

Advances in ionisation techniques for mass spectrometry-based omics research

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REVIEW

Advances in ionisation techniques for mass spectrometry-based omics research

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Abstract

Omics analysis by mass spectrometry (MS) is a vast field, with proteomics, metabolomics and lipidomics dominating recent research by exploiting biological MS ionisation techniques. Traditional MS ionisation techniques such as electrospray ionisation have limitations in analyte-specific sensitivity, modes of sampling and throughput, leading to many researchers investigating new ionisation methods for omics research. In this review, we examine the current landscape of these new ionisation techniques, divided into the three groups of (electro)spray-based, laser-based and other miscellaneous ionisation techniques. Due to the wide range of new developments, this review can only provide a starting point for further reading on each ionisation technique, as each have unique benefits, often for specialised applications, which promise beneficial results for different areas in the omics world.

KEYWORDS

acoustic droplet ejection, DESI, ionisation techniques, liquid MALDI, REIMS

1 | INTRODUCTION

Omics research is a wide-ranging topic, concerning vast datasets and complex biomolecular mixtures. Split into individual fields of study such as proteomics, lipidomics, metabolomics, the methods of analysis that can collect sufficient data for omic characterisation are specific and often limited (to a specific sub-field). One technique with unique and universal capabilities that facilitates omics research is mass spectrometry (MS). MS is a technique that exhibits high resolution, accuracy

and sensitivity, yet is typically limited by the ionisation source used. For example, an important part of lipidomics is the investigation of non-polar lipids, and therefore electrospray ionisation (ESI), which struggles with the use of non-polar solvents [1], is frequently dismissed for lipid analysis. Similarly, extremely complex mixtures as often seen in metabolomic and proteomic experiments are difficult to analyse without a prior separation step, such as liquid chromatography (LC) [2]. Hybridisation and coupling of techniques often result in advances but can also lead to limitations. In the case of coupling LC to MS, greater resolution by adding another separation dimension can be achieved but ionisation techniques such as matrix-assisted laser desorption/ionisation (MALDI) that are not easily on-line-coupled to LC will then often be excluded from the available toolbox of ionisation techniques.

In MS-based omics analysis, ESI is typically the ionisation technique of choice for a range of sample matrices and analytes [3, 4]. This has been bolstered by the ease of coupling ESI with LC, along

Abbreviations: CE, capillary electrophoresis; AP, atmospheric pressure; REIMS, rapid evaporative ionisation MS; LAP-MALDI, liquid AP-MALDI; HTS, high-throughput screening; 3-NBA, 3-nitrobenzyl alcohol; ETD, electron transfer dissociation; CAD, collision-activated dissociation; DPS, desalting paper spray; DESI, desorption electrospray ionisation; MSI, MS imaging; AFADESI, airflow-assisted DESI; LESA, liquid extraction surface analysis; ELDI, electrospray-assisted laser desorption ionisation; MALDESI, matrix-assisted laser desorption electrospray ionisation; LAESI, laser ablation electrospray ionisation; LSM, liquid support matrix; LA-REIMS, laser-assisted REIMS; ADE, acoustic droplet ejection; SESI, secondary electrospray ionisation; AMI, acoustic mist ionisation; OPI, open port interface; SAWN, surface acoustic wave-based nebulisation; MAIV, matrix-assisted ionisation vacuum.

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with ESI being a reliable soft ionisation technique that can interface under atmospheric pressure (AP) with a range of mass analysers. In recent years however, areas in which ESI is lacking have become more apparent. While ESI is typically robust and sensitive, it has a relatively low throughput due to the common use of relatively slow LC separation prior to ionisation [5]. ESI also has significant limitations when it comes to sample preparation. Considering nano-ESI in particular (with its smaller capillary internal diameter), sample matrices are heavily limited in their final composition as desalting steps are needed prior to the ionisation stage to avoid both salt precipitation on the ESI needle and ion suppression, further reducing throughput and potentially sensitivity due to suboptimal analyte recovery [6–9]. Additionally, ESI alone cannot perform MS imaging (aside from a few niche instrumental setups [10, 11]) and therefore cannot perform any omics analyses that require localisation without lengthy sample preparation procedures.

Due to these limitations, there has been a significant amount of research in recent years with the aim of improving upfront sample preparation using faster and more sensitive separation techniques [12], partially by miniaturisation [13, 14], enhancing post-ionisation separation [15, 16], expanding the analytical dimensionality and analyte range, and improving the MS data acquisition itself, again with a focus on sensitivity [17, 18] and speed, for example by increasing the throughput [19, 20] or the number of scans per second [21], as well as the down-stream data mining in all aspects, including MS data processing [22, 23], data searching [24–26] and again speed [27, 28].

Naturally, developing novel ionisation techniques and ion sources that can analyse various samples and sample matrices that were previously inaccessible via ESI can contribute to overcoming the limitations of ESI in omics research. As with the above improvements around ESI, novel ionisation techniques and their sources can have the potential of significantly addressing the analytical sensitivity and specificity, particularly if they are purposefully designed to overcome the short-comings or fill the gaps of ESI.

Current developments of novel ionisation methods relevant for MS-based omics research vary in scale. For example, some developments involve new computational abilities [29], while others are new (ion source) designs with unique functionalities, like different evaporative probes used in rapid evaporative ionisation MS (REIMS) [30]. In this review, we are considering only those advances which directly improve the ionisation or ion source, rather than any pre- or post-ionisation technique improvements. For the purposes of this review, novel ionisation techniques and ion sources have been divided up into three groups (as seen in Figure 1) – spray-based, laser-based and other ionisation techniques. Within these groups the various novel ionisation sources are generally designed for accommodating the same type of sample being introduced to the mass spectrometer. For example, spray-based ionisation techniques are typically able to analyse homogenised liquid samples directly or on-line with separation techniques based on liquid media such as LC, whereas laser-based ionisation techniques can provide MS imaging of solid (or frozen) samples for spatially defined omics analysis. Miscellaneous ionisation techniques have been developed that either modify or hybridise techniques, allowing for these new techniques to be tailored to specific analyses and analytes.

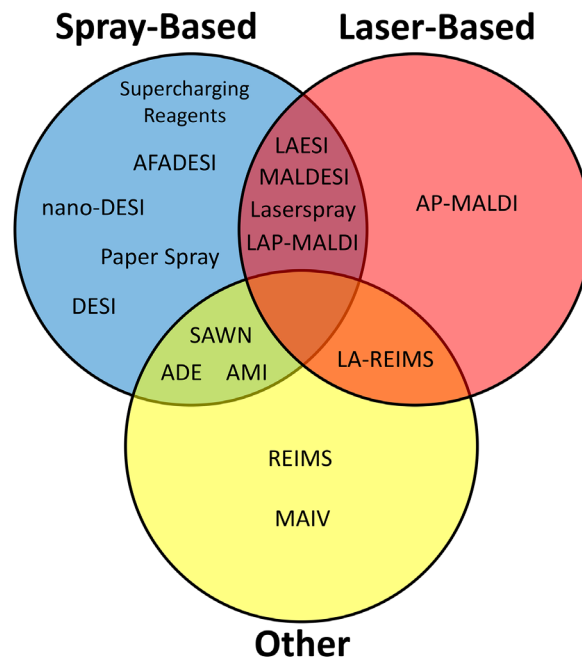


FIGURE 1 Classification of the ionisation techniques described within this review, according to spray-based, laser-based and other techniques. Note that the spray-based set consists of techniques the literature has discussed as being driven at least in part by a process similar to electrospray ionisation. While there are many more ionisation techniques available, the techniques mentioned in this review have significantly contributed to recent advances in the field of omics.

As the main ionisation technique in omics research, ESI has remained relatively static in terms of new developments in recent years. Most of the changes and associated targeted improvements in specific areas of ESI are with relation to sample additives and emitter shape [31–33]. While these advancements improved the analytical capabilities of ESI towards a wider variety of samples and analytes, they are not fundamentally new advancements of the technique itself.

MALDI has fundamentally made some greater advances such as liquid AP-MALDI (LAP-MALDI) as discussed below and in applications such as biotyping, imaging and high-throughput screening (HTS) in clinical and industrial application areas. In addition, there is a constant flow of developing new MALDI matrices [34, 35]. As with ESI (as discussed later in this review) there have also been many new ionisation methods, modifications and hybrid techniques that use MALDI (and/or ESI) as a starting point.

In this review, we will be assessing the current landscape of newly developed ionisation techniques, first looking at (electro)spray-based, then laser-based and finally other ionisation techniques in the context of omics research.

2 | SPRAY-BASED IONISATION TECHNIQUES

Spray-based ionisation techniques typically rely on the base principles of ESI; an electric field applied to a sprayed liquid sample can

generate charged gas-phase analyte ions that can be directed towards an MS inlet. Many of the techniques in this section were born from the need for specific analyses that ESI alone could not perform but have recently expanded in scope to a much wider variety of available analytes. While they are not universal ionisation techniques, they each offer unique advantages compared to ESI. It is also worth mentioning that while many of these techniques are not new, with most being first introduced in the early 2000s, there are regular updates and advances being provided by various groups for each technique. In this section, ESI-like spray techniques are considered. Some of these utilise laser-based desorption but those that potentially also exploit laser-based ionisation are considered in Section 3. Techniques that lead to ionisation during or after acoustic sample ejection are discussed in Section 4.

2.1 | Supercharging Reagents

Supercharging reagents are sample additives that are used typically with ESI to raise the surface tension of the sample solution. By raising the surface tension, the Rayleigh limit of the sprayed droplets is increased, leading to a greater charge density on the surface prior to coulombic explosion. Consequently, this generates a higher average charge state and the yield of higher charged ions [36]. This is desirable for many reasons; the mass measurement accuracy that can be obtained for lower m/z ions is often greater, high-mass ions can be brought within the m/z range of more mass analysers, and the fragmentation efficiency for tandem mass spectrometry (MS/MS) analyses becomes greatly improved. The addition of supercharging reagents occurs at the sample preparation stage and does not require any changes to the ion source. Thus, it can be easily applied to all commercial instrumentation without modifying any hardware. There are many other additives that can be beneficial to omic analyses, but supercharging reagents have a more direct and universal impact on the ionisation itself and are therefore listed here in the context of advances in MS ionisation.

The supercharging effects of glycerol and 3-nitrobenzyl alcohol (3-NBA) were already reported in 2001 [37]. Since then, more than 50 different supercharging reagents have been identified [36]. The increase in the charge state created by supercharging reagents has been exploited by multiple groups for the purposes of top-down proteomics. Li *et al.* applied 3-NBA to electron transfer dissociation (ETD) methodology for hydroxy radical protein foot printing [38]. Similarly, Zhang *et al.* increased the electron capture dissociation (ECD) and collision-activated dissociation (CAD) fragmentation rate of disulphide bonded proteins by the addition of sulpholane, allowing for greater sequence coverage [39]. Nshanian *et al.* explored the effects of supercharging reagents on reducing TFA-based ion suppression in LC-MS-based proteomics, allowing for higher yields of multiply protonated ion species and therefore higher resolutions [40]. Different supercharging reagents can also be used as a mixture to target and enhance specific charge states of target peptides, as demonstrated by Van Wanseele *et al.* [41]. Overall, there is a wealth of applications and

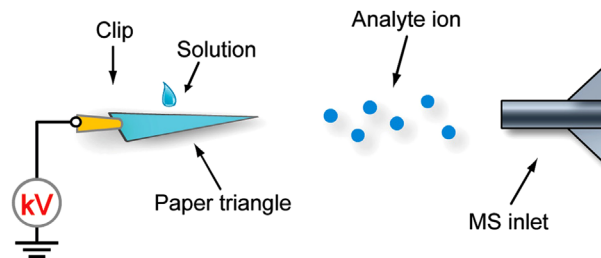


FIGURE 2 General schematic for paper spray ionisation. A paper triangle is wetted with the sample solution and connected to a high-voltage power supply. This causes a plume of charged droplets to be ejected from the tip of the paper triangle towards the mass spectrometer inlet. Adapted with permission from ref. [43]. Copyright 2010 American Chemical Society

a range of supercharging reagents, which have been recently reviewed by Abaye *et al.* [36].

2.2 | Paper spray

Paper spray ionisation is a low-cost, easily accessible technique, first developed by Wang *et al.* in 2010 [42]. As shown in Figure 2, an aliquot of liquid sample solution (or an adequate solvent, if the analyte is already on the paper) gets deposited onto a small paper triangle, which is then placed in front of the mass spectrometer inlet. An electric field is applied to the paper, initiating ionisation of the liquid sample from the paper's tip facing the mass spectrometer's inlet. Paper spray allows for quick and facile access to a variety of sampling methodologies inaccessible to many other ionisation techniques, such as the direct analysis of dried blood spots and separated compounds from a TLC strip, and surface analysis (e.g., by wiping a surface with the paper substrate) [42, 43]. It lends itself perfectly to metabolomic, lipidomic and proteomic analysis in clinical settings as biofluids can be deposited directly onto the paper substrate and then sent for analysis, increasing sample throughput (due to little sample preparation) and therefore diagnostic speed significantly [43].

While a range of applications were discussed in the original paper spray article, they were limited in scope because of significant ion suppression effects due to the substrates and elution solvents, resulting in low sensitivity [43, 44]. Advances in paper spray have therefore been primarily focused on improving sensitivity, while also aiming to expand the types of analytes accessible to the technique.

The (paper) substrate material has been a major focus for increasing sensitivity. Paper spray is not a 'one-type-fits-all' technique; different analyte classes have varying affinities for and interactions with different substrates [45]. While they cannot necessarily be called paper substrates, polymeric and non-porous materials have been used as substrates in paper spray. Recently, Teslin has been used as a substrate with the aim of rapidly detecting COVID-19 metabolomic biomarkers [46]. In theory, the silinol groups in the Teslin substrate allow for greater interaction of the analytes with the charged substrate, allowing for a greater number of molecules to be ionised compared to the

typical cellulose paper substrates [47]. A Teflon substrate with a conducting wire through the centre has been used to examine metabolites present in urine and saliva, with an S/N increase of at least 100-fold compared to cellulose paper [48]. Similarly, a poly(methyl methacrylate) substrate spiked with carbon nanotubes was used to analyse analyte types ranging from small molecules to mid-sized proteins with an ion signal intensity increase of 20–100 times that of cellulose paper [49].

Desalting paper spray (DPS) is another modification to paper spray that has been developed recently. By washing the substrate with an acetonitrile/water mixture prior to the analysis excess salts can be removed from the substrate. While initially utilised to reduce the analyte ion signal suppression observed in glycan and oligosaccharide analysis due to high levels of adduct ion formation [50, 51], this technique has been expanded into the realm of proteomics by using a polymer substrate coated with Nafion, a fluoropolymer. The proteomic profiles of saliva samples were examined, and the Nafion-coated substrates showed a desalting efficiency of up to 90% higher when compared to control substrates [52].

2.3 | Desorption electrospray ionisation (DESI)

Initially developed as an ambient ionisation technique for direct in vivo sampling and crude MS imaging (MSI) analysis in 2004 by Cooks and coworkers [53], the non-destructive nature of DESI was shown to offer distinct advantages within the field of MSI that MALDI (the leading MSI tool) could not match [54]. The primary advantage of DESI is that it does not necessarily require pre-treatment of a sample. A range of histologically compatible solvent systems are available that can be sprayed directly onto tissue samples for DESI MSI analysis, while MALDI MSI requires any tissue sample to be pre-treated with the MALDI matrix for effective ionisation to occur.

Since its inception, updates to the design of DESI sources and their coupling to MS instrumentation have been the key to unlocking access to higher spatial resolution, higher specificity and a wider range of applications. One area of these design updates lies within the design of the DESI emitter. Towers *et al.* modified a commercial DESI source by rerouting a high-voltage power supply through the solvent flow to create charged primary droplets, and by introducing a heated inlet capillary to aid desolvation of the secondary droplets [55]. With this setup alongside ion mobility spectrometry, it was possible to observe intact peptide and protein ion signals with a pixel resolution of 150 μm [55], an endeavour that had previously been extremely difficult outside of highly optimised conditions.

A modification to DESI was made by He *et al.* in 2011 [56], later called airflow-assisted DESI (AFADESI) [57]. It was introduced with the aim of achieving higher analytical sensitivities for remote DESI-like sampling by providing additional air flow to the mass spectrometer inlet, using a vacuum pump connected to a transport tube for efficient extraction of the DESI-produced sample material [56]. While initially shown to be useful in pharmaceutical and explosive residue analysis [56], AFADESI has also seen a number of novel uses in the field of omics.

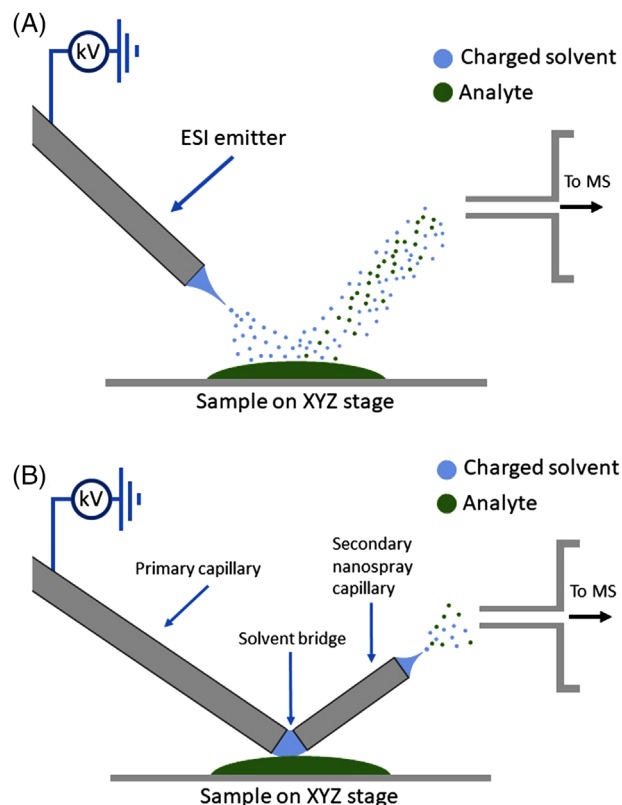


FIGURE 3 (A) Schematic of conventional DESI-MS with charged solvent droplets being (electro)sprayed onto the sample surface, rebounding towards the mass spectrometer inlet after desorbing sample material. (B) Schematic of nano-DESI, where a solvent bridge is formed between two sections of a sampling probe (a primary capillary and a secondary nanospray capillary), with the charged solvent desorbing sample material and emitting this in charged droplets from the end of the secondary nanospray capillary. DESI, desorption electrospray ionisation; MS, mass spectrometry

Tang and coworkers were able to image lipids at the isomeric level by exploiting the increased air exposure facilitated by the technique to accelerate oxidation reactions of lipids in mouse lung cancer tissues, providing the ability to differentiate between unsaturated lipid isomers [58]. Abliz and coworkers combined AFADESI with a reagent-spiked hydrogel derivatisation methodology, allowing for a large number of metabolites and lipids to be identified from a rat brain [59].

Another DESI modification that has seen an increase in uptake recently is nano-DESI [60, 61]. While DESI utilises a solvent spray at a distance from the substrate, nano-DESI employs a solvent bridge at the sample target between a primary capillary and a secondary nanospray capillary, resulting in a hybrid between liquid extraction surface analysis (LESA) and DESI [60, 62]. The differences between DESI and nano-DESI are displayed in more detail in Figure 3. Modifications to this technique have allowed for great increases in versatility. Cooper and coworkers utilised non-denaturing solvents alongside a shortened inlet capillary to gain high-resolution native proteomic profile images of intact proteins from kidney tissues [62] and brain tissues [63].

Lanekoff and coworkers further advanced nano-DESI with a technique they called pneumatically assisted nano-DESI [64], which has a similar relationship to nano-DESI as AFADESI has to DESI; pneumatically assisted nano-DESI allows for remote sampling of analytes at greater sensitivity. In pneumatically assisted nano-DESI, a nebuliser gas inlet is introduced to the secondary (nanospray) capillary. By directly introducing a gas flow through the nanospray capillary the probe can be placed further from the inlet and there is an enhanced sensitivity for metabolite species detection and a lower effect of probe-to-sample surface distance on the analyte signal intensity [64].

In general, the use of DESI in lipidomics has become prominent with advances in reactive DESI. Developed in 2009 by Nyadong *et al.* [65], reactive DESI employs solvent additives to form charged species from non-polar compounds such as lipids, which owing to their low polarity exhibit low detection sensitivity and poor solvent compatibility with commonly utilised polar solvent systems. For example, Nyadong *et al.* utilised various anions (Cl^- , Br^- , and CF_3COO^-) to form adducts with bromophycolides in algal tissues, greatly improving the previously poor detection sensitivity when using a polar spray solvent alone [65]. Lostun *et al.* utilised reactive DESI with a dicationic ion pairing compound named DC9, allowing for compounds normally only detectable in negative ionisation mode to be detectable in positive ionisation mode [66].

Hybridisation of DESI to other sources and analysers has also introduced new modalities for analysis. Wood and coworkers utilised an FT-ICR mass spectrometer equipped with both a DESI and MALDI source, generating increased lipid coverage of rat brain than either source individually, without the need to switch ion sources [54]. Pan and coworkers combined a standard DESI source with a photoionisation source to analyse lipids in mouse brain, revealing an increased number of identified lipids, in particular non-polar lipids, in both positive and negative mode compared to DESI alone [67]. Laskin and coworkers incorporated shear force microscopy into a nano-DESI probe, allowing for the probe to be kept a constant height from the substrate surface even when complex topography is present [68–70].

2.4 | Laser desorption/ESI hybrid techniques

Whereas DESI and paper spray have only slightly deviated from ESI with their instrumentation, other techniques have hybridised ESI, often with laser-based desorption techniques. Electrospray-assisted laser desorption ionisation (ELDI), described in 2005 by Shiea *et al.* as one of the first such techniques [71], combines an initial laser desorption/ionisation event with post-ionisation by ESI. In its initial design, it was more tailored towards the analysis of prepared sample droplets and solid samples [72], rather than directly analysing biological materials and tissues, which would be more suitable for MSI. Researchers soon made small adaptations to the methodology to make it more suitable for a wider variety of substrates and biological matrices, with further adaptations being made in more recent years.

2.4.1 | MALDESI

Matrix-assisted laser desorption electrospray ionisation (MALDESI), in practical terms a hybrid ionisation technique of MALDI and ESI, was developed in 2006 by Sampson *et al.* [73]. As with MALDI, pulsed laser irradiation [74] is focussed at either a dried sample droplet containing both analyte and matrix compound or a tissue sample doped with a matrix solution, causing material to be desorbed. Particularly for the analysis of crude samples such as tissue, mid-infrared (IR) lasers are typically used, exploiting the properties of water as a matrix [74]. Ionisation is achieved with ESI orthogonally applied to the desorbed plume, directing the desorbed/ionised analytes to the MS inlet [73]. Initial advances were successful in improving its applicability to MSI with the introduction of computer-controlled stages in 2013 by Robichaud *et al.* [75]. Sampson *et al.* widened the analyte and sampling types that MALDESI could access by introducing liquid-phase MALDESI, directly analysing the lipidome of milk and egg yolk [76]. In more recent years, however, its further development has been focused on optimising laser [77, 78] and solvent/matrix [74, 79, 80] parameters, typically to increase resolution and specificity towards previously inaccessible classes of molecules. The move towards using mid-IR lasers was found to decrease the amount of internal energy transferred to analyte molecules, making MALDESI (similar to IR-MALDI) more comparable to ESI in softness [81].

2.4.2 | LAESI

Laser ablation electrospray ionisation (LAESI) consists of a nearly identical setup as MALDESI and ELDI. First described by Nemes and Vertes in 2007, LAESI utilises mid-IR laser ablation orthogonal to an ESI source for the direct analysis of samples with a variety of surface features, as long as the samples have a sufficiently high water content [82]. In contrast to MALDESI, LAESI requires little to no sample preparation, which propels the technique towards rapid analysis of large sample sets [83–85]. However, recent IR-MALDESI studies using only the samples' water or solvent as matrix have shown that extremely high throughput can also be achieved by MALDESI as shown by the analysis of isocitrate dehydrogenase 1 (IDH1) kinetics assays [86]. In these cases, MALDESI and LAESI are virtually identical techniques.

One niche that suits LAESI well is the omics profiling of plants and foodstuffs due to its applicability to the uneven surfaces typically observed in these samples. Agtuca *et al.* used LAESI to analyse the metabolome of soybean root nodules, bypassing the extensive sample preparation that existing methodologies such as GC-MS, LC-MS and CE-MS require [87]. Taylor *et al.* were able to perform single cell metabolomics of *Allium cepa* (red onion) by combining LAESI with optical microscopy [88]. Alongside the profiling of small molecules, Zhou *et al.* performed some lipidomic profiling of different meat samples and were able to differentiate rapidly between meat from different species [89].

Other advances were made possible by further modifying the LAESI source. Vaikkinen *et al.* utilised a post-ESI heated nitrogen flow to improve the detection of low-polarity lipids, allowing these lipids to be detected alongside high-polarity compounds without the need of complementary analysis [90]. With a similar aim, Li *et al.* developed a solvent gradient methodology by replacing the ESI capillary with a glass theta capillary (a capillary with a central barrier to create two channels within, allowing for each channel to be filled with a different substance without them mixing prior to spraying). Each channel contained solvents of varying polarities, enabling the ionisation of species of both low and high polarities over a single acquisition [91]. It is worth mentioning again that ELDI and LAESI are virtually identical ionisation techniques, essentially only differentiated by small differences, for instance, in source geometries and laser wavelengths [92].

3 | LASER-BASED IONISATION TECHNIQUES

3.1 | Laserspray ionisation

Laserspray ionisation is a technique introduced by Trimpin *et al.* in 2010 with the aim of providing ESI-like spectra using standard MALDI sample preparation conditions [93]. In summary, a matrix/analyte mixture is co-crystallised onto a sample target that is placed in front of a heated ion transfer tube, which transfers the laser-desorbed sample material to the mass spectrometer inlet. Both AP and vacuum sources have been used for laserspray ionisation. In contrast to standard (AP-)MALDI and LAP-MALDI (see below), laserspray ionisation employs much higher laser fluences (≥ 10 kJ/m²). Initially used to investigate the formation of gas-phase ions with MALDI-like techniques, the technique was also used with ETD [93], for in situ protein analysis [94] and MSI [95]. However, not much research was recently published with this technique, potentially due to current advances in DESI and other AP-MALDI techniques, being less destructive in their approach.

3.2 | AP-MALDI

MALDI MS has long been one of the techniques of choice for automated high-throughput proteomics, due to its excellent sensitivity and analysis speed compared to other proteomic MS techniques [96]. It has been recognised as a complementary tool to ESI for proteomics analyses, particularly when coupled to LC [97].

AP-MALDI, first introduced by Laiko *et al.* in 2002, has further built upon this strength by allowing MALDI to analyse sample/analyte types that could not previously be effectively analysed when under vacuum, such as biological cells and tissues [98]. A major development that has supported the employment of AP-MALDI is the introduction of LAP-MALDI (see Figure 4 for a schematic). Rather than allowing a matrix/analyte droplet to cocrystallise on the target plate, typically the analyte solution is combined with a liquid support matrix (LSM) using a viscous support such as propylene glycol as well as dissolved matrix compounds as known from the conventional solid

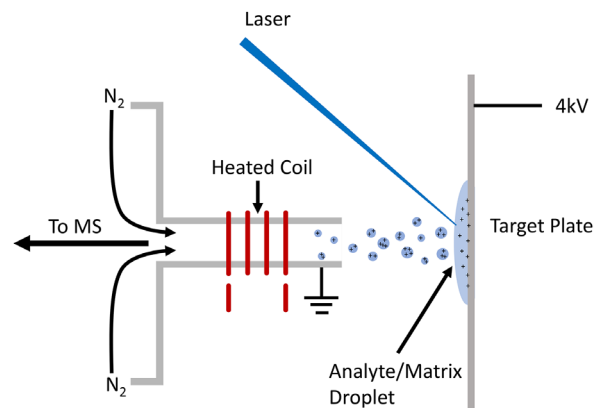


FIGURE 4 Schematic of the LAP-MALDI source as developed by Ryumin *et al.* [102]. The ion transfer tube combines high temperatures alongside a nitrogen gas counterflow to efficiently generate ESI-like multiply charged ions. ESI, electrospray ionisation; LAP-MALDI, liquid AP-MALDI; MALDI, matrix-assisted laser desorption/ionization

MALDI. This produces a stable self-healing droplet that remains liquid during the analysis and can last thousands of laser shots with little to no disruption to the ion signal. Early research demonstrated a 30-min acquisition of a single melittin-containing sample droplet (10 Hz laser pulse repetition rate) with no significant drop in ion signal intensity [99, 100].

In combination with a heated ion transfer tube, a beneficial effect of LAP-MALDI is the enhanced, ESI-like production of multiply charged ions, something not seen in conventional MALDI. While initially noticed in 2013 [99], the production of multiply charged ions was optimised in 2016 by Ryumin and Cramer by developing a modified AP-MALDI source for use on a commercial Q-TOF instrument with ion mobility separation [101]. Further optimisation of the heated ion transfer tube and gas counterflow during ion transfer led to a 14-fold increase in the ion yield of multiply protonated peptide analytes [102, 103]. Collision-induced dissociation (CID), a molecular fragmentation technique that improves in efficacy and sensitivity with an increase in charge state, was utilised with the LAP-MALDI setup to great effect, in structural lipidomics [104], protein/peptide identification [105] and disease diagnostics in combination with simultaneous multi-omic profiling [106]. Multi-omic profiling of a variety of sample types has been further developed and applied to microbial biotyping [107], speciation [108] and the early and cost-effective detection of mastitis from crude milk samples [109]. Owing to the high-throughput capabilities [106, 109, 110] of the technique combined with its extremely stable ion signal as well as new data acquisition software, record analysis speeds of up to 60 samples per second have been achieved [110, 111].

4 | OTHERS

4.1 | Rapid evaporative ionisation MS (REIMS)

REIMS enables direct sampling of difficult to handle samples and substrates such as biological tissues and fluids. First described in

2009 by Schäfer *et al.* [112], the technique originally employed an electro-surgical electrode that makes contact with the sample, rapidly evaporating biological material to generate gaseous molecular ions such as phospholipids [112, 113]. As part of the 'iKnife' technology, REIMS has demonstrated to be a hybrid surgical/analytical tool that can profile biological tissue during surgery [113–115], as well as rapidly profile the metabolome and lipidome of various crude biological fluids, like faeces, bile and urine [116].

Laser-assisted REIMS (LA-REIMS) is a sub-technique of REIMS first explored by Fatou *et al.* in 2016 [117], although not described as LA-REIMS at that time. Simply, the electrode probe is replaced with an infrared laser with the aim of exciting and evaporating water in the sample, allowing the technique to analyse samples with a high water content such as biofluids without the addition of a MALDI matrix [117, 118]. Van Meulebroek *et al.* successfully utilised LA-REIMS in profiling the metabolome of faeces in a similar way to an earlier (non-LA) REIMS study by Cameron *et al.*, although with a much higher sample throughput due to the lack of physical contact between the probe and the biofluid [116, 118]. Wijnant *et al.* developed an LA-REIMS methodology in complement to a UHPLC-HRMS method for metabolomic profiling of saliva samples, where LA-REIMS provided a rapid analysis platform much more suitable to clinical labs, demonstrating the proof of concept where the metabolomic profiles of normal weight and obese individuals were successfully differentiated [119]. However, these applications have all the same potential limitations of poor quantitation and ion suppression due to the lack of prior sample preparation and therefore matrix effects [120], which on the other hand provides simplicity, universality and speed [121].

4.2 | Acoustic ionisation techniques

One class of (AP) ionisation techniques that has seen more popularity in recent years is acoustic ionisation. The energy of acoustic waves typically produced by a transducer leads to the ejection of small amounts of liquid sample material from a well, allowing for rapid sample analyses from microtiter plates with little sample consumption making these techniques ideal for screening and profiling studies [122].

4.2.1 | Acoustic droplet ejection (ADE)

Acoustic techniques initially leading to the ejection of droplets are often called ADE. Although well known in the physical sciences, ADE only recently attracted attention in the field of MS, primarily for high-throughput applications. In 2015, Sinclair *et al.* [123] employed ADE and modulated the operating parameters of the transducer, thus creating a mist of smaller droplets after the initial ADE event. In one of the first studies, ionisation was achieved via a secondary electro-spray ionisation (SESI) source orthogonal to the plate with the sample wells, but subsequent setups directly applied a large voltage difference between the sample fluids in the well and the end of the transfer tube [123]. This setup, shown in Figure 5, allowed for a data acquisition

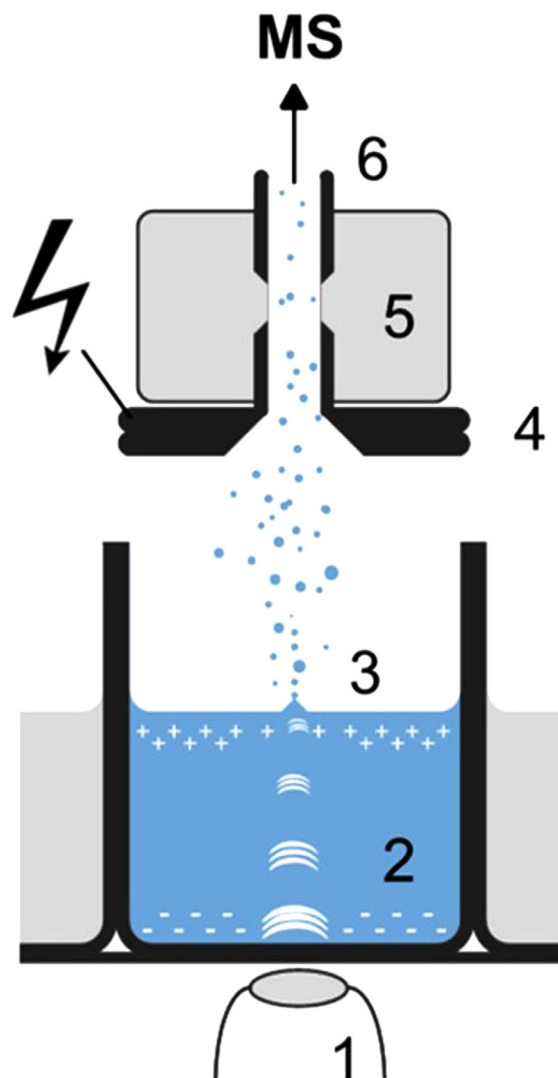


FIGURE 5 Schematic of the acoustic mist ionisation apparatus devised by Sinclair *et al.* The apparatus consists of a transducer (1) to initiate microdroplet formation from the sample liquid in the microtiter plate well (2). Droplets are formed at the sample's surface (3); their formation and analyte ionisation is supported by a high voltage applied to a cone in front of the mass spectrometer inlet (4). The microdroplets pass through a heated ion transfer tube (5) to promote desolvation, and finally towards the mass spectrometer inlet (6). Adapted with permission from ref. [124]. Copyright 2019 American Chemical Society

rate of >10,000 data points per hour, making it an excellent technique for high-throughput analysis. Later studies referred to this type of technique as acoustic mist ionisation (AMI) and applied it to enzymatic assay development for high-throughput biochemical screening, demonstrating more than 100,000 samples per day [124].

Bachman *et al.* explored the use of AMI in cellular metabolomics and lipidomics in 2020, successfully developing a generic cellular assay workflow, highlighting again the high-throughput suitability of the technique [125]. Smith *et al.* demonstrated its rapid analysis capabilities in a similar study with HepG2 cell lysates, obtaining a full data set of 2772 samples in under 5 h [126].

Covey and coworkers recently coupled an open port interface (OPI) to ADE, efficiently collecting and diluting the acoustically ejected (nL-)droplets in a solvent flow, which is then delivered to an ESI source [20, 127]. Various applications, from drug-drug interaction to pharmacokinetic and biomarker analysis, have been demonstrated [127]. Although this setup is also applicable to high-throughput analysis, its ultimate sampling rate is limited by sample diffusion within the transfer capillary to the ESI source.

4.2.2 | SAWN

Another recent acoustic ejection technique used in MS is surface acoustic wave-based nebulisation (SAWN), which was introduced in 2015 by Tveen-Jensen *et al.* and utilises a low-flow (0.5–30 $\mu\text{l}/\text{min}$) LC infusion onto a piezoelectric transducer, thus creating a very ‘soft’ nebulisation event. By providing less energy towards the nebulisation event compared to ESI, it was found that more labile compounds (e.g., phosphopeptides, serine-rich peptides, non-covalent complexes, etc.) could be generated with a much more uniform signal intensity across all elution times [128]. SAWN was utilised for bacterial biotyping by Liang *et al.* in 2018, successfully differentiating four gram-negative bacteria species via their lipid profiles [129].

4.3 | Matrix-assisted ionisation vacuum (MAIV)

Another relatively new technique is MAIV, first described by Trimpin and Inutan in 2013 [130]. MAIV is an ionisation method that applies no laser irradiation or high voltages, and simply requires an analyte/matrix (typically 3-nitrobenzotrile) mixture to be placed against the inlet cone of the mass spectrometer held at a vacuum. In one of the original publications, different methods of introducing the sample to the inlet cone were investigated, with equivalent ion counts between a KimWipe, the pointed tip of a strip of filter paper (similar to paper spray ionisation but without the high voltage), and a pipette tip [130]. This makes MAIV a versatile, low-cost technique, as samples can be prepared and analysed on a wide variety of common laboratory consumables. In 2015, the technique was advanced towards high-throughput analysis by Woodall *et al.* by placing either multi-channel pipette tips or filter paper onto an XYZ stage. Each sample spot is aligned with the inlet cone for between 1 and 5 s per sample, in theory allowing for up to 8000 samples per day. This was successfully used to analyse intact proteins and to perform top-down analysis of various peptides in the fmol/ μl to pmol/ μl range [131]. In 2016 Chen *et al.* utilised MAIV for post-translational modification (PTM) analysis and were able to detect intact proteins up to 18.7 kDa, up to a +18 charge state. The *in situ* analysis of peptides and proteins from rat brain tissue extracts were also performed, with a variety of singly charged and multiply charged peptides and proteins detected [132]. Most recently, Harding *et al.* were able to perform lipidomic MSI experiments of rat brain tissue using MAIV, optimising sample extraction into the 3-nitrobenzotrile matrix by the addition of 5% chloroform [133].

5 | CONCLUDING REMARKS

In this review, the landscape of MS ionisation techniques newly introduced for omics analyses over recent years has been examined. The number of these ionisation techniques now available and applied to the omics field are vast and are only growing larger. There have been many other, mainly ambient ionisation techniques that have been recently introduced to MS like plasma-based techniques, but many of these have not found their way into the omics world, let alone into large-scale and in-depth proteomics, which is still very much dominated by the two main soft ionisation techniques of MALDI and ESI – the latter being by far the preferred technique. However, as analytical techniques in the omics field are far from a one-size-fits-all approach, having a variety of ionisation techniques that are tailored for various sample conditions and target analytes is desirable. While many new ionisation techniques find it hard to compete with the softness of ESI and MALDI, many are further developments of these two or find their first competitive applications in areas such as metabolomics and lipidomics where the analytes are less demanding on the softness of the ionisation technique. However, many of these new techniques could also have the potential to be used for proteomics research, reducing the need to switch between sources and modes of analysis when performing multi-omics analysis. As can be seen by the growing range of commercial ion sources, there is a great demand for innovation in ion source development and fundamental research in ionisation techniques, with the ultimate aim to provide better solutions for the many unmet analytical needs in the omics.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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REFERENCES

1. Xia, B., Gao, Y., Ji, B., Ma, F., Ding, L., & Zhou, Y. (2018). Analysis of compounds dissolved in nonpolar solvents by electrospray ionization on conductive nanomaterials. *Journal of the American Society for Mass Spectrometry*, 29(3), 573–580. <https://doi.org/10.1007/s13361-017-1873-y>
2. Zhou, B., Xiao, J. F., Tuli, L., & Ransom, H. W. (2012). LC-MS-based metabolomics. *Molecular Biosystems*, 8(2), 470–481. <https://doi.org/10.1039/c1mb05350g>
3. Hale, O. J., & Cooper, H. J. (2020). *In situ* mass spectrometry analysis of intact proteins and protein complexes from biological substrates. *Biochemical Society Transactions*, 48(1), 317–326. <https://doi.org/10.1042/BST20190793>
4. Banerjee, S., & Mazumdar, S. (2012). Electrospray ionization mass spectrometry: A technique to access the information beyond the molecular weight of the analyte. *International Journal of Analytical Chemistry*, 2012, 282574. <https://doi.org/10.1155/2012/282574>

5. Kempa, E. E., Hollywood, K. A., Smith, C. A., & Barran, P. E. (2019). High throughput screening of complex biological samples with mass spectrometry – from bulk measurements to single cell analysis. *Analyst*, 144(3), 872–891. <https://doi.org/10.1039/C8AN01448E>
6. David, A., Abdul-Sada, A., Lange, A., Tyler, C. R., & Hill, E. M. (2014). A new approach for plasma (xeno)metabolomics based on solid-phase extraction and nanoflow liquid chromatography-nano electrospray ionisation mass spectrometry. *Journal of Chromatography A*, 1365, 72–85. <https://doi.org/10.1016/j.chroma.2014.09.001>
7. Tubaon, R. M., Haddad, P. R., & Quirino, J. P. (2017). Sample clean-up strategies for ESI mass spectrometry applications in bottom-up proteomics: Trends from 2012 to 2016. *Proteomics*, 17(20), 1700011–1700011. <https://doi.org/10.1002/pmic.201700011>
8. Zhang, Z., Pulliam, C. J., Flick, T., & Cooks, R. G. (2018). Electrophoretic desalting to improve performance in electrospray ionization mass spectrometry. *Analytical Chemistry*, 90(6), 3856–3862. <https://doi.org/10.1021/acs.analchem.7b04529>
9. Constantopoulos, T. L., Jackson, G. S., & Enke, C. G. (1999). Effects of salt concentration on analyte response using electrospray ionization mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 10(7), 625–634. [https://doi.org/10.1016/S1044-0305\(99\)00031-8](https://doi.org/10.1016/S1044-0305(99)00031-8)
10. Cahill, J. F., Kertesz, V., & Van Berkel, G. J. (2015). Characterization and application of a hybrid optical microscopy/laser ablation liquid vortex capture/electrospray ionization system for mass spectrometry imaging with sub-micrometer spatial resolution. *Analytical Chemistry*, 87(21), 11113–11121. <https://doi.org/10.1021/acs.analchem.5b03293>
11. Cahill, J. F., & Kertesz, V. (2018). automated optically guided system for chemical analysis of single plant and algae cells using laser microdissection/liquid vortex capture/mass spectrometry. *Frontiers in Plant Science*, 9, 1211–1211. <https://doi.org/10.3389/fpls.2018.01211>
12. Sarbu, M., & Zamfir, A. D. (2018). Modern separation techniques coupled to high performance mass spectrometry for glycolipid analysis. *Electrophoresis*, 39(9-10), 1155–1170. <https://doi.org/10.1002/elps.201700461>
13. Lion, N., Reymond, F., Girault, H. H., & Rossier, J. S. (2004). Why the move to microfluidics for protein analysis? *Current Opinion in Biotechnology*, 15(1), 31–37. <https://doi.org/10.1016/j.copbio.2004.01.001>
14. Juraschek, R., Dülcks, T., & Karas, M. (1999). Nanoelectrospray—more than just a minimized-flow electrospray ionization source. *Journal of the American Society for Mass Spectrometry*, 10(4), 300–308. [https://doi.org/10.1016/S1044-0305\(98\)00157-3](https://doi.org/10.1016/S1044-0305(98)00157-3)
15. McMahon, W. P., Dalvi, R., Lesniewski, J. E., Hall, Z. Y., & Jorabchi, K. (2020). Pulsed nano-ESI: Application in ion mobility-ms and insights into spray dynamics. *Journal of the American Society for Mass Spectrometry*, 31(3), 488–497. <https://doi.org/10.1021/jasms.9b00121>
16. Lanucara, F., Holman, S. W., Gray, C. J., & Evers, C. E. (2014). The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics. *Nature Chemistry*, 6(4), 281–294. <https://doi.org/10.1038/nchem.1889>
17. Wei, A. A. J., Joshi, A., Chen, Y., & McIndoe, J. S. (2020). Strategies for avoiding saturation effects in ESI-MS. *International Journal of Mass Spectrometry*, 450, 116306–116306. <https://doi.org/10.1016/j.ijms.2020.116306>
18. Janusson, E., Hesketh, A. V., Bamford, K. L., Hatlelid, K., Higgins, R., & McIndoe, J. S. (2015). Spatial effects on electrospray ionization response. *International Journal of Mass Spectrometry*, 388, 1–8. <https://doi.org/10.1016/j.ijms.2015.07.016>
19. Sawyer, W. S., Srikumar, N., Carver, J., Chu, P. Y., Shen, A., Xu, A., Williams, A. J., Spiess, C., Wu, C., Liu, Y., & Tran, J. C. (2020). High-throughput antibody screening from complex matrices using intact protein electrospray mass spectrometry. *Proceedings of the National Academy of Sciences of the United States of America*, 117(18), 9851–9856. <https://doi.org/10.1073/pnas.1917383117>
20. Häbe, T. T., Liu, C., Covey, T. R., Simon, R. P., Reindl, W., Büttner, F. H., Winter, M., Bischoff, D., Luippold, A. H., & Runge, F. (2020). Ultrahigh-throughput ESI-MS: Sampling pushed to six samples per second by acoustic ejection mass spectrometry. *Analytical Chemistry*, 92(18), 12242–12249. <https://doi.org/10.1021/acs.analchem.0c01632>
21. Hilmer, J. K., & Bothner, B. (2011). Physical signal modulation of time-of-flight mass analyzers increases precision and decreases noise. *Rapid Communications in Mass Spectrometry*, 25(6), 795–805. <https://doi.org/10.1002/rcm.4923>
22. Misra, B. B. (2021). New software tools, databases, and resources in metabolomics: Updates from 2020. *Metabolomics*, 17(5), 49–49. <https://doi.org/10.1007/s11306-021-01796-1>
23. Krasny, L., & Huang, P. H. (2021). Data-independent acquisition mass spectrometry (DIA-MS) for proteomic applications in oncology. *Molecular Omics*, 17(1), 29–42. <https://doi.org/10.1039/D0MO00072H>
24. Demichev, V., Messner, C. B., Vernardis, S. I., Lilley, K. S., & Ralser, M. (2020). DIA-NN: Neural networks and interference correction enable deep proteome coverage in high throughput. *Nature Methods*, 17(1), 41–44. <https://doi.org/10.1038/s41592-019-0638-x>
25. Cox, J., & Kruppa, G. (2020). Advanced computational methods drive large-scale data analysis in 4D-proteomics. *Genetic Engineering and Biotechnology*, 40(12), 22–23. <https://doi.org/10.1089/gen.40.12.07>
26. Tiwary, S., Levy, R., Gutenbrunner, P., Salinas Soto, F., Palaniappan, K. K., Deming, L., Berndl, M., Brant, A., Cimermanic, P., & Cox, J. (2019). High-quality MS/MS spectrum prediction for data-dependent and data-independent acquisition data analysis. *Nature Methods*, 16(6), 519–525. <https://doi.org/10.1038/s41592-019-0427-6>
27. Bian, Y., Zheng, R., Bayer, F. P., Wong, C., Chang, Y.-C., Meng, C., Zolg, D. P., Reinecke, M., Zecha, J., Wiechmann, S., Heinzlmeir, S., Scherr, J., Hemmer, B., Baynham, M., Gingras, A.-C., Boychenko, O., & Kuster, B. (2020). Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC-MS/MS. *Nature Communication*, 11(1), 157–157. <https://doi.org/10.1038/s41467-019-13973-x>
28. Cramer, R. (2020). High-speed analysis of large sample sets - how can this key aspect of the omics be achieved? *Molecular & Cellular Proteomics*, 19(11), 1760–1766. <https://doi.org/10.1074/mcp.P120.001997>
29. Britt, H. M., Cragolini, T., & Thalassinou, K. (2022). Integration of mass spectrometry data for structural biology. *Chemical Reviews*, 122, 7952–7986. <https://doi.org/10.1021/acs.chemrev.1c00356>
30. Balog, J., Sasi-Szabó, L., Kinross, J., Lewis, M. R., Muirhead, L. J., Veselkov, K., Mirnezami, R., Dezsó, B., Damjanovich, L., Darzi, A., Nicholson, J. K., & Takáts, Z. (2013). Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. *Science Translational Medicine*, 5(194), 194ra193–194ra193. <https://doi.org/10.1126/scitranslmed.3005623>
31. Gibson, G. T. T., Mugo, S. M., & Oleschuk, R. D. (2009). Nanoelectrospray emitters: Trends and perspective. *Mass Spectrometry Reviews*, 28(6), 918–936. <https://doi.org/10.1002/mas.20248>
32. Mao, P., Wang, H.-T., Yang, P., & Wang, D. (2011). Multinozzle emitter arrays for nanoelectrospray mass spectrometry. *Analytical Chemistry*, 83(15), 6082–6089. <https://doi.org/10.1021/ac2011813>
33. Mortensen, D. N., & Williams, E. R. (2014). Theta-glass capillaries in electrospray ionization: Rapid mixing and short droplet lifetimes. *Analytical Chemistry*, 86(18), 9315–9321. <https://doi.org/10.1021/ac502545r>
34. Leopold, J., Popkova, Y., Engel, K., & Schiller, J. (2018). Recent developments of useful MALDI matrices for the mass spectrometric characterization of lipids. *Biomolecules*, 8(4), 173–173. <https://doi.org/10.3390/biom8040173>
35. Calvano, C. D., Monopoli, A., Cataldi, T. R. I., & Palmisano, F. (2018). MALDI matrices for low molecular weight compounds: An endless

- story? *Analytical and Bioanalytical Chemistry*, 410(17), 4015–4038. <https://doi.org/10.1007/s00216-018-1014-x>
36. Abaye, D. A., Agbo, I. A., & Nielsen, B. V. (2021). Current perspectives on supercharging reagents in electrospray ionization mass spectrometry. *RSC Advances*, 11(33), 20355–20369. <https://doi.org/10.1039/D1RA00745A>
 37. Iavarone, A. T., Jurchen, J. C., & Williams, E. R. (2001). Supercharged protein and peptide ions formed by electrospray ionization. *Analytical Chemistry*, 73(7), 1455–1460. <https://doi.org/10.1021/ac001251t>
 38. Li, X., Li, Z., Xie, B., & Sharp, J. S. (2015). Supercharging by m-NBA improves ETD-based quantification of hydroxyl radical protein footprinting. *Journal of the American Society for Mass Spectrometry*, 26(8), 1424–1427. <https://doi.org/10.1007/s13361-015-1129-7>
 39. Zhang, J., Loo, R. R. O., & Loo, J. A. (2015). Increasing fragmentation of disulfide-bonded proteins for top-down mass spectrometry by supercharging. *International Journal of Mass Spectrometry*, 377, 546–556. <https://doi.org/10.1016/j.ijms.2014.07.047>
 40. Nshanian, M., Lakshmanan, R., Chen, H., Loo, R. R. O., & Loo, J. A. (2018). Enhancing sensitivity of liquid chromatography-mass spectrometry of peptides and proteins using supercharging agents. *International Journal of Mass Spectrometry*, 427, 157–164. <https://doi.org/10.1016/j.ijms.2017.12.006>
 41. Van Wansele, Y., Bongaerts, J., Segers, K., Viaene, J., De Bundel, D., Vander Heyden, Y., Smolders, I., & Van Eeckhaut, A. (2019). Assessing mixtures of supercharging agents to increase the abundance of a specific charge state of Neuromedin U. *Talanta*, 198, 206–214. <https://doi.org/10.1016/j.talanta.2019.01.098>
 42. Wang, H., Liu, J., Cooks, R. G., & Ouyang, Z. (2010). Paper spray for direct analysis of complex mixtures using mass spectrometry. *Angewandte Chemie (International ed in English)*, 49(5), 877–880. <https://doi.org/10.1002/anie.200906314>
 43. Liu, J., Wang, H., Manicke, N. E., Lin, J.-M., Cooks, R. G., & Ouyang, Z. (2010). Development, characterization, and application of paper spray ionization. *Analytical Chemistry*, 82(6), 2463–2471. <https://doi.org/10.1021/ac902854g>
 44. Ren, Y., Wang, H., Liu, J., Zhang, Z., Mcluckey, M. N., & Ouyang, Z. (2013). Analysis of biological samples using paper spray mass spectrometry: An investigation of impacts by the substrates, solvents and elution methods. *Chromatographia*, 76(19–20), 1339–1346. <https://doi.org/10.1007/s10337-013-2458-y>
 45. Colletes, T. C., Garcia, P. T., Campanha, R. B., Abdelnur, P. V., Romão, W., Coltro, W. K. T., & Vaz, B. G. (2016). A new insert sample approach to paper spray mass spectrometry: A paper substrate with paraffin barriers. *Analyst*, 141(5), 1707–1713. <https://doi.org/10.1039/C5AN01954K>
 46. De Silva, I. W., Nayek, S., Singh, V., Reddy, J., Granger, J. K., & Verbeck, G. F. (2020). Paper spray mass spectrometry utilizing Teslin® substrate for rapid detection of lipid metabolite changes during COVID-19 infection. *Analyst*, 145(17), 5725–5732. <https://doi.org/10.1039/D0AN01074J>
 47. De Silva, I. W., Converse, D. T., Juel, L. A., & Verbeck, G. F. (2019). A comparative study of microporous polyolefin silica-based paper and cellulose paper substrates utilizing paper spray-mass spectrometry in drug analysis. *Analytical Methods*, 11(24), 3066–3072. <https://doi.org/10.1039/C9AY00641A>
 48. Narayanan, R., Song, X., Chen, H., & Zare, R. N. (2020). Teflon spray ionization mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 31(2), 234–239. <https://doi.org/10.1021/jasms.9b00069>
 49. Song, X., Chen, H., & Zare, R. N. (2018). Conductive polymer spray ionization mass spectrometry for biofluid analysis. *Analytical Chemistry*, 90(21), 12878–12885. <https://doi.org/10.1021/acs.analchem.8b03460>
 50. Wang, Q., Bhattarai, M., Zhao, P., Alnsour, T., Held, M., Faik, A., & Chen, H. (2020). Fast and sensitive detection of oligosaccharides using desalting paper spray mass spectrometry (DPS-MS). *Journal of the American Society for Mass Spectrometry*, 31(10), 2226–2235. <https://doi.org/10.1021/jasms.0c00310>
 51. Chiu, K.-Y., Wang, Q., Gunawardena, H. P., Held, M., Faik, A., & Chen, H. (2021). Desalting paper spray mass spectrometry (DPS-MS) for rapid detection of glycans and glycoconjugates. *International Journal of Mass Spectrometry*, 469, 116688–116688. <https://doi.org/10.1016/j.ijms.2021.116688>
 52. Song, X., Mofidfar, M., & Zare, R. N. (2022). Introducing Nafion for in situ desalting and biofluid profiling in spray mass spectrometry in situ desalting and biofluid profiling in spray mass spectrometry. *Frontiers Chemistry*, 9, 807244. <https://doi.org/10.3389/fchem.2021.807244>
 53. Takáts, Z., Wiseman, J. M., Gologan, B., & Cooks, R. G. (2004). Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science*, 306(5695), 471–473. <https://doi.org/10.1126/science.1104404>
 54. Zemaitis, K. J., Izzydorczak, A. M., Thompson, A. C., & Wood, T. D. (2021). Streamlined multimodal DESI and MALDI mass spectrometry imaging on a singular dual-source FT-ICR mass spectrometer. *Metabolites*, 11(4), 253–253. <https://doi.org/10.3390/metabo11040253>
 55. Towers, M. W., Karancsi, T., Jones, E. A., Pringle, S. D., & Claude, E. (2018). Optimised desorption electrospray ionisation mass spectrometry imaging (DESI-MSI) for the analysis of proteins/peptides directly from tissue sections on a travelling wave ion mobility Q-ToF. *Journal of the American Society for Mass Spectrometry*, 29(12), 2456–2466. <https://doi.org/10.1007/s13361-018-2049-0>
 56. He, J., Tang, F., Luo, Z., Chen, Y., Xu, J., Zhang, R., Wang, X., & Abliz, Z. (2011). Air flow assisted ionization for remote sampling of ambient mass spectrometry and its application. *Rapid Communications in Mass Spectrometry*, 25(7), 843–850. <https://doi.org/10.1002/rcm.4920>
 57. Luo, Z., He, J., Chen, Y., He, J., Gong, T., Tang, F., Wang, X., Zhang, R., Huang, L., Zhang, L., Lv, H., Ma, S., Fu, Z., Chen, X., Yu, S., & Abliz, Z. (2013). Air flow-assisted ionization imaging mass spectrometry method for easy whole-body molecular imaging under ambient conditions. *Analytical Chemistry*, 85(5), 2977–2982. <https://doi.org/10.1021/ac400009s>
 58. Zhang, J., Huo, X., Guo, C., Ma, X., Huang, H., He, J., Wang, X., & Tang, F. (2021). Rapid imaging of unsaturated lipids at an isomeric level achieved by controllable oxidation. *Analytical Chemistry*, 93(4), 2114–2124. <https://doi.org/10.1021/acs.analchem.0c03888>
 59. Zang, Q., Wang, M., Zhu, Y., Wang, L., Luo, Z., Li, X., He, J., Zhang, R., & Abliz, Z. (2021). Enhanced on-tissue chemical derivatization with hydrogel assistance for mass spectrometry imaging. *Analytical Chemistry*, 93(46), 15373–15380. <https://doi.org/10.1021/acs.analchem.1c03118>
 60. Hsu, C.-C., Chou, P.-T., & Zare, R. N. (2015). Imaging of proteins in tissue samples using nanospray desorption electrospray ionization mass spectrometry. *Analytical Chemistry*, 87(22), 11171–11175. <https://doi.org/10.1021/acs.analchem.5b03389>
 61. Roach, P. J., Laskin, J., & Laskin, A. (2010). Nanospray desorption electrospray ionization: An ambient method for liquid-extraction surface sampling in mass spectrometry. *Analyst*, 135(9), 2233–2236. <https://doi.org/10.1039/C0AN00312C>
 62. Hale, O. J., & Cooper, H. J. (2021). Native mass spectrometry imaging of proteins and protein complexes by nano-DESI. *Analytical Chemistry*, 93, 4619–4627. <https://doi.org/10.1021/acs.analchem.0c05277>
 63. Sisley, E. K., Hale, O. J., Styles, I. B., & Cooper, H. J. (2022). Native ambient mass spectrometry imaging of ligand-bound and metal-bound proteins in rat brain. *Journal of the American Chemical Society*, 144, 2120–2128. <https://doi.org/10.1021/jacs.1c10032>
 64. Duncan, K. D., Bergman, H.-M., & Lanekoff, I. (2017). A pneumatically assisted nanospray desorption electrospray ionization source for increased solvent versatility and enhanced metabolite detection

- from tissue. *Analyst*, 142(18), 3424–3431. <https://doi.org/10.1039/C7AN00901A>
65. Nyadong, L., Hohenstein, E. G., Galhena, A., Lane, A. L., Kubanek, J., Sherrill, C. D., & Fernández, F. M. (2009). Reactive desorption electrospray ionization mass spectrometry (DESI-MS) of natural products of a marine alga. *Analytical and Bioanalytical Chemistry*, 394(1), 245–254. <https://doi.org/10.1007/s00216-009-2674-3>
66. Lostun, D., Perez, C. J., Licence, P., Barrett, D. A., & Ifa, D. R. (2015). Reactive DESI-MS imaging of biological tissues with dicationic ion-pairing compounds. *Analytical Chemistry*, 87(6), 3286–3293. <https://doi.org/10.1021/ac5042445>
67. Liu, C., Qi, F., & Pan, Y. (2021). Imaging of polar and nonpolar lipids using desorption electrospray ionization/post-photoionization mass spectrometry. In F.-F. Hsu (Ed.), *Mass spectrometry-based lipidomics: Methods and protocols* (pp. 285–298). Springer US.
68. Nguyen, S. N., Liyu, A. V., Chu, R. K., Anderton, C. R., & Laskin, J. (2017). Constant-distance mode nanospray desorption electrospray ionization mass spectrometry imaging of biological samples with complex topography. *Analytical Chemistry*, 89, 1131–1137. <https://doi.org/10.1021/acs.analchem.6b03293>
69. Yin, R., Kyle, J., Burnum-Johnson, K., Bloodsworth, K. J., Sussel, L., Ansong, C., & Laskin, J. (2018). High spatial resolution imaging of mouse pancreatic islets using nanospray desorption electrospray ionization mass spectrometry. *Analytical Chemistry*, 90(11), 6548–6555. <https://doi.org/10.1021/acs.analchem.8b00161>
70. Nguyen, S. N., Sontag, R. L., Carson, J. P., Corley, R. A., Ansong, C., & Laskin, J. (2018). Towards high-resolution tissue imaging using nanospray desorption electrospray ionization mass spectrometry coupled to shear force microscopy. *Journal of the American Society for Mass Spectrometry*, 29(2), 316–322. <https://doi.org/10.1007/s13361-017-1750-8>
71. Shiea, J., Huang, M.-Z., Hsu, H.-J., Lee, C.-Y., Yuan, C.-H., Beech, I., & Sunner, J. (2005). Electrospray-assisted laser desorption/ionization mass spectrometry for direct ambient analysis of solids. *Rapid Communications in Mass Spectrometry*, 19(24), 3701–3704. <https://doi.org/10.1002/rcm.2243>
72. Peng, I. X., Shiea, J., Loo, R. R. O., & Loo, J. A. (2007). Electrospray-assisted laser desorption/ionization and tandem mass spectrometry of peptides and proteins. *Rapid Communications in Mass Spectrometry*, 21(16), 2541–2546. <https://doi.org/10.1002/rcm.3154>
73. Sampson, J. S., Hawkridge, A. M., & Muddiman, D. C. (2006). Generation and detection of multiply-charged peptides and proteins by matrix-assisted laser desorption electrospray ionization (MALDESI) fourier transform ion cyclotron resonance mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 17(12), 1712–1716. <https://doi.org/10.1016/j.jasms.2006.08.003>
74. Nazari, M., & Muddiman, D. C. (2016). MALDESI: Fundamentals, direct analysis, and MS imaging. In R. Cramer (Ed.), *Advances in MALDI and laser-induced soft ionization mass spectrometry* (pp. 169–182). Springer International Publishing.
75. Robichaud, G., Barry, J. A., Garrard, K. P., & Muddiman, D. C. (2013). Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) imaging source coupled to a FT-ICR mass spectrometer. *Journal of the American Society for Mass Spectrometry*, 24(1), 92–100. <https://doi.org/10.1007/S13361-012-0505-9>
76. Sampson, J. S., Murray, K. K., & Muddiman, D. C. (2009). Intact and top-down characterization of biomolecules and direct analysis using infrared matrix-assisted laser desorption electrospray ionization coupled to FT-ICR mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 20(4), 667–673. <https://doi.org/10.1016/j.jasms.2008.12.003>
77. Ekelöf, M., Manni, J., Nazari, M., Bokhart, M., & Muddiman, D. C. (2018). Characterization of a novel miniaturized burst-mode infrared laser system for IR-MALDESI mass spectrometry imaging. *Analytical and Bioanalytical Chemistry*, 410(9), 2395–2402. <https://doi.org/10.1007/s00216-018-0918-9>
78. Bokhart, M. T., Manni, J., Garrard, K. P., Ekelöf, M., Nazari, M., & Muddiman, D. C. (2017). IR-MALDESI mass spectrometry imaging at 50 micron spatial resolution. *Journal of the American Society for Mass Spectrometry*, 28(10), 2099–2107. <https://doi.org/10.1007/s13361-017-1740-x>
79. Bagley, M. C., Ekelöf, M., & Muddiman, D. C. (2020). Determination of optimal electrospray parameters for lipidomics in infrared-matrix-assisted laser desorption electrospray ionization mass spectrometry imaging. *Journal of the American Society for Mass Spectrometry*, 31(2), 319–325. <https://doi.org/10.1021/jasms.9b00063>
80. Robichaud, G., Barry, J. A., & Muddiman, D. C. (2014). IR-MALDESI mass spectrometry imaging of biological tissue sections using ice as a matrix. *Journal of the American Society for Mass Spectrometry*, 25(3), 319–328. <https://doi.org/10.1007/s13361-013-0787-6>
81. Tu, A., & Muddiman, D. C. (2019). Internal energy deposition in infrared matrix-assisted laser desorption electrospray ionization with and without the use of ice as a matrix. *Journal of the American Society for Mass Spectrometry*, 30(11), 2380–2391. <https://doi.org/10.1007/s13361-019-02323-2>
82. Nemes, P., & Vertes, A. (2007). Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry, in vivo, and imaging mass spectrometry. *Analytical Chemistry*, 79(21), 8098–8106. <https://doi.org/10.1021/ac071181r>
83. Shrestha, B., & Vertes, A. (2014). High-throughput cell and tissue analysis with enhanced molecular coverage by laser ablation electrospray ionization mass spectrometry using ion mobility separation. *Analytical Chemistry*, 86(9), 4308–4315. <https://doi.org/10.1021/ac500007t>
84. Da Silva Lima, G., Franco Dos Santos, G., Ramalho, R. R. F., De Aguiar, D. V. A., Roque, J. V., Maciel, L. I. L., Simas, R. C., Pereira, I., & Vaz, B. G. (2022). Laser ablation electrospray ionization mass spectrometry imaging as a new tool for accessing patulin diffusion in mold-infected fruits. *Food Chemistry*, 373, 131490–131490. <https://doi.org/10.1016/j.foodchem.2021.131490>
85. Kulkarni, P., Wilschut, R. A., Verhoeven, K. J. F., Van Der Putten, W. H., & Garbeva, P. (2018). LAESI mass spectrometry imaging as a tool to differentiate the root metabolome of native and range-expanding plant species. *Planta*, 248(6), 1515–1523. <https://doi.org/10.1007/s00425-018-2989-4>
86. Radosevich, A. J., Pu, F., Chang-Yen, D., Sawicki, J. W., Talaty, N. N., Elsen, N. L., Williams, J. D., & Pan, J. Y. (2022). Ultra-high-throughput ambient MS: Direct analysis at 22 samples per second by infrared matrix-assisted laser desorption electrospray ionization mass spectrometry. *Analytical Chemistry*, 94(12), 4913–4918. <https://doi.org/10.1021/acs.analchem.1c04605>
87. Agtuca, B. J., Stopka, S. A., Evans, S., Samarah, L., Liu, Y., Xu, D., Stacey, M. G., Koppelaar, D. W., Paša-Tolić, L., Anderton, C. R., Vertes, A., & Stacey, G. (2020). Metabolomic profiling of wild-type and mutant soybean root nodules using laser-ablation electrospray ionization mass spectrometry reveals altered metabolism. *Plant Journal*, 103(5), 1937–1958. <https://doi.org/10.1111/tpj.14815>
88. Taylor, M. J., Liyu, A., Vertes, A., & Anderton, C. R. (2021). Ambient single-cell analysis and native tissue imaging using laser-ablation electrospray ionization mass spectrometry with increased spatial resolution. *Journal of the American Society for Mass Spectrometry*, 32(9), 2490–2494. <https://doi.org/10.1021/jasms.1c00149>
89. Zhou, W., Xia, L., Huang, C., Yang, J., Shen, C., Jiang, H., & Chu, Y. (2016). Rapid analysis and identification of meat species by laser-ablation electrospray mass spectrometry (LAESI-MS). *Rapid Communications in Mass Spectrometry*, 30(S1), 116–121. <https://doi.org/10.1002/rcm.7647>
90. Vaikkinen, A., Shrestha, B., Nazarian, J., Kostianinen, R., Vertes, A., & Kauppila, T. J. (2013). Simultaneous detection of nonpolar and polar

- compounds by heat-assisted laser ablation electrospray ionization mass spectrometry. *Analytical Chemistry*, 85(1), 177–184. <https://doi.org/10.1021/ac302432h>
91. Li, H., & Vertes, A. (2017). Solvent gradient electrospray for laser ablation electrospray ionization mass spectrometry. *Analyst*, 142(16), 2921–2927. <https://doi.org/10.1039/C7AN00819H>
 92. Barry, J. A., & Muddiman, D. C. (2011). Global optimization of the infrared matrix-assisted laser desorption electrospray ionization (IR MALDESI) source for mass spectrometry using statistical design of experiments. *Rapid Communications in Mass Spectrometry*, 25(23), 3527–3536. <https://doi.org/10.1002/rcm.5262>
 93. Trimpin, S., Inutan, E. D., Herath, T. N., & McEwen, C. N. (2010). Laser-spray ionization, a new atmospheric pressure MALDI method for producing highly charged gas-phase ions of peptides and proteins directly from solid solutions. *Molecular & Cellular Proteomics*, 9(2), 362–367. <https://doi.org/10.1074/mcp.M900527-MCP200>
 94. Inutan, E. D., Richards, A. L., Wager-Miller, J., Mackie, K., McEwen, C. N., & Trimpin, S. (2011). Laserspray ionization, a new method for protein analysis directly from tissue at atmospheric pressure with ultrahigh mass resolution and electron transfer dissociation. *Molecular & Cellular Proteomics*, 10(2), S1–S8. <https://doi.org/10.1074/mcp.M110.000760>
 95. Inutan, E. D., Wager-Miller, J., Mackie, K., & Trimpin, S. (2012). Laser-spray ionization imaging of multiply charged ions using a commercial vacuum MALDI ion source. *Analytical Chemistry*, 84(21), 9079–9084. <https://doi.org/10.1021/ac301665h>
 96. Cramer, R., & Corless, S. (2005). Liquid ultraviolet matrix-assisted laser desorption/ionization – mass spectrometry for automated proteomic analysis. *Proteomics*, 5(2), 360–370. <https://doi.org/10.1002/pmic.200400956>
 97. Hessler, B., Büttner, K., Hecker, M., & Becher, D. (2013). Global relative quantification with liquid chromatography-matrix-assisted laser desorption ionization time-of-flight (LC-MALDI-TOF)–cross-validation with LTQ-orbitrap proves reliability and reveals complementary ionization preferences. *Molecular & Cellular Proteomics*, 12(10), 2911–2920. <https://doi.org/10.1074/mcp.M112.023457>
 98. Laiko, V. V., Taranenko, N. I., Berkout, V. D., Yakshin, M. A., Prasad, C. R., Lee, H. S., & Doroshenko, V. M. (2002). Desorption/ionization of biomolecules from aqueous solutions at atmospheric pressure using an infrared laser at 3 μm . *Journal of the American Society for Mass Spectrometry*, 13(4), 354–361. [https://doi.org/10.1016/S1044-0305\(02\)00341-0](https://doi.org/10.1016/S1044-0305(02)00341-0)
 99. Cramer, R., Pirkel, A., Hillenkamp, F., & Dreisewerd, K. (2013). Liquid AP-UV-MALDI enables stable ion yields of multiply charged peptide and protein ions for sensitive analysis by mass spectrometry. *Angewandte Chemie (International ed in English)*, 52(8), 2364–2367. <https://doi.org/10.1002/anie.201208628>
 100. Ait-Belkacem, R., Dilillo, M., Pellegrini, D., Yadav, A., De Graaf, E. L., & McDonnell, L. A. (2016). In-source decay and pseudo-MS 3 of peptide and protein ions using liquid AP-MALDI. *Journal of the American Society for Mass Spectrometry*, 27(12), 2075–2079. <https://doi.org/10.1007/s13361-016-1511-0>
 101. Ryumin, P., & Cramer, R. (2016). Efficient production of multiply charged MALDI ions. In R. Cramer (Ed.), *Advances in MALDI and laser-induced soft ionization mass spectrometry* (pp. 37–47). Springer International Publishing.
 102. Ryumin, P., Brown, J., Morris, M., & Cramer, R. (2016). Investigation and optimization of parameters affecting the multiply charged ion yield in AP-MALDI MS. *Methods (San Diego, Calif.)*, 104, 11–20. <https://doi.org/10.1016/j.jymeth.2016.01.015>
 103. Ryumin, P., & Cramer, R. (2018). The composition of liquid atmospheric pressure matrix-assisted laser desorption/ionization matrices and its effect on ionization in mass spectrometry. *Analytica Chimica Acta*, 1013, 43–53. <https://doi.org/10.1016/j.aca.2018.01.070>
 104. Hale, O. J., & Cramer, R. (2018). Collision-induced dissociation of doubly-charged barium-cationized lipids generated from liquid samples by atmospheric pressure matrix-assisted laser desorption/ionization provides structurally diagnostic product ions. *Analytical and Bioanalytical Chemistry*, 410(5), 1435–1444. <https://doi.org/10.1007/s00216-017-0788-6>
 105. Ryumin, P., Brown, J., Morris, M., & Cramer, R. (2017). Protein identification using a nanoUHPLC-AP-MALDI MS/MS workflow with CID of multiply charged proteolytic peptides. *International Journal of Mass Spectrometry*, 416, 20–28. <https://doi.org/10.1016/j.ijms.2016.12.006>
 106. Hale, O. J., Morris, M., Jones, B., Reynolds, C. K., & Cramer, R. (2019). Liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry adds enhanced functionalities to MALDI MS profiling for disease diagnostics. *ACS Omega*, 4(7), 12759–12765. <https://doi.org/10.1021/acsomega.9b01476>
 107. Lellman, S. E., & Cramer, R. (2020). Bacterial identification by lipid profiling using liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. *Clinical Chemistry and Laboratory Medicine*, 58(6), 930–938. <https://doi.org/10.1515/cclm-2019-0908>
 108. Piras, C., Ceniti, C., Hartmane, E., Costanzo, N., Morittu, V. M., Roncada, P., Britti, D., & Cramer, R. (2020). Rapid liquid AP-MALDI MS profiling of lipids and proteins from goat and sheep milk for speciation and colostrum analysis. *Proteomes*, 8(3), 20–20. <https://doi.org/10.3390/proteomes8030020>
 109. Piras, C., Hale, O. J., Reynolds, C. K., Jones, A. K. B., Taylor, N., Morris, M., & Cramer, R. (2022). LAP-MALDI MS coupled with machine learning: An ambient mass spectrometry approach for high-throughput diagnostics. *Chemical Science*, 13(6), 1746–1758. <https://doi.org/10.1039/D1SC05171G>
 110. Krenkel, H., Hartmane, E., Piras, C., Brown, J., Morris, M., & Cramer, R. (2020). Advancing liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry toward ultrahigh-throughput analysis. *Analytical Chemistry*, 92(4), 2931–2936. <https://doi.org/10.1021/acs.analchem.9b05202>
 111. Krenkel, H., Brown, J., Richardson, K., Hoyes, E., Morris, M., & Cramer, R. (2022). Ultrahigh-throughput sample analysis using liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. *Analytical Chemistry*, 94(10), 4141–4145. <https://doi.org/10.1021/acs.analchem.1c05614>
 112. Schäfer, K.-C., Dénes, J., Albrecht, K., Szaniszló, T., Balog, J., Skoumal, R., Katona, M., Tóth, M., Balogh, L., & Takáts, Z. (2009). In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry. *Angewandte Chemie (International ed in English)*, 48(44), 8240–8242. <https://doi.org/10.1002/anie.200902546>
 113. Balog, J., Szaniszló, T., Schaefer, K.-C., Denes, J., Lopata, A., Godorhazy, L., Szalay, D., Balogh, L., Sasi-Szabo, L., Toth, M., & Takats, Z. (2010). Identification of biological tissues by rapid evaporative ionization mass spectrometry. *Analytical Chemistry*, 82(17), 7343–7350. <https://doi.org/10.1021/ac101283x>
 114. St John, E. R., Balog, J., Mckenzie, J. S., Rossi, M., Covington, A., Muirhead, L., Bodai, Z., Rosini, F., Speller, A. V. M., Shousha, S., Ramakrishnan, R., Darzi, A., Takats, Z., & Leff, D. R. (2017). Rapid evaporative ionisation mass spectrometry of electrosurgical vapours for the identification of breast pathology: Towards an intelligent knife for breast cancer surgery. *Breast Cancer Research*, 19(1), 59–59. <https://doi.org/10.1186/s13058-017-0845-2>
 115. Phelps, D. L., Balog, J., Gildea, L. F., Bodai, Z., Savage, A., El-Bahrawy, M. A., Speller, A. V., Rosini, F., Kudo, H., Mckenzie, J. S., Brown, R., Takáts, Z., & Ghaem-Maghami, S. (2018). The surgical intelligent knife distinguishes normal, borderline and malignant gynaecological tissues using rapid evaporative ionisation mass spectrometry (REIMS). *British Journal of Cancer*, 118(10), 1349–1358. <https://doi.org/10.1038/s41416-018-0048-3>

116. Cameron, S. J. S., Alexander, J. L., Bolt, F., Burke, A., Ashrafian, H., Teare, J., Marchesi, J. R., Kinross, J., Li, J. V., & Takáts, Z. (2019). Evaluation of direct from sample metabolomics of human feces using rapid evaporative ionization mass spectrometry. *Analytical Chemistry*, 91(21), 13448–13457. <https://doi.org/10.1021/acs.analchem.9b02358>
117. Fatou, B., Saudemont, P., Leblanc, E., Vinatier, D., Mesdag, V., Wisztorski, M., Focsa, C., Salzet, M., Ziskind, M., & Fournier, I. (2016). In vivo real-time mass spectrometry for guided surgery application. *Science Reports*, 6(1), 25919–25919. <https://doi.org/10.1038/srep25919>
118. Van Meulebroek, L., Cameron, S., Plekhova, V., De Spiegeleer, M., Wijnant, K., Michels, N., De Henauw, S., Lapauw, B., Takats, Z., & Vanhaecke, L. (2020). Rapid LA-REIMS and comprehensive UHPLC-HRMS for metabolic phenotyping of feces. *Talanta*, 217, 121043–121043. <https://doi.org/10.1016/j.talanta.2020.121043>
119. Wijnant, K., Van Meulebroek, L., Pomian, B., De Windt, K., De Henauw, S., Michels, N., & Vanhaecke, L. (2020). Validated ultra-high-performance liquid chromatography hybrid high-resolution mass spectrometry and laser-assisted rapid evaporative ionization mass spectrometry for salivary metabolomics. *Analytical Chemistry*, 92(7), 5116–5124. <https://doi.org/10.1021/acs.analchem.9b05598>
120. Arena, K., Rigano, F., Mangraviti, D., Cacciola, F., Occhiuto, F., Dugo, L., Dugo, P., & Mondello, L. (2020). Exploration of rapid evaporative-ionization mass spectrometry as a shotgun approach for the comprehensive characterization of *Kigelia africana* (Lam) Benth. Fruit. *Fruit Molecules*, 25(4), 962–962. <https://doi.org/10.3390/molecules25040962>
121. Robson, K., Birse, N., Chevillier, O., & Elliott, C. (2022). Metabolomic profiling to detect different forms of beef fraud using rapid evaporative ionisation mass spectrometry (REIMS). *NPJ Science of Food*, 6(1), 9–9. <https://doi.org/10.1038/s41538-022-00125-7>
122. Pu, F., Elsen, N. L., & Williams, J. D. (2020). Emerging chromatography-free high-throughput mass spectrometry technologies for generating hits and leads. *ACS Medicinal Chemistry Letters*, 11(11), 2108–2113. <https://doi.org/10.1021/acsmchemlett.0c00314>
123. Sinclair, I., Stearns, R., Pringle, S., Wingfield, J., Datwani, S., Hall, E., Ghislain, L., Majlof, L., & Bachman, M. (2015). Novel acoustic loading of a mass spectrometer: Toward next-generation high-throughput MS screening. *Journal of Laboratory Automation*, 21(1), 19–26. <https://doi.org/10.1177/2211068215619124>
124. Sinclair, I., Bachman, M., Addison, D., Rohman, M., Murray, D. C., Davies, G., Mouchet, E., Tonge, M. E., Stearns, R. G., Ghislain, L., Datwani, S. S., Majlof, L., Hall, E., Jones, G. R., Hoyes, E., Olechno, J., Ellson, R. N., Barran, P. E., Pringle, S. D.,... Wingfield, J. (2019). Acoustic mist ionization platform for direct and contactless ultrahigh-throughput mass spectrometry analysis of liquid samples. *Analytical Chemistry*, 91(6), 3790–3794. <https://doi.org/10.1021/acs.analchem.9b00142>
125. Bachman, M., Sinclair, I., Ivanov, D., & Wingfield, J. (2021). Information-rich high-throughput cellular assays using acoustic mist ionisation mass spectrometry. *Analyt*, 146(1), 315–321. <https://doi.org/10.1039/D0AN01519A>
126. Smith, M. J., Ivanov, D. P., Weber, R. J. M., Wingfield, J., & Viant, M. R. (2021). Acoustic mist ionization mass spectrometry for ultrahigh-throughput metabolomics screening. *Analytical Chemistry*, 93(26), 9258–9266. <https://doi.org/10.1021/acs.analchem.1c01616>
127. Zhang, H., Liu, C., Hua, W., Ghislain, L. P., Liu, J., Aschenbrenner, L., Noell, S., Dirico, K. J., Lanyon, L. F., Stepan, C. M., West, M., Arnold, D. W., Covey, T. R., Datwani, S. S., & Troutman, M. D. (2021). Acoustic ejection mass spectrometry for high-throughput analysis. *Analytical Chemistry*, 93(31), 10850–10861. <https://doi.org/10.1021/acs.analchem.1c01137>
128. Tveen-Jensen, K., Gesellchen, F., Wilson, R., Spickett, C. M., Cooper, J. M., & Pitt, A. R. (2015). Interfacing low-energy SAW nebulization with liquid chromatography-mass spectrometry for the analysis of biological samples. *Science Reports*, 5(1), 9736–9736. <https://doi.org/10.1038/srep09736>
129. Liang, T., Schneider, T., Yoon, S. H., Oyler, B. L., Leung, L. M., Fondrie, W. E., Yen, G., Huang, Y., Ernst, R. K., Nilsson, E., & Goodlett, D. R. (2018). Optimized surface acoustic wave nebulization facilitates bacterial phenotyping. *International Journal of Mass Spectrometry*, 427, 65–72. <https://doi.org/10.1016/j.ijms.2017.09.007>
130. Trimpin, S., & Inutan, E. D. (2013). New ionization method for analysis on atmospheric pressure ionization mass spectrometers requiring only vacuum and matrix assistance. *Analytical Chemistry*, 85(4), 2005–2009. <https://doi.org/10.1021/ac303717j>
131. Woodall, D. W., Wang, B., Inutan, E. D., Narayan, S. B., & Trimpin, S. (2015). High-throughput characterization of small and large molecules using only a matrix and the vacuum of a mass spectrometer. *Analytical Chemistry*, 87(9), 4667–4674. <https://doi.org/10.1021/ac504475x>
132. Chen, B., Lietz, C. B., Ouyang, C., Zhong, X., Xu, M., & Li, L. (2016). Matrix-assisted ionization vacuum for protein detection, fragmentation and PTM analysis on a high resolution linear ion trap-orbitrap platform. *Analytica Chimica Acta*, 916, 52–59. <https://doi.org/10.1016/j.aca.2016.02.018>
133. Harding, A., Hough, J., Curtis, C., Kinsman, D., & Clench, M. (2019). Matrix-assisted ionisation in vacuum mass spectrometry and imaging on a modified quadrupole-quadrupole-time-of-flight mass spectrometer. *Journal of Spectral Imaging*, 8, a12. <https://doi.org/10.1255/jsi.2019.a12>

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