On-Line Chromatography/ Dynamically Multiplexed Ion Mobility Time-of-Flight Mass Spectrometry for High Throughput Proteomics

Mikhail Belov, William Danielson, Yehia Ibrahim, David Prior, Erin Baker, Rui Zhao, Daniel López-Ferrer, Brianne Petritis, and Richard Smith
Pacific Northwest National Laboratory, Richland, WA

OVERVIEW

Ion Mobility Spectrometry–Time-of-Flight Mass Spectrometry (IMS-TOF MS) has been increasingly used in analysis of complex biological samples. A major challenge is to transform IMS-TOF MS to a high-sensitivity high-throughput platform for e.g. proteomics applications. Here we report on the development of a high-throughput high-sensitivity RPLC-IMS-TOF MS instrument, which addresses the low IMS duty cycle by using an efficient ion trapping and multiplexing with IMS. The system performance has been evaluated in experiments with complex biological samples.

INTRODUCTION

High-throughput detection of low abundance proteins from human bodily fluids has been challenged by the enormous complexity of such samples, wherein protein abundances span a dynamic range exceeding 10 orders of magnitude. A three-dimensional system incorporating reverse-phase capillary liquid chromatography (RPLC), Ion Mobility Spectrometry (IMS) and Time-of-Flight Mass Spectrometry (TOF MS) offers an increased analytical peak capacity at higher throughput and reproducibility. However, the inherently low ion utilization efficiency in the conventional IMS imposes constraints on detection of low abundance analytes in complex mixtures, particularly in conjunction with on-line RPLC.

We have earlier developed and integrated three advanced technologies, including efficient ion accumulation in the ion funnel trap prior to IMS separation [1], multiplexing of ion packet introduction into the IMS drift tube [2] and signal detection with an analog-to-digital converter (ADC) [3], into the IMS-TOF MS system.

In this work, we have combined dynamically multiplexed IMS-TOFMS [3] with on-line fast capillary RPLC and evaluated system performance in high-throughput analysis of the highly complex proteolytic digests.

METHODS

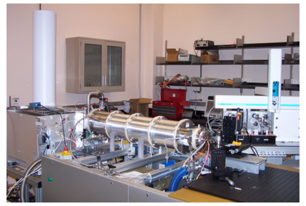


Figure 1. Photograph of the fully automated multidimensional separation system developed at PNNL. The system incorporates fast liquid chromatography (LC), ion mobility (IMS) and time-of-flight mass spectrometry (TOFMS).

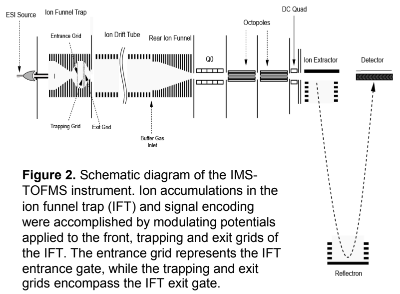


Figure 2. Schematic diagram of the IMS-TOFMS instrument. Ion accumulations in the ion funnel trap (IFT) and signal encoding were accomplished by modulating potentials applied to the front, trapping and exit grids of the IFT. The entrance grid represents the IFT entrance gate, while the trapping and exit grids encompass the IFT exit gate.

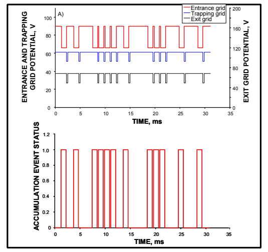


Figure 3. Timing diagrams of potentials applied to the grids of the ion funnel trap for ion accumulation and ion injection into the IMS drift tube

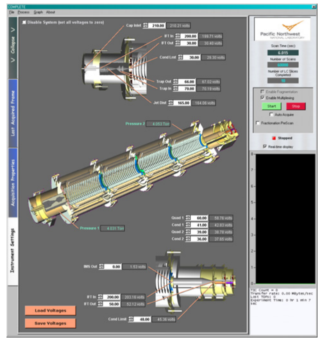
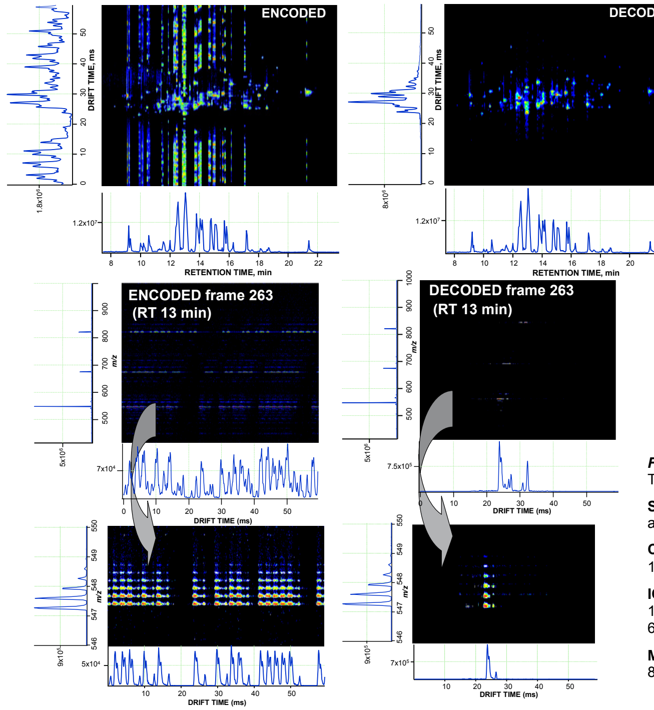


Figure 4. Instrument control software interface for adjustments of static voltages, generation of dynamic waveforms, data acquisition and visualization of spectra

- Online fast capillary LC coupled to IMS-TOFMS
- Signal encoding with IMS is performed based on total ion signal information from 100 ms prescans preceding each IMS-TOFMS acquisition and conducted in the continuous (no ion trapping) mode
- Signal reconstruction is accomplished in the batch processing mode using the inverse transform algorithm [2]

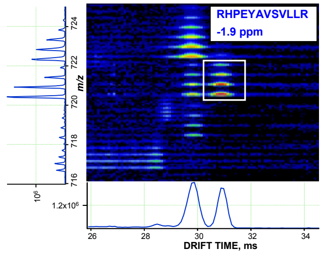
RESULTS

Tryptic Digest of Bovine Serum Albumin



PEPTIDE IDENTIFICATIONS FROM LC-MULTIPLEXED IMS-TOF ANALYSIS

PEPTIDE_SEQUENCE	ERROR,ppm	ABUNDANCE
ACFAVE	4.57	9370
AEFVEVTK	-1.33	327669
AFDEKL	2.44	21183
AFDEKLF	-0.29	134071
AFVDK	-6.22	60871
AKDAFLGSF	3.29	14986
AVSVLL	0.09	7348
AWSVAR	-1.08	8454
CCHGDLLECADDRADL	-2.15	35636
DAFLGSF	-0.12	5834
DAFLGSFL	-0.29	134071
DAFLGSFLYE	-1.43	15370
DAFLGSFLYEYSR	0.18	16651
DAIPENL	9.85	9365
DDPHACYSTVFDKL	7.5	6022
DLGEEHFK	-0.5	144511
DTHKSEIAHR	-5.5	73140
EACFAV	4.57	9370
EIARRHPYFY	0.17	200268
EYSR	1.6	9930
FKDLGEEHFK	0.35	6039237
FVEVTK	-6.82	127341
GEEHFK	-4.01	9794
GLVLIAF	-0.05	3798
GTRCCTKPESERMPCTE	-5.49	582145
HLVDEPQNLIK	-0.4	431516
HPEYAVSVLLR	-0.42	52477
HPYFYAPELL	-1.26	5071382
HPYFYAPELLYYANK	-2.29	193659
IAHRFK	-7.29	73379
ICDNQDTISSKLKE	2.73	24615
KDAIPENL	1.48	15098
KDLGEEHF	0.01	39292
KDLGEEHFK	-4.67	26752
KFWGK	0.06	667726
KQTALVELL	-0.76	360089
KQTALVELLK	-0.11	16381
KVPQVSTPTLVEVSR	-0.32	3387863
KVTKCCTESL	-7.28	50859
LCVLHE	-0.81	14158
LGEYGFQNAL	-1.21	50859
LGEYGFQNALIVR	-0.35	214370
LKHLVDEPQNL	-0.4	431516
LKHLVDEPQNLIK	-6.75	111300
LLYYANK	2.46	481462
LQQCPFDEHVKL	-5.36	1349527
LSHKDDSPDLPK	2.29	224943
LSQKFPK	-0.13	1347122
LVNELTEFAK	0.21	1421355
LVTDLTK	0.58	521965
LVVSTQTAL	-5.02	26539
LVVSTQTALA	0.22	1462243
…	…	…



mkwvtfisllllfssaysrgvfr**RDTHKSEIAHRFKDLGEEHFKGLVLIAF**sqy**LQQCPFDEHVKLVN**
ELTEFAKtcvadeshagcek**SLHTLFGDELCK**vaslretygdmadccekqepernecf**LSHKDDSPDL**
PKLKpdpntlcdefkadek**KFWGKYLYEIARRHPYFYAPELLYYANK**yngvfqeccqaedkgacllpk
ietmrekvlassarqrlrcasiqkfgeralk**AWSVARLSQKFPKAEFVEVTKLVTDLTKV**hke**CCHGD**
LLECADDRADLaky**ICDNQDTISSKLKE**ccdkpllekshciaeve**KDAIPENL**ppltadfaedkdvckn
yqe**AKDAFLGSFLYEYSRRHPEYAVSVLLR**lakeyeatleeccak**DDPHACYSTVFDKLKHLV**
DEPQNLIKqncdqfek**LGEYGFQNALIVRytrKVPQVSTPTLVEVSRSL**gk**VGTRCCTKPES**
ERMPCTEdyISLILNRLCVLHEktpv**SEKVTKCCTESLVNRRPCFSAL**tpdetyvpk**AFDEKL**
Ftfhadictlpdtekqik**KQTALVELLK**hkpkateeqlk**TVMENFVAFVDK**ccaaddk**EACFAVE**gpk**L**
VVSTQTALA

~60 % protein coverage

83 unique peptides, each observed in, at least, three consecutive TOF acquisitions

Figure 5. Analysis of a tryptic digest of Bovine Serum Albumin by dynamically multiplexed IMS-TOFMS in conjunction with on-line capillary RPLC

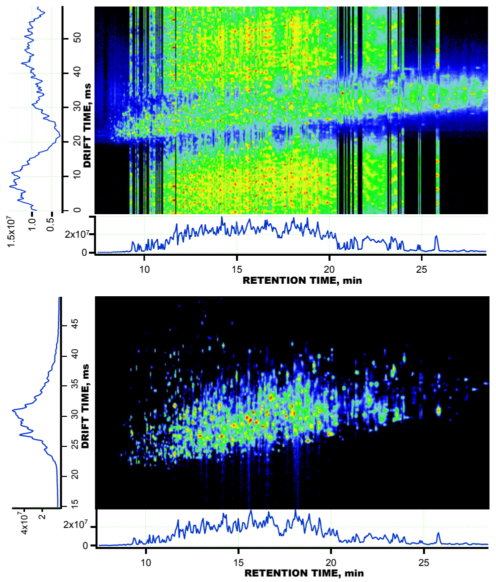
SAMPLE: tryptic digest of Bovine Serum Albumin at a concentration of 0.05 μg/μL, the total protein amount is 250 ng

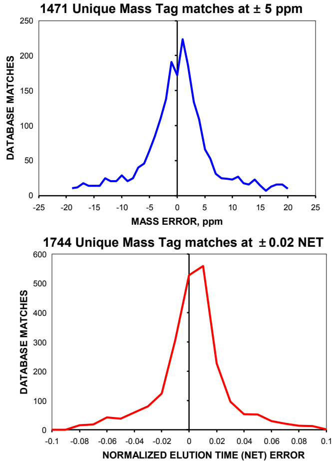
CHROMATOGRAPHY: fully automated 4-column reverse phase LC, 30 min gradient, 5000 psi, 15 cm-long, 75-μm i.d. packed with 3 μm C18 particles

ION MOBILITY: ion funnel trap followed by 88 cm-long drift tube filled with N_2 at a pressure of 4 torr; 1.8 kV drift tube bias; 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

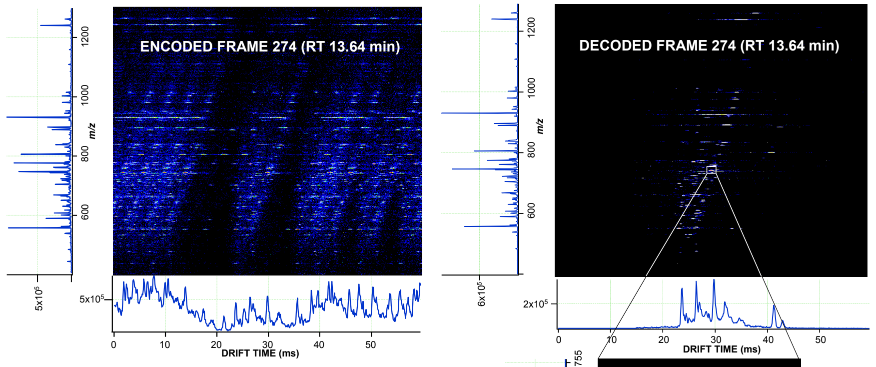
MASS SPECTROMETRY: Agilent 6220 TOFMS; mass accuracy of 2 ppm; mass resolving power of 8000

Tryptic Digest of Depleted Human Blood Plasma





1389 unique plasma peptides matched at ±5 ppm and ±0.02 NET



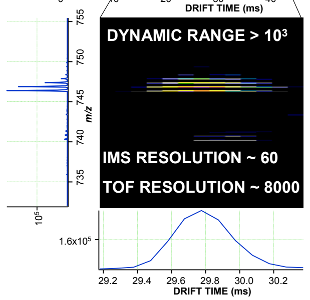


Figure 6. Online capillary LC-dynamically multiplexed IMS-TOF analysis of depleted human blood plasma

SAMPLE: 0.4 μg/μL human blood plasma depleted of the 12 most abundant proteins, the total protein amount is 2 μg.
Immunodepletion: GenWay Pre-packed Seppro mixed IgY12 LC10 Flow-Through
Concentration: Amicon 15 mL/5K MWCO
Digestion**:** 8M urea, 10 mM DTT, 40 mM iodoacetamide, 1:50 porcine trypsin:protein (w/w)
Cleanup**:** Discovery C18 (1 mL/100 mg)

CHROMATOGRAPHY, ION MOBILITY, MASS SPECTROMETRY: same as in Figure 5

CONCLUSIONS

1. Dynamically multiplexed IMS-TOFMS has been coupled to an on-line capillary RPLC
2. Challenges in reconstruction of signals from an ion source with varying ion production rates (e.g., LC) have been successfully addressed
3. Signal-to-noise ratios of low abundance peptides were found to increase proportionally to the square root of the number of ion packets injected into the drift tube on the time scale of a single IMS separation
4. Online capillary LC-dynamically multiplexed IMS-TOF MS has been evaluated in experiments with complex proteolytic digests (e.g., human blood plasma) and was found to yield an increased number of confident peptide identifications as compared to the conventional signal averaging approach

ACKNOWLEDGEMENTS

Portions of this research were supported by the NIH National Center for Research Resources (RR18522), National Cancer Institute (5R33CA126191 A) and the U.S. Department of Energy Biological and Environmental Research (DOE/BER).
Significant portions of the work were performed in the Environmental Molecular Science Laboratory, a DOE/BER national scientific user facility at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is operated for the DOE by Battelle under contract DE-AC05-76RLO-1830.

REFERENCES

1. Clowers, B.H.; Ibrahim, Y.M.; Prior, D.C.; Danielson, W.F.; Belov, M.E.; Smith, R.D. *Enhanced ion utilization efficiency using an electrodynamic ion funnel trap as an injection mechanism for ion mobility spectrometry.* Anal. Chem., 2008. **80**(3): p. 612-623.
2. Belov, M.E.; Buschbach, M.A.; Prior, D.C.; Tang, K.Q.; Smith, R.D. *Multiplexed ion mobility spectrometry-orthogonal time-of-flight mass spectrometry*. Anal. Chem., 2007. **79**(6): p. 2451-2462
3. Belov, M.E.; Clowers, B.H.; Prior, D.C.; Danielson, W.F.; Liyu, A.V.; Petritis, B.O.; Smith, R.D. *Dynamically multiplexed ion mobility time-of-flight mass spectrometry*. Anal. Chem., 2008. **80**(15): p. 5873-5883.

CONTACT: Mikhail Belov, Ph.D.
Biological Sciences Division, K8-98
Pacific Northwest National Laboratory
P.O. Box 999, Richland, WA 99352
E-mail: mikhail.belov@pnl.gov