AP MALDI-Quadrupole-Orbitrap MS Platform for High Spatial and High Mass Spectral Resolution In Situ Analysis of Biomolecules

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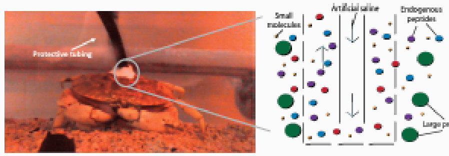
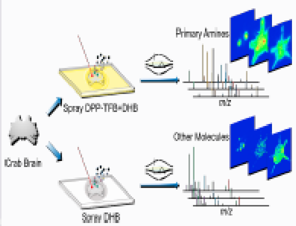
MassTech Inc. Breakfast Seminar, ASMS 2019
Atlanta, GA, USA, June 3, 2019

Li Research Group

Crustacean Neuropeptidomic Studies



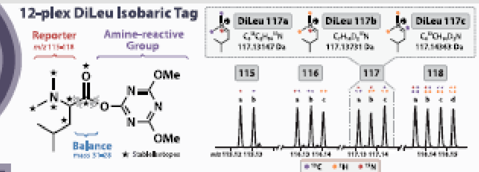


Neuropeptide changes induced by food intake, environmental stress, etc. in crustacean models are profiled and compared in this project. Microdialysis is performed to monitor neuropeptide secretion in live animals.

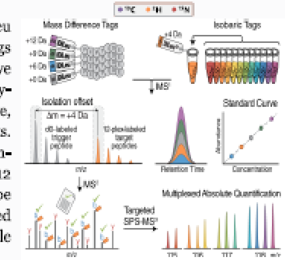
Research Focus

Development of Mass Spectrometry–Based Tools for Neuroscience and Bioanalytical Research

Quantitative MS Strategies



We created DiLeu isobaric and isotopic tags as a cost-effective alternative to widely-used, but expensive, commercial reagents. Paired with high-resolution MS, up to 12 biological samples can be simultaneously identified and quantified in a single experiment.



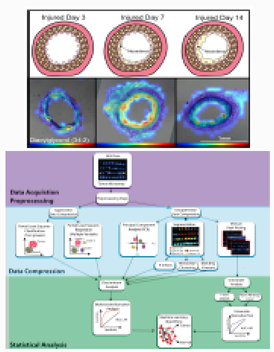
Group Accomplishments

Graduate students in the lab have received more than 130 awards, including multiple training grants (BTP, CBIT, CVRC, CNTP, METP), NSF and NIH fellowships. Our lab members have given more than 330 research presentations at national and international meetings. We have published more than 260 peer-reviewed research journal articles and successfully graduated 40 PhDs.

Our research has been supported by NIH, NSF, DOD, Sloan Research Foundation, American Society for Mass Spectrometry, UW-Madison School of Pharmacy, UW Carbone Cancer Center, UW Institute for Clinical and Translational Research, Wisconsin Alzheimer's Disease Research Center and Wisconsin Alumni Research Foundation.

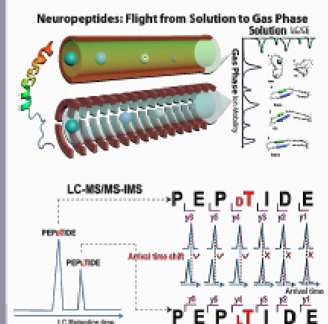
Mass Spectrometry Imaging

Mass spectrometric imaging enables scientists to obtain 2D and 3D maps of biological analytes in their native tissues without the need for antibodies. The Li group implements this technology to examine distribution patterns of neuropeptides, proteins, lipids, and metabolites in a variety of tissues.



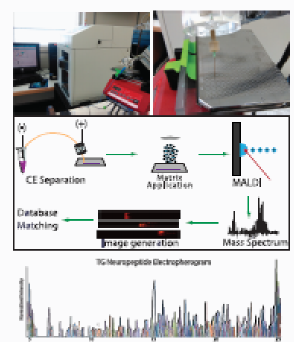
Ion Mobility-MS

IM-MS separates gas-phase ions by mass, charge, size, and shape through collisions with inert gas molecules. The Li group is using it to develop novel methods for glycan separation, peptide identification, and structural analyses.



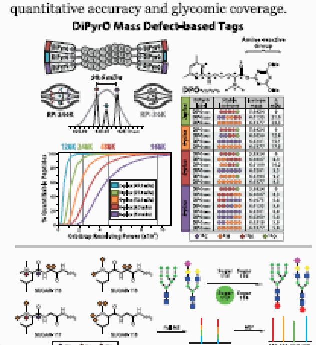
Capillary Electrophoresis MS

Capillary zone electrophoresis and capillary isoelectric focusing are being developed to couple with MS detection and MS imaging, both off-line and on-line, for enhanced neuropeptidomic and glycomic analysis.



Glycan Analysis

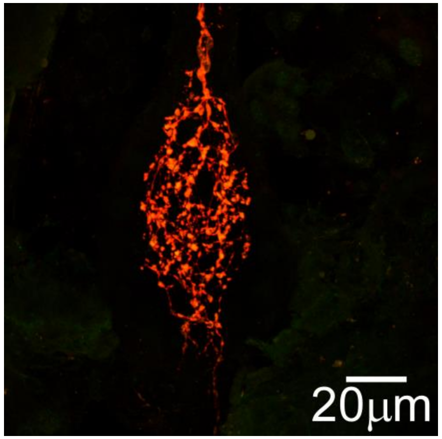
In conjunction with CE, LC, or IM-MS separation, our group investigates glycan structure and quantitation using commercial (e.g. aminoxyTMT) and novel labeling strategies. MultiNotch MS³ has been applied to improve quantitative accuracy and glycomic coverage.



Instrumentation

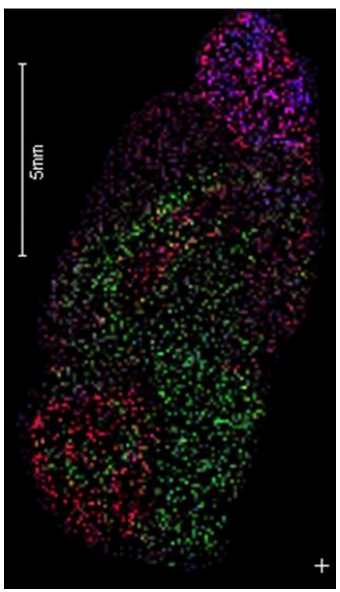
Thermo Q Exactive | Thermo Orbitrap Elite | Bruker UltrafleXtreme MALDI-TOF-TOF | Thermo Dionex LC | Thermo MALDI Orbitrap | Thermo Fusion Lumos | Waters Synapt G2 Q-TOF | Waters Acquity LC | Thermo Q Exactive HF | Bruker MaXis 4G Q-TOF | Bruker rapifleX MALDI-TOF-TOF

Molecular Imaging Strategies



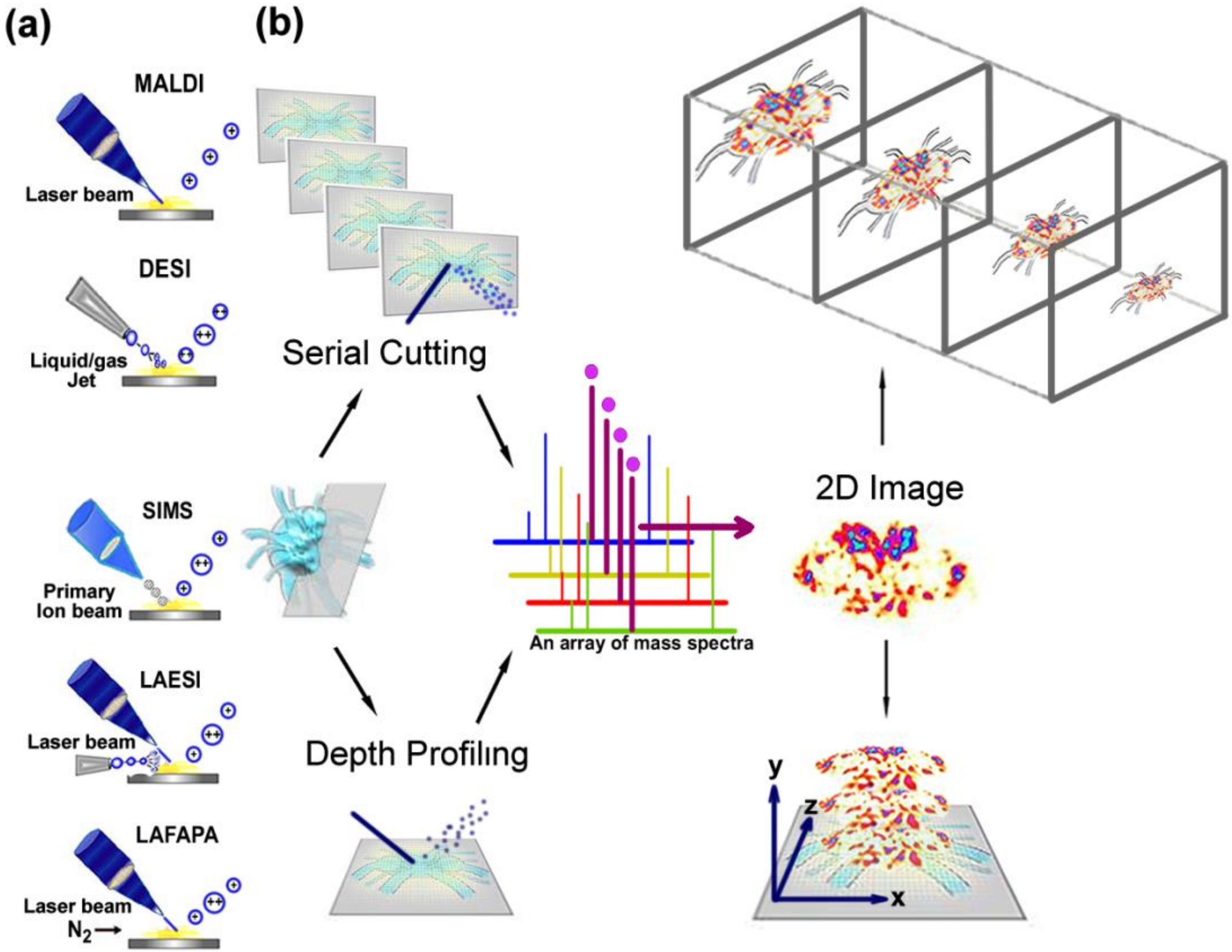
MS imaging advantages

- No labeling required
  - ✓ Biomolecules are functionally unmodified
- Image biomolecular modifications
  - ✓ PTM's
  - ✓ Metabolites
- Detailed information on molecular identity
- Large scope of different elements and molecules, discovery of unknowns
- Extend histopathology to a molecular level
- Multiplexing and highly parallel



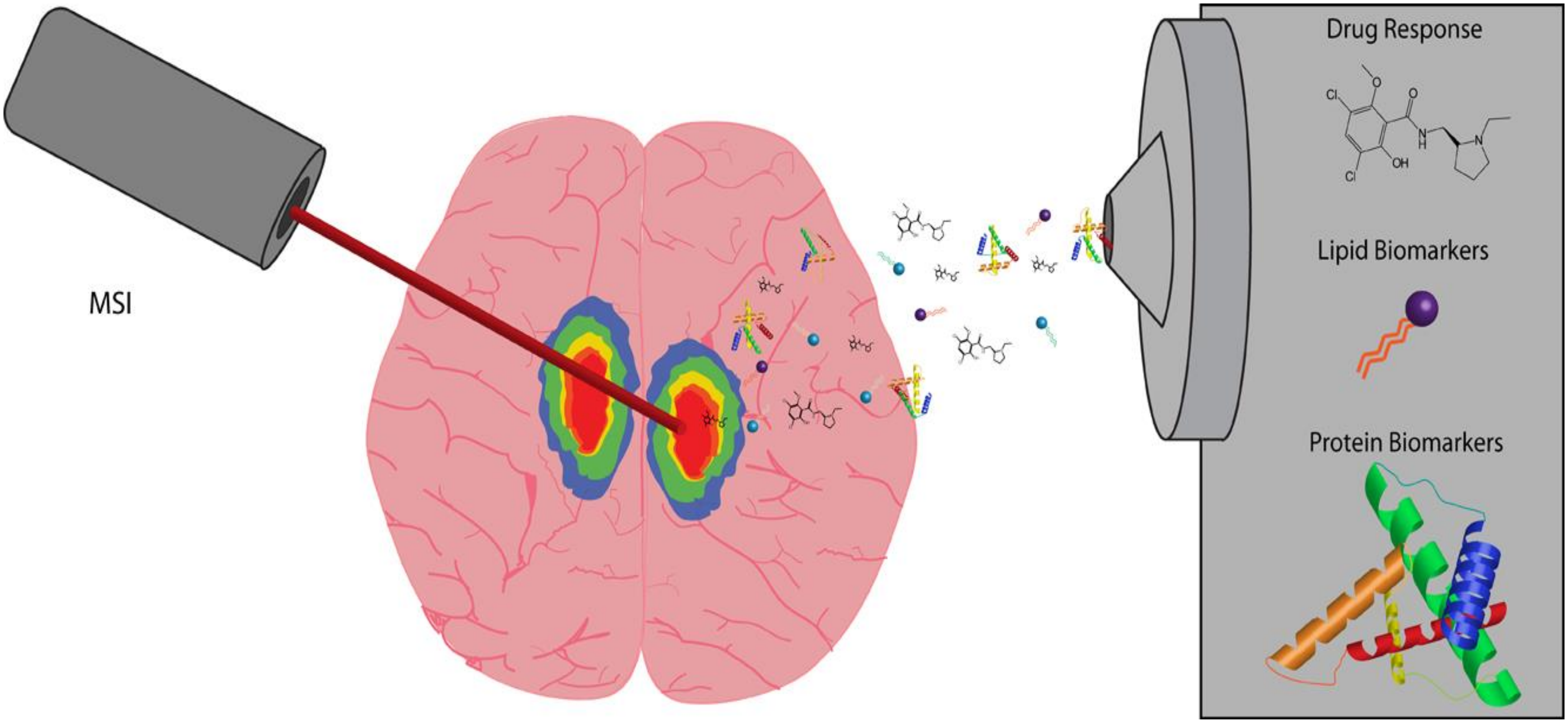
Imaging Mass Spectrometry
Liam A. McDonnell, Ron M. A. Heeren
Mass Spectrometry Reviews (2007) **26** 606-643

Mass spectral imaging: Ionization methods and tissue preparation strategies



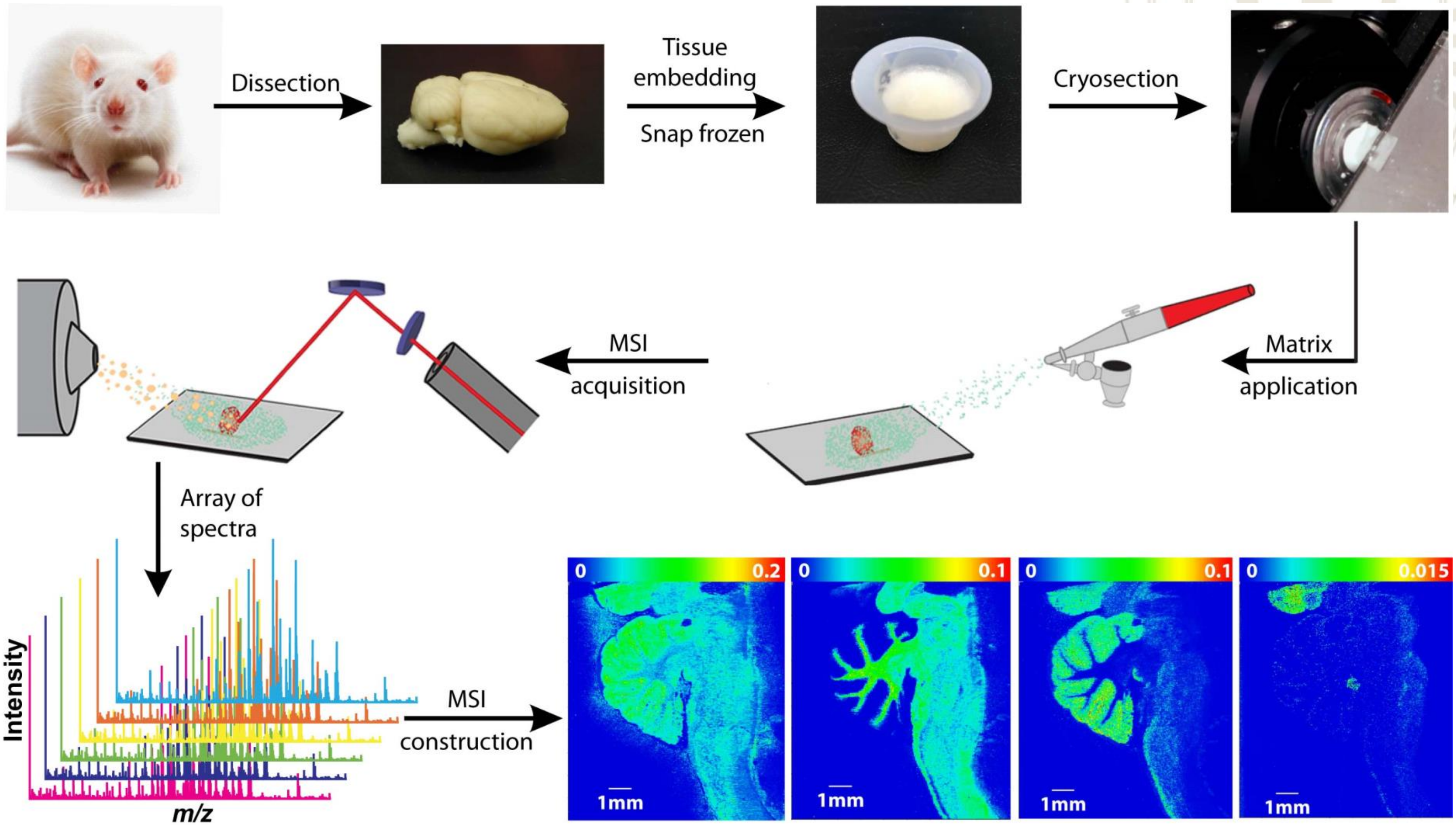
Ye, Greer, and Li, *Bioanalysis*, 3, 313-332 (2011)

With MALDI MSI, we can…



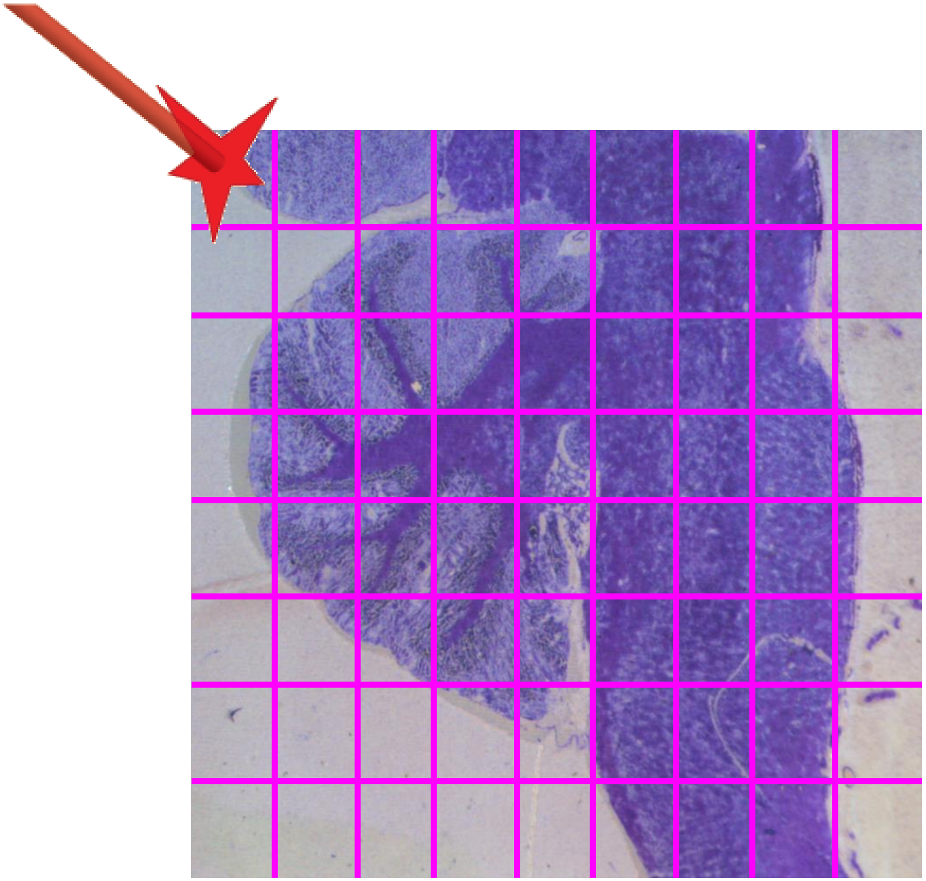
H. Ye, E. Gemperline, and L. Li (2013). A vision for better health: Mass spectrometry imaging for clinical diagnostics. *Clinica Chimica Acta*. *420*, 11-22.

Typical MS Imaging Workflow



Chen[#], Gemperline[#] & Li (2014), *Bioanalysis*, 6(4), 525-540; ([#]Co-First authors)
Angel & Caprioli (2013), *Biochemistry*, 52, 3818-3828; Gessel, Norris & Caprioli (2014), *J. Proteom.*, 107, 71-82.
Part of artwork by Dr. Erin Gemperline.

Image Acquisition Animation



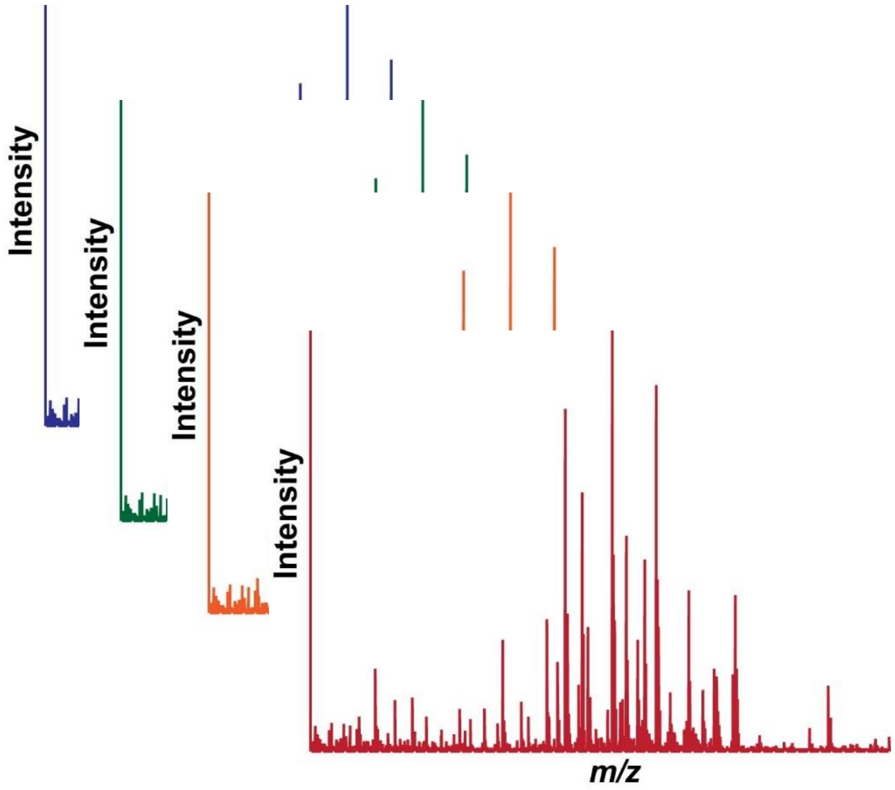
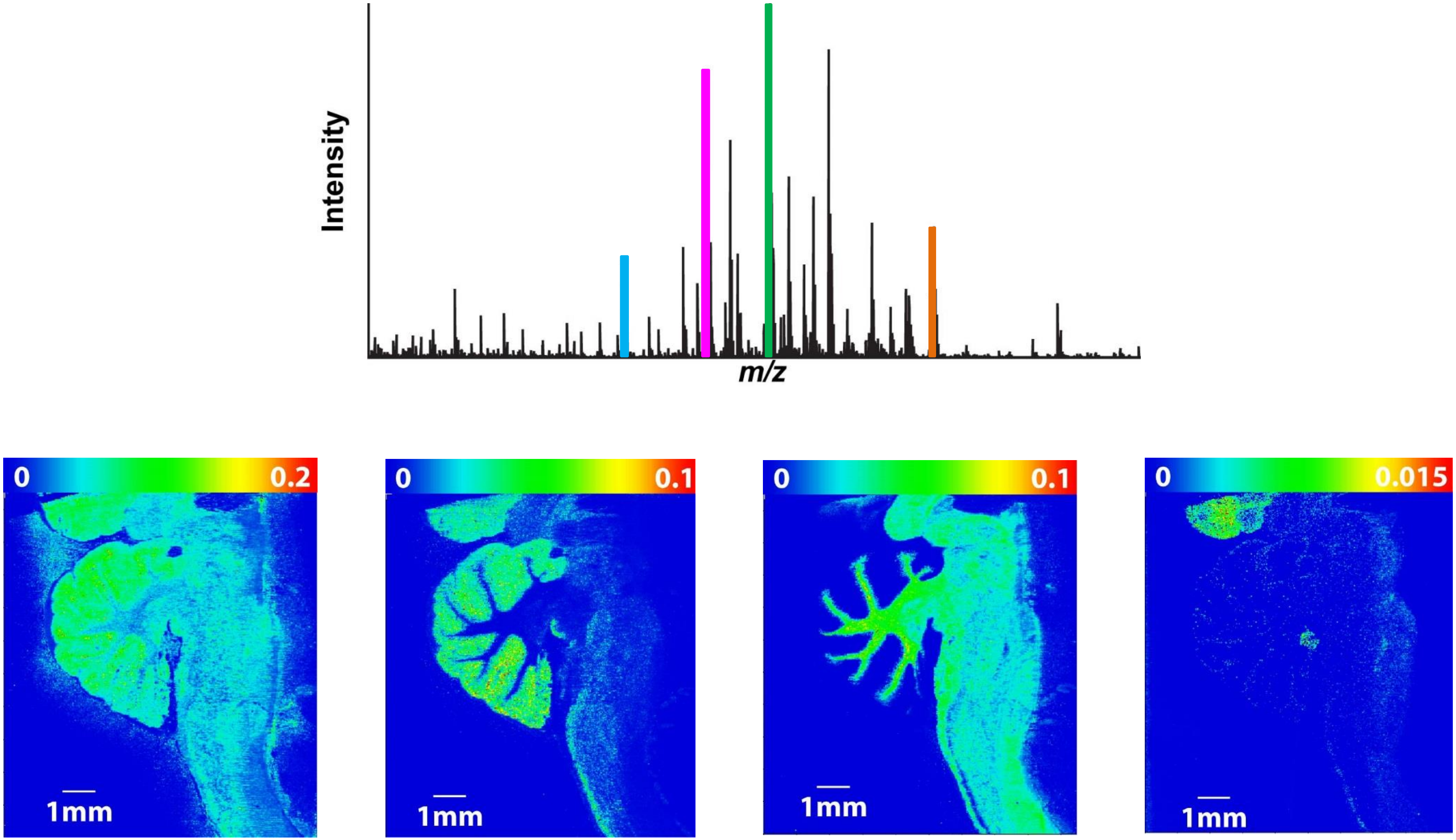


Image Data Analysis Animation



Ongoing Interest in MS Imaging

Project 1

Improve spatial resolution

Develop robust protocol

Project 2

Expand detection range of high resolution mass analyzer

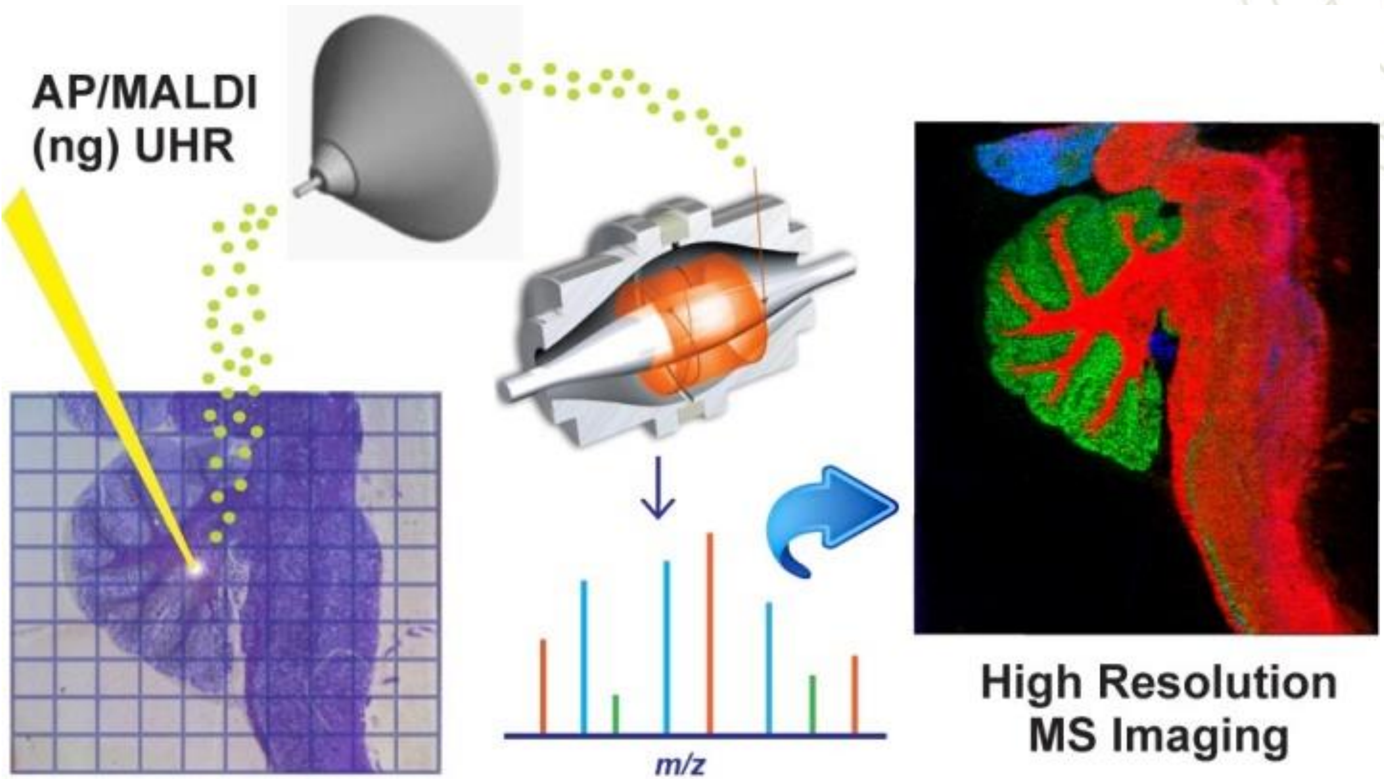
Project 3

Efficient biomolecule identification and structural elucidation

Applications

MS imaging of neuropeptides, glycans, lipids and metabolites

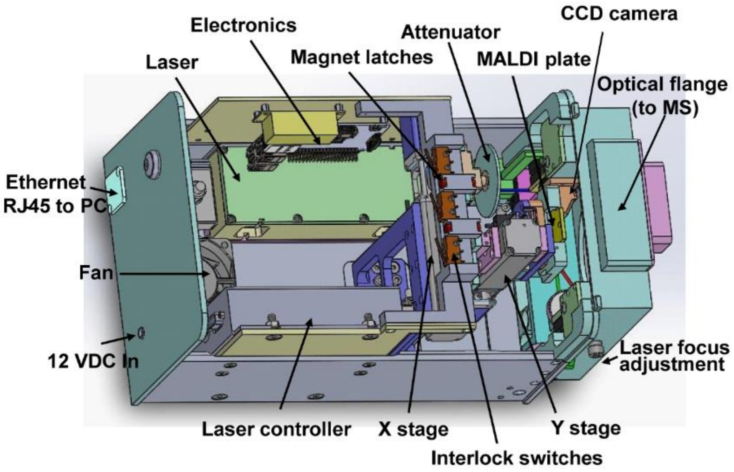
Project 1. A High Resolution Atmospheric Pressure MALDI-Quadrupole-Orbitrap Platform Enables *In Situ* Analysis of Biomolecules by Multi-Mode Ionization and Acquisition



AP/MALDI (ng) UHR source provided by MassTech.

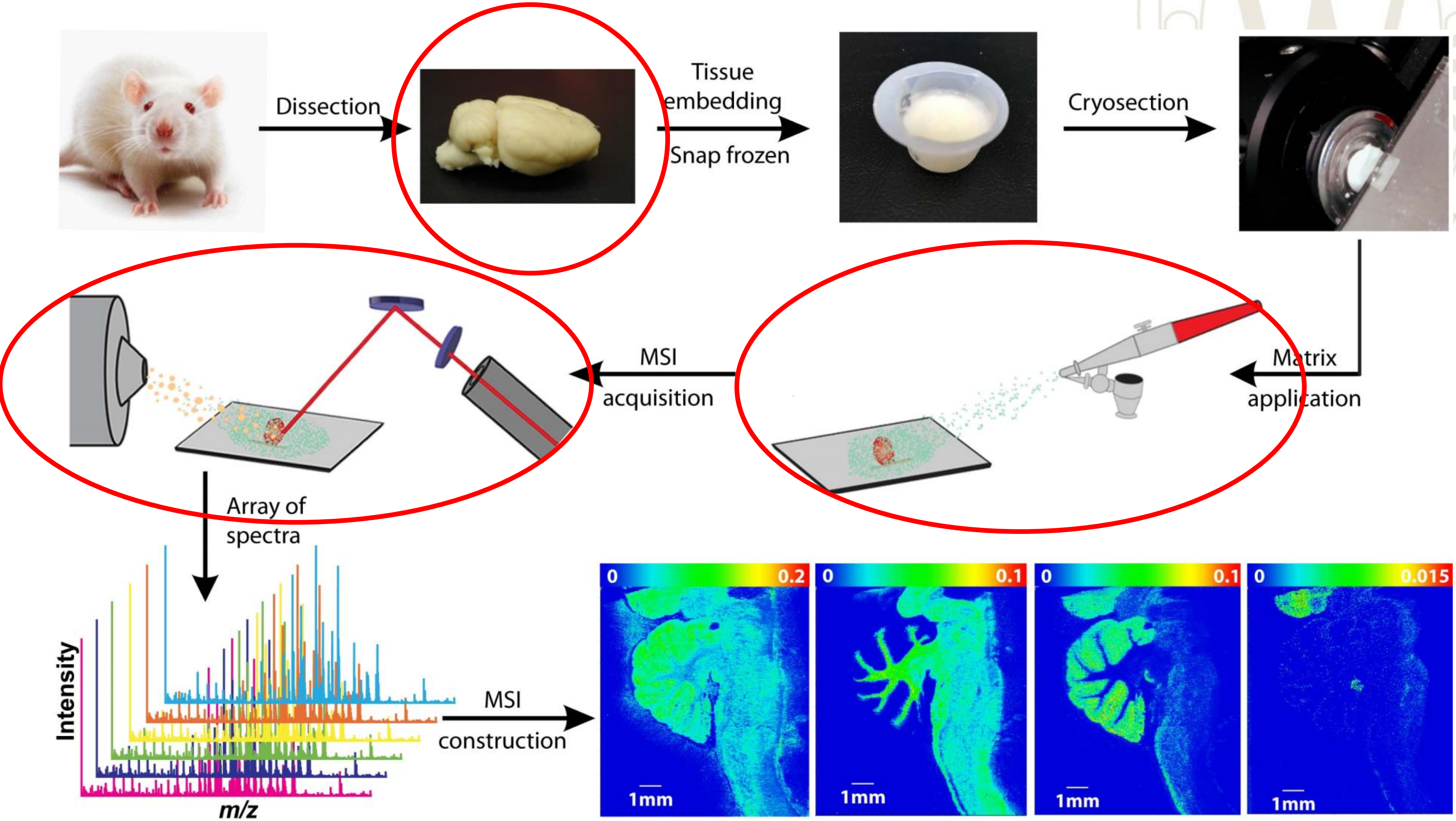
AP/MALDI-Q-Orbitrap Capability

- **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
- **Multiple types of ionization**
  - MALDI, novel ionizations for multiply charged ions (LSI and MAI/MAIV)
- **High resolution MS imaging**
  - In mass: 240k mass resolution at *m/z* 200
  - In space: < 10 µm spatial resolution



Laiko *et al.* (2000), *Analyt. Chem.,* 72, 652-657; Guenther *et al.,* (2011), *IJMS*, 305, 228-237.

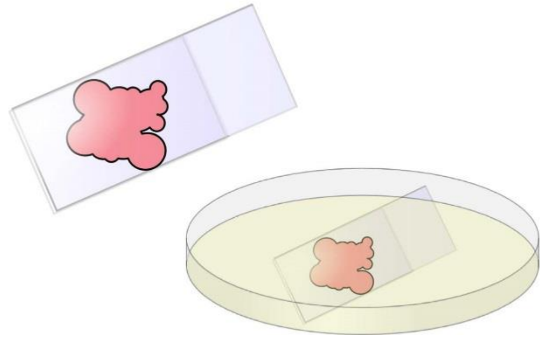
Optimization for MS Imaging



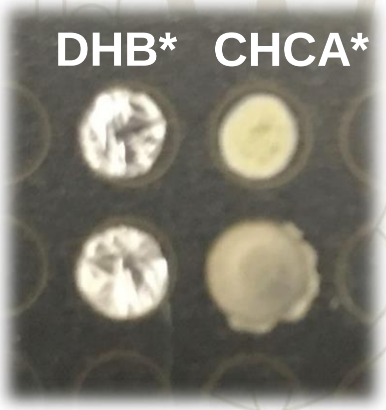
Chen#, Gemperline# & Li (2014), *Bioanalysis*, 6(4), 525-540; (#Co-First authors)
Angel & Caprioli (2013), *Biochemistry*, 52, 3818-3828; Gessel, Norris & Caprioli (2014), *J. Proteom.*, 107, 71-82.
Part of artwork by Dr. Erin Gemperline.

Optimization for MS Imaging

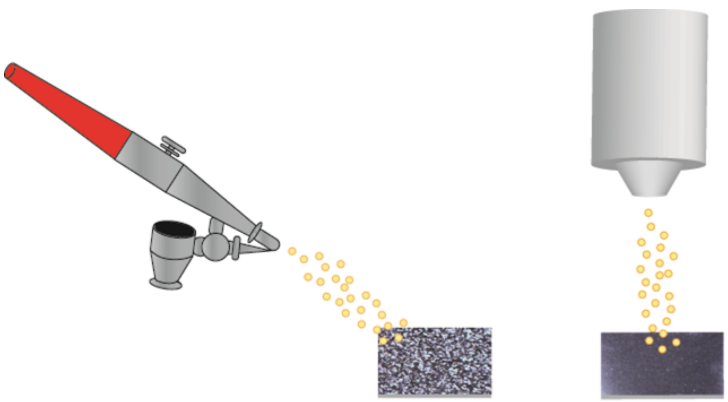
Protease Inhibition



Tissue Rinse



Matrix

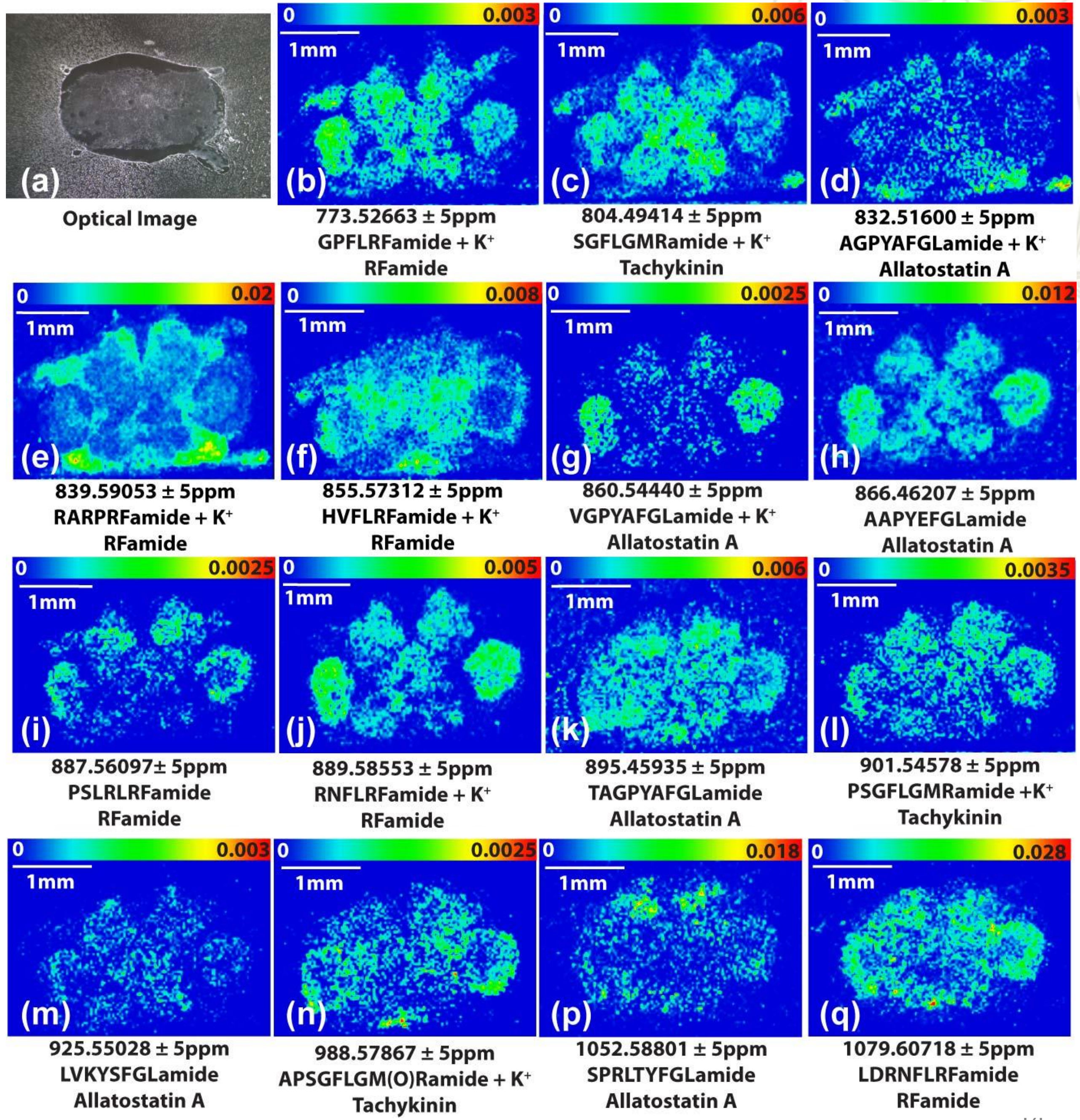


Matrix Application Method

Instrument

*DHB: 2,5-Dihydroxybenzoic acid
*CHCA: α-Cyano-4-hydroxycinnamic acid

High Resolution MSI of Neuropeptides in Crustacean Brain Tissue Section by AP MALDI Q-Orbitrap Platform



Chen et al., *Analytica Chimica Acta, 1007,* 16-25 (2018).

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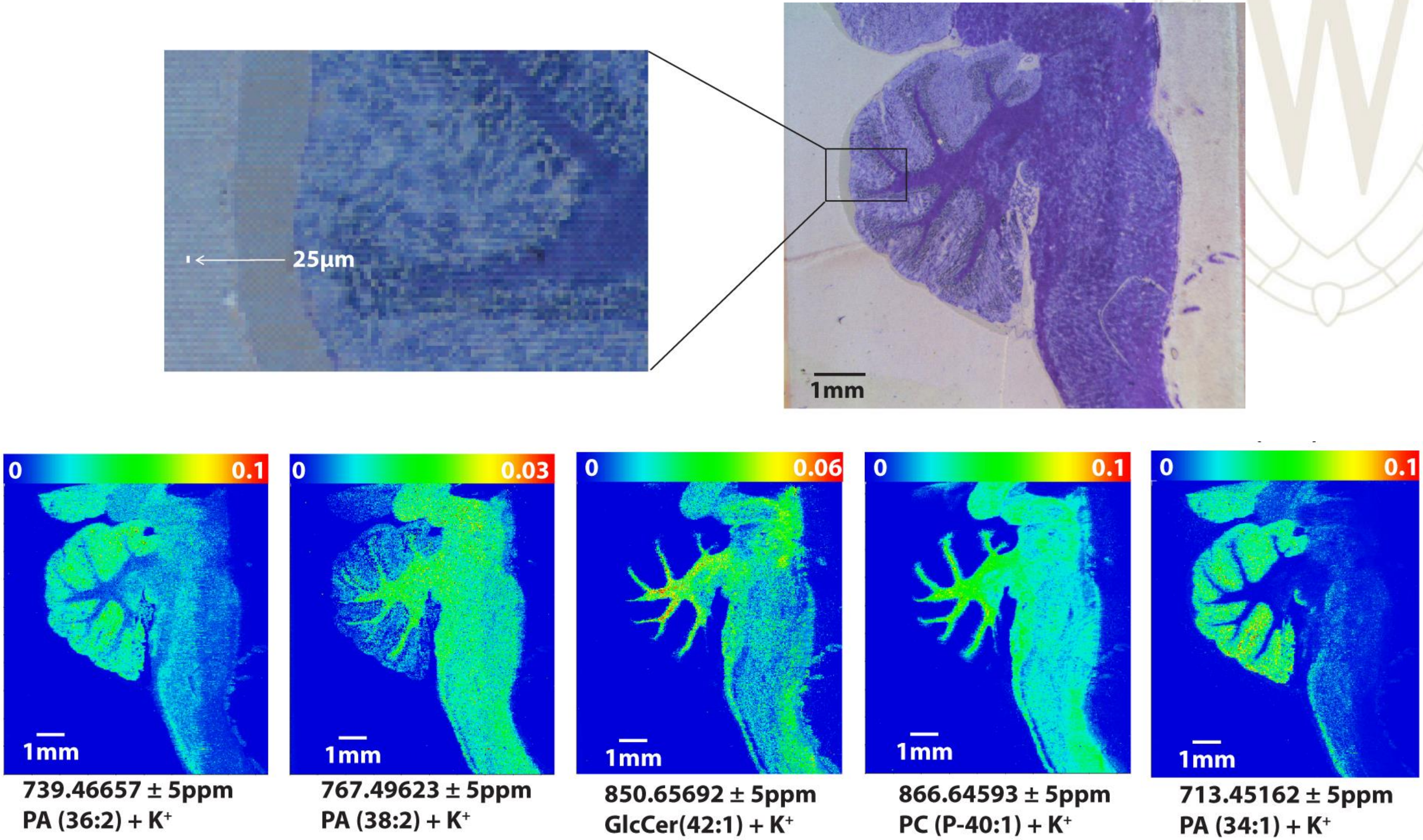
High Resolution MS Imaging



Chen et al., *Analytica Chimica Acta*, *1007*, 16-25 (2018).

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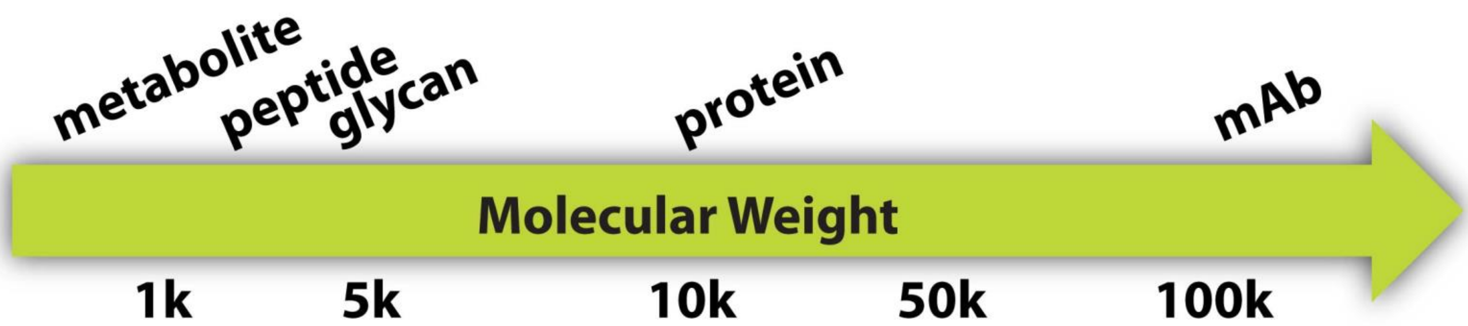
High Resolution Trade-off: Limited Mass Range

MALDI-LTQ-Orbitrap
***m/z* 4000**

QE HF
***m/z* 6000**

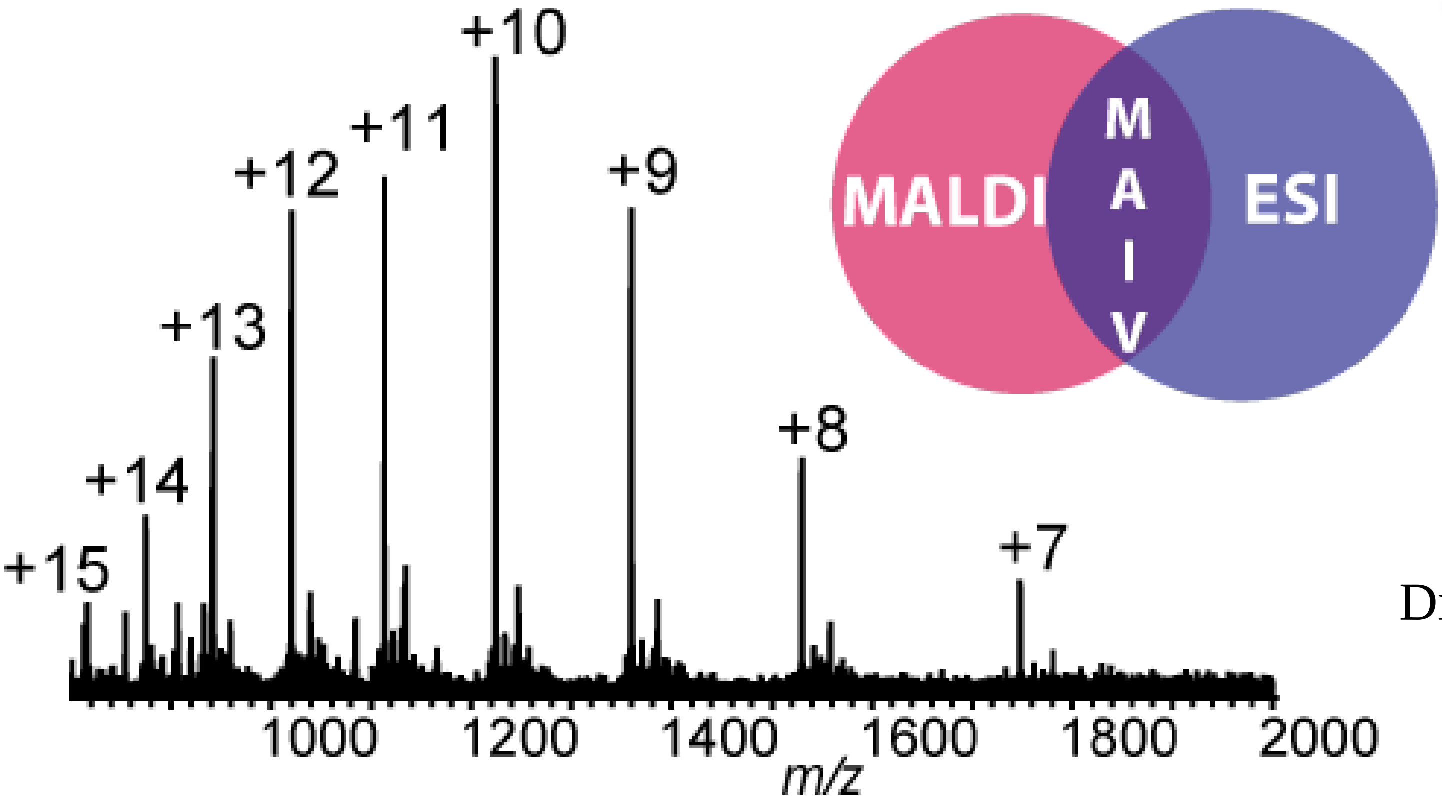
solariX XR 7T FTICR
***m/z* 10000**

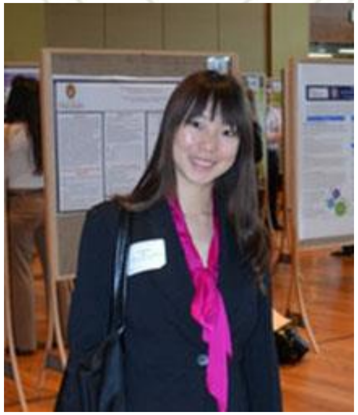


oxytocin 1.0K **bradykinin 1.1K** **neuropeptide Y 4.3K** **insulin 5.7K**

cytochrome C 12.3K **lysozyme 14.3K** **myoglobin 17.0K** **mAb 150K**

Project 2. "Magic" Ionization on High Performance Mass Spectrometers

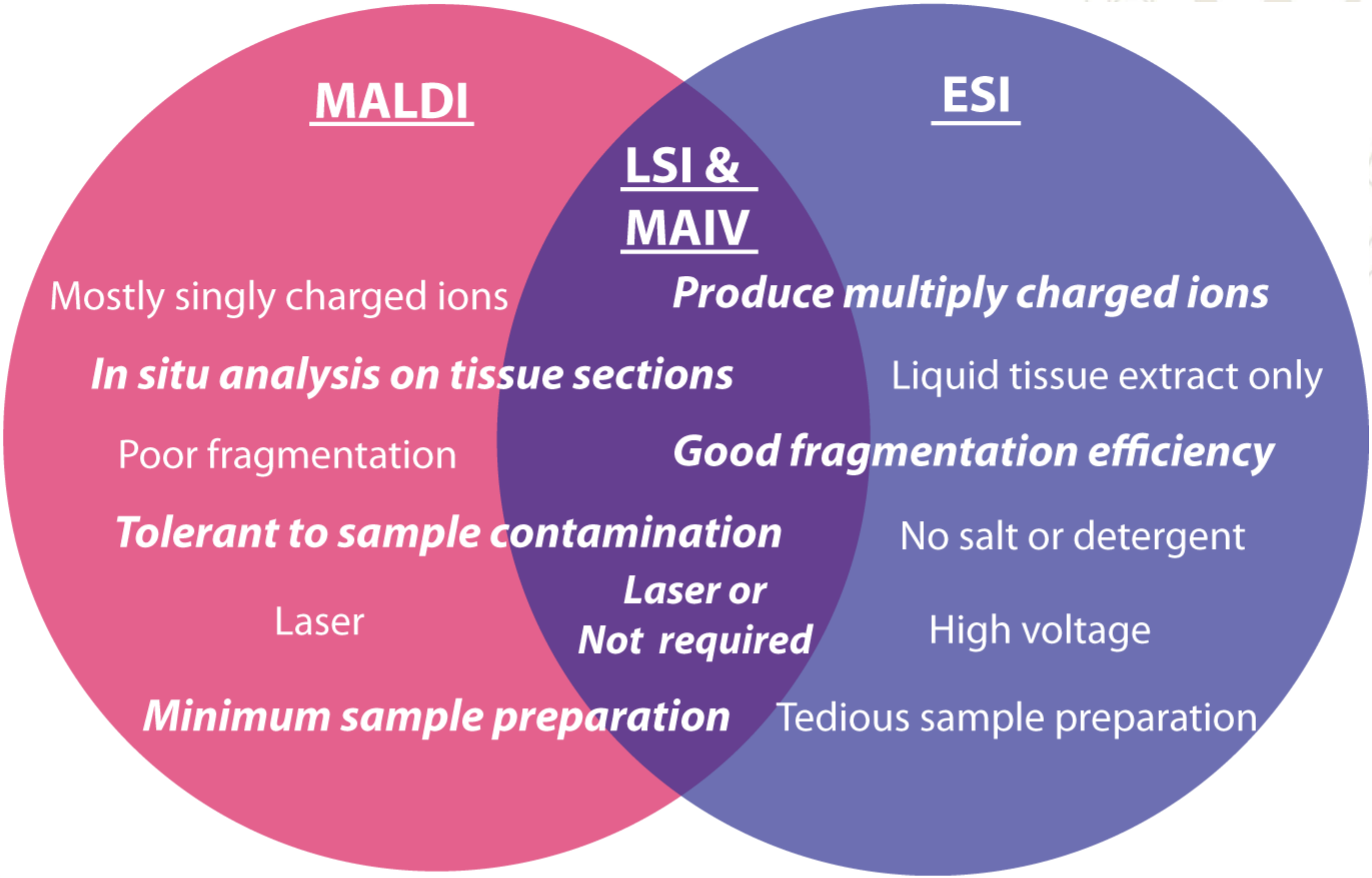




Dr. Bingming Chen

B.Chen, C.B. Lietz, C.Ouyang, X. Zhong, M. Xu and L. Li (2016). *Analyt. Chim. Acta*., vol 916. p.52-59; B.Chen, C.B. Lietz, L. Li (2014). *J. Am. Soc. Mass Spectrom.*, vol. 25 (12). *p. 2177-80;* B. Chen#, C. Ouyang#, Z. Tian, M. Xu and L. Li, *To be submitted to Analyt. Chim. Acta*. (#Co-First authors);

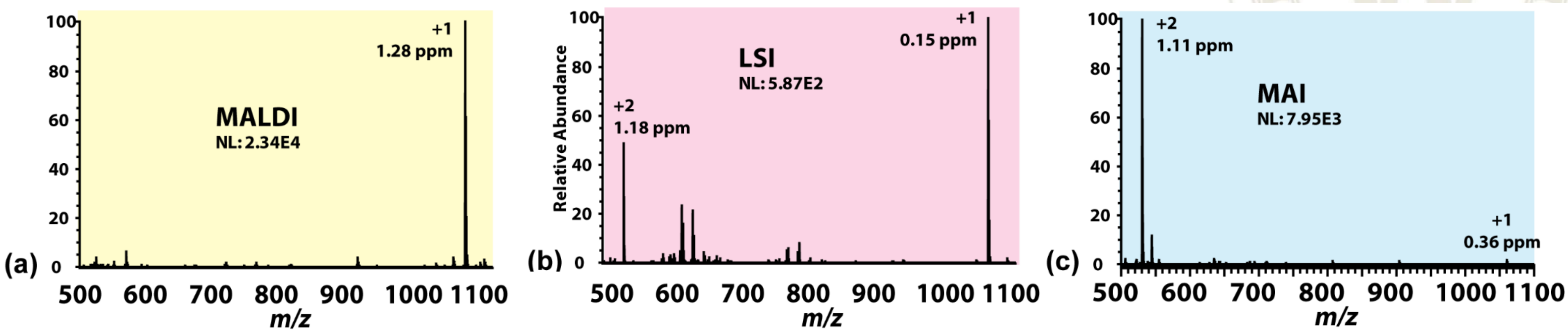
"Magic" Ionization – Multiply Charged MALDI



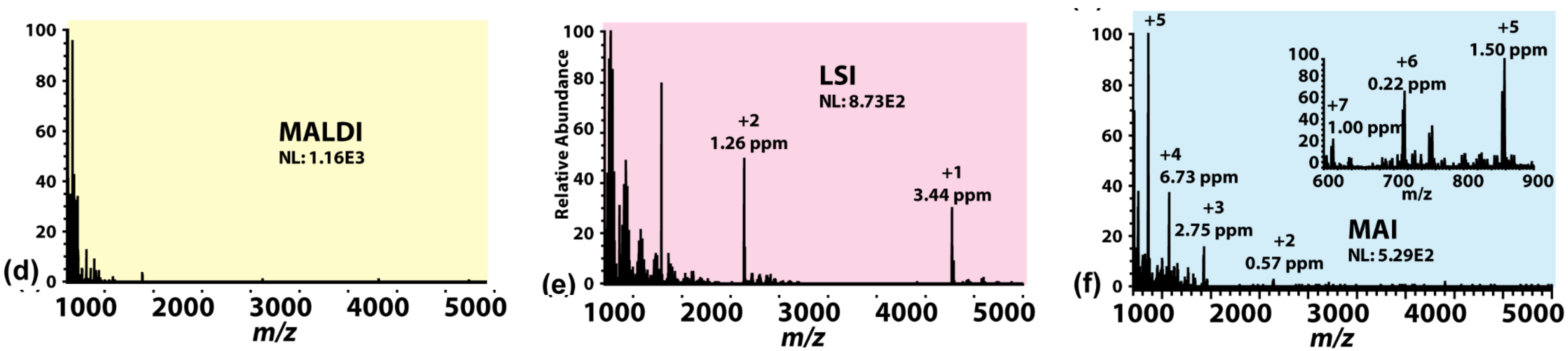
Chen, Lietz & Li, ***JASMS***, 2014, *25*, 2177-2180; **Chen** *et al.*, ***Analyt. Chim. Acta***, 2016, 916, 52-59; Trimpin, ***JASMS***, 2016, 27 (1), 4-21. Trimpin *et al.*, ***Mol Cell Proteomics*** 2010, *9*, 362-367; Inutan *et al.*, ***Mol Cell Proteomics*** 2011, *10*, M110 000760; Inutan & Trimpin, ***Mol Cell Proteomics*** 2013, 12, 792-796

Multiply Charged Ions on AP/MALDI MS

Bradykinin 1059.5608 Da



Neuropeptide Y 4269.0808 Da



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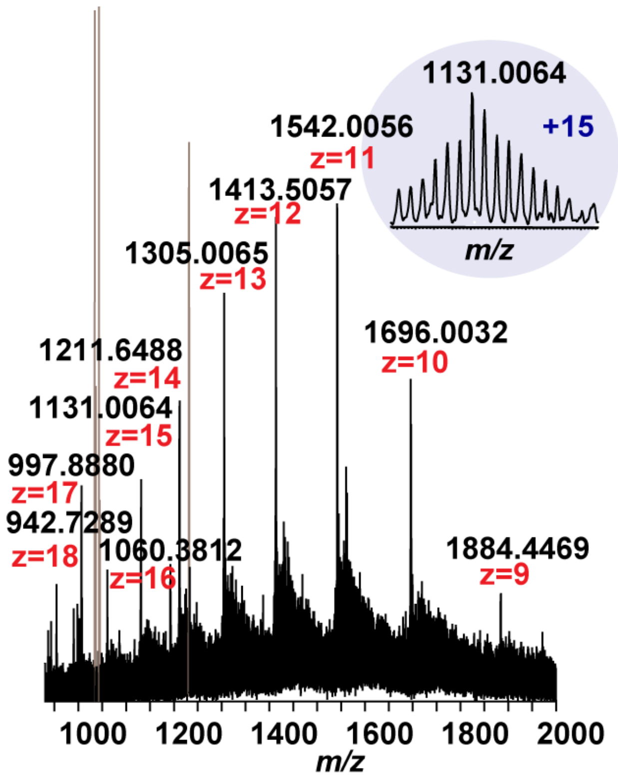
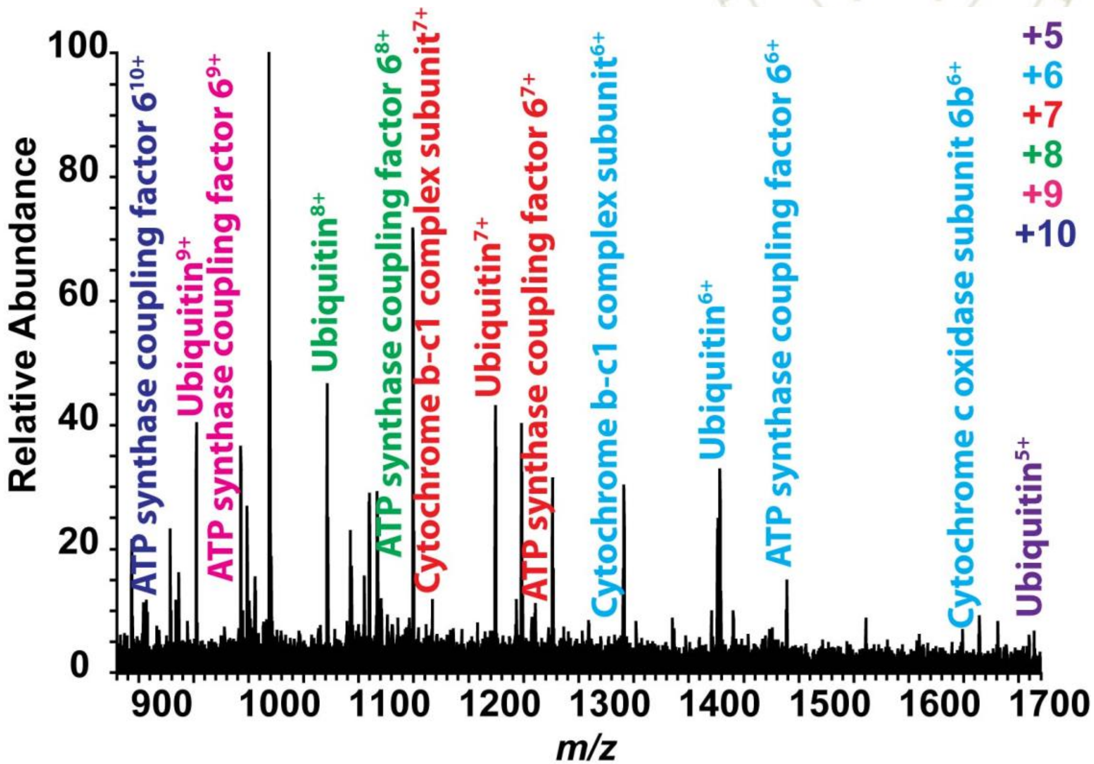
Extended Mass Range Enables Protein Detection

- **Myoglobin 17.0 kDa**

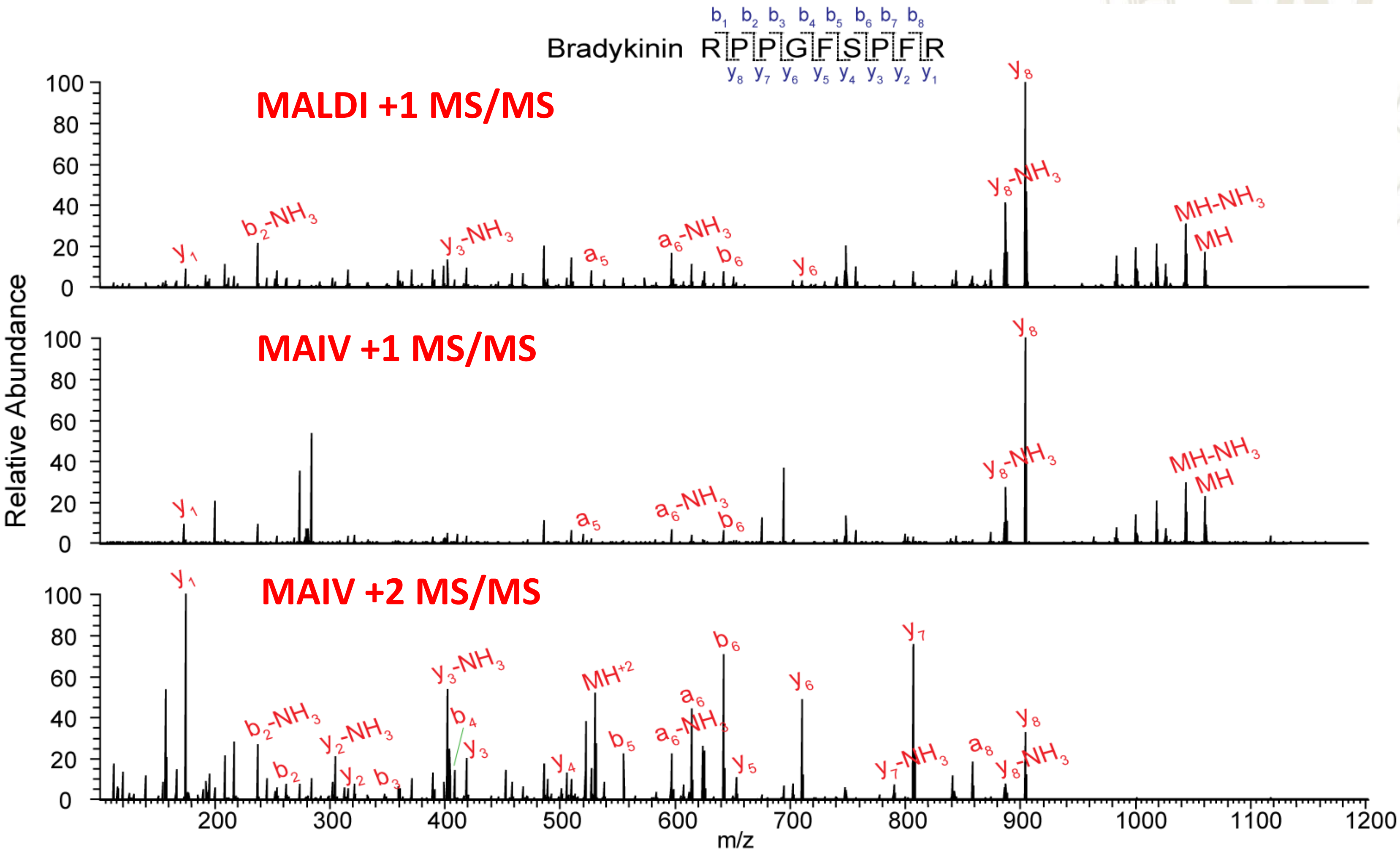


- **Rat brain protein extraction**



Chen *et al.*, ***Analyt. Chim. Acta***, 2016, 916, 52-59.

Improved Fragmentation: Bradykinin



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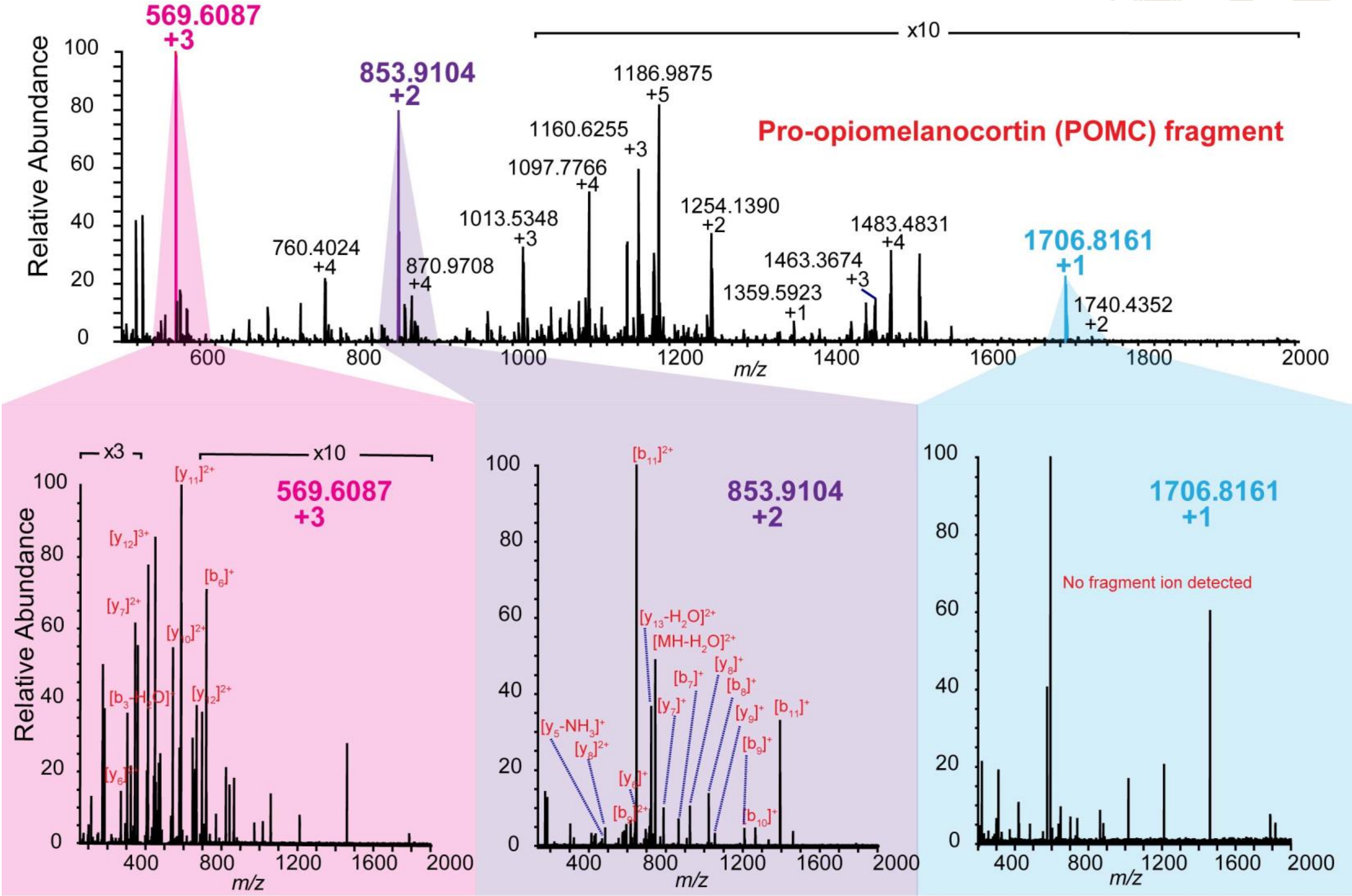
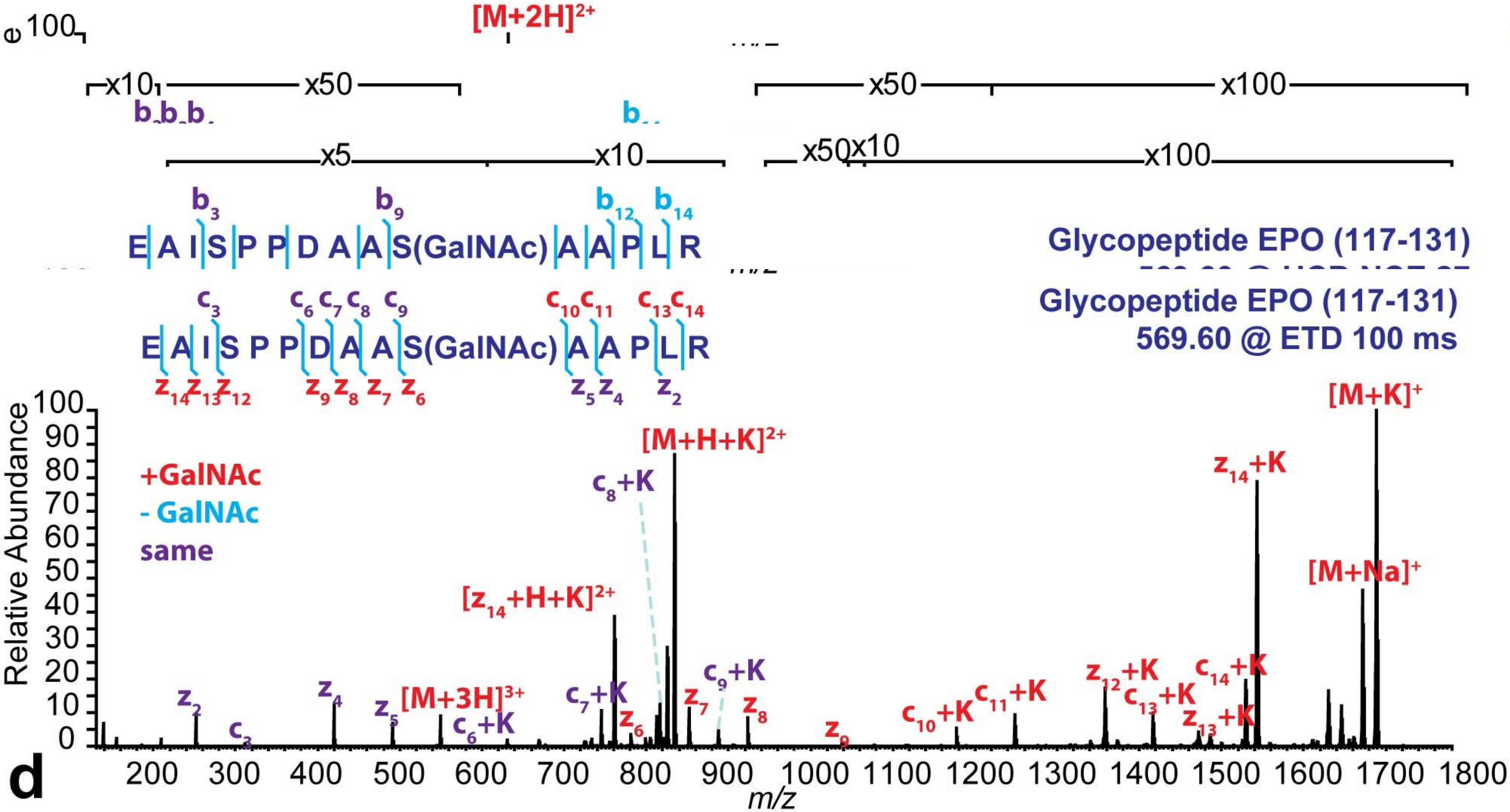
Improved Fragmentation Efficiency



Chen *et al.*, ***Analyt. Chim. Acta***, 2016, 916, 52-59.

Labile Post-Translational Modification Analysis



Project 1 & 2. HR^2 AP/MALDI MS Conclusions

Achieved HR^2 MS imaging

- In mass (240K at *m/z* 200)
- In space (< 10 µm)

Produced multiply charged ions

- Mass range expanded for protein detection
- Fragmentation efficiency improved
- Possible to study labile PTMs

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Ongoing Interest in MS Imaging

Project 1

Improve spatial resolution

Develop robust protocol

Project 2

Expand detection range of high resolution mass analyzer

Project 3

Efficient biomolecule identification and structural elucidation

Applications

MS imaging of neuropeptides, glycans, lipids and metabolites

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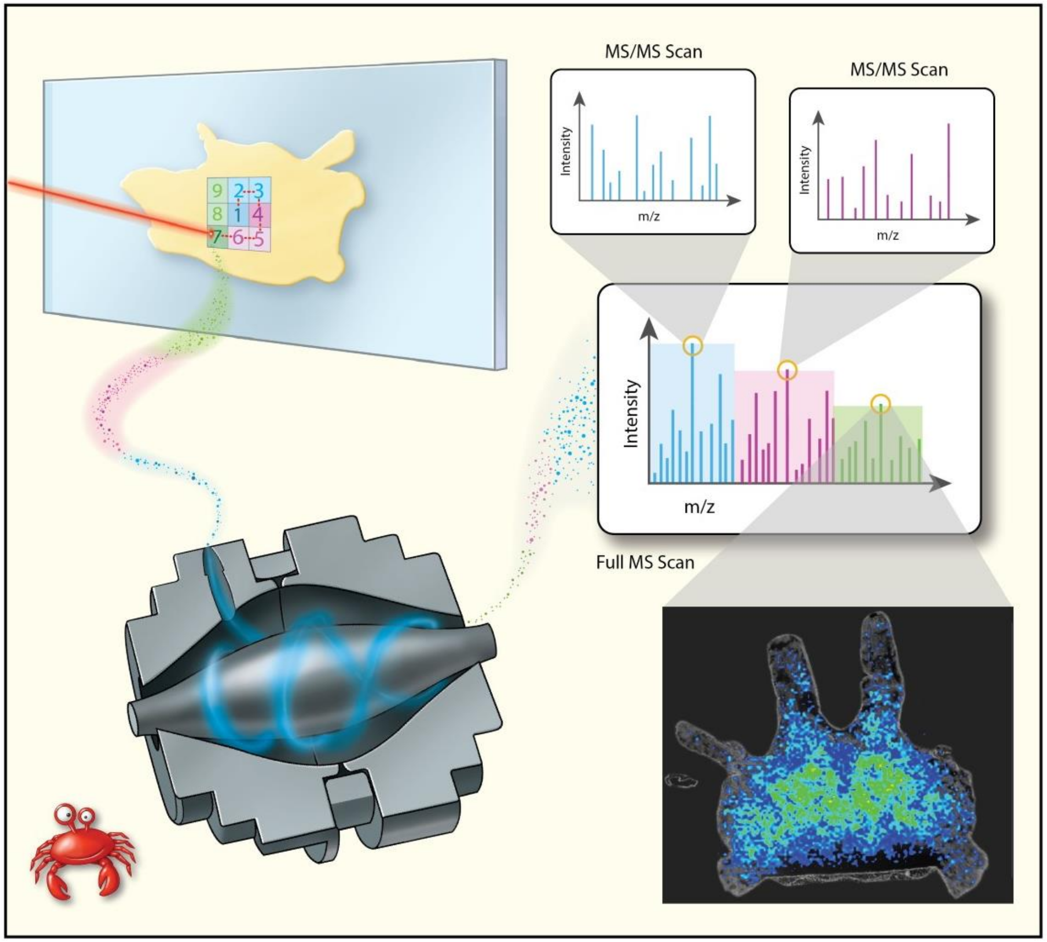
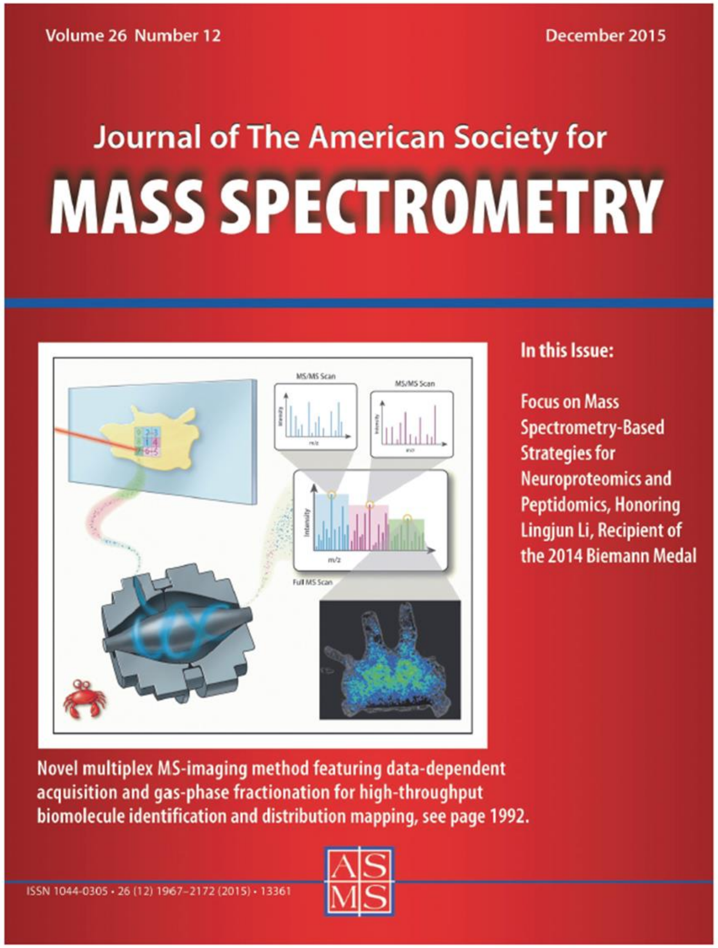
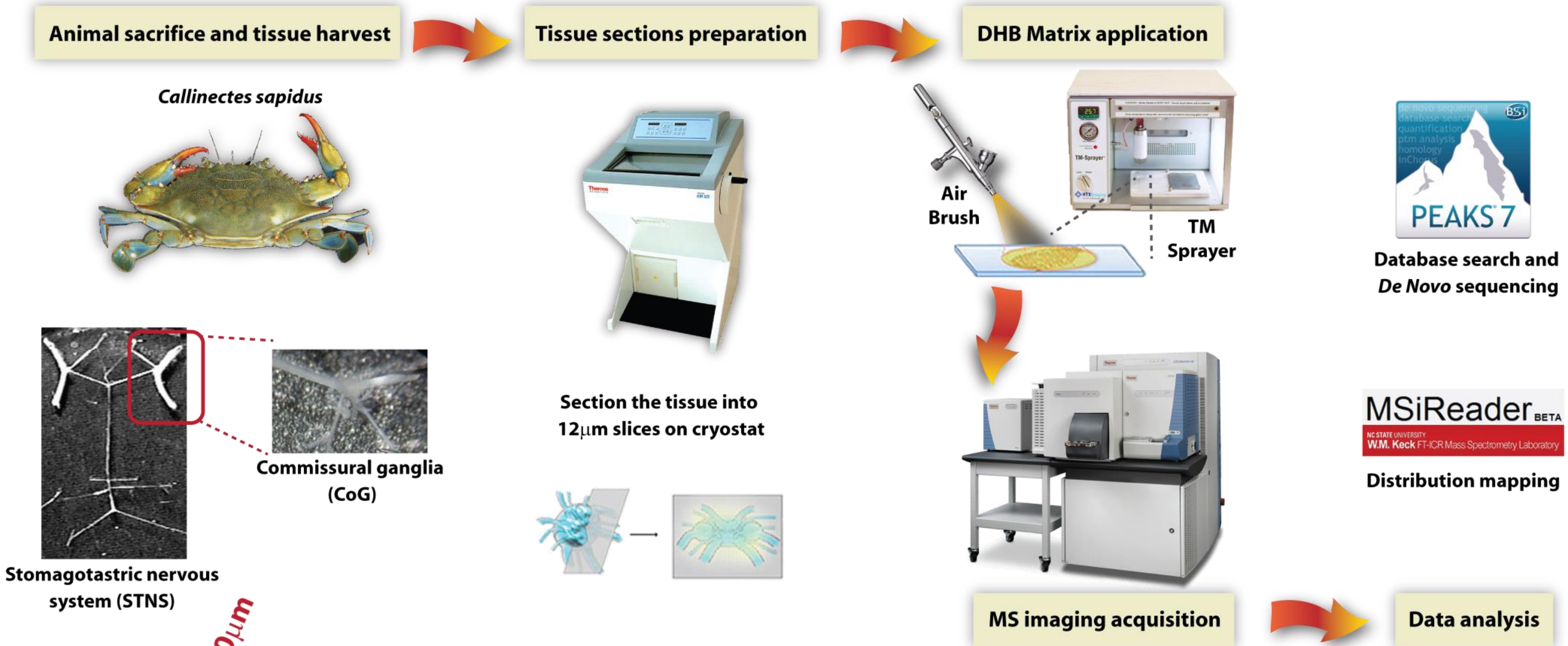
Project 3. High Throughput *In Situ* DDA Analysis of Neuropeptides by Coupling Novel Multiplex MS Imaging with Gas Phase Fractionation

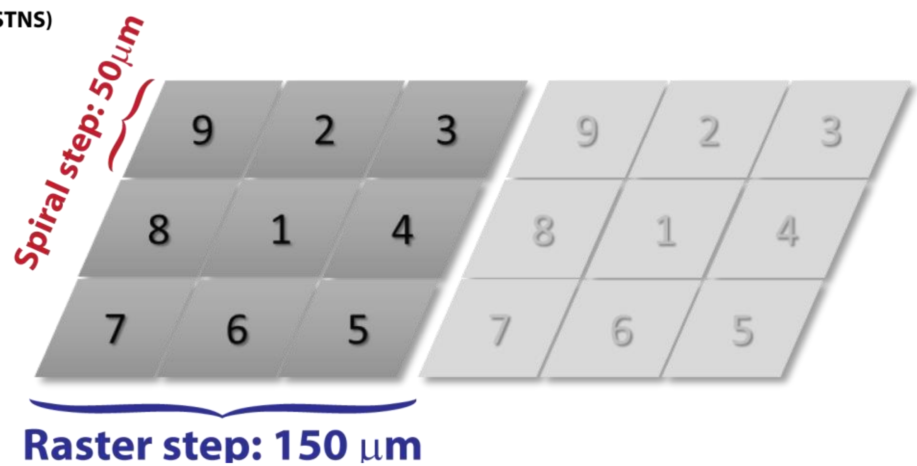




Chen[#], Ouyang[#] and Li (2015). High Throughput *In situ* DDA Analysis of Neuropeptides by Coupling Novel Multiplex Mass Spectrometric Imaging (MSI) with Gas-Phase Fractionation. *JASMS*, vol. 26 (12). p.1992-2001. Cover article ([#]Co-First authors), artwork by Sally Griffith-Oh

MSI Study of the Crustacean Stomatogastric Nervous System (STNS) by MALDI Orbitrap

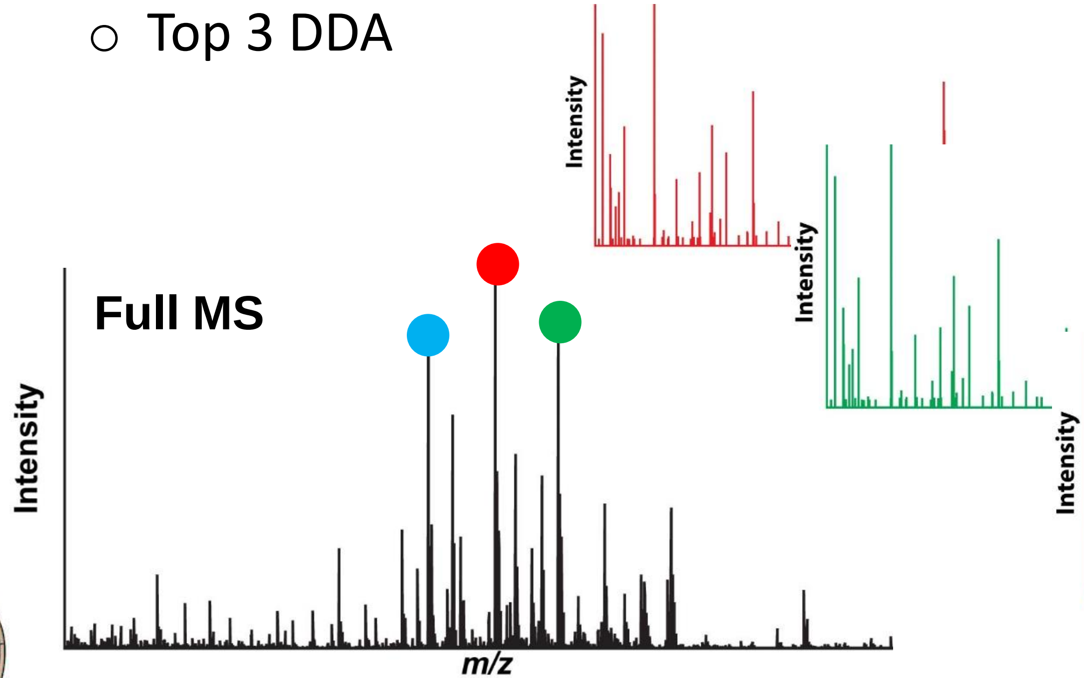


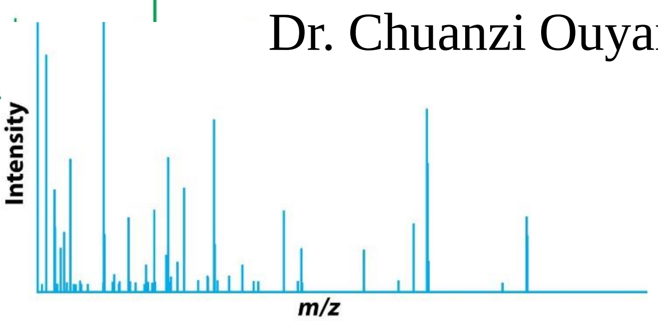


Spiral step 1: full MS scan in Orbitrap
Spiral steps 2-9: targeted tandem MS in ion trap

In Situ Biomolecule Identification

- **A major challenge in MS imaging**
  - Accurate mass matching: putative identification
  - Targeted MS/MS: confident identification and confirmation
- **Data dependent acquisition (DDA)**
  - Fragment top N ions after a full scan
  - Top 3 DDA

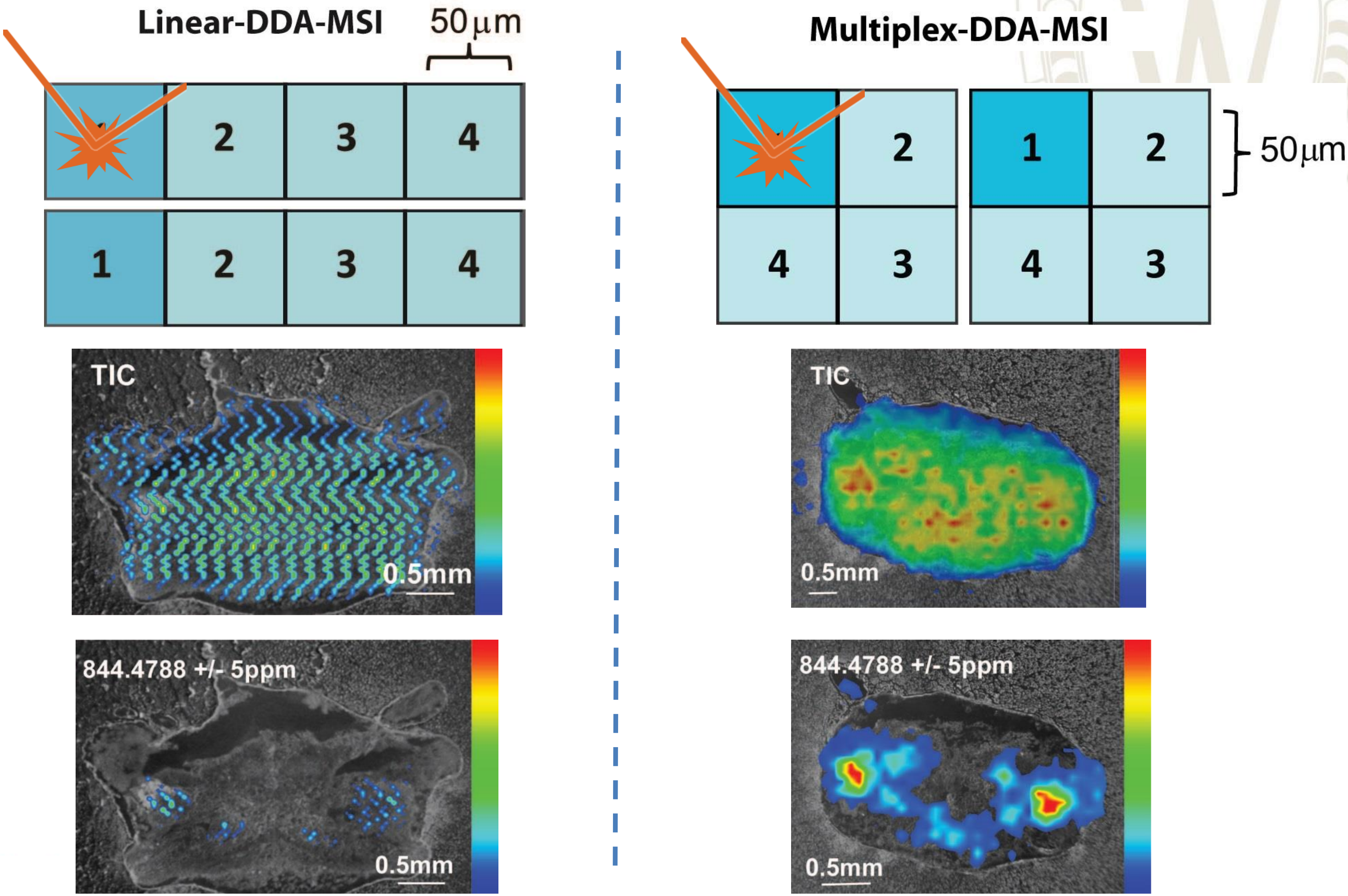




Dr. Chuanzi Ouyang

Chen[#]**,** Ouyang[#] and Li (2015).. *JASMS*, vol. 26 (12). p.1992-2001.

In Situ DDA: Linear vs. Multiplex MSI

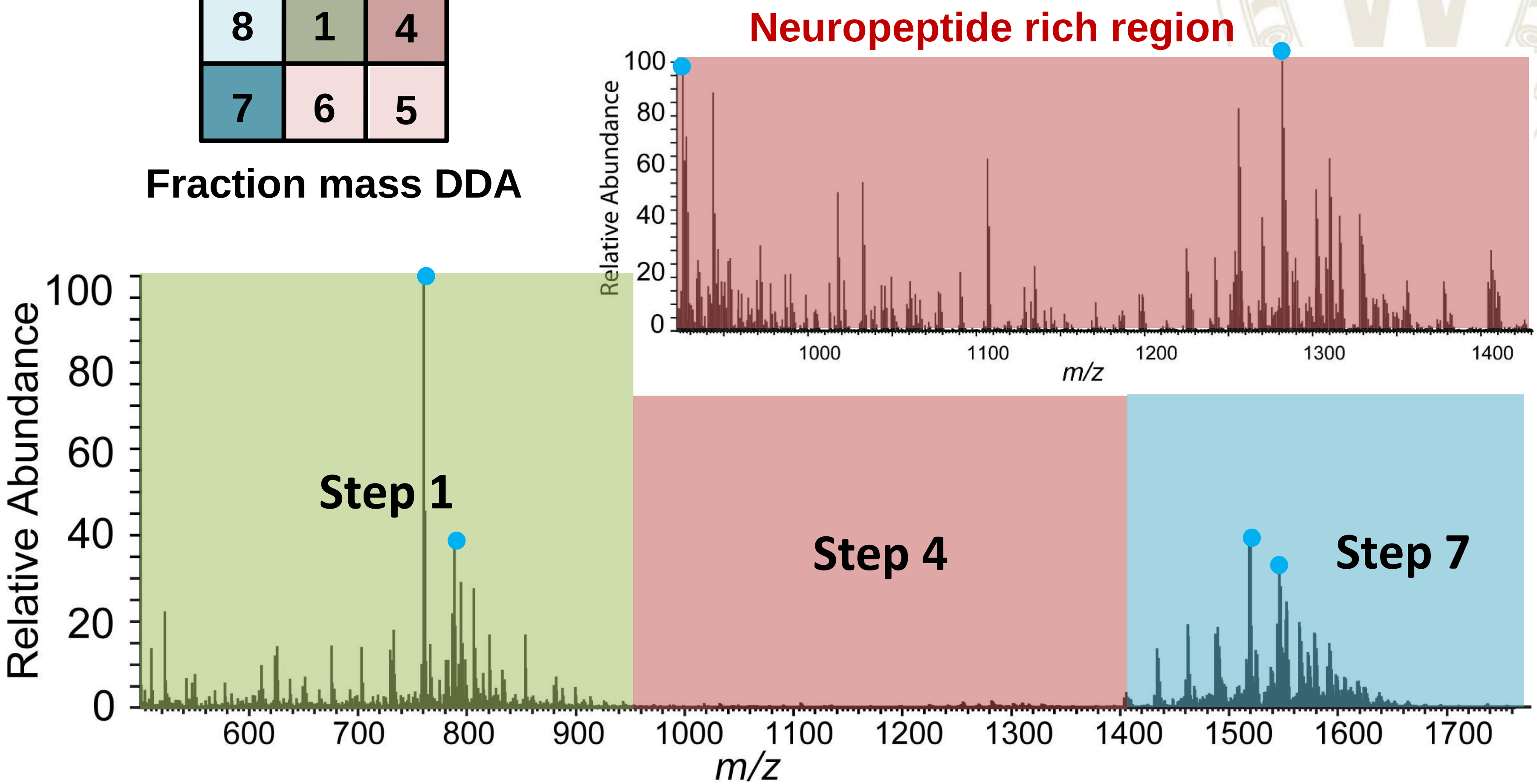


Chen[#], Ouyang[#] and Li (2015).. *JASMS*, vol. 26 (12). p.1992-2001. ([#]Co-First authors)

Improving MS^2 Precursor Selection Efficiency

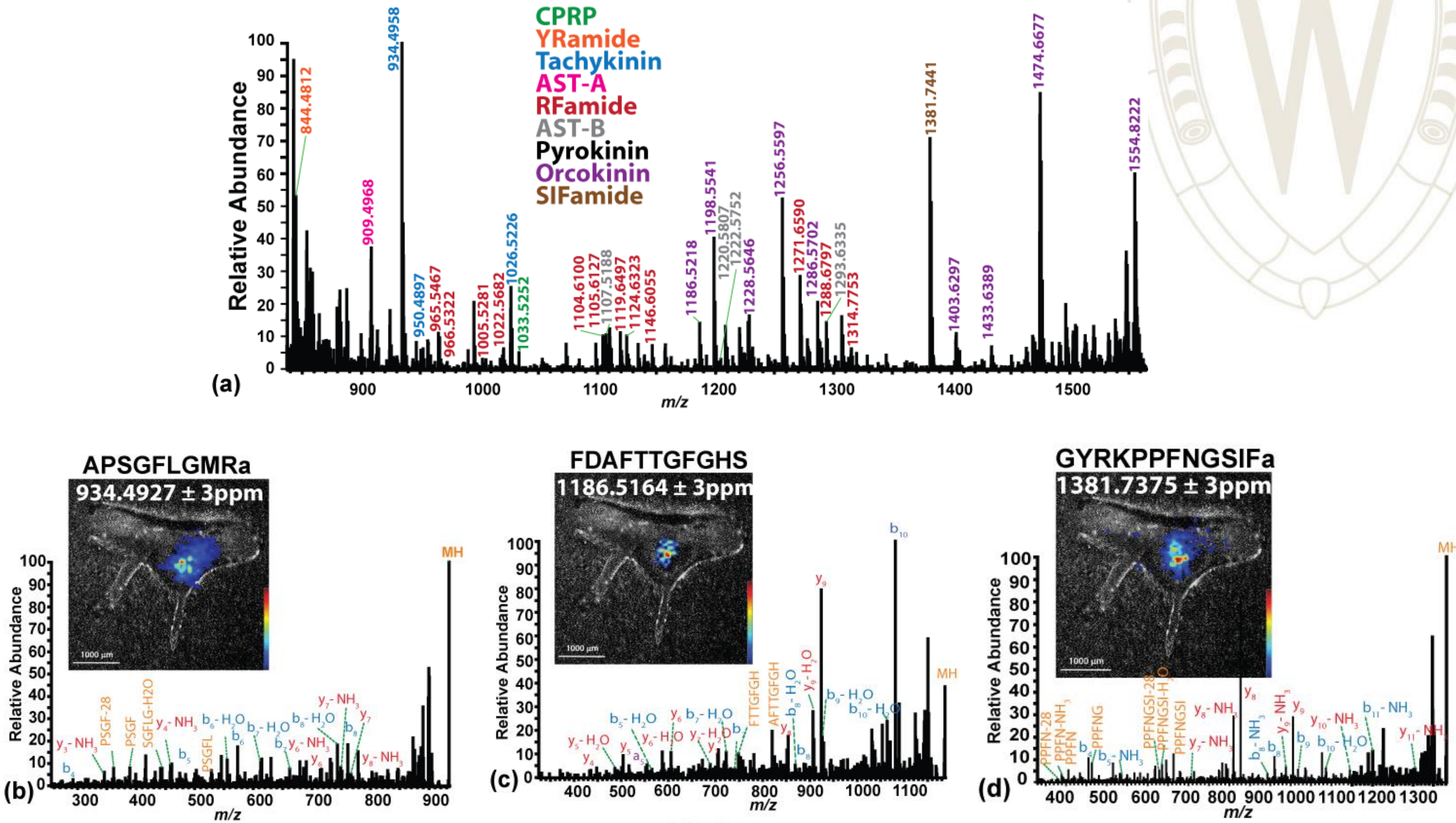
9	2	3
8	1	4
7	6	5

Fraction mass DDA



Chen#, Ouyang# and Li (2015).. *JASMS*, vol. 26 (12). p.1992-2001. (#Co-First authors)

In Situ DDA: Multiplex MSI Results



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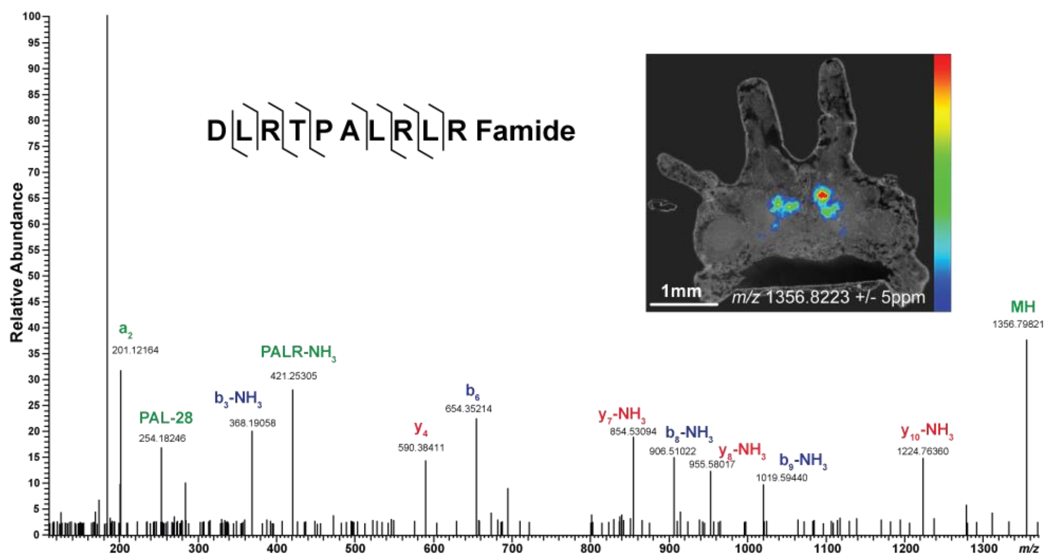
Chen[#], Ouyang[#] and Li (2015).. *JASMS*, vol. 26 (12). p.1992-2001. ([#]Co-First authors)

Multiplex DDA MSI Conclusions

- Simultaneous localization and identification
- Pseudo gas phase separation improved the precursor selection coverage
- Detection of novel neuropeptides by *de novo* sequencing
- **18** novel neuropeptides from crustacean neural tissue were identified by *in situ* MS/MS.

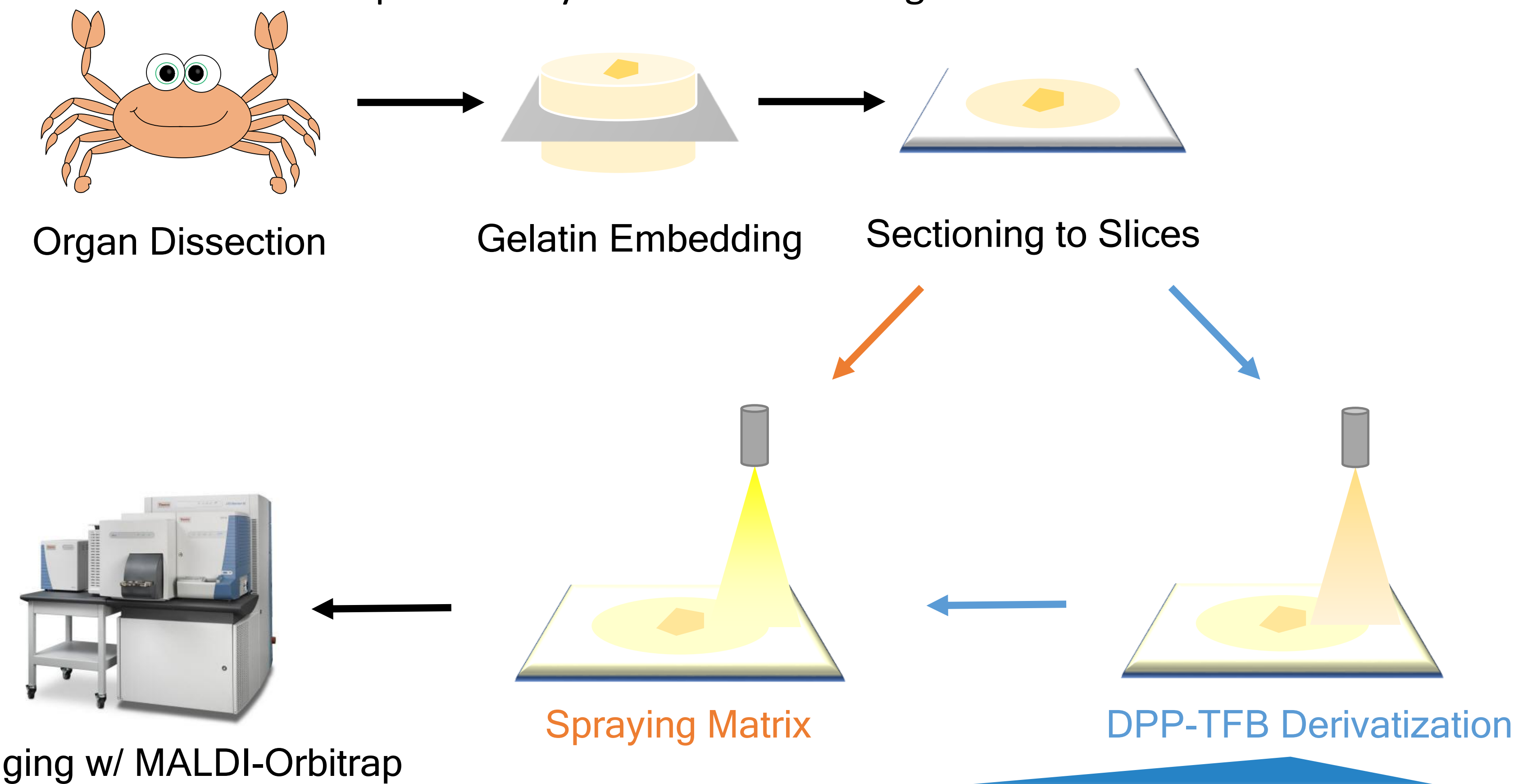


Novel RFamide identified in brain tissue of blue crab

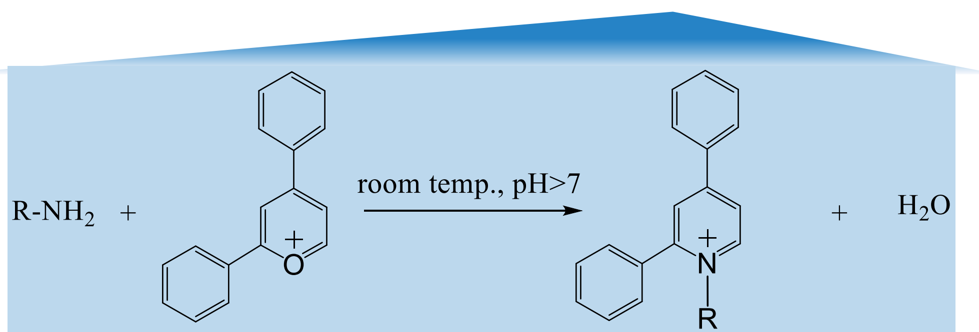
Chen[#], Ouyang[#] and Li (2015).. *JASMS*, vol. 26 (12). p.1992-2001. ([#]Co-First authors)

MALDI MSI Workflow

- Workflow to achieve complementary metabolite coverage



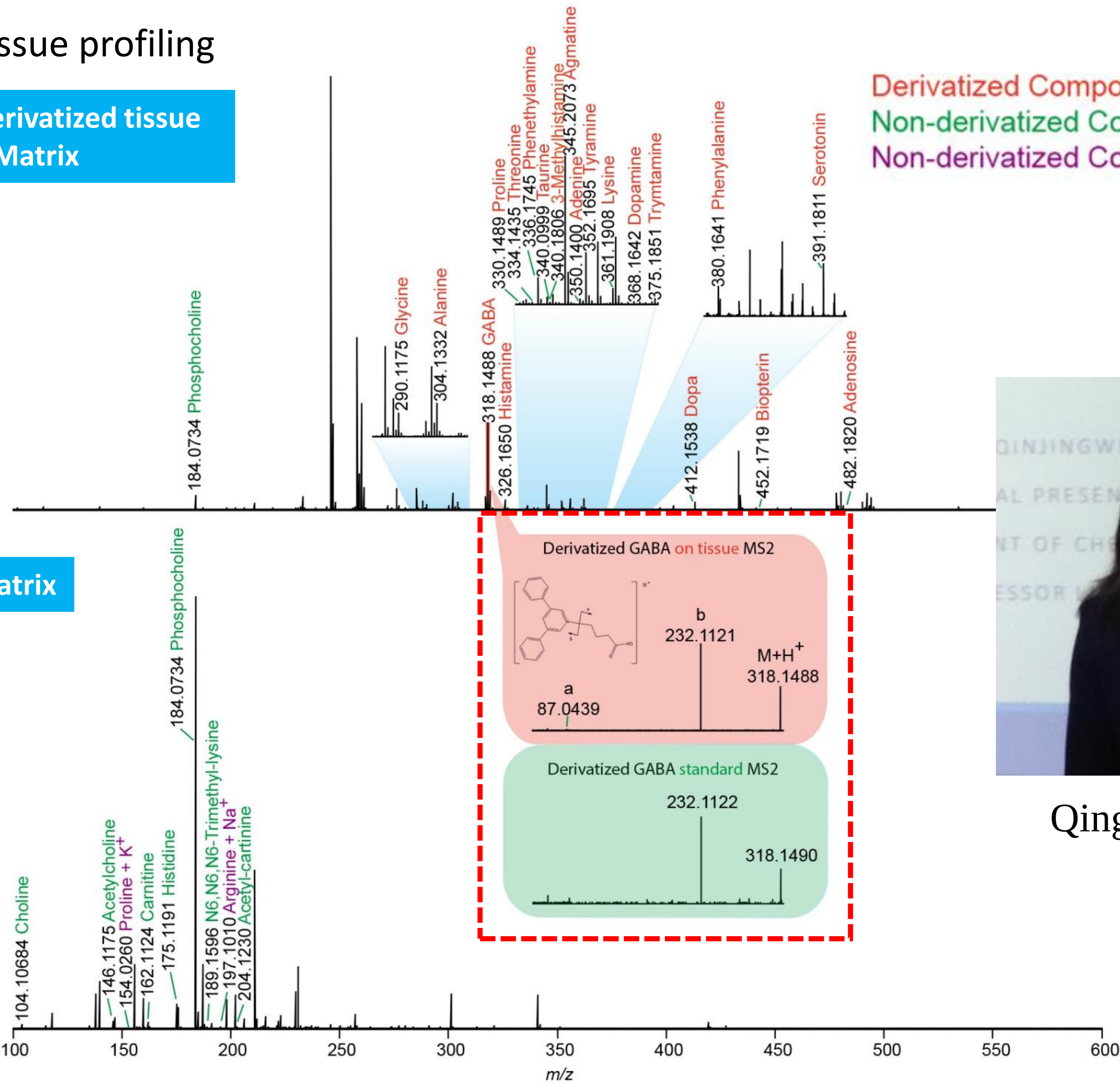
- **Metabolite MALDI MSI challenges**
  - Matrix interference
  - Low ionization efficiency



- On tissue profiling

Derivatized tissue + Matrix

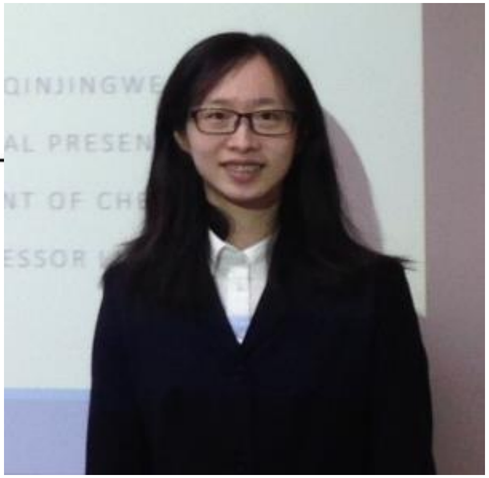
Matrix



Derivatized Compound + DPP^+

Non-derivatized Compound + H^+

Non-derivatized Compound + Na+/K+



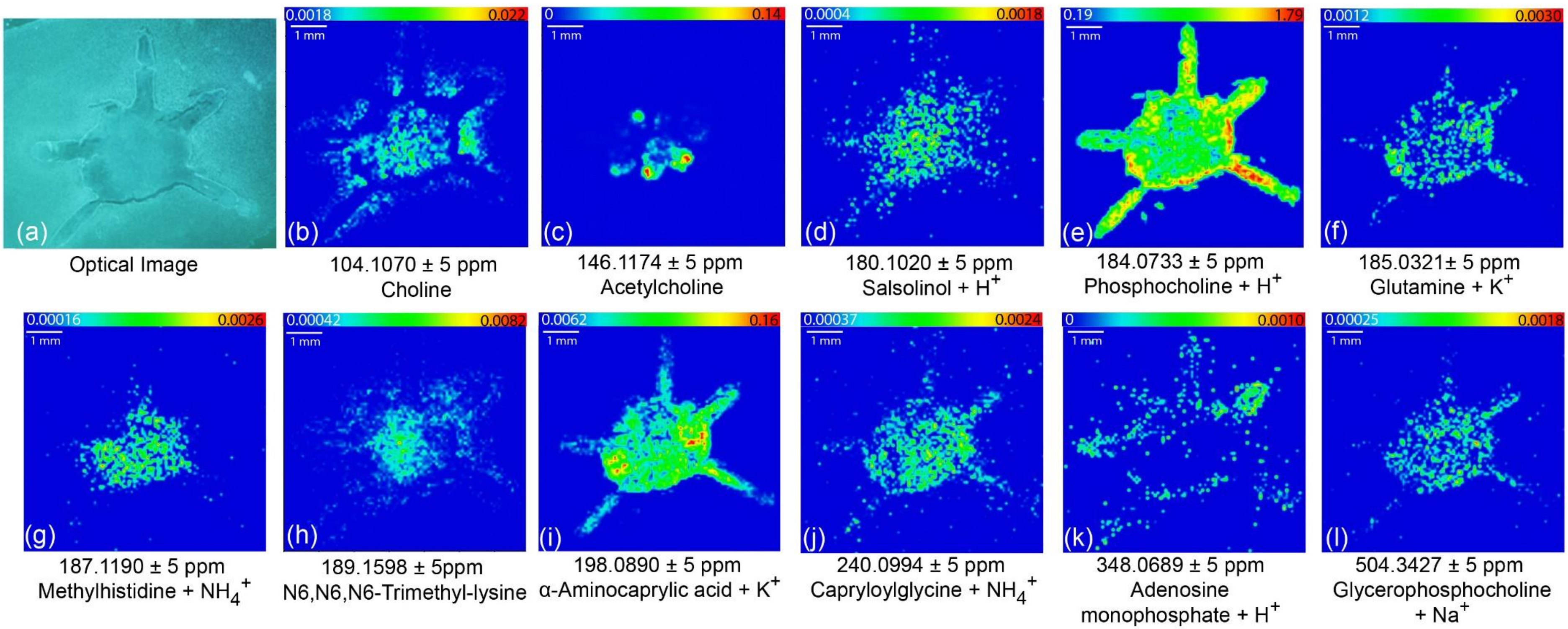
Qingjingwen Cao

Results

➢ Selected ion images from crab brain

Matrix

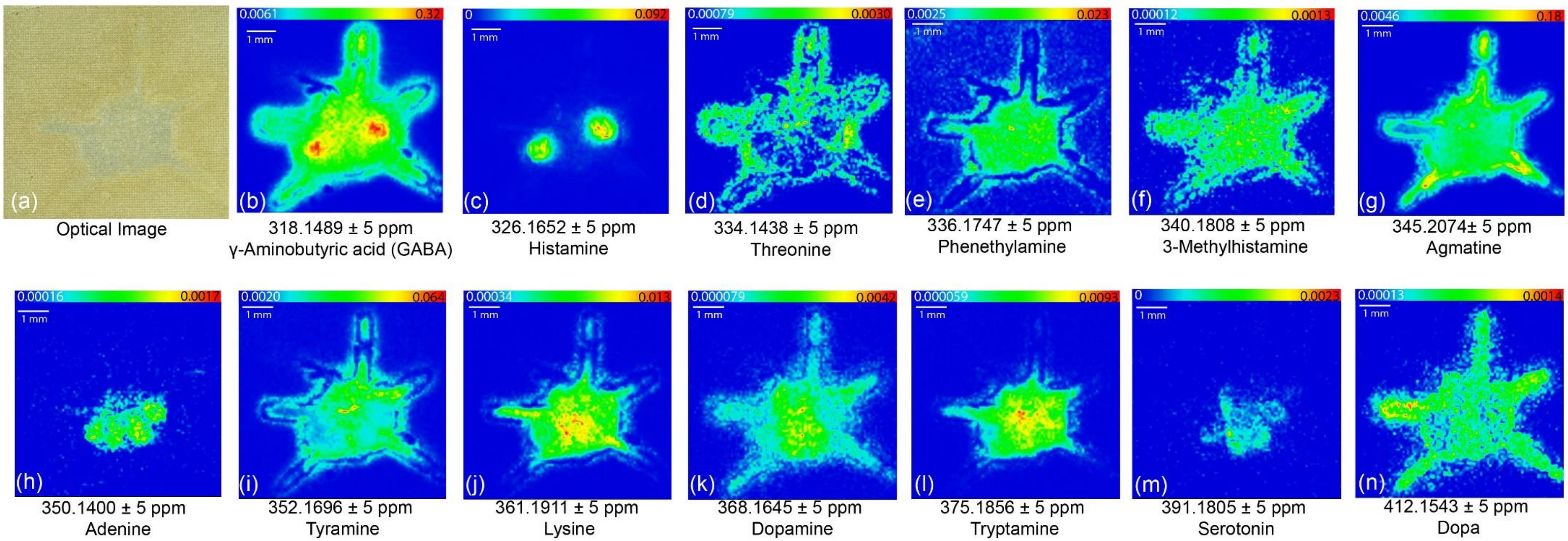
- Neurotransmitter: Acetylcholine
- Amino acids
- Lipids



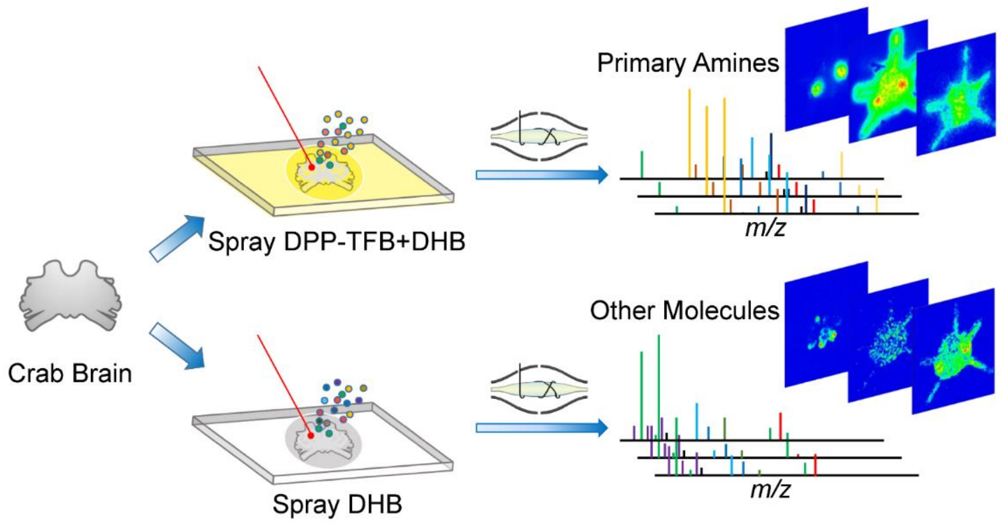
- Selected ion images from crab brain

Derivatized tissue + Matrix

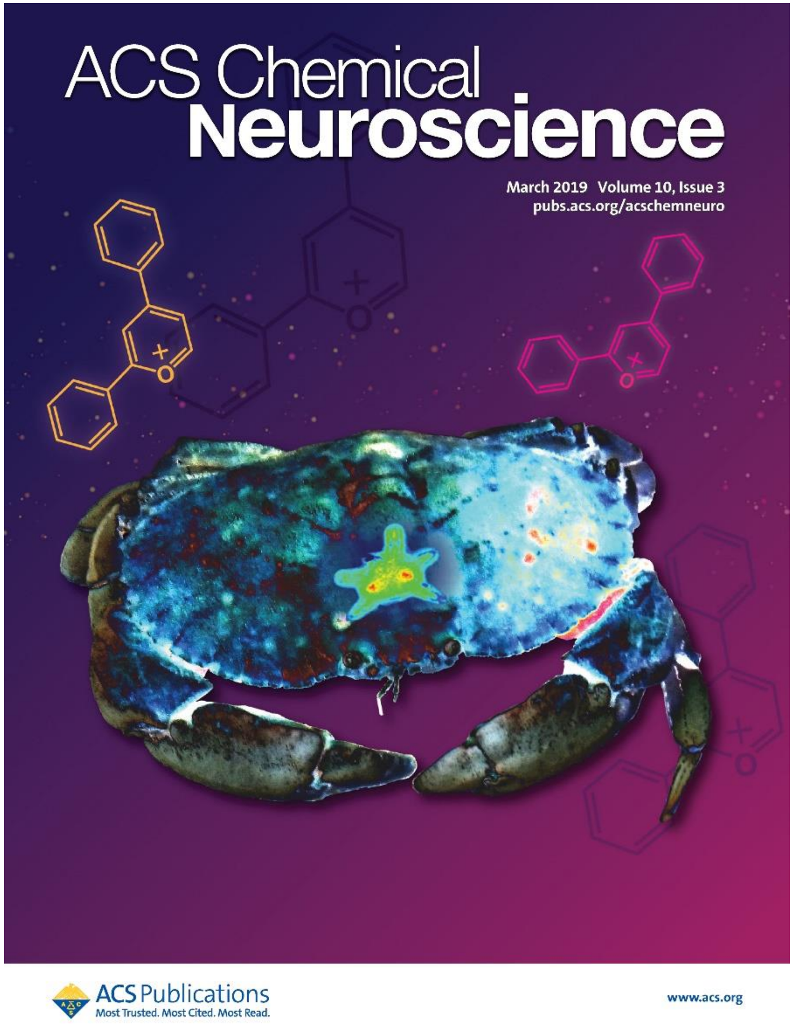
- Many more neurotransmitters!



MALDI MSI Visualization and Identification of Neurotransmitters in Crustacean Brain

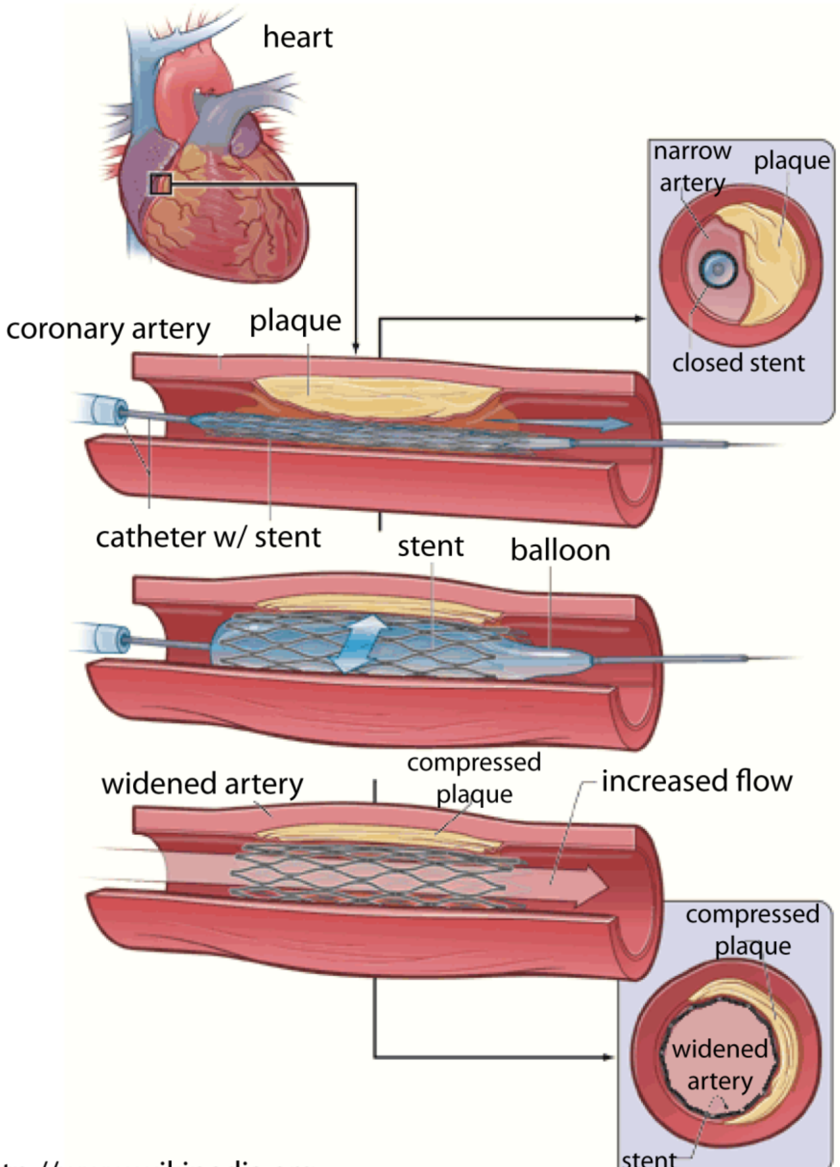


- On tissue chemical derivatization and reaction-free approaches enabled complementary signaling molecule visualization on crab brain sections via MALDI-LTQ-Orbitrap XL platform.
- Pyrylium salt served as a primary amine derivatization reagent and produced prominent signal enhancement of multiple neurotransmitters, including dopamine, serotonin, γ-aminobutyric acid and histamine that were not detected in underivatized tissues.
- Molecules with other functional groups, such as acetylcholine and phosphocholine, were directly imaged after matrix application.



Cao et al., *ACS Chem Neurosci* 2019 Mar 20;10(3):1222-1229. doi: 10.1021/acschemneuro.8b00730.

Restenosis: An unintended consequence



http://www.wikipedia.org

- Coronary atherosclerosis occurs when fatty plaques deposit on the interior wall of an artery and inhibits blood flow.
- Angioplasty is a common treatment wherein a balloon is inflated in the artery to clear the blockage.
- A stent is often placed after angioplasty to prevent future re-narrowing.

In collaboration with Dr. Craig Kent Lab

Rat Carotid Injury Model for Restenosis

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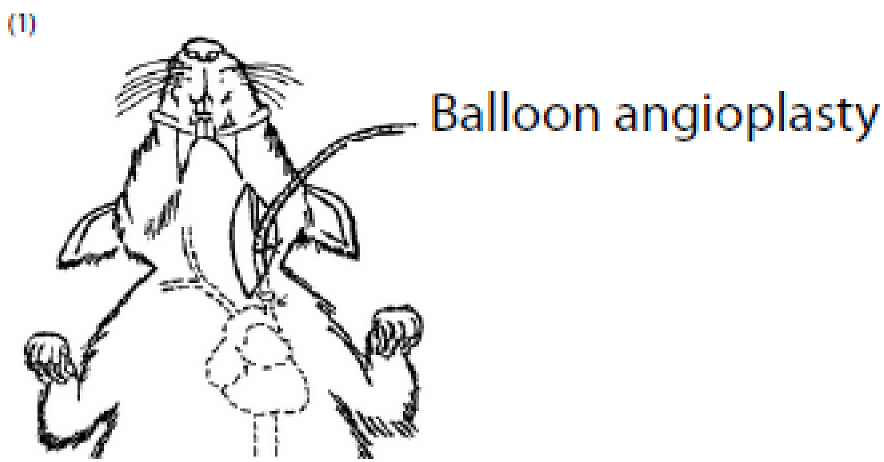
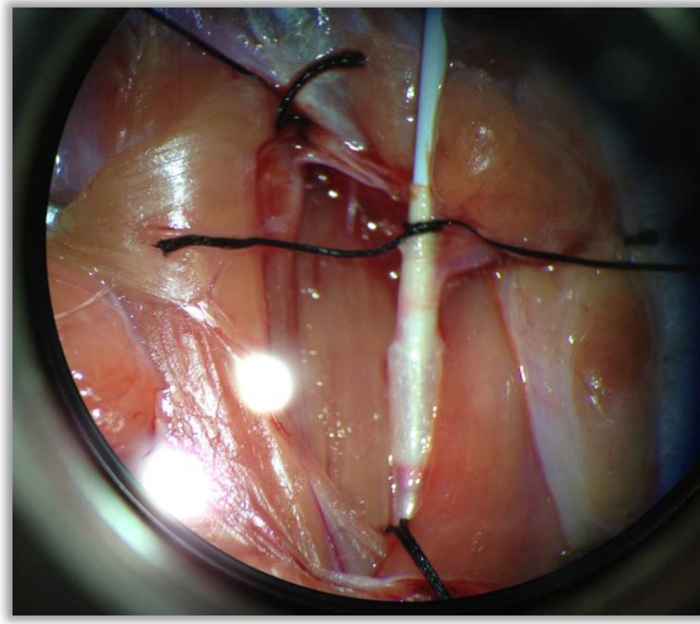
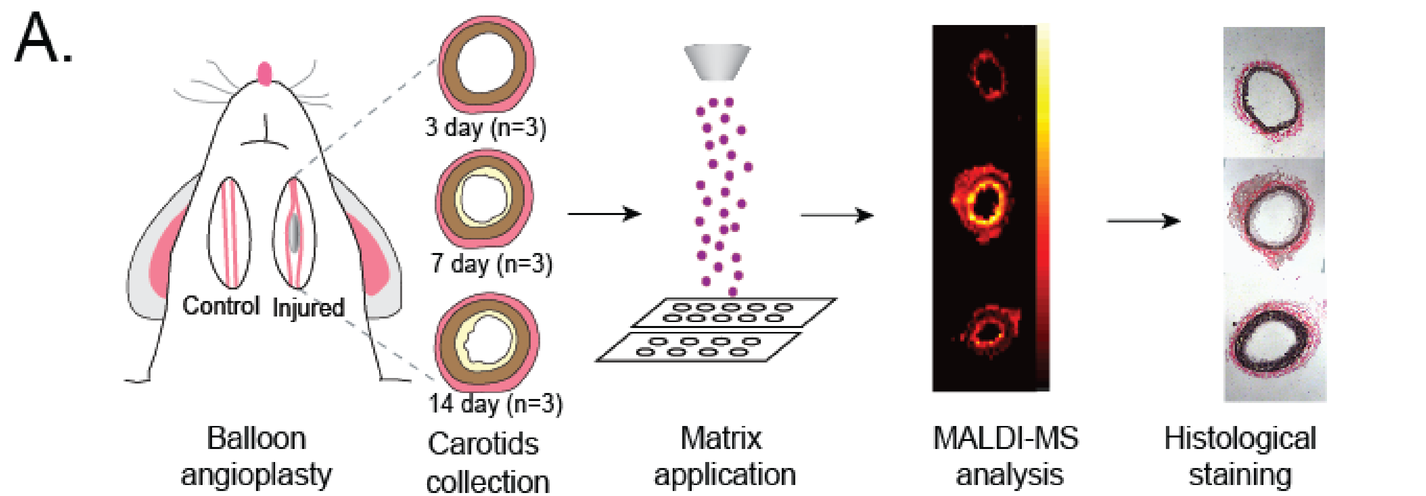




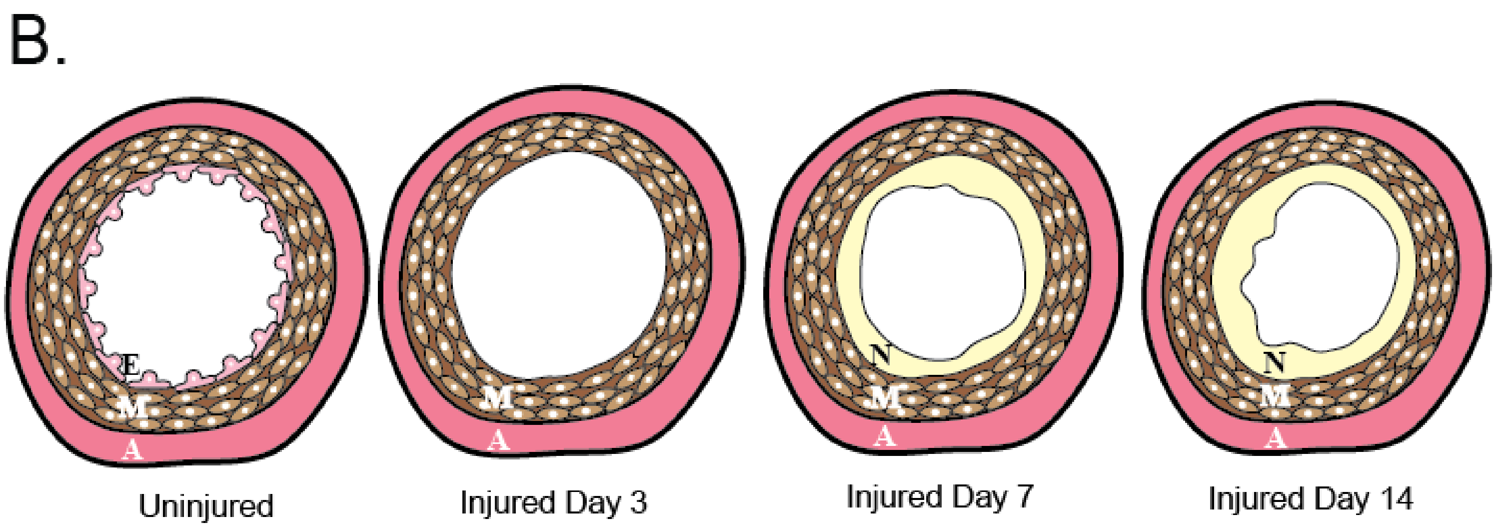
Left Carotid →	No treatment →	Self-control group
Right Carotid →	Balloon angioplasty →	Disease group

A rat model of restenosis is prepared by treating healthy rat carotid with balloon angioplasty. After surgery, the restenosis will gradually occur and rat carotid samples will be collected on 3, 7, 14 days after surgery, respectively.

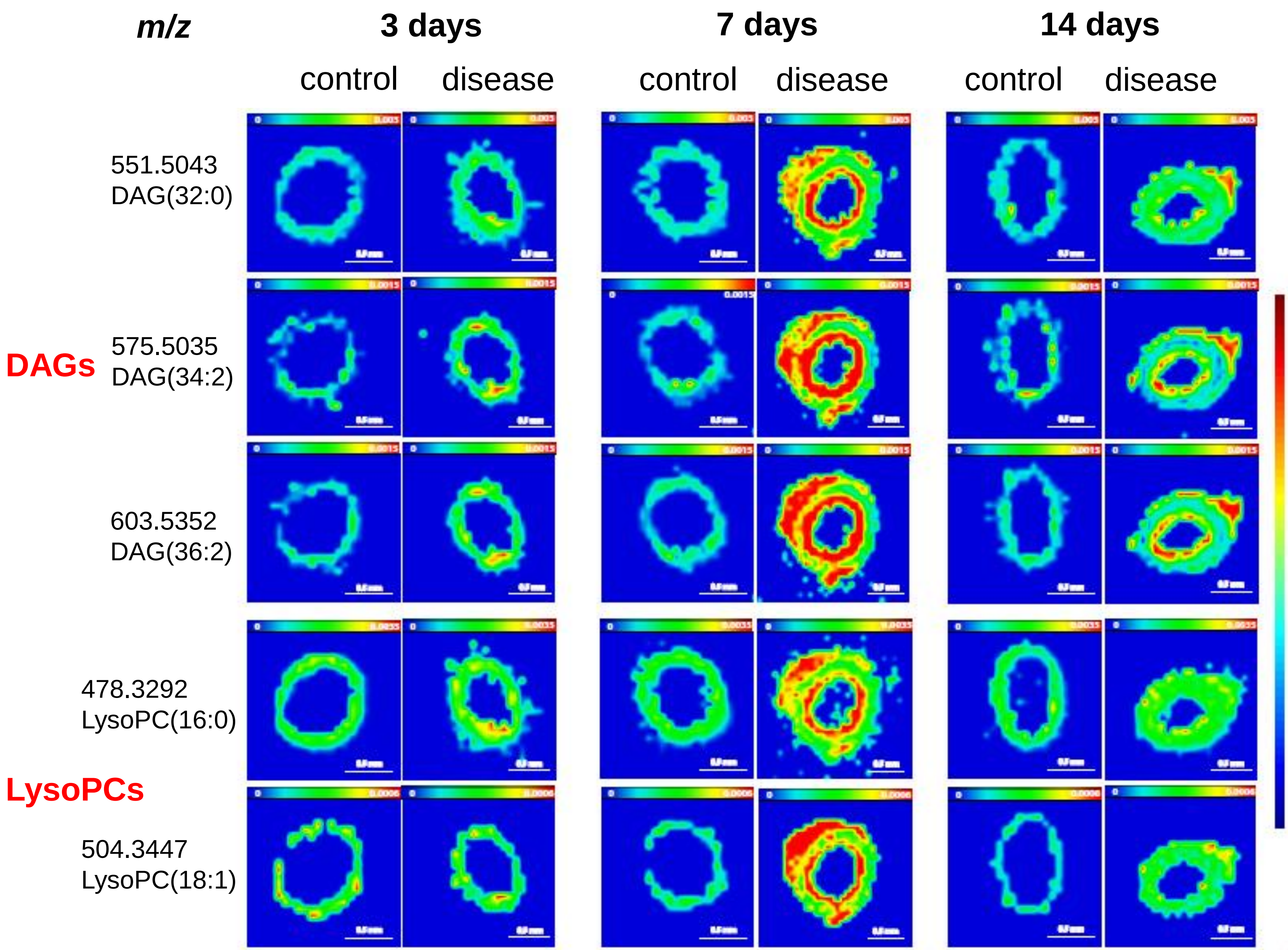
MALDI MSI Workflow to Reveal Dynamic Changes of Signaling Lipids in Restenosis



Yatao Shi

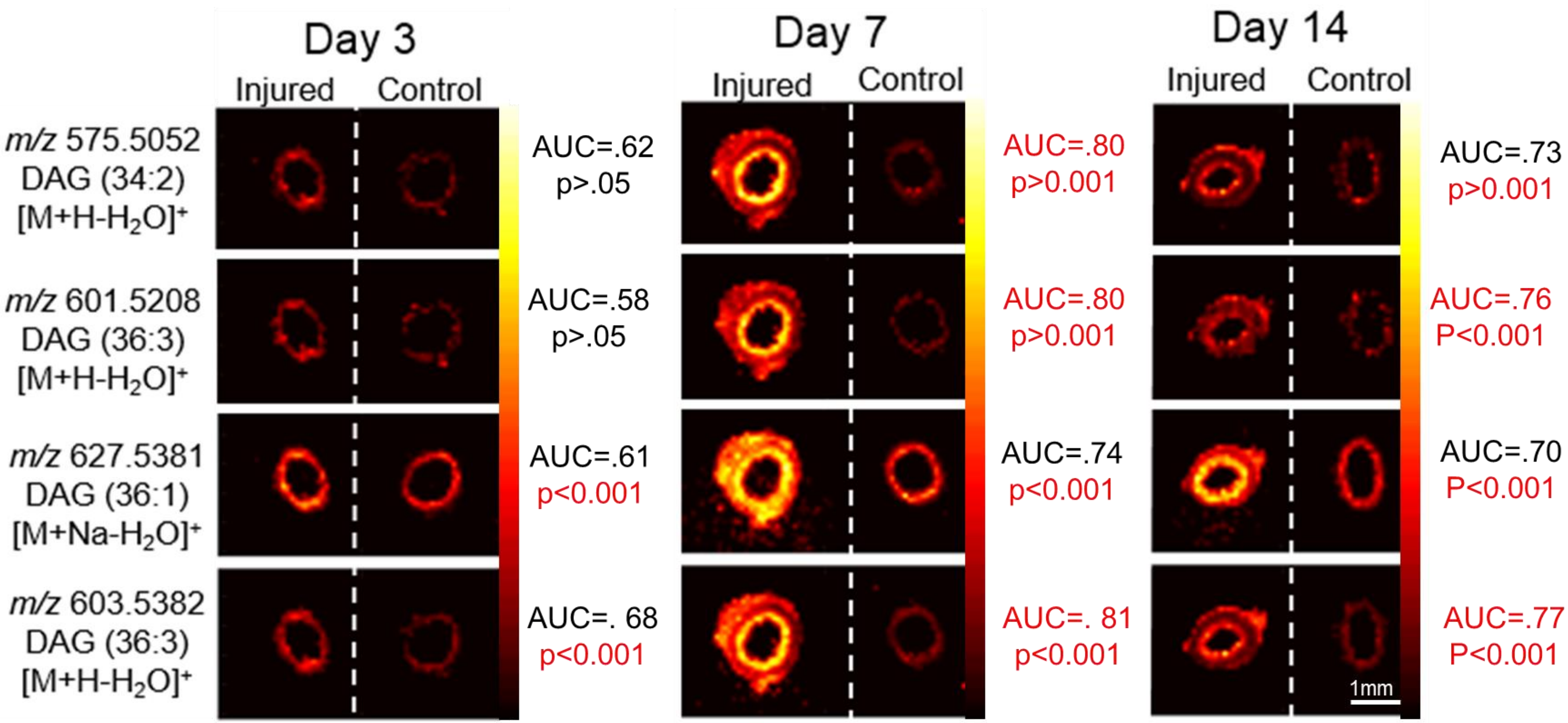


Jill Johnson



m/z
3 days
7 days
14 days
control
disease
control
disease
control
disease
DAGs
551.5043
DAG(32:0)
575.5035
DAG(34:2)
603.5352
DAG(36:2)
LysoPCs
478.3292
LysoPC(16:0)
504.3447
LysoPC(18:1)

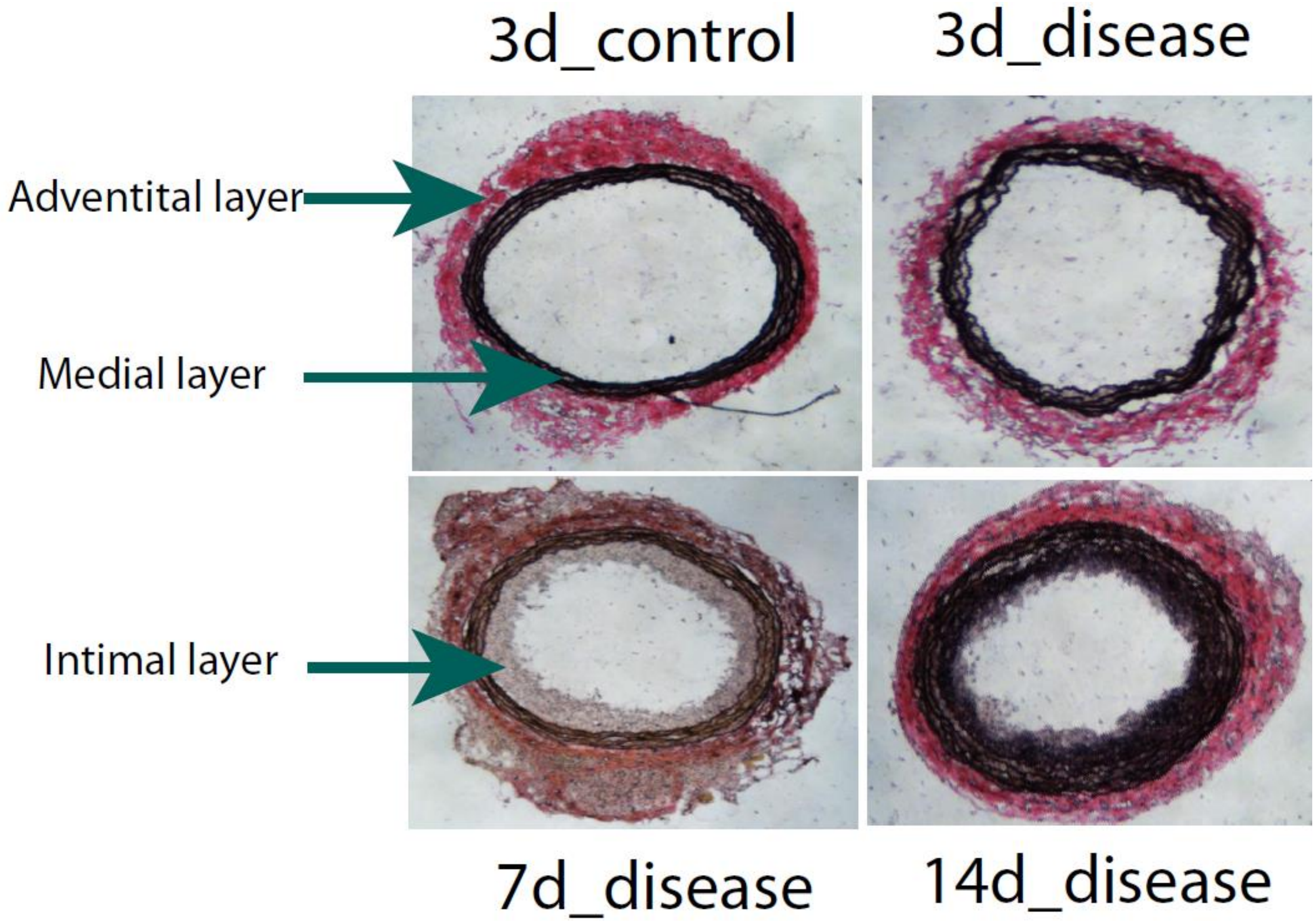
Diacylglycerols Upregulation: Statistics



[Mass Spectrometric Imaging Reveals Temporal and Spatial Dynamics of Bioactive Lipids in Arteries Undergoing Restenosis.]

Shi Y, Johnson J, Wang B, Chen B, Fisher GL, Urabe G, Shi X, Kent KC, Guo LW, Li L. *J Proteome Res.* 2019 Apr 5;18(4):1669-1678. doi: 10.1021/acs.jproteome.8b00941. Epub 2019 Mar 11.

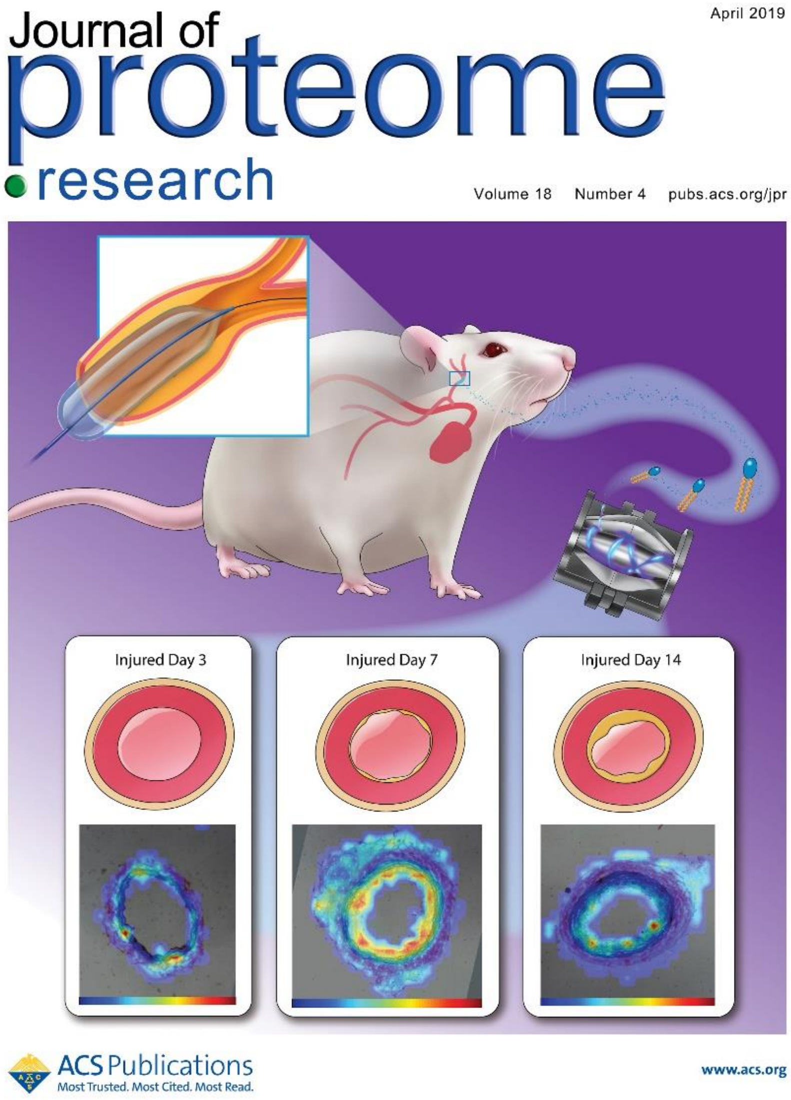
Verhoeff-van Gieson Staining

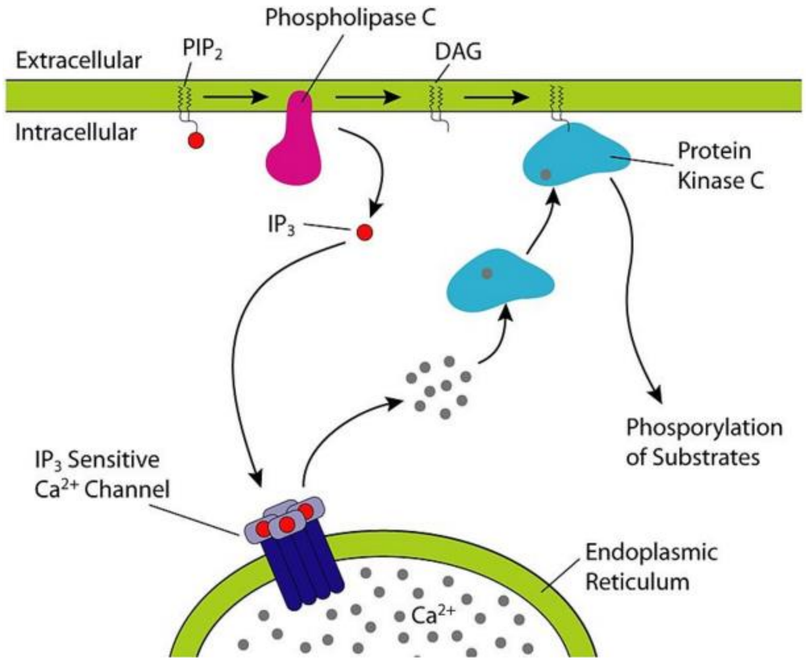


Bowen Wang

Dr. Xudong Shi

MALDI MSI Reveals Unique Neointimal Distributions of DAGs

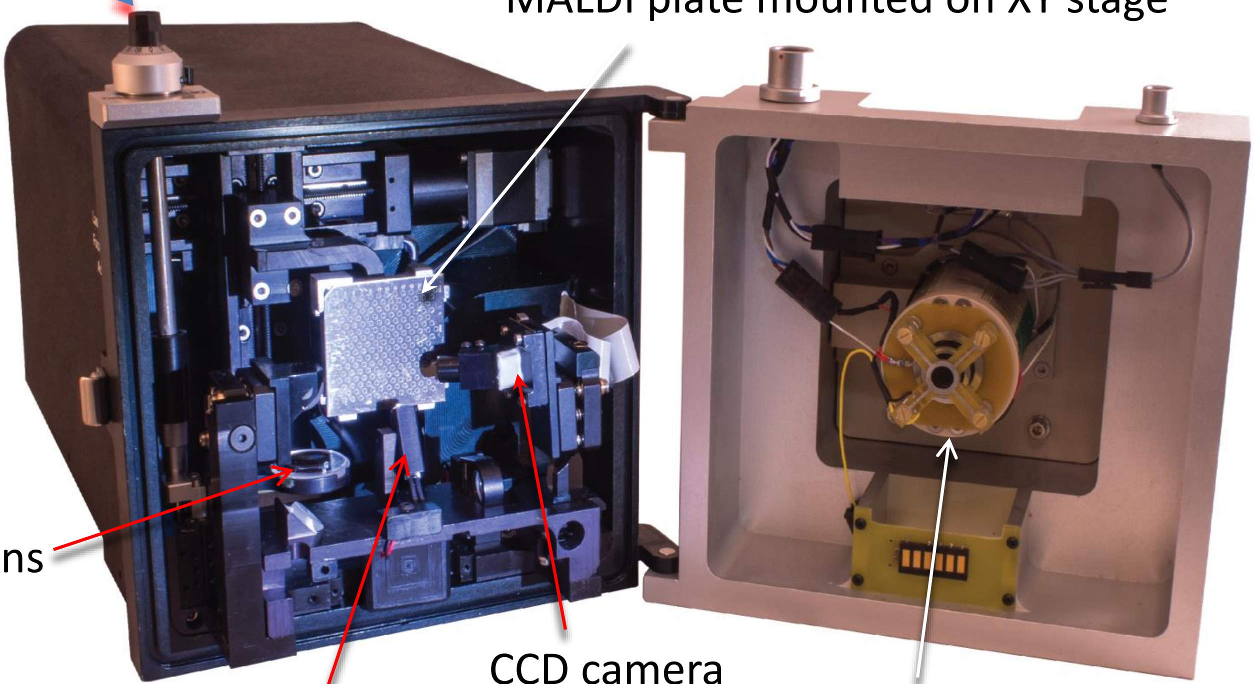




- DAGs were up-regulated during restenosis
- DAG-mediated signal pathway was proven to be involved in the process of restenosis
- LysoPCs are upregulated during the process of restenosis

Shi et al., *J Proteome Res* (2019).

SubAP-MALDI source



MassTech

A High Resolution SubAP/MALDI(ng) UHR Source Coupled to a Quadrupole-Orbitrap Platform for MSI

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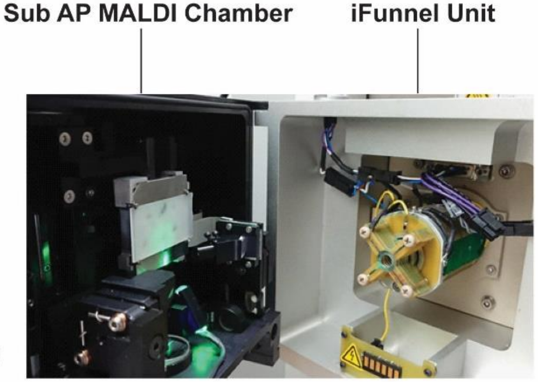
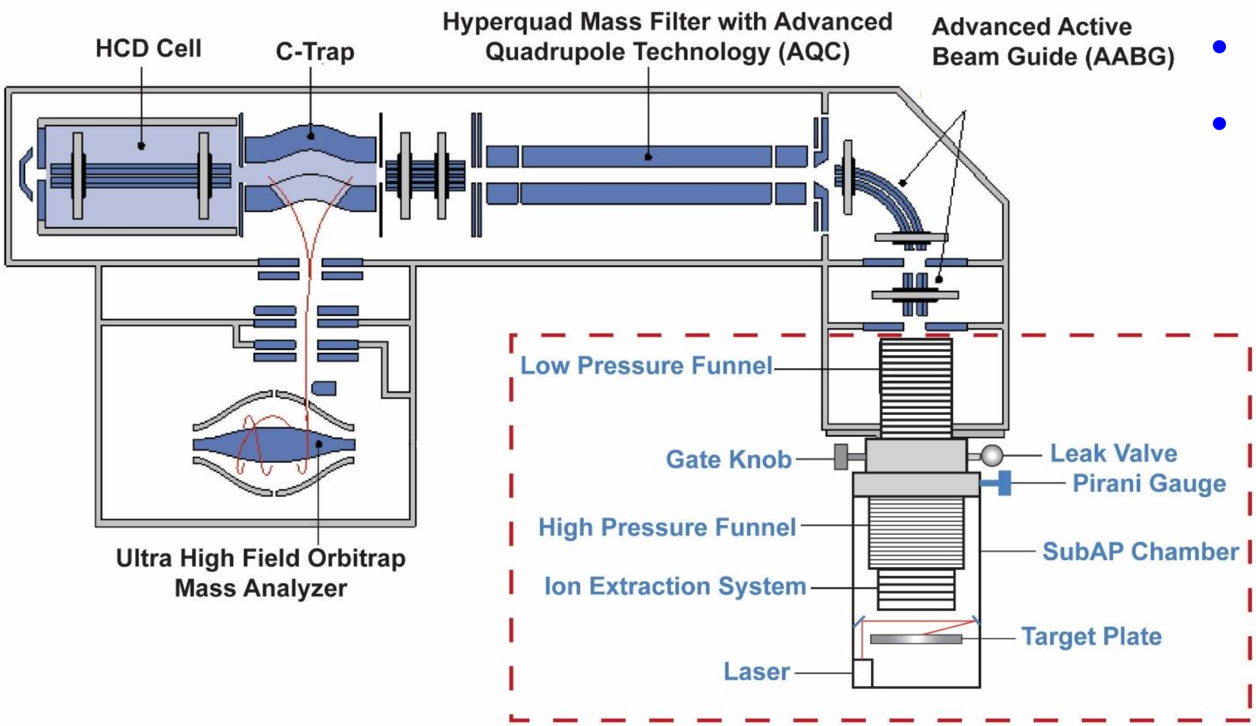
(a)

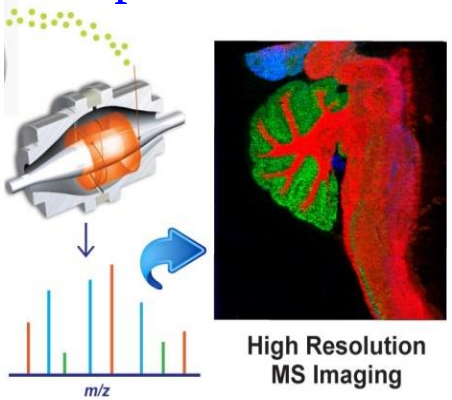
(b)



(c)



- Developed by MassTech
- Solid state laser
- <10 um laser diameter
- Up to 10 kHz firing rate
- Operated at 3~5 Torr
- Interfaced to QE-HF to achieve high resolution imaging in both space and mass



A High Resolution SubAP/MALDI-Q-Orbitrap Platform Enables *In Situ* Analysis of Biomolecules by Negative Mode Ionization and Acquisition

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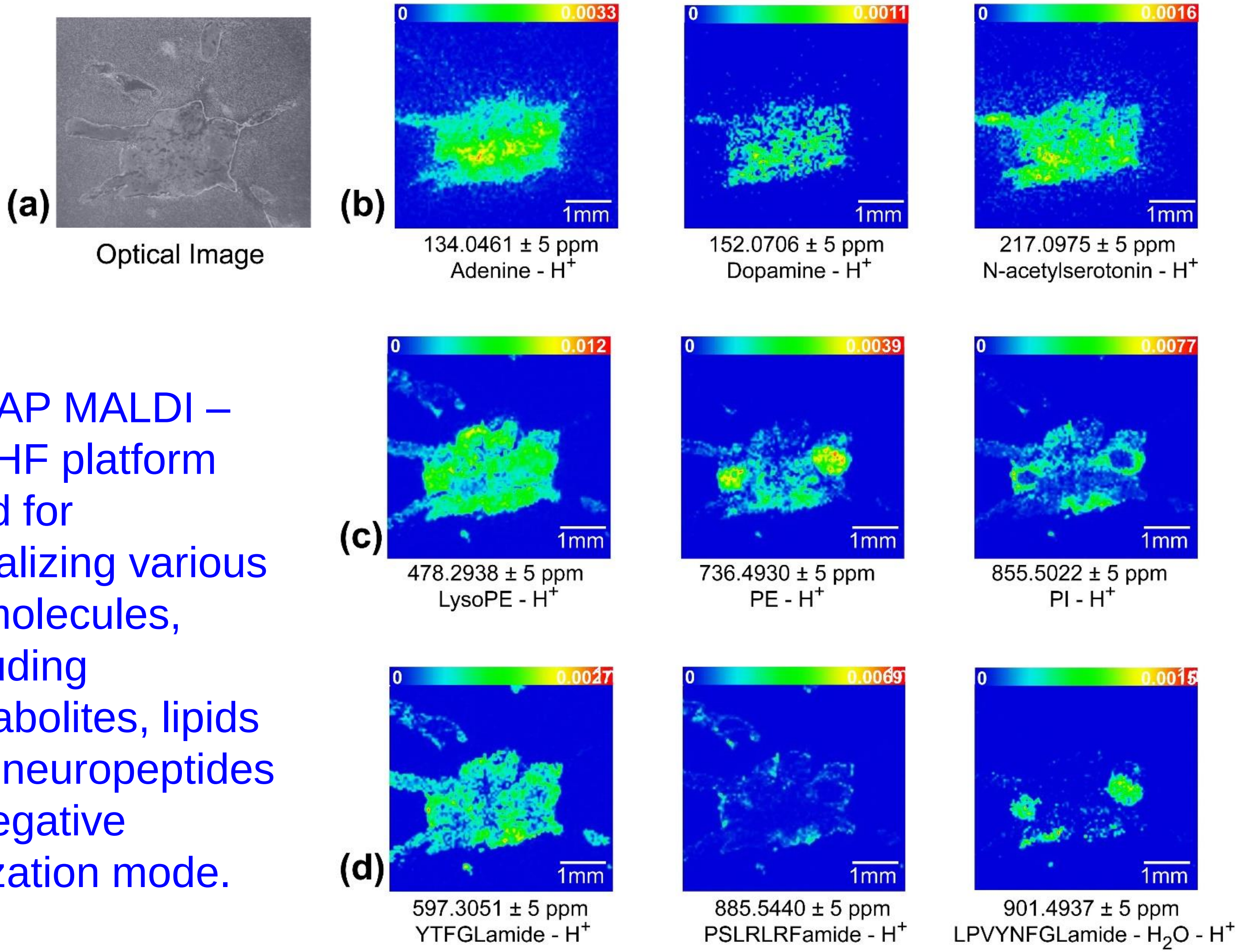

SubAP MALDI – QE HF platform used for visualizing various biomolecules, including metabolites, lipids and neuropeptides in negative ionization mode.

frontiers in Plant Science

ORIGINAL RESEARCH
published: 28 August 2018
doi: 10.3389/fpls.2018.01238

Check for updates

Comparison of Vacuum MALDI and AP-MALDI Platforms for the Mass Spectrometry Imaging of Metabolites Involved in Salt Stress in *Medicago truncatula*

Caitlin Keller[1], Junko Maeda[2], Dhileepkumar Jayaraman[3], Sanhita Chakraborty[4], Michael R. Sussman[5], Jeanne M. Harris[4], Jean-Michel Ané[2,3] and Lingjun Li[1,6]*

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Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is routinely used to determine the spatial distributions of various biomolecules in tissues. Recently, there has been an increased interest in creating higher resolution images using sources with more focused beams. One such source, an atmospheric pressure (AP) MALDI source from MassTech, has a laser capable of reaching spatial resolutions of 10 μm. Here, the AP-MALDI source coupled with a Q Exactive HF Orbitrap platform is compared to the commercial MALDI LTQ Orbitrap XL system using *Medicago truncatula* root nodules. AP-MALDI parameters, such as the S-lens value, capillary temperature, and spray voltage, were optimized on the Q Exactive-HF platform for optimal detection of plant metabolites. The performance of the two systems was evaluated for sensitivity, spatial resolution, and overall ability to detect plant metabolites. The commercial MALDI LTQ Orbitrap XL was superior regarding the number of compounds detected, as at least two times more *m/z* were detected compared to the AP-MALDI system. However, although the AP-MALDI source requires a spatial resolution higher than 10 μm to get the best signal, the spatial resolution at 30 μm is still superior compared to the 75 μm spatial resolution achieved on the MALDI platform. The AP-MALDI system was also used to investigate the metabolites present in *M. truncatula* roots and root nodules under high salt and low salt conditions. A discriminative analysis with SCiLS software

Dr. Caitlin Keller

A High Resolution AP/MALDI-Q-Orbitrap Platform Enables *In Situ* Analysis of Metabolites involved in *Medicago truncatula*

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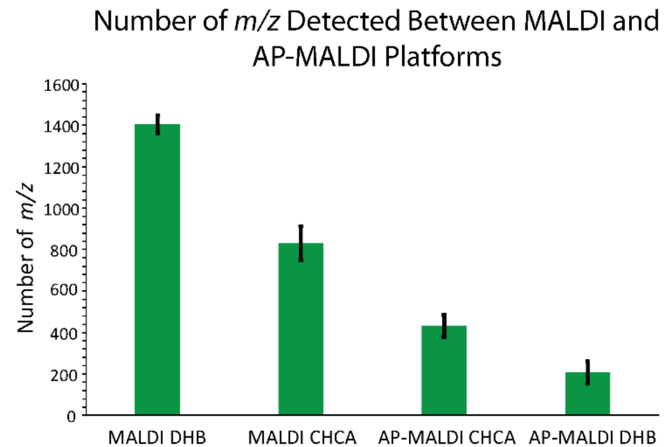
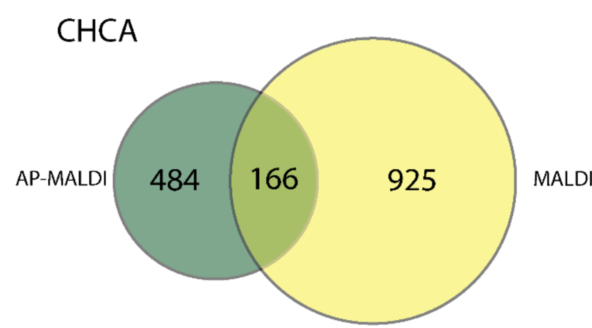
AP-MALDI-ORBITRAP-MSI

- Atmospheric pressure
- High spatial resolution

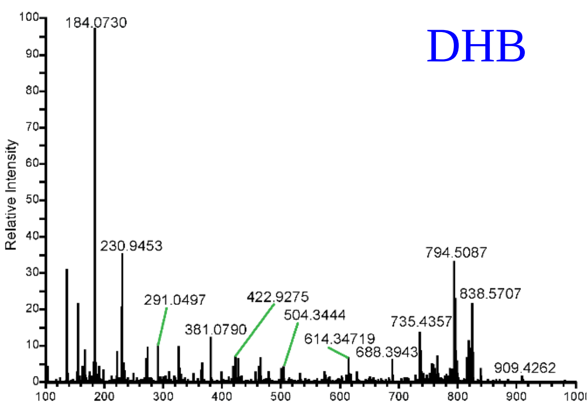
MALDI-Orbitrap-MSI

- Under vacuum
- Higher sensitivity

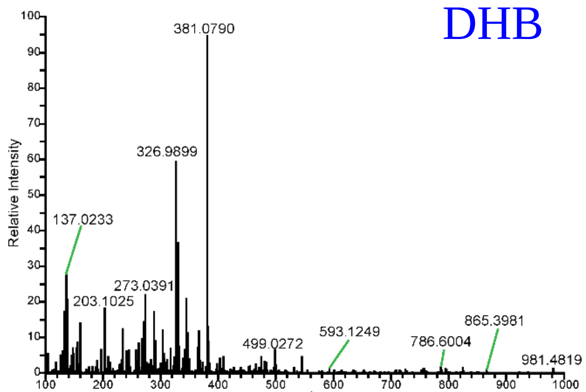




DHB

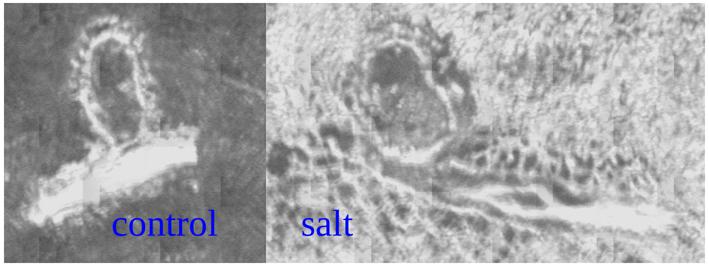


DHB

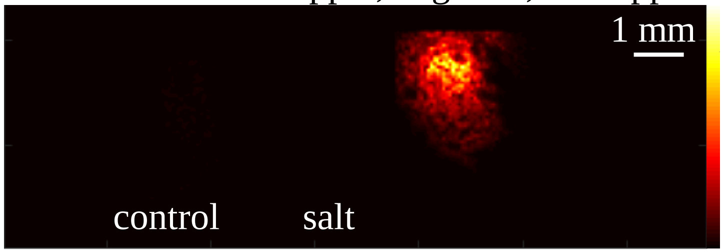


A High Resolution AP/MALDI-Q-Orbitrap Platform Enables *In Situ* Analysis of Metabolites involved in Salt Stress in *Medicago truncatula*

- Salt stress
  - Decreased plant growth
  - Poor development of symbiosis in root-nodule
  - Reduced nitrogen-fixation capacity
- Appearance of salt stress nodules: fewer, smaller, globular, white/brown



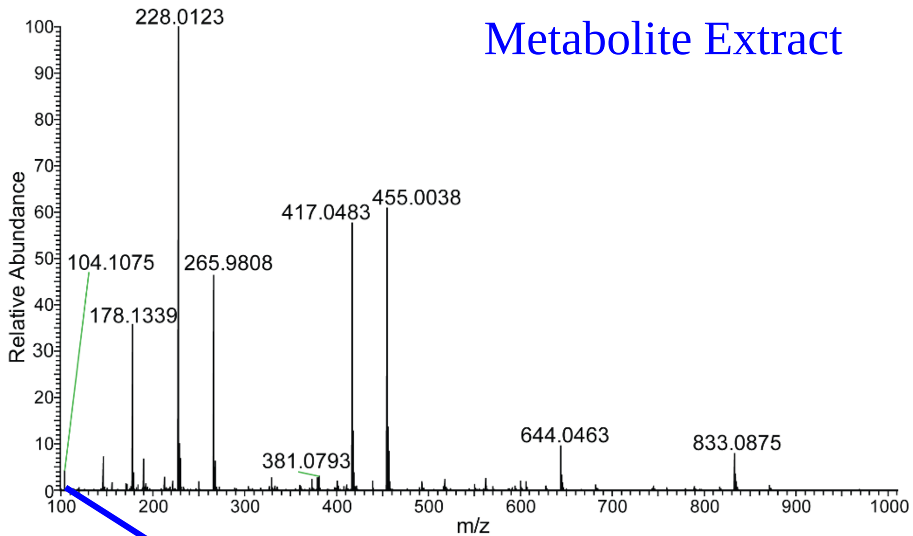
m/z 175.1186 +/- 5 ppm; Arginine, -1.81 ppm



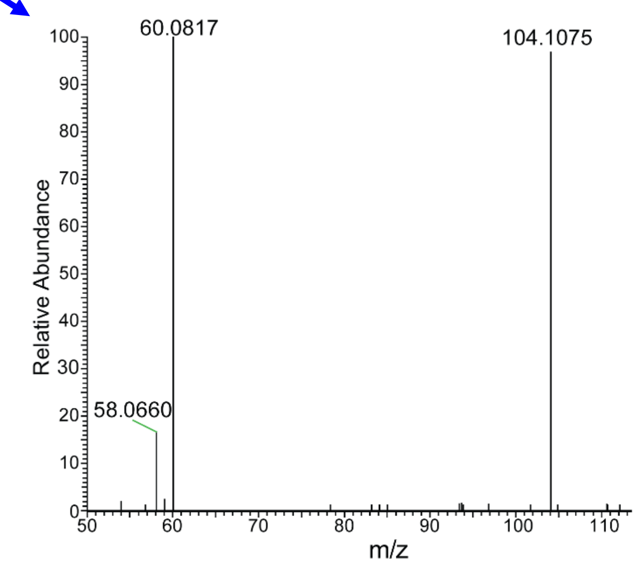
m/z; Retention time (min)	Distribution	AUC >0.75 Location	Identification; Adduct Identified	Literature Molecular Weight	Delta ppm
268.1034; 3.55	Control Nodule and Root	Root	Adenosine $[M+H]^+$	267.0968	-2.53
175.1186; 1.04	Salt Nodule	Nodule	Arginine $[M+H]^+$	174.1117	-1.81
965.5076; 20.46	Salt Root and Outer Nodule	Root	Soyasaponin I $[M+Na]^+$	942.5188	-0.44

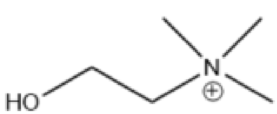
A High Resolution Sub-AP/MALDI-Q-Orbitrap Platform Enables Identification and *In Situ* Analysis of Metabolites in *Medicago Truncatula*

Metabolite Extract



MS/MS
HCD

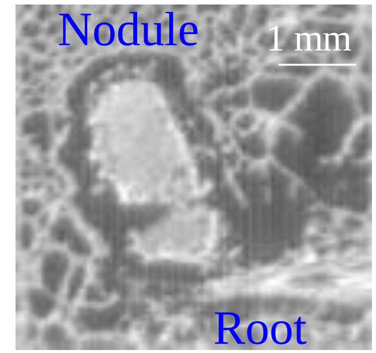




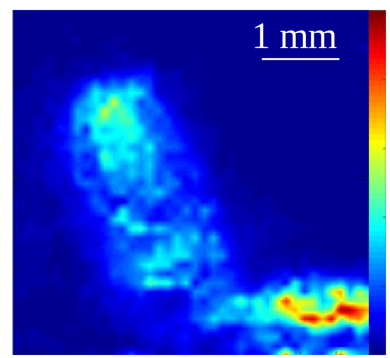
Choline

Imaging

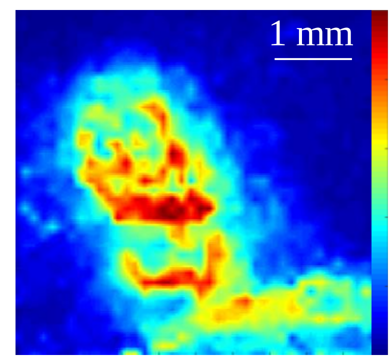
- 50 µm raster
- 16.91 minutes to acquire 2200 pixels



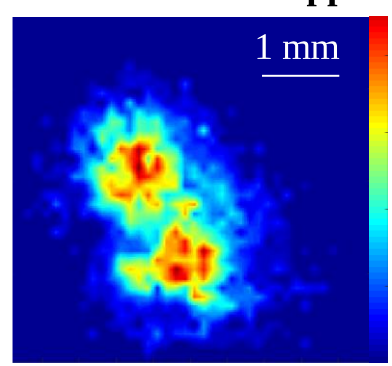
m/z **104.1077 +/- 5 ppm**



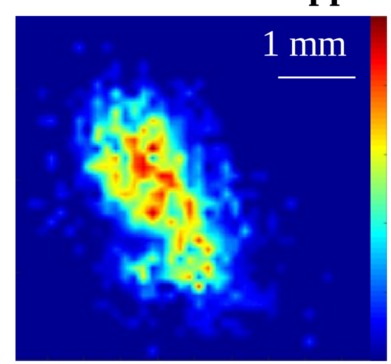
m/z **455.0043 +/- 5 ppm**



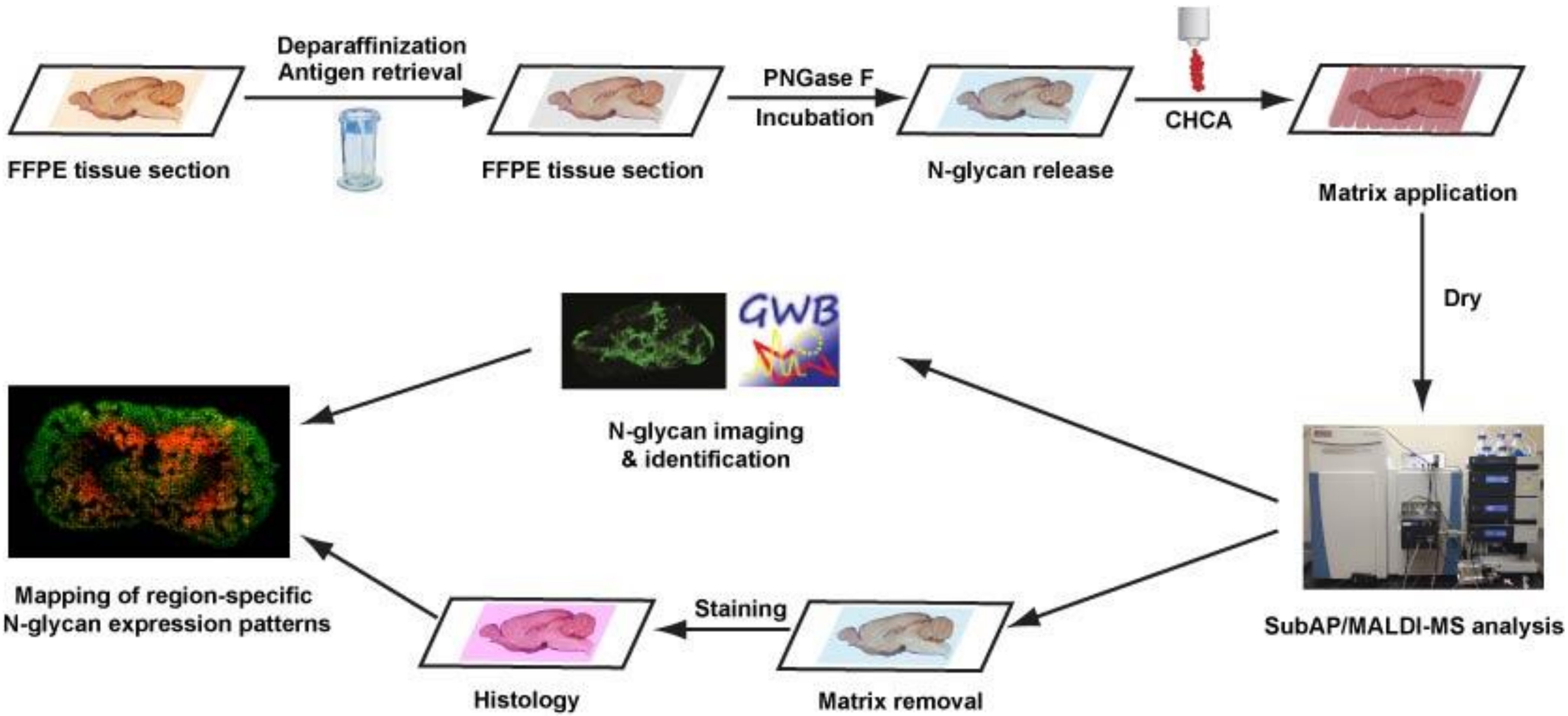
m/z **233.0525 +/- 5 ppm**



m/z **241.0691 +/- 5 ppm**

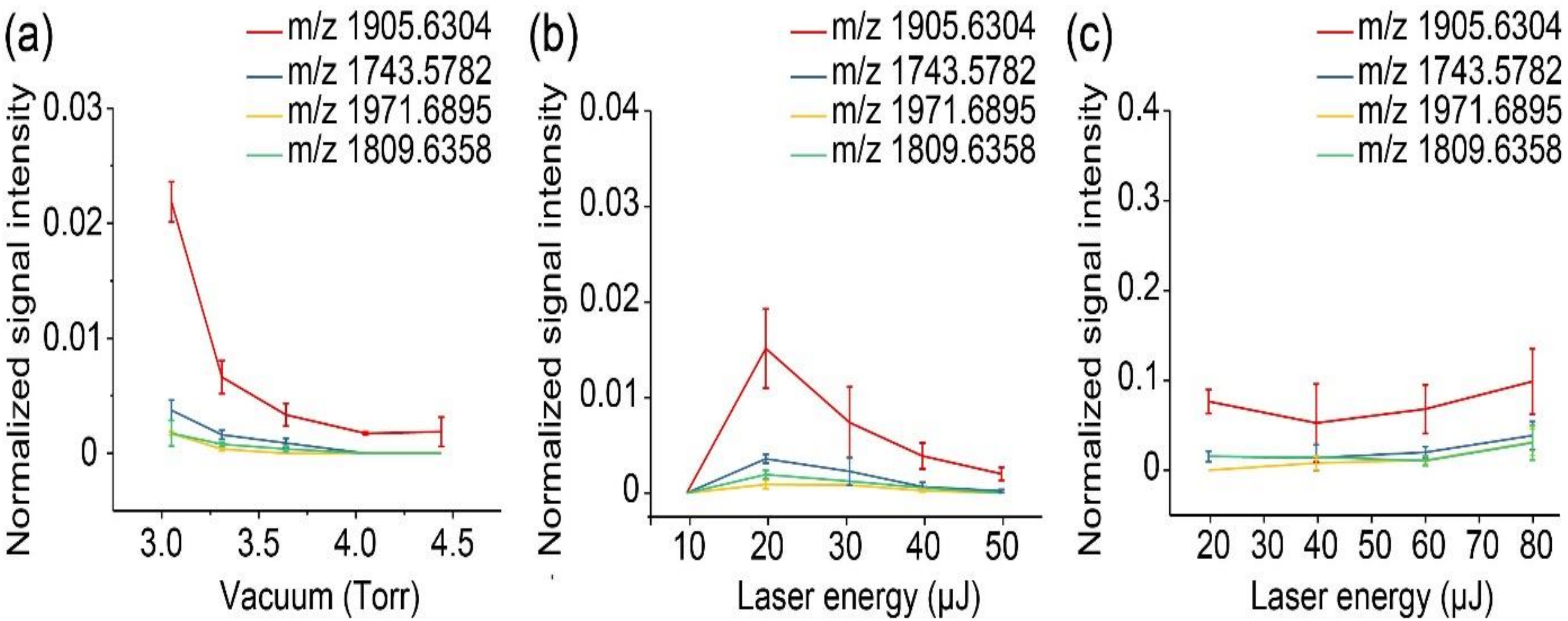


In Situ N-glycan Imaging of Mouse Brain with SubAP/MALDI-Q-Orbitrap Platform



SubAP/MALDI imaging of N-glycans from formalin-fixed, paraffin-embedded (FFPE) tissue sections

Optimization of the SubAP/MALDI Source Parameters for N-linked Glycan Analysis



(a) TIC-normalized signal intensities of N-glycans significantly dropped following the decrease of source vacuum; (b) When CHCA used as matrix, laser energy of 20μJ showed the highest TIC-normalized N-glycan signal intensities; (c) TIC-normalized N-glycan signal intensities were independent of laser energy when DHB was used.

In Situ N-glycan Imaging of Mouse Brain with SubAP/MALDI-Q-Orbitrap Platform

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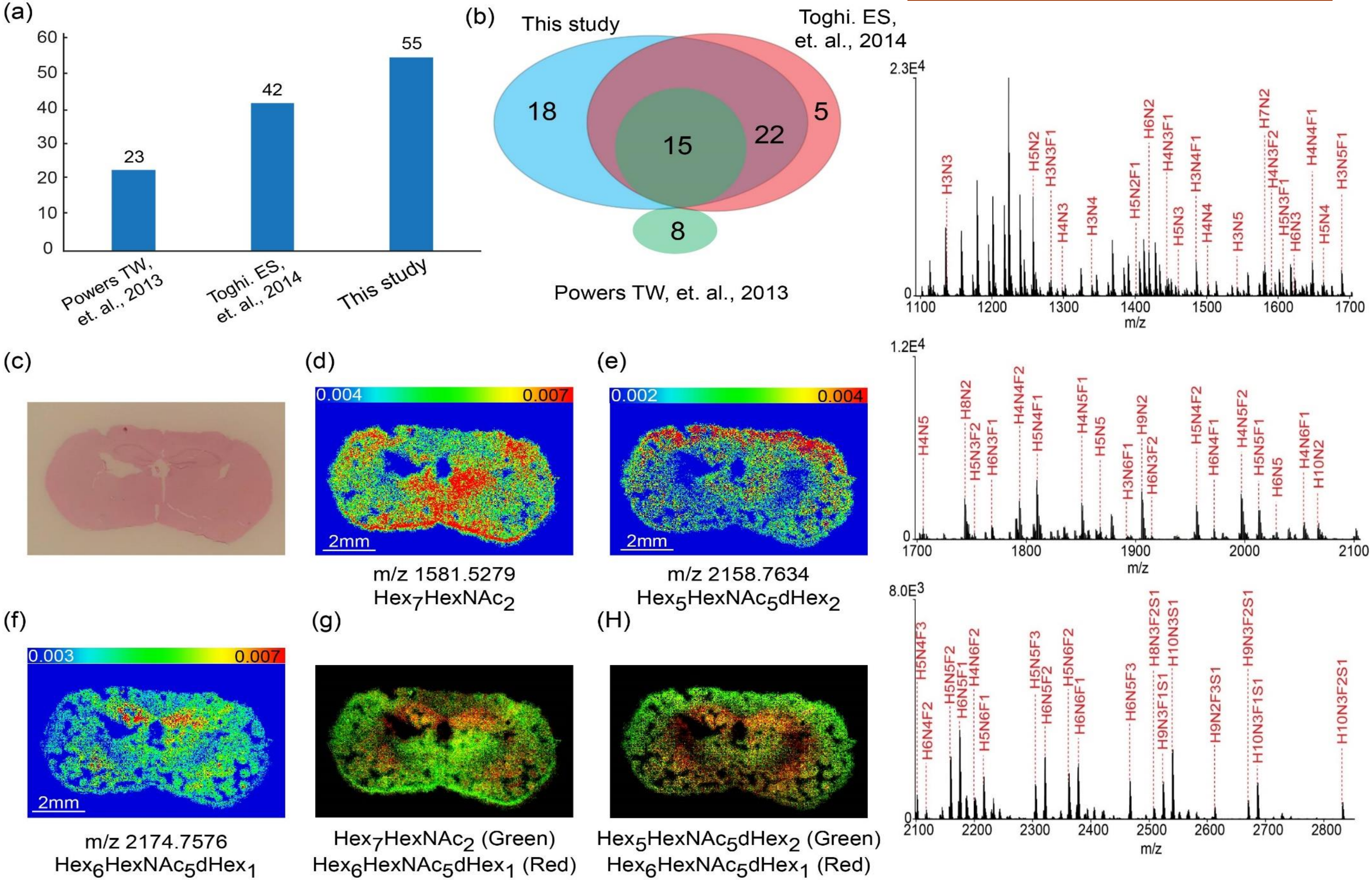
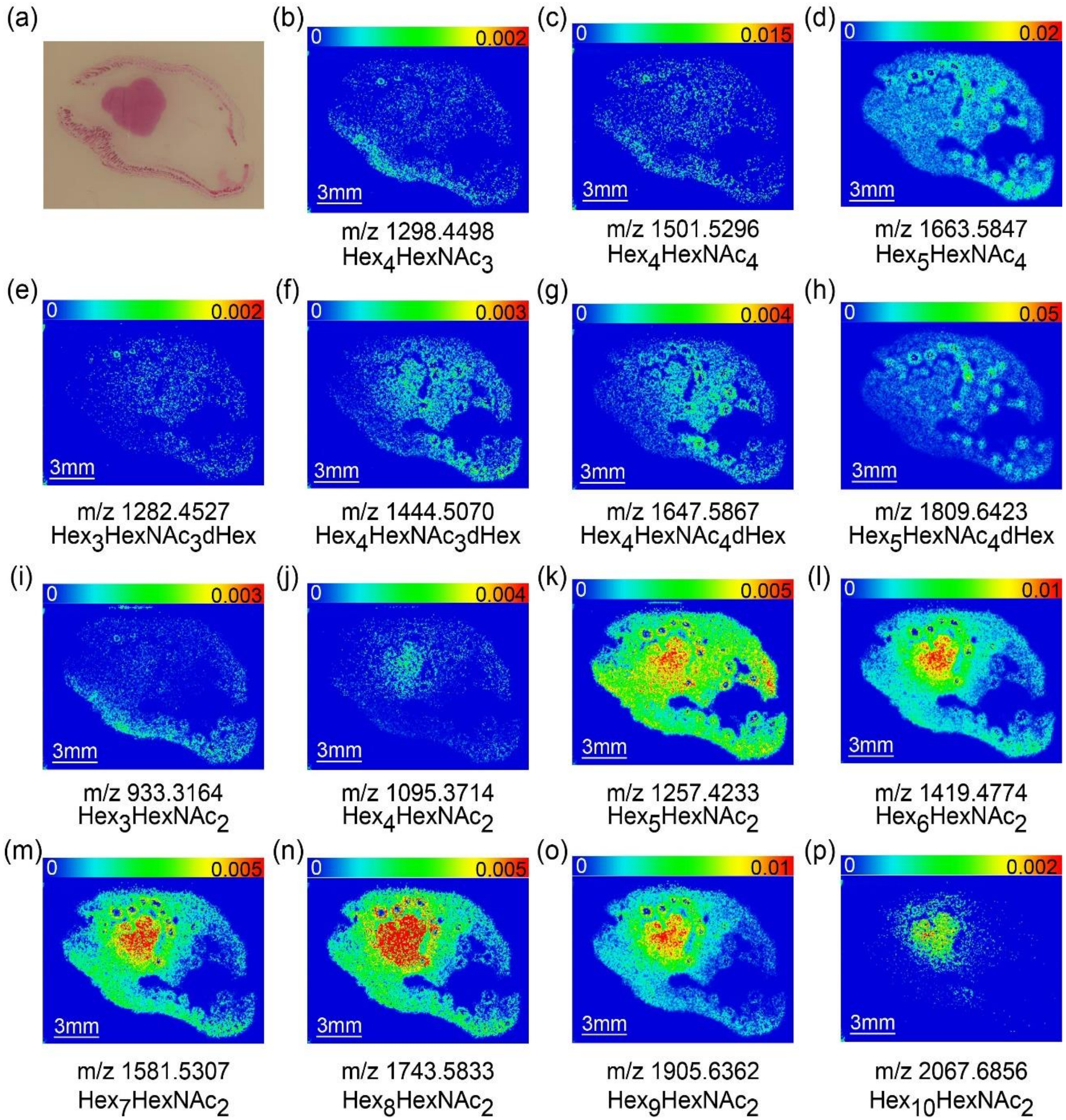
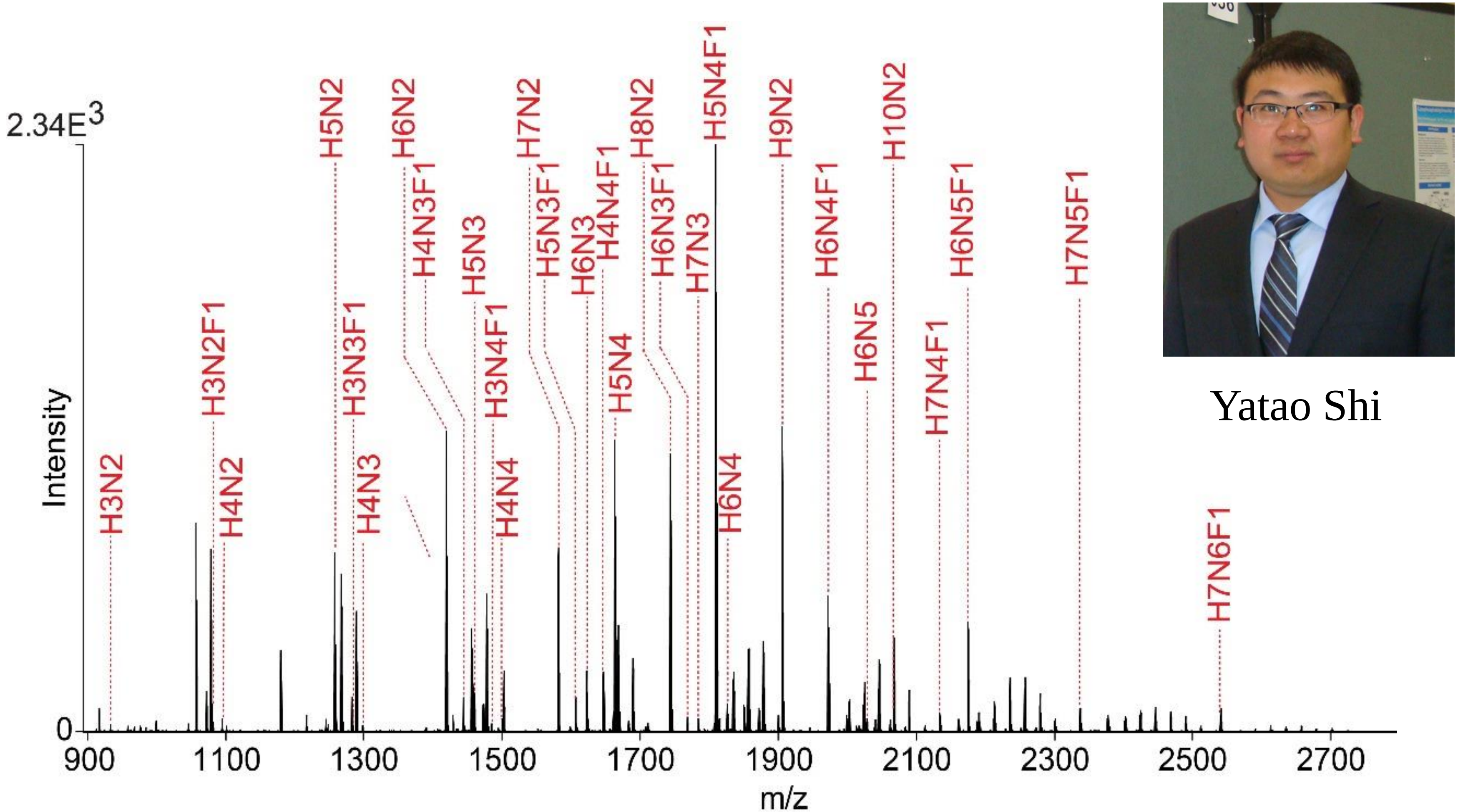


MSI of N-Glycan Analysis Reveals Distinct Distribution Patterns for Ovarian Cancer



Images of N-glycans showing different spatial distribution patterns on FFPE mouse tissue section with ovarian cancer. (a) H&E stained FFPE mouse tissue section with ovarian cancer. (b-h) Complex N-glycans showed similar distribution in cancer area in comparison to peripheral area; (i-p) High mannose N-glycans accumulated in cancer area except $Hex_3HexNAc_2$

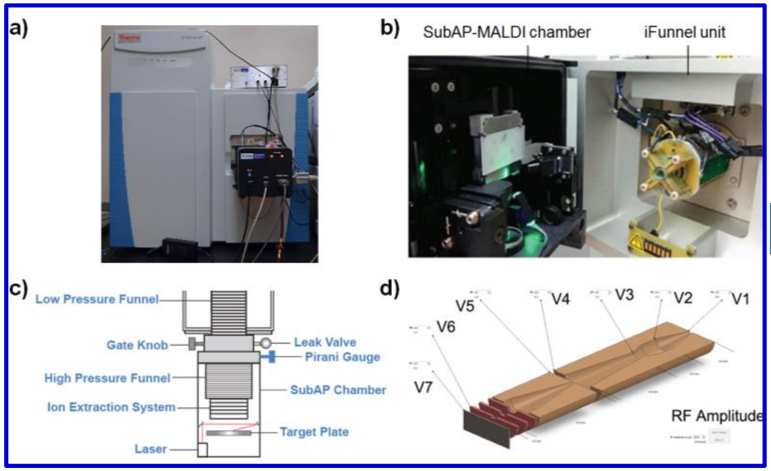
N-glycans detected from FFPE mouse tissue section with ovarian cancer



Yatao Shi

N-glycans detected from FFPE mouse tissue section with ovarian cancer. H: Hexose; N; N-Acetylglucosamine; F: Fucose; S: Sialic acid

AP-MALDI Sensitivity: SubAP + Ion Funnel + Optimization



SubAP-MALDI Platform

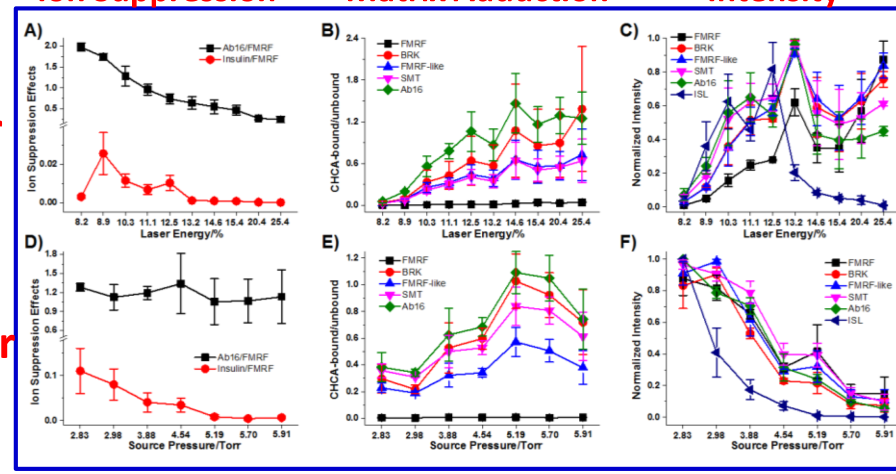
Laser

Gas/Torr

Ion Suppression

Matrix Adduction

Intensity

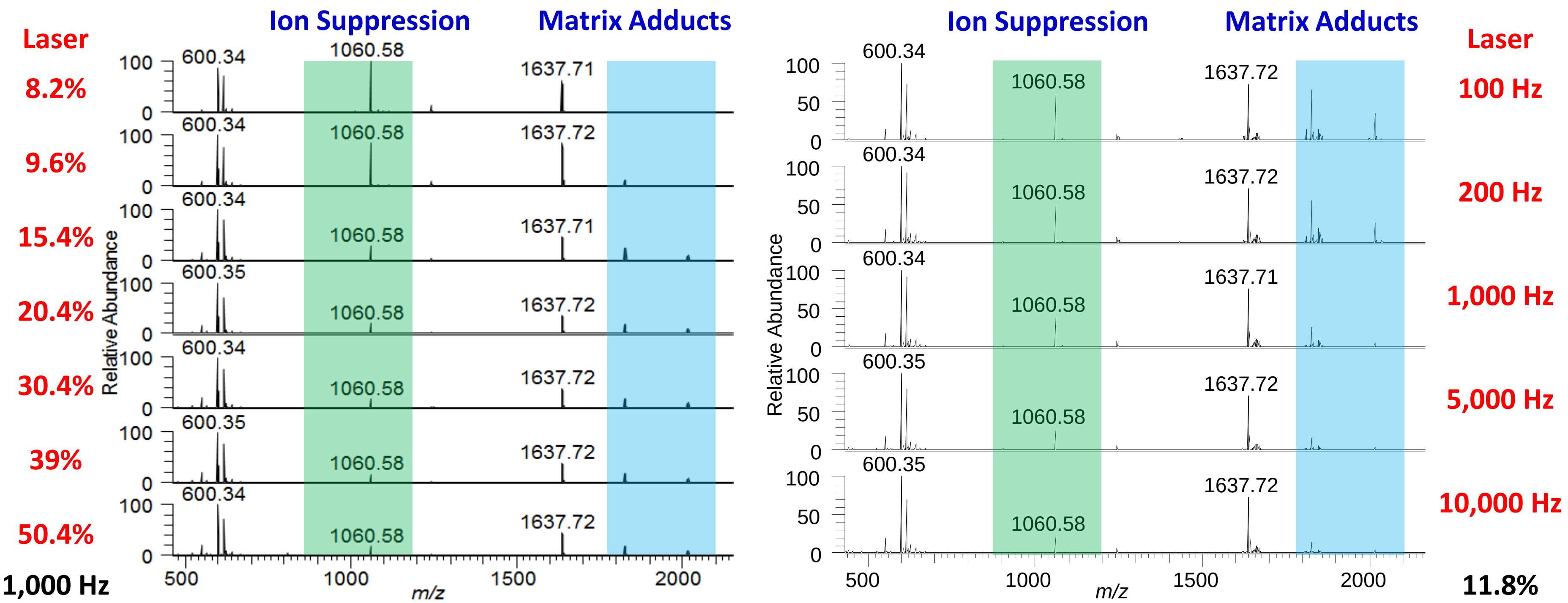


Optimization

- SubAP-MALDI was employed as a model system for AP-MALDI sensitivity study, due to its feasibility in parameter optimization.
- The sensitivity/performance of SubAP-MALDI system is laser energy- and gas pressure-dependent.
- In addition, laser firing frequency and ion funnel voltages are other important parameters to optimize.

Li, G., Cao, Q., Liu, Y., DeLaney, K., Tian, Z., Moskovets, E., & Li, L. (2019). Characterizing and alleviating ion suppression effects in atmospheric pressure matrix-assisted laser desorption/ionization. *Rapid Communications in Mass Spectrometry*, 33(4), 327-335.

SubAP-MALDI: Ion Suppression + Matrix Adducts



- Neuropeptide mixtures were used to observe the frequency- and laser energy-dependency of ion suppression effects and matrix adduction effects.
- Upon increasing laser energy, both ion suppression effects and matrix adduction increase.
- Upon increasing laser frequency, ion suppression increases but matrix adduction decreases.
- Optimized laser: frequency of ~1,000 Hz, energy less than ~15% (CHCA).

Li, G., Cao, Q., Liu, Y., DeLaney, K., Tian, Z., Moskovets, E., & Li, L. (2019). Characterizing and alleviating ion suppression effects in atmospheric pressure matrix-assisted laser desorption/ionization. *Rapid Communications in Mass Spectrometry*, 33(4), 327-335.

AP-MALDI imaging: reproducible lipid analysis of crab brain tissue section (#1)

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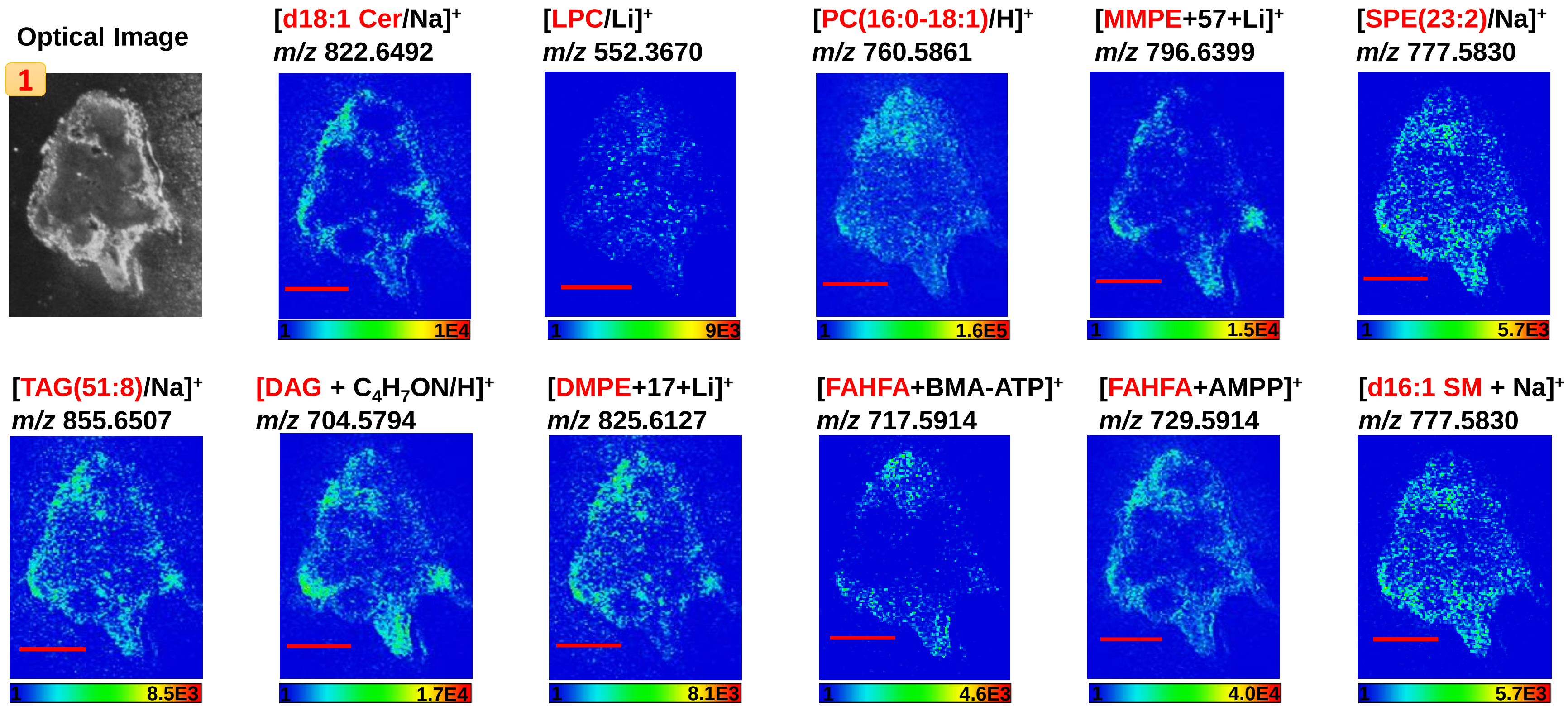

Lipid AP-MALDI-MSI, all ions selected with 10 ppm tolerance. Scale bar, 1 mm. Step size: 30 µm. Images are generated with the normalization to raw data in Thermo ImageQuest. In total, **68 lipids** are identified with accurate mass match, isotopic distribution, charge state comparison and S/N control (>3). **CHCA**, 20171206

AP-MALDI imaging: reproducible lipid analysis of crab brain tissue section (#2)

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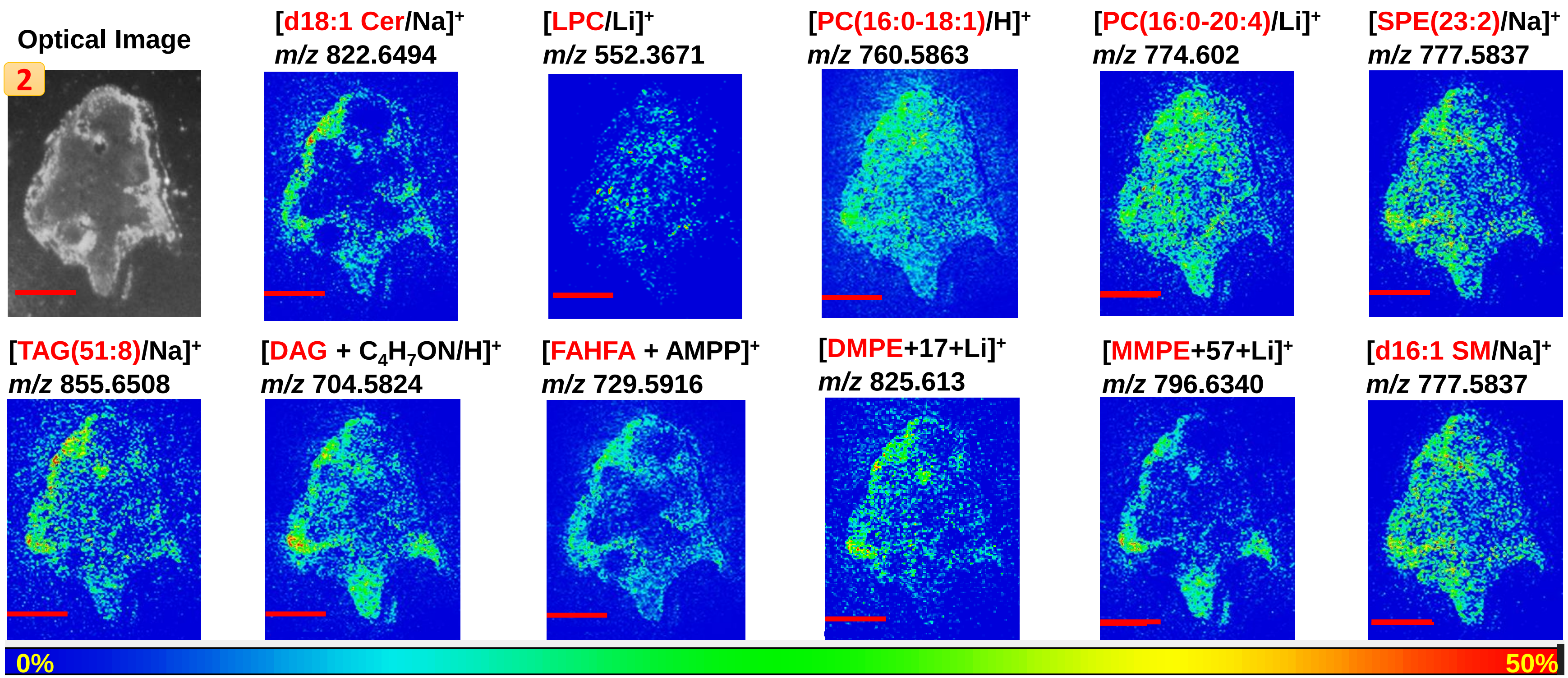


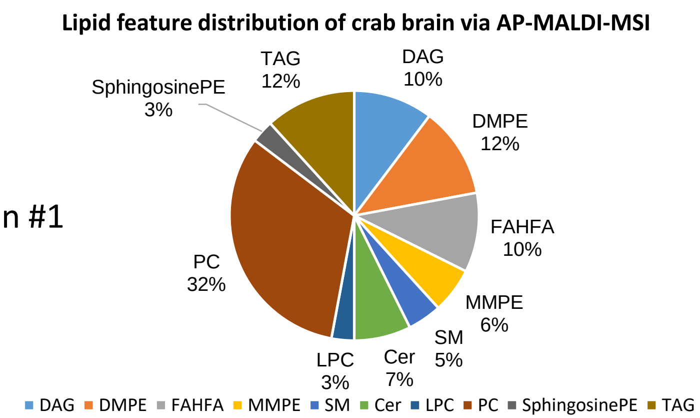
Lipid AP-MALDI-MSI, all ions selected with 10 ppm tolerance. Scale bar, 1 mm. Step size: 30 µm. Images are generated with the normalization to raw data in Thermo ImageQuest. In total, **77 lipids** are identified with accurate mass match, isotopic distribution, charge state comparison and S/N control (>3). **CHCA**, 20171206

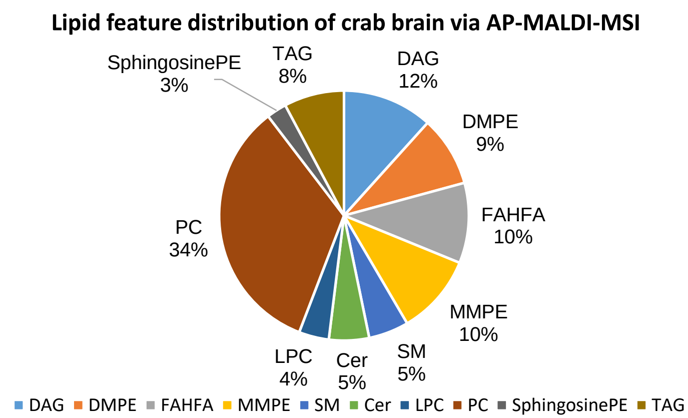
AP-MALDI: reliable/reproducible lipid imaging of crab brain tissue

Table 1. Lipid AP-MALDI-MSI analysis of crab brain tissue.

Tissue Section #	DAG	DMPE	FAHFA	MMPE	SM	Cer	LPC	PC	SphingosinePE	TAG	SUM
S1	7	8	7	4	3	5	2	22	2	8	**68**
S2	9	7	8	8	4	4	3	26	2	6	**77**
in total	9	9	8	8	4	6	3	29	2	8	**86**

Section #1





Section #2

- Both slides share similar lipid features and imaging patterns.

AP-MALDI: Multidimensional, High-resolution Visualization

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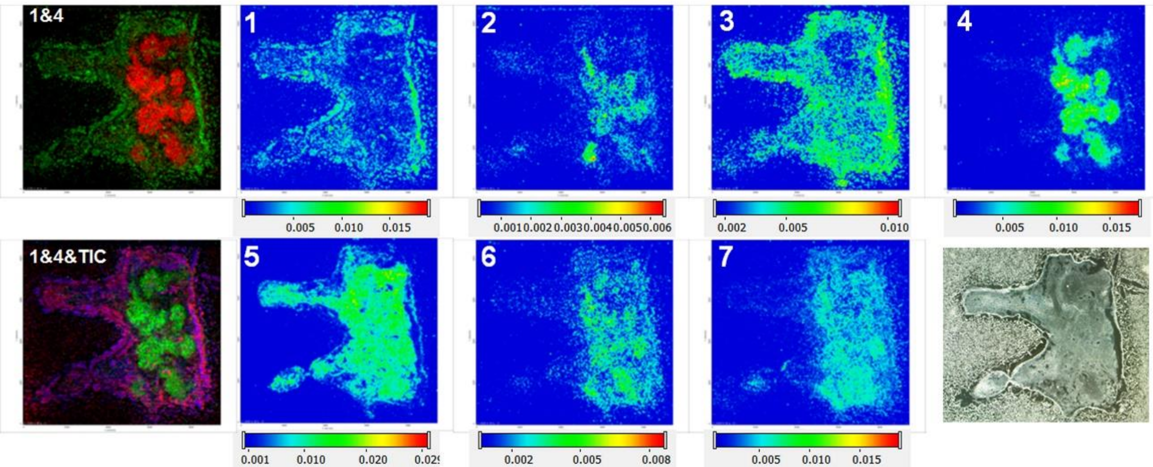
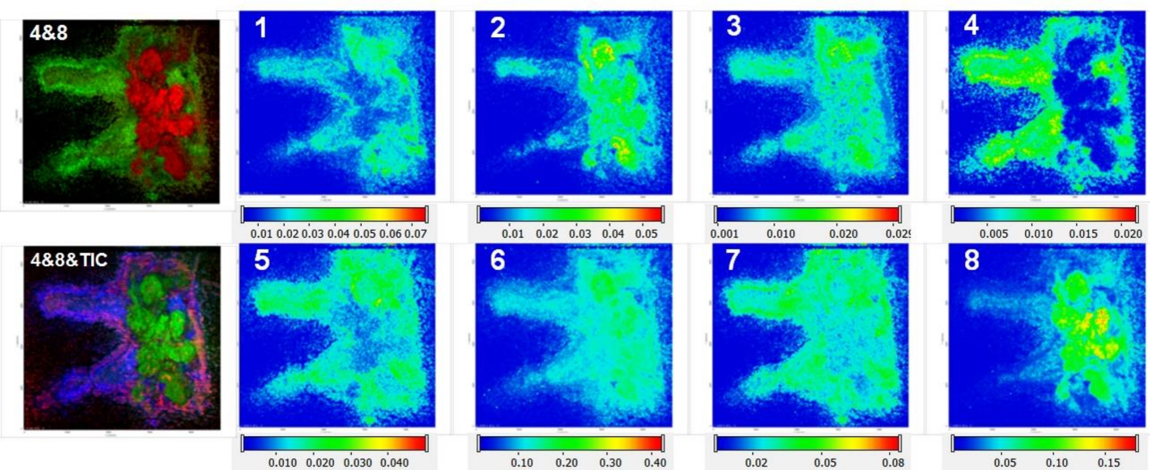


LPC

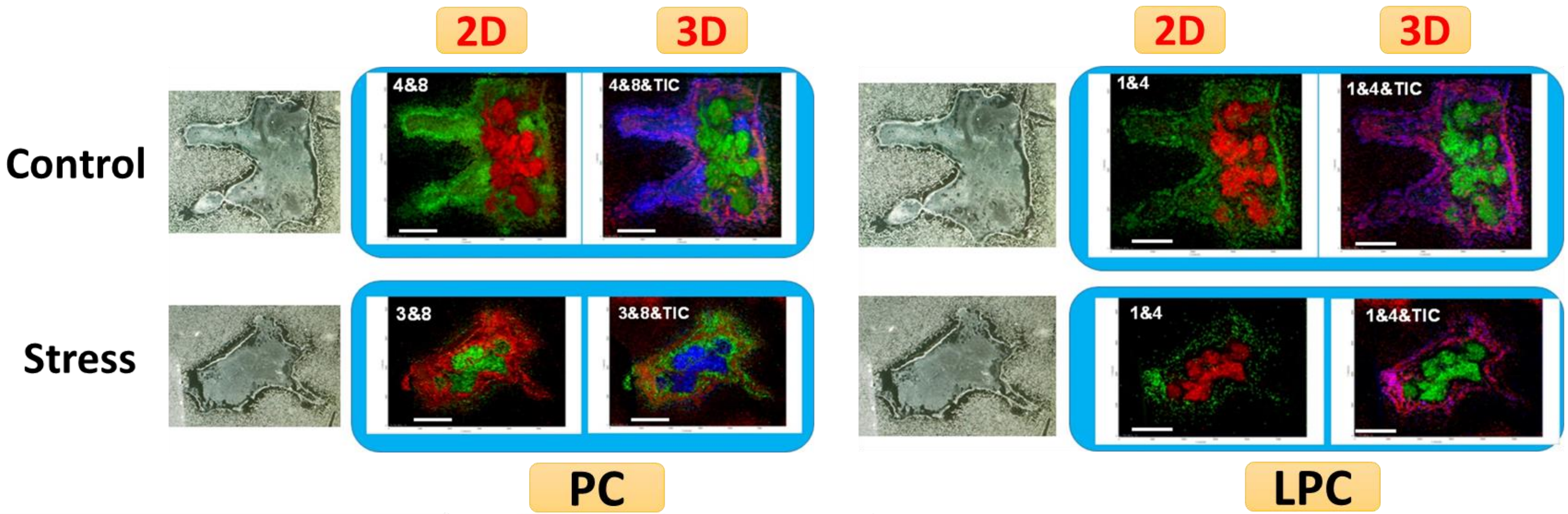
	Species	lipid features	calculated m/z	detected m/z	Delta m/z (ppm)	charge	S/N
1	LPC/Na+	A16:0	504.3424	504.3445	4.07	1	104.94
2	LPC/Li+	LPC18:3	524.3323	524.3348	4.79	1	34.08
3	LPC/Li+	X20:4	550.3479	550.3503	4.29	1	118.43
4	LPC/Li+	X20:3	552.3636	552.3660	4.36	1	86.49
5	LPC/Na+	P20:0	558.3894	558.3941	8.42	1	145.00
6	LPC/Li+	X22:4	578.3792	578.3815	3.91	1	42.41
7	LPC/Li+	X22:3	580.3949	580.3971	3.81	1	80.63

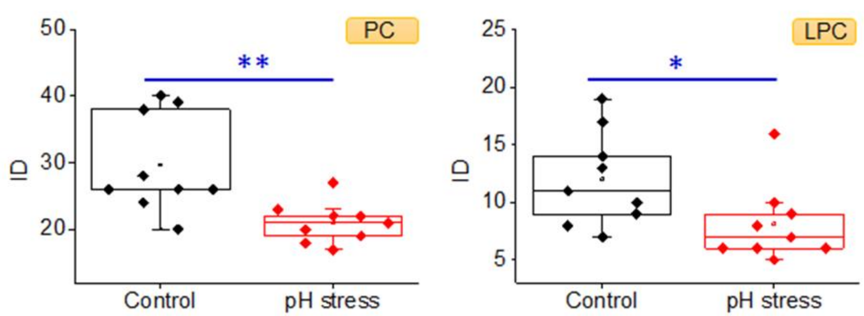


PC

	Species	lipid features	calculated m/z	detected m/z	Delta m/z (ppm)	charge	S/N
1	PC/H+	D16:1-16:0/D14:1-18:0	732.5538	732.5540	0.30	1	402.79
2	PC/H+	D16:0-16:0	734.5694	734.5686	1.13	1	281.44
3	PC/Li+	P16:0-18:1/A16:0-18:2	746.5670	746.5691	2.76	1	131.22
4	PC/H+	8:0/P18:0-16:0/A18:1-16:0/A16	746.6058	746.6060	0.24	1	145.54
5	PC/H+	D16:0-18:2	758.5694	758.5694	0.04	1	239.56
6	PC/H+	D16:0-18:1	760.5851	760.5846	0.63	1	2241.00
7	PC/H+	D18:0-18:2/D18:1-18:1	786.6007	786.6006	0.17	1	471.26
8	PC/H+	D18:0-18:1	788.6164	788.6158	0.74	1	1138.95

AP-MALDI: Multidimensional Visualization (Control vs. pH Stress)





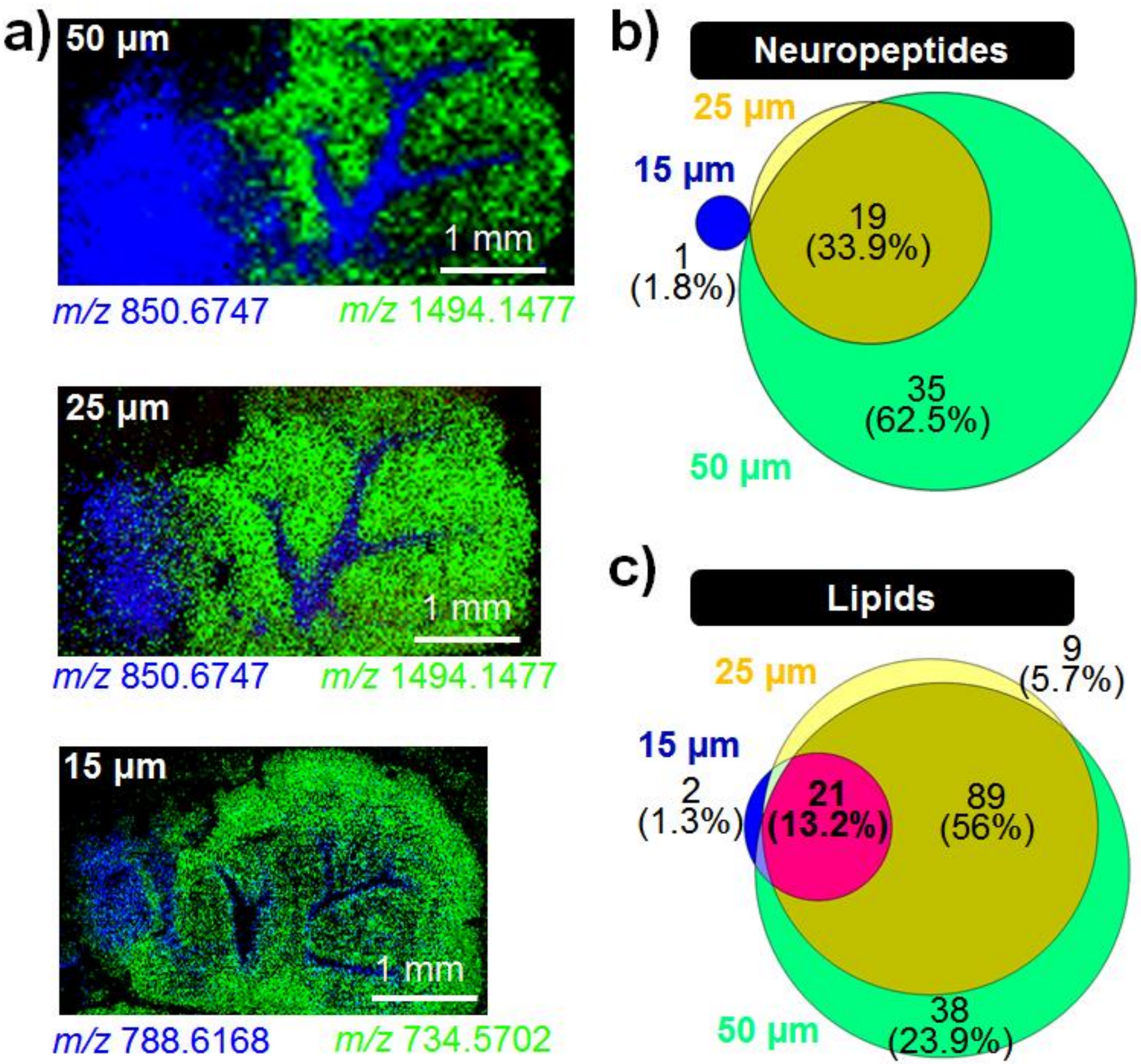
Statistical comparisons are performed using student's *t*-tests; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n.s., not significant. All error bars denote SD; n = 9.

Multidimensional AP-MALDI-MSI for high-resolution microstructure visualization.
Scale bar, 1000 µm. Crab brain. AP-MALDI, 30 µm step size.
2D, reconstruction of brain model by overlay of two lipids: like lipid 4 & lipid 8.
3D, reconstruction of brain model by overlay of three ions, two lipids + TIC.

- Lipids are down-regulated in response to pH stress.
- Neurons, just as imaged, shrink in response to pH stress.

High-resolution SubAP-MALDI-MSI

Spatial Resolution & Sensitivity



Mouse brain cerebellum tissue section MSI:

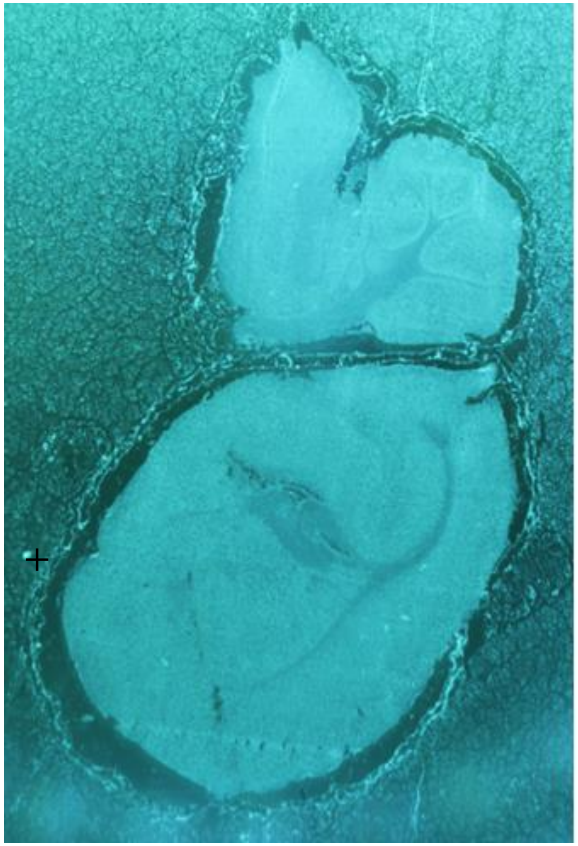
a) Step size:15/25/50 µm.
b) Neuropeptide distribution.
c) Lipid distribution.

Higher spatial resolution,
Lower sensitivity.

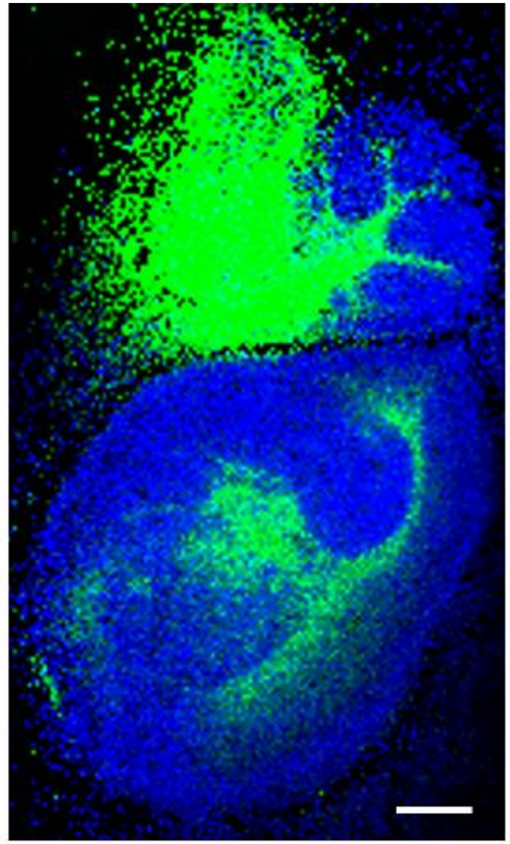
Optimal: 25 µm.
(lipids + neuropeptides)

High-resolution SubAP-MALDI-MSI

Mass Resolution & Sensitivity

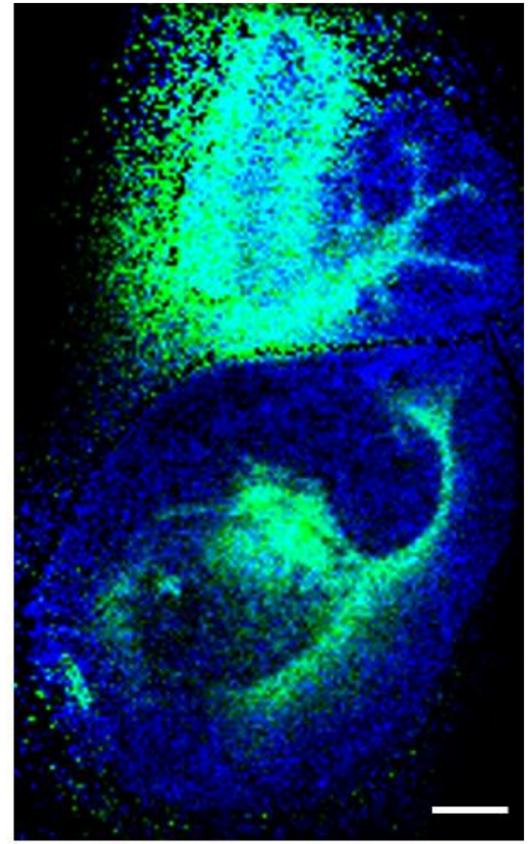


Optical image



TAG(52:9)/Na+
Lactosyle Ceramide(16:0)/Li+
m/z 867.6530 vs 867.6239

High mass resolution



LFDDFLRFamide/NH4+
[MMPE(38:4)+70+Li]+
m/z 1088.59 vs 822.647

neuropeptides+lipids

Nanosecond Photochemical Reaction (nsPCR) Enables On-demand Matrix Removal

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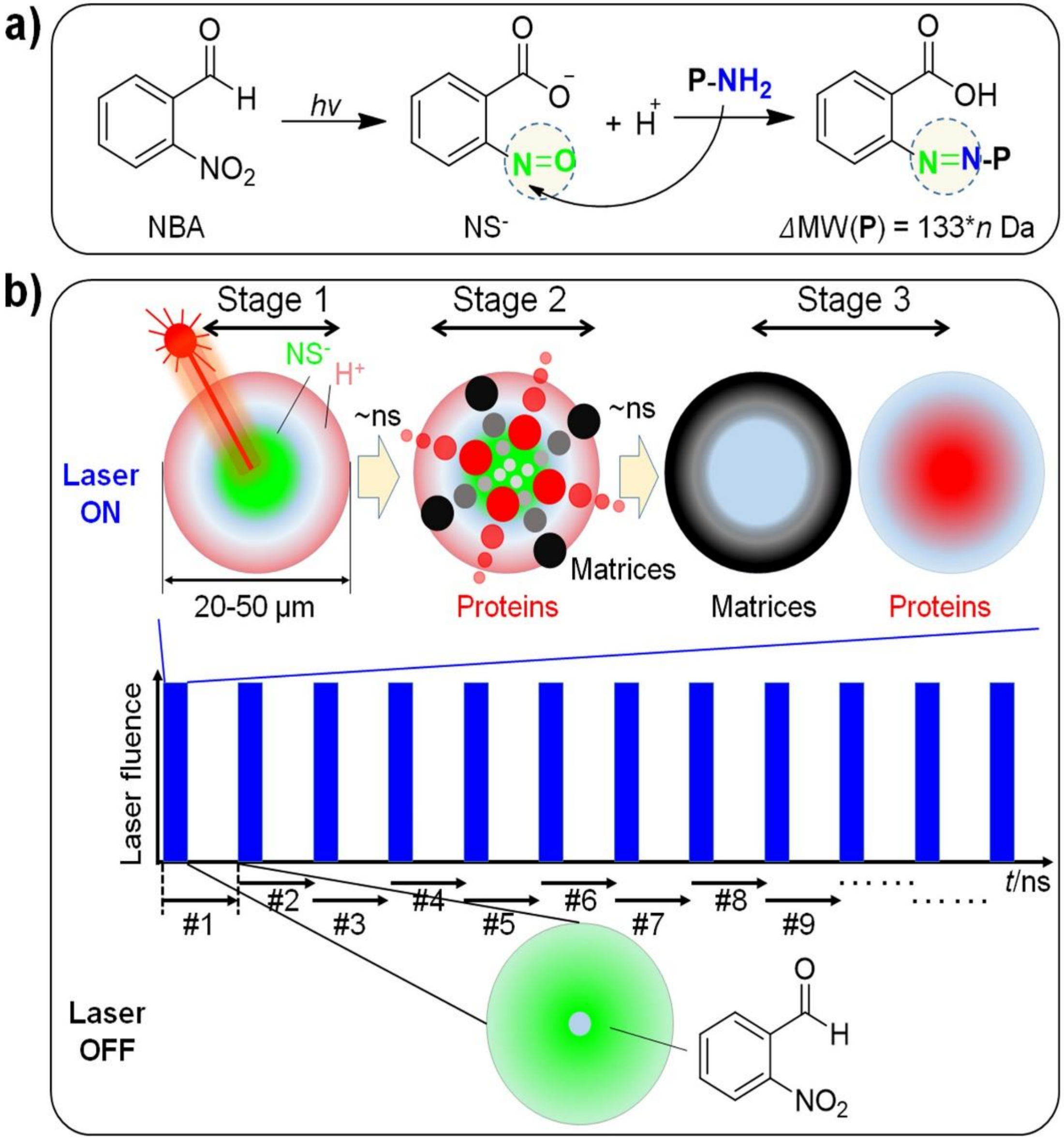
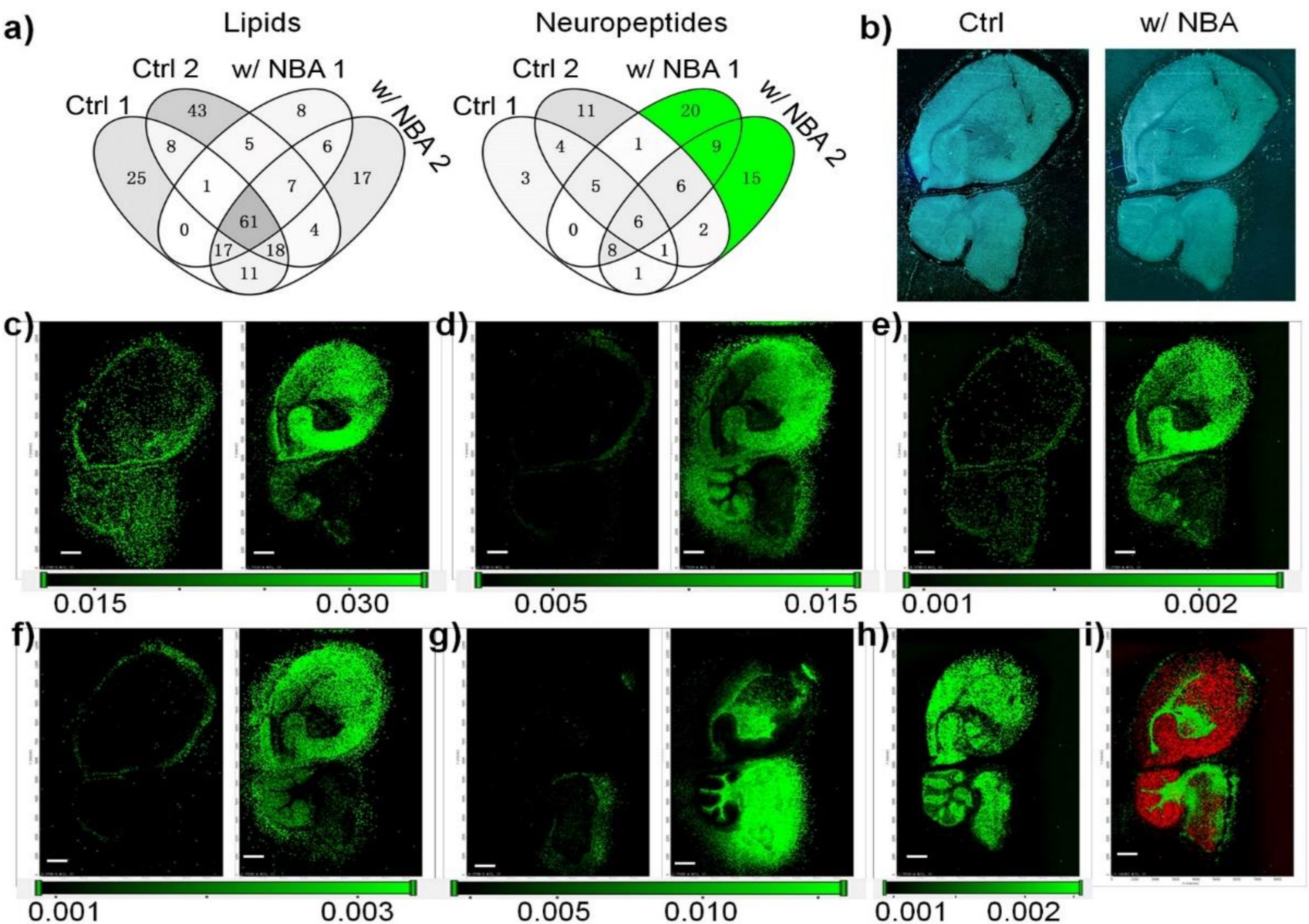


Dr. Gongyu Li

a) Nanosecond photochemistry on NBA. creating reactive NS$^-$ for localized micro-electric field.
b) On-demand three-stage matrix removal regulated by laser ON/OFF switch.

Enhanced Neuropeptide Identification and Visualization via nsPCR



Take Home Messages…

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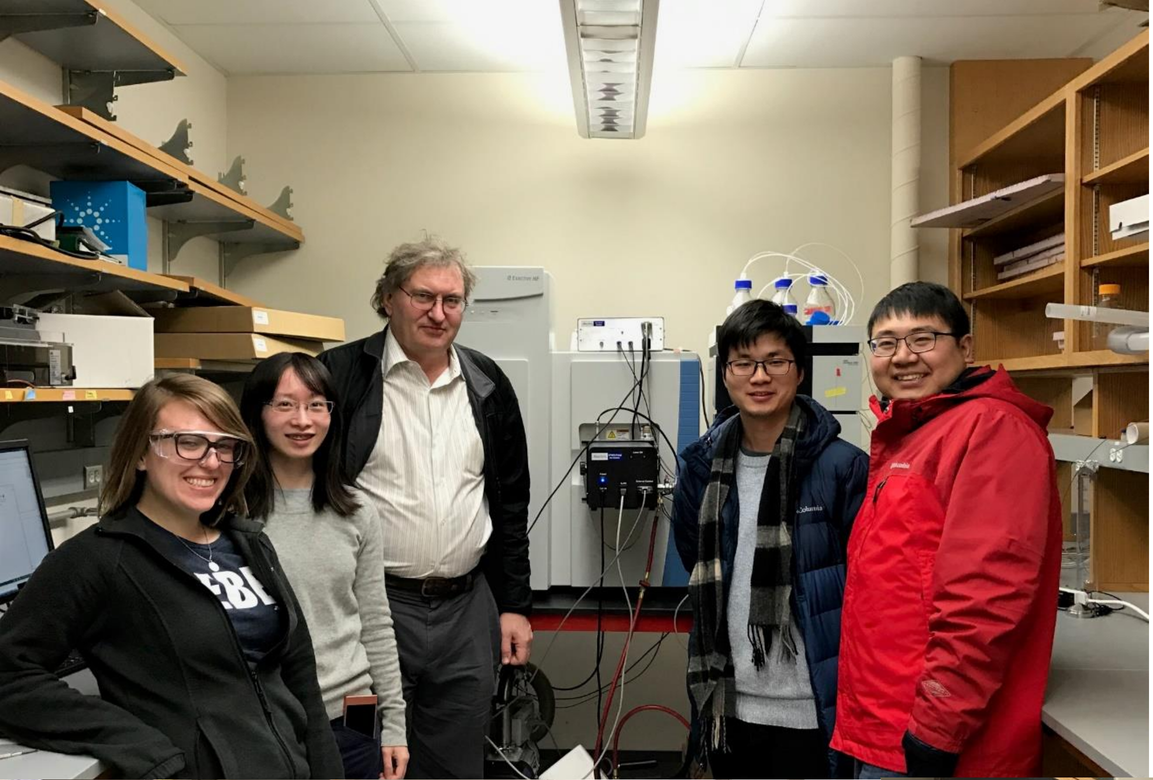
- LSI/MAIV-MS combines the benefit of MALDI and ESI to achieve expanded mass range, improved MS/MS efficiency, MS imaging capability and labile PTM protein analysis.

- Novel combination of multiplexed MSI with DDA on a MALDI Orbitrap platform enables enhanced *in situ* neuropeptide identification.

- AP/MALDI-Q-Orbitrap platform enables high resolution MSI in mass and space by multi-mode ionization and acquisition

- SubAP/MALDI-Q-Orbitrap platform provides promising solution to combine high spatial and mass spectral resolution with sensitivity for MSI of diverse biomolecules



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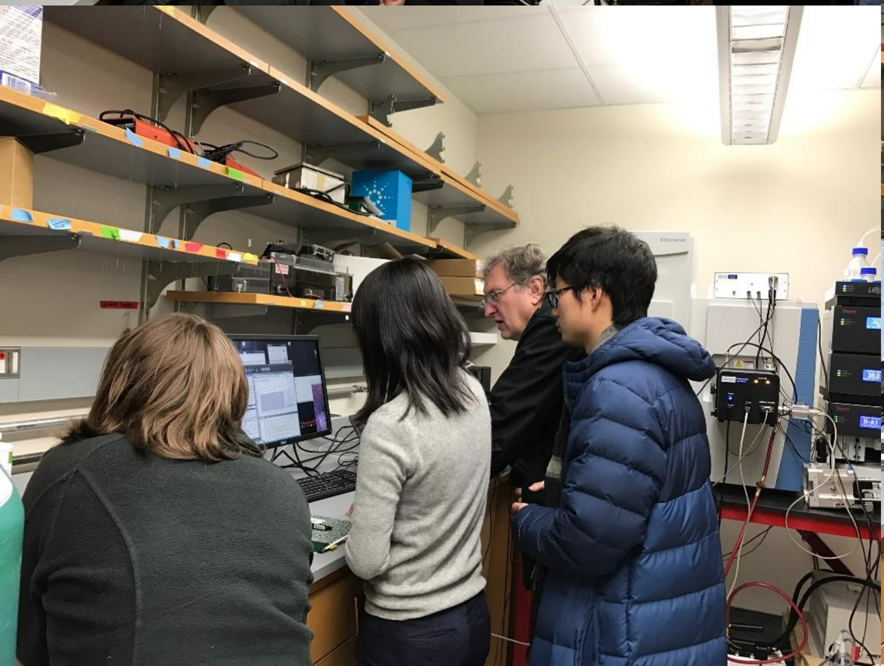

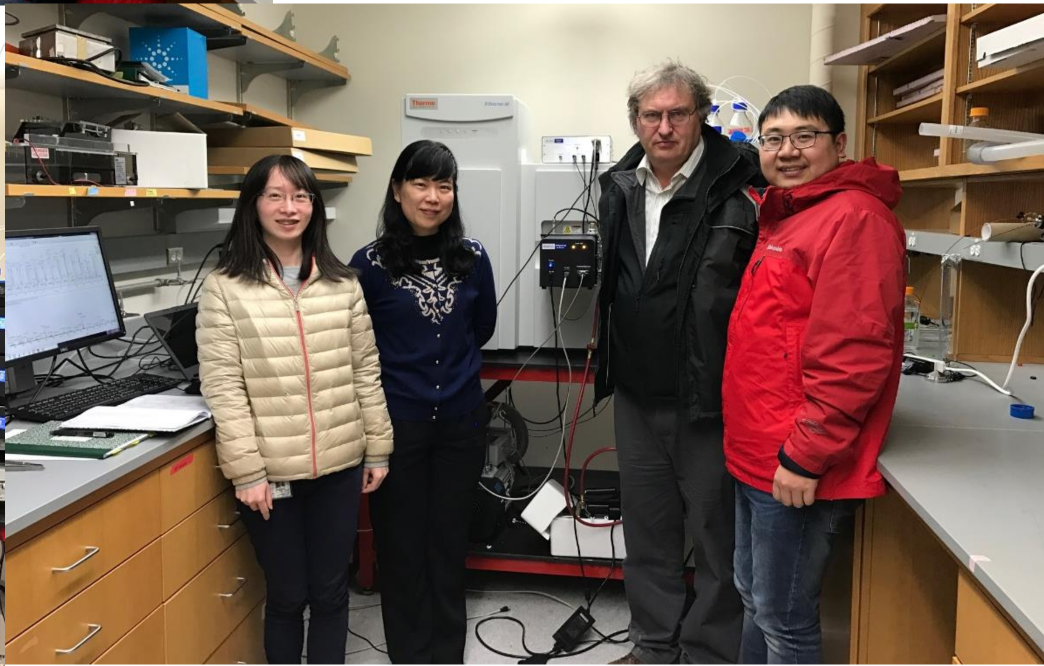

Li Lab

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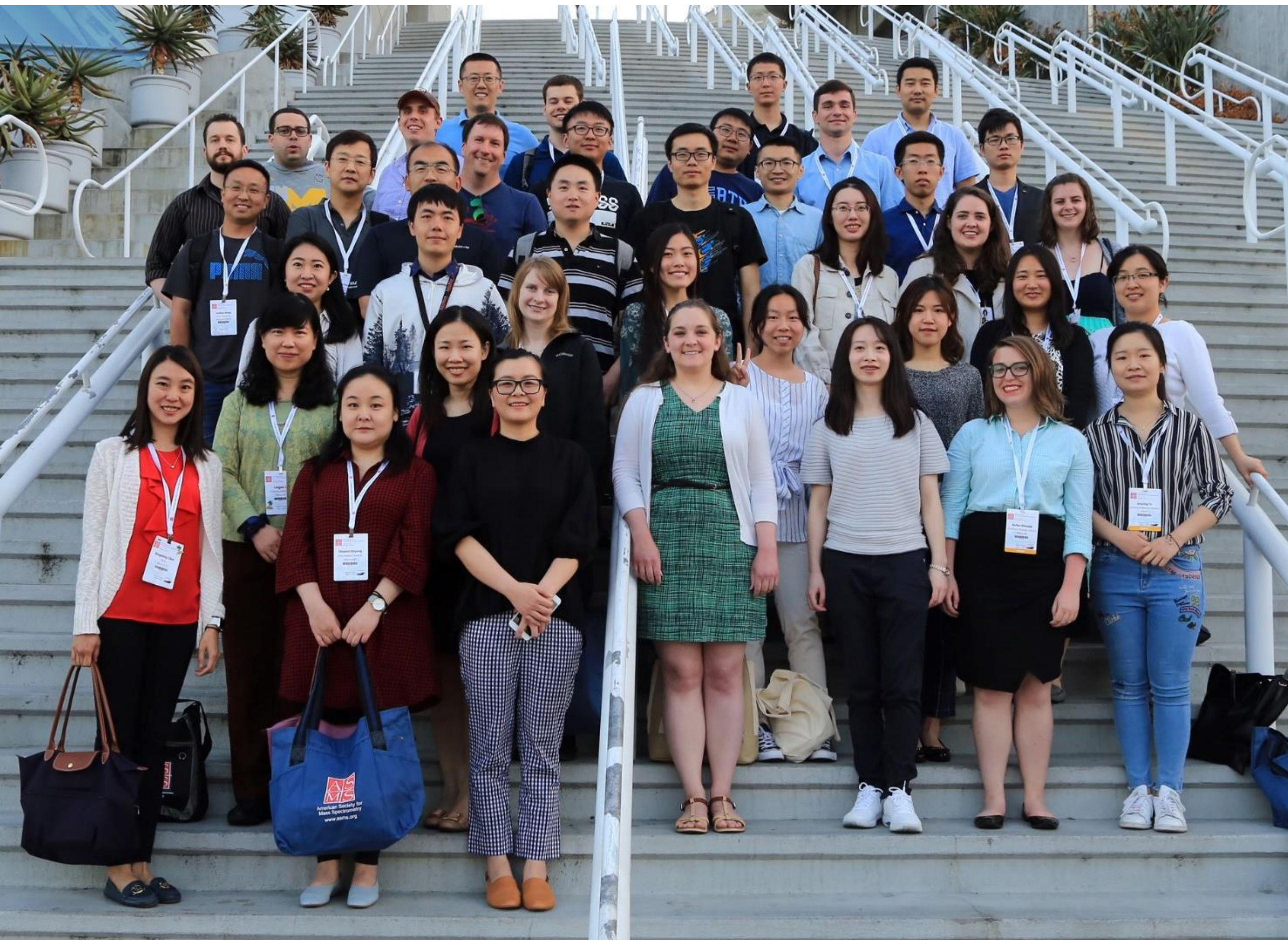
The Lab started in 2003; Successfully trained and graduated 43 Ph.D. students; Currently training 24 grad students; 5 postdocs; 6 undergrads; 1 HS



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IMPORTANT DEADLINES

August 9
Travel stipend application for students and post-docs

September 6
Abstract submissions for contributed posters and hot topic talks

September 10
Room reservations at Asilomar

September 13
Conference Registration

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Theodore Alexandrov (EMBL)
Per Andren (Uppsala University)
Josephine Bunch (The National Physical Laboratory, UK)
Zongwei Cai (Hong Kong Baptist University)
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