Biomedical "X-Omics" Research

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Introduction

The research activities in our group focus on analytical and biomedical areas. We aim to develop modern multidimensional mass spectrometry (MS) and separation technologies/techniques for analysis of complex sample mixtures.
Our laboratory is equipped with a 9.4 tesla superconducting/shielded magnet which is shared with two Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometers. These instruments are interfaced with separation techniques {*i.e.*, gas chromatograph (GC) and nano high performance liquid chromatograph (nano-HPLC)} for analysis of small and macromolecules. We have access to different ionization sources such as electron impact ionization (EI), chemical ionization (CI), electrospray ionization (ESI), and the newly developed radio-frequency ionization (RFI) as well as various ion fragmentation techniques such as collision induced dissociation (CID) and electron capture dissociation (ECD). Additionally we recently acquired a Waters Synapt G2-S Ion Mobility (IM) Time-of-Flight (TOF) MS equipped with a nano-ESI source and a nano-HPLC system. Specific research areas in our group include:

1) Gas-phase ion-molecule reactions and thermochemical studies (*e.g.*, hydrogen/deuterium exchange (HDX), proton affinity (PA) measurements, and multidimensional approaches)

2) Conformational analysis of proteins/peptides using HDX and IM-MS

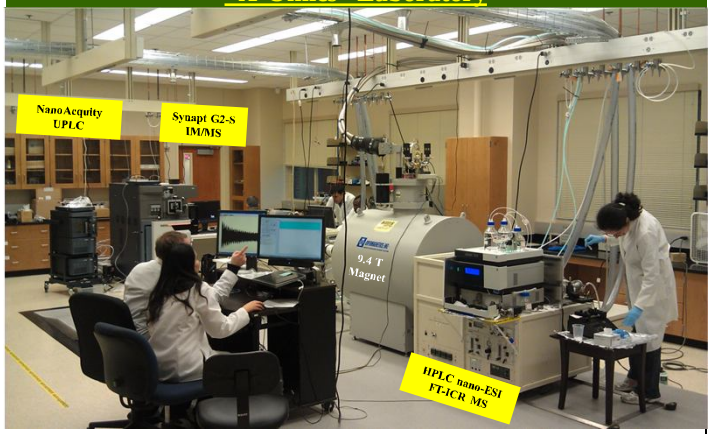
3) Cancer biomarker discovery through non-invasive biological fluid samplings such as human breath and saliva

4) "Metabolomics" and "proteomics" studies including stress/placebo biomarker discovery in human saliva

5) "Peptidomics" and mechanistic understanding of peptide fragmentation (using HDX and IM-MS)

6) Instrument development

"X-Omics" Laboratory



Novel Ionization Technique: Radio Frequency Ionization (RFI)

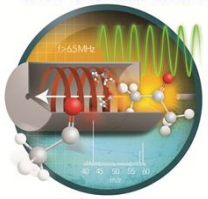


Figure 1. Radio frequency ionization (RFI) is a novel ionization technique that we recently introduced for mass spectrometry analysis of volatile and semi-volatile organic molecules. In RFI, a radio-frequency signal is used to ionize neutral organic molecules in the ultrahigh-vacuum region of Fourier-transform ion cyclotron resonance mass spectrometers (FT-ICR MS) .
Reference: Zekavat, B.; Solouki, T., *Angew. Chem. Int. Ed.*, **2013**, 52, 2426 –2429 (Highlighted as COVER PAGE!).

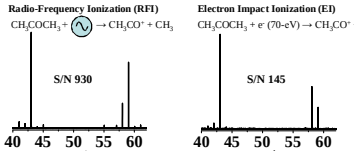


Figure 2. **(Left)** RFI/FT-ICR and **(right)** electron impact ionization (EI)/FT-ICR mass spectra of acetone. The ionization sensitivity (IS) of RFI is higher than the IS of conventionally used 70 eV EI for the analysis of volatile organic molecules (VOCs). For example, RFI yielded signal/noise (S/N) ratios roughly six times higher than those generated by EI (70 eV) for ionization of acetone.

We are exploring the application of RFI for MS fingerprinting of complex VOC mixtures in metabolomics (*e.g.*, enhanced characterization of human breath for disease biomarker discovery).

Disease Biomarker Discovery

Metabolomics: Human Breath Analysis

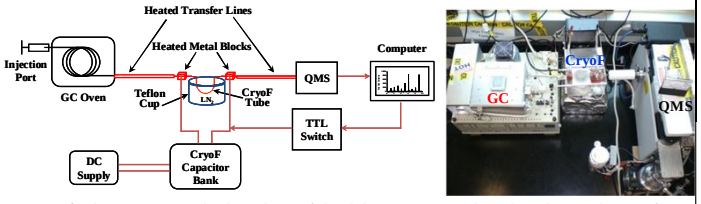


Figure 3. **(Left)** Schematic representation and **(right)** actual picture of a home-built GC/CryoF/QMS setup showing the gas chromatograph (GC), cryofocuser (CryoF), and quadrupole mass spectrometer (QMS). The GC separated analytes are pre-concentrated inside the cryofocuser tube before final injection into the MS ion source. Resistive heating, using capacitive discharging, is utilized for analyte desorption.

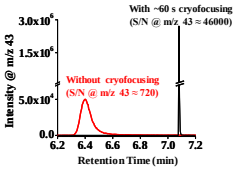


Figure 4. Selected ion chromatogram (SIC) (at m/z 43) GC/QMS obtained from the injection of ~3 nmoles of acetone without (red line) and with (black line) cryofocusing. An enhancement of ~64 folds in signal-to-noise ratio (S/N) was obtained using a post-column cryofocuser. Hence, conventional detection limits can be enhanced significantly using GC/CryoF/QMS.

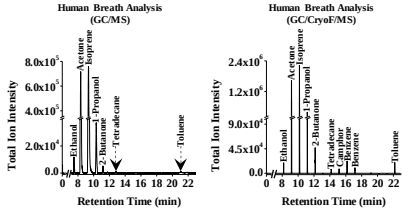
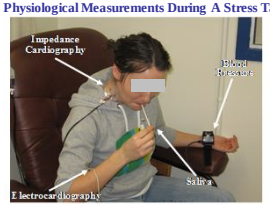


Figure 5. Total ion chromatograms (TICs) obtained from the analysis of a human breath sample using **(left)** GC/MS and **(right)** GC/GryoF/MS.

Proteomics: Stress/Placebo Biomarker Discover

Physiological Measurements During A Stress Task/Test



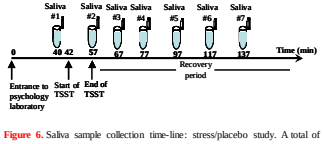


Figure 6. Saliva sample collection time-line: stress/placebo study. A total of seven saliva samples were collected during the ~140 min stress test.

Correlating the Physiological Measurements to Protein/Peptide Profiles

LC/FT-ICR MS

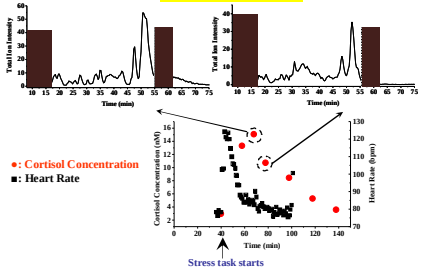


Figure 7. **(top)** Liquid chromatography/Fourier transform-ion cyclotron resonance mass spectrometry (LC/FT-ICR MS) chromatograms of samples #3 and 4 for a "healthy" human subject. **(bottom)** Plots of cortisol concentration (red circles) and heart rate (black rectangles) as a function of time before, during, and after the stress task/test. Physiological measurements (*e.g.*, heart rate) during the stress task/test can be correlated to the protein profile variations and cortisol concentrations (a previously established stress biomarker) for biomarker discovery (as single markers or panels of markers for diagnostic and prognostic purposes).

Conformational Analysis and Bioinformatics

Peptidomics: Peptide Fragmentation Mechanisms

Figure 8. Commonly Observed Fragment Ions in MS and MS[a] (a "Roepstorff-type" nomenclature for a hexapeptide).

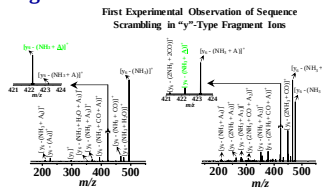
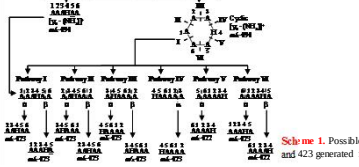
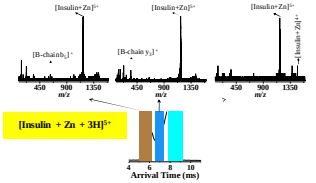


Figure 9. Collision-induced dissociation (CID) mass spectra of the *m/z*-isolated **(left)** $[y_6]^+$ (*m/z* 511) (generated from in-source fragmentation of AAAAHAA-NH_2 where "A" denotes a carbon thirteen (^{13}C) isotope on the alanine side chain) and **(right)** $[y_6 - (NH_3)]^+$ (*m/z* 494) (generated from CID of $[y_6]^+$). Insets show the expanded views of *m/z* range of 421 to 424 corresponding to the sequence scrambled $[y_6 - (NH_3 + \Delta)]^+$ fragment ion.
Reference: Miladi, M; Harper, B.; Solouki, T., *J. Am. Soc. Mass Spectrom.*, **2013,** under review.



Scheme 1. Possible pathways for the formation of fragment ions at *m/z* 422 and 423 generated from the CID of $[y_6]^+$ from AAAAHAA-NH_2.

Ion Mobility-Mass Spectrometry: Insulin versus Lispro



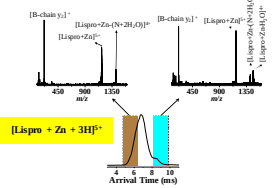


Figure 10. Post-IM/CID MS profiles of zinc-complexed species of **(left)** insulin ($[Insulin + Zn + 3H]^{5+}$) and **(right)** Lispro ($[Lispro + Zn + 3H]^{5+}$). Lispro is an insulin analogue with Pro28 → Lys28 and Lys29 → Pro29 substitutions in its B-chain. Insets represent the combined CID mass spectra for the shaded areas. Our preliminary Post-IM/CID results suggest that $[Insulin + Zn + 3H]^{5+}$ and $[Lispro + Zn + 3H]^{5+}$ have at least three and two gas-phase conformers, respectively. These observations suggest that the amino acid inversion causes less conformational variations in the Zn-complexed species of Lispro as compared to Insulin.

Chemometric Deconvolution of Overlapped Ion Mobility Profiles

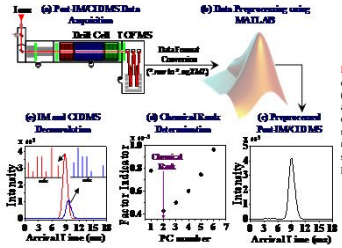


Figure 11. A chemometric data analysis approach for deconvolution of overlapped IM profiles. (a) Post-ion mobility collision induced dissociation (Post-IM/CID) is performed on a Waters Synapt IM-MS system. (b) *.raw* data are converted to *.mzXML* format and processed in MATLAB using an in-house developed data processing platform. (c) The processed data reveal mobility unresolved species. (d) Chemical rank is used to determine the number of mobility unresolved species. (e) The overlapped IM profiles and CID mass spectra are deconvoluted using a self-modeling mixture analysis technique.
References: (i) Zekavat, B.; Solouki, T., *J. Am. Soc. Mass Spectrom.*, **2012**, 23, 1873-1884.
(ii) Zekavat, B.; Miladi, M., Becker, C.; Munisamy, S. M.; Solouki, T., *J. Am. Soc. Mass Spectrom.*, **2013** accepted for publication.

Acknowledgements

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