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Mass Spectrometry Methods for Measuring Protein Stability

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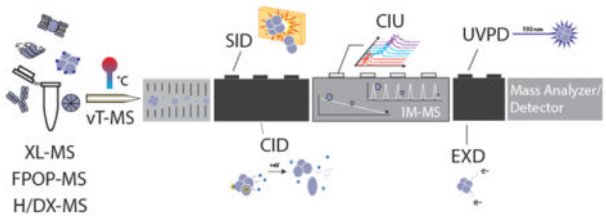
Abstract

Mass spectrometry is a central technology in the life sciences, providing our most comprehensive account of the molecular inventory of the cell. In parallel with developments in mass spectrometry technologies targeting such assessments of cellular composition, mass spectrometry tools have emerged as versatile probes of biomolecular stability. In this review, we cover recent advancements in this branch of mass spectrometry that target proteins, a centrally important class of macromolecules that accounts for most biochemical functions and drug targets. Our efforts cover tools such as hydrogen-deuterium exchange, chemical cross-linking, ion mobility, collision induced unfolding, and other techniques capable of stability assessments on a proteomic scale. In addition, we focus on a range of application areas where mass spectrometry-driven protein stability measurements have made notable impacts, including studies of membrane proteins, heat shock proteins, amyloidogenic proteins, and biotherapeutics. We conclude by briefly discussing the future of this vibrant and fast-moving area of research.

Graphical Abstract



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1. Introduction

Stability-shift measurements have been a key component of biochemical research since the 1920s. For example, early work by Eggerth and colleagues in the 1920s monitored the changes in *Escherichia coli* (*E. coli)* flocculation as a function of added colloidal proteins in suspension.[1] A follow up study further probed the stability of *E. coli* as a function of strain variation, suspension media, as well as storage conditions.[2] As such cellular stability shift assays gained importance, measurements targeting purified proteins soon surfaced. An example of such work is included in a series of publications by Northrop and colleagues wherein they explored the stability of trypsin by measuring its activity as a function of temperature.[3,4] Similar studies measuring protein activity as a function of pH, temperature, and aging were performed for a variety of enzymes such as pepsinogen,[5] and ribonuclease,[6] wherein a loss of enzymatic activity can be interpreted as a measurement of protein stability. Together, these types of studies helped build the foundations of modern biochemistry.

Later, spectrophotometry-based measurements emerged to enable in-depth investigations of protein stability. Early examples include absorbance spectrophotometry experiments, as demonstrated in early studies of rhodopsins,[7,8] and polarimetry measurements, which were used extensively to characterize the stabilities of collagen in response to pH,[9] temperature,[10] organic solvent,[11] or changes in primary sequence.[12] These spectrophotometric approaches typically yield global protein stability information. Although these techniques were deployed extensively in the characterization of protein targets such as myoglobin[13–16] and hemoglobin,[17] novel techniques were beginning to emerge which promised insights into intermediate conformers populated during protein unfolding.

Differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC) quickly became the primary approaches for studying protein stability. DSC measures the heat capacity (C_p), and transition/melting temperature (T_m) at which 50% of protein population is denatured, with higher T_m values are indicative of higher stability, which is employed in a comparative manner to quantitate the stabilizing/destabilizing effects of buffer composition,[18,19] protein-protein interactions,[20] ligand binding,[21–23] or mutation.[24] ITC measures changes in the thermodynamic properties of a protein sample upon the controlled addition of known ligands or binding partners. Changes in these properties, discussed in more detail in section 2 below, can be interpreted as changes in sample stability.[25–30] DSC and ITC remain standard techniques for protein stability measurements today.[31]

In the last three decades, techniques commonly used for small molecule structure determination such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) have developed into tools capable of targeting larger analytes such as intact proteins. Advancements in NMR spectroscopy enabled for the first time, higher resolution

measurements of protein secondary structure stability based on changes in chemical shifts of residue sidechains.[32–37] Similarly, the development of electrospray ionization (ESI)[38] proved crucial for the growth of MS as a probe of protein stability.[39,40] Since these early studies, researchers have sought to expand the MS toolbox to encompass a greater number of biophysical descriptors associated with protein conformation and folding (Figure 1).

Tandem MS (MS-MS or MS^2) approaches were utilized to determine primary sequence,[41] while charge state distributions (CSD)[40] were used to monitor protein structures incubated under different solvent conditions,[42–44] or temperatures.[45–51] These studies led to the realization that, under the appropriate conditions, ESI-MS was capable of transmitting non-covalent protein complexes in a manner closely mirroring their native states[52–56]. In the three decades since these initial discoveries, innovations in protein ionization[57,58] and instrumentation[59] have led to the emergence of native mass spectrometry, a technique capable of directly analyzing a wide array of multimeric protein machines,[55,60–62] including chaperones,[63,64,73–79,65–72] ribosomes,[80–82] and intact viral particles.[83–85]

MS-based techniques soon emerged capable of measuring protein stabilities. For example, covalent labeling techniques such as hydrogen-deuterium exchange MS (HDX-MS),[86] fast photochemical oxidation of proteins (FPOP),[87] and chemical cross-linking MS (CXL-MS)[88] are three such techniques which are capable of yielding protein structure and dynamics information at the level of individual amino acid residues within the targeted sequences. Briefly, these techniques covalently label proteins in solution, and the results of this labeling are then typically analyzed by bottom-up liquid chromatography MS (LC-MS) workflows. Such approaches can be used to directly assess the impacts of stress, ligand binding, or post-translational modification upon protein structure and stability within complex mixtures.

Another such approach, ion mobility-mass spectrometry (IM-MS), is capable of separating a variety of gas-phase ions based on their interactions with gaseous neutrals. IM-MS has been used to separate the electronic states of transition metals,[89–91] as well as the shapes of varying carbon clusters,[92–94] and PEG polymers.[95] Pioneering instrument development efforts combined ESI with IM-MS enabling the initial separations of intact protein ions,[96] and helped to establish a research area now referred to as gas-phase structural biology.[97] IM-MS technology provides the foundation for collision-induced unfolding (CIU) assays, wherein native-like protein ions are incrementally activated in the gas phase prior to IM separation. This results in gas phase unfolding of the ions, which is detected by IM as an increase in CCS.[98,99] CIU has been applied to a variety of biological targets[98] including biotherapeutics,[100–102] membrane proteins,[103–107] chaperone complexes,[108] and kinases.[109] This review will expand on the various gas phase stability measurement techniques summarized here, with focused sections covering applications targeting biotherapeutics, membrane proteins, intrinsically disordered proteins, and heat shock proteins.

2. Survey of MS-based Protein Stability Measurement Techniques

2.1 Reference Techniques for measuring protein Stability

Thermal stability is a key determinant of protein structure and function.[110] As such, stability measurements are extensively used throughout biochemistry, and especially in context

of pharmaceuticals, where protein stabilities are used to support the quality, safety and efficacy of biotherapeutics, or protein-based therapies, throughout their development.[111,112] Technologies such as circular dichroism (CD),[113] DSC,[111,114] ITC,[31] and differential scanning fluorimetry (DSF)[115] are widely used for the analysis of protein stabilities.[116] In particular DSC, ITC, and DSF function as reference protein stability probes due to their ability to directly track changes in thermodynamic stability[117–121].

Despite offering robust assessments of protein stability, the spectrometric and calorimetric methods briefly surveyed above often require large quantities of purified protein and lengthy acquisition times. In contrast, MS-based protein stability measurement techniques require substantially less sample and have the potential to be orders of magnitude faster when compared to their solution counterparts[122]. As indicated above, MS techniques also excel in extracting stability measurements from protein mixtures. Furthermore, when MS is combined with structurally sensitive labeling and separation techniques, such approaches can provide granular structural information that makes it possible to link specific regions of the protein sequence to changes in both protein structure and stability.

2.2 Mass Spectrometry-based Probes of Protein Stability

2.2.1 Native Mass Spectrometry

2.2.1.1 Introduction: For nearly three decades, a large number of MS based observations have indicated that protein complexes can be directly transferred into the gas phase in a manner that retains their native oligomeric states and ligand-binding properties.[123,124] From the initial reports of nMS measurements, questions have emerged regarding the structural and functional states of the proteins captured in flight. While many of these questions persist today, a number of reports have greatly informed our current understanding of gas-phase protein structures and their potential fidelity to native conformations. For example, it has been shown that gas-phase peptide and protein ions retain much of their structural integrity following soft-landing on a surface housed within MS equipment.[125] Specifically, apoferritin, a protein known for its iron-storage capabilities and cage-like structure was shown to retain its native configuration after transport in the gas-phase and soft-landing.[126] Also, ESI-MS has been employed to measure binding constants of protein ligand interactions.[127] Additionally, nIM-MS enables comparisons between measured CCS values and those estimated from high-resolution structure determination experiments (such as x-ray crystallography and NMR), revealing both strong correlations between kinetically-trapped gas-phase protein structures and those associated with their native conformational states, as well as specific areas of structural compaction.[128] For more details on how CCS measurements are obtained from nIM-MS and their application for structural refinement please refer to targeted reviews covering this topic.[129–131] Recently, the optimization of sample preparation, ionization, and ion transport conditions have advanced dramatically our knowledge of gas-phase biomolecular structure, and this area is covered by a number of excellent recent reviews.[128,132–138]

ESI has proven to be a key enabling technology for MS-based protein stability measurements.[139] The technique involves application of high voltage to a conductive emitter, resulting in the creation of charged populations of aerosolized droplets. Subsequent

droplet fission and evaporation events give rise to analyte ions that can be analyzed by MS with both positive and negative polarities. Typical ESI-MS experiments employ high source temperatures (> 100 °C), drying gas, and organic co-solvents in order to facilitate ion desolvation. Since such conditions can be detrimental to the preservation of native-like protein structures, nMS experiments utilize nanoESI (nESI), aqueous solutions and lower source temperatures (20 – 30 °C).[57] In nESI, smaller droplets are produced which allow for more complete protein ion desolvation, resulting in significantly improved mass resolution and dramatic reductions in artifact protein complex signals. The fundamental principles of ESI, along with recent advancements in its theory of operation and implementation, have been discussed in detail previously.[57,140–142]

2.2.1.2 Charge State Distributions as a Probe of Protein Stability: The native mass spectra of protein ions produced by nESI-MS typically reveals a series of ion signals corresponding to a narrow range of charge states, the magnitude of which correlate strongly with the available surface areas of the structures adopted by the analyte proteins in solution. As such, the charge state distribution (CSD) recorded for protein ions can be treated as a direct probe of protein solution structures, and measured over a wide array of preionization sample conditions.[143] By extension, such CSDs have been used as a measure of protein stability.[42,144] For example, Chowdhury *et al.* showed that at least three CSDs were observed in ESI-MS data recorded for bovine cytochrome c (cyt*c*) when incubated at pH values ranging from 2.6–5.2. Critically, the existence of at least three cyt*c* conformational states had been observed in both NMR and CD datasets.[42] Using a similar approach, Loo *et al.* demonstrated that ubiquitin CSDs could be varied based on the amount of organic solvent used, serving to denature the protein and alter its structure prior to ESI-MS.[144] In 1997, Konnerman & Douglas studied the CSDs of cyt*c* in detail and correlated their findings with both CD and DSF data.[145] Together, the studies discussed above laid the foundation for future developments in the application of CSD measurements to protein stability and structure.

Since their first use as a measure of protein structure and stability, CSD data have engendered questions surrounding the potential projection of solution-phase biophysical information into the gas-phase. Early IM-MS results for of cyt*c* showed that the +7 and +8 of the protein ions observed possessed more than one CCS value when compared to higher cyt*c* charge states.[146] Intrinsically disordered proteins (IDPs), which are often implicated in protein misfolding diseases, typically produce significantly wider CSDs and a greater plurality of CCS values for individual charge states than globular proteins studied by nIM-MS.[147,148] Storage time and conditions in the gas-phase can further influence the range of structures accessed by protein ions in a charge state dependent manner.[149] Taken together, the studies above strongly suggest that protein ion structure is a product of the conditions surrounding their preparation, creation, storage, and transport. All of the previous factors must be considered alongside properties such as charge state, stability, sequence, and native structure when evaluating relationships between solvated and gas-phase biophysical data.[150] For example, ubiquitin ions having identical charge states, but prepared through different routes, can display distinct stability differences.[151] As such, while CSD data has a

clear relationship to protein structure and stability in solution, the evaluation of associated gas-phase protein structure can prove more challenging.

2.2.2 Variable Temperature Mass Spectrometry—Variable temperature electrospray ionization (vT-ESI) is an analog of standard solution phase temperature annealing techniques, utilizing MS detection to monitor changes in protein structures (Figure 2A).[42] The experimental setup includes a heat-conductive material that encapsulates and houses the ESI emitter.[152] Throughout the experiment, higher currents are drawn through this housing, generating resistive heating in a controllable manner for the direct assessment of protein CSD,[153] and the oligomeric status of protein complexes.[154,155] The largest advantages of vT-ESI devices remains their ability to probe protein melting point (T_m) values that are comparable to reference technologies (see Section 2.1), without need of large amounts of purified sample (Figure 2A ii).[156–158] Recently, efforts in this space have been made to expand the structural information that can be obtained by vT-ESI-MS,[159] by incorporating IM-MS and detailed forms of data analysis in order to more deeply probe the connections between protein stability and gas-phase protein ion structure.[160,161]

2.2.3 Overview of MS-based Footprinting Methods—MS-based footprinting encompasses techniques that probe the solvent accessible surface area (SASA) of proteins using a wide range chemical modifications that act to shift the measured mass of an analyte in a manner dependent on its structure and stability (Figure 2B).[162,163] MS-enabled footprinting techniques offer high-throughput, amino-acid level resolution information, low limits of detection, and the ability to access protein stability values from within complex mixtures. In addition to the specific technologies covered in the sections below, a wide range of reagents, including carbene and diethylpyrocarbonate chemistries, that seek to comprehensively label solvent exposed residues within folded protein sequences.[162,163] Our coverage of MS-based footprinting tools will focus on specific labeling technologies and their applications to protein stability measurements, and will include examples from Hydrogen-Deuterium Exchange (HDX), chemical cross linking (CXL), and fast photo-oxidation of proteins (FPOP) data.

2.2.3.1 Hydrogen-Deuterium Exchange: HDX-MS enables the acquisition of protein structure and stability information through the exchange of labile hydrogens with deuterium in solution at the level of individual amino-acid residues.[164,165] The concept of HDX goes back to the late 1960s when it was first used in conjunction with NMR spectroscopy.[166] HDX exchange rates vary widely, and are based on the local environment of the backbone amide in question. Specifically, solvent accessibility and intramolecular hydrogen bonding can influence greatly the “protection factor” of an amino-acid residue in the context of HDX. In general, there are two procedures for measuring HDX by MS: Continuous-labeling and pulse-labeling. In continuous-labeling experiments, proteins are incubated with D_2O and then analyzed at fixed timepoints.[167] Continuous-labeling is primarily used to monitor slow (min – hours) structural transitions in intact proteins and is often performed using standard MS hardware.[166] In contrast, pulse-labeling HDX is capable of measuring faster transitions (seconds - hours), and functions by exposing samples briefly to a deuterium source following a structural pertubation.[168]

The MS equipment and techniques used to measure HDX are continually evolving. Key challenges include limiting the back-exchange of absorbed deuterium,[86,166] as well as improving both the throughput and coverage of the protein digestion step.[169,170] Many excellent reviews cover the current applications and practice of HDX-MS.[86,162,163,165,168,171–174] As such, the discussion below focuses mainly on the application of HDX-MS for the assessment of protein stability. The earliest examples of such experiments focus on model proteins, such as ubiquitin and lysozyme, with the latter protein probed both under conditions promoting intact and reduced disulfide bonds.[175] From these initial data, HDX-MS has grown significantly to encompass stability data targeting large proteins and their functional assemblies.

Specific examples of HDX-MS protein stability measurements in action span work associated with protein-ligand complexes, biotherapeutic antibodies, and protein-surface adsorption. In the latter area, HDX-MS has quantified the destabilization of proteins during surface adsorption,[176,177] and have extended to free energy assessments for myoglobin-silica adsorption events.[177] HDX-MS played a central role in the evaluation of the National Institute of Standards and Technology monoclonal antibody (NISTmAb) standard.[178] Specifically, HDX-MS revealed the role of net protein charge on the stability and aggregation of the NISTmAb, an insight verified using a battery of reference protein stability measurement tools. HDX-MS also led the way in uncovering the role of glycosylation in stabilizing mAb structure in general, linking the presence of high-mannose or complex glycans to stability increases in model immunoglobin G1 (IgG1) and IgG2 antibodies.[179] The influence of ligand binding on protein stability has also been examined in detail using HDX-MS.[180,181] For example, a recent study utilized HDX-MS to determine two hotspots adjacent to the core binding interface of the SARS-CoV-2 Spike Receptor Binding Domain and the human Angiotensin-converting enzyme 2 (hACE2) protein. These hotspots could represent potential targets for therapeutics that act to destabilize the spike protein-hACE2 interactions.[180]

Tandem MS (MS/MS) technologies are central to most high-resolution HDX-MS workflows. The migration of deuterium tags from their original labeling sites remains a challenge in evaluating residue-level HDX information. Such H/D scrambling is prevalent in datasets utilizing collision induced dissociation (CID) for ion activation,[182] but is dramatically lessened (or eliminated) for workflows using electron capture dissociation (ECD) or electron transfer dissociation (ETD).[164,183–185] Scrambling is caused by the increased vibrational energy and larger timescales of the CID process compared to ECD/ETD.[186] For example, an intact protein HDX-MS workflow incorporating ECD fragmentation has localized deuterium incorporation to residue pairs within the target sequence.[164,187] Clearly, avoiding protein digestion steps allows for both the acquisition of global exchange data while simultaneously limiting back-exchange due to the large decrease in sample preparation time and likelihood of back-exchange.[183] It is clear that the advantages associated with the direct sequencing of intact proteins will spur further advancements in HDX-MS technology.

The peak capacities of LC-MS methods associated with HDX-MS have a clear impact on the acquisition of protein stability information that can be accessed from larger protein systems.

As such, IM separation has been broadly deployed in HDX-MS workflows in order to expand the total number of resolved features accessed by HDX-MS technology.[188,189] The deconvolution of isotopic patterns for co-eluting peptides allows for accurate determination of exchange rates. In addition to HDX in solution, proteins can undergo HDX reactions with reagent gases and background neutrals while trapped in the gas phase. Such gas-phase HDX-MS experiments can be challenging to interpret, but a number of reports demonstrate how such HDX data, when combined with IM-MS, can provide synergistic datasets from which to resolve and identify the plurality of structural microstates that evolve during protein unfolding reactions.[190,191] Future work will undoubtedly reveal further complimentary aspects of IM-MS and HDX data for the evaluation of protein stability in even greater detail.

Lastly, HDX has been utilized to track shifts in protein stability associated with ligand-binding events, often as a function of ligand concentration or ligand to protein ratio. For example, using Stability of Unpurified Proteins from Rates of H/D Exchange (SUPREX) analyses, thermodynamic parameters have been evaluated for the binding of anions to ferric-binding protein.[192] Unlike, SUPREX, the Protein–ligand interactions by mass spectrometry, titration, and H/D exchange (PLIMSTEX) method does not need denaturants to obtain binding constant values for protein-ligand interactions using HDX-MS data.[193] For more information on HDX-MS studies of protein-small molecule binding and HDX-MS and other non-MS tools as a quantitative approaches for assessing protein stability, see the recent reviews by Williams[194] and Fordyce[195], respectively.

2.2.3.2 Chemical Crosslinking: Over the last 20 years, chemical crosslinking (CXL) combined with MS has proved to be an invaluable tool for evaluating the structures of protein complexes.[88] CXL-MS is most often accomplished by exposing proteins to compounds that possess two reactive sites spaced by a known distance. If both sites within the CXL reagent undergo a successful linking reaction with residues on the surface of the protein, then a covalent link between disparate regions of the protein can be established that, if located through MS/MS experiments, can reveal those sequence elements located nearby in the native protein structure. CXL-MS is also a potent tool for examining the stabilities stoichiometries, flexibilities, and binding interfaces associated with protein complexes.[196] Such CXL-MS datasets are most often used to create distance maps, a network of interactions between crosslinked proteins, that can be used as constraints for generating low-resolution models of protein assemblies.[197] Integration with nIM-MS data enables CXL-MS maps to directly account for protein complex stoichiometry, orientation, and size and thus improve the resolution of the models generated.[198,199] The gas-phase stabilities of cross-linked proteins and complexes reveal expected increases in stability, with greater increases provided by charged reagents.[198,199] Future developments in CXL chemistries and associated MS methods will clearly promote more detailed assessments of the stabilities of proteins and their associated complexes[200].

2.2.3.3 Fast Photochemical Oxidation of Proteins: Fast photochemical oxidation of proteins (FPOP) methods generate hydroxyl radicals that irreversibly label solvent-exposed amino-acid side chains within proteins for MS and MS/MS evaluation.[201,202] FPOP

reactions typically occur on the sub-millisecond timescale,[203,204] enabling wide ranging studies of protein stability.[201,205] Additionally, like other chemical footprinting techniques combined with MS detection, FPOP provides a unique set of restraints that can be used to generate, or improve, low-resolution protein structure models.[206] FPOP experiments often require laser irradiation within flow injection manifolds to control the oxidation reactions used for protein labeling.[207] More recent versions of FPOP operate without the need of lasers,[208] offering the potential to measure protein stabilities directly through the direct observation of protein oxidation rates,[209] and are moving towards *in vivo* proteome wide assessments of protein stability values. Future advancements in FPOP will likely continue along this trajectory, further enabling the measurement of protein and protein complex stabilities on a proteome scale.[210,211]

2.2.4 Overview of protein ion activation methods—The activation of gas-phase ions is central to a number of methods associated with assessing the stabilities of proteins.[212] Several approaches are available for increasing the internal energies of protein ions,[213] and if activated sufficiently, each method can provide unique patterns of fragment ions that, in many cases, can provide protein stability information. For example, collision induced dissociation (CID) often utilizes multiple low energy collisions with neutral gas, resulting in ions that undergo a relatively slow (microsecond timescale) accumulation of rotational/energy (Fig. 2C).[214] In contrast, surface induced dissociation (SID) utilizes fast (sub-microsecond) ion-surface collisions to impart rotational/vibrational energy to protein ions in a single step.[215] The different ion activation timescales accessed by CID and SID methods can lead to dramatically different fragmentation patterns in large protein complexes, with the former favoring the ejection of unfolded subunits and the latter producing multi-protein sub-assemblies as product ions.[215] ECD and ETD methods utilize electron capture/transfer and can produce fragment ions on shorter timescales than those accessed by CID and SID, leading to the rapid excitation of protein electronic states and the formation of product ions that can reveal unstructured regions within protein ions, adding further granularity to gas-phase protein stability assessment efforts.[216] Lastly, photo-activation of gas-phase ions provides a flexible platform for assessing protein stability values, with black body infrared dissociation (BIRD) and infra-red multi-photon dissociation (IRMPD) providing direct access to ion internal temperature information through a slow (microsecond) heating process, and thus, information regarding protein ion dissociation energetics. Ultra-violet photo-dissociation (UVPD), in contrast, produces a fast (sub-nanosecond) activation step primarily used to efficiently fragment the protein backbone. For each of the ion activation techniques listed above, the energy deposition timescale accessed dictates much of the eventual product ion population, as well as the utility of such data for protein stability measurments.[217,218]

2.2.4.1 The energetics of protein ion unfolding and dissociation in the gas phase: Since the inception of Rice–Ramsperger–Kassel–Marcus (RRKM) theory,[219] which provides access to micro-canonical rate constants and barrier heights associated with primarily small molecule unimolecular decay reactions, a detailed description of the energetics underlying molecular decomposition events observed by MS has been developed. A similarly detailed description has been sought for gas-phase protein ions, with most efforts focusing on

activation methods that utilize relatively slow energy addition steps.[217] For example, BIRD has been used to evaluate the energetic details associated with the gas-phase dissociation of both small and large molecules.[220] Specifically, BIRD measurements revealed exceptionally large pre-exponential factors associated with the temperature dependent rate contents determined for the dissociation of Shiga toxin oligomers, indicating the formation of highly disordered intermediates.[221] More recently, a framework for quantifying energy deposition during collisional activation has been described and used to evaluate the internal temperatures of protein complex ions undergoing SID.[222] In addition, this framework has been extended to evaluate the energetics associated with CID and CIU processes in protein ions.[223] Future efforts will seek to deploy such detailed knowledge of ion temperature to further evaluate the information content of gas-phase protein stability measurements.

2.2.5 Activation and Dissociation Techniques for Protein Stability Assessment

2.2.5.1 Collision Induced Dissociation: Early observations of protein complex CID[60,224,225,226] revealed product ion distributions dominated by highly-charged monomers and remaining oligomers stripped of both monomers and their associated charge. Such asymmetric charge partitioning between monomers and stripped oligomers can be rationalized based on the mobile proton model[227,228] coupled with the unfolding ejected monomers. Initial observations of asymmetrically charged CID product ion populations for protein complexes were rapidly broadened to include a wide range of protein assemblies, establishing the asymmetric charge partitioning model as the standard mechanism underlying protein complex CID.[150,221,229] Subsequent measurements indicated that this mechanism can be readily shifted to include folded monomers and sub-complexes, as well as peptide fragmentation channels, upon charge manipulation of the precursor complex.[230] A more detailed understanding of protein complex dissociation and unfolding was obtained by deploying IM-MS to monitor the sizes of collisionally-activated protein complexes.[133] Early IM-MS experiments focused on the tetrameric transthyretin (TTR), directly observing increases in IM drift time upon collisional activation that allowed protein unfolding to be confidently invoked within protein complex CID mechanisms for the first time.[231] In addition, these observations gave rise to current generation CIU technology (*see* Section 2.3.2), a method capable of directly assessing gas-phase protein stabilities.

Quantitative assessments of the dissociation pathways produced upon the collisional activation of protein complexes can be used to investigate the stabilities of target complexes, alongside subunit composition, topology and protein-protein interaction strengths.[232–234] Such an analysis was conducted for a series of small heat shock protein oligomers, and revealed the presence of different protein-protein interaction strengths and subunit stability values could be detected and quantified in such systems.[234] In a separate study, CID pathway analysis was used to assess the stabilities of TTR and one its amylogenic variants, ultimately determining that the wild type (WT) protein complex to be the more stable variant.[235] For further detail concerning the theory and practice of protein complex CID, a number of excellent review papers are available.[213,214,227,228,236–238]

The extent to which the CID mechanism can be altered through precursor charge manipulation remains an active area of research, and progress in this area may open new avenues for gas-phase protein stability measurements.[234,236–240] For example, a gas-phase corona discharge probe can be used to on-line charge reduction, and this approach can prepare ions that eject compact protein product ions upon CID over a wide range of oligomeric states and a masses (12 – 233 kDa).[240] Furthermore, in cases where no subunit was ejected, peptide fragmentation can be captured from such charge-reduced precursors.[230] Clearly, precise control over both the charge states and internal energies of proteins is necessary to maximally utilize CID for protein stability measurements.

2.2.5.2 Surface Induced Dissociation: In the context of protein stability measurements, SID is often utilized to evaluate the binding strength associated with protein-protein interfaces within larger assemblies.[215] Previous work has indicated that charge reduced complexes provide a dramatically improved ability to access to sub-complex product ions and a greater fidelity to the relative sizes of protein-protein interfaces.[241,242] SID has been deployed to evaluate the structures of tetrameric protein complexes in this fashion, finding strong correlations to SID product ion populations and the relative stabilities of the interfaces between dimers and monomers within such structures.[243–245] SID has also been used to detect differences in the stabilities of ligand-bound tetrameric protein complexes in a manner dependent upon ligand binding strength and location.[245] The stabilities and structures of large protein complexes, including the GroEL tetradecamer, have also been reported.[242] A broader evaluation of SID applications and instrumentation is available in recent reviews.[215,246]

2.2.5.3 Electron-Capture and Transfer Dissociation: ECD utilizes low energy electrons (1eV), (Figure 2C), whereas ETD uses anions to transfer electrons to precursors, both of which typically act to produce a similar population of product ions associated with protein backbone cleavage.[247,248] A large number of excellent reviews cover the theory and application of ECD and ETD technology, much of which is focused on protein squencing.[216,249–251] Many reports also focus on evaluating ECD/ETD fragmentation from the point of view of evaluating protein structure, as data strongly indicates that fragment ion production can be linked to unstructured regions within protein sequence or surface-accessible reaction sites.[216,252,253] The content below will focus primarily on the use of these tools for assessing protein stabilities.

ECD has been combined with collisional activation in order to survey the stabilities of multiple protein sytems.[254,255] For example, ECD has been used to evaluate the stability of the kinase inducible domain (KIX), revealing that its three helices unfold similarly in both the gas phase and in solution.[254] In a separate study, ECD data was able to differentiate horse and tuna heart cyt*c* on the basis of stability.[255] A large number of reports have focused on the ability of ECD to evaluate monomeric protein structures, and is this area is the subject of multiple excellent reviews.[216,252,253] Initial applications of ECD to multi-protein complexes focused on alcohol dehydrogenase (ADH) tetramer ions, which revealed the presence of an N-terminal fragmentation pattern correlating with those regions of its X-ray structure possessing high b-factors,[256] Atomic displacement parameter,

temperature factor or b-factor indicate regions of disorder in a crystal structure.[257] ECD fragmentation being produced from regions with high b-factors is an observation that was subsequently extended to other protein assemblies, indicating the utility of ECD to characterized disordered regions.[258–260] When combined with IM-MS, ECD acquired for protein complexes can provide a deeper level of structural insight associated with detected differences in protein stability.[151,261–265] For instance, the gas-phase unfolding of Hb tetramer ions was characterized by both IM and ECD, establishing that protein unfolding occurs first at the termini of both the α and β subunits of the complex, and that these unfolded regions are correlated with elevated b-factor regions within X-ray data.[266] As a further example, the structural differences between WT and mutants of the metamorphic protein lymphotactin have been quantified based on a combination of IM-MS and ECD data.[262] In another study, ECD fragmentation and IM-MS data streams were used to jointly constrain a coarse-grained model of variant apolipoprotein E oligomers, which together suggested the presence of a tetramer possessing C4 symmetry.[265] Finally, IM-MS combined with ECD was able to identified a compacted bound state alongside binding site information for a molecular tweezer-type compound attached to tau protein monomers.[264]

More recently, ETD fragmentation has been used to investigate protein stability and structure.[261] In an early set of experiments in this area, ETD data was collected on ADH tetramers.[256] In contrast to prior ECD work, ETD fragmentation was not correlated to regions of the sequence possessing enhanced b-factors, but instead was correlated with the solvent accessible surface area (SASA) of the complex. ETD methods have subsequently been extended to monitor the unfolding of both Concanavalin (ConA) and Hemoglobin (Hb) tetramer ions.[263] When combined with IM-MS, ECD acquired for protein complexes can provide a deeper level of structural insight associated with detected differences in protein stability.[151,260–264,267] For instance, the gas-phase unfolding of Hb tetramer ions was characterized by both IM and ECD, establishing that protein unfolding occurs first at the termini of both the α and β subunits of the complex, and that these unfolded regions are correlated with elevated b-factor regions within X-ray data.[260] As a further example, the structural differences between WT and mutants of the metamorphic protein lymphotactin have been quantified based on a combination of IM-MS and ECD data.[262] In another study, ECD fragmentation and IM-MS data streams were used to jointly constrain a coarse-grained model of variant apolipoprotein E oligomers, which together suggested the presence of a tetramer possessing C4 symmetry.[267] Finally, IM-MS combined with ECD was able to reveal a compacted bound state alongside binding site information, for a molecular tweezer-type compound attached to where in the structure the tweezer had bound to tau protein monomers.[264] Taken together, the studies above project a bright future for ECD/ETD methods as a vital technology for annotating the structural details associated with shifts in protein stability.

2.2.5.4 Ultraviolet Photodissociation: Through the absorption of monochromatic UV light, proteins and their assemblies can quickly cross the energy barriers associated with covalent bond dissociation, making UV photodissociation (UVPD) a useful technology for top-down protein sequencing applciations.[268] When applied to intact protein complexes, UVPD at lower laser powers engenders the formation of highly-charged unfolded protein

product ions and stripped complexes similar to those observed in CID datasets, but when laser fluence is increased, symmetric charge portioning among product ions is typically observed.[269] Recent work points to the potential of UVPD for characterizing of protein stability. For example, 193 nm UVPD has been used to assess the stabilities of a range of model protein tetramers.[270] Additionally, UVPD has been used to localize and characterize the conformational changes that occur upon ligand-binding.[266,271,272] Further, UVPD has detected the effects mutations within the proto-oncogene K-Ras and its kinase binding partner Raf.[273,274] Lastly, UVPD fragmentation has been observed to reflect IM-based measurements tracking protein unfolding in either solution or the gas phase.[275,276]Recent reviews are available covering advancements in UVPD technology.[268]

2.3 Ion Mobility Spectrometry

2.3.1 Fundamental principles and general Applications—IM is a technique that separates gas-phase protein structures based on their size and charge and has emerged as a useful technique for the characterization of protein structures and stabilities.[277] In a simple IM experiment, ions are collected in a pre-IM region, and released in concentrated ion packets into a drift tube filled with inert gas (typically N_2 or He). In the drift tube, ions are separated based on their mobility under the influence of a weak electric field. The amount of time an ion takes to traverse the drift tube, defined as drift time (DT), can be converted to a collision cross section (CCS), a parameter directly related to the size and shape of an ion.[278] Early native IM-MS (nIM-MS) data confirmed that gas phase ions retain compact native-like structures in the absence of bulk solvent.[231,239,279–281] Many IM analyzers are available and offer a range of capabilities for the detailed analysis of gas-phase protein structure and stability, primarily through CCS values, which can often be directly correlated to molecular models in order to assess protein structures.[132,282] When coupled to MS, CCS values can be correlated with ion compositions that reveal the influences of sequence changes[283,284], ligand binding[285], or post-translational modifications[286,287] on protein structure and stability.

2.3.2 Collision Induced Unfolding—Recent advancements in IM-MS technology have enabled the acquisition of both protein structure and stability information simultaneously through CIU experiments. CIU is accomplished by increasing the internal temperature of protein ions in a stepwise fashion prior to IM separation through exposure to activating collisions with a background gas (Figure 2E). Typically, large proteins will undergo several CIU transitions resulting in increased IM drift times. Plots of collision energy against IM drift time (or CCS) can be generated in order to capture and quantify the transitions detected. The resulting CIU ‘fingerprints’ have the ability to capture stability shifts associated with changes in protein domain structure[283,284,288–292], anion and cation adduction[293–295], disulfide bonding patterns[100], glycosylation[296], as well as ligand and cofactor binding[109,297–300]. Currently, efforts are being made to hyphenate CIU with online separation methods[301–303], expand the number of commercially available IM-MS instrument platforms capable of CIU[108,304–306], develop the technique for middle-level protein structure analysis[307], and to further illuminate the fundamental principles of CIU[222]. There have also been considerable efforts to integrate CIU with other structurally-sensitive MS-based probes,

producing datasets of high dimensionality and enabling a deeper level of protein structure analysis than was previously possible (Figure 3).

2.4 Proteome-wide Stability Measurements

An array of LC-MS technologies based on the bottom-up assessment of protein mixtures are currently providing exciting insights into protein stabilities across entire proteomes. Tools based on HDX and oxidative labeling technology are now used to regularly probe the impact of bioactive compounds on protein stabilities on a proteomic scale.[163,209,308] Other methods capable of comprehensively surveying protein stability seek to quantify the soluble proteins that remain following a thermal shock.[110,309] The remaining soluble, folded proteins are then quantified by LC-MS based proteomics. Using this method, protein stabilities have been captured across the cell cycle, revealing that protein stability is correlated with enzyme activity, DNA-binding, and protein complex formation. Similar thermal shock based proteome assays have been applied across 13 species covering all domains of life.[110] These experiments have produced the most comprehensive assessment of protein stability currently available, and the resulting ‘meltome’ atlas highlighted stability differences between protein classes, with those involved in cellular respiration granted particularly large stabilities. LC-MS analysis following the limited digestion of protein mixtures under native conditions with a non-specific protease prior to LC-MS interrogation is another widely used method to probe protein stabilities on a proteome-wide scale.[310] Stable proteins remain more protease resistant than those that are less stable, and thus methods associated with quantitative proteomics can readily quantify protein stabilities under such conditions. Finally, FPOP experiments have recently been carried out within cells, paving the way for a new generation of proteome-wide stability data. The technology relies on a Platform Incubator with movable XY stage (PIXY), which allows for both the growth of cultures and FPOP experiments to be carried out within the same optical bench.[311] Irradiation of one sample well takes 20s of analysis, which allows the study of protein folding and signaling in a time-dependent manner. For more details regarding current MS-based workflows for proteome-wide structural studies, a recent review is available.[312]

3. Biotherapeutics

Monoclonal antibodies, or mAbs, currently represent the largest and most successful class of biotherapeutics available.[313,314] Details surrounding the structures and immunological action of IgGs, the most common antibody isotype used for biotherapeutic development, can be found in previous reports.[315] Unlike small molecule therapeutics, IgGs are composed of over 12,000 atoms, thus dramatically increasing their relative complexities, and projecting myriad challenges for current pharmaceutical measurement science.[316–318] Specifically, the relative dynamism of mAbs (relating to their structures’ hinge region which provides flexibility allowing the structure to dynamically encompass a large conformational range of positions) creates difficulties in rapidly assessing higher order structure (HOS), a key factor associated with biotherapeutic safety and efficacy.[319–322] Structurally-sensitive MS methods have evolved into critical tools in the rapid analysis of mAb HOS characterization.[122] MS is compatible with a wide range of LC methods, and as such, LC-MS based methods are used throughout the pharmaceutical industry within multi-attribute monitoring (MAM)

workflows.[323–325] Furthermore, recent advancements in nMS and nIM-MS techniques have demonstrated potential for MAM characterization of mAb HOS, aggregation, degradation, and stability. Figure 4 illustrates the information content that can be achieved with thermal stability, MS, and IM-based methods.

3.1. Stability Analysis of Biotherapeutic Antibodies

3.1.1 Trends in MS-based mAb Stability data—MS-enabled stability assessments can be carried out in solution and in the gas-phase, depending upon the needs of the application in question. Variable temperature experiments are often used to monitor the shift from native mAb solution structures to unfolded structures and quantify melting point temperatures, Tm, while gas-phase technologies (e.g. IM-MS) can be deployed to detect changes in gas-phase mAb structures in a manner correlated with temperature changes in solution, or in a CIU mode (Fig 4A). Importantly, different mAb compositions and mass-resolved therapeutic modalities can be individually interrogated in MS-enabled stability assessments in manner that is challenging to replicate with other technology platforms. Biosimilar mAb products by design aim to recapitulate the Tm of their mAb reference. Shifts in variable temperature data can indicate either destabilized or stabilized structures, respectively (Fig 4B). Shifts in variable temperature MS data acquired for fusion protein samples will depend strongly on the type of fusion protein being measured (e.g. Fc vs Fab, smaller vs larger protein, etc.), but such assays can reveal both shifts in stabilities as well as new Tm features when compared with parent molecule data (Figure 4C). For variable temperature experiments targeting antibody-drug conjugates (ADCs), shifts in mAb stability can be tracked as more drug molecules and linkers are conjugated to the protein, which is often progressively destabilized as the drug-to-antibody (DAR) ratio is increased (Figure 4D). For bispecific mAbs, variable temperature MS data is expected to reveal a an intermediate stability relative to the Tm values recorded for parent mAb structures used in its generation (Figure 4E).

3.1.2. Standard and Variable Temperature MS—Clearly, the assessment of mAb primary structure remains a key MS-related task in biotherapeutic discovery and development, as such changes can induce altered therapeutic stability and efficacy profiles.[122,326,327,328,329,330] In concert with these standard MS measurements, variable temperature ESI-MS data is growing in utility in this area.[161] For example, such methods have been used to characterize changes in IgG stability through the detection of both degradation products and non-native disulfide bonds within mAb samples heated within the ESI source.[331] In general, MS-based methods have a central and growing role in assessing mAb HOS and stability.

3.1.3 Footprinting MS—Footprinting techniques such as covalent labelling, HDX, and FPOP provide a direct and granular assessment of antibody stabilities.[332] Covalent labelling measurements can reveal subtle changes to HOS,[333] structural integrity,[334] and antigen interactions.[162] HDX exchange methodologies are well established for the assessment of mAb stability,[335] and are increasingly used to determine elements of biotherapeutic HOS.[336,337] FPOP methods have also demonstrated their general utility in

the characterization mAb HOS,[338,339] and have specifically excelled in the area of epitope mapping.[340,341]

3.1.4 IM-MS and CIU—IM-MS and its associated methods have been shown to be able to differentiate between mAb subclasses based on disulfide bonding patterns,[100,284,342,343] differences between innovators and biosimilars,[323,344–347] glycosylation patterns,[100,296,348] HOS structure associated with domain exchange,[283] and ADC drug loadings.[101] Variable temperature IM-MS, achieved altering the temperature of the IM drift gas, can also be used to detect changes to antibody HOS.[349] In general, IM data has been particularly useful as a probe for subtle changes in HOS that may go undetected by MS alone[350] despite the fact that gas-phase mAbs typically undergo significant collapse in the gas-phase.[351]

In parallel with the above approaches, the capabilities of CIU to capture stability shifts associated with protein domain structure,[283,284,288–292] anion and cation adduction,[293–295] as well as ligand and cofactor binding in mAbs has been well documented.[109,297–300] Additionally, CIU has been shown to be able to track differences in mAb disulfide bonding[100] (Figure 5A), glycosylation patterns,[296] ADC drug loading[101] (Figure 5D), domain exchange[283] (Figure 5A), HDX-uptake,[344] light chain variants,[304] and bispecific stoichiometries[284,307] (Figure 5C), in addition to probing the subtle differences between innovator and biosimilar mAbs[323,346,347,352] (Figure 5B). IM-MS and CIU technologies are currently well positioned to provide information-rich, rapid assessments of mAb HOS across a wide range of therapeutic modalities.

4. Membrane Proteins

Membrane proteins (MPs) are important therapeutic targets which play vital roles in cellular function[353,354], they represent over 60% of therapeutic drug targets and nearly 80% of drugs approved by the FDA act on membrane proteins.[354–357] As the structure of a protein is closely linked to its function, the characterization of membrane proteins is vital to elucidating their involvement in disease and potential druggability.[358] Despite this, membrane proteins are underrepresented in structural databases due to challenges associated with their hydrophobicity and difficulty in obtaining high purity samples. Recent reviews form an excellent resource that outline both the current challenges and promise surrounding membrane protein structural biology.[359–365] Native MS has emerged as a method capable of handling the polydispersity of membrane proteins samples to yield details of their structure and function,[366] this section will discuss how MS has elucidated new information regarding membrane protein stability.

To conserve the native structure of membrane proteins in the absence of a cell membrane, multiple solubilization techniques have been developed. Historically, detergent micelles have been the most popular technique for solubilizing purified membrane proteins and they remain widely used today.[367,368] Detergents vary in structures and charges, but all possess the ability to form a micelle that can encapsulate membrane protein targets. The hydrophobic membrane protein residues interact with the hydrophobic tails of the detergent and the polar head groups of the detergent allow the complex to be solubilized[365,368,369]. Among the many newer approaches to create a lipid bilayer structure in contrast to the single

lipid layer created by micelles, bicelles and nanodiscs are most commonly used[370–372]. In bicelles, detergents are used to surround the edges of the bilayer to create a fluid, yet discrete, bilayer structure[373,374]. Other bilayer-based solubilization techniques include Styrene maleic acid lipid particles (SMALPs)[375,376], amphipols[377], and lipid vesicles or liposomes.[378] The details surrounding membrane mimetics are well covered in a number of recent review articles.[370,379–381]

4.1 Stability Analysis of Membrane Proteins

4.1.1. Mass Spectrometry—Mass spectrometry has recently emerged as a method especially suited for the analysis of native MPs, due in part to its ability to handle complex mixtures and lower sample concentrations.[366,382–390] For the purposes of this work we will focus on ESI (and nESI) methodologies, which dominate the analysis of native protein samples. MP ions generated through ESI or nESI for nMS analysis are most often still encapsulated in one of solubilization agents mentioned above, and therefore collisional activation must be applied to remove bound detergents or lipids from the target membrane protein ion[391]. Additionally, the independent solubilization agents themselves, *e.g.* detergents, also ionize and can thus result in an abundance of noise signals in the resulting native mass spectra. While time-of-flight (TOF) mass analyzers have shown great success in this field, higher resolution technologies, such as Orbitrap mass analyzers[392], can be helpful for resolving the intended membrane protein signal from noise. It is important to note that not all solubilization agents are equally effective in this endeavor, and screening detergents, solution conditions, and optimizing instrument parameters is a necessity for striking the delicate balance between the removal of solubilization agents and optimizing the stability of native MP structure[393]. However, with successful optimization, nMS has been used to study discrete lipid[393–397] and ligand binding[398] (Figure 6 A & F) events, as well as quantifying the thermodynamics associated with lipid binding[399–402] (Figure 6B), and specific protein-protein interactions associated with a wide range of MPs[403–405], and their functional assemblies[374,378,406–409] (Figure 6C).

4.1.2. Footprinting MS—Deeper structural insights can be gained from MP by deploying LC-MS techniques in combination with chemical labeling, where the solvent accessible sites of native MPs are labeled permanently or reversibly prior to digestion. HDX,[410–413] CXL,[414–416] and FPOP,[201,417–419] have all been used to probe MP tertiary structures, as well as the interactions between MPs and both protein binding partners and solubilization agents (Figure 6G). Much of the mechanics of MS-based footprinting tools targeting MPs remain similar to those directed towards water soluble protein systems, and typically provide a valuable readout capable of monitoring the conformational responses of MPs upon stimulation, both temporally and spatially. Among the labeling techniques surveyed here, FPOP has most often been applied to assess MP structure and stability. Favorable attributes of FPOP for MP-associated applications include its fast labeling times, the irreversible nature of the chemical modifications generated, the neutrality of pH maintained during the measurement, and its ability to access non-polar residues for labeling.[206,417]

4.1.3. IM-MS and CIU—The addition of IM separation coupled to MS is beneficial for nMS of MPs. Additionally, IM-MS platforms include supplemental trapping regions that can provide opportunities to perform collisional activation aimed at both the liberation of membrane proteins from their solubilization agents as well as the dissociation of detergent or lipid clusters, which can greatly increase signal quality. The energy experienced by ions in these trapping regions is a function of an accelerating potential (collision voltage, CV), and, ideally, optimized solubilization systems can be removed at relatively low CVs. At CVs higher than the threshold for removal of the solubilization agents, the membrane protein can experience CIU, and this unfolding can be tracked through the resulting IM arrival time distribution[98]. These CIU experiments are valuable for assessing the relative gas phase stability of membrane protein complexes[397,420] and have been used in the past to classify soluble protein systems[109]. The addition of IM to nMS allows for the elucidation on protein stability as a function of lipid and or ligand binding[103,400,420,421] (Figure 6F), amino acid sequence in relationship to disease pathologies[104,409] (Figure 6D), and lipid and or ligand binding locations[103] (Figure 6E).

The study of membrane proteins represents an exciting, high-risk, high-reward area of research with the potential for groundbreaking medical discoveries. Methods to study MP stability in native-like environments are essential to understanding how they perform their cellular functions and, as many MPs are implicated in human disease, how pharmaceuticals may be developed to correct their dysfunction. Due to their insolubility in aqueous solutions and the complex environments in which they exist natively, MPs are challenging analytes, and MS has emerged as frontier tool for determining their stabilities.

5. Amylodogenic Proteins

Amyloidogenic proteins are soluble proteins that can undergo conformational changes that result in the formation of amyloid fibrils which are typically characterized as highly organized states, rich in beta-sheet secondary structure.[422] Amyloids and amyloidogenic proteins are commonly associated with protein misfolding and a wide range of human diseases. The most well-known examples of amyloidogenic proteins include amyloid beta (Aβ) and α-synuclein (α-syn), key proteins associated with Alzheimer's disease (AD) and Parkinson's disease (PD) respectively. AD and PD together account for over 60% cases of dementia and created healthcare cost of over 400 billion USD in 2021 in the U.S.[423,424] However, not all amyloidogenic proteins are disease-related, as some produce functional amyloids that have well-defined physiological roles in many organisms, including in humans, bacteria and an array of animal species.[425] Interestingly, amyloid formation appears to be a general property of proteins. With the sufficient application of heat, mechanical, or pH-based stress, stable proteins, such as lysozymes, can misfold and form amyloid fibers.[426] The stability of amyloidogenic proteins related to human diseases is of great importance, as this characteristic is likely linked to their disaggregation and clearance *in vivo*. In this section, we will focus on how the stabilities of amyloidogenic proteins are studied through MS related techniques.

5.1. MS-related Stability Analysis of Amyloidogenic Proteins

Proteins prone to misfolding and amyloidogenesis present unique challenges in their stability measurements, as such systems aggregate to produce a wide range of oligomers, some of which are insoluble under the conditions designed to promote native protein structures. While most MS tools target only soluble oligomers for analysis, charge detection MS has been used to study the mass distribution and polymorphism of insoluble amyloid fibers.[427] Conventional mass spec measures m/z alone, with high molecular weight ions, the charge must be deducted from the charge state envelope, this resolution is often lacking for very large ions. CDMS allows for the simultaneous detection of m/z and the charge. This allows the centroid mass of individual ions to be calculated regardless of the complexity of the spectral features contained within the data analyzed. In addition, since CDMS allows for direct mass determinations, the technology enables larger ions to be measured than is typically achievable using standard MS technologies. CDMS is especially useful in interrogating the heterogeneous aggregates generated by amyloid proteins during fibril formation. For example, fibrils of Aβ1–42, tau and α-synuclein have been individually assessed by CDMS by Pansieri et al.[428] In combination of TEM data, CDMS mass distribution data revealed two bundles of Aβ1–42 with a low mass of 20 MDa and a high mass of 55 MDa. The authors also reported on the diversity of fibril mass and morphology of tau and α-synuclein.[428] A detailed review on CDMS and its various applications including amyloid proteins has been recently covered by Keifer et al.[429]

In general, native MS can transmit soluble protein oligomers in order to make targeted stability measurements linked to specific variants or ligand-bound oligomers.[430,431] (Figure 7A) Amyloidogenic proteins that natively occupy an assembly state can also be targeted by nMS. For example, amyloid inhibitors targeting the misfolding-prone L55P mutant of TTR have been screened using nMS, where the intensity of the intact tetramer was tracked, alongside subunit exchange dynamics, to assess potential compound efficacy.[432,433]

Identifying and quantifying the olgomeric species present in amyloidogenic protein samples is critically important for assessing the mechanisms surrounding amyloid-associated disease.[434,435] When probed by nMS, amyloidogenic proteins typically produce multimodal CSDs with the lower charge states correlating to compact, native-like conformers, and higher charge states corresponding to partially unfolded forms of the protein. The addition of IM separation prior to nMS has served to help quantify the conformational flexibility of amyloidogenic proteins.[436] (Figure 7B) Interestingly, IM-MS has supported the classification of many amyloidogenic proteins as intrinsically disordered proteins (IDP), due to their polydisperse distributions of ground state structures.[437–439] IM-based CCS information has been used to probe the stability of β-2 microglobulin (β-2m) through a series of titration experiments.[440] Barran *et al.* has reported stability changes associated with both WT and mutated p27 IDR, the disordered region of the cell cycle inhibitory protein p27kip1.[441] The use of IM-MS also enables CIU analysis (Figure 7C).[442] For example, Dong *et al.* employed CIU to track metallothioneins, a group of intrinsically disordered proteins, and their interactions with a series of metals, finding that stability changes can be used to separate the metal associated complexes that are otherwise difficult to distinguish based solely on their IM profiles.[443] Finally, Sanders *et al.* observed that binding of the

chaperon protein β-casein can stabilize α-lactalbumin from forming amyloids using CIU.[444] Overall, nMS, IM-MS, and CIU have provided key stability information for a wide range of amyloidogenic proteins and complexes.

If more localized stability information is desired, HDX-MS allows the exploration of the site-specific conformational status of amyloidogenic proteins (Figure 7D). An increasing number of reports have focused on HDX-based stability values, demonstrating the wide applicability of this technique. For instance, HDX-MS has been used to examine the stability of protofibrils associated with many amyloid-related proteins, including Aβ[445] and β-2m[446]. Moreover, pulsed HDX can capture Aβ stability data on the millisecond timescale, providing unique snapshots of the protein aggregation process. Pulsed HDX-MS is especially useful in studying proteins that undergo rapid aggregation, like $A\beta_{(1–42)}$, where continuous HDX-MS labeling methods have proven challenging to use in the same manner.[447] Lastly, both FPOP and covalent crosslinking can be readily coupled to MS for online evaluation of protein aggregation, and have been covered in depth by Johnson *et al.*[201] and O'Reilly *et al.*[448] respectively.

6. Heat Shock Proteins

Heat shock proteins (HSPs) are a class of molecular chaperones which particularly benefited from the emergence of nMS, and gas-phase methods for assessing protein stability. HSPs are biologically expressed in response to a variety of cellular stressors, and play critical roles in cell survival, preserving cellular homeostasis.[449] A class of smaller HSPs (sHSP) with monomeric molecular masses ranging from 12–42 kDa are largely ATP-independent chaperones which sequester early unfolded intermediates, or misfolded proteins in the cell to prevent aggregation.[450] All sHSPs share a conserved α-crystallin domain (ACD), and these domains interact to first form a dimer which then can be a part of a range of large heterogenous complexes often exceeding 1 MDa.[450] The inherent complexity of these mixtures has precluded their characterization by traditional biophysical techniques, however nMS enables the separation of these complexes via their molecular masses, enabling a detailed dissection of the sHSP assemblies which often coexist in solution. Historically, MS-enabled work in this space has been dominated by three main approaches: Tandem nMS experiments using CID, HDX-MS, and subunit exchange experiments evaluated by MS detection. In recent years, nIM-MS has also emerged for the assessment of sHSP 3D structure, given the heterogeneous nature of the complexes typically encountered in such samples. Most of the studies discussed in the section below integrate multiple MS-based data streams in order to comprehensively probe the stability of HSP complexes.

6.1. Stability Measurements Based on native and HDX-MS

The sHSP α-crystallin (αC) has been investigated extensively by MS leading to a wealth of structure and stability information for this otherwise refractory system. Found primarily in the eye lens, αC is a hetero-oligomer of αB-crystallin (αBC), and αA-crystallin (αAC). Large αC complexes act as ATP-independent chaperones to prevent the non-specific aggregation of β- and γ-crystallins, the main protein components of the eye lens.[451] Initial MS analysis of αC complexes primarily employed nMS and CID to dissect the

extreme complexity of αC samples. Gas phase activation by CID allowed the authors to deconvolute this protein mixture, demonstrating that αC hetero-oligomers ranging from 24-mers to 33-mers (up to ~1 MDa), co-exist in such samples.[452] In the years since, nMS in combination with CID[453] has been used to measure changes in the stabilities of αC complexes in response to post-translational modifications[454,455] or changes in primary sequence.[453,456,457] HDX-MS studies have also played a key role in characterizing the stability of αC complexes in response to heat shock, showing that despite the inherent thermos-stability encoded in sHSPs, αC undergoes a structural transition that weakens protein-protein interfaces when incubated at elevated temperatures.[458] Lastly, using nMS to track subunit exchange within large complexes, a 5 residue C-terminal truncation of one of the two αC subunits was revealed to retard complex formation substantially while also leading to a marked shift in the equilibrium of complex stoichiometry in order to significantly favor even numbered complexes.[453] Further subunit exchange MS experiments explored the varying stabilities of the individual αAC and αBC subunits,[459] as well as their stabilities in heterocomplexes in response to changes in temperature,[460] and pH.[461] Together tandem nMS, HDX, and subunit exchange are responsible for much that is currently understood surrounding αC oligomer structure and stability.

6.2. Other MS-Enabled Stability Measurements

As nMS-based approaches have gained recognition for their ability to characterize αCs, such tools were quickly adopted to characterize sHSP homologues from other organisms. Many of these studies utilize tandem nMS and subunit exchange to evaluate the stabilities sHSPs that contain α-Crystallin-like domains, and further established the mechanism by which these domains dictate sHSP complex formation and stability (Figure 8).[51,234,462–469] Furthermore, variable temperature ESI sources in conjunction with aforementioned approaches have been used to explore the impact of solution-phase temperature increases on the stability of sHSPs.[51,458,462,465] MS was also increasingly applied to the analysis of larger HSPs such as HSP70,[470,471] and HSP90,[472–475] which also oligomerize in a largely mono-disperse format to carry out their biological functions. Recent work has featured IM-MS to characterize the stabilities of sHSPs,[73,76,476–478] as well as larger HSPs,[479] often leveraging CIU[73,76,476,478] to study the gas phase stabilities of such assemblies. Methods such as tandem nMS and subunit exchange have been deployed on IM-MS instruments, providing increased peak capacity and the ability to record CCS values for the ions detected, thus aiding future efforts to model large oligomeric HSP assemblies. The recent release of a cyclic IM-MS (cIM) instrumentation capable of IM^n offers unique opportunities to deconvolution the intricately complex mixtures such as those observed for αC and other sHSP complexes. Overall, MS-based approaches have contributed substantially to our knowledge of HSPs, as well as other chaperones,[79,289,480–482] and these techniques are poised to continue playing a major role in the characterization of such complexes.

7. Conclusions and Future Outlook

Clearly, MS technologies are illuminating many previously hidden facets of protein stability. The range of technologies available, combined with the capabilities of MS to evaluate individual components within complex mixtures on the cellular scale, opens up new avenues

of investigation that will continue to be explored in the coming decades. As MS technology advances, armed with an ever-increasing appreciation for the correlations that exist between native proteins and their desolvated analogues, we can look forward to deeper, more granular assessments of protein stability shifts linked to a detailed view of protein structure. Ultimately, we can expect that MS-driven biophysical probes will give rise to comprehensive catalogs of protein stabilities across the universe of human proteoforms and structural states adopted under a wide range of conditions, ultimately leading to breakthroughs in biochemistry and the treatment of human disease.

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ABBREVIATIONS

Aβ	Amyloid Beta
ACD	α-Crystallin Domain
AD	Alzheimer's disease
ADCs	Antibody drug conjugates
ADH	Alcohol Dehydrogenase
BIRD	blackbody infrared radiative dissociation
CCS	Collision Cross Section
CD	Circular Dichroism
CID	Collision Induced Dissociation
CIU	Collision Induced Unfolding
CSD	Charge State Distribution
ConA	Concanavilin A
CV	Collision Voltage
DSC	Differential Scanning Calorimetry
DSF	Differential Scanning Fluorimetry
DT	Drift Time

ECD	Electron-Capture Dissociation
ESI	Electrospray Ionization
ETD	Electron-Transfer Dissociation
FDA	Food and Drug Administration
FPOP	Fast Photochemical Oxidation of Proteins
hACE2	human Angiotensin-converting Enzyme 2
Hb	Hemoglobin
HDX	Hydrogen Deuterium Exchange
HOS	High Order Structure
HSPs	Heat-Shock Proteins
ITC	Isothermal Titration Calorimetry
IM – MS	Ion Mobility - Mass Spectrometry
IR	Infrared
IRMPD	Infrared Multiphoton Dissociation
LC	Liquid Chromatography
mAb	Monoclonal Antibody
MAM	Multiple Attribute Monitoring
MP	Membrane Protein
MS	Mass Spectrometry
MS-MS	Tandem Mass Spectrometry
NISTmAb	National Institute of Standards and Technology Monoclonal Antibody
NMR	Nuclear Magnetic Resonance
nESI	Nano-electrospray Ionization
nIM-MS	Native Ion Mobility – Mass Spectrometry
nMS	Native Mass Spectrometry
PD	Parkinson’s Disease
PIXY	Platform Incubator with movable XY stage
RRKM	Rice–Ramsperger–Kassel–Marcus

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SASA	Solvent Accessible Surface Area
sHSP	Smaller Heat-Shock Proteins
SID	Surface-Induced Dissociation
SMALPs	Styrene Maleic Acid Lipid Particles
SUPREX	Stability of Unpurified Proteins from Rates of H/D Exchange
PLIMSTEX	Protein–ligand interactions by mass spectrometry, titration, and H/D exchange
TOF	Time-of-Flight
Tm	Melting Temperature
TTR	Transthyretin
vT	Variable Temperature
UV	Ultraviolet
UVPD	Ultraviolet Photodissociation
CXL	Chemical Cross-linking
αAC	αA-crystallin
αBC	αB-crystallin
αC	α-Crystallin
α-syn	α-synuclein

REFERENCES

(1). Eggerth AH; Bellows M The Flocculation of Bacteria by Proteins. J. Gen. Physiol 1922, 4, 669–680. [PubMed: 19871966]

(2). Eggerth AH Changes In The Stability And Potential Of Cell Suspensions I. The Stability And Potential Of Bacterium Coli. J. Gen. Physiol 1923, 6, 63–71. [PubMed: 19872050]

(3). Northrop JH; Kunitz M Crystalline Trypsin I. Isolation And Tests Of Purity. J. Gen. Physiol 1932, 16, 267–294. [PubMed: 19872705]

(4). Northrop JH Crystalline Trypsin Iv. Reversibility Of The Inactivation And Denaturation Of Trypsin By Heat. J. Gen. Physiol 1932, 16, 323–337. [PubMed: 19872708]

(5). Herriott RM Isolation, Crystallization, And Properties Of Swine Pepsinogen. J. Gen. Physiol 1938, 21, 501–540. [PubMed: 19873063]

(6). Kunitz M Crystalline Ribonuclease. J. Gen. Physiol 1940, 24, 15–32. [PubMed: 19873197]

(7). Radding CM; Wald G The Stability Of Rhodopsin And Opsin Effects Of PH And Aging. J. Gen. Physiol 1956, 39, 923–933. [PubMed: 13346045]

(8). Hubbard R The Thermal Stability Of Rhodopsin And Opsin. J. Gen. Physiol 1958, 42, 259–280. [PubMed: 13587911]

(9). Dick YP; Nordwig A Effect of PH on the Stability of the Collagen Fold. Arch. Biochem. Biophys 1966, 117, 466–468. [PubMed: 5972829]

(10). Woodlock AF; Harrap BS The Introduction Of Synthetic Cross-Links Into Tropocollagen And Their Effects On The Thermal Stability Of The Molecule. Int. J. Protein Res 1969, 1, 93–101. [PubMed: 5408673]

(11). Harrap BS The Effect Of Aliphatic Alcohols On The Thermal Stability Of Tropocollagen Under Acidic Conditions. Int. J. Protein Res 1969, 1, 245–252. [PubMed: 5408676]

(12). Rao NV; Harrington WF Relationship between the Positioning of Pyrrolidine Residues and the Stability of Collagen. J. Mol. Biol 1966, 21, 577–581. [PubMed: 19768873]

(13). Cann JR; Cann RL Contribution Of Aromatic Residue Interactions-to The Stability Of Myoglobin V. Enhancement By Aromatic Compounds Of The Rate Of Heat Denaturation. Biophys. J 1970, 10, 297–304. [PubMed: 5461769]

(14). Cann JR Contribution of Aromatic Residue Interactions to the Stability of Myoglobin. IV. Delineation of Binding Forces between Aromatic Compounds and Myoglobin. Biochemistry 1969, 8, 4036–4047. [PubMed: 5346385]

(15). Cann JR Contribution of Aromatic Residue Interactions to the Stability of Myoglobin. II. Enhancement by Aromatic Compounds of the Rate of Urea Denaturation. Biochemistry 1967, 6, 3427–3434. [PubMed: 5624039]

(16). Cann JR Contribution of Aromatic Residue Interactions to the Stability of Myoglobin. III. Molecular Complexes of Aromatic Compounds with Hemin, Hematoporphyrin, and Myoglobin. Biochemistry 1967, 6, 3435–3442. [PubMed: 6073029]

(17). Rieder RF Hemoglobin Stability: Observations on the Denaturation of Normal and Abnormal Hemoglobins by Oxidant Dyes, Heat, and Alkali. J. Clin. Invest 1970, 49, 2369–2376. [PubMed: 5480860]

(18). Delben F; Crescenzi V Thermal Denaturation of Lysozyme. A Differential Scanning Calorimetry Investigation. Biochim. Biophys. Acta - Protein Struct 1969, 194, 615–618.

(19). Delben F; Crescenzi V; Quadrifoglio F A Study Of The Thermal Denaturation Of Ribonuclease By Differential Scanning Calorimetry. Int. J. Protein Res 1969, 1, 145–149. [PubMed: 5408669]

(20). Ledward DA Scanning Calorimetric Studies of Some Protein-Protein Interactions Involving Myoglobin. Meat Sci 1978, 2, 241–249. [PubMed: 22055123]

(21). Donovan JW; Ross KD Increase in the Stability of Avidin Produced by Binding of Biotin. a Differential Scanning Calorimetric Study of Denaturation by Heat. Biochemistry 1973, 12, 512–517. [PubMed: 4683493]

(22). Borebaeck C; Mattiasson B A Study of Structurally Related Binding Properties of Concanavalin A Using Differential Scanning Calorimetry. Eur. J. Biochem 1980, 107, 67–71. [PubMed: 7398639]

(23). Takahashi K; Casey JL; Sturtevant JM Thermodynamics of the Binding of D-Glucose to Yeast Hexokinase. Biochemistry 1981, 20, 4693–4697. [PubMed: 7028093]

(24). Matthews CR; Crisanti MM; Gepner GL; Sturtevant JM; Velicelebi G Effect of Single Amino Acid Substitutions on the Thermal Stability of the α Subunit of Tryptophan Synthase. Biochemistry 1980, 19, 1290–1293. [PubMed: 6992862]

(25). Pfeil W Thermodynamics of α-Lactalbumin Unfolding. Biophys. Chem 1981, 13, 181–186. [PubMed: 7260334]

(26). Bhakuni V; Xie D; Freire E Thermodynamic Identification of Stable Folding Intermediates in the B-Subunit of Cholera Toxin. Biochemistry 1991, 30, 5055–5060. [PubMed: 2036374]

(27). Kuroki R; Kawakita S; Nakamura H; Yutani K Entropic Stabilization of a Mutant Human Lysozyme Induced by Calcium Binding. Proc. Natl. Acad. Sci 1992, 89, 6803–6807. [PubMed: 1495968]

(28). Zolkiewski M; Ginsburg A Thermodynamic Effects of Active-Site Ligands on the Reversible, Partial Unfolding of Dodecameric Glutamine Synthetase from Escherichia Coli: Calorimetric Studies. Biochemistry 1992, 31, 11991–12000. [PubMed: 1360813]

(29). Hamada D; Kidokoro S; Fukada H; Takahashi K; Goto Y Salt-Induced Formation of the Molten Globule State of Cytochrome c Studied by Isothermal Titration Calorimetry. Proc. Natl. Acad. Sci 1994, 91, 10325–10329. [PubMed: 7937949]

(30). Hiraga K; Yutani K A Thermodynamic Analysis of Conformational Change Due to the A2β2 Complex Formation of Tryptophan Synthase. Eur. J. Biochem 1996, 240, 63–70. [PubMed: 8797836]

(31). Jelesarov I; Bosshard HR Isothermal Titration Calorimetry and Differential Scanning Calorimetry as Complementary Tools to Investigate the Energetics of Biomolecular Recognition. J. Mol. Recognit 1999, 12, 3–18. [PubMed: 10398392]

(32). Ferguson RC; Phillips WD High-Resolution Nuclear Magnetic Resonance Spectroscopy. Science (80-.) 1967, 157, 257–267.

(33). Moore GR; Williams RJP The Stability of Ferricytochrome C. Eur. J. Biochem 1980, 103, 523–532. [PubMed: 6244161]

(34). Wüthrich K; Wagner G; Richarz R; Braun W Correlations between Internal Mobility and Stability of Globular Proteins. Biophys. J 1980, 32, 549–560. [PubMed: 7248460]

(35). Burns PD; La Mar GN Characterization of Conformational Heterogeneity in the Heme Pocket of Ferricytochrome c Using High Field Proton Nuclear Magnetic Resonance Spectroscopy. J. Biol. Chem 1981, 256, 4934–4939. [PubMed: 6262309]

(36). Seeholzer SH; Cohn M; Putkey JA; Means AR; Crespi HL NMR Studies of a Complex of Deuterated Calmodulin with Melittin. Proc. Natl. Acad. Sci 1986, 83, 3634–3638. [PubMed: 3459148]

(37). Yutani K; Akutsu H; Ogasahara K; Tsujita T; Kyogoku Y Proton Nuclear Magnetic Resonance Studies on the Wild-Type and Single Amino Acid Substituted Tryptophan Synthase .Alpha.-Subunits. Biochemistry 1987, 26, 5666–5671. [PubMed: 3314995]

(38). Yamashita M; Fenn JB Electrospray Ion Source. Another Variation on the Free-Jet Theme. J. Phys. Chem 1984, 88, 4451–4459.

(39). Meng CK; Mann M; Fenn JB Of Protons or Proteins - “A Beam’s a Beam for a” That.” (O.S. Burns).” Zeitschrift für Phys. D Atoms, Mol. Clust 1988, 10, 361–368.

(40). Fenn JB; Mann M; Meng CK; Wong SF; Whitehouse CM Electrospray Ionization for Mass Spectrometry of Large Biomolecules. Science. 1989, pp 64–71.

(41). Loo JA; Edmonds CG; Smith RD Primary Sequence Information from Intact Proteins by Electrospray Ionization Tandem Mass Spectrometry. Science (80-.) 1990, 248, 201–204.

(42). Chowdhury SK; Katta V; Chait BT Probing Conformational Changes in Proteins by Mass Spectrometry. J. Am. Chem. Soc 1990, 112, 9012–9013.

(43). Loo JA; Edmonds CG; Udseth HR; Smith RD Effect of Reducing Disulfide-Containing Proteins on Electrospray Ionization Mass Spectra. Anal. Chem 1990, 62, 693–698. [PubMed: 2327585]

(44). Loo JA; Loo RRO; Udseth HR; Edmonds CG; Smith RD Solvent-Induced Conformational Changes of Polypeptides Probed by Electrospray-Ionization Mass Spectrometry. Rapid Commun. Mass Spectrom 1991, 5, 101–105. [PubMed: 1666527]

(45). Mirza UA; Cohen SL; Chait BT Heat-Induced Conformational Changes in Proteins Studied by Electrospray Ionization Mass Spectrometry. Anal. Chem 1993, 65, 1–6. [PubMed: 8380538]

(46). El-Baba TJ; Woodall DW; Raab SA; Fuller DR; Laganowsky A; Russell DH; Clemmer DE Melting Proteins: Evidence for Multiple Stable Structures upon Thermal Denaturation of Native Ubiquitin from Ion Mobility Spectrometry-Mass Spectrometry Measurements. J. Am. Chem. Soc 2017, 139, 6306–6309. [PubMed: 28427262]

(47). Woodall DW; El-Baba TJ; Fuller DR; Liu W; Brown CJ; Laganowsky A; Russell DH; Clemmer DE Variable-Temperature ESI-IMS-MS Analysis of Myohemerythrin Reveals Ligand Losses, Unfolding, and a Non-Native Disulfide Bond. Anal. Chem 2019, 91, 6808–6814. [PubMed: 31038926]

(48). Brown CJ; Woodall DW; El-Baba TJ; Clemmer DE Characterizing Thermal Transitions of IgG with Mass Spectrometry. J. Am. Soc. Mass Spectrom 2019, 30, 2438–2445. [PubMed: 31363989]

(49). Woodall DW; Brown CJ; Raab SA; El-Baba TJ; Laganowsky A; Russell DH; Clemmer DE Melting of Hemoglobin in Native Solutions as Measured by IMS-MS. Anal. Chem 2020, 92, 3440–3446. [PubMed: 31990187]

(50). El-Baba TJ; Clemmer DE Solution Thermochemistry of Concanavalin A Tetramer Conformers Measured by Variable-Temperature ESI-IMS-MS. Int. J. Mass Spectrom 2019, 443, 93–100. [PubMed: 32226278]

(51). Sobott F; Benesch JLP; Vierling E; Robinson CV Subunit Exchange of Multimeric Protein Complexes: Real-Time Monitoring of Subunit Exchange between Small Heat Shock Proteins by Using Electrospray Mass Spectrometry. J. Biol. Chem 2002, 277, 38921–38929. [PubMed: 12138169]

(52). Katta V; Chait BT Observation of the Heme-Globin Complex in Native Myoglobin by Electrospray-Ionization Mass Spectrometry. J. Am. Chem. Soc 1991, 25000, 8534–8535.

(53). Ganem B; Li Y-T; Henion JD Observation of Noncovalent Enzyme-Substrate and Enzyme-Product Complexes by Ion-Spray Mass Spectrometry; 1991; Vol. 113.

(54). Ganem B; Li Y-T; Henion JD Detection of Noncovalent Receptor-Ligand Complexes by Mass Spectrometry. J. Am. Chem. Soc 1991, 113, 6294–6296.

(55). Light-Wahl KJ; Winger BE; Smith RD Observation of the Multimeric Forms of Concanavalin A by Electrospray Ionization Mass Spectrometry. J. Am. Chem. Soc 1993, 115, 5869–5870.

(56). Light-Wahl KJ; Schwartz BL; Smith RD Observation of the Noncovalent Quaternary Associations of Proteins by Electrospray Ionization Mass Spectrometry. J. Am. Chem. Soc 1994, 116, 5271–5278.

(57). Wilm M; Mann M Analytical Properties of the Nanoelectrospray Ion Source. Anal. Chem 1996, 68, 1–8. [PubMed: 8779426]

(58). Tahallah N; Pinkse M; Maier CS; Heck AJR The Effect of the Source Pressure on the Abundance of Ions of Noncovalent Protein Assemblies in an Electrospray Ionization Orthogonal Time-of-Flight Instrument. Rapid Commun. Mass Spectrom 2001, 15, 596–601. [PubMed: 11312509]

(59). Sobott F; Hernández H; McCammon MG; Tito MA; Robinson CV A Tandem Mass Spectrometer for Improved Transmission and Analysis of Large Macromolecular Assemblies. Anal. Chem 2002, 74, 1402–1407. [PubMed: 11922310]

(60). Light-Wahl KJ; Schwartz BL; Smith RD Observation of the Noncovalent Quaternary Associations of Proteins by Electrospray Ionization Mass Spectrometry. J. Am. Chem. Soc 1994, 116, 5271–5278.

(61). Ruotolo BT; Giles K; Campuzano I; Sandercock AM; Bateman RH; Robinson CV Biochemistry: Evidence for Macromolecular Protein Rings in the Absence of Bulk Water. Science (80-.) 2005, 310, 1658–1661.

(62). Park AY; Jergic S; Politis A; Ruotolo BT; Hirshberg D; Jessop LL; Beck JL; Barsky D; O'Donnell M; Dixon NE; Robinson CV A Single Subunit Directs the Assembly of the Escherichia Coli DNA Sliding Clamp Loader. Structure 2010, 18, 285–292. [PubMed: 20223211]

(63). Sobott F; Benesch JLP; Vierling E; Robinson CV Subunit Exchange of Multimeric Protein Complexes: Real-Time Monitoring Of Subunit Exchange Between Small Heat Shock Proteins By Using Electrospray Mass Spectrometry. J. Biol. Chem 2002, 277, 38921–38929. [PubMed: 12138169]

(64). Aquilina JA; Benesch JLP; Bateman OA; Slingsby C; Robinson CV Polydispersity of a Mammalian Chaperone: Mass Spectrometry Reveals the Population of Oligomers in AB-Crystallin. Proc. Natl. Acad. Sci 2003, 100, 10611–10616. [PubMed: 12947045]

(65). Lentze N; Aquilina JA; Lindbauer M; Robinson CV; Narberhaus F Temperature and Concentration-Controlled Dynamics of Rhizobial Small Heat Shock Proteins. Eur. J. Biochem 2004, 271, 2494–2503. [PubMed: 15182365]

(66). Aquilina JA; Benesch JLP; Ding LL; Yaron O; Horwitz J; Robinson CV Phosphorylation of AB-Crystallin Alters Chaperone Function through Loss of Dimeric Substructure. J. Biol. Chem 2004, 279, 28675–28680. [PubMed: 15117944]

(67). Aquilina JA; Benesch JLP; Lin LD; Yaron O; Horwitz J; Robinson CV Subunit Exchange of Polydisperse Proteins: MASS SPECTROMETRY REVEALS CONSEQUENCES OF AA-CRYSTALLIN TRUNCATION. J. Biol. Chem 2005, 280, 14485–14491. [PubMed: 15701626]

(68). Phillips JJ; Yao Z. ping; Zhang W; McLaughlin S; Laue ED; Robinson CV; Jackson SE Conformational Dynamics of the Molecular Chaperone Hsp90 in Complexes with a Co-Chaperone and Anticancer Drugs. J. Mol. Biol 2007, 372, 1189–1203. [PubMed: 17764690]

(69). den Engelsman J; Boros S; Dankers PYW; Kamps B; Vree Egberts WT; Böde CS; Lane LA; Aquilina JA; Benesch JLP; Robinson CV; de Jong WW; Boelens WC The Small Heat-Shock Proteins HSPB2 and HSPB3 Form Well-Defined Heterooligomers in a Unique 3 to 1 Subunit Ratio. J. Mol. Biol 2009, 393, 1022–1032. [PubMed: 19715703]

(70). Stengel F; Baldwin AJ; Painter AJ; Jaya N; Basha E; Kay LE; Vierling E; Robinson CV; Benesch JLP Quaternary Dynamics and Plasticity Underlie Small Heat Shock Protein Chaperone Function. Proc. Natl. Acad. Sci 2010, 107, 2007–2012. [PubMed: 20133845]

(71). Erba EB; Ruotolo BT; Barsky D; Robinson CV Ion Mobility-Mass Spectrometry Reveals the Influence of Subunit Packing and Charge on the Dissociation of Multiprotein Complexes. Anal. Chem 2010, 82, 9702–9710. [PubMed: 21053918]

(72). Aquilina JA; Shrestha S; Morris AM; Ecroyd H Structural and Functional Aspects of Hetero-Oligomers Formed by the Small Heat Shock Proteins AB-Crystallin and HSP27. J. Biol. Chem 2013, 288, 13602–13609. [PubMed: 23532854]

(73). Jovcevski B; Kelly MA; Aquilina JA; Benesch JLP; Ecroyd H Evaluating the Effect of Phosphorylation on the Structure and Dynamics of Hsp27 Dimers by Means of Ion Mobility Mass Spectrometry. Anal. Chem 2017, 89, 13275–13282. [PubMed: 29135234]

(74). Lai AL; Clerico EM; Blackburn ME; Patel NA; Robinson CV; Borbat PP; Freed JH; Gierasch LM Key Features of an Hsp70 Chaperone Allosteric Landscape Revealed by Ion-Mobility Native Mass Spectrometry and Double Electron-Electron Resonance. J. Biol. Chem 2017, 292, 8773–8785. [PubMed: 28428246]

(75). Dyachenko A; Tamara S; Heck AJR Distinct Stabilities of the Structurally Homologous Heptameric Co-Chaperonins GroES and Gp31. J. Am. Soc. Mass Spectrom 2019, 30, 7–15. [PubMed: 29736602]

(76). Collier MP; Alderson TR; Villiers C. P. de; Nicholls D; Gastall HY; Allison TM; Degiacomi MT; Jiang H; Mlynek G; Fürst DO; Ven P. F. M. van der; Djinovic-Carugo K; Baldwin AJ; Watkins H; Gehmlich K; Benesch JLP HspB1 Phosphorylation Regulates Its Intramolecular Dynamics and Mechanosensitive Molecular Chaperone Interaction with Filamin C. Sci. Adv 2019, 5, eaav8421. [PubMed: 31131323]

(77). Mitra R; Gadkari VV; Meinen BA; van Mierlo CPM; Ruotolo BT; Bardwell JCA Mechanism of the Small ATP-Independent Chaperone Spy Is Substrate Specific. Nat. Commun 2021, 12, 1–13. [PubMed: 33397941]

(78). Rostom AA; Robinson CV Detection of the Intact GroEL Chaperonin Assembly by Mass Spectrometry. J. Am. Chem. Soc 1999, 121, 4718–4719.

(79). Van Duijn E; Simmons DA; Van Den Heuvel RHH; Bakkes PJ; Van Heerikhuizen H; Heeren RMA; Robinson CV; Van Der Vies SM; Heck AJR Tandem Mass Spectrometry of Intact GroEL - Substrate Complexes Reveals Substrate-Specific Conformational Changes in the Trans Ring. J. Am. Chem. Soc 2006, 128, 4694–4702. [PubMed: 16594706]

(80). Benjamin DR; Robinson CV; Hendrick JP; Hartl FU; Dobson CM Mass Spectrometry of Ribosomes and Ribosomal Subunits. Proc. Natl. Acad. Sci. U. S. A 1998, 95, 7391–7395. [PubMed: 9636159]

(81). Rostom AA; Fucini P; Benjamin DR; Juenemann R; Nierhaus KH; Hartl FU; Dobson CM; Robinson CV Detection and Selective Dissociation of Intact Ribosomes in a Mass Spectrometer. Proc. Natl. Acad. Sci. U. S. A 2000, 97, 5185–5190. [PubMed: 10805779]

(82). McKay AR; Ruotolo BT; Ilag LL; Robinson CV Mass Measurements of Increased Accuracy Resolve Heterogeneous Populations of Intact Ribosomes. J. Am. Chem. Soc 2006, 128, 11433–11442. [PubMed: 16939266]

(83). Tito MA; Tars K; Valegard K; Hajdu J; Robinson CV Electrospray Time-of-Flight Mass Spectrometry of the Intact MS2 Virus Capsid. Journal of the American Chemical Society. April 12, 2000, pp 3550–3551.

(84). Uetrecht C; Barbu IM; Shoemaker GK; Van Duijn E; Heck AJ R. Interrogating Viral Capsid Assembly with Ion Mobility-Mass Spectrometry. Nat. Chem 2011, 3, 126–132. [PubMed: 21258385]

(85). van de Waterbeemd M; Snijder J; Tsvetkova IB; Dragnea BG; Cornelissen JJ; Heck AJR Examining the Heterogeneous Genome Content of Multipartite Viruses BMV and CCMV

by Native Mass Spectrometry. J. Am. Soc. Mass Spectrom 2016, 27, 1000–1009. [PubMed: 26926442]

(86). Hodge EA; Benhaim MA; Lee KK Bridging Protein Structure, Dynamics, and Function Using Hydrogen/Deuterium-Exchange Mass Spectrometry. Protein Sci. 2020, 29, 843–855. [PubMed: 31721348]

(87). Gau BC; Sharp JS; Rempel DL; Gross ML Fast Photochemical Oxidation of Protein Footprints Faster than Protein Unfolding. Anal. Chem 2009, 81, 6563–6571. [PubMed: 20337372]

(88). Sinz A; Arlt C; Chorev D; Sharon M Chemical Cross-Linking and Native Mass Spectrometry: A Fruitful Combination for Structural Biology. Protein Sci. 2015, 24, 1193–1209. [PubMed: 25970732]

(89). Kemper PR; Bowers MT State-Selected Mobilities of Atomic Cobalt Ions. J. Am. Chem. Soc 1990, 112, 3231–3232.

(90). Kemper PR; Bowers MT Electronic-State Chromatography: Application to First-Row Transition-Metal Ions; 1991; Vol. 9.

(91). Kemper PR; Bowers MT A Hybrid Double-Focusing Mass Spectrometer-High-Pressure Drift Reaction Cell to Study Thermal Energy Reactions of Mass-Selected Ions. J. Am. Soc. Mass Spectrom 1990, 1, 197–207.

(92). Von Helden G; Hsu M-T; Kemper PR; Bowers MT Structures of Carbon Cluster Ions from 3 to 60 Atoms: Linears to Rings to Fullerenes. J. Chem. Phys 1991, 95, 3835.

(93). Von Helden G; Kemper PR; Gotts NG; Bowers MT Isomers of Small Carbon Cluster Anions: Linear Chains with up to 20 Atoms. Science (80-.) 1993, 259, 1300–1302.

(94). Bowers MT; Kemper PR; Von Helden G; Van Koppen PAM Gas-Phase Ion Chromatography: Transition Metal State Selection and Carbon Cluster Formation. Science (80-.) 1993, 260, 1446–1451.

(95). Von Helden G; Wyttenbach T; Bowers MT Conformation of Macromolecules in the Gas Phase: Use of Matrix-Assisted Laser Desorption Methods in Ion Chromatography. Science (80-.) 1995, 267, 1483–1485.

(96). Clemmer DE; Hudgins RR; Jarrold MF Naked Protein Conformations: Cytochrome c in the Gas Phase. J. Am. Chem. Soc 1995, 117, 10141–10142.

(97). May JC; McLean JA Ion Mobility-Mass Spectrometry: Time-Dispersive Instrumentation. Anal. Chem 2015, 87, 1422–1436. [PubMed: 25526595]

(98). Dixit SM; Polasky DA; Ruotolo BT Collision Induced Unfolding of Isolated Proteins in the Gas Phase: Past, Present, and Future. Curr. Opin. Chem. Biol 2018, 42, 93–100. [PubMed: 29207278]

(99). Shelimov KB; Clemmer DE; Hudgins RR; Jarrold MF Protein Structure in Vacuo: Gas-Phase Conformations of BPTI and Cytochrome C. J. Am. Chem. Soc 1997, 119, 2240–2248.

(100). Tian Y; Han L; Buckner AC; Ruotolo BT Collision Induced Unfolding of Intact Antibodies: Rapid Characterization of Disulfide Bonding Patterns, Glycosylation, and Structures. Anal. Chem 2015, 87, 11509–11515. [PubMed: 26471104]

(101). Tian Y; Lippens JL; Netirojjanakul C; Campuzano IDG; Ruotolo BT Quantitative Collision-Induced Unfolding Differentiates Model Antibody–Drug Conjugates. Protein Sci 2019, 28, 598–608. [PubMed: 30499138]

(102). Hernandez-Alba O; Wagner-Rousset E; Beck A; Cianférani S Native Mass Spectrometry, Ion Mobility, and Collision-Induced Unfolding for Conformational Characterization of IgG4 Monoclonal Antibodies. Anal. Chem 2018, 90, 8865–8872. [PubMed: 29956914]

(103). Fantin SM; Parson KF; Niu S; Liu J; Polasky DA; Dixit SM; Ferguson-Miller SM; Ruotolo BT Collision Induced Unfolding Classifies Ligands Bound to the Integral Membrane Translocator Protein. Anal. Chem 2019, 91, 38.

(104). Fantin SM; Huang H; Sanders CR; Ruotolo BT Collision-Induced Unfolding Differentiates Functional Variants of the KCNQ1 Voltage Sensor Domain. J. Am. Soc. Mass Spectrom 2020, 01, 13.

(105). Laganowsky A; Reading E; Allison TM; Ulmschneider MB; Degiacomi MT; Baldwin AJ; Robinson CV Membrane Proteins Bind Lipids Selectively to Modulate Their Structure and Function. Nature 2014, 510, 172–175. [PubMed: 24899312]

(106). Liu Y; Cong X; Liu W; Laganowsky A Characterization of Membrane Protein-Lipid Interactions by Mass Spectrometry Ion Mobility Mass Spectrometry. J. Am. Soc. Mass Spectrom 2017, 28.

(107). Liu Y; LoCaste CE; Liu W; Poltash ML; Russell DH; Laganowsky A Selective Binding of a Toxin and Phosphatidylinositides to a Mammalian Potassium Channel. Nat. Commun. 2019 101 2019, 10, 1–9.

(108). Gadkari VV; Ramírez CR; Vallejo DD; Kurulugama RT; Fjeldsted JC; Ruotolo BT Enhanced Collision Induced Unfolding and Electron Capture Dissociation of Native-like Protein Ions. Anal. Chem 2020, 92, 15489–15496. [PubMed: 33166123]

(109). Rabuck JN; Hyung SJ; Ko KS; Fox CC; Soellner MB; Ruotolo BT Activation State-Selective Kinase Inhibitor Assay Based on Ion Mobility-Mass Spectrometry. Anal. Chem 2013, 85, 6995–7002. [PubMed: 23845095]

(110). Jarzab A; Kurzawa N; Hopf T; Moerch M; Zecha J; Leijten N; Bian Y; Musiol E; Maschberger M; Stoehr G; Becher I; Daly C; Samaras P; Mergner J; Spanier B; Angelov A; Werner T; Bantscheff M; Wilhelm M; Klingenspor M; Lemeer S; Liebl W; Hahne H; Savitski MM; Kuster B Meltome Atlas—Thermal Proteome Stability across the Tree of Life. Nat. Methods 2020, 17, 495–503. [PubMed: 32284610]

(111). Johnson CM Differential Scanning Calorimetry as a Tool for Protein Folding and Stability. Arch. Biochem. Biophys 2013, 531, 100–109. [PubMed: 23022410]

(112). Le Basle Y; Chennell P; Tokhadze N; Astier A; Sautou V Physicochemical Stability of Monoclonal Antibodies: A Review. J. Pharm. Sci 2020, 109, 169–190. [PubMed: 31465737]

(113). Joshi V; Shivach T; Yadav N; Rathore AS Circular Dichroism Spectroscopy as a Tool for Monitoring Aggregation in Monoclonal Antibody Therapeutics. Anal. Chem 2014, 86, 11606–11613. [PubMed: 25350583]

(114). Clas S; Dalton C; Hancock B Differential Scanning Calorimetry: Applications in Drug Development. Pharm. Sci. Technolo. Today 1999, 2, 311–320.

(115). Niesen FH; Berglund H; Vedadi M The Use of Differential Scanning Fluorimetry to Detect Ligand Interactions That Promote Protein Stability. Nat. Protoc 2007, 2, 2212–2221. [PubMed: 17853878]

(116). Temel DB; Landsman P; Brader ML Orthogonal Methods for Characterizing the Unfolding of Therapeutic Monoclonal Antibodies: Differential Scanning Calorimetry, Isothermal Chemical Denaturation, and Intrinsic Fluorescence with Concomitant Static Light Scattering, 1st ed.; Elsevier Inc., 2016; Vol. 567.

(117). Purdie N Circular Dichroism and the Conformational Analysis of Biomolecules Edited by Gerald D. Fasman (Brandeis University). Plenum Press: New York. 1996. x + 738 Pp. $125.00. ISBN 0–306-45142–5. J. Am. Chem. Soc 1996, 118, 12871–12871.

(118). Kelly SM; Jess TJ; Price NC How to Study Proteins by Circular Dichroism. Biochim. Biophys. Acta - Proteins Proteomics 2005, 1751, 119–139.

(119). Ionescu RM; Vlasak J; Price C; Kirchmeier M Contribution of Variable Domains to the Stability of Humanized IgG1 Monoclonal Antibodies. J. Pharm. Sci 2008, 97, 1414–1426. [PubMed: 17721938]

(120). Ejima D; Tsumoto K; Fukada H; Yumioka R; Nagase K; Arakawa T; Philo JS Effects of Acid Exposure on the Conformation, Stability, and Aggregation of Monoclonal Antibodies. Proteins Struct. Funct. Bioinforma 2006, 66, 954–962.

(121). Bruylants G; Wouters J; Michaux C Differential Scanning Calorimetry in Life Science: Thermodynamics, Stability, Molecular Recognition and Application in Drug Design. Curr. Med. Chem 2005, 12, 2011–2020. [PubMed: 16101501]

(122). Rathore D; Faustino A; Schiel J; Pang E; Boyne M; Rogstad S The Role of Mass Spectrometry in the Characterization of Biologic Protein Products. Expert Rev. Proteomics 2018, 15, 431–449. [PubMed: 29694790]

(123). Loo JA Observation of Large Subunit Protein Complexes by Electrospray Ionization Mass Spectrometry. J. Mass Spectrom 1995, 30, 180–183.

(124). Robinson CV; Radford SE Weighing the Evidence for Structure: Electrospray Ionization Mass Spectrometry of Proteins. Structure 1995, 3, 861–865. [PubMed: 8535780]

(125). Verbeck G; Hoffmann W; Walton B Soft-Landing Preparative Mass Spectrometry. Analyst 2012, 137, 4393–4407. [PubMed: 22900257]

(126). Mikhailov VA; Mize TH; Benesch JLP; Robinson CV Mass-Selective Soft-Landing of Protein Assemblies with Controlled Landing Energies. Anal. Chem 2014, 86, 8321–8328. [PubMed: 25026391]

(127). Kitova EN; El-Hawiet A; Schnier PD; Klassen JS Reliable Determinations of Protein-Ligand Interactions by Direct ESI-MS Measurements. Are We There Yet? J. Am. Soc. Mass Spectrom 2012, 23, 431–441. [PubMed: 22270873]

(128). Benesch JLP; Ruotolo BT Mass Spectrometry: Come of Age for Structural and Dynamical Biology. Curr. Opin. Struct. Biol 2011, 21, 641–649. [PubMed: 21880480]

(129). Marklund EG; Degiacomi MT; Robinson CV; Baldwin AJ; Benesch JLP Collision Cross Sections for Structural Proteomics. Structure 2015, 23, 791–799. [PubMed: 25800554]

(130). Gabelica V; Shvartsburg AA; Afonso C; Barran P; Benesch JLP; Bleiholder C; Bowers MT; Bilbao A; Bush MF; Campbell JL; Campuzano IDG; Causon T; Clowers BH; Creaser CS; De Pauw E; Far J; Fernandez-Lima F; Fjeldsted JC; Giles K; Groessl M; Hogan CJ; Hann S; Kim HI; Kurulugama RT; May JC; McLean JA; Pagel K; Richardson K; Ridgeway ME; Rosu F; Sobott F; Thalassinos K; Valentine SJ; Wyttenbach T Recommendations for Reporting Ion Mobility Mass Spectrometry Measurements. Mass Spectrom. Rev 2019, 38, 291–320. [PubMed: 30707468]

(131). Dodds JN; Baker ES Ion Mobility Spectrometry: Fundamental Concepts, Instrumentation, Applications, and the Road Ahead. J. Am. Soc. Mass Spectrom 2019, 30, 2185–2195. [PubMed: 31493234]

(132). Benesch JLP; Ruotolo BT; Simmons DA; Robinson CV Protein Complexes in the Gas Phase: Technology for Structural Genomics and Proteomics. Chem. Rev 2007, 107, 3544–3567. [PubMed: 17649985]

(133). Ruotolo BT; Hyung S-JJ; Robinson PM; Giles K; Bateman RH; Robinson CV Ion Mobility-Mass Spectrometry Reveals Long-Lived, Unfolded Intermediates in the Dissociation of Protein Complexes. Angew. Chemie - Int. Ed 2007, 46, 8001–8004.

(134). Heck AJR Native Mass Spectrometry: A Bridge between Interactomics and Structural Biology. Nat. Methods 2008, 5, 927–933. [PubMed: 18974734]

(135). Konijnenberg A; Butterer A; Sobott F Native Ion Mobility-Mass Spectrometry and Related Methods in Structural Biology. Biochim. Biophys. Acta - Proteins Proteomics 2013, 1834, 1239–1256.

(136). Leney AC; Heck AJR Native Mass Spectrometry: What Is in the Name? J. Am. Soc. Mass Spectrom 2017, 28.

(137). Konermann L Addressing a Common Misconception: Ammonium Acetate as Neutral PH “Buffer” for Native Electrospray Mass Spectrometry. J. Am. Soc. Mass Spectrom 2017.

(138). Poltash ML; McCabe JW; Shirzadeh M; Laganowsky A; Russell DH Native IM-Orbitrap MS: Resolving What Was Hidden. TrAC - Trends Anal. Chem 2020, 124, 115533.

(139). Fenn JB; Mann M; Meng CKAI; Wong SF; Whitehouse CM Electrospray Ionization for Mass Spectrometry of Large Biomolecules. Science (80-.) 1989, 246, 64–71.

(140). Konermann L; Ahadi E; Rodriguez AD; Vahidi S Unraveling the Mechanism of Electrospray Ionization. Anal. Chem 2013, 85, 2–9. [PubMed: 23134552]

(141). Wu C; Siems WF; Asbury GR; Hill HH Electrospray Ionization High-Resolution Ion Mobility Spectrometry-Mass Spectrometry. Anal. Chem 1998, 70, 4929–4938. [PubMed: 21644676]

(142). De La Mora JF Electrospray Ionization of Large Multiply Charged Species Proceeds via Dole’s Charged Residue Mechanism. Anal. Chim. Acta 2000, 406, 93–104.

(143). Mann M; Meng CK; Fenn JB Interpreting Mass Spectra of Multiply Charged Ions. Anal. Chem 1989, 61, 1702–1708.

(144). Loo JA; Loo RRO; Udseth HR; Edmonds CG; Smith RD Solvent-induced Conformational Changes of Polypeptides Probed by Electrospray-ionization Mass Spectrometry. Rapid Commun. Mass Spectrom 1991, 5, 101–105. [PubMed: 1666527]

(145). Konermann L; Douglas DJ Acid-Induced Unfolding of Cytochrome c at Different Methanol Concentrations: Electrospray Ionization Mass Spectrometry Specifically Monitors Changes in the Tertiary Structure. Biochemistry 1997, 36, 12296–12302. [PubMed: 9315869]

(146). Clemmer DE; Hudgins RR; Jarrold MF Naked Protein Conformations: Cytochrome c in the Gas Phase. J. Am. Chem. Soc 1995, 117, 10141–10142.

(147). Santambrogio C; Natalello A; Brocca S; Ponzini E; Grandori R Conformational Characterization and Classification of Intrinsically Disordered Proteins by Native Mass Spectrometry and Charge-State Distribution Analysis. Proteomics 2019, 19, 1800060.

(148). Stuchfield D; Barran P Unique Insights to Intrinsically Disordered Proteins Provided by Ion Mobility Mass Spectrometry. Curr. Opin. Chem. Biol 2018, 42, 177–185. [PubMed: 29428839]

(149). Myung S; Badman ER; Lee YJ; Clemmer DE Structural Transitions of Electrosprayed Ubiquitin Ions Stored in an Ion Trap over ~10 Ms to 30 S. J. Phys. Chem. A 2002, 106, 9976–9982.

(150). Jurchen JC; Garcia DE; Williams ER Further Studies on the Origins of Asymmetric Charge Partitioning in Protein Homodimers. J. Am. Soc. Mass Spectrom 2004, 15, 1408–1415. [PubMed: 15465353]

(151). Lermyte F; Łącki MK; Valkenborg D; Gambin A; Sobott F Conformational Space and Stability of ETD Charge Reduction Products of Ubiquitin. J. Am. Soc. Mass Spectrom 2017, 28, 69–76. [PubMed: 27495285]

(152). Mirza UA; Cohen SL; Chait BT Heat-Induced Conformational Changes in Proteins Studied by Electrospray Ionization Mass Spectrometry. Anal. Chem 1993, 65, 1–6. [PubMed: 8380538]

(153). Goodlelt DR; Ogorzalek Loo RR; Loo JA; Wahl JH; Udseth HR; Smith RD A Study of the Thermal Denaturation of Ribonuclease S by Electrospray Ionization Mass Spectrometry. J. Am. Soc. Mass Spectrom 1994, 5, 614–622. [PubMed: 24221963]

(154). Fändrich M; Tito MA; Leroux MR; Rostom AA; Hartl FU; Dobson CM; Robinson CV Observation of the Noncovalent Assembly and Disassembly Pathways of the Chaperone Complex MtGimC by Mass Spectrometry. Proc. Natl. Acad. Sci. U. S. A 2000, 97, 14151–14155. [PubMed: 11087821]

(155). Benesch JLP; Sobott F; Robinson CV Thermal Dissociation of Multimeric Protein Complexes by Using Nanoelectrospray Mass Spectrometry. Anal. Chem 2003, 75, 2208–2214. [PubMed: 12918957]

(156). Wang G; Abzalimov RR; Kaltashov IA Direct Monitoring of Heat-Stressed Biopolymers with Temperature-Controlled Electrospray Ionization Mass Spectrometry. Anal. Chem 2011, 83, 2870–2876. [PubMed: 21417416]

(157). El-Baba TJ; Woodall DW; Raab SA; Fuller DR; Laganowsky A; Russell DH; Clemmer DE Melting Proteins: Evidence for Multiple Stable Structures upon Thermal Denaturation of Native Ubiquitin from Ion Mobility Spectrometry-Mass Spectrometry Measurements. J. Am. Chem. Soc 2017, 139, 6306–6309. [PubMed: 28427262]

(158). El-Baba TJ; Clemmer DE Solution Thermochemistry of Concanavalin A Tetramer Conformers Measured by Variable-Temperature ESI-IMS-MS. Int. J. Mass Spectrom 2019, 443, 93–100. [PubMed: 32226278]

(159). Woodall DW; El-Baba TJ; Fuller DR; Liu W; Brown CJ; Laganowsky A; Russell DH; Clemmer DE Variable-Temperature ESI-IMS-MS Analysis of Myohemerythrin Reveals Ligand Losses, Unfolding, and a Non-Native Disulfide Bond. Anal. Chem 2019, 91, 6808–6814. [PubMed: 31038926]

(160). Raab SA; El-Baba TJ; Woodall DW; Liu W; Liu Y; Baird Z; Hales DA; Laganowsky A; Russell DH; Clemmer DE Evidence for Many Unique Solution Structures for Chymotrypsin Inhibitor 2: A Thermodynamic Perspective Derived from vT-ESI-IMS-MS Measurements. J. Am. Chem. Soc 2020, 142, 17372–17383. [PubMed: 32866376]

(161). McCabe JW; Shirzadeh M; Walker TE; Lin CW; Jones BJ; Wysocki VH; Barondeau DP; Clemmer DE; Laganowsky A; Russell DH Variable-Temperature Electrospray Ionization for Temperature-Dependent Folding/Refolding Reactions of Proteins and Ligand Binding. Anal. Chem 2021, 93, 6924–6931. [PubMed: 33904705]

(162). Limpikirati P; Liu T; Vachet RW Covalent Labeling-Mass Spectrometry with Non-Specific Reagents for Studying Protein Structure and Interactions. Methods 2018, 144, 79–93. [PubMed: 29630925]

(163). Liu XR; Zhang MM; Gross ML Mass Spectrometry-Based Protein Footprinting for Higher-Order Structure Analysis: Fundamentals and Applications. Chem. Rev 2020.

(164). Pan J; Han J; Borchers CH; Konermann L Hydrogen/Deuterium Exchange Mass Spectrometry with Top-down Electron Capture Dissociation for Characterizing Structural Transitions of a 17 KDa Protein. J. Am. Chem. Soc 2009, 131, 12801–12808. [PubMed: 19670873]

(165). Yan X; Watson J; Ho PS; Deinzer ML Mass Spectrometric Approaches Using Electrospray Ionization Charge States and Hydrogen-Deuterium Exchange for Determining Protein Structures and Their Conformational Changes. Mol. Cell. Proteomics 2004, 3, 10–23. [PubMed: 14623985]

(166). Wales TE; Engen JR Hydrogen Exchange Mass Spectrometry for the Analysis of Protein Dynamics. Mass Spectrom. Rev 2006, 25, 158–170. [PubMed: 16208684]

(167). Engen JR; Smithgall TE; Gmeiner WH; Smith DL Identification and Localization of Slow, Natural, Cooperative Unfolding in the Hematopoietic Cell Kinase SH3 Domain by Amide Hydrogen Exchange and Mass Spectrometry. Biochemistry 1997, 36, 14384–14391. [PubMed: 9398156]

(168). Konermann L; Simmons DA Protein-Folding Kinetics and Mechanisms Studied by Pulse-Labeling and Mass Spectrometry. Mass Spectrom. Rev 2003, 22, 1–26. [PubMed: 12768602]

(169). Hamuro Y; Zhang T High-Resolution HDX-MS of Cytochrome c Using Pepsin/Fungal Protease Type XIII Mixed Bed Column. J. Am. Soc. Mass Spectrom 2019, 30, 227–234. [PubMed: 30374663]

(170). Vorauer C; Wrigley MS; Rincon Pabon JP; Watson MJ; Mundorff CC; Weis DD; Guttman M Rapid Assessment of Pepsin Column Activity for Reliable HDX-MS Studies. J. Am. Soc. Mass Spectrom 2021.

(171). Wales TE; Engen JR Hydrogen Exchange Mass Spectrometry for the Analysis of Protein Dynamics. Mass Spectrom. Rev 2006, 25, 158–170. [PubMed: 16208684]

(172). Smith DL; Deng Y; Zhang Z Probing the Non-Covalent Structure of Proteins by Amide Hydrogen Exchange and Mass Spectrometry. J. Mass Spectrom 1997, 32, 135–146. [PubMed: 9102198]

(173). Engen JR; Komives EA Complementarity of Hydrogen/Deuterium Exchange Mass Spectrometry and Cryo-Electron Microscopy. Trends Biochem. Sci 2020, 1–13.

(174). Masson GR; Burke JE; Ahn NG; Anand GS; Borchers C; Brier S; Bou-Assaf GM; Engen JR; Englander SW; Faber J; Garlish R; Griffin PR; Gross ML; Guttman M; Hamuro Y; Heck AJR; Houde D; Iacob RE; Jørgensen TJD; Kaltashov IA; Klinman JP; Konermann L; Man P; Mayne L; Pascal BD; Reichmann D; Skehel M; Snijder J; Strutzenberg TS; Underbakke ES; Wagner C; Wales TE; Walters BT; Weis DD; Wilson DJ; Wintrode PL; Zhang Z; Zheng J; Schriemer DC; Rand KD Recommendations for Performing, Interpreting and Reporting Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS) Experiments. Nat. Methods 2019, 16, 595–602. [PubMed: 31249422]

(175). Katta V; Chait BT Hydrogen/Deuterium Exchange Electrospray Ionization Mass Spectrometry: A Method for Probing Protein Conformational Changes in Solution. J. Am. Chem. Soc 1993, 115, 6317–6321.

(176). Buijs J; Vera CC; Ayala E; Steensma E; Håkansson P; Oscarsson S Conformational Stability of Adsorbed Insulin Studied with Mass Spectrometry and Hydrogen Exchange. Anal. Chem 1999, 71, 3219–3225. [PubMed: 10450163]

(177). Buijs J; Ramström M; Danfelter M; Larsericsdotter H; Håkansson P; Oscarsson S Localized Changes in the Structural Stability of Myoglobin upon Adsorption onto Silica Particles, as Studied with Hydrogen/Deuterium Exchange Mass Spectrometry. J. Colloid Interface Sci 2003, 263, 441–448. [PubMed: 12909033]

(178). Hamuro Y; Derebe MG; Venkataramani S; Nemeth JF The Effects of Intramolecular and Intermolecular Electrostatic Repulsions on the Stability and Aggregation of NISTmAb Revealed by HDX-MS, DSC and NanoDSF. Protein Sci. 2021, No. January, 1–15.

(179). Fang J; Richardson J; Du Z; Zhang Z Effect of Fc-Glycan Structure on the Conformational Stability of IgG Revealed by Hydrogen/Deuterium Exchange and Limited Proteolysis. Biochemistry 2016, 55, 860–868. [PubMed: 26812426]

(180). Narang D; James DA; Balmer MT; Wilson DJ Protein Footprinting, Conformational Dynamics, and Core Interface-Adjacent Neutralization “Hotspots” in the SARS-CoV-2 Spike Protein Receptor Binding Domain/Human ACE2 Interaction. J. Am. Soc. Mass Spectrom 2021.

(181). West GM; Chien EYT; Katritch V; Gatchalian J; Chalmers MJ; Stevens RC; Griffin PR Ligand-Dependent Perturbation of the Conformational Ensemble for the GPCR B2 Adrenergic Receptor Revealed by HDX. Structure 2011, 19, 1424–1432. [PubMed: 21889352]

(182). Demmers JAA; Rijkers DTS; Haverkamp J; Killian JA; Heck AJR Factors Affecting Gas-Phase Deuterium Scrambling in Peptide Ions and Their Implications for Protein Structure Determination. J. Am. Chem. Soc 2002, 124, 11191–11198. [PubMed: 12224967]

(183). Charlebois JP; Patrie SM; Kelleher NL Electron Capture Dissociation and 13C,15N Depletion for Deuterium Localization in Intact Proteins after Solution-Phase Exchange. Anal. Chem 2003, 75, 3263–3266. [PubMed: 12964778]

(184). Wang Q; Borotto NB; Håkansson K Gas-Phase Hydrogen/Deuterium Scrambling in Negative-Ion Mode Tandem Mass Spectrometry. J. Am. Soc. Mass Spectrom 2019, 30, 855–863. [PubMed: 30805882]

(185). Abzalimov RR; Kaplan DA; Easterling ML; Kaltashov IA Protein Conformations Can Be Probed in Top-Down HDX MS Experiments Utilizing Electron Transfer Dissociation of Protein Ions Without Hydrogen Scrambling. J. Am. Soc. Mass Spectrom 2009, 20, 1514–1517. [PubMed: 19467606]

(186). Demmers JAA; Rijkers DTS; Haverkamp J; Killian JA; Heck AJR Factors Affecting Gas-Phase Deuterium Scrambling in Peptide Ions and Their Implications for Protein Structure Determination. J. Am. Chem. Soc 2002, 124, 11191–11198. [PubMed: 12224967]

(187). Pan J; Han J; Borchers CH; Konermann L Characterizing Short-Lived Protein Folding Intermediates by Top-down Hydrogen Exchange Mass Spectrometry. Anal. Chem 2010, 82, 8591–8597. [PubMed: 20849085]

(188). Cryar A; Groves K; Quaglia M Online Hydrogen-Deuterium Exchange Traveling Wave Ion Mobility Mass Spectrometry (HDX-IM-MS): A Systematic Evaluation. J. Am. Soc. Mass Spectrom 2017, 28, 1192–1202. [PubMed: 28374315]

(189). Iacob RE; Murphy III JP; Engen JR Ion Mobility Adds an Additional Dimension to Mass Spectrometric Analysis of Solution-Phase Hydrogen/Deuterium Exchange. Rapid Commun. Mass Spectrom 2008, 22, 2898–2904. [PubMed: 18727141]

(190). Robinson EW; Williams ER Multidimensional Separations of Ubiquitin Conformers in the Gas Phase: Relating Ion Cross Sections to H/D Exchange Measurements. J. Am. Soc. Mass Spectrom 2005, 16, 1427–1437. [PubMed: 16023362]

(191). Molano-Arevalo JC; Jeanne Dit Fouque K; Pham K; Miksovska J; Ridgeway ME; Park MA; Fernandez-Lima F Characterization of Intramolecular Interactions of Cytochrome c Using Hydrogen-Deuterium Exchange-Trapped Ion Mobility Spectrometry-Mass Spectrometry and Molecular Dynamics. Anal. Chem 2017, 89, 8757–8765. [PubMed: 28742962]

(192). Roulhac PL; Powell KD; Dhungana S; Weaver KD; Mietzner TA; Crumbliss AL; Fitzgerald MC SUPREX (Stability of Unpurified Proteins from Rates of H/D Exchange) Analysis of the Thermodynamics of Synergistic Anion Binding by Ferric-Binding Protein (FbpA), a Bacterial Transferring. Biochemistry 2004, 43, 15767–15774. [PubMed: 15595832]

(193). Zhu MM; Chitta R; Gross ML PLIMSTEX: A Novel Mass Spectrometric Method for the Quantification of Protein–Ligand Interactions in Solution. Int. J. Mass Spectrom 2005, 240, 213–220.

(194). McClure RA; Williams JD Impact of Mass Spectrometry-Based Technologies and Strategies on Chemoproteomics as a Tool for Drug Discovery. ACS Med. Chem. Lett 2018, 9, 785–791. [PubMed: 30128068]

(195). Atsavapranee B; Stark CD; Sunden F; Thompson S; Fordyce PM Fundamentals to Function: Quantitative and Scalable Approaches for Measuring Protein Stability. Cell systems. NLM (Medline) June 16, 2021, pp 547–560. [PubMed: 34139165]

(196). Soderblom EJ; Goshe MB Collision-Induced Dissociative Chemical Cross-Linking Reagents and Methodology: Applications to Protein Structural Characterization Using Tandem Mass Spectrometry Analysis. Anal. Chem 2006, 78, 8059–8068. [PubMed: 17134140]

(197). Rappsilber J The Beginning of a Beautiful Friendship: Cross-Linking/Mass Spectrometry and Modelling of Proteins and Multi-Protein Complexes. J. Struct. Biol 2011, 173, 530–540. [PubMed: 21029779]

(198). Samulak BM; Niu S; Andrews PC; Ruotolo BT Ion Mobility-Mass Spectrometry Analysis of Cross-Linked Intact Multiprotein Complexes: Enhanced Gas-Phase Stabilities and Altered Dissociation Pathways. Anal. Chem 2016, 88, 5290–5298. [PubMed: 27078797]

(199). Hanozin E; Grifnée E; Gattuso H; Matagne A; Morsa D; Pauw E De. Covalent Cross-Linking as an Enabler for Structural Mass Spectrometry. Anal. Chem 2019, 91, 12808–12818. [PubMed: 31490660]

(200). Cheung See Kit M; Carvalho VV; Vilseck JZ; Webb IK Gas-Phase Ion/Ion Chemistry for Structurally Sensitive Probes of Gaseous Protein Ion Structure: Electrostatic and Electrostatic to Covalent Cross-Linking. Int. J. Mass Spectrom 2021, 463, 116549. [PubMed: 33716558]

(201). Johnson DT; Di Stefano LH; Jones LM Fast Photochemical Oxidation of Proteins (FPOP): A Powerful Mass Spectrometry–Based Structural Proteomics Tool. J. Biol. Chem 2019, 294, 11969–11979. [PubMed: 31262727]

(202). Xu G; Chance MR Radiolytic Modification of Acidic Amino Acid Residues in Peptidies: Probes for Examining Protein-Protein Interactions. Anal. Chem 2004, 76, 1213–1221. [PubMed: 14987073]

(203). Chen J; Rempel DL; Gross ML Temperature Jump and Fast Photochemical Oxidation Probe Submillisecond Protein Folding. J. Am. Chem. Soc 2010, 132, 15502–15504. [PubMed: 20958033]

(204). Vahidi S; Konermann L Probing the Time Scale of FPOP (Fast Photochemical Oxidation of Proteins): Radical Reactions Extend Over Tens of Milliseconds. J. Am. Soc. Mass Spectrom 2016, 27, 1156–1164. [PubMed: 27067899]

(205). Gau BC; Sharp JS; Rempel DL; Gross ML Fast Photochemical Oxidation of Protein Footprints Faster than Protein Unfolding. Anal. Chem 2009, 81, 6563–6571. [PubMed: 20337372]

(206). Li KS; Shi L; Gross ML Mass Spectrometry-Based Fast Photochemical Oxidation of Proteins (FPOP) for Higher Order Structure Characterization. Acc. Chem. Res 2018, 51, 736–744. [PubMed: 29450991]

(207). Zhang B; Cheng M; Rempel D; Gross ML Implementing Fast Photochemical Oxidation of Proteins (FPOP) as a Footprinting Approach to Solve Diverse Problems in Structural Biology. Methods 2018, 144, 94–103. [PubMed: 29800613]

(208). Sharp JS; Chea EE; Misra SK; Orlando R; Popov M; Egan RW; Holman D; Weinberger SR Flash Oxidation (FOX) System: A Novel Laser-Free Fast Photochemical Oxidation Protein Footprinting Platform. J. Am. Soc. Mass Spectrom 2021.

(209). West GM; Tang L; Fitzgerald MC Thermodynamic Analysis of Protein Stability and Ligand Binding Using a Chemical Modification- and Mass Spectrometry-Based Strategy. Anal. Chem 2008, 80, 4175–4185. [PubMed: 18457414]

(210). Espino JA; Mali VS; Jones LM In Cell Footprinting Coupled with Mass Spectrometry for the Structural Analysis of Proteins in Live Cells. Anal. Chem 2015, 87, 7971–7978. [PubMed: 26146849]

(211). Espino JA; Zhang Z; Jones LM Chemical Penetration Enhancers Increase Hydrogen Peroxide Uptake in C. Elegans for in Vivo Fast Photochemical Oxidation of Proteins. J. Proteome Res 2020, 19, 3708–3715. [PubMed: 32506919]

(212). McLafferty FW Tandem Mass Spectrometry (MS/MS): A Promising New Analytical Technique for Specific Component Determination in Complex Mixtures. Acc. Chem. Res 1980, 13, 33–39.

(213). Brodbelt JS Ion Activation Methods for Peptides and Proteins. Anal. Chem 2016, 88, 30–51. [PubMed: 26630359]

(214). Mitchell Wells J; McLuckey SA Collision-Induced Dissociation (CID) of Peptides and Proteins. Methods Enzymol. 2005, 402, 148–185. [PubMed: 16401509]

(215). Stiving AQ; Vanaernum ZL; Busch F; Harvey SR; Sarni SH; Wysocki VH Surface-Induced Dissociation: An Effective Method for Characterization of Protein Quaternary Structure. Anal. Chem 2019, 91, 190–209. [PubMed: 30412666]

(216). Lermyte F; Valkenborg D; Loo JA; Sobott F Radical Solutions: Principles and Application of Electron-Based Dissociation in Mass Spectrometry-Based Analysis of Protein Structure. Mass Spectrom. Rev 2018, No. April 2017, 1–22.

(217). McLuckey SA; Goeringer DE Slow Heating Methods in Tandem Mass Spectrometry. J. Mass Spectrom 1997, 32, 461–474.

(218). Gross JH Mass Spectrometry; 2017; Vol. 56.

(219). Lifshitz C Recent Developments in Applications of RRKM-QET. Int. J. Mass Spectrom. Ion Process 1992, 118–119, 315–337.

(220). Wu R; McMahon TB Structures, Energetics, and Dynamics of Gas Phaseions Studied by FTICR and HPMS. Mass Spectrom. Rev 2009, 28, 546–585. [PubMed: 19353714]

(221). Felitsyn N; Kitova EN; Klassen JS Thermal Decomposition of a Gaseous Multiprotein Complex Studied by Blackbody Infrared Radiative Dissociation. Investigating the Origin of the Asymmetric Dissociation Behavior. Anal. Chem 2001, 73, 4647–4661. [PubMed: 11605843]

(222). Donor MT; Mroz AM; Prell JS Experimental and Theoretical Investigation of Overall Energy Deposition in Surface-Induced Unfolding of Protein Ions. Chem. Sci 2019, 10, 4097–4106. [PubMed: 31049192]

(223). Donor MT; Shepherd SO; Prell JS Rapid Determination of Activation Energies for Gas-Phase Protein Unfolding and Dissociation in a Q-IM-ToF Mass Spectrometer. J. Am. Soc. Mass Spectrom 2020, 31, 602–610. [PubMed: 32126776]

(224). Atha DH; Riggs A Tetramer Dimer Dissociation in Hemoglobin and the Bohr Effect. J. Biol. Chem 1976, 251, 5537–5543. [PubMed: 9390]

(225). Senear DF; Teller DC Thermodynamics of Concanavalin A Dimer-Tetramer Self-Association: Sedimentation Equilibrium Studies. Biochemistry 1981, 20, 3076–3083. [PubMed: 7248268]

(226). Schwartz BL; Bruce JE; Anderson GA; Steven A; Rockwood AL; Smith RD Dissociation of Tetrameric Ions of Noncovalent Streptavidin Complexes Formed by Electrospray Ionization. 1995.

(227). Tsaprailis G; Somogyi Á; Nikolaev EN; Wysocki VH Refining the Model for Selective Cleavage at Acidic Residues in Arginine-Containing Protonated Peptides. Int. J. Mass Spectrom 2000, 195–196, 467–479.

(228). Wysocki VH; Tsaprailis G; Smith LL; Breci LA Mobile and Localized Protons: A Framework for Understanding Peptide Dissociation. Journal of Mass Spectrometry. 2000.

(229). Jurchen JC; Garcia DE; Williams ER Origin of Asymmetric Charge Partitioning in the Dissociation of Gas-Phase Protein Homodimers. J. Am. Soc. Mass Spectrom 2003, 15, 1408–1415.

(230). Pagel K; Hyung SJ; Ruotolo BT; Robinson CV Alternate Dissociation Pathways Identified in Charge-Reduced Protein Complex Ions. Anal. Chem 2010, 82, 5363–5372. [PubMed: 20481443]

(231). Ruotolo BT; Hyung SJ; Robinson PM; Giles K; Bateman RH; Robinson CV Ion Mobility-Mass Spectrometry Reveals Long-Lived, Unfolded Intermediates in the Dissociation of Protein Complexes. Angew. Chemie - Int. Ed 2007, 46, 8001–8004.

(232). Benesch JLP Collisional Activation of Protein Complexes: Picking Up the Pieces. J. Am. Soc. Mass Spectrom 2009, 20, 341–348. [PubMed: 19110440]

(233). Erba EB; Ruotolo BT; Barsky D; Robinson CV Ion Mobility-Mass Spectrometry Reveals the Influence of Subunit Packing and Charge on the Dissociation of Multiprotein Complexes. Anal. Chem 2010, 82, 9702–9710. [PubMed: 21053918]

(234). Benesch JLP; Aquilina JA; Ruotolo BT; Sobott F; Robinson CV Tandem Mass Spectrometry Reveals the Quaternary Organization of Macromolecular Assemblies. Chem. Biol 2006, 13, 597–605. [PubMed: 16793517]

(235). Hyung S; Robinson CV; Ruotolo BT Gas-Phase Unfolding and Disassembly Reveals Stability Differences in Ligand-Bound Multiprotein Complexes. Chem. Biol 2009, 16, 382–390. [PubMed: 19389624]

(236). Jensen NJ; Tomer KB; Gross ML Gas-Phase Ion Decompositions Occurring Remote to a Charge Site. J. Am. Chem. Soc 1985, 107, 1863–1868.

(237). Palzs B; Suhal S Fragmentation Pathways of Protonated Peptides. Mass Spectrom. Rev 2005, 24, 508–548. [PubMed: 15389847]

(238). Jennings KR The Changing Impact of the Collision-Induced Decomposition of Ions on Mass Spectrometry. Int. J. Mass Spectrom 2000, 200, 479–493.

(239). Hyung SJ; Robinson CV; Ruotolo BT Gas-Phase Unfolding and Disassembly Reveals Stability Differences in Ligand-Bound Multiprotein Complexes. Chem. Biol 2009, 16, 382–390. [PubMed: 19389624]

(240). Bornschein RE; Ruotolo BT Ion Mobility-Mass Spectrometry of Charge-Reduced Protein Complexes Reveals General Trends in the Collisional Ejection of Compact Subunits. Analyst 2015, 140, 7020–7029. [PubMed: 26331159]

(241). Zhou M; Dagan S; Wysocki VH Impact of Charge State on Gas-Phase Behaviors of Noncovalent Protein Complexes in Collision Induced Dissociation and Surface Induced Dissociation. Analyst 2013, 138, 1353–1362. [PubMed: 23324896]

(242). Zhou M; Jones CM; Wysocki VH Dissecting the Large Noncovalent Protein Complex GroEL with Surface-Induced Dissociation and Ion Mobility-Mass Spectrometry. Anal. Chem 2013, 85, 8262–8267. [PubMed: 23855733]

(243). Quintyn RS; Zhou M; Dagan S; Finke J; Wysocki VH Ligand Binding and Unfolding of Tryptophan Synthase Revealed by Ion Mobility-Tandem Mass Spectrometry Employing Collision and Surface Induced Dissociation. Int. J. Ion Mobil. Spectrom 2013, 16, 133–143.

(244). Quintyn RS; Zhou M; Yan J; Wysocki VH Surface-Induced Dissociation Mass Spectra as a Tool for Distinguishing Different Structural Forms of Gas-Phase Multimeric Protein Complexes. Anal. Chem 2015.

(245). Quintyn RS; Yan J; Wysocki VH Surface-Induced Dissociation of Homotetramers with D2 Symmetry Yields Their Assembly Pathways and Characterizes the Effect of Ligand Binding -SI. Chem. Biol 2015, 22, 583–592. [PubMed: 25937312]

(246). Snyder DT; Panczyk EM; Somogyi A; Kaplan DA; Wysocki V Simple and Minimally Invasive SID Devices for Native Mass Spectrometry. Anal. Chem 2020, 92, 11195–11203. [PubMed: 32700898]

(247). Zubarev RA; Kelleher NL; McLafferty FW Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process. Journal of the American Chemical Society. April 8, 1998, pp 3265–3266.

(248). Zubarev RA; Horn DM; Fridriksson EK; Kelleher NL; Kruger NA; Lewis MA; Carpenter BK; McLafferty FW Electron Capture Dissociation for Structural Characterization of Multiply Charged Protein Cations. Anal. Chem 2000, 72, 563–573. [PubMed: 10695143]

(249). Cooper HJ; Håkansson K; Marshall AG The Role of Electron Capture Dissociation in Biomolecular Analysis. Mass Spectrom. Rev 2005, 24, 201–222. [PubMed: 15389856]

(250). Zhurov KO; Fornelli L; Wodrich MD; Laskay ÜA; Tsybin YO Principles of Electron Capture and Transfer Dissociation Mass Spectrometry Applied to Peptide and Protein Structure Analysis. Chem. Soc. Rev 2013, 42, 5014. [PubMed: 23450212]

(251). Zubarev RA; Haselmann KF; Budnik B; Kjeldsen F; Jensen F Towards an Understanding of the Mechanism of Electron-Capture Dissociation: A Historical Perspective and Modern Ideas. Eur. J. Mass Spectrom 2002, 8, 337–349.

(252). Schennach M; Breuker K Probing Protein Structure and Folding in the Gas Phase by Electron Capture Dissociation. J. Am. Soc. Mass Spectrom 2015, 26, 1059–1067. [PubMed: 25868904]

(253). Zhou M; Lantz C; Brown KA; Ge Y; Paša-Tolić L; Loo JA; Lermyte F Higher-Order Structural Characterisation of Native Proteins and Complexes by Top-down Mass Spectrometry. Chem. Sci 2020, 11, 12918–12936. [PubMed: 34094482]

(254). Breuker K; Brüschweiler S; Tollinger M Electrostatic Stabilization of a Native Protein Structure in the Gas Phase. Angew. Chemie - Int. Ed 2011, 50, 873–877.

(255). Schennach M; Breuker K Proteins with Highly Similar Native Folds Can Show Vastly Dissimilar Folding Behavior When Desolvated. Angew. Chemie - Int. Ed 2014, 53, 164–168.

(256). Zhang H; Cui W; Wen J; Blankenship RE; Gross ML Native Electrospray and Electron-Capture Dissociation in Fticr Mass Spectrometry Provide Top-down Sequencing of a Protein Component in an Intact Protein Assembly. J. Am. Soc. Mass Spectrom 2010, 21, 1966–1968. [PubMed: 20843701]

(257). Sun Z; Liu Q; Qu G; Feng Y; Reetz MT Utility of B-Factors in Protein Science: Interpreting Rigidity, Flexibility, and Internal Motion and Engineering Thermostability. Chem. Rev 2019.

(258). Zhang H; Cui W; Gross ML Native Electrospray Ionization and Electron-Capture Dissociation for Comparison of Protein Structure in Solution and the Gas Phase. Int. J. Mass Spectrom 2013, 354–355, 288–291.

(259). Zhang H; Cui W; Wen J; Blankenship RE; Gross ML Native Electrospray and Electron-Capture Dissociation FTICR Mass Spectrometry for Top-down Studies of Protein Assemblies. Anal. Chem 2011, 83, 5598–5606. [PubMed: 21612283]

(260). Cui W; Zhang H; Blankenship RE; Gross ML Electron-Capture Dissociation and Ion Mobility Mass Spectrometry for Characterization of the Hemoglobin Protein Assembly. Protein Sci. 2015, 24, 1325–1332. [PubMed: 26032343]

(261). Lermyte F; Konijnenberg A; Williams JP; Brown JM; Valkenborg D; Sobott F ETD Allows for Native Surface Mapping of a 150 KDa Noncovalent Complex on a Commercial Q-TWIMS-TOF Instrument. J. Am. Soc. Mass Spectrom 2014, 25, 343–350. [PubMed: 24408179]

(262). Harvey SR; Porrini M; Tyler RC; MacPhee CE; Volkman BF; Barran PE Electron Capture Dissociation and Drift Tube Ion Mobility-Mass Spectrometry Coupled with Site Directed Mutations Provide Insights into the Conformational Diversity of a Metamorphic Protein. Phys. Chem. Chem. Phys 2015, 17, 10538–10550. [PubMed: 25805055]

(263). Lermyte F; Sobott F Electron Transfer Dissociation Provides Higher-Order Structural Information of Native and Partially Unfolded Protein Complexes. Proteomics 2015, 15, 2813–2822. [PubMed: 26081219]

(264). Nshanian M; Lantz C; Wongkongkathep P; Schrader T; Klärner FG; Blümke A; Despres C; Ehrmann M; Smet-Nocca C; Bitan G; Loo JA Native Top-Down Mass Spectrometry and Ion Mobility Spectrometry of the Interaction of Tau Protein with a Molecular Tweezer Assembly Modulator. J. Am. Soc. Mass Spectrom 2019, 30, 16–23. [PubMed: 30062477]

(265). Wang H; Eschweiler JD; Cui W; Zhang H; Frieden C; Ruotolo BT; Gross ML Native Mass Spectrometry, Ion Mobility, Electron-Capture Dissociation, and Modeling Provide Structural Information for Gas-Phase Apolipoprotein E Oligomers. J. Am. Soc. Mass Spectrom 2019, 30, 876–885. [PubMed: 30887458]

(266). Santos IC; Brodbelt JS Structural Characterization of Carbonic Anhydrase-Arylsulfonamide Complexes Using Ultraviolet Photodissociation Mass Spectrometry. J. Am. Soc. Mass Spectrom 2021.

(267). Wang H; Eschweiler J; Cui W; Zhang H; Frieden C; Ruotolo BT; Gross ML Native Mass Spectrometry, Ion Mobility, Electron-Capture Dissociation, and Modeling Provide Structural Information for Gas-Phase Apolipoprotein E Oligomers. J. Am. Soc. Mass Spectrom 2019, 30, 876–885. [PubMed: 30887458]

(268). Brodbelt JS; Morrison LJ; Santos I Ultraviolet Photodissociation Mass Spectrometry for Analysis of Biological Molecules. Chem. Rev 2020, 120, 3328–3380. [PubMed: 31851501]

(269). Sipe SN; Brodbelt JS Impact of Charge State on 193 Nm Ultraviolet Photodissociation of Protein Complexes. Phys. Chem. Chem. Phys 2019, 21, 9265–9276. [PubMed: 31016301]

(270). Morrison LJ; Brodbelt JS 193 Nm Ultraviolet Photodissociation Mass Spectrometry of Tetrameric Protein Complexes Provides Insight into Quaternary and Secondary Protein Topology. J. Am. Chem. Soc 2016, 138, 10849–10859. [PubMed: 27480400]

(271). Cammarata M; Thyer R; Lombardo M; Anderson A; Wright D; Ellington A; Brodbelt JS Characterization of Trimethoprim Resistant: E. Coli Dihydrofolate Reductase Mutants by Mass Spectrometry and Inhibition by Propargyl-Linked Antifolates. Chem. Sci 2017, 8, 4062–4072. [PubMed: 29967675]

(272). Crittenden CM; Novelli ET; Mehaffey MR; Xu GN; Giles DH; Fies WA; Dalby KN; Webb LJ; Brodbelt JS Structural Evaluation of Protein/Metal Complexes via Native Electrospray

Ultraviolet Photodissociation Mass Spectrometry. J. Am. Soc. Mass Spectrom 2020, 31, 1140–1150. [PubMed: 32275426]

(273). Cammarata MB; Schardon CL; Mehaffey MR; Rosenberg J; Singleton J; Fast W; Brodbelt JS Impact of G12 Mutations on the Structure of K-Ras Probed by Ultraviolet Photodissociation Mass Spectrometry. J. Am. Chem. Soc 2016, 138, 13187–13196. [PubMed: 27665622]

(274). Mehaffey MR; Schardon CL; Novelli ET; Cammarata MB; Webb LJ; Fast W; Brodbelt JS Investigation of GTP-Dependent Dimerization of G12X K-Ras Variants Using Ultraviolet Photodissociation Mass Spectrometry. Chem. Sci 2019, 10, 8025–8034. [PubMed: 31853358]

(275). Theisen A; Black R; Corinti D; Brown J; Bellina B; Barran P Initial Protein Unfolding Events in Ubiquitin, Cytochrome c and Myoglobin Are Revealed with the Use of 213 Nm UVPD Coupled to IM-MS. J. Am. Soc. Mass Spectrom 2018, 30, 24–33. [PubMed: 29949061]

(276). Cammarata M; Lin KY; Pruet J; Liu HW; Brodbelt JS Probing the Unfolding of Myoglobin and Domain C of PARP-1 with Covalent Labeling and Top-down Ultraviolet Photodissociation Mass Spectrometry. Anal. Chem 2014, 86, 2534–2542. [PubMed: 24484264]

(277). Ben-Nissan G; Sharon M The Application of Ion-Mobility Mass Spectrometry for Structure/Function Investigation of Protein Complexes. Curr. Opin. Chem. Biol 2018, 42, 25–33. [PubMed: 29128665]

(278). Mason EA; McDaniel EW Transport Properties of Ions in Gases; 1988.

(279). Belov ME; Clowers BH; Prior DC; Danielson III WF; Liyu AV; Petritis BO; Smith RD Dynamically Multiplexed Ion Mobility Time-of-Flight Mass Spectrometry. Mol. Cell. Proteomics 2000, 72, 36.

(280). Ruotolo BT; Benesch JLP; Sandercock AM; Hyung S-J; Robinson CV Ion Mobility–Mass Spectrometry Analysis of Large Protein Complexes. Nat. Protoc 2008, 3, 1139–1152. [PubMed: 18600219]

(281). Bush MF; Hall Z; Giles K; Hoyes J; Robinson CV; Ruotolo BT Collision Cross Sections of Proteins and Their Complexes: A Calibration Framework and Database for Gas-Phase Structural Biology. Anal. Chem 2010, 82, 9557–9565. [PubMed: 20979392]

(282). Richardson K; Langridge D; Dixit SM; Ruotolo BT An Improved Calibration Approach for Traveling Wave Ion Mobility Spectrometry: Robust, High-Precision Collision Cross Sections. Anal. Chem 2021, 93, 3542–3550. [PubMed: 33555172]

(283). Watanabe Y; Vasiljevic S; Allen JD; Seabright GE; Duyvesteyn HME; Doores KJ; Crispin M; Struwe WB Signature of Antibody Domain Exchange by Native Mass Spectrometry and Collision-Induced Unfolding. Anal. Chem 2018, 90, 7325–7331. [PubMed: 29757629]

(284). Hernandez-Alba O; Wagner-Rousset E; Beck A; Cianférani S Native Mass Spectrometry, Ion Mobility and Collision Induced Unfolding for Conformational Characterization of IgG4 Monoclonal Antibodies. Anal. Chem 2018, 90, 8865–8872. [PubMed: 29956914]

(285). Debaene F; Bœuf A; Wagner-Rousset E; Colas O; Ayoub D; Corvaïa N; Van Dorsselaer A; Beck A; Cianférani S Innovative Native MS Methodologies for Antibody Drug Conjugate Characterization: High Resolution Native MS and IM-MS for Average DAR and DAR Distribution Assessment. Anal. Chem 2014, 86, 10674–10683. [PubMed: 25270580]

(286). Thompson NJ; Rosati S; Rose RJ; Heck AJR The Impact of Mass Spectrometry on the Study of Intact Antibodies: From Post-Translational Modifications to Structural Analysis. Chem. Commun 2013, 49, 538–548.

(287). Zhang H; Cui W; Gross ML Mass Spectrometry for the Biophysical Characterization of Therapeutic Monoclonal Antibodies. FEBS Lett. 2014, 588, 308–317. [PubMed: 24291257]

(288). Zhong Y; Han L; Ruotolo BT Collisional and Coulombic Unfolding of Gas-Phase Proteins: High Correlation to Their Domain Structures in Solution. Angew. Chemie Int. Ed 2014, 53.

(289). Dyachenko A; Tamara S; Heck AJR Distinct Stabilities of the Structurally Homologous Heptameric Co-Chaperonins GroES and Gp31. J. Am. Soc. Mass Spectrom 2019, 30, 7–15. [PubMed: 29736602]

(290). Chan DSH; Kavanagh ME; McLean KJ; Munro AW; Matak-Vinković D; Coyne AG; Abell C Effect of DMSO on Protein Structure and Interactions Assessed by Collision-Induced Dissociation and Unfolding. Anal. Chem 2017, 89, 9976–9983. [PubMed: 28803470]

(291). Zhang H; Liu H; Lu Y; Wolf NR; Gross ML; Blankenship RE Native Mass Spectrometry and Ion Mobility Characterize the Orange Carotenoid Protein Functional Domains. Biochim. Biophys. Acta - Bioenerg 2016, 1857, 734–739.

(292). Beynon RJ; Armstrong SD; Claydon AJ; Davidson AJ; Eyers CE; Langridge JI; Gómez-Baena G; Harman VM; Hurst JL; Lee V; McLean L; Pattison R; Roberts SA; Simpson DM; Unsworth J; Vonderach M; Williams JP; Woolerton YE Mass Spectrometry for Structural Analysis and Quantification of the Major Urinary Proteins of the House Mouse. Int. J. Mass Spectrom 2015, 391, 146–156.

(293). Han L; Hyung SJ; Ruotolo BT Bound Cations Significantly Stabilize the Structure of Multiprotein Complexes in the Gas Phase. Angew. Chemie - Int. Ed 2012, 51, 5692–5695.

(294). Han L; Hyung SJ; Mayers JJS; Ruotolo BT Bound Anions Differentially Stabilize Multiprotein Complexes in the Absence of Bulk Solvent. J. Am. Chem. Soc 2011, 133, 11358–11367. [PubMed: 21675748]

(295). Wagner ND; Kim D; Russell DH Increasing Ubiquitin Ion Resistance to Unfolding in the Gas Phase Using Chloride Adduction: Preserving More “Native-Like” Conformations Despite Collisional Activation. Anal. Chem 2016, 88, 5934–5940. [PubMed: 27137645]

(296). Tian Y; Ruotolo BT Collision Induced Unfolding Detects Subtle Differences in Intact Antibody Glycoforms and Associated Fragments. Int. J. Mass Spectrom 2018, 425, 1–9.

(297). Zhao B; Zhuang X; Bian X; Liu S; Liu Z; Song F Stabilities of Superoxide Dismutase and Metal-Free Superoxide Dismutase Studied by Electrospray Ionization-Ion Mobility-Mass Spectrometry. Rapid Commun. Mass Spectrom 2019, No. March, 894–896. [PubMed: 30861216]

(298). Huang Y; Salinas ND; Chen E; Tolia NH; Gross ML Native Mass Spectrometry, Ion Mobility, and Collision-Induced Unfolding Categorize Malaria Antigen/Antibody Binding. J. Am. Soc. Mass Spectrom 2017, 28, 2515–2518. [PubMed: 28875466]

(299). Hopper JTS; Oldham NJ Collision Induced Unfolding of Protein Ions in the Gas Phase Studied by Ion Mobility-Mass Spectrometry: The Effect of Ligand Binding on Conformational Stability. J. Am. Soc. Mass Spectrom 2009, 20, 1851–1858. [PubMed: 19643633]

(300). Beveridge R; Migas LG; Payne KAP; Scrutton NS; Leys D; Barran PE Mass Spectrometry Locates Local and Allosteric Conformational Changes That Occur on Cofactor Binding. Nat. Commun 2016, 7, 1–9.

(301). Ehkirch A; Hernandez-Alba O; Colas O; Beck A; Guillarme D; Cianférani S Hyphenation of Size Exclusion Chromatography to Native Ion Mobility Mass Spectrometry for the Analytical Characterization of Therapeutic Antibodies and Related Products. J. Chromatogr. B Anal. Technol. Biomed. Life Sci 2018, 1086, 176–183.

(302). Ehkirch A; Goyon A; Hernandez-Alba O; Rouviere F; D’Atri V; Dreyfus C; Haeuw JF; Diemer H; Beck A; Heinisch S; Guillarme D; Cianferani S A Novel Online Four-Dimensional SEC×SEC-IM×MS Methodology for Characterization of Monoclonal Antibody Size Variants. Anal. Chem 2018, 90, 13929–13937. [PubMed: 30371058]

(303). Deslignière E; Ehkirch A; Botzanowski T; Beck A; Hernandez-Alba O; Cianférani S Toward Automation of Collision-Induced Unfolding Experiments through Online Size Exclusion Chromatography Coupled to Native Mass Spectrometry. Anal. Chem 2020, 92, 12900–12908. [PubMed: 32886492]

(304). Vallejo DD; Polasky DA; Kurulugama RT; Eschweiler JD; Fjeldsted JC; Ruotolo BT A Modified Drift Tube Ion Mobility-Mass Spectrometer for Charge-Multiplexed Collision-Induced Unfolding. Anal. Chem 2019, 91, 8137–8146. [PubMed: 31194508]

(305). Zheng X; Kurulugama RT; Laganowsky A; Russell DH Collision-Induced Unfolding Studies of Proteins and Protein Complexes Using Drift Tube Ion Mobility-Mass Spectrometer. Anal. Chem 2020, 92, 7218–7225. [PubMed: 32338885]

(306). Eldrid C; Ben-Younis A; Ujma J; Britt H; Cragnolini T; Kalfas S; Cooper-Shepherd D; Tomczyk N; Giles K; Morris M; Akter R; Raleigh D; Thalassinos K Cyclic Ion Mobility-Collision Activation Experiments Elucidate Protein Behavior in the Gas Phase. J. Am. Soc. Mass Spectrom 2021.

(307). Botzanowski T; Hernandez-Alba O; Malissard M; Wagner-Rousset E; Deslignière E; Colas O; Haeuw JF; Beck A; Cianférani S Middle Level IM-MS and CIU Experiments for Improved

Therapeutic Immunoglobulin Subclass Fingerprinting. Anal. Chem 2020, 92, 8827–8835. [PubMed: 32453570]

(308). Ghaemmaghami S; Fitzgerald MC; Oas TG A Quantitative, High-Throughput Screen for Protein Stability. Proc. Natl. Acad. Sci. U. S. A 2000, 97, 8296–8301. [PubMed: 10890887]

(309). Becher I; Andrés-Pons A; Romanov N; Stein F; Schramm M; Baudin F; Helm D; Kurzawa N; Mateus A; Mackmull MT; Typas A; Müller CW; Bork P; Beck M; Savitski MM Pervasive Protein Thermal Stability Variation during the Cell Cycle. Cell 2018, 173, 1495–1507.e18. [PubMed: 29706546]

(310). Schopper S; Kahraman A; Leuenberger P; Feng Y; Piazza I; Müller O; Boersema PJ; Picotti P Measuring Protein Structural Changes on a Proteome-Wide Scale Using Limited Proteolysis-Coupled Mass Spectrometry. Nat. Protoc 2017, 12, 2391–2410. [PubMed: 29072706]

(311). Johnson DT; Punshon-Smith B; Espino JA; Gershenson A; Jones LM Implementing In-Cell Fast Photochemical Oxidation of Proteins in a Platform Incubator with a Movable XY Stage. Anal. Chem 2020, 92, 1691–1696. [PubMed: 31860269]

(312). Jones LM Mass Spectrometry-Based Methods for Structural Biology on a Proteome-Wide Scale. Biochem. Soc. Trans 2020, No. May, 945–954. [PubMed: 32412054]

(313). Lu RM; Hwang YC; Liu IJ; Lee CC; Tsai HZ; Li HJ; Wu HC Development of Therapeutic Antibodies for the Treatment of Diseases. Journal of Biomedical Science. BioMed Central Ltd. January 2020, pp 1–30.

(314). Ecker DM; Jones SD; Levine HL The Therapeutic Monoclonal Antibody Market. MAbs 2015, 7, 9–14. [PubMed: 25529996]

(315). Bhattacharya A Immunology Immunoglobulins : Structure and Function. Immunology 2008, 3–28.

(316). Van Beers MMC; Bardor M Minimizing Immunogenicity of Biopharmaceuticals by Controlling Critical Quality Attributes of Proteins. Biotechnol. J 2012, 7, 1473–1484. [PubMed: 23027660]

(317). Goetze AM; Schenauer MR; Flynn GC Assessing Monoclonal Antibody Product Quality Attribute Criticality through Clinical Studies. MAbs 2010, 2, 500–507. [PubMed: 20671426]

(318). Beck A; Wagner-Rousset E; Ayoub D; Van Dorsselaer A; Sanglier-Cianférani S Characterization of Therapeutic Antibodies and Related Products. Anal. Chem 2013, 85, 715–736. [PubMed: 23134362]

(319). Guddat LW; Herron JN; Edmundson AB Three-Dimensional Structure of a Human Immunoglobulin with a Hinge Deletion. Proc. Natl. Acad. Sci. U. S. A 1993, 90, 4271–4275. [PubMed: 8483943]

(320). Saphire EO; Parren PWHI; Pantophlet R; Zwick MB; Morris GM; Rudd PM; Dwek RA; Stanfield RL; Burton DR; Wilson IA Crystal Structure of a Neutralizing Human IgG against HIV-1: A Template for Vaccine Design. Science (80-.) 2001, 293, 1155–1159.

(321). Harris LJ; Skaletsky E; McPherson A Crystallographic Structure of an Intact IgG1 Monoclonal Antibody. J. Mol. Biol 1998, 275, 861–872. [PubMed: 9480774]

(322). Harris LJ; Larson SB; Hasel KW; McPherson A Refined Structure of an Intact IgG2a Monoclonal Antibody. Biochemistry 1997, 36, 1581–1597. [PubMed: 9048542]

(323). Beck A; Debaene F; Diemer H; Wagner-Rousset E; Colas O; Dorsselaer A. Van; Cianférani S Cutting-Edge Mass Spectrometry Characterization of Originator, Biosimilar and Biobetter Antibodies. Journal of Mass Spectrometry. 2015, pp 285–297. [PubMed: 25800010]

(324). Williams JG; Tomer KB Disposable Chromatography for a High-Throughput Nano-ESI/MS and Nano-ESI/MS-MS Platform. J. Am. Soc. Mass Spectrom 2004, 15, 1333–1340. [PubMed: 15337514]

(325). Sokolowska I; Mo J; Rahimi Pirkolachahi F; Mcvean C; Meijer LAT; Switzar L; Balog C; Lewis MJ; Hu P Implementation of a High-Resolution Liquid Chromatography-Mass Spectrometry Method in Quality Control Laboratories for Release and Stability Testing of a Commercial Antibody Product. Anal. Chem 2020, 92, 2369–2373. [PubMed: 31869206]

(326). Jiskoot W; Beuvery EC; de Koning AAM; Herron JN; Crommelin DJA Analytical Approaches to the Study of Monoclonal Antibody Stability. Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists. 1990, pp 1234–1241.

(327). Fornelli L; Srzentić K; Huguet R; Mullen C; Sharma S; Zabrouskov V; Fellers RT; Durbin KR; Compton PD; Kelleher NL Accurate Sequence Analysis of a Monoclonal Antibody by Top-Down and Middle-Down Orbitrap Mass Spectrometry Applying Multiple Ion Activation Techniques. Anal. Chem 2018, acs.analchem.8b00984.

(328). Melani RD; Srzentić K; Gerbasi VR; McGee JP; Huguet R; Fornelli L; Kelleher NL Direct Measurement of Light and Heavy Antibody Chains Using Ion Mobility and Middle-down Mass Spectrometry. MAbs 2019, 11, 1351–1357. [PubMed: 31607219]

(329). Donnelly DP; Rawlins CM; DeHart CJ; Fornelli L; Schachner LF; Lin Z; Lippens JL; Aluri KC; Sarin R; Chen B; Lantz C; Jung W; Johnson KR; Koller A; Wolff JJ; Campuzano IDG; Auclair JR; Ivanov AR; Whitelegge JP; Paša-Tolić L; Chamot-Rooke J; Danis PO; Smith LM; Tsybin YO; Loo JA; Ge Y; Kelleher NL; Agar JN Best Practices and Benchmarks for Intact Protein Analysis for Top-down Mass Spectrometry. Nat. Methods 2019, 16, 587–594. [PubMed: 31249407]

(330). Srzentić K; Fornelli L; Tsybin YO; Loo JA; Seckler H; Agar JN; Anderson LC; Bai DL; Beck A; Brodbelt JS; van der Burgt YEM; Chamot-Rooke J; Chatterjee S; Chen Y; Clarke DJ; Danis PO; Diedrich JK; D'Ippolito RA; Dupré M; Gasilova N; Ge Y; Goo YA; Goodlett DR; Greer S; Haselmann KF; He L; Hendrickson CL; Hinkle JD; Holt MV; Hughes S; Hunt DF; Kelleher NL; Kozhinov AN; Lin Z; Malosse C; Marshall AG; Menin L; Millikin RJ; Nagornov KO; Nicolardi S; Paša-Tolić L; Pengelley S; Quebbemann NR; Resemann A; Sandoval W; Sarin R; Schmitt ND; Shabanowitz J; Shaw JB; Shortreed MR; Smith LM; Sobott F; Suckau D; Toby T; Weisbrod CR; Wildburger NC; Yates JR; Yoon SH; Young NL; Zhou M Interlaboratory Study for Characterizing Monoclonal Antibodies by Top-Down and Middle-Down Mass Spectrometry. J. Am. Soc. Mass Spectrom 2020, 31, 1783–1802. [PubMed: 32812765]

(331). Brown CJ; Woodall DW; El-Baba TJ; Clemmer DE Characterizing Thermal Transitions of IgG with Mass Spectrometry. J. Am. Soc. Mass Spectrom 2019, 30, 2438–2445. [PubMed: 31363989]

(332). Tremblay CY; Limpikirati P; Vachet RW Complementary Structural Information for Stressed Antibodies from Hydrogen-Deuterium Exchange and Covalent Labeling Mass Spectrometry. J. Am. Soc. Mass Spectrom 2021, 32, 1237–1248. [PubMed: 33886284]

(333). Limpikirati P; Hale JE; Hazelbaker M; Huang Y; Jia Z; Yazdani M; Graban EM; Vaughan RC; Vachet RW Covalent Labeling and Mass Spectrometry Reveal Subtle Higher Order Structural Changes for Antibody Therapeutics. MAbs 2019, 11, 463–476. [PubMed: 30636503]

(334). Limpikirati PK; Zhao B; Pan X; Eyles SJ; Vachet RW Covalent Labeling/Mass Spectrometry of Monoclonal Antibodies with Diethylpyrocarbonate: Reaction Kinetics for Ensuring Protein Structural Integrity. J. Am. Soc. Mass Spectrom 2020, 31, 1223–1232. [PubMed: 32310649]

(335). Wei H; Mo J; Tao L; Russell RJ; Tymiak AA; Chen G; Iacob RE; Engen JR Hydrogen/Deuterium Exchange Mass Spectrometry for Probing Higher Order Structure of Protein Therapeutics: Methodology and Applications. Drug Discovery Today. 2014.

(336). Masson GR; Jenkins ML; Burke JE An Overview of Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS) in Drug Discovery. Expert Opin. Drug Discov 2017, 12, 981–994. [PubMed: 28770632]

(337). Huang RYC; Chen G Higher Order Structure Characterization of Protein Therapeutics by Hydrogen/Deuterium Exchange Mass Spectrometry. Anal. Bioanal. Chem 2014, 406, 6541–6558. [PubMed: 24948090]

(338). Li KS; Shi L; Gross ML Mass Spectrometry-Based Fast Photochemical Oxidation of Proteins (FPOP) for Higher Order Structure Characterization. Acc. Chem. Res 2018, 51, 736–744. [PubMed: 29450991]

(339). Zhang Y; Rempel DL; Zhang H; Gross ML An Improved Fast Photochemical Oxidation of Proteins (FPOP) Platform for Protein Therapeutics. J. Am. Soc. Mass Spectrom 2015, 26, 526–529. [PubMed: 25519854]

(340). Yan Y; Chen G; Wei H; Huang RYC; Mo J; Rempel DL; Tymiak AA; Gross ML Fast Photochemical Oxidation of Proteins (FPOP) Maps the Epitope of EGFR Binding to Adnectin. J. Am. Soc. Mass Spectrom 2014, 25, 2084–2092. [PubMed: 25267085]

(341). Jones LM; Sperry JB; Carroll JA; Gross ML Fast Photochemical Oxidation of Proteins for Epitope Mapping. Anal. Chem 2011, 83, 7657–7661. [PubMed: 21894996]

(342). Bagal D; Valliere-Douglass JF; Balland A; Schnier PD Resolving Disulfide Structural Isoforms of IgG2 Monoclonal Antibodies by Ion Mobility Mass Spectrometry. Anal. Chem 2010, 82, 6751–6755. [PubMed: 20704363]

(343). Debaene F; Wagner-Rousset E; Colas O; Ayoub D; Corvaïa N; Van Dorsselaer A; Beck A; Cianférani S Time Resolved Native Ion-Mobility Mass Spectrometry to Monitor Dynamics of IgG4 Fab Arm Exchange and "Bispecific" Monoclonal Antibody Formation. Anal. Chem 2013, 85, 9785–9792. [PubMed: 24007193]

(344). Upton R; Migas LG; Pacholarz KJ; Beniston RG; Estdale S; Firth D; Barran PE Hybrid Mass Spectrometry Methods Reveal Lot-to-Lot Differences and Delineate the Effects of Glycosylation on the Tertiary Structure of Herceptin®. Chem. Sci 2019, 10, 2811–2820. [PubMed: 30997002]

(345). Pisupati K; Tian Y; Okbazghi S; Benet A; Ackermann R; Ford M; Saveliev S; Hosfield CM; Urh M; Carlson E; Becker C; Tolbert TJ; Schwendeman SP; Ruotolo BT; Schwendeman A A Multidimensional Analytical Comparison of Remicade and the Biosimilar Remsima. Anal. Chem 2017, 89, 4838–4846. [PubMed: 28365979]

(346). Kang J; Kim SY; Vallejo D; Hageman TS; White DR; Benet A; Coghlan J; Sen KI; Ford M; Saveliev S; Tolbert TJ; Weis DD; Schwendeman SP; Ruotolo BT; Schwendeman A Multifaceted Assessment of Rituximab Biosimilarity: The Impact of Glycan Microheterogeneity on Fc Function. Eur. J. Pharm. Biopharm 2020, 146, 111–124. [PubMed: 31841688]

(347). Kang J; Halseth T; Vallejo D; Najafabadi ZI; Sen KI; Ford M; Ruotolo BT; Schwendeman A Assessment of Biosimilarity under Native and Heat-Stressed Conditions: Rituximab, Bevacizumab, and Trastuzumab Originators and Biosimilars. Anal. Bioanal. Chem 2020, 412, 763–775. [PubMed: 31853605]

(348). Atmanene C; Chaix D; Bessin Y; Declerck N; Van Dorsselaer A; Sanglier-Cianferani S Combination of Noncovalent Mass Spectrometry and Traveling Wave Ion Mobility Spectrometry Reveals Sugar-Induced Conformational Changes of Central Glycolytic Genes Repressor/DNA Complex. Anal. Chem 2010, 82, 3597–3605. [PubMed: 20361740]

(349). Pacholarz KJ; Peters SJ; Garlish RA; Henry AJ; Taylor RJ; Humphreys DP; Barran PE Molecular Insights into the Thermal Stability of MAbs with Variable-Temperature Ion-Mobility Mass Spectrometry. ChemBioChem 2016, 17, 46–51. [PubMed: 26534882]

(350). Campuzano IDG; Larriba C; Bagal D; Schnier PD Ion Mobility and Mass Spectrometry Measurements of the Humanized IgGk NIST Monoclonal Antibody. ACS Symp. Ser 2015, 1202, 75–112.

(351). Hansen K; Lau AM; Giles K; McDonnell JM; Struwe WB; Sutton BJ; Politis A A Mass-Spectrometry-Based Modelling Workflow for Accurate Prediction of IgG Antibody Conformations in the Gas Phase. Angew. Chemie - Int. Ed 2018, 57, 17194–17199.

(352). Pisupati K; Benet A; Tian Y; Okbazghi S; Kang J; Ford M; Saveliev S; Sen KI; Carlson E; Tolbert TJ; Ruotolo BT; Schwendeman SP; Schwendeman A Biosimilarity under Stress: A Forced Degradation Study of Remicade® and Remsima™. MAbs 2017, 9, 1197–1209. [PubMed: 28787231]

(353). Wallin E; Heijne G Von. Genome-Wide Analysis of Integral Membrane Proteins from Eubacterial, Archaean, and Eukaryotic Organisms. Protein Sci. 2008, 7, 1029–1038.

(354). Fagerberg L; Jonasson K; von Heijne G; Uhlén M; Berglund L Prediction of the Human Membrane Proteome. Proteomics 2010, 10, 1141–1149. [PubMed: 20175080]

(355). Overington JP; Al-Lazikani B; Hopkins AL How Many Drug Targets Are There? Nat. Rev. Drug Discov 2006, 5, 993–996. [PubMed: 17139284]

(356). Yıldırım MA; Goh K-I; Cusick ME; Barabási A-L; Vidal M Drug—Target Network. Nat. Biotechnol 2007, 25, 1119–1126. [PubMed: 17921997]

(357). Santos R; Ursu O; Gaulton A; Bento AP; Donadi RS; Bologa CG; Karlsson A; Al-Lazikani B; Hersey A; Oprea TI; Overington JP A Comprehensive Map of Molecular Drug Targets. Nat. Rev. Drug Discov 2016, 16, 19–34. [PubMed: 27910877]

(358). Dowhan W; Vitrac H; Bogdanov Mikhail. Lipid-Assisted Membrane Protein Folding and Topogenesis. Protein J 1234, 38, 274–288.

(359). AE R Membrane Protein Engineering to the Rescue. Biochem. Soc. Trans 2018, 46, 1541–1549. [PubMed: 30381335]

(360). Rawlings AE Membrane Proteins: Always an Insoluble Problem? Biochem. Soc. Trans 2016, 44, 790–795. [PubMed: 27284043]

(361). SA K; D S Dynamic Protein Interfaces and Conformational Landscapes of Membrane Protein Complexes. Curr. Opin. Struct. Biol 2020, 61, 191–197. [PubMed: 32036279]

(362). H Y; AD F Drugging Membrane Protein Interactions. Annu. Rev. Biomed. Eng 2016, 18, 51–76. [PubMed: 26863923]

(363). Gong J; Chen Y; Pu F; Sun P; He F; Zhang L; Li Y; Ma Z; Wang H Understanding Membrane Protein Drug Targets in Computational Perspective. Curr. Drug Targets 2018, 20, 551–564.

(364). Vinothkumar KR; Henderson R Structures of Membrane Proteins. Q. Rev. Biophys 2010, 43, 65. [PubMed: 20667175]

(365). Moraes I; Evans G; Sanchez-Weatherby J; Newstead S; Stewart PDS Membrane Protein Structure Determination - The next Generation. Biochim. Biophys. Acta - Biomembr 2014, 1838, 78–87.

(366). Bender J; Schmidt C Mass Spectrometry of Membrane Protein Complexes. Biol. Chem 2019, 400, 813–829. [PubMed: 30956223]

(367). Lacapère J-J; Pebay-Peyroula E; Neumann J-M; Etchebest C Determining Membrane Protein Structures: Still a Challenge! Trends Biochem. Sci 2007, 32, 259–270. [PubMed: 17481903]

(368). Haffke M; Duckely M; Bergsdorf C; Jaakola VP; Shrestha B Development of a Biochemical and Biophysical Suite for Integral Membrane Protein Targets: A Review. Protein Expr. Purif 2020, 167, 105545. [PubMed: 31778786]

(369). Cheng X; Kim J-K; Kim Y; Bowie JU; Im W Molecular Dynamics Simulation Strategies for Protein-Micelle Complexes. Biochim. Biophys. Acta 2016, 1858, 1566. [PubMed: 26679426]

(370). Warschawski DE; Arnold AA; Beaugrand M; Gravel A; Chartrand É; Marcotte I Choosing Membrane Mimetics for NMR Structural Studies of Transmembrane Proteins. Biochim. Biophys. Acta - Biomembr 2011, 1808, 1957–1974.

(371). Cross TA; Sharma M; Yi M; Zhou H-X Influence of Solubilizing Environments on Membrane Protein Structures. Trends Biochem. Sci 2011, 36, 117. [PubMed: 20724162]

(372). Lyukmanova EN; Shenkarev ZO; Khabibullina NF; Kopeina GS; Shulepko MA; Paramonov AS; Mineev KS; Tikhonov RV; Shingarova LN; Petrovskaya LE; Dolgikh DA; Arseniev AS; Kirpichnikov MP Lipid–Protein Nanodiscs for Cell-Free Production of Integral Membrane Proteins in a Soluble and Folded State: Comparison with Detergent Micelles, Bicelles and Liposomes. Biochim. Biophys. Acta - Biomembr 2012, 1818, 349–358.

(373). Dürr UHN; Gildenberg M; Ramamoorthy A The Magic of Bicelles Up Membrane Protein Structure. Chem. Rev 2012, 112, 6054. [PubMed: 22920148]

(374). Hutchison JM; Shih KC; Scheidt HA; Fantin SM; Parson KF; Pantelopulos GA; Harrington HR; Mittendorf KF; Qian S; Stein RA; Collier SE; Chambers MG; Katsaras J; Voehler MW; Ruotolo BT; Huster D; McFeeters RL; Straub JE; Nieh MP; Sanders CR Bicelles Rich in Both Sphingolipids and Cholesterol and Their Use in Studies of Membrane Proteins. J. Am. Chem. Soc 2020, 142, 12715–12729. [PubMed: 32575981]

(375). Lyons JA; Bøggild A; Nissen P; Frauenfeld J Saposin-Lipoprotein Scaffolds for Structure Determination of Membrane Transporters. Methods Enzymol 2017, 594, 85–99. [PubMed: 28779844]

(376). Jamshad M; Grimard V; Idini I; Knowles TJ; Dowle MR; Schofield N; Sridhar P; Lin Y; Finka R; Wheatley M; Thomas ORT; Palmer RE; Overduin M; Govaerts C; Ruysschaert JM; Edler KJ; Dafforn TR Structural Analysis of a Nanoparticle Containing a Lipid Bilayer Used for Detergent-Free Extraction of Membrane Proteins. Nano Res. 2015.

(377). Marty MT; Hoi KK; Robinson CV Interfacing Membrane Mimetics with Mass Spectrometry. Acc. Chem. Res 2016, 49, 2459–2467. [PubMed: 27736086]

(378). Chorev DS; Tang H; Rouse SL; Reddy Bolla J; Von Kügelgen A; Baker LA; Wu D; Gault J; Grünewald K; Bharat TAM; Matthews SJ; Robinson CV The Use of Sonicated Lipid Vesicles for Mass Spectrometry of Membrane Protein Complexes. Nat. Protoc

(379). Zhou H-X; Cross TA Influences of Membrane Mimetic Environments on Membrane Protein Structures. Annu. Rev. Biophys 2013, 42, 361–392. [PubMed: 23451886]

(380). Bayburt TH; Sligar SG Membrane Protein Assembly into Nanodiscs. FEBS Lett. 2010, 584, 1721–1727. [PubMed: 19836392]

(381). Nath A; Atkins WM; Sligar SG Applications of Phospholipid Bilayer Nanodiscs in the Study of Membranes and Membrane Proteins. Biochemistry 2007, 46, 2059–2069. [PubMed: 17263563]

(382). Barrera NP; Robinson CV Advances in the Mass Spectrometry of Membrane Proteins: From Individual Proteins to Intact Complexes. Annu. Rev. Biochem 2011, 80, 247–71. [PubMed: 21548785]

(383). Marty MT; Hoi KK; Robinson CV Interfacing Membrane Mimetics with Mass Spectrometry. Acc. Chem. Res 2016, 49, 2459–2467. [PubMed: 27736086]

(384). Barrera NP; Isaacson SC; Zhou M; Bavro VN; Welch A; Schaedler TA; Seeger MA; Miguel RN; Korkhov VM; van Veen HW; Venter H; Walmsley AR; Tate CG; Robinson CV Mass Spectrometry of Membrane Transporters Reveals Subunit Stoichiometry and Interactions. Nat. Methods 2009 68 2009, 6, 585–587.

(385). AJ B; DJ H; CV R Detergent Release Prolongs the Lifetime of Native-like Membrane Protein Conformations in the Gas-Phase. J. Am. Chem. Soc 2013, 135, 6078–6083. [PubMed: 23521660]

(386). Barrera NP; Zhou M; Robinson CV The Role of Lipids in Defining Membrane Protein Interactions: Insights from Mass Spectrometry. Trends Cell Biol. 2013, 23, 1–8. [PubMed: 22980035]

(387). Hellwig N; Peetz O; Ahdash Z; Tascó I; Booth PJ; Mikusevic V; Diskowski M; Politis A; Hellmich Y; Hä I; Reading E; Morgner N Native Mass Spectrometry Goes More Native: Investigation of Membrane Protein Complexes Directly from SMALPs †. Chem. Commun 2018, 54, 13702.

(388). Laganowsky A; Reading E; Hopper JTS; Robinson CV Mass Spectrometry of Intact Membrane Protein Complexes. Nat. Protoc 2013.

(389). Robinson CV From Molecular Chaperones to Membrane Motors: Through the Lens of a Mass Spectrometrist. Biochem. Soc. Trans 2017, 45, 251–260. [PubMed: 28202679]

(390). Calabrese AN; Radford SE Mass Spectrometry-Enabled Structural Biology of Membrane Proteins. Methods 2018.

(391). Borysik AJ; Hewitt DJ; Robinson CV Detergent Release Prolongs the Lifetime of Native-like Membrane Protein Conformations in the Gas-Phase. J. Am. Chem. Soc 2013, 135, 6078–6083. [PubMed: 23521660]

(392). Campuzano IDG; Li H; Bagal D; Lippens JL; Svitel J; Kurzeja RJM; Xu H; Schnier PD; Loo JA Native MS Analysis of Bacteriorhodopsin and an Empty Nanodisc by Orthogonal Acceleration Time-of-Flight, Orbitrap and Ion Cyclotron Resonance. Anal. Chem 2016, 88.

(393). Laganowsky A; Reading E; Hopper JTS; Robinson CV Mass Spectrometry of Intact Membrane Protein Complexes. Nat. Protoc 2013, 8, 639–651. [PubMed: 23471109]

(394). I L; MT D; S L; TD N; J G; E R; JTS H; NG H; P W; M C; A S; BA W; C K; PJ S; H B; JLP B; TM A; CV R Lipid Binding Attenuates Channel Closure of the Outer Membrane Protein OmpF. Proc. Natl. Acad. Sci. U. S. A 2018, 115, 6691–6696. [PubMed: 29891712]

(395). Kliman M; May JC; McLean JA Lipid Analysis and Lipidomics by Structurally Selective Ion Mobility-Mass Spectrometry. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 2011, 1811, 935–945.

(396). Gault J; Liko I; Landreh M; Shutin D; Reddy Bolla J; Jefferies D; Agasid M; Yen H-Y; G W Ladds MJ; Lane DP; Khalid S; Mullen C; Remes PM; Huguet R; McAlister G; Goodwin M; Viner R; Syka JE; Robinson CV Combining Native and 'Omics' Mass Spectrometry to Identify Endogenous Ligands Bound to Membrane Proteins. Nat. Methods

(397). Bolla JR; Agasid MT; Mehmood S; Robinson CV Membrane Protein–Lipid Interactions Probed Using Mass Spectrometry. 10.1146/annurev-biochem-013118-111508 2019, 88, 85–111.

(398). Gault J; Donlan JAC; Liko I; Hopper JTS; Gupta K; Housden NG; Struwe WB; Marty MT; Mize T; Bechara C; Zhu Y; Wu B; Kleanthous C; Belov M; Damoc E; Makarov A; Robinson CV High-Resolution Mass Spectrometry of Small Molecules Bound to Membrane Proteins Europe PMC Funders Group. Nat Methods 2016, 13, 333–336. [PubMed: 26901650]

(399). Cong X; Liu Y; Liu W; Liang X; Russell DH; Laganowsky A Determining Membrane Protein−Lipid Binding Thermodynamics Using Native Mass Spectrometry.

(400). Bechara CC; Robinson CV Different Modes of Lipid Binding to Membrane Proteins Probed by Mass Spectrometry. J. Am. Chem. Soc 2015, 137, 44.

(401). Cong X; Liu Y; Liu W; Liang X; Laganowsky A Allosteric Modulation of Protein-Protein Interactions by Individual Lipid Binding Events. Nat. Commun 2017, 8, 2203. [PubMed: 29259178]

(402). ZM M; JD Z; WA D; JS P Gas-Phase Protonation Thermodynamics of Biological Lipids: Experiment, Theory, and Implications. Anal. Chem 2020, 92, 10365–10374. [PubMed: 32628014]

(403). Keener JE; Zambrano DE; Zhang G; Zak CK; Reid DJ; Deodhar BS; Pemberton JE; Prell JS; Marty MT Chemical Additives Enable Native Mass Spectrometry Measurement of Membrane Protein Oligomeric State within Intact Nanodiscs. J. Am. Chem. Soc 2019, 141, 1054–1061. [PubMed: 30586296]

(404). Henrich E; Peetz O; Hein C; Laguerre A; Hoffmann B; Hoffmann J; Dö tsch V; Bernhard F; Morgner N Analyzing Native Membrane Protein Assembly in Nanodiscs by Combined Non-Covalent Mass Spectrometry and Synthetic Biology. Elife 2017, 6, 1–19.

(405). Sipe SN; Patrick JW; Laganowsky A; Brodbelt JS Enhanced Characterization of Membrane Protein Complexes by Ultraviolet Photodissociation Mass Spectrometry. Anal. Chem 2020, 92, 899. [PubMed: 31765130]

(406). Reading E; Hall Z; Martens C; Haghighi T; Findlay H; Ahdash Z; Politis A; Booth PJ Interrogating Membrane Protein Conformational Dynamics within Native Lipid Compositions. Angew. Chemie Int. Ed 2017, 56, 15654–15657.

(407). E R; TA W; I L; MT M; A L; DC R; CV R The Effect of Detergent, Temperature, and Lipid on the Oligomeric State of MscL Constructs: Insights from Mass Spectrometry. Chem. Biol 2015, 22, 593–603. [PubMed: 26000747]

(408). Konijnenberg A; Bannwarth L; Yilmaz D; Koçer A; Venien-Bryan C; Sobott F Top-down Mass Spectrometry of Intact Membrane Protein Complexes Reveals Oligomeric State and Sequence Information in a Single Experiment. Protein Sci. 2015, 24, 1292–1300. [PubMed: 25970171]

(409). SM F; KF P; P Y; B J; GC L; CR S; MD O; BT R Ion Mobility-Mass Spectrometry Reveals the Role of Peripheral Myelin Protein Dimers in Peripheral Neuropathy. Proc. Natl. Acad. Sci. U. S. A 2021, 118.

(410). Khakinejad M; Ghassabi Kondalaji S; Donohoe GC; Valentine SJ Ion Mobility Spectrometry-Hydrogen Deuterium Exchange Mass Spectrometry of Anions: Part 3. Estimating Surface Area Exposure by Deuterium Uptake. J. Am. Soc. Mass Spectrom 2016.

(411). Lau AM; Jia R; Bradshaw RT; Politis A Structural Predictions of the Functions of Membrane Proteins from HDX-MS. Biochem. Soc. Trans 2020, 48, 971. [PubMed: 32597490]

(412). Giladi M; Khananshvili D Hydrogen-Deuterium Exchange Mass-Spectrometry of Secondary Active Transporters: From Structural Dynamics to Molecular Mechanisms. Front. Pharmacol 2020, 0, 70.

(413). DP O; V H; A C; S B Hydrogen/Deuterium Exchange Mass Spectrometry for the Structural Analysis of Detergent-Solubilized Membrane Proteins. Methods Mol. Biol 2020, 2127, 339–358. [PubMed: 32112332]

(414). Weerasekera R; Schmitt-Ulms G Crosslinking Strategies for the Study of Membrane Protein Complexes and Protein Interaction Interfaces. Biotechnol. Genet. Eng. Rev 2006, 23, 41–62. [PubMed: 22530501]

(415). Debelyy MO; Waridel P; Quadroni M; Schneiter R; Conzelmann A Chemical Crosslinking and Mass Spectrometry to Elucidate the Topology of Integral Membrane Proteins. PLoS One 2017, 12.

(416). Corgiat BA; Nordman JC; Kabbani N Chemical Crosslinkers Enhance Detection of Receptor Interactomes. Front. Pharmacol 2014, 0, 171.

(417). Watkinson TG; Calabrese AN; Ault JR; Radford SE; Ashcroft AE FPOP-LC-MS/MS Suggests Differences in Interaction Sites of Amphipols and Detergents with Outer Membrane Proteins. J. Am. Soc. Mass Spectrom 2017, 28, 50–55. [PubMed: 27343183]

(418). Lu Y; Zhang H; Niedzwiedzki DM; Jiang J; Blankenship RE; Gross ML Fast Photochemical Oxidation of Proteins Maps the Topology of Intrinsic Membrane Proteins: Light-Harvesting Complex 2 in a Nanodisc. Anal. Chem 2016, 88, 8827–8834. [PubMed: 27500903]

(419). Zhou F; Yang Y; Chemuru S; Cui W; Liu S; Gross M; Li W Footprinting Mass Spectrometry of Membrane Proteins: Ferroportin Reconstituted in Saposin A Picodiscs. Anal. Chem 2021, acs.analchem.1c02325.

(420). Liu Y; Cong X; Liu W; Laganowsky A Characterization of Membrane Protein–Lipid Interactions by Mass Spectrometry Ion Mobility Mass Spectrometry. J. Am. Soc. Mass Spectrom 2017, 28, 579–586. [PubMed: 27924494]

(421). Sobott F; Konijnenberg A; Van Dyck JF; Kailing LL Extending Native Mass Spectrometry Approaches to Integral Membrane Proteins. Biol. Chem 2015, 396, 991–1002. [PubMed: 26352204]

(422). Chiti F; Dobson CM Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. Annu. Rev. Biochem 2017, 86, 27–68. [PubMed: 28498720]

(423). 2019 Alzheimer's Disease Facts and Figures. Alzheimers Dement 2019, 15, 321–387.

(424). Boiles A Parkinson's Disease Economic Burden on Patients, Families and the Federal Government Is $52 Billion, Doubling Previous Estimates | Parkinson's Disease https://www.michaeljfox.org/publication/parkinsons-disease-economic-burden-patients-families-and-federal-government-52-billion (accessed 2022 -01 -24).

(425). DM F; AV K; WE B; JW K Functional Amyloid--from Bacteria to Humans. Trends Biochem. Sci 2007, 32, 217–224. [PubMed: 17412596]

(426). Cao A; Hu D; Lai L Formation of Amyloid Fibrils from Fully Reduced Hen Egg White Lysozyme. Protein Sci. 2004, 13, 319–324. [PubMed: 14718651]

(427). Todd AR; Barnes LF; Young K; Zlotnick A; Jarrold MF Higher Resolution Charge Detection Mass Spectrometry. Anal. Chem 2020, 92, 11357–11364. [PubMed: 32806905]

(428). Pansieri J; Halim MA; Vendrely C; Dumoulin M; Legrand F; Sallanon MM; Chierici S; Denti S; Dagany X; Dugourd P; Marquette C; Antoine R; Forge V Mass and Charge Distributions of Amyloid Fibers Involved in Neurodegenerative Diseases: Mapping Heterogeneity and Polymorphism. Chem. Sci 2018, 9, 2791–2796. [PubMed: 29732065]

(429). Keifer DZ; Pierson EE; Jarrold MF Charge Detection Mass Spectrometry: Weighing Heavier Things. Analyst 2017, 142, 1654–1671. [PubMed: 28443838]

(430). Ashcroft AE Mass Spectrometry and the Amyloid Problem—How Far Can We Go in the Gas Phase? J. Am. Soc. Mass Spectrom 2010, 21, 1087–1096. [PubMed: 20363648]

(431). Nettleton EJ; Tito P; Sunde M; Bouchard M; Dobson CM; Robinson CV Characterization of the Oligomeric States of Insulin in Self-Assembly and Amyloid Fibril Formation by Mass Spectrometry. Biophys. J 2000, 79, 1053–1065. [PubMed: 10920035]

(432). McCammon MG; Scott DJ; Keetch CA; Greene LH; Purkey HE; Petrassi HM; Kelly JW; Robinson CV Screening Transthyretin Amyloid Fibril Inhibitors. Structure 2002, 10, 851–863. [PubMed: 12057199]

(433). Keetch CA; Bromley EHC; McCammon MG; Wang N; Christodoulou J; Robinson CV L55P Transthyretin Accelerates Subunit Exchange and Leads to Rapid Formation of Hybrid Tetramers. J. Biol. Chem 2005, 280, 41667–41674. [PubMed: 16219761]

(434). Sengupta U; Nilson AN; Kayed R The Role of Amyloid-β Oligomers in Toxicity, Propagation, and Immunotherapy. EBioMedicine 2016, 6, 42. [PubMed: 27211547]

(435). Laganowsky A; Liu C; Sawaya MR; Whitelegge JP; Park J; Zhao M; Pensalfini A; Soriaga AB; Landau M; Teng PK; Cascio D; Glabe C; Eisenberg D Atomic View of a Toxic Amyloid Small Oligomer. Science (80-.) 2012, 335, 1228–1231.

(436). Frimpong AK; Abzalimov RR; Uversky VN; Kaltashov IA Characterization of Intrinsically Disordered Proteins with Electrospray Ionization Mass Spectrometry: Conformational Heterogeneity of α-Synuclein. Proteins Struct. Funct. Bioinforma 2009, 78, NA-NA.

(437). C V; MI I; S S; B H; M P Characterization of Oligomerization-Aggregation Products of Neurodegenerative Target Proteins by Ion Mobility Mass Spectrometry. Methods Mol. Biol 2012, 896, 399–412. [PubMed: 22821540]

(438). Young LM; Cao P; Raleigh DP; Ashcroft AE; Radford SE Ion Mobility–Mass Spectrometry the Oligomeric Intermediates in Amylin Amyloid Formation andthe Mode of Action of Inhibitors. J. Am. Chem. Soc 2014, 136, 660. [PubMed: 24372466]

(439). Smith DP; Radford SE; Ashcroft AE Elongated Oligomers in B2-Microglobulin Amyloid Assembly Revealed by Ion Mobility Spectrometry-Mass Spectrometry. Proc. Natl. Acad. Sci 2010, 107, 6794–6798. [PubMed: 20351246]

(440). Smith DP; Giles K; Bateman RH; Radford SE; Ashcroft AE Monitoring Copopulated Conformational States During Protein Folding Events Using Electrospray Ionization-Ion Mobility Spectrometry-Mass Spectrometry. J. Am. Soc. Mass Spectrom 2007, 18, 2180–2190. [PubMed: 17964800]

(441). Beveridge R; Migas LG; Das RK; Pappu RV; Kriwacki RW; Barran PE Ion Mobility Mass Spectrometry Uncovers the Impact of the Patterning of Oppositely Charged Residues on the Conformational Distributions of Intrinsically Disordered Proteins. J. Am. Chem. Soc 2019, 141, 4908–4918. [PubMed: 30823702]

(442). Tian Y; Ruotolo BT The Growing Role of Structural Mass Spectrometry in the Discovery and Development of Therapeutic Antibodies. Analyst 2018, 143, 2459–2468. [PubMed: 29736508]

(443). Dong S; Wagner ND; Russell DH Collision-Induced Unfolding of Partially Metalated Metallothionein-2A: Tracking Unfolding Reactions of Gas-Phase Ions. Anal. Chem 2018, 90, 11856–11862. [PubMed: 30221929]

(444). Sanders HM; Jovcevski B; Carver JA; Pukala TL The Molecular Chaperone β-Casein Prevents Amorphous and Fibrillar Aggregation of α-Lactalbumin by Stabilisation of Dynamic Disorder. Biochem. J 2020, 477, 629. [PubMed: 31939601]

(445). Kheterpal Indu; Lashuel Hilal A.; Hartley Dean M.; Walz Thomas; Lansbury Peter T. Jr. and; Wetzel Ronald. Aβ Protofibrils Possess a Stable Core Structure Resistant to Hydrogen Exchange†. Biochemistry 2003, 42, 14092–14098. [PubMed: 14640676]

(446). Hoshino M; Katou H; Hagihara Y; Hasegawa K; Naiki H; Goto Y Mapping the Core of the β 2-Microglobulin Amyloid Fibril by H/D Exchange. Nat. Struct. Biol. 2002 95 2002, 9, 332–336.

(447). Zhang Y; Rempel DL; Zhang J; Sharma AK; Mirica LM; Gross ML Pulsed Hydrogen–Deuterium Exchange Mass Spectrometry Probes Conformational Changes in Amyloid Beta (Aβ) Peptide Aggregation. Proc. Natl. Acad. Sci 2013, 110, 14604–14609. [PubMed: 23959898]

(448). O'Reilly FJ; Rappsilber J Cross-Linking Mass Spectrometry: Methods and Applications in Structural, Molecular and Systems Biology. Nat. Struct. Mol. Biol. 2018 2511 2018, 25, 1000–1008.

(449). Lang BJ; Guerrero ME; Prince TL; Okusha Y; Bonorino C; Calderwood SK The Functions and Regulation of Heat Shock Proteins; Key Orchestrators of Proteostasis and the Heat Shock Response. Arch. Toxicol. 2021 956 2021, 95, 1943–1970.

(450). Reinle K; Mogk A; Bukau B The Diverse Functions of Small Heat Shock Proteins in the Proteostasis Network. J. Mol. Biol 2021, 167157. [PubMed: 34271010]

(451). Hayashi J; Carver JA The Multifaceted Nature of AB-Crystallin. Cell Stress Chaperones 2020, 25, 639–654. [PubMed: 32383140]

(452). Aquilina JA; Benesch JLP; Bateman OA; Slingsby C; Robinson CV Polydispersity of a Mammalian Chaperone: Mass Spectrometry Reveals the Population of Oligomers in AB-Crystallin. Proc. Natl. Acad. Sci 2003, 100, 10611–10616. [PubMed: 12947045]

(453). Aquilina JA; Benesch JLP; Lin LD; Yaron O; Horwitz J; Robinson CV Subunit Exchange of Polydisperse Proteins: MASS SPECTROMETRY REVEALS CONSEQUENCES OF AA-CRYSTALLIN TRUNCATION. J. Biol. Chem 2005, 280, 14485–14491. [PubMed: 15701626]

(454). Aquilina JA; Benesch JLP; Ding LL; Yaron O; Horwitz J; Robinson CV Phosphorylation of AB-Crystallin Alters Chaperone Function through Loss of Dimeric Substructure. J. Biol. Chem 2004, 279, 28675–28680. [PubMed: 15117944]

(455). Ecroyd H; Meehan S; Horwitz J; Aquilina JA; Benesch JLP; Robinson CV; Macphee CE; Carver JA Mimicking Phosphorylation of AB-Crystallin Affects Its Chaperone Activity. Biochem. J 2007, 401, 129–141. [PubMed: 16928191]

(456). Treweek TM; Rekas A; Lindner RA; Walker MJ; Aquilina JA; Robinson CV; Horwitz J; Perng M. Der; Quinlan RA; Carver JA R120G AB-Crystallin Promotes the Unfolding of Reduced α-Lactalbumin and Is Inherently Unstable. FEBS J 2005, 272, 711–724. [PubMed: 15670152]

(457). Jovcevski B; Andrew Aquilina J; Benesch JLP; Ecroyd H The Influence of the N-Terminal Region Proximal to the Core Domain on the Assembly and Chaperone Activity of AB-Crystallin. Cell Stress Chaperones 2018 235 2018, 23, 827–836.

(458). Hasan A; Yu J; Smith DL; Smith JB Thermal Stability of Human α-Crystallins Sensed by Amide Hydrogen Exchange. Protein Sci. 2004, 13, 332–341. [PubMed: 14739319]

(459). Morris AM; Aquilina JA Evidence for Specific Subunit Distribution and Interactions in the Quaternary Structure of α-Crystallin. Proteins Struct. Funct. Bioinforma 2010, 78, 2546–2553.

(460). Aquilina JA; Shrestha S; Morris AM; Ecroyd H Structural and Functional Aspects of Hetero-Oligomers Formed by the Small Heat Shock Proteins AB-Crystallin and HSP27. J. Biol. Chem 2013, 288, 13602–13609. [PubMed: 23532854]

(461). Baldwin AJ; Lioe H; Robinson CV; Kay LE; Benesch JLP AB-Crystallin Polydispersity Is a Consequence of Unbiased Quaternary Dynamics. J. Mol. Biol 2011, 413, 297–309. [PubMed: 21839090]

(462). Lentze N; Aquilina JA; Lindbauer M; Robinson CV; Narberhaus F Temperature and Concentration-Controlled Dynamics of Rhizobial Small Heat Shock Proteins. Eur. J. Biochem 2004, 271, 2494–2503. [PubMed: 15182365]

(463). Painter AJ; Jaya N; Basha E; Vierling E; Robinson CV; Benesch JLP Real-Time Monitoring of Protein Complexes Reveals Their Quaternary Organization and Dynamics. Chem. Biol 2008, 15, 246–253. [PubMed: 18355724]

(464). den Engelsman J; Boros S; Dankers PYW; Kamps B; Vree Egberts WT; Böde CS; Lane LA; Aquilina JA; Benesch JLP; Robinson CV; de Jong WW; Boelens WC The Small Heat-Shock Proteins HSPB2 and HSPB3 Form Well-Defined Heterooligomers in a Unique 3 to 1 Subunit Ratio. J. Mol. Biol 2009, 393, 1022–1032. [PubMed: 19715703]

(465). Benesch JLP; Aquilina JA; Baldwin AJ; Rekas A; Stengel F; Lindner RA; Basha E; Devlin GL; Horwitz J; Vierling E; Carver JA; Robinson CV The Quaternary Organization and Dynamics of the Molecular Chaperone HSP26 Are Thermally Regulated. Chem. Biol 2010, 17, 1008–1017. [PubMed: 20851350]

(466). Stengel F; Baldwin AJ; Painter AJ; Jaya N; Basha E; Kay LE; Vierling E; Robinson CV; Benesch JLP Quaternary Dynamics and Plasticity Underlie Small Heat Shock Protein Chaperone Function. Proc. Natl. Acad. Sci 2010, 107, 2007–2012. [PubMed: 20133845]

(467). Basha E; Jones C; Blackwell AE; Cheng G; Waters ER; Samsel KA; Siddique M; Pett V; Wysocki V; Vierling E An Unusual Dimeric Small Heat Shock Protein Provides Insight into the Mechanism of This Class of Chaperones. J. Mol. Biol 2013, 425, 1683–1696. [PubMed: 23416558]

(468). Heirbaut M; Lermyte F; Martin EM; Beelen S; Verschueren T; Sobott F; Strelkov SV; Weeks SD The Preferential Heterodimerization of Human Small Heat Shock Proteins HSPB1 and HSPB6 Is Dictated by the N-Terminal Domain. Arch. Biochem. Biophys 2016, 610, 41–50. [PubMed: 27717639]

(469). Thangaraj SK; James S; Rouvinen J; Jänis J Thermokinetic Analysis of Protein Subunit Exchange by Variable-Temperature Native Mass Spectrometry. Biochemistry 2019, 58, 5025–5029. [PubMed: 31790206]

(470). Morgner N; Schmidt C; Beilsten-Edmands V; Ebong IO; Patel NA; Clerico EM; Kirschke E; Daturpalli S; Jackson SE; Agard D; Robinson CV Hsp70 Forms Antiparallel Dimers Stabilized by Post-Translational Modifications to Position Clients for Transfer to Hsp90. Cell Rep. 2015, 11, 759–769. [PubMed: 25921532]

(471). Trcka F; Durech M; Vankova P; Chmelik J; Martinkova V; Hausner J; Kadek A; Marcoux J; Klumpler T; Vojtesek B; Muller P; Man P Human Stress-Inducible Hsp70 Has a High Propensity to Form ATP-Dependent Antiparallel Dimers That Are Differentially Regulated by Cochaperone Binding,. Mol. Cell. Proteomics 2019, 18, 320–337. [PubMed: 30459217]

(472). Phillips JJ; Yao Z. ping; Zhang W; McLaughlin S; Laue ED; Robinson CV; Jackson SE Conformational Dynamics of the Molecular Chaperone Hsp90 in Complexes with a Co-Chaperone and Anticancer Drugs. J. Mol. Biol 2007, 372, 1189–1203. [PubMed: 17764690]

(473). Karagöz GE; Duarte AMS; Ippel H; Uetrecht C; Sinnige T; Rosmalen M. van; Hausmann J; Heck AJR; Boelens R; Rüdiger SGD N-Terminal Domain of Human Hsp90 Triggers Binding to the Cochaperone P23. Proc. Natl. Acad. Sci 2011, 108, 580–585. [PubMed: 21183720]

(474). McLaughlin SH; Sobott F; Yao ZP; Zhang W; Nielsen PR; Grossmann JG; Laue ED; Robinson CV; Jackson SE The Co-Chaperone P23 Arrests the Hsp90 ATPase Cycle to Trap Client Proteins. J. Mol. Biol 2006, 356, 746–758. [PubMed: 16403413]

(475). Ebong I; Morgner N; Zhou M; Saraiva MA; Daturpalli S; Jackson SE; Robinson CV Heterogeneity and Dynamics in the Assembly of the Heat Shock Protein 90 Chaperone Complexes. Proc. Natl. Acad. Sci 2011, 108, 17939–17944. [PubMed: 22011577]

(476). Erba EB; Ruotolo BT; Barsky D; Robinson CV Ion Mobility-Mass Spectrometry Reveals the Influence of Subunit Packing and Charge on the Dissociation of Multiprotein Complexes. Anal. Chem 2010, 82, 9702–9710. [PubMed: 21053918]

(477). Santhanagopalan I; Degiacomi MT; Shepherd DA; Hochberg GKA; Benesch JLP; Vierling E It Takes a Dimer to Tango: Oligomeric Small Heat Shock Proteins Dissociate to Capture Substrate. J. Biol. Chem 2018, 293, 19511–19521. [PubMed: 30348902]

(478). Martin EM; Jackson MP; Gamerdinger M; Gense K; Karamonos TK; Humes JR; Deuerling E; Ashcroft AE; Radford SE Conformational Flexibility within the Nascent Polypeptide–Associated Complex Enables Its Interactions with Structurally Diverse Client Proteins. J. Biol. Chem 2018, 293, 8554–8568. [PubMed: 29650757]

(479). Lai AL; Clerico EM; Blackburn ME; Patel NA; Robinson CV; Borbat PP; Freed JH; Gierasch LM Key Features of an Hsp70 Chaperone Allosteric Landscape Revealed by Ion-Mobility Native Mass Spectrometry and Double Electron-Electron Resonance. J. Biol. Chem 2017, 292, 8773–8785. [PubMed: 28428246]

(480). Mitra R; Gadkari VV; Meinen BA; van Mierlo CPM; Ruotolo BT; Bardwell JCA Mechanism of the Small ATP-Independent Chaperone Spy Is Substrate Specific. Nat. Commun 2021, 12, 1–13. [PubMed: 33397941]

(481). Fan M; Rao T; Zacco E; Ahmed MT; Shukla A; Ojha A; Freeke J; Robinson CV; Benesch JL; Lund PA The Unusual Mycobacterial Chaperonins: Evidence for in Vivo Oligomerization and Specialization of Function. Mol. Microbiol 2012, 85, 934–944. [PubMed: 22834700]

(482). Zhou M; Jones CM; Wysocki VH Dissecting the Large Noncovalent Protein Complex GroEL with Surface-Induced Dissociation and Ion Mobility-Mass Spectrometry. Anal. Chem 2013, 85, 8262–8267. [PubMed: 23855733]

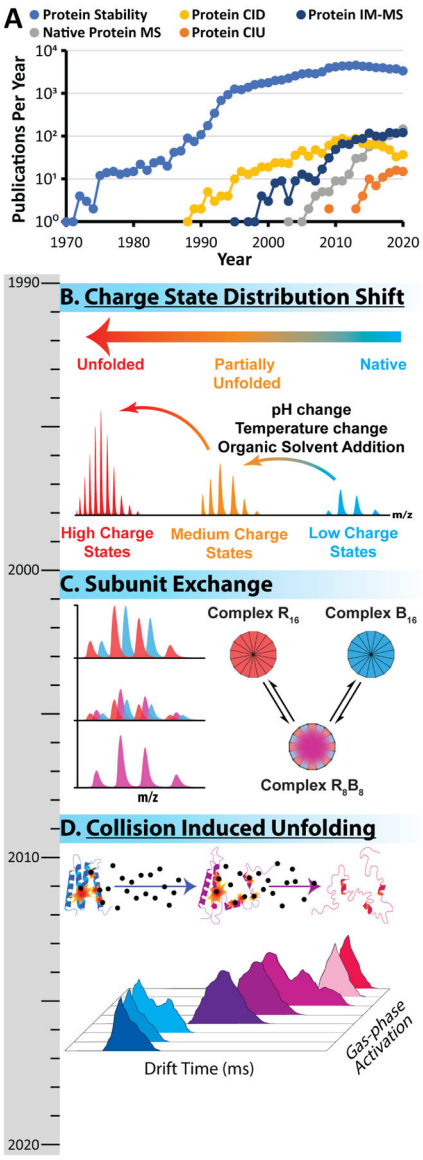

Figure 1.

A) The total number of publications in the pubmed.gov database for the search terms indicated in legend. **B)** The majority of early protein stability measurements conducted by MS (pre-2000) relied on the observation of CSD shifts. The blue CSD represents a “native” protein possessing lower charge states. Upon denaturation, the protein unfolds presenting a CSD centered at higher charge (lower m/z), occupying partially unfolded structures (orange CSD) enroute to a fully unfolded population (red CSD). **C)** Native MS emerged in the early 2000s, and one of the initial biological systems studied by this approach were α-crystallin proteins. MS is able to track the exchange between co-incubated homo-oligomers, which eventually leads to the formation of hetero-oligomers. **D)** Building upon these initial measurements, CIU employs gas phase activation in conjunction with IM, to observe the gas phase change in structure due to collisional heating. This approach is growing rapidly and is being applied to a broad variety of biological systems.

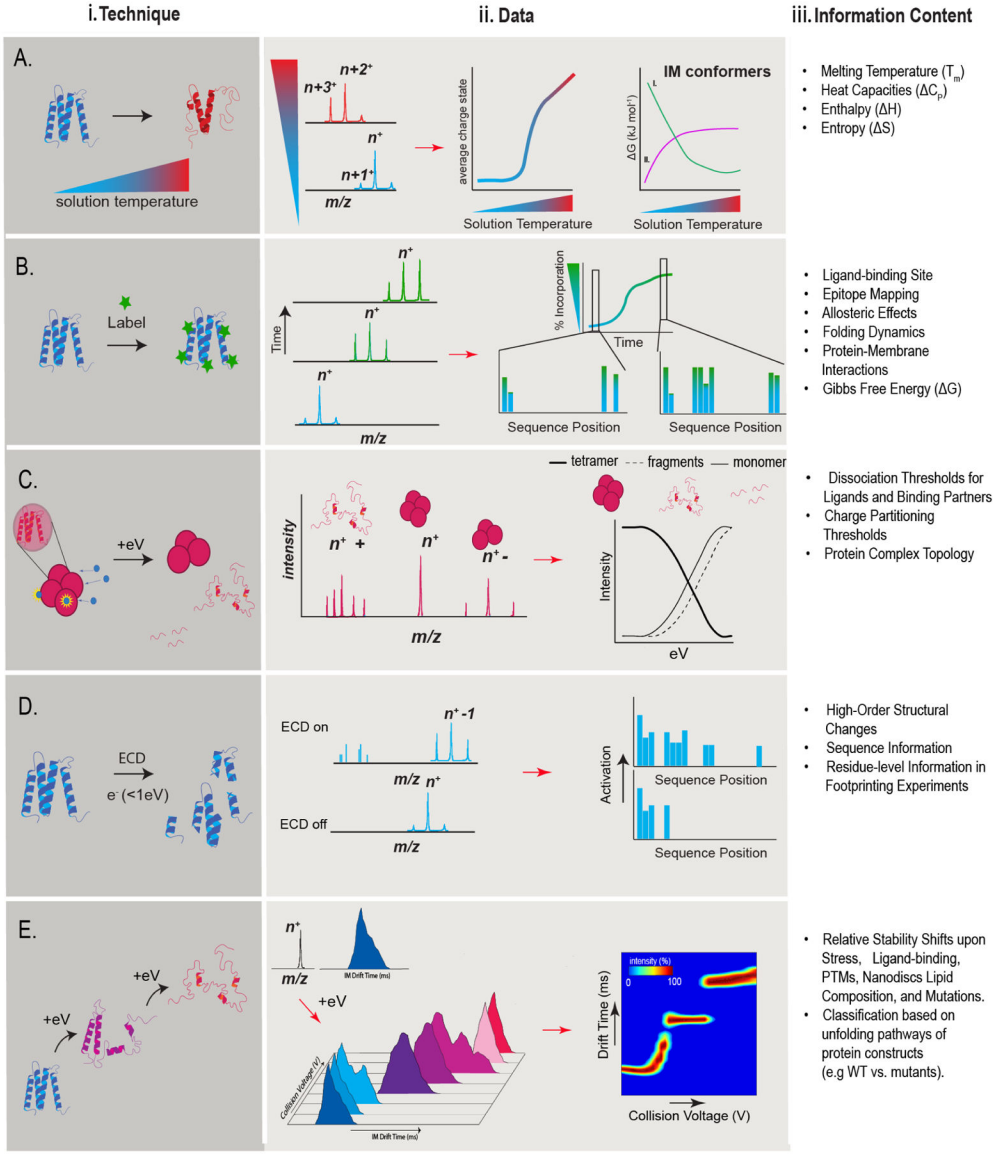

Figure 2. Mass-Spectrometry Techniques for Protein Conformational Stability Measurements. For each technique described, there is a corresponding summary figure, **ii)** data and **iii)** information content column. **A) Variable Temperature**. A protein experiences a temperature gradient as it is introduced into the mass spectrometer. A shift in CSD indicates conformational changes. By monitoring IM conformers, insights into thermodynamic values can be made. **B) Footprinting** uses selective or non-selective reagents (green stars) to monitor changes to the solvent-accessible regions of a protein. **C) Electron Capture Dissociation** is a fragmentation technique where proteins are exposed to low energy electrons, which are captured and produce backbone fragmentation. ECD fragmentation patterns can changed based on the precursor conformation and charge state. **D) Collision Induce Dissociation** allows the evaluation of protein complex subunit stoichiometry and composition. By monitoring protein ejection from protein complex precursors, dissociation thresholds can be determined and related to the stabilities of subunits and interfaces. **E)**

Collision Induced Unfolding is an IM-MS technique where a protein conformation is monitored as its internal energy is increased using collisional activation. A shift in voltage required for eliciting the unfolding transitions observed is indicative of stability shifts when comparing between fingerprints of different states (e.g. apo vs holo).

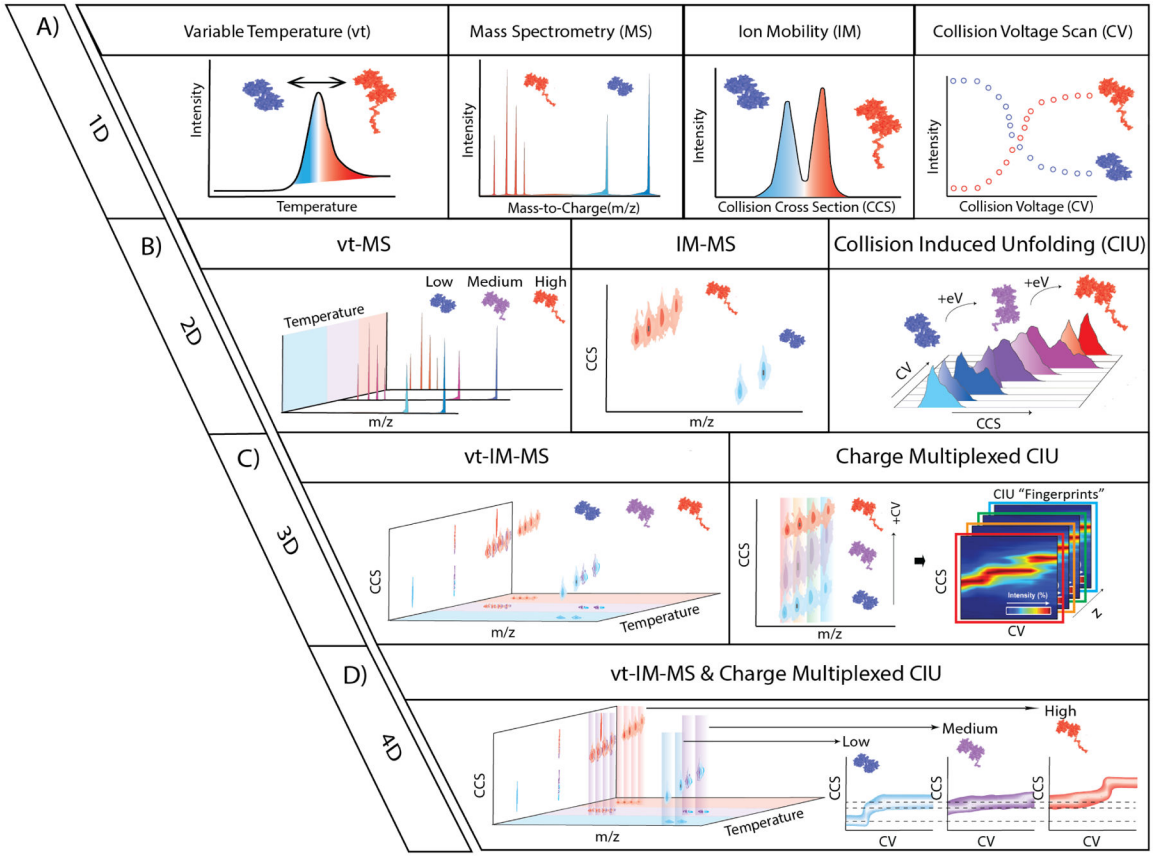

Figure 3:

Scaling the dimensionality of MS-based protein stability measurements. A) One-dimensional assays of protein stability can include variable temperature, mass spectrometry, ion mobility, and collision voltage scanning. B) Hyphenation of the 1-D techniques in A provides access to powerful 2-D techniques such as vt-MS, IM-MS, and CIU that can separate and generate a wide range of unfolded protein structures. C) Further enhancements can be achieved with 3-D hyphenated techniques based on those shown in A and B above. D) A logical extension of the techniques shown in C leads to a "4-D" assay of protein stability governed by MS methods, capable of measuring changes in protein stability across multiple phases/conditions simultaneously.

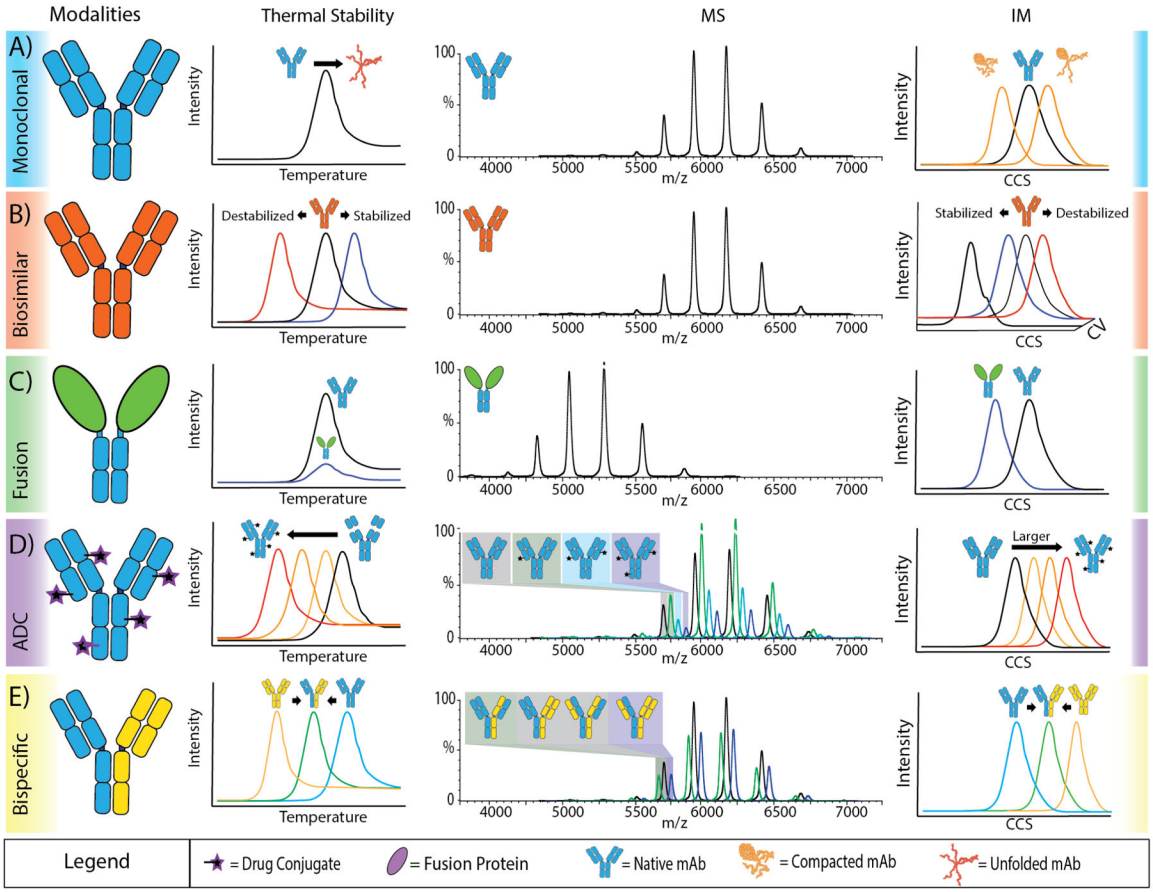

Figure 4.

Data and information content that can be expected from variable temperature, MS and IM datasets for the following biotherapeutic modalities: **A)** mAbs, **B)** biosimilars, **C)** fusion proteins, **D)** antibody-drug conjugates, and **E)** bispecific antibodies. Generally, for variable temperature experiments shifts to lower T_m values indicate a decrease in stability and higher values indicate an increase in stability. Changes in mass spectrometry generally indicate different structures or stoichiometries. For IM shifts to lower CCS values indicate more compact structures while larger values indicate larger, often unfolded structures. By applying activation energy and monitoring unfolding, ex. Biosimilar IM, shifts in stability can be monitored by shifts in the IM peak relative to the activation energy.

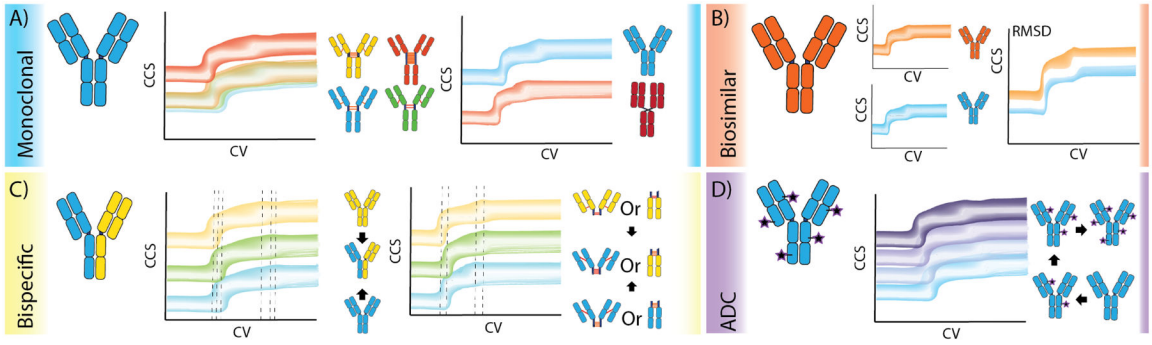

Figure 5. CIU applications for biotherapeutic mAbs.

A) Differentiation of monoclonal IgG subclasses by disulfide bonding patterns and difference in CIU unfolding due to domain exchange. **B)** Biosimilar antibodies have qualitatively similar fingerprints, but contemporary CIU analyses are able to quantitate subtle differences in stability. **C)** Bispecific antibodies present CIU characteristics centered between the precursor structures. **D)** Shifts in CCS and stability can be quantified as a function of increasing drug load in ADC biotherapeutics.

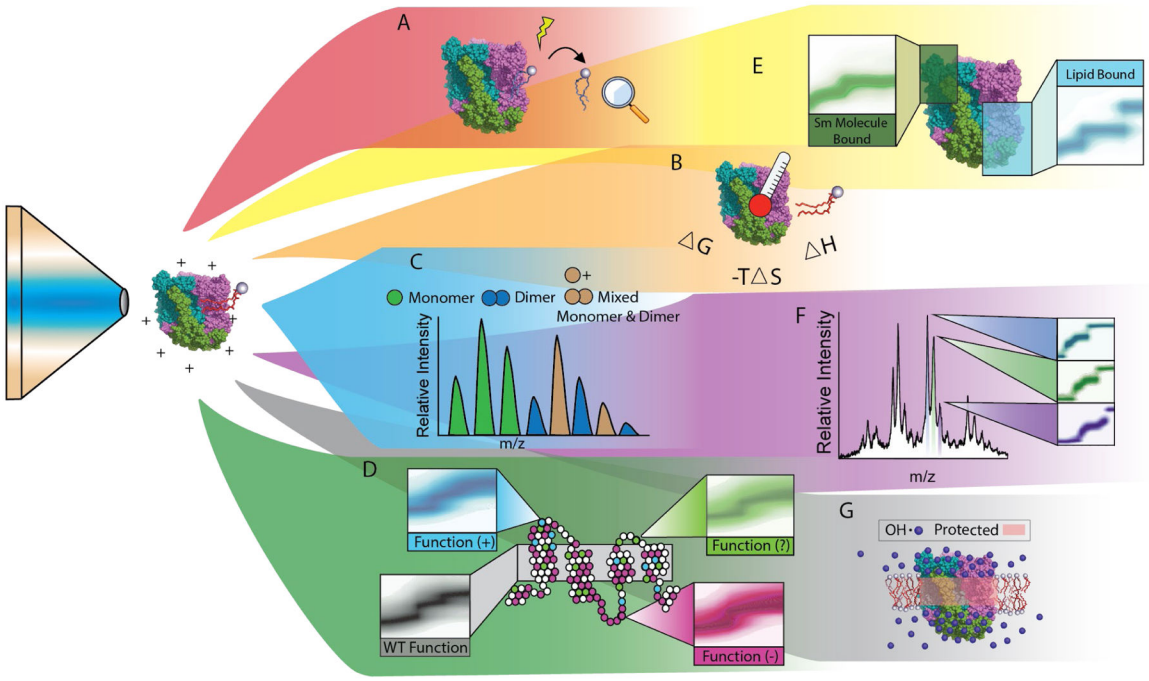

Figure 6. Summary of various MS-related measurements associated with MP stability assessments.

A) identification of endogenous lipid binding **B)** thermodynamics of lipid binding to membrane protein **C)** oligomeric state assignment **D)** evaluation of disease state mutations in amino acid sequence **E)** site-selective ligand binding events **F)** resolving multiple simultaneous ligand bound states **G)** oxidative labeling.

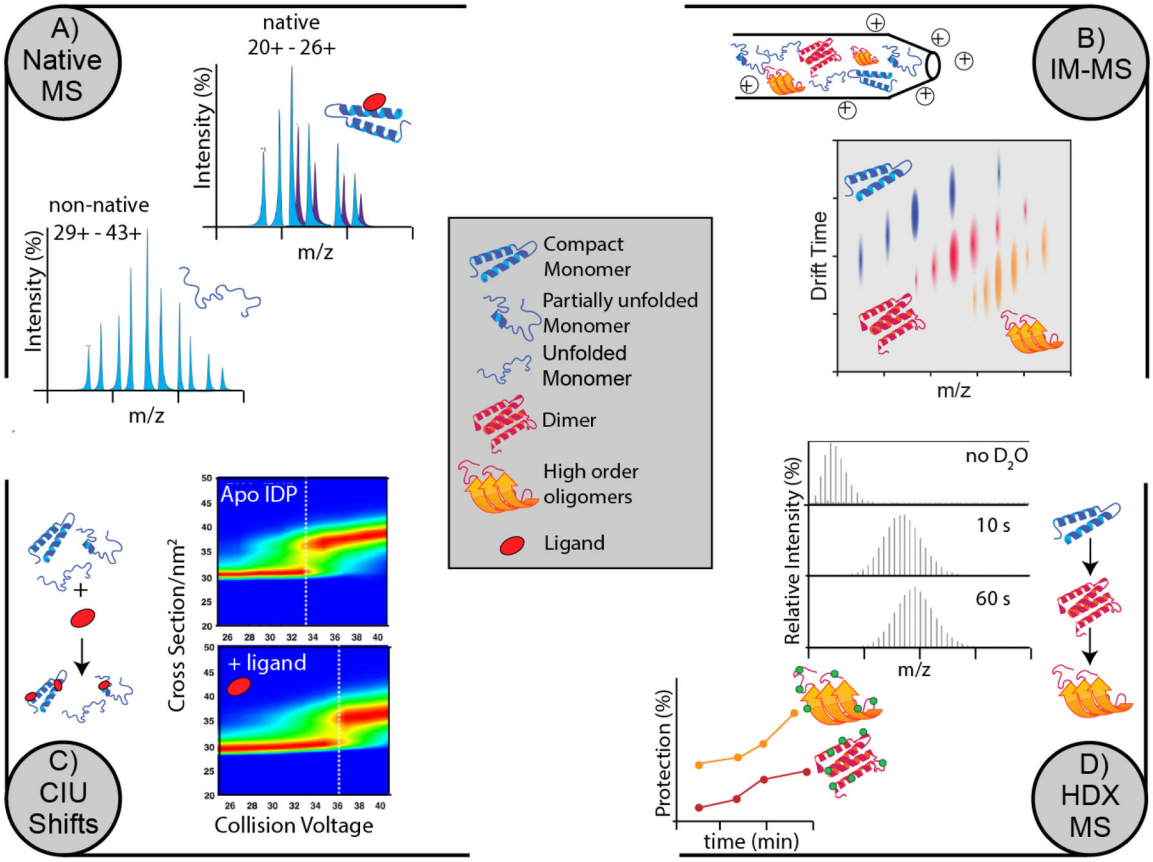

Figure 7.

Overview of select MS techniques that allow direct stability measurements with challenging amyloidogenic proteins A) Native MS usually produces a narrow range of charge states compare to non-native MS. Native MS can retain the native structures of amyloidogenic proteins and even the non-covalent complexes formed through ligand or protein binding through gentle ionization parameters. B) nESI needle is filled with a mixture of different oligomers of an amyloidogenic protein. IM-MS is able to separate the complex population of oligomers in drift time space based on their size, charge and shape. C) CIU shifts otherwise known as CIU50 values can be obtained through a series of IM-MS experiments at increasing collision energy. If a ligand binding event caused an amyloid protein to increase in stability, CIU50 values will reflect this increase. D) HDX-MS can capture localized information, in as little as few milliseconds or as long as days, allowing us to take snapshots of an amyloid aggregation process

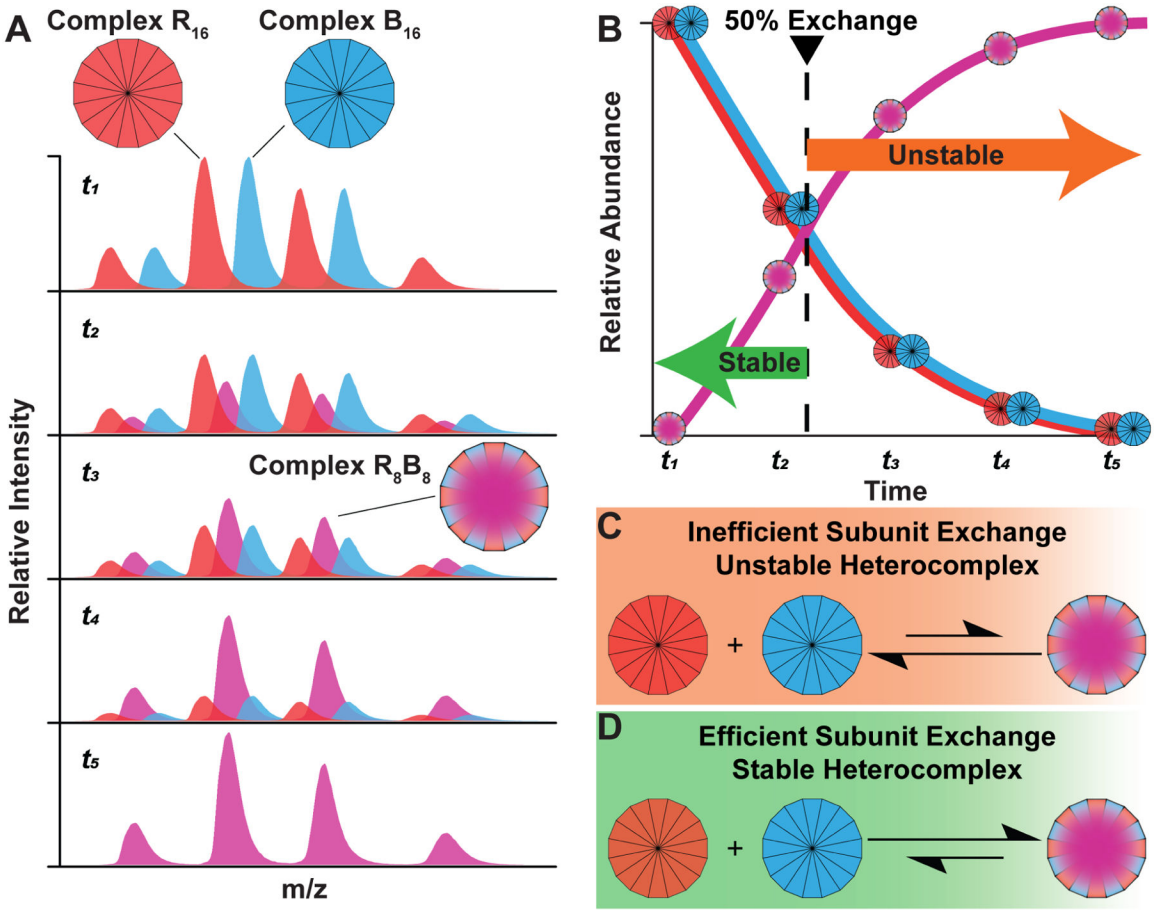

Figure 8. Subunit Exchange between sHSP Oligomers Illuminated through MS.

A) Two sHSP homo-oligomers (R and B, numbers indicated the number of subunits within the example oligomers) with suspected hetero-oligomeric interactions are co-incubated in solution, and MS data is collected at various time points (t1, t2, t3 etc.). **B)** The MS data is quantified versus time to measure the decay of homo-oligomers and the growth in abundance of hetero-oligomeric sHSP species. The rate of this conversion from homo-oligomers to hetero-oligomers can be measured as a function of time, and can serve as an indication or stability. **C)** When hetero-oligomeric sHSP complexes are stable their subunit exchange is expected to occur quickly. **D)** Conversely, when hetero-oligomeric complexes are unstable, the equilibrium shifts in the direction of homo-oligomers.