Vol. 11, No. 4, 2020
ISSN 2233-4203/ e-ISSN 2093-8950
REVIEW www.msletters.org | Mass Spectrometry Letters

Advances in Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS)-Based Techniques for Elucidating Higher-Order Protein Structures

Jongcheol Seo*

Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang 37673, Korea

Received September 13, 2020; Revised November 13, 2020; Accepted November 17, 2020
First published on the web December 31, 2020; DOI: 10.5478/MSL.2020.11.4.65

Abstract : Despite its great success in the field of proteomics, mass spectrometry has limited use for determining structural details of peptides, proteins, and their assemblies. Emerging ion mobility spectrometry-mass spectrometry has enabled us to explore the conformational space of protein ions in the gas phase, and further combinations with the gas-phase ion spectroscopy and the collision-induced unfolding have extended its abilities to elucidating the secondary structure and local details of conformational transitions. This review will provide a brief introduction to the combined approaches of IMS-MS with gas-phase ion infrared spectroscopy or collision-induced unfolding and their most recent results that successfully revealed higher-order structural details.

Keywords : protein structure, ion mobility spectrometry-mass spectrometry, ion spectroscopy, collision-induced unfolding

Introduction

Elucidating the structures of proteins and their assemblies are of great significance in the field of structural biophysics, since their structures are directly related to functions in diverse biological processes. Typically, various experimental techniques, such as nuclear magnetic resonance (NMR), X-ray scattering and crystallography, electron microscopy, and many more have been used actively to determine the high-resolution, atom-level structural details of proteins.[1-4] However, they often have limited sensitivity, and therefore require high sample densities. Furthermore, complicated sample preparation steps such as isolating and crystallizing proteins are necessary for crystallography, which decreases analytical throughput significantly.

Mass spectrometry (MS) has been extremely successful for the past several decades. Its unprecedented sensitivity, speed, accuracy, high tolerance of sample complexity, together with mass-selective isolation and fragmentation capabilities, make MS become the prime method for determining the primary structure (amino acid sequence) and providing post-translational modification of peptides and proteins.[5-7] However, MS alone is hardly applicable to obtaining higher-order structural information such as secondary structures, folding state, conformations, and topologies, since the molecular mass values do not provide any three-dimensional arrangements of atoms and molecules unless they are combined with fragmentation methods. To investigate higher-order structures, MS have been coupled to several experimental methods which include hydrogen/deuterium exchange (HDX) and non-statistical dissociation techniques such as UV photodissociation (UVPD) and electron capture/transfer dissociations (ECD/ETD).[8,9] However, they also need to dissociate intact peptide/protein ions, and the structural information is indirectly gathered from the molecular mass of fragment ions.

Alternatively, experimental approches using chemical cross-linking have been successful for investigating the folded structures and quaternary structures of proteins.[10] Forming covalent bonds between specific functional groups in proximity fixes the three-dimentional structures and protein-protein interactions, and therefore enables us to capture the higher order structural details using mass spectrometry. However, still the enzymatic digestion is necessary to confirm the interconnected residues, and therefore structural information is obtained indirectly from the peptide fragments, not from the intact protein.

Recently, emerging ion mobility spectrometry-mass spectrometry (IMS-MS) in combination with native MS has enabled to obtain three-dimensional sizes and shapes of intact biomolecular ions.[11-13] As shown in Figure 1, IMS can separate protein and peptide ions by collision cross sections (CCSs) that are directly related to their

Open Access

*Reprint requests to Jongcheol Seo
E-mail: jongcheol.seo@postech.ac.kr

All MS Letters content is Open Access, meaning it is accessible online to everyone, without fee and authors' permission. All MS Letters content is published and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org /licenses/by/3.0/). Under this license, authors reserve the copyright for their content; however, they permit anyone to unrestrictedly use, distribute, and reproduce the content in any medium as far as the original authors and source are cited. For any reuse, redistribution, or reproduction of a work, users must clarify the license terms under which the work was produced.

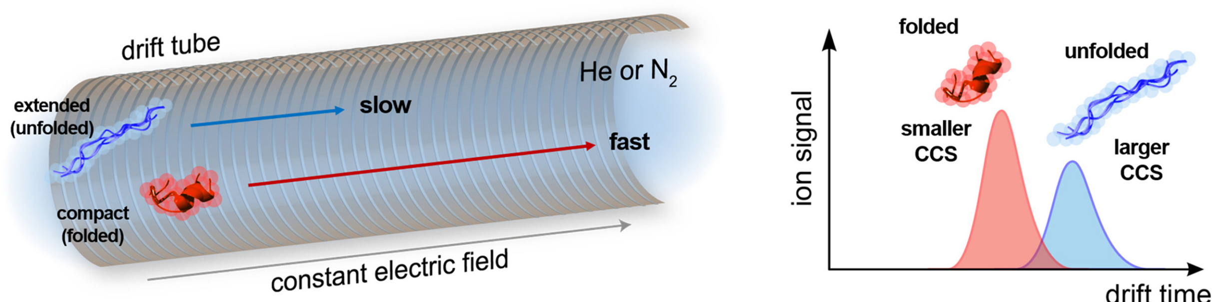

Figure 1. Schematic illustration of ion mobility spectrometry for distinguishing protein conformers.

conformation, compactness, folding state, and shapes. Distinguishable conformers yield separated peaks at the arrival time distribution (ATD) obtained by ion mobility separation, and the arrival time of each conformer can be converted to the CCS values. The measured CCS values can be used to determine protein structures precisely if the proper theoretical molecular modeling is followed. For last decades, therefore, IMS-MS has been widely and successfully applied to the structural investigation of diverse proteins and their assemblies, which include the self-assembled amyloidogenic peptide and protein oligomers,[14-21] intrinsically disordered proteins,[22,23] various protein-ligand complexes, and even vast complexes of proteins in the membrane.[24,25]

However, IMS-MS is not directly sensitive to the secondary structural elements because the measured CCS can only provide rough information about size and shape. Furthermore, the folding details cannot be extracted from the measured CCS value alone. Therefore, additional experimental techniques have been further combined with IMS-MS in order to elucidate higher-order structural details.

This review discusses the most recent advances that combine IMS-MS with other experimental techniques in order to obtain secondary, tertiary structural details and topological information of proteins. The gas-phase ion infrared (IR) spectroscopy that implements infrared multiple photon dissociation (IRMPD) is coupled to IMS-MS for deducing secondary structural details, while the collision-induced unfolding (CIU) is added for examining conformational and topological details. The unique capability of IMS-MS to isolate biomolecular ions of specific ion mobility as well as *m*/*z* enables us to apply those techniques mass- and conformer-selectively.

Gas-phase ion IR spectroscopy

IR spectroscopy is sensitive to the secondary structure of proteins. Interactions and chemical environments of characteristic vibrational modes such as C=O stretching (amide I) and N-H bending (amide II) in the peptide bond vary by secondary structural elements, and therefore each secondary structural element, an α-helix, a β-sheet, random coils, and turns, has its diagnostic band position for amide I and II. For example, an α-helix has an amide I band at ~1655 cm^{-1}, while an antiparallel β-sheet has two diagnostic amide I bands at 1620–1640 cm^{-1} and ~1690 cm^{-1}.

Acquiring IR spectra of molecular ions, however, is not trivial because the extremely low density of molecular ions due to the space-charge effect prevents observing measurable attenuations of incident IR photons to determine absorbance. Therefore, an alternative approach should be employed, which observes the 'action' of the molecular ions upon IR photon absorption. One representative action is the dissociation of molecular ions as a result of absorbing multiple IR photons, which is known as infrared multiple photon dissociation (IRMPD).[26] Molecular ions absorb multiple IR photons if and only if irradiated IR photon energy is in resonance with molecular vibrations, which causes the subsequent dissociation of molecular ions. MS is capable of monitoring the fragment and precursor ions, and a gas-phase ion IR spectrum can be obtained by recording dissociation yield as a function of irradiated IR photon energy, as shown in Figure 2. After introduced in the early 1990's first,[27,28] this IRMPD spectroscopy of molecular ions has been applied to various molecular ions which include small organic molecules including polyaromatic hydrocarbons,[29,30] amino acid monomers, dimers, and oligomers,[31-34] peptides and proteins,[35,36] and many more.

Recently, von Helden and co-workers successfully applied IMS-MS and IRMPD spectroscopy to confirm whether the native secondary structures of medium-sized proteins can be retained after the electrospray ionization for the first time.[37] Two proteins: myoglobin and β-lactoglobulin, which are representative examples of α-helix and β-sheet-rich proteins, respectively, were investigated. The ion mobility- and *m*/*z*-selected protein ions were interrogated by IRMPD to obtain *m*/*z*- and conformer-specific IR spectra of myoglobin and β-lactoglobulin ions. The measured gas-phase IR spectra have diagnostic IR bands at ~1655 cm^{-1} (α-helix) and ~1638 cm^{-1} (β-sheet) for the myoglobin and β-lactoglobulin ions, when those ions

 ©Korean Society for Mass Spectrometry

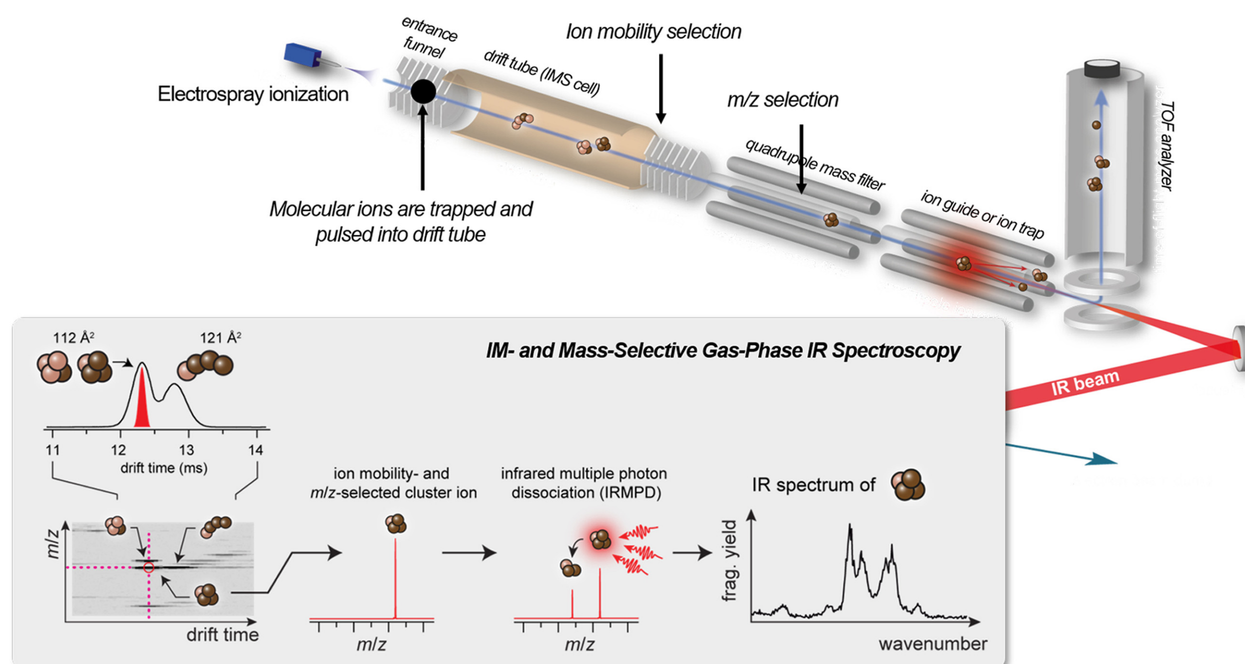

Figure 2. Schematic illustration of ion mobility- and mass-selective infrared multiple photon dissociation (IRMPD) spectroscopy implemented in the Fritz Haber Institute of the Max Planck Society. An intense, tunable IR beam is supplied by the FHI IR free electron laser facility.

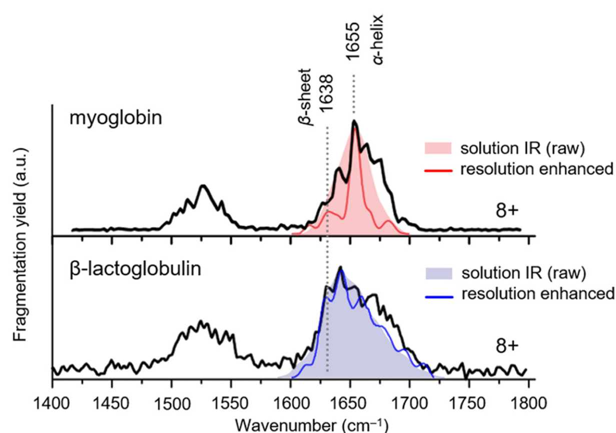

Figure 3. IRMPD spectra of myoglobin and β-lactoglobulin ions of 8+ charge state. IR spectra measured under the native condition in solution are shown together. Those 8+ protein ions were confirmed to have compact conformation whose CCS values are close to the theoretically predicted CCS values of native structures in solution.

have CCS values similar to the predicted for their native structures (Figure 3). This result indicates that secondary structures of native proteins can be retained in the gas phase after the ionization, and therefore supports the IMS-MS based structural studies in the field of structural biophysics. Furthermore, it is shown that the gas-phase ion IR spectroscopy can be a potent add-on to IMS-MS for obtaining the conformer-specific secondary structural information of protein ions for the first time.

This approach was further applied to investigate the amyloidogenic peptide oligomers. Since the formation of amyloid fibril involves both peptide aggregations and extensive secondary structural transitions to repeating β-sheets, monitoring β-sheet formation during the aggregation is critical to understand the mechanism and underlying force of the amyloid fibril formation. Combining IMS-MS and gas-phase IR spectroscopy, von Helden, Pagel, and co-workers succeed to measure the onset of β-sheet formation directly in early soluble oligomers of the insulin fragment VEALYL and its variant, YVEALL.[38] Especially for the YVEALL pentamer, which has four different conformers, IR features for the β-sheet increase as the CCS increases, suggesting the pentameric assembly is the onset of the amyloid fibril formation for the YVEALL peptide (Figure 4). This result is the first direct observation and confirmation of secondary structural transitions of amyloidogenic peptide oligomers in the field of mass spectrometry. It strongly supports the interpretation in the recent IMS-MS studies of amyloid systems, which suppose the positive correlation between the CCS increase and β-sheet formations.[16,18-21] Moreover, this demonstrates how the combination of gas-phase IR spectroscopy and IMS-MS can be a great asset in this research field.

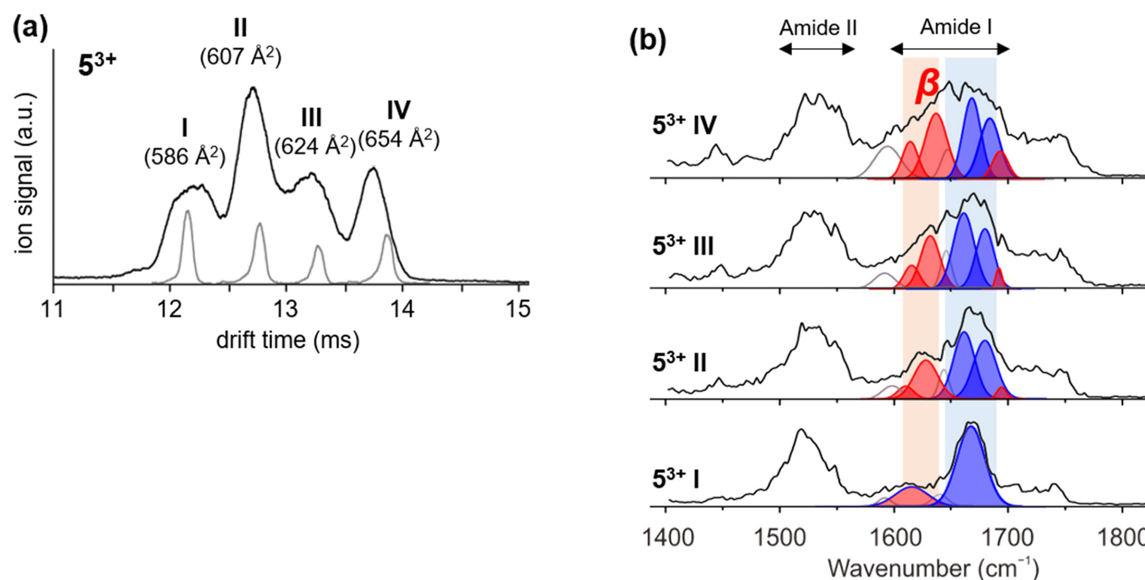

Figure 4. (a) Arrival time distribution of triply-protonated YVEALL pentamer (**5^{3+}**) showing four different conformers (**I**–**IV**), and (b) their conformer-selective IRMPD spectra. Color-coded gaussian envelopes stand for the IR bands assigned to the unordered (blue) and the antiparallel β-sheet (red), respectively.

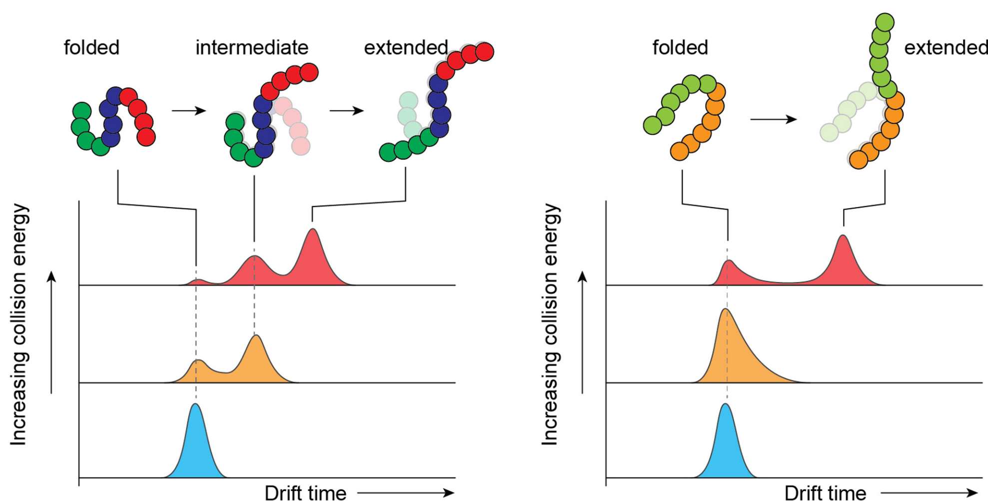

Figure 5. Collision-induced unfolding for determining the conformational details.

Collisional activation IMS-MS (Collision-induced unfolding)

Collisional activation has been one of the most common methods that interrogate isolated biomolecular ions in MS, especially to examine the stability as well as the structure of protein and peptide ions. Representatively, many tandem mass spectrometry (MS/MS) approaches utilize collisional activations (collision-induced dissociation, CID) to induce peptide bond dissociation for the peptide sequencing.

Meanwhile, collisional activations followed by IMS-MS can be used to probe tertiary/quaternary structures, conformations, and topologies of protein ions, if they are not providing sufficient energy to induce the covalent bond dissociation.[39-41] The increased internal energy leads the protein ions to undergo unfolding that can be probed by monitoring CCS in IM-MS. This collision-induced unfolding (CIU) often generates a series of partially-folded intermediates that enable us to gain insights into the structural/conformational details as well as unfolding mechanism and dynamics, as illustrated in Figure 5. Hence, CIU has been shedding light on the structural studies of many

 ©Korean Society for Mass Spectrometry

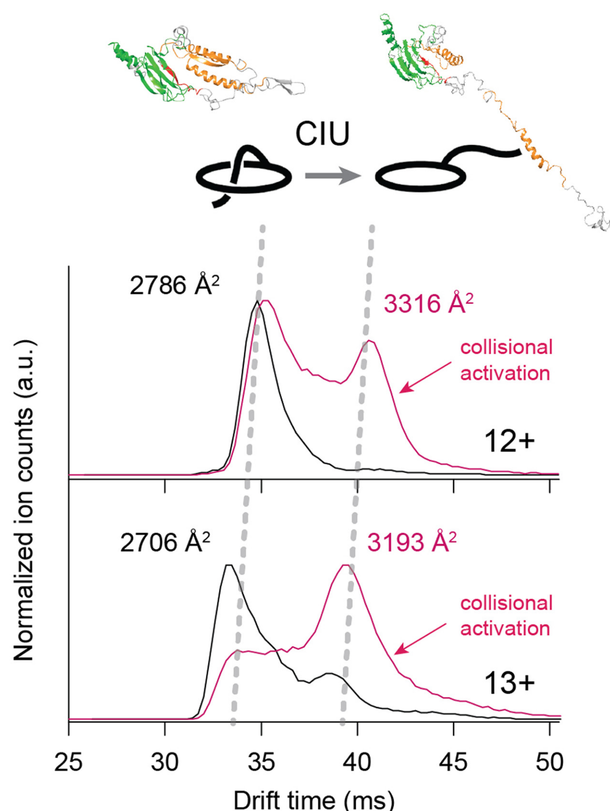

Figure 6. The IMS data of 12+ and 13+ lasso protein ions without collisional activation (black) and with collisional activation (red), which indicate conformational transitions (a threaded lasso → an extended tadpole) due to the collision-induced unfolding.

complex protein systems. Representatively, Ruotolo and co-workers were successfully applied CIU to distinguish several immunoglobulin G (IgG) subtypes that show minor structural differences based on their disulfide bond numbers and patterns.[41] Many other examples using CIU also include structural/conformational works for glycoproteins, protein-protein or protein-ligand complexes, and amyloid protein assemblies.[42-44]

Most recently, CIU combined with IMS-MS has proved its capability to examine topological and conformational differences of a specifically designed functional protein. Seo, Wenbin and co-workers characterized the conformation of a topologically engineered protein that has a lasso topology.[45] As shown in Figure 6, the IMS-MS result suggests the presence of two or more conformers for the lass protein: i) a threaded lasso with and without tail unfolding, and ii) an extended tadpole. The CIU result shows the direct conformational transitions from a threaded lasso to an extended tadpole and no further unfolding for the tadpole, further proving the lasso topology and the presence of its two distinct conformations.

This IMS-MS and CIU are expected to be further combined with other structural methods such as chemical cross-linking. Chemically cross-linked proteins are supposed to undergo the limited unfolding under the CIU. Therefore, the selective cross-linkings between specific residues possibly enable us to unveil which amino acid residues and their interactions are closely related to a specific folding mode.

Concluding Remarks

In summary, the emerging IMS-MS has dramatically extended the realm of MS in the field of investigating protein structures. Combining other experimental techniques such as the gas-phase ion IR spectroscopy and the CIU enhanced the utilities of IMS-MS much further, and therefore the elucidating higher-order structures and conformational details of proteins becomes feasible using IMS-MS. These approaches are also combined with other structural proteomics techniques such as HDX, chemical cross-linkings, and even several fragmentation methods to gain further details of protein foldings and interactions. However, gas-phase ion spectroscopy and CIU are still far from becoming routine methods for protein structures. A highly intense IR light source is necessary for the gas-phase ion IR spectroscopy, and low resolving power of IMS limit the use of CIU for determining precise conformational details. Nevertheless, these combined approaches in IMS-MS will be very promising in the research fields of protein structures, with the future advances in the IR light source and the action spectroscopic methods, and the development of high-resolution IMS-MS instruments.

Acknowledgments

The author is grateful for the support from the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (Grant No. 2019R1C1C1C11008414).

Conflict of Interest

The author declares no conflict of interest.

References

1. Cavalli, A.; Salvatella, X.; Dobson, C. M.; Vendruscolo, M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, 104, 9615, DOI: 10.1073/pnas.0610313104.
2. Clore, G. M.; Gronenborn, A. M. *Science* **1991**, 252, 1390, DOI: 10.1126/science.2047852.
3. Pfeffer, S.; Mahamid, J. *Curr. Opin. Struct. Biol.* **2018**, 52, 111, DOI: 10.1016/j.sbi.2018.08.009.
4. Shi, Y. *Cell* **2014**, 159, 995, DOI: 10.1016/j.cell.2014.10.051.

5. Nilsson, T.; Mann, M.; Aebersold, R.; Yates, J. R.; Bairoch, A.; Bergeron, J. J. M. *Nat. Methods* **2010**, 7, 681, DOI: 10.1038/nmeth0910-681.
6. Pandey, A.; Mann, M. *Nature* **2000**, 405, 837, DOI: 10.1038/35015709.
7. Yates, J. R.; Ruse, C. I.; Nakorchevsky, A. *Annu. Rev. Biomed. Eng.* **2009**, 11, 49, DOI: 10.1146/annurev-bioeng-061008-124934.
8. Engen, J. R. *Anal. Chem.* **2009**, 81, 7870, DOI: 10.1021/ac901154s.
9. Oh, H. B.; Moon, B. *Mass Spectrom. Rev.* **2015**, 34, 116, DOI: 10.1002/mas.21426.
10. Liu, F.; Heck, A.J.R. *Curr. Opin. Struct. Biol.* **2015**, 35, 100, DOI: 10.1016/j.sbi.2015.10.006.
11. Ben-Nissan, G.; Sharon, M. *Curr. Opin. Chem. Biol.* **2018**, 42, 25, DOI: 10.1016/j.cbpa.2017.10.026.
12. Wyttenbach, T.; Bowers, M. T. *Annu. Rev. Phys. Chem.* **2007**, 58, 511, DOI: 10.1146/annurev.physchem.58.032806.104515.
13. Bohrer, B.C.; Merenbloom, S.I.; Koeniger, S.L.; Hilderbrand, A.E.; Clemmer, D.E. *Annu. Rev. Anal. Chem.* **2008**, 1, 293, DOI: 10.1146/annurev.anchem.1.031207.113001.
14. Bernstein, S.L.; Dupuis, N.F.; Lazo, N.D.; Wyttenbach, T.; Condron, M.M.; Bitan, G.; Teplow, D.B.; Shea, J.-E.; Ruotolo, B.T.; Robinson, C.V.; Bowers, M.T. *Nat. Chem.* **2009**, 1, 326, DOI: 10.1038/nchem.247.
15. Dupuis, N.F.; Wu, C.; Shea, J.-E.; Bowers, M.T. *J. Am. Chem. Soc.* **2009**, 131, 18283, DOI: 10.1021/ja903814q.
16. Bleiholder, C.; Dupuis, N.F.; Wyttenbach, T.; Bowers, M.T. *Nat. Chem.* **2011**, 3, 172, DOI: 10.1038/nchem.945.
17. Hoffmann, W.; von Helden, G.; Pagel, K. *Curr. Opin. Struct. Biol.* **2017**, 46, 7, DOI: 10.1016/j.sbi.2017.03.002.
18. Do, T. D.; Economou, N. J.; Chamas, A.; Buratto, S. K.; Shea, J.-E.; Bowers, M. T. *J. Phys. Chem. B* **2014**, 118, 11220, DOI: 10.1021/jp506258g.
19. Do, T. D.; LaPointe, N. E.; Economou, N. J.; Buratto, S. K.; Feinstein, S. C.; Shea, J.-E.; Bowers, M. T. *J. Phys. Chem. B* **2013**, 117, 10759, DOI: 10.1021/jp406066d.
20. Do, T. D.; LaPointe, N. E.; Sangwan, S.; Teplow, D. B.; Feinstein, S. C.; Sawaya, M. R.; Eisenberg, D. S.; Bowers, M. T. *J. Phys. Chem. B* **2014**, 118, 7247, DOI: 10.1021/jp502473s.
21. Young, L. M.; Cao, P.; Raleigh, D. P.; Ashcroft, A. E.; Radford, S. E. *J. Am. Chem. Soc.* **2014**, 136, 660, DOI: 10.1021/ja406831n.
22. Han, J. Y.; Choi, T. S.; Heo, C. E.; Son, M. K.; Kim, H. I. *Mass Spectrom. Rev.* **2019**, 38, 483, DOI: 10.1002/mas.21596.
23. Lee, S. J. C.; Lee, J. W.; Choi, T. S.; Jin, K. S.; Lee, S.; Ban, C.; Kim, H. I. *Anal. Chem.* **2014**, 86, 1909, DOI: 10.1021/ac404132g.
24. Ruotolo, B. T.; Benesch, J. L. P.; Sandercock, A. M.; Hyung, S.-J.; Robinson, C. V. *Nat. Protoc.* **2008**, 3, 1139, DOI: 10.1038/nprot.2008.78.
25. Lanucara, F.; Holman, S. W.; Gray, C. J.; Eyers, C. E. *Nat. Chem.* **2014**, 6, 281, DOI: 10.1038/nchem.1889.
26. Oomens, J.; Sartakov, B.G.; Meijer, G.; von Helden, G. *Int. J. Mass Spectrom.* **2006**, 254, 1, DOI: 10.1016/j.ijms.2006.05.009.
27. Shin, S. K.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1990**, 112, 2066, DOI: 10.1021/ja00162a004.
28. Shin, S. K.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1990**, 112, 2057, DOI: 10.1021/ja00162a003.
29. Banisaukas, J.; Szczepanski, J.; Eyler, J.; Vala, M.; Hirata, S.; Head-Gordon, M.; Oomens, J.; Meijer, G.; Von Helden, G. *J. Phys. Chem. A* **2003**, 107, 782, DOI: 10.1021/jp0219754.
30. Oomens, J.; Meijer, G.; von Helden, G. *J. Phys. Chem. A* **2001**, 105, 8302, DOI: 10.1021/jp0110455.
31. Kong, X.; Tsai, I.A.; Sabu, S.; Han, C.-C.; Lee, Y.T.; Chang, H.-C.; Tu, S.-Y.; Kung, A.H.; Wu, C.-C. *Angew. Chem. Int. Ed.* **2006**, 45, 4130, DOI: 10.1002/anie.200600597.
32. Oh, H.-B.; Lin, C.; Hwang, H. Y.; Zhai, H.; Breuker, K.; Zabrouskov, V.; Carpenter, B. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **2005**, 127, 4076, DOI: 10.1021/ja040136n.
33. Seo, J.; Hoffmann, W.; Malerz, S.; Warnke, S.; Bowers, M. T.; Pagel, K.; von Helden, G. *Int. J. Mass Spectrom.* **2018**, 429, 115, DOI: 10.1016/j.ijms.2017.06.011.
34. Wu, R.; McMahon, T. B. *J. Am. Chem. Soc.* **2007**, 129, 4864, DOI: 10.1021/ja068715a.
35. Polfer, N. C. *Chem. Soc. Rev.* **2011**, 40, 2211, DOI: 10.1039/C0CS00171F.
36. Polfer, N. C.; Oomens, J. *Mass Spectrom. Rev.* **2009**, 28, 468, DOI: 10.1002/mas.20215.
37. Seo, J.; Hoffmann, W.; Warnke, S.; Bowers, M. T.; Pagel, K.; von Helden, G. *Angew. Chem. Int. Ed.* **2016**, 55, 14173, DOI: 10.1002/anie.201606029.
38. Seo, J.; Hoffmann, W.; Warnke, S.; Huang, X.; Gewinner, S.; Schöllkopf, W.; Bowers, M. T.; von Helden, G.; Pagel, K. *Nat. Chem.* **2017**, 9, 39, DOI: 10.1038/nchem.2615.
39. Hoffmann, W.; Hofmann, J.; Pagel, K. *J. Am. Soc. Mass Spectrom.* **2014**, 25, 471 DOI: 10.1021/jasms.8b04709.
40. Hyung, S.-J.; Robinson, C. V.; Ruotolo, B. T. *Chem. Biol.* **2009**, 16, 382, DOI: 10.1016/j.chembiol.2009.02.008.
41. Tian, Y.; Han, L.; Buckner, A. C.; Ruotolo, B. T. *Anal. Chem.* **2015**, 87, 11509, DOI: 10.1021/acs.analchem.5b03291.
42. Laganowsky, A.; Reading, E.; Allison, T.M.; Ulmschneider, M.B.; Degiacomi, M.T.; Baldwin, A.J.; Robinson, C.V. *Nature* **2014**, 510, 172, DOI: 10.1038/nature13419.
43. Liu, Y.; Cong, X.; Liu, W.; Laganowsky, A. *J. Am. Soc. Mass Spectrom.* **2017**, 28, 579, DOI: 10.1007/s13361-016-1555-1.
44. Liu, Y.; LoCaste, C. E.; Liu, W.; Poltash, M. L.; Russell, D. H.; Laganowsky, A. *Nat. Comm.* **2019**, 10, 1352, DOI: 10.1038/s41467-019-09333-4.
45. Liu, Y.; Wu, W.-H.; Hong, S.; Fang, J.; Zhang, F.; Liu, G.-X.; Seo, J.; Zhang, W.-B. *Angew. Chem. Int. Ed.* **2020**, 59, 19153, DOI: 10.1002/anie.202006727.

©Korean Society for Mass Spectrometry