FACES OF MASS SPECTROMETRY

Lingjun Li

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A Mentor to Many

Just a few months ago, Lingjun Li, a professor of Pharmaceutical Sciences and Chemistry at the University of Wisconsin (UW)-Madison for the last 16 years, hooded her 40th student.

"Interacting with so many great, young minds is what keeps me coming in every day. When I started my lab, I thought that I would focus on great science. But one of the most exciting and rewarding things has been training the next generation and having the privilege to impact their careers."

And it looks like Li will be busy for another decade or so: she currently has another 20 graduate students and several postdocs collecting and analyzing data.

Li grew up in academia—both of her parents are professors—and attended Beijing University of Technology before starting graduate research at the University of Illinois at Urbana-Champaign (UIUC). She credits three mentors, doctoral supervisor Jonathan Sweedler, and postdoctoral mentors Richard Smith at Pacific Northwest National Lab and Eve Marder at Brandeis University, with cementing mass spectrometry research with neuroscience. Li's contribution has been to find new bioactive peptides and help transform people's thinking about neuropeptide family organization and functional consequences of neuropeptide multiplicity, leading to more than 250 peer-reviewed publications.

Her work in developing chemical tools to study neuropeptides has gained notice, including a National Science Foundation CAREER Award (2005), an Alfred P. Sloan Research Fellowship (2006), and most recently she received the ASMS Biemann Medal (2014). She was named one of the top 50 most influential women in the analytical sciences and was included in the 2016 Analytical Scientist Power List. Since 2018, she has held the Charles Melbourne Johnson Distinguished Chair in Pharmaceutical Sciences at UW-Madison.

What is your research in mass spectrometry focused on?

One area that our group is best known for is developing multi-faceted mass spec tools to study neuropeptides, a big class of signaling molecules that is present in all nervous systems. There are a lot of challenges to studying these bioactive molecules because they are oftentimes active at very low abundance, the chemical diversity is enormous, and they are highly dynamic. By developing these diverse and complementary chemical tools, including chemical derivatization methods for improved *de novo* sequencing, *in vivo* microdialysis to follow temporal dynamics, and mass spec imaging to map the spatial distribution of these signaling molecules, we have discovered over 300 novel neuropeptides over the past decade. Another area of our focus has been the development of novel chemical tags to facilitate high throughput quantitative proteomics, metabolomics and glycomics studies.

What was your path into science?

When I was a little girl, I always enjoyed school and loved learning new things. Because I was brought up in the university environment—both of my parents are material science professors at Tsinghua University in Beijing—I knew that I wanted to be a scientist very early on.

I majored in environmental analytical chemistry in college, and I spent two and a half years as a research scientist at the Research Center for Eco-Environmental Sciences, Chinese Academy of Science. I was first exposed to a high resolution GC-MS instrument during a three-month internship opportunity at Environment Canada. I really started to study biological MS in a very inter-disciplinary environment when I started graduate school with Jonathan Sweedler at UIUC. My training focused on single cell MS with an invertebrate model system—a sea slug—to study neuropeptides in the model neurons [Li, L., *et al., Anal. Chem.*, **71**, 5451-5458 (1999); Li, L. *et al., Trends Biotechnol.*, **18**, 151-160 (2000)].

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How did you continue to develop your research?

As a post doc, I continued to work at UIUC but also launched into working with two other mentors, Dick Smith where I learned high performance proteomics mass spectrometry, and Eve Marder, an eminent neuroscientist who works on the neurophysiology of crustaceans. Her lab is interested in the very small neural circuit in the stomach of the crabs and lobsters and how it generates rhythmic activity via neuromodulation.

When I started my own lab, I merged MS analytical tool development with testing of novel bioactive peptides on crustaceans through a long-term collaboration with Eve Marder and other neuroscientists. The real challenge for studying crustaceans is that, compared to other model organisms, they do not have a sequenced genome, thus lacking a protein database. So we have to rely on very high quality MS based sequencing. We developed a number of chemical derivatization methods that really changed our ability to discover novel peptides: formaldehyde labeling for *de novo* sequencing to improve fragmentation [Fu and Li, *Anal. Chem.* **77**, 7783-7795 (2005)] and, over the past decade, a series of novel chemical tags [Xiang *et al., Anal. Chem.* **82**, 2817-2825 (2010); Frost *et al., Anal. Chem.* **87**, 1646-1654 (2015); Frost *et al., Anal. Chem.* **89**, 10798-10805 (2017)]. We are now developing even higher 'plexing for high-throughput quantitative analysis, initially with neuropeptides and now with proteomics and glycomics.

How did your work change the field?

Prior to gaining information through mass spectrometry, neuroscientists thought they had well characterized the system in crustaceans with 20 or so neuromodulators utilized by a small, 30 motor neuron circuit. But, all of a sudden, our work found hundreds of neuropeptides that transformed the understanding of the chemical complexity of neuromodulation and peptide family organization. In one family, we found 30 or so different chemical isoforms that only differ from each other by one amino acid [Ma *et al., J. Proteome Res*. **8**, 2426-2437 (2009)].

A lot of our analytical method development is directed to solving a biological problem. For example, we want to know about inter-cell signaling molecules, so we develop *in vivo* microanalysis tools to monitor peptide secretion over an extended period of time and under different physiological conditions. However, crustaceans are marine animals, and the samples are high in salt, so we have to improve detection because peptide release is probably less than 10% compared with those expressed in tissue. We developed an affinity enhanced *in vivo* microdialysis method using magnetic nanoparticles that link to an antibody that recognized a particular peptide family to improve peptide collection [Schmerberg and Li, *Anal. Chem.*, **85**, 915-922 (2013)].

What research excites you right now?

Our tool development enables both basic fundamental research and clinical translational discovery. We have expanded into neurodegenerative diseases to try to discover diagnostic biomarkers in Alzheimer's disease. If we could identify early disease signatures, before the preclinical stage, it will help many people. We are focusing on glycoproteins or glycosylation pattern changes, that could be implicated in cancer, cardiovascular disease and neurodegeneration. With the development of new methods and available new instrumentation, we are able to discover novel glycosylation occurring in well-known peptide hormones. For example, even though insulin is so well studied, there was no literature on its unique glycosylation; with the advancement of mass spectrometry, we pinpointed the site of these unique modifications and reported on the first endogenous O-glycosylated insulin [Yu *et al., Anal Chem*. **89**, 9184-9191 (2017)]. We are currently also exploring the use of ion mobility MS to differentiate peptide epimers and localize the site of D-amino acids in a peptide.

What do you see as the biggest challenge in your career?

I think, initially, my biggest challenge was overcoming cultural differences. But I was fortunate to have very supportive mentors throughout my graduate and post doc years. I think it's important to find something that you really love and are passionate about because a positive attitude is very important in a career. As a PI, balancing fundamental and translational research can be challenging in the current funding environment, but you just have to find the things that you are excited about and keep going.

What do you enjoy outside of the lab?

Music, gardening, traveling—anything that keeps me relaxed and refreshed. One of my hobbies is to take pictures of food that I have when traveling. I have a deep appreciation of all of the great food from different regions and try to recreate meals at home. I also play table tennis. I have been playing since primary school in China—we had a concrete table outside the classroom that was always very crowded—but I now play a few times a week with my husband in our basement.