



Customer Insights

- Top-Down Proteomics: An Emerging Technique Driven By Cutting-Edge Mass Spectrometry

Top-Down Proteomics: An Emerging Technique Driven by Cutting-Edge Mass Spectrometry

High resolution and extreme resolution mass spectrometry instrumentation facilitates multidisciplinary research by the Ge lab, University of Wisconsin Madison, a leader in the field of top-down proteomics



Professor Ying Ge's Ge Lab at the University of Wisconsin benefits from three key instruments for their capabilities for high resolution data acquisition.

The University of Wisconsin, Madison

Based in Madison, Wisconsin, The University of Wisconsin (UW) is a top 10 research university, with over \$1 billion in annual research expenditure, making it 6th in the United States for volume of research undertaken. UW has a highly collaborative approach on campus, with faculties from various departments sharing knowledge and expertise across numerous disciplines. The Chemistry Department is the largest, comprising approximately 30-40 separate faculties with 300-400 students. Biological and chemical research also spans the medical and vet schools, priming researchers for cutting-edge discoveries and forming a good position for translational and clinical research.

Professor Ying Ge is an Associate Professor in the Department of Cell Regenerative Biology, and the Department of Chemistry, as well as the Director of Mass Spectrometry in the Human Proteomics Program of the School of Medicine and Public Health at UW-Madison. Her research in cardiac systems biology is multidisciplinary, which utilizes the collaborative ethos of the university. Her group consists of one postdoctoral associate, 11 graduate students, five undergraduate students, and three visiting scholars. With over 70 peer-reviewed publications to her name, Professor Ge is an established leader in the field of proteomics with a special focus on cardiovascular systems biology, and her

group relies on the most sophisticated equipment to push the field of top-down proteomics into the realm of mainstream science.

The Research

The Ge lab aims to understand the molecular and cellular mechanisms underlying cardiovascular diseases, using cutting-edge technology such as high-resolution mass spectrometry to study comparative proteomics and metabolomics together with biochemical and physiological studies. Professor Ge's research focus on top-down mass spectrometry-based proteomics with the goal to better understand heart diseases is unparalleled by any other group. The more conventional bottom-up approach to proteomics is arguably less powerful, as it involves digesting the protein into many smaller peptides, thereby complicating the proteome and reducing the level of obtainable information, as only a small fraction of peptides are recovered. Top-down proteomics allows more powerful, reliable data to be obtained, as it directly analyzes intact proteins before fragmentation and allow analysis of unique proteoforms using mass spectrometry. Mass spectrometry-based top-down proteomics has unique advantages for unraveling the molecular complexity, quantifying proteoforms, deep sequencing of intact proteins, mapping modification sites with full sequence coverage, discovering unexpected modifications, identifying and quantifying positional isomers and determining the order of multiple modifications.

Many proteins are affected by post-translational modifications (PTMs), such as glycosylation and phosphorylation. PTMs can modulate protein activity and could therefore crucially link to many human diseases: their identification is greatly improved through top-down proteomics as the analytical processes involved preserve protein modifications, making the technique useful in probing modified proteins as potential disease biomarkers. Innovative integrated top-down proteomics can enable researchers to examine biomarkers in healthy and diseased models, to characterize any protein modifications present.

Despite top-down proteomics emerging as an increasingly popular technique, there are still some important challenges to overcome before the method becomes fully robust. One such challenge, protein solubility, is being tackled by Professor Ge's group by the development of novel top-down mass spectrometry-compatible surfactants, which can solubilize all protein categories effectively. The group is also developing novel strategies for high-resolution multi-dimensional liquid chromatography, to address the present issue of protein separation. Sample preparation, detection of large-sized proteins and a lack of software integration are additional challenges. Most routine labs are yet to adopt the approach because of this, but Professor Ge is positive that "we are in the best position right now to integrate these technologies with biology and medicine. At the moment, top-down proteomics is still challenging, but we can present solutions to address the challenges to help the community adapt to the top-down methods."

Funding and Investment

Professor Ge's research spans multiple disciplines, which cuts across the traditional boundaries of chemistry, biology, and medicine, with the aim to further the understanding of the molecular basis of cardiovascular diseases. Her group's focus on proteomics stems from the requirement for better understanding of how molecules interact as a system, in order to elucidate cellular system functions in health and disease. Professor Ge describes the process of selecting an instrument capable of accomplishing these goals:

"We wrote an application for a National Institute of Health (NIH) high-end instrument grant that was fortunately awarded. This award gave us the chance to purchase high resolution FTMS instruments, which are extremely beneficial to top-down mass spectrometry-based proteomics. When we got the funding we then carefully evaluated all of the high-end high-resolution instruments available on the market. Bruker instruments gave us a pleasant surprise in terms of the resolution and ease of use. They really captured the hearts of my students who operated the instruments during the demo, especially the TOF instruments! ..."

".. The amount of data they got just from the two-week demo trip was incredible."



As a result of the successful demonstration, Professor Ge decided to purchase the high-end extreme resolution solarix XR™ (FTMS) together with the two ultra-high resolution (UHR) Q-TOF (Impact II and Maxis II) instruments, which are now central to the strength of proteomics and mass spectrometry on campus. The nature of the group's work demands extreme and ultra-high resolution mass spectrometry techniques, which Professor Ge accesses through her collaboration with Bruker. The Q-TOF has surprisingly sophisticated capabilities for proteoform profiling, which allows intact protein masses to be measured to facilitate disease biomarker discovery, whilst the FT-ICR-MS provides the eXtreme Resolution (XR) necessary to resolve large proteins to ensure the success in top-down proteomics.

Instruments

Professor Ge's lab uses three key instruments for top-down proteomics: the solarix XR™ (FTMS), Maxis II and Impact II Q-TOF, each of which provides different benefits for her work. Since the beginning of her academic career, Professor Ge used Fourier Transform mass spectrometry (FTMS), and the ultra-high resolution and high sensitivity still makes it the best instrument for characterizing large proteins through a top-down approach, and differentiates it from other mass spectrometers currently in use on the UW-Madison campus. Professor Ge uses this instrument for translational and clinical research to understand the basis of human diseases, as well as identifying potential molecular biomarkers for treatment of disease. The FTMS instrument is housed in the Human Proteomics Program (HPP) Mass Spectrometry Core Facility, and is shared by users across the UW campus.

Technology across the field has improved dramatically: "In comparison to the mass spectrometry instruments ten years ago, those

available today are phenomenal. There's a day and night difference between their capabilities for high-resolution data acquisition ..."

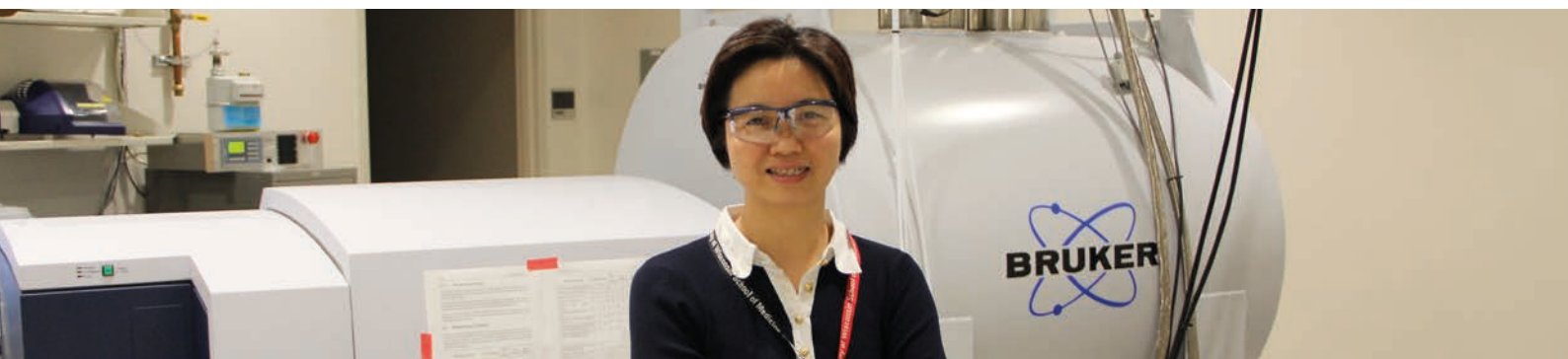
"It is now very likely that more and more proteomics labs will adapt the top-down approach soon, as the advanced instruments make it so much easier."

Campus-Wide Collaboration

The overarching aim of Professor Ge's research is to advance the understanding of the molecular basis of diseases and nurture the development of new methods for early diagnosis, prevention and better treatment of cardiovascular diseases. The group extracts intact proteins and metabolites from tissues and cells to identify, characterize and quantify them for proteoform profiling. Their studies uncover changes in the proteome and metabolome in response to external and internal stress factors, which would be lost using traditional bottom-up approaches.

Currently the group's research is split in two main directions: 1) studying cardiac myofilaments through top-down comparative proteomics, to establish a protein modification map in normal and disease models, and 2) studying cardiac regenerative biology using integrated proteomic and metabolomic approaches to evaluate the efficacy of stem cell therapies for heart failure treatment.

Professor Ge's research group enjoys collaborations widely spanning academia and industry. As director of the Human Proteomics Program at UW-Madison, Professor Ge is a strong advocate for a user-friendly facility that

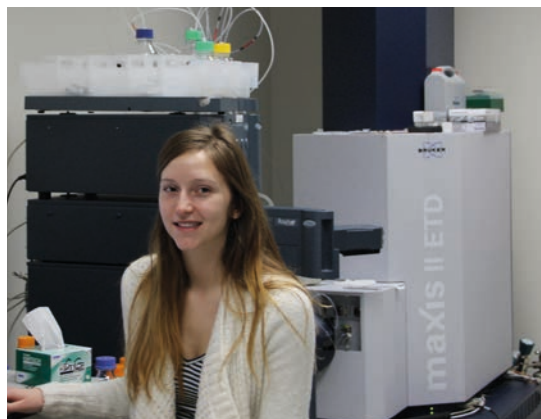


offer users training and access to the instruments so the instrumentation her lab uses is shared with the whole campus. The high-end solariX XR™, Bruker's FTMS instrument, in particular is well-utilized across the campus including School of Medicine and Public Health, College of Letter and Sciences, School of Engineering, School of Pharmacy, College of Agriculture and Life Sciences, and School of Veterinary Medicine. The extreme resolution provided by the solariX XR™ enables accurate, high quality mass measurements.

In addition to academia, Professor Ge's lab collaborates with industry partners, particularly in the pharmaceutical field. Scientists from this industry visit the university to use the instruments for a broad range of applications, but most commonly for large protein (top-down proteomics) and small molecule (metabolites, natural products) experiments, and antibody-drug conjugates research. Professor Ge describes how this partnership is mutually beneficial:

"The university is really the place for disseminating new ideas and technologies to the broad users in the community. We have achieved isotopic resolution for antibodies using the solariX XR™, and our industry collaborators are very impressed with the data. For natural product and small molecule characterization, ...

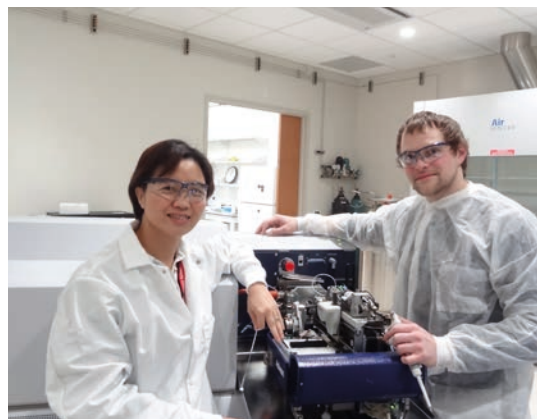
"... we showed that the extreme resolution capabilities with Bruker solariX XR™ are beyond what can be achieved by other instruments."



Access to superior mass spectrometry instrumentation is vital for the pharmaceutical industry. From Professor Ge's perspective, she can share her knowledge and expertise with the community, and importantly these collaborations generate revenue and bring business to the facility, which is essential for its continued research excellence.

The Research Challenges

Top-down proteomics is poised to move from academic curiosity to routine adoption: the key challenges which previously made the approach too difficult for most labs are now being addressed by advanced research groups such as that of Professor Ge. Specifically, her research group is employing a multi-pronged approach to address these challenges in a comprehensive manner by developing new MS-compatible surfactants for protein solubilisation (Chang et al. J. Proteome Res., 2015, 14, 1587-1599), new strategies for multi-dimensional chromatography separation of proteins (Valeja et al. Anal. Chem. 2015, 87, 5363-5371), novel nanomaterials for enrichment of low-abundance proteins (Hwang et al. J. Am. Chem. Soc., 2015, 137, 2432-2435), and a new comprehensive software package for top-down proteomics (Cai et al., Mol. Cell. Proteomics, 2016, 15, 703-14). Front end sample preparation and back end data analysis challenges are being overcome. But another industry-wide challenge facing proteomics and metabolomics research is the high degree of complexity and dynamic nature of the proteome and metabolome. High resolution and extreme resolution mass spectrometry instrumentation can address this issue; optimized for top-down



comparative proteomics and metabolomics, the instrumentation capabilities are now being tackled as sophisticated packages are brought to the market:

“Companies like Bruker are doing an excellent job of optimizing their instruments for top-down proteomics ...”

“... One of the previous challenges with the top-down approach was the instrument capabilities. Most mass spectrometers are designed for bottom-up. With the availability of these new instruments specially tailored for top-down, it’s really an exciting era for top-down researchers, or those keen to enter the field.” Professor Ge explains.

Current Performance

Bruker is optimizing its instruments in line with the needs of Professor Ge’s lab for top-down proteomics development. In addition to data quality and quantity, instrument uptime is a dominating factor for considering an instrument’s quality. When equipment breaks down or has to be switched off frequently or for long periods of time for maintenance and cleaning, the productivity of the lab dramatically decreases, affecting research output. So using instruments with minimal downtime has a positive impact on academic workload. Professor Ge explains how Bruker’s zero-downtime instruments benefit her work:

“The new generation of mass spectrometers are amazing. We are lucky to have both Q-TOF instruments (Impact II and Maxis II) and FTMS (solariX XR™); we use TOF for more routine analysis and high throughput proteomics, which you can do on a daily basis – unimaginable compared to 10 years ago! During the past year of owning the impact II, Maxis II and solariX XR™ we have barely had any downtime, the instrument essentially works 24/7. Indeed, we have already obtained enormous amount of data for publications.”

In collaborations such as the one between Bruker and Professor Ge’s lab, feedback is essential for Bruker to continue optimizing their instruments for Ge’s increasingly complex needs: “We as a community usually shout out to companies like Bruker to inform them of our innovative new approaches to a problem, and rely on them to provide us with an instrument with the correct capabilities and compatibility for intact protein detection. Bruker heard our needs and came up with a solution incredibly quickly, giving leverage to the top-down consortium.” Professor Ge explains,

“With the rapid advancements in technology, the major bottlenecks are now being overcome”

Training and Support

Staff employed by the UW-Madison Human Proteomics Program’s mass spectrometry facility are dedicated to training users across the university. This forms a highly diverse collaboration:

“The impact we have generated at UW-Madison with these instruments is huge. In the past years, our facility has trained many ‘super-users’, meaning they can operate the instruments independently. This works really well, the instruments are so robust they can be used around the clock!” explains Professor Ge. “All these newly trained scientists now have the experience using excellent equipment, and could go on to purchase similar instruments in their future job positions.”

The solariX XR™ is more sophisticated than the other instruments, so more time is required to learn how to use it:

“We are lucky to have a facility for training mass spectrometry users. The TOF instruments typically take one to two weeks for students with no previous experience to learn to operate, which is amazingly quick. The students really love this instrument. The solariX XR™ takes a little longer to grasp, but one user with no prior mass spectrometry experience can now independently operate this sophisticated instrument.”

Future

"This year we expect to publish a number of papers using data acquired with Bruker instruments. The data output is incredible, and the quality is unmatched." Professor Ge predicts, with emphasis on the fact that this degree of lab productivity would not be possible without the highly advanced equipment they use. Professor Ge's work sets a precedent for the nature of the research to come: through her innovative solutions to top-down challenges and her passion for communicating to the community, top-down proteomics will soon break into the mainstream.

"Researchers in proteomics and mass spectrometry heavily rely on the quality of the instruments..."

"..The role that instrument capabilities play in academic life is so important -- excellent instruments can boost the productivity and ultimately, accelerate the success of our career! From the bottom of our hearts, we're so happy with our instruments and really want to thank Bruker."



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