High-Throughput Proteomics Platform and Software for Improved Biomarker Discovery

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Overview

- 30-min LC-IMS-MS analyses were performed on depleted human plasma spiked with 10 non-plasma peptides to characterize sensitivity, quantitation, and confidence of peptide identifications with both signal averaging and dynamic multiplexing approaches
- Non-plasma peptides were spiked into human plasma at 5 different concentration levels
- 10 technical replicates of each sample were performed in each approach, and peak intensity was plotted as a function of concentration to characterize dynamic range and sensitivity
- Key differences between dynamic multiplexing and signal averaging IMS-MS approaches are explained

Introduction

Mass spectrometry (MS)-based technologies are playing a growing role in the discovery of new candidate biomarkers for human and environmental health. However, in spite of significant advances, MS technologies remain challenged by biofluid samples such as blood plasma where protein concentrations span a dynamic range >10 orders of magnitude and inadequate measurement throughput hinders the ability to effectively account for biological diversity.

In response to this shortcoming, we are developing a new proteomics platform that combines fast LC, ion mobility separations (IMS) and MS. By spreading ions through a range of IMS drift times so they do not all arrive simultaneously at the detector, this new platform allows the detection of much lower concentration species and the ability to use shorter LC gradient times for higher-throughput analyses (**Figure 1**). Since potential candidate biomarkers are expected at low concentrations and require the examination of many samples, LC-IMS-TOF MS has enormous potential for improving biomarker discovery and verification.

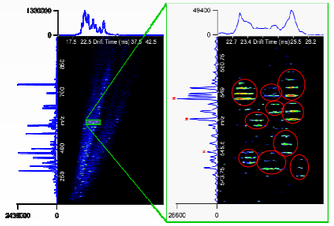


Figure 1. Selected LC elution time region from a complex sample of 20 proteins covering 10^8 dynamic range. Results show IMS-TOF MS can detect more proteins in the sample than FT MS. Ten peptides are easily detected in the zoomed region of the IMS spectra, but only 3 can be deisotoped and effectively identified by MS only (shown by *).

Methods

Experimental

- Samples: 10 non-plasma peptides spiked (at 5 levels shown in **Table 1**) into 0.4 µg/µL human blood plasma depleted of the 12 most abundant proteins
- 10 technical replicates of each sample were analyzed using LC-IMS-TOF MS (30-min gradient) with both signal averaging and dynamic multiplexing [1] approaches to determine peak intensity coefficient of variation (CV) values

Table 1. Spiking levels and non-plasma peptides spiked into human plasma

Spiking Levels (nM)	Non-plasma Peptides
1, 10, 50, 100, 1000	Fibrinopeptide A, (D-Ala-6) LHRH, Bradykinin Fragment 1-7, Renin Substrate Porcine, Diazepam Binding Inhibitor, Syntide 2, Des Pro Ala Bradykinin, Tyr C Peptide, Osteocalcin Fragment 7-19 Human, Dynorphin A Porcine Fragment 1-13

Table 2. Average peak intensity CV values (%) for all 10 non-plasma peptides using the signal averaging and dynamic multiplexing approaches

Method	Average Peak Intensity CV Value (%) at Each Concentration				
	1 nM	10 nM	50 nM	100 nM	1000 nM
Signal Averaging	17.1	15.9	13.1	8.9	5.9
Dynamic Multiplexing	15.2	12.9	9.4	6.8	4.7

Informatics

- THRASH [2] algorithm implemented in Decon2LS [3] used to deisotope data
- Deisotoped ions are grouped into features utilizing drift time, monoisotopic mass and elution time information
- VIPER used to match features to sample information for identifications

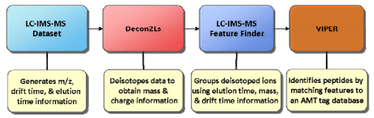


Figure 2. Data analysis flowchart

Results

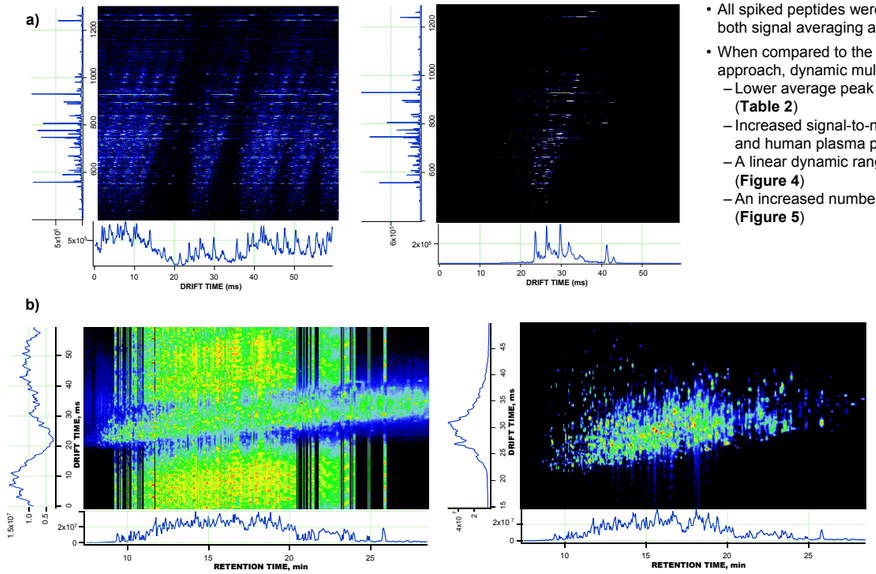


Figure 3. Dynamically multiplexed IMS-TOF analysis of the spiked human blood plasma sample for **a)** a single region of the LC run and **b)** the whole run where the encoded (left) and decoded (right) spectra are shown.

- All spiked peptides were detected at each concentration using both signal averaging and dynamic multiplexing (**Figure 3**)
- When compared to the conventional signal averaging approach, dynamic multiplexing showed:
  - Lower average peak intensity CV values at all spiking levels (**Table 2**)
  - Increased signal-to-noise ratios for low abundance spiked and human plasma peptides
  - A linear dynamic range for the concentration range examined (**Figure 4**)
  - An increased number of identified human plasma proteins (**Figure 5**)

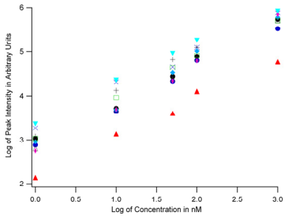


Figure 4. The concentration versus peak intensity plot for all 10 spiked peptides. All peptides had R^2 values of ~0.99 indicating the LC-IMS-TOF MS had a linear dynamic range for the concentration range studied.

LC-IMS-TOF MS Platform

High pressure automated LC system

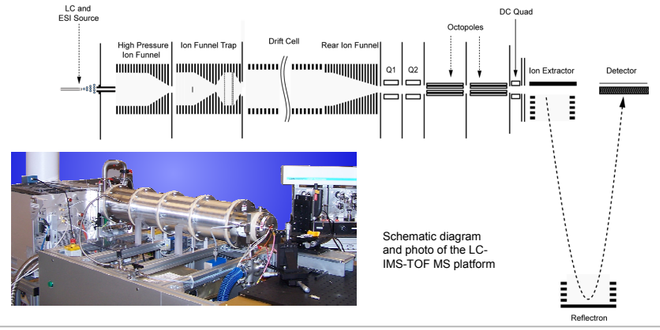
- Two 1.5 mL stainless mixers
- Four 15-cm long, 75 µm i.d. reversed-phase capillary columns packed in-house with porous 3 µm C18 bonded particles
- Each column has a chemically-etched 20-µm i.d. fused-silica emitter

Future source advances

- Near perfect efficiency nanoESI-MS SPIN interface
  J. Page, et al., *Anal. Chem.* 2008, *80:* 1800-1805
- High sensitivity droplet based microfluidics
  R. Kelly, et al., *Angew. Chem. Int. Ed.* 2009, *48:* 6832-6835

IMS-TOF MS [4-6]

- High-pressure converging hourglass ion funnel focuses and trap ions prior to ion injection
- 1-meter IMS drift cell
- Second high-pressure ion funnel focuses the diffuse ion beam post-IMS separation
- Encoding was dynamically varied between signal averaging, 4-bit, 5-bit and 6-bit pseudo-random sequences; each 60-ms long IMS frame was acquired over 3 s
- Orthogonal Agilent TOF MS provides high mass measurement accuracy after IMS separation



Schematic diagram and photo of the LC-IMS-TOF MS platform

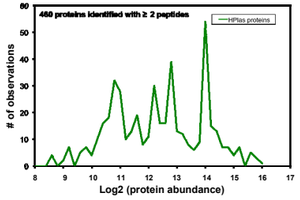


Figure 5. Protein observations at each abundance generated using DAnTE [7]. 460 human plasma proteins were identified from the multiplexed IMS-TOF data using the criteria of at least 2 peptides/protein, a mass error tolerance of ≤5 ppm, and a NET tolerance of <2%. Protein intensities have a dynamic range of 10^3.

Conclusions

- Online capillary LC-dynamically multiplexed IMS-TOF MS was evaluated with human blood plasma spiked with non-plasma peptides
- When compared to the conventional signal averaging approach, dynamic multiplexing yielded an increased number of confident human plasma peptide identifications, a higher signal-to-noise ratio for low abundance peptides, a linear dynamic range, and lower peak intensity CV values
- Future developments (in progress) anticipated to further improve IMS-MS performance include:
  - Implementation of data analysis that incorporates peptide IMS drift times for feature discrimination (more confident identification)
  - Implementation of IMS-MS RF-heating/fragmentation to provide more confident identifications
  - Extended dynamic range, high resolution, and greater mass accuracy TOF MS detection

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