Surface-assisted laser desorption/ionization mass spectrometry

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Laser desorption/ionization mass spectrometry (MS) is rapidly growing in popularity as an analytical characterization method in several fields. The technique shot to prominence using matrix-assisted desorption/ionization for large biomolecules (>700 Da), such as proteins, peptides and nucleic acids. However, because the matrix, which consists of small organic molecules, is also ionized, the technique is of limited use in the low-molecular-mass range (<700 Da). Recent advances in surface science have facilitated the development of matrix-free laser desorption/ionization MS approaches, which are referred to here as surface-assisted laser desorption/ionization (SALDI) MS. In contrast to traditional matrix-assisted techniques, the materials used for SALDI-MS are not ionized, which expands the usefulness of this technique to small-molecule analyses. This review discusses the current status of SALDI-MS as a standard analytical technique, with an emphasis on potential applications in proteomics.

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Mass spectrometry (MS) is a classic analytical chemistry technique that has seen an amazing rise in popularity in the last decade with a clearly discernable shift towards biochemical applications. According to many researchers in the field, this technique has become the cornerstone of the proteomics revolution because it enables rapid access to accurate information on protein identification, sequence and quantitation. While several ionization methods are currently in use, two modes dominate in commercial instruments used for proteomic analyses. These are matrix-assisted laser desorption/ionization (MALDI) (FIGURE 1) [1–4] and electrospray ionization (ESI) [5] MS, both of which have their merits. For instance, ESI-MS enables easier online coupling to pre-MS separations, such as high-performance liquid chromatography (LC) or capillary electrophoresis (CE). This has proved to be particularly useful in multidimensional LC-based whole proteome studies [6], although robotic MALDI-plate spotters such as the ProBot™ are now commercially available and have facilitated increased MALDI usage in proteomic experiments requiring faster throughput. A comparison of the two ionization methods demonstrates that both are equally successful in protein identification, with differences such as more unique peptide molecular weight matches with MALDI time-of-flight (TOF)-based mapping [7]. A large part of the success of the robust MALDI platform is due to the ease of sample preparation and tolerance of salts as well as common biochemical buffer components. In addition, while tandem MS (MS/MS) (or multiple rounds of MS cycles usually involving fragmentation) is relatively routine for protein and/or peptide identification, it is also an integral component of recently developed quantitative MS strategies such as iTRAQ™ [8]. In these strategies, analysis of isobaric tagged samples in MALDI mode allows for the examination of every peptide eluting out of the high-performance LC, as compared with ESI-MS where a predetermined choice must be made, which is typically based on the two most intense peaks in any particular MS scan. Furthermore, one of the most significant advantages of MALDI-MS is the use of samples in the solid state, which enables the samples to be archived and revisited for additional levels of analyses. Advances in interfaces to upstream

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separations and other biochemical approaches, including those for quantitative analysis, have recently made MALDI-MS an indispensable tool in the modern proteomics laboratory [9–13].

In MALDI, a matrix that typically consists of small organic acids is added to an analyte to act as an intermediate for absorbing energy at the laser wavelength used for excitation and then subsequently transferring the energy to an analyte. The choice of a matrix for a specific analyte is a critical parameter, and generally specific to a given class of materials. For example, proteins and peptides are most frequently analyzed using 3,5-dimethoxy-4-hydroxycinnamic (sinapinic) acid, or α-cyano-4-hydroxycinnamic acid, respectively. Although effective for proteomic analysis, the use of a matrix to receive energy from the laser and transfer it to a crystallized analyte has some disadvantages. In particular, matrix molecules are ionized along with the analyte of interest, which generates a significant background signal of small matrix ions that suppresses and hinders small-molecule analyses. In addition, all of the advantages described above for MALDI could be significantly improved by eliminating the matrix required for ionization of analytes by generalizing the laser desorption/ionization (LDI) technique, as well as increasing the speed at which sample measurements are made. This presents a significant need to develop novel platforms for matrix-free or surface-assisted LDI-MS techniques.

Surface-assisted laser desorption/ionization mass spectrometry

Surface-assisted laser desorption/ionization (SALDI) MS is a term originally used for MS analysis involving desorption/ionization from particles of, for example, cobalt or carbon suspended in solution [2,14]. Since this work was reported, several different materials have been used to characterize small molecules using LDI-MS. These include, but are not entirely limited to, organic materials, such as carbon nanotubes (CNTs) [15–20] and polymers [21–24], inorganic materials [25], such as silicon [26–28] and metal oxides [29,30], as well as composite materials, which consist of an organic matrix embedded in an inorganic supporting material [31–34]. This review will make a distinction between techniques where matrix materials are ionized along with analytes of interest, as in MALDI-MS, and techniques in which the matrix is not ionized and is only used to promote the ionization of analytes. This latter technique is defined as SALDI for the purposes of this review.

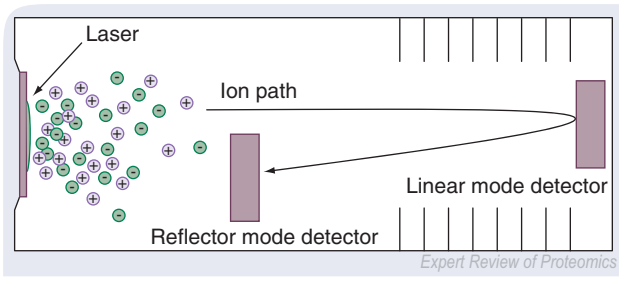


Figure 1. Schematic of laser desorption/ionization time-of-flight mass spectrometry. Laser excitation of samples (or sample–matrix mixture in the case of matrix-assisted laser desorption/ionization) results in desorption of ionized species that then drift into the time-of-flight tube and are detected in either linear or reflector mode.

Carbon materials

Carbon assembled into fullerenes and nanotubes has been used successfully to promote ionization of small analytes without matrix interference in the low-mass range. Carbon assemblies have been proposed to both absorb energy from the exciting ultraviolet (UV) laser and then couple the energy to an analyte in close contact to promote ionization and desorption processes, similar to a typical MALDI matrix. Carbon fullerenes and functionalized carbon fullerenes have been used for the mass spectral characterization of small molecules, but its low sensitivity has limited this material's use as an effective matrix [35–37]. As an alternative to carbon fullerenes, researchers have recently begun studying CNTs as a SALDI matrix. Xu and coworkers first reported the use of CNTs prepared from coal by an arc discharge method for SALDI-MS [19]. Thin films of CNTs shown in FIGURE 2A were prepared by evaporative deposition from a toluene solution containing nanotubes onto a standard MALDI plate [17]. CNTs were shown to detect as little as 50 amol of small molecules (<1200 Da) with lower laser power thresholds compared with standard MALDI techniques [19]. Chen and coworkers extended the mass range of CNTs prepared from a NaH-treated anodic aluminum oxide template by adding a high concentration of citrate buffer, which acts as a proton source and suppresses alkali cation adducts, to solutions containing small peptides and proteins [20]. In this way, small peptides at a concentration of a few femtomol, as well as cytochrome c (12 kDa), could be detected. It was also demonstrated that CNTs can be used to preconcentrate analytes from a solution containing potentially interfering species, such as surfactants, based on electrostatic and hydrophobic interactions between citrate-coated nanotubes and analyte [20]. A solution containing the analytes of interest was mixed with a solution of citrate-coated nanotubes and then separated by centrifugation. The CNTs were resuspended and deposited on a standard MALDI plate for LDI-MS analysis. This preconcentration method was also useful for analyzing a tryptic digest of cytochrome c. In a similar fashion, Pan and coworkers have used unfunctionalized CNTs to selectively extract small molecules from a mixture based on strong hydrophobic interactions between the CNT and molecules, followed by LDI mass characterization [16]. Addition of glycerol and/or sucrose was required to achieve the highest signal-to-noise ratio as well as the greatest signal resolution and intensity.

There are two disadvantages of the use of CNTs for LDI-MS. First, CNTs are not covalently attached to the MALDI plate, and can therefore fly from the surface and foul the TOF detector over time. Second, CNTs are not easily dissolved in aqueous solutions, which limits their use in proteomic analyses. However, CNTs have recently been immobilized in a polyurethane adhesive prior to sample spotting to prevent CNTs from flying off the MALDI support surface (FIGURES 2B & C) [18]. The structure of the immobilized CNTs was similar to CNTs without added polyurethane, and the immobilized CNTs were just as effective as CNTs deposited without the additive. Immobilized nanotubes were able to characterize small peptides and neutral

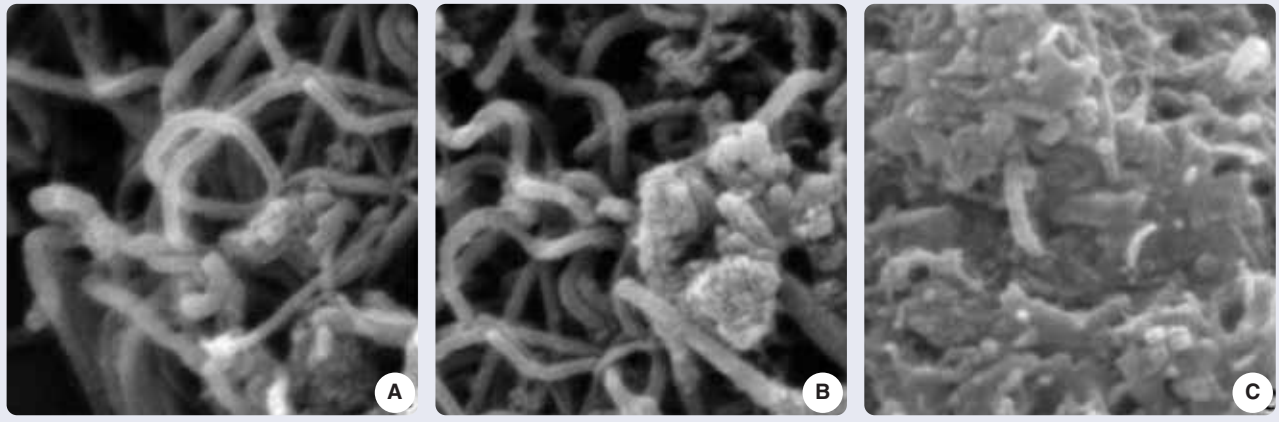


Figure 2. Scanning electron microscopy images of (A) multiwalled carbon nanotube layer, (B) a nanotube layer in the presence of an immobilizing polymer and (C) a nanotube layer immobilized in a polymer after sample spotting. Reprinted with permission from [18] © 2005, Elsevier, The Netherlands.

carbohydrates (which are generally difficult to observe by LDI) in solution, as well as glucose in urine samples. In addition, immobilization allows for fast spotting techniques, such as electrospray sample deposition, which forgoes searching for a 'sweet spot', as well as facilitating post-source decay experiments. In a slight variation on the above work, carbon graphite microparticles have also been dispersed in an active polymer, Naifion®, to promote desorption and ionization of analytes [38]. The authors demonstrated that the carbon particles act to absorb and transfer energy to the analytes, while the polymer, in this case Naifion, acts to donate protons to the analyte to promote ionization.

For improved CNT analysis capabilities in aqueous environments, some researchers have recently explored the use of oxidized CNTs, which are prepared by chemical oxidation in nitric acid, for SALDI-MS [15,17]. Oxidized CNTs have been demonstrated to form higher quality films compared with unfunctionalized CNTs, making them easier to manipulate. Due to their hydrophilic nature, samples also crystallize more uniformly, which leads to improved laser shot-to-shot reproducibility, allowing semiquantitative analysis to be performed, which is a significant advantage over nonoxidized nanotubes. Furthermore, addition of a proton source is not necessary when using oxidized CNTs, as the oxidized surface has numerous protonated hydroxyl (-OH) and carboxy (-COOH) groups that can donate a proton to an analyte.

Polymeric materials

UV-absorbing oligomers and polymers have recently been used by several groups to promote ionization of analytes for LDI-MS [21–24]. As is the case for CNTs, the oligomers were either deposited directly on a standard MALDI substrate to form a crystalline-like film, followed by spotting of the analyte, or co-dissolved with the analyte, then spotted together on a sample plate prior to LDI-MS analysis. An advantage of these polymeric materials is that sample signals in positive-ion mode are observed without a protonation or metal ion adduction step, as is generally necessary for other surface ionization techniques. A charge transfer mechanism has been proposed to account for the ionization mechanism using these types of materials [22]. This ionization process has proved to be particularly useful for the characterization of nonpolar compounds, which are generally difficult to characterize by LDI-MS techniques. In negative-ion mode, the dominant mechanism still appears to follow a deprotonation process to form ions. Woldegiorgis and coworkers reported that small oligomers used for LDI-MS analyses were ionized, similar to a typical matrix, along with the analyte, but did not fragment and, as such, did not appear to interfere or suppress the mass spectrum of small molecules (<350 Da) [21]. Several neutral organometallic ferrocene-type complexes as well as charged organic acids were also characterized using this method at the 50-pmol range [22]. Soltzberg and coworkers used similar, but larger, alkyl-substituted thiophene polymers for SALDI-MS of small aromatic complexes [23]. The sensitivity of this technique was limited to approximately 10 nmols, compared with attomole/femtomole sensitivity using typical MALDI matrices. Peterson and coworkers have also reported the use of copolymer and polymer blends based on methacrylate monomers deposited on standard MALDI plates for SALDI-MS analysis of small molecules [24]. UV-initiated polymerizations enabled the authors to prepare an ordered polymer array on which to spot samples. The hydrophobic nature of the methacrylate polymers allowed approximately 1-µl sample volumes to be spotted and concentrated in specific areas, which improved the overall signal and spot-to-spot reproducibility. At laser fluencies used for typical MALDI-MS, the films were effective at characterizing several small organic molecules. Furthermore, at ambient conditions, the polymer films were found to be stable for almost a month without a noticeable change in analyte signal. Varying the average pore size in the polymer films by changing the polymer mixtures was also demonstrated to have an affect on desorption and ionization processes. A pore size of approximately 200 nm was found to be optimal for energy transfer and desorption of the analyte for the small molecules reported [24].

Semiconducting materials for surface-assisted laser desorption/ionization mass spectrometry

Inorganic and composite materials have also been extensively studied for SALDI-MS. These materials may potentially be more useful for SALDI analyses because they are generally more stable and robust and, in some cases, have a more labile surface chemistry for analyte-specific applications compared with most of the organic materials described earlier.

The most successful surface to promote LDI processes identified to date is porous silicon. Work by Wei and coworkers reinvigorated the area of surface-assisted MS when they reported that electrochemically etched silicon was effective for SALDI-MS [28]. Their invention, termed desorption/ionization on silicon (DIOS), opened a new area of research in the field. DIOS films are now sold commercially by Waters, Inc. and are used extensively for the characterization of molecules (<6000 Da) without the need for a matrix. However, the initial DIOS films suffered from a short active life [39], due to the chemistry of exposed silicon hydride on the etched surface [40]. Since then, the working life-time has been improved to at least 9 months by oxidation of the hydride surface to form a thin SiO_x film and self-assembly of alkyl silanes on the oxidized surface [41]. Furthermore, under special storage conditions, the modified porous silicon films may be used up to 1 year after the substrates are prepared [42]. An advantage of the DIOS chips is the ability to modify the surface chemistry for a variety of LDI-MS applications, which are described in more detail later (see Proteomic applications) [43,44].

Cuiffi and coworkers have also recently applied a plasma deposition method [45] to prepare an array of nonporous columns of semiconducting material, such as silicon, that are perpendicular to a substrate surface for SALDI-MS [26]. The columns were reported to be 3–10-nm thick with a variable spacing/void distance [45]. Currently, germanium columnar/void films prepared by this method are commercially available under the name of Quickmass® from Shimadzu Corp. These films are reportedly stable under ambient conditions for over 1 year without observable loss to its LDI-MS activity, providing an archival capability that is comparable to the functionalized DIOS chips described earlier [46]. Since these films have only recently become available, little work has been reported on their use to date. In a related class of materials, silicon in the form of nanowires has recently been used as a matrix for SALDI-MS [47]. Silicon nanowires, approximately 1 µm in height and 10–40-nm thick were catalyzed by gold deposited on a surface to grow silicon nanowires perpendicular to the substrate [47]. Compared with MALDI and DIOS, the silicon nanowire bundles needed significantly less laser energy to promote ionization of attomolar quantities of small organic molecules. The mechanistic reasons for the lower energy threshold value are not entirely clear at this time.

Sol-gel films

Sol-gel films are prepared by an initial hydrolysis step in a solution of molecular precursors (e.g., tetraethylorthosilicate), followed by a condensation step to form a porous 3D network. Prior to complete condensation, solutions may be deposited onto surfaces to form a thin film by dip-coating or spin-coating with good control over film thickness. Silica $(SiO_2)_x$ alone does not absorb UV light suitably efficiently for LDI-MS; however, it may be doped with or deposited on an absorbing material, which, by itself, is not an active surface for SALDI-MS (e.g., planar silicon) in order to yield an effective SALDI surface [26]. In an early example of SALDI-MS using a silica-based film, Chen and coworkers used graphite drawn by a soft carbon pencil on silica to analyze small molecules dissolved in a sucrose, glucose and methanol solution [30]. Building on these results, Hoang and coworkers doped active carbon into a silica sol-gel for the characterization of several organoselenium compounds [48]. In an interesting combination of MALDI and SALDI, Lin and coworkers have doped silica with standard MALDI matrices, such as 2,5-dihydroxybenzoic acid (DHB) and 3,4-diaminobenzoic acid (DABA) for MS analyses [31–34] The silica-encapsulated organic matrix molecules absorb and transfer energy to analytes spotted on their surfaces, without being significantly ionized when the concentration of matrix is less than 10,000 parts per million [34]. It has been possible to characterize peptides and proteins up to 16 kDa at a concentration of 81 fmol using these composite materials, which is noted by the authors to be approximately five- to ten-times less sensitive than standard MALDI procedures. The DABA-doped silica films were demonstrated to characterize oligonucleotides as large as a 72-mer, with a limit of detection for a 24-mer at 20 fmol [31]. The amino groups of the encapsulated DABA also effectively desalted the oligonucleotide, which almost completely minimized the observance of salt adducts in the mass spectrum. These amorphous silica films are effective as SALDI surfaces for at least 1 week, although the signal intensity decreases during this time. The relatively short useful lifetime of these sol-gel materials is presumably due to increased condensation within the silica sol-gel network as it ages over time. These results also indicated the need for a porous network to obtain significant ionization and desorption, similar to that described earlier for polymer films.

As already identified, long-term stability of sol-gel-derived films is an obstacle to overcome. To address this, the authors have recently studied the use of mesoporous silica thin films deposited on silicon substrates for SALDI-MS characterization of small molecules [49]. The mesoporous films, prepared by an evaporation-induced self-assembly process [50] and subsequent UV exposure [51] are simple to produce, require no special handling or storage and are stable at ambient laboratory conditions for at least several months. The authors have observed mass spectra of several low-molecular-weight molecules desorbed from mesoporous silica films without added matrix. Fonash and coworkers have hypothesized that silica deposited on planar silicon helps couple the laser light to the silicon surface to promote ionization of deposited analytes [46]. Drug candidates such as fosmidomycin have been subjected to SALDI-MS analysis as a quality control step prior to use in inhibition experiments in Dattelbaum and Iyer's laboratory. In addition,

Koppisch and Iyer have routinely used SALDI-MS on mesoporous silica for the structural characterization of siderophores, which are small iron carriers secreted by various bacteria to acquire iron from the surrounding milieu. Many siderophores are nonribosomal peptides and are structurally diverse [52]. MS fragmentation patterns shown in FIGURE 3 provide accurate identification of motifs, which, in turn, help assign the final structure of a given siderophore.

Unlike silica, titania $(TiO_2)_x$ strongly absorbs UV light and, therefore, has also been investigated as a potential material for SALDI-MS [53]. Amorphous titania has been prepared with a molecular imprint of α-cyclodextrin to extract the α-form of cyclodextrin from a mixture of cyclodextrins. However, this sol-gel process resulted in the presence of many Ti_xO_y peaks in the mass spectrum, which limited the usefulness of amorphous titania. These peaks could be removed by thermally treating the titania films to form a crystalline anatase phase prior to LDI-MS analysis. Citrate buffer was also required as a proton source to characterize several proteins, including trypsinogen (24 kDa), which is the largest protein characterized by any SALDI-MS technique reported to date. Tryptic digests of cytochrome c were also performed on these nanocrystalline titania chips, with more peaks observed at a lower signal-to-noise ratio compared with MALDI-MS [29]. The substrate was also reported to have an effect on the ionization/desorption process. Aluminum substrates were found to be more effective than glass for ionization, which may be attributed to the higher thermal conductivity properties of aluminum. The addition of tungsten ions into the titania framework has proved to be effective for SALDI analysis of a small membrane protein, gramicidin (~1140 Da) [54]. Although the mechanism for desorption/ionization from these materials is unclear, the significance of the porous framework was demonstrated, as a greater signal (by ~2 orders of magnitude) was obtained from the ordered porous films compared with the signal from a nonporous film of the same material.

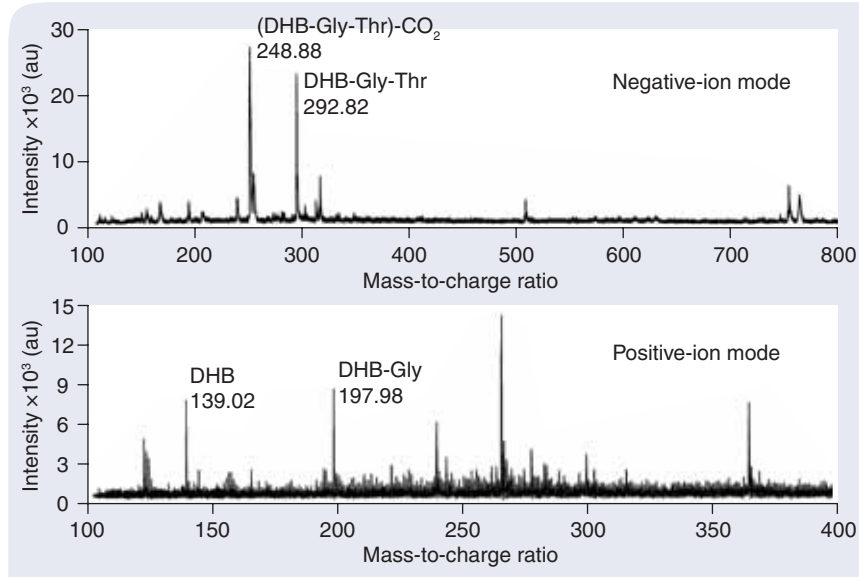


Figure 3. Surface-assisted laser desorption/ionization mass spectrometry of bacillibactin hydrolysate on nanoporous silica films. Subunits of bacillibactin were identified by analyzing the hydrolysate in positive- and negative-ion mode, firmly establishing the components of this siderophore. au: Arbitrary units; DHB: 2,5-dihydroxybenzoic acid.

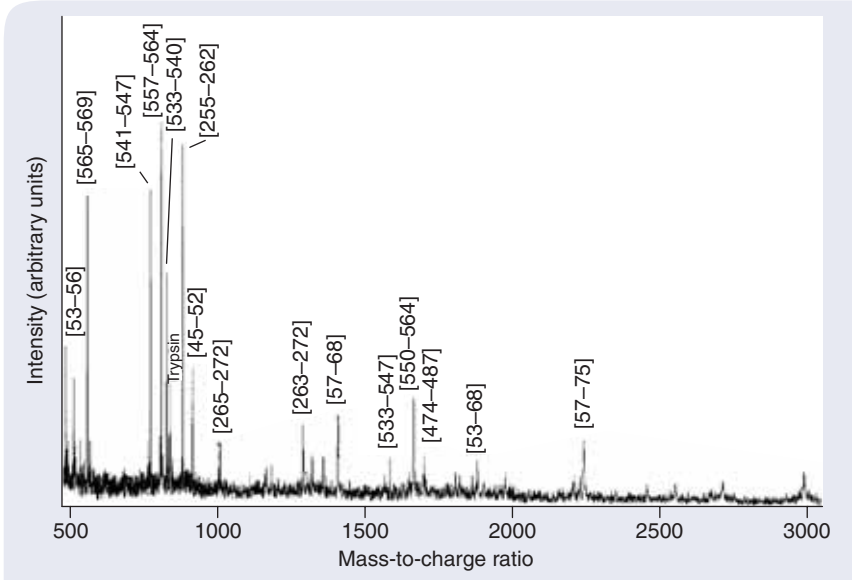
Proteomic applications

While part of the motivation to develop (and the most significant impact of) SALDI-MS approaches is to facilitate small-molecule analysis, several studies have demonstrated effective desorption/ionization of biological molecules for proteomic applications. The most common sample in routine proteomic analyses is a tryptic digest of specific proteins or a mixture of proteins. The resultant peptide mass map (PMM) is usually sufficient to identify the protein by performing database searches against theoretical digests of protein sequences. In some of the earliest examples with DIOS, Wei and coworkers analyzed mixtures of peptides, even in the presence of saturating concentrations of salt [28]. In the same study, excellent resolution was obtained with angiotensin, comparable to that obtained through regular MALDI-MS. These observations were substantiated by studies in the authors' laboratory using an alternative SALDI platform, mesoporous silica on planar silicon, where several peptides were analyzed with high resolution [49]. Tryptic digests of bovine serum albumin, cytochrome c, β-lactoglobulin and other proteins have also been analyzed on the DIOS chip [40], as well as on a titania sol-gel film [29]. While the signal-to-noise ratios in both cases are clearly sufficient to identify the proteins, the data presented do not address the sensitivity of the desorption/ionization process. A comparison between various matrices and the titania film suggests that the SALDI approach can avoid problems associated with matrix suppression [29]. For instance, several peaks that were not seen using standard matrices, such as sinapinic acid, α-cyano-4-hydroxycinnamic acid and DHB, were observed when a cytochrome c digest was analyzed on the titania film. However, it appears that a few peaks are also only observed when a matrix is used, although the peaks in each case were sufficient for protein identification. In other studies, a standard MS peptide calibration mixture on a DIOS chip with ions under 3 kDa were analyzed with sufficient resolution to observe an isotopic envelope in the approximately 2700-Da mass range [55].

In addition to PMM, another important area of proteomics is to functionally characterize proteins or, more specifically, ascertain enzymatic activity of specific proteins. While MS has not been the traditional choice for these analyses, SALDI-MS now offers an attractive alternative to labeling-based

approaches. An example of this approach is demonstrated in experiments conducted by Lewis and coworkers, where the authors performed an activity assay for acetylcholinesterase on a DIOS chip [55]. The authors were able to identify the substrate and products of the enzyme reaction in the same spot without any additional steps. Furthermore, trypsin digestion of acetylcholinesterase was achieved on the DIOS chip within 4 h, and the resultant peptides were sufficient for unambiguous identification of the protein. In a similar fashion, the authors performed PMM of several other proteins on the DIOS chip, as shown in FIGURE 4.

These experiments highlight an important application for high-throughput proteomics studies; that of having trypsin-functionalized chips on which a protein sample could be spotted, digested and analyzed without any additional treatment. In addition, the ability to immobilize enzymes in active form and measure their activity enables high-throughput assays, such as a MS plate reader described by Shen and coworkers, to rapidly screen enzymes and their inhibitors at sampling rates of up to 38 inhibitors per minute on current instrumentation [56]. Meng and coworkers describe another interesting use of the hydride-covered porous silicon surface [43,44]. The authors functionalized the porous silicon surface using biotin, coated the surface with avidin and then used the chip as an affinity-capture device for biotinylated ligands, including peptides. The use of reversible Diels–Alder reactions to attach molecules enables the detachment and detection of these molecules using LDI-MS. This scheme was used to probe the active site of glutathione *S*-transferase by affinity enrichment on a biotinylated chip, which resulted in an estimated 100-fold enhancement in sensitivity compared with underivatized sample.



Rank	Probability	Protein description
1	0.64	PEN3_ADE02 PENTON PROTEIN (VIRON COMPONENT III BASE PROTEIN)
2	0.36	PEN3_ADE05 PENTON PROTEIN (VIRON COMPONENT III BASE PROTEIN)
3	2.1^{e-17}	(M55059) HEMAGGLUTININ [influenza type A]

Figure 4. Desorption/ionization on silicon mass spectrometry data from an adenovirus penton protein digest and subsequent identification of the correct serotype. Reprinted with permission from [55] © 2003, Elsevier, The Netherlands.

Expert commentary & five-year view

In a relatively short period of time, SALDI-MS has gained significant attention as a technique with diverse potential. A wide range of molecules, such as peptides, nucleic acids and products of enzyme reactions, has been effectively analyzed. In addition to the obvious advantages of avoiding matrix peak interference, SALDI-MS platforms offer an endless range of functionalization options. As the field progresses towards more functional studies, the authors envisage increased use of SALDI-MS for functional characterization of proteins. In the authors' opinion, surfaces with embedded enzymes will form a major component of proteomic analyses on SALDI chips, especially for high-throughput screening of agonist–antagonist libraries or for studying the rate of inhibition of enzyme reactions. Such analyses in a high-throughput format will be of great benefit to the pharmaceutical industry in drug candidate screening. Wall and coworkers performed a detailed comparison of DIOS-TOF-MS versus conventional LC/MS/MS, and concluded that, while LC/MS/MS yields better dynamic range, the DIOS-TOF-MS method provides adequately precise and rapid measurements for enzyme inhibition in a relatively simple assay format [57]. Furthermore, although robotic plate spotters are capable of mixing LC or CE elutants with a matrix solution, the use of a SALDI support, which removes the need for a matrix, will significantly simplify these types of experiments. This will be particularly advantageous for CE separations using LDI-MS as the detection mode (CE-MS), where use of a T-junction to bring in a matrix solution can result in a backflow and subsequent loss of resolution. The success of SALDI-MS platforms with post-source decay analyses suggests that sufficient parent ion intensity can be obtained to perform subsequent fragmentation, thus enabling the effective use of these platforms on newer generation MALDI-TOF/TOF instruments.

However, SALDI-MS is still relatively immature as a proteomics technique, despite several studies that have been published to date on protein and peptide analyses using these platforms. To a certain extent, this may be due to a limited number of commercially available surfaces. In addition, a large part of current proteomics approaches involve large-scale proteome mapping, which is easily achieved using available approaches and, thus, a specific need for SALDI-MS has not yet been established in this area. Another significant challenge facing SALDI-MS for proteomic analysis is the size limitation, which is approximately 6 kDa for commercially available substrates. This limitation may be due to poor energy transfer process between the surface and analytes, and may potentially be improved by optimization of pore sizes within the film. Some of the surfaces described earlier can characterize larger proteins, but have short shelf lives, which limits their potential use. A stable SALDI substrate that can be used to characterize both small and large molecular complexes at the same time would be extremely beneficial for functional proteomic work.

Key issues

- Matrix-free laser desorption/ionization mass spectrometry (MS) has been shown to facilitate analysis of small molecules, peptides, amino acids, drugs and metabolites.
- A variety of materials, including carbon nanotubes, porous silicon and sol-gel composite materials, have been studied for surface-assisted laser desorption/ionization (SALDI) MS; two materials are currently commercially available, desorption/ionization on silicon (Waters, Inc.) and Quickmass® (Shimadzu Corp.).
- SALDI-MS significantly simplifies the interfacing of upstream separations with laser desorption MS.
- Functionalization of SALDI surfaces with embedded enzymes will form a major component of proteomic analyses on SALDI chips, especially, for example, in high-throughput screening of agonist–antagonist libraries or for studying the rate of inhibition of enzyme reactions.
- While lower mass range analysis is now well established for SALDI substrates, a major challenge is to extend the applicability of this technique to size regimes accessible by typical matrix-assisted laser desorption/ionization experiments.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Karas M, Bachmann D, Bahr U, Hillenkamp F. Martrix-assisted ultraviolet-laser desorption of nonvolatile compounds. *Int. J. Mass Spectrom.* 78, 53–68 (1987).

•• First description of matrix-assisted laser desorption/ionization (MALDI) as we know it today. Seminal work firmly established MALDI as a technique for biomolecular analysis.

2 Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T. Protein and polymer analyses up to m/z 100,000 by laser ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 2, 151–153 (1988).

• Tanaka, who received a Nobel prize in chemistry for his work on developing laser ionization techniques, describes ionization of proteins as large as 100 kDa.

3 Hillenkamp F, Karas M, Holtkamp D, Klusener P. Energy deposition in ultraviolet laser desorption mass spectrometry of biomolecules. *Int. J. Mass Spectrom.* 69, 265–276 (1986).

4 Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding. *Anal. Chem.* 60, 2299–2301 (1988).

5 Fenn J, Mann M, Meng C, Wong S, Whitehouse C. Electrospray ionization for mass spectrometry of large biomolecules. *Science* 246, 64–71 (1989).

• First significant article on the characterization of large biomolecules (oligonucleotides and proteins as large as 130 kDa) using electrospray ionization (ESI) mass spectrometry (MS). Fenn, a pioneer of ESI-MS for protein analysis, shared a Nobel prize in chemistry with Tanaka.

6 Washburn MP, Wolters D, Yates JR III. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nature Biotechnol.* 19, 242–247 (2001).

• Pioneering article on multidimensional liquid chromatography with online MS for large-scale proteome analysis, which is now a standard approach in most proteomics laboratories.

7 Lim H, Eng J, Yates JR III *et al.* Identification of 2D-gel proteins: a comparison of MALDI/TOF peptide mass mapping to mu LC-ESI tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* 14, 957–970 (2003).

8 DeSouza L, Diehl G, Rodrigues MJ *et al.* Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with multidimensional liquid chromatography and tandem mass spectrometry. *J. Proteome Res.* 4, 377–386 (2005).

9 Zhang N, Li N, Li L. Liquid chromatography MALDI MS/MS for membrane proteome analysis. *J. Proteome Res.* 3, 719–727 (2004).

10 Zuberovic A, Ullsten S, Hellman U, Markides KE, Bergquist J. Capillary electrophoresis off-line matrix-assisted laser desorption/ionisation mass spectrometry of intact and digested proteins using cationic-coated capillaries. *Rapid Commun. Mass Spectrom.* 18, 2946–2952 (2004).

11 Hardt M, Witkowska HE, Webb S *et al.* Assessing the effects of diurnal variation on the composition of human parotid saliva: quantitative analysis of native peptides using iTRAQ reagents. *Anal. Chem.* 77, 4947–4954 (2005).

12 Chen H-S, Rejtar T, Andreev V, Moskovets E, Karger BL. Electrospray interfacing of polymer microfluidics to MALDI-MS. *Electrophoresis* 26, 3631–3640 (2005).

13 Chen H-S, Rejtar T, Andreev V, Moskovets E, Karger BL. High-speed, high-resolution monolithic capillary LC-MALDI MS using an off-line continuous deposition interface for proteomic analysis. *Anal. Chem.* 77, 2323–2331 (2005).

14 Sunner J, Dratz E, Chen YC. Graphite surface-assisted laser desorption/ionization time-of-flight mass spectrometry of peptides and proteins from liquid solutions. *Anal. Chem.* 67, 4335–4342 (1995).

15 Pan CS, Xu SY, Hu LG *et al.* Using oxidized carbon nanotubes as matrix for analysis of small molecules by MALDI-TOF MS. *J. Am. Chem. Soc.* 16, 883–892 (2005).

16 Pan CS, Xu SY, Zou HF, Guo Z, Zhang Y, Guo BC. Carbon nanotubes as adsorbent of solid-phase extraction and matrix for laser desorption/ionization mass spectrometry. *J. Am. Chem. Soc.* 16, 263–270 (2005).

17 Ren SF, Guo YL. Oxidized carbon nanotubes as matrix for matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis of biomolecules. *Rapid Commun. Mass Spectrom.* 19, 255–260 (2005).

18 Ren SF, Zhang L, Cheng ZH, Guo YL. Immobilized carbon nanotubes as matrix for MALDI-TOF-MS analysis: applications to neutral small carbohydrates. *J. Am. Chem. Soc.* 16, 333–339 (2005).

19 Xu S, Li Y, Zou H, Qiu J, Guo Z, Guo B. Carbon nanotubes as assisted matrix for laser desorption/ionization time-of-flight mass spectrometry. *Anal. Chem.* 75, 6191–6195 (2003).

20 Chen WY, Wang LS, Chiu HT, Chen YC, Lee CY. Carbon nanotubes as affinity probes for peptides and proteins in MALDI MS analysis. *J. Am. Chem. Soc.* 15, 1629–1635 (2004).

21 Woldegiorgis A, von Kieseritzky F, Dahlstedt E, Hellberg J, Brinck T, Roeraade J. Polymer-assisted laser desorption/ionization analysis of small molecular weight compounds. *Rapid Commun. Mass Spectrom.* 18, 841–852 (2004).

22 Woldegiorgis A, Lowenhielm P, Bjork A, Roeraade J. Matrix-assisted and polymer-assisted laser desorption/ionization time-of-flight mass spectrometric analysis of low molecular weight polystyrenes and polyethylene glycols. *Rapid Commun. Mass Spectrom.* 18, 2904–2912 (2004).

23 Soltzberg LJ, Patel P. Small molecule matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a polymer matrix. *Rapid Commun. Mass Spectrom.* 18, 1455–1458 (2004).

24 Peterson DS, Luo QZ, Hilder EF, Svec F, Frechet JMJ. Porous polymer monolith for surface-enhanced laser desorption/ionization time-of-flight mass spectrometry of small molecules. *Rapid Commun. Mass Spectrom.* 18, 1504–1512 (2004).

25 Kinumi T, Saisu T, Takayama M, Niwa H. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using an inorganic particle matrix for small molecule analysis. *J. Mass Spectrom.* 35, 417–422 (2000).

26 Cuiffi JD, Hayes DJ, Fonash SJ, Brown KN, Jones AD. Desorption-ionization mass spectrometry using deposited nanostructured silicon films. *Anal. Chem.* 73, 1292–1295 (2001).

•• Demonstrated the use of nonporous columnar silicon for surface-assisted laser desorption/ionization (SALDI) MS. This process led to the Quickmass® chip offered by Shimadzu Corp.

27 Finkel NH, Prevo BG, Velev OD, He L. Ordered silicon nanocavity arrays in surface-assisted desorption/ionization mass spectrometry. *Anal. Chem.* 77, 1088–1095 (2005).

28 Wei J, Buriak JM, Siuzdak G. Desorption-ionization mass spectrometry on porous silicon. *Nature* 399, 243–246 (1999).

•• First demonstration of nanostructured silicon for SALDI-MS. This work led to the first commercially available chip (desorption/ionization on silicon, Waters, Inc.) for SALDI-MS.

29 Chen C, Chen Y. Desorption/ionization mass spectrometry on nanocrystalline titania sol-gel-deposited films. *Rapid Commun. Mass Spectrom.* 18, 1956–1964 (2004).

• Demonstrated desorption/ionization of the largest protein (~24 kDa) by a SALDI technique to date.

30 Chen YC, Wu JY. Analysis of small organics on planar silica surfaces using surface-assisted laser desorption/ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 15, 1899–1903 (2001).

31 Chen WY, Chen YC. Reducing the alkali cation adductions of oligonucleotides using sol-gel-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* 75, 4223–4228 (2003).

32 Ho KC, Lin YS, Chen YC. Laser desorption/ionization mass spectrometry on sol-gel-derived dihydroxybenzoic acid isomeric films. *Rapid Commun. Mass Spectrom.* 17, 2683–2687 (2003).

33 Lin YS, Yang CH, Chen YC. Glass-chip-based sample preparation and on-chip tryptic digestion for matrix-assisted laser desorption/ionization mass spectrometric analysis using a sol. *Rapid Commun. Mass Spectrom.* 18, 313–318 (2004).

34 Lin YS, Chen YC. Laser desorption/ionization time-of-flight mass spectrometry on sol-gel-derived 2,5-dihydroxybenzoic acid film. *Anal. Chem.* 74, 5793–5798 (2002).

35 Hopwood F, Michalak L, Alderdice D, Fisher K, Willett G. C-60-assisted laser desorption/ionization mass spectrometry in the analysis of phosphotungstic acid. *Rapid Commun. Mass Spectrom.* 8, 881–885 (1994).

36 Shiea J, Huang J, Teng C, Jeng J, Wang L, Chiang L. Use of a water-soluble fullerene derivative as precipitating reagent and matrix-assisted laser desorption/ionization matrix to selectively detect charged species in aqueous solutions. *Anal. Chem.* 75, 3587–3595 (2003).

37 Michalak L, Fisher K, Alderdice D, Jardine D, Willett G. C^{60}-assisted laser desorption-ionization mass spectrometry. *Org. Mass Spectrom.* 29, 512–515 (1994).

38 Kalkan AK, Fonash SJ. Carbon/Nafion® nanocomposite thin films as potential matrix-free laser desorption-ionization mass spectroscopy substrates. *Mater. Res. Soc. Symp. Proc.* 788, 595–600 (2004).

39 Kruse RA, Li X, Bohn PW, Sweedler JV. Experimental factors controlling analyte ion generation in laser desorption/ionization mass spectrometry on porous silicon. *Anal. Chem.* 73, 3639–45 (2001).

40 Shen ZX, Thomas JJ, Averbuj C *et al.* Porous silicon as a versatile platform for laser desorption/ionization mass spectrometry. *Anal. Chem.* 73, 612–619 (2001).

41 Trauger SA, Go EP, Shen Z *et al.* High sensitivity and analyte capture with desorption/ionization mass spectrometry on silylated porous silicon. *Anal. Chem.* 76, 4484–4489 (2004).

42 Credo G, Hewitson H, Benevides C, Bouvier ESP. Development of a porous silicon product for small molecule mass spectrometry. *Mater. Res. Soc. Symp. Proc.* 471–476 (2004).

43 Meng J, Siuzdak G, Finn M. Affinity mass spectrometry from a tailored porous silicon surface. *Chem. Commun.* 2108–2109 (2004).

44 Meng JC, Averbuj C, Lewis WG, Siuzdak G, Finn MG. Cleavable linkers for porous silicon-based mass spectrometry. *Angew. Chem. Int. Ed.* 43, 1255–1260 (2004).

45 Nam WJ, Bae S, Kalkan AK, Fonash SJ. Nano- and microchannel fabrication using column/void network deposited silicon. *J. Vac. Sci. Technol. A* 19, 1229–1233 (2001).

46 Kalkan AK, Henry MR, Li HD *et al.* Biomedical/analytical applications of deposited nanostructured Si films. *Nanotechnology* 16, 1383–1391 (2005).

47 Go EP, Apon JV, Luo G *et al.* Desorption/ionization on silicon nanowires. *Anal. Chem.* 77, 1641–1646 (2005).

48 Hoang TT, Chen YF, May SW, Browner RF. Analysis of organoselenium compounds in human urine using active carbon and chemically modified silica sol-gel surface-assisted laser desorption/ionization high-resolution time-of-flight mass spectrometry. *Anal. Chem.* 76, 2062–2070 (2004).

49 Dattelbaum A, Iyer S. Mesoporous silica for desorption-ionization mass spectrometry. In: *Technical Proceedings of the 2005 NSTI Nanotechnology Conference and Trade Show, Volume 1* NSTI, USA, 225–228 (2005).

50 Lu Y, Ganguli R, Drewien CA *et al*. Continuous formation of supported cubic and hexagonal mesoporous films by sol–gel dip-coating. *Nature* 389, 364–368 (1997).

51 Dattelbaum AM, Amweg ML, Yee C, Ecke LE, Shreve AP, Parikh AN. Photochemical pattern transfer and enhancement of thin film silica mesophases. *Nano Lett.* 3, 719 (2003).

52 Sigel A, Sigel H (Eds). *Metal Ions in Biological Systems Volume 35: Iron Transport and Storage in Microorganisms, Plants and Animals.* Marcel Dekker, Inc. NY, USA, 37–66 (1998).

53 Chen CT, Chen YC. Molecularly imprinted TiO_2-matrix-assisted laser desorption/ionization mass spectrometry for selectively detecting α-cyclodextrin. *Anal. Chem.* 76, 1453–1457 (2004).

54 Yuan MJ, Shan Z, Tian BZ, Tu B, Yang PY, Zhao DY. Preparation of highly ordered mesoporous WO_3–TiO_2 as matrix in matrix-assisted laser desorption/ionization mass spectrometry. *Microporous Mesoporous Mater.* 78, 37–41 (2005).

55 Lewis WG, Shen ZX, Finn MG, Siuzdak G. Desorption/ionization on silicon (DIOS) mass spectrometry: background and applications. *Int. J. Mass Spectrom.* 226, 107–116 (2003).

56 Shen Z, Go EP, Gamez A *et al.* A mass spectrometry plate reader: monitoring enzyme activity and inhibition with a desorption/ionization on silicon (DIOS) platform. *Chembiochem* 5, 921–927 (2004).

57 Wall DB, Finch JW, Cohen SA. Quantification of codeine by desorption/ionization on silicon time-of-flight mass spectrometry and comparisons with liquid chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* 18, 1403–1406 (2004).

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