# Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 20 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

**UW-Madison Department of Chemistry**
**1101 University Avenue**
**Madison, WI 53706-1396**
**Contact: Prof. Bassam Z. Shakhashiri**
**bassam@chem.wisc.edu**
**www.scifun.org**

May 2014

# Development of Matrix-Assisted Laser Desorption/Ionization and Liquid Chromatography - Electrospray Ionization Based Mass Spectrometric Techniques for Characterizing and Quantifying Endogenous and Therapeutic Biomolecules

By
Bingming Chen

A dissertation submitted in partial fulfillment of
the requirements for the degree of

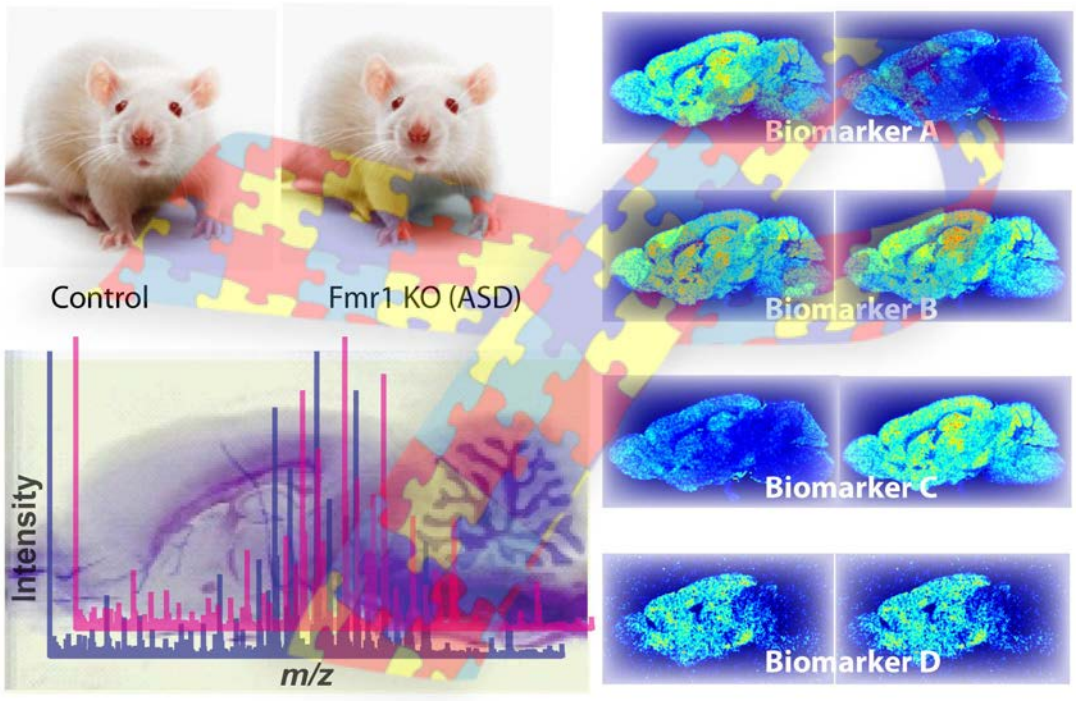
Doctor of Philosophy
(Pharmaceutical Sciences)

at the
UNIVERSITY OF WISCONSIN-MADISON
2017

Date of final oral examination: March 24th, 2017

The dissertation is examined by the following members of the Final Oral Committee:
Lingjun Li, Professor, School of Pharmacy and Department of Chemistry
Robert G. Thorne, Assistant Professor, School of Pharmacy
Paul C. Marker, Professor, School of Pharmacy
Hrissanthi Ikonomidou, Professor, Department of Neurology
Antony O. Stretton, Professor, Department of Zoology

# Chapter 8
# Probing Neuropeptidomic Changes Related to Autism Spectrum Disorders via Mass Spectrometry Imaging
# For the Wisconsin Initiative for Science Literacy




**Author contribution:** study was designed by B. Chen, H. Ikonomidou and L. Li; analytical experiment was performed by B. Chen; animal experiment was performed by H. Ikonomidou; data was analyzed by B. Chen; manuscript was written by B. Chen and edited by L. Li.

**Abstract**

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders that have prevalence of 1 in 68 children. Despite its high prevalence, doctors solely depend on behavioral tests for diagnosis, which could be biased and inaccurate. Up to today, we have no clue about the exact cause of ASD: we are not sure whether it is caused by a change in protein or a gene mutation. Therefore, we are interested in searching for molecules, which can serve as a target of ASD. We hope that the presence or absence of these target molecules can indicate whether a patient has ASD without performing behavioral testing. Furthermore, we hope to develop treatments that target these molecules and alleviate or cure ASD. These target molecules are called biomarkers. In order to look for potential biomarkers, we will use mass spectrometry (MS) imaging technique, which is a powerful tool to localize a wide range of biomolecules simultaneously on tissue. It is particularly valuable in tissue biomarker discovery as it not only tells us what the molecule is, but also where the molecule is located. In this study, we developed a MS imaging method to search for biomarkers in some lab mice with ASD and compared them with normal, healthy mice. Several molecules appear differently in brains of autistic and healthy mice. These molecules can be potential biomarkers for ASD after additional verification studies.

**What is Autism Spectrum Disorders (ASD)?**

ASD are common neurobehavioral disorders in children that impair the ability to interact and communicate. Patients with ASD tend to have reduced motor coordination and impaired intellectual level. However, some children with ASD can be very talented in a special area, such as math, art, music or visual skills. There are more than 200 thousand US cases each year, and they are more common among boys. Based on statistics from U.S. Centers of Disease Control and Prevention, 1 out of 42 boys and 1 out of 189 girls are diagnosed with ASD in the United States. Although the exact cause of ASD is not known, researchers consider that some

interference in the brain before or right after birth can lead to the disease. In most cases, toddlers start showing obvious symptoms or signs of ASD between 12 and 18 months, but in some cases, it can be as late as 2 years. The diagnosis of ASD is entirely based on behavioral tests in three categories: impairments in social interaction, impairment in communication and restricted, repetitive and stereotyped patterns of behavior. Studies find that early diagnosis and early intervention for children with ASD are highly effective ways to improve IQ, communication skill and social interaction. Although we now know that several genes are related to ASD, the exact cause of the disease is still unknown. Also, we do not have an effective treatment that targets a specific molecule to help alleviate or cure the symptoms. Therefore, we are very interested in looking for target molecules that play important roles in the development of ASD. We can then use them as a marker for diagnosis and drug targets. These molecules are called biomarkers, which will be introduced in the next section.

**What is a biomarker?**

A biomarker is a biomolecule that can be used for disease diagnosis or as a target for drug development. It could be any biomolecule, such as proteins, metabolites and lipids. Biomarker discovery usually goes through stages of discovery, verification and qualification (Figure 1). During the discovery stage, we compare a small number of samples (~10) to examine all possible molecules and pick out potential biomarker candidates. During the verification stage, we only examine the biomarker candidates which have been identified during the discovery stage. At this stage, we use a larger sample set (~100) and eliminate most of these candidates that do not meet the requirement. At the qualification stage, we use a very large sample set (~1000) to further confirm and validate the chosen biomarkers. After this final stage, a biomolecule officially becomes a biomarker for certain disease or biological state.

Our work here focuses on the discovery stage and we use mice models instead of human samples for all experiments. During the discovery stage, animal models can be used instead of human clinical samples, especially when human clinical samples are difficult to obtain due to technical or ethical difficulties. Using animal models allows better understanding of disease without adding risk of human harm. Here, we use a mouse model called "*Fmr1* KO" mouse to mimic ASD condition. The *Fmr1* KO mice displayed ASD-like symptoms, such as repetitive behavior, anxiety and impaired social behavior.


**Figure 1**. Three essential stages for biomarker discovery.

**What is mass spectrometry (MS) and mass spectrometry imaging (MSI)**

Mass spectrometry (MS) is a commonly used technique that measures the mass of biomolecules. A mass spectrometer is usually composed of four parts: ion source, mass analyzer, detector and computer (**Figure 2**). It offers speed and sensitivity to detect hundreds of molecules at once. To be more precise, a mass spectrometer measures the mass-to-charge ratio (*m/z*) of a molecule. Therefore, a molecule has to become positively or negatively charged, called ionization, before a mass spectrometer "sees" it. The ionization process happens in the ion source. The mass analyzer is then used to separate the mixture according to *m/z*, which is then detected in the detector. The signal is converted to a spectrum in the computer for us to further analyze the result. There are usually two types of MS analyses, which are full MS and tandem MS

fragmentation (**Figure 3**). During a full MS analysis, the mass spectrometer sees almost everything in our samples. This step gives us a basic idea about what molecules are present in our sample. During a tandem MS fragmentation analysis, some molecules that we are interested in are selected and shredded into pieces, allowing us to know the structure of the molecule.

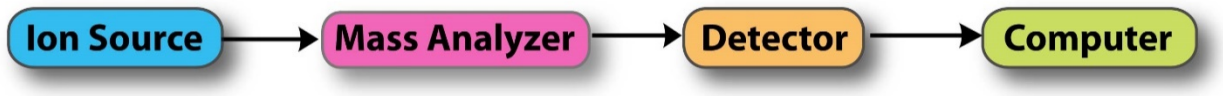



**Figure 2.** Main components of a mass spectrometer.

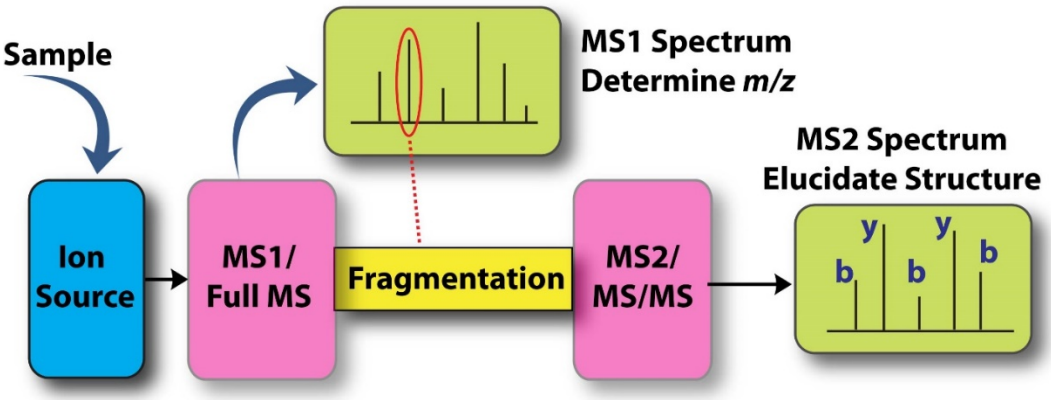



**Figure 3.** Full MS and tandem MS fragmentation illustration.

Now that we have learnt some concepts about MS, let's explore a very exciting area of study in MS: MS imaging. It is a powerful technique that not only gives me the mass of a molecule, but also a picture of its location on tissue. I put together a typical workflow of MS imaging in **Figure 4**. During the experiment, I slice tissues into very thin slices (12 micrometer, similar to the diameter of a human hair) in a freezer and lay the slices on a microscope slide. Then, I coat the slide with a homogenous layer of small molecule, called matrix, that helps the ionization process. The sample preparation process usually takes about 2 hours to finish. Then, I can load the slide into a mass spectrometer, choose the area of interest to analyze and start the automatic data acquisition. The resulting imaging data are analyzed by specialized software to

construct the distribution pictures. studies on MS imaging have grown rapidly in the last two decades (**Figure 5**). More than 700 papers are published each year on the topic of MS imaging.

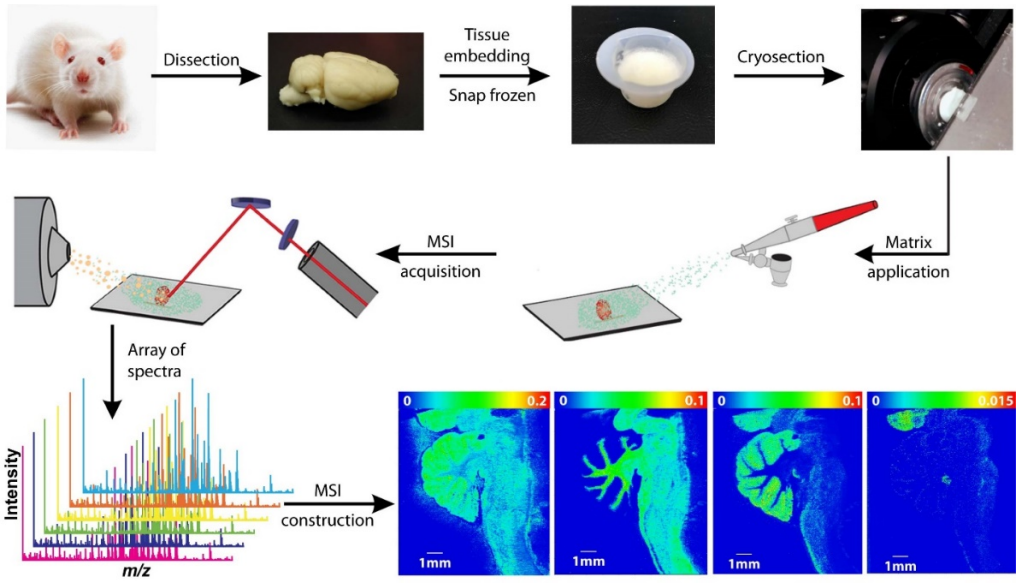



**Figure 4.** A typical workflow of MS imaging.

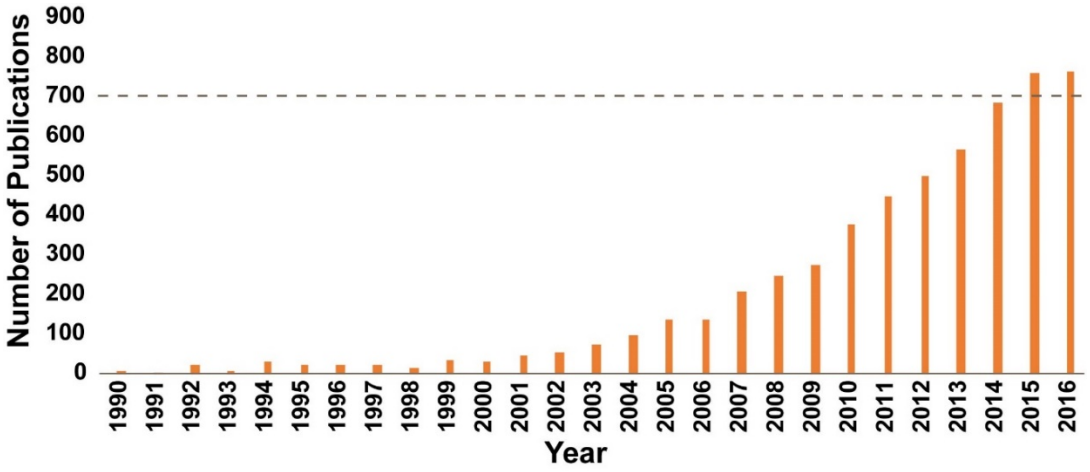



**Figure 5.** Number of publication MS imaging since 1990. Data were collected from PubMed search of “mass spectrometry imaging” or “imaging mass spectrometry” from 1990 to 2016.

**Result of my research**

In my study of ASD biomarkers, I used MS imaging to look at the neuropeptide distribution on brain sections of autistic mice (*Fmr1* KO) and compared with healthy/control mice. Neuropeptide is a small protein-like molecule, which helps neurons to communicate and

transfer signal. We are interested in studying neuropeptide as potential ASD biomarker, as it is involved in a variety of brain functions, including social behavior and learning. The neuropeptides that behave differently were listed as biomarker candidates. I detected hundreds of biomolecules on the brain tissue sections. Among them, ten neuropeptides revealed obviously different abundances between control and autistic mice brain sections. **Figure 6** shows three representative neuropeptide MS images. A fragment of neuroendorcrine protein 7B2 at *m/z* 1772.97 only shows up in the control brain section. In contrast, a fragment of secretogranin-2 and pro-opiomelanocortin only show up in the *Fmr1* KO brain section. These results were verified by three technical replicates. A more systematic approach with five biological replicates is currently under investigation.

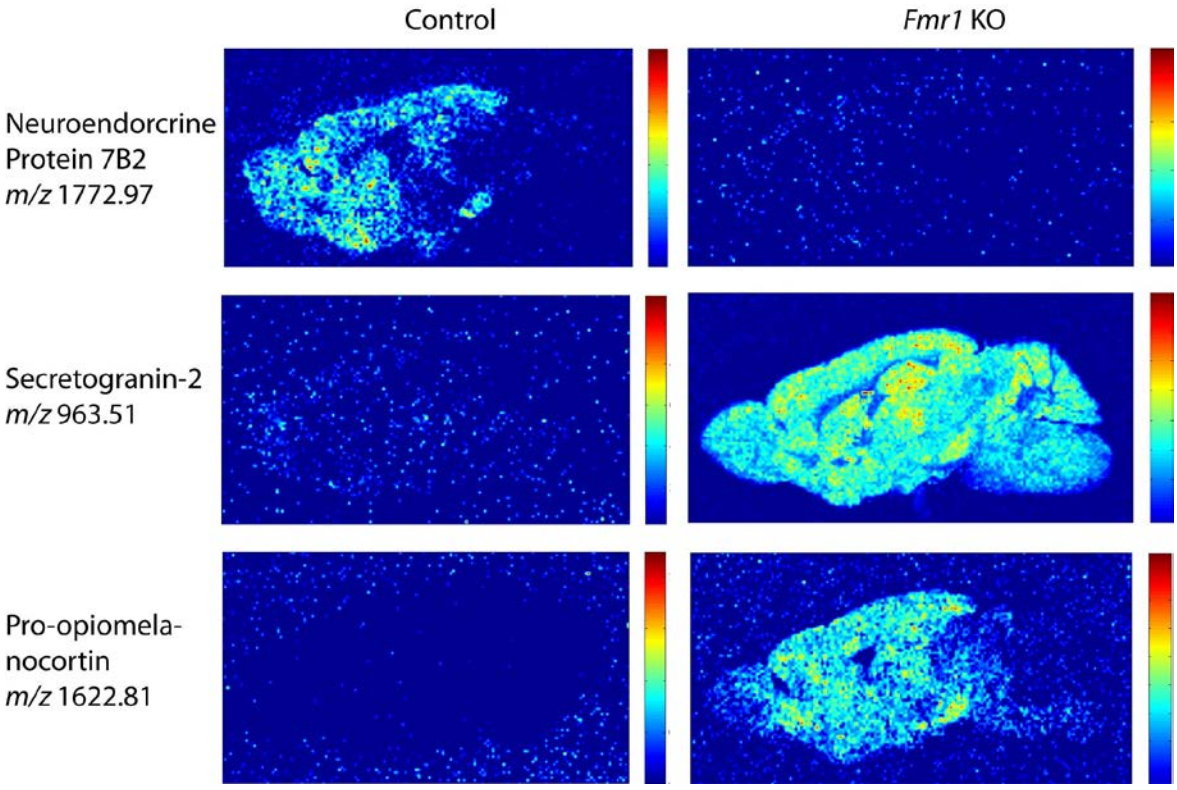



**Figure 6.** Selected neuropeptide MS images that show obvious differences between control and *Fmr1* KO mice brain sections.

Finally, I compiled a list of neuropeptides that show obvious differences between control

and *Fmr1* KO mice brain sections in the MS imaging experiment in **Table 1**. Overall, four neuropeptides show obviously higher abundance in control samples and six neuropeptides show obviously higher abundance in *Fmr1* KO sample.

**Table 1.** List of neuropeptides that reveal obvious differences between control and *Fmr1* KO mice brain sections in MS imaging experiment.

| **Intensity: control > *Fmr1* KO** | | |
|---|---|---|
| ***m/z*** | **Precursor name** | **Peptide name** |
| 746.489 | Neurotensin | N/A |
| 1570.905 | Proenkephalin-B | Dynorphin B |
| 1772.973 | Neuroendorcrine protein 7B2 | N/A |
| 1522.830 | Orexin | N/A |
| **Intensity: *Fmr1* KO > control** | | |
| 866.433 | Protachykinin-1 | N/A |
| 963.509 | Secretogranin-2 | N/A |
| 984.530 | Proenkephalin-A | N/A |
| 1100.606 | Proenkephalin-B | Beta-neoendorphin |
| 1622.807 | Pro-opiomelanocortin | N/A |
| 2180.084 | Secretogranin-1 | N/A |

The MS images provide a lot of useful information about these neuropeptides. The imaging result not only shows the presence or absence of certain neuropeptides between samples, but also provides distribution information. For example, some neuropeptides are located all over the brain, while others are in specific areas, such as the olfactory bulb or cerebellum. Based on this information, biologists can associate the neuropeptide distribution with brain areas and their functions in order to predict how the peptides might affect autistic symptom. This study will hopefully increase the understanding of ASD underlying mechanisms and identify putative biomarkers as diagnosis and therapeutic targets.