Bioanalytic applications of mass spectrometry

Catherine E Costello

Mass spectrometry has suddenly expanded out of research and assay laboratories into biology, medicine and therapeutics. Electrospray ionization and matrix-assisted laser desorption/ionization yield increased mass-range and sensitivity, leading to novel applications and sparking new analyzer designs, software, and robotics.

Addresses
Mass Spectrometry Resource, Departments of Biochemistry and Biophysics, Boston University School of Medicine, 715 Albany Street, R-806, Boston, MA 02118-2526, USA; e-mail: cecmsms@bu.edu

Current Opinion in Biotechnology 1999, **10**:22–28

http://biomednet.com/elecref/0958166901000022

 ISSN 0958-1669

Abbreviations

CID	collision-induced decomposition
ESI	electrospray ionization
FT-ICR	Fourier-transform ion cyclotron resonance
HPLC	high performance LC
LC	liquid chromatography
MALDI	matrix-assisted laser desorption/ionization
MS	mass spectrometry
MS^n	tandem mass spectrometry
PSD	post-source decay
SORI	sustained off-resonance irradiation
TOF	time-of-flight

Introduction

In recent years, mass spectrometry (MS) has moved at an extraordinary pace, expanding out of the realm of physics and chemical research and applications for routine tests in clinical, industrial and regulatory assay laboratories and into the worlds of biology, medicine and therapeutics [1••]. Two developments fueled this transition: electrospray ionization (ESI) [2] and matrix-assisted laser desorption/ionization (MALDI) [3]. Suddenly, the mass range for analyses increased to well beyond 100 kDa, and the sample requirement fell below the picomole level. The capabilities and needs of these techniques have sparked new analyzer designs that deliver increased resolution and/or improved capability for multistage (tandem) mass spectrometry (MS^n), dedicated and Internet-based interactive software, and the implemention of robotics for high-throughput analyses. This review outlines the status of bioanalytical MS after this period of rapid growth and provides the reader with links to literature sources.

Applications showing strong development in the past two years include determinations of biopolymer structures and the constitution of macromolecular assemblies. Proteins have received priority but progress in other areas is also dramatic, as is growth in the understanding of interactions within and among biopolymers.

Instrumentation

MALDI employs a pulsed laser, for example, a nitrogen laser operating in the ultraviolet wavelength range (337 nm) or an Er-YAG laser operating in the infrared range (2.94 μm). The plume of desorbed ions is compatible with a time-of-flight (TOF) analyzer, but the energy spread among the ions leads to broad peaks and thus limits both resolution and mass assignment accuracy. To counter this problem by allowing equilibration of the energy distribution until it falls within a narrow range prior to ion acceleration, TOF instruments now use delayed extraction (DE) [4]. The TOF analyzer is relatively inexpensive and simple to operate; with DE, its mass resolution exceeds 1:10,000.

MALDI imparts little excess energy, but some molecular ions do undergo metastable decomposition during transit through the analyzer. To gain structural details, for example, peptide amino acid sequences, this fragmentation is recorded by operation of a reflection TOF analyzer in the post-source decay (PSD) mode [5••]. In this mode, a precursor molecular ion is selected and the transmission of the analyzer is adjusted in steps that allow its fragment ions (whose lower energies correspond to the same fraction of the original mass that each retains) to reach the detector. Assembly of the data recorded at several steps produces a PSD spectrum that displays the full set of fragment ions. PSD precursor ion selection has ~1:100 resolution.

For ESI MS analysis [6••], analyzer developments have included introduction of the Q-TOF (Micromass, Inc.), an instrument that combines a two-stage quadrupole with a reflectron TOF (current price >$500,000). In the MS/MS mode, the transmission of the Q-TOF is 10–100 times that of triple quadrupole instruments; the Q-TOF measures femtomole samples with ≥0.1 Da accuracy. Other vendors have introduced single-stage ESI-TOF instruments for under $200,000; it is expected that competition will soon lower the price for the hybrid instruments. Quadrupole ion traps have growing importance, in part because they easily perform multistage mass spectral (MS^n) analysis; they also can cost <$200,000.

For analyses of complex mixtures where high resolution (≥300,000) spectra, isotope-resolved precursor ion selection and accurate (low ppm) mass assignments in the MS^n mode are necessary, the Fourier-transform ion cyclotron resonance (FT-ICR) method excels in resolution, mass accuracy and ion control. It is, therefore, moving rapidly into the applications area [7]. FT-ICR MS prices begin around $400,000 and increase with the number of options and magnetic field strength, and may exceed $1M.

Sample introduction methods

Better and more sophisticated methods for sample introduction that are being developed include surface selection/modification for MALDI and microscale techniques for sample separation, cleanup and/or deposition. Bioactive MALDI probe tips enable a variety of experiments, from digestions with surface-bound proteases or glycosidases to investigations of affinity and higher-order structure [8•]. For peptide mapping, micro-liquid chromatography (LC) has been combined with on-line membrane blotting, followed by off-line IR-MALDI [9]. A clever approach that maximizes sample utilization combines Edman sequencing of high performance LC (HPLC) eluent fraction collected on polyvinylidine fluoride (PVDF) membranes with trapping the flow-through material on Teflon^{TM} membranes for UV-MALDI [10•].

Protein identification by a combination of MALDI-TOF MS and 2D-gels has been reviewed recently [11]. Hillenkamp and co-workers [12••] used IR-MALDI for analysis of proteins separated by 2D-PAGE and transferred to PVDF membranes. Ogorzalek-Loo *et al.* [13] produced '2D' protein analyses by UV-MALDI mass-mapping directly from thin gels, thus adding molecular weight information to the characterization based on mobility in the electric field. Others have described microelution/preconcentration methods prior to MALDI analysis to clean up subpicomole protein samples recovered from SDS-PAGE [14,15]. MALDI-TOF MS is also being used to directly image peptides and proteins in biological samples [16••].

HPLC systems were immediately combined with ESI MS. On-line LC-MS systems are even programmed to control fraction collectors [17]. With nanospray, low-flow (1–100 nL/min) systems are also compatible; these maximize sensitivity by providing the most efficient ionization of small sample amounts (fmol–pmol) eluting from capillary columns. Digestion of proteins in water containing 50% ^{18}O followed by capillary HPLC ESI MS/MS analysis can thereby provide sensitive, rapid *de novo* peptide sequencing [18••,19•].

An integrated microscale HPLC-ESI source interfaced to a quadrupole ion trap has been used to analyze peptides obtained from in-gel digestion of 50–500 fmol protein spots [20•]. Micro-ESI that incorporates capillaries packed with reversed-phase media has enabled high-sensitivity FT-ICR MS analysis of neuropeptides; the components (500 amol/μl) were detected with 1 μL/min flow rate [21•].

Aebersold and co-workers [22], having demonstrated that capillary zone electrophoresis (CZE)-micro-ESI permits tandem MS analysis at the femtomole level, have now undertaken an ambitious program that involves on-chip chromatography interfaced to ESI MS. This approach minimizes sample-handling losses and decrease solvent volumes; it should lend itself to automated operations. Karger's group [23•] has incorporated tryptic digestion into on-chip ESI MS analysis. Microdialysis has enabled on-line coupling of capillary isoelectric focusing (CIEF) with ESI on a sector instrument fitted with an array detector for selected-ion monitoring of proteins at the low and sub-picomole level [24]; in this technique, detection time is dedicated to sampling a few substance-specific m/z values and sensitivity is substantially increased over that achieved when continuous scanning over the full mass range is employed.

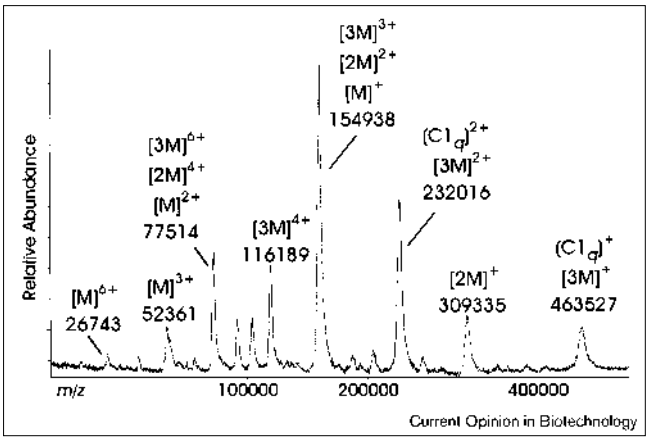
Studies of intact proteins

The new MS methods are being employed for structural determinations and purity evaluation of protein-based drugs or drug systems. Within a study aimed at developing a monoclonal antibody-based method for delivering radionuclides to cancer tissue, ESI MS has been utilized to compare wild-type recombinant streptavidin, genetically-engineered analogs and commmercial streptavidin proteins [25]. Although the proteins had similar HPLC and SDS-PAGE behavior, they could be distinguished by both isoelectric focusing (IEF), which provides high-resolution separation based on pI, and ESI MS, which provides molecular weight information. ESI MS showed that even the commercial proteins were heterogeneous, due to both amino- and carboxy-terminal truncations.

The high resolution of the FT-ICR instrument allows the charge states and masses of molecular and fragment ions to be assigned with confidence [26••]. Sustained off-resonance irradiation (SORI) collision-induced decomposition (CID) of multiply-charged protein molecular ions yields fragments that may be reassembled to provide full sequence information, a method that McLafferty has called 'top-down' sequencing [26••]. Decomposition of ions in the FT-ICR cell can also be accomplished by IR multiphoton dissociation (IRMPD) [27••], electron dissociation [28] or blackbody IR radiative dissociation (BIRD) [26••]. Protein conformations can be probed by hydrogen/deuterium (H/D) exchange studies that demonstrate the surface availability of exchangeable hydrogens [29,30]. To counter the decrease in signal-to-noise and reduced ability to resolve components of similar molecular weights that result from the broad natural isotopic distributions of large molecules, a technique that enriches the ^{12}C and ^{14}N isotopes has been introduced. Organisms grown on isotope-depleted media produce proteins with narrow isotopic distributions and thus both assignment of the molecular weight and mixture analyses are facilitated [31•].

The identification of proteins is greatly aided by access to the large databases now readily available over the Internet. Through an iterative approach, mapping of tryptic (or other) peptides obtained by digestion of proteins recovered from PAGE or other separation leads to efficient retrieval of identical (or related) proteins from the databases, even when the separated fractions contain multiple components [32••].

Figure 1



Infrared MALDI mass spectrum of C1*q* complement protein (Er-YAG laser, 2.94 μm, succinic acid matrix, Vision 2000 RTOF MS). Symbols: $(C1q)^{n+}$, $[3M]^{n+}$, various charge states of the intact complex, M_r 465 kDa; $[M]^{n+}$, hexameric subunit, M_r 155 kDa. (X Hronowski and CE Costello, unpublished data).

Post-translational modifications can be determined by MS by shifts in the molecular weight of the intact protein or peptide and by protease digestion followed by MS to locate and characterize the modified sites [33••]. During LC-MS of a digest, it is helpful to search for unique fragment ions (e.g. *m/z* 79 [PO_3^-] and 97 [$H_2PO_4^-$] that are characteristic of a phosphate group, or *m/z* 204 [internal $HexNAc^+$] and 366 [internal $HexHexNAc^+$] that signal the presence of an N-acetyl hexosamine moiety) to locate peptides that bear specific modifications. Protease digestions, in conjunction with antigen binding and MALDI or ESI and MS/MS, can locate epitopes of proteins retrieved from complex media (e.g. cell lysates [34•]), even if heavily glycosylated (e.g. HIV-gp120 envelope glycoprotein [35]).

MALDI and ESI MS methods are now widely employed for recognition and location of amino acid mutations in native and recombinant proteins and peptides. Variant forms of porcine surfactant polypeptide C have been structurally determined by nanospray ESI MS and CID MS/MS on an orthogonal acceleration TOF instrument [36]. Their heterogeneity could be assigned to truncation and elongation of the 35-residue polypeptide chain, methionine oxidation, cysteine-palmitoylation at various levels and methylation. Both in-source (cone voltage) fragmentation and CID were employed and several charge states of the molecular ions were selected as precursors before the full structural details of all components could be established.

A substantial effort in the pharmaceutical industry is presently focused on combinatorial chemistry. Evaluation of the product mix (peptides, carbohydrates, oligonucleotides, etc.) depends heavily on high-throughput MS and MS/MS and, therefore, on the design of high-capacity autosamplers, robotic manipulations and efficient data processing and analysis [37••,38•,39,40].

Protein assemblies

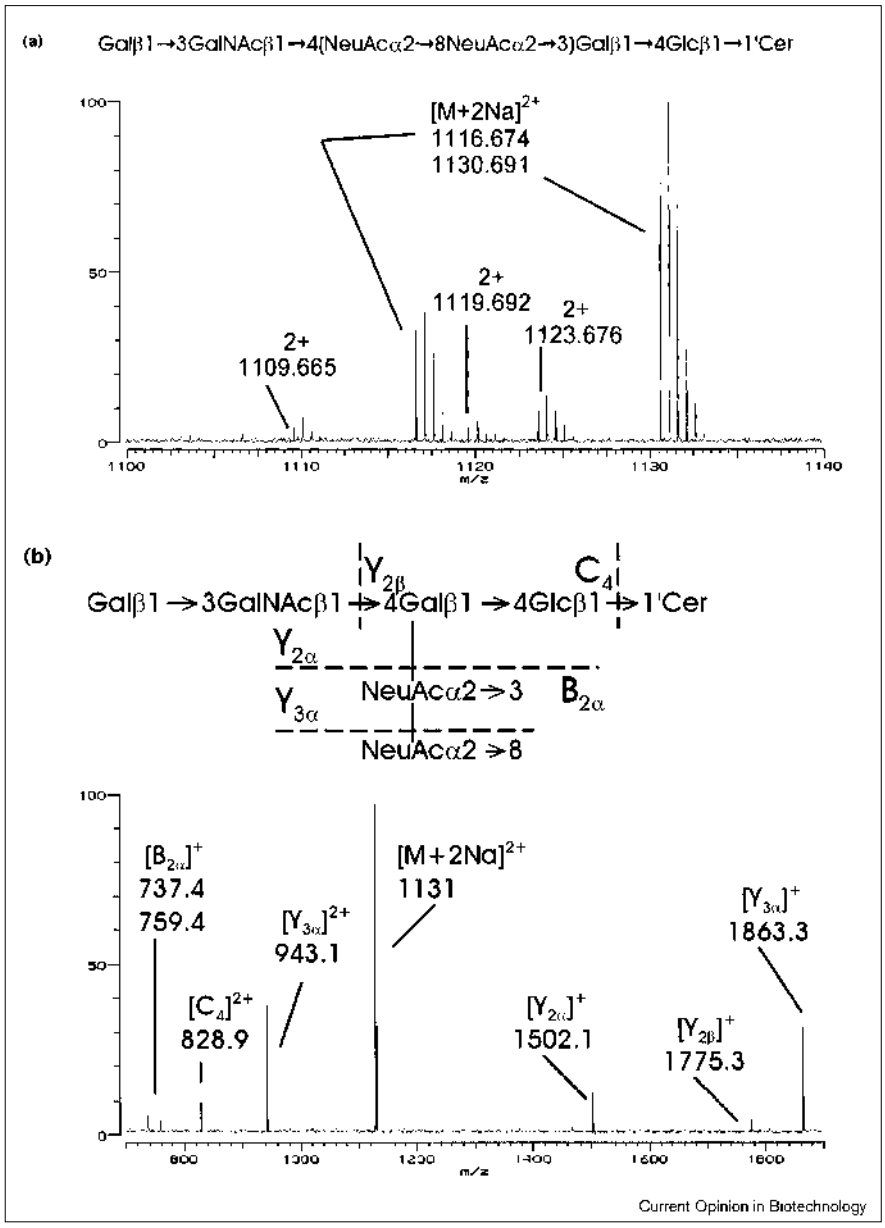
That mass spectrometric observation of noncovalent complexes is now possible with ESI [41••] is frequently reported and, despite the greater difficulty of stabilizing such complexes in the presence of high-energy irradiation and excited matrix molecules, a few investigators have also had success in this area using MALDI [42•,43•]. This opens the way to investigations of intermolecular interactions. Great care must be exercised to assure that the systems are studied under biologically relevant conditions.

Low temperature micro-ESI MS using a capillary kept at 45°C, has been used to study the calcium-dependent interaction of calmodulin with two small amphipathic peptides, calmodulin-dependent protein kinase II and mellittin [44•]. The calmodulin–peptide–metal stoichiometry could be rapidly determined as 1:1:4 for both peptides.

For the wild-type and two amyloidogenic mutants (Val30→Met and Leu55→Pro) of the 14 kDa plasma protein transthyretin, nanospray ESI MS has recently been used to assess the relative proportions of the monomeric and tetrameric forms, and to determine the influence of water- and thyroxine-binding on the tetramer stability [45•]. The amyloidogenic Val30→Met variant monomer underwent more extensive H/D exchange and thus was shown to be substantially more unfolded than wild-type protein. Such studies of protein (un)folding may help to elucidate the mechanism(s) that lead to fibril formation from transthyretin and other normally soluble proteins. Amyloid plaque formation by aberrant proteins is a characteristic of several well-known but incompletely understood neurodegenerative disorders, including Alzheimer's disease and Creutzfeld-Jakob's disease.

We are using several mass spectral approaches to investigate in detail the structures of collagens, structural proteins that are highly post-translationally modified and form extensive triple helices that may contain intra- and intermolecular crosslinks. In the course of these investigations, we have found that the triple helix domain, even when non-covalently bound, has sufficient stability to allow its observation by MALDI [46]. Other protein assemblies with collagen-like triple helix domains can also be observed. Figure 1 shows the IR MALDI-reflectron TOF mass spectrum we obtained for complement C1*q*, a noncovalent cluster of three hexameric subunits, each containing six ~26 kDa chains that are associated through a combination of disulfide bonds and noncovalent interactions [47]. The weight of the intact cluster is ~465 kDa. Several charge states of C1*q* and the hexameric subunit are observed, as indicated on the spectrum.

Figure 2



ESI FT-ICR mass spectra of permethylated ganglioside G_{D1b}, M_r 2150 Da and 2178 Da. (Calculated for $[M+2Na]^{2+}$, *m/z* 1116.672, 1130.687. Observed *m/z* 1116.674, 1130.691, external calibration). Sample introduced at 1 µL/min by flow injection, total ganglioside concentration 2 pmol/µl in 1:3 MeOH/0.1N NaOH, sampling time 1 s, sample used for data acquisitions was 20 fmol. IonSpec Ultima FT-ICR MS, 4.7 T actively-shielded magnet. **(a)** Molecular ion region of the ESI mass spectrum, showing $[M+2Na]^{2+}$ for each of the homologs. Low abundance peaks at *m/z* 1109 and 1123 indicate partial de-esterification and the ion at *m/z* 1119 corresponds to the $[M+H+Na]^{2+}$ of the higher homolog. **(b)** SORI-CID spectrum of the $[M+2Na]^{2+}$ isotopic cluster of the higher homolog, shown over the region *m/z* 700–1900. Peak assignments are indicated on the scheme. (W Mühlecker and CE Costello, unpublished data).

The varied interactions of bacteriophage T4regA protein with a set of RNAs have been explored by ESI FT-ICR MS [48]. The order of competitive binding was established and information about the binding domain was obtained in a SORI-CID experiment. The components of multiprotein complexes can be rapidly identified by a combination of affinity chromatography steps and MS-based sequence determinations and comparison with databases, as was recently demonstrated in a study of the yeast U1 small nuclear ribonucleoprotein complex [49••].

Oligonucleotides

For oligonucleotide analyses, modified surfaces confer selectivity. The stability of their abundant phosphate anions motivates extensive use of negative ion measurements. With delayed extraction, MALDI resolution, sensitivity and mass accuracy are similar to that obtained for peptides; fragmentation that permits oligonucleotide sequencing can be enhanced by selection of a MALDI matrix that fosters metastable decomposition and ajustment of delay time to maximize fragment ion yield [50]. Transfer of tRNAs from a PAGE gel to a nitrocellulose membrane, followed by dissolving the bit of membrane holding the spot in matrix solution and performing MALDI-TOF MS has provided 2D-analysis at the 40 pmol level [51]. IR MALDI is particularly useful for molecular weight determinations of large oligonucleotides: the spectra of synthetic DNA, restriction enzyme

fragments of plasmid DNA and of RNA transcripts up to 2180 nucleotides have recently been reported [52••]. The ability to handle large DNAs and RNAs should facilitate the high-throughput screening necessary, for example, for genotyping and clinical diagnosis. Several laboratories are investigating oligonucleotide PSD fragmentation. Photodissociation of ions trapped in an ESI FT-ICR MS cell gives clear sequence ions whose exact masses can be accurately assigned using stable peptide molecular ions as internal standards [32••].

Oligosaccharides and glycoconjugates

Oligosaccharides and their conjugates provide challenges to the analyst because they have many possible isomeric forms, contain very labile bonds and usually occur in nature as mixtures of closely related compounds. Glycoproteins often contain multiple modified sites, each bearing a different set of glycoforms, whose distribution may change as a function of age, growth conditions, immune state, tissue location, and so on. For release of *N*-linked oligosaccharides from glycoproteins prior to MALDI-TOF MS analysis, Papac *et al.* [53•] have described a high-throughput microscale method that employs a 96-well plate. Charlwood *et al.* [54] have chosen instead a method that carries out the release in microcapillaries. Rouse *et al.* [55••] have developed MALDI-PSD analyses for isomer differentiation among oligosaccharide pools released from glycoproteins. Although these approaches are highly useful for batch-to-batch monitoring of recombinant glycoproteins, they do sacrifice site-specific information that is available when protease digestion precedes carbohydrate release. Loss of this information may be acceptable in the quality-control situation if the ambiguity does not impinge on the therapeutic value of the product, but for structure-activity studies it is important to distinguish among the glycoforms present at each site. Medzihradszky *et al.* [56•] recently employed reversed-phase capillary HPLC combined with ESI-orthogonal acceleration-TOF MS for the individual characterization of more than twenty *N*- and *O*-linked sites of the 909 amino acid B-domain of the recombinant human Factor VIII. The sample requirement was only 5% of that used for earlier studies on a sector instrument, and minor components were more easily assigned. Isomer assignments, however, depended on prior knowledge of expected glycoforms rather than on MS/MS of these samples. Vouros and co-workers [57•] have shown that multistage MS^n, performed in an ion trap, facilitates isomer differentiation.

Penn *et al.* [58•] provided a comparison of the MALDI and ESI FT-ICR MS results obtained for several native gangliosides. The ESI spectra showed less fragmentation, but the MALDI spectra were easier to obtain. We find that, with FT-ICR MS, as with other MS methods, there are advantages to derivatization of oligosaccharides and glycoconjugates: increase in both sensitivity and informative fragmentation [59,60•]. Figure 2a shows the region containing the doubly-charged $[M+2Na]^{2+}$ molecular ions in the ESI MS spectrum of a few picomoles of the permethylated ganglioside G_{D1b}. Figure 2b shows a portion of the SORI-CID spectrum obtained for the $[M+2Na]^{2+}$ of the higher homolog. In a further experiment (not shown), the MS^3 spectrum of the $[C_4]^{2+}$ ion, corresponding to the intact oligosaccharide, provided further details about the sugar sequence. These spectra illustrate the high sensitivity and mass accuracy of the FT-ICR MS approach. As these instruments become more available, the experimental approaches should continue to be refined, optimizing results for different sample classes and illuminating rules for spectral interpretation.

Conclusion

It is clear that the rapid growth phase of bioanalytical mass spectrometry has not yet reached its peak. It is likely that the next decade will also be one of expansion, as the technique becomes more widely known in the biological community and the technology becomes yet more powerful, user-friendly and capable of automation. Critical to this progress is continuance of fruitful communication among research mass spectrometry laboratories, manufacturers and investigators throughout the range of life sciences.

Acknowledgements

The author is grateful to her colleagues X Hronowski and W Mühlecker for acquiring the spectra shown in Figures 1 and 2, respectively, to R Nelson for providing the complement C1q sample, to the NIH National Center for Research Resources Grant No. RR10888 for support of her laboratory, and to Thermo BioAnalysis, Inc. for loan of the Vision 2000 RTOF MS.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Burlingame AL, Boyd RK, Gaskell SJ: **Mass spectrometry.** *Anal Chem* 1998, **70**:647R-716R.
This is a biennial review of the current literature of mass spectrometry.

2. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM: **Electrospray ionization for the mass spectrometry of large biomolecules.** *Science* 1989, **246**:64-71.

3. Karas M, Hillenkamp F: **Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons.** *Anal Chem* 1988, **60**:2299-2301.

4. Takach EJ, Hines WM, Patterson DH, Juhasz P, Falick AM, Vestal ML, Martin SA: **Accurate mass measurements using MALDI-TOF with delayed extraction.** *J Protein Chem* 1997, **16**:363-369.

5. •• Spengler B: **Post-source decay analysis in matrix-assisted laser desorption/ionization mass spectrometry of biomolecules.** *J Mass Spectrom* 1997, **32**:1019-1036.
A tutorial on post-source decay MS including experimental details and a guide to interpretation of spectra.

6. •• Gaskell SJ: **Electrospray: principles and practice.** *J Mass Spectrom* 1997, **32**:677-688.
A review with extensive diagrams and examples to introduce ESI MS and provide many examples of applications.

7. Marshall AG, Hendrickson CL, Jackson GS: **Fourier transform ion cyclotron resonance mass spectrometry: a primer.** *Mass Spectrom Rev* 1998, **17**:1-35.

8. Nelson R: **The use of bioreactive probes in protein**
• **characterization.** *Mass Spectrom Rev* 1997, **16**:353-376.
A discussion of principles, preparation and illustrations of applications of MALDI probes with surfaces having specific activities for individual biopiolymers or sets of molecules with a common structural feature.

9. Eckerskorn C, Strupat K, Kellermann J, Lottspeich F, Hillenkamp F: **High sensitivity peptide mapping by micro-LC with on-line membrane blotting and subsequent detection by scanning-IR-MALDI mass spectrometry.** *J Protein Chem* 1997, **16**:349-362.

10. Stevenson TL, Loo JA, Greis KD: **Coupling capillary high**
• **performance liquid chromatography to matrix-assisted laser desorption/ionization mass spectrometry and N-terminal sequencing of peptides *via* automated microblotting onto membrane substrates.** *Anal Biochem* 1998, **262**:99-109.
Title adequately covers content of paper. The method advances sensitivity and simplifies sample handling.

11. Jungblut P, Thiede B: **Protein identification from 2-DE gels by MALDI mass spectrometry.** *Mass Spectrom Rev* 1997, **16**:145-162.

12. Eckerskorn C, Strupat K, Schleuder S, Hochsttrasser D, Sanche J-C,
•• Lottspeich F, Hillenkamp F: **Analysis of proteins by direct-scanning infrared-MALDI mass spectrometry after 2D-PAGE separation and electroblotting.** *Anal Chem* 1997, **69**:2888-2992.
This is the latest contribution from the group that has led in the development of methods for IR-MALDI MS analysis of proteins separated on gels and transferred to membranes.

13. Ogorzalek-Loo RR, Stevenson TI, Mitchell C, Loo JA, Andrews PC: **Mass spectrometry of proteins directly from polyacrylamide gels.** *Anal Chem* 1996, **68**:1910-1917.

14. Cohen SL, Chait BT: **Mass spectrometry of whole proteins eluted from sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels.** *Anal Biochem* 1997, **247**:257-267.

15. Winston RL, Fitzgerald MC: **Concentration and desalting of protein samples for mass spectrometry analysis.** *Anal Biochem* 1998, **262**:83-85.

16. Caprioli RM, Farmer TB, Gile J: **Molecular imaging of biological**
•• **samples: localization of peptides and proteins using MALDI-TOF MS.** *Anal Chem* 1997, **69**:4751-4760.
Molecular imaging of the spatial distribution of biopolymers in biological tissues is sure to be a growing field; this paper represents a strong early contribution to the area.

17. Drexler DM, Tiller PR: **'Intelligent' fraction collection to improve structural characterization by mass spectrometry.** *Rapid Commun Mass Spectrom* 1998, **12**:895-900.

18. Shevchenko A, Chernushevich IV, Ens W, Standing KG, Thomson B,
•• Wilm M, Mann M: **Rapid '*de novo*' peptide sequencing by a combination of nanoelectrospray, isotopic labeling and a quadrupole/time-of-flight mass spectrometer.** *Rapid Commun Mass Spectrom* 1997, **11**:1015-1024.
This paper results from a collaboration between instrumental designers who have the most insight into orthogonal ESI-TOF MS and the group which has been a leader in high sensitivity profiling of peptides and proteins.

19. Qin J, Herring CJ, Zhang X: ***De novo* peptide sequencing in an ion**
• **trap mass spectrometer with ^{18}O labeling.** *Rapid Commun Mass Spectrom* 1998, **12**:209-216.
Protease digestion in the presence of $H_2{}^{18}O$ allows differentiation of amino- and carboxy-terminal fragment ion series. This paper combines this digestion procedure with mass analysis in a quadrupole ion trap.

20. Davis MT, Lee T: **Rapid protein identification using a microscale**
• **electrospray LC/MS system on an ion trap mass spectrometer.** *J Am Soc Mass Spectrom* 1998, **9**:194-201.
This contribution combines expertise in sample handling, derivatization and degradation procedures with straightforward MS analysis on a benchtop instrument.

21. Emmett MR, White FM, Hendrickson CL, Shi SD-H, Marshall AG:
• **Application of micro-electrospray liquid chromatography techniques to FT-ICR MS to enable high sensitivity biological analysis.** *J Am Soc Mass Spectrom* 1998, **9**:333-340.
The sensitivity and specificity developed in this methodology should allow *in vivo* ESI MS measurements and tissue- or cell-specific profiling of neuropeptides.

22. Figeys D, van Oostveen I, Ducret A, Aebersold R: **Protein identification by capillary zone electrophoresis/microelectrospray ionization-tandem mass spectrometry at the subfemtomole level.** *Anal Chem* 1996, **68**:1822-1828.

23. Xue Q, Dunayevskiy YM, Foret F, Karger BL: **Integrated multichannel**
• **microchip electrospray ionization mass spectrometry: analysis of peptides from on-chip tryptic digestion of melittin.** *Rapid Commun Mass Spectrom* 1997, **11**:1253-1256.
The procedures described here minimize sample requirement and manipulation and could soon lead to highly automated methods.

24. Lamoree MH, van der Hoeven RAM, Tjaden UR, van der Greef J: **Application of microdialysis for on-line coupling of capillary isoelectric focusing with electrospray mass spectrometry on a magnetic sector instrument.** *J Mass Spectrom* 1998, **33**:453-460.

25. Wilbur DS, Stayton PS, To R, Buhler KR, Klumb LA, Hamlin DK, Stray JE, Vessella RL: **Streptavidin in antibody pretargeting. Comparison of a recombinant streptavidin with two streptavidin mutant proteins and two commercially available streptavidin proteins.** *Bioconjug Chem* 1998, **9**:100-107.

26. Williams ER: **Tandem FTMS of large biomolecules.** *Anal Chem*
•• 1998, **70**:179A-185A.
An introduction to the powerful techniques of FT-ICR MS with numerous examples of applications to protein characterization.

27. Hofstadler SA, Griffey RH, Pasa-Tolic L, Smith RD: **The use of a**
•• **stable internal mass standard for accurate mass measurements of oligonucleotide fragment ions using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry with infrared multiphoton dissociation.** *Rapid Commun Mass Spectrom* 1998, **12**:1400-1404.
This very sophisticated method capitalizes on the relative stability of protein ions compared to those of oligonucleotides in order to provide exceptionally accurate mass assignments for the oligonucleotide fragments.

28. Zubarev RA, Kelleher NL, McLafferty FW: **Electron capture dissociation of multiply charged protein cations, a non-ergodic process.** *J Am Chem Soc* 1998, **120**:3265-3266.

29. Wood TD, Chorush RA, Wampler FM III, Little DP, O'Connor PB, McLafferty FW: **Gas-phase folding and unfolding of cytochrome c cations.** *Proc Natl Acad Sci USA* 1995, **92**:2451-2454.

30. Green MK, Lebrilla CB: **Ion-molecule reactions as probes of gas-phase structures of peptides and proteins.** *Mass Spectrom Rev* 1997, **16**:53-71.

31. Zubarev RA, Demirev PA: **Isotope depletion of large biomolecules:**
• **implications for molecular mass measurements.** *J Am Soc Mass Spectrom* 1998, **9**:149-156.
Replacement of the broad natural distribution of isotopes into a few peaks yields an increase in signal-to-noise ratio for proteins and reduces the possibility of overlap by signals from multiple components of a complex mixture.

32. Jensen ON, Podtelejnikov AN, Mann M: **Identification of the**
•• **components of simple protein mixtures by high-accuracy peptide mass mapping and database searching.** *Anal Chem* 1997, **69**:4741- 4750.
Results from MS experiments may now be easily compared with frequently updated information easily available on the Internet; the approach described here provides rapid identification of many proteins at the level normally recovered from PAGE gels.

33. Annan RS, Carr SA: **The essential role of mass spectrometry in**
•• **characterizing protein structure: mapping posttranslational modifications.** *J Protein Chem* 1997, **16**:391-402.
Because the majority of proteins are post-translationally modified, rapid and sensitive methods for handling the assignment of structural modifications are needed; this contribution summarizes the present state of these techniques.

34. Yu L, Gaskell SJ, Brookman JL: **Epitope mapping of monoclonal**
• **antibodies by mass spectrometry: identification of protein antigens in complex biological systems.** *J Am Soc Mass Spectrom* 1998, **9**:208-215.
Antigen–antibody interactions are often stable enough to survive ionization and/or proteolysis. New MS methods reviewed here take advantage of the stability of these interactions to localize the active sites.

35. Jeyarajah S, Parker CE, Sumner MT, Tomer KB: **Matrix-assisted laser desorption ionization/mass spectrometry mapping of human immunodeficiency virus gp120 epitopes recognized by a limited polyclonal antibody.** *J Am Soc Mass Spectrom* 1998, **9**:157-165.

36. Griffiths WJ, Gustafsson M, Yang Y, Curstedt T, Sjovall J, Johansson J: **Analysis of variant forms of porcine surfactant polypeptide-C by nano-electrospray mass spectrometry.** *Rapid Commun Mass Spectrom* 1998, **12**:1104-1114.

37. Loo JA: **Mass spectrometry in the combinatorial chemistry**
•• **revolution.** *Eur Mass Spectrom* 1997, **3**:93-104.
Up-to-date review of the ways in which MS can contribute to the rapid structural assignment of products and a guide to the design considerations for such experiments.

38. Tutko DC, Henry KD, Winger BE, Stout H, Hemling M: **Sequential**
• **mass spectrometry and MS^n analyses of combinatorial libraries using automated matrix-assisted laser desorption/ionization Fourier transform mass spectrometry.** *Rapid Commun Mass Spectrom* 1998, **12**:335-338.
The first discussion of the practical implementation of FT-ICR MS for combinatorial chemistry product characterization in the setting of a pharmaceutical industry laboratory.

39. Wang T, Zeng L, Strader T, Burton L, Kassel DB: **A new ultra-high throughput method for characterizing combinatorial libraries incorporating a multiple probe autosampler coupled with flow injection mass spectrometry analysis.** *Rapid Commun Mass Spectrom* 1998, **12**:1123-1129.

40. Berlin K, Jain RK, Tetzlaff C, Steinbeck C, Richert C: **Spectrometrically monitored selection experiments: quantitative laser desorption mass spectrometry of small chemical libraries.** *Chem Biol* 1997, **4**:63-67.

41. Loo J: **Studying noncovalent protein complexes by electrospray**
•• **ionization mass spectrometry.** *Mass Spectrom Rev* 1997, **16**:1-23.
A current review of the techniques, advantages and possible pitfalls in the study of noncovalent complexes by ESI MS, written by one of the most established practitioners of this approach.

42. Moniatte M, Lesieur C, Vécsey-Semjén B, Buckley JT, Pattus F, van
• der Goot R, Van Dorsselaer FG: **Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry in the subunit stoichiometry study of high-mass non-covalent complexes.** *Int J Mass Spectrom Ion Processes* 1997, **169/170**:179-199.
A review of MALDI MS analyses of noncovalent complexes, with extensive examples from the authors' laboratory. This paper appears in a special double volume dedicated to MALDI MS that contains a large number of significant contributions by leaders in the field.

43. Gruic-Sovulj I, Lüdemann H-C, Hillenkamp F, Weygand-Durševic I,
• Kucan Z, Peter-Katalinic J: **Detection of noncovalent tRNA-aminoacyl-tRNA synthetase complexes by matrix-assisted laser desorption/ionization mass spectrometry.** *J Biol Chem* 1997, **272**:32084-32091.
Biologically relevant protein–nucleic acid complexes with molecular weights near 100 kDa were observed.

44. Veenstra TD, Tomlinson AJ, Benson L, Kumar R, Naylor S: **Low**
• **temperature aqueous electrospray ionization mass spectrometry of noncovalent complexes.** *J Am Soc Mass Spectrom* 1998, **9**:580-584.
Many practical details of the ESI MS experiment optimized for observation of noncovalent complexes are discussed.

45. Nettleton EJ, Sunde M, Lai Z, Kelly JW, Dobson CM, Robinson CV:
• **Protein subunit interactions and structural integrity of amyloidogenic transthyretins: evidence from electrospray mass spectrometry.** *J Mol Biol* 1998, **281**:553-564.
This paper correlates X-ray data with observations of interactions among protein subunit components determined by ESI MS, and the influence of amino acid substitutions on the multimer stability.

46. Zaia J, Hronowski XL, Costello CE: **Infrared and ultraviolet MALDI-TOF observation of collagen monomers and noncovalent trimers.** In *Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics*: *1997 June 1–4; Palm Springs.* Edited by Giordani AB. American Society for Mass Spectrometry; 1997:367.

47. Nelson RW, Krone JR, Tubbs KA, Dogruel D: **Mass spectrometric methods for biomolecular characterization.** In *Proceedings of the NATO workshop on New Methods for the Study of Molecular Aggregates*. Edited by Ens W, Standing KG, Chevnushevich IV. Dordrecht: Kluwer Academic Publishers; 1998:225-238.

48. Liu C, Pasa-Tolic L, Hofstadler SA, Harms AC, Smith RD, Kang CH, Sinha N: **Probing RegA/RNA interactions using electrospray ionization-Fourier transform ion cyclotron resonance-mass spectrometry.** *Anal Biochem* 1998, **262**:67-76.

49. Neubauer G, Gottschalk A, Fabrizio, P, Séraphin B, Lührmann R,
•• Mann M: **Identification of the proteins of the yeast U1 small nuclear ribonucleoprotein complex by mass spectrometry.** *Proc Natl Acad Sci USA* 1997, **94**:385-390.
One of the first examples of the assignment of the components of an entire multiprotein complex based on MS combined with database searching.

50. Juhasz P, Roskey MT, Smirnov IP, Haff LA, Vestal ML, Martin SA: **Applications of delayed extraction matrix-assisted laser desorption ionization time-of-flight mass spectrometry to oligonucleotide analysis.** *Anal Chem* 1996, **68**:941-946.

51. Wei J, Lee CS: **Polyacrylamide gel electrophoresis coupled with matrix-assisted laser desorption/ionization mass spectrometry for tRNA mutant analysis.** *Anal Chem* 1997, **69**:4899-4904.

52. Berkenkamp S, Kirpekar F, Hillenkamp F: **Infrared MALDI mass**
•• **spectrometry of large nucleic acids.** *Science* 1998, **281**:260-262.
This paper significantly extends the mass range for oligonucleotide analysis.

53. Papac DI, Briggs JB, Chin ET, Jones AJS: **A high-throughput**
• **microscale method to release N-linked oligosaccharides from glycoproteins for matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis.** *Glycobiology* 1998, **8**:445-454.
The analysis of glycoforms in an industrial setting requires development of methods that are rapid and amenable to automation. This paper demonstrates that techniques similar to those used for protein characterization are feasible for quality-control screening of glycoproteins.

54. Charlwood J, Birrell H, Camilleri P: **Efficient carbohydrate release, purification and derivatization.** *Anal Biochem* 1998, **262**:197-200.

55. Rouse JC, Strang AM, Yu W, Vath JE: **Isomeric differentiation of**
•• **asparagine-linked oligosaccharides by matrix-assisted laser desorption-ionization postsource decay time-of-flight mass spectrometry.** *Anal Biochem* 1998, **256**:33-46.
Careful determinations of relative abundances of peaks in the PSD mass spectra of native oligosaccharides give reliable indications of glycoform distributions.

56. Medzihradszky KF, Besman MJ, Burlingame AL: **Reverse-phase**
• **capillary high performance liquid chromatography/high performance electrospray ionization mass spectrometry: an essential tool for the characterization of complex glycoprotein digests.** *Rapid Commun Mass Spectrom* 1998, **12**:472-478.
The sample size requirement and analysis time for glycoprotein glycosylation site mapping are reduced by utilization of microscale separation methods and ESI Q-TOF mass analysis.

57. Weiskopf AS, Vouros P, Harvey DJ: **Characterization of**
• **oligosaccharide composition and structure by quadrupole ion trap mass spectrometry.** *Rapid Commun Mass Spectrom* 1997, **11**:1493-1504.
Multistage mass analysis of oligosaccharides allows determination of branching patterns; identical substructures give equivalent spectra at any stage of the analysis.

58. Penn SG, Cancilla MT, Green MK, Lebrilla CB: **Direct comparison of matrix-assisted laser desorption/ionization and electrospray ionization in the analysis of gangliosides by Fourier transform mass spectrometry.** *Eur Mass Spectrom* 1997, **3**:67-79.

59. Solouki T, Reinhold BB, Costello CE, O'Malley M, Guan S, Reinhold VN, Marshall AG: **Electrospray ionization and matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry of permethylated oligosaccharides.** *Anal Chem* 1998, **70**:857-864.

60. Perreault H, Hronowski XL, Koul O, Street J, McCluer RH, Costello
• CE: **High sensitivity mass spectral characterization of glycosphingolipids from bovine erythrocytes, mouse kidney and fetal calf brain.** *Int J Mass Spectrom Ion Processes* 1997, **169/170**:351-370.
MALDI TOF and PSD MS of derivatized glycosphingolipids provide structural information at the low and subpicomole level that enable study of fucosyl transferase pathways in the developing brain. High energy CID on a four-sector instrument provides further information when larger samples are available.