MALDI-TOF mass spectrometry in the 21st century

Maria Emilia Dueñas and **Matthias Trost** (Newcastle University, UK)

This year marks the 20th anniversary of Koichi Tanaka securing the Nobel Prize in chemistry, shared with John Fenn and Kurt Wüthrich, for demonstrating the applicability of laser technology to analyze biological macromolecules. The principle of laser desorption is fundamental for many of today's analytical methods such as matrix-assisted laser desorption/ionization (MALDI). In this article, we provide an overview of MALDI and highlight the power, versatility and range of applications within the biochemistry community.

The controversy of the 2002 Nobel Prize for laser desorption mass spectrometry

In 2002, the Nobel Prize for chemistry 'for the development of methods for identification and structure analyses of biological macromolecules' was awarded to Kurt Wüthrich for the development of NMR spectroscopy, to John Fenn for developing electrospray ionization mass spectrometry and to Koichi Tanaka for developing desorption mass spectrometry. Whilst the awards to Wüthrich and Fenn were applauded by most, the award to Tanaka raised a strong debate in the mass spectrometry community. Many scientists in the field believed that the award failed to recognize the achievement of Franz Hillenkamp and Michael Karas on the development of matrix-assisted laser desorption/ionization (MALDI). The arguments were based on the fact that MALDI, which is now widely adopted, was developed prior to Tanaka's alternative method, soft laser desorption. Moreover, unlike MALDI, soft laser desorption was never widely adopted by the community.

In the early 2000s, the application of MALDI in the bio- and life sciences declined due to the increased use of electrospray ionization liquid chromatography mass spectrometry (ESI LC-MS). However, in the last decade, due to the advances in sample preparation and instrumentation, MALDI has once again become an increasingly popular tool for a range of applications.

What is MALDI-TOF mass spectrometry?

MALDI is a soft ionization method that uses laser irradiation to ionize intact (bio-)molecules which have been co-crystallized in a matrix of (usually) phenolic organic acids (Figure 1). Laser ablation of the dried spot enables transfer of both matrix and analyte molecules into the gas phase. Matrix molecules readily absorb the laser irradiation and enter an excited state which enables a proton transfer between excited matrix ions and vaporized analyte molecules, thereby ionizing them. Analyte ions are then accelerated by an electric field to enter the mass analyzer, in most cases, a time-of-flight (TOF) mass spectrometer. A TOF analyzer works on the principle that accelerated ions from the laser pulse in the MALDI source will enter a long, field-free tube in vacuum. As the ions are accelerate in the same electric field, they will separate in the field-free region according to their mass-to-charge (m/z) ratio. Thus, when ions hit the detector at the end of the flight-tube, the m/z ratio of the ions can be determined by calculating the time it takes them to fly through the tube.

The main advantages of MALDI-TOF MS are that it is robust, (relatively) insensitive to buffer components and contaminants and particularly fast. For example, MS is often not compatible with biochemical assays that contain high concentration of detergents and buffering agents as these can induce ion suppression, which is specifically problematic with ESI LC-MS. Moreover, in terms of the speed, newer instruments deploy laser shots at 10 kHz, allowing for less than 1 second per sample.

MALDI-TOF MS biotyping

A well-established application for MALDI-TOF MS is the classification of micro-organisms, also known as biotyping, which was first presented in 1996. Microbe classification is achieved by identifying protein biomarkers from intact microorganisms to generate a signature spectral mass fingerprint. Typically, this fingerprint reflects the unique profiles of highly abundant ribosomal protein profiles of a bacterial species. For classification, the specific spectral fingerprint is then compared against a database of known bacteria genus spectra to identify an unknown species. This allows fast, inexpensive, sensitive and robust genus and sub-species determination in clinical microbiology.

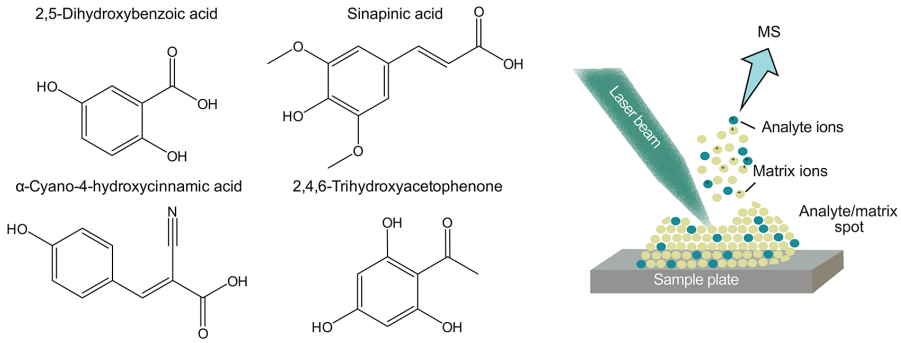

Figure 1. Chemical structures of used MALDI matrices (left) and schematic diagram of MALDI (right).

MALDI-TOF MS imaging

In 1997, Richard Caprioli first developed MALDI MS imaging and its application to molecular map tissues in biology and medicine. MALDI is used to acquire two-dimensional arrays of hundreds of thousands of mass spectra over a tissue sample to study numerous diseases and therapeutics. This allows one to map the localization of chemical species within a sample in a two-dimensional map. Since then, this technique has been widely adopted not only in the mass spectrometry community but also in biomarker discovery and clinical research. MALDI MS imaging paints a molecular picture by utilizing the versatility, selectivity and sensitivity of MALDI while preserving the spatial information inherent to the sample. This technology is used to visualize proteins, peptides, small molecules and drugs directly on tissue specimens, in applications ranging from the drug discovery pipeline (to study the localization of therapeutics and diseases), to forensic science (to examine evidence such as fingerprints, hair and fibres) and cancer research (providing histological information to find new biomarkers).

MALDI-TOF MS in drug discovery

In recent years, we and others have utilized the high speed of MALDI mass spectrometers for drug discovery. A MALDI plate with 1536 samples can routinely be analyzed in less than 10 minutes on some high-end instruments. This led to the development of enzymatic *in vitro* drug discovery assays where both the substrate and the product are analyzed. As these assays are label-free, they have often a higher physiological relevance than assays using fluorescently labelled read-outs. Moreover, recent data showed that one could combine the strengths of MALDI MS imaging and the speed of drug discovery to conduct cellular-based assays on a few thousand cells per well and measure specific biomolecules during phenotypic screening.

Due to the rise of high-throughput methods in the bio- and life sciences, MALDI-TOF MS will endure to have an important place in the toolbox of biochemists in the future.

Future outlook

As a field that is under continuous development, the MALDI MS community still needs to overcome many challenges. These are mainly connected with ion suppression issues, management and interpretation of large and complex datasets and inter-laboratory standardization. Recent advancements in sample preparation, instrumentation and applications of MALDI-TOF MS have allowed this technology to evolve during the last decades. For example, MS-based read-outs are susceptible to isobaric interference, which can result in unreliable results (i.e., false-positives in a drug screening campaign). MALDI-TOF MS coupled to ion mobility separation (IMS) had demonstrated significant utility for separation of isobaric species in the gas phase. By separating on the basis of collisional cross-section differences between ions, molecules can be separated based on their charge, shape and size. Incorporating both MALDI and IMS has increased the separation and identification of analytes, which not only simplifies the data interpretation, but also decreases false-positives and increases the confidence in results. MALDI-2 is another powerful advancement that will revolutionize the field in the upcoming years. So far, the development of MALDI-2, where a second laser is used to initiate an additional ionization process produced by the first MALDI process, has enhanced the sensitivity and dimensionality of this technology. ■

Further Reading

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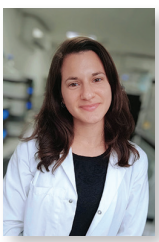

Maria Emilia Dueñas completed her B.S. in chemistry at the Universidad San Francisco de Quito (Ecuador) in 2013. In 2018, she obtained her PhD degree in analytical chemistry from Iowa State University (USA) after working in Dr Young Jin Lee's group. Her research focused on advancing the field of metabolomics using high-spatial resolution MALDI mass spectrometry imaging. She is currently a Marie Skłodowska-Curie Fellow at Newcastle University in Professor Matthias Trost's Laboratory of Biomedical Mass Spectrometry, where she leads the Drug Discovery group. Email: maria.duenas@newcastle.ac.uk

Matthias Trost studied chemistry in Freiburg, Germany, and Manchester, UK. He received a PhD in 2004 from the Helmholtz Centre for Infection Research, Braunschweig, Germany, for research on the proteome of the human pathogen Listeria monocytogenes *and* Listeria*-infected host cells. In 2010, Matthias became Programme Leader and Head of Proteomics in the MRC Protein Phosphorylation and Ubiquitylation Unit at the University of Dundee, Scotland, UK. Since 2017, he is Professor of Proteomics in the Faculty of Medical Sciences at Newcastle University, UK. Email: Matthias.Trost@newcastle.ac.uk*

