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Revealing the fates of proteins in the gas phase

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ABSTRACT

The ability to observe intact proteins by native mass spectrometry allows measurements of size, oligomeric state, numbers and types of ligands and post translational modifications bound, among many other characteristics. These studies have the potential to, and in some cases are, advancing our understanding of the role of structure in protein biology and biochemistry. However, there are some long-unresolved questions about to what extent solution-like structures persist without solvent in the vacuum of the mass spectrometer. Strong evidence from multiple sources over the years has demonstrated that well-folded proteins maintain native-like states if care is taken during sample preparation, ionization, and transmission through the gas phase. For partially unfolded states, dynamic and disordered proteins, and other important landmarks along the protein folding/unfolding pathway, caution has been urged in the interpretation of the results of native ion mobility/mass spectrometric data. New gas-phase tools allow us to provide insight into these questions with *in situ, in vacuo* labeling reactions delivered through ion/ion chemistry. This Young Scientist Perspective demonstrates the robustness of these tools in describing native-like structure as well as possible deviations from native-like structure during native ion mobility/mass spectrometry. This Perspective illustrates some of the changes in structure produced by the removal of solvent and details some of the challenges and potential of the field.

1. Introduction

1.1. Flying elephants

The history of forming bare ions by spray-based droplet methods, including the works of Malcolm Dole [1], Iribarne and Thompson [2], Vestal and coworkers [3], Lidija Gall [4], and John Fenn [5] is well known by members of this community. Since the application of electrospray ionization was reported by multiple research groups, mass spectrometers have been measuring a new state of matter: the flying elephant [5]. Indeed, the field of native mass spectrometry (nMS) had its beginnings in the early portion of the 1990s when naked gas phase protein ions [6] and non-covalent complexes [7,8] of proteins were observed intact in mass spectrometers. One of the earliest observations was that the number of charges on the ions and the shapes of the charge state distributions depend strongly on the solution conditions from which the proteins were electrosprayed, suggesting a link between solution phase structure and m/z for protein ions [9]. Not much later investigators began to couple electrospray to ion mobility spectrometry coupled with mass spectrometry (IM/MS), a technique that separates ions by size and shape to charge ratio, observing that the overall shape and size of gas-phase protein ions could be measured [10]. Many groundbreaking experiments were done in this area including increasing protein ions' kinetic energy into the mobility cells to see how protein conformations change as a function of collisional energy [11], as well as observing changes in the ion mobility spectra as a function of the makeup of the electrospray solvent [12]. Exciting current research in nMS and nIM/MS includes probing membrane proteins and the lipids and other ligands that stabilize them [13–15], sequencing and identifying membrane proteins directly from cellular membranes [16], thermodynamics applied to the study of the myriad important solution conformations in protein folding and protein dynamics and the binding of ligands in stabilizing and activating protein/protein complexes [17,18], the characterization of gas-phase stabilities for screening biotherapeutics, including fusion proteins [19], and RNAs [20], and the study of massive virus particles and other megadalton plus biomolecules via charge detection mass spectrometry and allied techniques [21,22].

1.2. Do proteins retain Solution structures during and following ionization?

By 1997 Joe Loo, at Parke-Davis/Pfizer at the time, had written a review on the application of electrospray (ESI) mass spectrometry to studying noncovalent protein complexes [23]. In this review he added

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an important fourth S to the three Ss of Fred McLafferty (speed, sensitivity, selectivity), stoichiometry. Under stoichiometric nMS measurements, Loo included the number of ligands bound to a protein and the oligomeric order of a protein-protein complex (i.e., quaternary structure). Later, the identification of the number and types of protein modifications for intact proteins gave rise to the idea of protofeorms [24] (multiple protein forms encoded by the same canonical DNA sequence but with functional differences). Loo also noted that at that present time there were "three camps of opinion [regarding ESI for studying protein complexes]: believers, non-believers, and undecided." IM/MS studies [25] as well as indirect information provided by electron-based fragmentation methodologies [26] revealed that in fact proteins do retain important elements of tertiary and quaternary structure in the gas phase over the time scale of IM/MS experiments [27].

However, there are many studies that show important differences. Protein ions are significantly compacted in the gas phase when compared to structures derived from X-ray crystallography [28–30]. Additionally, the change in dielectric between gas phase and solution phase results in much stronger electrostatic interactions including coulombic repulsion which was shown to lead to the expansion of structures for example, in higher charge states of ubiquitin as observed by Bowers in coworkers by IM/MS [31]. Breuker and McLafferty rephrased Loo in their 2008 paper to state, "for how long under what conditions and to what extent can solution structure be retained without solvent?" [32].

This question is perhaps even more important for investigating steps along the so-called protein folding funnel. These important steps include partially folded and unfolded states [33], as well as intrinsically disordered or dynamic proteins (which some estimate to make up to 40 % of protein in human cells) [34], and toxic oligomers and aggregates. Though important discoveries have been made in these areas, there have also been important warnings from the community, including statements that there are no or few connections between gas phase and solution structure for more unfolded or unstructured proteins, or for natively folded proteins that are sprayed from denaturing conditions, citing different ESI mechanisms for compact proteins versus more extended species [35]. An important statement was made by Vahidi and Konermann where caution was urged in the interpretation of IM/MS data for partially disordered proteins due to the governance of charge on overall gas phase structure [36]. However, they were optimistic that "future developments will result in the emergence of more robust strategies for the interrogation of non-native conformers by gas phase methods."

1.3. Technologies and our role

There have been developments of quite a few gas-phase technologies to give additional information to IM/MS alone (other than the before mentioned electron fragmentation techniques). These include photodissociation techniques [37], [38] ion/molecule reactions [39] including hydrogen deuterium exchange [40], and other methods that have been performed in the gas phase for the purpose of interrogating gas-phase structures. Unlike traditional experiments, these experiments require that the entire protein be transferred intact into the gas phase. My start in this research area was at Purdue University in the research laboratory of Scott McLuckey, where the ability to form covalent bonds in the gas phase through ion/ion reactions between ions of opposite and unequal charge inside of an ion trap mass spectrometer (forming the covalent product from a strong electrostatically-bound ion/ion complex via low-energy collision induced dissociation (CID) [41]) had recently been demonstrated. At the time I was tasked with using sulfo-NHS (sulfo-N-hydroxysuccinimide) based crosslinking reagents to intramolecularly crosslink the small protein ubiquitin in the gas phase. We discovered that the crosslinking process was not random. Our identifications showed tendencies of sulfo-EGS (ethylene glycol bis(sulfosuccinimidyl succinate)) to react in specific positions, suggesting preferred structures or conformational arrangements [42]. It was at this time when I became familiar with the scientific literature on the study of gaseous protein ions. I became interested in both exploiting mass spectrometry as a structural biology technology as well as determining whether these gas-phase measurements yielded accurate descriptors of solution-like states.

In my time as a postdoc and staff scientist in the laboratory of Richard Smith at Pacific Northwest National Laboratory, I actively worked with ion mobility spectrometry [43] including high resolution ion mobility separations of proteins. Therefore, the first goals in my independent career were to establish methodology for gas-phase protein structural interrogation and to provide insights into which aspects of protein structure are retained in mass spectrometry measurements, fulfilling the hope (or so I thought) of Vahidi and Konermann for a more robust strategy for the interrogation of non-native conformers. In this Young Scientist Feature, unlike a traditional review, I will primarily focus on contributions from our research laboratory in the field of gas-phase protein structure.

2. Development of a platform for gas-phase structural biology

2.1. Coupling ESI/ESI ion/ion reactions with a Commercial ion mobility/mass spectrometer

A suitable platform to perform these ion/ion reaction studies for determining gaseous phase structures for proteins needs to have three essential components. The first component is the ability to somehow pulse anions and cations into the instrument [44], the second is the ability to store both ion polarities simultaneously in an ion trap [45], and third, the ability to explore the overall structure with a complementary method. Due to these factors, I decided to purchase a Waters Synapt G2-Si q/IM/TOF instrument with electron transfer dissociation (ETD, a kind of ion/ion reaction) enabled and ion mobility enabled. However, out of the box the instrument was not suitable for these ESI/ESI studies. With help from Jeff Brown and Lindsay Morrison at Waters [46] the route to enabling ESI/ESI ion/ion reactions on the Synapt was made clear. The first implementation used the lockspray source, which uses a sample capillary as well as a lockspray reference capillary for internal mass calibration. The baffle between the two sprayers was removed, and the voltage for the ETD needle was placed on the lockspray capillary, while the lockspray capillary voltage was removed. In this way we could use the embedded ETD software modules to control the application of voltages to the ion sources synchronously with the anion and cation fill steps in the ETD sequencing. Thus, instead of trapping an ETD reagent, we trapped the ESI-generated anions followed by transmission of cations into the ion trap (Fig. 1). The trap is located after the quadrupole which allowed us to mass select both the reagent and protein ion. The ion/ion reaction products were released from the trap and separated based upon the number of sequential reagents (i.e., by post ion/ion charge state) by IM/MS. Fragmenting the ion/ion products after the ion mobility separation allows for the mobility alignment of fragment ions to their precursors. A similar strategy could have been to employ an additional quadrupole mass analyzer to select ion/ion products, or to use a linear ion trap for MS^n, as has been done in other setups [42]. One of the issues with our setup is that we were limited to the power supply for discharge ionization to use as the reagent ESI supply, so we used the Waters Research Enabled Software (WRENS) to allow us to more precisely choose the exact voltage applied to the anion sprayer and exceed the typical limitation of 2 kV. However, timing the pulsing of the electrospray sources with trapping was challenging, leading us to eventually use an external power supply that was triggered by the changing of polarities in the ion trap [47]. We adapted the same setup for the nanolockspray source allowing us to perform ion/ion reactions at nanoflow rates or via static nanoelectrospray with pulled borosilicate glass capillaries.

We published our initial adaptation of the instrument for ESI/ESI

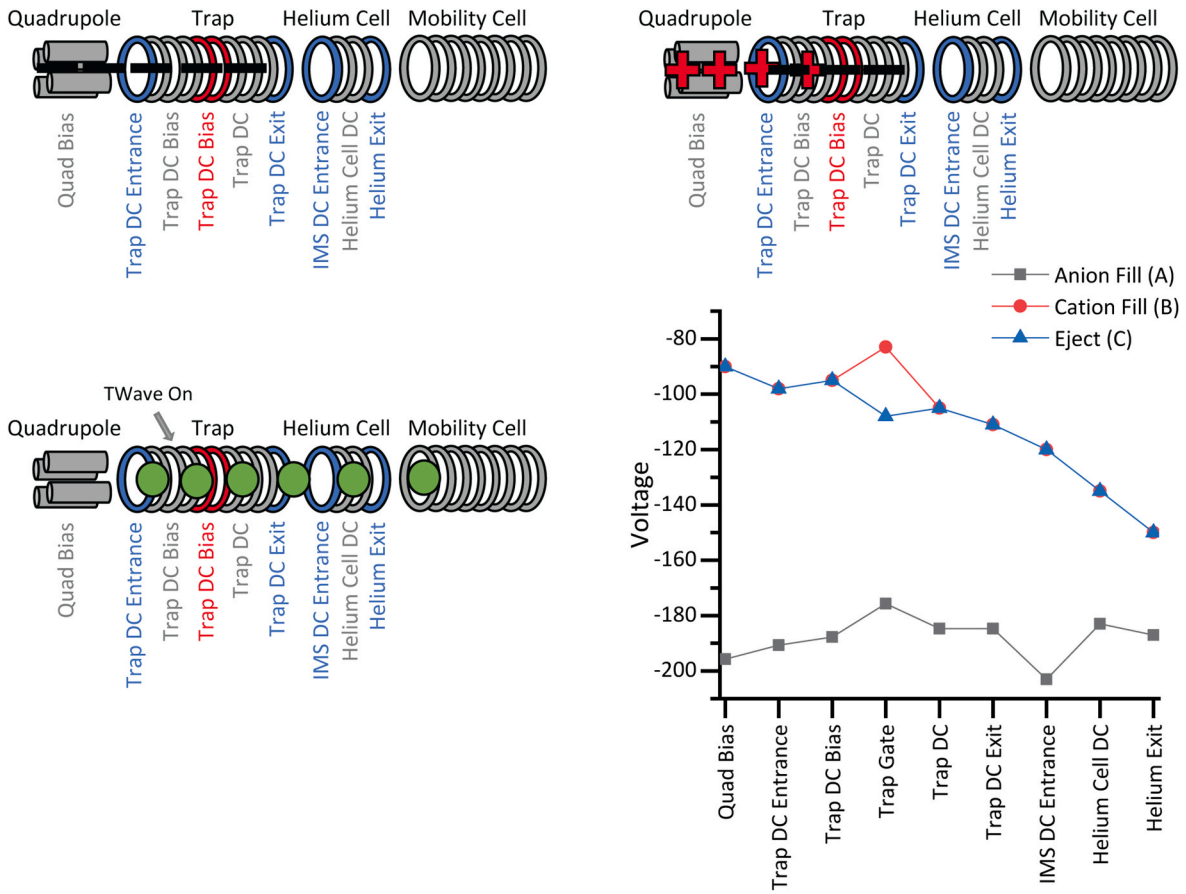

Fig. 1. A cartoon of the region of the instrument involved in performing the ion/ion reactions. The labels below the ion optics correspond to the locations where DC voltages are applied during the experiment (not shown for the mobility cell). A. Anions (black minus signs) are mass selected in the quadrupole and injected into the trap. B. Cations (red plus signs) are isolated and injected to the trap. The trap gate prevents cations from being released. Ion populations overlap and the ion/ion reaction proceeds to products (green circles). C. Product ions are pulsed out of the trap with a 500 ms pulse of the trap gate and drift through the ion mobility cell. Products will traverse the transfer cell and the time-of-flight mass analyzer (not shown). D. Voltage diagram for the three states of the trap during the ion/ion reaction. Reprinted from *International Journal of Mass Spectrometry*, 444, Dueling electrospray implemented on a traveling-wave ion mobility/time-of-flight mass spectrometer: Towards a gas-phase workbench for structural biology, 116177–116185, Copyright 2019, with permission from Elsevier.

ion/ion chemistry in 2019 in IJMS in a special issue honoring Scott McLuckey on the topic of probing biomolecules in the gas phase [46]. Our findings were neither controversial nor unexpected. Reactions proceeded with increased spatial and phase overlap between cations and anions [48], with additional collisional cooling with higher gas pressures, better confinement of both polarities of ions in the same space with higher RF amplitudes, and longer reaction periods via reduced traveling wave heights [49] enabling more extensive reactions. One of the difficulties that we did have, however, was in observing efficient formation of the covalent reaction between protein cations and regent anions. With typically used helium cell pressures, flow rates of greater than 100 mL per minute, collisionally activating the ion/ion complex formed by reaction of the 6^+ charge state ion of ubiquitin with the sulfobenzoyl-HOAT (1-Hydroxy-7-azabenzotriazole) anion [50] resulted in simply the loss of the reagent, yielding the mass and charge difference nominally equal to the loss of a proton from the unreacted 6^+ ion. To favor the covalent reaction channel, which would be observed as a neutral loss of the m/z equal to the formula weight of the HOAT reactive moiety, we significantly decreased gas flows into both the helium cell and ion mobility cells to 20 mL per minute flows or lower, allowing us to use much lower collision energies and observe the covalent product. Ubiquitin and cytochrome C were chosen as model proteins since they have both been extensively characterized in solution and gas phases, providing robust measurements and calculations for us to validate our methodology against.

The product from covalent ion/ion reactions has been determined by the McLuckey group to be favored under low energy activation over a longer time periods, suggesting that these processes occur at far lesser energy than do process such as CID [51]. Fig. 2 shows the ion/ion reaction where formation of the covalent product was strongly favored over loss of the reagent. We used HOAT esters instead of NHS esters, as they have been shown to react much more quickly, and in our experiments, kinetics drives the observed product [50]. We used CID following the mobility separation of each ion/ion product to localize the binding of the sulfobenzoic acid and formation of new amide bonds with either arginine or lysine, demonstrating the potential utility of our new method and platform.

2.2. *Covalent and electrostatic ion/ion reactions with ubiquitin*

Again, by performing CID in the transfer cell of the instrument, which follows the mobility cell, we can detect b and y-type fragment ions as a function of the specific ion/ion reaction product, if the ion/ion products were separable by mobility. We determined sites of covalent modification by determining which fragments included the mass of the covalent modification and which did not. With this workflow, we investigated whether our method was sensitive to changes in protein structure. Therefore, we performed ion/ion reactions with the 5^+ and 6^+ charge states formed from aqueous ammonium acetate solutions of ubiquitin, allowing us to localize labeling to lysine 29 and arginine 54

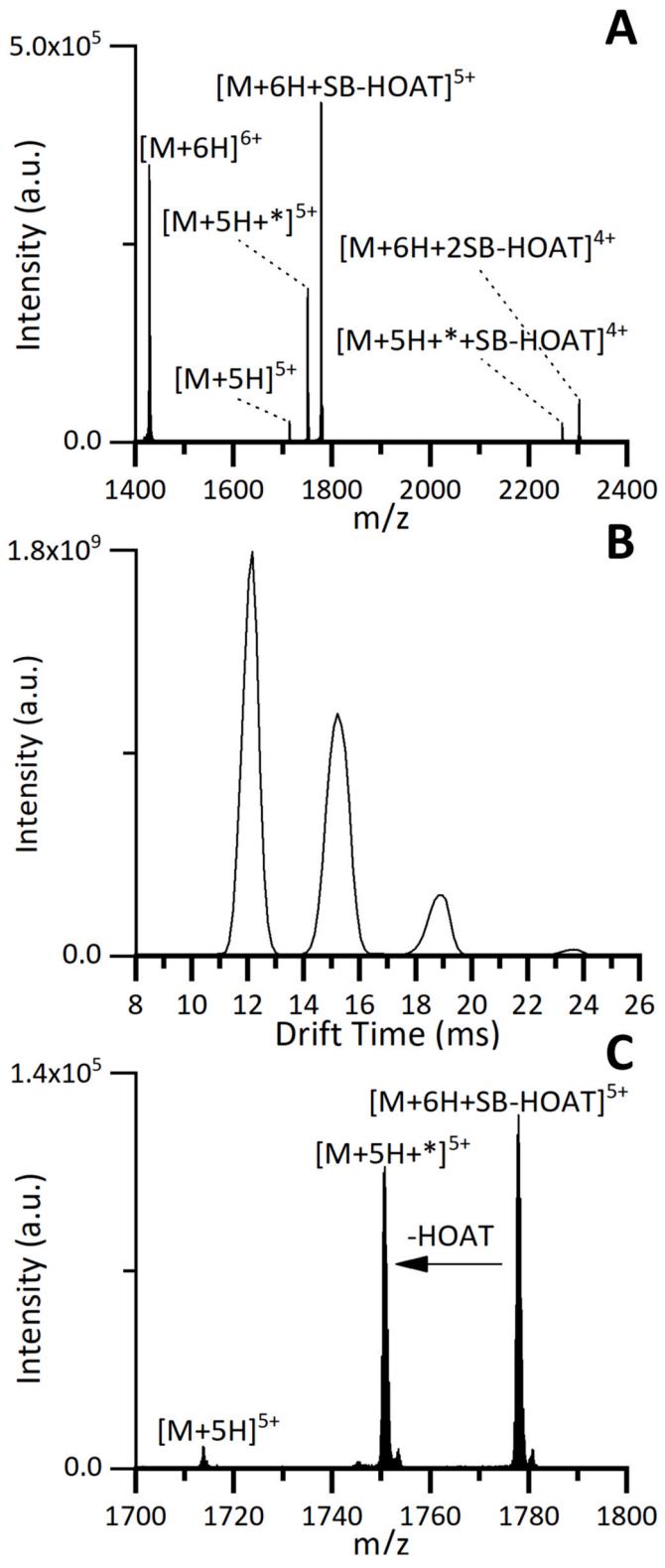

Fig. 2. A. Mass spectrum from the ion/ion reaction of ubiquitin 6^+ with sulfo-benzoyl HOAT- (SB-HOAT). **B.** ATD of ion/ion reaction in A. with reduced pressures in the helium cell and IM cell and 40V trap cell bias. **C.** Mass spectrum corresponding to 14.517–15.899 ms in **B.** The * denotes covalent addition of the sulfo-benzoyl moiety through observation of the neutral loss of HOAT. Reprinted from *International Journal of Mass Spectrometry,* 444, Dueling electrospray implemented on a traveling-wave ion mobility/time-of-flight mass spectrometer: Towards a gas-phase workbench for structural biology, 116177–116185, Copyright 2019, with permission from Elsevier.

for both of these species [52]. (While solution phase covalent labeling through NHS, HOAT or other activated ester amine-reactive reagents is not reactive with arginine, because nearly all arginine residues will be protonated at biological pH, in the gas phase, there are often more ionizable sites than are ionizing protons, especially under nMS ESI conditions. The McLuckey group previously determined that in fact deprotonated arginine residues were very reactive towards NHS-ester ion/ion chemistry, with the observation of covalent modification of arginine containing peptides in the gas phase.) [53] When we examined the 7^+ and 8^+ charge states, the reagents that were electrostatically bound to ubiquitin ions were lost during transmission through the instrument, likely due to the lack of available sites of modification with the greater number of protons, preventing covalent product formation, or via changes in structure driven by coulombic repulsion in gas phase putting too much distance between the electrostatic binding site and covalently reactive residues [54,55].

We also sought to determine whether our ion/ion reactions, the activation energies associated with these reactions, and timescales of our experiments were competitive with collision induced unfolding (CIU) type processes [56] or were necessarily preceded by collision induced unfolding prior to labeling. We used an Eyring activation enthalpy and entropy treatment with computations developed by the Prell group in their Ion Spa simulation program, developed to measure activation and thermodynamics for ions in various parts of mass spectrometers [57]. Our results showed that covalent bond formation was favored at low energies, with an entropic/temperature dependent activation barrier, and unfolding was favored at higher energies [58]. We were also able to determine that covalent reactions took place without applying any collisional energy beyond the kinetic energy necessary for ion transmission, albeit this process was very slow. These data suggested that covalent bond formation was not preceded by unfolding, and that the structural insights formed from these methods inform on well-folded native-like states if measured in low energy conditions with ESI from aqueous ammonium acetate solutions.

2.3. *Electrostatic ion/ion crosslinking of folded cytochrome C ions*

Finally, upon modifying our instrument with the ExD cell from e-MSion following our ion mobility cell [59], we could localize covalent and noncovalent modifications via electron capture dissociation (ECD) [47]. The ability to localize noncovalent modifications by ECD has been extensively demonstrated in the literature. Noncovalently bound ligands [60] and labile post translational modifications (PTMs) [61] are conserved under electron capture dissociation. By conducting ion/ion reactions with disulfonate salts, with sulfonate groups spaced by different length linker groups, we could form two-sided salt bridges with positively charged residues, electrostatically crosslinking them. Linker distances were obtained by performing geometry optimizations using density functional theory (Fig. 3A and B) [62]. We then used these different electrostatic crosslinkers as distance restraints for modeling gaseous structures. Since we wanted to avoid structures that were artifacts of overcrosslinking, we only examined single crosslinks at a time. However, by using variable lengths, our hypothesis was that we could probe different regions/interactions of the protein. We first tried these experiments with cytochrome C 7^+ charge state which is generated from nESI of cytochrome C from aqueous ammonium acetate. The ion/ion products between this charge state of cytochrome C and the dianionic disulfonate linkers were 5^+ ions, so, as a control, we used 1H,1H,2H, 2H-perfluoro-1-octanol (PFO), an effective proton transfer reagent [63], to generate cytochrome C 5^+ for comparison of ECD fragmentation with the disulfonate-modified proteins.

We determined the binding sites of each of the electrostatic crosslinkers via ECD of the ion/ion reaction products; and other than using ECD instead of CID after ion mobility, the workflow was the same as for the ubiquitin studies mentioned previously. We first examined the crystal structure of cytochrome C and found that all the crosslinks were

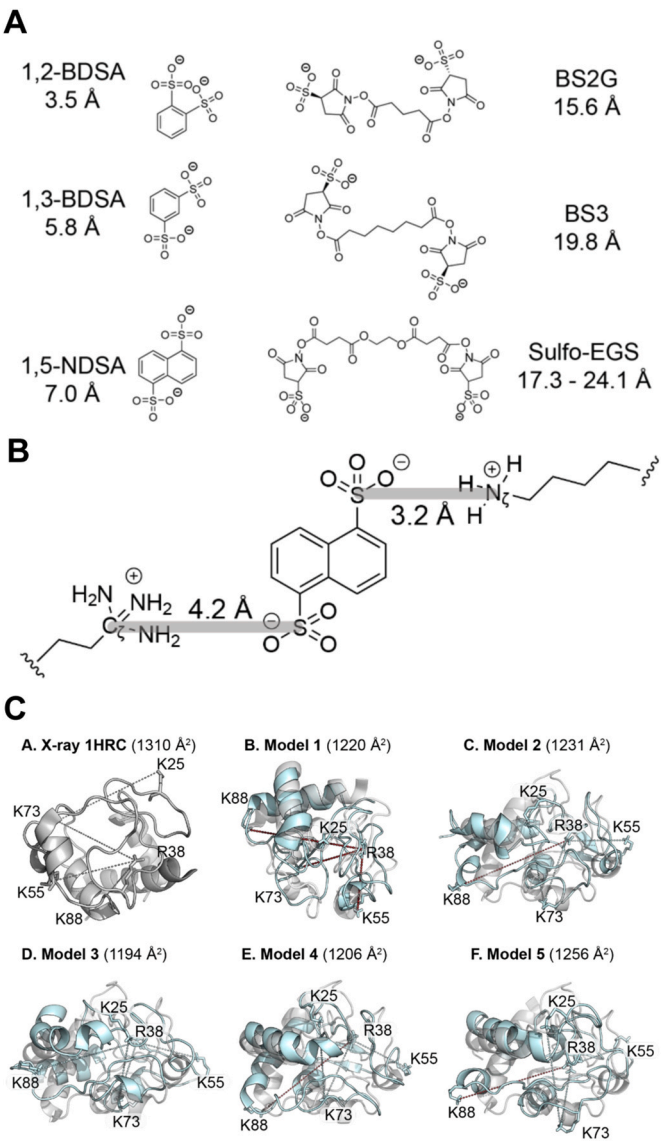

Fig. 3. A. Predicted lengths of the disulfonate electrostatic crosslinkers. **B.** Predicted distance between the lysine nitrogen and sulfonate sulfur and arginine C_ζ and sulfonate sulfur. **C.** X-ray structure of horse heart cytochrome C (gray) overlaid with five gas-phase MD replicates (cyan) with crosslinks labeled (dotted lines and residue single letters and positions). Adapted with permission from *Analytical Chemistry,* 94, Application of Multiple Length Cross-linkers to the Characterization of Gaseous Protein Structure, 13301–13310, Copyright 2022, American Chemical Society.

over length. However, this is not surprising, as previous literature has reported that proteins in the gas phase can compact significantly, including cytochrome C [64]. Therefore we modeled gas phase structures of cytochrome C produced by a molecular dynamics method developed for replicating experimental observations of protein compaction in the gas phase [30,64], applying our crosslinking distances as restraints (Fig. 3C). We found that this produced structures with collision cross sections (CCS) similar to measured cross sections [65], as well as satisfying many of the restraints for five replicates. We interpreted these results as evidence for multiple coexisting conformers being crosslinked in the gas phase, as evidenced by the variety of structures with varying CCS that satisfy restraints. Tandem ion mobility "slicing" experiments of cytochrome C have revealed that native IM mobility CCS distributions are in fact comprised of many non-interconverting structures [66], supporting our interpretation. We also observed decreased overlap with the X-ray structure for the random coil regions, demonstrating compaction in the gas phase.

3. Solution to gas structural transitions of chemically unfolded proteins: ubiquitin and cytochrome C

3.1. Unfolded ubiquitin in the gas phase

Satisfied that our experiments were both structurally representative and in strong agreement with previous ion mobility results, we moved on to investigating partially and fully unfolded systems as this is where the debate on whether the gas-phase structures represent solution-phase structures is still unresolved [36]. Again, we used ubiquitin as our first model system ionized from both neutral aqueous ammonium acetate and acidic water/methanol (i.e., pH 3) solutions [67]. We used sulfo-EGS crosslinker (i.e., the two sulfonate groups) as an electrostatic crosslinker (*vide supra*) as well as used both electrostatic binding and covalent binding site localization of sulfobenzoyl HOAT (electrostatic binding of the sulfonate to a positively charged residue and covalent addition of sulfobenzoate to deprotonated lysine/arginine/N-terminus). We found that the aqueous 6^+ charge state was cross-linked by sulfo-EGS from proline 19 to arginine 42 and by sulfobenzoyl-HOAT from lysine 29 to proline 19 and arginine 54 to lysine 63. However, for the 6^+ produced from acidic water/methanol solution, linking sites were different. Sulfo-EGS linked lysine 33 to arginine 72, while SBHOAT links lysine 48 to lysine 11 and arginine 54 to lysine 63. Gas phase molecular dynamics were performed starting from the so-called A state [68], where ubiquitin is partially folded in the N-terminal half and unfolded on the C terminus. Clustering the MD trajectory resulted in three clusters of varying overall size and shape. Our crosslinking results agreed most strongly with the most compact of these three clustered structures, evidence that even under the denaturing conditions, the lower charge states can produce more compact structures in the gas phase, which has also been demonstrated by CCS [68]. However, there are still significant differences between the gas-phase ubiquitin structures from electrosprayed from pH 7 and pH 3, methanolic solutions as revealed by our crosslinking experiments.

3.2. Effects of Solution unfolding of cytochrome C on gas-phase structure Elucidated by ECD

Next, we decided to compare solution versus gas phase unfolding of cytochrome C. Solutions of aqueous cytochrome C with 10 %, 5 %, and 0 % by volume acetic acid solutions teed into the infusion line at the same flow rate as the protein were directly sampled by applying an ESI voltage to the pulled distal end of the infusion line. We also used typical pressures in the ion mobility cell and much lower pressures (see article for details) [69] in order that the ions experience different electric field to drift gas number density ratios (E/N). In this way we could compare both unfolding through changing pH as well as unfolding through collisional activation in the gas phase (i.e., CIU [70]). By increasing the E/N, collisions occurred at higher than thermal energies, resulting in increased ion internal temperatures [71]. We first examined these differences by observing the arrival time distributions with the lower E/N and higher E/N settings. At high fields, the arrival time distributions of the 9^+ charge of cytochrome C were identical regardless of which acidity of solution it was mixed with. However, using lower E/N, it was observed that the distribution began to show the presence of more intermediate and extended conformers as a function of decreasing pH, evidence that at relatively low fields some memory of the solution structure is maintained even for partially unfolded states.

To provide higher resolution structural information, we applied ECD following the ion mobility separation of cytochrome C 9^+, since the 9^+ charge state was observed from all the solution conditions used. Unfolding of cytochrome C under low pH has been shown by fluorescence spectroscopy to include the dissociation of the heme iron to the distal methionine 80 residue [72]. We observed that under relatively

low fields, there was very little fragmentation (virtually none for pH 7) between cysteine 14, the first heme-bound cysteine, and methionine 80. This suggested that for 0 % and 5 % acid, most solution structures likely had these bonds intact. With 10 % acid teed into the infusion line, there was extensive fragmentation throughout the entire sequence, indicating that indeed the methionine 80 to iron bond was likely broken, allowing for the liberation of ECD fragments that had been held together by the iron to methionine 80 bond and the cysteine 14 and 17 to heme bond. This phenomenon was much like how salt bridges reduce the amount of ECD fragmentation observed for a native-like protein [73]. However, when the high field was used in the mobility cell, extensive fragmentation regardless of the originating solution conditions was observed. In fact, cytochrome C 9^+ ions electrosprayed from pH 7.0 solutions showed more extensive sequence coverage when passed through the mobility cell at high E/N than was observed for the 10 % mixed acetic acid case at low field. This evidence suggests that coulombic unfolding in the gas phase and solution unfolding may follow different mechanisms, especially for ions with charge states higher than the lowest, most compact states. Finally, a manuscript is in preparation detailing changes in crosslinking, and thus structure, in solution and the gas phase for cytochrome C electrosprayed from solutions stabilizing native and partially unfolded states [74].

4. Natively unfolded proteins

4.1. Gaseous ensembles of alpha synuclein

Finally, we examined a protein that occupies many conformational configurations in solution, the intrinsically disordered protein (IDP) alpha synuclein (αSN). αSN forms toxic oligomers and fibrils involved in Parkinson's disease and other dementias [75]. Solution studies have revealed long range interactions between the C terminus and central non-amyloid component (NAC)/fibril core region [76]. The N terminal region contains positively and negatively charged amino acids as well as some hydrophilic residues, the NAC is hydrophobic, and the C terminal region is strongly anionic. Thus, charge-charge interactions and hydrophobic interactions drive the ensemble of solution structures that coexist. We used in-solution crosslinking, and with the combination of crosslinking and molecular modeling [77], we generated an ensemble very similar to the one measured by spin-labeled NMR [78]. We used a combined top-down and bottom-up crosslinking method that allowed us to place the greatest confidence in crosslinks found in both modes. We did not want to over constrain the molecule, so we used a limited amount of crosslinking (one crosslink per protein) in these modes.

While our in-solution values were very close to the radius of gyration and end to end distances provided by the NMR ensemble, when solvent is removed, charge-charge interactions are expected to be much stronger and can possibly lead to an over compaction of the protein versus the solution state [29,64,79]. When analyzed in positive mode, the CCS distributions of αSN are more compact than those predicted directly from the NMR ensemble. However, when examining the protein in negative mode (as αSN is an overall anionic protein), the overall CCS distribution matched much more closely to the cross sections predicted from the NMR solution distribution. While perhaps intuitively this is not an unexpected result, we sought to use our suite of gas-phase crosslinks along with the solution relaxation approximation (SRA) developed by the Bleiholder group [80] to understand what aspects of the cations in the gas phase were very different than solution structures [77]. We found two structural trends (Fig. 4). For a low charge state, such as the 8^+ charge state, the radius of gyration was much lower than predicted from the NMR data, our crosslinking-derived ensembles, and small angle X-ray scattering measurements [81]. The molecular descriptors of the 11^+ charge state, which is the dominant charge state in our distribution, are a closer match to solution ensembles. Higher charge states show expansion in the radius of gyration and in the N-terminus to C-terminus distances from what would be expected for solution ensembles. Also, we

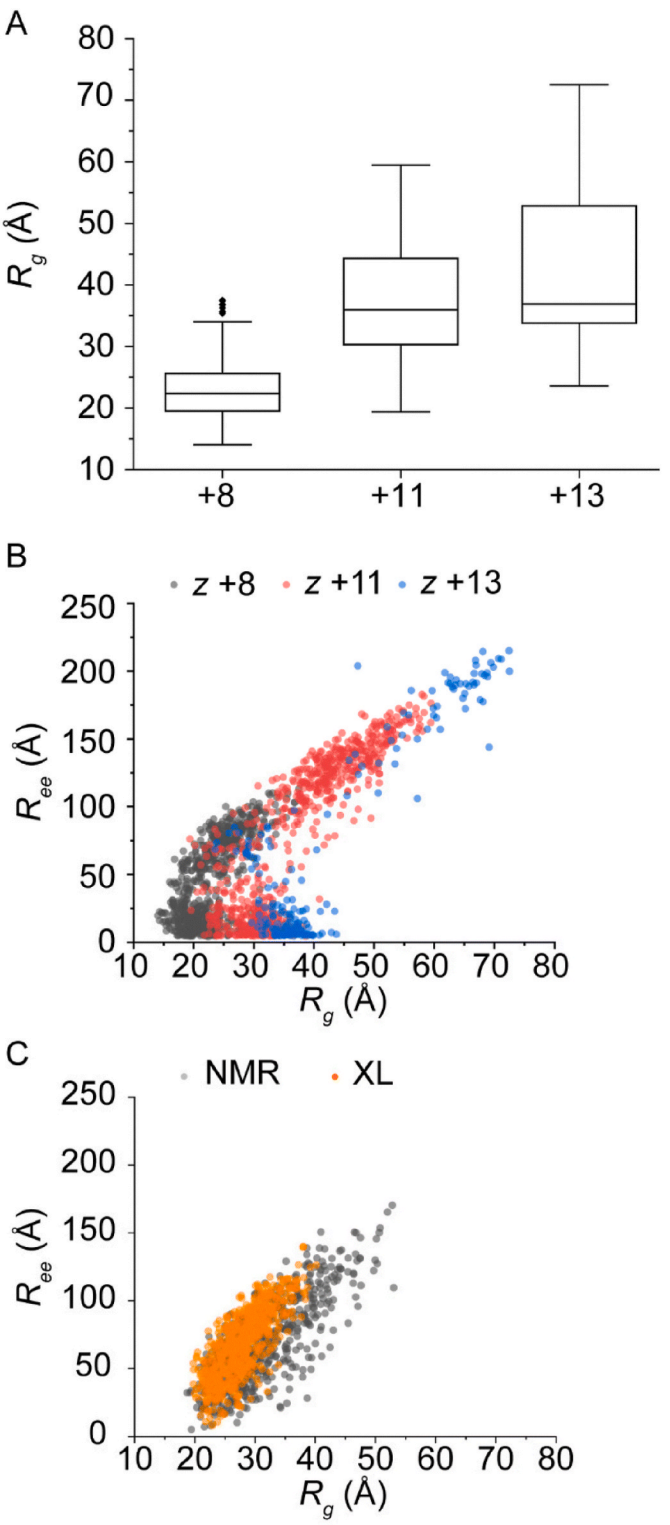

Fig. 4. Comparison of gas phase structural ensembles for z = 8^+, 11^+ and 13^+ calculated using the SRA and solution ensembles with distance restraints obtained by spin label NMR and the unbiased XL-native MS/MS data. **A.** Box and whisker plots of the calculated R_g distributions for SRA 8^+, 11^+ and 13^+ ensembles, **B.** R_{ee} vs. R_g for SRA 8^+, 11^+ and 13^+ ensembles (labeled in black, red, and blue respectively). **C.** R_{ee} vs. R_g for NMR (gray) and XL-native MS/MS (orange) ensembles. Reprinted with permission from *Analyst*, 149, The role of solvation on the conformational landscape of α-synuclein, 125–136, Copyright 2024, Royal Society of Chemistry.

observed a very strange structural feature for the gas-phase ensembles. There is a subset of structures that have seemingly typical radii of gyration (from about 15 to 40 Å) but extremely small end to end distances (below 25 Å) (Fig. 4 B). This was a trend that was not observed for a solution structures (Fig. 4C). Upon examining the gas phase structures that were found to comply with crosslinking distances restraints (Fig. 5 B and D), we noticed much more compacted structures including head to tail cyclized structures, structures very much unlike what was observed from the experimental solution crosslinking and NMR ensembles. Although structural trends with αSN observed with solution studies can be observed in the gas phase, such as compaction of structures (i.e., decrease in CCS) upon binding metals and at low pH, the actual αSN structures themselves seem to diverge largely from the solution ensemble.

5. Conclusions, challenges, and outlook

Our studies have reinforced the idea that ion mobility is a useful structural method. Iteratively determining if changes in structure occur upon new intra- or intermolecular interactions from changes in solution states is an important task for IM/MS practitioners, especially for biomolecules too large or dynamic for study by "gold standard" biophysical methods. Our studies with native-like ubiquitin and cytochrome *c* ions gave direct experimental evidence that not only are gas-phase native-like ions compact, the backbone of these proteins themselves do not unfold or refold on the timescales of IM/MS experiments. However, it is best not to interpret these data as producing identical structural features between gas phase and solution, as shown by the observations of compaction with ion mobility and gas-phase crosslinking. Though our crosslinking data provided evidence that gas-phase ions of ubiquitin from denaturing solution were distinct from those from native-like conditions, this does not mean that the unfolded structures are the same in solution as the gas-phase. Our studies with cytochrome *c* solution versus gas-phase unfolding revealed that while trends occurring in solution unfolding can be uncovered with gas-phase methods, treating gas-phase and solution-phase unfolding as similar processes is not supported by experimental evidence. In some cases, the gas-phase structures do not reflect solution structures, found to be especially true when proteins are ionized in the opposite polarity than their net polarity in solution, such as was the case with the anionic protein αSN electrosprayed as a cation, although both the charge state distribution and ion mobility data for αSN cations clearly reflect its disordered nature in solution. Fortunately, many important structural discoveries do not rely on exact matches in structure between the solution and gas phase. These include stability studies, like CIU [70] and variable temperature electrospray [82], where important information can be gleaned and minute structural differences that are either impossible or difficult to observe with condensed-phase methods can be readily detected. Finally, the

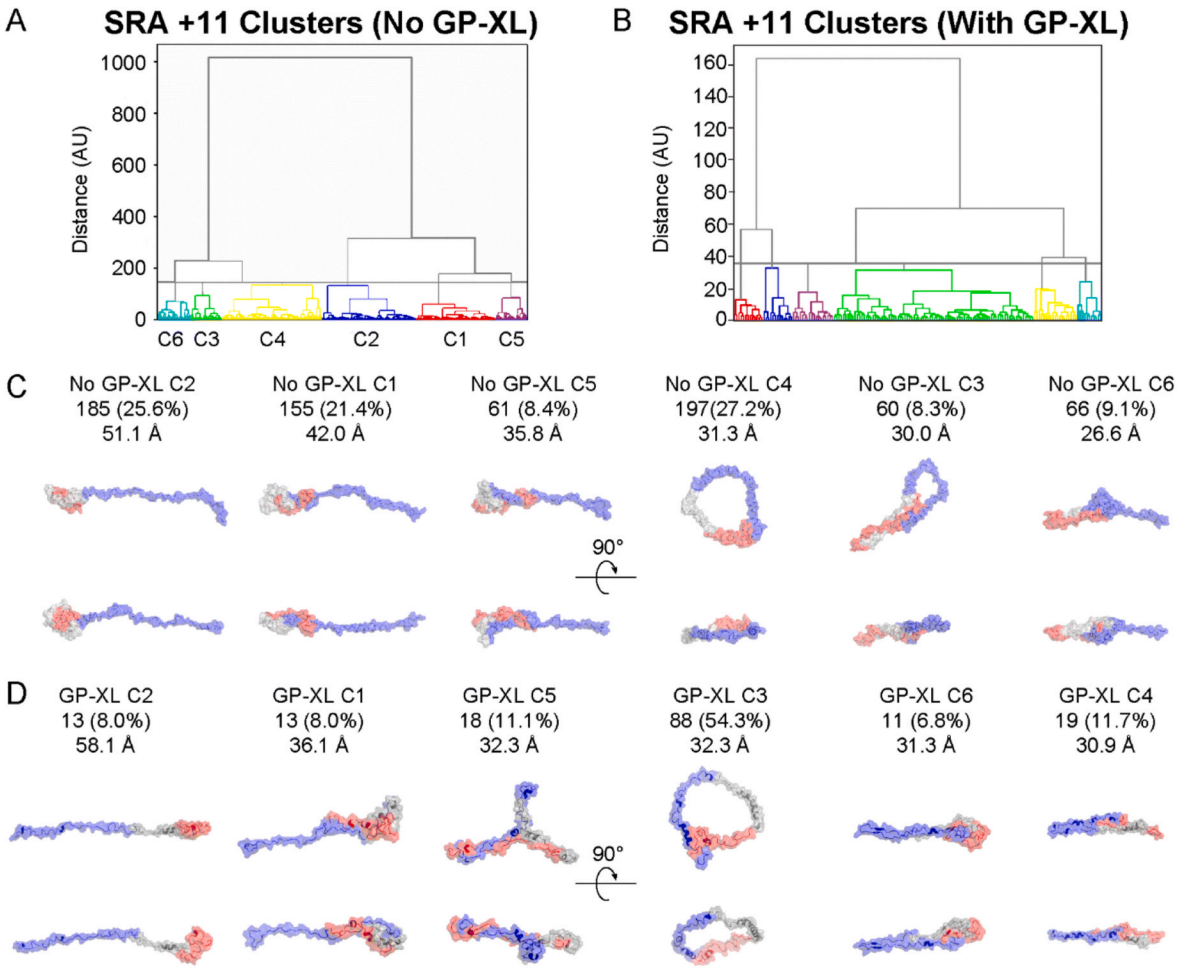

Fig. 5. Clustered SRA +11 conformational ensembles prior to (n = 724) and post (n = 126) filtering using gas phase XL (GP-XL) restraints. Cluster dendrograms show distribution of conformational space into specific clusters **A** before and **B** after GP-XL filtering, respectively. Representative conformers for each cluster, rotated 90° along the x-axis and the αSN structure is colored as blue (N-terminal region, residues 1–60), gray (nonamyloid-β component, residues 61–95) and red (C-terminal tail, residues 96–140) before **C** and after **D** GP-XL filtering respectively. Reprinted with permission from *Analyst*, 149, The role of solvation on the conformational landscape of α-synuclein, 125–136, Copyright 2024, Royal Society of Chemistry.

analytical utility of gas-phase protein structural measurements is not hindered by the inability to determine exact three-dimensional structures.

Challenges for using gas-phase chemistry for structural biology include the need for increased sequence coverage with intact protein fragmentation, the dilution of signal into multiple channels, and the availability of instrumentation for gas-phase chemistry. However, the outlook is very strong, as many of these problems are actively being solved with innovative ion traps, new fragmentation methods for intact proteins, the ability to accumulate and store huge numbers of ions, and the support of ion/ion chemistry by several vendors. An exciting development is the application of ion mobility to separate fragment ions of proteins, followed by fragmentation of the fragments to provide for more localized label and crosslinking site identifications, using ion mobility and first generation fragments like how LC is used to separate tryptic peptides prior to tandem MS [83]. Recently, electron and photon-based fragmentation methods have become available on nearly every mass spectrometry vendor's flagship platform [84], [-87] allowing top-down label/crosslink site identifications to be performed in many laboratories. Thermo, Bruker, and Waters provide ion/ion reactions with several of their instrument platforms, and Sciex instrumentation has been adapted for ion/ion chemistry [88–91]. Finally, Structures for Lossless Ion Manipulations (SLIM) provide platforms for simultaneous storage and transmission of cations and anions [92,93], the buildup of massive ion populations to enhance sensitivity [94], and ultra-high ion mobility resolution [95]. We hope that incorporating these technological advances will allow this line of research to continue to provide a greater understanding of gas-phase protein structures, helping discover and eliminate biases created by measuring proteins in the absence of solvent, and provide novel avenues for exploring protein-protein complexes.

CRediT authorship contribution statement

Ian K. Webb: Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

[1] M. Dole, L.L. Mack, R.L. Hines, R.C. Mobley, L.D. Ferguson, M.B. Alice, Molecular Beams of Macroions, J. Chem. Phys. 49 (5) (1968) 2240–2249, https://doi.org/10.1063/1.1670391(acccessed6/2/2024.

[2] J.V. Iribarne, B.A. Thomson, On the evaporation of small ions from charged droplets, J. Chem. Phys. 64 (6) (1976) 2287–2294, https://doi.org/10.1063/1.432536(acccessed6/2/2024.

[3] C.R. Blakley, J.J. Carmody, M.L. Vestal, A new soft ionization technique for mass spectrometry of complex molecules, J. Am. Chem. Soc. 102 (18) (1980) 5931–5933, https://doi.org/10.1021/ja00538a050.

[4] A.N. Verenchikov, N.V. Krasnov, V.A. Shkurov, Electrospray ionization developed by Lidija Gall's group, Int. J. Mass Spectrom. 490 (2023) 117067, https://doi.org/10.1016/j.ijms.2023.117067.

[5] J.B. Fenn, Electrospray wings for molecular elephants (Nobel lecture), Angew Chem. Int. Ed. Engl. 42 (33) (2003) 3871–3894, https://doi.org/10.1002/anie.200300605.

[6] R.D. Smith, J.A. Loo, C.G. Edmonds, C.J. Barinaga, H.R. Udseth, New developments in biochemical mass spectrometry: electrospray ionization, Anal. Chem. 62 (9) (1990) 882–899, https://doi.org/10.1021/ac00208a002.

[7] B. Ganem, Y.T. Li, J.D. Henion, Detection of noncovalent receptor-ligand complexes by mass spectrometry, J. Am. Chem. Soc. 113 (16) (1991) 6294–6296, https://doi.org/10.1021/ja00016a069.

[8] V. Katta, B.T. Chait, Observation of the heme-globin complex in native myoglobin by electrospray-ionization mass spectrometry, J. Am. Chem. Soc. 113 (22) (1991) 8534–8535, https://doi.org/10.1021/ja00022a058.

[9] S.K. Chowdhury, V. Katta, B.T. Chait, Probing conformational changes in proteins by mass spectrometry, J. Am. Chem. Soc. 112 (24) (1990) 9012–9013, https://doi.org/10.1021/ja00180a074.

[10] D.E. Clemmer, R.R. Hudgins, M.F. Jarrold, Naked protein conformations - cytochrome-C in the gas-phase, J. Am. Chem. Soc. 117 (40) (1995) 10141–10142, https://doi.org/10.1021/ja00145a037.

[11] K.B. Shelimov, M.F. Jarrold, Conformations, unfolding, and refolding of apomyoglobin in vacuum: an activation barrier for gas-phase protein folding, J. Am. Chem. Soc. 119 (13) (1997) 2987–2994, https://doi.org/10.1021/ja962914k.

[12] J. Li, J.A. Taraszka, A.E. Counterman, D.E. Clemmer, Influence of solvent composition and capillary temperature on the conformations of electrosprayed ions: unfolding of compact ubiquitin conformers from pseudonative and denatured solutions, Int. J. Mass Spectrom. 185–187 (1999) 37–47, https://doi.org/10.1016/s1387-3806(98)14135-0.

[13] L. Han, L. Nguyen, E.N. Schmidt, M. Esmaili, E.N. Kitova, M. Overduin, M. S. Macauley, J.S. Klassen, How Choice of model membrane affects protein–glycosphingolipid interactions: insights from native mass spectrometry, Anal. Chem. 94 (46) (2022) 16042–16049, https://doi.org/10.1021/acs.analchem.2c03067.

[14] J. Lyu, T. Zhang, M.T. Marty, D. Clemmer, D.H. Russell, A. Laganowsky, Double and triple thermodynamic mutant cycles reveal the basis for specific MsbA-lipid interactions, Elife 12 (2024) RP91094, https://doi.org/10.7554/eLife.91094.

[15] W. Liu, H.S. Jayasekera, J.D. Sanders, G. Zhang, R. Viner, M.T. Marty, Online buffer exchange enables automated membrane protein analysis by native mass spectrometry, Anal. Chem. 95 (47) (2023) 17212–17219, https://doi.org/10.1021/acs.analchem.3c02164.

[16] C.A. Lutomski, T.J. El-Baba, J.D. Hinkle, I. Liko, J.L. Bennett, N.V. Kalmankar, A. Dolan, C. Kirschbaum, K. Greis, L.H. Urner, et al., Infrared multiphoton dissociation enables top-down characterization of membrane protein complexes and G protein-coupled receptors, Angew. Chem. Int. Ed. 62 (36) (2023) e202305694, https://doi.org/10.1002/anie.202305694(acccessed2024/07/25.

[17] H. Pan, S.A. Raab, T.J. El-Baba, S.R. Schrecke, A. Laganowsky, D.H. Russell, D. E. Clemmer, Variation of CI-2 conformers upon addition of methanol to water: an IMS-MS-based thermodynamic analysis, J. Phys. Chem. 127 (45) (2023) 9399–9408, https://doi.org/10.1021/acs.jpca.3c03651.

[18] T. Walker, H.M. Sun, T. Gunnels, V. Wysocki, A. Laganowsky, H. Rye, D. Russell, Dissecting the thermodynamics of ATP binding to GroEL one nucleotide at a time, ACS Cent. Sci. 9 (3) (2023) 466–475, https://doi.org/10.1021/acscentsci.2c01065.

[19] R.C. Villafuerte-Vega, H.W. Li, A.E. Bergman, T.R. Slaney, N. Chennamsetty, G. Chen, L. Tao, B.T. Ruotolo, Ion mobility-mass spectrometry and collision-induced unfolding rapidly characterize the structural polydispersity and stability of an fc-fusion protein, Anal. Chem. 96 (24) (2024) 10003–10012, https://doi.org/10.1021/acs.analchem.4c01408.

[20] A.G. Anders, E.D. Tidwell, V.V. Gadkari, M. Koutmos, B.T. Ruotolo, Collision-induced unfolding reveals disease-associated stability shifts in mitochondrial transfer ribonucleic acids, J. Am. Chem. Soc. 146 (7) (2024) 4412–4420, https://doi.org/10.1021/jacs.3c09230.

[21] E. Deslignière, A. Rolland, E. Ebberink, V. Yin, A.J.R. Heck, Orbitrap-based mass and charge analysis of single molecules, Acc. Chem. Res. 56 (12) (2023) 1458–1468, https://doi.org/10.1021/acs.accounts.3c00079From.NLM.

[22] M.F. Jarrold, Applications of charge detection mass spectrometry in molecular biology and biotechnology, Chem. Rev. 122 (8) (2022) 7415–7441, https://doi.org/10.1021/acs.chemrev.1c00377.

[23] J.A. Loo, Studying noncovalent protein complexes by electrospray ionization mass spectrometry, Mass Spectrom. Rev. 16 (1) (1997) 1–23, https://doi.org/10.1002/(SICI)1098-2787(1997)16:1<1::AID-MAS1>3.0.CO;2-L(acccessed2024/06/06.

[24] L.M. Smith, N.L. Kelleher, Consortium for Top Down, P. Proteoform: a single term describing protein complexity, Nat. Methods 10 (3) (2013) 186–187, https://doi.org/10.1038/nmeth.2369.

[25] B.T. Ruotolo, K. Giles, I. Campuzano, A.M. Sandercock, R.H. Bateman, C. V. Robinson, Evidence for macromolecular protein rings in the absence of bulk water, Science 310 (5754) (2005) 1658–1661, https://doi.org/10.1126/science.1120177.

[26] K. Breuker, H. Oh, D.M. Horn, B.A. Cerda, F.W. McLafferty, Detailed unfolding and folding of gaseous ubiquitin ions characterized by electron capture dissociation, J. Am. Chem. Soc. 124 (22) (2002) 6407–6420, https://doi.org/10.1021/ja012267j.From.NLM.

[27] T. Wyttenbach, M.T. Bowers, Structural stability from solution to the gas phase: native solution structure of ubiquitin survives analysis in a solvent-free ion mobility-mass spectrometry environment, J. Phys. Chem. B 115 (42) (2011) 12266–12275, https://doi.org/10.1021/jp206867a.

[28] Z. Hall, A. Politis, M.F. Bush, L.J. Smith, C.V. Robinson, Charge-state dependent compaction and dissociation of protein complexes: insights from ion mobility and

molecular dynamics, J. Am. Chem. Soc. 134 (7) (2012) 3429–3438, https://doi.org/10.1021/ja2096859.
[29] J.R. Jhingree, B. Bellina, K.J. Pacholarz, P.E. Barran, Charge mediated compaction and rearrangement of gas-phase proteins: a case study considering two proteins at opposing ends of the structure-disorder continuum, J. Am. Soc. Mass Spectrom. 28 (7) (2017) 1450–1461, https://doi.org/10.1007/s13361-01716921PubMed.
[30] A.D. Rolland, L.S. Biberic, J.S. Prell, Investigation of charge-state-dependent compaction of protein ions with native ion mobility–mass spectrometry and theory, J. Am. Soc. Mass Spectrom. 33 (2) (2022) 369–381, https://doi.org/10.1021/jasms.1c00351.
[31] T. Wyttenbach, M.T. Bowers, Structural stability from solution to the gas phase: native solution structure of ubiquitin survives analysis in a solvent-free ion mobility-mass spectrometry environment, J. Phys. Chem. B 115 (42) (2011) 12266–12275, https://doi.org/10.1021/jp206867a.
[32] K. Breuker, F.W. McLafferty, Stepwise evolution of protein native structure with electrospray into the gas phase, 10(-12) to 10(2) s, Proc Natl Acad Sci U S A 105 (47) (2008) 18145–18152, https://doi.org/10.1073/pnas.0807005105.
[33] D.E. Clemmer, D.H. Russell, E.R. Williams, Characterizing the conformationome: toward a structural understanding of the proteome, Acc. Chem. Res. 50 (3) (2017) 556–560, https://doi.org/10.1021/acs.accounts.6b00548.
[34] A.K. Dunker, J.D. Lawson, C.J. Brown, R.M. Williams, P. Romero, J.S. Oh, C. J. Oldfield, A.M. Campen, C.M. Ratliff, K.W. Hipps, et al., Intrinsically disordered protein, J. Mol. Graph. Model. 19 (1) (2001) 26–59, https://doi.org/10.1016/s10933263(00)00138-8From.NLM.
[35] L. Konermann, H. Metwally, Q. Duez, I. Peters, Charging and supercharging of proteins for mass spectrometry: recent insights into the mechanisms of electrospray ionization, Analyst 144 (21) (2019) 6157–6171, https://doi.org/10.1039/c9an01201j, 10.1039/C9AN01201J.
[36] S. Vahidi, B.B. Stocks, L. Konermann, Partially disordered proteins studied by ion mobility-mass spectrometry: implications for the preservation of solution phase structure in the gas phase, Anal. Chem. 85 (21) (2013) 10471–10478, https://doi.org/10.1021/ac402490r.
[37] H. Oh, K. Breuker, S.K. Sze, Y. Ge, B.K. Carpenter, F.W. McLafferty, Secondary and tertiary structures of gaseous protein ions characterized by electron capture dissociation mass spectrometry and photofragment spectroscopy, Proc. Natl. Acad. Sci. USA 99 (25) (2002) 15863–15868, https://doi.org/10.1073/pnas.212643599(acccessed2024/06/06.
[38] J. Seo, W. Hoffmann, S. Warnke, M.T. Bowers, K. Pagel, G. von Helden, Retention of native protein structures in the absence of solvent: a coupled ion mobility and spectroscopic study, Angew. Chem. Int. Ed. 55 (45) (2016) 14173–14176, https://doi.org/10.1002/anie.201606029(acccessed2024/06/06.
[39] R.R.O. Loo, J.A. Loo, H.R. Udseth, J.L. Fulton, R.D. Smith, Protein structural effects in gas phase ion/molecule reactions with diethylamine, Rapid Commun. Mass Spectrom. 6 (3) (1992) 159–165, https://doi.org/10.1002/rcm.1290060302(acccessed2024/06/06.
[40] D. Suckau, Y. Shi, S.C. Beu, M.W. Senko, J.P. Quinn, F.M. Wampler, F. W. McLafferty, Coexisting stable conformations of gaseous protein ions, Proc. Natl. Acad. Sci. USA 90 (3) (1993) 790, https://doi.org/10.1073/pnas.90.3.790.
[41] H. Han, S.A. McLuckey, Selective covalent bond formation in polypeptide ions via gas-phase ion/ion reaction chemistry, J. Am. Chem. Soc. 131 (36) (2009) 12884–12885, https://doi.org/10.1021/ja904812d.
[42] I.K. Webb, M. Mentinova, W.M. McGee, S.A. McLuckey, Gas-phase intramolecular protein crosslinking via ion/ion reactions: ubiquitin and a homobifunctional sulfo-NHS ester, J. Am. Soc. Mass Spectrom. 24 (5) (2013) 733–743, https://doi.org/10.1007/s13361-013-0590-4, journal article.
[43] Y.M. Ibrahim, A.M. Hamid, L. Deng, S.V. Garimella, I.K. Webb, E.S. Baker, R. D. Smith, New frontiers for mass spectrometry based upon structures for lossless ion manipulations, Analyst 142 (7) (2017) 1010–1021, https://doi.org/10.1039/c7an00031f, 10.1039/C7AN00031F.
[44] J.M. Wells, P.A. Chrisman, S.A. McLuckey, "Dueling" ESI: instrumentation to study ion/ion reactions of electrospray-generated cations and anions, J. Am. Soc. Mass Spectrom. 13 (6) (2002) 614–622, https://doi.org/10.1016/S1044-0305(01)00364-6.
[45] S.A. McLuckey, J.L. Stephenson Jr., Ion/ion chemistry of high-mass multiply charged ions, Mass Spectrom. Rev. 17 (6) (1998) 369–407, https://doi.org/10.1002/(SICI)1098-2787(1998)17:6<369::AID-MAS1>3.0.CO;2-J.
[46] I.K. Webb, L.J. Morrison, J. Brown, Dueling electrospray implemented on a traveling-wave ion mobility/time-of-flight mass spectrometer: towards a gas-phase workbench for structural biology, Int. J. Mass Spectrom. 444 (2019) 116177–116185, https://doi.org/10.1016/j.ijms.2019.116177. ARTN 116177.
[47] M. Cheung See Kit, V.V. Carvalho, J.Z. Vilseck, I.K. Webb, Gas-phase ion/ion chemistry for structurally sensitive probes of gaseous protein ion structure: electrostatic and electrostatic to covalent cross-linking, Int. J. Mass Spectrom. 463 (2021) 116549–116559, https://doi.org/10.1016/j.ijms.2021.116549.
[48] S.A. McLuckey, T.-Y. Huang, Ion/ion reactions: new chemistry for analytical MS, Anal. Chem. 81 (21) (2009) 8669–8676, https://doi.org/10.1021/ac9014935.
[49] F. Lermyte, T. Verschueren, J.M. Brown, J.P. Williams, D. Valkenborg, F. Sobott, Characterization of top-down ETD in a travelling-wave ion guide, Methods 89 (2015) 22–29, https://doi.org/10.1016/j.ymeth.2015.05.019.
[50] J. Bu, Z. Peng, F. Zhao, S.A. McLuckey, Enhanced reactivity in nucleophilic acyl substitution ion/ion reactions using triazole-ester reagents, J. Am. Soc. Mass Spectrom. 28 (7) (2017) 1254–1261, https://doi.org/10.1007/s13361-017-1613-3, journal article.
[51] J. Bu, C.M. Fisher, J.D. Gilbert, B.M. Prentice, S.A. McLuckey, Selective covalent chemistry via gas-phase ion/ion reactions: an Exploration of the energy surfaces associated with N-hydroxysuccinimide ester reagents and primary amines and guanidine groups, J. Am. Soc. Mass Spectrom. 27 (6) (2016) 1089–1098, https://doi.org/10.1007/s13361-016-1359-3, journal article.
[52] V.V. Carvalho, M.C. See Kit, I.K. Webb, Ion mobility and gas-phase covalent labeling study of the structure and reactivity of gaseous ubiquitin ions electrosprayed from aqueous and denaturing solutions, J. Am. Soc. Mass Spectrom. 31 (5) (2020) 1037–1046, https://doi.org/10.1021/jasms.9b00138.
[53] W.M. McGee, M. Mentinova, S.A. McLuckey, Gas-phase conjugation to arginine residues in polypeptide ions via N-hydroxysuccinimide ester-based reagent ions, J. Am. Chem. Soc. 134 (28) (2012) 11412–11414, https://doi.org/10.1021/ja304778j.
[54] I. Sinelnikov, E.N. Kitova, J.S. Klassen, Influence of Coulombic repulsion on the dissociation pathways and energetics of multiprotein complexes in the gas phase, J. Am. Soc. Mass Spectrom. 18 (4) (2007) 617–631, https://doi.org/10.1016/j.jasms.2006.11.006FromNLM.
[55] C.J. Hogan Jr., J.A. Carroll, H.W. Rohrs, P. Biswas, M.L. Gross, Combined charged residue-field emission model of macromolecular electrospray ionization, Anal. Chem. 81 (1) (2009) 369–377, https://doi.org/10.1021/ac8016532.
[56] Y. Zhong, L. Han, B.T. Ruotolo, Collisional and Coulombic unfolding of gas-phase proteins: high correlation to their domain structures in solution, Angew Chem. Int. Ed. Engl. 53 (35) (2014) 9209–9212, https://doi.org/10.1002/anie.201403784FromNLM.
[57] M.T. Donor, S.O. Shepherd, J.S. Prell, Rapid determination of activation energies for gas-phase protein unfolding and dissociation in a Q-IM-ToF mass spectrometer, J. Am. Soc. Mass Spectrom. 31 (3) (2020) 602–610, https://doi.org/10.1021/jasms.9b00055.
[58] M. Cheung See Kit, S.O. Shepherd, J.S. Prell, I.K. Webb, Experimental determination of activation energies for covalent bond formation via ion/ion reactions and competing processes, J. Am. Soc. Mass Spectrom. 32 (9) (2021) 2313–2321, https://doi.org/10.1021/jasms.1c00025.
[59] J.P. Williams, L.J. Morrison, J.M. Brown, J.S. Beckman, V.G. Voinov, F. Lermyte, Top-down characterization of denatured proteins and native protein complexes using electron capture dissociation implemented within a modified ion mobility-mass spectrometer, Anal. Chem. 92 (5) (2020) 3674–3681, https://doi.org/10.1021/acs.analchem.9b04763.
[60] K.F. Haselmann, T.J.D. Jørgensen, B.A. Budnik, F. Jensen, R.A. Zubarev, Electron capture dissociation of weakly bound polypeptide polycationic complexes, Rapid Commun. Mass Spectrom. 16 (24) (2002) 2260–2265, https://doi.org/10.1002/rcm.853.
[61] A. Stensballe, O.N. Jensen, J.V. Olsen, K.F. Haselmann, R.A. Zubarev, Electron capture dissociation of singly and multiply phosphorylated peptides, Rapid Commun. Mass Spectrom. 14 (19) (2000) 1793–1800, https://doi.org/10.1002/1097-0231(20001015)14:19<1793::AID-RCM95>3.0.CO;2-Q.
[62] M. Cheung See Kit, I.K. Webb, Application of multiple length cross-linkers to the characterization of gaseous protein structure, Anal. Chem. 94 (39) (2022) 13301–13310, https://doi.org/10.1021/acs.analchem.2c03044.
[63] Y. Xia, X. Liang, S.A. McLuckey, Pulsed dual electrospray ionization for ion/ion reactions, J. Am. Soc. Mass Spectrom. 16 (11) (2005) 1750–1756, https://doi.org/10.1016/j.jasms.2005.07.013.
[64] A.D. Rolland, J.S. Prell, Computational insights into compaction of gas-phase protein and protein complex ions in native ion mobility-mass spectrometry, Trends Analyt. Chem. 116 (2019) 282–291, https://doi.org/10.1016/j.trac.2019.04.023PubMed.
[65] K.B. Shelimov, D.E. Clemmer, R.R. Hudgins, M.F. Jarrold, Protein structure in vacuo: gas-phase conformations of BPTI and cytochrome c, J. Am. Chem. Soc. 119 (9) (1997) 2240–2248, https://doi.org/10.1021/ja9619059.
[66] C. Eldrid, J. Ujma, S. Kalfas, N. Tomczyk, K. Giles, M. Morris, K. Thalassinos, Gas phase stability of protein ions in a cyclic ion mobility spectrometry traveling wave device, Anal. Chem. 91 (12) (2019) 7554–7561, https://doi.org/10.1021/acs.analchem.8b05641.
[67] M.C.S. Kit, V.V. Carvalho, J.Z. Vilseck, I.K. Webb, Gas-phase ion/ion chemistry for structurally sensitive probes of gaseous protein ion structure: electrostatic and electrostatic to covalent cross-linking, Int. J. Mass Spectrom. 463 (2021) 116549, https://doi.org/10.1016/j.ijms.2021.116549.
[68] H. Shi, D.E. Clemmer, Evidence for two new solution states of ubiquitin by IMS-MS analysis, J. Phys. Chem. B 118 (13) (2014) 3498–3506, https://doi.org/10.1021/jp4097327PubMed.
[69] R.L. Cain, I.K. Webb, Online protein unfolding characterized by ion mobility electron capture dissociation mass spectrometry: cytochrome C from neutral and acidic solutions, Anal. Bioanal. Chem. 415 (5) (2023) 749–758, https://doi.org/10.1007/s00216-022-04501-w.
[70] S.M. Dixit, D.A. Polasky, B.T. Ruotolo, Collision induced unfolding of isolated proteins in the gas phase: past, present, and future, Curr. Opin. Chem. Biol. 42 (2018) 93–100, https://doi.org/10.1016/j.cbpa.2017.11.010.
[71] V.D. Gandhi, L. Hua, X. Chen, M. Latif, C. Larriba-Andaluz, A critical review of the two-temperature theory and the derivation of matrix elements. High field ion mobility and energy calculation for all-atom structures in light gases using a 12-6-4 potential, Talanta Open 7 (2023) 100191, https://doi.org/10.1016/j.talo.2023.100191.
[72] W. Colón, G.A. Elöve, L.P. Wakem, F. Sherman, H. Roder, Side chain packing of the N- and C-terminal helices plays a critical role in the kinetics of cytochrome c folding, Biochemistry 35 (17) (1996) 5538–5549, https://doi.org/10.1021/bi960052u.
[73] O.S. Skinner, F.W. McLafferty, K. Breuker, How ubiquitin unfolds after transfer into the gas phase, J. Am. Soc. Mass Spectrom. 23 (6) (2012) 1011–1014, https://doi.org/10.1007/s13361-012-0370-6FromNLM.

[74] R.L. Cain, I.K. Webb, Comparison of solution and gas phase structural ensembles of denatured cytochrome C, in: American Society for Mass Spectrometry Annual Conference, Anaheim, CA, 2024.
[75] L. Stefanis, alpha-Synuclein in Parkinson's disease, Cold Spring Harb. Perspect. Med. 2 (2) (2012) a009399, https://doi.org/10.1101/cshperspect.a009399PubMed.
[76] M.M. Dedmon, K. Lindorff-Larsen, J. Christodoulou, M. Vendruscolo, C.M. Dobson, Mapping long-range interactions in α-synuclein using spin-label NMR and ensemble molecular dynamics simulations, J. Am. Chem. Soc. 127 (2) (2005) 476–477, https://doi.org/10.1021/ja044834j.
[77] M. Cheung See Kit, T.C. Cropley, C. Bleiholder, C.D. Chouinard, F. Sobott, I. K. Webb, The role of solvation on the conformational landscape of α-synuclein, Analyst 149 (1) (2024) 125–136, https://doi.org/10.1039/D3AN01680C, 10.1039/D3AN01680C.
[78] J.R. Allison, P. Varnai, C.M. Dobson, M. Vendruscolo, Determination of the free energy landscape of α-synuclein using spin label nuclear magnetic resonance measurements, J. Am. Chem. Soc. 131 (51) (2009) 18314–18326, https://doi.org/10.1021/ja904716h.
[79] S. Warnke, G. von Helden, K. Pagel, Protein structure in the gas phase: the influence of side-chain microsolvation, J. Am. Chem. Soc. 135 (4) (2013) 1177–1180, https://doi.org/10.1021/ja308528d.
[80] C. Bleiholder, F.C. Liu, Structure relaxation approximation (SRA) for elucidation of protein structures from ion mobility measurements, J. Phys. Chem. B 123 (13) (2019) 2756–2769, https://doi.org/10.1021/acs.jpcb.8b11818.
[81] A.S. Morar, A. Olteanu, G.B. Young, G.J. Pielak, Solvent-induced collapse of α-synuclein and acid-denatured cytochrome c, Protein Sci. 10 (11) (2001) 2195–2199, https://doi.org/10.1110/ps.24301(acccessed2024/06/21.
[82] J.W. McCabe, M. Shirzadeh, T.E. Walker, C.-W. Lin, B.J. Jones, V.H. Wysocki, D. P. Barondeau, D.E. Clemmer, A. Laganowsky, D.H. Russell, Variable-temperature electrospray ionization for temperature-dependent folding/refolding reactions of proteins and ligand binding, Anal. Chem. 93 (18) (2021) 6924–6931, https://doi.org/10.1021/acs.analchem.1c00870.
[83] K.A. Graham, V.J. Grisolia, N.B. Borotto, Mobility-assisted pseudo-MS3 sequencing of protein ions, J. Am. Soc. Mass Spectrom. (2024), https://doi.org/10.1021/jasms.4c00148.
[84] D. Papanastasiou, D. Kounadis, A. Lekkas, I. Orfanopoulos, A. Mpozatzidis, A. Smyrnakis, E. Panagiotopoulos, M. Kosmopoulou, M. Reinhardt-Szyba, K. Fort, et al., The omnitrap platform: a versatile segmented linear ion trap for multidimensional multiple-stage tandem mass spectrometry, J. Am. Soc. Mass Spectrom. 33 (10) (2022) 1990–2007, https://doi.org/10.1021/jasms.2c00214.
[85] J.B. Shaw, W. Liu, Y.V. Vasil′ev, C.C. Bracken, N. Malhan, A. Guthals, J. S. Beckman, V.G. Voinov, Direct determination of antibody chain pairing by top-down and middle-down mass spectrometry using electron capture dissociation and ultraviolet photodissociation, Anal. Chem. 92 (1) (2020) 766–773, https://doi.org/10.1021/acs.analchem.9b03129.
[86] M. Lanzillotti, J.S. Brodbelt, Comparison of top-down protein fragmentation induced by 213 and 193 nm UVPD, J. Am. Soc. Mass Spectrom. 34 (2) (2023) 279–285, https://doi.org/10.1021/jasms.2c00288.
[87] T. Baba, P. Ryumin, E. Duchoslav, K. Chen, A. Chelur, B. Loyd, I. Chernushevich, Dissociation of biomolecules by an intense low-energy electron beam in a high sensitivity time-of-flight mass spectrometer, J. Am. Soc. Mass Spectrom. 32 (8) (2021) 1964–1975, https://doi.org/10.1021/jasms.0c00425.
[88] N.M. Riley, C. Mullen, C.R. Weisbrod, S. Sharma, M.W. Senko, V. Zabrouskov, M. S. Westphall, J.E.P. Syka, J.J. Coon, Enhanced dissociation of intact proteins with high capacity electron transfer dissociation, J. Am. Soc. Mass Spectrom. 27 (3) (2016) 520–531, https://doi.org/10.1007/s13361-015-1306-8.
[89] F. Lermyte, F. Sobott, Electron transfer dissociation provides higher-order structural information of native and partially unfolded protein complexes, Proteomics 15 (16) (2015) 2813–2822, https://doi.org/10.1002/pmic.201400516.
[90] Y. Xia, X. Liang, S.A. McLuckey, Pulsed dual electrospray ionization for ion/ion reactions, J. Am. Soc. Mass Spectrom. 16 (11) (2005) 1750–1756, https://doi.org/10.1016/j.jasms.2005.07.013.
[91] J.T. Specker, S.L. Van Orden, M.E. Ridgeway, B.M. Prentice, Identification of phosphatidylcholine isomers in imaging mass spectrometry using gas-phase charge inversion ion/ion reactions, Anal. Chem. 92 (19) (2020) 13192–13201, https://doi.org/10.1021/acs.analchem.0c02350.
[92] I.K. Attah, S.V.B. Garimella, I.K. Webb, G. Nagy, R.V. Norheim, C.E. Schimelfenig, Y.M. Ibrahim, R.D. Smith, Dual polarity ion confinement and mobility separations, J. Am. Soc. Mass Spectrom. 30 (6) (2019) 967–976, https://doi.org/10.1007/s13361-019-02138-1.
[93] S.V.B. Garimella, I.K. Webb, A. Prabhakaran, I.K. Attah, Y.M. Ibrahim, R.D. Smith, Design of a TW-SLIM module for dual polarity confinement, transport, and reactions, J. Am. Soc. Mass Spectrom. 28 (7) (2017) 1442–1449, https://doi.org/10.1007/s13361-017-1680-5.
[94] L. Deng, Y.M. Ibrahim, S.V.B. Garimella, I.K. Webb, A.M. Hamid, R.V. Norheim, S. A. Prost, J.A. Sandoval, E.S. Baker, R.D. Smith, Greatly increasing trapped ion populations for mobility separations using traveling waves in structures for lossless ion manipulations, Anal. Chem. 88 (20) (2016) 10143–10150, https://doi.org/10.1021/acs.analchem.6b02678.
[95] L. Deng, I.K. Webb, S.V.B. Garimella, A.M. Hamid, X. Zheng, R.V. Norheim, S. A. Prost, G.A. Anderson, J.A. Sandoval, E.S. Baker, et al., Serpentine ultralong path with extended routing (SUPER) high resolution traveling wave ion mobility-MS using structures for lossless ion manipulations, Anal. Chem. 89 (8) (2017) 4628–4634, https://doi.org/10.1021/acs.analchem.7b00185.

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