (IonSpec, Lake Forest, CA). Ions were electrosprayed by applying a potential of 1.6 kV on the HPLC chip, and these ions were accumulated in the Q3 region for 2s. The typical resolving power observed in the mass spectra was between 75,000-150,000 in broadband mode (m/z 185 – 2000). MS/MS was performed by using IRMPD.

Peptide identification was confirmed in parallel using a Q-ToF Global mass spectrometer (Waters, Milford, MA).

Microfluidic HPLC Chips

The experimental HPLC chip system (Agilent, Santa Clara, CA) (Yin et al., 2005) consists of an enrichment column and an LC column packed with either 3.5 μ m C18 or C8 particles, depending upon the analyte (i.e., peptides vs. intact proteins). The eluent, flowing at 250 nL/min, is electrosprayed from the chip through an 8 μ m i.d. tip. An Agilent 1100 quaternary pump delivered solvent during sample loading and a nanoLC 1100 pump delivered the gradient.

Data Analysis

The data were analyzed by using the FT DocViewer program (IonSpec). Total ion chromatograms were converted with Visual Basic scripts to ASCII formatted lists containing deconvoluted MWs and retention times. These data were directly imported into software developed in-house for retention time-dependent database searching (vide infra).

RESULTS

MASS SPECTROMETRY

Resolving Power and Mass Accuracy

- Resolving powers achieved with FT-ICR were between 75,000-150,000 in broadband mode (m/z 185 – 2000).
- Mass accuracy ranged from 1-10 ppm in MS mode (external calibration) and from 5-15 ppm in MS/MS mode (external calibration).
- Duty cycles ranged from 2.5 to 4.5s per MS scan.

C-18 HPLC CHIP

Retention Time Reproducibility (Figure 2)

- Retention times for peptide ions from both a simple BSA digest, and even a much more complex HeLa cell lysate digest, have RSD values of <1%

Figure 2. (A) Six (6) overlaid chromatograms of a tryptic digest of HeLa cell lysate collected over 3 days. The precision of the retention times for 30 selected peptide ions is tabulated. Several proteins were identified in this mixture and their identities confirmed by LC-Q-ToF MS/MS, including α -enolase, 60 kDa heat shock protein, and Histone H2A type 1-B.

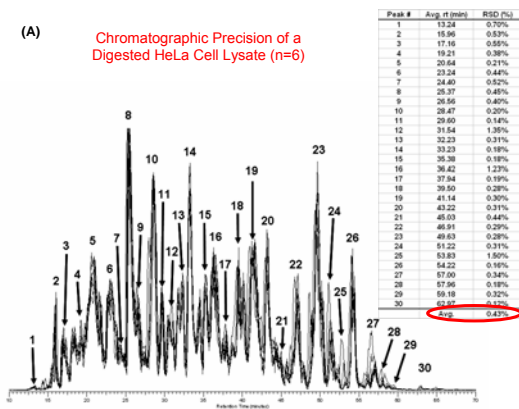
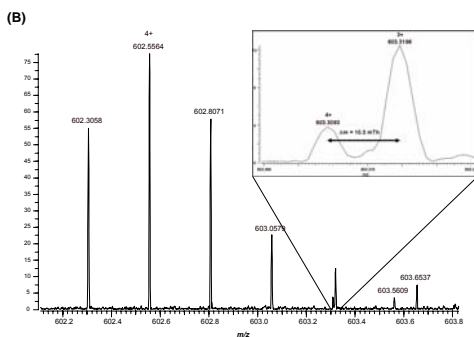


Figure 2. (B) A HPLC Chip -QFT mass spectrum from the HeLa cell lysate digest (RT = 15.56 min) showing a pair of co-eluting peptides whose isotopic envelopes overlap. The m/z difference between the overlapping portions of their envelopes is 10.3 mTh, requiring a resolving power of at least 60k to differentiate. The actual resolving power was approximately 145k.

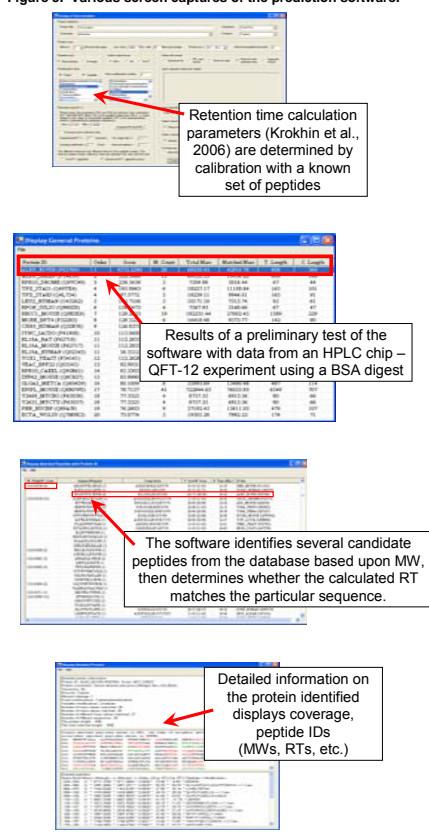


RESULTS

Combined High Precision HPLC with the Mass Accuracy of FT-ICR MS

- With mass accuracy of 1-10 ppm in MS mode (external calibration) and chromatographic precision of less than 1% RSD, we have been developing software to combine these two advantages for peptide identification via retention time prediction (Krokhin et al., 2006) with accurate mass tagging (Conrads et al., 2000).
- As stated previously by Spengler (2004), the use of very accurate mass measurements can be used to identify the amino acid composition of a peptide. However, without taking other factors into account (i.e., existence of peptides in databases, chromatographic properties of the peptide, etc.), such strategies are difficult to implement successfully.
- The software described here combines mass accuracy and retention time prediction in searches against protein databases.

Figure 3. Various screen captures of the prediction software.



ACKNOWLEDGMENTS

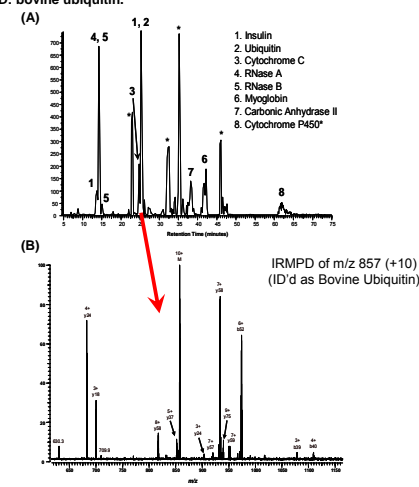
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RESULTS

“Top-Down” Sequencing – Protein Mixtures with C-8 HPLC Chip Protein Mixture Analyses

- Retention time reproducibility is also excellent with the C-8 HPLC chip.
- On-column injections of less than 50 fmol/protein yielded abundant ion signals (Figure 4A).

Figure 4. (A) Total ion chromatogram of an 8-protein mixture eluted from a C-8 HPLC chip. 50 fmol of each protein was injected on-column, with the exception of 7 and 8 (125 fmol injected, each). (B) An MSMS spectrum obtained after isolation of an ion of m/z 857 (+10 charge state) in Q2, followed by IRMPD in-cell. A database search (Mascot, SwissProt) of these fragment ions yielded the correct ID: bovine ubiquitin.



- Data-dependent acquisition (DDA) of an 8-Protein Mixture
- As indicated in Figure 4, the combination of the C-8 HPLC chip with the QFT-12 enables for DDA experiments with intact proteins in a DDA-style “top-down” experiment. (Kelleher et al., 1999)
- Smaller proteins, such as ubiquitin (8.6 kDa) (Figure 4B) have been successfully analyzed. Larger proteins may require the acquisition of several MS/MS scans, which could be afforded by manipulation of the gradient/flow rate from the C-8 HPLC chip.
- Use of ProSight PTM (Taylor et al., 2003) for interpretation of top-down experiments has yielded moderate success.

CONCLUSIONS & FUTURE WORK

- The combination of the HPLC chips and QFT-12 has provided excellent sensitivity, retention time reproducibility, mass accuracy, and resolving power.
- We have successfully demonstrated this software for identifying a simple mixture.
- We are currently refining our software to maximize its ability to integrate peptide retention time and accurate mass. Identification of peptides from more complex mixtures, such as the HeLa cell lysate digest, should then be feasible, especially if 2D LC is also performed.
- Development of a reliable source of an internal calibrant is underway.

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