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Electron Detachment Dissociation and Negative Ion Infrared Multiphoton Dissociation of Electrosprayed Intact Proteins

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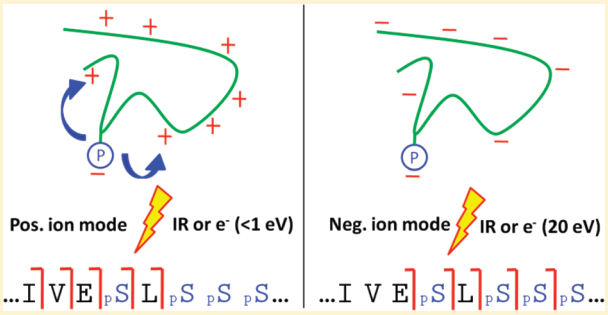
Supporting Information

ABSTRACT: In top-down proteomics, intact gaseous proteins are fragmented in a mass spectrometer by, e.g., electron capture dissociation (ECD) to obtain structural information. By far, most top-down approaches involve dissociation of protein cations. However, in electrospray ionization of phosphoproteins, the high acidity of phosphate may contribute to the formation of intramolecular hydrogen bonds or salt bridges, which influence subsequent fragmentation behavior. Other acidic proteins or proteins with regions containing multiple acidic residues may also be affected similarly. Negative ion mode, on the other hand, may enhance deprotonation and unfolding of multiply phosphorylated or highly acidic protein regions. Here, activated ion electron detachment dissociation (AI-EDD) and negative ion infrared multiphoton dissociation (IRMPD) were employed to investigate the fragmentation of intact proteins, including multiply phosphorylated β-casein, calmodulin, and glycosylated ribonuclease B. Compared to AI-ECD and positive ion IRMPD, AI-EDD and negative ion IRMPD provide complementary protein sequence information, particularly in regions with high acidity, including the multiply phosphorylated region of β-casein.



The development of modern mass spectrometers together with the rapid advances in genome sequencing have enabled the field of proteomics. Two complementary approaches, i.e.,"bottom-up"[1] and "top-down"[2,3] analysis have evolved for proteomic research. The more widely utilized bottom-up approach targets peptides with masses less than a few kDa. Such peptide mixtures are either naturally present or generated by protease digestion. Top-down proteomics, on the other hand, involves introduction of undigested proteins into the mass spectrometer. Masses of whole proteins are measured and further dissociation of protein ions (tandem mass spectrometry or MS/MS) provides structural information. Several MS/MS strategies are employed in top-down proteomics, including collision activated dissociation (CAD),[4−9] infrared multiphoton dissociation (IRMPD),[10] blackbody infrared radiative dissociation (BIRD),[11,12] electron capture dissociation (ECD),[13−16] and electron transfer dissociation (ETD).[17] The three former techniques are referred to as "slow-heating" methods[18] because activation of precursor ions is achieved by accumulation of energy from multiple collisions with collision gas molecules or from absorption of multiple infrared photons. Due to intramolecular vibrational energy redistribution (IVR),[19] vibrational energy gained from collisions or from photon absorption is redistributed to thermodynamically favored fragmentation pathways. Thus, facile losses of labile post-translational modifications (PTMs) frequently dominate MS/MS spectra of peptides.[20] For example, for peptides phosphorylated on serine or threonine, abundant loss of phosphate and/or phosphoric acid is observed.[21,22] There are examples showing that labile PTM loss is less frequent when intact proteins (>9 kDa) are fragmented.[23,24] This difference between bottom-up and top-down analysis may be due to gas-phase protein higher-order structures that stabilize PTMs and thus favor fragmentation at backbone amide bonds rather than PTM loss.[25] In contrast to vibrational activation of even-electron protein ions, ECD involves gas-phase radical ion chemistry[13,26] following capture of low-energy electrons (<2 eV) by multiply protonated peptide and protein ions. Backbone N−C_α bonds are cleaved to produce c- and $z^\bullet$-type ions without loss of PTMs.[27−29] Analogous fragmentation behavior is observed in electron transfer dissociation (ETD), which also involves radical intermediates but from ion−ion reactions.[30]

Phosphorylation is one of the most common PTMs and plays critical roles in various biological processes such as cell signaling.[31] Top-down proteomics provides benefits in the characterization of phosphoproteins, including determination of phosphorylation sites and possible quantification of site-specific phosphorylation.[32,33] ECD and ETD, in particular, provide extensive backbone cleavages without phosphate or phosphoric acid loss.[28,30,34]

To date, the vast majority of top-down approaches involves positive ion mode in which proteins are typically electrosprayed in acidified solvent. Protein N-termina and basic amino acid residues are protonated or become protonated during the

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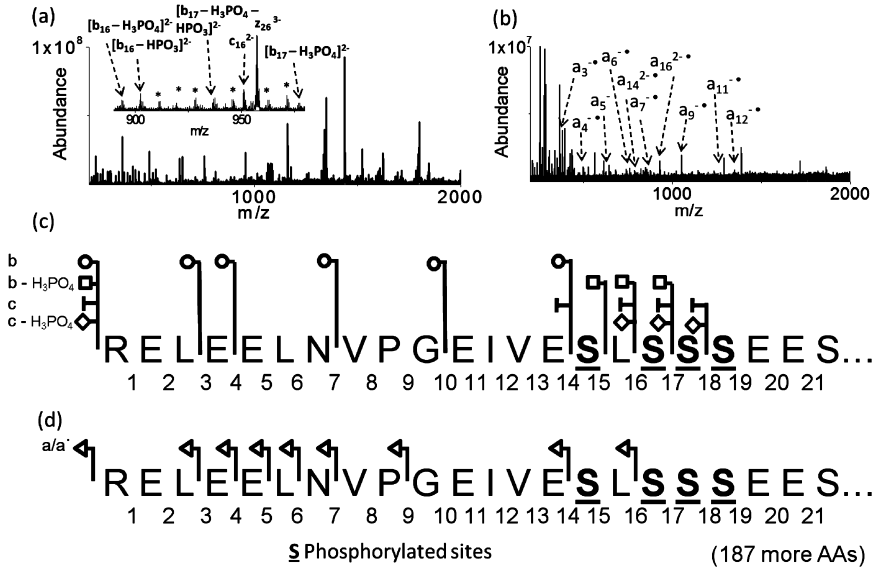

Figure 1. (a) Negative ion IRMPD of all observed charge states (15- to 26-) of β-casein anions generated by electrospray ionization. Inset: partial IRMPD spectrum. Noise peaks are labeled with asterisks. (b) AI-EDD of all observed charge states of β-casein anions generated by electrospray ionization. Only *a*-type fragments are labeled. Spectra are summed over 256 scans. (c) Fragmentation map from (a). (d) Fragmentation map from (b). Only the first 22 amino acids are shown.

electrospray process. However, for phosphorylated proteins, the high acidity of phosphate affects the overall protonation following electrospray ionization. Furthermore, salt bridges may form between negatively charged phosphates and protonated groups if favorable steric configurations exist.[35,36] Because ECD at conventional low electron energies does not disrupt noncovalent interactions,[37−39] backbone cleavages near phosphorylated sites may not be observed. Lack of such cleavages is unfortunate because they are frequently required to precisely assign phosphorylation sites. Higher electron energy can improve ECD efficiency, presumably by disrupting noncovalent bonds and unfolding peptide/protein gas-phase structure.[36] Alternatively, an infrared laser pulse or collisions with neutral gas before or after electron irradiation may unfold the gas-phase protein structure and consequently increase ECD backbone cleavage coverage, an approach termed "activated ion" ECD (AI-ECD).[40,41] In "plasma" ECD,[42] protonated protein cations are activated by gas collisions. In addition, electrons are decelerated and forced to travel in the same direction as the precursor ions to maximize the cross-section of the ion-electron reaction. However, even after AI-ECD or plasma ECD, backbone cleavages near phosphorylated sites are scarcer than in nonphosphorylated protein regions.[42]

Negative ion mode, in which proteins are typically electrosprayed in basic solvent, promotes deprotonation of protein C-termina, acidic PTMs, acidic amino acid residues, and to a certain extent, backbone amides. In regions with multiple acidic PTMs or acidic amino acid residues, electrostatic forces may contribute to unfolding of the local noncovalent tertiary structure, thus potentially enhancing the observation of backbone cleavages from such regions compared to positive ion mode. Formation of salt bridges may also be circumvented because protonation is suppressed by the basic electrospray solvent. In bottom-up approaches, CAD and IRMPD have been demonstrated to generate useful structural information for deprotonated peptides.[43−47] However, vibrational activation of peptide anions typically results in abundant internal fragments, neutral losses, and side chain losses, in addition to the backbone cleavages that dominate in positive ion mode.[45,47] Thus, MS/MS spectra are more complex, and less useful structural information is generated in negative ion mode. Electron detachment dissociation (EDD)[48] operates in negative ion mode and involves higher electron energy (>10 eV) than ECD. EDD has been shown to yield mainly $a^{\bullet}$- and *x*-type product ions for peptides and proteins,[48−50] mainly *d*- and *w*-type ions for nucleic acids,[51−54] and extensive glycosidic and cross-ring fragments for oligosaccharides.[55,56] However, one drawback of EDD of polypeptides is its low fragmentation efficiency, compared to other fragmentation methods.[57]

Top-down analysis of large biomolecules in negative ion mode has only recently begun to be explored. Taucher and Breuker reported ∼90% sequence coverage for an 18.2 kDa ribonucleic acid in negative ion CAD[52] and McLuckey and co-workers demonstrated ∼60% sequence coverage for a 25 kDa intact tRNA.[58] Very recently, Breuker and co-workers explored EDD for top-down protein analysis and reported ∼19% sequence coverage for an acidic 147-residue protein.[50] However, to our knowledge, negative ion top-down analysis of proteins with acidic modifications, including phosphorylation and glycosylation, has not previously been explored. Here, we employ negative ion IRMPD and activated ion electron detachment dissociation (AI-EDD) for top-down analysis of three proteins, including multiply phosphorylated β-casein, the acidic protein calmodulin, and glycosylated ribonuclease B.

INFRARED MULTIPHOTON DISSOCIATION OF β-CASEIN ANIONS

Figure 1a shows negative ion IRMPD of β-casein anions. Intact β-casein was electrosprayed at 0.1 mg/mL from a solution containing 1:1 v/v water/acetonitrile with 0.25% piperidine, and all charge states were irradiated with a 180 ms laser pulse (7.5 W). IRMPD of the individual charge states 15^- and 22^- yielded virtually identical spectra but with lower signal-to-noise ratio than IRMPD of all charge states. N-terminal *b*- and *c*-type ions dominate the IRMPD spectrum, as summarized in Figure 1c, but extensive internal fragments were also observed. The

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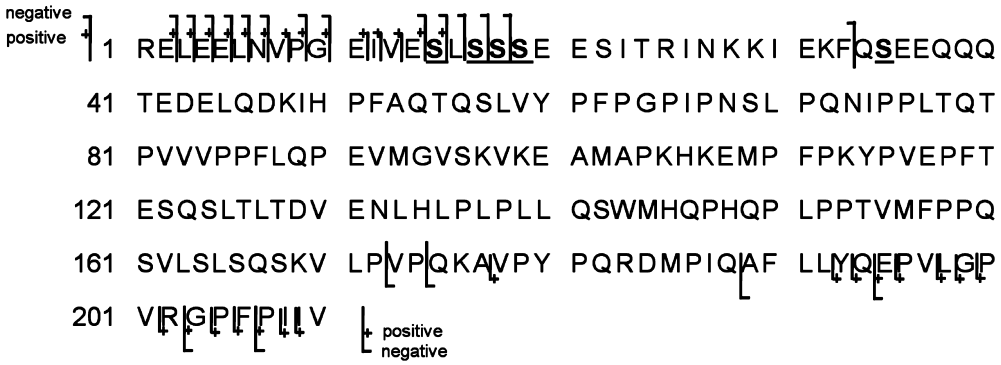

Figure 2. Comparison of β-casein fragmentation patterns in negative and positive ion mode. All backbone cleavages from AI-EDD and negative ion IRMPD are labeled "negative", and all backbone cleavages from AI-ECD and positive ion IRMPD are labeled "positive".

overall sequence coverage was 7% (15 backbone cleavages out of 208). However, extensive fragmentation was observed within the region Glu-14 to Glu-21, which contains seven acidic or phosphorylated side chains out of eight amino acids. Glu-14 to Glu-21 has a pI of 1.6 as predicted by Scansite Molecular Weight and Isoelectric Point Calculator (http://scansite.mit.edu/cgi-bin/calcpi). Partial phosphate loss is observed for both *b*- and *c*-type ions. Phosphorylation sites at Ser-15, Ser-17, Ser-18, and Ser-19 are clearly identified by *c*-type fragments and a combination of *b*- and *b*-H_3PO_4-type ions also provides identification of phosphorylation sites at Ser-15, Ser-17, and Ser-18.

ACTIVATED ION ELECTRON DETACHMENT DISSOCIATION OF β-CASEIN ANIONS

AI-EDD of β-casein anions is shown in Figure 1b. All observed charge states were irradiated with a 90 ms IR laser pulse (7.5 W), followed by a beam of ~19 eV electrons for 1s. AI-EDD of β-casein yields predominantly *a*-type radical ions corresponding to backbone cleavage close to the N-terminus, as shown in Figure 1d. The EDD fragmentation mechanism for peptides has been investigated by Kjeldsen, et al. and Anusiewicz, et al. by computational approaches.[59,60] In their work, it was proposed that negative charges are located on backbone nitrogen atoms and that electron ejection occurs from such deprotonated backbone nitrogens. These authors showed that formation of $a/x^{\bullet}$ product ions is both thermodynamically and kinetically less favored than formation of $a^{\bullet}/x$ product ions. In a recent investigation of protein, EDD $a/x^{\bullet}$ product ions were not reported.[50] However, even-electron ions a_4, and a_9 and radical $x_2^{\bullet}$ and $x_3^{\bullet}$ ions (not labeled in Figure 1b due to space limitations) were observed in our EDD spectra. One hypothesis is that even-electron *a*-type ions are from hydrogen transfer events, similar to those observed in ECD.[61,62] Another hypothesis is that, even though formation of $a/x^{\bullet}$ product ions is less favored, it is still statistically possible, noting that electron irradiation time in our experiments was 1 s, rather than the 100−300 ms used by Ganisl et al.[50] Neutral CO_2 loss, which was reported as a major fragmentation pathway by Ganisl et al., was not observed in our experiments, even for product ions with terminal glutamic acid residues. However, the product ion abundance in our experiments was rather low, and thus, fragments corresponding to CO_2 loss may be below our detection limit. Ganisl et al. also reported that $a^{\bullet}/x$ product ion formation was facilitated by neighboring basic residues. However, the fragments observed in our study did not result from cleavage near lysine or arginine. Compared to negative ion IRMPD, AI-EDD provides less sequence coverage, ~5% (10/208 backbone cleavages observed). However, the total number of peaks in the AI-EDD spectrum corresponds to 28% of the number of peaks (with an S/N ratio >2) in the IRMPD spectrum. Thirteen percent of the peaks in the AI-EDD spectrum are sequence informative compared to 5% in IRMPD. Thus, AI-EDD results are more facile to interpret. The simplicity of EDD spectra and the specificity of *a*-type backbone cleavage appear advantageous compared to IRMPD although EDD fragmentation efficiency is low.

COMPARISON BETWEEN NEGATIVE AND POSITIVE ION MODE

Positive ion IRMPD and AI-ECD were applied to β-casein for comparison with the negative ion results. Positive ion IRMPD cleaved 21 backbone amide bonds, with extensive internal fragments, water losses, and phosphoric acid losses. Under our instrumental conditions, AI-ECD cleaved 26 backbone amine bonds. The combination of IRMPD and AI-ECD provided 29 backbone cleavages. Previously reported AI-ECD and plasma ECD of intact β-casein provided 87 backbone cleavages.[34] In negative ion mode, the combination of AI-EDD and IRMPD yielded 20 backbone cleavages. As shown in Figure 2, AI-EDD and negative ion IRMPD provided nine unique backbone cleavages compared to AI-ECD and positive ion IRMPD. In particular, negative ion IRMPD yielded extensive fragmentation within the highly acidic region Glu-14 to Glu-21. Four phosphorylated sites were directly identified by backbone cleavages in this region. The latter analysis was not feasible in positive ion mode with our instrument, nor from previously published AI-ECD.[34] Plasma ECD data[42] located all phosphorylation sites; however, it did not provide backbone cleavages between each phosphorylated residue.

Calmodulin (pI = 4.0) and glycosylated ribonuclease B (RNaseB, pI = 8.6) were also investigated in both positive and negative ion top down MS/MS. For calmodulin, AI-ECD and positive ion IRMPD provided 40 backbone cleavages out of 148 possible peptide bonds. AI-EDD and negative ion IRMPD yielded an additional six backbone cleavages, as shown in Figure S1 (Supporting Information). For RNase B, AI-ECD and positive ion IRMPD provided 41 backbone cleavages out of 124 possible peptide bonds whereas AI-EDD and negative ion IRMPD yielded an additional two backbone cleavages, as shown in Figure S2 (Supporting Information). Intact RNase B was previously examined with ion trap-CAD in negative ion mode by Chrisman and McLuckey.[63] This protein contains four disulfide bonds (C26−C84, C40−C95, C58−C110, and C65−

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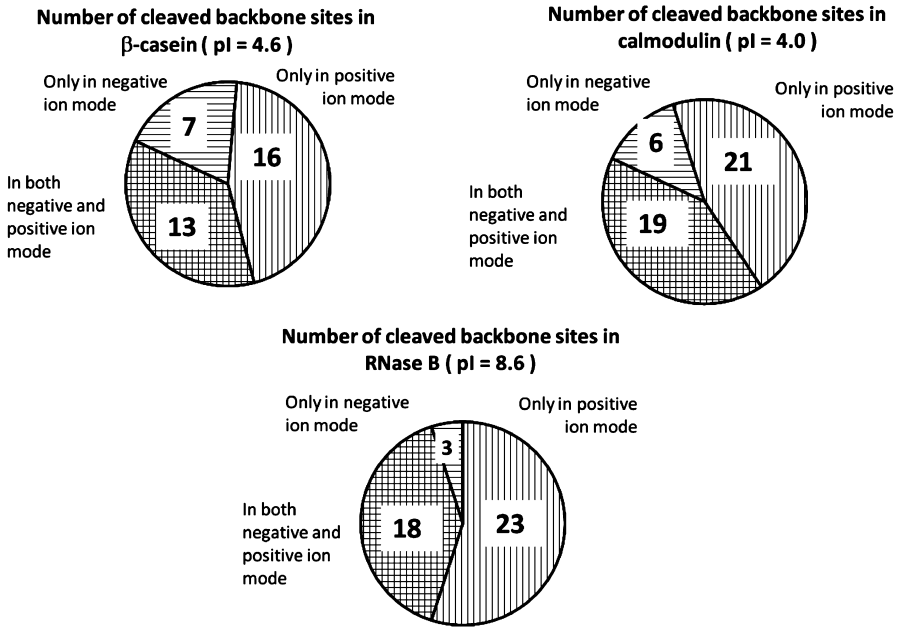

Figure 3. Comparison of the numbers of backbone cleavages in negative and positive ion mode top down MS/MS of (a) β-casein; (b) calmodulin; and (c) RNase B.

C72) and one glycosylated amino acid (N34). In the negative ion CAD spectrum, major product ions corresponded to c/z-type ions near cysteines, including five out of eight possible backbone bond cleavages N-terminal to cysteine. Backbone fragments retaining the glycan were also observed. In contrast, we only observed one backbone bond cleavage N-terminal to cysteine (between Y25 and C26) following negative ion IRMPD. The observed y_{28} and y_{29} ions indicate that the C58−C110 disulfide bond was cleaved whereas glycan retention could not be evaluated due to low sequence coverage. The observed differences between previously reported negative ion CAD and our negative ion IRMPD may be due to secondary fragmentation in the latter technique: first generation product ions remain in the laser beam and can become further activated whereas they are off-resonance in ion trap-CAD and thus do not further dissociate. In our IRMPD spectrum, only 4% of the peaks (S/N > 2) are sequence informative.

A summary of observed negative and positive ion mode fragmentation for all three investigated proteins is shown in Figure 3. For β-casein and calmodulin, which are both acidic proteins, negative ion dissociation, particularly IRMPD, provides more complementary sequence information than for the basic protein RNase B. AI-EDD of β-casein, calmodulin, and RNase B yielded similar numbers of backbone $a^{\bullet}/x$ ions. In all three cases, backbone cleavages were mainly observed close to the protein termini. Kjeldsen et al. reported that EDD backbone cleavages in peptides are favored near acidic residues.[60] In contrast, such a preference was not observed in EDD of the proteins ubiquitin and melittin.[50] In our work, the largest $a^{\bullet}$ ion observed from β-casein was $a^{\bullet}_{16}$. For this protein, 11 of the first 16 (i.e., 69%) possible backbone cleavages, counting from the N-terminus, represent cleavage N- or C-terminal to acidic residues (D, E, and pS). Among the observed eight backbone cleavages in this region, five (i.e., 63%) represent backbone cleavage close to acidic residues. For calmodulin, 62% of the first 13 possible backbone cleavages are close to acidic residues and, among the observed nine backbone cleavages, 56% are close to acidic residues. For RNase B, 50% of the first 16 possible backbone cleavages are close to acidic residues and, among the observed nine backbone cleavages, 33% are close to acidic residues. Thus, for these proteins, a cleavage preference near acidic residues was not apparent. However, a general analysis was hampered by the low sequence coverage. As for the previously mentioned proposed favored cleavage near basic residues,[50] all three proteins investigated here contain similar total numbers of K and R residues: 15, 14, and 14, respectively. The total numbers of $a^{\bullet}/x$-type backbone bond cleavages were similar as well (10, 9, and 10, respectively). Further, the N-terminal regions of these three protein sequences contain few K and R residues. Thus, a clear correlation between $a^{\bullet}/x$ cleavage and the presence of basic residues is difficult to derive.

CONCLUSIONS

Top-down protein analysis in negative ion mode was investigated by employing two dissociation techniques on three intact proteins. In general, negative ion IRMPD and AI-EDD provided complementary sequence information compared to positive ion IRMPD and AI-ECD. However, less overall sequence coverage was seen in negative ion mode. More complementary fragments were observed in negative ion mode for the acidic proteins β-casein and calmodulin than for the basic protein RNase B. A possible explanation for this observation may be that salt-bridge formation, which can reduce the observation of backbone fragments, is favored in positive ion mode for acidic proteins. In particular, IRMPD of β-casein, with multiple phosphorylated sites close to each other, yielded backbone cleavages between the first four phosphorylated amino acids from the N-terminus. Such cleavages, which are essential to precisely locate phosphorylation sites, were not observed in positive ion IRMPD nor AI-ECD but were partially observed in previous plasma ECD.[42]

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■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Chait, B. T. *Science* **2006**, *314*, 65−66.
(2) Kelleher, N. L.; Lin, H. Y.; Valaskovic, G. A.; Aaserud, D. J.; Fridriksson, E. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **1999**, *121*, 806−812.
(3) McLafferty, F. W.; Fridriksson, E. K.; Horn, D. M.; Lewis, M. A.; Zubarev, R. A. *Science* **1999**, *284*, 1289−1290.
(4) Loo, J. A.; Edmonds, C. G.; Smith, R. D. *Science* **1990**, *248*, 201−204.
(5) Smith, R. D.; Loo, J. A.; Barinaga, C. J.; Edmonds, C. G.; Udseth, H. R. *J. Am. Soc. Mass. Spectrom.* **1990**, *1*, 53−65.
(6) Stephenson, J. L.; McLuckey, S. A. *Anal. Chem.* **1998**, *70*, 3533−3544.
(7) Stephenson, J. L.; Cargile, B. J.; McLuckey, S. A. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 2040−2048.
(8) Reid, G. E.; Wu, J.; Chrisman, P. A.; Wells, J. M.; McLuckey, S. A. *Anal. Chem.* **2001**, *73*, 3274−3281.
(9) Loo, J. A.; Quinn, J. P.; Ryu, S. I.; Henry, K. D.; Senko, M. W.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 286−289.
(10) Little, D. P.; Speir, J. P.; Senko, M. W.; Oconnor, P. B.; McLafferty, F. W. *Anal. Chem.* **1994**, *66*, 2809−2815.
(11) Price, W. D.; Schnier, P. D.; Williams, E. R. *Anal. Chem.* **1996**, *68*, 859−866.
(12) Ge, Y.; Horn, D. M.; McLafferty, F. W. *Int. J. Mass Spectrom.* **2001**, *210*, 203−214.
(13) Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *J. Am. Chem. Soc.* **1998**, *120*, 3265−3266.
(14) Zubarev, R. A.; Horn, D. M.; Fridriksson, E. K.; Kelleher, N. L.; Kruger, N. A.; Lewis, M. A.; Carpenter, B. K.; McLafferty, F. W. *Anal. Chem.* **2000**, *72*, 563−573.
(15) McLafferty, F. W.; Horn, D. M.; Breuker, K.; Ge, Y.; Lewis, M. A.; Cerda, B.; Zubarev, R. A.; Carpenter, B. K. *J. Am. Soc. Mass. Spectrom.* **2001**, *12*, 245−249.
(16) Ge, Y.; Lawhorn, B. G.; ElNaggar, M.; Strauss, E.; Park, J. H.; Begley, T. P.; McLafferty, F. W. *J. Am. Chem. Soc.* **2002**, *124*, 672−678.
(17) Coon, J. J.; Ueberheide, B.; Syka, J. E. P.; Dryhurst, D. D.; Ausio, J.; Shabanowitz, J.; Hunt, D. F. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 9463−9468.
(18) McLuckey, S. A.; Goeringer, D. E. *J. Mass Spectrom.* **1997**, *32*, 461−474.
(19) Stannard, P. R.; Gelbart, W. M. *J. Phys. Chem.* **1981**, *85*, 3592−3599.
(20) McLachlin, D. T.; Chait, B. T. *Curr. Opin. Chem. Biol.* **2001**, *5*, 591−602.
(21) Carr, S. A.; Huddleston, M. J.; Annan, R. S. *Anal. Biochem.* **1996**, *239*, 180−192.
(22) Annan, R. S.; Carr, S. A. *Anal. Chem.* **1996**, *68*, 3413−3421.
(23) Meng, F. Y.; Cargile, B. J.; Miller, L. M.; Forbes, A. J.; Johnson, J. R.; Kelleher, N. L. *Nat. Biotechnol.* **2001**, *19*, 952−957.
(24) Reid, G. E.; Stephenson, J. L.; McLuckey, S. A. *Anal. Chem.* **2002**, *74*, 577−583.
(25) Siuti, N.; Kelleher, N. L. *Nat. Methods* **2007**, *4*, 817−821.
(26) Kruger, N. A.; Zubarev, R. A.; Carpenter, B. K.; Kelleher, N. L.; Horn, D. M.; McLafferty, F. W. *Int. J. Mass Spectrom.* **1999**, *182*, 1−5.
(27) Kelleher, R. L.; Zubarev, R. A.; Bush, K.; Furie, B.; Furie, B. C.; McLafferty, F. W.; Walsh, C. T. *Anal. Chem.* **1999**, *71*, 4250−4253.
(28) Stensballe, A.; Jensen, O. N.; Olsen, J. V.; Haselmann, K. F.; Zubarev, R. A. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1793−1800.
(29) Hakansson, K.; Cooper, H. J.; Emmett, M. R.; Costello, C. E.; Marshall, A. G.; Nilsson, C. L. *Anal. Chem.* **2001**, *73*, 4530−4536.
(30) Syka, J. E. P.; Coon, J. J.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 9528−9533.
(31) Hunter, T. *Cell* **2000**, *100*, 113−127.
(32) Mann, M.; Ong, S. E.; Gronborg, M.; Steen, H.; Jensen, O. N.; Pandey, A. *Trends Biotechnol.* **2002**, *20*, 261−268.
(33) Breuker, K.; Jin, M.; Han, X. M.; Jiang, H. H.; McLafferty, F. W. *J. Am. Soc. Mass. Spectrom.* **2008**, *19*, 1045−1053.
(34) Shi, S. D. H.; Hemling, M. E.; Carr, S. A.; Horn, D. M.; Lindh, I.; McLafferty, F. W. *Anal. Chem.* **2001**, *73*, 19−22.
(35) Woods, A. S.; Ferre, S. *J. Proteome. Res.* **2005**, *4*, 1397−1402.
(36) Creese, A. J.; Cooper, H. F. *J. Am. Soc. Mass. Spectrom.* **2008**, *19*, 1263−1274.
(37) Haselmann, K. F.; Jorgensen, T. J. D.; Budnik, B. A.; Jensen, F.; Zubarev, R. A. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 2260−2265.
(38) Jackson, S. N.; Dutta, S.; Woods, A. S. *J. Am. Soc. Mass. Spectrom.* **2009**, *20*, 176−179.
(39) Xie, Y. M.; Zhang, J.; Yin, S.; Loo, J. A. *J. Am. Chem. Soc.* **2006**, *128*, 14432−14433.
(40) Horn, D. M.; Ge, Y.; McLafferty, F. W. *Anal. Chem.* **2000**, *72*, 4778−4784.
(41) Chalmers, M. J.; Hakansson, K.; Johnson, R.; Smith, R.; Shen, J. W.; Emmett, M. R.; Marshall, A. G. *Proteomics* **2004**, *4*, 970−981.
(42) Sze, S. K.; Ge, Y.; Oh, H. B.; McLafferty, F. W. *Anal. Chem.* **2003**, *75*, 1599−1603.
(43) Steinborner, S. T.; Bowie, J. H. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1243−1247.
(44) Bowie, J. H.; Brinkworth, C. S.; Dua, S. *Mass Spectrom. Rev.* **2002**, *21*, 87−107.
(45) Ewing, N. P.; Cassady, C. J. *J. Am. Soc. Mass. Spectrom.* **2001**, *12*, 105−116.
(46) Kalli, A.; Hakansson, K. *Int. J. Mass Spectrom.* **2007**, *263*, 71−81.
(47) Flora, J. W.; Muddiman, D. C. *Anal. Chem.* **2001**, *73*, 3305−3311.
(48) Budnik, B. A.; Haselmann, K. F.; Zubarev, R. A. *Chem. Phys. Lett.* **2001**, *342*, 299−302.
(49) Haselmann, K. F.; Budnik, B. A.; Kjeldsen, F.; Nielsen, M. L.; Olsen, J. V.; Zubarev, R. A. *Eur. J. Mass Spectrom.* **2002**, *8*, 117−121.
(50) Ganisl, B.; Valovka, T.; Hartl, M.; Taucher, M.; Bister, K.; Breuker, K. *Chem.—Eur. J.* **2011**, *17*, 4460−4469.
(51) Yang, J.; Mo, J. J.; Adamson, J. T.; Hakansson, K. *Anal. Chem.* **2005**, *77*, 1876−1882.
(52) Taucher, M.; Breuker, K. *J. Am. Soc. Mass. Spectrom.* **2010**, *21*, 918−929.
(53) Yang, J.; Hakansson, K. *J. Am. Soc. Mass. Spectrom.* **2006**, *17*, 1369−1375.
(54) Mo, J. J.; Hakansson, K. *Anal. Bioanal. Chem.* **2006**, *386*, 675−681.
(55) Wolff, J. J.; Amster, I. J.; Chi, L. L.; Linhardt, R. J. *J. Am. Soc. Mass. Spectrom.* **2007**, *18*, 234−244.
(56) Adamson, J. T.; Hakansson, K. *J. Am. Soc. Mass. Spectrom.* **2007**, *18*, 2162−2172.
(57) Kweon, H. K.; Hakansson, K. *J. Proteome. Res.* **2008**, *7*, 749−755.
(58) Huang, T. Y.; Liu, J. A.; McLuckey, S. A. *J. Am. Soc. Mass. Spectrom.* **2010**, *21*, 890−898.
(59) Anusiewicz, I.; Jasionowski, M.; Skurski, P.; Simons, J. *J. Phys. Chem. A* **2005**, *109*, 11332−11337.
(60) Kjeldsen, F.; Silivra, O. A.; Ivonin, I. A.; Haselmann, K. F.; Gorshkov, M.; Zubarev, R. A. *Chem.—Eur. J.* **2005**, *11*, 1803−1812.

(61) O'Connor, P. B.; Lin, C.; Cournoyer, J. J.; Pittman, J. L.; Belyayev, M.; Budnik, B. A. *J. Am. Soc. Mass. Spectrom.* **2006**, *17*, 576–585.
(62) Savitski, M. M.; Kjeldsen, F.; Nielsen, M. L.; Zubarev, R. A. *J. Am. Soc. Mass. Spectrom.* **2007**, *18*, 113–120.
(63) Chrisman, P. A.; McLuckey, S. A. *J. Proteome. Res.* **2002**, *1*, 549–557.