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Sensitivity and Robustness Enhancements by Using a V-Shape Ion Funnel in FTICR-MS

Liulin Deng,^{†,§} Xiangfeng Chen,^{*,†,‡} Wan Li,[†] Ze Wang,[†] Yiling Elaine Wong,[†] and T.-W. Dominic Chan^{*,†}

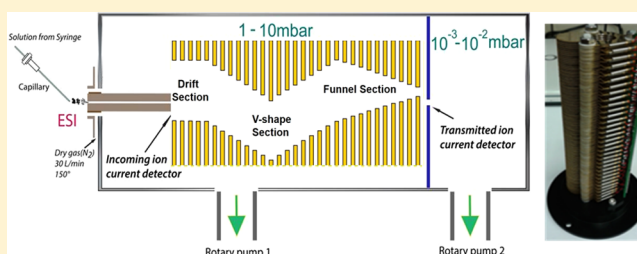
[†]Department of Chemistry, The Chinese University of Hong Kong, Hong Kong SAR, P. R. China

[‡]Shandong Academy of Sciences, Jinan, Shandong, 250014, P. R. China

[§]Biological Sciences Division and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352, United States

Supporting Information

ABSTRACT: In this paper, a new configuration of the ion funnel interface (i.e., V-shape ion funnel (V-IF)) for high ion transmission efficiency and robustness enhancement was developed and implemented on FTICR-MS. The performance of the V-IF was compared with that of a home-built orthogonal ion funnel. An order of magnitude of improvement in sensitivity was achieved for various peptides and proteins. The performance of the instrument was maintained for a long period by neutral molecule removal. Other ion transmission patterns, such as gentle ion transmission, adduct ion removal, and radio frequency (RF)-driven collision induced dissociation (CID), was also realized in V-IF by varying the RF potentials. V-IF is believed to be a novel ion guide that has promising applications in mass spectrometry.



Electrospray ionization (ESI)¹ is one of the most important ion generation methods in mass spectrometry for large biomolecules such as peptides (or proteins)² and DNA.^{3,4} Although ESI is believed to have fairly high ionization efficiency, only a small fraction (approximately 0.01–0.1%) of analyte ions is successfully transported to the mass analyzer.^{5–7} A large fraction of the ions is believed to be lost when passing through the conductance limiting orifices. In low vacuum regions, conventional electrostatic ion optical devices, such as the Einzel lens,^{8–10} are ineffective in focusing the ion beam presumably because of the ion–molecule collisions and space charge effect. The situation is alleviated by using multipole ion guides (e.g., quadrupole, hexapole, and octapole).^{11,12} By using radiofrequency (RF) potentials, ions can be manipulated, focused, and transmitted in medium vacuum conditions ($<10^{-4}$ mbar). The skimmer cone (SC) is normally used to transport ions from the first vacuum chamber with high pressure (10^{-1} mbar to 10 mbar) to the second vacuum chamber with low pressure (10^{-3} to 10^{-4} mbar). Without any ion focusing properties, the SC is believed to be the sensitivity bottleneck.

Gerlich demonstrated that ions can be efficiently confined and transported through a series of stacked ring electrodes with a fixed ring internal diameter by using RF electric fields with reverse polarity on adjacent electrodes.¹³ The design can create a “pseudo-potential” that can achieve a steep potential gradient near every electrode and a near-field free region over most of the internal volume. A novel development of this technique was the electrodynamic ion funnel (IF) pioneered by Smith and co-workers.^{14–22} This electrodynamic IF has a funnel-shaped

channel formed by internal apertures of ring electrodes from the ion inlet toward the outlet end. IF was able to capture, focus, and transport ions in high efficiency by radially applying RF potentials combined with an axial direct current (DC) voltage gradient. An increase of over an order of magnitude in sensitivity for various peptides and proteins was demonstrated.^{15,17}

In ESI, highly charged droplets are generated and undergo a series of evaporation and coulombic explosion to reduce the droplet mass and charge, respectively.²³ A relevant problem with the ESI source originates from the presence of solvent-related ions^{24,25} and both droplet- and residue-related ion species,^{26–28} which lead to an increase of contamination and chemical noise if partially transmitted into the mass spectrometer. A significant quantity of gas can also enter the vacuum system. Thus, the removal of the accompanying gas from the ions in the pathways prior to the mass analyzer is essential.²⁹ Many designs in ESI sources or ion guide devices, such as z-spray and step-wave (Waters, Milford, MA, U.S.A.), off-axis ion funnel (Bruker Daltonics Inc., Billerica, MA), and iFunnel (Agilent Technologies, MD), have been developed and commercialized for effective removal of neutral molecules.

In this study, we present a new ion guide device named the V-shaped ion funnel (V-IF), as well as an orthogonal ion funnel

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(or-IF) for comparison, to reduce the entrance gas flow and remove the neutral contaminants from the sample solvent. The ion transmission efficiency of these interfaces for various samples can be maintained because of the effective RF confinement in the radial direction and the application of the appropriate DC potentials. The other ion transmission characteristics of V-IF, including adduct ion retain/removal and ion dissociation, were also investigated.

EXPERIMENTAL SECTION

Chemicals. Peptide and protein samples over a wide mass range were used to evaluate the ion transmission properties of or-IF and V-IF interfaces. PHE-VAL, substance P, angiotensin II, ubiquitin, cytochrome c, myoglobin, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All samples were used without further purification.

Ion Funnel Design. Orthogonal ion funnel (or-IF) was simply designed by perpendicularly aligning the ion funnel device to the dielectric capillary (Figure 1A). The V-shape ion

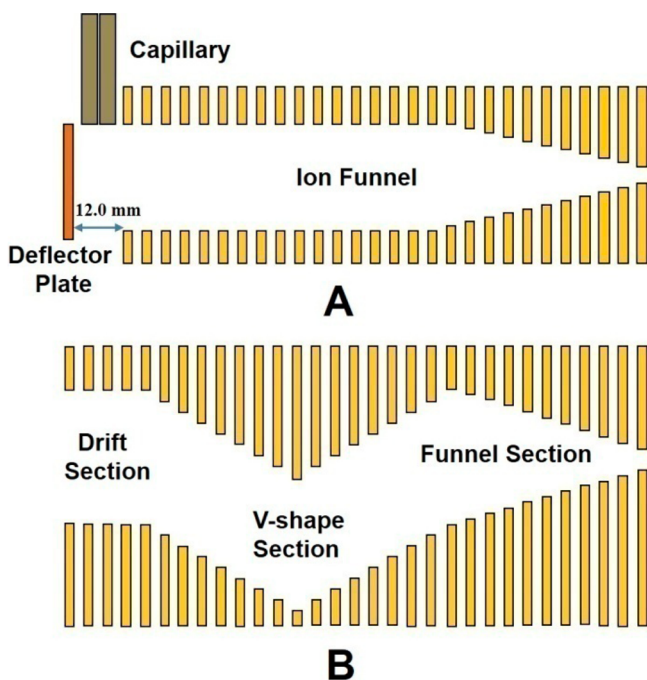


Figure 1. Schematics of the (A) or-IF interface and (B) V-IF interface (not in scale).

funnel (V-IF) was designed by using a “V” shape channel to transmit ions rather than a straight channel used in the traditional ion funnel. It had three sections: drift section, V-shape section, and funnel section, as shown in Figure 1B. The ion trajectories were simulated using SIMION 8.0. The hard sphere, elastic, ion-neutral collision model developed by Appelhans and Dahl was chosen.³⁰ Cytochrome c ions with various charge states were selected to simulate the ion trajectories for the V-IF device. The details of the funnel fabrication and ion trajectory simulation process were shown in the Supporting Information.

Instrumentation. Measurements of the ion currents transmitted through the dielectric capillary, or-IF, and V-IF were made using an independent setup as shown in Figure S-1. All mass spectra were obtained by using an APEX47e FTICR-MS (APEX III, Bruker Instrument Inc., Boston, MA) equipped

with an unshielded 4.7 T superconducting magnet and a homemade microspray ion source. The or-IF and V-IF were used to replace the traditional skimmer cone interface (Figure S-2). The or-IF and V-IF were operated at the same pressure as the traditional skimmer cone interface. The hexapole generally worked in trapping mode which could accumulate ions for a certain period (typically 1.0 s) to increase the sensitivity of FTICR-MS. Sample solutions were infused by a Harvard syringe pump (South Natick, MA) at a flow rate of 10 $\mu\text{L}/\text{h}$ through a fused-silica capillary tip (150 μm o.d., 50 μm i.d., Polymicro Technologies, AZ, USA). The ESI tip was connected to the grounded syringe and the entrance of the dielectric capillary was floated to a negative potential (~ -3000 V). The capillary inlet was heated by a flow of dry nitrogen gas at a temperature of 250 $^{\circ}\text{C}$. The distance between the end of the ESI tip and the heated metal capillary cap was about 1.0 mm.

RESULTS AND DISCUSSION

Ion Trajectory Simulation. V-IF functions as a “beam stopper” that can block gas flow by using a V-shaped channel. The neutral contaminants from the sample solvent accompanying the gas molecules will hit the electrodes and be pumped away. The transmission direction of the ion beam can be changed at the corners of the V-shaped channel by the RF potential. Ions can be confined and transmitted without any losses. The trajectories of the cytochrome c ions in V-IF are shown in Figure S-3. The results show that the ions can be transmitted to the exit of V-IF with high efficiency because of the strong strength of the RF potential (e.g., 600 kHz, 100 V_{pp}). The axial DC voltage gradient (E_{VIF}) is insensitive to the ion transmission.

Ion Transmission Efficiency. Molecular ions with sizes ranging from a small molecule (PHE-VAL) to a large biomolecule (BSA) were used to test the ion transmission efficiency of the designed IFs. Figures S4–S6 depict the ion transmission efficiencies of or-IF and V-IF for various samples with the changes in RF amplitude. It can be found that the ion transmission efficiencies of V-IF can reach 60–80% and remain constant as long as the RF amplitude is larger than 40 V_{pp} . No evident ion discrimination was observed, which indicates that V-IF can simultaneously transmit various sample ions in the same conditions and has the potential to couple with various mass analyzers. Effective RF potential can confine ion beams in a radial direction, which is dependent on both the RF frequency and amplitude. The ion transmission efficiencies for various samples were measured by adjusting the RF amplitude at different frequencies (Figure S-7). High ion transmission efficiencies were obtained when the appropriate RF frequency and amplitude were applied. Insufficiently strong RF potentials (e.g., 250 kHz, $< 10 V_{\text{pp}}$ and 600 kHz, $< 40 V_{\text{pp}}$, etc.) cannot confine ion beams effectively, thus leading to low ion transmission efficiencies. Extremely high RF potentials (e.g., 150 kHz, $> 150 V_{\text{pp}}$, etc.) would reduce the ion transmission efficiencies presumably because of ion losses at the exit where a high RF potential exists.

Collisions between the analyte ions and residual gas molecules are advantageous for ion desolvation but can dramatically reduce ion transmission efficiency. Figure S-8 describes the ion transmission efficiencies for various samples depending on the pressure by using a fixed RF potential (600 kHz, 190 V_{pp}). The ion transmission efficiencies for all samples decreased from 60–80% to 20–40% when the pressure increased from 1.0 to 8.0 mbar. The pressure plays a key role

in ion transmission. When the pressure increased, the effectiveness of the ion confinement by the effective RF potential is reduced, thus leading to increased ion losses. The situation can be improved by accordingly increasing the RF frequency and amplitude. The ion transmission efficiencies for various samples were also tested by using different E_{VIF} values with a specific RF potential (Figure S-9). The ion transmission efficiencies do not significantly change as long as the DC gradient is larger than 3 V/cm, thus implying that the DC gradient is not a key parameter for ion transmission.

Sensitivity Improvement. Table S-1 describes the S/N ratio enhancements of the most abundant ion peaks in the mass spectra acquired by using V-IF and or-IF for various peptide and protein samples. The mass spectra are shown in Figure S-10. The S/N ratios are enhanced by an order of magnitude for substance P, angiotensin II doubly charged ions, and cytochrome c multiple charged ions as compared to the traditional SC. An enhancement of 5.2-times was obtained for PHE-VAL singly charged ions by using V-IF, which may be caused by the entrance gas dynamic effect.

Robustness Enhancement. Contamination is a common problem in ESI-MS. The transfer of excessive solvent and matrix molecules into ion optical devices can quickly and dramatically decrease the overall sensitivity of the mass spectrometers. The jet disruptor reported by Smith and co-workers³¹ was used in an ion funnel interface to enhance the dispersion of the directed gas flow from a multicapillary inlet and reduce the pumping speed requirement. However, the gas flow can still drive the neutral contaminants to transmit into the following ion optics behind the ion funnel. In our design, the V-IF was fabricated to reduce the contamination to the hexapole. As shown in Figure 2, the normalized intensities of m/z 674 ion

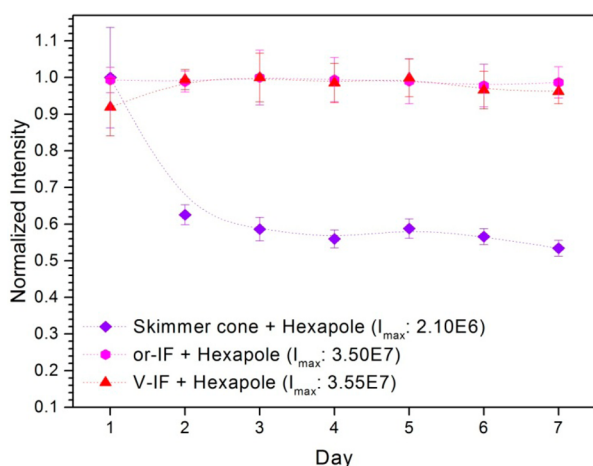


Figure 2. Normalized intensities of the doubly charged ion m/z 674 ($1.0 \mu\text{M}$ substance P) peak in the mass spectra obtained by using the traditional SC, or-IF, and V-IF coupled with a hexapole trapping mode over a week.

($1.0 \mu\text{M}$ substance P) peak in the mass spectra acquired by using V-IF and or-IF are kept constant over 7 days. However, the normalized intensities quickly decrease by approximately 45% and then remain constant over days using the original SC interface. Therefore, V-IF can effectively eliminate neutral molecules, thus reducing the hexapole contamination. The device can extend the robustness of the entire instrument without any significant change in performance over a long period.

Adduct Ion Retain and Removal. Adduct ions for high-mass samples can be retained in the transporting process by using IF under weak RF potentials because of their gentle ion transmission property. Increasing the RF potential can efficiently transform the adduct ions to parent ions.¹⁷ The experiments show that V-IF has a similar ion transmission property; i.e., it can protect the adducting species. The mass spectra for $29.0 \mu\text{M}$ myoglobin were obtained by using a V-IF interface at 250 kHz with different RF amplitudes (Figure 3A,B). Notably, Figure 3A consists of several ion clusters of

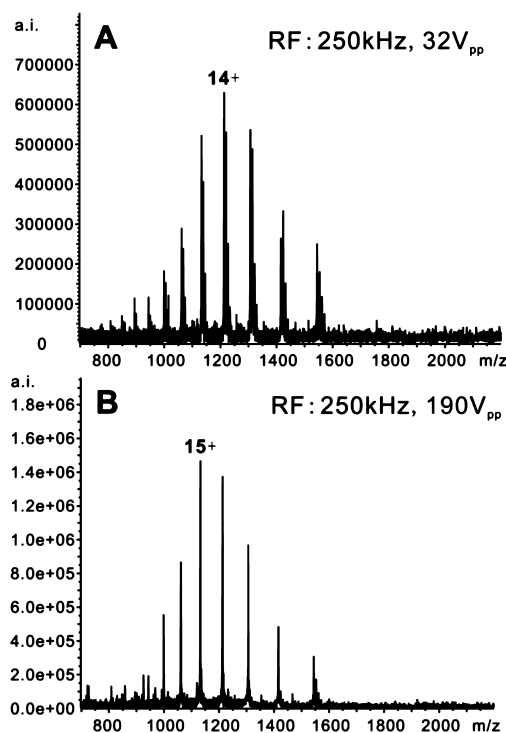


Figure 3. Mass spectra for a $29.0 \mu\text{M}$ myoglobin sample solution acquired by using V-IF (5 V/cm and 2.3 mbar) at 250 kHz with RF amplitudes of (A) 32 V_{pp} and (B) 190 V_{pp} .

myoglobin ions. These adduct ions suggested that sulfate and/or phosphate impurities present in the myoglobin ions can produce some sulfuric and/or phosphoric acid that may bind to the accessible basic sites of the myoglobin ions.³² Once the RF amplitude increases to 190 V_{pp} , the adducting species will be completely removed by collisional activation (Figure 3B). The peak intensities were observed to be approximately two times of those in low RF amplitude mode.

Comparisons between V-IF and Other IFs. The design of the “V” shape channel instead of the straight channel used in conventional IF can block neutrals more effectively. There are several advantages as compared with conventional IF. Structurally, the design is simple by stacking the electrode rings with progressive displacement of the central opening. It is not necessary to modify the other hardware including the source manifold and the electronics of the instrument. For instance, the or-IF is built by aligning the capillary perpendicularly to the conventional IF. A deflector is needed to push ions into the IF channel orthogonally. In terms of the design, the vacuum chamber must be modified to mount the capillary flange. One more DC power supply is required for providing a DC for the deflection. Some portion of ions (about 10%) may be lost during the ion deflection process. For the

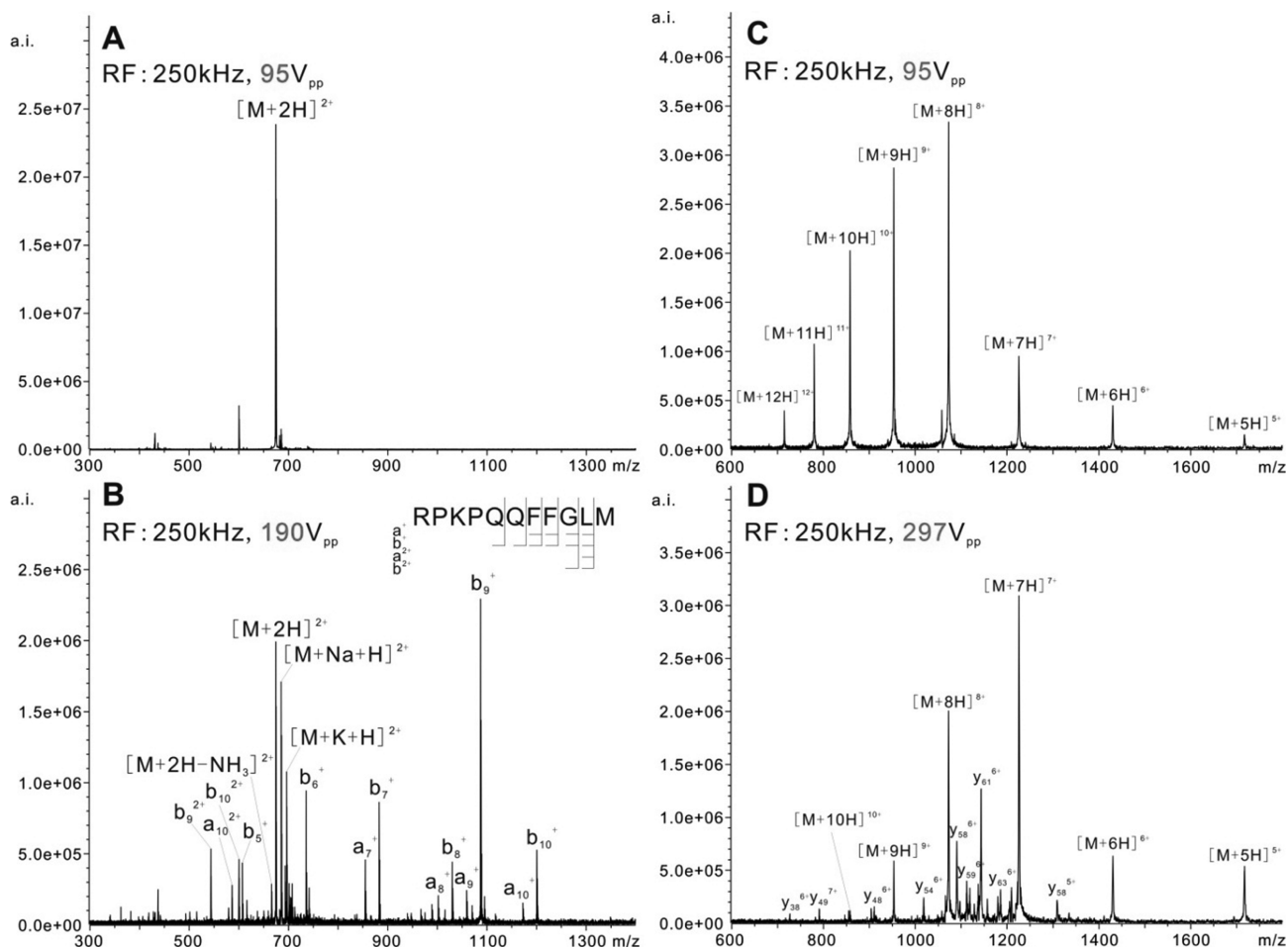


Figure 4. Mass spectra for 1.0 μM substance P obtained at (A) 95 V_{pp} and (B) 190 V_{pp} and 30.0 μM ubiquitin obtained at (C) 95 V_{pp} and (D) 297 V_{pp} using V-IF (250 kHz, 5 V/cm, and 2.3 mbar).

commercial off-axis IF device, it consists of a high pressure ion funnel (HPIF) and a conventional IF. They are off axis aligned to eliminate the neutrals. However, it needs two ion funnel setups which have a different number of electrodes and voltage parameters. The HPIF requires higher RF frequency and amplitude to achieve high ion transmission efficiencies for different ions. Therefore, two sets of the RF power supplies are needed to support the off-axis IF device. Since the HPIF and conventional IF are working in different pressure regions, one more vacuum chamber is needed and other hardware such as the pumping port, roughing pump, and DC power supplies are also needed. In summary, the V-IF is simple in design and easy to operate. The performance is better than the other IFs.

RF-Driven Collision Induced Dissociation. Here, we developed a technique to induce ion dissociation (i.e., RF-driven collision induced dissociation (CID)) in V-IF. Figure 4A,B presents the mass spectra for a 1.0 μM substance P sample solution obtained by V-IF at 250 kHz with RF amplitudes of 95 and 190 V_{pp} , respectively. No fragment ion was observed because the strength of the RF potential was insufficient. Once the RF amplitude increases to 190 V_{pp} , numerous fragment ions are generated due to the collisional activation assisted by RF heating at the corners and exit of V-IF. It was found that *b*-type fragment ions accompanying some adduct parent ions are produced. The mass spectra of 30.0 μM ubiquitin are also obtained at 250 kHz with various RF amplitudes (Figure 4C,D). A few *y*-type fragment ions covering

a certain *m/z* range (800–1200 Da) are produced when the RF amplitude is set to 297 V_{pp} , which reaches the limit of the RF power supply. The RF-driven CID is achieved by using high RF potential to activate the ions with higher energy collisions with the residual gas molecules. In transmission mode of V-IF, the RF amplitude is low and ions are confined efficiently and delivered gradually to go through the entire channel. When increasing the RF amplitude, the ions are activated and the amplitude of ion motion will increase and multiply collide with the residue gas molecules. In-source collision induced dissociation (CID) can be realized at the exit of the IF by increasing the DC potential between the exit electrode of the IF and the conductance limiting plate (Figure S-11). The distances between the IF exit plate to the conductance limit plate is generally very short (i.e., 1.0 mm); fragment ions formed might not be focused efficiently in this region. However, the product ions formed by RF-driven CID in V-IF can be confined and transmitted. Figure S-12 shows the intensities of *a*-/*b*-ions generated by the DC-driven CID (in-source) and RF-driven CID conditions under the same electrospray ionization conditions. It can be seen that the intensities of product ions in RF-driven CID were generally higher than that of DC-driven CID.

CONCLUSIONS

To improve the sensitivity and extend the robustness of the FTICR-MS instrument, a novel ion guide V-IF was developed

and evaluated by offline and online experiments. For comparison, a prototype or-IF was built and characterized. High ion transmission efficiencies for various peptide and protein samples were achieved with appropriate DC and RF potentials in a broad pressure range. An order of magnitude of sensitivity enhancement for peptides and proteins was obtained. The neutral molecules can be effectively eliminated to significantly improve the robustness of the instrument using V-IF. V-IF has the ability to preserve the noncovalent bonds by using low RF potentials and efficiently removing the adducting species in a high RF amplitude mode. Furthermore, RF-driven CID is achieved by using V-IF for various peptides and proteins by applying high RF potentials.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01828.

Designs of V-IF and or-IF; ion trajectory simulation; ion current measurements; ion transmission efficiency of or-IF; samples and ESI conditions; additional figures and a table (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: twdchan@cuhk.edu.hk.

*E-mail: xiangfchensdas@163.com.

Notes

The authors declare no competing financial interest.

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