Development of a Multi-Channel Ion Mobility-Mass Spectrometer for High-Throughput Interrogation of Cellular Response

Jody C. May[1,3,4], Sevugarajan Sundarapandian[1,3,4], Jeffrey R. Enders[1,3,4], Kevin T. Seale[2,3], John P. Wikswo[2,3] and John A. McLean[1,3,4]

***Short Abstract* — An 8-channel (octuplexed) ion mobility-mass spectrometer is being developed to meet the demands for highly sensitive, fast temporal response analysis of cellular secretions. Parallel analysis approaches allow for a number of novel experimental methods including sample partitioning (throughput and temporal resolution), interleaved control experiments (validation) and real-time calibration runs (measurement accuracy). The 8-channel instrument will incorporate a completely parallel, dispersive multidimensional (size and mass) analysis and is designed for complete 2D spectral acquisitions in under *ca.* 1 min with near real time response. The challenges, both technical and conceptual, will be presented in light of current progress.**

***Keywords* — Multiplexed Ion Mobility-Mass Spectrometer, High-Throughput, Secretome, Cellular Response.**

I. Introduction and Purpose

The cellular secretion profile is highly complex both in terms of molecular diversity and the high dynamic range of concentrations represented. Mass spectrometry (MS) has been utilized successfully as a high-throughput (<1 min) analysis for secretion analytes, however, significant sample cleanup (extraction and separation) is required prior to MS [1,2]. Ion mobility (IM) is a complementary technology to MS that provides a rapid (μs-ms) post-ionization separation on the basis of analyte size. IM combined with MS (IM-MS) improves the MS analysis by partitioning signal from noise and providing chemical class-specific correlation information for analyte identification purposes [3,4].

Both partially multiplexed LC-MS and fully multiplexed MS have been developed [7-9]. Here we describe a completely multiplexed IM-MS instrument where 8 separate IM and MS analysis stages are run in parallel under a common vacuum system and electronics. A number of methods can be utilized with this octuplexed IM-MS, including (i) 8 identical experiments summed to improve sensitivity (ion counts/second) (ii) interleaved experiments with standards/controls, and (iii) 8 separate experiments, such as fraction analysis or offset temporal response from a cellular excretion.

Acknowledgements: This work is funded by a pilot project grant from the Vanderbilt Institute of Chemical Biology (VICB), Vanderbilt Institute for Integrative Biosystems Research and Education (VIIBRE), the Defense Threat Reduction Agency (HDTRA-09-1-0013), and the NIH National Institute on Drug Abuse (RC2DA028981).

[1]Department of Chemistry, Vanderbilt University, Nashville, TN.
E-mail: jody.c.may@vanderbilt.edu
[2]Department of Biomedical Engineering, Vanderbilt University.
[3]Vanderbilt Institute for Integrative Biosystems Research and Education.
[4]Vanderbilt Institute for Chemical Biology.

II. Description of Instrumentation and Methods

The 8-channel (8x) IM-MS instrument is being developed in modules so that each component can be tested and validated before assembly. Each module is designed *in silico* (CAD) and undergoes rigorous computer modeling for gas flow (COMSOL) and ion trajectory (SIMION) dynamics prior to fabrication. This is a critical strategy for developing any complex instrument platform. The instrument modules are (i) 8x electrospray source (ii) 8x ion funnel for ion focusing and temporal modulation (iii) 8x IM spectrometer (iv) 8x converging ion funnel array, (v) ion focusing/shaping optics, (vi) orthogonal time-of-flight MS with 8 discrete ion beams, and (vii) an 8-anode time-to-digital ion detector. An 8x Faraday ion detector is also utilized for testing purposes.

III. Conclusion

Theoretical design and simulation considerations are presented. Currently, modules (i), (ii) and (iii) have been designed and constructed and the custom vacuum system is being fabricated for initial testing.

References

[1] Kalkum M, Lyon GJ, Chait BT (2003) Detection of Secreted Peptides by Using Hypothesis Driven Multistage MS *PNAS* **100**, 2795-2800.
[2] Xiao Z, et al. (2008) Analysis of the Extracellular Matrix and Secreted Vesicle Proteomes by MS, In *Methods in Molecular Biology* vol. 428 (Vlahou A, Ed) Humana Press, Totowa, NJ pp. 231-244.
[3] McLean JA, et al. (2005) Ion Mobility-Mass Spectrometry: A New Paradigm for Proteomics, *Int. J. Mass Spectrom.* **240**, 301-315.
[4] McLean JA (2009) The Mass-Mobility Correlation Redux: The Conformational Landscape of Anhydrous Biomolecules, *J. Amer. Soc. Mass Spectrom.* **20**, 1775-1781.
[5] Zeng L, Kassel DB (1998) Developments of a Parallel HPLC/Mass Spectrometry System for the Analytical Characterization of Combinatorial Libraries. *Anal. Chem.* **70**, 4380-4388.
[6] Feng B, et al. (2001) Integrated Ten-Pump, Eight-Channel Parallel LC/MS System for High-Throughput Analysis of Proteins. *Anal. Chem.* **73**, 5691-5697.
[7] Tabert AM, et al. (2006) Multiplexed Rectilinear Ion Trap MS for High-Throughput Analysis, *Anal. Chem.* **78**, 4830-4838.