



Dr. Zheng-Jiang Zhu
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of Sciences

Customer Insights

- Metabolic phenotyping of disease using IM-MS

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Researchers at the Zhu Laboratory for Mass Spectrometry and Metabolomics, Chinese Academy of Sciences, are using Bruker solutions to cultivate cutting-edge research in metabolomics.



Working with Bruker

The work of Dr. Zheng-Jiang Zhu and his team has led to recent breakthroughs in advancing metabolomics research. However, Dr. Zhu's relationship with Bruker began much earlier in his career when he worked with the company's MS instrumentation. The launch of the Bruker timsTOF Pro sparked his interest in testing the instrument to see how it addressed challenges in the metabolomics field:

"Since 2013, my team has been testing and comparing a lot of different instruments for metabolomics research. We were looking for technologies that could meet the future needs of the field. The Bruker timsTOF Pro offers huge advantages for metabolomics in the instrument's design and performance."

Metabolic Phenotyping of Disease at the Chinese Academy of Sciences

The Zhu Laboratory for Mass Spectrometry and Metabolomics focuses on the development of mass spectrometry (MS) metabolomics technologies, including liquid chromatography (LC)-MS techniques, metabolite spectral database and data processing software. Affiliated with the Interdisciplinary Research Center on Biology and Chemistry (IRCBC), Shanghai Institute of Organic Chemistry (SIOC) at the Chinese Academy of Sciences (CAS), researchers at the Zhu Lab develop data processing programs and/or build metabolite database for automated data processing (both quantitative and qualitative analyses). The team is also exploring new techniques such as data-independent acquisition (DIA) and ion mobility spectrometry (IMS)-MS techniques for metabolomics and lipidomics, as well as expanding their research into the metabolic phenotyping of various challenging diseases.

The lab also focuses on aging and aging-dependent neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease. Researchers use MS and metabolomics tools to understand metabolic remodeling during aging, and metabolism regulation of aging and aging-related diseases.

Dr. Zheng-Jiang Zhu, Principal Investigator and Director of Metabolomics Research Center of Interdisciplinary Research Center on Biology and Chemistry in CAS, describes the laboratory's role:

"We have about 10 graduate students in the laboratory who are working on their PhDs, as well as laboratory technicians, post-docs and staff scientists. All our projects are related to MS and metabolomics, with each person focusing on their specific projects, including applications for aging-related diseases like Alzheimer's, as well as clinical applications."

MS-based techniques for metabolomics

Metabolites are the chemical entities that are transformed during metabolism, and they provide a functional readout of cellular biochemistry. With MS-based metabolomics techniques, thousands of metabolites can now be quantitatively measured from minimal amounts of biological material, which has thereby enabled systems-level analyses. By performing global metabolite profiling, new discoveries linking cellular pathways to biological mechanism are being revealed and are shaping the understanding of cell biology, physiology and medicine.

Although relatively new compared with its genomic and proteomic predecessors, research in metabolomics has already led to the discovery of biomarkers for disease diagnosis, fundamental insights into cellular biochemistry, and clues related to disease pathogenesis. Dr. Zhu describes the quick adoption of metabolomics in these research fields:

“Ten years ago, you heard a lot about genomics and proteomics; now you hear more about metabolomics as more people are becoming interested in new applications that use this technology. One indication is that the number of metabolomics studies in publications is growing very fast, now even catching up with published work on proteomics, and that has created a lot of

awareness. About 5-10 years ago, people in the field were focused on developing the technology for metabolomics. Now, scientists are looking at the potential applications in different areas – not just biologists, but also doctors and clinicians, drug discovery researchers and others.”

One of the biggest challenges facing metabolomics research is reliable identification to ensure proper interpretation of data. Due to the chemical similarity of metabolites (as isobars and isomers), identification by MS or chromatography alone can be difficult. Ion mobility spectrometry - mass spectrometry (IMS-MS) has emerged as a powerful technology to address these issues, and the use of IMS-MS in untargeted metabolomics has shown that it improves the separation of metabolite isomers and generation of multidimensional data to support the identification of metabolites. Dr. Zhu explains how this affects metabolomics research:

“In most cases, it is a challenge to differentiate between isomers because they can have the same mass, charge, and physical properties, but vary widely in function and recognition. Separation and identification of these complex isomeric materials can be exceedingly time-consuming and resource intensive. So that’s why we started looking for new technology.”

The search led Dr. Zhu to test and eventually purchase the Bruker timsTOF Pro instrument in July 2020.



Benefits of the Bruker timsTOF Pro

The Bruker timsTOF Pro uses trapped ion mobility spectrometry (TIMS), a separation technique in gas phases where ions are propelled through the TIMS tunnel by a gas flow. An electrical field controls each ion from moving beyond a position defined by the ion's mobility, where the push it experiences from the gas flow matches the force of the electrical field. Ramping down the electrical field allows selectively releasing ions from the TIMS tunnel according to their mobility.

The unique TIMS design allows researchers to reproducibly measure the collisional cross section (CCS) values for all detected ions, and those can be used to further increase the system's selectivity, enabling more and more reliable relative quantitation information from complex samples and short gradient analyses.

The introduction of the novel Parallel Accumulation Serial Fragmentation (PASEF) acquisition method to the MS/MS design allows for ions to be accumulated in the front section, while ions in the rear section are sequentially released depending on their ion mobility. By using a dual TIMS analyzer, ions may be nearly continuously queued for accumulation, sorting and elution by mobility, allowing a duty cycle near 100% (Figure 1)

The CAS research team found the performance of the Bruker timsTOF Pro allowed routine measurements of IM information and enabled high-quality 4D feature alignment in retention time, m/z and IM. Achieving this depth offers completely new possibilities to analyze large sample cohorts of hundreds to thousands of samples in metabolomics. Dr. Zhu describes the advantages:

“The Bruker timsTOF Pro’s combination of high scanning speed and the ability to generate CCS values is a unique combination, which can significantly improve our capabilities in high-throughput metabolomics.”

Most of the time we analyze hundreds of samples per day in just 12 minutes/sample with the timsTOF Pro. With the timsTOF Pro 2, I think we could reduce that to 6 minutes per sample, and maybe as fast as 2 minutes per sample. Other instruments can accommodate high-throughput analyses, but they sacrifice sensitivity. The timsTOF Pro enables us to improve scan speed, while also getting very rich information. Another benefit is the new dimension of information provided by CCS, which can be used to further increase the system’s selectivity, enabling more and more reliable relative quantitation information from complex samples and short gradient analyses. It helps to differentiate isomers that cannot be achieved in LC-MS. That’s why we need IM in metabolomics research.”

Working with Bruker

Since developing the first tests with the timsTOF Pro, researchers at the Zhu Lab have worked closely with Bruker as they evaluate how the instrument can help with the laboratory's array of projects. Dr. Zhu describes this relationship:

“We have frequent technical meetings with Bruker to communicate and problem solve any issues we have.”



Bruker is very supportive. We also have a very good collaboration with their team on our work in bioinformatics.

Additionally, the feedback we provide them is very helpful as they learn more about how we use the instrument for our research purposes. That's valuable for both organizations."

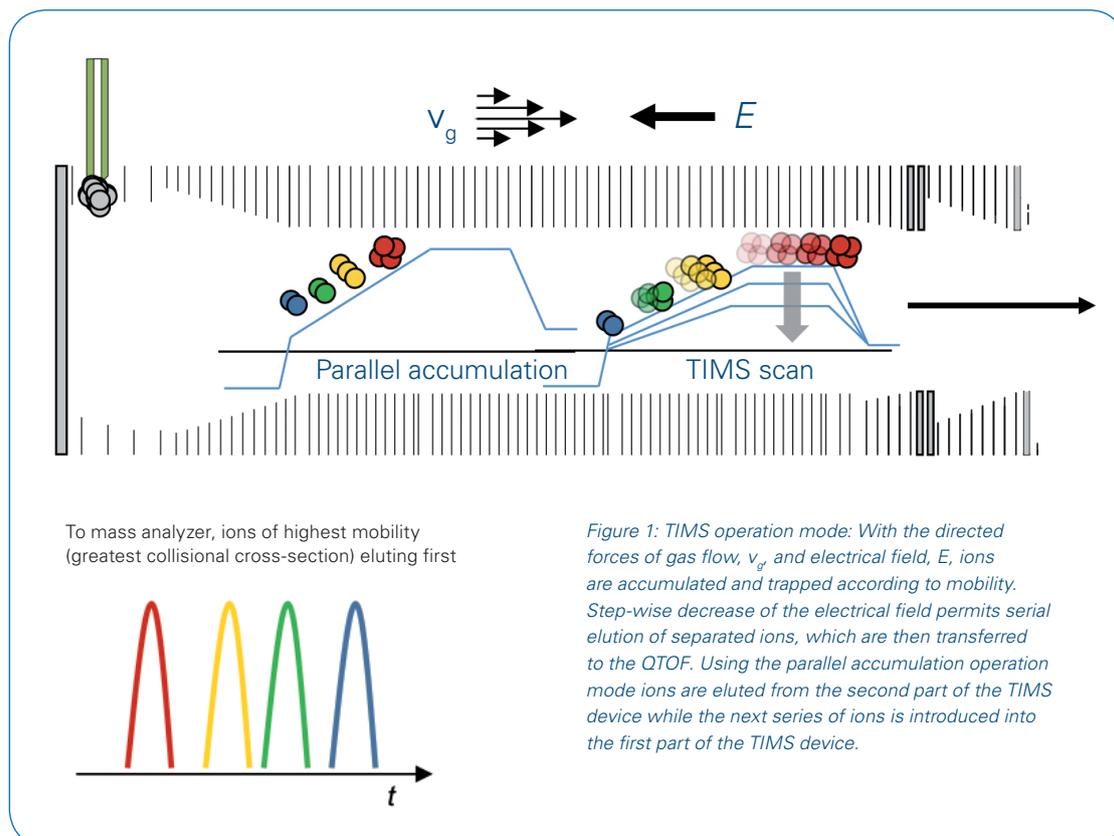
Metabolomics research on aging-related diseases

The research in Dr. Zhu's lab focuses on the development of MS-based metabolomics technologies and metabolic phenotyping of various challenging diseases, mainly focusing on aging and aging-dependent neurodegenerative diseases such as:

- Metabolic remodeling/homeostasis during aging
- Metabolism regulation of aging in *Drosophila melanogaster*
- Metabolite-protein interaction in neurodegenerative diseases
- Metabolite biomarker discovery for the diagnosis of Alzheimer's Disease.

Scientists at the Zhu Lab believe a comprehensive study of metabolites at the system level may give a holistic view of the challenging questions associated with diseases related to aging. Among their achievements in the field, the team has developed new metabolomic techniques using IMS-MS instrumentation such as the Bruker timsTOF Pro. Dr. Zhu describes this research:

"We study aging using fruit flies as a model because the lifespan is about 60 days, and we also use mice to measure how metabolites change during the aging process. We probe the



metabolic indications in inter-organ communications – for example, how the brain communicates with the liver. We found that when the animal is young, inter-organ communication is very good. But during the aging process, this communication or metabolic homeostasis is largely destroyed. And we think that disconnect might have a metabolic factor. Research can help improve metabolic health and even expand the lifespan for these animal models. Metabolites are decreasing with age in animal models, so supplementing those metabolites may slow the aging process.”

Clinical metabolomics

Scientists at the Zhu Lab are looking towards a future where high-throughput metabolomics is used in clinical settings. Currently, the laboratory’s researchers work closely with hospitals in Shanghai to obtain samples to test for metabolomic differences in patients with specific diseases or conditions, which could eventually lead to improved treatments. Dr. Zhu outlines how his team’s metabolomics research can help in a clinical setting:

“We collect samples from patients with colon rectal cancer, for example, who are undergoing different types of chemotherapy. Some people respond to the treatments, their tumors shrink, and they have better recovery. But some people do not respond well to chemotherapy, and the tumor continues to grow and even metastasize to other organs or tissues. We’re trying to measure the difference in their metabolic profiles and identify why some people respond to those treatments so well, and why other individuals do not.”

Dr. Zhu and his team believe that the combination of IMS-MS with automatic sample preparation and injection technologies will enable highly efficient and large-scale metabolomics in clinical settings. As a result, eventually this research could lead to improved treatments in the form of personalized medicine based on the patient’s metabolic profile. He explains:

“Often, and especially in traditional Chinese medicine, you use medicine to cure a disease, but you don’t know why it cures it. That is why we’re working on fostering collaborations between our laboratory and doctors in hospitals. Biomarkers can predict whether patients will have an effective or practical response to the treatment.

Ultimately, we see the application to precision or personalized medicine, where doctors can predict whether a treatment is beneficial to a patient or not.”

Future steps

With the improved performance of IMS-MS instruments like the Bruker timsTOF Pro, Dr. Zhu sees new possibilities for metabolomics research. The upcoming improvements in databases and the software tools for processing IMS-MS data are the next steps in his laboratory’s efforts to harness the potential for their research. Additionally, with the ultra-high separation speed of IM, Dr. Zhu sees the technology’s potential to replace LC as the field continues to work towards achieving high-throughput metabolomics analyses. He explains:

“Especially for complex materials, like those found in Chinese medicine, IMS-MS has the capability to identify compounds that cannot be analyzed with other technology. Currently, about 80 percent of the instruments used in metabolomics are LC-MS systems. I think in the next 5-10 years, almost all of them will be replaced by IMS-MS.”

The availability of high-resolution IMS-MS will open new possibilities for the application of the technique in metabolomics. In addition, Dr. Zhu and his team see potential in coupling IMS-MS instruments with different ionization sources, as well as enabling imaging analysis of metabolites. He describes the future possibilities of the technology:

“Metabolomics has moved from technology development to applications where we’re looking how the capabilities of the technique can advance in other areas—including environmental, drug discovery, clinical, etc.”

As IMS-MS becomes more mature, we’ll see a shift from the traditional uses of metabolomics today to other applications, and many new fields will emerge.”

For more information about the timsTOF Pro, please visit <https://www.bruker.com/products/mass-spectrometry-and-separations/lc-ms/o-tof/timstof-pro.html>.

References

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General Reference

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