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Review Article

In situ mass spectrometry analysis of intact proteins and protein complexes from biological substrates

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Advances in sample preparation, ion sources and mass spectrometer technology have enabled the detection and characterisation of intact proteins. The challenges associated include an appropriately soft ionisation event, efficient transmission and detection of the often delicate macromolecules. Ambient ion sources, in particular, offer a wealth of strategies for analysis of proteins from solution environments, and directly from biological substrates. The last two decades have seen rapid development in this area. Innovations include liquid extraction surface analysis, desorption electrospray ionisation and nanospray desorption electrospray ionisation. Similarly, developments in native mass spectrometry allow protein–protein and protein–ligand complexes to be ionised and analysed. Identification and characterisation of these large ions involves a suite of hyphenated mass spectrometry techniques, often including the coupling of ion mobility spectrometry and fragmentation techniques. The latter include collision, electron and photon-induced methods, each with their own characteristics and benefits for intact protein identification. In this review, recent developments for *in situ* protein analysis are explored, with a focus on ion sources and tandem mass spectrometry techniques used for identification.

Introduction

Analysis of proteins *in situ* has become a focus in the development of many mass spectrometer technologies, especially ion sources. *In situ* analysis is attractive as information may be obtained rapidly, with little to no sample preparation. Consequently, many developments have been made with robotic systems. A further benefit is the opportunity to analyse proteins directly from their physiological environment (body fluids, tissue, etc.), which allows spatial information about the protein distribution to be recorded, so-called mass spectrometry imaging (MSI). MSI has typically been the realm of matrix-assisted laser desorption/ionisation (MALDI) MS [1], an ionisation technique that is usually performed under vacuum conditions; however, MALDI MSI generally requires complementary methods to identify the proteins detected since MALDI-generated protein ions are predominantly in low charge states and are thus not amenable to efficient fragmentation for top-down identification [2]. The high m/z of the MALDI-generated protein ions also necessitates the use of specific, high m/z capable mass spectrometers, which are not necessary for ionisation techniques that produce highly charged ions. As such, developments in soft and ambient ionisation techniques that do produce highly charged ions are now showing their value for intact protein analysis, and without the time-consuming sample preparation required for MALDI.

Identification of proteins may be performed in a ‘top-down’ methodology, whereby the intact proteins are ionised and then fragmented within the mass spectrometer to provide sequence information. The fragments generated depend on the fragmentation technique used. Protein identification is achieved by automated searching of the fragment m/z values against protein databases. Alternatively, digestion by specific enzymes, such as trypsin, enable ‘bottom-up’ proteomics; the peptides produced by protein digestion are analysed and identified, then the source protein is determined by matching to

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sequences in a database. On-tissue digestion followed by MALDI MS allows for bottom-up protein identification from peptides but generally results in some loss of integrity of protein spatial distribution. Bottom-up proteomics may also result in missing information such as the connectivity of labile post-translational modifications (PTMs), presence of single-nucleotide polymorphisms and ambiguity as to the source of redundant peptides [3]. Multiple alternatives to MALDI for *in situ* protein analysis now exist, many taking advantage of the highly charged ions produced by electrospray ionisation (ESI)-like processes. This review covers these alternative techniques and related technologies that allow for *in situ* protein detection and identification.

Ambient ionisation

In situ mass spectrometry analysis has benefitted from the many ambient ionisation techniques that have been developed in the last two decades. The analysis is performed at atmospheric pressure and usually without the constraints on sample size and sample state imposed by *in vacuo* ion sources. Here, we focus on those techniques that have been applied to the analysis of intact proteins.

Liquid junction surface sampling

Many liquid extraction-based ion sources for mass spectrometry are described in the literature, many leveraging the flexibility and sensitivity of solvent-based extraction with subsequent ESI. ESI has a 30-year heritage of protein analysis [5,6] and resulted in one-quarter of the Nobel Prize in Chemistry in 2002. It was later developed into nanoESI, dramatically reducing droplet size, improving sensitivity and salt tolerance at a much-reduced sample consumption rate [7]. NanoESI is the major form of the technique used for biomolecule analysis today. The various liquid extraction techniques include liquid extraction surface analysis (LESA) [8], nanospray desorption electrospray ionisation (nano-DESI) and devices such as the FlowProbe (Prosolia Inc. Indianapolis, IN) and the MasSpec Pen [9]. In the last decade, these techniques have become established for analysis of small molecules, metabolites and lipids directly from biological samples (LESA [10–12], nano-DESI [13–15], FlowProbe [16], MasSpec Pen [17]), but *in situ* protein analysis has proven a greater challenge.

Liquid extraction surface analysis (LESA)

LESA (Figure 1A) is perhaps the most established liquid extraction method for *in situ* protein analysis, and was successfully commercialised in a robotic sampling system by Advion (Ithaca, NY) building on work by Kertesz

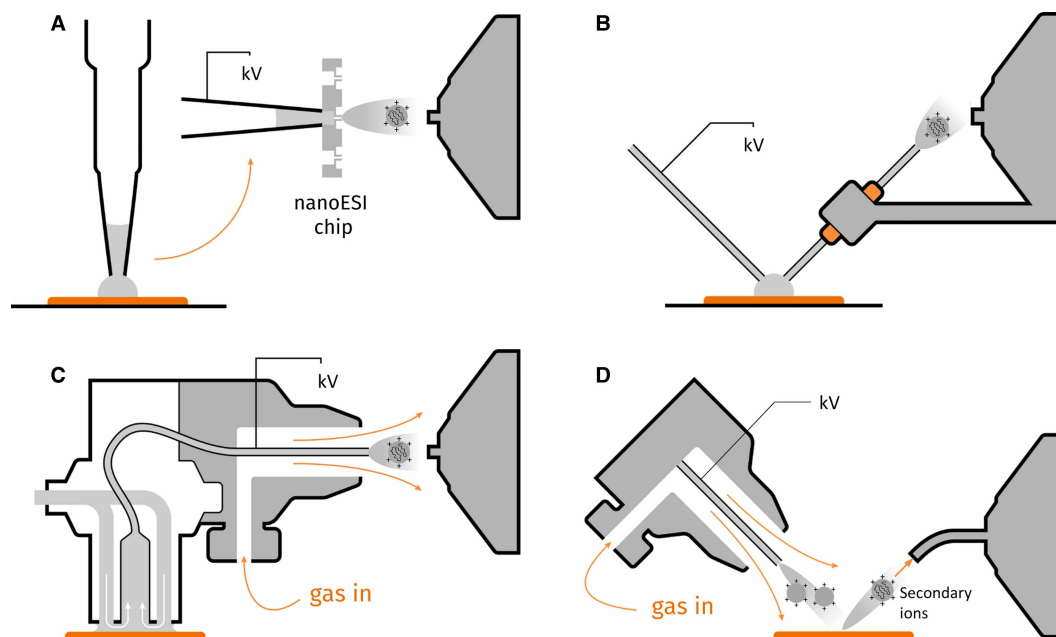


Figure 1. Schematics of ambient ion sources.

(A) LESA, (B) nano-DESI, (C) FlowProbe and (D) DESI. Adapted from reference [4] (DOI: 10.1002/jms.4087) under the Creative Commons Attribution Licence (CC-BY) (<http://creativecommons.org/licenses/by/4.0/>).

et al. [8] on liquid microjunction surface sampling probes (LMJ-SSP). Other LESA robotic systems have been developed, enabling online chromatographic separation, laser-guided sampling probe height and more rapid sampling [18,19]. Since LESA is a commercially available, automatable technique based on the robustness of ESI, and requires little to no sample preparation, it presents the most accessible *in situ* analysis technique considered here.

Typically, LESA is performed by dispensing solvent from a pipette tip to form a liquid junction between the tip and the sample surface, allowing analytes to dissolve. A variant of the technique is contact LESA [20], in which the pipette tip maintains contact with the sample surface during extraction, confining the extraction solvent and sampling a smaller area. Contact LESA enables improved spatially-specific sampling since an area $\sim 400\ \mu\text{m}$ in diameter is sampled, compared with $\sim 1\ \text{mm}$ for LESA. After a defined period (seconds), the solvent is aspirated back into the pipette tip, which is then moved to the mass spectrometer inlet for nanoESI. Direct coupling to high-performance liquid chromatography for analyte separation and concentration prior to mass analysis is also possible and can be performed with commercial systems [18,21]. Recent bottom-up and top-down proteomics studies have been performed in this way and provide complementary data to conventional MSI experiments, with higher spatial resolution ($\sim 110\ \mu\text{m}$) recently achieved with the microLESA setup [22–24]. Since the spatial resolution is largely governed by the available compatible pipette tips, modified systems demonstrating LESA at smaller sampling areas may be developed in the future.

LESA has been demonstrated for protein analysis from a range of biological substrates. Initial work on LESA MS of dried blood spots revealed its potential for direct detection of variants of haemoglobin [25]. Protein extraction and analysis by LESA MS using denaturing solvent conditions enabled the distinction between multiple Gram-positive and Gram-negative bacteria by direct sampling of the colonies [26]. In that work, top-down MS/MS identification was performed for 40 proteins. Further analysis of dried blood spots has been performed using bottom-up [27] and top-down [28] proteomics workflows. Top-down analysis of blood spots under non-denaturing ‘native’ conditions allowed the detection of heme-bound haemoglobin complexes, most interestingly the heterotetramer, $(\alpha\beta^{2\text{H}})_2$ [29]. Several studies have used LESA for intact protein analysis directly from thin tissue sections. Smaller proteins are often reported; analysis of mouse tissues resulted in the detection of proteins up to $\sim 20\ \text{kDa}$, including calmodulin ($16.8\ \text{kDa}$) and α -globin ($15.0\ \text{kDa}$) identified by LESA tandem mass spectrometry (see the section below) [30]. Different proteins may be detected based on tissue storage protocol, as evidenced by the analysis of heat-treated versus fresh frozen tissue [31].

An inherent challenge of *in situ* analysis is the complexity of the resulting sample. To address that challenge, LESA has been coupled to high-field asymmetric waveform ion mobility spectrometry (FAIMS) [32]. FAIMS can improve the signal-to-noise ratio for protein ion signals by filtering out interfering signals arising from other extracted analytes from both tissue [30,31,33] and bacterial colonies [33,34]. Travelling wave ion mobility spectrometry (TWIMS) [35] has been combined with LESA MS [36] to determine the 3D shape of protein ions (in the form of collision cross sections) but its utility as a FAIMS-like filter has yet to be explored. Other ion mobility separation techniques, for example, trapped ion mobility spectrometry (TIMS) [37], may offer similar advantages. A recent study also investigated the ability to derive quantitative measurements of protein concentration directly from tissue during LESA MS experiments [38].

In native MS, information on the solution-phase state of proteins is inferred by the analysis of protein ions in the gas phase [39]. Native MS is achieved by the use of non-denaturing, physiologically mimicking solvent systems, soft ionisation and desolvation conditions and careful optimisation of instrument voltages. Effective desolvation is key for achieving accurate mass measurements and charge state determination [40]. Native MS under optimised conditions preserves protein quaternary structure, the result of delicate non-covalent interactions, allowing the analysis of folded proteins and protein–ligand complexes [40–42]. Native MS is almost universally performed by nanoESI, although other ion sources have been investigated [43–45], and can be combined with LESA by use of an appropriate solvent system. Native LESA MS was demonstrated for the analysis of purified protein complexes exceeding $800\ \text{kDa}$ from a glass substrate [46]. Analysis from tissue enabled small, intact proteins to be detected as well as the $64\ \text{kDa}$ tetrameric haemoglobin complex [36]. Native LESA MS was recently demonstrated for analysis of proteins and complexes from thin tissue sections on a high mass range Orbitrap mass spectrometer [47]. Proteins exceeding $70\ \text{kDa}$ were detected. In that work, a LESA extraction solvent containing the detergent C8E4, often used for native MS of membrane proteins [48], was also considered and proteins up to $47\ \text{kDa}$ were detected. The combination of detergent and high mass range MS resulted in richly populated mass spectra, including peaks corresponding to protein complexes such as the $42.6\ \text{kDa}$ reactive intermediate deiminase A homotrimer.

Flow-based techniques

Nano-DESI (Figure 1B) is a continuous flow-based technique that has been reported for high spatial resolution (<20 μm) surface sampling of biological samples [49]. Nano-DESI may be automated, and requires no substantial sample preparation; however, the best performance of the nano-DESI probe has been shown to require technical skill and supplementary apparatus and there is currently no commercial implementation [50].

Despite its name, nano-DESI is more similar to LESA than DESI in terms of analyte extraction mechanism, but differs in that the solvent flows continuously. A liquid microjunction is formed between two fused silica capillaries and the sample surface. Solvent flows from the incident capillary, the material is dissolved from the surface and the sample flows through the second capillary to the mass spectrometer inlet to be ionised by nanoESI. To effectively maintain the liquid microjunction, incremental modifications have been made to nano-DESI ion sources since its inception. These modifications include automatic readjustment of the *z*-axis of the stage for samples with complex topography [49,50,51], and have been demonstrated to provide consistent spatial resolution of up to 10 μm over a period of many hours. This spatial resolution is in the realm of all but the most sophisticated MALDI imaging experiments but without the requirement for matrix deposition [52]. Much of nano-DESI research has focused on lipid analysis, and the highest spatial resolution values are reported for such analytes; however, nano-DESI was combined with light microscopy to detect denatured proteins including haemoglobin, ubiquitin and β -thymosin 4 [53]. The same experimental setup was recently used to profile proteins and peptides in leech tissue [54]. Nano-DESI MSI of proteins up to 15 kDa in healthy and tumour-ridden regions of the mouse brain (spatial resolution $\sim 200\ \mu\text{m}$) has also been reported [55]. To date, nano-DESI has not been reported for native protein analysis.

The FlowProbe (Figure 1C, Prosolia, Indianapolis, IN) [56,57] is a commercial system taking advantage of continuous solvent flow and liquid microjunction formation between the probe tip and sample surface. Protein analysis directly from rat brain sections resulted in the detection of 84 multiply charged ion signals, attributable to proteins [58]. FAIMS was used to improve the signal to noise ratio for protein signals. Protein MSI was also performed on mouse brain, allowing detection of small, abundant proteins like ubiquitin (8.6 kDa) directly from tissue [59]. The authors concluded that while raster sampling provided spatial resolution down to $50\ \mu\text{m} \times 600\ \mu\text{m}$, this was to the detriment of ion image accuracy. It has also been noted that the polyimide coating of the probe capillaries is subject to swelling as a result of the acetonitrile which constitutes a major component of the most effective denaturing protein sampling solvent blends [59].

Remote, liquid droplet-based sampling has also been demonstrated in the form of the MasSpec Pen. This device is currently designed with sampling location diameters of 1.5–5.0 mm [9]. Negative ion mode analysis of human breast tissue sections enabled the detection of β -thymosin 4 (4.96 kDa) but larger proteins have yet to be reported [9]. Another study [60] focusing on the analysis of intact proteins directly from cell suspensions incorporated online electroporation and electrophoresis into a nanoESI-like setup to release proteins for analysis. This approach enabled the analysis of proteins up to 44 kDa. Electroporation/electrophoresis could be applied to other ion sources discussed above. Another ion source, the open port sampling interface (OPSI) [61] has been demonstrated for recombinant protein standard analysis.

Desorption techniques

DESI is now routinely applied to the analysis of lipids and metabolites, with recent studies investigating lipidosis within rodent lungs [62], and lipidomic and metabolic profiling of kidney cancers [63,64]. Few studies have focused on the analysis of intact proteins by DESI (Figure 1D) suggesting it remains a considerable challenge. DESI is a relatively mature technique for *in situ* analysis of lipids and the intellectual property rights were recently acquired by Waters Corporation (<https://waterscorporation.gcs-web.com/news-releases/news-release-details/waters-bolsters-mass-spectrometry-imaging-portfolio-acquisition>), suggesting continued commercial interest in its development. DESI currently requires a level of expertise to operate effectively as there are many parameters to optimise, particularly for *in situ* protein analysis, with only a few examples published to date.

Optimised DESI conditions taking advantage of TWIMS and providing spatial resolution of $\sim 150\ \mu\text{m}$ for protein analysis were reported recently [65]. TWIMS enabled the separation of the more intense lipid signals from the lower abundance, isobaric protein signals, an approach that will likely be important for many direct tissue analysis implementations. Similarly, the combination of DESI and FAIMS enabled the detection of protein signals from mouse (brain, kidney) and human (ovarian, breast) tissues on Orbitrap mass spectrometers [66]. Ultraviolet photodissociation (UVPD) and collision-induced dissociation (CID) were used for top-down

identifications directly by DESI MS. Interestingly, the optimised conditions for both DESI studies report a heated mass spectrometer inlet, but differ in the reported optimal DESI spray voltage (2.5–5.0 kV [65] and 1 kV [66]). Serine dissolved in the DESI solvent reportedly improved protein desalting when analysing standards deposited on a surface [67]. It has also been reported that humidity affects spray stability in negative mode [68]. Clearly, DESI requires further investigation to develop our understanding for protein analysis and this is certainly a barrier to its adoption in routine or high-throughput analysis environments. One study has reported DESI for native protein analysis of purified proteins, successfully ionising large non-covalent complexes such as GroEL_{14-mer} [69]. Insoluble membrane proteins were also analysed by the addition of detergents to the DESI solvent system. Currently, native DESI has not been performed for *in situ* analysis of proteins in tissue.

Tu and Muddiman [70] recently reported that infrared matrix-assisted laser desorption/ionisation (IR-MALDESI) was suitable for intact protein complex analysis. The technique uses an infrared laser to desorb material, rather than a solvent spray, with electrospray post-ionisation. While not demonstrated for *in situ* analysis, IR-MALDESI of holo-myoglobin was demonstrated. IR-MALDESI is already used as an MSI ion source and makes use of water as a matrix, so the application to *in situ* analysis is a logical next step.

Analysis of material by laser ablation/droplet capture was demonstrated in a spatially resolved proteomics strategy [71]. When combined with a nanoPOTS workflow, hundreds of proteins were identified from 50 µm diameter laser-dissected tissue samples; however, the workflow inherently relies on enzymatic digestion and liquid chromatography separation to achieve these numbers.

Intact protein identification

Intact protein analysis is dependent on a top-down approach to protein identification. Tandem mass spectrometry (MS/MS) techniques may be used individually, or in combination, to fragment intact protein ions within the mass spectrometer. The fragment ions provide sequence information for the protein [72]. The most commonly available MS/MS method is CID. Protein ions are collided with a neutral gas, typically nitrogen or argon. The energy from these collisions is redistributed throughout the ion and ultimately results in peptide bond cleavage throughout the protein backbone. While it is generally less efficient for larger proteins, CID produces extremely predictable fragmentation patterns predominantly featuring b and y ions (see Figure 2) [72,73]. A variant of CID found on Orbitrap mass spectrometers is higher-energy collision dissociation (HCD) [74]. HCD produces similar fragment ion spectra to CID. *In situ* top-down identification of proteins has been reported for FlowProbe and CID [58], nano-DESI with CID [54], DESI with CID [66] and LESA with CID [26,75] and HCD [34].

Electron-mediated techniques such as electron transfer dissociation (ETD) [76] and electron capture dissociation (ECD) [77] produce fragment ion spectra featuring c and z ions (see Figure 2), resulting from fragmentation of N-Cα bonds in the polypeptide chain. Since ETD and ECD are reduction processes [78], multiply charged precursor ions are a necessity and this is an advantage if using an ion source that produces multiply charged ions. For large proteins, ETD and ECD tend to produce greater sequence coverage than solely collision-based techniques since fragmentation occurs randomly throughout the polypeptide chain; this is particularly apparent with supplemental activation [79]. Importantly, these techniques are able to retain labile PTMs such as phosphorylation and glycosylation [80–83]. ETD and ECD are also able to cleave bonds in the

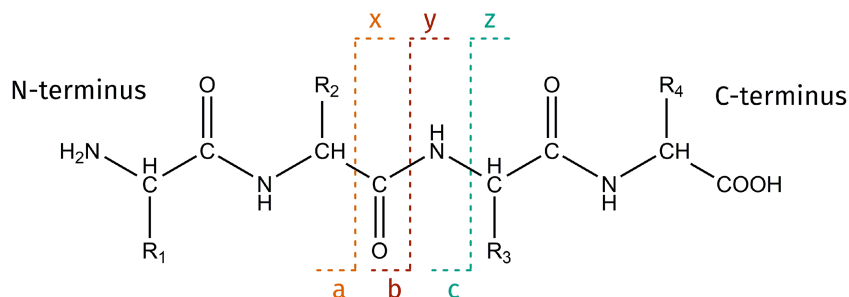


Figure 2. Annotation of peptide fragment ions according to [73].

The a/b/c ions are N-terminal fragments, while x/y/z fragments are C-terminal. CID and HCD predominantly result in b and y ions; electron-mediated techniques, c and z ions; and UVPD, a complex mixture of a/b/c, x/y/z.

protein backbone without dissociation of non-covalent interactions, e.g. salt bridges, in native MS [84,85]. The electron-mediated technique, electron ionisation dissociation (EID) also produces a wealth of a/b/c and x/y/z fragment ions from intact proteins [86]. LESA coupled to liquid chromatography separation and top-down ETD MS/MS has been used to provide protein identification information to complement separately collected high-resolution MALDI imaging data from mouse pup sections.[23] LESA ETD MS/MS also enabled the identification of two variants of fatty acid-binding protein in human liver [75].

UVPD involves the absorbance of UV photons by protein ions, causing a transition to an excited electronic state and subsequent fragmentation. UVPD spectra are richly populated with a/b/c and x/y/z ions due to fragmentation occurring throughout the polypeptide chain. UVPD is still a relatively niche MS/MS method, with limited commercial availability, but research continues to demonstrate its utility for intact protein analysis [66,87,88]. Currently, only DESI has been demonstrated for *in situ* protein sequencing by UVPD [66].

Manual interpretation of top-down MS/MS data is an intensive process and is thus ideally performed by bioinformatics tools capable of automatic, unsupervised identification. The intact mass of the protein is usually determined by deconvolution of the charge state distribution of the multiply charged precursor ions, or by direct calculation if an accurate mass and charge state can be determined. Assuming MS/MS spectra of sufficient quality are obtainable by fragmenting the precursor ions, automatic peak detection, accurate mass measurement and charge state assignment are powerful for unambiguous identification. Many of the same considerations as bottom-up proteomics database searching also apply to top-down searches; e.g. keeping the database focused to the appropriate proteomes to minimise search time, applying scoring to assess identification probability. Top-down MS/MS must also consider labile PTMs and bound ligands (under native conditions). Tools designed for top-down MS/MS interpretation and database searching include ProSightPC [89] (now marketed by Thermo Scientific), TopPIC [90], SpectroGene [91], MSPathFinder [92], pTop [93] and TopMG [94]. ProSightPC has been integrated with Proteome Discoverer (Thermo Scientific) and considering the most recent Orbitrap MS instrumental developments bring improvements to intact protein analysis, indicates substantial commercial interest in developing top-down MS/MS into a routine procedure.

Perspectives

- *In situ* analysis of intact proteins offers the opportunity for direct analysis of proteins with reduced sample preparation compared with LC-MS/MS methods. Since spatial information may also be retained, it is possible to assign location, conformation and mass from a single experiment, i.e. gain a wealth of information for the investigation of protein function.
- The challenge of protein dynamic range within cells remains considerable for direct analysis techniques and suggests why many of the studies accounted for here report the same proteins. *In situ* protein analysis must progress beyond these to become a more useful tool. Ion mobility separation has already demonstrated its value for improving the numbers of detected proteins and will undoubtedly continue to be useful as the field progresses.
- Much of the potential value of intact protein mass spectrometry lies in the analysis of large protein complexes and low abundance species to aid our understanding of diseases and their treatments. Further development of the current tools and methods, and perhaps even entirely novel techniques, will be needed to achieve *in situ* analysis of these species and realise that goal.

Abbreviations

CID, collision-induced dissociation; DESI, desorption electrospray ionisation; ECD, electron capture dissociation; EID, electron ionisation dissociation; ESI, electrospray ionisation; ETD, electron transfer dissociation; FAIMS, high-field asymmetric waveform ion mobility spectrometry; HCD, higher-energy collisional dissociation; IMS, ion mobility spectrometry; IRMPD, infrared multiphoton dissociation; LESA, liquid extraction surface analysis; LMJ-SS, liquid microjunction-surface analysis; *m/z*, mass-to-charge ratio; MALDI, matrix-assisted laser

desorption/ionisation; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MSI, mass spectrometry imaging; nano-DESI, nanospray desorption electrospray ionisation; nanoESI, nanoelectrospray ionisation; OPSI, open port sampling interface; TIMS, trapped ion mobility spectrometry; TWIMS, travelling wave ion mobility spectrometry; UVPD, ultraviolet photodissociation.

Author Contributions

O.J.H. and H.J.C. wrote the manuscript.

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

References

- Addie, R.D., Balluff, B., Bovee, J.V., Morreau, H. and McDonnell, L.A. (2015) Current state and future challenges of mass spectrometry imaging for clinical research. *Anal. Chem.* **87**, 6426–6433 <https://doi.org/10.1021/acs.analchem.5b00416>
- Ryan, D.J., Spraggins, J.M. and Caprioli, R.M. (2019) Protein identification strategies in MALDI imaging mass spectrometry: a brief review. *Curr. Opin. Chem. Biol.* **48**, 64–72 <https://doi.org/10.1016/j.cbpa.2018.10.023>
- Yates, J.R., Ruse, C.I. and Nakorchevsky, A. (2009) Proteomics by mass spectrometry: approaches, advances, and applications. *Annu. Rev. Biomed. Eng.* **11**, 49–79 <https://doi.org/10.1146/annurev-bioeng-061008-124934>
- Kocurek, K.I., Griffiths, R.L. and Cooper, H.J. (2018) Ambient ionisation mass spectrometry for in situ analysis of intact proteins. *J. Mass Spectrom.* **53**, 565–578 <https://doi.org/10.1002/jms.4087>
- Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. and Whitehouse, C.M. (1989) Electrospray ionization for mass spectrometry of large biomolecules. *Science* **246**, 64–71 <https://doi.org/10.1126/science.2675315>
- Mann, M. (2019) The ever expanding scope of electrospray mass spectrometry—a 30 year journey. *Nat. Commun.* **10**, 3744 <https://doi.org/10.1038/s41467-019-11747-z>
- Wilm, M. and Mann, M. (1996) Analytical properties of the nanoelectrospray ion source. *Anal. Chem.* **68**, 1–8 <https://doi.org/10.1021/ac9509519>
- Kertesz, V. and Van Berkel, G.J. (2010) Fully automated liquid extraction-based surface sampling and ionization using a chip-based robotic nanoelectrospray platform. *J. Mass Spectrom.* **45**, 252–260 <https://doi.org/10.1002/jms.1709>
- Zhang, J., Rector, J., Lin, J.Q., Young, J.H., Sans, M., Katta, N. et al. (2017) Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. *Sci. Transl. Med.* **9**, eaan3968 <https://doi.org/10.1126/scitranslmed.aan3968>
- Lopez-Clavijo, A.F., Griffiths, R.L., Goodwin, R.J.A. and Cooper, H.J. (2018) Liquid extraction surface analysis (LESA) electron-induced dissociation and collision-induced dissociation mass spectrometry of small molecule drug compounds. *J. Am. Soc. Mass Spectrom.* **29**, 2218–2226 <https://doi.org/10.1007/s13361-018-2042-7>
- Swales, J.G., Tucker, J.W., Spreadborough, M.J., Iverson, S.L., Clench, M.R., Webborn, P.J. et al. (2015) Mapping drug distribution in brain tissue using liquid extraction surface analysis mass spectrometry imaging. *Anal. Chem.* **87**, 10146–10152 <https://doi.org/10.1021/acs.analchem.5b02998>
- Zong, L., Pi, Z., Liu, S., Xing, J., Liu, Z. and Song, F. (2018) Liquid extraction surface analysis nanospray electrospray ionization based lipidomics for in situ analysis of tumor cells with multidrug resistance. *Rapid Commun. Mass Spectrom.* **32**, 1683–1692 <https://doi.org/10.1002/rcm.8229>
- Burnum-Johnson, K.E., Baker, E.S. and Metz, T.O. (2017) Characterizing the lipid and metabolite changes associated with placental function and pregnancy complications using ion mobility spectrometry-mass spectrometry and mass spectrometry imaging. *Placenta* **60**, S67–S72 <https://doi.org/10.1016/j.placenta.2017.03.016>
- Yin, R., Kyle, J., Burnum-Johnson, K., Bloodsworth, K.J., Sussel, L., Ansong, C. et al. (2018) High spatial resolution imaging of mouse pancreatic islets using nanospray desorption electrospray ionization mass spectrometry. *Anal. Chem.* **90**, 6548–6555 <https://doi.org/10.1021/acs.analchem.8b00161>
- Bergman, H.M., Lindfors, L., Palm, F., Kihlberg, J. and Lanehoff, I. (2019) Metabolite aberrations in early diabetes detected in rat kidney using mass spectrometry imaging. *Anal. Bioanal. Chem.* **411**, 2809–2816 <https://doi.org/10.1007/s00216-019-01721-5>
- Olson, M.T., Baxi, A., ElNaggar, M., Umbricht, C., Yergey, A.L. and Clarke, W. (2016) Morphologically compatible mass spectrometric analysis of lipids in cytological specimens. *J. Am. Soc. Cytopathol.* **5**, 3–8 <https://doi.org/10.1016/j.jasc.2015.10.001>
- Sans, M., Zhang, J., Lin, J.Q., Feider, C.L., Giese, N., Breen, M.T. et al. (2019) Performance of the MasSpec Pen for rapid diagnosis of ovarian cancer. *Clin. Chem.* **65**, 674–683 <https://doi.org/10.1373/clinchem.2018.299289>
- Oberlies, N.H., Knowles, S.L., Amrine, C.S.M., Kao, D., Kertesz, V. and Raja, H.A. (2019) Droplet probe: coupling chromatography to the in situ evaluation of the chemistry of nature. *Nat. Prod. Rep.* **36**, 944–959 <https://doi.org/10.1039/C9NP00019D>
- Karki, S., Meher, A.K., Inutan, E.D., Pophristic, M., Marshall, D.D., Rackers, K. et al. (2019) Development of a robotics platform for automated multi-ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 1–11 <https://doi.org/10.1002/rcm.8449>

- 20 Randall, E.C., Bunch, J. and Cooper, H.J. (2014) Direct analysis of intact proteins from *Escherichia coli* colonies by liquid extraction surface analysis mass spectrometry. *Anal. Chem.* **86**, 10504–10510 <https://doi.org/10.1021/ac503349d>
- 21 Van Berkel, G.J. and Kertesz, V. (2013) Continuous-flow liquid microjunction surface sampling probe connected on-line with high-performance liquid chromatography/mass spectrometry for spatially resolved analysis of small molecules and proteins. *Rapid Commun. Mass Spectrom.* **27**, 1329–1334 <https://doi.org/10.1002/rcm.6580>
- 22 Lamont, L., Baumert, M., Ogrinc Potocnik, N., Allen, M., Vreeken, R., Heeren, R.M.A. et al. (2017) Integration of ion mobility MS(E) after fully automated, online, high-resolution liquid extraction surface analysis micro-liquid chromatography. *Anal. Chem.* **89**, 11143–11150 <https://doi.org/10.1021/acs.analchem.7b03512>
- 23 Ryan, D.J., Nei, D., Prentice, B.M., Rose, K.L., Caprioli, R.M. and Spraggins, J.M. (2018) Protein identification in imaging mass spectrometry through spatially targeted liquid micro-extractions. *Rapid Commun. Mass Spectrom.* **32**, 442–450 <https://doi.org/10.1002/rcm.8042>
- 24 Ryan, D.J., Patterson, N.H., Putnam, N.E., Wilde, A.D., Weiss, A., Perry, W.J. et al. (2019) MicroLESA: integrating autofluorescence microscopy, in situ micro-digestions, and liquid extraction surface analysis for high spatial resolution targeted proteomic studies. *Anal. Chem.* **91**, 7578–7585 <https://doi.org/10.1021/acs.analchem.8b05889>
- 25 Edwards, R.L., Griffiths, P., Bunch, J. and Cooper, H.J. (2014) Compound heterozygotes and beta-thalassemia: top-down mass spectrometry for detection of hemoglobinopathies. *Proteomics* **14**, 1232–1238 <https://doi.org/10.1002/pmic.201300316>
- 26 Kocurek, K.I., Stones, L., Bunch, J., May, R.C. and Cooper, H.J. (2017) Top-down LESEA mass spectrometry protein analysis of gram-positive and gram-negative bacteria. *J. Am. Soc. Mass Spectrom.* **28**, 2066–2077 <https://doi.org/10.1007/s13361-017-1718-8>
- 27 Rosting, C., Yu, J. and Cooper, H.J. (2018) High field asymmetric waveform ion mobility spectrometry in nontargeted bottom-up proteomics of dried blood spots. *J. Proteome Res.* **17**, 1997–2004 <https://doi.org/10.1021/acs.jproteome.7b00746>
- 28 Griffiths, R.L., Dexter, A., Creese, A.J. and Cooper, H.J. (2015) Liquid extraction surface analysis field asymmetric waveform ion mobility spectrometry mass spectrometry for the analysis of dried blood spots. *Analyst* **140**, 6879–6885 <https://doi.org/10.1039/C5AN00933B>
- 29 Martin, N.J., Griffiths, R.L., Edwards, R.L. and Cooper, H.J. (2015) Native liquid extraction surface analysis mass spectrometry: analysis of noncovalent protein complexes directly from dried substrates. *J. Am. Soc. Mass Spectrom.* **26**, 1320–1327 <https://doi.org/10.1007/s13361-015-1152-8>
- 30 Griffiths, R.L., Creese, A.J., Race, A.M., Bunch, J. and Cooper, H.J. (2016) LESEA FAIMS mass spectrometry for the spatial profiling of proteins from tissue. *Anal. Chem.* **88**, 6758–6766 <https://doi.org/10.1021/acs.analchem.6b01060>
- 31 Griffiths, R.L., Simmonds, A.L., Swales, J.G., Goodwin, R.J.A. and Cooper, H.J. (2018) LESEA MS imaging of heat-preserved and frozen tissue: benefits of multistep static FAIMS. *Anal. Chem.* **90**, 13306–13314 <https://doi.org/10.1021/acs.analchem.8b02739>
- 32 Buryakov, I.A., Krylov, E.V., Nazarov, E.G. and Rasulev, U.K. (1993) A new method of separation of multi-atomic ions by mobility at atmospheric-pressure using a high-frequency amplitude-asymmetric strong electric-field. *Int. J. Mass Spectrom. Ion Process.* **128**, 143–148 [https://doi.org/10.1016/0168-1176\(93\)87062-W](https://doi.org/10.1016/0168-1176(93)87062-W)
- 33 Sarsby, J., Griffiths, R.L., Race, A.M., Bunch, J., Randall, E.C., Creese, A.J. et al. (2015) Liquid extraction surface analysis mass spectrometry coupled with field asymmetric waveform ion mobility spectrometry for analysis of intact proteins from biological substrates. *Anal. Chem.* **87**, 6794–6800 <https://doi.org/10.1021/acs.analchem.5b01151>
- 34 Kocurek, K.I., May, R.C. and Cooper, H.J. (2019) Application of high-field asymmetric waveform ion mobility separation to LESEA mass spectrometry of bacteria. *Anal. Chem.* **91**, 4755–4761 <https://doi.org/10.1021/acs.analchem.9b00307>
- 35 Giles, K., Pringle, S.D., Worthington, K.R., Little, D., Wildgoose, J.L. and Bateman, R.H. (2004) Applications of a travelling wave-based radio-frequency-only stacked ring ion guide. *Rapid Commun. Mass Spectrom.* **18**, 2401–2414 <https://doi.org/10.1002/rcm.1641>
- 36 Griffiths, R.L., Sisley, E.K., Lopez-Clavijo, A.F., Simmonds, A.L., Styles, I.B. and Cooper, H.J. (2019) Native mass spectrometry imaging of intact proteins and protein complexes in thin tissue sections. *Int. J. Mass Spectrom.* **437**, 23–29 <https://doi.org/10.1016/j.ijms.2017.10.009>
- 37 Fernandez-Lima, F., Kaplan, D.A., Suetering, J. and Park, M.A. (2011) Gas-phase separation using a trapped ion mobility spectrometer. *Int. J. Ion Mobil. Spectrom.* **14**, 93–98 <https://doi.org/10.1007/s12127-011-0067-8>
- 38 Havlikova, J., Randall, E.C., Griffiths, R.L., Swales, J.G., Goodwin, R.J.A., Bunch, J. et al. (2019) Quantitative imaging of proteins in tissue by stable isotope labeled mimetic liquid extraction surface analysis mass spectrometry. *Anal. Chem.* **91**, 14198–14202 <https://doi.org/10.1021/acs.analchem.9b04148>
- 39 Leney, A.C. and Heck, A.J. (2017) Native mass spectrometry: what is in the name? *J. Am. Soc. Mass Spectrom.* **28**, 5–13 <https://doi.org/10.1007/s13361-016-1545-3>
- 40 Snijder, J., Rose, R.J., Veessler, D., Johnson, J.E. and Heck, A.J. (2013) Studying 18 MDa virus assemblies with native mass spectrometry. *Angew. Chem. Int. Ed. Engl.* **52**, 4020–4023 <https://doi.org/10.1002/anie.201210197>
- 41 Ruotolo, B.T., Benesch, J.L., Sandercock, A.M., Hyung, S.J. and Robinson, C.V. (2008) Ion mobility-mass spectrometry analysis of large protein complexes. *Nat. Protoc.* **3**, 1139–1152 <https://doi.org/10.1038/nprot.2008.78>
- 42 van Duijn, E., Barendregt, A., Synowsky, S., Versluis, C. and Heck, A.J. (2009) Chaperonin complexes monitored by ion mobility mass spectrometry. *J. Am. Chem. Soc.* **131**, 1452–1459 <https://doi.org/10.1021/ja8055134>
- 43 Beaufour, M., Ginguene, D., Le Meur, R., Castaing, B. and Cadene, M. (2018) Liquid native MALDI mass spectrometry for the detection of protein–protein complexes. *J. Am. Soc. Mass Spectrom.* **29**, 1981–1994 <https://doi.org/10.1007/s13361-018-2015-x>
- 44 Peetz, O., Hellwig, N., Henrich, E., Mezhyrova, J., Dotsch, V., Bernhard, F. et al. (2019) LILBID and nESI: different native mass spectrometry techniques as tools in structural biology. *J. Am. Soc. Mass Spectrom.* **30**, 181–191 <https://doi.org/10.1007/s13361-018-2061-4>
- 45 Hale, O.J. and Cramer, R. (2019) Atmospheric pressure ultraviolet laser desorption and ionization from liquid samples for native mass spectrometry. *Anal. Chem.* **91**, 14192–7 <https://doi.org/10.1021/acs.analchem.9b03875>
- 46 Mikhailov, V.A., Griffiths, R.L. and Cooper, H.J. (2017) Liquid extraction surface analysis for native mass spectrometry: Protein complexes and ligand binding. *Int. J. Mass Spectrom.* **420**, 43–50 <https://doi.org/10.1016/j.ijms.2016.09.011>
- 47 Griffiths, R.L., Konijnenberg, A., Viner, R. and Cooper, H.J. (2019) Direct mass spectrometry analysis of protein complexes and intact proteins up to >70 kDa from tissue. *Anal. Chem.* **91**, 6962–6966 <https://doi.org/10.1021/acs.analchem.9b00971>
- 48 Reading, E., Liko, I., Allison, T.M., Benesch, J.L., Laganowsky, A. and Robinson, C.V. (2015) The role of the detergent micelle in preserving the structure of membrane proteins in the gas phase. *Angew. Chem. Int. Ed. Engl.* **54**, 4577–4581 <https://doi.org/10.1002/anie.201411622>

- 49 Lanekoff, I., Heath, B.S., Liyu, A., Thomas, M., Carson, J.P. and Laskin, J. (2012) Automated platform for high-resolution tissue imaging using nanospray desorption electrospray ionization mass spectrometry. *Anal. Chem.* **84**, 8351–8356 <https://doi.org/10.1021/ac301909a>
- 50 Nguyen, S.N., Sontag, R.L., Carson, J.P., Corley, R.A., Ansong, C. and Laskin, J. (2018) Towards high-resolution tissue imaging using nanospray desorption electrospray ionization mass spectrometry coupled to shear force microscopy. *J. Am. Soc. Mass Spectrom.* **29**, 316–322 <https://doi.org/10.1007/s13361-017-1750-8>
- 51 Nguyen, S.N., Liyu, A.V., Chu, R.K., Anderton, C.R. and Laskin, J. (2017) Constant-distance mode nanospray desorption electrospray ionization mass spectrometry imaging of biological samples with complex topography. *Anal. Chem.* **89**, 1131–1137 <https://doi.org/10.1021/acs.analchem.6b03293>
- 52 Rompp, A. and Spengler, B. (2013) Mass spectrometry imaging with high resolution in mass and space. *Histochem. Cell Biol.* **139**, 759–783 <https://doi.org/10.1007/s00418-013-1097-6>
- 53 Hsu, C.C., White, N.M., Hayashi, M., Lin, E.C., Poon, T., Banerjee, I. et al. (2013) Microscopy ambient ionization top-down mass spectrometry reveals developmental patterning. *Proc. Natl Acad. Sci. U.S.A.* **110**, 14855–14860 <https://doi.org/10.1073/pnas.1310618110>
- 54 Hsu, C.C., Baker, M.W., Gaasterland, T., Meehan, M.J., Macagno, E.R. and Dorrestein, P.C. (2017) Top-down atmospheric ionization mass spectrometry microscopy combined with proteogenomics. *Anal. Chem.* **89**, 8251–8258 <https://doi.org/10.1021/acs.analchem.7b01096>
- 55 Hsu, C.C., Chou, P.T. and Zare, R.N. (2015) Imaging of proteins in tissue samples using nanospray desorption electrospray ionization mass spectrometry. *Anal. Chem.* **87**, 11171–5 <https://doi.org/10.1021/acs.analchem.5b03389>
- 56 Kertesz, V., Ford, M.J. and Van Berkel, G.J. (2005) Automation of a surface sampling probe/electrospray mass spectrometry system. *Anal. Chem.* **77**, 7183–7189 <https://doi.org/10.1021/ac0510742>
- 57 Van Berkel, G.J., Kertesz, V. and King, R.C. (2009) High-throughput mode liquid microjunction surface sampling probe. *Anal. Chem.* **81**, 7096–7101 <https://doi.org/10.1021/ac901098d>
- 58 Feider, C.L., Elizondo, N. and Eberlin, L.S. (2016) Ambient ionization and FAIMS mass spectrometry for enhanced imaging of multiply charged molecular ions in biological tissues. *Anal. Chem.* **88**, 11533–11541 <https://doi.org/10.1021/acs.analchem.6b02798>
- 59 Griffiths, R.L., Randall, E.C., Race, A.M., Bunch, J. and Cooper, H.J. (2017) Raster-mode continuous-flow liquid microjunction mass spectrometry imaging of proteins in thin tissue sections. *Anal. Chem.* **89**, 5683–5687 <https://doi.org/10.1021/acs.analchem.7b00977>
- 60 Li, G., Yuan, S., Zheng, S., Liu, Y. and Huang, G. (2018) In situ living cell protein analysis by single-step mass spectrometry. *Anal. Chem.* **90**, 3409–3415 <https://doi.org/10.1021/acs.analchem.7b05055>
- 61 Van Berkel, G.J., Kertesz, V. and Boeltz, H. (2017) Immediate drop on demand technology (I-DOT) coupled with mass spectrometry via an open port sampling interface. *Bioanalysis* **9**, 1667–1679 <https://doi.org/10.4155/bio-2017-0104>
- 62 Dexter, A., Steven, R.T., Patel, A., Dailey, L.A., Taylor, A.J., Ball, D. et al. (2019) Imaging drugs, metabolites and biomarkers in rodent lung: a DESI MS strategy for the evaluation of drug-induced lipidosis. *Anal. Bioanal. Chem.* **411**, 8023–8032 <https://doi.org/10.1007/s00216-019-02151-z>
- 63 Vijayalakshmi, K., Shankar, V., Bain, R.M., Nolley, R., Sonn, G.A., Kao, C.S. et al. (2019) Identification of diagnostic metabolic signatures in clear cell renal cell carcinoma using mass spectrometry imaging. *Int. J. Cancer* <https://doi.org/10.1002/ijc.32843>
- 64 Zhang, J., Li, S.Q., Lin, J.Q. and Yu W. L.S.E. (2019) Mass spectrometry imaging enables discrimination of renal oncocytoma from renal cell cancer subtypes and normal kidney tissues. *Cancer Res.* <https://doi.org/10.1158/0008-5472.CAN-19-2522>
- 65 Towers, M.W., Karancsi, T., Jones, E.A., Pringle, S.D. and 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. *J. Am. Soc. Mass Spectrom.* **29**, 2456–2466 <https://doi.org/10.1007/s13361-018-2049-0>
- 66 Garza, K.Y., Feider, C.L., Klein, D.R., Rosenberg, J.A., Brodbelt, J.S. and Eberlin, L.S. (2018) Desorption electrospray ionization mass spectrometry imaging of proteins directly from biological tissue sections. *Anal. Chem.* **90**, 7785–7789 <https://doi.org/10.1021/acs.analchem.8b00967>
- 67 Javanshad, R., Honarvar, E. and Venter, A.R. (2019) Addition of serine enhances protein analysis by DESI-MS. *J. Am. Soc. Mass Spectrom.* **30**, 694–703 <https://doi.org/10.1007/s13361-018-02129-8>
- 68 Feider, C.L., DeHoog, R.J., Sans, M., Zhang, J., Krieger, A. and Eberlin, L.S. (2019) DESI spray stability in the negative ion mode is dependent on relative humidity. *J. Am. Soc. Mass Spectrom.* **30**, 376–380 <https://doi.org/10.1007/s13361-018-2105-9>
- 69 Ambrose, S., Housden, N.G., Gupta, K., Fan, J., White, P., Yen, H.Y. et al. (2017) Native desorption electrospray ionization liberates soluble and membrane protein complexes from surfaces. *Angew. Chem. Int. Ed. Engl.* **56**, 14463–8 <https://doi.org/10.1002/anie.201704849>
- 70 Tu, A. and 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. *J. Am. Soc. Mass Spectrom.* **30**, 2380–2391 <https://doi.org/10.1007/s13361-019-02323-2>
- 71 Fatou, B., Wisztorski, M., Focsa, C., Salzet, M., Ziskind, M. and Fournier, I. (2015) Substrate-mediated laser ablation under ambient conditions for spatially-resolved tissue proteomics. *Sci. Rep.* **5**, 18135 <https://doi.org/10.1038/srep18135>
- 72 Johnson, R.S., Martin, S.A., Biemann, K., Stults, J.T. and Watson, J.T. (1987) Novel fragmentation process of peptides by collision-induced decomposition in a tandem mass spectrometer: differentiation of leucine and isoleucine. *Anal. Chem.* **59**, 2621–2625 <https://doi.org/10.1021/ac00148a019>
- 73 Roepstorff, P. and Fohlman, J. (1984) Proposal for a common nomenclature for sequence ions in mass spectra of peptides. *Biomed. Mass Spectrom.* **11**, 601 <https://doi.org/10.1002/bms.1200111109>
- 74 Olsen, J.V., Macek, B., Lange, O., Makarov, A., Horning, S. and Mann, M. (2007) Higher-energy C-trap dissociation for peptide modification analysis. *Nat. Methods* **4**, 709–712 <https://doi.org/10.1038/nmeth1060>
- 75 Sarsby, J., Martin, N.J., Lalor, P.F., Bunch, J. and Cooper, H.J. (2014) Top-down and bottom-up identification of proteins by liquid extraction surface analysis mass spectrometry of healthy and diseased human liver tissue. *J. Am. Soc. Mass Spectrom.* **25**, 1953–1961 <https://doi.org/10.1007/s13361-014-0967-z>
- 76 Syka, J.E., Coon, J.J., Schroeder, M.J., Shabanowitz, J. and Hunt, D.F. (2004) Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc. Natl Acad. Sci. U.S.A.* **101**, 9528–9533 <https://doi.org/10.1073/pnas.0402700101>
- 77 Zubarev, R.A., Kelleher, N.L. and McLafferty, F.W. (1998) Electron capture dissociation of multiply charged protein cations. A nonergodic process. *J. Am. Chem. Soc.* **120**, 3265–3266 <https://doi.org/10.1021/ja973478k>
- 78 Lermyte, F., Valkenborg, D., Loo, J.A. and Sobott, F. (2018) Radical solutions: Principles and application of electron-based dissociation in mass spectrometry-based analysis of protein structure. *Mass Spectrom. Rev.* **37**, 750–771 <https://doi.org/10.1002/mas.21560>

- 79 Riley, N.M., Westphall, M.S. and Coon, J.J. (2018) Sequencing larger intact proteins (30–70 kDa) with activated ion electron transfer dissociation. *J. Am. Soc. Mass Spectrom.* **29**, 140–149 <https://doi.org/10.1007/s13361-017-1808-7>
- 80 Sze, S.K., Ge, Y., Oh, H. and McLafferty, F.W. (2002) Top-down mass spectrometry of a 29-kDa protein for characterization of any posttranslational modification to within one residue. *Proc. Natl Acad. Sci. U.S.A.* **99**, 1774–1779 <https://doi.org/10.1073/pnas.251691898>
- 81 Chi, A., Huttenhower, C., Geer, L.Y., Coon, J.J., Syka, J.E., Bai, D.L. et al. (2007) Analysis of phosphorylation sites on proteins from *Saccharomyces cerevisiae* by electron transfer dissociation (ETD) mass spectrometry. *Proc. Natl Acad. Sci. U.S.A.* **104**, 2193–2198 <https://doi.org/10.1073/pnas.0607084104>
- 82 Schroeder, M.J., Webb, D.J., Shabanowitz, J., Horwitz, A.F. and Hunt, D.F. (2005) Methods for the detection of paxillin post-translational modifications and interacting proteins by mass spectrometry. *J. Proteome Res.* **4**, 1832–1841 <https://doi.org/10.1021/pr0502020>
- 83 Cooper, H.J., Hakansson, K. and Marshall, A.G. (2005) The role of electron capture dissociation in biomolecular analysis. *Mass Spectrom. Rev.* **24**, 201–222 <https://doi.org/10.1002/mas.20014>
- 84 Zhang, Z., Browne, S.J. and Vachet, R.W. (2014) Exploring salt bridge structures of gas-phase protein ions using multiple stages of electron transfer and collision induced dissociation. *J. Am. Soc. Mass Spectrom.* **25**, 604–613 <https://doi.org/10.1007/s13361-013-0821-8>
- 85 Xie, Y., Zhang, J., Yin, S. and Loo, J.A. (2006) Top-down ESI-ECD-FT-ICR mass spectrometry localizes noncovalent protein-ligand binding sites. *J. Am. Chem. Soc.* **128**, 14432–3 <https://doi.org/10.1021/ja063197p>
- 86 Li, H., Sheng, Y., McGee, W., Cammarata, M., Holden, D. and Loo, J.A. (2017) Structural characterization of native proteins and protein complexes by electron ionization dissociation-mass spectrometry. *Anal. Chem.* **89**, 2731–2738 <https://doi.org/10.1021/acs.analchem.6b02377>
- 87 Morrison, L.J. and Brodbelt, J.S. (2016) 193 nm ultraviolet photodissociation mass spectrometry of tetrameric protein complexes provides insight into quaternary and secondary protein topology. *J. Am. Chem. Soc.* **138**, 10849–10859 <https://doi.org/10.1021/jacs.6b03905>
- 88 Greisch, J.F., Tamara, S., Scheltema, R.A., Maxwell, H.W.R., Fagerlund, R.D., Fineran, P.C. et al. (2019) Expanding the mass range for UVPD-based native top-down mass spectrometry. *Chem Sci.* **10**, 7163–7171 <https://doi.org/10.1039/C9SC01857C>
- 89 Zamdborg, L., LeDuc, R.D., Glowacz, K.J., Kim, Y.B., Viswanathan, V., Spaulding, I.T. et al. (2007) Prosight PTM 2.0: improved protein identification and characterization for top down mass spectrometry. *Nucleic Acids Res.* **35**, W701–W706 <https://doi.org/10.1093/nar/gkm371>
- 90 Kou, Q., Xun, L. and Liu, X. (2016) TopPIC: a software tool for top-down mass spectrometry-based proteoform identification and characterization. *Bioinformatics* **32**, 3495–3497 <https://doi.org/10.1093/bioinformatics/btw398>
- 91 Kolmogorov, M., Liu, X. and Pevzner, P.A. (2016) SpectroGene: a tool for proteogenomic annotations using top-down spectra. *J. Proteome Res.* **15**, 144–151 <https://doi.org/10.1021/acs.jproteome.5b00610>
- 92 Park, J., Piehowski, P.D., Wilkins, C., Zhou, M., Mendoza, J., Fujimoto, G.M. et al. (2017) Informed-proteomics: open-source software package for top-down proteomics. *Nat. Methods* **14**, 909–914 <https://doi.org/10.1038/nmeth.4388>
- 93 Sun, R.X., Luo, L., Wu, L., Wang, R.M., Zeng, W.F., Chi, H. et al. (2016) Ptop 1.0: a high-accuracy and high-efficiency search engine for intact protein identification. *Anal. Chem.* **88**, 3082–3090 <https://doi.org/10.1021/acs.analchem.5b03963>
- 94 Kou, Q., Wu, S., Tolic, N., Pasa-Tolic, L., Liu, Y. and Liu, X. (2017) A mass graph-based approach for the identification of modified proteoforms using top-down tandem mass spectra. *Bioinformatics* **33**, 1309–1316 <https://doi.org/10.1093/bioinformatics/btw806>