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# MALDI mass spectrometry in medical research and diagnostic routine laboratories

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ABSTRACT

Franz Hillenkamp and Michael Karas in the eighties of the last century invented and developed the matrix-assisted laser-desorption/ionization mass spectrometry (MALDI-MS). Very soon after their invention, MALDI became one of the main tools for the identification of proteins and therefore for proteomics. With MALDI, not only peptides and proteins but also other larger biomolecules like glycans, lipids, and nucleotides are detectable. Thus, it is not surprising that MALDI has become a powerful instrument in medical research and today is well established in routine medical laboratories. This review highlights the first pioneering years after the invention of MALDI-MS with respect to its impact on applications in medicine. Furthermore, the most important developments of MALDI-based techniques in medical research and routine laboratories are reviewed, such as MALDI mass spectrometric imaging and identification of bacteria.



**Contents**



## 1. Introduction

After the development of the main principle of the matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) by Franz Hillenkamp and Michael Karas in 1985 [1], the group reported the detection of labile biopolymers – proteins – with this approach for the first time in 1988 [2]. This paved the way for mass spectrometry in life sciences including medicine. The soft desorption and ionization by MALDI is achieved by irradiation of crystals consisting of a MALDI matrix compound and the analytes with laser light of a wavelength close to that of the absorption maximum of the MALDI matrix. As MALDI matrices, small organic acids like 2,5-dihydroxy benzoic acid (DHB) with chromophores, absorbing UV-light in the range of 320–340 nm, are used. The matrix molecules in the crystals irradiated with the UV-laser absorb the energy of the laser, causing an immediate and rapid transfer of the matrix molecules from the solid phase into the gas phase. This phe-

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nomenon catapults the analytes into the gas phase as well. Since the wavelengths of the absorption maxima of the UV spectra of most of the biomolecules are not close to the wavelength of the laser, the uptake of energy usually is low: reducing the probability of fragmentation. In addition, the adiabatic expansion of the matrix molecules from the crystals into the gas phase causes cooling thereby minimizing fragmentation of labile analytes. Thus most of the labile biomolecule ions are getting in an intact form into the gas phase. Analyte ions are already present in the MALDI matrix crystals but are also formed in the gas phase. An in-depth review about desorption and ionization processes of MALDI is given by Knochenmuss [3]. The main charge state of MALDI ions is one, thus making interpretation of MALDI spectra relatively easy. In the case of well-desalted samples, analyte ions are protonated ($[M+H]^+$) and often present with a single signal for a defined analyte (if the spectra are not zoomed in for displaying the isotopic distribution). The presence of sodium and potassium ions can result in the formation of several adduct ions from one single defined analyte represented by corresponding signals $[M+Na]^+$,$[M+K]^+$ and$[M+H]^+$, thereby lowering the detection limit. Thus careful and complete desalting of the samples prior to MALDI-MS is recommendable and part of many protocols.

The choice of the proper MALDI matrix with respect to an individual biomolecule class is of most importance as demonstrated by the following anecdote. In the early years of MALDI the group of Karas and Hillenkamp extensively analyzed biologically active fractions purified from human platelets and other cells without any success over a period of several years. After changing to the MALDI matrix hydroxy-picolinic acid (HPA); a matrix for the analysis of nucleotides, lots of signals appeared in the MALDI spectra of these biologically active fractions. By the information of the molecular weights derived from these MALDI spectra and additional UV spectroscopic data, the biologically active molecules were identified as small nucleotides diadenosine-pentaphosphate ($Ap_5A$) and diadenosine-hexaphosphate ($Ap_6A$) [4], which cannot be detected with the matrix DHB.

For getting deeper insights into the mechanisms of MALDI ionization and desorption as well as advantages and limitations of various matrices with respect to the biomolecules of interest, the following reviews are recommended [5–10], which should be read carefully, before starting MALDI analysis of samples in life science including medicine. MALDI has become a success story for biomedical applications not only because diverse classes of biomolecules can be detected in an intact form because of the soft desorption and ionization processes but also because sample preparation for MALDI-MS is relatively simple and spectra are easy to interpret.

## 2. MALDI-MS for the analysis of proteins

Although Franz Hillenkamp was not a biochemist, he was obviously aware of the huge potency of MALDI for bioanalytics after his ground-breaking publication from 1988, which was cited in approximately 4000 articles at the end of June 2016. Consequently, he supervised PhD students, together with Ute Bahr and Michael Karas, on topics focusing on the MALDI mass spectrometry (MS) of some main classes of biomolecules. He thus opened this method for research and diagnostic purposes in medicine which will be highlighted in this review

Arnd Ingendoh started in the group of Franz Hillenkamp in 1988 and focused his work on MALDI mass spectrometry (MS) of proteins [11–21]. In his first publication in 1989, he demonstrated the detection of proteins of masses from 100 000 to 250 000 Da (β-D-galactosidase, glucose isomerase, and catalase), using nicotinic acid as MALDI matrix and a 266-nm light of a Nd-YAG laser [22]. In a following paper, he showed that MALDI-MS is a very sensitive method for detecting proteins [12], which is one of the major beneficial properties of MALDI awakening the interests of scientists in the field of life-sciences including medicine. Measurement of intact proteins with MALDI-MS became the most successful application in routine medical diagnostics and revolutionized identification of bacterial strains in microbiology [23]. For identification of bacteria with MALDI-MS, patient samples are transferred to a culture medium supporting bacterial growth and then incubated. After defined incubation times a small part of a bacterial plaque is transferred to a MALDI target, MALDI matrix added and the dried crystals analyzed with a MALDI-MS instrument. The resulting spectra show signal patterns in the range from 1 to 15 kDa, many of them representing ribosomal proteins. For identification of the strains, the patterns are compared with spectral libraries of defined bacteria [24]. Even bacterial strains which show only very small differences in their genomes can be differentiated and identified as shown by Christner et al. [25]. They were able to identify Shiga-Toxigenic Escherichia coli (ESTEC) by two MALDI signals representing two small proteins, unique to ESTEC.

Kerstin Strupat, another PhD student of Michael Karas and Franz Hillenkamp, together with Christoph Eckerskorn, demonstrated that proteins blotted to membranes can also be detected with MALDI [26–30].

A method for the quantification of target proteins in plasma or serum performable with MALDI-MS was developed by Anderson, who termed it “the stable isotope standards and capture by antipeptide antibodies – SISCAPA”. This method was developed with the aim to transfer it into the routine diagnostic. SISCAPA consists of four steps: (1) digestion of all proteins within the sample with trypsin; (2) addition of stable isotope labelled internal standard peptides; (3) enrichment of the peptides of interest by immobilized specific antibodies; (4) measurement with MALDI-MS (Fig. 1). The quantity of the target protein is calculated by the signal intensities of the target peptide ion and its heavy isotope labelled counterpart (internal standard peptide). This method allows shorter analysis time because long separation gradients with reversed phase and/or ion exchange chromatography are not needed [31]. Alternate to MALDI-MS, the quantification can be achieved by liquid chromatography coupled to a tandem mass spectrometer (LC–MS/MS) using multiple reaction monitoring (MRM). Inspired by the work of Borchers on immuno-MALDI (iMALDI) [32] and the work of Nelson on mass spectrometric immunoassay (MSIA), Anderson compared the typical SISCAPA-MRM setup with MALDI-MS analysis and concluded that it is advantageous, because the MALDI-MS measurement is much faster than the LC–MS/MS analysis. In cases where SISCAPA is specific enough to eliminate the need for chromatographic separation prior to MS analysis, SISCAPA MALDI-MS provides a simple and precise method and may be applicable for diagnostic purposes in clinical routine laboratories in the future [33,34]. However, since the development of anti-peptide antibodies is difficult and expensive it is questionable if this method will be successful on a long term.

## 3. MALDI-MS for the analysis of peptides

In close proximity to Franz Hillenkampís institute (Institute of Medical Physics), one of the authors of this review (H. Schlüter), was located in the institute for biochemistry and in the medical faculty of the University of Münster from 1988 to 1996. At that time he was searching for unknown vasoconstrictors potentially responsible for causing hypertension according to a hypothesis of Zidek [35]. The coworker of Zidek got aware in 1989 of the MALDI technique and its high sensitivity towards the detection of peptides. Since many vasoconstrictors like angiotensin-II were known to be peptide hormones, the author of this review asked Michael Karas and Franz