Published in final edited form as:

Anal Chem. 2020 January 07; 92(1): 252–266. doi:10.1021/acs.analchem.9b05014.

Recent Developments in Gas-Phase Ion/Ion Reactions for Analytical Mass Spectrometry

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Graphical Abstract

INTRODUCTION TO GAS-PHASE ION/ION REACTIONS

While the study of reactions between oppositely-charged ions has a long history, particularly within the context of plasmas and interstellar chemistry, the exploitation of such reactions for analytical applications was made possible with the advent of electrospray ionization (ESI).^{1,2} ESI provides a means for generating abundant multiply-charged ions from a wide variety of molecular classes. It was then possible to use ESI to generate multiply-charged analyte or reagent ions for reactions with ions of opposite polarities without resulting in complete neutralization. The first description of ion/ion reaction studies using ESI was given by Loo et al. using a Y-tube reactor leading to the inlet of an atmosphere/vacuum interface coupled with a quadrupole mass filter.^{3,4} This was an early example of ion/ion chemistry being effected prior to sampling ions into a mass spectrometer. The first implementation of ion/ion reactions within the context of a MSⁿ experiment was demonstrated at Oak Ridge National Lab.^{5,6} This was accomplished through the use of electrodynamic ion traps, which allow ions of opposite polarity to be stored in overlapping regions of space. Since those

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The authors declare no competing financial interest.

early reports, the use of ion/ion reactions, both *in vacuo* and *ex vacuo*, within the context of molecular analysis has expanded significantly.

A number of characteristics make ion/ion reactions attractive for analytical mass spectrometry. For example, given the long-range Coulomb attraction associated with oppositely-charged ions, the cross-sections for ion/ion collisions are very large such that reactions can be driven on the millisecond to sub-millisecond time scales, depending upon ion densities and extents of ion overlap. Another important characteristic is that mutual neutralization is highly exothermic for virtually all combinations of oppositely-charged gas-phase ion/ion reactions. Hence, ion/ion reactions are highly efficient and always result in some kind of reaction. Given the diversity of ion types that can be generated by the suite of ionization methods now available, the range of ion/ion reactions that can be effected is extremely large, even larger than that of ion/molecule reactions, which are limited by volatility constraints. In the case of ion/ion reactions that take place within a tandem mass spectrometer, such as an ion trap or hybrid instrument, the fact that ions are readily manipulated on a time-dependent basis (i.e., they can be selected or ejected either selectively or non-selectively) enables a high degree of control over the identities of the reactants and the times over which they are exposed to one another.

The attractive characteristics of ion/ion reactions for multiple analytical applications have been illustrated in reports going back to the original works described above. Many of these have been reviewed previously.⁷⁻⁹ In this review, we emphasize developments that have taken place largely within the past decade with particular emphasis on the last five years. These have included developments in tools used to implement ion/ion reactions for one or more types of mass spectrometry experiments, an expansion in the range of ion/ion reaction types, and the growth in analytical applications of ion/ion chemistry. The review is organized with descriptions of instrument development, proton transfer chemistry, electron transfer chemistry, and reactions that can proceed only through long-lived complexes.

DEVELOPMENTS IN ION/ION REACTION INSTRUMENTATION

Scientific progress is directly related to and dependent on the development of instrumentation. In this respect, the increasing adoption of ion/ion reactions as an analytical tool within the past half-decade stems from the development of novel instrumentation equipped to perform such experiments. The commercialization of electron transfer dissociation (ETD), and more recently proton transfer charge reduction (PTCR), has emerged as, perhaps, the most significant advancement in ion/ion reaction instrumentation, bringing ion/ion reaction capabilities to laboratories around the world. Previously, research in the area of ion/ion reaction chemistry was exclusively limited to home-built instruments or heavily modified commercial instruments. The evolution of such instrumentation has been reviewed.⁷⁻¹⁰ In this section, rather than providing a complete history of ion/ion reaction instrumentation, we focus on the recent developments since the last comprehensive review of instrumentation in 2008.¹⁰ These developments are described in the context of three categories: ion/ion reactions performed outside the mass spectrometer, ion/ion reactions performed *in vacuo*, and the analysis of high *m/z* ions.

Front-end Ion/Ion Reaction Instrumentation

Several criteria must be met for a gas-phase ion/ion reaction to occur, chief among them is the temporal and spatial overlap of oppositely charged ions. In the case of reactions proceeding at or near atmospheric pressure, this is accomplished prior to the introduction of the ions into the vacuum system. As such, the modifications required to perform gas-phase ion/ion chemistry at atmospheric pressure are localized to the ionization region, specifically prior to the entrance to the atmosphere/vacuum interface. Recently, there have been several reports of modified or repurposed commercial ionization sources used for front-end ion/ion reactions.¹¹⁻¹³

Two reports of instrumentation highly analogous to that described by L. M. Smith and co-workers^{14,15} involved the modification of commercial ionization sources. In one report, the ESI source of a LC/MS platform was outfitted with a polonium-210 (²¹⁰Po) α -particle source.¹¹ The emitted α -particles initiate ion/molecule reactions involving ambient gas and solvent molecules generating ions of both polarities. Subsequent reactions between the α -particle progeny ions and the analyte ions generated via ESI result in charge reduced analyte ions, which are then sampled into the mass spectrometer for mass analysis. In another report, both an ESI and a nESI source of a traveling wave ion mobility mass spectrometer (TWIM MS) were modified to contain a corona discharge probe.¹² The discharge generates anions from a nitrogen gas supply that is flowed over the platinum wire/discharge plate. Ensuing proton transfer ion/ion reactions result in the charged reduced protein species. The extent of charge reduction can be modulated by varying the flow rate of nitrogen or the applied voltage to the discharge needle. Robb et al. have demonstrated similar charge reduction reactions by simply repurposing a commercial atmospheric pressure ECD source.¹³

In addition to modifying commercial ionization sources as described above, commercial sources can be replaced altogether with modular front-end ionization sources designed to perform ion/ion reactions. For example, the Brodbelt group constructed a free-standing dual ionization source reactor that can be mounted to the front-end of any mass spectrometer.¹⁶ The reactor consists of two electrosonic spray ionization sources fixed to a U-shaped rail. Permitting enhanced control, the angle of the two sources relative to one another and relative to the inlet of the mass spectrometer can be adjusted (Figure 1). The first source is integrated with the instrument such that the voltage and polarity are controlled with the instrument software. The voltage of the second source is supplied by an external dual polarity high voltage power supply.

With this setup, ion/ion reactions are conducted in dual spray mode where the two sources are operated simultaneously. One source generates a plume of multiply charged peptide cations while the other generates a mixture of singly and doubly charged reagent anions. The two sources are oriented at an angle such that collisions between oppositely charged droplets, pseudodroplets, and gas-phase ions are maximized. Experimental evidence suggests that the reactions proceed exclusively through an ion/ion mediated pathway; the abundance of the peptide/reagent complex is directly related to the anion source voltage. Additionally, when the anion source voltage is zero, reminiscent of an ion/molecule reaction, the peptide/reagent complex is not observed. Stutzman and co-workers have performed similar experiments to demonstrate the charge reduction of synthetic polymers with a bipolar

dual spray setup.¹⁷ Despite the setup being highly analogous to the dual spray reactor described above, we note a lack of conclusive evidence suggesting exclusively ion/ion chemistry. Nonetheless, it represents an intriguing avenue for charge state manipulation performed *ex vacuo*.

***In Vacuo* Ion/Ion Reaction Instrumentation**—While instrumentation equipped to perform front-end ion/ion reactions offer some advantages (i.e. compatibility with any mass analyzer, simplicity of modifications, etc.), the vast majority of ion/ion reactions are performed *in vacuo*. There are several distinct advantages to performing experiments within the confines of an electrodynamic trap: (1) independent control/optimization of reactant species, (2) well defined reaction conditions, and (3) MS^n capabilities in conjunction with ion/ion reactions. Ion/ion reactions performed within the mass spectrometer have historically been conducted in either a 3-D quadrupole ion trap or a 2-D linear ion trap. Compared to 3-D traps, linear ion traps have a larger ion capacity and are more efficiently coupled to ionization sources and other devices. Consequently, apart from an IT-IM-TOF instrument which couples a 3-D ion trap with an ion-mobility cell,¹⁸ reactions performed in linear ion traps constitute the bulk of recent literature.

Much of the work surrounding ion/ion reactions performed in both quadrupolar and hexapolar linear ion traps originates from the development and optimization of the now commercialized ETD instruments. ETD equipped instrumentation has been discussed in detail by Riley and Coon.¹⁹ Therefore, it is outside the scope of this review to thoroughly discuss the instrumentation and methodological developments of ETD. The ion traps in which ETD reactions are performed, however, can serve as reaction regions for any ion/ion reaction. In this context, some of the same instrumentation is mentioned as it pertains to ion/ion reactions other than ETD.

As mentioned above, any electrodynamic ion trap with mutual storage capabilities can be used to perform ion/ion experiments, provided that ions of opposite polarity can be generated and transported to the same trap. This can be accomplished by the generation of oppositely-charged ions from two different sources and subsequently introducing ions through two separate orifices or through a common orifice. Instruments of both types have been described in recent years. He et al. described a dual polarity ion trap mass spectrometer of the former type.²⁰ The instrument consists of two ionization sources and two sets of ion optical systems separated by a segmented linear ion trap in the middle. Ions are generated using the two sources and can be transported to the linear ion trap where the reaction proceeds. Application of dipolar DC to one rod set enables the simultaneous detection of positive and negative ions of the same reaction experiment.

Lin et al. also described an instrument which ions are introduced through separate orifices.²¹ Their home-built mass spectrometer consists of two discontinuous atmospheric pressure interfaces (DAPI) 180° from one another, one rectilinear ion trap, and one detector. Analyte ions are generated by DAPI I, introduced into the trap through its respective orifice, and, if desired, isolated using custom waveforms. Reagent anions generated by DAPI II are then introduced into the trap. Next, the two populations are allowed to react. However, opening the pinch valve of the DAPI results in an increased pressure of the trap which, in turn, could

have deleterious effects on the ion/ion reactions. In fact, the proton transfer product ion spectrum shows two low abundance fragment ions which likely originate from the energy deposition by gas flow resulting from the sudden change in pressure from opening of DAPI II. A static higher base pressure is more desirable as it serves to enhance ion/ion reaction efficiency. However, Campbell and Hager have demonstrated ion/ion reactions in a low pressure reaction cell.²² They created an evanescent ion/ion reaction region within the low pressure region of Q3 of a hybrid triple quadrupole/linear ion trap mass spectrometer by adding DC and RF voltages to the optical components surrounding Q3 and by adding a pulsed valve for the addition of a cooling gas. The results of the ion/ion reactions performed in this low pressure region were similar to that of the higher pressure region.

Early versions of ETD equipped instrumentation contained a chemical ionization source at the rear of the instrument.^{23,24} Once generated, the reagent radical anions are delivered through the C-trap and into the segmented linear ion trap where the ETD reaction would take place. It wasn't long before this platform was used for proton transfer reactions.²⁵ In 2013, Hunt and co-workers described a front-end ETD source.²⁶ Here, cations and anions can be generated and introduced through a common orifice at the front of the instrument. Since its inception, the front-end chemical ionization source has been implemented on a number of research grade instruments including the 21 Tesla (T) Fourier transform-ion cyclotron resonance (FTICR) mass spectrometer at the National High Magnetic Field Laboratory.²⁷ Additionally, this source serves as the basis for the newest Orbitrap hybrid systems, including those with PTCR capabilities.

A common characteristic of all the instruments described above is the use of a linear ion trap as the reaction vessel. Ion/ion reactions are not restricted to linear ion traps though. The use of a traveling wave (T-wave) cell as a reaction vessel has been reported.²⁸⁻³¹ Cations and anions are generated at the front of the instrument. The optical polarities are alternately switched, and the quadrupole ion guide is set to transmit either exclusively precursor cations or reagent anions into the T-wave trap cell. The reaction proceeds in this cell as the ion populations are propelled through one another by the T-wave. Similarly, a traveling wave structure for lossless ion manipulations (TW SLIM) device capable of performing ion/ion reactions has been described.³² Here, one SLIM surface contains four alternating phase RF electrodes interweaved with three traveling wave electrodes (Figure 2a). A second SLIM surface contains an identical configuration carrying the opposite phase on every RF electrode as shown in Figure 2b. Simulations suggest ion/ion reactions in this device are feasible but have yet to be demonstrated experimentally.

Analysis of High m/z Product Ions from Ion/Ion Reactions

Ion/ion reaction product ions can be one or more orders of magnitude higher in m/z than the reactant ions. This is especially true for proton transfer ion/ion reactions involving proteins of relatively high mass (e.g. > 30 kDa). There is, however, a number of challenges associated with generating and analyzing high mass-to-charge ions, both directly from electrospray ionization and from ion/ion proton transfer reactions. In the case of an ion/ion reaction experiment in trapping instruments, the upper m/z limit is dependent on (1) the detector response, (2) the range over which ions of disparate m/z can be mutually stored, and (3) the

efficiency in which high m/z ions can be mass selectively ejected towards the detector. Our group has demonstrated a waveform switching technique that directly addresses the third challenge.³³ A schematic of the instrument is provided in Figure 3. A high frequency sine wave of 1.008 MHz is applied to the ring electrode during ion injection and mutual storage. After the ion/ion reaction period, a low frequency square wave is applied to the end cap electrodes, the sine wave is turned off, and product ions are mass selectively ejected via a frequency scan of the square wave. Switching from a high frequency sine wave operation of the trap to a low frequency square wave scan during mass analysis eliminates an upper m/z limit associated with voltage constraints. Additionally, the reduction in frequency places the product ions at higher q values, thereby, generating deeper well depths and smaller ion packets despite using lower amplitudes. Using this method, the upper m/z limit of the ion/ion reaction was improved by a factor of 2-3.

In addition to the development in high m/z analysis described above, recent modifications to the Orbitrap mass spectrometer platform have been aimed at increasing the transmission, isolation, fragmentation, and resolution of high m/z ions.³⁴⁻³⁸ Modifications include orthogonal ion injection into the instrument to reduce instrument contamination, in-source trapping to facilitate ion desolvation and to minimize the ions' axial momentum, reduction of the RF frequencies applied to the bent flatapole, quadrupole, transfer multipole, C-trap, and HCD-cell, lengthening ion injection times from the C-trap to the Orbitrap mass analyzer, and changes to the image current preamplifier to detect a broader range of ion frequencies. Implementation of these modifications have enabled the detection of high m/z ions up to m/z 70,000.³⁹ To date, no ion/ion reactions have been performed on this platform. However, it stands to reason these developments can lend themselves to eventually couple ion/ion reactions to high mass-to-charge protein or protein complex ions.

PROTON TRANSFER ION/ION REACTIONS

The first proton transfer ion/ion reactions were conducted in the early 1990's using the front-end Y-tube reactor.^{3,4} Since then, altering ion charge states via gas-phase proton transfer has developed into a robust and mature technique that has found utility in biological mass spectrometry, primarily in the charge state manipulation of proteins. Much of the early work devoted to proton transfer for protein analysis was performed in 3-D ion traps.^{6,40-43} As the resolution of ion traps is generally limited, identification of ion charges greater than two can be a nontrivial task when adjacent charge states from an analyte of interest are not clearly identifiable. Proton transfer ion/ion reactions, therefore, have historically been used to simplify spectra containing highly charged ions by decreasing ion charges to mostly 1+ or 2+ and by dispersing ions across a wide mass-to-charge range. Increased resolving power can enable charge state determination via measurement of the isotope spacings within a single charge state. Yet, it has been shown that even high-resolution instruments, such as the Orbitrap and FT-ICR mass spectrometers, can benefit from using proton transfer reactions when complex mixtures of ions are present.⁴⁴ In recent years, as the complexity of protein mixtures subjected to ESI has increased, there has been an extension of proton transfer workflows to high-resolution instruments. Applications of proton transfer ion/ion reactions include MS^n product ion analysis, precursor ion charge state manipulation, and precursor ion signal concentration. Additionally, proton transfer reactions are used in structural proteomic

studies. The following sections feature recent work describing proton transfer reactions in all these application areas.

Product Ion Analysis

Several activation methods, including electron transfer dissociation and ultraviolet photodissociation (UVPD), have emerged with a common objective to enhance structural characterization by maximizing the number of sequence informative product ions. However, fragment ions produced using these techniques typically result in a product ion spectrum in which fragments are confined to a relatively narrow m/z range centered about the precursor. When combined with proteins of relatively large size and the multiplicity of fragment ion charge states, the resulting product ion spectrum can be quite complex. In 2005, the Hunt group used sequential ion/ion reactions (i.e. electron transfer dissociation followed by proton transfer reactions) for protein identification.²⁵ ETD of the $[M+13H]^{13+}$ charge state of ubiquitin performed on a Finnigan LTQ mass spectrometer generated a spectrum that was uninterpretable. However, using proton transfer on the ETD products, approximately 90% of the residues could be sequenced. The utility of this ETD-proton transfer workflow is further demonstrated by the identification of forty-six *E. coli* 70S ribosomal proteins.⁴⁵

Expanding upon the work performed in ion trap mass spectrometers,^{25,46-48} Hunt and co-workers have recently coupled the ETD-proton transfer workflow with Orbitrap mass analysis.⁴⁹ In this experiment, a multiply charged protein ion was isolated and fragmented by electron transfer dissociation, resulting in a complex product ion spectrum, as shown in Figure 4a. Following ETD, product ions were charge reduced via proton transfer to sulfur hexafluoride radical anions. Figures 4b - 4e show the resulting charge reduced spectra under identical ETD parameters but increasing proton transfer reaction durations. Interestingly, the most informative reaction duration was observed to be the 20 ms ion/ion proton transfer reaction. ETD followed by proton transfer on the Orbitrap platform has been used in only a few other studies,⁵⁰⁻⁵² likely due to the lack of a commercial instrument optimized to perform both types of ion/ion reactions. An increase in the number of applications utilizing ETD followed by proton transfer can be anticipated with the commercialization of PTCR on ETD equipped instruments.

Precursor Charge State Manipulation

In addition to reducing spectral complexity during product ion analysis, proton transfer can be used to produce simpler spectra during MS^1 experiments. Laszlo and Bush demonstrate that proton transfer can increase accuracy in assigning charge states of native proteins and protein complexes generated via ESI.⁵³ With ESI under denaturing conditions, as the size of the analyte increases, the propensity for generating multiply charged ions increases potentially creating overlap between charge state distributions of different species even for relatively simple mixtures. For complex mixtures, the overlap can be quite extensive, complicating data interpretation. This problem is exacerbated with polydisperse polymers, where the distributions are broad spanning, in some cases, tens of thousands of Da. Recently, proton transfer ion/ion reactions have been used to alleviate the spectral congestion of large synthetic polymers.^{12,13} In those studies, extensive charge reduction via proton transfer was shown to enable facile data interpretation for polymers up to 40 kDa. Other ion/ion charge

reduction strategies have been reported for polymer characterization, though it is unclear if the charge reduction occurs via proton transfer.^{11,17}

Most examples described above (i.e. product ion analysis and polymer analysis) utilize proton transfer reactions to charge reduce analyte ions to mainly the 1+ charge state. However, in some scenarios, it is desirable to perform less extensive charge reduction. For instance, our group has used proton transfer reactions to manipulate precursor ion charge in order to study the charge state dependent collisional activation of proteins.⁵⁴⁻⁵⁹ This technique has been adopted to study the charge state dependent fragmentation of proteins utilizing ETD and UVPD activation methods.⁶⁰⁻⁶³

In 2016, the Brodbelt group reported on the photodissociation patterns of native proteins following gas-phase proton transfer.⁶² While only minor changes in fragmentation were observed, this report demonstrates another attractive application for proton transfer reactions. ESI of bovine superoxide dismutase (SOD)/CuZn complexes under native conditions generates the mass spectrum shown in the top panel of Figure 5a. Close examination of the peak at approximately m/z 3140 shows an overlap of 5+ monomers with 10+ dimers (Figure 5b, top). This overlap inhibits the independent MS/MS analysis of the monomer or dimer distribution. Proton transfer, on the other hand, can generate purified distributions of monomer and dimer. For example, a clean 5+ monomer distribution and a clean 10+ dimer distribution is generated from proton transfer of the 12+/6+ distribution (Figure 5, middle row) and from proton transfer from the 11+ dimer (Figure 5, bottom row), respectively. Here, proton transfer is used to purify charge state distributions in the gas-phase. Gas-phase charge state purification via proton transfer reactions involving protein mixtures under denaturing conditions has also been demonstrated,^{41,42} and the Coon group extended this approach to proteomic quantitation with isobaric tagging.^{64,65}

The kinetics of ion/ion reactions can play a major role in determining product ion distributions, particularly when mixtures of ions of widely different charges are subjected simultaneously to reaction. Again, using the SOD/CuZn data as an illustrative case, when both the 12+ dimer and 6+ monomer are subjected to proton transfer ion/ion reactions, the resulting spectrum shows only a 5+ monomer distribution with no evidence of the isobaric 10+ dimer species. This follows from the charge squared dependence associated with ion/ion reaction rates.^{40,66} That is to say, the sequential charge reduction reactions of the 12+ dimer react at a faster rate than the 6+ monomer species. Consequently, the entire 10+ population is depleted, having reacted to lower charge states, leaving a relatively enriched purified monomer distribution.

A degree of control over ion/ion reaction kinetics can be effected in a process termed “ion parking.”⁶⁷ This is accomplished by applying a low amplitude auxiliary RF signal at the secular frequency of the desired product ion. The applications of ion parking are numerous. Many of the studies in the past decade involving ion/ion reactions utilized ion parking to enhance sensitivity, including some examples discussed above.^{51,61,62} Recently, we have used ion parking to concentrate precursor ion signal prior to moving the ions to a m/z region likely to maximize cleavage at aspartic acid and proline residues.⁶⁸ The Hunt group implemented “parallel ion parking”^{69,70} during the on-line HPLC, data-dependent MS/MS

analysis of the *E. coli* ribosome (Figure 6).⁷¹ Here, a broadband parallel ion parking waveform is applied to concentrate ion signal without *a priori* knowledge of the protein (compare signal intensities of Figures 6a and 6b). Campbell and Le Blanc employed ion parking to impart an additional degree of selectivity during protein quantitation,⁷² and in 2008, L. M. Smith and co-workers combined fixed-charge derivatization and ion parking to generate an abundant precursor at a charge state not generated under conventional electrospray conditions.⁷³ Ion parking provides a high degree of flexibility in mixture analysis applications due both to the concentration of multiple charge states into one or a few charge states, which is attractive for sensitivity, and the simplification of the precursor ion spectrum, which can improve specificity.

Structural Proteomic Studies

Native mass spectrometry is increasingly being adopted as a tool for structural biology, allowing researchers to extract more information from a mass spectrometry experiment than simply molecular weight.⁷⁴⁻⁷⁷ Coupling ion-mobility with native MS facilitates native protein and protein complex collisional cross section measurements. Those measurements, however, are obtained from the gas-phase structures of the analyte, and unfortunately, there is still a great deal of ambiguity regarding the relation of gas-phase structure with those observed in solution. Expanding upon the early work of Badman and co-workers,^{18,78} the Bush group is combining native mass spectrometry, gas-phase proton transfer, and ion-mobility to study structure/charge relationships and protein folding.⁷⁹⁻⁸³ Lermyte et al. perform similar experiments, but, in their case, an electron transfer reagent anion, 1,4-dicyanobenzene, is used for charge reduction.⁸⁴ Using 1,4-dicyanobenzene leads to a combination of electron transfer and proton transfer.

ELECTRON TRANSFER ION/ION REACTIONS

Because of the implementation of ETD across multiple commercially available instrument platforms, electron transfer ion/ion reactions can be considered the most commonly employed type of ion/ion reaction. The ETD process begins with an ion/ion reaction involving the transfer of an electron from a reagent radical anion to a multiply charged analyte cation, *viz.*:

Fragmentation of the charge-reduced product via radical directed mechanisms results, in the case of multiply-charged polypeptide cations, in c- and z[•]-type fragment ions and extensive primary sequence information.²³ Since its introduction in commercially available instruments, ETD has become a standard approach for the structural characterization of bio-ions. The majority of electron transfer ion/ion reactions are discussed in the context of ETD and negative electron transfer dissociation (NETD). Consequently, ETD and NETD have been extensively reviewed,^{19,85-88} with the latest review being published in 2018.¹⁹ Therefore, ETD is not discussed here. Instead, we highlight other electron transfer ion/ion reactions and their analytical utilities. Specifically, charge reduction via electron transfer and charge transfer dissociation are discussed.

Electron Transfer Charge Reduction

Electron transfer from a reagent radical anion to a multiply charged cation does not always lead to spontaneous dissociation to generate product ions. Non-dissociative electron transfer, often referred to as “electron transfer no dissociation” (ETnoD), gives rise to an intact charge reduced product ion. The extent of ETnoD has been shown to be dependent on several factors including protein ion conformation, precursor ion charge state, degree of supplemental activation, etc.^{84,89} This non-dissociative channel is undesirable when ETD is the objective, yet several studies exploit the formation of ETnoD products. The Kaltashov group, for example, employ native electrospray ionization in combination with electron transfer⁹⁰⁻⁹³ or electron capture^{94,95} charge reduction to reduce the spectral complexity of heterogeneous samples. Figure 7 shows the native ESI mass spectrum of an antithrombin-III/heparin complex (AT/heparin) in gray. Mass selection of the precursor ion and subsequent electron transfer charge reduction generates the product ion spectra shown in pink and cyan (Figure 7). The value of this strategy stems from the ability to correctly assign charges from the resulting mass spectrum that is reduced in spectral complexity. By selecting a narrower population of precursor ions, interpretable mass information can be extracted (compare magenta and cyan traces).⁹¹ Lermyte et al. performed similar electron transfer ion/ion reactions, though, in their case, precursor ions were reduced to the 1+ charge state.⁹⁶

Beyond charge assignment and mass determination, electron transfer charge reduction has proven to be useful in both protein conformational studies and in the generation of metal species in unusual oxidation states. In the former case, the effect of charge reduction on protein conformation has been studied by coupling ETnoD with ion-mobility, providing insights into the importance of net charge in the gas-phase on the extent of protein compaction.^{84,97,98} In the latter case, the transfer of a single electron from a radical anion to a metal-ligand complex, $M(II)L_x$, serves to reduce the metal forming the $M(I)L_x$ complex ion. Gronert first used this technique to study the reactivity of metals in the 1+ oxidation state towards allyl iodide.⁹⁹ Later, Oomens and co-workers used gas-phase reduction via electron transfer to study the coordination environment of several reduced metals and their ligands.^{100,101}

Charge Transfer Dissociation

Charge transfer dissociation (CTD), pioneered by the Jackson lab, is another electron transfer based fragmentation technique. Unlike most ion/ion and ion/electron fragmentation techniques in which reactions proceed through cation/anion interactions, the electron transfer of CTD occurs between two cationic species.¹⁰² Reactions of ions of like charge are characterized by a large Coulomb barrier that must be overcome by a large relative translation. Specifically, analyte cations are irradiated with a beam of 6 keV helium cations, leading to the abstraction of an electron by the helium ion:

Ultimately, this electron hole initiates radical directed fragmentation. Using cations at 6 keV overcomes the electrostatic repulsive barrier associated with cation/cation collisions.

Additionally, helium is intentionally chosen as the cation of choice for CTD due to its adiabatic recombination energy of 24.6 eV, the largest of any 1+ ion, which drives the electron abstraction process from other singly charged cations. In addition to the one-electron oxidation pathway discussed above, experimental evidence exists to suggest a one-step two-electron oxidation pathway.¹⁰³ Compared to ETD, CTD is a unique high energy dissociation method with the advantage of inducing fragmentation of singly charged precursor ions. To date, CTD has been applied to the analysis of peptides,¹⁰²⁻¹⁰⁴ phospholipids,¹⁰⁵ and oligosaccharides.^{106,107}

REACTIONS THAT PROCEED THROUGH COMPLEX FORMATION

Gas-phase ion/ion reactions between oppositely charged ions proceed through the formation of a Coulombically-bound orbit.¹⁰⁸ Initially, the distance between the ions in the orbit can exceed that necessary for chemistry to occur. As translational energy is removed via collisions and/or tidal effects,¹⁰⁹ the size of the orbit decreases until either a small charged particle (i.e. proton or electron) is transferred at a crossing point on the potential energy surface or a physical collision occurs resulting in formation of a collision complex. While all types of ion/ion reactions can take place through complex formation, many reaction types can only take place through complex formation. More so, complex lifetime is largely determined by the strength of the electrostatic interactions, the number of degrees of freedom of the complex, and both the emissive and collisional cooling rates. Examples of ion/ion reactions that can proceed only through a complex include, but are not limited to, metal ion transfer, charge inversion, and covalent chemistries. Recent work from all areas are highlighted below.

Metal Ion Transfer

Ion/ion reactions involving metal-ligand complexes can be used to insert metal cations into analyte ions. These reactions have been demonstrated in both polarities:

where M represents the analyte, Met represents the metal cation, L represents a singly charged anionic ligand, and Phen represents the neutral 1,10-phenanthroline ligand.¹¹⁰⁻¹¹⁵ In the case where the analyte is a singly deprotonated anion, ion/ion reaction with a doubly charged cationic metal phenanthroline complex results in the charge inversion of the analyte:

This case will be discussed in further detail in the charge inversion subsection below.

The reaction presented in Equation 3, for example, has been used to incorporate gold cations into disulfide containing polypeptides using AuCl_2^- .¹¹⁶⁻¹¹⁸ In all cases, gold cationization showed a preference for cleavage of disulfide bonds upon collisional activation, in contrast with the collisional activation of the corresponding protonated species. Recently, gold (I) cations have been incorporated into polypeptides lacking disulfide bonds.^{119,120} It was found that gold (I) cationization facilitates peptide oxidation at a neutral lysine residue via the loss of gold hydride and a molecule of ammonia. The resulting structure contains a fixed charge cyclic imine, which weakens the adjacent amide bond. Upon subsequent activation, facile fragmentation N-terminal to the oxidized residue is observed.¹¹⁹ This so called “weak-spot” at lysine was exploited for cyclic peptide analysis providing a site-specific ring opening pathway. Collisional activation of the oxidized cyclic peptide was shown to lead to unambiguous sequence information.¹²⁰

Whereas metal transfer from a reagent to the analyte is highlighted above, gas-phase ion/ion reactions for selective alkali metal removal have also been demonstrated.^{121,122} Luongo et al. investigated a series of weakly coordinating anions and demonstrated that, of the reagents examined, carborane anions (e.g., $\text{CHB}_{11}\text{Cl}_{11}^-$) were the most selective for the removal of alkali metals.¹²² Later, Betancourt et al. simplified the electrospray mass spectra of Polysorbate 80 via ion/ion reaction with carborane anions.¹²³ ESI of Polysorbate 80 shows overlapping distributions from multiple ion types and multiple charge states below m/z 1400. The ion/ion reaction between Polysorbate 80 cations and carborane anions pushed the charge states to largely singly charged species. That is to say, doubly charged species adducted one carborane anion and triply charged species adducted two carborane anions. Broadband collisional activation of the product ions results in the removal of the metal cations with carborane. The product ion spectrum is less congested than the electrospray mass spectrum and shows clear distributions of singly charged, metal cationized species.¹²³

Charge Inversion

Multiple charges (i.e. two or more) can be transferred during a single intimate collision between two ions. For example, ion/ion reaction between a singly protonated phosphatidylethanolamine (PE) and doubly deprotonated 1,4-phenylenedipropionic acid (PDPA) generates the singly deprotonated PE via the transfer of two protons from the PE cation to the PDPA anion.¹²⁴ In this case, the charge of the PE analyte ion is inverted from positive to negative polarity. Charge inversion reactions of both ion polarities have been reported.^{125,126} Our group has been investigating the utility of charge inversion ion/ion reactions and have demonstrated their applications in sulfo- and phosphopeptide characterization,^{127,128} concentration of multiple cation types to a single anion type,¹²⁹ and reduction of chemical noise.¹³⁰ Sequential charge inversion reactions have been used to increase ion charge.^{131,132}

Charge inversion has proven particularly useful in altering the ion type of fatty acids and glycerophospholipids to yield structurally informative ion types. For example, Stutzman et al. used PDPA dianions to convert phosphatidylcholine (PC) monocations to the structurally informative $[\text{PC} - \text{CH}_3]^-$ anion species. Ion/ion reaction between PC cations and PDPA

dianions formed the $[\text{PC} - \text{H} + \text{PDPA}]^-$ complex anion, and subsequent CID of the charge inverted anion resulted in concomitant proton transfer and methyl cation transfer.¹³³ Here, the demethylated PC anions afforded acyl chain information, whereas the PC monocations fragment predominantly to give a choline head group product ion. Reactions with PDPA have also been applied to isomeric mixtures of PC and PE lipids. Here, an isomeric mixture of PC and PE cations are reacted in the gas-phase with PDPA dianions, ultimately resulting in the chemical separation of PC and PE upon charge inversion due to the proton/methyl cation transfer unique to PC cations and the double proton transfer pathway undertaken by PE cations.¹²⁴ While a $[\text{PE} - \text{H} + \text{PDPA}]^-$ complex was not directly observed, the $[\text{PE} - \text{H}]^-$ product anion is not likely to be formed from two consecutive single-proton transfer reactions as neutralization would occur after the first reaction and ionization of the neutral from a second encounter is improbable. Rather, two proton transfer reactions within a single collision complex with subsequent dissociation of the complex is far more likely. Combining charge inversion with other techniques, like trimethylation enhancement using ^{13}C -diazomethane (^{13}C -TrEnDi) or the Paternò-Büchi reaction, can result in enhanced structural characterization of PC and PE phospholipids.^{134,135}

In the above cases, charge inversion was used to convert phospholipid cations to structurally informative anions. Recently, gas-phase charge inversion ion/ion reactions have also been applied to convert fatty acid anions to metalated cations.^{136,137} These reactions have successfully been applied in a shotgun (i.e., direct infusion ESI-MS) approach for fatty acid (FA) profiling, permitting unambiguous FA identification, isomeric distinction, and relative quantitation of isomeric FA. In the special case of magnesium phenanthroline complexes, charge inversion analogous to that of Equation 5 can be achieved where only two phenanthroline ligands are lost:

Singly deprotonated FA anions, either derived from non-esterified (i.e., free) FA or complex lipid decomposition via solution-based hydrolysis or gas-phase broadband collisional activation, undergo charge inversion when subjected to reactions with tris-phenanthroline magnesium dications to generate $[\text{FA} - \text{H} + \text{MgPhen}]^+$ cations. Subsequent isolation and ion-trap CID of the charge-inverted FA complex cation yields a reproducible, predictable product ion spectrum, that upon spectral matching to a developed FA mass spectral library composed of $[\text{FA} - \text{H} + \text{MgPhen}]^+$ product ion spectra, facilitates localization of carbon-carbon double bond positions and confident FA identification. Furthermore, using multiple linear regression analysis paired with the library data and in conjunction with data derived from FA mixtures, relative abundances of isomeric FA can be sensitively determined (Figure 8).¹³⁷ Collectively, this approach provides a relatively rapid and sensitive approach to lipid analysis based entirely on gas-phase chemistries.

Oxidation Reactions

Reduction/oxidation reactions in terms of electron transfer have been discussed above. However, oxidation can also be discussed in terms of oxygen transfer (i.e. oxidation is the gain of oxygen) and hydrogen transfer (i.e. oxidation is the loss of hydrogen). The gas-phase oxidation of multiply protonated peptide ions via oxygen transfer has recently been described. The periodate anion, IO_4^- , was used to selectively oxidize methionine residues, and to a lesser extent, tryptophan residues,¹³⁸ alkylated cysteine residues,¹³⁹ and disulfide bonds.¹⁴⁰ Under favorable conditions, the periodate anion can be used to oxidize neutral basic residues in the gas-phase.¹⁴¹

In the case of oxygen transfer to disulfide bonds, subsequent activation of the $[\text{M} + \text{H} + \text{O}]^+$ species results in the cleavage of the oxidized disulfide bonds at the $\text{S}(\text{O})\text{—S}$ bond or the $\text{C—S}(\text{O})$ bond, generating a fragmentation pattern indicative of the presence of a disulfide linkage.¹⁴⁰ Tryptic digestion of disulfide intact lysozyme yields a total of three peptides; two peptides contain one intermolecular disulfide bond and one peptide contains one intermolecular and one intramolecular disulfide bond. The product ion spectra from the activation of the three oxidized species are shown in Figure 9. In all cases, cleavage of the intermolecular disulfide bond is evidenced by the presence of the A and B chain peptides.

Similar to the periodate anion, a suite of reagents derived from persulfate can be used to oxidize peptides in the gas-phase; HSO_5^- generates $[\text{M} + \text{H} + \text{O}]^+$, HS_2O_8^- generates $[\text{M} - \text{H}]^+$ and $[\text{M} + \text{H} + \text{O}]^+$, and $\text{SO}_4^{\cdot-}$ generates $[\text{M}]^{+\cdot}$.¹⁴² Notably, the sulfate radical anion, denoted $\text{SO}_4^{\cdot-}$, is used to generate molecular radical cations. For polypeptides/proteins, CID of radical cations can give rise to the loss of radical side chains from several amino acids, forming dehydroalanine.⁸⁷ Fragmentation of peptide ions containing dehydroalanine has been noted to produce abundant c- and/or z- ions N-terminal to dehydroalanine.¹⁴³ In 2017, Peng et al. introduced dehydroalanine into multiply protonated ubiquitin ions via ion/ion reaction with $\text{SO}_4^{\cdot-}$.¹⁴⁴ The so called “dehydroalanine effect” upon fragmentation led to site specific c-/z- fragment ions. In addition to the sulfate radical anion, other reagent ions have been described to generate radical containing analyte ions via ion/ion reaction.^{114,115,145} Whatever the reagent, though, introduction of selective cleavage at dehydroalanine may prove useful in several situations. For example, a database search incorporating dehydroalanine fragmentation could provide enhanced specificity in top-down workflows.

Covalent Chemistry: Bond Formation in the Gas-Phase

A relatively new area of ion/ion reaction research involves the formation of covalent bonds in the gas-phase. While some of the examples above (e.g., oxidation via oxygen transfer) involved the formation of new covalent bonds, not all oxidation examples included the formation of new bonds and were therefore not discussed in the context of covalent chemistry. The first selective covalent bond forming ion/ion reaction was reported in 2009 using 4-formyl-1,3- benzenedisulfonic acid (FBDSA) to generate a Schiff base.¹⁴⁶ Much of the early work surrounding Schiff base formation via ion/ion reactions with FBDSA and the monosulfonic acid derivative, 2-formylbenzenemonosulfonic acid (FBMSA) has been previously reviewed.⁹ However, since that review, there have been several reports surrounding Schiff base formation via ion/ion reaction. In one example, Wang et al.

identified a rearrangement of Schiff base modified peptide ions that is 19 Da lower in mass than the protonated or deprotonated peptide, which could be mistaken as a water loss.¹⁴⁷ The Brodbelt group has demonstrated how ion/ion Schiff base formation can be used to increase UVPD efficiency compared to the unmodified peptide ions by binding a UV chromophore to the polypeptide.¹⁶ Lastly, the Brodbelt group has also shown how the electrostatic interactions of the sulfonate group of FBDSA with basic residues can be used to retain the phosphate group upon CID of phosphopeptide cations.¹⁴⁸

In the gas-phase, N-hydroxysuccinimide (NHS) ester-based reagents have been utilized to selectively derivatize nucleophilic sites, such as unprotonated arginine residues and unprotonated primary amines, in the gas-phase. Like Schiff base formation, ion/ion reactions involving NHS ester reagents have been reviewed.⁹ Thereafter, Bu and co-workers explored the potential energy surfaces associated with NHS ester reagents and primary amines or guanidine groups.¹⁴⁹ In that work, they explored two scenarios: (1) a case where the transition state barrier to covalent modification is relatively high and (2) a case where the transition state barrier is low and covalent reaction efficiencies are high. Interestingly, the efficiencies of covalent reactions in the former case could be significantly increased by implementing a long, slow-heating activation step prior to complex dissociation.¹⁴⁹ In later work, Bu and co-workers examined new reagents that exhibited enhanced reactivity towards amines and guanidines, attributed to the lower transition state barrier using triazole ester based reagent ions.¹⁵⁰ In addition to reactivity towards primary amines and guanidines, the gas-phase reactivity of both NHS esters and triazole esters towards carboxylates has been described in recent years.^{151,152} The latter chemistry has proven useful for the identification carboxylate groups present in salt bridges and/or zwitterions.¹⁵²

A variety of other ion/ion reactions have been described that result in covalent bond formation including alkyl cation transfer,¹⁵³ 1,3-dipolar cycloaddition (i.e. click chemistry),¹⁵⁴ and reactions with Woodward's reagent K (*wrk*).¹⁵⁵ The equivalent solution-phase chemistry of *wrk* was harnessed to prepare a reagent anion for the C-terminal peptide extension via gas-phase ion/ion reactions.¹⁵⁶ Our group also demonstrated the gas-phase N-terminal peptide extension using NHS reagents.¹⁵⁷ In all cases of gas-phase covalent bond formation, reactions were fast and efficient. Additionally, gas-phase ion/ion reactions offer a degree of selectivity that is not obtained with solution-phase reactions. Specifically, reactants can be isolated using a quadrupole mass filter prior to reaction, and the extent of reaction can be controlled.

REAGENT ION SELECTION

Mutual storage of oppositely charged ions can result in competitive reaction processes (e.g., proton transfer versus electron transfer) leading to several different product ions, where the major reaction pathways are dependent on both the analyte ion and reagent ion. For example, ETD sequence coverage is influenced by precursor ion charge^{60,158-160}, size,¹⁶¹ and structure.^{162,163} In another example, sodium cationized arginine residues are reactive towards NHS reagent anions while protonated arginine residues are not reactive.¹⁶⁴ In cases where the analyte ion-type is not readily varied, the partitioning of reaction products is determined by the characteristics of the reagent ion. This latter scenario is of particular

interest, as the analyte ion-type is determined by the ionization method and, in most cases, the ionization method is selected based on the approach that leads to the greatest analyte ion yield. Thus, it is important to understand the characteristics of reagent ions that influence favored reaction pathways in order to efficiently transform the analyte ion to a product ion-type of interest.¹⁶⁵ Here, several reagent characteristics as they pertain to different reaction types are discussed.

Electron transfer dissociation has been used extensively due to its ability to generate a high degree of structural information from bio-ions. The ideal electron transfer reagent would exclusively transfer an electron to generate fragment ions with minimal formation of non-dissociative product ions. However, depending on the nature of the electron transfer reagent ion, proton transfer can compete with electron transfer.^{89,166,167} In fact, a variety of ETD reagents have been explored and, to the best of our knowledge, every ETD anion has shown some degree of proton transfer.^{23,25,166-168} In recognition of the importance of the reagent in ETD, much attention has been devoted to studying ETD reagents.^{48,89,166-169} The likelihood of observing electron transfer is governed largely by the Franck-Condon overlap between the reagent anion and corresponding neutral and the electron affinity of the corresponding neutral.¹⁶⁷ If these parameters are not optimized, that is to say, if the Franck-Condon overlap is minimal and the electron affinity is relatively large, proton transfer will tend to dominate.¹⁶⁷

As highlighted in the above proton transfer section, there are scenarios where proton transfer is highly desirable. In such experiments there should be little contribution from fragmentation. Generally, fragmentation is not observed following proton transfer from a multiply protonated bio-ion to a singly charged reagent anion. Conversely, protonation of a multiply charged anion via ion/ion reaction has been shown to result in fragmentation and the extent of fragmentation was found to be related to the reaction exothermicity.¹⁷⁰ Fragmentation could be minimized by reducing the proton affinity of the cationic reagent.¹⁷⁰ Additionally, for proton transfer, it is often desirable that upon neutralization of the reagent ion, no long-lived complexes survive. Using the experiment performed by Bush and co-workers as an example, proton transfer was used to increase the accuracy of charge state assignment.⁵³ Here, if the reagent was “sticky” and a long-lived complex was formed, the mass of the reagent would be added to the analyte with each reaction. Of course, if every reaction resulted in adduct formation, the mass of the analyte could be readily deduced, yet not all cases result in this ideal scenario. Adduct formation in proton transfer experiments can be especially problematic if the reaction results in a mixture of product ion types (i.e. proton transfer and adduct formation). This situation has been observed with the iodide anion, I^- .¹⁷¹ When examining a mixture of product ion types, and without *a priori* knowledge of the analyte, deducing mass information may be challenging.

Several types of gas-phase ion/ion reactions rely on long-lived complex formation. One example is covalent modification in the gas-phase. Han et al. demonstrated the importance of forming a long-lived complex during Schiff base modification reactions.¹⁴⁶ The sulfonate group of singly deprotonated 4-formyl-1,3-benzenedisulfonic acid (FBDSA) interacts strongly with the doubly protonated peptide ion. This interaction allows enough time for the imine bond formation to occur. When reagents with carboxylate groups were used, which

interact less strongly with cations, proton transfer was the sole process observed likely due to a lower barrier from proton transfer relative to that for Schiff base formation.¹⁴⁶ Sulfonate groups and fixed-charge ammonium ions are widely used to promote long-lived complexes. In addition to a sticky group, reagents for covalent modification require a reactive site that undergo chemical reactions with the analyte ion.

The above examples discussed characteristics crucial to generating the product ion-type of interest. For instance, an electron transfer experiment cannot be successful if the reagent ion results only in proton transfer. Sometimes, there are cases in which multiple reagents lead to comparable results and selecting a reagent often comes down to subtle, yet important, differences. Sulfo-benzoyl-1-hydroxy-7-azabenzotriazole ester (HOAt), sulfo-benzoyl-1-hydroxybenzotriazole ester (HOBt), and sulfo-benzoyl-N-hydroxysuccinimide ester (NHS) all undergo acyl substitution reactions at primary amines and guanidine in the gas-phase.¹⁵⁰ Figure 10 demonstrates that HOAt and HOBt are more reactive towards amines than their NHS counterpart, yet all reagents result in a covalently modified product ion shaded in yellow. The enhanced reactivity results in less selectivity. Choosing triazole ester reagents versus NHS ester reagents will depend on the experiment. In another example, calcium, strontium, barium, and magnesium phenanthroline complexes can be used to charge invert monounsaturated FAs and identify the site of unsaturation.¹³⁶ However, the magnesium phenanthroline complex was the preferred reagent as it showed the lowest degree of water adduction in the collision cell from adventitious water, and therefore resulted in more straightforward product ion spectra.¹³⁶ Prentice et al. demonstrated the use of reagent cluster ions for multiple modifications performed in one ion/ion collision.¹⁷² Similar results could be obtained via sequential ion/ion reactions. The difference between the two, however, is the charge “cost.” Using the reagent cluster ion results in the reduction of the analyte charge by only one charge whereas consecutive reactions require a charge for each encounter, thus limiting the extent to which multiple modifications can be performed. It is clear that for a given analyte ion, the identity of the reagent ion is crucial. Therefore, choosing the correct reagent is key for any experiment.

FUTURE OUTLOOK

Reactions that are fast, efficient, and provide useful information have a long history in analytical chemistry. Over the past twenty-five years, gas-phase ion/ion reactions have proven to be fast, efficient, and remarkably powerful as means for converting analyte ions into forms that facilitate analysis and/or structural characterization. Single proton transfer applications to simplify mixture analysis, to concentrate charge into a one or a few species, to generate charge states that are not formed directly, etc. were the first to receive extensive attention. Following the discovery of electron transfer reagents by the Hunt group, attention was extended to structural characterization applications analogous to those employing electron capture. The commercial introduction of products that support ETD experiments greatly expanded the availability of ion/ion reactions to the analytical mass spectrometry community. The commercial availability of instruments that are also optimized for proton transfer ion/ion reactions will likely lead to growing use of charge manipulation applications, particularly in top-down proteomics applications.

Single proton transfer and electron transfer ion/ion reactions are relatively mature in terms of analytical applications. Reactions that require the formation of a long-lived complex, such as charge inversion, metal ion transfer, and selective covalent reaction, on the other hand, are far less explored. However, they illustrate the diversity of reaction types that can be accessed via ion/ion reactions. No instrument vendor currently supports these reaction types as the ion sources generally used to generate proton transfer and electron transfer reagents are not suitable for the generation of reagent ions that lead to these reaction types. Two ESI sources have generally been used to generate the reactants for charge inversion, metal transfer, and covalent reaction. However, commercial platforms that include a separate ion source for mass calibration purposes can, in principle, be adapted for dual-ESI ion/ion reaction experiments, as recently described by Webb et al.³¹ As more laboratories gain access to such capabilities, the range of reactions and applications thereof are likely to grow significantly. As analytical mass spectrometry continues to expand to larger analytes and more complex mixtures, reactions that enhance specificity, simplify mixture analysis, and facilitate structural characterization will find use. Novel gas-phase ion/ion reactions for analytical applications in the future are likely to process through long-lived complex formation.

ACKNOWLEDGMENTS

The authors would like to acknowledge all group members, specifically Caitlin Randolph, for their helpful discussions and contributions towards this review. We would also like to acknowledge support for ion/ion reaction research in our laboratory from NIH Grants GM R37-45372 and GM R01-118484 for protein and lipid chemistries, respectively, and from Sciex for instrument development efforts.

Biography

Scott A. McLuckey received a B.S. degree in chemistry from Westminster College in New Wilmington, PA in 1978 and a Ph.D. degree in chemistry from Purdue University in 1982, with Professor Graham Cooks serving as thesis advisor. Following a one-year post-doctoral appointment at the F.O.M. Institute for Atomic and Molecular Physics, he joined the Analytical Chemistry Division at Oak Ridge National Laboratory as a Wigner Fellow, which later transitioned into a staff position. He spent sixteen years at Oak Ridge having served as a group leader and section head. In 2000, he joined the faculty at Purdue University as a full professor and was named John A. Leighty Distinguished Professor in 2008. His mass spectrometry related research has been recognized with the 2012 Field and Franklin Award in mass spectrometry, the 2016 ASMS Distinguished Contribution Award, and the 2016 Thompson Medal from the International Mass Spectrometry Foundation.

David J. Foreman obtained his B.S. in chemistry and a minor in mathematics from Merrimack College in North Andover, MA in 2011. He worked in the pharmaceutical industry for four years before beginning his graduate studies at Purdue University in 2015. He is currently a Ph.D. candidate in the research laboratory of Scott A. McLuckey. His research is focused on the introduction of selective fragmentation pathways into bio-ions via gas-phase ion chemistry.

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Figure 1.

Dual source reactor used to perform ion/ion reactions (a) mounted to the front end of a mass spectrometer and (b) free standing to be adapted to any mass spectrometer platform.

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Figure 2.

(a) Schematic of the SLIM boards used for dual polarity ion confinement looking at the x-y plane (left) and z-y plane (right). (b) 3-D view of the SLIM device showing the ion conduits. The green line represents the equidistant line between the SLIM surfaces. Reprinted by permission from Springer Nature: Journal of the American Society for Mass Spectrometry, Garimella, S. V. B.; Webb, I. K.; Prabhakaran, A.; Attah, I. K.; Ibrahim, Y. M.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* 2017, 28, 1442-1449 (ref 32). Copyright 2017.

Figure 3.

Schematic of the instrumental setup for the waveform switching experiments. The sine wave is applied to the ring electrode and the square wave is applied to the end cap electrodes.

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Figure 4.

ETD product ion spectrum of the $[M + 26H]^{26+}$ charge state of apomyoglobin followed by sequential ion/ion proton transfer (IIP) reaction for (a) 0 ms, (b) 20 ms, (c) 40 ms, (d) 80 ms, and (e) 160 ms. Reprinted from *Int. J. Mass Spectrom.*, Vol. 377, Anderson, L. C.; English, A. M.; Wang, W.; Bai, D. L.; Shabanowitz, J.; Hunt, D. F. Protein derivatization and sequential ion/ion reactions to enhance sequence coverage produced by electron transfer dissociation mass spectrometry, pp. 617–624 (ref 49). Copyright 2015, with permission from Elsevier.

Figure 5.

Mass spectra of SOD/CuZn complexes (a) as observed under native MS conditions (top), isolation and 25 ms proton transfer reaction of the 12+/6+ species (middle), and isolation and 25 ms proton transfer reaction of the 11+ species (bottom) with (b) a 60 m/z wide zoomed view of the 10+/5+ region. Adapted from Holden, D. D.; Brodbelt, J. S. *Anal. Chem.* 2016, 88, 12354-12362 (ref 62). Copyright 2016 American Chemical Society.

Figure 6. MS/MS analysis scheme of 50S L22 *E. coli* ribosomal protein. (a) Positive electrospray mass spectrum. (b) 100 ms proton transfer and parallel ion parking of the 600 – 1000 m/z isolation window. (c) HCD product ion spectrum of the data-dependently selected 10+ precursor. Reproduced from Ugrin, S. A.; English, A. M.; Syka, J. E. P.; Bai, D. L.; Anderson, L. C.; Shabanowitz, J.; Hunt, D. F. *J. Am. Soc. Mass Spectrom.* 2019 DOI: [10.1007/s13361-019-02290-8](https://doi.org/10.1007/s13361-019-02290-8) (ref 71).

Figure 7.

The electrospray mass spectrum of a AT/heparin complex is shown in gray. Ion charge state assignment is facilitated by limited electron transfer charge reduction using wide (pink) and narrow (cyan) precursor ion selection windows. The species labeled A, B, and C represent resolved charge-reduced species present in the precursor isolation windows. Reproduced from Zhao, Y.; Abzalimov, R. R.; Kaltashov, I. A. *Anal. Chem.* 2016, 88, 1711-1718 (ref 91). Copyright 2016 American Chemical Society.

Figure 8.

Product ion spectra of $[18:1 - H + MgPhen]^+$ for the isomeric mixture of 18:1 *n*-9/*n*-7 at the molar ratios of (a) 5/95 and (b) 95/5. Product ions used in the multiple linear regression for relative quantitation are shown in red. Calculated molar ratios (mean ± standard deviation, *n* = 3) are shown in green. The lightning bolt corresponds to the species subjected to CID.

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Figure 9.

Collisional activation of the ion/ion reaction complex formed between the reaction of the periodate anion with (a) doubly protonated doubly GYSLGNWVCAAK/CK, (b) triply protonated CELAAAMK/GCR, and (c) triply protonated WWCNDGR/NLCNIPCSALLSSDITASVNCAK. The degree signs correspond to water losses, and the lightning bolts correspond to the species subjected to CID. Reproduced from Pilo, A. L.; McLuckey, S. A. *Anal. Chem.* 2016, 88, 8972-8979 (ref 140). Copyright 2016 American Chemical Society.

Figure 10.

Product ion spectrum from CID of the complex formed between doubly protonated KGAGGKGAGGKL and (a) HOAt, (b) HOBt, and (c) NHS. The m/z region corresponding to proton transfer is shaded in red, the m/z region corresponding to covalent modification is shaded in yellow, and the m/z region corresponding to the complex ion is shaded in green. Reprinted by permission from Springer Nature: Journal of the American Society for Mass Spectrometry, Bu, J.; Peng, Z.; Zhao, F.; McLuckey, S.A. *J. Am. Soc. Mass Spectrom.* 2017, 28, 1254-1261 (ref 150). Copyright 2017.