Dr. Zhongyi Cheng
PTM Bio

Customer Insights

- Trapped ion mobility spectrometry (TIMS) supports dedicated proteomics services in China

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Experts at PTM Bio facilitate biomedical research using Bruker's timsTOF Pro

Working with Bruker
Dr. Zhongyi Cheng, Chief Executive Officer, combines his expertise in proteomics and post-translational modifications (PTMs) with advanced technology from Bruker:

"Trapped ion mobility spectrometry (TIMS) with the timsTOF Pro enables us to identify many more proteins using less sample than our previous methods – it's extremely powerful."

Protein services at PTM Bio

PTM Bio is a proteomics-oriented precision medicine company founded in 2011, based in Hangzhou, China, which aims to transform the way human diseases are understood, diagnosed, and treated, by developing and using cutting-edge proteomics technology to examine protein profiles, histone epigenetic marks, and post-translational modifications (PTMs).

PTM Bio provides a variety of services to the biomedical research and biopharmaceutical communities, offering its state-of-the-art mass spectrometry (MS) instrumentation and expertise to deliver high quality data and insights to industry, academia, and government organizations. These insights offer the ability to gain a deeper understanding of the functional characterization of drug targets, to mitigate risk and generate more predictable outcomes to work towards a precision medicine approach.

The company's key protein services include protein identification, quantitative proteomics, and whole proteome analysis, in addition to PTM discovery services and proprietary antibody generation. PTM Bio analyzes a broad range of sample types, from animal and plant tissue, to bacteria and body fluids.

PTM Bio owns a leading mass spectrometry-based integrated proteomics and epigenetics technology platform with industry leading sensitivity and accuracy. The development of this platform is the result of the dedicated work of the Mass Spectrometry Department, comprised of approximately 90 staff. This department links with the sales team, which connects PTM Bio's clients, including academic researchers and clinicians, with advanced technological solutions that address key challenges facing mechanism study and translational medicine.

Powerful MS technology

Proteins are the most relevant functional molecule of all diseases; almost all biological products, drug targets, and diagnostic reagents are proteins. Reliable identification and characterization of proteins and peptides is therefore crucial for biomedical research, which requires advanced analytical tools such as MS. Over the last few years, trapped ion mobility spectrometry (TIMS) combined with QTOF-MS as on Bruker's timsTOF Pro, has become an increasingly popular MS method to identify proteins, quantify protein expression, discover new biomarkers, and characterize therapeutic antibodies.

TIMS is a gas-phase separation technique, which resolves sample complexity with an added dimension of separation in addition to high performance liquid chromatography (HPLC) and MS, increasing peak capacity and confidence in compound characterization. Time-of-flight mass spectrometry (TOF-MS) is an approach used to capture a broad molecular weight range of signals at high scan rates. Combining the ion focusing effect from TIMS with ultra-high resolution and high-speed TOF technology enables the discovery of low level biologically relevant proteins which, currently, non-TIMS MS systems cannot detect. The overall speed advantage is unparalleled compared to other techniques combined with MS, such as drift tube ion mobility methods.

The ability to separate ions by mobility using TIMS boosts sensitivity and provides additional selectivity, because the ions are separated by a fourth parameter – their collisional cross section (CCS). Compact ions with a small CCS drift faster than extended ions with a large CCS, effectively allowing ions to be separated by shape, as well as retention time, mass-to-charge (*m/z*) ratio, and intensity. Known as 4D-Proteomics™, this approach enables the separation of proteins or peptides with similar molecular weights, which would otherwise show as the same peak on the mass spectra.

Dr. Cheng explains how this technology has propelled PTM Bio's protein services:

"TIMS is a really helpful analytical technique. People working in MS-based proteomics want to identify more and more proteins, to get the whole picture of biological processes.

Compared with our previous MS technologies, TIMS can identify more proteins in exactly the same conditions. It is a very powerful technique for quantification as well."

Working with Bruker

In order to expand the proteomics platform, PTM Bio installed Bruker's timsTOF Pro in Spring 2019, and now there are 5 timsTOF Pro systems in total. Customers working with the company looking to acquire deeper coverage of proteome with higher accuracy and sensitivity from various samples specifically requested analyses with the timsTOF Pro. Dr. Cheng explains:

"The results that customers were getting before did not provide them with perfect answers. We found out that there are many proteins we could not find when we first analyzed the samples, that we now can with the timsTOF Pro – that's why our clients are very satisfied with the results we get from the Bruker instrument.

*The timsTOF Pro can quantify **more** using **less** sample and improve the likelihood of finding important proteins for different aims. I admire the ability of the Bruker timsTOF Pro to do this – it is extremely advantageous for the proteomics community."*

The timsTOF Pro is powered by Parallel Accumulation–Serial Fragmentation (PASEF) technology, which boosts research capabilities due to an unprecedented, uncompromised combination of speed, sensitivity, selectivity, and robustness. TIMS combined with PASEF greatly enhances peak capacity and speeds up MS/MS data acquistion. These features enable analytical results from 20-minute gradients that previously took more than one hour to achieve. Due to the extremely high data acquisition speed provided by PASEF, without a loss in sensitivity and resolution, typical proteomic analyses can be performed even faster, increasing the throughput of biological experiments considerably. In addition, PTMs can be better separated from each other using TIMS, resulting in a higher identification rate.

Post-translational modification analysis

PTMs are involved in most cellular processes and increase the functional diversity of the proteome. These modifications impact almost all aspects of normal cell biology and pathogenesis. The analysis of protein PTMs is challenging; regular analysis often leads to incomplete amino acid sequence coverage and insufficient understanding of the crosstalk and selectivity of different PTMs and their impacts on cellular regulatory networks.

Over the years, PTM Bio has developed highly efficient MS-based proteomics technology for system-wide identification and quantification of PTM substrate proteins and mapping of PTM sites. This integrated platform – founded on the understanding of the functional relevance of PTMs in cellular physiology – has profoundly benefited biomedical and pharmaceutical research, as demonstrated by continuous high-profile publications and long-term collaborations with top drug discovery organizations.

PTM Bio offers a broad range of PTM discovery services, facilitated by TIMS TOF-MS. These services include: global PTM mapping at the whole proteome level for both well-known PTMs such as acetylation, phosphorylation, methylation, and ubiquitination, and newly identified PTMs including crotonylation, succinylation, propionylation, butyrylation, and malonylation; PTM identification, to detect a variety of PTMs on proteins of interest; quantitative PTM profiling to understand dynamics and dysregulation pathways; and an all-in-one service, which covers cell culture, affinity enrichment, LC-MS/MS analysis, and bioinformatics.

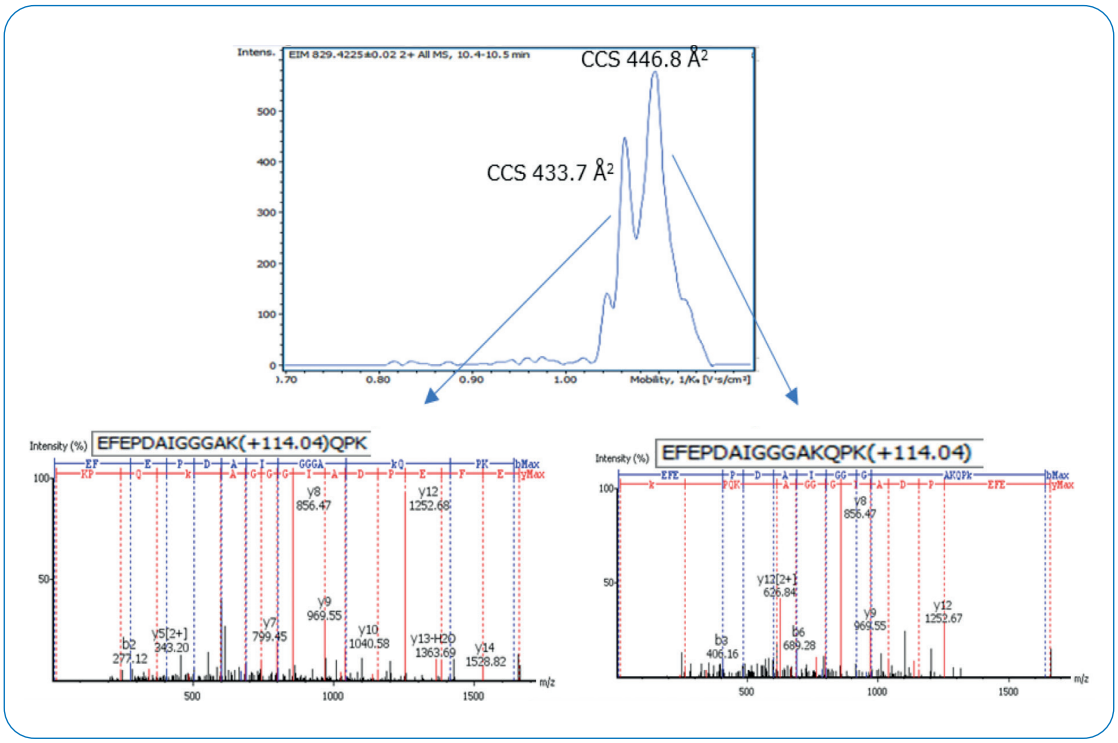

Figure 1: The separation of the two co-eluting isobaric ubiquitinated peptides with different modification sites by ion mobility (A). The extracted ion mobilogram for 829.4115 m/z at 10.45 min. The isomers can be resolved by collisional cross section (CCS) value (B). Non-chimeric MS/MS spectra of the two site-specific ubiquitinated peptides.

Ubiquitination is a PTM that is essential for regulating key biological processes, including protein degradation, immune response, signal transduction and DNA repair. MS-based proteomics platforms have proven to be a powerful tool enabling proteome wide profiling of PTMs at the site-specific level. However, the characterization of global ubiquitination is still challenging owing to the sub-stoichiometric abundance of this PTM and co-elution of ubiquitinated peptides only differing in the position of the modified site. In collaboration with Bruker, PTM Bio performed an in-depth characterization of enriched ubiquitinated peptides from human cells by TIMS and PASEF [1]. Ubiquitinated peptides were enriched from HeLa cell lysates with the PTM Bio enrichment kit (CAT PTM-1104) and analyzed by LC MS/MS using a Bruker nanoElute LC coupled to the timsTOF Pro.

More than 17,000 ubiquitination sites were identified from four HeLa cell samples with only 50 min gradients, corresponding to 5093 ubiquitinated proteins. The additional dimension of separation provided by TIMS enabled the identification and quantification of co-eluting isobaric peptides with different modification sites (Figure 1). The high affinity enrichment kit and high sensitivity of the timsTOF Pro enabled highly reproducible qualitative and quantitative identification of site-specific PTM isomers.

Acetylation is another well-known PTM that plays an essential role in cellular processes, including transcriptional regulation, cell cycles, and apoptosis. However, the low abundance and high dynamic range of acetylation make thorough MS-based profiling particularly challenging. PTM Bio's high affinity lysine acetylation enrichment kit, together with TIMS and PASEF technology, can overcome such challenges and enable the comprehensive characterization of acetylation.

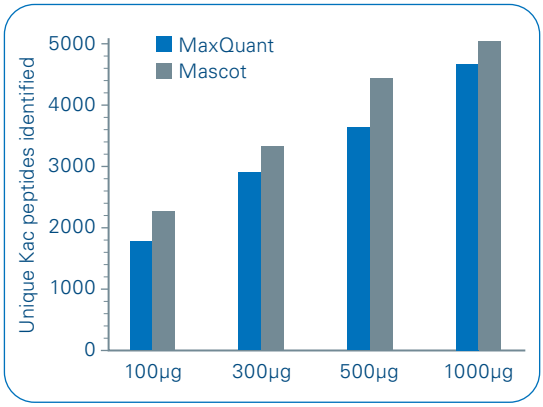

Figure 2: Identification of the acetylated lysine peptides with four different starting amounts (mouse liver).

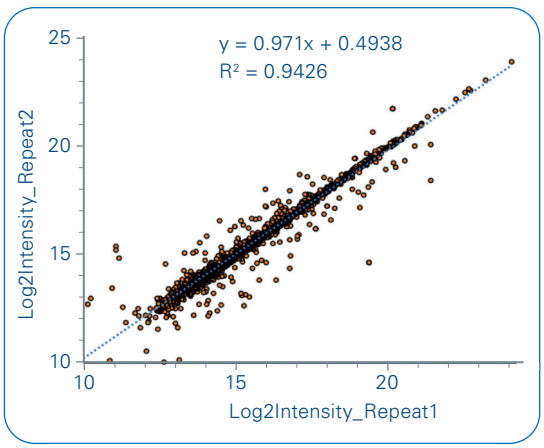

Figure 3: Biological reproducibility of the acetylated lysine peptides (mouse liver).

In one study, acetylated peptides were enriched from mouse liver and rice leaf samples with the PTM Bio lysine acetylation enrichment kit (CAT PTM-104) [2]. All digests were analyzed with the timsTOF Pro equipped with the nano-Elute UHPLC, resulting in over 1800 acetylated lysine peptides being identified from 100 µg of starting material over a 50-min gradient in mouse liver samples, and nearly 5000 acetylated lysine peptides could be identified with 1000 µg of starting material (Figure 2). Figure 3 shows the high reproducibility of identification made possible with the enrichment kit and timsTOF Pro.

For rice leaf sample analysis, 10,000 lysine acetylated sites were identified in a single shot over 4800 protein groups (50-min gradient).

Advances in clinical research

Direct protein analysis from tissue or biofluids raises a variety of analytical challenges. Protein expression varies depending on the genetic background of an individual, but also on time, localization, and as a physiological response to external stimuli. The dynamic nature of the proteome is further exacerbated by the effects of PTMs, leading to a variety of proteoforms being expressed from one protein, each with dedicated biological activity. Understanding the role of proteoforms in health and disease using TIMS TOF-MS is making considerable contributions to personalized medicine. For example, the speed and robustness of modern TIMS spectrometers are vital for analyzing large sample cohorts for clinical research.

A key service that PTM Bio provides is to profile proteome and different PTMs from clinical samples, such as blood, urine, cells, and tissues. With such analysis using the timsTOF Pro following by bioinformatic data mining, researchers can gather information about individual biological responses to diseases with the aim of developing personalized treatment options.

Looking ahead

The addition of the timsTOF Pro to the PTM Bio portfolio has expanded the company's business opportunities, as Dr. Cheng describes:

"I am very thankful for the timsTOF Pro. It is not only extremely powerful for our research, but when clinicians and researchers have heard that we have this instrument, they want to work and collaborate with us. That is really amazing.

We were the first company in China to install a timsTOF Pro – we are very proud of that. We would like to acquire more in the future to increase capacity for our clients."

For more information about PTM Bio, please visit www.ptmbiolabs.com/.

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

References

[1] Du X, Zhu J, Bu C, Zhou L, Liu X, Chen N, Mullens C, Kaspar-Schoenefeld S, and Koch H (2020). *Trapped Ion Mobility Spectrometry and PASEF Enables In-Depth Characterization of Protein Ubiquitination*, Poster WP 504, ASMS 2020.

2. Liu X, Zhu J, Bu C, Zhou L, Chen N, Du X, Ghose S, Kaspar-Schoenefeld S, and Koch H (2020). *High sensitivity lysine acetylation profiling with Trapped Ion Mobility Spectrometry and PASEF*, Poster WP 507, ASMS 2020.

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Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology.

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