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Improved structural elucidation of peptide isomers and their receptors using advanced ion mobility-mass spectrometry

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ABSTRACT

Ion mobility-mass spectrometry (IM-MS) has emerged as a highly important analytical technique for the structural elucidation of a wide range of biomolecules, including neuropeptides, proteins and protein complexes. However, though the inherent value presented by powerful ion mobility techniques has garnered significant interest and spurred on the development of novel and increasingly powerful IM-MS regimes, modern commercial implementations continually fall short in the effort to fully resolve subtle structural variations of biologically-relevant analytes, including peptide stereoisomers. Presented here is a review of emerging instrumentation, computational strategies, and methods of data analysis vital to the integration and development of IM-MS workflows for structural characterization. Furthermore, we explore the usefulness of ion mobility techniques in probing structurally-critical biomolecules, examining the ability of current methods to elucidate neuropeptide stereoisomers and receptors.

1. Introduction

Neuropeptides play indispensable roles in regulating many essential physiological functions through interactions with their corresponding receptors [1] and extensive research efforts have revealed that post-translational modifications (PTMs) of these signaling neuropeptides directly impact their biological activities. Post-translational modifications are well understood to drastically increase sample complexity and provide a rich landscape of biomolecules with differentiated function, but the profound impact of modifications is only fully appreciated when considering the heterogeneous collection of neuropeptides that may exist as isomers. Such peptide isomers can be generated from sequence scrambling [2], variations in PTM position [3,4], naturally occurring residue isomers (i.e. leucine vs isoleucine, aspartic acid vs isoaspartic acid, etc.) [5] and racemization of certain residues [6,7]. As isomerization-based PTMs reveal no mass shift between modified and unmodified peptides, identification of these species through mass spectrometry (MS) and tandem MS (MS^n) techniques is highly limited and supplemental separation strategies are required in order to discriminate ionic species on the basis of structure [5]. While capillary electrophoresis and liquid chromatography have been employed for the efficient separation of peptide isomers [8–12], ion mobility-mass spectrometry (IM-MS) represents an emerging method of choice for structural discrimination of these isomers according to their size and shape due to its efficient separation timescale and small sample requirement [2–4,13–18].

Among the numerous isomerization-based PTMs, an area of topical interest and concern is that involving the enzymatic conversion of amino acids from L- to D-conformation. The discovered quantity of these aptly named D-amino acid-containing peptides (DAACPs) is rapidly expanding and these molecules are noted to be largely implicated in unique biological functions. Over 30 DAACPs have been discovered from a range of animal species, including sea slug *Aplysia californica* [19,20], lobster *Homarus americanus* [21], crayfish *Procambarus clarkii* [22], octopus *Octopus minor* [23], platypus *Ornithorhynchus anatinu* [24,25], venom from cone snail [26], funnel web spider *Agelenopsis aperta* [27] and South American tree frog *Phyllomedusa sauvagei* [28]. Interestingly, while DAACPs are regularly detected in animal venoms, the D-isomerization of amino acid residues have also been reported in human ageing proteins from various tissues, including tooth, skin and brain [29]. Most DAACPs are believed to be enzymatically generated in animal cells [6,30], but the amino acid chiral inversion of several age-dependent proteins in human tissues has also been linked to a

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non-enzymatic covalent modification [29,31]. Signaling molecules, a class to which all neuropeptides belong, display great dependence on structural conformation for appropriate recognition and binding with their receptor. This now stated, the small conformational change of a single amino acid chiral inversion often presents significant alteration to downstream processes. DAACPs – often containing only a single D-amino acid in most neuropeptides – have been shown to have longer lifetime due to greater resistance to endogenous enzymes, which only recognize proteins and peptides comprised exclusively of L-amino acids [32,33]. And additionally, stereoisomer analogues of endogenous neuropeptides have been found to bear enhanced biological activities in many cases, as observed through dramatically increased receptor-binding affinity and selectivity [6,34]. Interestingly, neuropeptides where all amino acid residues have been chirally inverted to the D-conformation display reduced levels of toxicity [35], whereas similar neuropeptides with only partial inversion display the opposite trend [6,30]. These noted observations indicate that increased detection and recognition of chiral residue effects could potentially be useful for later drug discovery. Collectively, the controversial role of chirality in neuropeptides suggests the importance of developing new strategies that allow researchers to study these structurally similar peptides with improved resolution and specificity. Here, we provide an overview of several recent reports describing the method development and research progress of structural elucidation of neuropeptide epimers and their activity in binding with receptors.

Amongst the multidisciplinary techniques developed or applied to distinguish neuropeptide epimers, ion mobility-mass spectrometry (IM-MS) has emerged as a method of choice due to its rapid acquisition, low sample consumption, and high resolution for both mass and gas-phase conformation determination [36]. During IM-MS experiments, ions are separated based on differences in shape and charge as they travel against a region of inert buffer gas under the influence of an electric field. Several types of ion mobility spectrometry (Fig. 1) have been integrated with MS, such as drift tube ion mobility spectrometry (DTIMS) [36–38], high-field asymmetric waveform ion mobility spectrometry (FAIMS) [39], traveling wave ion mobility spectrometry (TWIMS) [40], and trapped ion mobility spectrometry (TIMS) [41].

IM-MS can be used to separate and characterize a wide range of biomolecules, including peptides, proteins and protein complexes [42–48]. Compared to liquid-phase separations, IM-MS offers the opportunity to capture and manipulate transient intermediates not stable enough to be identified and characterized in solution [46]. However, as protein isoforms often exhibit great similarity in collisional cross section – often with differences lower than 2% – there is a profound need for improved IM-MS instrument resolution [49]. Collision-induced unfolding (CIU) [50] has been integrated into IM-MS, adding a resolving dimension and enabling complete differentiation of protein conformations indistinguishable through CCS measurements alone. Method progressions like CIU speak to the ever-increasing sophistication for IM-MS analyses, and such technical designs are even further enhanced through the development of multi-functional data collection and interpretation [49,51–53], as demonstrated in a growing number of examples [54–56]. Moreover, IM-MS separation of ionized species typically occurs on the order of milliseconds in the gas phase [57]. This small timescale is important as it allows for ion mobility separation to be implemented between LC and MS, bridging the gap between the minute and microsecond timescales for separation and detection. As seen here, IM-MS is highly useful for applications in native MS protein structure elucidation, and also may be implemented into many standard LC-MS/MS procedures commonly used in -omics workflows [58].

Given the aforementioned biological significance and inherent technological challenges in distinguishing neuropeptide isomers, it is of great necessity to survey and highlight the recently emerging IM-MS strategies that have proven useful for neuropeptide isomer separation, discovery, and functional investigation. With the continuous development of modern bioanalytical techniques, previously unappreciated roles for peptide isomers in biological processes have been revealed [38]. In this review, we will focus our

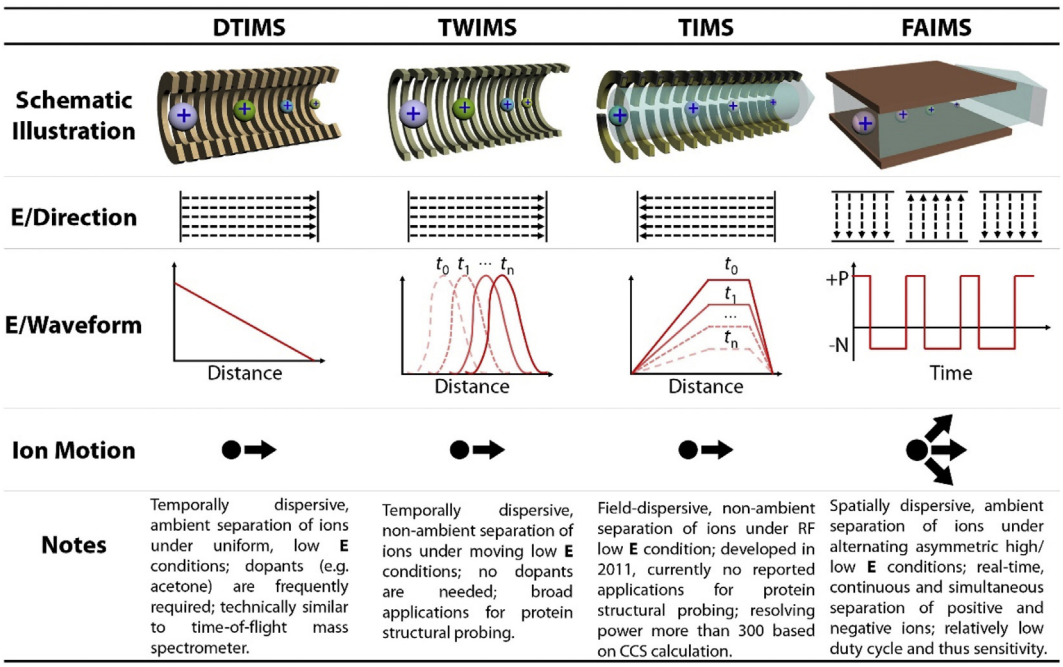

Fig. 1. Major IM-MS regimes currently commercially available for peptide isomer analysis. Four types of ion mobility setups (DTIMS/TWIMS/TIMS/FAIMS) are schematically illustrated with the electrode design and buffer gas (if applicable, shadowed region). Vector descriptions of electric field (E, direction and waveform) and ion motion are also provided.

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discussion on IM-MS characterization of bioactive DAACPs (Fig. 2A), peptide isomers involved in various biological processes (Fig. 2B and C), and structural elucidation of both neuropeptide epimers and their receptors. Novel insights and future directions for IM-MS of peptide isomers – for example, the study of the effects of residue chirality on peptide epimer oligomerization and their impact on receptor recognition and binding – will also be discussed.

2. Structural separation of peptide isomers by IM-MS

2.1. D-Amino acid-containing peptides (DAACPs) (Fig. 2A)

In nature, only a small number of amino acid residues in neuropeptides are observed to be D-isomerized, whereas almost all proteins and peptides are formed exclusively by L-amino acids. As a result, high resolution techniques such as IM-MS are necessary to unambiguously resolve the subtle conformational variations between DAACPs and their counterparts. However, the necessity of techniques such as IM-MS for D-peptide analysis would not be fully recognized if not for the significant contributions from numerous multi-disciplinary researchers. Bai et al. previously reviewed the history of DAACPs and highlighted several analytical tools for the separation and detection of neuropeptide epimers from animal sources [6], while Ollivaux et al. reviewed the natural occurrence and functional significance of DAACPs, various pathways of the biogenesis of D-residues, and also discussed the spatiotemporal characteristics, cellular and enzyme specificities and known mechanisms of the peptidyl aminoacyl L-to D-isomerization [7]. Further still, Sweedler and coworkers pioneered the MS-based DAACP research, reporting on non-targeted discovery through a suite of biochemical tools including capillary electrophoresis, enzymatic screening, chiral amino acid analysis and LC-MS [11,33,59].

The earliest trials of tandem MS strategies for DAACP analysis were documented in 2004 when Adams et al. used electron capture dissociation (ECD) as the first tandem MS technique to distinguish a single D-amino acid in a protein [60]. In this study, chiral recognition was defined by the degree of chiral distinction, $R_{chiral} = R_D/R_L$, where $R_{D/L}$ was measured by the ratio of the abundances of z_{i8}^{+} to z_{i9}^{+} ions generated from ECD. This parameter of chiral distinction can be used to quantitatively characterize the degree of D-isomerization of amino acids [60,61]. Serafin et al. reported the first collision activated dissociation (CAD)-based tandem MS strategy for differentiation of single amino acid D-isomerized peptides from all L-form counterparts [62]. Through combining the statistical calculations and rationally comparing the ratios of fragment ion abundances between stereoisomers, they observed statistically significant differences between Ser- D-isomerized amyloid β fragment (Aβ17-29) and its epimers. Metal complexation was also used to allow enantiomer separation of Aβ17-29, allowing for discussion of a putative CAD fragmentation pathway [62]. In parallel, Julian's group has extended the tandem MS strategy from CID to radical-directed dissociation, which has also been demonstrated as effective in discriminating DAACPs from their epimers [9,63]. Also, tandem MS strategies have been developed to distinguish peptide epimers on a MALDI TOF/TOF instrument with the use of appropriate fragmentation and/or metal adducts [64,65].

Alternatively, IM-MS has grown to be a direct, rapid and effective method of choice for peptide isomer discrimination via the direct measurements of CCS values, accomplished by converting drift time into cross-sectional values via a physical model. For this reason, IM-MS provides more straightforward structural information of the observed peptide compared to traditional tandem MS strategies. Moreover, augmentation of IM-MS instruments bears gradually improved performance in distilling valuable structural information from neuropeptide epimers. Instrument augmentation is typically facilitated through equipping additional or more powerful resolving units and activation components. Inspired by pioneering efforts in DAACP analysis using tandem MS strategies and IM-MS strategies, Li and coworkers previously demonstrated a site-specific strategy to rapidly and reliably localize the D-amino acids in DAACPs by using TWIMS [17]. In this strategy (Fig. 3B), peptide epimers isolated from complex mixtures through reversed-phase liquid chromatography were subjected to collision-induced dissociation (CID) and subsequent fragment ions were structurally monitored by IM-MS. D-amino acid residues were then localized by evaluating arrival time/CCS shift of fragment ions derived from DAACPs and their corresponding epimers. This rational design revealed isomerization of L-to D-Phe at the third residue of the crustacean hyperglycemic hormones. In the same vein, a recent combinatorial CID-IM-MS study on a 29-residue DAACP further highlighted the advantages of online interfacing of IM and tandem MS for epimer separation, sequencing, and even relative quantification [16].

A higher resolution IM-MS platform such as trapped-ion mobility spectrometry (TIMS) mass spectrometry has been utilized to achieve enhanced separation of these highly similar stereoisomers [16], in some cases with the assistance from metal adducts (Fig. 3A). By using TIMS-based IM-MS, Fouque et al. reported the baseline separation of some DAACPs from their counterparts – even with theoretical CCS differences as small as 1% [16]. This experiment was remarkable in that it achieved both a CCS-

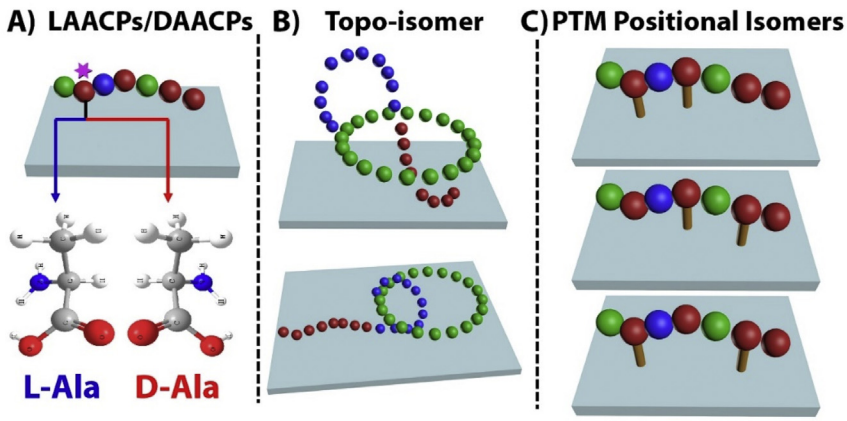

Fig. 2. Schematic illustrations for peptide isomerization involved in this review. A) DAACPs with single D-amino acid residue, B) Lasso peptides, and C) PTM positional isomers.

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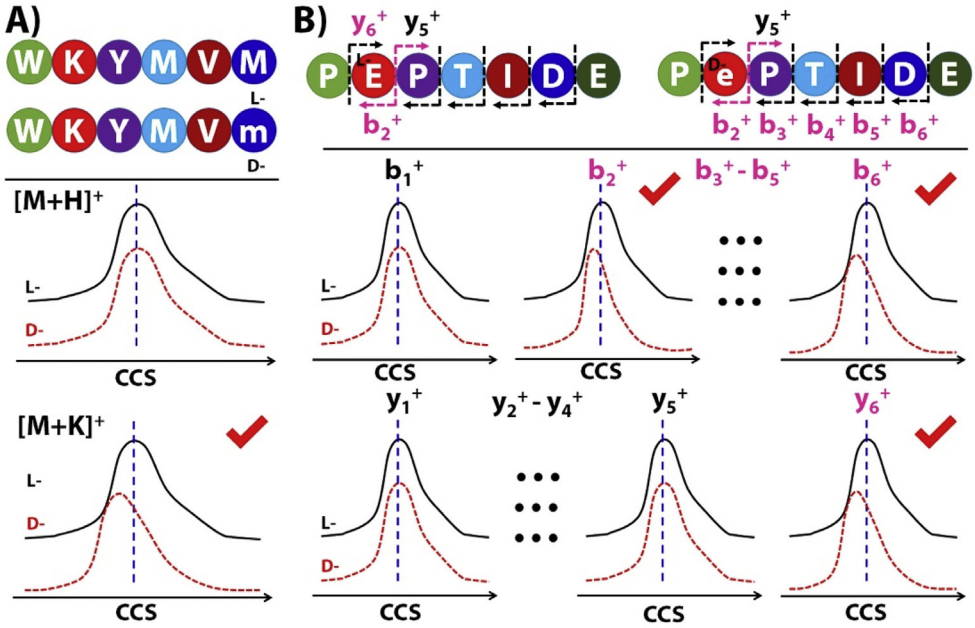

Fig. 3. Two typical strategies in IM-MS analysis of peptide isomers: A) metal adduction to amplify the structural differences between isomers and B) tandem MS technique that enables differentiation and localization of the D-amino acid residues.

based resolving power as high as 340 and reliable quantification of a 29-residue DAACP. As a note, the structural effects of alkali metal adducts on peptides have been systematically studied by Clemmer and coworkers through IM-MS [66]. In another report, Pang et al. documented enhanced structural differences of metal adducted peptide oligomer epimers induced by D-isomerization of amino acid residues, which were investigated on a TWIMS platform [67]. In a recent study, the Sweedler and Bowers groups collaborated in performing conformational investigations of a D-peptide, GdFFD, using a lab-built DTIMS [38]. Negative polarity-nanoelectrospray ionization was used to generate ions suitable for ion mobility separation in their lab-built instrument. Their investigation established a model for the study of steric effects on peptide activity in binding and regulating neuropeptide receptors, one of which was the *Aplysia* achatin-like neuropeptide receptor (apALNR). This study revealed a new avenue to detail the effects of chirality on neuropeptide's structure and activity, which may be more appreciated than the identification of DAACPs as extensive biological and pharmaceutical applications are now anticipated.

The preceding lists of studies are only recent examples for DAACP analysis by using IM-MS, selected from numerous reports utilizing a wide range of analytical strategies. As a typical peptide isomerization, D-/L-substitution at one or several amino acid residues of a neuropeptide plays a pivotal biological role — such as enhanced bioactivity — [59] and separation of such relevant isomeric species represents an area of broad interests in analytical chemistry. Similarly, other PTMs that induce peptide isomerization can also be resolved by IM-MS, as shown below.

2.2. Topology-isomeric peptides (Fig. 2B)

Lasso peptides, a typical class of topologically isomeric peptides, are ribosomally synthesized and post-translationally modified bioactive molecules [68,69]. Lasso peptides are named as such due to the formation of a macrocyclic ring at the N-terminus, through which the isomerized C-terminus is threaded. The diversity in Lasso peptide structures can be accurately imagined when considering the size of ring that may be formed and the number of residues that could exist in the D-confirmation. Because lasso peptides are often low in abundance and high resolution is paramount for meaningful observation, IM-MS for structural characterization of lasso peptides has grown in popularity over the past ten years and significant efforts have been devoted to the development of suitable strategies for lasso peptide analysis with improved resolving power. Specifically, charge state increments have been found to enhance IM separation efficiency as exemplified in TWIMS and DTIMS separation of lasso peptides and their branched-cyclic topoisomers [15,70]. Metal binding has also been used to enhance the topoisomer separations on a TIMS instrument, though this study primarily focused on nonspecific metalation between Lasso peptides and alkali cations [14]. Complementary to these techniques, Afonso's group reported a multidimensional strategy comprised of mean charge divided by mass, relative range of CCS and mean ion mobility peak width [71]. By distributing these three dimensions in space, it was possible to observe clear clustering patterns between the lasso peptides and branched-cyclic peptides, which suggested a new effective strategy to discriminate mechanically interlocked topologically isomeric peptides. Notably, the same group also reported the utility of CID and ETD for structural elucidation of lasso peptides and their epimers [72].

2.3. PTM-isomeric peptides (Fig. 2C)

Proteins and peptides are frequently modified post-translation on their primary amino acid sequence, altering their activities under different conditions. One such PTM is phosphorylation, which, in many cases, may occur at multiple residues within a peptide or protein. For this reason, it is common to observe mixtures of localization isomers in phosphoproteins and phosphopeptides featuring more than one phosphorylated residue [4]. Conventional liquid-phase separation techniques such as LC [12,73] or CE [74] often lack ability to separate isomeric phosphopeptides - as the weak polar phosphate-hydrophobic stationary phase interaction leads to co-elution, or require very sophisticated design to separate on a timescale of minutes with a compromised resolving power. In addition, gas-phase tandem MS strategies employed in DAACP

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discrimination – such as those discussed previously – are not that effective in localization of phosphorylation positional isomers, due to low fragmentation efficiency common to low charge state phosphopeptides.

Offering relief from some analytical challenges, IM-MS has been used for rapid, efficient separation of positional isomers – where the only structural difference is the location of phosphate group on the peptide backbone [4,18,75]. While the role of phosphorylation in compacting peptide conformation has been observed in several ion mobility instruments, it seems that DTIMS achieves the best performance in distinguishing positional isomers when considering sensitivity and resolving power together [4,75]. A recent report has demonstrated improved ion mobility separation efficiency, sensitivity and throughput for phosphopeptides through integration of LC into an ultrahigh-resolution ion mobility separation cell [8].

In addition to phosphorylation, peptides modified through scrambled disulfide bond formation or glycosylation have also been successfully separated and characterized through IM-MS. Disulfide bonds play an important role in stabilizing peptides and facilitating conformational folding, as supported by previous efforts in disulfide peptide sequencing [76–80], and disulfide-scrambling is also readily observed in MS analysis of disulfide bond-containing peptides and proteins [81,82]. More recently, glycan isomer analysis received increased attention due to implication of importance in many biological processes including cell recognition, molecular trafficking and signal transduction. A previous review from Chen et al. highlighted recent advances in IM-MS for improved structural characterization of glycosylated species and noted that improvements in IM-MS for glycan and glycoconjugate isomer differentiations have been exemplified through the development of high-resolution instruments, optimization of charge state or ionization polarity mode, glycan labeling and integration with orthogonal separation techniques. Readers interested in these aspects may find details in the aforementioned review [83].

3. Structural elucidation of receptors for peptide isomers by IM-MS

It has been widely acknowledged that peptides and proteins cannot fulfill their functions alone. Instead, they require coordinated interactions with surrounding factors, including metal ions, small molecules and large protein receptors [84–86]. Therefore, it is highly desirable to develop strategies that enable structural and functional characterization of interactions between DAACPs and their receptors. A key challenge associated with the study of the DAACP-receptor interaction is the difficulty in obtaining receptor protein crystals with sufficient purity and quantity for physiological characterization. Faced with this challenge, IM-MS emerges as a method of choice due to its aforementioned advantages over other biophysical techniques, including high sensitivity, high analytical speed, and enriched structural information at the molecular scale.

Both neuropeptide lifetime and receptor specificity are reported to be related to the chirality of amino acid residues. Utilizing molecular and physiological characterization techniques such as quantitative polymerase chain reaction and *in situ* hybridization experiments, Checco et al. recently observed interactions between a set of DAACPs and their receptors in *Aplysia californica* [1]. More recently, the same group reported a new neuropeptide isomer receptor, a specific G protein-coupled receptor, as an allatotropin-related peptide (ATRP) receptor [87]. It has been surprisingly observed that both L-ATRP and D2-ATRP activate this ATRP receptor but D2-ATRP appears to have a longer lifetime. In addition to molecular characterization, structural elucidation through IM-MS was utilized, for the first time, to gain greater insight into the role of three-dimensional conformation in regulating DAACPs' activity with receptors [38]. A lab-built DTIMS aids in the creation of a predictive model for DAACPs' activities in combination with MD-based computational modeling (Fig. 4B–D), which makes the structural understanding of DAACP-receptor interactions attainable even without the high amount of protein crystals for structural data acquisition. Although computational modeling and cell-based receptor activity assays provide useful information to interrogate DAACP-receptor interaction, additional tools and strategies are needed to enhance our understanding of its functional interaction especially in a protein/receptor-centered manner.

4. Instrumentation and computational modeling

While great attention has been paid to improvements in multifaceted IM-MS techniques, there exists a continual pursuit for greater resolving power in mobility instruments. Current commercial instruments are largely based on TWIMS, FAIMS or DTIMS technologies, which are generally limited by either their resolving power (~40–300) or sensitivity, though recent coupling of TIMS with TOF or Orbitrap instruments has offered substantially improved resolution [16]. Despite these advancements, on-going efforts are directed towards achieving a better balance between resolving power and sensitivity as most current instrumental developments struggle to maintain sensitivity levels while improving resolving power. The development of the Structures for Lossless Ion Manipulations (SLIM) technology by the Smith group represents a promising solution in that endeavor, though current SLIM implementations leave plenty of space for sensitivity improvement [88–91]. A recent study by the group reported on the use of SLIM technology to differentiate minor structural differences in D-isomerized amino acid residues of amyloid β (Aβ) peptide [92]. In addition to the almost baseline separation of Aβ peptide isomers, the SLIM technology also enabled probing of structural effects on Aβ fragments as a result of positional D-isomerization. Furthermore, SLIM can also be used for effective separation of many other isomers, suggesting its utility and versatility to be incorporated into various MS platforms [93]. Meanwhile, differential ion mobility spectrometry (DMS)/FAIMS has also been increasingly implemented in peptide isomer separations, including isomeric glycopeptide separation and localization of PTMs [3,13,94,95]. Specifically, seven positional isomers of a tryptic peptide with a nitrated tyrosine residue were fully resolved by using a FAIMS instrument with a resolving power as high as ~300 [3]. TIMS has also been demonstrated in multiple cases for its versatility in peptide isomer separation [14,41,96]. For example, a TIMS-based separation and identification workflow was established for distinguishing lasso peptides from branched cyclic peptides, which is analytically challenging as these two isomers only demonstrate conformational differences when tertiary structure is preserved [14].

In addition to hardware development and improvement, computational modeling and simulations can offer important complementary information to aid in structural assignment of complex peptide/protein systems. Molecular dynamics (MD) simulations, based on classical dynamics and the assumptions that biological activity is the result of time-dependent interactions between molecules, permit the study of complex static and dynamic biological properties and processes including protein stability, conformational change, folding/unfolding, molecular recognition, and the like. As an example of use case, MD can aid in drug design through combination with X-ray crystallography, NMR and even IM-MS [97]. Determining where drug molecules bind and how they exert their effects can be used to describe the mechanism by which drugs binding to one end of the receptor causes the other end to change its shape [98]. Since its advent in the 1950s, several

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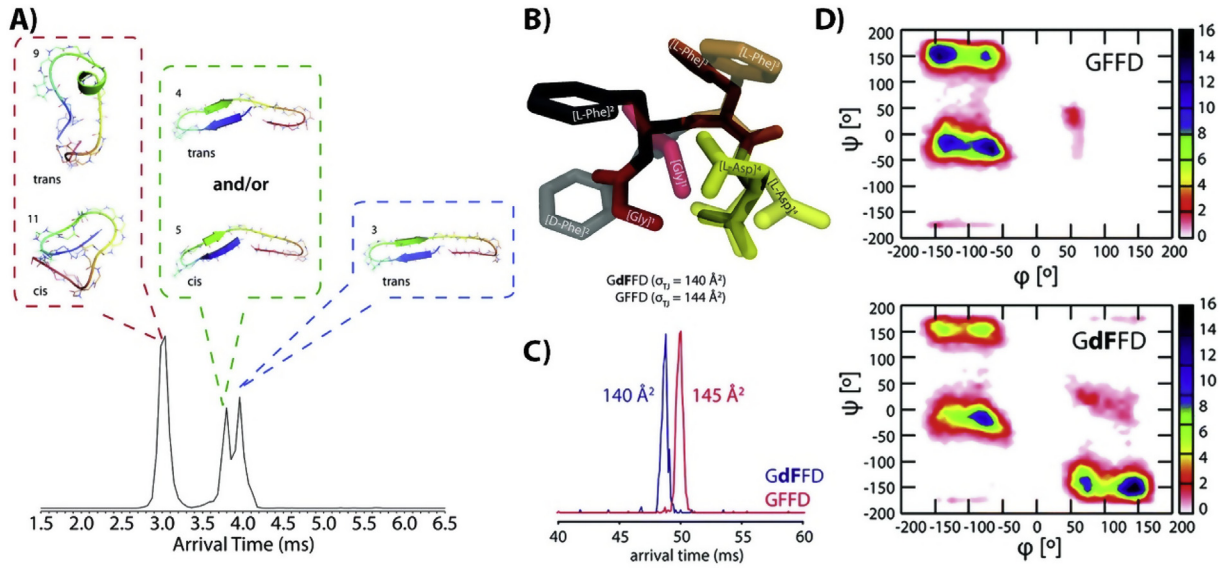

Fig. 4. Computational modeling combining with IM-MS for enhanced structural analysis of peptide isomers. A) Replica-exchange molecular dynamics (REMD)-based MD simulation and MaxCluster analysis of the CCS-filtered ensembles providing a versatile strategy to confirm IM-MS results on cis/trans peptide stereoisomers. B-D) REMD-based MD simulations on a DAACP, GdFFD and its counterpart (GFFD). B) Overlaid structures of GdFFD (semi-transparent) and GFFD (solid). Hydrogens are omitted for clarity. C) Arrival time distributions of singly charged species ($z = -1$, $m/z = 484$) of separate samples of the peptides GdFFD (blue) and GFFD (red). D) Ramachandran plots of GFFD and GdFFD structures obtained from T-REMD. The peptide concentration is 50 μM in water. σ_{TJ} is the theoretical CCS obtained from the trajectory method. Reproduced from C.B. Lietz, Z.W. Chen, C.Y. Son, X.Q. Pang, Q. Cui, L.J. Li, Analyst 141 (2016) 4863 (ref 98), and T.D. Do, J.W. Checco, M. Tro, J.E. Shea, M.T. Bowers, J.V. Sweedler, Phys. Chem. Chem. Phys. 20 (2018) 22047 (ref 17), with permission of the Royal Society of Chemistry.

software packages have been developed for MD simulations: CHARMM, which uses experimental enthalpies, GROMOS, and AMBER, which uses experimental free energies.

Up to now, the majority of MD simulations have been applied to peptide structure studies, with only a small portion covering stereo-inversed peptides [38,99,100]. Lietz et al. previously implemented MD-based theoretical calculations (Fig. 4A) to support and/or clarify IM-MS signatures of cis/trans conformations of proline isomerization in a neuropeptide. By investigating the differences between experimentally measured conformation and MD simulation results, this study highlighted the importance of computational modeling in IM-MS structural assignments [101]. As such, combined use of IM-MS and computational modeling strategies, such as MD and other simplified molecular modeling tools like coarse-grained modeling for larger protein complexes is expected to bring improved structural assignments [102].

5. Concluding remarks

In this review, we summarize recent progress in advancing IM-MS for improved structural analysis of peptide isomers and their receptors through discussing isomeric heterogeneity, high-performance ion mobility instrumentation, and innovative implementation of computational modeling. There are remaining challenges and limitations to each facet of this integrated platform, including the need to balance between sensitivity and resolving power for the IM-MS instrumentation, the limited MS-compatible solvent/buffer conditions that enable analysis of highly hydrophobic peptides and proteins, and the need for advanced computing power and force field accuracy to improve the efficiency and speed of molecular dynamic simulations and other computational modeling strategies. Overall, enhanced separation and structural signatures provided by improved IM-MS measurements and theoretical calculation from computational modeling provide an invaluable platform not only for peptide structural analysis, but also for rational drug design and rapid inhibitor screening that may help to accelerate drug discovery and therapeutic development process.

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References

[1] J.W. Checco, G. Zhang, W.D. Yuan, K. Yu, S.Y. Yin, R.H. Roberts-Galbraith, P.M. Yau, E.V. Romanova, J. Jing, J.V. Sweedler, Molecular and physiological characterization of a receptor for d-amino acid-containing neuropeptides, ACS Chem. Biol. 13 (5) (2018) 1343.
[2] C. Jia, Z. Wu, C.B. Lietz, Z. Liang, Q. Cui, L. Li, Gas-phase ion isomer analysis reveals the mechanism of peptide sequence scrambling, Anal. Chem. 86 (6) (2014) 2917.
[3] A.A. Shvartsburg, A.J. Creese, R.D. Smith, H.J. Cooper, Separation of a set of peptide sequence isomers using differential ion mobility spectrometry, Anal. Chem. 83 (18) (2011) 6918.
[4] Y.M. Ibrahim, A.A. Shvartsburg, R.D. Smith, M.E. Belov, Ultrasensitive identification of localization variants of modified peptides using ion mobility spectrometry, Anal. Chem. 83 (14) (2011) 5617.
[5] E.T. Jansson, Strategies for analysis of isomeric peptides, J. Sep. Sci. 41 (1) (2018) 385.
[6] L. Bai, S. Sheeley, J.V. Sweedler, Analysis of endogenous D-amino acid-containing peptides in metazoa, Bioanal. Rev. 1 (1) (2009) 7.
[7] C. Ollivaux, D. Soyez, J.Y. Toullec, Biogenesis of D-amino acid containing peptides/proteins: where, when and how? J. Sep. Sci. 20 (8) (2014) 595.
[8] C.D. Chouinard, G. Nagy, I.K. Webb, T. Shi, E.S. Baker, S.A. Prost, T. Liu, Y.M. Ibrahim, R.D. Smith, Improved sensitivity and separations for phosphopeptides using online liquid chromotography coupled with structures for lossless ion manipulations ion mobility-mass spectrometry, Anal. Chem. 90 (18) (2018) 10889.

[9] Y. Tao, R.R. Julian, Identification of amino acid epimerization and isomerization in crystallin proteins by tandem LC-MS, Anal. Chem. 86 (19) (2014) 9733.
[10] A.V. Patel, T. Kawai, L. Wang, S.S. Rubakhin, J.V. Sweedler, Chiral measurement of aspartate and glutamate in single neurons by large-volume sample stacking capillary electrophoresis, Anal. Chem. 89 (22) (2017) 12375.
[11] S.A. Sheeley, H. Miao, M.A. Ewing, S.S. Rubakhin, J.V. Sweedler, Measuring D-amino acid-containing neuropeptides with capillary electrophoresis, Analyst 130 (8) (2005) 1198.
[12] D. Singer, J. Kuhlmann, M. Muschket, R. Hoffmann, Separation of multiphosphorylated peptide isomers by hydrophilic interaction chromatography on an aminopropyl phase, Anal. Chem. 82 (15) (2010) 6409.
[13] A.J. Creese, H.J. Cooper, Separation and identification of isomeric glycopeptides by high field asymmetric waveform ion mobility spectrometry, Anal. Chem. 84 (5) (2012) 2597.
[14] K.J. Dit Fouque, J. Moreno, J.D. Hegemann, S. Zirah, S. Rebuffat, F. Fernandez-Lima, Identification of lasso peptide topologies using native nano-electrospray ionization-trapped ion mobility spectrometry-mass spectrometry, Anal. Chem. 90 (8) (2018) 5139.
[15] K. Jeanne Dit Fouque, C. Afonso, S. Zirah, J.D. Hegemann, M. Zimmermann, M.A. Marahiel, S. Rebuffat, H. Lavanant, Ion mobility-mass spectrometry of lasso peptides: signature of a rotaxane topology, Anal. Chem. 87 (2) (2015) 1166.
[16] K. Jeanne Dit Fouque, A. Garabedian, J. Porter, M. Baird, X. Pang, T.D. Williams, L. Li, A. Shvartsburg, F. Fernandez-Lima, Fast and effective ion mobility-mass spectrometry separation of d-amino-acid-containing peptides, Anal. Chem. 89 (21) (2017) 11787.
[17] C. Jia, C.B. Lietz, Q. Yu, L. Li, Site-specific characterization of (D)-amino acid containing peptide epimers by ion mobility spectrometry, Anal. Chem. 86 (6) (2014) 2972.
[18] A.A. Shvartsburg, D. Singer, R.D. Smith, R. Hoffmann, Ion mobility separation of isomeric phosphopeptides from a protein with variant modification of adjacent residues, Anal. Chem. 83 (13) (2011) 5078.
[19] W. Bawab, E. Querido, P. Crine, L. DesGroseillers, Identification and characterization of aminopeptidases from Aplysia californica, Biochem. J. 286 (3) (1992) 967.
[20] F. Morishita, Y. Nakanishi, S. Kaku, Y. Furukawa, S. Ohta, T. Hirata, M. Ohtani, Y. Fujisawa, Y. Muneoka, O. Matsushima, A novel D-amino-acid-containing peptide isolated from Aplysia heart, Biochem. Biophys. Res. Commun. 240 (2) (1997) 354.
[21] D. Soyez, F. Van Herp, J. Rossier, J.P. Le Caer, C.P. Tensen, R. Lafont, Evidence for a conformational polymorphism of invertebrate neurohormones. D-amino acid residue in crustacean hyperglycemic peptides, J. Biol. Chem. 269 (28) (1994) 18295.
[22] A. Yasuda, Y. Yasuda, T. Fujita, Y. Naya, Characterization of crustacean hyperglycemic hormone from the crayfish (Procambarus clarkii): multiplicity of molecular forms by stereoinversion and diverse functions, Gen. Comp. Endocrinol. 95 (3) (1994) 387.
[23] E. Iwakoshi, M. Hisada, H. Minakata, Cardioactive peptides isolated from the brain of a Japanese octopus, Octopus minor, Peptides 21 (5) (2000) 623.
[24] A.M. Torres, I. Menz, P.F. Alewood, P. Bansal, J. Lahnstein, C.H. Gallagher, P.W. Kuchel, D-Amino acid residue in the C-type natriuretic peptide from the venom of the mammal,Ornithorhynchus anatinus, the Australian platypus, FEBS Lett. 524 (1–3) (2002) 172.
[25] A.M. Torres, C. Tsampazi, D.P. Geraghty, P.S. Bansal, P.F. Alewood, P.W. Kuchel, D-amino acid residue in a defensin-like peptide from platypus venom: effect on structure and chromatographic properties, Biochem. J. 391 (Pt 2) (2005) 215.
[26] S. Dutertre, N.G. Lumsden, P.F. Alewood, R.J. Lewis, Isolation and characterisation of conomap-Vt, a D-amino acid containing excitatory peptide from the venom of a vermivorous cone snail, FEBS Lett. 580 (16) (2006) 3860.
[27] S. Heck, C. Siok, K. Krapcho, P. Kelbaugh, P. Thadeio, M. Welch, R. Williams, A. Ganong, M. Kelly, A. Lanzetti, a. et, Functional consequences of post-translational isomerization of Ser46 in a calcium channel toxin, Science 266 (5187) (1994) 1065.
[28] K. Richter, R. Egger, G. Kreil, D-alanine in the frog skin peptide dermorphin is derived from L-alanine in the precursor, Science 238 (4824) (1987) 200.
[29] S. Ritztimme, M. Collins, Racemization of aspartic acid in human proteins, Ageing Res. Rev. 1 (1) (2002) 43.
[30] G. Kreil, D-amino acids in animal peptides, Annu. Rev. Biochem. 66 (1997) 337.
[31] N. Fujii, Y. Kaji, N. Fujii, D-Amino acids in aged proteins: analysis and biological relevance, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 879 (29) (2011) 3141.
[32] S. Ha, I. Kim, T. Takata, T. Kinouchi, M. Isoyama, M. Suzuki, N. Fujii, Identification of D-amino acid-containing peptides in human serum, PLoS One 12 (12) (2017), e0189972.
[33] M.A. Ewing, J. Wang, S.A. Sheeley, J.V. Sweedler, Detecting D-amino acid-containing neuropeptides using selective enzymatic digestion, Anal. Chem. 80 (8) (2008) 2874.
[34] M. Garton, S. Nim, T.A. Stone, K.E. Wang, C.M. Deber, P.M. Kim, Method to generate highly stable D-amino acid analogs of bioactive helical peptides using a mirror image of the entire PDB, Proc. Natl. Acad. Sci. U. S. A. 115 (7) (2018) 1505.
[35] S. Dutta, A.R. Foley, C.J.A. Warner, X. Zhang, M. Rolandi, B. Abrams, J.A. Raskatov, Suppression of oligomer formation and formation of non-toxic fibrils upon addition of mirror-image Abeta42 to the natural l-enantiomer, Angew. Chem. Int. Ed. 56 (38) (2017) 11506.
[36] P. Dugourd, R.R. Hudgins, D.E. Clemmer, M.F. Jarrold, High-resolution ion mobility measurements, Rev. Sci. Instrum. 68 (2) (1997) 1122.
[37] H.H. Hill, W.F. Siems, R.H.S. Louis, D.G. McMinn, Ion mobility spectrometry, Anal. Chem. 62 (23) (1990) 1201A.
[38] T.D. Do, J.W. Checco, M. Tro, J.E. Shea, M.T. Bowers, J.V. Sweedler, Conformational investigation of the structure-activity relationship of GdFFD and its analogues on an achatin-like neuropeptide receptor of Aplysia californica involved in the feeding circuit, Phys. Chem. Chem. Phys. 20 (34) (2018) 22047.
[39] R.W. Purves, R. Guevremont, S. Day, C.W. Pipich, M.S. Matyjaszczyk, Mass spectrometric characterization of a high-field asymmetric waveform ion mobility spectrometer, Rev. Sci. Instrum. 69 (12) (1998) 4094.
[40] A.A. Shvartsburg, R.D. Smith, Fundamentals of traveling wave ion mobility spectrometry, Anal. Chem. 80 (24) (2008) 9689.
[41] F. Fernandez-Lima, D.A. Kaplan, J. Suetering, M.A. Park, Gas-phase separation using a trapped ion mobility spectrometer, Int. J. Ion Mobil. Spectrom. 14 (2) (2011) 93.
[42] T. Wyttenbach, G. vonHelden, M.T. Bowers, Gas-phase conformation of biological molecules: Bradykinin, J. Am. Chem. Soc. 118 (35) (1996) 8355.
[43] S. Koneshan, J.C. Rasaiah, R.M. Lynden-Bell, S.H. Lee, Solvent structure, dynamics, and ion mobility in aqueous solutions at 25 °C, J. Phys. Chem. B 102 (21) (1998) 4193.
[44] A.S. Woods, M. Ugarov, T. Egan, J. Koomen, K.J. Gillig, K. Fuhrer, M. Gonin, J.A. Schultz, Lipid/peptide/nucleotide separation with MALDI-ion mobility-TOF MS, Anal. Chem. 76 (8) (2004) 2187.
[45] C.S. Kaddis, S.H. Lomeli, S. Yin, B. Berhane, M.I. Apostol, V.A. Kickhoefer, L.H. Rome, J.A. Loo, Sizing large proteins and protein complexes by electrospray ionization mass spectrometry and ion mobility, J. Am. Soc. Mass Spectrom. 18 (7) (2007) 1206.
[46] B.C. Bohrer, S.I. Merenbloom, S.L. Koeniger, A.E. Hilderbrand, D.E. Clemmer, Biomolecule analysis by ion mobility spectrometry, Annu. Rev. Anal. Chem. 1 (2008) 293.
[47] M.L. Zhu, B. Bendiak, B. Clowers, H.H. Hill, Ion mobility-mass spectrometry analysis of isomeric carbohydrate precursor ions, Anal. Bioanal. Chem. 394 (7) (2009) 1853.
[48] F. Lanucara, S.W. Holman, C.J. Gray, C.E. Eyers, The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics, Nat. Chem. 6 (4) (2014) 281.
[49] J.D. Eschweiler, J.N. Rabuck-Gibbons, Y. Tian, B.T. Ruotolo, CIUSuite: a quantitative analysis package for collision induced unfolding measurements of gas-phase protein ions, Anal. Chem. 87 (22) (2015) 11516.
[50] 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.
[51] C. Bleiholder, T. Wyttenbach, M.T. Bowers, A novel projection approximation algorithm for the fast and accurate computation of molecular collision cross sections (I). Method, Int. J. Mass Spectrom. 308 (1) (2011) 1.
[52] E.G. Marklund, M.T. Degiacomi, C.V. Robinson, A.J. Baldwin, J.L. Benesch, Collision cross sections for structural proteomics, Structure 23 (4) (2015) 791.
[53] M.T. Marty, A.J. Baldwin, E.G. Marklund, G.K. Hochberg, J.L. Benesch, C.V. Robinson, Bayesian deconvolution of mass and ion mobility spectra: from binary interactions to polydisperse ensembles, Anal. Chem. 87 (8) (2015) 4370.
[54] Y. Tian, L. Han, A.C. Buckner, B.T. Ruotolo, Collision induced unfolding of intact antibodies: rapid characterization of disulfide bonding patterns, glycosylation, and structures, Anal. Chem. 87 (22) (2015) 11509.
[55] J.D. Eschweiler, R.M. Martini, B.T. Ruotolo, Chemical probes and engineered constructs reveal a detailed unfolding mechanism for a solvent-free multi-domain protein, J. Am. Chem. Soc. 139 (1) (2017) 534.
[56] J.N. Rabuck-Gibbons, J.M. Lodge, A.K. Mapp, B.T. Ruotolo, Collision-induced unfolding reveals unique fingerprints for remote protein interaction sites in the KIX regulation domain, J. Am. Soc. Mass Spectrom. 30 (1) (2018) 94.
[57] J.A. McLean, B.T. Ruotolo, K.J. Gillig, D.H. Russell, Ion mobility–mass spectrometry: a new paradigm for proteomics, Int. J. Mass Spectrom. 240 (3) (2005) 301.
[58] B.X. MacLean, B.S. Pratt, J.D. Egertson, M.J. MacCoss, R.D. Smith, E.S. Baker, Using skyline to analyze data-containing liquid chromatography, ion mobility spectrometry, and mass spectrometry dimensions, J. Am. Soc. Mass Spectrom. 29 (11) (2018) 2182.
[59] I. Livnat, H.C. Tai, E.T. Jansson, L. Bai, E.V. Romanova, T.T. Chen, K. Yu, S.A. Chen, Y. Zhang, Z.Y. Wang, D.D. Liu, K.R. Weiss, J. Jing, J.V. Sweedler, A d-amino acid-containing neuropeptide discovery funnel, Anal. Chem. 88 (23) (2016) 11868.
[60] C.M. Adams, F. Kjeldsen, R.A. Zubarev, B.A. Budnik, K.F. Haselmann, Electron capture dissociation distinguishes a single D-amino acid in a protein and probes the tertiary structure, J. Am. Soc. Mass Spectrom. 15 (7) (2004) 1087.
[61] C.M. Adams, R.A. Zubarev, Distinguishing and quantifying peptides and proteins containing D-amino acids by tandem mass spectrometry, Anal. Chem. 77 (14) (2005) 4571.

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[62] S.V. Serafin, R. Maranan, K.L. Zhang, T.H. Morton, Mass spectrometric differentiation of linear peptides composed of L-amino acids from isomers containing one D-amino acid residue, Anal. Chem. 77 (17) (2005) 5480.
[63] Y. Tao, N.R. Quebbemann, R.R. Julian, Discriminating D-amino acid-containing peptide epimers by radical-directed dissociation mass spectrometry, Anal. Chem. 84 (15) (2012) 6814.
[64] L. Bai, E.V. Romanova, J.V. Sweedler, Distinguishing endogenous D-amino acid-containing neuropeptides in individual neurons using tandem mass spectrometry, Anal. Chem. 83 (7) (2011) 2794.
[65] J. Koehbach, C.W. Gruber, C. Becker, D.P. Kreil, A. Jilek, MALDI TOF/TOF-Based approach for the identification of D- amino acids in biologically active peptides and proteins, J. Proteome Res. 15 (5) (2016) 1487.
[66] J.M. Dilger, S.J. Valentine, M.S. Glover, M.A. Ewing, D.E. Clemmer, A database of alkali metal-containing peptide cross sections: influence of metals on size parameters for specific amino acids, Int. J. Mass Spectrom. 330–332 (2012) 35.
[67] X.Q. Pang, C.X. Jia, Z.W. Chen, L.J. Li, Structural characterization of monomers and oligomers of D-amino acid-containing peptides using T-wave ion mobility mass spectrometry, J. Am. Soc. Mass Spectrom. 28 (1) (2017) 110.
[68] J.D. Hegemann, M. Zimmermann, S. Zhu, H. Steuber, K. Harms, X. Xie, M.A. Marahiel, I.-I.I.I. Xanthomonins, A new class of lasso peptides with a seven-residue macrolactam ring, Angew. Chem. Int. Ed. 53 (8) (2014) 2230.
[69] T.A. Knappe, U. Linne, S. Zirah, S. Rebuffat, X. Xie, M.A. Marahiel, Isolation and structural characterization of capistruin, a lasso peptide predicted from the genome sequence of Burkholderia thailandensis E264, J. Am. Chem. Soc. 130 (34) (2008) 11446.
[70] K. Jeanne Dit Fouque, H. Lavanant, S. Zirah, J. Lemoine, S. Rebuffat, J.C. Tabet, A. Kulesza, C. Afonso, P. Dugourd, F. Chirot, Gas-phase conformations of capistruin - comparison of lasso, branched-cyclic and linear topologies, Rapid Commun. Mass Spectrom. 29 (15) (2015) 1411.
[71] K.J.D. Fouque, H. Lavanant, S. Zirah, J.D. Hegemann, M. Zimmermann, M.A. Marahiel, S. Rebuffat, C. Afonso, Signatures of mechanically interlocked topology of lasso peptides by ion mobility-mass spectrometry: lessons from a collection of representatives, J. Am. Soc. Mass Spectrom. 28 (2) (2017) 315.
[72] K. Jeanne Dit Fouque, H. Lavanant, S. Zirah, J.D. Hegemann, C.D. Fage, M.A. Marahiel, S. Rebuffat, C. Afonso, General rules of fragmentation evidencing lasso structures in CID and ETD, Analyst 143 (5) (2018) 1157.
[73] P. Langlais, L.J. Mandarino, Z. Yi, Label-free relative quantification of co-eluting isobaric phosphopeptides of insulin receptor substrate-1 by HPLC-ESI-MS/MS, J. Am. Soc. Mass Spectrom. 21 (9) (2010) 1490.
[74] E. Ollikainen, A. Bonabi, N. Nordman, V. Jokinen, T. Kotiaho, R. Kostiainen, T. Sikanen, Rapid separation of phosphopeptides by microchip electrophoresis-electrospray ionization mass spectrometry, J. Chromatogr. A 1440 (2016) 249.
[75] M.S. Glover, J.M. Dilger, M.D. Acton, R.J. Arnold, P. Radivojac, D.E. Clemmer, Examining the influence of phosphorylation on peptide ion structure by ion mobility spectrometry-mass spectrometry, J. Am. Soc. Mass Spectrom. 27 (5) (2016) 786.
[76] C.N. Cramer, C.D. Kelstrup, J.V. Olsen, K.F. Haselmann, P.K. Nielsen, Complete mapping of complex disulfide patterns with closely-spaced cysteines by in-source reduction and data-dependent mass spectrometry, Anal. Chem. 89 (11) (2017) 5949.
[77] S. Lu, S.B. Fan, B. Yang, Y.X. Li, J.M. Meng, L. Wu, P. Li, K. Zhang, M.J. Zhang, Y. Fu, J. Luo, R.X. Sun, S.M. He, M.Q. Dong, Mapping native disulfide bonds at a proteome scale, Nat. Methods 12 (4) (2015) 329.
[78] G. Li, J. Pei, Y. Yin, G. Huang, Direct sequencing of a disulfide-linked peptide with electrospray ionization tandem mass spectrometry, Analyst 140 (8) (2015) 2623.
[79] G. Li, Y. Yin, G. Huang, Increased disulfide peptide sequence coverage via "cleavage ON/OFF" switch during nanoelectrospray, RSC Adv. 4 (103) (2014) 59650.
[80] Y. Xia, R.G. Cooks, Plasma induced oxidative cleavage of disulfide bonds in polypeptides during nanoelectrospray ionization, Anal. Chem. 82 (7) (2010) 2856.
[81] J. Echterbille, L. Quinton, N. Gilles, E. De Pauw, Ion mobility mass spectrometry as a potential tool to assign disulfide bonds arrangements in peptides with multiple disulfide bridges, Anal. Chem. 85 (9) (2013) 4405.
[82] M. Yang, C. Dutta, A. Tiwari, Disulfide-bond scrambling promotes amorphous aggregates in lysozyme and bovine serum albumin, J. Phys. Chem. B 119 (10) (2015) 3969.
[83] Z. Chen, M.S. Glover, L. Li, Recent advances in ion mobility-mass spectrometry for improved structural characterization of glycans and glycoconjugates, Curr. Opin. Chem. Biol. 42 (2018) 1.
[84] G. Li, S. Yuan, S. Zheng, Y. Liu, G. Huang, In situ living cell protein analysis by single-step mass spectrometry, Anal. Chem. 90 (5) (2018) 3409.
[85] G. Li, S. Yuan, S. Zheng, Y. Chen, Z. Zheng, Y. Liu, G. Huang, The effect of salts in promoting specific and competitive interactions between zinc finger proteins and metals, J. Am. Soc. Mass Spectrom. 28 (12) (2017) 2658.
[86] G. Li, S. Yuan, Y. Pan, Y. Liu, G. Huang, Binding states of protein-metal complexes in cells, Anal. Chem. 88 (22) (2016) 10860.
[87] J.W. Checco, G. Zhang, W.D. Yuan, Z.W. Le, J. Jing, J.V. Sweedler, Aplysia allatotropin-related peptide and its newly identified D-amino acid-containing epimer both activate a receptor and a neuronal target, J. Biol. Chem. 293 (43) (2018) 16862.
[88] A.V. Tolmachev, I.K. Webb, Y.M. Ibrahim, S.V. Garimella, X. Zhang, G.A. Anderson, R.D. Smith, Characterization of ion dynamics in structures for lossless ion manipulations, Anal. Chem. 86 (18) (2014) 9162.
[89] X. Zhang, S.V. Garimella, S.A. Prost, I.K. Webb, T.C. Chen, K. Tang, A.V. Tolmachev, R.V. Norheim, E.S. Baker, G.A. Anderson, Y.M. Ibrahim, R.D. Smith, Ion trapping, storage, and ejection in structures for lossless ion manipulations, Anal. Chem. 87 (12) (2015) 6010.
[90] S.V.B. Garimella, A.M. Hamid, L. Deng, Y.M. Ibrahim, I.K. Webb, E.S. Baker, S.A. Prost, R.V. Norheim, G.A. Anderson, R.D. Smith, Squeezing of ion populations and peaks in traveling wave ion mobility separations and structures for lossless ion manipulations using compression ratio ion mobility programming, Anal. Chem. 88 (23) (2016) 11877.
[91] A.M. Hamid, Y.M. Ibrahim, S.V. Garimella, I.K. Webb, L. Deng, T.C. Chen, G.A. Anderson, S.A. Prost, R.V. Norheim, A.V. Tolmachev, R.D. Smith, Characterization of traveling wave ion mobility separations in structures for lossless ion manipulations, Anal. Chem. 87 (22) (2015) 11301.
[92] X. Zheng, L. Deng, E.S. Baker, Y.M. Ibrahim, V.A. Petyuk, R.D. Smith, Distinguishing d- and l-aspartic and isoaspartic acids in amyloid beta peptides with ultrahigh resolution ion mobility spectrometry, Chem. Commun. 53 (56) (2017) 7913.
[93] L. Deng, Y.M. Ibrahim, E.S. Baker, N.A. Aly, A.M. Hamid, X. Zhang, X. Zheng, S.V.B. Garimella, I.K. Webb, S.A. Prost, J.A. Sandoval, R.V. Norheim, G.A. Anderson, A.V. Tolmachev, R.D. Smith, Ion mobility separations of isomers based upon long path length structures for lossless ion manipulations combined with mass spectrometry, ChemistrySelect 1 (10) (2016) 2396.
[94] A.A. Shvartsburg, Y. Zheng, R.D. Smith, N.L. Kelleher, Separation of variant methylated histone tails by differential ion mobility, Anal. Chem. 84 (15) (2012) 6317.
[95] M.A. Baird, A.A. Shvartsburg, Localization of post-translational modifications in peptide mixtures via high-resolution differential ion mobility separations followed by electron transfer dissociation, J. Am. Soc. Mass Spectrom. 27 (12) (2016) 2064.
[96] J.A. Silveira, M.E. Ridgeway, M.A. Park, High resolution trapped ion mobility spectrometery of peptides, Anal. Chem. 86 (12) (2014) 5624.
[97] S.A. Sievers, J. Karanicolas, H.W. Chang, A. Zhao, L. Jiang, O. Zirafi, J.T. Stevens, J. Munch, D. Baker, D. Eisenberg, Structure-based design of non-natural amino-acid inhibitors of amyloid fibril formation, Nature 475 (7354) (2011) 96.
[98] R.O. Dror, A.C. Pan, D.H. Arlow, D.W. Borhani, P. Maragakis, Y. Shan, H. Xu, D.E. Shaw, Pathway and mechanism of drug binding to G-protein-coupled receptors, Proc. Natl. Acad. Sci. U. S. A. 108 (32) (2011) 13118.
[99] A.M. Brown, D.R. Bevan, Molecular dynamics simulations of amyloid beta-peptide (1-42): tetramer formation and membrane interactions, Biophys. J. 111 (5) (2016) 937.
[100] A. Oda, K. Kobayashi, O. Takahashi, Comparison of molecular dynamics simulation methods for amyloid beta(1-42) monomers containing D-aspartic acid residues for predicting retention times in chromatography, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 879 (29) (2011) 3337.
[101] C.B. Lietz, Z. Chen, C. Yun Son, X. Pang, Q. Cui, L. Li, Multiple gas-phase conformations of proline-containing peptides: is it always cis/trans isomerization? Analyst 141 (16) (2016) 4863.
[102] J.D. Eschweiler, A.T. Frank, B.T. Ruotolo, Coming to grips with ambiguity: ion mobility-mass spectrometry for protein quaternary structure assignment, J. Am. Soc. Mass Spectrom. 28 (10) (2017) 1991.

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