ESI-FTICR Mass Spectrometry Employing Data-Dependent External Ion Selection and Accumulation

Richard Harkewicz, Mikhail E. Belov, Gordon A. Anderson, Ljiljana Paša-Tolić, Christophe D. Masselon, David C. Prior, Harold R. Udseth, and Richard D. Smith

The Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland Washington, USA

Data-dependent external *m/z* selection and accumulation of ions is demonstrated in use with ESI-FTICR instrumentation, with two different methods for ion selection being explored. One method uses RF/DC quadrupole filtering and is described in use with an 11.5 tesla (T) FTICR instrument, while the second method employs RF-only resonance dipolar excitation selection and is described in use with a 3.5 T FTICR instrument. In both methods ions are data-dependently selected on the fly in a linear quadrupole ion guide, then accumulated in a second linear RF-only quadrupole trap that immediately follows. A major benefit of ion preselection prior to external accumulation is the enhancement of ion populations for low-level species. This development is expected to expand the dynamic range and sensitivity of FTICR for applications including analysis of complex polypeptide mixtures (e.g., proteomics). (J Am Soc Mass Spectrom 2002, 13, 144–154) © 2002 American Society for Mass Spectrometry

The application of mass spectrometry to the study of large biomolecules has greatly benefited from the incorporation of electrospray ionization (ESI) [1, 2]. Electrospray ionization has the advantage of producing multiply charged ions with minimal fragmentation, placing even the heaviest intact biomolecules within the most effective mass-to-charge (*m/z*) region (approximately 500 to 2000) of most mass spectrometers. These features of ESI combined with the precise mass measurement capabilities, ultra-high resolving power, and high sensitivity of Fourier transform ion cyclotron resonance mass spectrometry (FTICR) [3–5] have resulted in an extremely powerful tool for the analysis of biomolecules. The utility of ESI-FTICR has been even further enhanced through its successful interfacing with on-line liquid chromatography (LC) [6–8] and capillary electrophoresis (CE) [9, 10].

Due to the atmospheric pressure at which ESI sources operate and the ultra-high vacuum requirements for optimal FTICR performance ($\sim 10^{-9}$ torr), the ESI source is typically separated from the ICR cell by several stages of differential pumping, allowing it to be well outside the strong magnetic field of the ICR cell. Electrostatic [11] or RF multipole [12] ion guides are used to transport ions through these stages (and the magnet fringing fields) and into the ICR cell. Used in this mode, either type of ion guide might be described as having a passive role since its function is basically to transfer ions from the source to the cell. Once inside the ICR cell, methods such as gated trapping [13], side-kick trapping [14], and gas-pulse assisted accumulated trapping [15] can be used for capturing the ions.

It would be beneficial to utilize the ion guide of FTICR instruments in a more active role for the accumulation and selection of ions external of the ICR cell. External accumulation converts the continuous mode ESI source into a pulsed ion source, which is more compatible with the sequential nature of an FTICR experiment. Senko *et al*. demonstrated the external accumulation of ions in the RF-only octopole of a dual octopole injection system of an FTICR mass spectrometer [8]. After external accumulation, ions are transferred to the ICR cell and captured using gated trapping, without the necessity of a high pressure gas pulse. Having the external accumulation event occur simultaneously with the ICR excite/detect events can result in a nearly 100% duty cycle. The resulting increased spectrum acquisition rate is especially beneficial for applications involving on-line separations. By comparison, using conventional in-trap gas-assisted accumulation it is possible to miss sample components eluted during higher resolution separation.

Wang et al. [16] recently demonstrated external mass-selective accumulation in the octopole ion guide

Published online December 3, 2001

Address reprint requests to Dr. R. D. Smith, Environmental Molecular Sciences Laboratory, MSIN K8-98, Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA 99352, USA. E-mail: rd_smith@pnl.gov

1044-0305/02/$20.00
PII S1044-0305(01)00343-9

Received April 19, 2001
Revised October 9, 2001
Accepted October 9, 2001

of an FTICR instrument through application of superimposed RF and DC potentials during ion accumulation, achieving a mass resolving power of approximately 10. We have recently reported on selective ion accumulation in the external quadrupole ion guide of our 3.5 tesla FTICR mass spectrometer [17]. Prior to entering a linear quadrupole ion trap where the ions were accumulated, ions were preselected using either RF/DC quadrupole mass filtering with a mass resolution of approximately 100 or RF-only resonance dipolar excitation [18] with a mass resolution greater than 30. Though lower in resolution compared to RF/DC mass filtering, RF-only resonance dipolar excitation provides the capability for simultaneous ejection of multiple species across the mass spectrum. Ejection of the most abundant ions was shown to expand the dynamic range of the instrument by up to two orders of magnitude [17]. This expansion of dynamic range by filling the ICR cell with ions having smaller relative intensities is similar to that demonstrated earlier by Bruce et al. [19] for dynamic range enhancement using colored noise waveforms for magnetron ejection of the most abundant species. However, the benefits inherent to external accumulation are additionally gained.

While the external ion guide selection process can be performed in a purely manual mode where the DC/RF levels or RF-only resonance dipolar excitation levels are predetermined and set by the operator, it is useful to automate this process to work on the fly such that a current MS acquisition can be used to automatically determine ion selection for the MS acquisition(s) which immediately follows, particularly in combination with on-line separations. For example, an initial nonselective acquisition can be used to identify *m/z* peaks above a set threshold, then a set of acquisitions immediately following are selective for individual peaks (i.e., starting with the most abundant peak). Berg et al. [20] and Senko et al. [21] demonstrated the use of data-dependent spectral acquisition with FTICR instruments which served to maximize MS/MS data collection with minimal operator intervention. These works utilized data-dependent generated SWIFT [22] waveforms for in-cell isolation of precursor ions.

In the present work, we demonstrate two techniques for ESI-FTICR using data-dependent external selective ion accumulation, gaining additional benefits from the use of external accumulation. One method employs an RF/DC quadrupole filter and the other is based on RF-only resonance dipolar excitation. In both methods, ions are data-dependently selected (or ejected from) an external quadrupole ion guide and then accumulated in a linear RF-only quadrupolar ion trap that immediately follows the selection quadrupole. Application of these methods is demonstrated useful for increasing the sensitivity and expanding the dynamic range of FTICR measurements with significant gains predicted for applications that include analysis of complex polypeptide mixtures (e.g., proteomics).

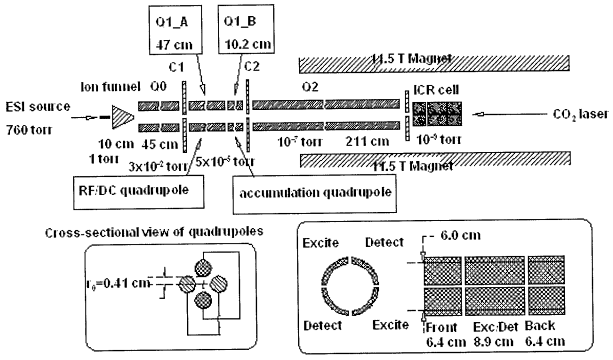

Figure 1. Drawing of the ion optics for the 11.5 T FTICR instrument (not to scale). Listed are the lengths of the quadrupole ion guide elements and the pressures in the various differentially pumped regions. Q1_A is operated in an RF/DC mode for *m/z* filtering, Q1_B functions as a linear quadrupole ion trap for ion accumulation. C1, C2, and C3 are aluminum conductance limits.

Experimental

Data-Dependent External Ion Selection Employing an RF/DC Filter

The experiments described employing data-dependent external ion selection using an RF/DC quadrupole filter were performed with an 11.5 tesla FTICR mass spectrometer designed and constructed at our laboratory. The spectrometer's passively shielded 11.5 tesla (T) horizontal bore solenoidal magnet (Magnex Scientific, Abingdon, UK) has a 20.3 cm diameter room temperature bore. The original electrostatic ion guide system described earlier [23] has been modified [17, 24] and is currently used for the 3.5 T system used in this work for ion selection employing RF-only resonant dipolar excitation. The spectrometer uses six differential vacuum pumping stages, which drop the pressure from atmosphere at the ESI source inlet to ultra-high vacuum in the ICR cell region. A drawing of the ion optics for this system is shown in Figure 1. The ESI source incorporates a heated stainless steel inlet capillary used for droplet desolvation (0.084 cm i.d., 160 °C) and an ion funnel assembly [25, 26] (funnel length is 10 cm). A collisional RF-only quadrupole ion guide (Q0, 45 cm in length) is coupled at the exit of the ion funnel. The outlet end of the heated inlet capillary, the ion funnel, and Q0 are differentially evacuated by three of the six pumping stages. The capillary region is evacuated by an 84-l/s roots pump (model RUVAC WA251, Leybold, Export, PA), the ion funnel region by a 24-l/s mechanical pump (model 2063, Alcatel, Hingham, MA), and the Q0 region by a 27-l/s molecular drag pump (model 5030CP, Alcatel). The exit of Q0 is isolated from the next downstream vacuum section by a 0.3 cm diameter orifice aluminum conductance limit (C1).

Referring to Figure 1, the vacuum region containing Q1_A and Q1_B is evacuated by a 2,400 l/s diffusion pump with a water cooled baffle (model VHS-6/336,

Varian, Lexington, MA). In this study, Q1_A (47 cm in length) served as an RF/DC quadrupole filter and Q1_B (10.2 cm in length) as a linear quadrupole accumulation trap; further description of these two quadrupole devices is provided below. The exit of Q1_B is isolated from the next downstream vacuum section by a 0.3 cm diameter orifice aluminum conductance limit (C2). The vacuum region containing RF-only quadrupole ion guide Q2 (211 cm in length) is also evacuated by a 2,400 l/s diffusion pump with a water cooled baffle and is isolated from the ICR cell ultra-high vacuum region by a 0.4 cm diameter orifice aluminum conductance limit (C3).

Quadrupole elements Q0, Q1_A, and Q1_B all have field radii of 0.413 cm and were machined from high quality 0.953 cm diameter nonmagnetic 316 stainless steel rod. Q2 also has a 0.413 cm field radius and was machined from 0.953 cm diameter titanium rod (Ed Fagan Inc., Los Alamitos, CA). All the quadrupole elements, with the exception of Q1_A, are driven by the same RF power supply (1 MHz). The capability is provided to drive the different quadrupole elements at different RF amplitude levels (typically Q0 is driven at 250 $V_{p\text{-}p}$, while Q1_B and Q2 are driven each at 425 $V_{p\text{-}p}$).

The ICR cell ultra-high vacuum region is evacuated by a cryogenic system consisting of an inner and outer tubular aluminum cryo-shield (100 K) which surrounds a tubular copper cryo-panel (16 K), all attached to a two stage 50W/12.5W He cryogenic cold head (model RGD1245, Leybold, Export, PA). The panel/shield assembly surrounds the entire length of the last quadrupole ion guide section (Q2). The panel/shield, Q2, and the ICR cell are all contained within a custom-made all-titanium vacuum chamber (Ability Engineering Technology, South Holland, IL). As shown in Figure 1 the system incorporates an open ended cylindrical ICR cell (6.0 cm i.d.; 6.4 cm, 8.9 cm, 6.4 cm: Length of front trap, excite/detect electrodes, back trap respectively) fabricated from aluminum stock that was then gold plated. The front and back trap plates are segmented and capacitively coupled through an RC circuit to the central excite electrodes to provide a linearized excitation. The ICR cell assembly is mounted on a titanium flange. An in-house built ICR cell detection preamplifier [27] is mounted on the atmosphere-side of the titanium flange. An Odyssey data station (Finnigan Corp., San Jose, CA) is used to generate experimental control scripts and to acquire data from the ICR cell preamplifier.

This system was also designed for in-cell infrared multiphoton dissociation (IRMPD) [28, 29]. A BaF_2 window (Bicron Harshaw, Solon, OH) mounted on the rear of the titanium flange permits a beam from a CO_2 IR laser (model 48-2W-25W, Synrad, Mukilteo, WA) to enter the ICR cell region of the spectrometer. The MS/MS of data-dependently externally selected and accumulated ions is demonstrated in this work.

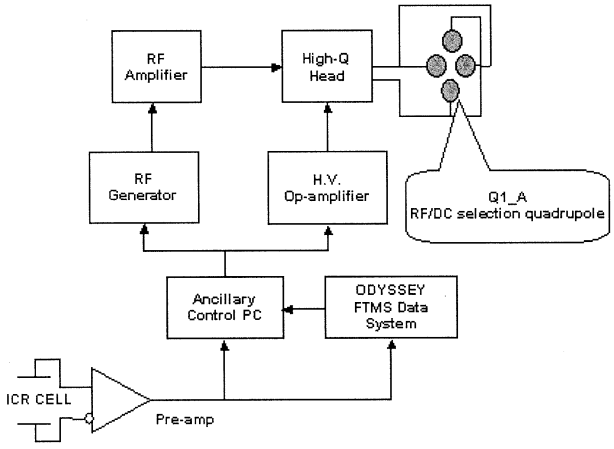

Figure 2. Block diagram of the 11.5T FTICR electronic hardware used for data-dependent external *m/z* selection (RF/DC mode) and accumulation. Refer to text for further details.

Data-Dependent Selection Hardware

In this work, the Q1_A section of the quadrupole ion guide was operated in an RF/DC mode for *m/z* selection of ions. A linear quadrupole ion trap (Q1_B) immediately following was used for ion accumulation. The electronic hardware employed for *m/z* selection of ions based on data recorded from a previous MS acquisition is diagrammed in Figure 2. The Odyssey data system is used to acquire data and trigger an ancillary control PC (Dell Optiplex Gxa 64 MB RAM, Austin, TX) to record raw data in parallel with the Odyssey system. The PC, using the PC based FTICR software program ICR-2LS, converts the raw data into a mass spectrum from which a peak is selected for isolation as described below. Based on a programmed calibration, analog control voltages are generated by a PC I/O card (model PCI-6110E; National Instruments, Austin, TX) and used to define the RF and DC voltages for the quadrupole mass filter based on the selected *m/z* value. The RF analog control voltage is used to drive the amplitude modulation input of an RF generator (model HP33120A; Hewlett-Packard, Loveland, CO), which then drives a 150W RF amplifier (model 150A100A; Amplifier Research, Souderton, PA). The amplifier output is used to drive an RF high-Q head (built in-house using air core inductors and air variable capacitors). The DC analog control voltage is used to drive a high-voltage op-amplifier (built in-house) which contains two outputs, one with a $+10\times$ gain, the other with a $-10\times$ gain. The high-voltage op-amplifier incorporates an additional offset feature that allows the user to initially define a DC offset voltage that is folded into the +DC/−DC outputs. These +DC/−DC voltages are coupled to the RF high-Q head. The quadrupole *m/z* filter is operated at an RF frequency of 540 kHz. PC control software was written using Microsoft Visual Basic version 5.0 and Borland C version 4.5.

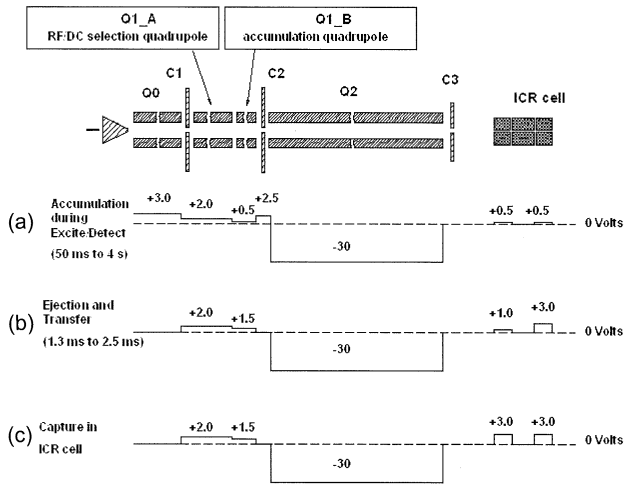

Figure 3. Depiction of the DC operating potentials used on the 11.5T FTICR instrument for (**a**) externally accumulating (**b**) ejecting/transferring ions from linear quadrupole ion trap Q1_B. Refer to text for further details, and (**c**) trapping in the FTICR cell.

External Ion Accumulation

Figure 3 depicts the sequence and working DC potentials used for ion accumulation in the linear quadrupole ion trap (Q1_B) along with DC offset potentials used for the other ion guide elements. Ions are accumulated in a potential well created by a lower DC offset potential on Q1_B, while keeping higher DC potentials on Q1_A and conductance limit C2. These potentials are shown in Figure 3a as Q1_A, Q1_B, C2: +2.0 V, +0.5 V, +2.5 V, respectively. In Figure 3b, ions are ejected from Q1_B by raising its DC offset potential to +1.5 V and dropping the potential of C2 to 0 V. Note that during ion transfer Q0 is lowered to 0 V to prevent any passage of ions from the continuous ESI source. During the ejection/transfer event the ICR front trap is kept at +1.0 V and the rear trap at +3.0 V. Ions are captured in the ICR cell by rapidly raising its front trap potential to +3.0 V, i.e., by gated trapping (Figure 3c). Note that after the gated trapping event, both front and rear trap potentials are lowered to +0.5 V for optimal detection and prior to the next ejection/transfer event ions are purged from the trap by applying +9 V/−9 V trapping potential for a brief period.

Ion accumulation times typically range from 50 ms to a few s (depending on the concentration of injected sample and ion intensity) and as stated earlier, can occur simultaneously during part of or the entire ICR excite/detect event. Ion transfer times range from 1.3 ms to 2.5 ms. It is observed that the shorter transfer time preferentially favors lower *m/z* ions, while the longer transfer time favors the higher *m/z* because of the different time of flights for these species. In general, however, a ~1.6 ms transfer time yields a broadly useful *m/z* representation and was used in this work. We also observe that the gated trapping of externally accumulated ions produces a relatively high quality ICR signal, without the necessity of pulsed gas introduction for ion cooling, consistent with other observations [8].

Data-Dependent External Ion Selection Employing RF-Only Resonant Dipolar Excitation

The present studies also employed data-dependent external selection using RF-only resonant dipolar (or quadrupolar) excitation using a 3.5 tesla FTICR mass spectrometer designed and constructed at our laboratory [17, 23, 24]. The most noted difference between this system and the system described above is the resonant dipolar ejection capability using this instrument's selection quadrupole and its use of a segmented accumulation quadrupole followed by an electrostatic ion guide. In common with the other system, this instrument's ESI source incorporates a heated metal capillary/ion funnel assembly [25, 26] coupled to a collisional focusing RF-only quadrupole ion guide. The ions exit this quadrupole through a 0.20 cm diameter orifice conductance limit to a set of three quadrupoles (0.413 cm field radii, 0.953 cm rod diameter) used for ion guiding, selection, and accumulation and referred to as ion guiding, selection, and accumulation quadrupoles, respectively. The 15 cm long ion guiding quadrupole is driven by a commercial RF-power supply (Extranuclear Labs, Pittsburgh, PA) at an RF amplitude of 560 $V_{p\text{-}p}$ and a frequency of 2.1 MHz. The 10 cm long selection quadrupole is enclosed in a differentially pumped vacuum region, which allows for a pressure adjustment in the range of 10^{-2} to 10^{-4} torr without affecting the pressure in the adjacent quadrupole sections of the interface. The selection quadrupole is driven by a high-Q head (built in-house using air core inductors and air variable capacitors) which is controlled by a function generator (Model HP 33120A, Hewlett-Packard, Loveland, CO) and RF-amplifier (Model 100A150A, Amplifier Research, Souderton, PA). To implement selection based on RF-only resonant dipolar excitation, two opposite rods of the selection quadrupole are coupled to the secondary coil of an auxiliary 1:1 transformer. The center point of the transformer secondary coil is driven by the main RF-drive at amplitudes ranging from 300 $V_{p\text{-}p}$ to 1200 $V_{p\text{-}p}$ and frequencies ranging from 500 kHz to 700 kHz. The resonant dipolar excitation voltages are applied to the primary coil of the transformer. These auxillary or tickle voltages were synthesized with our ICR-2LS software program and generated using a plug-in PC DAC board. In some experiments a function generator (Model HP 33120A, Hewlett-Packard, Loveland, CO), operating at a single frequency, was used. The final quadrupole of the interface, used for ion accumulation, is 10 cm long and segmented into 22 sections to provide an axial electric field for prompt ion ejection. A high-Q head was used to drive

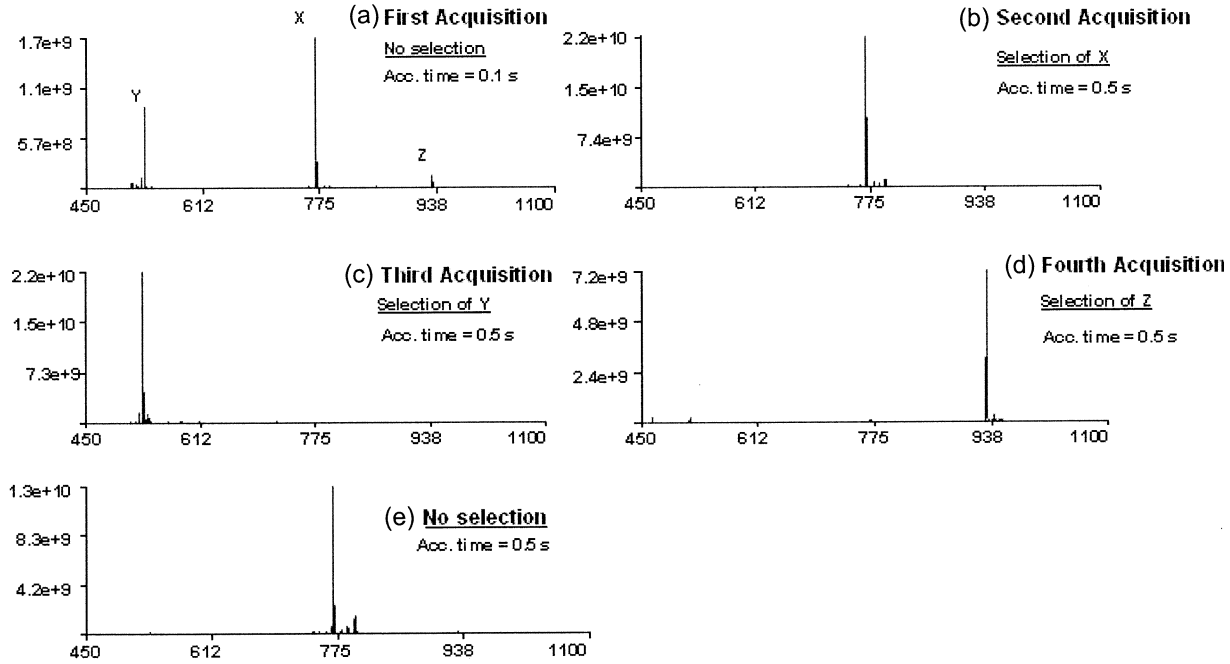

Figure 4. Data-dependent selection and accumulation acquisition series for a mixture of three peptides. Peaks labeled X, Y, and Z are fibrinopeptide 2+, bradykinin 2+, and γ-endorphin 2+, respectively. Information obtained in first acquisition spectrum (**a**) was used to generate the second through fourth acquisition spectra (**b**, **c**, **d**). Spectrum shown in (**e**) depicts the unwanted discrimination effect that occurs if a long accumulation time, without prior selection, is employed.

the accumulation quadrupole at RF amplitude of 220 $V_{p\text{-}p}$ and a frequency of 525 kHz. The RF voltage applied to the quadrupole segments is decoupled from the applied DC voltages through an RC circuit.

Ions ejected from the segmented accumulation quadrupole are focused by an electrostatic ion guide into a dual cylindrical FTICR cell maintained at a pressure of 8×10^{-10} torr. The designs of the electrostatic ion guide and the FTICR cell have been described earlier [23].

An Odyssey data station (Finnigan Corp., San Jose, CA) is used to generate experimental control scripts and to acquire data from the ICR cell preamplifier.

Data-dependent selective ion accumulation is implemented using parallel data acquisition with a PC. A 12-bit ADC, triggered by the Odyssey data station, is used to acquire time-domain signals, which are automatically converted to *m/z* spectra by the ICR-2LS software program. An in-house program is used to generate resonant dipolar excitation frequencies used to eject specific ions. For example, a nonselective acquisition is first obtained from which the most intense *m/z* peaks (based on a threshold setting) are designated. These designated peaks are then used to generate resonant dipolar excitation frequencies, which are used during a selective acquisition, which immediately follows. A superposition of resonant sine waveforms can be used for ejecting multiple peaks simultaneously. Such selective ejection is performed with a mass resolution greater than 30 and in a data-dependent manner.

Chemicals, Cell Culture, and Capillary HPLC System

Three test solutions were used for direct infusion consisting of 1μg each of three peptides dissolved in 1 ml of a water:methanol:acetic acid solution (49:49:2 vol %); the peptides used were (1) bradykinin, fibrinopeptide A, and γ-endorphin, (2) rennin inhibitor, fibrinopeptide A, and γ-endorphin, and (3) bradykinin, gramicidin S, and angiotensin I. The solutions were directly infused into the ESI sources at a flow rate of 0.3 μl/min using a syringe pump (Harvard Apparatus, South Natick, MA).

HPLC/FTICR MS data sets were obtained using a capillary LC system based on a high pressure syringe pump (ISCO Inc., Lincoln, NE) [30] using a polypeptide mixture obtained from the proteolysis of a cell lysate (work described using the 11.5 tesla FTICR instrument) and a 0.01 mg/ml bovine serum albumin tryptic digest solution (work described using the 3.5 tesla FTICR instrument). The proteolysis of the *D. radiodurans* R1 strain cell lysate (i.e., genome sequenced strain) purchased from American Type Culture Collection (Manassas, VA) and cultured on plates containing minimal media. Inoculation cultures were grown for approximately 72 hours (i.e., to post-stationary phase). Cells were harvested by centrifugation at 14,000 × g for 3 min (centrifuge model 5415C, Eppendorf, Westbury, NY). The cells were resuspended in 200 μL of 50 mM Tris-HCL (pH 8.0) and lysed by mechanical agitation using 0.1 mm diameter zirconium/silica beads (Biospec

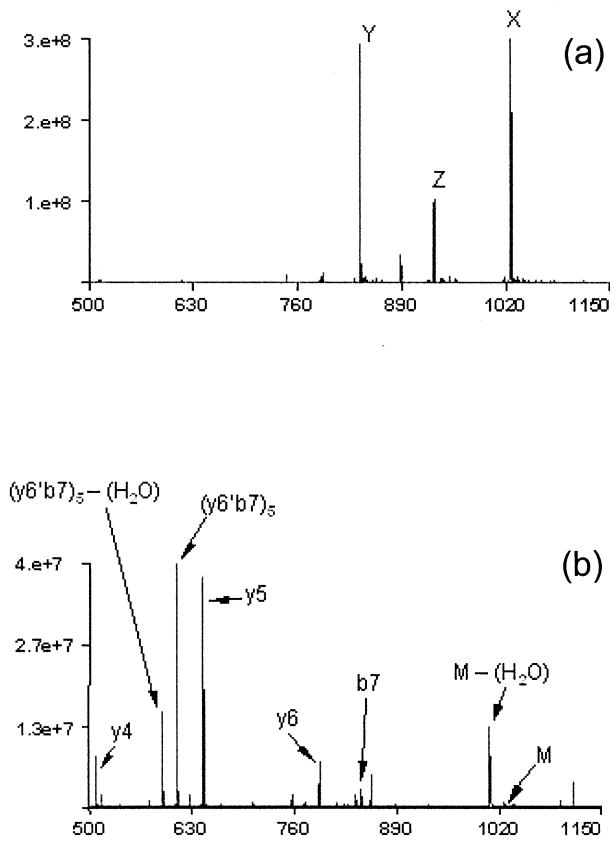

Figure 5. Demonstration of data-dependent external selection/accumulation MS/MS. Peaks labeled X, Y, and Z are rennin inhibitor 1+, fibrinopeptide A 2+, and γ-endorphin 2+, respectively. The spectrum in (**a**) is the result of a nonselective acquisition; all three peptides visibly present. An RF/DC selective acquisition immediately followed, with data-dependent selection of the most abundant species (X) taking place. After an external selective accumulation period (0.5s), the ion packet was ejected and transferred to the ICR cell, trapped, subjected a 0.25 s infrared laser pulse, and then excited and detected. The spectrum in (*b*) shows the identifiable rennin inhibitor fragments.

Products, Bartlesville, OK) and a Mini-Beadbeater (Biospec Products) operated at 4,500 rpm for 90 s. Protein concentration was determined using the BCA assay kit (Pierce, Rockford, IL). The proteins were denatured (urea, DDT, boiling) and then digested overnight (at 37 °C) using modified trypsin (Promega, Madison, WI). This was followed by overnight dialysis (Tris-HCl, ph 5.0, 4 °C) and ultracentrifugation at 100,000 × g for 30 min (centrifuge model Optima TL, Beckman Instruments, Palo Alto, CA).

The tryptic digest solutions were injected into a capillary column (150 μm i.d., 360 μm OD, 50 cm long), packed in-house and containing 5 μm diameter Jupiter C_{18} resin (Phenomenex, Torrance, CA). A solvent gradient was used to elute the polypeptides, solvent A being 0.2% acetic acid, 0.1% TFA, water (vol %) and solvent B being 80% acetonitrile, 10% water, 0.1% TFA (vol %). The peptides were eluted using a linear gradient of 0 to 80% solvent B over 120 min. Solvents were delivered to the capillary column at a pressure of 5000 psi using two Isco model 100 DM pumps controlled by an Isco series D controller and a LC Packings Accurate microflow processor splitter (LC Packings, San Francisco, CA) resulting in a capillary flow rate of ~1 μL/min.

Results and Discussion

RF/DC Quadrupole Selection

The spectra in Figure 4a, b, c, and d, (first through fourth acquisition) show the data-dependent selection/accumulation series of acquisitions obtained using one of the peptide mixture solutions (peaks labeled X, Y, and Z are fibrinopeptide 2+, bradykinin 2+, and γ-endorphin 2+ respectively). The first acquisition (Figure 4a) involved no selection; here the *m/z* filter quadrupole Q1_A operated in an RF-only mode to allow sampling of all species present in the mixture. Information from this initial nonselective acquisition was used with the data-dependent algorithm to generate the second (Figure 4b), third (Figure 4c), and fourth (Figure 4d) acquisitions (in that order). The algorithm used the peaks and their relative intensities (information obtained in the first acquisition), then *m/z* selective acquisitions immediately followed starting with the most abundant peak (X), followed by the second most abundant (Y), then the third most abundant (Z), and so forth. A feature of the approach was that the same peak was selected no more than once in a single acquisition series and a file was created specifying the peaks selected and the order of selection.

In the example shown here, during the initial nonselective acquisition the peaks resulted from an accumulation of ions in Q1_B for a period of 0.1 s. During the *m/z* selective acquisitions, the accumulation time was increased to 0.5 s, resulting in a significant S/N increase (in Figure 4 note the y-axis scale, arbitrary units, and the increase in the second through fourth acquisitions compared to the first). A significant finding is that the signal enhancement observed by using a longer accumulation time during the *m/z* selective acquisitions is not observed if only a longer accumulation time is used during a nonselective acquisition (Figure 4e). This spectrum was obtained using the same conditions as in Figure 4a, only a longer accumulation time was used (0.5 s compared to the 0.1 s). A discrimination effect suppresses peaks Y and Z. A similar phenomenon has been observed in our laboratory with other FTICR instruments employing external ion accumulation, and is the subject of further studies [31]. Thus, the *m/z* selection improves the S/N obtainable for the selected *m/z* range(s) by enabling longer accumulation events without undesired effects due to excessive space charge.

Data-dependent external selection and accumulation is attractive for FTICR MS/MS because in such a scheme the parent ion can be externally selected and accumulated, transferred to the ICR trap and then

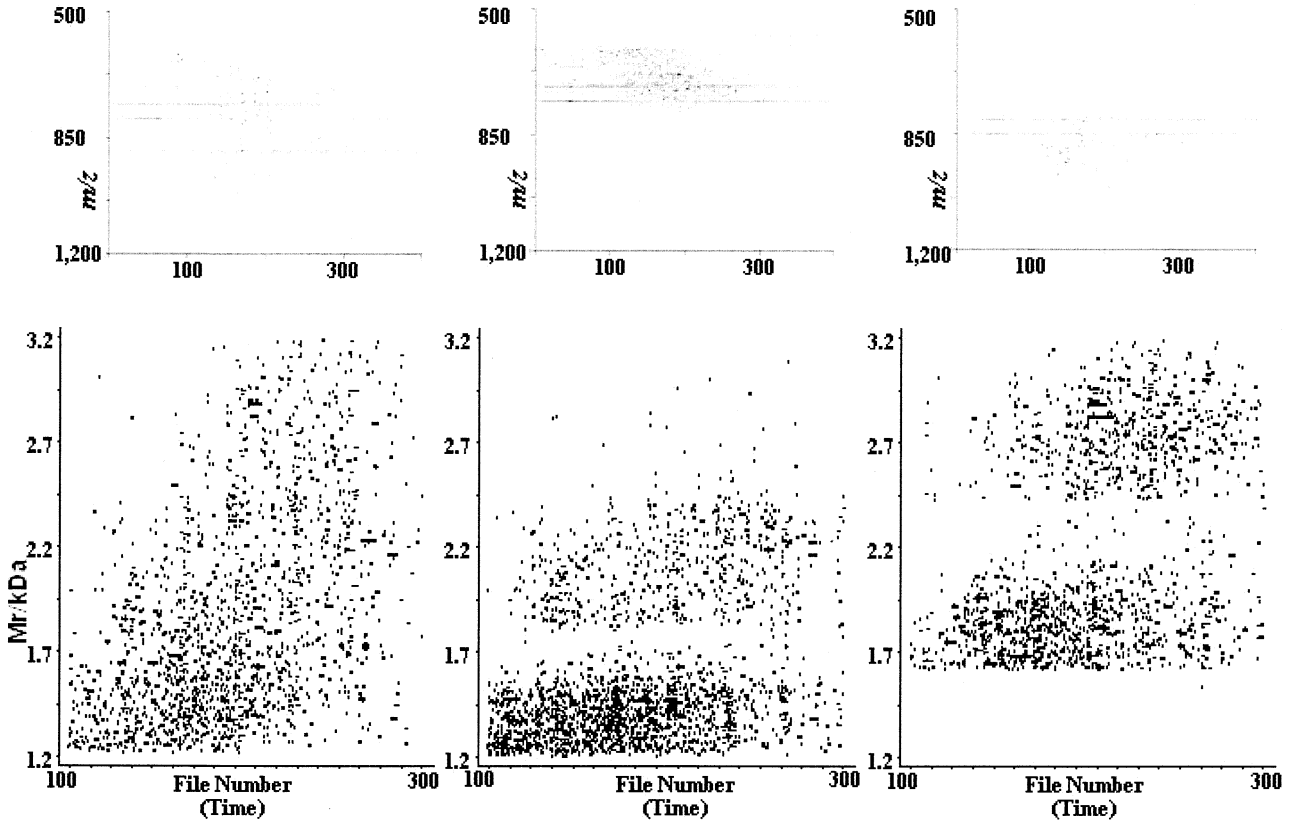

Figure 6. Results obtained using *m/z* notch mode of selective accumulation during the LC separation of *D. radiodurans* proteolytic cell lysate. The 2-D *m/z* (top) and deconvoluted mass spectra (bottom), as a function of file number (time), shown for the boardband (left), *m/z* notch 600 to 800 (center), and *m/z* notch 800 to 1000 (right). Refer to text for further details.

fragmented using a laser pulse prior to excite/detect events without the need for gas-pulsing. Figure 5 shows results obtained using the rennin inhibitor, fibrinopeptide A, and γ-endorphin mixture (peaks X, Y, and Z respectively). Figure 5a is the result of a 0.1 s nonselective acquisition; all three peptides are visibly present. A selective acquisition immediately followed, with data-dependent selection for the most abundant species (X) taking place. After an external selective accumulation period (0.5 s), the ion packet was ejected and transferred to the ICR cell, trapped, subjected a 0.25 s infrared laser pulse, excited and detected. Figure 5b shows the identifiable rennin inhibitor fragments. Using the algorithm described above, the next acquisition could be selective for the next most abundant peak (Y), etc. Note that the nonselective acquisition used to identify the peak for isolation need only occur at the start of an acquisition series. As such, this method provides an increase in duty cycle since external selection and accumulation of the next in-line parent ion occurs concurrently with the fragmentation and excite/detection of the last in-line parent.

The mass spectra obtained from the digest of a single protein can be quite complex, with numerous *m/z* peaks throughout a spectrum and the potential complexity grows for protein mixtures (i.e., digestion of a whole cell lysate). As such, the utility of mass selective RF/DC filtering prior to accumulation for such a sample is limited, since only one *m/z* species or continuous *m/z* range can be preselected and accumulated per acquisition. When working with such samples, another approach could be to employ the filter so as to provide low resolution selection. In such a scheme, the parameters of the RF- and DC fields are chosen so that the quadrupole filter has a stability region just below the apex of the ion stability diagram. Therefore, the filter allows passage of a fixed *m/z* range of ions into the accumulation quadrupole trap (e.g., *m/z* of 600 to 800, 800 to 1000). Figure 6 shows results obtained using this *m/z* notch approach of selective accumulation during the LC separation of *D. radiodurans* proteolytic cell lysate. For this the Odyssey data station script was programmed to record 300 files, each file having three acquisition parts: A broadband mode acquisition during the first third (left column in Figure 6), followed by an *m/z* 600 to 800 notch acquisition during the middle third (center column in Figure 6), followed by an *m/z* 800 to 1000 notch acquisition during the last third (right column in Figure 6); accumulation times were 0.1 s, 0.5 s, and 0.5 s respectively. The Figure shows the 2-D display and deconvoluted and processed mass information spectra for each of these operational modes. The

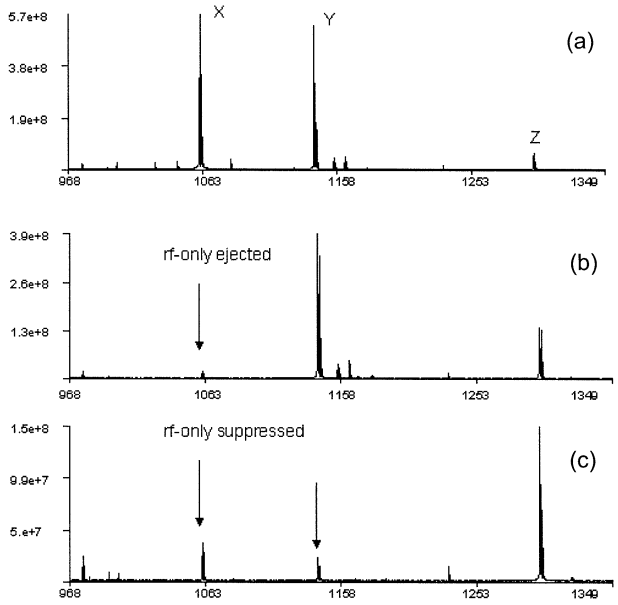

Figure 7. Data-dependent RF-only resonant dipolar ejection and accumulation acquisition series using a mixture of three peptides. Peaks labeled X, Y, and Z are bradykinin 1+, gramicidin S 1+, and angiotensin I 1+, respectively. (**a**) Broadband acquisition mode, (**b**) selective ejection of X, (**c**) selective suppression of X and Y.

number of unique polypeptide spots in the left spectrum compared to the middle and right spectra shows an approximate doubling of the number of species detected, demonstrating the clear benefit of *m/z* selection prior to accumulation because of the enhancement of low-level species.

We are currently implementing a data-dependent variable accumulation time capability. In such a scheme, the broadband acquisition can be used to quantify the total ion current (TIC) within designated *m/z* notches and used to set the accumulation time employed for the subsequent selective acquisitions (i.e., a lower overall TIC would result in a longer accumulation time). This should help avoid discrimination in the accumulation quadrupole that can occur, even during a selective *m/z* notch, if too long an accumulation time is used [32].

RF-Only Resonant Dipolar Selection

When analyzing complex protein digests, simultaneous and selective suppression of several higher abundance peaks spread out across the mass spectrum can be advantageous. As was highlighted above, higher abundance species may discriminate against lower abundant ions in the external accumulation trap, thus reducing the number of detected isotopic distributions during a capillary LC/ESI FTICR run. Additionally, by ejecting higher abundance species and accumulating lower abundance ions for a longer accumulation period in the external linear quadrupole trap, one can effectively expand the dynamic range of the FTICR measurement: in effect the dynamic range of the external trap becomes

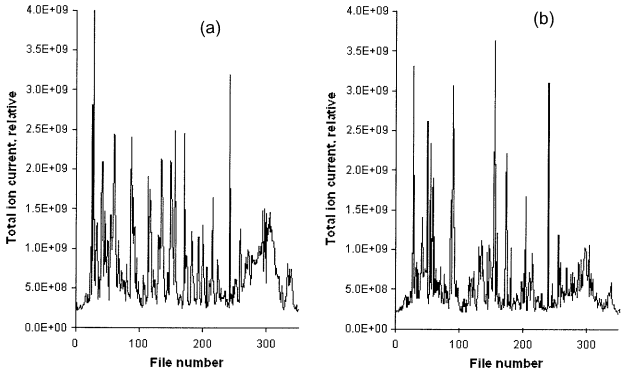

Figure 8. Results showing the total ion current chromatograms obtained using a 0.01 mg/mL bovine serum albumin tryptic digest solution acquired during a two acquisition sequence capillary LC/ESI FTICR run. (**a**) Broadband mode acquisition (first half of each of the 350 files), (**b**) selective mode acquisition (second half of each of the 350 files). Refer to text for further details.

the limiting factor [17]. Since RF/DC mass filtering is limited to one *m/z* region of the mass spectrum at a time, RF-only resonant dipolar excitation appears to be a complementary broad-band approach.

In the first region of the ion stability diagram at $q < 0.4$, ion motion can be presented as a superposition of rapid oscillations and a smooth drift in a harmonic pseudopotential well. The latter motion for a specific *m/z* is characterized by a unique fundamental resonant frequency, Ω, which can be estimated as follows [18, 33]:

$$\Omega = \frac{q}{\sqrt{8}}\,\omega_0 \tag{1}$$

$$q = \frac{4zeV_{rf}}{m\omega_0^2 r_0^2} \tag{2}$$

where q is the Mathieu parameter, V_{rf} is the peak-to-ground RF-amplitude, z is the ion charge state, e is the elementary charge, m is the ion mass, ω_0 is the RF field angular frequency, and r_0 is the quadrupole inscribed radius.

Based on eqs 1 and 2, the mass-to-charge ratio of a particular ion (m/z_i), is uniquely related to the fundamental resonant frequency of the ion's oscillation in the effective potential well, Ω_i. If a superposition of sine waveforms at frequencies of Ω_1, $\Omega_2 \ldots \Omega_n$ is applied to the pair of opposite rods of the selection quadrupole as an auxiliary or tickle voltage (this auxiliary voltage in addition to the main RF drive voltage defined by V_{rf} and ω_0), the ions with the corresponding m/z_1, $m/z_2 \ldots m/z_n$ will be ejected from the quadrupole simultaneously without infringing upon transmission of other ions, provided sufficient mass resolution is available.

It is noteworthy that eq 1 is valid only at lower ion populations in the selection quadrupole when the effective potential governed by:

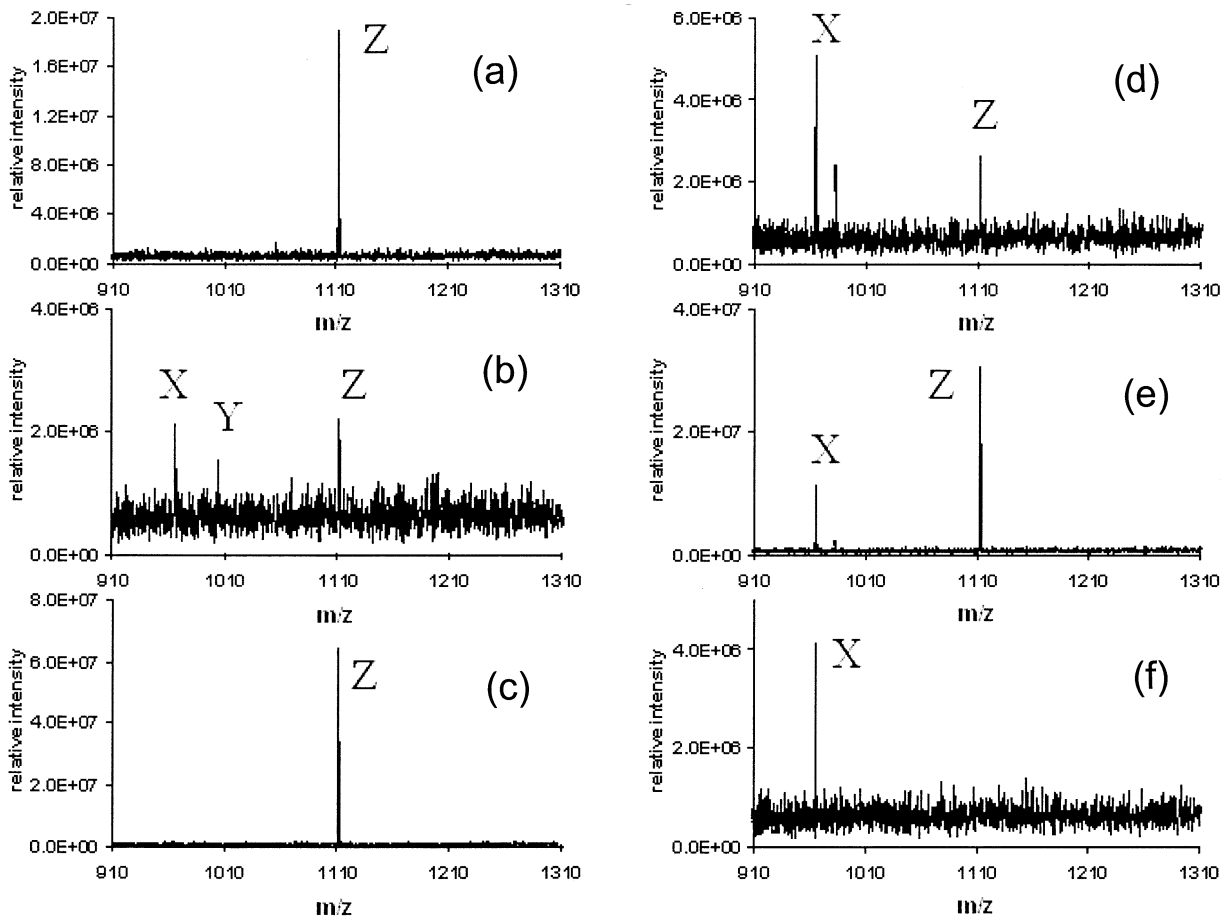

Figure 9. Results showing portions of the mass spectra acquired during a two-acquisition sequence capillary LC/ESI FTICR analysis using a 0.01 mg/mL bovine serum albumin tryptic digest solution. (**a**), (**c**) and (**e**) obtained using broadband mode acquisition (first half of files 135, 136, 137 respectively) and (**b**), (**d**) and (**f**) obtained using RF-only resonant dipolar ejection of most abundant peak (second half of files 135, 136, 137 respectively). Refer to text for further details.

$$V^*(r) = \frac{z^2e^2V_{rf}{}^2r^2}{m\omega_0{}^2r_0{}^4} \quad (3)$$

is a quadratic function of the radial coordinate of an ion, r.

Filling the selection quadrupole with higher space charge results in distortion of the effective potential, e.g., the appearance of a flat bottom potential well, and a subsequent increase in the resonant frequencies needed for resonant dipolar excitation. Under high space charge conditions using an excitation frequency defined by eq 1 would result in the beating oscillation of excited ions, where the ion cloud radius would increase and decrease with a period determined by the difference between the calculated resonant frequency, Ω_1, and some true resonant frequency, Ω_1* [17].

To avoid excessive space charge in the selection quadrupole, ions are excited and ejected during transmission through the selection quadrupole. The RF field angular frequency, ω_0, and RF-amplitude, V_{rf}, in the selection quadrupole is chosen to correspond to $q \sim 0.7$ for the lowest m/z species.

Figure 7 shows portions of the mass spectra obtained using one of the peptide mixture solutions (X, Y, and Z are bradykinin 1+, gramicidin S 1+, and angiotensin I 1+, respectively). The mass spectra were acquired during a three acquisition sequence that included broadband acquisition (Figure 7a), a selective acquisition with ejection of the most abundant species (Figure 7b), and a selective acquisition with suppression of the two most abundant ion peaks (Figure 7c). External accumulation time for all acquisitions was 0.1 s. Resonant frequencies for RF-only dipolar excitation were identified during the broadband acquisition and the corresponding species were data-dependently ejected during selective acquisitions, which immediately followed.

Having evaluated the RF-only data-dependent mode using direct infusion with the myoglogin and the three peptide mixture, this approach was applied in a capillary LC/FTICR study of 0.01 mg/mL bovine serum albumin (BSA) tryptic digest solution. An Odyssey data station script was programmed to record 350 files, each file containing both the broadband mode and selective mode acquisitions. The broadband mode employed a

5 s accumulation time and the selective mode employed an 8 s accumulation time. During the broadband mode a most abundant peak was identified and this species was then ejected during the selective mode acquisition that immediately followed. Figure 8 shows the recorded total ion current (TIC) chromatograms, for the broadband (Figure 8a) and selective (Figure 8b) mode acquisitions.

Figure 9 shows portions of the mass spectra recorded during file numbers 135, 136, and 137. Mass spectra in Figures 9a, c, and e (first half of 135, 136, 137 respectively) were acquired using the broadband mode, while mass spectra in Figure 9b, d, and f (second half of 135, 136, 137 respectively) were acquired using the selective mode. It is worth noting the peptide ions (peaks at *m/z* 964.4, 982.1, and 1112.4 labeled X, Y, and Z, respectively) have similar elution times, however, the lower abundant species (X and Y) are not evident in the mass spectra acquired during broadband mode (Figure 9a and c). The presence of these peaks becomes evident only after selective ejection (or suppression) of the most abundant species —peak Z (Figure 9b and d). Once the intensity of the lower abundant species is increased, all the peptide ions are detected during a broadband acquisition (Figure 9e). The appearance of the lower abundance species (X and Y) during the selective accumulation scans is most likely due to the expansion of the accumulation quadrupole dynamic range resulting from the ejection of the most abundant species (Z). Selective ejection of the most abundant species followed by accumulation of lower abundance species for extended periods results in the increased number of the detected isotopic distributions and can potentially be applied for enhanced characterization of proteomes.

Conclusions

The data-dependent external *m/z* selection and accumulation of ions with ESI-FTICR mass spectrometry instrumentation has been demonstrated using two different modes of ion selection prior to accumulation in an external linear quadrupole trap. Results using both direct infusion and capillary LC separation methods demonstrate a major benefit of ion preselection prior to external accumulation is the enhancement of low level species. While the levels of resolution obtained here appear modest, they are suitable for most applications. Coupling external selective accumulation with data-dependent control appears especially attractive with on-line separations. Application of these methods is predicted to provide significant gains in sensitivity and dynamic range that include analysis of complex polypeptide mixtures (e.g., for proteomics). Indeed, in other work we demonstrate the significant gains that can be realized when we apply a method derivative of this work termed dynamic range enhancement applied to mass spectrometry (DREAMS) with FTICR to increase the dynamic range of proteome-wide measurements [34].

Acknowledgments

The authors wish to thank Dr. Mary Lipton, Dr. Yufeng Shen, and Mr. Nikola Tolić for their contributions to this work. We also wish to thank the United States Department of Energy Office of Biological and Environmental Research for support of this work. Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the U.S. Department of Energy under contract DE-AC06-76RLO 1830.

References

1. Henry, K. D.; Williams, E. R.; Wang, B-H; McLafferty, F. W.; Shabanowitz, J.; Hunt, D. F. Fourier-Transform Mass Spectrometry of Large Molecules by Electrospray Ionization. *Proc. Natl. Acad. Sci. U.S.A.* **1989,** *86,* 9075–9078.
2. Smith, R. D.; Loo, J. A. Ogorzalek; Loo, R. R.; Busman, M.; Udseth, H. R. Principles and Practice of Electrospray Ionization-Mass Spectrometry for Large Polypeptides and Proteins. *Mass Spectrom. Rev.* **1991,** *10,* 359–451.
3. Marshall, A. G.; Verdun, F. R. Fourier Transforms in NMR, Optical, and Mass Spectrometry: A User's Handbook. Elsevier: Amsterdam, 1990.
4. Marshall, A. G.; Guan, S. Advantages of High Magnetic Field for Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1996,** *10,* 1819–1823.
5. Hendrickson, C. L.; Emmett, M. R. Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Annu. Rev. Phys. Chem.* **1999,** *50,* 517–536.
6. Stockton, G. W.; Meek, J. T.; Millen, W. G.; Wayne, R. S. FTICR/MS: Analytical Applications of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Asamoto, B., Ed.; VCH: New York, 1991.
7. Stacey, C. C.; Kruppa, G. H.; Watson, C. H.; Wronka, J.; Laukien, F. H.; Banks, J. F.; Whitehouse, C. M. Reverse-Phase Liquid Chromatography Electrospray-Ionization Fourier-Transform Mass Spectrometry in the Analysis of Peptides. *Rapid Commun. Mass Spectrom.* **1994,** *8,* 513–516.
8. 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.
9. Hofstadler, S. A.; Wahl, J. H.; Bruce, J. E.; Smith, R. D. On-Line Capillary Electrophoresis with Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Chem. Soc.* **1993,** *115,* 6983–6984.
10. Jensen, P. K.; Paša-Tolić, L.; Anderson, G. A.; Horner, J. A.; Lipton, M. S.; Bruce, J. E.; Smith, R. D. Probing Proteomes Using Capillary Isoelectric Focusing-Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* **1999,** *71,* 2076–2084.
11. Kofel, P.; Alleman, M.; Kellerhals, H.; Wancek, K. P. External Generation of Ions in ICR Spectrometry. *Int. J. Mass Spectrom. Ion Proc.* **1985,** *65,* 97–103.
12. McIver, R. T.; Hunter, R. L.; Bowers, W. D. Coupling a Quadrupole Mass Spectrometer and a Fourier Transform Mass Spectrometer. *Int. J. Mass Spectrom. Ion Proc.* **1985,** *64,* 67–77.
13. Alford, J. M.; Williams, P. E.; Trevor, D. J.; Smalley, R. E. Metal Cluster Ion Cyclotron Resonance Combining Supersonic Metal Cluster Beam Technology with FT-ICR. *Int. J. Mass Spectrom. Ion Proc.* **1986,** *72,* 33–51.
14. Caravatti, P. *U.S.A. Patent No. 4,924,089*; issued May 1990.
15. Beu, S. C.; Senko, M. W.; Quinn, J. P.; McLafferty, F. W. Improved Fourier-Transform Ion-Cyclotron-Resonance Mass

Spectrometry of Large Biomolecules. *J. Am. Soc. Mass Spectrom.* **1993,** *4,* 190–192.
16. Wang, Y.; Shi, S. D.-H.; Hendrickson, C. L.; Marshall, A. G. Mass Selective Ion Accumulation and Fragmentation in a Linear Octopole Ion Trap External to a Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer. *Int. J. Mass Spectrom. Ion Proc.* **2000,** *198,* 113–120.
17. 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.
18. Campbell, J. M.; Collings, B. A.; Douglas, D. J. A New Linear Ion Trap Time-of-Flight System with Tandem Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1998,** *12,* 1463–1474.
19. Bruce, J. E.; Anderson, G. A.; Smith, R. D. "Colored" Noise Waveforms and Quadrupole Excitation for the Dynamic Range Expansion of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* **1996,** *68,* 534–541.
20. Berg, C., Speir, P., Kruppa, G., Afzaal, S., Laukien, F. Automated MS/MS for FT-ICR-MS. *Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics*; Orlando, FL, 1998; p 515.
21. Senko, M. W., White, F., Syka, J. E. P., Shabanowitz, J., Hunt, D. F., Marto, J. A. Data Dependent Acquisition for Peptide Sequence Analysis on a FT/ICR Mass Spectrometer. *Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics*; Dallas, TX, 1999; pp 2500–2501.
22. Marshall, A. G.; Wang, T.-C. L.; Chen, L.; Ricca, T. L. Tailored Excitation for Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Chem. Soc.* **1985,** *107,* 7893–7897.
23. Gorshkov, M. V.; Paša-Tolić, L.; Udseth, H. R.; Anderson, G. A.; Huang, B. M.; Bruce, J. E.; Prior, D. C.; Hofstadler, S. A.; Tang, L.; Chen, L.-Z.; Willet, J. A.; Rockwood, A. L.; Sherman, M.; Smith, R. D. Electrospray Ionization-Fourier Transform Ion Cyclotron Resonance Mass Spectrometry at 11.5 Tesla: Instrument Design and Initial Results. *J. Am. Soc. Mass Spectrom.* **1998,** *9,* 692–700.
24. Belov, M. E.; Gorshkov, M. V.; Udseth, H. R.; Anderson, G. A.; Smith, R. D. Zeptomole-Sensitivity Electrospray Ionization-Fourier Transform Ion Cyclotron Resonance. *Anal. Chem.* **2000,** *72,* 2271–2279.
25. Belov, M. E.; Gorshkov, M. V.; Udseth, H. R.; Anderson, G. A.; Tolmachev, A. V.; Prior, D. C.; Harkewicz, R.; Smith, R. D. Initial Implementation of an Electrodynamic Ion Funnel With Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2000,** *11,* 19–23.
26. Kim, T.; Tolmachev, A. V.; Harkewicz, R.; Anderson, G. A.; Udseth, H. R.; Futrell, J. H.; Smith, R. D. Design and Implementation of a New Electrodynamic Ion Funnel. *J. Anal. Chem.* **2000,** *72,* 2247–2255.
27. Anderson, G. A., Bruce, J. E., Hofstadler, S. A., Rockwood, A. L., Smith, R. D. Low Noise ICR Detection Electronics. *Proceedings of the 43rd ASMS Conference on Mass Spectrometry and Allied Topics*; Atlanta, GA, 1995; p. 1093.
28. Little, D. P.; Speir, J. P.; Senko, M. W.; O'Connor, P. B.; McLafferty, F. W. Infrared Multiphoton Dissociation of Large Multiply Charged Ions for Biomolecule Sequencing. *Anal. Chem.* **1994,** *66,* 2809–2815.
29. Masselon, C. D.; Anderson, G. A.; Harkewicz, R.; Bruce, J. E.; Paša-Tolić, L.; Smith, R. D. Accurate Mass Multiplexed Tandem Mass Spectrometry for High-Throughput Polypeptide Identification from Mixtures. *Anal. Chem.* **2000,** *72,* 1918–1924.
30. Shen, Y.; Tolić, N.; Rui, Z.; Paša-Tolić, L.; Li, L.; Berger, S. J.; Harkewicz, R.; Anderson, G. A.; Belov, M. E.; Smith, R. D. High-Throughput Proteomics Using High-Efficiency Multiple-Capillary Liquid Chromatography with On-Line High-Performance ESI FTICR Mass Spectrometry. *Anal. Chem.* **2001,** *73,* 3011–3021.
31. Belov, M. E.; Nikolaev, E. N.; Harkewicz, R.; Masselon, C. D.; Alving, K.; Smith, R. D. Ion Discrimination During Ion Accumulation in a Quadrupole Interface External to a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. *Int. J. Mass Spectrom.* **2001,** *208,* 205–225.
32. Belov, M. E.; Nikolaev, E. N.; Alving, K.; Smith, R. D. A New Technique for Unbiased External Ion Accumulation in a Quadrupole Two-Dimensional Ion Trap for Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Rapid. Commun. Mass Spectrom.* **2001,** *15,* 1172–1180.
33. Dehmelt, H. G. Radiofrequency Spectroscopy of Stored Ions I: Storage. *Adv. Atom. Mol. Phy.* **1967,** *3,* 53–72.
34. Belov, M. E., Anderson, G. A., Angell, N. H., Shen, Y., Tolić, N., Udseth, H. R., Smith, R. D. Dynamic Range Enhancement Applied to Mass Spectrometry (DREAMS) Based Upon Data-Dependent Selective External Ion Accumulation in Capillary LC-FTICR for Enhanced Proteome Characterization. *Anal. Chem.* **2001,** *73,* 5052–5060.