A High Resolution Atmospheric Pressure MALDI-Quadrupole-Orbitrap Platform Enables *In Situ* Analysis of Biomolecules by Multi-Mode Ionization and Acquisition

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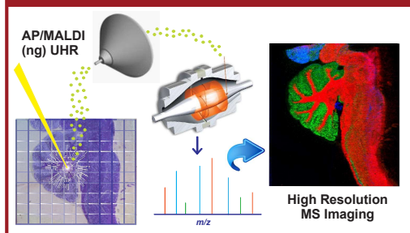
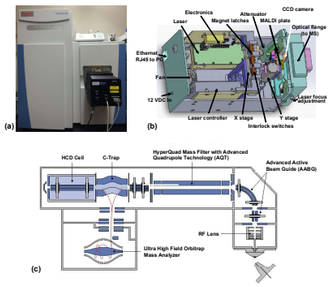
Overview



- Multiple types of ionization techniques, including MALDI, laserspray ionization and matrix assisted ionization have been achieved on the AP/MALDI-Q-Orbitrap platform to extend detection mass range and improve fragmentation efficiency.
- High resolution mass spectrometry imaging has been performed in mass (240k mass resolution at *m/z* 200) and space (< 10 µm spatial resolution).
- Multiple acquisitions modes, including high resolution accurate mass (HRAM) full MS, targeted MS/MS, data dependent acquisition (DDA) and parallel reaction monitoring (PRM), allow in depth deciphering of complex biological samples.

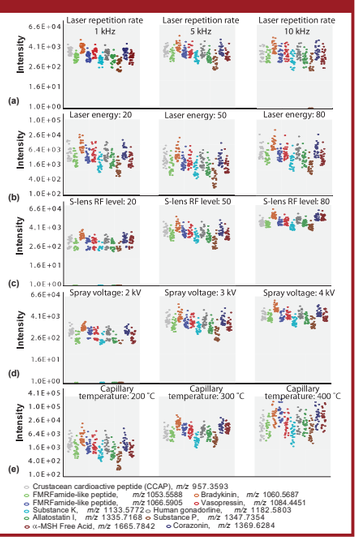
Introduction



- **AP/MALDI: an alternative to vacuum MALDI**
  - Interchangeable with ESI source
  - Ease of sample introduction and handling at AP condition
  - Capable of analyzing volatile molecules
  - Optimized source geometry design and ion transfer efficiency
- **AP/MALDI (ng) UHR source**
  - Developed by MassTech Inc. (Columbia, MD)
  - Equipped with a solid state 355 nm Nd:YAG laser
  - Output frequency up to 10 kHz
- **Multiple types of ionization**
  - MALDI & novel ionization techniques for multiply charged ions
- **High resolution MS imaging**
  - In mass: 240 K mass resolution at *m/z* 200
  - In space: < 10 µm spatial resolution

Method & Optimization

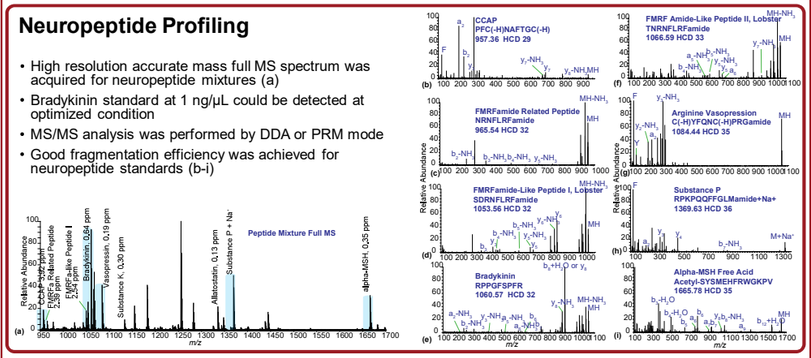
- **Matrix solutions**
  - α-cyano-4-hydroxycinnamic acid (CHCA): 10 mg/mL in ACN:EtOH:H_2O:FA (v/v/v/v) 84:13:2.997:0.003
  - 2,5-dihydroxybenzoic acid (DHB): 150 mg/mL in MeOH:H_2O:FA (v/v/v) 49.95:49.95:0.1
  - 2-nitrophloroglucinol (2-NPG): 12.5 mg/mL in ACN:H_2O:FA (v/v/v) 69.93:29.97:0.1
  - 3-nitrobenzonitrile (3-NBN): 50 mg/mL in ACN:H_2O:FA (v/v/v) 62.46:37.46:0.08
- **Neuropeptide and protein standards**
  - Serial dilutions of bradykinin, neuropeptide Y and insulin standards (10 µM, 1 µM, 100 nM and 10 nM) were dissolved and diluted in ACN:H_2O:FA (v/v/v) 49.95:49.95:0.1 solution
  - Master mix was prepared by mixing neuropeptide standards at 0.87 µg/mL for parameter optimization
- **Animal experiments**
  - Animal experiments were performed following institutional guidelines (UW-Madison, IACUC)
  - Tissue neuropeptides were extracted with chilled acidified methanol (MeOH: H_2O:AA (v/v/v) 90:9:1)
  - Tissue sections were prepared by embedding tissues in gelatin solution, snap freezing, cryosectioning into 12 µm slices and thaw mounting onto glass slides. Matrix was applied by TM Sprayer for tissue imaging.
- **Parameter optimizations**
  - Source and instrument parameters were carefully optimized using neuropeptide master mix
  - Laser rate (1, 5, 10 kHz), laser energy (20, 50, 80%), S-lens RF level (20, 50, 80), plate voltage (2, 3, 4 kV) and capillary temperature (200, 300, 400 °C) were tested.
  - Results were summarized on the right panel



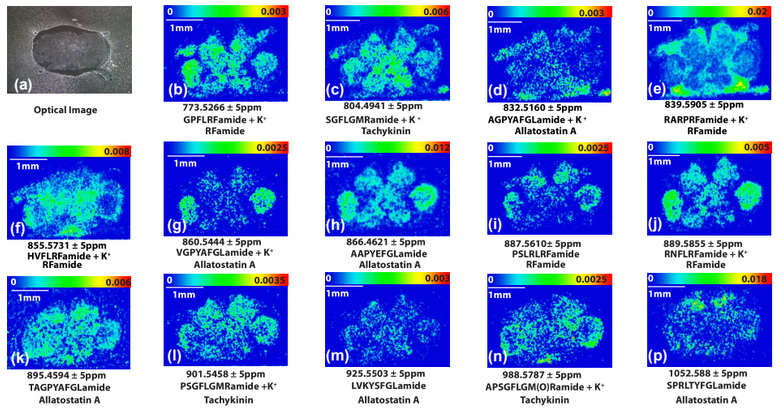
High Resolution MS Profiling & Imaging

Neuropeptide Profiling

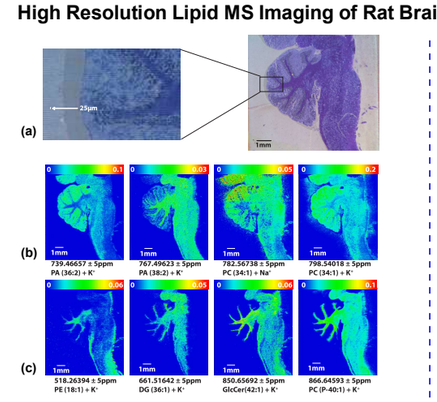
- High resolution accurate mass full MS spectrum was acquired for neuropeptide mixtures (a)
- Bradykinin standard at 1 ng/µL could be detected at optimized condition
- MS/MS analysis was performed by DDA or PRM mode
- Good fragmentation efficiency was achieved for neuropeptide standards (b-i)



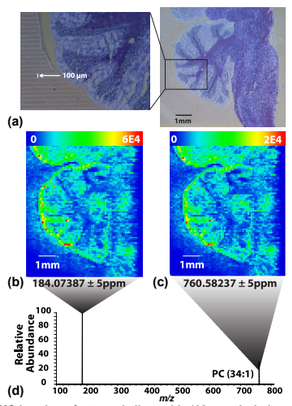
Neuropeptide MS Imaging of Crustacean Neuronal Tissues



High Resolution Lipid MS Imaging of Rat Brain



High resolution MSI of rat cerebellum section with 25 µm pixel size. (a) Microscopic optical image of cresyl violet stained rat cerebellum after MSI acquisition. The laser burnt mark could be observed on the zoomed in image. The distance between each line is 25 µm. (b) Selected ion images that have distributions all around the tissue. (c) Selected ion images that have distribution only in the center of cerebellum. Identifications were tentatively assigned by database search with a mass tolerance of 10 ppm in METLIN. All MSIs were normalized to TIC and presented without smoothing

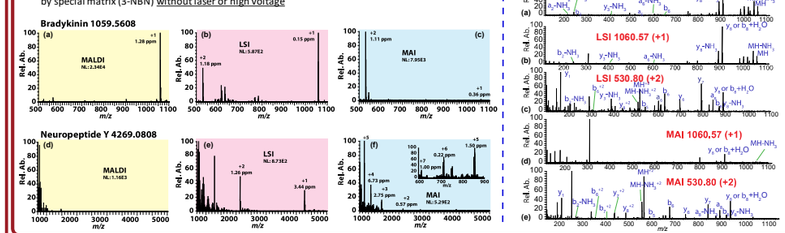


MS/MS imaging of rat cerebellum with 100 µm pixel size. (a) Microscopic optical image of cresyl violet stained rat cerebellum after MSI acquisition. The laser burnt mark could be observed on the zoomed in optical image on the left. The distance between each line is 100 µm. (b) Fragment ion image at *m/z* 184.07. (c) Parent ion image at *m/z* 760.58, which was identified to be phosphatidylcholine (34:1). (d) MS/MS spectrum of *m/z* 760.58 with NCE 18 and mass window of 3 *m/z*.

Novel Ionization Techniques

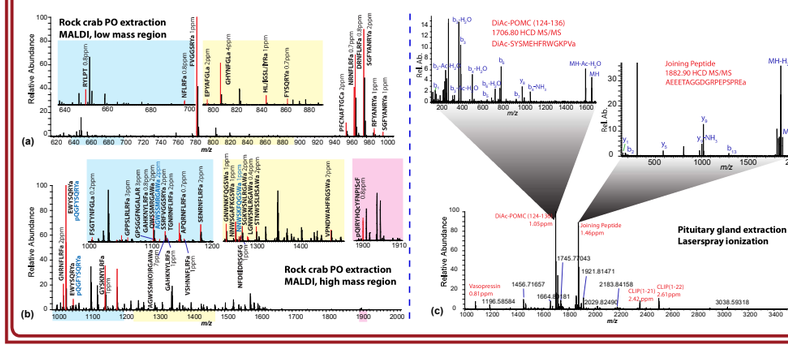
Comparisons of MALDI, Laserspray Ionization and Matrix Assisted Ionization

- **Laserspray ionization (LSI)** - produces multiply charged ions in MALDI source by laser ablation and special matrix (2-NPG)
- **Matrix assisted ionization (MAI)** - produces ESI-like multiply charged ions in MALDI or ESI source by special matrix (3-NBN) without laser or high voltage



Laserspray Ionization and MALDI Analyses of Tissue Extract

- Neuropeptide extraction of Rock crab pericardial organ (PO)
- Ionization technique: MALDI
- Neuropeptide extraction of rat pituitary gland
- Ionization technique: laserspray ionization



Conclusion & Future Directions

- AP/MALDI (ng) UHR source, developed by MassTech Inc., is a high resolution atmospheric pressure MALDI source, which is interchangeable with ESI source and has a laser spot of less than 10 µm.
- In this study, this source was coupled to QE HF to achieve high resolution analysis in both space and mass.
- Multiple ionization modes, including MALDI, LSI and MAI have been successfully performed in this platform without instrument modification. By generating multiply charged ions under LSI and MAI conditions, the fragmentation efficiencies have been improved and the detection range of Orbitrap has been expanded.
- Full MS, targeted MS/MS, DDA and PRM acquisitions were achieved on standard neuropeptide mixtures and tissue sections. Due to the high mass accuracy of Orbitrap, biomolecules were identified based on accurate mass matching with extremely low ppm error. Identities of selected ions were also verified by MS/MS analysis. High resolution full MS and MS/MS images were obtained on crustacean and rat tissues.
- Overall, the AP/MALDI-Q-Orbitrap is a powerful instrument platform that is capable of multi-ionization and multi-acquisitions for various samples. It could be an attractive alternative to other high resolution MALDI instruments.

Acknowledgements

The authors would like to acknowledge MassTech Inc. for providing the AP-MALDI (ng) UHR source, especially Dr. Vladimir M. Doroshenko, Dr. Victor V. Laiko and Dr. Eugene Moskovets for their generous technical supports. The authors would also like to thank Dr. Robert Thorne's laboratory at University of Wisconsin-Madison for providing rat tissue samples. This work was supported in part by NIH R56MH110215. The MALDI-Orbitrap was purchased through an NIH shared instrument grant (NCRR S10RR029531). B. Chen acknowledges a 2017 ASMS Graduate Student Award.