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► **To cite this version:**

Elisabetta Boeri Erba, Luca Signor, Carlo Petosa. Exploring the structure and dynamics of macromolecular complexes by native mass spectrometry. *Journal of Proteomics*, 2020, 222, pp.103799. 10.1016/j.jprot.2020.103799 . hal-02864550

HAL Id: hal-02864550

<https://hal.univ-grenoble-alpes.fr/hal-02864550v1>

Submitted on 22 Aug 2022

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Exploring the structure and dynamics of macromolecular complexes by native mass spectrometry

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Acknowledgements

We acknowledge the MS facility of the Grenoble Instruct-ERIC center (ISBG; UMS 3518 CNRS-CEA-UGA-EMBL) within the Grenoble Partnership for Structural Biology (PSB). Platform access was supported by FRISBI (ANR-10-INBS-05-02) and GRAL, a project of the University Grenoble Alpes graduate school (Ecoles Universitaires de Recherche) CBH-EUR-GS (ANR-17-EURE-0003). The IBS acknowledges integration into the Interdisciplinary Research Institute of Grenoble (IRIG, CEA).

Declaration of interest

No conflict to declare.

Abstract

Mass spectrometry (MS) is an effective approach for determining the mass of biomolecules with high accuracy, sensitivity and speed. Over the past 25 years, MS performed under non-denaturing conditions ("native MS") has been successfully exploited to investigate non-covalently associated biomolecules. Here we illustrate native MS applications aimed at studying protein-ligand interactions and structures of biomolecular assemblies, including both soluble and membrane protein complexes. Moreover, we review how the partial dissociation of holo-complexes can be used to determine the stoichiometry of subunits and their topology. We also describe "native top-down MS", an approach based on Fourier Transform MS (FT MS), whereby non-covalent interactions are preserved while covalent bonds are selectively fragmented. Overall, native MS plays an increasingly important role in integrative structural biology, helping researchers to elucidate the three dimensional architecture of intricate macromolecular complexes.

Keywords

Native mass spectrometry (MS), native top-down MS, protein-ligand interactions, macromolecular complexes, stoichiometry, 2D interaction map, integrative structural biology

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Abstract

Mass spectrometry (MS) is an effective approach for determining the mass of biomolecules with high accuracy, sensitivity and speed. Over the past 25 years, MS performed under non-denaturing conditions – native MS – has been successfully exploited to investigate non-covalently associated biomolecules. Here we illustrate the use of native MS to study protein-ligand interactions and structures of biomolecular assemblies, including both soluble and membrane protein complexes. Moreover, we review how the partial dissociation of holo-complexes can be used to determine the stoichiometry of subunits and their topology. We also describe native top-down MS, whereby non-covalent interactions are firstly preserved and then covalent bonds are selectively broken. This allows the detailed probing of intersubunit and subunit-ligand interactions within a biomolecular assembly. Overall, native MS plays an increasingly important role in integrative structural biology, helping researchers to elucidate the three dimensional architecture of intricate macromolecular complexes.

Keywords

Top-down mass spectrometry (MS), protein-ligand interactions, macromolecular complexes, stoichiometry, 2D interaction map, integrative structural biology

1. Introduction

Knowledge of the three-dimensional (3D) structure of a macromolecular complex is often critical for achieving a detailed understanding of its biological role and of the molecular mechanisms that underlie its functional activity. Traditionally, methods such as X-ray crystallography, Nuclear Magnetic Resonance (NMR) spectroscopy and cryo-electron microscopy (cryo-EM) have been used to decipher the atomic organization of biomolecules. Recently, mass spectrometry (MS) has emerged as an important tool in structural proteomics for investigating 3D structures [1-5]. Several MS-based methods have been introduced for monitoring the structural and conformational changes of biomolecules and for characterizing their non-covalent interactions both in well-defined biochemical conditions and in complex biological environments such as cell lysates and intact cells. These techniques include MS coupled to: chemical cross-linking (XL) [6], hydrogen/deuterium exchange (HDX) [7-9], hydroxyl radical footprinting (HRF) [10, 11], limited [12] or pulse proteolysis [13], cellular thermal shift assay (CETSA) [14], Drug Affinity Responsive Target Stability (DARTS) [15, 16] and Stability of Proteins from Rates of Oxidation (SPROX) [16]. XL-MS allows the identification of spatially proximal residues and can be used to validate an experimentally determined 3D structure or to deduce the structure of a complex for which the individual subunit structures are known [17]. MS combined with HDX, HRF or limited proteolysis can be used to identify residues whose surface accessibility becomes altered upon complex formation, thereby providing information on intermolecular interfaces and on the conformational dynamics of complexes. MS coupled to pulse proteolysis, CETSA, DARTS and SPROX can identify the protein targets of small-molecule ligands.

Below we review the role of native MS in investigating the structure and dynamics of biomolecular assemblies, including soluble and membrane protein complexes. In particular, we describe the different approaches used to assess the stoichiometry and topology of non-covalent complexes. These include the partial dissociation of assemblies under controlled conditions in the gas phase and in solution. Moreover, we present recent progress in native top-down MS, whereby both covalent bonds and non-covalent interactions (i.e., primary and quaternary structures) can be investigated using the same mass spectrometer. Finally, we outline prospects for the future development of native MS and its application to the study of macromolecular complexes.

2. Native MS and its role in investigating macromolecular complexes

Over the last 25 years native MS (also known as structural MS [18]) has emerged as a key tool for investigating macromolecular and protein-ligand complexes because it allows non-covalent interactions (hydrogen bonds and van der Waals, electrostatic, hydrophobic and π -interactions) to be preserved during experimental analysis [19-21]. This distinguishes native MS from other types of biological MS (such as MS-based proteomics) which generally require denaturing conditions that disrupt such interactions. Using native MS one can determine the mass and stoichiometry of intact macromolecular complexes, identify direct interactions between their components and, in the case of multi-subunit assemblies, identify stable subcomplexes and assign the relative position (core vs. periphery) of subunits [22-27] (**Figure 1**). One can deduce the hierarchy of an assembly pathway by mixing subunits in a stepwise manner [18] or by using different sample buffer conditions to induce a change in oligomeric state [28, 29]. Equilibrium dissociation (K_d) constants for protein-protein and protein-ligand interactions can also be determined by native MS [30-32]. The K_d values obtained by native MS were consistent with those obtained by isothermal titration calorimetry (ITC) [30-33]. Native MS combined with “double-mutant cycle” analysis successfully determined pairwise interaction energies [33]. Native MS coupled with ion mobility (IM; MS-related abbreviations are summarised in **Table 1**) allows one to investigate the shape of macromolecular assemblies [34-38], and has been combined with bioinformatics to study the evolutionary history of protein complexes [17, 39].

3. Strengths and limitations of native MS

Native MS presents several advantages compared to other structural approaches. First, it can be used to study a wide diversity of biological samples that differ in mass, polydispersity, symmetry and dynamic flexibility [2]. Second, native MS does not require samples to be labelled or crosslinked. Third, different oligomeric states can be investigated simultaneously. Specific information is obtained for each individual species present, without data being averaged over different species. Therefore, the dynamics of quaternary structure can be studied in real time [40, 41]. Finally, native MS represents a quite sensitive approach. Successful analyses have been reported in which only a few microliters of sample at relatively low (μM) concentration were required.

Nonetheless, native MS experiments can be biased because (as all MS analyses) they are performed under vacuum conditions and so macromolecular complexes are detected in the gas phase [42, 43]. Therefore, the relative abundances of detected assemblies may differ from those in solution because distinct complexes may present different ionisation, transmission and detection probabilities [30]. Moreover, hydrophobic bonds are weaker in the gas phase than in solution, whereas electrostatic interactions become stronger. Therefore, certain assemblies need to be stabilized by crosslinking to enable their detection [44]. Nevertheless, computational and experimental data indicate that the transition from solution to the gas phase does not drastically alter biomolecules [45, 46]. For instance, enzymes such as lysozyme and trypsin retain their catalytic activity after their ionisation, mass selection, and soft-landing onto surfaces [47]. Also, when solution conditions such as pH and concentration were modified, the gas-phase spectra correspondingly changed [30]. Changes in MS spectra that mirror pH changes have been recently confirmed using designed homo-trimers (3-mers) and hetero-dimers (2-mers) engineered to undergo a pH-responsive conformational change upon protonation of buried histidine residues [48]. These complexes were stable above pH 6.5, but underwent cooperative, large-scale conformational changes when the pH was lowered, as shown by native MS and size-exclusion chromatography [48]. Recent evidence regarding the stability of folded biomolecules in vacuum has been obtained by low-energy electron holography [49]. Using electrospray ionisation (see next section) and soft-landing, two monomeric (1-mer) proteins (cytochrome C and BSA) and a hetero-4-mer (haemoglobin) were deposited on freestanding graphene. Then, sub-nanometer resolution images of individual biomolecules were obtained. These low-energy electron micrographs showed that the samples were in a folded state after being subjected to ultrahigh vacuum [49].

To conclude, native MS presents many advantages for investigating biomolecular assemblies in terms of accuracy, selectivity, sensitivity and speed of analyses. However, the limitations of native MS should be kept in mind when the results of such experiments are evaluated.

4. Preserving non-covalent interactions in the gas phase

Native MS requires a gentle ionisation of macromolecular complexes that preserves non-covalent interactions. Although in some cases this has been achieved using matrix-assisted laser desorption/ionization (MALDI) [50-54], by far the more prevalent method is electrospray ionisation (ESI) [55-57], which more easily preserves non-covalent interactions. ESI-MS investigations can be influenced by the nature of the intermolecular interactions, by the composition, ionic strength and pH of the sample buffer, and by the voltages and pressures within the mass spectrometer. Consequently, it is important to consider these parameters during data acquisition and analysis. Unlike other types of ESI-MS analyses that require the use of acidic conditions or organic solvents, native MS experiments are usually performed using volatile buffers such as ammonium acetate [58], ethylenediammonium diacetate [59] or alkylammonium acetate [60]. Typically the sample buffer is exchanged immediately prior to native MS analysis [23], although in some cases the sample purification protocol may also require modification.

Generally, non-covalent assemblies are investigated using nano-electrospray ionisation (nano-ESI), which requires a sample emitter with an orifice diameter (1–10 μm) smaller than that used for conventional ESI (approximately 100 μm) [61, 62]. The small opening enables

the use of a low flow rate (20-200 nL/min) to analyse a 2-5 μL sample volume at micromolar concentrations [63]. Recently, 0.5 μm emitter tips have been introduced to study proteins and their complexes using buffers containing non-volatile salts (such as Na^+ or K^+) at 135-150 mM concentration that more closely resemble the intracellular environment [64-66]. Therefore, experiments can be performed utilizing widely used biochemical buffers, such as phosphate and Tris. It has been hypothesized that the ESI droplets generated by submicrometer emitters contain a lower salt-to-biomolecule ratio than those generated by traditional nano-ESI needles [64]. Another advantage of submicrometer ESI tips is that they do not require buffer exchange into volatile solutions. These emitter tips have also been used for the study of membrane proteins [67]. Overall, controlled experimental conditions using appropriate buffers and ionisation conditions allow the maintenance of weak non-covalent interactions during native MS experiments.

5. Native MS experiments are performed using Q-TOF and Orbitrap instruments

Native MS requires mass spectrometers able to transmit and detect large fragile assemblies. Early studies showed that relatively small complexes could be analysed by conventional nano-ESI-spectrometers [68-71] (see **Figure 2** for timeline). However, larger complexes (>60 kDa) generate ions with mass-to-charge (m/z) ratios above 4000 that exceed the detection limit of such instruments. This led in the 2000s to customise nano-ESI-quadrupole-time-of-flight (Q-TOF) mass spectrometers to detect non-covalent complexes [43, 72]. The use of modified Q-TOFs made it possible to investigate remarkably massive particles, such as an 18 MDa viral capsid [73], and to analyse membrane protein complexes [74-80] (see section 11 below). Fourier transform (FT) based mass spectrometers have also successfully been used to study non-covalent interactions. These include Orbitrap instruments [81-84], which in 2012 were used to analyse intact protein complexes 150-800 kDa in mass [85-88], and were subsequently utilized to study virus-like nanoparticles with a mass up to 4.5 MDa [89]. To perform such analyses, the Orbitrap m/z range was extended up to 40000 to transmit “heavy ions” [85, 90]. Using only a 1 nM sample concentration, this modified instrument reached an excellent resolution at high m/z , deciphering different glycosylated forms of IgG antibodies [86]. Such high resolution enabled the characterisation of the type (ADP or ATP) and number of nucleotides bound to a large assembly such as GroEL (800 kDa) [85]. The performance of Orbitrap instruments utilised for native MS is continuously being improved by different groups [42, 91-93]. For example, in 2017 a Q Exactive Plus™ mass spectrometer was further modified to enhance its sensitivity and increase transmission up to 70 000 m/z [92, 93].

6. Dissociation of macromolecular complexes in the gas phase and subunit fragmentation

Tandem MS (also called MS^2 or MS/MS) is based on two MS steps, whereby selected ions are broken down in the gas phase and the product ions are analysed [94]. It is possible to distinguish between (a) the breaking of non-covalent interactions to dissociate complexes and (b) the fragmentation of covalent interactions to study the primary sequences of subunits and their post-translation modifications (PTMs). Here we outline the most common dissociation/fragmentation methods, including collision-induced dissociation [known as Higher Energy Collisional Dissociation (HCD) in Orbitrap instruments [42, 91]], surface-induced dissociation, photodissociation and electron-based dissociation techniques (e.g., electron-capture and electron-transfer dissociation) [95-97].

(i) Collision-induced dissociation (CID). Normally, dissociation/fragmentation experiments require that a single ion population, characterised by a specific mass (m) and charge state (z) and referred to as a “precursor ion”, is selected according to its m/z ratio. In the case of CID, when non-covalent assemblies are investigated, a precursor ion is selected according to its m/z ratio in a first mass analyser (e.g., a quadrupole in the case of Q-TOF instruments) and then accelerated toward a cell filled with a collision gas such as argon or xenon. Precursor ions are subjected to numerous, low-energy collisions that convert part of

their kinetic energy into vibrational energy, causing the internal energy of the precursor ions to increase. Since many small steps of energy conversion take place, CID is considered a “slow heating” process [98]. Non-covalent interactions are broken by collisions and the dissociation products are further analysed (e.g., in a TOF region). With some exceptions [99-101], 1-meric proteins are ejected from biomolecular assemblies one after the other and this phenomenon allows the assessment of stoichiometry with high accuracy. Ion charges are distributed across the dissociated particles in a highly asymmetric manner. For instance, a dodecamer (12-mer) generates highly charged 1-mers and lowly charged “stripped” 11-mers that can further dissociate into 1-mers and 10-mers [99].

The dissociation behaviour of assemblies during CID has been studied, but many questions remain open [29, 59, 102-104]. Behaviour can be influenced by sample features such as charge density, salt bridges, flexibility of subunits, isoelectric point (pI) and strength of intersubunit interfaces [99-101, 105]. For example, the pI of subunits has been shown to affect the dissociation of protein complexes [105]. Leney studied two 6-mers (i.e., phycoerythrin and allophycocyanin), composed of alternating alpha and beta subunits [105]. The subunits of the phycoerythrin presented identical pIs and in the CID experiments both types of subunits were ejected. In contrast, the alpha and beta subunits of allophycocyanin showed distinct pIs and only alpha 1-mers were detected upon CID. CID is very useful for investigating biomolecular complexes because it provides an insight into the location (core vs. periphery) of subunits within the complex [106], as peripheral subunits are expelled at lower energy compared to the core subunits [18]. CID can also provide important structural information such as subunit interactions and their relative spatial arrangements [99, 100].

(ii) Surface-induced dissociation (SID). In the case of SID, an ion population is accelerated toward a surface [97, 98, 107]. The non-covalent interactions are broken by collision against the surface and the masses of the dissociation products are further assessed using, for example, a TOF analyzer [108] or an Orbitrap [109]. Compared to the slow-heating effects of CID, SID is regarded as a fast, single-step, and energetic activation process [98]. SID makes non-covalent complexes dissociate into folded subunits that carry ion charges proportional to their mass without major structural rearrangements. This means that ion charges are distributed across the dissociated particles in a symmetric manner and that oligomers (as opposed to only 1-mers in the case of CID) are ejected from assemblies. For instance, a 4-mer composed of a 2-mer of 2-mers could be dissociated into individual 2-mers at low SID energy, and these in turn could be further dissociated into 1-mers at higher SID energy [108].

(iii) Photodissociation (PD). PD is based on the use of photons irradiated on target ions [95, 97, 110]. Different sources of photons have been used. For instance, ultraviolet (UV) lasers can deposit a large amount of energy into ions, allowing dissociation and fragmentation pathways different from those generated by other MS² methods. Remarkably, it has been shown that ion charges can be distributed across the dissociated particles in both symmetric and asymmetric manners by modulating the laser power [103]. Moreover, UVPD induced covalent bond cleavage of the 1-meric subunits, most frequently within highly flexible loop regions.

(iv) Electron-based techniques. In techniques such as electron-capture dissociation (ECD) [111-114] and electron-transfer dissociation (ETD) [96, 115], the fragmentation of biomolecules is based on the addition of electrons to the target molecules. In the case of ECD low-energy photoelectrons are released and captured by even-electron (M+nH)ⁿ⁺ biomolecular ions (peptides, proteins or macromolecular assemblies) [116, 117]. Thus, radical charge-reduction products (M+nH)^{(n-1)+•} are generated and selective cleavage of the N-C α bond, a highly bond specific process, takes place. ETD is a fragmentation method based on the transfer of electrons originating from radical anions [96, 118, 119]. Similarly to ECD, N-C α bonds are broken in ETD experiments. Both ECD and ETD allow non-covalent bonds and PTMs to be preserved [120, 121], whereas covalent interactions such as peptide bonds are fragmented. The mechanism of ECD and ETD has been extensively studied

theoretically and experimentally, but remains a subject of debate [116]. ECD and ETD are mainly used in FT instruments, but can also be incorporated in ion traps [113], [114, 122] IM mass spectrometers (such as Synapt® instruments [123]) and Q-TOF mass spectrometers. For instance, ETD was utilised to sequence fragments of a non-covalent protein complex (ADH) using a Q-TOF instrument [124].

To conclude, a diversity of dissociation/fragmentation methods are available that enable one to dissociate non-covalent assemblies or fragment covalent bonds with distinct outcomes. These techniques are of key importance for confirming stoichiometry, studying protein-ligand interactions and determining protein sequences and PTMs (see sections 7, 9 and 12 below).

7. Native top-down MS to study non-covalent interactions

In the late 1990s the use of ECD led to the introduction of top-down MS, an approach to efficiently characterise intact proteins [125]. Unlike bottom-up proteomics that requires samples to be digested enzymatically [126, 127], samples subjected to top-down MS are analysed as intact molecules and fragmented inside appropriate mass spectrometers [128]. Top-down MS allows the investigation of different proteoforms [129, 130], protein conformations and PTMs [131-135]. Recent developments allowed native top-down MS experiments to be performed to study intact assemblies and to localize ligand binding sites on proteins [19, 127, 136-139].

These experiments require three different analysis steps [42, 91, 127, 136, 140] (Figure 1). In the first (MS) step, the mass of an intact macromolecular complex is assessed. In the second (MS²) step, protein assemblies are often dissociated by CID and 1-mers are ejected. This step allows the stoichiometry of the complex to be confirmed [127]. In the final (MS³) step, the ejected 1-mers are fragmented by backbone cleavage using various methods such as ECD or UVPD. This last step is useful to investigate the primary sequence of the ejected subunits of interest. According to the type of dissociation/fragmentation utilised, non-covalent interactions can be preserved and covalent ones broken [19, 141-143].

The complexity of samples that can be analysed by this approach has increased over the years. In early studies, small non-covalent complexes [144] and a protein-ligand complex [145] were fragmented using ECD. Since 2010, the approach has been applied to large non-covalent assemblies [63, 146-148]. Specifically, a 4-meric yeast alcohol dehydrogenase (ADH, 147 kDa) was analysed using an ECD and ESI-FT-ICR instrument [146]. By breaking covalent bonds within monomers, Gross and coworkers fragmented ADH subunits without disrupting non-covalent interactions. This was the first example in which information on both the primary and quaternary structures was obtained using the same instrument.

A recent example illustrates the use of native top-down MS and of nanodiscs to study a copper-dependent membrane metalloenzyme, particulate methane monooxygenase (pMMO), composed of three subunits (PmoA, PmoB, and PmoC), each present in three copies [149]. Using traditional biochemical and biophysical approaches, the assessment of the stoichiometry of metal centers and their localization represents a major challenge for large, multisubunit metalloprotein complexes, particularly when these cannot be heterologously expressed. In the case of pMMO, spectroscopic and crystallographic studies provided inconclusive information regarding the metal stoichiometry [149]. Using the three step MS-based approach (MS, MS², MS³, see above), it was possible to assess the copper stoichiometry of the pMMO subunits and to investigate their PTMs. In an MS experiment performed to assess the total mass of the pMMO holocomplex, the observed mass of the main species was 559.7 Da lower than the theoretical one. Subsequently, the possible presence of PTMs and metal cofactors was investigated by MS² and MS³ experiments. In MS², subunits were ejected from the intact pMMO and their masses were determined. The PmoA subunit had an experimental mass 89.1 Da smaller than that expected, consistent with removal of the first methionine and acetylation of the new N-terminus. This hypothesis was confirmed by MS³. Similarly, these experiments revealed a methylated lysine within PmoB and the truncation of six N-terminal residues from PmoC. Moreover, they confirmed

the presence of a Cu(II) ion in PmoB and revealed an additional Cu(II) ion bound to PmoC that was shown in activity assays to be important for enzyme activity.

To conclude, native top-down MS experiments combine the ability to study non-covalent assemblies with the ability to fragment individual subunits using using a single mass spectrometer.

8. Dissociation of macromolecular complexes in solution

In addition to gas-phase dissociation, macromolecular complexes can also be dissociated in solution. Destabilising agents such as dimethyl sulfoxide (DMSO) or methanol or an increase in buffer ionic strength are used to selectively break intermolecular interactions within a complex before introducing the sample into the mass spectrometer [24]. In-solution dissociation of hetero-complexes allows one to identify direct interactions between subunits by generating subcomplexes [105, 150-152]. The analysis of these subcomplexes can lead to a two-dimensional interaction map that subsequently can be combined with other structural data to try to infer 3D organization. In-solution dissociation has also been exploited to elucidate the assembly and disassembly pathways of certain complexes, revealing that these pathways recapitulate the evolutionary routes by which different quaternary structures arise [39, 153, 154]. In summary, the ability to dissociate assemblies in solution is highly useful for elucidating intersubunit interactions, assembly/disassembly pathways and molecular evolutionary history.

9. Soluble protein-ligand interactions studied by native MS

The use of native MS to analyse non-covalent interactions between proteins and small ligands has been extensively reviewed [19, 34, 37, 136, 155-157]. Here we outline a few examples mainly in the context of structural studies [59, 158-162]. For instance, native MS was combined with X-ray crystallography to study two nickel import proteins of *Staphylococcus aureus* [163]. One of these was successfully crystallised bound to a Ni-(L-His)₂ ligand. The other protein could not be crystallised, but native MS showed that it bound Ni(II) ions via a distinct His-dependent chelator [163]. In another example, native MS combined with IM was used to quantify the stabilizing effects of ligands on soluble proteins, and of lipid binding on membrane proteins [164].

In many cases, the identification of ligand-binding sites within macromolecular complexes made use of appropriate dissociation/fragmentation techniques such as ECD, SID and UVPD (see above) [165-170]. In one of the first examples of non-covalent binding studied by ECD, published in 2002, homo-2-mer formation by different peptides and the binding of two glycopeptide antibiotics, vancomycin and eremomycin, to their bacterial tripeptide target were investigated [144]. These experiments allowed the authors to localize the binding sites and determine primary sequence information.

In a recent example, ECD was utilised to investigate the interaction between tau, a protein implicated in neurodegenerative disorders such as Alzheimer's disease, and a synthetic "molecular-tweezer" compound (CLR01) that inhibits the aggregation of amyloidogenic polypeptides through hydrophobic and electrostatic interactions [143]. Using ECD, Nshanian *et al.* identified phosphorylation sites on tau and localized the binding site of CLR01 to a 38-residue microtubule-binding region within the protein, providing novel insights into the mechanism of CLR01-based inhibition. Another example from the Loo laboratory combined ECD with CID to localize the binding sites for cobalt and manganese in an intrinsically disordered protein, α -synuclein [142].

The usefulness of SID was demonstrated in a recent study involving two homo-5-meric complexes, C-reactive protein (CRP) and cholera toxin B (CTB) [141]. These proteins have different ligand binding site arrangements: the site is located within each subunit in CRP, but between adjacent subunits in CTB. The authors were unable to obtain information on ligand location using CID, which caused ligand-bound 5-mers to dissociate into 4-mers and ligand-free 1-mers in a manner suggesting that ligands were lost or migrated away from their binding site. In contrast, SID of 5-mers led to the dissociation into 1-mers, 2-mers, 3-mers and 4-mers. The predominant species detected for each CRP *n*-mer contained *n* ligands,

whereas CTB n -mers contained $n-1$, n and $n+1$ ligands, consistent with the known ligand binding site locations of these proteins [141].

Another fast activation technique, UVPD, has also been proved useful for investigating ligand binding sites [171, 172]. For instance, UVPD-MS was used to investigate the catalytic cycle of adenylate kinase (AK) [172]. This enzyme, which catalyzes the interconversion of adenine nucleotides via phosphoryl transfer from ATP to AMP to generate ADP, undergoes a large conformational change from an open inactive state to a closed active state. Native MS and top-down UVPD-MS were used to investigate different complexes of AK with four ligands (AMP, ADP, ATP, and a small-molecule inhibitor) to monitor different steps of the catalysis. Holo fragment ions produced in UVPD experiments revealed specific regions of AK that exhibited substantial differences in fragmentation throughout the catalytic cycle. In particular, changes in backbone cleavages involving three α -helices and adenosine binding regions of AK indicated that these elements were in different structural microstates as AK progressed through its enzymatic cycle. Moreover, enhanced fragmentation of the loop binding the phosphates of ATP suggested that the metal ion cofactor Mg^{2+} increases the flexibility of this loop, consistent with a role for Mg^{2+} in disrupting interactions between AK side chains and ligand phosphate groups. Changes in backbone cleavage efficiency also corroborated the hypothesis that a specific conserved residue (Arg138) stabilizes the donor phosphoryl group during catalysis.

Overall, the above examples illustrate that native MS combined with dissociation/fragmentation experiments represents a versatile approach for studying protein-ligand interactions and characterising binding sites.

10. Employing native MS to probe the structure of soluble protein complexes

Numerous studies exemplify the great ability of native MS to elucidate structural features of soluble protein assemblies (reviewed in [18, 90, 106, 173-179]). The examples below illustrate how native MS can be integrated with other structural approaches, provide valuable knowledge when high-resolution structural data are unavailable, and elucidate assembly pathways of macromolecular complexes.

(i) Native MS complements other structural methods

MS based-structural proteomics plays an increasingly important role in characterising the architecture of non-covalent assemblies, complementing traditional approaches [178, 180, 181]. 3D models of macromolecular complexes have been deduced by combining MS experiments with data from crystallography, NMR, small-angle X-ray scattering and EM [28, 182-188]. Computational methods have been developed for merging native MS information with other MS-based data (such as IM-MS and chemical crosslinking) to generate pseudo-atomic models of biomolecular assemblies [189-192].

For instance, native MS was used in combination with EM to characterise protein cages formed by lumazine synthase, a bacterial enzyme that self-assembles into a 60-subunit dodecahedral shell (~1 MDa) [193]. Sasaki and colleagues engineered two variants of this enzyme that resulted in much larger assemblies of either ~3 MDa or ~6 MDa. Accurate mass measurements by native MS revealed that the smaller assembly comprised 180 subunits and that the larger assembly formed a distribution of particles comprising 240-420 subunits.

Native MS was combined with X-ray crystallography in structure/function studies of a kinase, Receptor Interacting protein kinase 2 (RIP2). This study investigated the wildtype (WT) form of the RIP2 kinase domain as well as two kinase-dead point mutants [194]. While both point mutants showed suppressed autophosphorylation activity, native MS revealed that they differed significantly in oligomeric state. The WT and one mutant (D146K) formed stable 2-mers, whereas the other mutant (K47R) was in a 1-mer/2-mer equilibrium. Interestingly, crystallography revealed that the D146K and K47R mutants adopted the active and inactive conformations, respectively. The combined findings provided valuable insights by revealing a link between dimerization and kinase activation [194].

Native MS has also been used to shed light on the dynamics of macromolecular assemblies. For instance, transthyretin (TTR), an amyloidogenic protein, has been extensively studied by this technique [40, 41, 195-197]. Native MS allowed scientists to

assess the effect of point mutations and to monitor the exchange of unlabelled and labelled TTR subunits over time. In a recent report, MS-based subunit exchange experiments combined with neutron crystallography data and modelling studies led to propose a novel mechanism of TTR fibrillation [40] (**Figure 3**).

Large (several MDa) viral assemblies have also been successfully analysed by native MS [90, 198-203] as well as by other MS-based methods such as charge detection MS [204-206] and nanomechanical resonator MS (NEMS) [207] (see below). For instance, the bacteriophage HK97 was investigated by native MS and HDX MS, label-free quantitative proteomics and single-particle cryo-EM to gain insights into the function of the HK97 protease during maturation of the bacteriophage capsid [199]. The masses of protease-free and protease-containing procapsids and of the mature capsid (17.9, 21.4 and 12.9 MDa, respectively) were determined by native MS, allowing for the number of protease molecules in the procapsid and the efficiency of procapsid cleavage to be evaluated.

(ii) Native MS provides useful structural information in the absence of high-resolution 3D structures

Native MS provides important structural information even when no high-resolution 3D data are available [151, 208-212]. For instance, native MS was combined with size exclusion chromatography and multi-angle light scattering (SEC-MALS), isothermal titration calorimetry (ITC), epifluorescence microscopy and *in vivo* functional studies to investigate a bacterial complex called MukBEF, a member of the Structural Maintenance of Chromosomes (SMC) complexes involved in chromosome organization and segregation [213]. The MukBEF complex is formed by an ATPase, MukB, which associates with two other proteins, MukE and MukF. When the three proteins were incubated with ADP, three major complexes were detected: a 2-mer (MukB₂), 6-mer (MukE₄:MukF₂) and 8-mer (MukB₂:MukE₄:MukF₂). In the presence of a non-hydrolysable ATP analogue, an additional 10-mer (MukB₄:MukE₄:MukF₂) was detected. Combined with additional biophysical data, these results shed important light on the architecture of the MukBEF complex and the changes induced upon ATP binding and hydrolysis [213].

Another interesting example showed that the energy of SID experiments, which informs on the relative strength of intermolecular interfaces (see above), can be used as an experimental restraint to improve the ability of computational protein-protein docking studies to predict the quaternary structure of protein complexes. Such studies are useful when structural data are unavailable for the complex but NMR or crystal structures are individually available for the constituent subunits [214].

Excellent recent reviews discuss the application of IM-MS and molecular modelling to study unknown structures [192, 215]. In one example, the Ruotolo laboratory investigated a labile 18-subunit assembly, a urease pre-activation complex from *Klebsiella aerogenes* [216]. By combining previously reported SAXS and chemical crosslinking data with IM-MS data and molecular modeling, the authors identified a discrete population of putative structures that was consistent with all the available data for this 610-kDa assembly [216]. A second example involves studies of the plant photoreceptor UVR8, a homo-2-meric protein that dissociates into 1-mers upon UV-B light activation [217]. The authors used IM-MS and molecular dynamics simulations to study the UVR8 β -propeller core domain, whose crystal structure is known, and the full-length protein that includes intrinsically disordered N- and C-terminal regions. The authors found that the full-length 2-mer exists in two conformational families that differ in the conformation of the terminal regions. Upon UV illumination the C-terminal tail promotes the destabilization of the core domain of the UVR8 1-mer.

(iii) Native MS allows the investigation of assembly pathways

An advantage of native MS is the ability to detect several different oligomeric and compositional states simultaneously and to monitor how the distribution of these states changes in real time. This makes native MS ideally suited for studying the assembly and disassembly pathway of macromolecular complexes [73, 218, 219]. Native MS was combined with cryo-EM to investigate the assembly behaviour of a circadian oscillator from

cyanobacteria composed of three proteins, KaiC, KaiB, and KaiA [187]. Incubation of the three purified recombinant proteins with ATP reconstitutes the oscillator, which can maintain a stable rhythm autonomously for weeks *in vitro*. Native MS experiments were used to monitor how the stoichiometry and phosphorylation state of Kai protein complexes changed during the circadian cycle. These experiments revealed the formation of more than 10 different complexes (including KaiC₆, KaiC₆A₂, KaiC₆A₄, KaiC₆B₁, KaiC₆B₆ and KaiC₆B₆A_{2n}, n=1-6) that ranged in mass from ~350 to ~820 kDa over the course of 24 h (**Figure 4**). The data allowed the authors to identify conditions in which the KaiA-binding site of the KaiC₆B₆ complex was fully occupied, allowing them to determine the cryo-EM structure of a KaiC₆B₆A₁₂ complex. The combined native MS and cryo-EM data allowed them to propose a detailed model for the cyclic phosphorylation-dependent assembly of the protein oscillator.

11. Investigation of membrane protein complexes and their binding to ligands

In the last fifteen years, interactions involving membrane proteins have been studied by MS [1, 220-223], including protein-drug interactions [74, 224], protein-lipid complexes [75, 225-232] and multiprotein assemblies [76, 77, 149, 179]. Heteromeric membrane protein complexes containing both soluble and transmembrane subunits were successfully analysed by native MS [78, 79, 233-235] (**Table 2**). Here we point out a few of the most recent studies.

Native MS was instrumental in deciphering the role of lipids in tuning different conformational states of membrane proteins and the transfer of small molecules or drugs through membranes [224, 236-241]. For instance, native MS was used to investigate the bacterial ATP-Binding Cassette (ABC) transporter P-glycoprotein (P-gp) and its binding to lipids, nucleotides, and drugs in real time [242]. By determining lipid binding rates and apparent K_d values, it was shown that P-gp preferentially binds anionic (versus zwitterionic) phospholipids and short-chain (versus long-chain) cardiolipins, and that the interaction with cardiolipin is enhanced by the binding of cyclosporin A, a P-gp inhibitor. Other native MS studies of bacterial ABC transporters revealed the significance of specific annular lipids in the ATPase activity of TmrAB [227], the propensity of the lipid-A flippase MsbA to bind negatively charged phosphatidyl glycerol lipids [229], and the influence of substrate and nucleotide binding on complex formation by the molybdate importer ModBC-A and by the vitamin B12 importer BtuCD-F [230].

Native MS was also recently used to study a member of the mycobacterial membrane protein Large (MmpL) transporters from *Mycobacterium tuberculosis* [243]. These transporters shuttle fatty acids and lipid components to the mycobacterial cell wall, which is abundant in mycolic acids (MA), and are critical for mycobacterial physiology and pathogenesis. Native MS analysis revealed that the transporter MmpL3 was able to specifically bind phosphatidylethanolamine, a previously unknown MmpL3 ligand, as well as trehalose monomycolates (TMMs), which are precursors of MA-containing trehalose dimycolates (TDMs), but was unable to bind TDMs [243].

Native MS was also helpful to study allosteric effects concerning lipid interactions with the ammonia channel AmtB from *E. coli* [241]. Specifically, the authors investigated mixtures of a fluorescent-labelled cardiolipin (TFCDL) with one of six other lipids and determined K_d values for AmtB binding up to a total of five lipid molecules. This allowed the authors to estimate how some lipid-protein interactions influenced the binding of other lipid types to AmtB, revealing for example that TFCDL exhibits positive and negative allostery on the binding of phosphatidylethanolamine and phosphatidic acid lipids, respectively [241].

Several MS-based studies employed detergents (such as β-octylglucoside and dodecylmaltoside) to maintain membrane protein complexes in their native oligomeric state. The development of detergent-free alternatives indicated that native forms of proteins and macromolecular assemblies can also be preserved if lipid bicelles, amphipols or nanodiscs are used [1, 244-247]. Native MS and nanodiscs were used to study the interactions between glycolipids and two toxins (i.e., the B subunit homo-5-mers of cholera toxin and heat labile toxin) and their relative affinities ranked by screening the proteins against different glycolipid-incorporating nanodiscs [248]. Small, soluble protein-lipid complexes (~3

nm), named picodiscs, were exploited for time-resolved enzymatic studies, antibody-antigen interaction assays and bacterial toxin-glycolipid interaction analyses [249].

In 2018 a detergent-free approach was developed for obtaining mass spectra of biomolecular complexes from native membranes without chemical disruption [250]. Lipid vesicles isolated from prokaryotic and eukaryotic membranes were disrupted and multiple protein complexes ejected “directly” into the instrument gas phase [250]. Quantitative proteomics, lipidomics and native MS allowed the authors to identify numerous complexes, including a chaperone-porin complex in the outer *E. coli* membrane, different subassemblies of multidrug efflux pumps in the inner membrane, and a complex between F_1F_0 ATP synthase and the protein translocation channel SecYEG.

12. Analysis of macromolecular complexes by hybrid MS-based methods

Hybrid MS-based methods address biological problems through the use of more than one MS approach and instrument. For example, van de Waterbeemd *et al.* combined bottom-up proteomics, top-down MS and native MS to study ribosomal particles from bacteria, plants and humans [93, 251]. Using proteomics, they identified the ribosomal proteins and their PTMs. Using top-down MS, they assessed the presence of different proteoforms (carrying multiple PTMs and truncations) [129, 135]. Using MS³, they determined the composition and stoichiometry of intact ribonucleoprotein complexes, including the human 40S ribosomal subunit bound to viral internal ribosome entry site (IRES) RNA elements.

Several studies have reported native MS analyses of membrane proteins and membrane macromolecular coupled with lipidomics. The latter approach enables LC-MS/MS-based identification of extracted lipids to characterize the function of the bound lipidome [229, 250, 252]. A hybrid MS approach has also been used to study the heterogeneity of monoclonal antibodies [253]. Specifically, native MS was combined with targeted glycan profiling (the MS/MS-based investigation of glycans released by PNGase F digestion). Native MS analysis provided a general overview of the glycoform distribution and indicated the coexistence of antibody modifications such as glycations and truncations. Profiling of released glycans elucidated the linkages within carbohydrate residues and enabled the differentiation of structural isomers.

Overall, these examples illustrate the utility of hybrid MS-based methods to gather insights on inter-subunit interactions within macromolecular complexes, on primary sequences of subunits and on features of bound molecules such as metals, lipids and glycans.

13. Concluding remarks and outlook

Twenty five years ago, the impressive results achieved using bottom-up proteomics firmly established MS as a key technology for biological research [254-257]. At that time, MALDI- and ESI-MS exhibited excellent sensitivity, exquisite accuracy of mass measurement and high speed of data acquisition [258, 259]. Currently, bottom-up proteomics allows one to exhaustively describe the complete proteome of a model organism in a few hours [257] as well as to profile multiple cancer proteomes per mass spectrometer per day, paving the way for proteomics applications in personalized cancer medicine [260]. Soon it may even be possible to profile the proteome of individual cells [261]. Although the first MS studies of non-covalent interactions were reported over two decades ago, [68-71], native MS is currently far less widely used than bottom-up proteomics. Nevertheless, native MS-based studies have steadily gained momentum in the field of integrative structural biology thanks to its wide applicability, speed of analysis, sensitivity and selectivity [179, 262-265]. This latter feature is especially valuable, as it enables several species with different masses to be simultaneously analysed and separated. Indeed, the fact that mass selectivity was recently exploited to image individual proteins and a protein complex by low-energy electron holography demonstrate that MS can be exploited to purify heterogeneous assemblies for structural studies [49].

A key strength of native MS that will prove increasingly useful is its ability to inform on the identity, solubility, oligomeric state, and stability of overexpressed biomolecules directly in crude cell lysates and culture media (i.e., without the need for purification) [266-268]. Moreover, the investigation of endogenous macromolecular assemblies without the need for their recombinant over-expression in prokaryotic and in eukaryotic hosts is also emerging. Several examples of endogenous complexes studied by native MS point to an increasingly important role in deciphering the function and regulation of PTMs, cofactors and transient complexes assembled in a cellular milieu [150, 250, 251, 263, 269, 270].

The use of native MS to investigate endogenous complexes will undoubtedly improve with technical advances that allow the enhanced purification yield of complexes present in cells at their natural expression level [271, 272] and with the development of new instruments having increased sensitivity, resolution and ionisation efficiency. Orbitrap mass spectrometers already present an improvement in sensitivity and resolution compared to Q-TOF instruments [85, 86, 88]. Novel ionisation approaches are of key importance for increasing the amount of sample introduced in mass spectrometers [273-278]. Moreover, new detectors advance the applicability of MS to study biological nanoparticles. For instance, nanoelectromechanical systems (NEMS) detect large masses with unprecedented sensitivity, requiring only a few hundred single-molecule adsorption events to detect megadalton molecules [207, 279]. Recently, empty and DNA-filled bacteriophage T5 capsids with masses up to 105 MDa were investigated, using less than 1 picomole of biological material and reaching an instrument resolution above 100 [207].

Novel detectors were also developed for charge detection mass spectrometry (CDMS), whereby the m/z and z are simultaneously measured for each ion [206, 280-282]. Recently CDMS was combined with cryo-EM, lipid and glycan analysis to study Sindbis viruses (>50 MDa) produced from arthropod and vertebrate hosts [283]. These studies revealed differences in lipid composition between the Sindbis viruses that influenced the assembly, budding and stability of viral particles, explaining differences in infectivity [283]. CDMS was also combined with transmission EM to investigate the heterogeneity and polymorphism of amyloid fibers [284, 285].

A key area that requires improvement is data analysis software. Currently, some MS software packages are available for the characterisation of macromolecular complexes (e.g., [286-289]), but manual evaluation remains critical. Programs as effective as those developed for bottom-up proteomics will enable high-throughput studies of non-covalent assemblies.

Overall, multiple complementary techniques are often necessary to decipher the structures of challenging macromolecular complexes. The examples cited in this review suggest that native MS will play an increasingly important role in such structural studies.

References

- [1] Calabrese A.N. and Radford S.E. Mass spectrometry-enabled structural biology of membrane proteins. *Methods*, 2018; 147: 187-205.
- [2] Kaur U., Johnson D.T., Chea E.E., Deredge D.J., Espino J.A., and Jones L.M. Evolution of Structural Biology through the Lens of Mass Spectrometry. *Anal Chem*, 2019; 91: 142-155.
- [3] Kaur U., Meng H., Lui F., Ma R., Ogburn R.N., Johnson J.H.R., Fitzgerald M.C., and Jones L.M. Proteome-Wide Structural Biology: An Emerging Field for the Structural Analysis of Proteins on the Proteomic Scale. *J Proteome Res*, 2018; 17: 3614-3627.

- [4] Kondrat F.D., Struwe W.B., and Benesch J.L. Native mass spectrometry: towards high-throughput structural proteomics. *Methods Mol Biol*, 2015; 1261: 349-71.
- [5] Petrotchenko E.V. and Borchers C.H. Modern mass spectrometry-based structural proteomics. *Adv Protein Chem Struct Biol*, 2014; 95: 193-213.
- [6] Piotrowski C. and Sinz A. Structural Investigation of Proteins and Protein Complexes by Chemical Cross-Linking/Mass Spectrometry. *Adv Exp Med Biol*, 2018; 1105: 101-121.
- [7] Kochert B.A., Jacob R.E., Wales T.E., Makriyannis A., and Engen J.R. Hydrogen-Deuterium Exchange Mass Spectrometry to Study Protein Complexes. *Methods Mol Biol*, 2018; 1764: 153-171.
- [8] Kostyukevich Y., Acter T., Zhrebker A., Ahmed A., Kim S., and Nikolaev E. Hydrogen/deuterium exchange in mass spectrometry. *Mass Spectrom Rev*, 2018; 37: 811-853.
- [9] Mistarz U.H., Chandler S.A., Brown J.M., Benesch J.L.P., and Rand K.D. Probing the Dissociation of Protein Complexes by Means of Gas-Phase H/D Exchange Mass Spectrometry. *J Am Soc Mass Spectrom*, 2019; 30: 45-57.
- [10] Maleknia S.D. and Downard K.M. Protein Footprinting with Radical Probe Mass Spectrometry- Two Decades of Achievement. *Protein Pept Lett*, 2019; 26: 4-15.
- [11] Rajabi K., Ashcroft A.E., and Radford S.E. Mass spectrometric methods to analyze the structural organization of macromolecular complexes. *Methods*, 2015; 89: 13-21.
- [12] Feng Y., De Franceschi G., Kahraman A., Soste M., Melnik A., Boersema P.J., de Laureto P.P., Nikolaev Y., Oliveira A.P., and Picotti P. Global analysis of protein structural changes in complex proteomes. *Nat Biotechnol*, 2014; 32: 1036-44.
- [13] Chang Y., Schleich J.P., VerHeul R.A., and Park C. Simplified proteomics approach to discover protein-ligand interactions. *Protein Sci*, 2012; 21: 1280-7.
- [14] Prabhu N., Dai L., and Nordlund P. CETSA in integrated proteomics studies of cellular processes. *Curr Opin Chem Biol*, 2019; 54: 54-62.
- [15] Drewes G. and Knapp S. Chemoproteomics and Chemical Probes for Target Discovery. *Trends Biotechnol*, 2018; 36: 1275-1286.
- [16] McFedries A., Schwaid A., and Saghatelian A. Methods for the elucidation of protein-small molecule interactions. *Chem Biol*, 2013; 20: 667-73.
- [17] Politis A. and Schmidt C. Structural characterisation of medically relevant protein assemblies by integrating mass spectrometry with computational modelling. *J Proteomics*, 2018; 175: 34-41.
- [18] Sharon M. How far can we go with structural mass spectrometry of protein complexes? *J Am Soc Mass Spectrom*, 2010; 21: 487-500.

- [19] Allison T.M. and Bechara C. Structural mass spectrometry comes of age: new insight into protein structure, function and interactions. *Biochem Soc Trans*, 2019; 47: 317-327.
- [20] Leney A.C. and Heck A.J. Native Mass Spectrometry: What is in the Name? *J Am Soc Mass Spectrom*, 2017; 28: 5-13.
- [21] Tong W. and Wang G. How can native mass spectrometry contribute to characterization of biomacromolecular higher-order structure and interactions? *Methods*, 2018; 144: 3-13.
- [22] Boeri Erba E. and Petosa C. The emerging role of native mass spectrometry in characterising the structure and dynamics of macromolecular complexes. *Protein Sci*, 2015.
- [23] Boeri Erba E., Signor L., Oliva M.F., Hans F., and Petosa C. Characterizing Intact Macromolecular Complexes Using Native Mass Spectrometry. *Methods Mol Biol*, 2018; 1764: 133-151.
- [24] Hernandez H. and Robinson C.V. Determining the stoichiometry and interactions of macromolecular assemblies from mass spectrometry. *Nat Protoc*, 2007; 2: 715-26.
- [25] Liko I., Allison T.M., Hopper J.T., and Robinson C.V. Mass spectrometry guided structural biology. *Curr Opin Struct Biol*, 2016; 40: 136-144.
- [26] Lossl P., Brunner A.M., Liu F., Leney A.C., Yamashita M., Scheltema R.A., and Heck A.J. Deciphering the Interplay among Multisite Phosphorylation, Interaction Dynamics, and Conformational Transitions in a Tripartite Protein System. *ACS Cent Sci*, 2016; 2: 445-55.
- [27] Olshina M.A. and Sharon M. Mass Spectrometry: A Technique of Many Faces. *Q Rev Biophys*, 2016; 49.
- [28] Macek P., Kerfah R., Boeri Erba E., Crublet E., Moriscot C., Schoehn G., Amero C., and Boisbouvier J. Unraveling self-assembly pathways of the 468-kDa proteolytic machine TET2. *Sci Adv*, 2017; 3: e1601601.
- [29] Yewdall N.A., Allison T.M., Pearce F.G., Robinson C.V., and Gerrard J.A. Self-assembly of toroidal proteins explored using native mass spectrometry. *Chem Sci*, 2018; 9: 6099-6106.
- [30] Boeri Erba E., Barylyuk K., Yang Y., and Zenobi R. Quantifying protein-protein interactions within noncovalent complexes using electrospray ionization mass spectrometry. *Anal Chem*, 2011; 83: 9251-9.
- [31] Gabelica V., Rosu F., and De Pauw E. A simple method to determine electrospray response factors of noncovalent complexes. *Anal Chem*, 2009; 81: 6708-15.
- [32] Gavriilidou A.F.M., Holding F.P., Coyle J.E., and Zenobi R. Application of Native ESI-MS to Characterize Interactions between Compounds Derived from Fragment-Based

- Discovery Campaigns and Two Pharmaceutically Relevant Proteins. *SLAS Discov*, 2018; 23: 951-959.
- [33] Sokolovski M., Cveticanin J., Hayoun D., Korobko I., Sharon M., and Horovitz A. Measuring inter-protein pairwise interaction energies from a single native mass spectrum by double-mutant cycle analysis. *Nat Commun*, 2017; 8: 212.
- [34] Ben-Nissan G. and Sharon M. The application of ion-mobility mass spectrometry for structure/function investigation of protein complexes. *Curr Opin Chem Biol*, 2018; 42: 25-33.
- [35] Chandler S.A. and Benesch J.L. Mass spectrometry beyond the native state. *Curr Opin Chem Biol*, 2018; 42: 130-137.
- [36] Dixit S.M., Polasky D.A., and Ruotolo B.T. Collision induced unfolding of isolated proteins in the gas phase: past, present, and future. *Curr Opin Chem Biol*, 2018; 42: 93-100.
- [37] Eschweiler J.D., Kerr R., Rabuck-Gibbons J., and Ruotolo B.T. Sizing Up Protein-Ligand Complexes: The Rise of Structural Mass Spectrometry Approaches in the Pharmaceutical Sciences. *Annu Rev Anal Chem (Palo Alto Calif)*, 2017; 10: 25-44.
- [38] Poltash M.L., McCabe J.W., Shirzadeh M., Laganowsky A., Clowers B.H., and Russell D.H. Fourier Transform-Ion Mobility-Orbitrap Mass Spectrometer: A Next-Generation Instrument for Native Mass Spectrometry. *Anal Chem*, 2018; 90: 10472-10478.
- [39] Ahnert S.E., Marsh J.A., Hernandez H., Robinson C.V., and Teichmann S.A. Principles of assembly reveal a periodic table of protein complexes. *Science*, 2015; 350: aaa2245.
- [40] Yee A.W., Aldeghi M., Blakeley M.P., Ostermann A., Mas P.J., Moulin M., de Sanctis D., Bowler M.W., Mueller-Dieckmann C., Mitchell E.P., Haertlein M., de Groot B.L., Boeri Erba E., and Forsyth V.T. A molecular mechanism for transthyretin amyloidogenesis. *Nat Commun*, 2019; 10: 925.
- [41] Yee A.W., Moulin M., Breteau N., Haertlein M., Mitchell E.P., Cooper J.B., Boeri Erba E., and Forsyth V.T. Impact of Deuteration on the Assembly Kinetics of Transthyretin Monitored by Native Mass Spectrometry and Implications for Amyloidoses. *Angew Chem Int Ed Engl*, 2016; 55: 9292-6.
- [42] Belov M.E., Damoc E., Denisov E., Compton P.D., Horning S., Makarov A.A., and Kelleher N.L. From protein complexes to subunit backbone fragments: a multi-stage approach to native mass spectrometry. *Anal Chem*, 2013; 85: 11163-73.
- [43] Sobott F., Hernandez H., McCammon M.G., Tito M.A., and Robinson C.V. A tandem mass spectrometer for improved transmission and analysis of large macromolecular assemblies. *Anal Chem*, 2002; 74: 1402-7.

- [44] Bich C., Baer S., Jecklin M.C., and Zenobi R. Probing the hydrophobic effect of noncovalent complexes by mass spectrometry. *J Am Soc Mass Spectrom*, 2010; 21: 286-9.
- [45] Meyer T., de la Cruz X., and Orozco M. An atomistic view to the gas phase proteome. *Structure*, 2009; 17: 88-95.
- [46] Seo J., Hoffmann W., Warnke S., Bowers M.T., Pagel K., and von Helden G. Retention of Native Protein Structures in the Absence of Solvent: A Coupled Ion Mobility and Spectroscopic Study. *Angew Chem Int Ed Engl*, 2016; 55: 14173-14176.
- [47] Ouyang Z., Takats Z., Blake T.A., Gologan B., Guymon A.J., Wiseman J.M., Oliver J.C., Davisson V.J., and Cooks R.G. Preparing protein microarrays by soft-landing of mass-selected ions. *Science*, 2003; 301: 1351-4.
- [48] Boyken S.E., Benhaim M.A., Busch F., Jia M., Bick M.J., Choi H., Klima J.C., Chen Z., Walkey C., Mileant A., et al. De novo design of tunable, pH-driven conformational changes. *Science*, 2019; 364: 658-664.
- [49] Longchamp J.N., Rauschenbach S., Abb S., Escher C., Latychevskaia T., Kern K., and Fink H.W. Imaging proteins at the single-molecule level. *Proc Natl Acad Sci U S A*, 2017; 114: 1474-1479.
- [50] Bolbach G. Matrix-assisted laser desorption/ionization analysis of non-covalent complexes: fundamentals and applications. *Current pharmaceutical design*, 2005; 11: 2535-57.
- [51] Chen F., Gulbakan B., Weidmann S., Fagerer S.R., Ibanez A.J., and Zenobi R. Applying mass spectrometry to study non-covalent biomolecule complexes. *Mass Spectrometry Reviews*, 2015; 35: 48-70.
- [52] Madler S., Boeri Erba E., and Zenobi R. MALDI-ToF mass spectrometry for studying noncovalent complexes of biomolecules. *Top Curr Chem*, 2013; 331: 1-36.
- [53] Wang J.S., Whitehead S.N., and Yeung K.K. Detection of Amyloid Beta (A β) Oligomeric Composition Using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS). *J Am Soc Mass Spectrom*, 2018; 29: 786-795.
- [54] Beaufour M., Ginguene D., Le Meur R., Castaing B., and Cadene M. Liquid Native MALDI Mass Spectrometry for the Detection of Protein-Protein Complexes. *Journal of the American Society for Mass Spectrometry*, 2018; 29: 1981-1994.
- [55] Kebarle P. and Verkerk U.H. Electrospray: from ions in solution to ions in the gas phase, what we know now. *Mass Spectrom Rev*, 2009; 28: 898-917.
- [56] Wilm M. Principles of electrospray ionization. *Mol Cell Proteomics*, 2011; 10: M111 009407.

- [57] Yue X., Vahidi S., and Konermann L. Insights into the mechanism of protein electrospray ionization from salt adduction measurements. *J Am Soc Mass Spectrom*, 2014; 25: 1322-31.
- [58] Konermann L. Addressing a Common Misconception: Ammonium Acetate as Neutral pH "Buffer" for Native Electrospray Mass Spectrometry. *Journal of the American Society for Mass Spectrometry*, 2017; 28: 1827-1835.
- [59] Dyachenko A., Gruber R., Shimon L., Horovitz A., and Sharon M. Allosteric mechanisms can be distinguished using structural mass spectrometry. *Proc Natl Acad Sci U S A*, 2013; 110: 7235-9.
- [60] Zhuang X., Gavriilidou A.F.M., and Zenobi R. Influence of Alkylammonium Acetate Buffers on Protein-Ligand Noncovalent Interactions Using Native Mass Spectrometry. *Journal of the American Society for Mass Spectrometry*, 2017; 28: 341-346.
- [61] Wilm M. and Mann M. Electrospray and Taylor-Cone theory, Dole's beam of macromolecules at last. *Int. J. Mass Spectrom. Ion Proc.*, 1994; 136: 167-180.
- [62] Wilm M. and Mann M. Analytical properties of the nanoelectrospray ion source. *Anal Chem*, 1996; 68: 1-8.
- [63] Zhang H., Cui W., Gross M.L., and Blankenship R.E. Native mass spectrometry of photosynthetic pigment-protein complexes. *FEBS Lett*, 2013.
- [64] Susa A.C., Xia Z., and Williams E.R. Small Emitter Tips for Native Mass Spectrometry of Proteins and Protein Complexes from Nonvolatile Buffers That Mimic the Intracellular Environment. *Anal Chem*, 2017; 89: 3116-3122.
- [65] Susa A.C., Xia Z., and Williams E.R. Native Mass Spectrometry from Common Buffers with Salts That Mimic the Extracellular Environment. *Angew Chem Int Ed Engl*, 2017; 56: 7912-7915.
- [66] Xia Z., DeGrandchamp J.B., and Williams E.R. Native mass spectrometry beyond ammonium acetate: effects of nonvolatile salts on protein stability and structure. *Analyst*, 2019; 144: 2565-2573.
- [67] Susa A.C., Lippens J.L., Xia Z., Loo J.A., Campuzano I.D.G., and Williams E.R. Submicrometer Emitter ESI Tips for Native Mass Spectrometry of Membrane Proteins in Ionic and Nonionic Detergents. *J Am Soc Mass Spectrom*, 2018; 29: 203-206.
- [68] Smith R.D. and Light-Wahl K.J. The observation of non-covalent interactions in solution by electrospray ionization mass spectrometry: promise, pitfalls and prognosis. *Biological mass spectrometry*, 1993; 22: 493-501.
- [69] Katta V. and Chait B.T. Observation of the heme-globin complex in native myoglobin by electrospray-ionization mass spectrometry. *J Am Chem Soc*, 1991; 113: 8535-7.

- [70] Ganem B., Li Y.T., and Henion J.D. Detection of noncovalent receptor ligand complexes by mass spectrometry. *J Am Chem Soc*, 1991; 113: 6294-6.
- [71] Ganem B., Li Y.T., and Henion J.D. Observation of noncovalent enzyme-substrate and enzyme-product complexes by ion-spray mass spectrometry. *J Am Chem Soc*, 1991; 113: 7818-9.
- [72] van den Heuvel R.H., van Duijn E., Mazon H., Synowsky S.A., Lorenzen K., Versluis C., Brouns S.J., Langridge D., van der Oost J., Hoyes J., and Heck A.J. Improving the performance of a quadrupole time-of-flight instrument for macromolecular mass spectrometry. *Anal Chem*, 2006; 78: 7473-83.
- [73] Snijder J., Rose R.J., Veessler D., Johnson J.E., and Heck A.J. Studying 18 MDa virus assemblies with native mass spectrometry. *Angew Chem Int Ed Engl*, 2013; 52: 4020-3.
- [74] Ilag L.L., Ubarretxena-Belandia I., Tate C.G., and Robinson C.V. Drug binding revealed by tandem mass spectrometry of a protein-micelle complex. *J Am Chem Soc*, 2004; 126: 14362-3.
- [75] Demmers J.A., van Dalen A., de Kruijff B., Heck A.J., and Killian J.A. Interaction of the K⁺ channel KcsA with membrane phospholipids as studied by ESI mass spectrometry. *FEBS Lett*, 2003; 541: 28-32.
- [76] Lengqvist J., Svensson R., Evergren E., Morgenstern R., and Griffiths W.J. Observation of an intact noncovalent homotrimer of detergent-solubilized rat microsomal glutathione transferase-1 by electrospray mass spectrometry. *J Biol Chem*, 2004; 279: 13311-6.
- [77] Esteban O., Bernal R.A., Donohoe M., Videler H., Sharon M., Robinson C.V., and Stock D. Stoichiometry and localization of the stator subunits E and G in *Thermus thermophilus* H⁺-ATPase/synthase. *J Biol Chem*, 2008; 283: 2595-603.
- [78] Barrera N.P., Di Bartolo N., Booth P.J., and Robinson C.V. Micelles protect membrane complexes from solution to vacuum. *Science*, 2008; 321: 243-6.
- [79] Barrera N.P., Isaacson S.C., Zhou M., Bavro V.N., Welch A., Schaedler T.A., Seeger M.A., Miguel R.N., Korkhov V.M., van Veen H.W., Venter H., Walmsley A.R., Tate C.G., and Robinson C.V. Mass spectrometry of membrane transporters reveals subunit stoichiometry and interactions. *Nat Methods*, 2009; 6: 585-7.
- [80] Zhou M., Morgner N., Barrera N.P., Politis A., Isaacson S.C., Matak-Vinkovic D., Murata T., Bernal R.A., Stock D., and Robinson C.V. Mass spectrometry of intact V-type ATPases reveals bound lipids and the effects of nucleotide binding. *Science*, 2011; 334: 380-5.
- [81] Makarov A. Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis. *Anal Chem*, 2000; 72: 1156-62.

- [82] Makarov A., Denisov E., Kholomeev A., Balschun W., Lange O., Strupat K., and Horning S. Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer. *Anal Chem*, 2006; 78: 2113-20.
- [83] Makarov A., Denisov E., Lange O., and Horning S. Dynamic range of mass accuracy in LTQ Orbitrap hybrid mass spectrometer. *J Am Soc Mass Spectrom*, 2006; 17: 977-82.
- [84] Mohr J., Swart R., Samonig M., Bohm G., and Huber C.G. High-efficiency nano- and micro-HPLC--high-resolution Orbitrap-MS platform for top-down proteomics. *Proteomics*, 2010; 10: 3598-609.
- [85] Rose R.J., Damoc E., Denisov E., Makarov A., and Heck A.J. High-sensitivity Orbitrap mass analysis of intact macromolecular assemblies. *Nat Methods*, 2012; 9: 1084-6.
- [86] Rosati S., Rose R.J., Thompson N.J., van Duijn E., Damoc E., Denisov E., Makarov A., and Heck A.J. Exploring an orbitrap analyzer for the characterization of intact antibodies by native mass spectrometry. *Angew Chem Int Ed Engl*, 2012; 51: 12992-6.
- [87] Compton P.D. and Kelleher N.L. Spinning up mass spectrometry for whole protein complexes. *Nat Methods*, 2012; 9: 1065-6.
- [88] Whitelegge J. Intact protein mass spectrometry and top-down proteomics. *Expert Rev Proteomics*, 2013; 10: 127-9.
- [89] Snijder J., van de Waterbeemd M., Damoc E., Denisov E., Grinfeld D., Bennett A., Agbandje-McKenna M., Makarov A., and Heck A.J. Defining the stoichiometry and cargo load of viral and bacterial nanoparticles by Orbitrap mass spectrometry. *J Am Chem Soc*, 2014; 136: 7295-9.
- [90] Snijder J. and Heck A.J. Analytical approaches for size and mass analysis of large protein assemblies. *Annu Rev Anal Chem (Palo Alto Calif)*, 2014; 7: 43-64.
- [91] Ben-Nissan G., Belov M.E., Morgenstern D., Levin Y., Dym O., Arkind G., Lipson C., Makarov A.A., and Sharon M. Triple-Stage Mass Spectrometry Unravels the Heterogeneity of an Endogenous Protein Complex. *Anal Chem*, 2017; 89: 4708-4715.
- [92] Fort K.L., van de Waterbeemd M., Boll D., Reinhardt-Szyba M., Belov M.E., Sasaki E., Zschoche R., Hilvert D., Makarov A.A., and Heck A.J.R. Expanding the structural analysis capabilities on an Orbitrap-based mass spectrometer for large macromolecular complexes. *Analyst*, 2017; 143: 100-105.
- [93] van de Waterbeemd M., Fort K.L., Boll D., Reinhardt-Szyba M., Routh A., Makarov A., and Heck A.J. High-fidelity mass analysis unveils heterogeneity in intact ribosomal particles. *Nat Methods*, 2017; 14: 283-286.

- [94] Benesch J.L., Aquilina J.A., Ruotolo B.T., Sobott F., and Robinson C.V. Tandem mass spectrometry reveals the quaternary organization of macromolecular assemblies. *Chem Biol*, 2006; 13: 597-605.
- [95] Brodbelt J.S. Ion Activation Methods for Peptides and Proteins. *Anal Chem*, 2016; 88: 30-51.
- [96] Lermyte F., Valkenburg D., Loo J.A., and Sobott F. Radical solutions: Principles and application of electron-based dissociation in mass spectrometry-based analysis of protein structure. *Mass Spectrom Rev*, 2018; 37: 750-771.
- [97] Stiving A.Q., VanAernum Z.L., Busch F., Harvey S.R., Sarni S.H., and Wysocki V.H. Surface-Induced Dissociation: An Effective Method for Characterization of Protein Quaternary Structure. *Anal Chem*, 2019; 91: 190-209.
- [98] Zhou M. and Wysocki V.H. Surface induced dissociation: dissecting noncovalent protein complexes in the gas phase. *Acc Chem Res*, 2014; 47: 1010-8.
- [99] Boeri Erba E., Ruotolo B.T., Barsky D., and Robinson C.V. Ion mobility-mass spectrometry reveals the influence of subunit packing and charge on the dissociation of multiprotein complexes. *Anal Chem*, 2010; 82: 9702-10.
- [100] Hall Z., Hernandez H., Marsh J.A., Teichmann S.A., and Robinson C.V. The role of salt bridges, charge density, and subunit flexibility in determining disassembly routes of protein complexes. *Structure*, 2013; 21: 1325-37.
- [101] Pagel K., Hyung S.J., Ruotolo B.T., and Robinson C.V. Alternate dissociation pathways identified in charge-reduced protein complex ions. *Anal Chem*, 2010; 82: 5363-72.
- [102] Loo R.R. and Loo J.A. Salt Bridge Rearrangement (SaBRe) Explains the Dissociation Behavior of Noncovalent Complexes. *J Am Soc Mass Spectrom*, 2016; 27: 975-90.
- [103] Morrison L.J. and Brodbelt J.S. 193 nm Ultraviolet Photodissociation Mass Spectrometry of Tetrameric Protein Complexes Provides Insight into Quaternary and Secondary Protein Topology. *J Am Chem Soc*, 2016; 138: 10849-59.
- [104] Tamara S., Dyachenko A., Fort K.L., Makarov A.A., Scheltema R.A., and Heck A.J. Symmetry of Charge Partitioning in Collisional and UV Photon-Induced Dissociation of Protein Assemblies. *J Am Chem Soc*, 2016; 138: 10860-8.
- [105] Leney A.C. Subunit pI Can Influence Protein Complex Dissociation Characteristics. *J Am Soc Mass Spectrom*, 2019; 30: 1389-1395.
- [106] Lorenzen K. and van Duijn E. Native mass spectrometry as a tool in structural biology. *Curr Protoc Protein Sci*, 2010; Chapter 17: Unit17 12.
- [107] Harvey S.R., Liu Y., Liu W., Wysocki V.H., and Laganowsky A. Surface induced dissociation as a tool to study membrane protein complexes. *Chem Commun (Camb)*, 2017; 53: 3106-3109.

- [108] Quintyn R.S., Yan J., and Wysocki V.H. Surface-Induced Dissociation of Homotetramers with D2 Symmetry Yields their Assembly Pathways and Characterizes the Effect of Ligand Binding. *Chem Biol*, 2015; 22: 583-92.
- [109] VanAernum Z.L., Gilbert J.D., Belov M.E., Makarov A.A., Horning S.R., and Wysocki V.H. Surface-Induced Dissociation of Noncovalent Protein Complexes in an Extended Mass Range Orbitrap Mass Spectrometer. *Anal Chem*, 2019; 91: 3611-3618.
- [110] Brodbelt J.S. Photodissociation mass spectrometry: new tools for characterization of biological molecules. *Chem Soc Rev*, 2014; 43: 2757-83.
- [111] Zubarev R.A. Electron-capture dissociation tandem mass spectrometry. *Curr Opin Biotechnol*, 2004; 15: 12-6.
- [112] Zubarev R.A., Kelleher N.L., and McLafferty F.W. Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process. *J. Am. Chem. Soc.*, 1998: 3265-66.
- [113] Ding L. and Brancia F.L. Electron capture dissociation in a digital ion trap mass spectrometer. *Anal Chem*, 2006; 78: 1995-2000.
- [114] Voinov V.G., Deinzer M.L., Beckman J.S., and Barofsky D.F. Electron capture, collision-induced, and electron capture-collision induced dissociation in Q-TOF. *J Am Soc Mass Spectrom*, 2011; 22: 607-11.
- [115] Skinner O.S., McAnally M.O., Van Duyne R.P., Schatz G.C., Breuker K., Compton P.D., and Kelleher N.L. Native Electron Capture Dissociation Maps to Iron-Binding Channels in Horse Spleen Ferritin. *Anal Chem*, 2017; 89: 10711-10716.
- [116] Oh H.B. and Moon B. Radical-driven peptide backbone dissociation tandem mass spectrometry. *Mass Spectrom Rev*, 2015; 34: 116-32.
- [117] Wang H., Eschweiler J., Cui W., Zhang H., Frieden C., Ruotolo B.T., and Gross M.L. 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.
- [118] Syka J.E., Coon J.J., Schroeder M.J., Shabanowitz J., and Hunt D.F. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc Natl Acad Sci U S A*, 2004; 101: 9528-33.
- [119] Riley N.M. and Coon J.J. The Role of Electron Transfer Dissociation in Modern Proteomics. *Anal Chem*, 2018; 90: 40-64.
- [120] Kjeldsen F., Giessing A.M., Ingrell C.R., and Jensen O.N. Peptide sequencing and characterization of post-translational modifications by enhanced ion-charging and liquid chromatography electron-transfer dissociation tandem mass spectrometry. *Anal Chem*, 2007; 79: 9243-52.

- [121] Chi A., Huttenhower C., Geer L.Y., Coon J.J., Syka J.E., Bai D.L., Shabanowitz J., Burke D.J., Troyanskaya O.G., and Hunt D.F. Analysis of phosphorylation sites on proteins from *Saccharomyces cerevisiae* by electron transfer dissociation (ETD) mass spectrometry. *Proc Natl Acad Sci U S A*, 2007; 104: 2193-8.
- [122] Tsybin Y.O., Fornelli L., Stoermer C., Luebeck M., Parra J., Nallet S., Wurm F.M., and Hartmer R. Structural analysis of intact monoclonal antibodies by electron transfer dissociation mass spectrometry. *Anal Chem*, 2011; 83: 8919-27.
- [123] Lermyte F., Verschueren T., Brown J.M., Williams J.P., Valkenburg D., and Sobott F. Characterization of top-down ETD in a travelling-wave ion guide. *Methods*, 2015; 89: 22-9.
- [124] Lermyte F., Konijnenberg A., Williams J.P., Brown J.M., Valkenburg D., and 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: 343-50.
- [125] Kelleher N.L., Lin H.Y., Valaskovic G.A., Aaserud D.J., Fridriksson E.K., and McLafferty F.W. Top Down versus Bottom Up Protein Characterization by Tandem High-Resolution Mass Spectrometry. *J. Am. Chem. Soc.*, 1999: 806-12.
- [126] Chait B.T. Chemistry. Mass spectrometry: bottom-up or top-down? *Science*, 2006; 314: 65-6.
- [127] Lermyte F., Tsybin Y.O., O'Connor P.B., and Loo J.A. Top or Middle? Up or Down? Toward a Standard Lexicon for Protein Top-Down and Allied Mass Spectrometry Approaches. *J Am Soc Mass Spectrom*, 2019; 30: 1149-1157.
- [128] Kelleher N.L. Top-down proteomics. *Anal Chem*, 2004; 76: 197A-203A.
- [129] Smith L.M. and Kelleher N.L. Proteoform: a single term describing protein complexity. *Nat Methods*, 2013; 10: 186-7.
- [130] Smith L.M. and Kelleher N.L. Proteoforms as the next proteomics currency. *Science*, 2018; 359: 1106-1107.
- [131] Armirotti A. and Damonte G. Achievements and perspectives of top-down proteomics. *Proteomics*, 2010; 10: 3566-76.
- [132] Cui W., Rohrs H.W., and Gross M.L. Top-down mass spectrometry: recent developments, applications and perspectives. *Analyst*, 2011; 136: 3854-64.
- [133] Garcia B.A. What does the future hold for Top Down mass spectrometry? *J Am Soc Mass Spectrom*, 2010; 21: 193-202.
- [134] Siuti N. and Kelleher N.L. Decoding protein modifications using top-down mass spectrometry. *Nat Methods*, 2007; 4: 817-21.
- [135] Smith L.M., Thomas P.M., Shortreed M.R., Schaffer L.V., Fellers R.T., LeDuc R.D., Tucholski T., Ge Y., Agar J.N., Anderson L.C., et al. A five-level classification system for proteoform identifications. *Nat Methods*, 2019.

- [136] Li H., Nguyen H.H., Ogorzalek Loo R.R., Campuzano I.D.G., and Loo J.A. An integrated native mass spectrometry and top-down proteomics method that connects sequence to structure and function of macromolecular complexes. *Nat Chem*, 2018; 10: 139-148.
- [137] Polasky D.A., Lermyte F., Nshanian M., Sobott F., Andrews P.C., Loo J.A., and Ruotolo B.T. Fixed-Charge Trimethyl Pyrylium Modification for Enabling Enhanced Top-Down Mass Spectrometry Sequencing of Intact Protein Complexes. *Anal Chem*, 2018; 90: 2756-2764.
- [138] Donnelly D.P., Rawlins C.M., DeHart C.J., Fornelli L., Schachner L.F., Lin Z., Lippens J.L., Aluri K.C., Sarin R., Chen B., et al. Best practices and benchmarks for intact protein analysis for top-down mass spectrometry. *Nat Methods*, 2019; 16: 587-594.
- [139] Schachner L.F., Ives A.N., McGee J.P., Melani R.D., Kafader J.O., Compton P.D., Patrie S.M., and Kelleher N.L. Standard Proteoforms and Their Complexes for Native Mass Spectrometry. *J Am Soc Mass Spectrom*, 2019; 30: 1190-1198.
- [140] Skinner O.S., Haverland N.A., Fornelli L., Melani R.D., Do Vale L.H.F., Seckler H.S., Doubleday P.F., Schachner L.F., Szrentic K., Kelleher N.L., and Compton P.D. Top-down characterization of endogenous protein complexes with native proteomics. *Nat Chem Biol*, 2018; 14: 36-41.
- [141] Busch F., VanAernum Z.L., Ju Y., Yan J., Gilbert J.D., Quintyn R.S., Bern M., and Wysocki V.H. Localization of Protein Complex Bound Ligands by Surface-Induced Dissociation High-Resolution Mass Spectrometry. *Anal Chem*, 2018; 90: 12796-12801.
- [142] Wongkongkathep P., Han J.Y., Choi T.S., Yin S., Kim H.I., and Loo J.A. Native Top-Down Mass Spectrometry and Ion Mobility MS for Characterizing the Cobalt and Manganese Metal Binding of alpha-Synuclein Protein. *J Am Soc Mass Spectrom*, 2018; 29: 1870-1880.
- [143] Nshanian M., Lantz C., Wongkongkathep P., Schrader T., Klarner F.G., Blumke A., Despres C., Ehrmann M., Smet-Nocca C., Bitan G., and Loo J.A. 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.
- [144] Haselmann K.F., Jorgensen T.J., Budnik B.A., Jensen F., and Zubarev R.A. Electron capture dissociation of weakly bound polypeptide polycationic complexes. *Rapid Commun Mass Spectrom*, 2002; 16: 2260-5.
- [145] Xie Y., Zhang J., Yin S., and Loo J.A. Top-down ESI-ECD-FT-ICR mass spectrometry localizes noncovalent protein-ligand binding sites. *J Am Chem Soc*, 2006; 128: 14432-3.

- [146] Zhang H., Cui W., Wen J., Blankenship R.E., and Gross M.L. 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: 1966-8.
- [147] Zhang H., Cui W., Wen J., Blankenship R.E., and Gross M.L. Native electrospray and electron-capture dissociation FTICR mass spectrometry for top-down studies of protein assemblies. *Anal Chem*, 2011; 83: 5598-606.
- [148] Li H., Wongkongkathep P., Van Orden S.L., Ogorzalek Loo R.R., and Loo J.A. Revealing Ligand Binding Sites and Quantifying Subunit Variants of Noncovalent Protein Complexes in a Single Native Top-Down FTICR MS Experiment. *J Am Soc Mass Spectrom*, 2014.
- [149] Ro S.Y., Schachner L.F., Koo C.W., Purohit R., Remis J.P., Kenney G.E., Liauw B.W., Thomas P.M., Patrie S.M., Kelleher N.L., and Rosenzweig A.C. Native top-down mass spectrometry provides insights into the copper centers of membrane-bound methane monooxygenase. *Nat Commun*, 2019; 10: 2675.
- [150] Hernandez H., Dziembowski A., Taverner T., Seraphin B., and Robinson C.V. Subunit architecture of multimeric complexes isolated directly from cells. *EMBO Rep*, 2006; 7: 605-10.
- [151] Zhou M., Sandercock A.M., Fraser C.S., Ridlova G., Stephens E., Schenauer M.R., Yokoi-Fong T., Barsky D., Leary J.A., Hershey J.W., Doudna J.A., and Robinson C.V. Mass spectrometry reveals modularity and a complete subunit interaction map of the eukaryotic translation factor eIF3. *Proc Natl Acad Sci U S A*, 2008; 105: 18139-44.
- [152] Song Y., Nelp M.T., Bandarian V., and Wysocki V.H. Refining the Structural Model of a Heterohexameric Protein Complex: Surface Induced Dissociation and Ion Mobility Provide Key Connectivity and Topology Information. *ACS Cent Sci*, 2015; 1: 477-487.
- [153] Levy E.D., Boeri Erba E., Robinson C.V., and Teichmann S.A. Assembly reflects evolution of protein complexes. *Nature*, 2008; 453: 1262-5.
- [154] Marsh J.A., Hernandez H., Hall Z., Ahnert S.E., Perica T., Robinson C.V., and Teichmann S.A. Protein complexes are under evolutionary selection to assemble via ordered pathways. *Cell*, 2013; 153: 461-70.
- [155] Vivat Hannah V., Atmanene C., Zeyer D., Van Dorsselaer A., and Sanglier-Cianferani S. Native MS: an 'ESI' way to support structure- and fragment-based drug discovery. *Future Med Chem*, 2010; 2: 35-50.

- [156] Niu S., Rabuck J.N., and Ruotolo B.T. Ion mobility-mass spectrometry of intact protein--ligand complexes for pharmaceutical drug discovery and development. *Curr Opin Chem Biol*, 2013; 17: 809-17.
- [157] Schmidt C. and Robinson C.V. Dynamic protein ligand interactions--insights from MS. *FEBS J*, 2014; 281: 1950-64.
- [158] Ritschel T., Atmanene C., Reuter K., Van Dorsselaer A., Sanglier-Cianferani S., and Klebe G. An integrative approach combining noncovalent mass spectrometry, enzyme kinetics and X-ray crystallography to decipher Tgt protein-protein and protein-RNA interaction. *J Mol Biol*, 2009; 393: 833-47.
- [159] Cousido-Siah A., Ayoub D., Berberian G., Bollo M., Van Dorsselaer A., Debaene F., DiPolo R., Petrova T., Schulze-Briese C., Olieric V., et al. Structural and functional studies of ReP1-NCXSQ, a protein regulating the squid nerve Na⁺/Ca²⁺ exchanger. *Acta Crystallogr D Biol Crystallogr*, 2012; 68: 1098-107.
- [160] Laventie B.J., Potrich C., Atmanene C., Saleh M., Joubert O., Viero G., Bachmeyer C., Antonini V., Mancini I., Cianferani-Sanglier S., et al. p-Sulfonato-calix[n]arenes inhibit staphylococcal bicomponent leukotoxins by supramolecular interactions. *Biochem J*, 2013; 450: 559-71.
- [161] Marcotte D., Zeng W., Hus J.C., McKenzie A., Hession C., Jin P., Bergeron C., Lugovskoy A., Enyedy I., Cuervo H., et al. Small molecules inhibit the interaction of Nrf2 and the Keap1 Kelch domain through a non-covalent mechanism. *Bioorg Med Chem*, 2013; 21: 4011-9.
- [162] Ren C., Bailey A.O., VanderPorten E., Oh A., Phung W., Mulvihill M.M., Harris S.F., Liu Y., Han G., and Sandoval W. Quantitative Determination of Protein-Ligand Affinity by Size Exclusion Chromatography Directly Coupled to High-Resolution Native Mass Spectrometry. *Anal Chem*, 2019; 91: 903-911.
- [163] Lebrette H., Borezee-Durant E., Martin L., Richaud P., Boeri Erba E., and Cavazza C. Novel insights into nickel import in *Staphylococcus aureus*: the positive role of free histidine and structural characterization of a new thiazolidine-type nickel chelator. *Metallomics*, 2015.
- [164] Allison T.M., Reading E., Liko I., Baldwin A.J., Laganowsky A., and Robinson C.V. Quantifying the stabilizing effects of protein-ligand interactions in the gas phase. *Nat Commun*, 2015; 6: 8551.
- [165] Liu H. and Hakansson K. Divalent metal ion-peptide interactions probed by electron capture dissociation of trications. *J Am Soc Mass Spectrom*, 2006; 17: 1731-41.
- [166] Erales J., Gontero B., Whitelegge J., and Halgand F. Mapping of a copper-binding site on the small CP12 chloroplastic protein of *Chlamydomonas reinhardtii* using top-

- down mass spectrometry and site-directed mutagenesis. *Biochem J*, 2009; 419: 75-82, 4 p following 82.
- [167] Yin S. and Loo J.A. Elucidating the site of protein-ATP binding by top-down mass spectrometry. *J Am Soc Mass Spectrom*, 2010; 21: 899-907.
- [168] Marty M.T., Zhang H., Cui W., Blankenship R.E., Gross M.L., and Sligar S.G. Native mass spectrometry characterization of intact nanodisc lipoprotein complexes. *Anal Chem*, 2012; 84: 8957-60.
- [169] Wills R.H., Habtemariam A., Lopez-Clavijo A.F., Barrow M.P., Sadler P.J., and O'Connor P.B. Insights into the binding sites of organometallic ruthenium anticancer compounds on peptides using ultra-high resolution mass spectrometry. *J Am Soc Mass Spectrom*, 2014; 25: 662-72.
- [170] Li H., Snelling J.R., Barrow M.P., Scrivens J.H., Sadler P.J., and O'Connor P.B. Mass spectrometric strategies to improve the identification of Pt(II)-modification sites on peptides and proteins. *J Am Soc Mass Spectrom*, 2014; 25: 1217-27.
- [171] Mehaffey M.R., Sanders J.D., Holden D.D., Nilsson C.L., and Brodbelt J.S. Multistage Ultraviolet Photodissociation Mass Spectrometry To Characterize Single Amino Acid Variants of Human Mitochondrial BCAT2. *Anal Chem*, 2018; 90: 9904-9911.
- [172] Mehaffey M.R., Cammarata M.B., and Brodbelt J.S. Tracking the Catalytic Cycle of Adenylate Kinase by Ultraviolet Photodissociation Mass Spectrometry. *Anal Chem*, 2018; 90: 839-846.
- [173] Sharon M. Biochemistry. Structural MS pulls its weight. *Science*, 2013; 340: 1059-60.
- [174] Konijnenberg A., Butterer A., and Sobott F. Native ion mobility-mass spectrometry and related methods in structural biology. *Biochim Biophys Acta*, 2012.
- [175] Benesch J.L. and Ruotolo B.T. Mass spectrometry: come of age for structural and dynamical biology. *Curr Opin Struct Biol*, 2011; 21: 641-9.
- [176] Heck A.J. Native mass spectrometry: a bridge between interactomics and structural biology. *Nat Methods*, 2008; 5: 927-33.
- [177] Gulbakan B., Barylyuk K., and Zenobi R. Determination of thermodynamic and kinetic properties of biomolecules by mass spectrometry. *Curr Opin Biotechnol*, 2014; 31C: 65-72.
- [178] Robinson C.V. From molecular chaperones to membrane motors: through the lens of a mass spectrometrist. *Biochem Soc Trans*, 2017; 45: 251-260.
- [179] Robinson C.V. Mass spectrometry: From plasma proteins to mitochondrial membranes. *Proc Natl Acad Sci U S A*, 2019; 116: 2814-2820.

- [180] Schmidt C. and Urlaub H. Combining cryo-electron microscopy (cryo-EM) and cross-linking mass spectrometry (CX-MS) for structural elucidation of large protein assemblies. *Curr Opin Struct Biol*, 2017; 46: 157-168.
- [181] Hoffmann W., von Helden G., and Pagel K. Ion mobility-mass spectrometry and orthogonal gas-phase techniques to study amyloid formation and inhibition. *Curr Opin Struct Biol*, 2017; 46: 7-15.
- [182] Nematollahi L.A., Garza-Garcia A., Bechara C., Esposito D., Morgner N., Robinson C.V., and Driscoll P.C. Flexible stoichiometry and asymmetry of the PIDDosome core complex by heteronuclear NMR spectroscopy and mass spectrometry. *J Mol Biol*, 2015; 427: 737-752.
- [183] Schuller J.M., Beck F., Lossl P., Heck A.J., and Forster F. Nucleotide-dependent conformational changes of the AAA+ ATPase p97 revisited. *FEBS Lett*, 2016; 590: 595-604.
- [184] Casanal A., Kumar A., Hill C.H., Easter A.D., Emsley P., Degliesposti G., Gordiyenko Y., Santhanam B., Wolf J., Wiederhold K., Dornan G.L., Skehel M., Robinson C.V., and Passmore L.A. Architecture of eukaryotic mRNA 3'-end processing machinery. *Science*, 2017; 358: 1056-1059.
- [185] Fagerlund R.D., Wilkinson M.E., Klykov O., Barendregt A., Pearce F.G., Kieper S.N., Maxwell H.W.R., Capolupo A., Heck A.J.R., Krause K.L., Bostina M., Scheltema R.A., Staals R.H.J., and Fineran P.C. Spacer capture and integration by a type I-F Cas1-Cas2-3 CRISPR adaptation complex. *Proc Natl Acad Sci U S A*, 2017; 114: E5122-E5128.
- [186] De Nardis C., Lossl P., van den Biggelaar M., Madoori P.K., Leloup N., Mertens K., Heck A.J., and Gros P. Recombinant Expression of the Full-length Ectodomain of LDL Receptor-related Protein 1 (LRP1) Unravels pH-dependent Conformational Changes and the Stoichiometry of Binding with Receptor-associated Protein (RAP). *J Biol Chem*, 2017; 292: 912-924.
- [187] Snijder J., Schuller J.M., Wiegard A., Lossl P., Schmelling N., Axmann I.M., Plitzko J.M., Forster F., and Heck A.J. Structures of the cyanobacterial circadian oscillator frozen in a fully assembled state. *Science*, 2017; 355: 1181-1184.
- [188] Fisher G.L., Pastrana C.L., Higman V.A., Koh A., Taylor J.A., Butterer A., Craggs T., Sobott F., Murray H., Crump M.P., Moreno-Herrero F., and Dillingham M.S. The structural basis for dynamic DNA binding and bridging interactions which condense the bacterial centromere. *Elife*, 2017; 6.
- [189] Politis A., Stengel F., Hall Z., Hernandez H., Leitner A., Walzthoeni T., Robinson C.V., and Aebersold R. A mass spectrometry-based hybrid method for structural modeling of protein complexes. *Nat Methods*, 2014; 11: 403-6.

- [190] Politis A., Schmidt C., Tjioe E., Sandercock A.M., Lasker K., Gordiyenko Y., Russel D., Sali A., and Robinson C.V. Topological Models of Heteromeric Protein Assemblies from Mass Spectrometry: Application to the Yeast eIF3:eIF5 Complex. *Chem Biol*, 2014.
- [191] Campuzano I.D.G., Sobott F., and van Stipdonk M.J. Editorial and Review: 30th ASMS Sanibel Conference on Mass Spectrometry-Computational Modelling in Mass Spectrometry and Ion Mobility: Methods for Ion Structure and Reactivity Determination. *J Am Soc Mass Spectrom*, 2018; 29: 2283-2286.
- [192] Marklund E.G. and Benesch J.L. Weighing-up protein dynamics: the combination of native mass spectrometry and molecular dynamics simulations. *Curr Opin Struct Biol*, 2019; 54: 50-58.
- [193] Sasaki E., Bohringer D., van de Waterbeemd M., Leibundgut M., Zschoche R., Heck A.J., Ban N., and Hilvert D. Structure and assembly of scalable porous protein cages. *Nat Commun*, 2017; 8: 14663.
- [194] Pellegrini E., Signor L., Singh S., Boeri Erba E., and Cusack S. Structures of the inactive and active states of RIP2 kinase inform on the mechanism of activation. *PLoS One*, 2017; 12: e0177161.
- [195] McCammon M.G., Scott D.J., Keetch C.A., Greene L.H., Purkey H.E., Petrassi H.M., Kelly J.W., and Robinson C.V. Screening transthyretin amyloid fibril inhibitors: characterization of novel multiprotein, multiligand complexes by mass spectrometry. *Structure*, 2002; 10: 851-63.
- [196] Keetch C.A., Bromley E.H., McCammon M.G., Wang N., Christodoulou J., and Robinson C.V. L55P transthyretin accelerates subunit exchange and leads to rapid formation of hybrid tetramers. *J Biol Chem*, 2005; 280: 41667-74.
- [197] Marcoux J., Mangione P.P., Porcari R., Degiacomi M.T., Verona G., Taylor G.W., Giorgetti S., Raimondi S., Sanglier-Cianferani S., Benesch J.L., et al. A novel mechano-enzymatic cleavage mechanism underlies transthyretin amyloidogenesis. *EMBO Mol Med*, 2015; 7: 1337-49.
- [198] Bereszczak J.Z., Barbu I.M., Tan M., Xia M., Jiang X., van Duijn E., and Heck A.J. Structure, stability and dynamics of norovirus P domain derived protein complexes studied by native mass spectrometry. *J Struct Biol*, 2012; 177: 273-82.
- [199] Veessler D., Khayat R., Krishnamurthy S., Snijder J., Huang R.K., Heck A.J., Anand G.S., and Johnson J.E. Architecture of a dsDNA viral capsid in complex with its maturation protease. *Structure*, 2014; 22: 230-7.
- [200] Uetrecht C. and Heck A.J. Modern biomolecular mass spectrometry and its role in studying virus structure, dynamics, and assembly. *Angew Chem Int Ed Engl*, 2011; 50: 8248-62.

- [201] van de Waterbeemd M., Snijder J., Tsvetkova I.B., Dragnea B.G., Cornelissen J.J., and Heck A.J. Examining the Heterogeneous Genome Content of Multipartite Viruses BMV and CCMV by Native Mass Spectrometry. *J Am Soc Mass Spectrom*, 2016; 27: 1000-9.
- [202] Weiss V.U., Pogan R., Zoratto S., Bond K.M., Boulanger P., Jarrold M.F., Lykтей N., Pahl D., Puffler N., Schelhaas M., Selivanovitch E., Uetrecht C., and Allmaier G. Virus-like particle size and molecular weight/mass determination applying gas-phase electrophoresis (native nES GEMMA). *Anal Bioanal Chem*, 2019; 411: 5951-5962.
- [203] Kondylis P., Schlicksup C.J., Zlotnick A., and Jacobson S.C. Analytical Techniques to Characterize the Structure, Properties, and Assembly of Virus Capsids. *Anal Chem*, 2019; 91: 622-636.
- [204] Pierson E.E., Keifer D.Z., Asokan A., and Jarrold M.F. Resolving Adeno-Associated Viral Particle Diversity With Charge Detection Mass Spectrometry. *Anal Chem*, 2016; 88: 6718-25.
- [205] Keifer D.Z., Motwani T., Teschke C.M., and Jarrold M.F. Acquiring Structural Information on Virus Particles with Charge Detection Mass Spectrometry. *J Am Soc Mass Spectrom*, 2016; 27: 1028-36.
- [206] Keifer D.Z. and Jarrold M.F. Single-molecule mass spectrometry. *Mass Spectrom Rev*, 2017; 36: 715-733.
- [207] Dominguez-Medina S., Fostner S., Defoort M., Sansa M., Stark A.K., Halim M.A., Vernhes E., Gely M., Jourdan G., Alava T., Boulanger P., Masselon C., and Hentz S. Neutral mass spectrometry of virus capsids above 100 megadaltons with nanomechanical resonators. *Science*, 2018; 362: 918-922.
- [208] Chaix D., Ferguson M.L., Atmanene C., Van Dorsselaer A., Sanglier-Cianferani S., Royer C.A., and Declerck N. Physical basis of the inducer-dependent cooperativity of the Central glycolytic genes Repressor/DNA complex. *Nucleic Acids Res*, 2010; 38: 5944-57.
- [209] Atmanene C., Chaix D., Bessin Y., Declerck N., Van Dorsselaer A., and 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: 3597-605.
- [210] Jore M.M., Lundgren M., van Duijn E., Bultema J.B., Westra E.R., Waghmare S.P., Wiedenheft B., Pul U., Wurm R., Wagner R., et al. Structural basis for CRISPR RNA-guided DNA recognition by Cascade. *Nat Struct Mol Biol*, 2011; 18: 529-36.
- [211] Staals R.H., Zhu Y., Taylor D.W., Kornfeld J.E., Sharma K., Barendregt A., Koehorst J.J., Vlot M., Neupane N., Varossieau K., et al. RNA targeting by the type III-A CRISPR-Cas Csm complex of *Thermus thermophilus*. *Mol Cell*, 2014; 56: 518-30.

- [212] Hernychova L., Rosulek M., Kadek A., Mareska V., Chmelik J., Adamkova L., Grobarova V., Sebesta O., Kukacka Z., Skala K., Spiwok V., Cerny J., and Novak P. The C-type lectin-like receptor Nkrp1b: Structural proteomics reveals features affecting protein conformation and interactions. *J Proteomics*, 2019; 196: 162-172.
- [213] Rajasekar K.V., Baker R., Fisher G.L.M., Bolla J.R., Makela J., Tang M., Zawadzka K., Koczy O., Wagner F., Robinson C.V., Arciszewska L.K., and Sherratt D.J. Dynamic architecture of the Escherichia coli structural maintenance of chromosomes (SMC) complex, MukBEF. *Nucleic Acids Res*, 2019.
- [214] Seffernick J.T., Harvey S.R., Wysocki V.H., and Lindert S. Predicting Protein Complex Structure from Surface-Induced Dissociation Mass Spectrometry Data. *ACS Cent Sci*, 2019; 5: 1330-1341.
- [215] Eschweiler J.D., Frank A.T., and Ruotolo B.T. Coming to Grips with Ambiguity: Ion Mobility-Mass Spectrometry for Protein Quaternary Structure Assignment. *J Am Soc Mass Spectrom*, 2017; 28: 1991-2000.
- [216] Eschweiler J.D., Farrugia M.A., Dixit S.M., Hausinger R.P., and Ruotolo B.T. A Structural Model of the Urease Activation Complex Derived from Ion Mobility-Mass Spectrometry and Integrative Modeling. *Structure*, 2018; 26: 599-606 e3.
- [217] Camacho I.S., Theisen A., Johannissen L.O., Diaz-Ramos L.A., Christie J.M., Jenkins G.I., Bellina B., Barran P., and Jones A.R. Native mass spectrometry reveals the conformational diversity of the UVR8 photoreceptor. *Proc Natl Acad Sci U S A*, 2019; 116: 1116-1125.
- [218] Shoemaker G.K., van Duijn E., Crawford S.E., Uetrecht C., Baclayon M., Roos W.H., Wuite G.J., Estes M.K., Prasad B.V., and Heck A.J. Norwalk virus assembly and stability monitored by mass spectrometry. *Mol Cell Proteomics*, 2010; 9: 1742-51.
- [219] Snijder J., Uetrecht C., Rose R.J., Sanchez-Eugenia R., Marti G.A., Agirre J., Guerin D.M., Wuite G.J., Heck A.J., and Roos W.H. Probing the biophysical interplay between a viral genome and its capsid. *Nat Chem*, 2013; 5: 502-9.
- [220] Konijnenberg A., Bannwarth L., Yilmaz D., Kocer A., Venien-Bryan C., and 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: 1292-300.
- [221] Kar U.K., Simonian M., and Whitelegge J.P. Integral membrane proteins: bottom-up, top-down and structural proteomics. *Expert Rev Proteomics*, 2017; 14: 715-723.
- [222] van Dyck J.F., Konijnenberg A., and Sobott F. Native Mass Spectrometry for the Characterization of Structure and Interactions of Membrane Proteins. *Methods Mol Biol*, 2017; 1635: 205-232.

- [223] Rodenburg R.N.P., Snijder J., van de Waterbeemd M., Schouten A., Granneman J., Heck A.J.R., and Gros P. Stochastic palmitoylation of accessible cysteines in membrane proteins revealed by native mass spectrometry. *Nat Commun*, 2017; 8: 1280.
- [224] Gault J., Donlan J.A., Liko I., Hopper J.T., Gupta K., Housden N.G., Struwe W.B., Marty M.T., Mize T., Bechara C., et al. High-resolution mass spectrometry of small molecules bound to membrane proteins. *Nat Methods*, 2016; 13: 333-6.
- [225] Laganowsky A., Reading E., Allison T.M., Ulmschneider M.B., Degiacomi M.T., Baldwin A.J., and Robinson C.V. Membrane proteins bind lipids selectively to modulate their structure and function. *Nature*, 2014; 510: 172-5.
- [226] Bechara C. and Robinson C.V. Different modes of lipid binding to membrane proteins probed by mass spectrometry. *J Am Chem Soc*, 2015; 137: 5240-7.
- [227] Bechara C., Noll A., Morgner N., Degiacomi M.T., Tampe R., and Robinson C.V. A subset of annular lipids is linked to the flippase activity of an ABC transporter. *Nat Chem*, 2015; 7: 255-62.
- [228] Liu Y., Cong X., Liu W., and Laganowsky A. Characterization of Membrane Protein-Lipid Interactions by Mass Spectrometry Ion Mobility Mass Spectrometry. *J Am Soc Mass Spectrom*, 2017; 28: 579-586.
- [229] Gupta K., Li J., Liko I., Gault J., Bechara C., Wu D., Hopper J.T.S., Giles K., Benesch J.L.P., and Robinson C.V. Identifying key membrane protein lipid interactions using mass spectrometry. *Nat Protoc*, 2018; 13: 1106-1120.
- [230] Fiorentino F., Bolla J.R., Mehmood S., and Robinson C.V. The Different Effects of Substrates and Nucleotides on the Complex Formation of ABC Transporters. *Structure*, 2019; 27: 651-659 e3.
- [231] Song W., Yen H.Y., Robinson C.V., and Sansom M.S.P. State-dependent Lipid Interactions with the A2a Receptor Revealed by MD Simulations Using In Vivo-Mimetic Membranes. *Structure*, 2019; 27: 392-403 e3.
- [232] Robinson C.V., Rohacs T., and Hansen S.B. Tools for Understanding Nanoscale Lipid Regulation of Ion Channels. *Trends Biochem Sci*, 2019; 44: 795-806.
- [233] Zhou M., Politis A., Davies R.B., Liko I., Wu K.J., Stewart A.G., Stock D., and Robinson C.V. Ion mobility-mass spectrometry of a rotary ATPase reveals ATP-induced reduction in conformational flexibility. *Nat Chem*, 2014; 6: 208-15.
- [234] Housden N.G., Hopper J.T., Lukoyanova N., Rodriguez-Larrea D., Wojdyla J.A., Klein A., Kaminska R., Bayley H., Saibil H.R., Robinson C.V., and Kleanthous C. Intrinsically disordered protein threads through the bacterial outer-membrane porin OmpF. *Science*, 2013; 340: 1570-4.

- [235] Cong X., Liu Y., Liu W., Liang X., and Laganowsky A. Allosteric modulation of protein-protein interactions by individual lipid binding events. *Nat Commun*, 2017; 8: 2203.
- [236] Gupta K., Donlan J.A.C., Hopper J.T.S., Uzdavinys P., Landreh M., Struwe W.B., Drew D., Baldwin A.J., Stansfeld P.J., and Robinson C.V. The role of interfacial lipids in stabilizing membrane protein oligomers. *Nature*, 2017; 541: 421-424.
- [237] Bolla J.R., Sauer J.B., Wu D., Mehmood S., Allison T.M., and Robinson C.V. Direct observation of the influence of cardiolipin and antibiotics on lipid II binding to MurJ. *Nat Chem*, 2018; 10: 363-371.
- [238] Liko I., Degiacomi M.T., Lee S., Newport T.D., Gault J., Reading E., Hopper J.T.S., Housden N.G., White P., Colledge M., et al. Lipid binding attenuates channel closure of the outer membrane protein OmpF. *Proc Natl Acad Sci U S A*, 2018; 115: 6691-6696.
- [239] Dijkman P.M., Castell O.K., Goddard A.D., Munoz-Garcia J.C., de Graaf C., Wallace M.I., and Watts A. Dynamic tuneable G protein-coupled receptor monomer-dimer populations. *Nat Commun*, 2018; 9: 1710.
- [240] Yen H.Y., Hoi K.K., Liko I., Hedger G., Horrell M.R., Song W., Wu D., Heine P., Warne T., Lee Y., et al. PtdIns(4,5)P₂ stabilizes active states of GPCRs and enhances selectivity of G-protein coupling. *Nature*, 2018; 559: 423-427.
- [241] Patrick J.W., Boone C.D., Liu W., Conover G.M., Liu Y., Cong X., and Laganowsky A. Allostery revealed within lipid binding events to membrane proteins. *Proc Natl Acad Sci U S A*, 2018; 115: 2976-2981.
- [242] Marcoux J., Wang S.C., Politis A., Reading E., Ma J., Biggin P.C., Zhou M., Tao H., Zhang Q., Chang G., Morgner N., and Robinson C.V. Mass spectrometry reveals synergistic effects of nucleotides, lipids, and drugs binding to a multidrug resistance efflux pump. *Proc Natl Acad Sci U S A*, 2013; 110: 9704-9.
- [243] Su C.C., Klenotic P.A., Bolla J.R., Purdy G.E., Robinson C.V., and Yu E.W. MmpL3 is a lipid transporter that binds trehalose monomycolate and phosphatidylethanolamine. *Proc Natl Acad Sci U S A*, 2019; 116: 11241-11246.
- [244] Hopper J.T., Yu Y.T., Li D., Raymond A., Bostock M., Liko I., Mikhailov V., Laganowsky A., Benesch J.L., Caffrey M., Nietlispach D., and Robinson C.V. Detergent-free mass spectrometry of membrane protein complexes. *Nat Methods*, 2013; 10: 1206-8.
- [245] Calabrese A.N., Watkinson T.G., Henderson P.J., Radford S.E., and Ashcroft A.E. Amphipols Outperform Dodecylmaltoside Micelles in Stabilizing Membrane Protein Structure in the Gas Phase. *Anal Chem*, 2014.

- [246] Marty M.T., Hoi K.K., Gault J., and Robinson C.V. Probing the Lipid Annular Belt by Gas-Phase Dissociation of Membrane Proteins in Nanodiscs. *Angew Chem Weinheim Bergstr Ger*, 2016; 128: 560-564.
- [247] Campuzano I.D., Li H., Bagal D., Lippens J.L., Svitel J., Kurzeja R.J., Xu H., Schnier P.D., and Loo J.A. Native MS Analysis of Bacteriorhodopsin and an Empty Nanodisc by Orthogonal Acceleration Time-of-Flight, Orbitrap and Ion Cyclotron Resonance. *Anal Chem*, 2016; 88: 12427-12436.
- [248] Leney A.C., Fan X., Kitova E.N., and Klassen J.S. Nanodiscs and electrospray ionization mass spectrometry: a tool for screening glycolipids against proteins. *Anal Chem*, 2014; 86: 5271-7.
- [249] Leney A.C., Rezaei Darestani R., Li J., Nikjah S., Kitova E.N., Zou C., Cairo C.W., Xiong Z.J., Prive G.G., and Klassen J.S. Picodiscs for facile protein-glycolipid interaction analysis. *Anal Chem*, 2015; 87: 4402-8.
- [250] Chorev D.S., Baker L.A., Wu D., Beilsten-Edmands V., Rouse S.L., Zeev-Ben-Mordehai T., Jiko C., Samsudin F., Gerle C., Khalid S., Stewart A.G., Matthews S.J., Grunewald K., and Robinson C.V. Protein assemblies ejected directly from native membranes yield complexes for mass spectrometry. *Science*, 2018; 362: 829-834.
- [251] van de Waterbeemd M., Tamara S., Fort K.L., Damoc E., Franc V., Bieri P., Itten M., Makarov A., Ban N., and Heck A.J.R. Dissecting ribosomal particles throughout the kingdoms of life using advanced hybrid mass spectrometry methods. *Nat Commun*, 2018; 9: 2493.
- [252] Bolla J.R., Agasid M.T., Mehmood S., and Robinson C.V. Membrane Protein-Lipid Interactions Probed Using Mass Spectrometry. *Annu Rev Biochem*, 2019; 88: 85-111.
- [253] Yang Y., Wang G., Song T., Lebrilla C.B., and Heck A.J.R. Resolving the micro-heterogeneity and structural integrity of monoclonal antibodies by hybrid mass spectrometric approaches. *MAbs*, 2017; 9: 638-645.
- [254] Chait B.T. Mass spectrometry--a useful tool for the protein X-ray crystallographer and NMR spectroscopist. *Structure*, 1994; 2: 465-7.
- [255] Wang R. and Chait B.T. High-accuracy mass measurement as a tool for studying proteins. *Curr Opin Biotechnol*, 1994; 5: 77-84.
- [256] Chait B.T. and Kent S.B. Weighing naked proteins: practical, high-accuracy mass measurement of peptides and proteins. *Science*, 1992; 257: 1885-94.
- [257] Aebersold R. and Mann M. Mass-spectrometric exploration of proteome structure and function. *Nature*, 2016; 537: 347-55.
- [258] Tsarbopoulos A., Karas M., Strupat K., Pramanik B.N., Nagabhushan T.L., and Hillenkamp F. Comparative mapping of recombinant proteins and glycoproteins by

- plasma desorption and matrix-assisted laser desorption/ionization mass spectrometry. *Anal Chem*, 1994; 66: 2062-70.
- [259] Fenn J.B., Mann M., Meng C.K., Wong S.F., and Whitehouse C.M. Electrospray ionization for mass spectrometry of large biomolecules. *Science*, 1989; 246: 64-71.
- [260] Doll S., Gnad F., and Mann M. The Case for Proteomics and Phospho-Proteomics in Personalized Cancer Medicine. *Proteomics Clin Appl*, 2019; 13: e1800113.
- [261] Doerr A. Single-cell proteomics. *Nat Methods*, 2019; 16: 20.
- [262] Ward A.B., Sali A., and Wilson I.A. Biochemistry. Integrative structural biology. *Science*, 2013; 339: 913-5.
- [263] Faini M., Stengel F., and Aebersold R. The Evolving Contribution of Mass Spectrometry to Integrative Structural Biology. *J Am Soc Mass Spectrom*, 2016; 27: 966-74.
- [264] Perrakis A., Musacchio A., Cusack S., and Petosa C. Investigating a macromolecular complex: the toolkit of methods. *J Struct Biol*, 2011; 175: 106-12.
- [265] Mikhailov V.A., Mize T.H., Benesch J.L., and Robinson C.V. Mass-selective soft-landing of protein assemblies with controlled landing energies. *Anal Chem*, 2014; 86: 8321-8.
- [266] Gan J., Ben-Nissan G., Arkind G., Tarnavsky M., Trudeau D., Noda Garcia L., Tawfik D.S., and Sharon M. Native Mass Spectrometry of Recombinant Proteins from Crude Cell Lysates. *Anal Chem*, 2017; 89: 4398-4404.
- [267] Cveticanin J., Netzer R., Arkind G., Fleishman S.J., Horovitz A., and Sharon M. Estimating Interprotein Pairwise Interaction Energies in Cell Lysates from a Single Native Mass Spectrum. *Anal Chem*, 2018; 90: 10090-10094.
- [268] Ben-Nissan G., Vimer S., Warszawski S., Katz A., Yona M., Unger T., Peleg Y., Morgenstern D., Cohen-Dvashi H., Diskin R., Fleishman S.J., and Sharon M. Rapid characterization of secreted recombinant proteins by native mass spectrometry. *Commun Biol*, 2018; 1: 213.
- [269] Chorev D.S., Moscovitz O., Geiger B., and Sharon M. Regulation of focal adhesion formation by a vinculin-Arp2/3 hybrid complex. *Nat Commun*, 2014; 5: 3758.
- [270] Hernandez H., Makarova O.V., Makarov E.M., Morgner N., Muto Y., Krummel D.P., and Robinson C.V. Isoforms of U1-70k control subunit dynamics in the human spliceosomal U1 snRNP. *PLoS One*, 2009; 4: e7202.
- [271] Gavin A.C., Maeda K., and Kuhner S. Recent advances in charting protein-protein interaction: mass spectrometry-based approaches. *Curr Opin Biotechnol*, 2011; 22: 42-9.

- [272] Domanski M., Molloy K., Jiang H., Chait B.T., Rout M.P., Jensen T.H., and LaCava J. Improved methodology for the affinity isolation of human protein complexes expressed at near endogenous levels. *Biotechniques*, 2012; 0: 1-6.
- [273] Trimpin S. and Inutan E.D. New ionization method for analysis on atmospheric pressure ionization mass spectrometers requiring only vacuum and matrix assistance. *Anal Chem*, 2013; 85: 2005-9.
- [274] Wang B., Tisdale E., Trimpin S., and Wilkins C.L. Matrix-assisted ionization vacuum for high-resolution Fourier transform ion cyclotron resonance mass spectrometers. *Anal Chem*, 2014; 86: 6792-6.
- [275] Yoon S.H., Huang Y., Edgar J.S., Ting Y.S., Heron S.R., Kao Y., Li Y., Masselon C.D., Ernst R.K., and Goodlett D.R. Surface acoustic wave nebulization facilitating lipid mass spectrometric analysis. *Anal Chem*, 2012; 84: 6530-7.
- [276] Huang Y., Yoon S.H., Heron S.R., Masselon C.D., Edgar J.S., Turecek F., and Goodlett D.R. Surface acoustic wave nebulization produces ions with lower internal energy than electrospray ionization. *J Am Soc Mass Spectrom*, 2012; 23: 1062-70.
- [277] Heron S.R., Wilson R., Shaffer S.A., Goodlett D.R., and Cooper J.M. Surface acoustic wave nebulization of peptides as a microfluidic interface for mass spectrometry. *Anal Chem*, 2010; 82: 3985-9.
- [278] Pramanik A., Hauf W., Hoffmann J., Cernescu M., Brutschy B., and Braun V. Oligomeric structure of ExbB and ExbB-ExbD isolated from *Escherichia coli* as revealed by LILBID mass spectrometry. *Biochemistry*, 2011; 50: 8950-6.
- [279] Hanay M.S., Kelber S., Naik A.K., Chi D., Hentz S., Bullard E.C., Colinet E., Duraffourg L., and Roukes M.L. Single-protein nanomechanical mass spectrometry in real time. *Nat Nanotechnol*, 2012; 7: 602-8.
- [280] Contino N.C., Pierson E.E., Keifer D.Z., and Jarrold M.F. Charge detection mass spectrometry with resolved charge states. *J Am Soc Mass Spectrom*, 2013; 24: 101-8.
- [281] Pierson E.E., Keifer D.Z., Selzer L., Lee L.S., Contino N.C., Wang J.C., Zlotnick A., and Jarrold M.F. Detection of late intermediates in virus capsid assembly by charge detection mass spectrometry. *J Am Chem Soc*, 2014; 136: 3536-41.
- [282] Todd A.R. and Jarrold M.F. Dramatic Improvement in Sensitivity with Pulsed Mode Charge Detection Mass Spectrometry. *Anal Chem*, 2019.
- [283] Dunbar C.A., Rayaprolu V., Wang J.C., Brown C.J., Leishman E., Jones-Burrage S., Trinidad J.C., Bradshaw H.B., Clemmer D.E., Mukhopadhyay S., and Jarrold M.F. Dissecting the Components of Sindbis Virus from Arthropod and Vertebrate Hosts: Implications for Infectivity Differences. *ACS Infect Dis*, 2019; 5: 892-902.

- [284] Doussineau T., Mathevon C., Altamura L., Vendrely C., Dugourd P., Forge V., and Antoine R. Mass Determination of Entire Amyloid Fibrils by Using Mass Spectrometry. *Angew Chem Int Ed Engl*, 2016; 55: 2340-4.
- [285] Pansieri J., Halim M.A., Vendrely C., Dumoulin M., Legrand F., Sallanon M.M., Chierici S., Denti S., Dagany X., Dugourd P., Marquette C., Antoine R., and Forge V. Mass and charge distributions of amyloid fibers involved in neurodegenerative diseases: mapping heterogeneity and polymorphism. *Chem Sci*, 2018; 9: 2791-2796.
- [286] Morgner N. and Robinson C.V. Massign: an assignment strategy for maximizing information from the mass spectra of heterogeneous protein assemblies. *Anal Chem*, 2012; 84: 2939-48.
- [287] Tseng Y.H., Uetrecht C., Heck A.J., and Peng W.P. Interpreting the charge state assignment in electrospray mass spectra of bioparticles. *Anal Chem*, 2011; 83: 1960-8.
- [288] Reid D.J., Diesing J.M., Miller M.A., Perry S.M., Wales J.A., Montfort W.R., and Marty M.T. MetaUniDec: High-Throughput Deconvolution of Native Mass Spectra. *J Am Soc Mass Spectrom*, 2019; 30: 118-127.
- [289] Marty M.T. Eliminating Artifacts in Electrospray Deconvolution with a SoftMax Function. *J Am Soc Mass Spectrom*, 2019.
- [290] Rozen S., Tieri A., Ridner G., Stark A.K., Schmalzer T., Ben-Nissan G., Dubiel W., and Sharon M. Exposing the subunit diversity within protein complexes: a mass spectrometry approach. *Methods*, 2013; 59: 270-7.
- [291] Tian Y., Simanshu D.K., Ascano M., Diaz-Avalos R., Park A.Y., Juraneck S.A., Rice W.J., Yin Q., Robinson C.V., Tuschl T., and Patel D.J. Multimeric assembly and biochemical characterization of the Trax-translin endonuclease complex. *Nat Struct Mol Biol*, 2011; 18: 658-64.
- [292] Sharon M., Mao H., Boeri Erba E., Stephens E., Zheng N., and Robinson C.V. Symmetrical modularity of the COP9 signalosome complex suggests its multifunctionality. *Structure*, 2009; 17: 31-40.
- [293] Yamashita M. and Fenn J.B. Electrospray ion source. Another variation on the free-jet theme. *The Journal of Physical Chemistry*, 1984; 88: 4451-4459.
- [294] Wilm M. and Mann M. Electrospray and Taylor-Cone theory, Dole's beam of macromolecules at last? *International Journal of Mass Spectrometry and Ion Processes*, 1994; 136: 167-180.
- [295] Wiedenheft B., van Duijn E., Bultema J.B., Waghmare S.P., Zhou K., Barendregt A., Westphal W., Heck A.J., Boekema E.J., Dickman M.J., and Doudna J.A. RNA-guided complex from a bacterial immune system enhances target recognition through seed sequence interactions. *Proc Natl Acad Sci U S A*, 2011; 108: 10092-7.

- [296] Butterer A., Pernstich C., Smith R.M., Sobott F., Szczelkun M.D., and Toth J. Type III restriction endonucleases are heterotrimeric: comprising one helicase-nuclease subunit and a dimeric methyltransferase that binds only one specific DNA. *Nucleic Acids Res*, 2014; 42: 5139-50.
- [297] Thompson N.J., Merdanovic M., Ehrmann M., van Duijn E., and Heck A.J. Substrate occupancy at the onset of oligomeric transitions of DegP. *Structure*, 2014; 22: 281-90.
- [298] Konijnenberg A., Yilmaz D., Ingolfsson H.I., Dimitrova A., Marrink S.J., Li Z., Venien-Bryan C., Sobott F., and Kocer A. Global structural changes of an ion channel during its gating are followed by ion mobility mass spectrometry. *Proc Natl Acad Sci U S A*, 2014; 111: 17170-5.

Figure captions

Figure 1. Native MS and “native top-down MS” experiments provide key knowledge about non-covalent assemblies.

Grey boxes depict a holocomplex and its derivative components, including subcomplexes, stripped complexes, individual subunits and polypeptide fragments. MS measurements of these species provide information regarding dynamics (green box), binding interactions (blue box) and structural organization (purple box) of a macromolecular complex. Coloured arrows indicate the flow of information.

The mass of an intact assembly is determined by native MS (MS mode). High-performance liquid chromatography (HPLC) performed under denaturing conditions followed by MS measurements yields the experimental mass of each individual subunit [23, 290]. Experimentally assessing the masses of individual subunits is important since the measured masses often differ from the theoretical ones because of unexpected truncations and post-translational modifications. Destabilising conditions [e.g., dimethyl sulfoxide (DMSO) or methanol] allow one to partially dissociate the complex in solution before loading the sample into the mass spectrometer. These subcomplexes (such as 2-mers and 3-mers) enable one to identify direct interactions between proteins [105, 151, 152]. This type of experiment also assesses the stability of interfaces between subunits [153][154]. In MS² experiments, non-covalent bonds are broken and assemblies are dissociated in the gas phase [19, 43]. According to the type of dissociation methods, 1-mers or oligomers can be ejected from the assembly (see section 6 of text). In MS³ experiments of “native top-down MS” [42, 91, 136, 140], subunits are fragmented by backbone cleavage using various methods (e.g., ECD or UVPD) (see section 7). These experiments provide access to information about primary sequence, PTMs and sites of protein-ligand interactions [141-143, 171, 172]

Dynamics. The dynamic behaviour of complexes can be studied by MS. For instance, the presence of different oligomeric states and their changes in equilibrium induced by different solution pH values and concentrations can be monitored by MS [28, 30, 48]. By incubating light and heavy isoforms of a protein (such as those labelled with ¹³C and ¹⁵N), the changes in subunit composition can be studied as a function of time. The subunit exchange revealed

distinct behaviour for wild-type and mutant proteins (e.g., involved in amyloidosis [40, 196]). Native MS can be instrumental in providing information about allostery [59, 241].

Binding. The experimental masses assessed by native MS, HPLC-MS, dissociation in solution and MS² allow one to determine the direct interactions between proteins [105, 150-152], stability of interfaces between subunits [39, 153, 154] and stoichiometry of subunits [23, 150]. The MS³ experiments [42, 91, 136, 140] provide access to information about primary sequence, PTMs and sites of protein-ligand interactions [141-143, 162, 171, 172].

Organization. Overall, the data collected during native MS and “native top-down MS” experiments allows the reconstruction of a two dimensional (2D) map of the interactions between subunits within a macromolecular complex. These data can be combined with structural information (obtained by X-ray crystallography and SAXS) [28, 182-188], to model a 3D architecture of an assembly [189, 291]. Moreover, individual proteins or subcomplexes can be mixed in solution to assess the formation of (sub-) complexes. In this case, a mass shift is detected [292] and allows one to characterise the assembly pathway of a complex and its evolution [39, 153, 154].

Figure 2. Timeline of MS-based investigations of non-covalent assemblies

ESI [259, 293] and nano-ESI [294] paved the way for the advent of native MS. For the first time, the MS investigation of macromolecular complexes was performed in 1991 and electron capture dissociation (ECD) was developed in 1998 [112]. Non-covalent complexes were fragmented by ECD in 2002 [144] and during the same year MS² experiments on protein complexes were described [43]. The first ECD fragmentation of a ligand-protein complex was reported in 2006 [145]. After four years, intact protein complexes were fragmented by ECD [146] and using an Orbitrap instrument subunits ejected from intact protein assemblies were broken down in 2013 during MS³ experiments [42]. In 2018 NEMS measurements of T5 bacteriophage capsids took place [207] and during the same year membrane proteins were successfully ejected from native membranes [250].

Figure 3. Native MS experiments to study the kinetics of dissociation of transthyretin (TTR).

Dissociation of 4-meric TTR is thought to be a key step in the formation of amyloid fibrils. Using native MS, the exchange of TTR subunits was monitored for 11 days. In particular, the effect of two mutations (i.e., S52P and T119M) and that of a drug (tafamidis) on the rate of dissociation of TTR were assessed. A-D) Mass spectra of subunit exchange experiments when deuterated S52P TTR was mixed with hydrogenated (A) WT, (B) S52P, (C) T119M, and (D) S52P bound to tafamidis, respectively. The spectra were recorded at the beginning of the reaction (left panel) and after 11 days (right panel). The *mass-to-charge* ratio is indicated as *m/z*. E) Scheme of the native MS experiments: 4H (hydrogenated) and 4D (deuterated) 4-mers were mixed in equal parts; following dissociation, four hetero- 4-meric species were formed. F) Results of the subunit exchange experiments of D-S52P with H-WT, H-S52P, H-T119M, and H-S52P bound to tafamidis, respectively (from the left panel to the right one). Changes in relative abundance of homo- and hetero- 4-meric species over the course of 11 days are shown, along with an estimate of their association/dissociation rates. These figures were reproduced with permission from [40], ©(2019) Nature publications, under a Creative Commons Attribution 4.0 International License.

Figure 4. The dynamic assembly of KaiCBA can be efficiently monitored during native MS experiments.

A) Native mass spectra of the circadian oscillator recorded at 30°C. Peaks corresponding to KaiC, KaiCA, KaiCB, and KaiCBA are highlighted with different colours. The relative signal

intensity (RSI) is shown on the y axis and the m/z on the x axis. B) Labelled mass spectrum of Kai system after 12 hours of incubation at 30°C. The identified Kai complexes are schematically drawn and colour-coded as follows: KaiC₆, blue and green; KaiA, yellow; KaiB, pink. In the spectrum the peaks corresponding to the Kai complexes are labelled with different coloured diamonds and circles. Light pink diamonds: KaiC₆; pink diamonds: KaiC₆A₂; light violet circles: KaiC₆B₁ and KaiC₆B₆; violet circle: KaiC₆B₆; blue KaiC₆B₆A₄; light blue: KaiC₆B₆A₆. This figure was reproduced with permission from [187]© (2017) The American Association for the Advancement of Science.

Table 1. Summary of main abbreviations mentioned in the text.

Acronym	Technique	Description
CID (or CAD)	Collision induced dissociation (Collisionally activated dissociation)	Approach to dissociate ions in the gas phase, whereby ions are accelerated by an electrical potential and collide with neutral gas molecules such as argon or xenon.
ECD	Electron capture dissociation	Technique for fragmenting ions in the gas phase. Ions directly interact with low-energy free electrons.
ESI	Electrospray ionisation	Soft ionisation technique whereby ions are produced in the gas phase by applying a voltage to a solution of sample and creating an aerosol.
ETD	Electron transfer dissociation	Approach for fragmenting ions in the gas phase by transferring electrons to them from a radical anion (e.g., anthracene and azobenzene).
FT MS	Fourier Transform based MS	Technique for determining the m/z ratio of ions through the frequency signals that the ions generate within the instrument. The mass spectrum is derived by applying a Fourier transform to the frequency data.
FT-ICR	Fourier Transform ion cyclotron resonance	Type of FT-based MS in which mass spectra are determined from the cyclotron resonance frequencies produced by ions when they rotate in a magnetic field.
HCD	Higher energy C-trap (or collisional) dissociation	A CID technique specific to Orbitrap instruments in which the fragmentation of ions takes place outside the Orbitrap analyser.
HDX	Hydrogen/deuterium exchange	Technique used to monitor covalently bonded hydrogen atoms replaced by deuterium atoms. Overall, it can be utilized to investigate conformations of individual proteins and macromolecular complexes.
IM	Ion mobility	Technique that separates molecular ions in the gas phase based on their mobility in a buffer gas under the influence of a weak electric field.
ISCID	In-source collision induced dissociation	Type of CID whereby ions are fragmented in the source region of a mass spectrometer.
IRMPD	Infrared multiphoton dissociation	Approach to fragment ions in the gas phase by the absorption of multiple infrared photons.
MALDI	Matrix-assisted laser desorption/ionisation	Soft ionisation technique whereby molecules are embedded in a solid organic acid (known as matrix) and subsequently desorbed and ionised by a pulse of laser light.
MS/MS or MS ²	Tandem MS	Method of analysis involving two stages of MS selection. The first MS stage separates sample components according to their m/z . During the second MS stage, the selected ions are subsequently subjected to fragmentation/dissociation and mass spectra of the products are obtained.
MS ³	-	Using Orbitrap instruments, the 1-mers, ejected during MS ² experiments, are fragmented by backbone cleavage using various methods such as ECD or UVPD.
-	Orbitrap	A FT-based analyser composed of an inner spindle-like electrode and an outer barrel-like one. Ions are trapped in an orbital motion around the spindle and the frequency signals, which arise from the resulting image current, are utilised for calculating mass spectra.
NEMS	nanoelectromechanical systems	A nanoscale device that resonates at high frequency and functions as a highly sensitive mass sensor. When species are adsorbed on NEMS, the sensors register changes in frequencies that are directly proportional to the mass of the adsorbed species.
Q	Quadrupole	An analyser composed of four parallel metal rods to which a radio frequency (RF) voltage and direct current (DC) voltage are applied. For a given ratio of voltages, only ions with the appropriate m/z ratio can travel through the analyser, while others present unstable trajectories and collide with the rods.
SID	Surface-induced dissociation	Technique for dissociating ions in the gas phase, whereby ions are accelerated to collide against a surface such as that self-assembled monolayers of CF ₃ (CF ₂) ₁₀ CH ₂ CH ₂ S- on gold serve.
TOF	Time-of-flight	A type of analyser in which ions are accelerated in an electric field and then allowed to drift through a field-free region to a detector. The square of the time taken to reach the detector is directly proportional to the m/z

of the ions.

UVPD

Ultraviolet photodissociation

Absorption of photons by gas-phase ions leads to energization that induces the fragmentation of the ions.

Table 2. Examples of soluble and membrane protein complexes analysed by native MS

Complex	Mass (kDa)	Outcome of analysis	Prior atomic structure known	References
Soluble Complexes				
Cascade	405	Two dimensional map	N	[210]
Csy (CRISPR system yersinia)	350	“ “ “	N	[295]
EcoP15I, EcoPI, PstII	63-311	Functional insights	N	[296]
Structural Maintenance of Chromosomes complex (SMC)	424-589	“ “	N	[213]
Kinase domain of RIP2 (receptor Interacting protein 2)	34-69	“ “	Y	[194]
Lumazine synthase	3000-6000	“ “	Y	[193]
Transthyretin	55	“ “	Y	[40]
Bacteriophage HK97 capsid	18000	Assembly pathway	N	[73]
TrV virions (from <i>Triatoma infestans</i>)	8300	“ “	N	[219]
Norwalk virus-like particles	10100	“ “	N	[218]
Kai system	624-823	“ “	Y	[187].
HK97 Prohead-1 particles	~21400	“ “	Y	[199]
DegP oligomers	143-575	Model for the transition between the resting and active states of an enzyme	Y	[297]
Protruding (P) domain of the norovirus capsid protein oligomers	72-1361	Discovery of multiple oligomeric states controlled by buffer conditions (e.g., pH)	Y	[198]
Membrane Complexes				
<i>E. coli</i> Translocon (ColE9-Im9 complex, BtuB, OmpF trimer, and TolB)	296	Functional insights	Y	[234]
MscL	78	“ “	Y	[298]
DgkA, pSRII, LacY-GFP	13-78	Reconstitution in detergent, amphipols, bicelles and nanodiscs	Y	[244]

PagP and OmpT, Mhp1 and GalP	20-54	Same as above.	Y	[245]
Many complexes	7-809	Complexes ejected from native membranes into the gas phase	Y	[250]
ATP-Binding Cassette transporter P-glycoprotein (P-gp)	141-147	Ligand binding affinities	Y	[242]
MsbA	134	“ “ “	Y	[229].
ModBC-A and BtuCD-F	29-159	“ “ “	Y	[230]
MscL, AqpZ and AmtB	85-126	“ “ “	Y	[225]
B subunits of cholera and heat labile toxins	58-62	“ “ “	Y	[248]
Mycobacterial membrane protein Large 3	83-110	“ “ “	Y	[243]
AmtB	127	Allosteric interactions	Y	[241].







