Analysis of Intact Protein Isoforms by Mass Spectrometry

The diverse proteome of an organism arises from such events as single nucleotide substitutions at the DNA level, different RNA processing, and dynamic enzymatic post-translational modifications. This minireview focuses on the measurement of intact proteins to describe the diversity found in proteomes. The field of biological mass spectrometry has steadily advanced, enabling improvements in the characterization of single proteins to protein forms derived from cells or tissues. In this minireview, we discuss the basic technology for “top-down” intact protein analysis. Furthermore, examples of studies involved with the qualitative and quantitative analysis of full-length polypeptides are provided.

Introduction to Intact Protein Mass Spectrometry

Goals of modern biological and biomedical research include characterizing and ultimately treating human disease. Different diseases may be characterized by DNA, RNA, and proteins with regard to observed malfunctions and phenotypes at the molecular level. The field of functional genomics has progressed to “rapid” whole genome sequencing. Large-scale mapping of genetic information catalogues mutations and polymorphisms that can translate into proteome variation. RNA processing increases protein variation through basic transcription or alternative splicing (Fig. 1). To determine the significance of shotgun proteomics-type experiments, the identified proteins may often be sorted into “interaction networks” based on known interactions or changes in regulation upon different disease types or perturbations (Fig. 1). Analysis of the proteome of an organism presents a difficult challenge due to the complexity and often the distribution of gene products into different protein forms. In contrast to genomics and RNA analysis, comprehensive mapping of the proteome of an organism and its dynamic variation is further complicated by the enzymatic (or chemical) addition/deletion of post-translational modifications (PTMs).

The control and regulation of protein expression stem largely from the control of RNA expression from coded DNA and, as such, the control of different isoforms. The term “isoforms,” as has been recommended by the International Union of Pure and Applied Chemistry (IUPAC), refers to protein forms that have high sequence identity and arise from the same gene family or polymorphisms. Thus, the variation for protein isoforms arises from “genetic” sources by this definition. The term “protein species” is suggested to refer to highly related protein forms that differ due to PTMs and alternative splicing (Fig. 1) (1, 2). Colloquially, most use the term “isoform” or “variant” to refer to a mixture of all these sources of molecular variation at the protein level. Here, we focus on modern protein analysis and, more specifically, the identification and characterization of whole protein molecules by MS. This has come to be known as “top-down” (TD) intact protein analysis (3–5).

Proteins may also be routinely analyzed by “bottom-up” (BU) proteomics, in which the proteins are digested with a protease prior to peptide detection and protein identification by peptide sequencing with MS/MS (supplemental Fig. 1). The digested samples produce a complex mixture of peptides between ~500 and 3500 Da that are usually separated by single mode or multidimensional chromatography. On-line precursor mass measurement, along with the MS/MS “fragmentation” spectrum, typically allows for the inference of peptides by mapping to protein databases and the probability of such. The peptides are matched to proteins along with the probability of the protein being a true identification. Without the aid of additional information, often only the gene family can be identified by BU proteomics.

Furthermore, several isoforms may be associated with a gene family, and the individual isoforms may produce peptides with identical sequences (6). Although isoforms, sequence, and PTM information may be lost in the context of describing the full proteome, BU proteomics affords higher proteome coverage in comparison with TD proteomics (7). One way to convey this, in biological terms, is in the challenge of analyzing isoforms created by evolution in the process of microbial speciation. Using simple mass analysis of whole ribosomal proteins, the sequence variation imbedded in the mass of the whole protein can be used to create a new type of phylogenetic tree (8).

As mentioned above, TD proteomics has yet to reach the same proteome coverage as BU proteomics. This is further exacerbated by non-routine TD analysis of proteins above 50 kDa in a high-throughput discovery mode. However, intact protein analysis is more likely to result in measurement of var-

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1 The on-line version of this article (available at http://www.jbc.org) contains supplemental data and Fig. 1.

2 The abbreviations used are: PTM, post-translational modification; TD, top-down; BU, bottom-up; GE, gel electrophoresis; ESI, electrospray ionization; RPLC, reversed-phased LC; DIGE, two-dimensional difference GE; FT-ICR, Fourier transform ion cyclotron resonance; LTQ, linear trapping quadrupole; ECD, electron capture dissociation; CID, collision-induced dissociation; nLC, nano-LC; cMyBP-C, cardiac myosin-binding protein C; cTnI, cardiac troponin I.
iation from coding polymorphisms, alternative splicing, and diverse PTM changes, which has been demonstrated in several targeted studies on single proteins or those present in modest mixtures (9–12).

If we loosen our definition of TD proteomics to include the classic approach of two-dimensional gel electrophoresis (GE), one can see that this strategy of molecular analysis has been used for quite some time (13). However, to identify proteins with two-dimensional GE, interesting “spots” are analyzed by BU proteomics. Thus, this minireview will include some information on hybrid techniques such as two-dimensional GE with BU proteomics for protein identification (14). However, much of the review under “Technology for Intact Proteins: Ionization, Separations, Instrumentation, and Informatics” describes separations that are “solution-based” platforms, which maintain proteins in solution. This strategy preserves the proteins in their intact mature form for mass measurement and direct sequencing in a mass spectrometer (5).

Covering the continuum of sample complexity, from single protein to protein complexes to whole cells and tissues, helps to define the workflows and platforms required for analysis. For “targeted” TD intact protein analysis, “high sequence coverage” of single proteins or protein complexes can be obtained, whereas high proteome coverage can be obtained for global analysis of cellular and tissue lysates. Global proteome analysis is often associated with high-throughput technologies because several different components may be identified over a relatively short amount of time. Targeted TD analysis is not intended to mean low-throughput. With regard to global TD analysis, increasing the number of identified isoforms per experiment is desirable to describe multiple isoforms at the same time, affording the ability to perform experiments in discovery mode. Thus, high-throughput TD includes the analysis of proteomes from complex samples, whereas targeted TD analysis is performed on single or simple mixtures of proteins (Fig. 2). As the duty cycle for instruments has improved over the last 10 years, high-throughput TD proteomics has continued to become increasingly possible for discovery and quantitation experiments in a fashion that is starting to approach BU proteomics. Below, we will expand upon the different separations, instrumentation, and informatics that have been developed to achieve TD proteomics in targeted or high-throughput contexts.

**Technology for Intact Proteins: Ionization, Separations, Instrumentation, and Informatics**

Many of the breakthroughs in biological MS, i.e. intact protein analysis, were achieved due to the advent of soft ionization...
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Techniques such as electrospray ionization (ESI) (15) and MALDI (16, 17). A basic description of these ionization techniques can be found in the supplemental data. Briefly, ESI allows for samples to be directly infused or analyzed on-line by reversed-phase LC (RPLC). This unique capability allows for proteins to be maintained in the “solution phase” for MS analysis. Proteins may also be separated by different modes such as those based on charge, strong cation exchange, or weak anion exchange or those based on normal-phase separations with hydrophilic interaction chromatography. Other reviews have outlined successful applications of different separation modes (18, 19). Furthermore, non-particle base preparative (solution) isoelectric focusing (20) and gel-eluted liquid fraction entrapment electrophoresis (21) have recently provided modes of separation similar to acrylamide gels but result in recovery of proteins in the solution phase with high yields. In contrast to RPLC, each of the above techniques is usually performed off-line to MS because of incompatibility with ESI. Thus, samples are separated with an orthogonal method such as RPLC for direct infusion ESI-MS or by on-line RPLC-ESI-MS (22–25). Especially for complex protein samples, improved separations will always improve the MS analysis (19).

As mentioned above, intact proteins may also be separated by slab GE, although not considered TD analysis in the traditional sense. Slab gels are the cheapest and most common way to separate proteins by size and/or isoelectric point. Proteins are detected by staining dyes, covalently attached fluorescent dyes, radiolabeling, or immunoblotting (26, 27) or are identified by BU MS. One of the main strengths of slab gel intact protein separations is quantitation by two-dimensional difference GE (DIGE) (28). The drawback for this detection scheme is that the PTM site location, full sequence, and/or full isomorph information may be lost. Solution phase-based TD proteomics also provides quantitation platforms with chemical and metabolic labeling schemes. Akin to fluorescent labeling for DIGE, analysis of yeast by $^{14}$N/$^{15}$N metabolic labeling may provide relative quantitation across different treatments (29). More recently, Muddiman and co-workers (30) performed stable isotope labeling with amino acids in cell culture (known as SILAC) with Aspergillus flavus and human embryonic stem cells to yield relative quantitation of whole proteins.

Traditionally, TD intact protein analysis has been most successful with ESI Fourier transform ion cyclotron resonance (FT-ICR) MS. The FT-ICR mass spectrometer is still currently the highest resolution instrument available for sample analysis (31). The analytical advantages of FT-ICR-MS for large biomolecules have been reviewed previously (9, 19, 31). FT-ICR-MS can provide mass error measurement of <2 ppm for proteins up to 25 kDa. This mass accuracy is often required for unambiguous characterization, especially when the mass shift may be associated with deamidation (Δ = 1 Da on an ~15-kDa protein) (32), the difference between acetylation and trimethylation (Δ = 0.0363 Da on fragments) (33), or reduced/oxidized disulfide bonds (Δ = 2 Da on an ~14-kDa protein) (34). Furthermore, when analyzing fragment ion spectra (i.e. dissociation of the intact protein into fragments for mass measurement), high mass accuracy is desirable for high confidence, and high resolving power is advantageous for resolving overlapping fragment peaks (35). FT-ICR-MS has set the standard for the requirements for accurate and precise mass measurement. Recently, a second high-resolution instrument, the linear trapping quadrupole (LTQ) orbitrap mass spectrometer, akin to FT-ICR technology but lacking a superconducting magnet, was applied to the analysis of intact proteins (25, 36–38).

Although not typically associated with high-resolution intact protein analysis, TOF mass spectrometers have also been used to characterize proteins. For example, MALDI-TOF-MS analysis for protein mapping has been developed for such applications as biodetection (39). Thus, intact protein mass spectra may be compared across sample types by MALDI-TOF-MS. Another application includes tissue imaging, by which MALDI-TOF-MS can provide highly sensitive and specific spatial analysis of biomolecules based on mass (40–42). The experiments add another dimension to the investigation of protein arrangements in all types of biological tissues, such as diagnosis from resections (43).

Each of the different mass analyzers described above may fragment proteins through different methods based on activation using thermal heating (collisions with gas) or electron capture/transfer. Fragment ions report on the protein primary sequence and PTM site location. One of the first successful implementations of protein fragmentation includes electron capture dissociation (ECD) and FT-ICR-MS on ubiquitin and cytochrome c (44). ECD fragments proteins by ultra-fast dissociation of the amino acid backbone, thus generating several product ions along the backbone of the protein. Often, ECD produces the most sequence information for characterization of intact proteins. Furthermore, ECD is usually associated with single or simple mixtures of proteins.

Not mentioned above are the LTQ or quadrupole mass analyzers. LTQ (45) and quadrupole analyzers are not normally associated with intact mass measurement as stand-alone mass analyzers; however, the development of hybrid instruments (two mass analyzers placed in-line), i.e. LTQ-FT-ICR, LTQ orbitraps, quadrupole TOF, and TOF/TOF (46), has improved throughput by thermal activation with collision-induced dissociation (CID). Ion trap hybrid instruments fragment proteins in the LTQ or quadrupole analyzer prior to transfer to a second higher resolution mass analyzer. Electron transfer dissociation (47) is another fragmentation method, akin to ECD, that takes place in an LTQ analyzer as opposed to ICR cells. Also, higher energy CID has been implemented on LTQ orbitraps (48).

In comparison with ECD, thermal activation produces fewer fragments with increased amino acid specificity. An early implementation of thermal activation includes CID fragmentation by Loo et al. in 1990 (49). Other early forms of protein fragmentation methods include in-source, infrared multiphoton (50, 51), and surface off-resonance irradiation (52) dissociation. During the last 10 years, all of the described fragmentation methods have been improved. For example, funnel skimmer dissociation, akin to in-source dissociation (53, 54), and CID (55) continue to be implemented across instrument types; however, fragmentation is still limited to specific amino acids and often only in the proximity of the N or C terminus of proteins.
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Hybrid instruments have also resulted in gains to “scan speed” for intact protein analysis. For example, a linear octopole was placed in-line with an FT-ICR mass spectrometer to detect 101 whole proteins from 5 to 59 kDa, with many PTMs and protein forms identified from the methanogen Methanosarcina acetivorans (56). Another implementation included CID with an LTQ-FT-ICR mass spectrometer. This instrument was similar to the linear octopole; however, the number of ions transferred into the ICR cell (or orbitrap) can be controlled (57, 58). Another advantage of the improved duty cycle of the new generation of hybrid instruments becomes particularly important as the resolution of intact protein separation has improved with reversed-phase nano-LC (nLC; i.e. flow rates at 300–500 nl/min with capillary columns). Currently, high-throughput MS analysis with on-line nLC can provide routine analysis of proteins up to 35 kDa (22, 23). LTQ orbitraps have yet to reach the same intact mass plateaus as ICR mass analyzers (in terms of resolving power); however, they are very promising for TD proteomics analysis (59).

On-line fragmentation of intact proteins can also be achieved with TOF instrumentation (60). Resemann et al. (61) reported the ability to unambiguously sequence a 13-kDa fragment of a variable region of an antibody through the use of MALDI ion-source decay in a TOF/TOF hybrid instrument. TOF/TOF hybrid instruments (two TOF mass analyzers) have also been found to be useful for some proteins between 5 and 15 kDa (62). A second type of TOF hybrid instrument includes quadrupole TOF research performed by McLuckey and co-workers (63). They were able to analyze fragmentation spectra with relatively lower resolving power through reducing the complexity of fragmentation spectra by collapsing the charge states of fragments to mainly singly charged species though the use of fast ion-ion reactions (64). All of the described instrumentation has generated new types of data to be extracted and analyzed. The complexity of the data has resulted in new programs for sequencing proteins.

Several advances in protein identification and instrument control have also improved the throughput of TD intact protein analysis. Intact and fragment masses are characterized by mapping calculated sequence tags or fragment ions to databases with known protein sequences or by heuristic analysis. ProSight was the first program to provide algorithms for single and high-throughput protein analysis (65). More recently, other algorithms such as Big Mascot (53) and PIITA (precursor ion-independent TD algorithm) (66) have been developed to provide general software packages for intact protein analysis. TOF/TOF hybrid instruments have also found development of software for interpretation of small proteins in regard to food-borne pathogens (62). With regard to instrument control software, fraction collection followed by direct infusion MS with a robot provides automated control of all steps from sample addition to data analysis. In short, fractions were collected off-line with a split flow directed toward the instrument for intact mass profiling. The information from the chromatogram generated a feedback loop for the robot to infuse the sample with intelligent MS data acquisition that included mass isolation and fragmentation. Fragment ions were then “signal-averaged” for increased sensitivity and mass accuracy with direct infusion ESI-FT-ICR (67, 68).

Other versions of this experiment include that carried out by Lubman and co-workers (69), who achieved high-resolution separation by coupling solution isoelectric focusing and non-porous RPLC to MALDI-TOF-MS. This pseudo three-dimensional separation afforded mass resolution (from the TOF analyzer) that surpassed that of SDS-PAGE. Furthermore, the group also provided identifications through off-line digestion of the fractions collected from the front end separation. With regard to other forms of automation, Pasa-Tolic and co-workers (70) combined intact protein profiling by on-line nLC-FT-ICR-MS and split-flow fraction collection. The integrated workflow was also implemented to identify phosphorylation sites with BU proteomics as a second dimension to TD analysis.

Applications of Intact Protein MS

High-throughput characterization of isoforms and PTMs may reveal incorrect database predictions. For example, Ferguson et al. (71) detected 99 proteins from M. acetivorans, 15 of which were shown to have translational start sites that were mispredicted. Also, the study identified five unannotated proteins and another set of proteins from incorrect reading frames. In another high-throughput study on human primary leukocytes harvested by leukoreduction, Roth et al. (72) were able to report on “the proteotype” of the diverse human proteome by high-throughput characterization of 133 proteins (>600 unique intact masses), 32 of which had PTMs, SNPs, or were detected as proteolysis products. Other studies continue to improve lower limits for characterization. For example, with only 500 ng of material, Lourette et al. (73) were able to profile and semiquantitate over 250 oxidized and nitrated calmodulin forms in activated macrophages through nano-LC-MS. There are other fine examples of high-throughput characterization of intact proteins and isoforms; however, we now turn to more targeted studies.

Fraction collection by liquid chromatography prior to direct infusion may provide highly purified proteins for targeted characterization. For example, Ryan et al. (74) characterized salivary proteins from the cystatin family, identifying signal peptide cleavages, disulfide bonds, and phosphorylation locations after off-line prefraccionation. Furthermore, three SNPs on two members were detected for this protein family. In another study, Ge et al. (77) identified >150 possible sites for phosphorylation of cardiac myosin-binding protein C (cMyBP-C) on the full-length and truncated isoforms. By achieving unit mass resolution and high mass accuracy for the 115-kDa full-length form of cMyBP-C, they were able to identify multiple phosphoprotein forms. Furthermore, sites for phosphorylation were localized to Ser-283, Ser-292, and Ser-312 after fragmentation analysis. In comparison with the wild-type forms, the recombinant truncated forms of cMyBP-C studied had dramatically altered phosphorylation profiles. In another series of studies, Ayaz-Guner et al. (76) identified bisphosphorylation of Ser-22 and Ser-23 in mouse cardiac troponin I (cTnI) in a wild-type rather than a transgenic mouse. Certain sites were shown to be phosphorylated in vitro that were not observed in vivo (Fig. 3, upper left panel). As a last example, Zhang et al. (75) reported
that swine cTnI had the same Ser-22/Ser-23 phosphorylation and N-terminal acetylation but also revealed the localization of a novel V116A SNP genetic variant. Many TD studies have provided results on highly purified proteins or families of proteins. For example, therapeutic antibodies represent a class of single proteins that have grown in relevance to biologics and the biotechnology industry (78).

With regard to biologics, TD intact protein analysis may be applied with other techniques to characterize the expressed antibody. Zhang et al. (48) and Bondarenko et al. (79) characterized monoclonal immunoglobulin G by direct infusion and on-line reversed-phase chromatography with an LTQ orbitrap. The result was the mass analysis of large antibodies, along with the detection of different glycoforms (Fig. 3, upper right panel). Similar experiments have also been performed on TOF instruments, however, with less sequence information and at lower resolution (80). There are many other publications that tackle the challenging problems of therapeutics. A recent review reports on the analysis of biologics and antibodies by MS (81).

TD intact protein analysis may aid in the early decision process to streamline costs associated with drug development, especially with regard to therapeutics and antibodies. Furthermore, there are many other therapeutic proteins that may be characterized by TD intact protein analysis includes histones, their isoforms, and their dynamic post-translational modifications. Histone MS, in cohort with other techniques, has informed the selection of different methyl- and acetyltransferases as “druggable” targets.

Histones, the protein building blocks of chromatin, are a class of complex protein isoforms (H1, H2A, H2B, H3, and H4 plus all of the other possible forms) with dynamic PTM changes depending on the expression state of a cell. All histone families have been successfully characterized by TD analysis to reveal protein forms and dynamic PTMs (82). Some of the many examples from our group include the gene-specific characterization of human histone H2B (83) and the H2A gene family.

**FIGURE 3. Four examples of intact protein analysis are presented.** The complete findings are described in text. *Upper left panel*, the data resulted in the characterization of cTnI with several phosphorylations (76). *Upper right panel*, spectra include those obtained by the analysis of antibodies with an LTQ orbitrap and the final deconvoluted spectra, including the detection of glycan forms (48). *Lower left panel*, analysis of histones that were derived from MLL cell lines and had changes in MMSET (a histone methyltransferase) expression (82). Up-regulation of MMSET expression increased the methylation states of H3.1, H3.2, and H3.3. *Lower right panel*, capability of imaging whole tissue using whole protein MS, with the spatial resolution combined with detection of protein across the molecular mass range of 1000–30,000 Da (43).
(84), quantitation of human H4 isoforms (85), specific methylation of H4K20 (86, 87), and characterization and quantitation of H3 modification in human cells (88). More recently, Martinez-Garcia et al. (82) provided a direct picture of the change in methylation of H3.1, H3.2, and H3.3 based on MMSET (a histone methyltransferase) and the lack of or presence of such (Fig. 3, lower left panel). With regard to the other proteins associated with chromatin, Garcia and co-workers (89) also performed extensive work on histones and protein classes such as high-mobility groups and the characterization of PTM dynamics. Furthermore, Coon and co-workers (90) extended histone TD proteomics to analysis with orbitraps and the identification of 74 unique histone forms. There are other examples of protein families and the TD analysis of their isoforms and PTM status. However, we now turn our attention to plant proteome applications.

Many of the described platforms may also be applied to plant proteome analysis and, more specifically, membrane proteins. The analysis of integral membrane proteins is difficult due to their hydrophobic nature. Proteins of this subset are often analyzed with variations in mobile-phase conditions to improve solubility (91). Some examples include the analysis of several chloroplast proteins, resulting in the mapping of a variety of PTMs and enzymatic cofactors (92, 93). Additionally, 11 integral membrane proteins identified from red algal Photosystem II (a 750-kDa complex) resulted in the characterization of several PTMs (94). The use of organic solvents for membrane protein extraction followed by separation by hydrophilic interaction chromatography has also been described (95, 96). These studies resulted in the identification of several integral membrane proteins from mitochondria.

As a second platform for analysis of the plant proteome, genetics coupled to DIGE can be used to investigate the genetic architecture of complex phenotypes (Fig. 4) (97). Cilia et al. (97) used DIGE to phenotype sister aphid F2 genotypes that segregated in their ability to transmit plant viruses. The study revealed phenotyping and virus transmission information. Results included the description of genetic heterogeneity in the aphid’s bacterial endosymbiont (Buchnera aphidicola) co-segregating with the virus transmission phenotype and heritable bacterial and aphid protein isoforms linked to the virus transmission phenotype and to specific virus transmission barriers within the aphid. There have been other elegant DIGE/BU experiments performed on humans and other species that are beyond the scope of this minireview. As mentioned above, tissue imaging by MALDI-TOF-MS is also another form of intact protein analysis.

Tissue analysis by MALDI-TOF-MS results in the mapping of protein species for different clinical applications (Fig. 3, lower right panel) (43). Intact protein imaging from tissue is often followed by TD or BU protein characterization, unless the protein of interest has a known molecular weight a priori. For example, Schey and co-workers (98) have imaged ocular lenses and retinal tissue from human, bovine, and rabbit. This study resulted in spatial resolution and mapping of the distribution of the G-protein-coupled receptor in the rabbit retina, and an integral membrane protein (AQP0) in human and bovine lenses could be mapped. Imaging normally consists of the analysis of soluble proteins; however, the preparation in their study also allowed for the analysis of integral membrane proteins (98). Imaging can also be used for tissues, as Cazares et al. (99) compared tissue slices from benign and cancerous prostate tissues and found that MEKK2 (MAPK/ERK kinase kinase 2) was prevalent in the cancerous tissue. As an additional example of profiling cancer, Rauser et al. (100) reported on the usefulness of MS imaging for breast cancer and human EGF receptor 2 with regard to decision making and therapeutics. To confirm the findings, the group also accessed TD and BU proteomics techniques to fully identify the proteins.

Conclusions

This minireview has briefly covered the broad field of intact protein analysis by TD MS. Measurement of intact proteins (isoforms and protein species) should result in stronger correlations between MS data and complex phenotypes. To the extent that the above hypothesis is true, TD proteomics and its
focus on analyzing protein molecules in an isoform-resolved manner will help make the interface between biological MS and translational biomedicine more efficient. TD protein analysis has been driven by biological diversity to have many different platforms. Furthermore, examples were presented that illustrate the usefulness of different platforms. TD proteomics can reveal the rich isoform and PTM diversity found in nature and the human body.

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REFERENCES

1. Jungblut, P. R., Holzhütter, H. G., Apweiler, R., and Schlüter, H. (2008) Chem. Cent. J. 2, 16
2. Schlüter, H., Apweiler, R., Holzhütter, H. G., and Jungblut, P. R. (2009) Chem. Cent. J. 3, 11
3. Siuti, N., and Kelleher, N. L. (2007) Nat. Methods 4, 817–821
4. Kellie, J. F., Tran, J. C., Lee, J. E., Ahlf, D. R., Thomas, H. M., Ntai, I., Catherman, A. D., Durbin, K. R., Zamborg, L., Vellaiachamy, A., Thomas, P. M., and Kelleher, N. L. (2010) Mol. Biol. Cell. 6, 1532–1539
5. Kelleher, N. L., Lin, H. Y., Valaskovic, G. A., Aaserud, D. J., Fridriksson, E. K., and McLafferty, F. W. (1999) J. Am. Soc. Mass Spectrom. 12, 806–812
6. Nesvizhskii, A. I., and Aebersold, R. (2005) Mol. Cell. Proteomics 4, 1419–1440
7. Wiśniewski, J. R., Zougman, A., Nagaraj, N., and Mann, M. (2009) Nat. Methods 6, 359–362
8. Wynne, C., Edwards, N. J., and Fenselau, C. (2010) Proteomics 10, 3631–3643
9. Kelleher, N. L. (2004) Anal. Chem. 76, 196A–203A
10. Chait, B. T. (2006) Science 314, 65–66
11. McLafferty, F. W., Breuer, K., Jin, M., Han, X., Infusini, G., Jiang, H., Kong, X., and Begley, T. P. (2007) FEBS J. 274, 6256–6266
12. Breuer, K., Jin, M., Han, X., Jiang, H., and McLafferty, F. W. (2008) J. Am. Soc. Mass Spectrom. 19, 1045–1053
13. Marouga, R., David, S., and Hawkins, E. (2005) Anal. Bioanal. Chem. 382, 669–678
14. Tannu, N. S., and Hemby, S. E. (2006) Nat. Protoc. 1, 1732–1742
15. Fenn, J. B., Mann, M., Meng, C. K., Wong, S. F., and Whitehouse, C. M. (1989) Science 246, 64–71
16. Tanaka, K., Waki, H., Ido, Y., Akita, S., Yoshida, Y., Yoshida, T., and Matsu, T. (1988) Rapid Commun. Mass Spectrom. 2, 151–153
17. Karas, M., and Hillenkamp, F. (1988) Anal. Chem. 60, 2299–2301
18. Wang, H., and Hanash, S. (2005) Mass Spectrom. Rev. 24, 413–426
19. Yates, J. R., Ruse, C. I., and Nakorchevsky, A. (2009) Annu. Rev. Biomed. Eng. 11, 49–79
20. Tran, J. C., and Doucette, A. A. (2008) J. Proteome Res. 7, 1761–1766
21. Tran, J. C., and Doucette, A. A. (2008) Anal. Chem. 80, 1568–1573
22. Lee, S. W., Berger, S. J., Martinovic, S., Pasa-Tolić, L., Andersson, G. A., Shen, Y., Zhao, R., and Smith, R. D. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 5942–5947
23. Lee, J. E., Kelleie, J. F., Tran, J. C., Tipton, J. D., Catherman, A. D., Thomas, H. M., Ahlf, D. R., Durbin, K. R., Vellaiachamy, A., Ntai, I., Marshall, A. G., and Kelleher, N. L. (2009) J. Am. Soc. Mass Spectrom. 20, 2183–2191
24. Li, W., Hendrickson, C. L., Emmett, M. R., and Marshall, A. G. (1999) Anal. Chem. 71, 4397–4402
25. Mohr, J., Swart, R., Samonig, M., Böhm, G., and Huber, C. G. (2010) Proteomics 10, 3598–3609
26. Patton, W. F. (2002) J. Chromatogr. B 771, 3–31
