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

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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, whereas protein abundances span a dynamic range exceeding 10 orders of magnitude. A three-dimensional system incorporating reverse-phase capillary liquid chromatography (RPLC), time-of-flight mass spectrometry (MS) and Ion Mobility Spectrometry (IMS) and Time-of-Flight Mass Spectrometry (TOF MS) offers an increased analytical peak capacity at higher throughput and reproduction. 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 capillary RPLC.

We have earlier developed and integrated three advanced technologies, including efficient ion accumulation in the ion funnel stage prior to IMS separation [5], multiplexing of ion packed introduction into the IMS drift tube [2] and signal detection with an analog-to-digital converter (ADC) [5], into the IMS-TOF MS system.

In this work, we have combined dynamically multiplexed IMS-TOF MS [5] with on-line fast capillary RPLC and evaluated system performance in high-throughput analysis of the highly complex proteolytic digests.

METHODS

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 peptide ions (e.g. 25 ppm) increased 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 National Cancer Institute at the National Institutes of Health under contract DE-AC05-76RLO-1830.

REFERENCES

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