Construction of a Hybrid Quadrupole/Fourier Transform Ion Cyclotron Resonance Mass Spectrometer for Versatile MS/MS Above 10 kDa

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Technological advancements including an open-cylindrical Penning trap with capacitively coupled ICR cell, selective ion accumulation with a resolving quadrupole, and a voltage gradient used during ion extraction from an octopole ion trap, have individually improved dynamic range and sensitivity in Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). Documented here is a new instrument utilizing these technologies toward the robust detection and fragmentation of biomolecules >10 kDa. Up to 55-fold enhancement in ion population by selective ion accumulation combined with 10- to 20- fold signal-to-noise improvement by application of a DC voltage gradient to an accumulation octopole during the ion transfer event offers improved signal-to-noise (or speed) of MS/MS experiments, for proteins from *Methanococcus jannaschii* and *Saccharomyces cerevisiae* whole cell lysates. After external quadrupole filtering with a 40 *m/z* window, three proteins were fragmented (and identified) in parallel from the database of *Methanococcus jannaschii*. Electron capture dissociation (ECD) of an intact yeast protein provides extensive sequence information resulting in a high degree of localization for an N-terminal acetylation. Hybrid fragmentation, infrared multiphoton dissociation (IRMPD) followed by low energy electrons (ECD), with the electron source located laterally off the z-axis and external to the magnet bore, presents a strategy for identification of proteins by means of the sequence tag approach. Automated implementation of diverse MSⁿ approaches in a Q-FTMS instrument promises to help realize "top-down" proteomics in the future. (J Am Soc Mass Spectrom 2004, 15, 1099–1108) © 2004 American Society for Mass Spectrometry

Mass spectrometers with increasing resolving power and sensitivity have been devised over the past 20 years to analyze ions created by electrospray ionization (ESI) [1] and matrix-assisted laser desorption ionization (MALDI) [2], as recently reviewed [3]. Since its introduction in late 1973, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has emerged as a performance leader with illustrations of low attomole sensitivity for proteins [4],

8×10^6 resolving power at 8.6 kDa [5], MS/MS above 10 kDa [6], and measurement of thousands of components as a complex mixture [7, 8].

In the mid- to late 1990s, major performance boosts for FT-ICR MS resulted from access to higher magnetic field [9, 10]. Additional advances included external ion accumulation [11], insertion of an external quadrupole for mass selection prior to ion accumulation and detection [12–14], and application of a dc voltage gradient to facilitate ion transfer to the ICR cell [12, 15]. Dissociation techniques such as collision-assisted dissociation (CAD) [16], infrared multiphoton dissociation (IRMPD) [17], and electron capture dissociation (ECD) [18] can provide extensive backbone bond cleavage for proteins [19] and DNA oligomers [20] for

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Figure 1. Schematic representation of the quadrupole/Fourier transform ion cyclotron resonance hybrid mass spectrometer designed for versatile MS/MS and improved dynamic range by means of m/z -selective ion accumulation external to the superconducting magnet bore.

highly efficient analysis of biomolecules in a “top-down” fashion [21, 22].

Here we describe the design and characterization of a quadrupole/FT-ICR mass spectrometer hybrid (Q-FTMS), incorporating an external quadrupole mass filter [12–14] with a 9.4 tesla actively-shielded magnet, a dc gradient external accumulation octopole [15], and an open-ended capacitively coupled cylindrical cell divided axially into five segments. These features allow for order-of-magnitude increases in dynamic range or experimental speed during top-down sequence analysis of intact proteins from *Methanococcus jannaschii* and *Saccharomyces cerevisiae*. Also, a dispenser cathode displaced from the magnetic field (z -) axis for sequential IRMPD and ECD facilitates hybrid MS³ fragmentation modes applied to gas-phase dissociation of intact proteins.

Experimental

The Q-FTMS instrument, which was constructed at the National High Magnetic Field Laboratory (NHMFL), was designed to incorporate a Bruker (Billerica, MA) 9.4 tesla (T) actively shielded magnet. The system design was based largely upon a 9.4 T passively shielded instrument described previously [11, 13]. The vacuum system design layout, created in AutoCAD 2000 (AutoDesk, San Rafael, CA), is shown schematically in Figure 1.

The experimental event sequence, shown in Figure 2a, is controlled by a MIDAS data station [23]. Ions are accumulated [11] in an 18 cm octopole (1.5 MHz at 500 V_{p-p} , –10 V dc offset) after traversing through the quadrupole (ABB Extrel trifilter (Pittsburgh, PA), 20 cm long $\times$ 9.5 mm diameter rods, controlled by a 1.2 MHz 300 Watt QC150 RF power supply, –25 V offset) in either rf-only or mass selection mode. Nitrogen or helium gas (~1 mTorr) facilitates accumulation in this region. For improved transmission through the quadrupole, ions are collisionally axialized in a 20 cm octopole (1.5 MHz at 500 V_{p-p} , –10 V dc offset at 1

mTorr, located behind the skimmer) for 100 ms prior to transfer through the quadrupole. The transfer is then repeated (see Figure 2) as necessary to accumulate the desired ion population. A DC voltage gradient in the accumulation octopole, generated by applying a dc potential of 40–100 V to angled wires placed between adjacent rods of the accumulation octopole, enhances the transfer efficiency of ions into the ICR cell by an order of magnitude [15]. The ICR cell is an open-ended cylinder (38 cm long, 7 cm i.d.) and is divided axially into five segments. The inner trapping electrodes are capacitively coupled (10 nF capacitors and 10 K resistors, with connections outside the vacuum chamber) to the central excitation electrodes [24]. Differential pumping for the vacuum system is accomplished by a variety of roughing and ultrahigh vacuum pumps with ultimate base pressure less than 1.0×10^{-9} Torr at the ICR cell.

For MS/MS or MSⁿ experiments, IRMPD, collisional dissociation in the accumulation region [25, 26], and/or ECD were used. Modified event sequences for octopole dissociation and IRMPD (or ECD) are shown in Figure 2b and c, respectively. For ECD, a dispenser cathode source of low energy electrons (HeatWave Labs, Watsonville, CA) [27] is mounted 20 cm outside the magnet bore and displaced laterally by 1.5 cm from the z -axis to allow the IR beam from a 75 Watt (10.6 μ m) CO₂ laser (Synrad Inc., Mukilteo, WA) to pass on-axis. For optimal electron capture (at 2.5 V on all trapping electrodes), the cathode was heated to ~1000 °C and biased at 2.75 V to induce an electron current of ~80 nA through the cell (measured on conductance limit 5). A grid potential is cycled from +10 V to –10 V to accelerate electrons to the cell. The typical irradiation period was 5–100 ms. Dissociation in the accumulation region was induced by dropping the axial dc offset of the accumulation octopole to <–25 V (at static pressure) during the transfer event from the first octopole. In MS/MS/MS experiments, either CAD or IRMPD was

Figure 2. (a) Experimental event sequence for the Q-FTMS hybrid. Ions are collisionally axialized in the focusing octopole, transported through the quadrupole (rf-only or rf/dc mode), accumulated in the accumulation octopole, and finally transported to the cell for excitation and detection. (b) Lowering the axial offset of the accumulation octopole to <-25 V during the ion accumulation step (versus -10 V during normal accumulation) induces collisional dissociation. (c) IRMPD and ECD occur during the delay between the gated trapping and excitation events.

followed by SWIFT [28] isolation of a fragment ion and then ECD.

Experimental mass peaks lists, generated by a modified version of the THRASH algorithm [29], were analyzed for b, y, c, and z[•] ions and sequence tag information [30, 31] derived either by hand or with custom software for database retrieval and protein characterization available at <http://prosightptm.scs.uiuc.edu/> [32]. The probability scores reported are with 50 ppm fragment ion tolerance, to accommodate externally calibrated ions, with a ± 1000 Da search window around the candidate protein mass to accommodate shifts associated with post-translational modifications. The theoretical masses for b/y and c/z[•] fragment ions were calculated by the Ion Predictor program available in the Prosight PTM software and database suite [32]. Mass accuracies for all data are external calibration based on bovine ubiquitin except where system performance is evaluated, in which case internal calibration is performed. The italicized number located after the reported M_r indicates the mass difference between the reported isotopic peak and the monoisotopic value (in units of 1.00235).

Bovine ubiquitin was obtained from Sigma Aldrich [St. Louis, MO] and dissolved in a 78:20:2 $\text{CH}_3\text{CN}:\text{H}_2\text{O}:\text{CH}_3\text{COOH}$ electrospray solution to a concentration of $\sim 1 \mu\text{M}$. Protein samples from *Methanococcus jannaschii* [33] and *Saccharomyces cerevisiae* (yeast) were produced by two dimensional fractionation of cell lysates by continuous-elution gel electrophoresis (PAGE) with an acid-labile surfactant (ALS) added to facilitate subsequent reversed-phase liquid chromatography (RPLC)

[34]. These ALS-PAGE/RPLC fractions were resuspended in electrospray solution and analyzed with the Q-FTMS described above.

Results and Discussion

Instrument Baseline Performance

The functionality and performance of ESI-FTMS at magnetic fields above 7 Tesla has been well documented [5, 8–11, 23, 35, 36]. Figure 3a reflects the performance of the new instrument for bovine ubiquitin (8.5 kDa). Multiple charge states are observed with a base peak S/N ratio of 851 for a single scan (1 μM solution, 2 s accumulation and 0.3 $\mu\text{L}/\text{min}$ flow rate). Accurate isotopic distribution at a resolving power, $m/\Delta m_{50\%} = 112,000$ (in which $\Delta m_{50\%}$ is the magnitude-mode FT-ICR mass spectral peak full width at half-maximum height) resulted in an average mass accuracy to within 1.5 ppm for the charge states shown.

External Capacitive Coupling

Reduction of axial ejection from the ICR cell results in a 12 $\times$ and 2 $\times$ sensitivity enhancement at 0.5 V and 2.5 V trapping potentials, respectively, for multiple charge states of bovine ubiquitin (Figure 3b). As expected, capacitive coupling gives the greatest improvement in S/N ratio for ions of lower m/z [37]. The integrated (over all charge states) signal for the coupled cell at 0.5 V trapping voltage is comparable to that for the non-

Figure 3. (a) ESI FT mass spectrum of bovine ubiquitin after external capacitive coupling of the excitation and trapping electrodes of the ICR cell. (b) Relative abundance changes for charge states of ubiquitin upon changing trap electrode voltages and capacitive coupling (on or off) during the ion storage and excitation/detection portion of the experimental event sequence. Data were compiled from 10 averaged scans. (c) Mass selection of singly oxidized 11+ charge state of ubiquitin, bottom (4 s accumulation, 1 scan) with the same m/z window in the broadband spectrum, top (0.1 s accumulation, 1 scan). (d) Mass selection of the 18+ charge state of horse heart myoglobin (bottom) with a 100 V axial DC potential applied to angled wires in the accumulation octopole (middle) and with the outermost trapping electrode potentials at 7 V dc in the presence of N_2 collision gas pulsed into the ICR cell during ion transfer from the octopole (top).

coupled signal at 2.5 V trapping voltage. The data shows that at lower trap potentials the ICR detection is limited by axial ejection and thus a capacitively coupled cell will have a lower detection limit ultimately extending its dynamic range. The improvement at lower trapping voltage is beneficial for large biomolecule analysis because high electrostatic trapping potentials can cause closely spaced isotopic peaks to coalesce [38–41].

Q-FTMS Performance Enhancement

The 11+ charge state of singly-oxidized ubiquitin (+16 Da) was chosen to test the m/z selectivity of the quadrupole mass filter. Figure 3c shows a 7.5-fold improvement in signal-to-noise ratio for quadrupole-selected singly-oxidized ubiquitin relative to the same ions transmitted through the same quadrupole in rf-only

Figure 4. Transmission for the mass selected 11+ charge state of bovine ubiquitin as a function of the mass selection window size.

mode. This selection of ions at a resolving power (RP) of ~ 650 is consistent with standard quadrupole performance. Overall transmission efficiency is consistent with results in Figure 4 which shows the absolute signal level for the 11+ charge state of ubiquitin versus the mass selection window size ($\Delta m/z$). A 2 fold difference in signal exists between the $\Delta(m/z) = 5$ and $\Delta(m/z) = 60$ mass selection windows; however, the signal magnitude quickly drops for window size below $\Delta m/z = 5$.

The increased signal measured for the larger $\Delta(m/z)$ windows versus that observed in the broadband spectrum is consistent with samples high in complexity (i.e., chemical noise) and/or S/N. For example, a complex RPLC sample of *Methanococcus jannaschii* proteins was collected and interrogated with the quadrupole in adjacent $\Delta(m/z) = 40$ segments (14 segments total) resulting in an average ~ 24 -fold increase in S/N for proteins (ranging from 4 to >200 -fold) compared to the standard ESI/FTMS configuration for a single scan. For one such $\Delta(m/z) = 40$ window, a 55-fold S/N enhancement reveals more than three times as many isotopic clusters for proteins ranging from 7 to 21 kDa (Figure 5a versus 5b). A total of 22.6 min was required to sample the intact spectrum in 40 m/z segments (as opposed to 3.1 min for the standard experiment, 50 scans); however, with S/N improvements associated with the quadrupole experiment it would require ~ 575 scans (35 min) to achieve similar results from the intact spectrum. The improved dynamic range associated by segmenting the intact spectrum of complex samples is attributed to a reduced ion population in the storage octopole, allowing for more efficient accumulation per unit time for ions of interest as well as reduced multipole fragmentation associated with high ion population [42, 43]. Also, lowering the charge density within the cell (attributed primarily to suppression effects from electrospray when accumulating in the broadband mode) can increase observed S/N (for long detection periods) by reducing Coulombic interaction between ion clouds resulting in damping of the coherent cyclotron motion [38, 44].

Effect of DC Gradient during Ion Extraction from a Multipole Storage Device

Application of a dc voltage to angled wires positioned between adjacent rods of the accumulation octopole generates an axial potential gradient throughout the octopole and improves the efficiency of subsequent capture and trapping in the ICR cell by about an order of magnitude [15]. This observation is shown for mass selected ions for which a ten-fold increase in S/N ratio is achieved for mass-selected 18+ charge state myoglobin ions, Figure 3d (middle). Conversely, an FT-ICR mass spectrum of a given signal-to-noise ratio may be obtained with ~ 10 -fold fewer ions (and thus correspondingly shorter external ion accumulation period).

For optimal sensitivity of mass-selected 13+ and 7+ ions of ubiquitin, we observe a $\sim 300 \mu s$ difference in ion transfer time from the accumulation octopole to the cell. That result agrees with theoretical simulations that predict increased mass discrimination arising from variation in the ejection profile for ions initially located at different axial positions the storage octopole [15], as well as variation in time-of-flight from the octopole to the ICR cell.

S/N Improvement for Mass-Selected Ions in a Five-Segment ICR Cell

Application of a 7 V dc voltage to the outermost trapping electrodes during the gated trapping event increases FT-ICR MS S/N ratio by a factor of ~ 5 with respect to grounded outer electrodes. Collision gas present in the cell (1×10^{-8} torr; 3 s pump down) improved S/N to ~ 10 -fold, Figure 3d (top) during gated trapping with the outer electrodes. Thus, the outer trapping electrodes allow for increased trapping efficiency whereas the collision gas improved detection efficiency by damping the z-axis trapping motion to the center of the cell. Peak splitting and frequency shifts were observed if the outer trapping electrode dc voltages were not lowered to at least that of the inner trapping potentials prior to detection (no pulsed gas; data not shown).

Protein Fragmentation and Identification in Parallel with Notch-Selected Protein Ions

For one of the $\Delta(m/z) = 40$ quadrupole mass-selected segments described above, the spectrum (Figure 5c) revealed 7 quasimolecular species with M_r values from 17–39 kDa. Subsequent fragmentation of all proteins present in the $880 < m/z < 920$ segment resulted in a spectrum (Figure 5d) with 206 distinct fragments. Entry of those fragment masses into the Retriever algorithm of ProSight PTM [32] yields three high scoring proteins with 6, 5, and 10 b/y ions matching three 50S ribosomal proteins (± 15 ppm): MJ0543, MJ0471, and MJ0472, with N-terminal methionine loss observed for the first two. No further identifications with P-scores < 0.01 ($> 99\%$

Figure 5. (a) Expansion of a 60 m/z section segment of the broadband spectrum from a *Methanococcus jannaschii* RPLC fraction (3.7 s scan time; 50 scans). (b) The same $\Delta(m/z) = 60$ segment after quadrupole enhanced ion accumulation ($\Delta(m/z) = 40$ segment, 9.7 s scan time; 10 scans). (c) $\Delta(m/z) = 60$ segment from the same sample with subsequent IRMPD fragmentation of all intact proteins in parallel [shown in (d), 25 scans]. Identified proteins are MJ0543 (X, Exptl. 19,431.8-0 kDa, Theoret. 19432.5-0 kDa), MJ0471 (O, Exptl. 20,511.3-0 kDa, Theoret. 20,511.1-0), and MJ0472 (asterisk, Exptl. 17,263.0-0 kDa, Theoret. 17,263.6-0 kDa).

confidence) could be made. Stepwise progression through a given spectrum of intact proteins could serve to identify most of the constituent proteins in parallel, thereby increasing overall experimental throughput and allowing for inclusion of more fragmentation scans and thus increasing the number of identified proteins from a given initial total amount of protein sample.

Identification of Modified Yeast Proteins by ECD

In one ALS-PAGE/RPLC sample isolated from *S. cerevisiae* cells, ESI-FTMS analysis yielded a single component, 11,596.7-0 (Figure 6a). SWIFT isolation of its 15+ ions followed by ECD gave an MS/MS spectrum (Figure 6b) with 375 resolved isotopic clusters resulting in 123 discrete fragment ion mass values. The “absolute mass” search function [32] in ProSight PTM was able to

tion. IRMPD of SWIFT-isolated ubiquitin 12+ ions in the ICR cell or dissociation in the accumulation region of quadrupole mass-selected ubiquitin 11+ ions produced cleavages at 33 and 57%, respectively, of the potential backbone sights in separate experiments (Figure 7a and c). After IRMPD, the y58⁸⁺ ions were SWIFT isolated for subsequent ECD induced cleavage at 49/57 backbone sites (Figure 7b). After collisional dissociation in the accumulation octopole, ECD for the y24⁴⁺ ion (2.7 kDa) gave complete sequence coverage (Figure 7d). By comparison, ECD of intact ubiquitin typically yields ~50–60% of the possible backbone cleavages with the present instrument (no pulsed gas; data not shown). Thus longer sequence tags are generated by first break-

Figure 7. MSⁿ experiments for electrosprayed intact bovine ubiquitin. (a) IRMPD for the SWIFT-isolated 12+ charge state. (b) ECD for y₅₈⁸⁺ ions SWIFT-isolated by IRMPD in (a). (c) Collisional dissociation in the accumulation octopole of quadrupole-selected 11+ ions. (d) ECD for SWIFT-isolated y₂₄⁴⁺ ions generated in (c).

ing intact proteins into smaller b/y fragments, and then applying ECD. Such MS³ experiments are supported by the hybrid search mode of ProSight PTM through first compiling sequence tags, then fragment ions from MS/MS.

Conclusions

We have constructed an electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer improved by a 5-segmented capacitively coupled ICR

cell, selective ion accumulation with a notch-filtering quadrupole, a voltage gradient during ion extraction from an octopole ion trap, and an analyzer geometry to provide for hybrid IRMPD/ECD MSⁿ in quick succession. These diverse improvements combine to significantly boost the overall performance of FT-ICR MS, especially valuable for extending the throughput and dynamic range of Top Down mass spectrometry. The improved sensitivity, experimental speed, and versatile ion fragmentation will drive efficient analysis of ever more complex mixtures of ever larger biomolecules.

Finally, the hybrid MS³ experiments, including ECD, will improve the likelihood of correct protein assignment by extension of the sequence tag approach to top-down proteomics.

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References

- Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. Electrospray Ionization—Principles and Practice. *Mass Spectrom. Rev.* **1990**, *9*, 37–70.
- Karas, M.; Hillenkamp, F. Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10,000 Daltons. *Anal. Chem.* **1988**, *60*, 2299–2301.
- Kelleher, N. L. From Primary Structure to Function: Biological Insights from Large-Molecule Mass Spectra. *Chem. Biol.* **2000**, *7*, R37–R45.
- Valaskovic, G. A.; Kelleher, N. L.; McLafferty, F. W. Attomole Protein Characterization by Capillary Electrophoresis-Mass Spectrometry. *Science* **1996**, *273*, 1199–1202.
- Shi, S. D.; Hendrickson, C. L.; Marshall, A. G. Counting Individual Sulfur Atoms in a Protein by Ultrahigh-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: Experimental Resolution of Isotopic Fine Structure in Proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11532–11537.
- McLafferty, F. W. High-Resolution Tandem FT Mass-Spectrometry above 10-Kda. *Acc. Chem. Res.* **1994**, *27*, 379–386.
- Hughey, C. A.; Rodgers, R. P.; Marshall, A. G. Resolution of 11,000 Compositionally Distinct Components in a Single Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrum of Crude Oil. *Anal. Chem.* **2002**, *74*, 4145–4149.
- McLafferty, F. W.; Fridriksson, E. K.; Horn, D. M.; Lewis, M. A.; Zubarev, R. A. Biochemistry—Biomolecule Mass Spectrometry. *Science* **1999**, *284*, 1289–1290.
- Marshall, A. G. Milestones in Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Technique Development. *Int. J. Mass Spectrom.* **2000**, *200*, 331–356.
- Marshall, A. G.; Guan, S. H. Advantages of High Magnetic Field for Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1819–1823.
- Senko, M. W.; Hendrickson, C. L.; Emmett, M. R.; Shi, S. D. H.; Marshall, A. G. External Accumulation of Ions for Enhanced Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 970–976.
- Belov, M. E.; Nikolaev, E. N.; Anderson, G. A.; Auberry, K. J.; Harkewicz, R.; Smith, R. D. Electrospray Ionization-Fourier Transform Ion Cyclotron Mass Spectrometry Using Ion Preselection and External Accumulation for Ultrahigh Sensitivity. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 38–48.
- Hendrickson, C. L. Q.; J. P.; Emmett, M. R.; Marshall, A. G. *Proceedings of the 48th ASMS Conference*, Long Beach, CA; June 2000; pp 834–835.
- Brock, A.; Horn, D. M.; Peters, E. C.; Shaw, C. M.; Ericson, C.; Phung, Q. T.; Salomon, A. R. An Automated Matrix-Assisted Laser Desorption/Ionization Quadrupole Fourier Transform Ion Cyclotron Resonance Mass Spectrometer for “Bottom-Up” Proteomics. *Anal. Chem.* **2003**, *75*, 3419–3428.
- Wilcox, B. E.; Hendrickson, C. L.; Marshall, A. G. Improved Ion Extraction from a Linear Octopole Ion Trap: SIMION Analysis and Experimental Demonstration. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 1304–1312.
- Senko, M. W.; Speir, J. P.; McLafferty, F. W. Collisional Activation of Large Multiply-Charged Ions Using Fourier-Transform Mass-Spectrometry. *Anal. Chem.* **1994**, *66*, 2801–2808.
- Little, D. P.; Speir, J. P.; Senko, M. W.; Oconnor, P. B.; McLafferty, F. W. Infrared Multiphoton Dissociation of Large Multiply-Charged Ions for Biomolecule Sequencing. *Anal. Chem.* **1994**, *66*, 2809–2815.
- Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process. *J. Am. Chem. Soc.* **1998**, *120*, 3265–3266.
- Sze, S. K.; Ge, Y.; Oh, H.; McLafferty, F. W. Top-Down Mass Spectrometry of a 29-kDa Protein for Characterization of Any Posttranslational Modification to Within One Residue. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1774–1779.
- Little, D. P.; Aaserud, D. J.; Valaskovic, G. A.; McLafferty, F. W. Sequence Information from 42–108-mer DNAs (Complete for a 50-mer) by Tandem Mass Spectrometry. *J. Am. Chem. Soc.* **1996**, *118*, 9352–9359.
- Kelleher, N. L.; Lin, H. Y.; Valaskovic, G. A.; Aaserud, D. J.; Fridriksson, E. K.; McLafferty, F. W. Top Down Versus Bottom up Protein Characterization by Tandem High-Resolution Mass Spectrometry. *J. Am. Chem. Soc.* **1999**, *121*, 806–812.
- Meng, F. Y.; Cargile, B. J.; Miller, L. M.; Forbes, A. J.; Johnson, J. R.; Kelleher, N. L. Informatics and Multiplexing of Intact Protein Identification in Bacteria and the Archaea. *Nat. Biotechnol.* **2001**, *19*, 952–957.
- Senko, M. W.; Hendrickson, C. L.; PasaTolic, L.; Marto, J. A.; White, F. M.; Guan, S. H.; Marshall, A. G. Electrospray Ionization Fourier Transform Ion Cyclotron Resonance at 9.4 T. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1824–1828.
- Beu, S. C.; Laude, D. A. Elimination of Axial Ejection During Excitation with a Capacitively Coupled Open Trapped-Ion Cell for Fourier-Transform Ion-Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* **1992**, *64*, 177–180.
- Danell, R. M.; Easterling, M. L.; Van Orden, S.; Berg, C. B.; Anderson, J.; Meier, J.; Speir, P. Automated Protein Identification Using Data-Dependent Q-FTMS. *Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics*, Montreal, Canada; June 2003.
- McFarland, M. A.; Hendrickson, C. L.; Marshall, A. G. Ion “Threshing”: Collisionally-Activated Dissociation in an External Octopole Ion Trap by Oscillation of an Axial Electric Potential Gradient. *Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics* Montreal, Canada; June 2003.
- Haselmann, K. F.; Budnik, B. A.; Olsen, J. V.; Nielsen, M. L.; Reis, C. A.; Clausen, H.; Johnsen, A. H.; Zubarev, R. A. Advantages of External Accumulation for Electron Capture Dissociation in Fourier Transform Mass Spectrometry. *Anal. Chem.* **2001**, *73*, 2998–3005.
- Guan, S. H.; Marshall, A. G. Stored Waveform Inverse Fourier Transform (SWIFT) Ion Excitation in Trapped-Ion Mass Spectrometry: Theory and Applications. *Int. J. Mass Spectrom. Ion Processes* **1996**, *158*, 5–37.

29. Horn, D. M.; Zubarev, R. A.; McLafferty, F. W. Automated Reduction and Interpretation of High Resolution Electrospray Mass Spectra of Large Molecules. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 320–332.
30. Mortz, E.; O'Connor, P. B.; Roepstorff, P.; Kelleher, N. L.; Wood, T. D.; McLafferty, F. W.; Mann, M. Sequence Tag Identification of Intact Proteins by Matching Tandem Mass Spectral Data Against Sequence Data Bases. *Proceedings of the National Academy of Sciences of the United States of America*; 1996, **93**, pp 8267–9267.
31. Mann, M.; Wilm, M. Error Tolerant Identification of Peptides in Sequence Databases by Peptide Sequence Tags. *Anal. Chem.* **1994**, *66*, 4390–4399.
32. Taylor, G. K.; Kim, Y. B.; Forbes, A. J.; Meng, F. Y.; McCarthy, R.; Kelleher, N. L. Web and Database Software for Identification of Intact Proteins Using “Top Down” Mass Spectrometry. *Anal. Chem.* **2003**, *75*, 4081–4086.
33. Mukhopadhyay, B.; Johnson, E. F.; Wolfe, R. S. Reactor-Scale Cultivation of the Hyperthermophilic Methanarchaeon *Methanococcus jannaschii* to High Cell Densities. *Applied Environ. Microbiol.* **1999**, *65*, 5059–5065.
34. Meng, F. Y.; Cargile, B. J.; Patrie, S. M.; Johnson, J. R.; McLoughlin, S. M.; Kelleher, N. L. Processing Complex Mixtures of Intact Proteins for Direct Analysis by Mass Spectrometry. *Anal. Chem.* **2002**, *74*, 2923–2929.
35. Kelleher, N. L.; Senko, M. W.; Siegel, M. M.; McLafferty, F. W. Unit Resolution Mass Spectra of 112 kDa Molecules with 3 Da Accuracy. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 380–383.
36. Li, W. Q.; Hendrickson, C. L.; Emmett, M. R.; Marshall, A. G. Identification of Intact Proteins in Mixtures by Alternated Capillary Liquid Chromatography Electrospray Ionization and LC ESI Infrared Multiphoton Dissociation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* **1999**, *71*, 4397–4402.
37. Huang, S. K.; Rempel, D. L.; Gross, M. L. Mass-Dependent Z-Excitation of Ions in Cubic Traps Used in FTMS. *Int. J. Mass Spectrom. Ion Processes* **1986**, *72*, 15–31.
38. Mitchell, D. W.; Smith, R. D. Cyclotron Motion of 2 Coulombically Interacting Ion Clouds with Implications to Fourier-Transform Ion-Cyclotron Resonance Mass-Spectrometry. *Phys. Rev. E* **1995**, *52*, 4366–4386.
39. Pasa-Toli, C. L.; Huang, Y.; Guan, S.; Kim, H. S.; Marshall, A. G. Ultrahigh-Resolution Matrix-Assisted Laser Desorption/Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectra of Peptides. *J. Mass Spectrom.* **1995**, *30*, 825–833.
40. Naito, Y.; Inoue, M. Peak Confluence Phenomenon in Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Soc. Mass Spectrom. Japan.* **1994**, *42*(1), 9.
41. Huang, J. Y.; Tiedemann, P. W.; Land, D. P.; McIver, R. T.; Hemminger, J. C. Dynamics of Ion Coupling in an Ftms Ion-Trap and Resulting Effects on Mass-Spectra, Including Isotope Ratios. *Int. J. Mass Spectrom. Ion Processes* **1994**, *134*, 11–21.
42. Sannes-Lowery, K. A.; Hofstadler, S. A. Characterization of Multipole Storage Assisted Dissociation: Implications for Electrospray Ionization Mass Spectrometry Characterization of Biomolecules. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 1–9.
43. Belov, M. E.; Gorshkov, M. V.; Udseth, H. R.; Smith, R. D. Controlled Ion Fragmentation in a 2-D Quadrupole Ion Trap for External Ion Accumulation in ESI FTICR Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 1312–1319.
44. Li, Y. Z.; Hunter, R. L.; McIver, R. T. Ultrahigh-Resolution Fourier Transform Mass Spectrometry of Biomolecules Above m/z 5000. *Int. J. Mass Spectrom. Ion Processes* **1996**, *158*, 175–188.