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Critical Review

The Growing Role of Structural Mass Spectrometry in the Discovery and Development of Therapeutic Antibodies

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The comprehensive structural characterization of therapeutic antibodies is of critical importance for the successful discovery and development of such biopharmaceuticals, yet poses many challenges to modern measurement science. Mass spectrometry has evolved into a rapid and sensitive tool for assessing the structures, stabilities, and dynamics of such proteins. Here, we review the current state-of-the-art mass spectrometry technologies focusing on the characterization of antibody-based therapeutics. We conclude by discussing the future of structural mass spectrometry, and its role in enabling the biopharmaceutical pipeline.

1 Introduction

Over the past few decades, biopharmaceuticals have emerged as an exceptionally important class of therapies, evidenced by the number of approved therapies in this class for indication ranging from cancers to autoimmune diseases.¹ White biopharmaceuticals represent a diverse group of molecular subclasses, monoclonal antibodies (mAbs) and related $\frac{26}{26}$ therapeutics, such as antibody-drug conjugates (ADCs) an bispecific antibodies (bsAbs), are undoubtedly the mo promising and fastest growing of these subclasses, owing their high specificity, high efficacy and fewer side effects.^{2–6} the benefits of biopharmaceuticals are often attributed totheir complex molecular compositions and divers conformations, the challenging task of their comprehensive biophysical characterization is exceptionally important during discovery and development.

17 Mass spectrometry has emerged to produce a family of 18 methods aimed at addressing the structural complexity of biopharmaceuticals. With concomitant advances in sensitivity, resolution, accuracy, and speed, MS has been widely deployed for the characterization of therapeutic mAbs. In addition to elucidating mAb primary structures, MS methods are capable of probing the higher order structures and dynamics of therapeutic mAbs. In this review, we will focus on recent progress in the development of structural MS tools for the characterization of mAbs and mAb-related therapeutics (Figure 1). Structural MS refers to those MS-based tools focused on the biophysical characterization of protein samples, including the extraction of 3D structure information from MS datasets. In discussing this work, we aim to illustrate the versatility of MS in context of mAb structural characterization. We conclude by discussing the future potential of structural MS in the context of rapidly evolving biopharmaceutical analysis workflows.

Sequencing Intact Antibodies

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Fig. 1 A schematic diagram illustrating four key structural MS-based technologies used for the characterization of mAbs and mAb-related therapeutics: Top-down MS for sequencing and PTM analysis (*upper-left*), Native MS for assessing intact mass, target binding stoichiometry, and oligomer populations (*bottom-left*), Chemical labeling MS for probing the conformation and dynamics of mAbs (*upper-right*), and ion mobility-MS for characterizing higher order structures and stabilities (*bottom-right*).

Typically, "bottom-up" protein sequencing methods, involvide the reduction, alkylation and proteolytic digestion prior B3LC/MS/MS, are used to interrogate the primary structures 34 therapeutic mAbs.⁷ Although such strategies are we3/5 established, quantifying all the post-translationally modified (PTM) or degraded states for a given mAb can be challengible \mathbf{B} using such approaches.⁸ Top-down mass spectrometry can $a_{2}8$ to overcome these challenges by directly introducing intade mAbs into gas phase for PTM assessment and sequencing 0 Ideally, each mAb isoform is isolated and analyzed individual 4/1 for a more comprehensive analysis of mAb PTMs ar4d2 sequence variants. Despite advances in top-down sequenci Ag technology, complete mAb sequences remain challenging 4d obtain partially due to the highly structured regions 45 antibody domains protected by disulfide bonds. As such6 middle-down approaches, which involve cleaving mAbs in 47 large peptide fragments via limited enzymatic digestion f48 tandem MS analysis, are often used to supplement both to \$9 down and bottom-up sequencing data. (Figure. 2) Tbe application of both top-down and middle-down MS workflows toward mAb analysis have been extended through the use 52 high-resolution MS and a range of ion activation technologies3 For example, high resolution Orbitrap and Fourier transform4 ion cyclotron resonance(FT-ICR) ^{10,11} are both leveraged 55 order to reduce mass overlaps within the complex spect5a resulting from intact protein fragmentation events. In addition 7 such platforms are often equipped with a broad range of $i \overline{5} \delta$ activation technologies, ranging from slow-heating techniques9 *e.g.* collision induced dissociation (CID), to rapid ion activatiapproaches, e.g. ultraviolet photodissociation (UVPD), wi61 each providing complementary sets of mAb fragments and

enable substantially increased protein sequence coverage values. $^{\rm 12}$

Early top-down analyses of mAbs revealed that the variable regions of mAbs can be rapidly characterized by performing insource CID fragmentation followed by tandem MS, in partnership with accurate time-of-flight (ToF) measurements of the intact mAb.¹¹ This approach was then further developed by increasing the transmission efficiency for intact mAbs on hybrid linear quadrupole ion trap-Orbitrap (LTQ-Orbitrap) platforms, enabling both intact mass and sequencing data to be acquired on a single platform.¹³ Although this approach was able to differentiate IgG2 disulfide isoforms, as well as glutamine and pyro-glutamate variants, the mAb sequence coverage obtained was limited. Workflows incorporating middle-down analyses have achieved greater sequence coverages, permitting the identification of site-specific methionine oxidations¹⁴, as well as detecting drug-productrelated impurities and variants¹⁵.

For both top-down and middle-down MS mAb analysis, electron-based ion activation methods have been used to produce extensive sequence-informative fragmentation and breaking disulfide bonds while retaining thermally labile PTMs. For example, online liquid chromatography (LC) has been coupled to electron transfer dissociation (ETD) and high-resolution ToF-MS for the comprehensive top-down structural characterization of two different mAbs.¹⁶ This workflow yielded quantitatively improved sequence coverage when compared to previous protocols, ranging from 15 to 21%, including sequencing information from mAb constant regions that proved transparent to previous CID experiments¹³. Subsequently, a hybrid Orbitrap FTMS workflow incorporating ETD was described and used to sequence samples of

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Adalimumab (the biotherapeutic Humira from AbbVie) at L30
 timescales, achieving ca. 33% sequence coverage.¹⁷ Wh84
 impressive, this level of sequence coverage has prov32
 challenging to improve upon in recent top-down sequenci33
 experiments, owing primarily to the significant gas-pha344
 stabilities of mAb ions.

In order to further improve mAb sequence data, middledown ETD-MS data has been integrated with previous described top-down protocols to produce double tBe6 sequence coverage compared to previous top-down only analyses. Importantly, the incorporation of middle-down sequencing data has served to unlock sequence information from the entirety of the mAb complementarity determini $\tilde{\chi}$ regions (CDRs), measure mAb glycoforms, and characterize Lys-clipped variants.¹⁸ Another approach to broadening mAb sequence coverage in both top-down and middle-down M_{2} sequencing experiments is to move to alternative ignactivation technologies. For example, top-down sequencing at mAbs with electron capture dissociation (ECD) on an FT-ICR M_{46}^{SS} platform has been shown to provide a greater number of total cleavages than analogous CID or ETD experiments performed $\frac{1}{48}$ on quadrupole (Q)-ToF-MS platforms, and a comparable number of cleavages when compared to ETD sequencies performed using Orbitrap MS.¹⁰ In addition, 193-nm UVPD has been reported to offer detailed sequence analysis for intact mAbs, as well as PTM site localization.¹⁹ The acquisition speed of the UVPD tandem MS experiment makes it an attractive $\check{\mu}$ option for performing rapid mAb sequencing experiments $\tilde{\mathfrak{G5}}$ an LC timescale. Further development of hybrid MS igr

activation techniques, such as a combination of ETD and higher-energy collision dissociation (EThcD)²⁰ and ETD combined with UVPD (ETUVPD)²¹, promises to further drive the performance of both top-down and middle-down mAb sequence analysis in the future.

Measuring the Stoichiometries of Antibodyassociated Complexes

The introduction of soft ionization sources, such as matrixassisted laser desorption ionization (MALDI)²² and electrospray ionization (ESI)²³, over thirty years ago enabled the transfer of large biomolecules to gas phase in their intact form, and have significantly strengthened various MS methods used to study biological molecules.^{24,25} While the vast majority of mAb sequencing experiments use ions produced under denaturing conditions in order to improve sequence coverage, native MS experiments seek to make mass measurements of mAbs while preserving their structure under native conditions.²⁶ The vast majority of all native MS experiments utilize ESI-based approaches to form ions directly from native-like solutions, where pH and ionic strength can be easily controlled to produce conditions that preserve mAb structure and function.²⁷To enhance the intensities of mAb signals in native MS experiments, Nano-ESI (nESI), utilizing a miniaturized ESI emitter and nL/min flow rates is often used in order to reduce ESI droplet sizes, increase overall ionization efficiency, and increase the overall tolerance of the ion source for salts and other common biotherapeutic excipients.²⁸ Ammonium



Fig. 2 A schematic diagram comparing Top-Down (*Left*) and Middle-Down (*middle*) MS workflows with Bottom-Up MS protocols (*Right*) for mAb sequencing. For bottom-up MS approaches, proteins are digested into small peptides for LC separation and MS analysis, where peptides are selected and sequenced. Some labile PTMs may be lost during bottom-up workflows. In top-down MS, all proteoforms are directly sequenced in the gas-phase using advanced MS/MS strategies. For middle-down workflows, MS/MS analysis is performed on large fragments or mAb subunits after limited proteolysis in order to maximize both sequence coverage and PTM retention. (LC – light chain; Fd – heavy chain fragment generated from reduction of the antigen binding fragment; Fc/2 – heavy chain fragment obtained after reducing the Fc fragment.)

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1 acetate salts are often used to establish the ionic strength f582 samples to be analyzed by native MS due to their genetation 3 volatility. Owing to their folded conformations, fewer to 60 charges are deposited on the mAb, resulting in a narrow61 4 5 charge envelope shifted towards greater mass-to-charge (m/ a^2 6 values when compared to MS data often acquired under 7 denaturing conditions as part of top-down sequencified 8 experiments. Thus, the equipment used for native Nots 9 experiments are typically modified in order to maximize the 10 transmission of high m/z ions. Owing to their wide availabba 11 mass ranges, modified Q-ToF instruments have been the dominant platform for native MS.^{29,30} More recently, Orbitrad 12 and FT-ICR mass analyzers have been described for robuz0 13 native MS experiments, offering higher resolving power than 14 typical Q-ToF measurments.^{31–34} 15 72

In the context of mAb analysis, native MS provides 16 17 accurate intact masses as well as information on glycofor74 heterogeneity, antibody-antigen binding, and any oligomer75 18 states present.^{35–38} For example, native MS data acquir \overline{a} 19 20 using a modified Orbitrap platform has been used to assign and quantify the heterogeneous glycoforms within a mAB 21 sample.³⁶ In these spectra, a mass resolving power of up 7922 23 12000 at an m/z of 6000 could be achieved, allowing for t 24 confident assignment of antibody glycoforms. In addition 81 the identification of PTM states, high-resolving power nati 82 25 MS has also been demonstrated to both qualitatively and 26 quantitatively characterize antibody mixutres.^{39,40} 27 F84 example, Q-ToF based native MS has been used to resolve a 28 29 quantify nine out of ten antibodies present within a mixture6 30 whereas such a mixture could not be similarly unraveled 87 cation exchange chromatography.³⁹ Furthermore, using hig8831 resolving power native MS, a mixture containing fifte 32 different antibodies, with mass differences ranging from 20.90 33 to 1149.41 Da were baseline resolved.⁴⁰ Triplicate native Ngs 34 measurements showed excellent quantitative reproducibili 92 35 36 exhibiting less than 1.2% relative error in the ion intens 37 values recorded for the resolved mAbs. 94

38 The ability to preserve noncovalent protein-prote 39 interactions during the ESI process in native MS workflo@6 40 enables the direct measurement of antibody-antigen binding 41 stoichiometries and stabilities. Pioneering work in this area³⁸ demonstrated that complexes formed between the 42 43 recombinant V antigen (rV), a 37-kDa protein secreted by 98 pestis, and its complimentary mAb could be readily detected 44 and characterized. These native MS measurements revealed 10045 that the rV antigen forms a tightly associated dimer 10146 micromolar concentrations, that a 1:2 binding stoichiometr $\bar{\gamma}$ 47 prevalent for the antibody:antigen complex, and quantified 48 the resulting antibody-antigen binding specificity. Later work 49 used native MS to investigate the immune complex formed 50 between the recombinant JAM-A protein, as well as 10651 antigenic protein (Ag) overexpressed in tumor cells, with both 52 murine and humanized mAbs.⁴¹ These data were used 10853 determine both the mAb:antigen binding stoichiometry and 54 selectivity, revealing similar values for both humanized $\bar{a}\bar{n}\bar{d}$ 55 murine mAbs. As above, the advent of higher resolving power $\mathbf{T}_{\mathbf{T}}$ 56 57 native MS platforms has also been leveraged for the analysis of

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antibody-antigen complexes.³⁶ Native MS is also a useful tool for characterizing antibody aggregates, which are common degradation products for therapeutic proteins, causing activity loss, decreased solubility, and enhanced unwanted immunogenicity. Because aggregation can occur during production, formulation and storage, it is critical to monitor aggregate formation through multiple stages of biopharmaceutical development. То this end, the chromatographic separation of protein oligomers was integrated with native MS in order to successfully detect soluble mAb oligomers induced by pH-stress.³⁵ In addition, native MS has been used to analyze the antigen binding stoichiometry of a functional IgG hexamer.⁴² The resulting large multi-protein complex was further characterized by tandem MS, which provided critical information on the spatial arrangement and stoichiometry of the subunits within the assembly.

Antibody related drug products, such as bsAbs and ADCs, have also recently been characterized by native MS workflows. For instance, native MS was used to monitor Fab-arm exchange, a physiological process in which portions of two IgG4 mAbs recombine to form a chimeric bsAbs. ⁴³ Fab-arm exchange was mimicked in vitro through the addition of a mild reducing agent, and the dissociation kinetics of IgG4 were monitored by native MS. The results highlighted the importance of the C_H3 domain in the process that gives rise to the ultimate chimeric bsAbs. Native MS was also used to characterize cysteine-linked ADCs, yielding average drug-toantibody ratio (DAR) values comparable to more time consuming hydrophobic interaction chromatography (HIC) analyses.^{44,45} Recent work has also demonstrated the benefits of native MS for characterizing highly heterogeneous lysinelinked ADCs.^{46,47} Average DAR values can be accurately deduced from native MS spectra collected for deglycoslyated lysine-linked ADC samples using high resolving power native MS. Furthermore, charge reduction approaches coupled to native MS analysis of ADCs has been used to reduce spectral complexity and decrease mass overlaps for the broadband measurement of highly accurate DAR values.⁴⁶

Probing the Higher Order Structures of Therapeutic Antibodies

A detailed understanding of higher order structure is critically important for developing protein therapeutics. For example, mAb misfolding can lead to a loss of antigen binding affinity, as well as altered aggregation and degradation pathways, all combining to give rise to reduced mAb efficacy and increased immunogenicity.^{48,49} Hydrogen/deuterium exchange (HDX) coupled to MS has been used for over twenty-five years to study the dynamics of proteins in solution,^{50–52} and is now increasingly applied to mAb analysis. Modern HDX-MS experiments can quantify the flexibility and stability of mAbs at the intact protein, peptide, and amino acid-level (Figure. 3). HDX-MS workflows are typically initiated through the exchange of labile backbone amide hydrogens by diluting

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1 protein samples into a D_2O -containing buffer, which 322 quenched by lowering the pH after a fixed amount of exchanged 3 time. The amount of deuterium uptake can be assessed B_{4} 4 both top-down and bottom-up workflows, utilizing rap35 5 activation tools in MS/MS mode experiments to asse36 6 exchange levels for individual residues within the proteb7 7 while the latter approach is currently more common 38 8 deployed. As the HDX rate is related to protein foldide 9 structure and dynamics, differences in deuterium uptake lev 40 10 can be mapped on to protein structures to identify epitopes 41 antigen-antibody interactions, as well as examine local 11 12 conformational changes of mAbs provoked ₿⁄3

fucose from the native population of antibody glycoforms did not lead to detectable changes in mAb conformation.

ADCs have also been broadly characterized by HDX-MS, where comparative data can uncover alterations in mAb dynamics perpetrated by both inter-chain disulfide reduction and the presence of conjugated drug molecules.⁵⁸ HDX-MS has also been used to assess antibody aggregates, aimed at understanding operative mechanism of mAb selfassociation.^{59–61} For example, HDX-MS analysis of Bevacizumab aggregates induced from multiple freeze/thaw cycles were observed to possess exchange profiles indistinguishable from native mAbs, whereas a similar analysis of thermally-induced



Fig. 3 A generalized workflow for chemical labeling-based structural MS experiments. In the workflow shown, two antibodies are exposed to a chemical label before quenching the labeling reactions. The labeled antibody is then subjected to proteolytic digestion, followed by MS analysis. The mass of each peptide is tracked at each time point and presented as kinetic plot. The data are processed to compare different mAb samples and search of variations in mAb structure and flexibility. If the mAb structure is known, or if a structural model is available, molecular modeling can be performed in order to map conformational differences.

modifications.53,54 13

44 14 HDX-MS can be employed to assess mAb conformatio45 15 and dynamics upon chemical modification, offering benefits 46 16 both therapeutic design and quality control protocols. F47 17 local conformational dynamics of an IgG1 antibody.⁵⁵ Chang49 18 19 in mAb conformation related to deglycosylation web 20 examined using differential HDX-MS analysis, revealing tvbd 21 regions within IgG1 that possess altered protection and web2 22 rationalized as critical to FcγRIII receptor binding. Tb3 23 conformational effects of other PTMs, such as galactosylation 4 24 fucosylation, methionine oxidation, aspartic ас**Бо**Б 25 isomerization, and asparagine deamidation have also be 56 investigated by HDX-MS.56,57 In particular, HDX-MS h57 26 27 revealed that the complete galactosylation in IgG1, where 518 28 mAb glycoforms contain a terminal galactose, results in 59 29 increase in structural rigidity within the C_{H2} domains in 6a30 manner correlated with Fc receptor binding affinity. 61 31 contrast, this same study demonstrated that the removal 62 63

aggregates revealed large changes in exchange behavior within mAb CDR regions.⁵⁹ Distinct mechanisms for the above stressinduced aggregation events can be extracted directly from the collected data, further highlighting the capabilities of comparative HDX-MS analysis. More recently, the combination of HDX-MS and a spatial aggregation propensity (SAP) algorithm allows identification of aelf-association hotspots in a mAb CDR region, underlining the potential of HDX-MS analysis to direct engineering of therapeutic antibodies in discovery and early development stage.⁶¹ Furthermore, newly developed HDX-MS strategies along with the traditional method have been shown to provide useful insights into the formulation development of mAbs.62-64

A separate chemical reactivity-based approach for monitoring protein structure utilizes oxidation chemistry to label protein side chains in a manner correlated with their solvent accessibility. Typically in such oxidative footprinting experiments, susceptible amino acid side chains are irreversibly labeled through hydroxyl radical mediated oxidation at submillisecond time scales. The products of this

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oxidation chemistry are then recorded by MS, providibe information complementary to HDX-MS experiments.⁶⁵ Wh the details concerning available experimental workflows abd general applications of oxidative labeling techniques has been covered by recent reviews, $^{66-68}$ here we focus on tbe uses of this technology for therapeutic mAb analysis. In 6a manner similar to HDX, the fast photochemical oxidation 61 proteins (FPOP) has also been used to assess mAb higher ord62 structures and map antibody epitopes. For example, rece68 work describes the utilization of FPOP for characterizing Ig 624 disulfide variants, in which such data identified locas conformational changes in the mAb hinge regions, as well 65 detecting altered protein dynamics within the CDRs for Ig 627 mutants in comparison with wild type.⁶⁹ This study featured the integration of multiple complementary MS-based methods in order to rapidly characterize antibody mutants. Specifically, FPOP data was supported by both top-down and ion mobility-mass spectrometry (IM-MS) analyses in order to provide a comprehensive view of both mAb conformation and composition. In addition, FPOP has been used to determine the specific residues involved in the epitopes for an antiinterleukin-23 (anti-IL-23) antibody.⁷⁰ Although oxidative labeling techniques have not been as widely used in biopharmaceutical industry as HDX due to ongoing challenges associated with automated sample preparation and data processing, it is clear that continuing efforts will integrate this family of tools into the ever evolving roadmap for biopharmaceutical discovery and development.

29 Simultaneously Assessing the Size, Shape and 30 Stability of Intact Antibodies

Ion mobility (IM) can rapidly separate ions based on their charges and shapes in gas phase under the influence of a weak electric field.^{71,72} IM separation can be performed on a wide range of platforms combined with MS detection, such as the drift tubes (DTs) 73,74 and travelling wave ion mobility (TWIM) separators ^{75–77}. In a typical IM experiment, packets of ions are introduced into an ion guide pressurized with inert neutral gas under the influence of a relatively weak electric field. The larger, more-elongated ions collide more frequently with these gas molecules, and thus take a longer time to traverse the IM separator when compared to smaller and more-compact ions. The output of IM separations is the orientationally-averaged ion-neutral collision cross sections (CCS) for the ions analyzed, and this information can be readily extracted either directly from ion arrival times, or through careful calibration with ions of known CCS.⁷⁸ Furthermore, theoretical CCSs can be computed from protein structure models, as well as used as constraints for molecular dynamics simulations, enabling the detailed assessment of protein structural states in the gas phase.⁷⁹⁻⁸¹ IM-MS has been employed for the structural analysis of proteins and protein complexes, the separation of small molecule pharmaceutical compounds, the resolution effective isomeric metabolites, the deconvolution of complex polymer MS spectra, the identification of carbohydrate structures, and

the interrogation of multi-protein complex topology.^{82–86} Furthermore, once isolated in the gas phase, ions can be collisionally heated and unfolded in an effort to both record protein stabilities and use gas-phase unfolding patterns as a means of differentiating iso-CCS ions.^{87,88} Such collisioninduced unfolding (CIU) methods are often used in partnership with IM-MS data in order to study protein higher order structures.

While IM is just beginning to be used to analyze mAb structure and stability, a number of reports showcased the ability of IM-MS to separate structural isoforms of antibodybased therapeutics. For example, early results in this area illustrated that IM can rapidly differentiate IgG2 disulfide-



Fig. 4 An example of collision-induced unfolding (CIU) analysis for IgG isoforms. Intact IgG1 (A) and IgG4 (D) are collisionally heated and undergo unfolding (B, E) in the gas phase prior to IM measurement. The IM data are then extracted in order to generate a plot of IM drift time against collision voltage projected as a contour plot (C, F). Once complied, this CIU fingerprint data are compared in order to detect differences in mAb (G). Figure C and F are adapted with permission from Reference 97. Copyright @2015 American Chemical Society.

bonding structure based isoforms.⁸⁹ IM-MS data has also shown that intact mAbs are more conformationally diverse than proteins or protein complexes of comparable sizes, as

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represented by the peak widths achieved during ISA 1 separation.⁹⁰ This work, as well as a later report combining 2 CCS data from both DT, TWIM devices with molecular 3 4 dynamics simulations strongly indicates that mAb ions undergo 5 significant compaction in the gas phase, centering on the hinge region of the mAb structure.⁹¹ In recent work, a combination 6 of IM-MS and HDX-MS was used to probe the global and local 7 dynamics of a series of IgG1 Fc variants.⁹² While IM data were 8 nearly identical for lower charge states of three IgG1 9 variants were, significant differences were observed in the IM 10 11 data acquired for higher charge states. Overall, the IM-MS data 12 indicated that the IgG1 Fc mutants were more susceptible gas-phase unfolding when compared to wild type mAb 13 'n consistent with their stabilities in solution. IM-MS data for 14 intact therapeutic antibodies have also been used to rapidly $\frac{1}{2}$ 15 16 assess the similarity of innovator mAbs and their biosimilars. 17 In general, antibody isoforms that exhibit CCS difference 18 of greater than 3% can be routinely resolved by separation.^{77,78,94,95} 19 In many cases, however, local 20 conformational changes caused by PTMs or mutations can b 21 too subtle to be captured by IM separation alone. In suc cases, CIU can be used to rapidly resolve such conformational 22 states through differences in their unfolding patterns and 23 24 stabilities in the gas phase. CIU data is frequently displayed 25 a 'fingerprint', where the IM drift times or CCS values and plotted against the collision voltages used to heat ions and 26 generate protein unfolding.(Figure 4) Such experiments have 27 been used for a broad array of applications, and the general 28 29 utility of CIU in the context of small molecule drug discover and development has been previously reviewed. 30 31 Relatively recently, CIU data has been shown to quantitative discriminate between IgG subtypes that differ only in terms 87 32 their disulfide bonding.¹⁰² For example, IgG1 and IgG4 possess 33 the same number of inter-chain disulfide bonds, and only 34 35 differ in the disulfide connectivity pattern between their heavy and light chains. In both cases, three main features were 36 37 observed during CIU. However, detailed comparisons enabled by CIUSuite software¹⁰³ revealed clear differences within the 38 39 CIU datasets. Continuing work in this area has seen CIU used to differentiate innovator and biosimilar preparations of 40 93 infliximab, in which minor differences in mAb glycosylation and 41 42 glycation across multiple sample lots produced measurabed shifts in mAb unfolding. $^{\rm 104}$ More recently, the combination 9543 44 native IM-MS and CIU distinguished complexes formed 45 different epitopes.¹⁰⁵ 46

47 In addition to coupling with native MS for intact protein 46 48 analysis, IM-MS has also been used extensively to separate 47 49 and analyze complex peptide carbohydrate mixtures. The 48 50 potential of IM-MS to distinguish lot-to-lot variability within 49 51 mAb N-glycosylation profiles was recently reported 1001^{100} 50 Although such techniques have not been applied 102 52 51 53 therapeutic proteins yet, the utility of IM-MS for in-depty 52 54 structural analysis of carbohydrate and glycoconjugate has 53 been illustrated generally, thus illuminate the clear benefits 5 55 54 that such workflows will provide for the characterization 106 56 therapeutic antibodies 107-109 55 57 107 6

Conclusions and Outlook

Structural mass spectrometry offers a variety of approaches for the in-depth characterization of therapeutic antibodies. Such technologies can probe all levels of mAb structure, including their primary structures and PTMs, enabling the detailed assessment of mAb variants within complex mixtures. Higher-order structure data can be extracted directly from MSbased technologies as well, enabling the stoichiometry of antibody-antigen complexes, mAb stability, dynamics, and overall size to be assessed rapidly from relatively impure samples.

There are many challenges associated with the further development of structural MS techniques for mAb analysis. Clearly, therapeutic proteins equally modified on different sites are exceptionally challenging to separate by MS alone, greatly complicating their differential analysis. Furthermore, the data interpretation steps for many structural MS techniques continues to be a bottle-neck generally, but also specifically in the context of mAb analyses. In addition, automated sample handling and high-throughput sample delivery systems have yet to be completely integrated with structural MS workflows. Despite the clear synergy of structural MS-based approaches, integration of these data types has remained challenging. Clearly, the facile integration of such data would enable generating high-quality structural for large biopharmaceuticals that models resist characterization by NMR or X-ray crystallography. Overall, we expect that continued developments in all of these areas will further drive our ability to discover and develop the next generation of therapeutic antibodies, as well as substantially improve our ability to assess biosimilars, thus enabling the continued growth of this exciting class of therapeutics and their profound impact on human health.

Conflicts of Interest

There are no conflicts of interest to declare.

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References

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- G. Walsh, Nat. Biotechnol., 2014, 32, 992-1000.
- J. M. Reichert, MAbs, 2012, 4, 413-415.
- D. M. Ecker, S. D. Jones and H. L. Levine, MAbs, 2015, 7, 9-14.
- A. M. Scott, J. D. Wolchok and L. J. Old, Nat. Rev., 2012, 12, 278-287.
- R. V. J. Chari, M. L. Miller and W. C. Widdison, Angew. Chem. Int. Ed. Engl., 2014, 53, 3796-827.
- J. M. Reichert, MAbs, 2017, 9, 167–181.

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2013, 85, 11163-11173.

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D. Renc, G. P., Pies, D. Liu, L. Y. Shih, A. C. Michols, M. J. Ste Trechelt, D. N. Brems and P. V. Bondernko, <i>Anal. Chem.</i> , 2001, 32 , 126–1368. J. Stronger, M. J. Stema, <i>Anal. Biochem.</i> , 2006, 356 , 22-3061 S. S. Fodor and Z. Zhang, <i>Anal. Biochem.</i> , 2006, 356 , 22-3061 S. S. Kotter, Y. L. Kelleher, F. Badala, M.G. S. Marshall, <i>Anal. Chem.</i> , 2013, 85 , 239–4246. S. Gommun., 2014, 445 , 683–633. G. S. Notari, N. J. Rose, N. J. Thompson, E. Van Duijn, E. D. Warker, V. Makarov and A. I. B. Heck, <i>Angew. Chemie. Int. Ed.</i> , 2012, 51 , 1292–12956. Y. Mao, S. G. Valeja, J. C. Rouse, C. L. Hendrickson and A. 65 Thompson, S. Rosati and A. J. R. Heck, <i>Methods</i> , 2014, 55 , 11–7. S. Bronbelt, <i>Anal. Chem.</i> , 2016, 88 , 30–51. G. J. Zhang, J. Am. Soc. Mass Spectrom., 2009, 70 J. Jahag, J. C. Wonker, F. Guy, C. Becker, J. Zhao, H. M. J. Zhang, J. M. Shaneem and Y. H. Liu, <i>Anal. Chem.</i> , 2014, 51 , 197–203. M. Hirksky, L. J. Mass Spectrom., 2019, 51 , 243, 120–243. J. Waller, H. Liu, M. Shaneem and Y. H. Liu, <i>Anal. Chem.</i> , 2015, 87 , 914–921. Y. O. Tsybin, M. Cerwai, J. M. Shaneem and Y. H. Liu, <i>Anal. Chem.</i> , 2017, 85 , 7914–921. Y. O. Tsybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Deras, J. M. Wurm and R. Hartmer, <i>Anal. Chem.</i> , 2015, 88 , 914–927. S. J. Droteneilic, C. Stoermer, M. Luebeck, J. Deras, J. M. Wurm and R. Har		•	
Filtering, V. Cup, J. Weih, K. E. Bankersmap, W. Cup, J. Weih, K. E. Bankersmap, W. Cup, J. Weih, K. E. Bankersmap, W. Cup, J. Weih, K. E. Bankersmap, M. Cup, J. Weih, K. E. Griffin, E. D. Williamson, J. Stonat, M. J. Masarov, and J. Zhang, J. Am, Soc. Mass Spectrom, 2009, 70 V. Bonderk, M. T. P. Scond, V. Zubrouskov, A. Mass Spectrom, 2009, 70 S. Rosati, R. J. I. Thompson, S. Rosati and A. J. R. Heck, Methods, 2014, 51, 11–7. V. Sonderk, M. J. Moss Spectrom, 2009, 70 S. Rosati, N. J. Thompson, A. Barendregt, L. J. Hendriks, J. De Kuil, M. Throsby, E. Van Duijn and A. J. R. Heck, Mass 2014, 61, 107–201. J. Zhang, J. Lu and V. Katal, J. Mass Spectrom, 2019, 45, 72 S. Rosati, N. J. Thompson, S. Rosati and A. J. R. Heck, Mathods, 2014, 61, 107–201. J. Zhang, J. Lu and V. Katal, J. Mass Spectrom, 2019, 45, 72 S. Rosati, N. J. Thompson, J. Barendregt, L. J. Hendriks, J. De Kuil, M. Throsby and J. J. R. Heck, Mass Spectrom, 2019, 45, 727–723. J. Lornelli, D. Ayoub, K. Atikov, X. Liu, E. Damoc, F. M. Thomas, N. L. Kelleer, And. Chem, 2015, 87, 619–7201. S. Rosati, M. J. Thompson, J. Barendregt, L. J. Hendriks, J. De Kuil, M. Massard, B. N. J. Kelek, And. Chem, 2012, 84, 7227–7232. J. Chorenill, J. Massong, M. J. Kelek,	D. Ren, G. D. Pipes, D. Liu, L. Y. Shih, A. C. Nichols, M. J. 58	8 0 24	J. Am. Soc. Mass Spectrom., 2010, 21 , 1966–1968.
 Janderen, J. 2017, 52, 3396–300. K. Korker, Y. Linge, L. Yan Duija, P. T. Kasper, R. J. Vreeken, A. D. Ketherman, O. S. Skinner, N. L. Kelleher, F. Badala, K.G. K. Kirker, Y. J. Miller, L. Wand, Y. L. Kasper, R. J. Vreeken, A. J. K. Heck and W. Jiskout, <i>Pharm. Res.</i>, 2010, 27, 2137–2204. Sonstit, R. J. Rose, N. J. Thompson, E. Van Duijn, F. Casper, R. J. Vreeken, A. J. K. Heck and W. Jiskout, <i>Pharm. Res.</i>, 2010, 27, 2137–2204. Sonstit, R. J. Rose, N. J. Thompson, E. Van Duijn, F. Casper, R. J. Vreeken, A. J. K. Heck and W. Jiskout, <i>Pharm. Res.</i>, 2010, 27, 2137–2204. Sonstit, R. J. Rose, N. J. Thompson, E. Van Duijn, F. Casper, R. J. Vreeken, A. J. K. Heck, <i>Angew. Chemic - Int. Ed.</i>, 2012, 51, 1292–1296. Sonstit, N. J. Rose, N. J. Thompson, E. Van Duijn, F. Casper, R. J. Vreeken, M. J. Strobeth, <i>Anal. Chem.</i>, 2017, 85, 305–305. J. Berobeth, <i>Anal. Chem., Soc. Mass Spectrom.</i>, 2009, 70 J. Sharot, J. L. J. Mang, J. M. Sonsema and Y. L. Tabaro, J. K. Heck, <i>Anal. Chem.</i>, 2012, 84, 127–203. J. Sharot, J. L. J. Mang, J. J. Moss Spectrom., 2010, 77, 213 S. Roatti, N. J. Thompson, L. J. Hendriks, J. De Kruif, M. Throsby, E. Van Duijn and A. J. R. Heck, <i>Nahstrow</i>, and Y. C. Tybin, <i>H. Liu, And. Chem.</i>, 2013, 85, 11–7. J. Sharot, J. L. Wurm and R. Hartmer, <i>Anal. Chem.</i>, 2017, 79 L. Fornelli, C. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 L. Fornelli, C. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 L. Fornelli, C. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 L. Fornelli, C. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 K. Kata, J. Mastrov and Y. O. Tybin, J. 818 Bayana and J. S. Brodbelt, <i>Anal. Chem.</i>, 2016, 88, 1697–1048. L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 8	Discham 2000 202 12 21	0 0	Angl. Cham. 2011 92 EE08 E606
3. Youdi and Z. Lindig, Junit, Bucker, N. L., Kelleher, F. Baddal, K.C. 3. R. Heck and W. Jiskot, Pharm. Res., 2010, 27 , 2137–2204. Nouri-mahdavi and D. A. Stoof, B. Storker, N. L. Kelleher, F. Baddal, K.C. A. J. K. Heck and W. Jiskot, Pharm. Res., 2010, 27 , 2137–2204. Numeri-mahdavi and D. A. Badof, B. Sorker, J. Storker, K. J. Rosser, A. J. K. Heck and W. Jiskot, Pharm. Res., 2010, 27 , 2137–2204. Storker, J. Thompson, E. Van Duijn, E. Numeri-matrix J. J. Mass 239–4246. Genes, J. J. Berdink, J. J. Berdink, J. J. Mass Spectrom, 2009, 70 N. J. Thompson, S. Rossti and A. J. R. Heck, Angew. Chemie- int E. d., 2012, 51 , 1292–12936. V. Sondarenko, T. P. Second, V. Zabrouskov, A. A. Basker, J. J. Miller, N. Walker, K. F. Griffin, E. D. Williamson, J. Despervoux-Hill, R. W. Titball and C. V. Robinson, J. J. J. Hendriks, J. Metsor, J. L. J. Marking, J. L. Keil, M. Timosy, E. Van Duijn and A. J. R. Heck, Anal. Chem., 2013, 83 , 112–20. V. O. Taybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Parra77 Genes, Y. M. Hubas, J. J. Keil, M. Timosy, E. Van Duijn and A. J. R. Heck, Mab, 2014, G. J. Petruit, M. Timosy, E. Van Duijn and A. J. R. Heck, Mab, 2014, G. J. Corvaia, A. Van Dorssie, J. M. Berker, J. G. J. Van Den Keil, J. J. Evronelli, E. Darnoc, P. M. Thomas, N. L. Kelleher, K. Sold, Cell J. J. Toomson, J. Marker, W. Hubar, J. C. Krumanen, E. Wagner-Rousset, J. Malie, F. M. Van Den K. K. K. Storture, 2009, 81 , 6364–6373. J. Fornelli, C. Janoc, P. M. Thomas, N. L. Kelleher, K. Sold, Cell J. J. Toomson, J. And Chem., 2013, 85 , 1699–704. Genes, Y. M. Yue J. M. L. Parren and A. J. R. Heck, K. Storture, 2011, 91 , 573–574. <	S Endor and 7 7bang Angl Pincham 2006 256 292 206	0 1 25	P. Kükror, V. Eiling, E. Van Duijn, D. T. Kasper, P. I. Vroekon
 A. B. Contamination D. A. Raoof, <i>Biochem. Biophys. Res.</i> 63 <i>Commun.</i>, 2014, 445, 683–693. <i>Commun.</i>, 2014, 445, 683–693. <i>Commun.</i>, 2014, 445, 683–693. <i>Commun.</i>, 2014, 445, 683–693. <i>Scosti, R. J. Rose, N. J. Thompson, E. Van Duijn, E. S. The teck inform transformation of the state of the sta</i>	A D Catherman O S Skinner N I Kelleher F Badalà Kh	2 2	A L R Heck and W liskoot Pharm Res 2010 27 2197-
 Kasari Mandar Barbar, Massari K. J., Kasari K. J., Kasari K. J., Kose, N. J. Thompson, E. Van Duijn, E. Sanosti R. J. Rose, N. J. Thompson, E. Van Duijn, E. Sanosti R. J. Rose, N. J. Thompson, E. Van Duijn, E. Sanosti R. J. Rose, N. J. Thompson, E. Van Duijn, E. Sanosti R. J. Rose, N. J. Thompson, E. Van Duijn, E. Sanosti R. J. Rose, N. J. Thompson, E. Van Duijn, E. Sanosti R. J. Rose, N. J. Thompson, S. Rosati and A. J. R. Heck, <i>Andexov</i> and A. J. R. Heck, <i>Mathods</i>, 2014, 55 (1-7). Sanosti R. J. Jana, <i>J. Am. Soc. Mass Spectrom</i>, 2009, 70 (20), 1415–1424. J. Zhang, H. Lu and V. Katta, <i>J. Mass Spectrom</i>, 2009, 70 (20), 1415–1424. J. Zhang, H. Lu and V. Katta, <i>J. Mass Spectrom</i>, 2010, 45, 73 (20), 48 (20), 2014, 81, 530–5309. J. Zhang, H. Lu and V. Katta, <i>J. Mass Spectrom</i>, 2010, 45, 73 (20), 48 (20), 2014, 81, 530–5309. J. Zhang, H. Lu and V. Katta, <i>J. Mass Spectrom</i>, 2010, 45, 73 (20), 48 (20), 2014, 81, 530–5309. J. Zhang, H. Lu and Y. Katta, <i>J. Mass Spectrom</i>, 2010, 45, 73 (20), 48 (20), 2014, 21 (20), 2014, 21 (20), 2014, 21 (20), 2014, 201	Nouri-mahdavi and D. A. Raoof <i>Biochem Biophys Res</i>	2	22014
 J. S. Rodali, J. J. C. Rouse, C. L. Hendrickson and A. 65 Marshall, Anol. Chem., 2013, 85, 4239–4246. J. S. Rodbelt, Anol. Chem., 2013, 85, 4239–4246. S. Rodbelt, Anol. Chem., 2013, 85, 4239–4246. S. Rodbelt, Anol. Chem., 2016, 88, 30–51. S. Rodbelt, Anol. Chem., 2016, 88, 30–51. J. S. Rodbelt, Anol. Chem., 2016, 88, 30–51. J. S. Rodbelt, Anol. Chem., 2016, 88, 30–51. J. S. Rodbelt, Anol. Chem., 2016, 89, 30–51. J. S. Rodbelt, M. Throshy, A. Makarov, A. B. Sepertour, Hill, R. W. Titball and C. V. Robinson, Biophys. J. 2001, 81, 3503–3509. J. R. Heck, Mads. 2014, 61, 197–203. J. S. Rodbelt, J. M. Shameem and Y. H. Liu, Anol. Chem., 705 J. S. Naller, F. M. Wurm and R. Hartmer, Anal. Chem., 2017, 84, 7227–7232. N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby and A. J. R. Heck, Mads. 2014, 6, 197–203. C. Attmanene. E. Wagner-Rousset, M. Milssard, B. Chol, A. Robert, N. Corvaia, A. Van Dorsselear, A. Beck and S. Sanglier-Ganiferani, Anal. Chem., 2013, 85, 1636–6373. J. Fornelli, D. Ayoub, K. Akikov, A. Beck and Y. O. Tsybin, J. 88 Proteomics, 2017, 1159, 67–76. L. Garnen, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2016, 88, 59 Proteomics, 2017, 159, 67–76. J. Chem., 2018, 48, 61080–12. J. Chem., 2018, 40, 2002, 40, 27–31. J. Chem., 2015, 46, 192–203. J. Chem., 2014, 266, 5005–5102. R. Camman, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2015, 85, 100–10677. J. Chem., 2014, 266, 3005–122. J. Chenthann, J. Cheman, J. S. Brodbelt, Anal. Chem., 20	Commun 2014 445 683–693	2 2 36	S Rosati R I Rose N I Thompson F Van Duijn F
 Harshall, And. Chem., 2015, 85, 4239–4246. Z. Thang and B. Shah, Anal. Chem., 2016, 88, 30–51. S. Brobelt, Anal. Chem., 2016, 88, 30–51. S. Brobelt, Anal. Chem., 2016, 88, 30–51. J. Zhang, J. Am. Soc. Mass Spectrom., 2009, 70 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 712 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 723 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 723 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 723 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 723 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 730 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 730 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 730 J. Zhang, H. Lu and V. Katta, J. Mass Spectrom., 2010, 45, 730 J. Zhang, H. Lu and Y. Katta, J. Mass Spectrom., 2010, 45, 730 J. Zhang, H. Lu and Y. Katta, J. Mass Spectrom., 2011, 78 S. Nallet, F. M. Wurra and R. Hartmer, Anal. Chem., 2011, 78 S. Nallet, F. M. Wurra and R. Hartmer, Anal. Chem., 2011, 78 S. Maller, J. M. Wurra and R. Hartmer, Anal. Chem., 2011, 78 S. Bass, A. S. H. Bassen, and Y. O. Tsybin, M. Samg, Bassen, B. Chu, M. Bassen, B. Chou, R. Kattawa, Y. A. Beck and Y. O. Tsybin, M. Samg, B. Chu, Anal, Chem., 2015, 87, 910–910 L. Fornelli, D. Ayoub, K. Alzikov, X. Euker, P. M. L. Valler Rev. L. S. Cortam and A. J. R. Heck, Mass Spectrom., 2001, 86, 10674–10683. L. Fornelli, D. Ayoub, K. Alzikov, X. Lui, E. Damoc, P. A. Strophen, M. L. Parten and A. J. R. Heck, Structure, 4004–4013. J. Rennon, M. Barth, M. Masong, P. A. Strophen, M. L. Strophen, S. P. Wang, M. Shanian, J. L. Lapren, 2013, 86, 10674–10683. J. Rennon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2015, 41, 1274–12	Y Mao S G Valeia I C Rouse C L Hendrickson and A 6	- 30 5	Damoc F Denisov A Makarov and A L B Heck Angew
 J. Zhang and B. Shah, <i>Anal. Chem.</i>, 2017, 95 5723–5729. 67 N. J. Thompson, S. Rosati and A. J. R. Heck, <i>Methods</i>, 2014, 65, 11–7. N. J. Thompson, S. Rosati and A. J. R. Heck, <i>Methods</i>, 2014, 65, 11–7. N. J. Thompson, S. Rosati and A. J. R. Heck, <i>Methods</i>, 2014, 65, 11–7. N. J. Thompson, S. Rosati and A. J. R. Heck, <i>Methods</i>, 2014, 65, 11–7. N. J. Thompson, S. Rosati and A. J. R. Heck, <i>Methods</i>, 2014, 65, 11–7. J. Bang, H. Liu and V. Katta, <i>J. Mass Spectrom</i>, 2010, 45, 27 J. Bang, H. Liu and V. Katta, <i>J. Mass Spectrom</i>, 2010, 45, 27 J. Bang, H. Liu and V. Katta, <i>J. Mass Spectrom</i>, 2010, 45, 27 J. Chernelli, C. Stoermer, M. Luebeck, J. Para, J. T. J. Van Duris and A. J. R. Heck, <i>Anal. Chem.</i>, 2017, 46, 727–723. V. O. Tsybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Para, J. T. Sager, J. J. A. Hendriks, J. De Kruif, M. Throsby, and A. J. R. Heck, <i>Mals</i>, 2014, 6, 197–203. C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A. Robert, N. Corvaia, A. Van Dorsselaer, A. Beck and S. Sanglier-Clainferiani, <i>Anal. Chem.</i>, 2009, 81, 6364–6373. L. Fornelli, C. Stoermer, M. Luebeck, J. Para, J. R. Heck, <i>Malacrow</i>, R. Makarov, A. B. Seddelt, <i>Anal. Chem.</i>, 2016, 88, 3021–311, 158–67. L. Fornelli, D. Ayoub, K. Aizikov, A. Beck and Y. O. Tsybin, <i>Mol. Cell</i>. L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. Stroeter, M. Makarov, A. Beck and Y. O. Tsybin, <i>B. Romon, D. B. Holden and J. S. Brodbelt, Anal. Chem.</i>, 2016, 88, 10970–1097. J. Chenn, 2014, 86, 10077–1087. J. Chenn, 2004, M. Saspectrom, <i>Neor</i>, 1987, 753–568. J. Chenn, 2004, K. Aizikov, X. Liu, E. Damoc, P. A. Stroeter, J. Bash, J. Macrow, J. S. F. Wong and C. M. 990 J. R. Engen, Man, C. Kem ang S. F. Wong and C. M. 991 <li< td=""><td>Marshall Angl Chem 2013 85 4239-4246</td><td>6</td><td>Chemie - Int Ed 2012 51 12992–12996</td></li<>	Marshall Angl Chem 2013 85 4239-4246	6	Chemie - Int Ed 2012 51 12992–12996
 L. Brodell, Andi. Chem., 2016, 83, 30–51. S. Brodbell, Andi. Chem., 2016, 83, 30–51. S. Brodbell, Andi. Chem., 2016, 83, 30–51. S. Brodbell, Andi. Chem., 2016, 83, 30–51. M. a Tito, J. Miller, N. Walker, K. F. Griffin, E. D. Williamson, D. Despervux-Hill, R. W. Titball and C. V Robinson, Biophys. J., 2001, 81, 3503–3509. S. Rosati, N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby, E. Van Duijn and A. J. R. Heck, Made, Chem., 2012, 84, 7227–7232. N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby, E. Van Duijn and A. J. R. Heck, Mabs, 2014, 6, 197–203. N. J. Thompson, L. J. a Hendrik, J. De Kruif, M. Malissard, B. Chol, A. Robert, N. Corvaia, A. Van Dorsselaer, A. Beck and S. Sanglier-Clanferani, Anal. Chem., 2013, 84, 193–8927. S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 85. S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 85. S. Nallet, F. M. Wurm and R. Hartmer, And. Chem., 2011, 84. S. Forbeili, E. Damoc, P. M. Thomas, N. L. Kelleher, K. 804. J. Fornelli, D. Ayoub, K. Akikov, A. Beck and Y. O. Tsybin, Md. Cell Strain, Anal. Chem., 2014, 86, 3005–12. L. Fornelli, D. Ayoub, K. Akikov, X. Liu, E. Damoc, P. A. 87. J. Cortamand J. S. Brodbelt, Anal. Chem., 2016, 88. Stohurman, P. W. H. I. Parren and A. J. R. Heck, Strature, 2011, 19, 1274–1282. L. Fornelli, D. Ayoub, K. Akikov, X. Liu, E. Damoc, P. A. 87. J. R. Lanon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2016, 88. Stohurman, P. W. H. I. Parren and A. J. R. Heck, Strature, 2014, 86, 1007–10977. L. Schurman, P. W. H. I. Parren and A. J. R. Heck, Strature, 2014, 86, 1007–10977. L. Shennon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2017, 85. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94<	7 Zhang and B Shah Angl Chem 2007 79 5723–5729 6	0 7 37	N Thompson S Rosati and A R Heck Methods 2014
P. V. Bondarenko, T. P. Second, V. Zabrouskov, A. A. 69 38 Makarov and Z. Zhang, J. Am. Soc. Mass Spectrom., 2009, 70 D. Despevroux-Hill, R. W. Titball and C. V. Robinson, Biophys. J. 2001, 81, 3503–3509. J. Zhang, H. Liu and V. Katta, J. Mass Spectrom., 2010, 45, 72 39 S. Rosati, N. J. Thompson, A. Barendregt, L. J. A. Hendriks, A. B. H. Bakker, J. De Krulf, M. Throsby, E. Van Duijn and A. J. R. Heck, Anal. Chem., 2012, 49, 7227–7232. Mueller, H. Li, M. Shameem and Y. H. Liu, Anal. Chem., 75 40 N. J. Thompson, L. J. a Hendriks, J. De Krulf, M. Throsby and A. J. R. Heck, Mal. Sci 14, 6, 197–203. S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2017, 178 C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A. Robert, N. Corvaia, A. Van Dorsselaer, A. Beck and S. Sanglier-Clainferiani, Anal. Chem., 2009, 81, 6364–6373. L. Fornelli, C. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 42 A. Dirkenw, Makarov, A. Beck and Y. O. Tsybin, Mol. Cell 43 Proteomics, 2017, 159, 67-76. 82 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 44 J. Cherm., 2014, 86, 3005–12. 86 J. C. Cottham and J. S. Brodbelt, Anal. Chem., 2016, 88. 52 Schurman, P. W. H. I. Partern and A. J. R. Pecker, Makarov, A. Beck and Y. O. Tsybin, J. 86 52 J. C. Cottham and J. S. Brodbelt, Anal. Chem., 2016, 88. 52	L. S. Brodbelt, Anal. Chem., 2016, 88 , 30–51.	8	65. 11–7.
 Makarov and Z. Zhang, J. Am. Soc. Mass Spectrom., 2009, 70 Zb, Jahang, H. Liu and V. Katta, J. Mass Spectrom., 2010, 45, 72 Jahang, H. Liu and V. Katta, J. Mass Spectrom., 2010, 45, 72 S. Rossi, H. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby, E. Van Duijn and A. J. R. Heck, Malo. Chem., 2012, 46, 7227–723. N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby, and A. J. R. Heck, Malo. Chem., 2012, 46, 7227–723. N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby, and A. J. R. Heck, Malo. Chem., 2012, 46, 7227–723. N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby and A. J. R. Heck, Malo. Chem., 2012, 46, 7227–723. N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby and A. J. R. Heck, Malo. Chem., 2014, 66, 1039–817. C. Attmanene, E. Wagner-Rousset, M. Malisard, B. Chol, A. Robert, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. Sanglier-Clanférani, Anal. Chem., 2015, 87, 6095–6102. R. J. Rose, A. F. Labrijn, E. T. J. Van Den Bremer, S. Loverix, I. Lasters, P. H. C. Van Berkel, J. G. J. Van De Winkel, J. Schuurman, P. W. H. I. Parren and A. J. R. Heck, Katarov, A. Beck and Y. O. Tsybin, M. 82 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 J. R. Leak, Marov, M. Beck and Y. O. Tsybin, M. 88 Schuurman, P. W. H. I. Parren and A. J. R. Heck, Malor, M. M. Sanda, J. Chem., 2014, 86, 1067–1087. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 90 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 90 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 90 <li< td=""><td>P. V. Bondarenko, T. P. Second, V. Zabrouskov, A. A.</td><td>9 38</td><td>M. a Tito, I. Miller, N. Walker, K. F. Griffin, F. D. Williamson,</td></li<>	P. V. Bondarenko, T. P. Second, V. Zabrouskov, A. A.	9 38	M. a Tito, I. Miller, N. Walker, K. F. Griffin, F. D. Williamson,
20, 1415–1424. 71 Biophys. J., 2001, 81, 3503–3509. J. Zhang, H. Liu and V. Katta, J. Moss Spectrom., 2010, 45,72 3 S. Rosati, N. J. Thompson, A. Barendregt, L. J. A. Hendriks, J. E. Yan Duijn and A. J. R. Heak, <i>Anal. Chem.</i> , 2012, 84, 7227–7232. Mueller, H. Li, M. Shameem and Y. H. Liu, <i>Anal. Chem.</i> , 75 M. J. R. Heck, <i>Anal. Science</i> , 10, 10, 40, 40, 10, 10, 10, 10, 10, 10, 10, 10, 10, 1	Makarov and Z. Zhang, J. Am. Soc. Mass Spectrom. 2009.7	0	D. Despevroux-Hill, R. W. Titball and C. V Robinson.
J. Zhang, H. Liu and V. Katta, J. Mass Spectrom., 2010, 45,72 39 S. Rosati, N. J. Thompson, A. Barendregt, L. J. A. Hendriks, 112–20. J. Wang, C. Wynne, F. Gu, C. Becker, J. Zhao, H. M. 74 J. R. Heck, <i>Anal. Chem.</i> , 2012, 48, 7227–732. Mueller, H. Li, M. Shameem and Y. H. Liu, <i>Anal. Chem.</i> , 75 40 J. R. Heck, <i>Anal. Chem.</i> , 2012, 48, 7227–732. Y. O. Tsybin, J. Fornelli, C. Stoermer, M. Luebeck, J. Parra7 41 C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A. S. Nallet, F. M. Wurm and R. Hartmer, <i>Anal. Chem.</i> , 2011, 78 79 Sanglier-Cianférani, <i>Anal. Chem.</i> , 2009, 81, 6364–6373. J. E. Fornelli, C. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 42. A. Dyachenko, G. Wang, M. Belov, A. Makarov, R. N. de Jong, E. T. J. van den Bremer, P. W. H. J. Parren and A. J. R. Heck, <i>Anal. Chem.</i> , 2013, 81, 6059–6102. J. Cortamin, J. J. Strodbelt, <i>Anal. Chem.</i> , 2016, 88 52 Schuurman, P. W. H. J. Parren and A. J. R. Heck, <i>Structure</i> , 2014, 86, 1097–10977. S. Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 88 Schuurman, P. W. H. J. Parren and A. J. R. Heck, <i>Structure</i> , 2014, 86, 1097–10977. S. Perzennic, S. D. Bachmann, U. Bahr and F. Hillenkamp, <i>Int. J.</i> 91 J. Chen, S. Yin, Y. Wu and J. Ouyang, <i>Anal. Chem.</i> , 2013, 85, 1099–704. Proteinics, 2017, 159, 67–76. 91 J. Chem., 2014, 86, 10674–10683. J. Chem., 2017, 86, 10674–10683. J. R.	20 . 1415–1424. 7	1	Biophys. J., 2001. 81 , 3503–3509.
112-20. 73 A. B. H. Bakker, J. De Kruif, M. Throsby, E. Van Duijn and A. 112-20. A. B. H. Bakker, J. De Kruif, M. Throsby, E. Van Duijn and A. 112-20. J. R. Heck, Anal. Chem., 2011, 84, 7227-7232. Nueller, H. Li, M. Shameem and Y. H. Liu, Anal. Chem., 75 A. N. J. Stybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Parra77 C. S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 76 C. S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 76 C. A. B. J. Backard, J. V. Drossbard, A. Van Dorsselaer, A. Beck and S. Sanglier-Cianférani, Anal. Chem., 2009, 81, 6364–6373. A. D. Yachenko, G. Wang, M. Belov, A. Makarov, R. N. de Jong, E. T. J. van Den Bremer, P. W. H. I. Parren and A. J. R. Proteomics, 2012, 11, J758–67. St. Heck, Anal. Chem., 2013, 85, 7069–5012. L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 Schuurman, P. W. H. I. Parren and A. J. R. Heck, Structure, 2011, 19, 1274–1282. V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2014, 86, 1007–10077. Stass Spectrom. Ion Process., 1987, 78, 53–68. 54 J. B. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2014, 86, 1007–10077. Stass Spectrom., 2007, 78, 466, 4–71. 54 J. B. Leo, Mass Spectrom., Nam, 2006, 78, 78, 53–68. 59 52 52 54 54	J. Zhang, H. Liu and V. Katta, J. Mass Spectrom., 2010, 45.7	2 39	S. Rosati, N. J. Thompson, A. Barendregt, L. J. A. Hendriks.
D. Wang, C. Wynne, F. Gu, C. Becker, J. Zhao, H. M. 74 Mueller, H. Li, M. Shameem and Y. H. Liu, Anal. Chem., 75 40 Jolts, 87, 914–921. 76 Y. O. Tsybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Parra, 77 71 S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 78 78 S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 78 79 S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 78 79 S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 78 79 S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 78 79 Sagler-Clarifferiani, Anal. Chem., 2008, 81, 636–6373. 82 L. Fornelli, D. Ayoub, K. Alzikov, A. Beck and Y. O. Tsybin, M. Ce/B1 76 Proteomics, 2011, 19, 758–67. 82 L. Fornelli, D. Ayoub, K. Alzikov, A. Beck and Y. O. Tsybin, M. Ce/B1 76 Proteomics, 2017, 159, 67–76. 84 L. Fornelli, D. Ayoub, K. Alzikov, X. Liu, E. Damoc, P. A. 87 44 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2014, 86, 1909–704. 76 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 2014, 86, 1907–10977. 79 J. B. Fenn, M. Mann, C. K. Maeng, S. F. Wong and C. M. 76 Mutehouse, Science, 1989, 246, 64–71. <	112–20. 73	3	A. B. H. Bakker, J. De Kruif, M. Throsby, E. Van Duijn and A.
 Mueller, H. Li, M. Shameem and Y. H. Liu, <i>Anal. Chem.</i>, 75 Mueller, H. Li, M. Shameem and Y. H. Liu, <i>Anal. Chem.</i>, 75 N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby and A. J. R. Heck, <i>Mabs</i>, 2014, 66, 197–203. C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A. Robert, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. Sanglier-Clanférani, <i>Anal. Chem.</i>, 2009, 81, 6364–6373. C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A. Robert, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. Sanglier-Clanférani, <i>Anal. Chem.</i>, 2009, 81, 6364–6373. L. Fornelli, C. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 L. Fornelli, D. Ayoub, K. Alzikov, A. Beck and Y. O. Tsybin, <i>Mol. Cell</i> L. Fornelli, D. Ayoub, K. Alzikov, X. Liu, E. Damoc, P. A. 87 L. Cornell, D. Ayoub, K. Alzikov, X. Liu, E. Damoc, P. A. 87 L. Fornelli, D. Ayoub, K. Alzikov, X. Liu, E. Damoc, P. A. 87 R. Cannon, D. D. Holden and J. S. Brodbelt, <i>Anal. Chem.</i>, 2014, 86, 10670–10977. J. B. Cannon, D. D. Holden and J. S. Brodbelt, <i>Anal. Chem.</i>, 90 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. R. Heck,	D. Wang, C. Wynne, F. Gu, C. Becker, J. Zhao, H. M. 74	4	J. R. Heck, Anal. Chem., 2012, 84 , 7227–7232.
2015, 87, 914–921. 76 A. J. R. Heck, MAbs, 2014, 6, 197–203. Y. O. Tsybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Parra, T 11 C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A. S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2017, 83 83, 8919–8927. 12 L. Fornelli, E. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 24 24 Arizkov, E. Densov, A. Makarov and Y. O. Tsybin, Mol. Cell 27 28 Proteomics, 2012, 11, 1758–67. 82 24 A. Dyachenko, G. Wang, M. anden Bremer, P. W. H. I. Parren and A. J. R. Heck, Anal. Chem., 2015, 87, 6095–6102. R. J. Roma, D. J. S. Brodbelt, Anal. Chem., 2016, 88, 85 85 2011, 19, 1274–1282. V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88, 85 2011, 19, 1274–1282. 2011, 19, 1274–1282. L. Fornelli, D. Ayoub, K. Alzikov, X. Liu, E. Damoc, P. A. 87 44 15 F. Debanen, A. Beevin, F. Wagner-Rousset, O. Colas, D. Proteomics, 2017, 159, 67–76. 89 45 F. Debanen, A. Beevin, F. Wagner-Rousset, O. Colas, D. Ans. Spectrom., Don Process., 1987, 78, 53–68. 93 16997–10977. 10 Mass Spectrom, Rev., 1997, 16, 1–23. 47 10, G. Campuzano, C. Netrojanakul, M. Nshanian, J. L. 1199, 1274–1282. J. Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. <td>Mueller, H. Li, M. Shameem and Y. H. Liu, Anal. Chem., 7</td> <td>5 40</td> <td>N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby and</td>	Mueller, H. Li, M. Shameem and Y. H. Liu, Anal. Chem., 7	5 40	N. J. Thompson, L. J. a Hendriks, J. De Kruif, M. Throsby and
Y. O. Tsybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Parra, 77 41 C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A. S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011, 78 83, 8919–8927. Sanglier-Clanférani, Anal. Chem., 2009, 81, 6364–6373. L. Fornelli, D. Dayoub, K. Alizkov, A. Beck and Y. O. Tsybin, Mol. Cells Proteomics, 2012, 11, 1758–67. 84 J. Fornelli, D. Ayoub, K. Alizkov, A. Beck and Y. O. Tsybin, Mol. Cells R. J. Rose, A. F. Labrijn, E. T. J. Van Den Bremer, S. Loverix, A. Van Derusselaer, A. Makarov and A. J. R. Heck, Anal. Chem., 2014, 86, 3005–12. 84 V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88, 85 85 Quo4-d013. 84 Pertornics, 2017, 159, 67–76. 89 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 44 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 45 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 47 Mittehouse, Science, 1989, 246, 64–71. 94 J. a Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 J. R. Heck, Nat. Methods, 2008, 59, 27–933. 98 P. Sebarle and U. H. Verkerk,	2015, 87 , 914–921. 70	6	A. J. R. Heck, <i>MAbs</i> , 2014, 6 , 197–203.
S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011,78 Robert, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 83 , 8919–8927. 79 L. Fornelli, E. Damoc, P. M. Thomas, N. L. Kelleher, K. 42 Azikov, E. Denisov, A. Makarov and Y. O. Tsybin, Mol. Cell 13 Proteomics, 2012, 11 , 1758–67. 82 L. Fornelli, D. Ayoub, K. Alizikov, A. Beck and Y. O. Tsybin, Mol. Cell 14 Proteomics, 2014, 16 , 3005–12. 14 Y. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88 , 85 Schuurman, P. W. H. I. Parren and A. J. R. Heck, <i>Structure</i> , 2011, 19 , 1274–1282. L. Fornelli, D. Ayoub, K. Alizikov, X. Liu, E. Damoc, P. A. 87 44 Perverner, A. Makarov, A. Beck and Y. O. Tsybin, J. 86 Perverner, A. Makarov, A. Beck and Y. O. Tsybin, J. 86 Perverner, A. Makarov, A. Beck and Y. O. Tsybin, J. 87 Perverner, A. Makarov, A. Beck and Y. O. Tsybin, J. 86 Proteomics, 2017, 159 , 67–76. 89 J. R. Connon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 901 15 J. Aleo, Mass, Spectrom. Ion Process., 1987 , 78 , 53–68. 93 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 44	Y. O. Tsybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Parra,7	7 41	C. Atmanene, E. Wagner-Rousset, M. Malissard, B. Chol, A.
83, 8919–8927. 79 Sanglier-Clanférani, Anal. Chem., 2009, 81, 6364–6373. L. Fornelli, E. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 A. Dyachenko, G. Wang, M. Belov, A. Makarov, R. N. de Azikov, E. Denisov, A. Makarov, and Y. O. Tsybin, Mol. Cells 1008, E. T. J. van den Bremer, P. W. H. I. Parren and A. J. R. Proteomics, 2012, 11, 1758–67. 82 Heck, Anal. Chem., 2015, 87, 6095–6102. R. J. Rose, A. F. Labrijn, E. T. J. Van Den Bremer, S. Loverix, 1. Lasters, P. H. C. Van Berkel, J. G. J. Van De Winkel, J. V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88 85 V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88 85 Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 86 Proteomics, 2017, 159, 67–76. 89 45 Proteomics, 2017, 159, 67–76. 89 45 Proteomics, 2017, 159, 67–76. 89 45 N. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int J. 92 46 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int J. 92 47 Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 J. B. Loo, Int J. Mass Spectrom, Rev., 1997, 16, 1–23. 94 J. a Loo, Int J. Mass Spectrom, Rev., 1997, 16, 1–23. 94 J. a Loo, Int J. Mass Spectrom, Rev., 2000, 200, 175–186.	S. Nallet, F. M. Wurm and R. Hartmer, Anal. Chem., 2011,7	8	Robert, N. Corvaïa, A. Van Dorsselaer, A. Beck and S.
L. Fornelli, E. Damoc, P. M. Thomas, N. L. Kelleher, K. 80 42 A. Dyachenko, G. Wang, M. Belov, A. Makarov, R. N. de Aizikov, E. Denisov, A. Makarov and Y. O. Tsybin, <i>Mol. Cell</i> S 82 A. Dyachenko, G. Wang, M. Belov, A. Makarov, R. N. de Jordenics, 2012, 11 , 1758–67. 82 Heck, <i>Anal. Chem.</i> , 2015, 87 , 6095–6102. L. Fornelli, D. Ayoub, K. Aizikov, A. Beck and Y. O. Tsybin, <i>83</i> 85 Schuurman, P. W. H. I. Parren and A. J. R. Heck, <i>Anal. Chem.</i> , 2014, 86 , 3005–12. V. C. Cotham and J. S. Brodbelt, <i>Anal. Chem.</i> , 2016, 88 , 85 Schuurman, P. W. H. I. Parren and A. J. R. Heck, <i>Structure</i> , 2011, 19 , 1274–1282. L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 J. R. Cannon, D. D. Holden and J. S. Brodbelt, <i>Anal. Chem.</i> , 90 Schuurman, P. W. H. I. Parren and A. J. R. Heck, <i>Structure</i> , 2011, 19 , 1274–1282. J. R. Cannon, D. D. Holden and J. S. Brodbelt, <i>Anal. Chem.</i> , 90 Spectrom. Jon Process., 1987, 78 , 53–68. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Whitehouse, <i>Science</i> , 1989, 246 , 64–71. 95 J. a. Loo, <i>Int. J. Mass Spectrom.</i> , 2000, 00 , 175–186. 97 J. J. Ao, <i>Mas Spectrom.</i> , 2000, 200 , 175–186. 97 J. J. R. Heck, <i>Nat. Methods</i> , 2008, 5 , 927–933. 98 J. R. Herk, <i>Nat. Methods</i> , 20008, 5 , 927–933. 98	83 , 8919–8927. 7	9	Sanglier-Cianférani, Anal. Chem., 2009, 81 , 6364–6373.
Aizikov, E. Denisov, A. Makarov and Y. O. Tsybin, Mol. Cel81 Jong, E. T. J. van den Bremer, P. W. H. I. Parren and A. J. R. Proteomics, 2012, 11, 1758–67. 82 L. Fornelli, D. Ayoub, K. Aizikov, A. Beck and Y. O. Tsybin, 83 43 Anal. Chem., 2014, 86, 3005–12. 84 V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88, 85 Schuurman, P. W. H. I. Parren and A. J. R. Heck, Structure, 2011, 19, 1274–1282. L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 Proteomics, 2017, 159, 67–76. 89 Proteomics, 2017, 159, 67–76. 89 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 901 Agoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 2014, 86, 10970–10977. 91 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 47 J. a. Loo, Int. J. Mass Spectrom. Rev., 1997, 76, 53–68. 93 J. a. Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 Y. Kebarle and U. L. Verkerk, Mass Spectrom., 2000, 200, 175–186. 97 A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 Y. Kebarle and U. L. Verkerk, Mass Spectrom., 2000, 206, 5, 927–933. 98 S. Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. <td< td=""><td>L. Fornelli, E. Damoc, P. M. Thomas, N. L. Kelleher, K. 80</td><td>0 42</td><td>A. Dyachenko, G. Wang, M. Belov, A. Makarov, R. N. de</td></td<>	L. Fornelli, E. Damoc, P. M. Thomas, N. L. Kelleher, K. 80	0 42	A. Dyachenko, G. Wang, M. Belov, A. Makarov, R. N. de
Proteomics, 2012, 11, 1758–67. 82 L. Fornelli, D. Ayoub, K. Aizikov, A. Beck and Y. O. Tsybin, 83 43 Anal. Chem., 2014, 86, 3005–12. 84 V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88, 85 85 4004–4013. 86 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 44 J. Romon, D. Makarov, A. Beck and Y. O. Tsybin, J. 86 Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 86 Poteomics, 2017, 159, 67–76. 89 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 Youb, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 2014, 86, 10970–10977. 91 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. a. Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a. Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 97 J. B. Fenn, M. Mann, C. K. Meng, S. 5, 927–933. 98 S. Sepectrom., 2000, 200, 175–186. 97 A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 S. Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. 98, 898–917. J. B. Kenramawy, K. W. M. Siu and B. a Thomson, J. Am. Subl 9	Aizikov, E. Denisov, A. Makarov and Y. O. Tsybin, Mol. Cel8	1	Jong, E. T. J. van den Bremer, P. W. H. I. Parren and A. J. R.
L. Fornelli, D. Ayoub, K. Aizikov, A. Beck and Y. O. Tsybin, J. 43 R. J. Rose, A. F. Labrijn, E. T. J. Van Den Bremer, S. Loverix, Anal. Chem., 2014, 86 , 3005–12. V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88 , 85 55 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 J. Roranon, D. A. Beck and Y. O. Tsybin, J. 86 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 94 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 94 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 94 J. R. Cannon, C. D. Holden and J. S. Brodbelt, Anal. Chem., 90 94 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Whitehouse, Science, 1989, 246, 64–71. 95 J. a Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a Loo, Int. J. Mass Spectrom. Rev., 2000, 200, 175–186. 97 A. J. R. Heck, Nat. Methods, 2008, S, 927–933. 98 S. Spottri, H. Hernández, M. G. McCammon, M. a. Tito af403 58 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D	Proteomics, 2012, 11, 1758–67. 82	2	Heck, Anal. Chem., 2015, 87 , 6095–6102.
Anal. Chem., 2014, 86, 3005–12. 84 I. Lasters, P. H. C. Van Berkel, J. G. J. Van De Winkel, J. V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88, 85 Schuurman, P. W. H. I. Parren and A. J. R. Heck, Structure, 2011, 19, 1274–1282. L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 86 L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 88 Proteomics, 2017, 159, 67–76. 89 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 2014, 86, 10970–10977. 91 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 Mithebouse, Science, 1989, 246, 64–71. 95 J. a Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 10. D. G. Campuzano, C. Netirojjanakul, M. Nshanian, J. L. J. a Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, P. Kebarle and U. H. Verkerk, Mass Spectrom., 2009, 99 48 A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb01 6. F. Pirrone, R. E. Iacob and J. R. Engen, Anal. Chem., 2015, 8, 124–217. S. V. No Diversen, C. Versluis, S. J. J. Brouns, D. 100 49 A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb01 58	L. Fornelli, D. Ayoub, K. Aizikov, A. Beck and Y. O. Tsybin, 82	3 43	R. J. Rose, A. F. Labrijn, E. T. J. Van Den Bremer, S. Loverix,
V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88, 85 Schuurman, P. W. H. I. Parren and A. J. R. Heck, Structure, 2011, 19, 1274–1282. U. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 44 Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 88 16.99–704. Proteomics, 2017, 159, 67–76. 89 45 F. Debaene, A. Bœuf, E. Wagner-Rousset, O. Colas, D. J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 901 91 Cianférani, Anal. Chem., 2014, 86, 10674–10683. M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 40 J. Macoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 Corvaïa, A. Van Dorsselaer, A. Beck and S. Cianférani, And. Chem., 2017, acs. analchem.7b03021. J. a. Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 Lippens, D. P. A. Kligour, S. L. Van Orden and J. Loo, Anal. J. a. Loo, Int. J. Mass Spectrom. Rev., 2000, 200, 175–186. 97 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, 482–490. I. a. Kaltashov, C. E. Bobst, R. A. Kbzalimov, G. Wang, B. Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom, 2005, 16, 1702–7. 102 50 Yonwsky, K. U. M. Siu and B. a Thomson, J. A. M. 106 57, 99–118.	Anal. Chem., 2014, 86 , 3005–12. 84	4	I. Lasters, P. H. C. Van Berkel, J. G. J. Van De Winkel, J.
4004-4013. 86 2011, 19, 1274-1282. L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 44 Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 88 1. Chen, S. Yin, Y. Wu and J. Ouyang, Anal. Chem., 2013, 85, 1699–704. Proteomics, 2017, 159, 67–76. 89 45 F. Debaene, A. Bœuf, E. Wagner-Rousset, O. Colas, D. J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 Ayoub, N. Corvaia, A. Van Dorsselaer, A. Beck and S. 2014, 86, 10970–10977. 91 Cianférani, Anal. Chem., 2014, 86, 10674–10683. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Protein Sci. 2015, 24, 1210–1223. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Protein Sci. 2013, 24, 1210–1223. J. a Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 Lippens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, Anal. J. a Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, 482–490. B. 8989–917. 100 49 I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sp01 Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 50 V. Katta, B. T. Chait and S. Carr, Rapid Commun. Mass	V. C. Cotham and J. S. Brodbelt, Anal. Chem., 2016, 88, 8	5	Schuurman, P. W. H. I. Parren and A. J. R. Heck, Structure,
L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 87 44 J. Chen, S. Yin, Y. Wu and J. Ouyang, Anal. Chem., 2013, 85, Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 88 1699–704. Proteomics, 2017, 159, 67–76. 89 45 J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 2014, 86, 10970–10977. 91 Cianférani, Anal. Chem., 2014, 86, 10674–10683. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 Corvaïa, A. Van Dorsselaer, A. Beck and S. Cianférani, J. J. Loo, Mass Spectrom. Rev., 1997, 16, 1–23. 95 47 I. D. G. Campuzano, C. Netirojjanakul, M. Nshanian, J. L. J. a Loo, Int. J. Mass Spectrom. 2000, 200, 175–186. 97 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, A. S. Heck, Nat. Methods, 2008, 5, 927–933. 98 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. V. Katta, B. T. Chait and S. Carr, Rapid Commun. Mass Spectrom., 2005, 16, 1702–7. 102 50 F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito ald03 Spectrom., 1991, 5, 214–217.	4004–4013. 8	6	2011, 19 , 1274–1282.
Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 88 1699–704. Proteomics, 2017, 159 , 67–76. 89 45 F. Debaene, A. Bœuf, E. Wagner-Rousset, O. Colas, D. J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem.90 Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 2014, 86 , 10970–10977. 91 Cianférani, Anal. Chem., 2014, 86 , 10674–10683. M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Mass Spectrom. Ion Process., 1987, 78 , 53–68. 93 Corvaïa, A. Van Dorsselaer, A. Beck and S. Cianférani, Protein Sci., 2015, 24 , 1210–1223. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Protein Sci., 2015, 24 , 1210–1223. Whitehouse, Science, 1989, 246 , 64–71. 95 47 I. D. G. Campuzano, C. Netirojjanakul, M. Nshanian, J. L. J. a. Loo, Int. J. Mass Spectrom. Rev., 1997, 16 , 1–23. 96 Lippens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, Anal. J. a. Loo, Int. J. Mass Spectrom. Rev., 2009, 99 8 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28 , P. K. Belse and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 1 . a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28 , P. K. Subgauna, S. Matass Spectrom., 2005, 16	L. Fornelli, D. Ayoub, K. Aizikov, X. Liu, E. Damoc, P. A. 8	7 44	J. Chen, S. Yin, Y. Wu and J. Ouyang, Anal. Chem., 2013, 85,
Proteomics, 2017, 159, 67–76. 89 45 F. Debaene, A. Bœuf, E. Wagner-Rousset, O. Colas, D. J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 2014, 86, 10970–10977. 91 Cianférani, Anal. Chem., 2014, 86, 10674–10683. M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 Corvaïa, A. Van Dorsselaer, A. Beck and S. Cianférani, J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Protein Sci., 2015, 24, 1210–1223. Whitehouse, Science, 1989, 246, 64–71. 95 47 I. D. G. Campuzano, C. Netirojjanakul, M. Nshanian, J. L. J. a Loo, Mass Spectrom. Rev., 1997, 16, 1–23. 96 Lippens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, Anal. J. a. Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 Chem., 2017, acs.analchem.7b03021. A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009,99 482–490. I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. S. Sebott, H. Hernández, M. G. McCammon, M. a. Tito alu33 Spectrom., 1991, 5, 214–217. S0 V. Katta, B. T. Chait and S. Carr, Rap	Pevzner, A. Makarov, A. Beck and Y. O. Tsybin, J. 8	8	1699–704.
J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem., 90 Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S. 2014, 86, 10970–10977. 91 M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Whitehouse, Science, 1989, 246, 64–71. 95 J. a Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009,99 48 A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb01 94 Mass Spectrom., 2005, 16, 1702–7. 100 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106	Proteomics, 2017, 159 , 67–76. 8	9 45	F. Debaene, A. Bœuf, E. Wagner-Rousset, O. Colas, D.
2014, 86, 10970–10977. 91 Cianférani, Anal. Chem., 2014, 86, 10674–10683. M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 Corvaïa, A. Van Dorsselaer, A. Beck and S. Cianférani, J. Protein Sci., 2015, 24, 1210–1223. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Protein Sci., 2015, 24, 1210–1223. Whitehouse, Science, 1989, 246, 64–71. 95 47 J. a Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a. Loo, Int. J. Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a. Loo, Int. J. Mass Spectrom. Rev., 2009, 200, 175–186. 97 A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 48 A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb@1 Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 50 F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito a403 51 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 </td <td>J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem.9</td> <td>0</td> <td>Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S.</td>	J. R. Cannon, D. D. Holden and J. S. Brodbelt, Anal. Chem.9	0	Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck and S.
M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92 46 J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 Yenter Science, 1989, 246, 64–71. 94 J. a Loo, Mass Spectrom. Rev., 1997, 16, 1–23. 96 Hillepens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, Anal. J. a. Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 28, 898–917. 100 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 28, 898–917. 100 49 I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb01 Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 50 F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito alú03 Spectrom., 1991, 5, 214–217. G. V. Robinson, Anal. Chem., 2002, 74, 1402–1407. 104 51 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 52 Synowsky, K. Lorenzen, C. Versluis, S. J. Broun	2014, 86 , 10970–10977. 99	1	Cianférani, Anal. Chem., 2014, 86 , 10674–10683.
Mass Spectrom. Ion Process., 1987, 78, 53–68. 93 Corvaïa, A. Van Dorsselaer, A. Beck and S. Cianférani, J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Protein Sci., 2015, 24, 1210–1223. Whitehouse, Science, 1989, 246, 64–71. 95 47 I. D. G. Campuzano, C. Netirojjanakul, M. Nshanian, J. L. J. a Loo, <i>Int. J. Mass Spectrom. Rev.</i> , 1997, 16, 1–23. 96 Lippens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, <i>Anal.</i> J. a. Loo, <i>Int. J. Mass Spectrom. Rev.</i> , 2000, 200, 175–186. 97 Chem., 2017, acs.analchem.7b03021. A. J. R. Heck, <i>Nat. Methods</i> , 2008, 5, 927–933. 98 48 A. S. De Groot and D. W. Scott, <i>Trends Immunol.</i> , 2007, 28, 98–917. A. B. Feramawy, K. W. M. Siu and B. a Thomson, <i>J. Am.</i> Sbôl 99 482–490. 1. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. B. El-Faramawy, K. W. M. Siu and B. a Thomson, <i>J. Am.</i> Sbôl Baykal and S. Wang, <i>Biotechnol. Adv.</i> , 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 V. Katta, B. T. Chait and S. Carr, <i>Rapid Commun. Mass</i> F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito aû03 Spectrom., 1991, 5, 214–217. V. Katta, B. T. Chait and S. Carr, <i>Rapid Commun. Mass</i> Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 52 J. R. Engen and T. E. Wales, <i>Annu. Rev. Anal. Chem.</i> , 2015, 8, 127–148.	M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, Int. J. 92	2 46	J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N.
J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94 Protein Sci., 2015, 24, 1210–1223. Whitehouse, Science, 1989, 246, 64–71. 95 47 J. a Loo, Mass Spectrom. Rev., 1997, 16, 1–23. 96 J. a. Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 48 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 482–490. 1. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 Y. Katta, B. T. Chait and S. Carr, Rapid Commun. Mass Spectrom., 2005, 16, 1702–7. 102 Y. Kobinson, Anal. Chem., 2002, 74, 1402–1407. 104 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Yonowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 H. H. Van Den Heuvel, E. Denisov, A. Makarov and A. J. R. 109 51 H. Kei, J. Mot, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. R. J. Rose, E. Damoc, E. Denisov, P. D. Compton, S. 111 Heck, Nat. Methods, 2012, 9,	Mass Spectrom. Ion Process., 1987, 78 , 53–68. 93	3	Corvaïa, A. Van Dorsselaer, A. Beck and S. Cianférani,
Whitehouse, Science, 1989, 246, 64–71. 95 47 I. D. G. Campuzano, C. Netirojjanakul, M. Nshanian, J. L. J. a Loo, Mass Spectrom. Rev., 1997, 16, 1–23. 96 Lippens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, Anal. J. a. Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 Chem., 2017, acs.analchem.7b03021. A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, 482–490. P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009,999 1. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 Mass Spectrom., 2005, 16, 1702–7. 102 50 V. Katta, B. T. Chait and S. Carr, Rapid Commun. Mass F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito a103 Spectrom., 1991, 5, 214–217. G. F. Pirrone, R. E. Iacob and J. R. Engen, Anal. Chem., 2015, R. H. H. Van Den Heuvel, E. Van Duijn, H. Mazon, S. A. 105 87, 99–118. Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 52 Langridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, Ark007 8, 127–148. R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R.109 H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. R. J. Rose, F. Damoc, E. De	J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. 94	4	Protein Sci., 2015, 24 , 1210–1223.
J. a Loo, Mass Spectrom. Rev., 1997, 16, 1–23. 96 Lippens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, Anal. J. a. Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 Chem., 2017, acs.analchem.7b03021. A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 48 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009, 99 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, 482–490. 28, 898–917. 100 49 I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb01 Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 50 F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito a103 Spectrom., 1991, 5, 214–217. C. V. Robinson, Anal. Chem., 2002, 74, 1402–1407. 104 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Sungridge, J. Van Der Oost, J. Hoyes and A. J. R. H	Whitehouse, <i>Science</i> , 1989, 246 , 64–71. 99	5 47	I. D. G. Campuzano, C. Netirojjanakul, M. Nshanian, J. L.
J. a. Loo, Int. J. Mass Spectrom., 2000, 200, 175–186. 97 Chem., 2017, acs.analchem.7b03021. A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 48 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009,999 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, 482–490. 28, 898–917. 100 49 I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 50 V. Katta, B. T. Chait and S. Carr, Rapid Commun. Mass F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito a103 Spectrom., 1991, 5, 214–217. S. Perrone, R. E. Iacob and J. R. Engen, Anal. Chem., 2015, 87, 99–118. Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 52 J. R. Engen and T. E. Wales, Annu. Rev. Anal. Chem., 2015, 8, 127–148. Chem., 2006, 78, 7473–7483. 108 53 H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. E. Iacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95– 102. M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 54 D. D. Weis, Hydrogen Exchange Mass Spectrometry of Proteins, John Wiley & Sons, Ltd, Chichester. UK. 2016.	J. a Loo, <i>Mass Spectrom. Rev.</i> , 1997, 16 , 1–23. 90	6 7	Lippens, D. P. A. Kilgour, S. L. Van Orden and J. Loo, Anal.
A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 48 A. J. R. Heck, Nat. Methods, 2008, 5, 927–933. 98 48 P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009,99 48 A. S. De Groot and D. W. Scott, Trends Immunol., 2007, 28, 482–490. 28, 898–917. 100 49 I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222. Mass Spectrom., 2005, 16, 1702–7. 102 50 V. Katta, B. T. Chait and S. Carr, Rapid Commun. Mass F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito at 103 Spectrom., 1991, 5, 214–217. G. F. Pirrone, R. E. Iacob and J. R. Engen, Anal. Chem., 2015, 87, 99–118. Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 52 J. R. Engen and T. E. Wales, Annu. Rev. Anal. Chem., 2015, 81, 127–148. Chem., 2006, 78, 7473–7483. 108 53 H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. E. Iacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95– Heck, Nat. Methods, 2012, 9, 1084–1086. 110 102. M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 54 Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112 54 D. D. Weis, Hydrogen Exchange Mass Spectrometry of Proteins, John Wiley & Sons, Ltd, Chichester, UK. 2016.	J. a. Loo, Int. J. Mass Spectrom., 2000, 200 , 175–186. 9	/	<i>Chem.</i> , 2017, acs.analchem.7b03021.
P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009,99 482–490. 28, 898–917. 100 49 A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb01. I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B. Mass Spectrom., 2005, 16, 1702–7. 102 50 F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito a103 Spectrom., 1991, 5, 214–217. C. V. Robinson, Anal. Chem., 2002, 74, 1402–1407. 104 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 Sumaridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, Arth07 8, 127–148. Chem., 2006, 78, 7473–7483. 108 R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R.109 H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. H. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.	A. J. R. Heck, <i>Nat. Methods</i> , 2008, 5 , 927–933.	8 48 0	A. S. De Groot and D. W. Scott, <i>Trends Immunol.</i> , 2007, 28 ,
28, 898–917.10049A. El-Faramawy, K. W. M. Siu and B. a Thomson, J. Am. Sb0.1I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B.Mass Spectrom., 2005, 16, 1702–7.102F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito al 03Spectrom., 1991, 5, 214–217.C. V. Robinson, Anal. Chem., 2002, 74, 1402–1407.104R. H. H. Van Den Heuvel, E. Van Duijn, H. Mazon, S. A.105Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D.106Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D.106Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D.106Langridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, Art 0753R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R. 10953H. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.110M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 11254	P. Kebarle and U. H. Verkerk, Mass Spectrom. Rev., 2009,99	9	482–490.
A. El-Faramawy, K. W. M. Slu and B. a Thomson, J. Am. S00.1Baykal and S. Wang, Biotechnol. Adv., 2012, 30, 210–222.Mass Spectrom., 2005, 16, 1702–7.10250F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito a103Spectrom., 1991, 5, 214–217.C. V. Robinson, Anal. Chem., 2002, 74, 1402–1407.104R. H. H. Van Den Heuvel, E. Van Duijn, H. Mazon, S. A.105Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D.106Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D.106Langridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, Anton53R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R. 10953H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R.E. Iacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95–Heck, Nat. Methods, 2012, 9, 1084–1086.110M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 11254	28 , 898–917. 100	0 49 1	I. a. Kaltashov, C. E. Bobst, R. R. Abzalimov, G. Wang, B.
Mass Spectrom., 2005, 16, 1702–7. 102 50 V. Katta, B. T. Chait and S. Carr, Rapid Commun. Mass F. Sobott, H. Hernández, M. G. McCammon, M. a. Tito a103 Spectrom., 1991, 5, 214–217. G. F. Pirrone, R. E. Iacob and J. R. Engen, Anal. Chem., 2015, R. H. H. Van Den Heuvel, E. Van Duijn, H. Mazon, S. A. 105 S7, 99–118. Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 52 Langridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, Antol 7 8, 127–148. Chem., 2006, 78, 7473–7483. 108 53 R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R.109 H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. E. Iacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95– 102. M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 M. Engen, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112 D. D. Weis, Hydrogen Exchange Mass Spectrometry of	A. El-Faramawy, K. W. M. Slu and B. a Thomson, J. Am. SDO.	1 2 F0	Baykai and S. Wang, <i>Biotechnol. Adv.</i> , 2012, 30 , 210–222.
 Spectrom., 1991, 5, 214–217. Spectrom., 1991, 5, 214–217. Spectrom., 1991, 5, 214–217. Spectrom., 1991, 5, 214–217. G. F. Pirrone, R. E. Iacob and J. R. Engen, Anal. Chem., 2015, 87, 99–118. J. R. Engen and T. E. Wales, Annu. Rev. Anal. Chem., 2015, 81, 27–148. Stangridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, And 7 R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R. 109 Heck, Nat. Methods, 2012, 9, 1084–1086. M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. E. Iacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95– 102. D. D. Weis, Hydrogen Exchange Mass Spectrometry of Proteins, John Wiley & Sons, Ltd. Chichester. UK. 2016. 	Mass Spectrom., 2005, 16 , 1702–7. 10.	2 50 2	V. Katta, B. I. Chait and S. Carr, <i>Rapid Commun. Mass</i>
C. V. Robinson, Andr. Chem., 2002, 74, 1402–1407. 104 S1 G. F. Pirrone, R. E. facob and J. R. Engen, Andr. Chem., 2015, R. H. H. Van Den Heuvel, E. Van Duijn, H. Mazon, S. A. 105 87, 99–118. Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D. 106 52 Langridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, And 07 8, 127–148. Chem., 2006, 78, 7473–7483. 108 53 R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R.109 H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R. H. Kei, Nat. Methods, 2012, 9, 1084–1086. 110 102. M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 54 Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112 54 D. D. Weis, Hydrogen Exchange Mass Spectrometry of	F. Soboli, H. Hernandez, M. G. McCammon, M. a. Hio allo	Э Л г1	Specirom, 1991, 5 , 214–217.
K. H. H. Vall Den Heuvel, E. Van Dulji, H. Mazon, S. A.10557, 95–118.Synowsky, K. Lorenzen, C. Versluis, S. J. J. Brouns, D.10652Langridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, Ard 07J. R. Engen and T. E. Wales, Annu. Rev. Anal. Chem., 2015,R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R. 10953Heck, Nat. Methods, 2012, 9, 1084–1086.110M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111Starren, A. Makarov and N. L. Kelleher, Anal. Chem., 11254B. D. Weis, Hydrogen Exchange Mass Spectrometry of Proteins, John Wiley & Sons, Ltd, Chichester, UK. 2016.	C. V. Robilisofi, Anal. Chem., 2002, 74 , 1402–1407. 104	4 JI 5	97 00_119
Syntowsky, R. Euferland, C. Versidis, S. J. Brouns, D.10032J. R. Engen and T. E. Wales, Annu. Rev. Annu. Chem., 2013,Langridge, J. Van Der Oost, J. Hoyes and A. J. R. Heck, Andu?8, 127–148.Chem., 2006, 78, 7473–7483.108R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R.109H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R.E. lacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95–Heck, Nat. Methods, 2012, 9, 1084–1086.110M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112D. D. Weis, Hydrogen Exchange Mass Spectrometry of Proteins, John Wiley & Sons, Ltd, Chichester, UK. 2016.	Synowsky, K. Loronzon, C. Vorshuis, S. L. L. Brouns, D. 10.	5 52	67, 55-110.
Chem., 2006, 78, 7473–7483.10853R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R.109H. Wei, J. Mo, L. Tao, R. J. Russell, A. A. Tymiak, G. Chen, R.E. Jacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95–Heck, Nat. Methods, 2012, 9, 1084–1086.110M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112D. D. Weis, Hydrogen Exchange Mass Spectrometry of	Langridge L Van Der Oost L Heves and A. L. P. Heck Add	0 32 7	9 127-149
R. J. Rose, E. Damoc, E. Denisov, A. Makarov and A. J. R.109E. Iacob and J. R. Engen, Drug Discov. Today, 2014, 19, 95–Heck, Nat. Methods, 2012, 9, 1084–1086.110M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.111Herning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112D. D. Weis, Hydrogen Exchange Mass Spectrometry of	Chem 2006 78 7473-7483	, 8 53	H Wei Mo Tao R Russell & A Tumiak G Chen P
Heck, Nat. Methods, 2012, 9, 1084–1086.110102.M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S.11154Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112Proteins, John Wiley & Sons, Ltd, Chichester. UK. 2016.	R Rose F Damor F Denisov A Makarovand A P 100	9	F Jacob and L R Engen Drug Discov Today 2014 10 05-
M. E. Belov, E. Damoc, E. Denisov, P. D. Compton, S. 111 54 Horning, A. A. Makarov and N. L. Kelleher, <i>Anal. Chem.</i> , 112 Proteins, John Wiley & Sons, Ltd, Chichester. UK. 2016.	Heck Nat Methods 2012 9 1084-1086 11	0	102
Horning, A. A. Makarov and N. L. Kelleher, Anal. Chem., 112 Proteins, John Wiley & Sons, Ltd, Chichester. UK. 2016.	M. F. Belov, F. Damoc F. Denisov, P. D. Compton, S. 11	- 1 54	D. D. Weis, Hydrogen Exchange Mass Spectrometry of
	Horning, A. A. Makarov and N. L. Kelleher, <i>Anal. Chem.</i> , 11.	2	Proteins, John Wiley & Sons, Ltd, Chichester, UK, 2016.

D. Houde, J. Arndt, W. Domeier, S. Berkowitz and J. R. Engen, Anal. Chem., 2009, 81, 2644-2651.

 Journal Name

2					
2	1	56	D. Houde, Y. Peng, S. a Berkowitz and J. R. Engen, Mol. Ce	9.8 77	Y. Zhong, SJ. Hyung and B. T. Ruotolo, Analyst, 2011, 136,
2	2		Proteomics, 2010, 9 , 1716–1728.	59	3534–3541.
4	3	57	A. Zhang, P. Hu, P. MacGregor, Y. Xue, H. Fan, P. Suchecki	50 78	M. F. Bush, Z. Hall, K. Giles, J. Hoyes, C. V. Robinson and B.
5	4		L. Olszewski and A. Liu, Anal. Chem., 2014, 86, 3468–75.	51	T. Ruotolo, Anal. Chem., 2010, 82, 9557–9565.
6	5	58	L. Y. Pan, O. Salas-Solano and J. F. Valliere-Douglass, Anal	5 2 79	A. A. Shvartsburg and M. F. Jarrold, Chem. Phys. Lett.,
7	6		Chem., 2014, 86 , 2657–64. 6	53	1996, 261 , 86–91.
8	7	59	A. Zhang, S. K. Singh, M. R. Shirts, S. Kumar and E. J.	54 80	M. F. Mesleh, J. M. Hunter, A. A. Shvartsburg, G. C. Schatz
9	8		Fernandez, <i>Pharm. Res.</i> , 2012, 29 , 236–50.	55	and M. F. Jarrold, J. Phys. Chem., 1996, 100, 16082–16086.
10	9	60	R. E. Iacob, G. M. Bou-Assaf, L. Makowski, J. R. Engen, S. A	66 81	J. L. P. Benesch and B. T. Ruotolo, Curr. Opin. Struct. Biol.,
11	10		Berkowitz and D. Houde, J. Pharm. Sci., 2013, 102, 4315–6	57	2011, 21 , 641–649.
12	11		4329. 6	58 82	B. T. Ruotolo, J. L. P. Benesch, A. M. Sandercock, SJ.
13	12	61	C. L. Dobson, P. W. A. Devine, J. J. Phillips, D. R. Higazi, C. 6	59	Hyung and C. V Robinson, Nat. Protoc., 2008, 3, 1139–52.
17	13		Lloyd, B. Popovic, J. Arnold, A. Buchanan, A. Lewis, J. 7	70 83	J. M. Koomen, B. T. Ruotolo, K. J. Gillig, J. A. McLean, D. H.
14	14		Goodman, C. F. van der Walle, P. Thornton, L. Vinall, D. 7	71	Russell, M. Kang, K. R. Dunbar, K. Fuhrer, M. Gonin and J.
15	15		Lowne, A. Aagaard, LL. Olsson, A. Ridderstad Wollberg, E	2	A. Schultz, Anal. Biognal. Chem., 2002, 373 , 612–617.
16	16		Welsh T K Karamanos C L Pashley M G Jadanza N $\overline{A7}$	73 84	A Arcella G Portella M I Ruiz R Fritia M Vilaseca V
17	17		Ranson A E Ashcroft A D Kippen T I Vaughan S E 7	7 <u>4</u>	Gabelica and M. Orozco, J. Am. Chem. Soc. 2012 134
18	18		Radford and D. C. Lowe, Sci. Rep. 2016 6 28644	75	6506_6606
19	10	62	L Arora L M Hickov P Majumdar P Estandiany S M 7	76 or	Li B Bondiak W E Sigms D B Cang and H H Hill
20	20	02	J. Alora, J. W. Hickey, R. Wajulluar, R. Estallulary, S. W. J.		Angl Cham 2012 95 2760 2760
21	20		Bishop, H. S. Samra, C. R. Middaugh, D. D. Weis and D. B. 7	70 oc	Andi. Chem., 2013, 85 , 2760–2769.
22	21	62	Volkin, <i>MADS</i> , 2015, 7, 525–539.		F. Lanucara, S. W. Holman, C. J. Gray and C. E. Eyers, Nat.
22	22	63	D. Houde, Z. E. Nazari, G. M. Bou-Assaf, A. S. Weiskopf and	19	Chem., 2014, 6 , 281–94.
25	23		K. D. Rand, J. Am. Soc. Mass Spectrom., 2016, 27, 669–676	KU 87	K. B. Shelimov and M. F. Jarrold, J. Am. Chem. Soc., 1997,
24	24	64	J. Arora, S. B. Joshi, C. R. Middaugh, D. D. Weis and D. B. &	31	119 , 2987–2994.
25	25		Volkin, J. Pharm. Sci., 2017, 106 , 1508–1518.	32 88	SJ. Hyung, C. V Robinson and B. T. Ruotolo, Chem. Biol.,
26	26	65	D. M. Hambly and M. L. Gross, J. Am. Soc. Mass Spectrom	\$3	2009, 16 , 382–90.
27	27		2005, 16 , 2057–2063.	34 89	D. Bagal, J. F. Valliere-Douglass, A. Balland and P. D.
28	28	66	G. Xu and M. R. Chance, <i>Chem. Rev.</i> , 2007, 107 , 3514–	35	Schnier, Anal. Chem., 2010, 82 , 6751–5.
29	29		3543. 8	36 90	K. J. Pacholarz, M. Porrini, R. a Garlish, R. J. Burnley, R. J.
30	30	67	L. Konermann, B. B. Stocks, Y. Pan and X. Tong, Mass	37	Taylor, A. J. Henry and P. E. Barran, Angew. Chem. Int. Ed.
31	31		<i>Spectrom. Rev.</i> , 2009, 47 , n/a-n/a.	38	Engl., 2014, 53 , 7765–9.
37	32	68	J. B. Sperry and L. M. Jones, in <i>Biophysical Methods for</i>	39 91	I. D. G. Campuzano, C. Larriba, D. Bagal and P. D. Schnier, in
22	33		Biotherapeutics, John Wiley & Sons, Inc., Hoboken, NJ,	90	ACS Symposium Series, 2015, vol. 1202, pp. 75–112.
33	34		USA, 2014, pp. 151–172.	91 92	M. J. Edgeworth, J. J. Phillips, D. C. Lowe, A. D. Kippen, D. R.
34	35	69	L. M. Jones, H. Zhang, W. Cui, S. Kumar, J. B. Sperry, J. a	92	Higazi and J. H. Scrivens, Angew. Chemie Int. Ed., 2015, 54,
35	36		Carroll and M. L. Gross, J. Am. Soc. Mass Spectrom., 2013	93	15156–15159.
36	37		24 , 835–45.	94 93	A. Beck, F. Debaene, H. Diemer, E. Wagner-Rousset, O.
37	38	70	J. Li, H. Wei, S. R. Krystek, D. Bond, T. M. Brender, D.	95	Colas, A. Van Dorsselaer and S. Cianférani, J. Mass
38	39		Cohen, J. Feiner, N. Hamacher, J. Harshman, B. YC. Huar	€ €	Spectrom. 2015. 50 . 285–297.
39	40		S H Julien 7 Jin K Moore J Mueller C Noriega P	97 94	M E Bush I D G Campuzano and C V Robinson Angl
40	41		Seiwal P Shennard B Stevens G Chen A A Tymiak M ^C	98	Chem 2012 84 7124–7130
41	42		L Gross and L A Schneeweis Angl Chem 2017 89	99 as	R Salbo M E Rush H Naver I Campuzano C V
40 1	/12		2250_2258 110 L. A. Schneeweis, Anui. Chem., 2017, 05, 11	0	Robinson Bettersson T D ørgensen and K E
42	10	71	E A Mason and E W McDaniel Transport Bronarties of)0)1	Hasalmann Banid Commun Mass Spectrom 2012 26
43	44	/1	Long in Cases Wiley VCH Verlag CmbH 8 Co. KCaA 10	12	1191 1102
44	45		In Gases, Wiley-VCH Verlag GmbH & CO. KGaA, IC)2 00	1181–1193.
45	40	70	Weinneim, FRG, 1988.	13 96	L. Han, S. J. Hyung, J. J. S. Mayers and B. T. Ruotolo, <i>J. Am.</i>
46	47	72	A. B. Kanu, P. Dwivedi, M. Tam, L. Matz and H. H. Hill, J. 10)4)5	Chem. Soc., 2011, 133 , 11358–11367.
47	48		Mass Spectrom., 2008, 43, 1–22.	15 97 NG	L. Han, S. J. Hyung and B. I. Ruotolo, Angew. Chemie - Int.
48	49	73	T. Wyttenbach and M. Bowers, Int J Mass Spectrom, 2001,)6 	Ed., 2012, 51 , 5692–5695.
49	50		212 , 13–23. 10	98	J. N. Rabuck, S. Hyung, K. S. Ko, C. C. Fox, M. B. Soellner
50	51	74	S. R. Harvey, C. E. MacPhee and P. E. Barran, <i>Methods</i> , 10	NR N	and B. T. Ruotolo, Anal. Chem.
51	52		2011, 54 , 454–461. 10	99 99	A. Laganowsky, E. Reading, T. M. Allison, M. B.
51	53	75	S. D. Pringle, K. Giles, J. L. Wildgoose, J. P. Williams, S. E.11	LO	Ulmschneider, M. T. Degiacomi, A. J. Baldwin and C. V
52	54		Slade, K. Thalassinos, R. H. Bateman, M. T. Bowers and J14	1	Robinson, <i>Nature</i> , 2014, 510 , 172–5.
53	55		Scrivens, Int. J. Mass Spectrom., 2007, 261, 1–12. 11	L 2 100	Y. Zhong, L. Han and B. T. Ruotolo, Angew. Chem. Int. Ed.
54	56	76	K. Giles, J. P. Williams and I. Campuzano, Rapid Communa	13	Engl., 2014, 53 , 9209–12.
55	57		Mass Spectrom., 2011, 25, 1559–1566. 11	L 4 101	S. Niu, J. N. Rabuck and B. T. Ruotolo, Curr. Opin. Chem.
56					

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1		ARTIC	E
2	1		<i>Biol.</i> , 2013, 17 , 809–17.
3	2	102	Y. Tian, L. Han, A. C. Buckner and B. T. Ruotolo, Anal.
4	3		Chem., 2015, 87 , 11509–11515.
5	4	103	J. D. Eschweiler, J. N. Rabuck-Gibbons, Y. Tian and B. T.
6	5		Ruotolo, Anal. Chem., 2015, 87, 11516–11522.
7	6	104	K. Pisupati, Y. Tian, S. Okbazghi, A. Benet, R. Ackermann,
8	7		M. Ford, S. Saveliev, C. M. Hosfield, M. Urh, E. Carlson, C.
9	8		Becker, T. J. Tolbert, S. P. Schwendeman, B. T. Ruotolo and
10	9		A. Schwendeman, Anal. Chem., 2017, 89, 4838–4846.
11	10	105	Y. Huang, N. D. Salinas, E. Chen, N. H. Tolia and M. L. Gross,
12	11	100	J. Am. Soc. Mass Spectrom., 2017, 24–27.
13	12	106	C. W. N. Damen, W. Chen, A. B. Chakraborty, M. van
14	15		Dosternout, J. R. Mazzeo, J. C. Gebler, J. H. M. Schellens, H.
15	14		20 2021–2033
16	16	107	Y Huang A Gelb and F Dodds Curr Metabolomics 2014
17	17	107	1 , 291–305.
18	18	108	P. Both, A. P. Green, C. J. Gray, R. Šardzík, J. Voglmeir, C.
19	19		Fontana, M. Austeri, M. Rejzek, D. Richardson, R. A. Field,
20	20		G. Widmalm, S. L. Flitsch and C. E. Eyers, Nat Chem, 2014,
21	21		6 , 65–74.
22	22	109	J. Hofmann, H. S. Hahm, P. H. Seeberger and K. Pagel,
23	23		Nature, 2015, 526 , 241–244.
24	24		
25			
26			
27			
28			
29			
30			
31			
32			
33			
34 25			
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