

Cite this: *RSC Med. Chem.*, 2025, 16, 5114

Toward routine utilisation of native mass spectrometry as an enabler of contemporary drug development

Louise M. Sternicki ^{ab} and Sally-Ann Poulsen ^{*ab}

As therapeutic modalities increasingly diversify, the need for biophysical tools for routine characterisation of the underlying biomolecular targets and their noncovalent interactions is growing. In this Opinion article we discuss the role of native mass spectrometry (nMS), a mass spectrometry technique where the intact biomolecule and its noncovalent interactions are preserved during the analysis, to gain important insights to guide drug discovery and development. We conclude that nMS is one of the most powerful technologies available with potential to rapidly advance multiple stages of therapeutic discovery and development, yet it is arguably underutilised. Specifically, we highlight how nMS may progress research for contemporary therapeutic modalities including those implicated in targeted protein degradation, fragment-based drug discovery and mRNA therapies.

Received 15th July 2025,
Accepted 25th July 2025

DOI: 10.1039/d5md00617a

rsc.li/medchem

Introduction

The development and implementation of enabling technologies in drug discovery is critical to provide advances that match the demands of an increasingly diverse landscape of therapeutic modalities. Native mass spectrometry (nMS) is a powerful biophysical technique that provides the mass of intact biomolecules in their native folded state including their noncovalent interactions with key binding partners.^{1–5} Building on decades of pioneering research to develop instruments and experimental methods, nMS has slowly but steadily transitioned from a niche and specialist MS technique to a method whereby today's trained MS practitioners are from diverse research backgrounds.^{1,6–9} Furthermore, the wider medicinal chemistry community now can perform high resolution and high accuracy mass measurements of diverse biomolecular systems using commercially available MS infrastructure commonly found within an organisation's core facility. Our group has a long-standing research interest in the application of nMS in the context of the ever-changing landscape of modern drug discovery, including with targeted protein degradation, fragment-based drug discovery and RNA-targeting. In this Opinion article our intention is to place a spotlight on the potential of nMS methods to address the analytical demands of discovery and development presented by modern

therapeutic modalities, bridging small molecule approaches for modulating protein and oligonucleotide targets and larger biologics such as antibodies or mRNA.

nMS can be considered gas-phase structural biology,^{5,8,10} with biomolecules and their noncovalent complexes analysed from volatile 'physiological' solution conditions (typically 100–200 mM ammonium acetate and neutral pH) that are ionised and transferred to the gas phase for detection. The nMS experimental readout is a mass spectrum comprising peaks corresponding to the sample components measured as multiply charged ions with a mass (m) to charge (z) ratio, m/z , providing an indirect measurement of mass. The m/z value is, however, straightforward to convert to molecular weight (MW) provided the observed signals can be uniquely mapped to a charge state z using a process termed deconvolution,¹¹ with the MW affording the sample component's identity, Fig. 1.¹² The broad scale of the m/z readout enables the multiple solution components (*i.e.*, multiple m/z values) to be readily distinguished and identified from a single mass spectrum. This ability of nMS to simultaneously detect all species present in each sample is critical for the applications discussed herein. Furthermore, nMS analysis of complex samples comprising noncovalent interactions can provide access to metrics including binding strength, stoichiometry, thermodynamic parameters and kinetics, in addition to other high-order structural information relevant to the interaction such as conformational changes, stoichiometries or oligomeric state.^{1,7,11,13} Advantages of the nMS technique to complement other methods for direct measurements of biomolecular interactions include speed and automation, low sample consumption, and label free measurements direct

^a Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, Queensland 4222, Australia. E-mail: s.poulsen@griffith.edu.au

^b School of Environment and Science, Griffith University, Brisbane, Queensland 4111, Australia



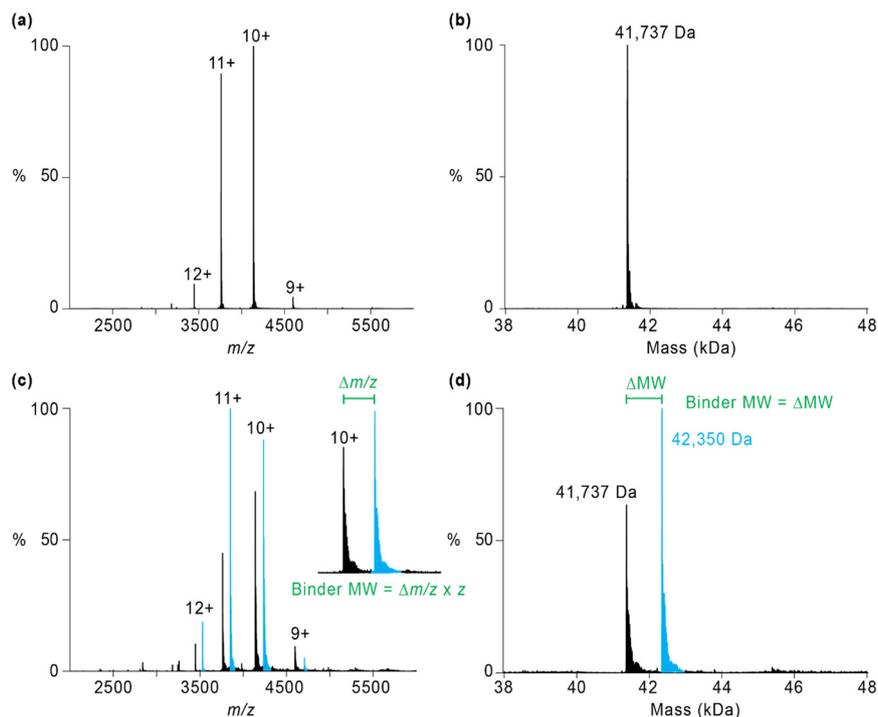


Fig. 1 Schematic of raw (x axis is m/z) and deconvoluted (x axis is mass (kDa)) nMS data of a purified biomolecule sample without (a and b) or with an added interacting partner molecule or binder (c and d). The unbound biomolecule is the black trace; the bound biomolecule is the blue trace. Deconvolution provides the MW of the biomolecule species (bound and unbound) in the sample. Green annotations in (c and d) demonstrate the calculation of the MW (= identity) of the binder is possible using either raw or deconvoluted nMS data.

from a sample solution. These advantages cannot be understated, particularly when the biomolecules of interest can only be generated in limited amounts, are heterogeneous, not amenable to covalent modification or other labelling and/or not able to crystallise. Under such circumstances nMS can offer an unrivalled scope across the therapeutic landscape when compared to other structural biology techniques in use.

Characterisation of biomolecules using native mass spectrometry

Biomolecular characterisation by nMS is typically performed using nano-electrospray ionisation (nanoESI) where single-use small diameter spray capillaries or emitters are used to introduce the sample into the instrument in place of standard infusion ESI.^{14–16} NanoESI hardware enables reduced sample flow rates and smaller initial droplet sizes, this in turn enables gentler mass spectrometry instrument parameters for desolvation and ionisation and fewer charge states that collectively improve the preservation of weak noncovalent interactions.^{14–16} The use of nanoESI nMS can make possible the production of mass spectra using only a few picomoles of native biomolecule, vital when working with biochemical species which are difficult to produce in the quantities required for standard ESI or clinical samples with limited, non-replenishable supply. Importantly, nanoESI provides better sample tolerance to nonvolatile

salts that are common in biomolecule samples and overall increased sensitivity of measurements. Even so, biomolecule analysis with nMS usually requires ‘buffer exchange’ of the sample into a volatile solution that is compatible with nMS prior to analysis, a time-consuming step when performed offline (manually) that negates the benefits of automation of other steps of the nMS analysis and also limits nMS applications with less stable biomolecules, Fig. 2(a).¹⁷ This drawback has been addressed with automated and rapid (<5 minute) online buffer exchange (OBE) directly coupled with nMS, demonstrating an avenue for high sample throughput¹⁸ and also improved compatibility for low stability samples as they are out of their preferred non-volatile storage buffer for a shorter period of time prior to nMS analysis, Fig. 2(b).

Another approach circumventing the requirement for tedious manual buffer exchange is the use of submicron emitters to introduce the biological sample into the MS instrument, where the even smaller diameter openings (nm diameters) improve tolerance to salt, desalting and desolvation, such that it makes possible the analysis of biomolecule samples direct from high salt biological buffers (e.g., Tris, HEPES, NaCl) without buffer exchange, Fig. 2(c).¹⁹ More, recently this has also been demonstrated using theta emitters that comprise two internal channels, with the analyte in biological buffer in one channel and ammonium acetate solution in the second channel, and mixing of the samples occurring at the point of nanoESI, Fig. 2(c).²⁰ While



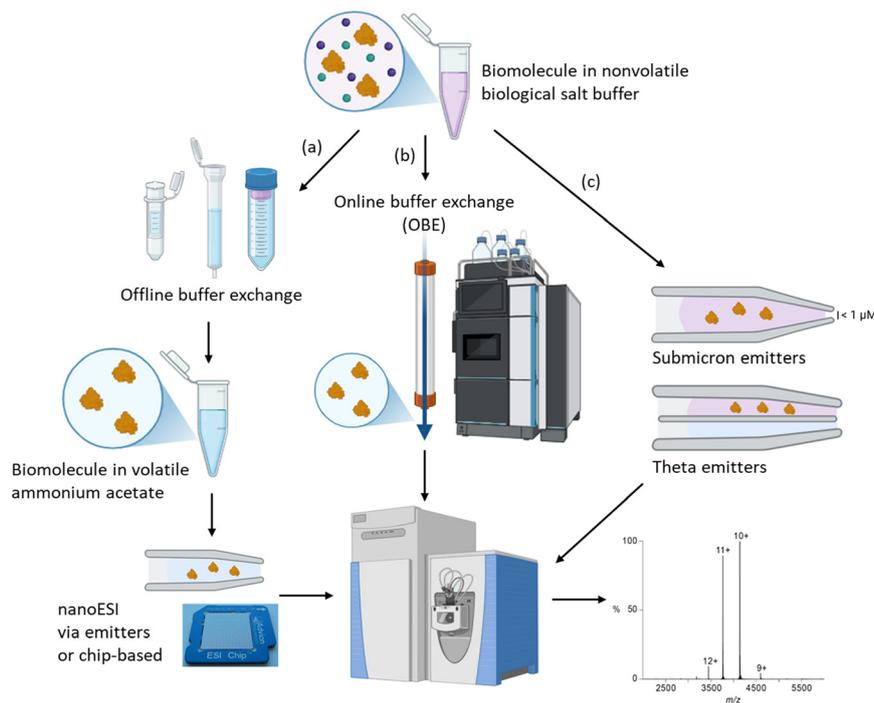


Fig. 2 Alternative workflows for nMS analysis of biomolecules where samples are typically exchanged from their nonvolatile biological buffers into volatile ammonium acetate. Buffer exchange methods may be (a) manual offline buffer exchange methods (e.g., centrifugal spin columns, gravity size exclusion or dialysis); or (b) semi-automated/automated online buffer exchange *via* size exclusion chromatography directly coupled to the MS; or (c) alternatively, altered emitters (submicron or theta emitters) have allowed nMS analysis of biomolecules directly from the nonvolatile storage buffer.

the use of modified (theta or submicron) emitters is yet to be demonstrated with a broad range of analytes, with the emitters typically produced in-house and workflows low in throughput, they may in future help to expand access of nMS analysis to biomolecules including those not stable in traditional volatile nMS buffers. Automation of nMS using chip-based nanoESI (Advion Nanomate)²¹ has dramatically increased throughput to improve the speed of applications to biomolecular screening, while the chip design comprises individual nozzles that eliminate carryover effects between samples, a further advantage when high quality data is sought.^{6,21}

One of the core parameters that nMS can inform that is of relevance in drug discovery is binding affinity (K_D) as both the unbound and bound species in a sample can be measured simultaneously. The K_D may be determined using a traditional titration approach or alternatively a more rapid single concentration approach. The choice of which approach to deploy may depend on the level of detail required from the measurement, with rapid triaging of a screening library possible with the single concentration K_D method and a more in-depth characterisation of interactions possible with a titration K_D . For the single concentration approach to give meaningful K_D values it needs the biomolecule and its binding partner to be at relevant concentrations respective to their binding affinity, with the use of control systems of known K_D values best practice to validate the method.⁷

These quantitative approaches were recently reviewed in full, and we direct interested readers to this review.⁷

Advances in instrument hardware^{2,6} and charge detection mass spectrometry^{22–24} now enable characterisation of biomolecules from heterogeneous populations with essentially unrestricted upper size limits. Using commercial instruments, megadalton protein assemblies up to 18 MDa have been successfully characterised² with mass analysis of intact mRNA analysis recently reaching ~ 3 MDa.²⁵ These large and heterogeneous systems still predominantly remain in the realms of mass spectrometry specialists, with advanced operational experience and expertise needed to unravel the information present within the spectral complexity. An important consideration for wider use is a need to establish and harmonise fundamental methods for these sample types as a safeguard against misinterpretation of complex spectra that could lead to discrepancies or errors entering and contaminating the scientific literature.¹¹ In this Opinion we will focus on application of nMS to three contemporary therapeutic modalities, targeted protein degradation, fragment-based drug discovery and mRNA therapies. These are reflective of drug discovery approaches where nMS is not used extensively but we anticipate that the benefits of nMS approaches can have a major impact in the future to complement other more common biophysical approaches and further advance progress towards new medicines, Fig. 3.



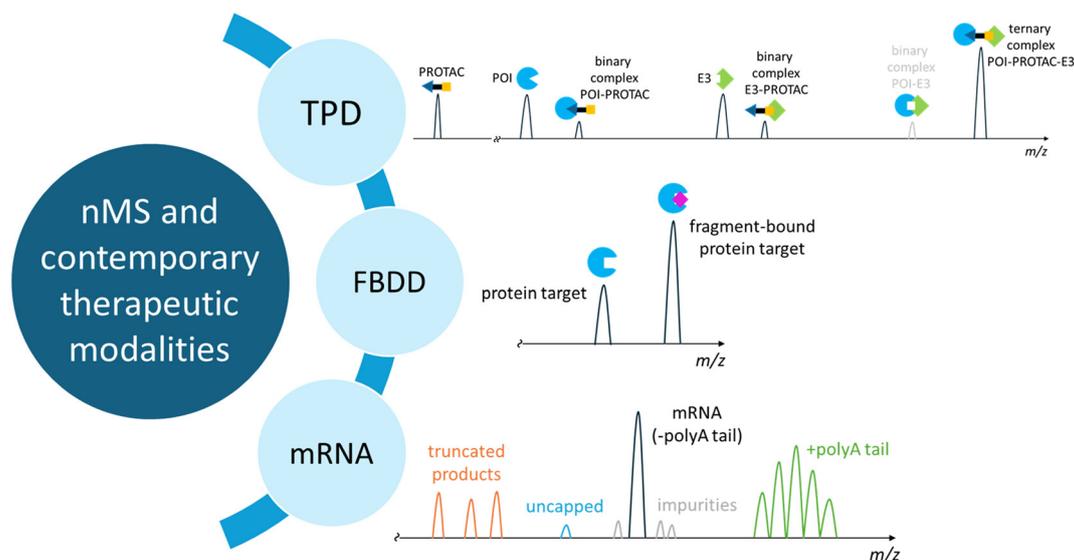


Fig. 3 Application of native mass spectrometry (nMS) with contemporary therapeutic modalities of targeted protein degradation (TPD), fragment-based drug discovery FBDD and mRNA. PROTAC: PROteolysis TArgeting Chimera; POI: protein of interest; E3: E3 ubiquitin ligase.

nMS and targeted protein degradation

Targeted protein degradation is one of the most compelling contemporary approaches for therapeutic drug discovery against proteins that drive disease, with >25 degrader drug candidates currently in clinical trials.^{26,27} There are two prominent subclasses of degraders: PROteolysis TArgeting Chimeras (PROTACs) and molecular glues (MGs), both are small molecules that hijack the endogenous ubiquitin–proteasome system to induce ubiquitination of a target protein, marking it for degradation by the proteasome. This mechanism of action leads to a longer duration of action than classical small molecule inhibitors as well as the ability to target and degrade previously considered undruggable proteins, hence, the attractiveness and strong interest in this method.²⁶

PROTACs comprise two distinct parts, one that recruits the target protein (also called the neo-substrate) and a second that recruits an E3 ligase, with the two parts covalently linked. MGs comprise a single pharmacophore that directly recruits and binds to both the target protein and the E3 ligase. The formation of the ternary complex comprising the E3 ligase, degrader molecule and protein target, is the driver for productive target degradation. Characterising this critical interaction is immensely important for degrader discovery and development, yet there is a paucity of direct, sensitive, systematic and quantitative methods to do so, with a reliance on protein crystallisation or stringent sample preparation for X-ray crystallography/cryo-electron microscopy, or protein modification needed for alternative biophysical methods (*e.g.*, SPR) or cell-based techniques (*e.g.*, NanoLuciferase and HaloTag reporters). This presents a significant barrier for those working to develop degraders as there is a vast number of proteins of interest that may be too flexible or too dynamic to crystallise or that are not amenable to modification as it

interferes with function or structure. Recently we reviewed the reported contributions of nMS to targeted protein degradation,⁹ while small in number the samples comprised three different PROTACs^{28–30} and seven different MGs^{31,32} using proteins expressed from *E. coli* or SF9 insect cells. The nMS data was acquired on a range of different commercially available instruments, with samples in 100–200 mM ammonium acetate and measured ternary complexes ranging in size from ~57 kDa to 180 kDa. Since our 2023 review (also the first review on this topic), we have identified several new studies that use nMS to characterise PROTAC or MG mediated ternary complexes.^{33–36} There are also two additional reported reviews on the topic,^{37,38} possibly a reflection of the important value of the emerging contribution of nMS with targeted protein degradation.

We anticipate that as more E3 ligases are discovered (so far only a handful of the >600 E3 ligases are in use with targeted protein degradation) and where the samples associated are more complex and more challenging for ternary complexes to be characterised by X-ray crystallography or cryo-electron microscopy, that nMS will become invaluable as an alternative structural biology method. We also foresee that because nMS could study more ternary complexes with different ligands more routinely that it could play an important role to triage the best candidate ligands to advance to X-ray crystallography or cryo-electron microscopy analysis where comprehensive ligand screening is not viable (*e.g.*, sample quantity, time or cost are prohibitive), hence, providing a substantial platform for the field to advance. Noting the first PROTAC ternary complex crystal structure was only published in 2017,³⁹ this is particularly an avenue where nMS has potential to generate protein ternary complex interaction data at scale to fast-track future degrader development. Specifically, nMS could assist with the stoichiometry and identity of complex constituents and



quantifying binding (K_D s) using a combination of established commercial software or opensource software.

Native charge detection mass spectrometry (nCDMS) where the masses of individual ions are determined by simultaneous measurement of their mass-to-charge ratio (m/z) and charge (z)^{40–42} may also facilitate production of high-resolution spectra to accurately characterise high mass degrader complexes. With CDMS the amplitude of the image current generated by each individual ion corresponds to peak intensity and makes possible a measure of ion charge (z). With both m/z and z available for all measured ions accurate mass distributions can be determined for heterogeneous and/or high molecular weight species that are typically not amenable for meaningful analysis with standard nMS.^{40–42} The caveat with this is the same as for other biophysical methods, trained users with a strong understanding of the underlying principles of the method are integral for the contribution of quality data to support research endeavours. Although other proximity inducing agents such as protein–protein interaction stabilisers are not discussed here, nMS has been used to characterise ternary complexes of these agents,⁴³ and it stands to reason that nMS could provide opportunities to accelerate broadly the development of reagents that act *via* a proximity associated mechanism.

nMS and fragment-based drug discovery

Fragment based drug discovery (FBDD) is another therapeutic approach where bridging the divide between small molecule libraries and their downstream therapeutic development can be enhanced by adopting nMS. An underlying principle of FBDD is that fragments of interest bind weakly but optimally to their target, with weak binding necessitating sensitive biophysical techniques to identify hits from non-binding fragments.^{44,45} Fragment hits are commonly identified using biophysical methods to screen curated fragment libraries, either commercially available or commercial-in-confidence libraries where the underlying library construction is of intrinsic value.^{46,47} A key advantage of fragment screening is the efficient coverage of chemical space that fragments provide due to their small molecular size, with complete screening campaigns possible using substantially smaller (by number) compound libraries than is typically needed for success from high throughput screening (HTS) libraries made up of larger mass compounds.⁴⁸

We first reviewed the contributions of nMS to guiding fragment screening for hit identification in 2013 (ref. 49) and subsequently published an update in 2023.⁵⁰ These reviews provide both a comprehensive perspective on the early^{51–54} and then later adoption of nMS for fragment screening.^{55–59} We concluded that nMS is particularly well suited for supporting fragment screening campaigns by allowing the pivotal detection of weakly binding ligands (binding constants as low as mM), and nMS even supports screening of pooled fragments or multiplexed proteins. Despite the advantages, nMS is not used to the extent of the more

common biophysical methods, including those where there may be significant challenges such as X-ray crystallography, that requires proteins suitable for crystallisation, or NMR, where proteins are commonly isotopically labelled and high fragment stock concentrations are needed, that can frequently meet with insufficient solubility leaving many fragments unsuitable. The trend in use of different biophysical methods for fragment screening has been recorded by informal polls of the Practical Fragments Blog community in 2011, 2013, 2016, 2019 and 2024.⁶⁰ The most common methods are consistently X-ray crystallography, NMR and SPR, but MS approaches (comprising all MS-based approaches, not just nMS) have risen notably from <10% of users in 2011 to >25% in 2024. At the time of writing there are seven approved fragment-derived drugs and more than 50 fragment-derived compounds in various stages of clinical development,^{47,61–63} with nMS not yet playing a substantial role in those examples, but a situation we expect will change for future FBDD-derived drugs.

Recently, the use of covalent fragments for FBDD has emerged,^{64,65} with covalent binding altering the technical considerations for biophysical screening requirements as compared to noncovalent fragment binding. Our group recently developed a nMS workflow for electrophilic fragment screening of pooled fragments.⁶⁶ The screening method also enabled identification of the modified protein residue by utilising mutant proteins and supported direct simultaneous observation of orthosteric (noncovalent) and covalent fragment binding, not possible with denaturing MS methods. This powerful capability of nMS could greatly accelerate discovery of covalent drug discovery where there is a genuine need for screening technologies to characterise concurrent binding and support better understanding of covalent binding.

nMS and mRNA as biotherapeutics

Despite the fast-moving landscape of mRNA development, product quality specifications (identity, quantity and purity) are not established for mRNA therapeutics. This is a significant issue as there is an expectation of consistent quality control as has been in place (and is expected) for alternative biologics such as protein-based therapeutics. The current draft guidelines released by the United States Pharmacopeia, August 2024 ‘Analytical Procedures for Quality of mRNA vaccines and therapeutics, 3rd edition’⁶⁷ and the European Medicines Agency, March 2025 ‘Guideline on the quality aspects of mRNA vaccines’⁶⁸ identify a pressing need for higher resolution analytical approaches that can monitor the integrity of the whole ‘intact’ mRNA. The challenge for implementing mRNA quality specifications is partly owing to technical and infrastructure considerations that are substantially different to those needed for protein-based biotherapeutics. The unique critical quality attributes (CQAs) of the mRNA therapeutic include the 5′-cap, the 3′-poly(A) tail length and heterogeneity, nucleotide modifications, and the



overall mRNA identity and integrity. These CQAs significantly impact the mRNA stability, translational efficiency and efficacy. Furthermore, all mRNA-based vaccines on the market or in clinical trials are manufactured using *in vitro* transcription (IVT), with both shorter and extended RNA byproducts formed in the IVT reaction. These byproducts equate to impurities, and they can adversely impact mRNA production costs, efficacy and safety. To provide insight on the CQAs, as well as the identity and quantity of mRNA impurities (either during development, production or in the final product), current analytical methods must be improved or novel analytical tools introduced.^{67,68}

To date there are only a few published examples of mRNA analysis by nMS.^{25,69} Genentech described the use of nMS to measure a 683 nt IVT mRNA and the heterogeneity that arose from the addition of a 3' poly(A) tail. Analysis of the mRNA without the 3'-poly(A) tail (mRNA-) revealed a single product with a mass of 224 080 kDa.⁶⁹ Addition of the 3'-poly(A) tail resulted in a 783 nt mRNA (mRNA+), which nMS confirmed had the expected mass difference that corresponded with the addition of the polyA tail. nMS of the mRNA+ also displayed higher heterogeneity due to the partially resolved variability in the number of adenosines constituting the 3'-poly(A) tail. Analysis of both species revealed MWs 3–4 kDa higher than expected due to the presence of noncovalently bound nucleotide fragments (*i.e.*, aborted transcripts), while in the mRNA+ sample a small number of minor variants 2.5 kDa larger were also observed, which corresponded to a small number of additional nucleotides (<10) and were hypothesised to be small dsRNA 3'-loop back byproducts. nMS analysis (at isotopic resolution) of the products from cleavage of the 3'-poly(A) tail by T1 RNase revealed a distribution from 95 to 110 adenosine residues. The approach of RNase cleavage prior to nMS analysis simplified analysis (lower mass, reduced heterogeneity) and facilitated a more detailed assessment of the polyA tail modification compared to intact analysis of the full-length mRNA.

In an academic-industry collaboration Heck and colleagues together with Pfizer characterised intact mRNA-based therapeutics without digestion using both nMS (mRNA <1 MDa) and charge detection mass spectrometry (CDMS; mRNA >1 MDa).²⁵ This study was inspired by the need for strategies specific for mRNA analytical challenges and the corresponding very limited tools available to characterise therapeutic mRNA.⁷⁰ Through analysis of a panel of different mRNAs (ranging from 858 to 9400 nucleotides (283 kDa–3 MDa)) they demonstrated that nMS can reliably characterise the mass of mRNAs up to 1000 nucleotides, although this is considered mid-size mRNA. CDMS was used to access accurate mass measurements for higher mass mRNAs. For this an organic co-solvent was required to denature the RNA and give higher charged state species for improved ion behaviour, increased signal-to-noise and reduced charge uncertainty, leading to an overall increase in mass accuracy. They highlight the challenges for intact RNA analysis, that although shared with protein

samples, is exacerbated by the inherent heterogeneity of mRNA as well as higher propensity of salt adducts (interferents of nMS) as a consequence of the negatively charged RNA backbone.²⁵ An important take home message commented on in this study was 'it should be noted that it is often difficult to find a fit-for-all MS method that would allow for optimal transmission and desolvation of all species from low-molecular-weight-species to high-molecular-weight species', a reminder how biophysical methods used in combination and *via* collaboration are a way forward to strengthen drug discovery.

Outlook and broader application of nMS in other contemporary drug discovery

The nMS applications covered in this Opinion were selected to put a spotlight on nMS as it relates to contemporary drug development. That said, we believe that the coverage we provided is the tip of the iceberg, and that nMS is genuinely underutilised in drug discovery and development. We wish to acknowledge other pioneering efforts of researchers in industry and academia advancing drug discovery for other challenging systems including membrane proteins,^{71–76} transient protein–protein interactions,^{77–82} therapeutic antibodies^{83–86} and gene delivery vectors.^{87–89} We recognise that long standing challenges remain for wider uptake of nMS in drug discovery – these are both technical (*e.g.*, affordable, commercially available and user-friendly instruments) and nontechnical (policies and environments that support research and research training across disciplines). As nMS may be used in concert with a vast array of allied methods (*e.g.*, ion mobility and collision-induced unfolding, hydrogen–deuterium exchange, collision dissociation, top-down sequencing) and methods in development (*e.g.*, soft landing nMS whereby analysed molecules are gently landed and collected post-MS analysis for further investigation, most recently optimised for cryo-electron microscopy structural characterisation)^{90–94} there is the promise of even greater advanced capability than we have discussed, for example with membrane proteins, the emergence of RNA targeting therapies, antibodies and antibody–drug conjugates, virus like particles and many other biomolecules. Additionally, recent advances in nMS are moving from purified biomolecules characterised in isolation, to endogenous biomolecules analysed in semi-purified or entirely native environments at their natural abundances and with their natural proteoforms and/or post-translational modifications, facilitating improved maintenance of relevant biomolecular structure and function, an especially important consideration for membrane proteins.^{78,95–101}

We expect to witness advanced nMS becoming more common practice instead of residing predominantly in specialist laboratories using bespoke in-house modified



instruments. We caution that as more researchers affiliated with drug discovery adopt nMS, that the community that has pioneered nMS will be presented with a greater need to establish standardised practices for the various steps of nMS analysis and continue to address the challenges of throughput, data reproducibility, data analysis and sharing so that it is fit-for-purpose in the fast pace of drug discovery and development settings. We hope to see training of tomorrow's nMS professionals so that they can provide the human element to critical nMS infrastructure, for example engaged in research directly or providing access to expertise in core analytical facilities in academia and industry, ultimately increasing the impact of nMS. Despite challenges, the applications of nMS in drug discovery and development has progressed markedly in recent decades, and with so much to offer for biomolecular analysis the future appears very bright indeed.

Data availability

As an Opinion there are no new data generated or shared.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors gratefully acknowledge support of the Clive and Vera Ramaciotti Foundation (Biomedical Research Award, grant number 2023BRA19), the Australian Government Australian Research Council's Industrial Transformation Training Centre grant (grant number IC180100021) and Therapeutic Innovation Australia for their native mass spectrometry research. The authors also acknowledge the use of <https://biorender.com> for the preparation of Fig. 2 and table of contents graphic.

References

- H. M. Britt and C. V. Robinson, Traversing the drug discovery landscape using native mass spectrometry, *Curr. Opin. Struct. Biol.*, 2025, **91**, 102993, DOI: [10.1016/j.sbi.2025.102993](https://doi.org/10.1016/j.sbi.2025.102993).
- S. Tamara, M. A. den Boer and A. J. R. Heck, High-Resolution Native Mass Spectrometry, *Chem. Rev.*, 2022, **122**(8), 7269–7326, DOI: [10.1021/acs.chemrev.1c00212](https://doi.org/10.1021/acs.chemrev.1c00212).
- E. Largy, A. König, A. Ghosh, D. Ghosh, S. Benabou, F. Rosu and V. Gabelica, Mass Spectrometry of Nucleic Acid Noncovalent Complexes, *Chem. Rev.*, 2022, **122**(8), 7720–7839, DOI: [10.1021/acs.chemrev.1c00386](https://doi.org/10.1021/acs.chemrev.1c00386).
- S. A. Hofstadler and K. A. Sannes-Lowery, Applications of ESI-MS in drug discovery: interrogation of noncovalent complexes, *Nat. Rev. Drug Discovery*, 2006, **5**(7), 585–595, DOI: [10.1038/nrd2083](https://doi.org/10.1038/nrd2083).
- J. L. P. Benesch and B. T. Ruotolo, Mass spectrometry: come of age for structural and dynamical biology, *Curr. Opin. Struct. Biol.*, 2011, **21**(5), 641–649, DOI: [10.1016/j.sbi.2011.08.002](https://doi.org/10.1016/j.sbi.2011.08.002).
- I. D. G. Campuzano and J. A. Loo, Evolution of Mass Spectrometers for High m/z Biological Ion Formation, Transmission, Analysis and Detection: A Personal Perspective, *J. Am. Soc. Mass Spectrom.*, 2025, **36**(4), 632–652, DOI: [10.1021/jasms.4c00348](https://doi.org/10.1021/jasms.4c00348).
- J. L. Bennett, G. T. Nguyen and W. A. Donald, Protein–small molecule interactions in native mass spectrometry, *Chem. Rev.*, 2021, **122**(8), 7327–7385.
- I. D. G. Campuzano and W. Sandoval, Denaturing and Native Mass Spectrometric Analytics for Biotherapeutic Drug Discovery Research: Historical, Current, and Future Personal Perspectives, *J. Am. Soc. Mass Spectrom.*, 2021, **32**(8), 1861–1885, DOI: [10.1021/jasms.1c00036](https://doi.org/10.1021/jasms.1c00036).
- L. M. Sternicki and S.-A. Poulsen, Native Mass Spectrometry: Insights and Opportunities for Targeted Protein Degradation, *Anal. Chem.*, 2023, **95**(51), 18655–18666.
- J. L. P. Benesch, B. T. Ruotolo, D. A. Simmons and C. V. Robinson, Protein Complexes in the Gas Phase: Technology for Structural Genomics and Proteomics, *Chem. Rev.*, 2007, **107**(8), 3544–3567, DOI: [10.1021/cr068289b](https://doi.org/10.1021/cr068289b).
- V. Yin, P. W. A. Devine, J. C. Saunders, A. Barendregt, F. Cusdin, A. Ristani, A. Hines, S. Shepherd, M. Dembek, C. L. Dobson, J. Snijder, N. J. Bond and A. J. R. Heck, Stochastic assembly of biomacromolecular complexes: impact and implications on charge interpretation in native mass spectrometry, *Chem. Sci.*, 2023, **14**(35), 9316–9327, DOI: [10.1039/D3SC03228K](https://doi.org/10.1039/D3SC03228K).
- M. T. Marty, Fundamentals: How Do We Calculate Mass, Error, and Uncertainty in Native Mass Spectrometry?, *J. Am. Soc. Mass Spectrom.*, 2022, **33**(10), 1807–1812, DOI: [10.1021/jasms.2c00218](https://doi.org/10.1021/jasms.2c00218).
- C. Ren, A. O. Bailey, E. VanderPorten, A. Oh, W. Phung, M. M. Mulvihill, S. F. Harris, Y. Liu, G. Han and W. Sandoval, Quantitative Determination of Protein-Ligand Affinity by Size Exclusion Chromatography Directly Coupled to High-Resolution Native Mass Spectrometry, *Anal. Chem.*, 2019, **91**(1), 903–911, DOI: [10.1021/acs.analchem.8b03829](https://doi.org/10.1021/acs.analchem.8b03829).
- R. Juraschek, T. Dülcks and M. Karas, Nanoelectrospray—More than just a minimized-flow electrospray ionization source, *J. Am. Soc. Mass Spectrom.*, 1999, **10**(4), 300–308, DOI: [10.1016/S1044-0305\(98\)00157-3](https://doi.org/10.1016/S1044-0305(98)00157-3).
- K. De Vriendt, K. Sandra, T. Desmet, W. Nerinckx, J. Van Beeumen and B. Devreese, Evaluation of automated nano-electrospray mass spectrometry in the determination of non-covalent protein–ligand complexes, *Rapid Commun. Mass Spectrom.*, 2004, **18**(24), 3061–3067, DOI: [10.1002/rem.1728](https://doi.org/10.1002/rem.1728).
- J. T. S. Hopper and C. V. Robinson, Mass Spectrometry Quantifies Protein Interactions—From Molecular Chaperones to Membrane Porins, *Angew. Chem., Int. Ed.*, 2014, **53**(51), 14002–14015, DOI: [10.1002/anie.201403741](https://doi.org/10.1002/anie.201403741).
- Y. Li, W. Li, Y. Zheng, T. Wang, R. Pu and Z. Zhang, Desalting strategies for native mass spectrometry, *Talanta*, 2025, **281**, 126824, DOI: [10.1016/j.talanta.2024.126824](https://doi.org/10.1016/j.talanta.2024.126824).



- 18 Z. L. VanAernum, F. Busch, B. J. Jones, M. Jia, Z. Chen, S. E. Boyken, A. Sahasrabudhe, D. Baker and V. H. Wysocki, Rapid online buffer exchange for screening of proteins, protein complexes and cell lysates by native mass spectrometry, *Nat. Protoc.*, 2020, **15**(3), 1132–1157, DOI: [10.1038/s41596-019-0281-0](https://doi.org/10.1038/s41596-019-0281-0).
- 19 A. C. Susa, Z. Xia and E. R. Williams, Small Emitter Tips for Native Mass Spectrometry of Proteins and Protein Complexes from Nonvolatile Buffers That Mimic the Intracellular Environment, *Anal. Chem.*, 2017, **89**(5), 3116–3122, DOI: [10.1021/acs.analchem.6b04897](https://doi.org/10.1021/acs.analchem.6b04897).
- 20 J. B. Hatvany, E.-L. P. Olsen and E. S. Gallagher, Characterizing Theta-Emitter Generation for Use in Microdroplet Reactions, *J. Am. Soc. Mass Spectrom.*, 2024, **35**(12), 2926–2933, DOI: [10.1021/jasms.4c00262](https://doi.org/10.1021/jasms.4c00262).
- 21 G. A. Schultz, T. N. Corso, S. J. Prosser and S. Zhang, A Fully Integrated Monolithic Microchip Electrospray Device for Mass Spectrometry, *Anal. Chem.*, 2000, **72**(17), 4058–4063, DOI: [10.1021/ac000325y](https://doi.org/10.1021/ac000325y).
- 22 S. D. Fuerstenau and W. H. Benner, Molecular weight determination of megadalton DNA electrospray ions using charge detection time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.*, 1995, **9**(15), 1528–1538, DOI: [10.1002/rcm.1290091513](https://doi.org/10.1002/rcm.1290091513).
- 23 N. C. Contino, E. E. Pierson, D. Z. Keifer and M. F. Jarrold, Charge Detection Mass Spectrometry with Resolved Charge States, *J. Am. Soc. Mass Spectrom.*, 2013, **24**(1), 101–108, DOI: [10.1007/s13361-012-0525-5](https://doi.org/10.1007/s13361-012-0525-5).
- 24 A. G. Elliott, S. I. Merenbloom, S. Chakrabarty and E. R. Williams, Single Particle Analyzer of Mass: A Charge Detection Mass Spectrometer with a Multi-Detector Electrostatic Ion Trap, *Int. J. Mass Spectrom.*, 2017, **414**, 45–55, DOI: [10.1016/j.ijms.2017.01.007](https://doi.org/10.1016/j.ijms.2017.01.007).
- 25 E. Deslignière, L. F. Barnes, T. W. Powers, O. V. Friese and A. J. R. Heck, Characterization of intact mRNA-based therapeutics by charge detection mass spectrometry and mass photometry, *Mol. Ther. - Methods Clin. Dev.*, 2025, **33**(2), 101454, DOI: [10.1016/j.omtm.2025.101454](https://doi.org/10.1016/j.omtm.2025.101454).
- 26 D. Chirnomas, K. R. Hornberger and C. M. Crews, Protein degraders enter the clinic - a new approach to cancer therapy, *Nat. Rev. Clin. Oncol.*, 2023, **20**(4), 265–278, DOI: [10.1038/s41571-023-00736-3](https://doi.org/10.1038/s41571-023-00736-3).
- 27 C. Crowe, M. A. Nakasone, S. Chandler, C. Craigon, G. Sathe, M. H. Tatham, N. Makukhin, R. T. Hay and A. Ciulli, Mechanism of degrader-targeted protein ubiquitination, *Sci. Adv.*, 2024, **10**(41), ead06492, DOI: [10.1126/sciadv.ado6492](https://doi.org/10.1126/sciadv.ado6492).
- 28 R. Beveridge, D. Kessler, K. Rumpel, P. Ettmayer, A. Meinhart and T. Clausen, Native Mass Spectrometry Can Effectively Predict PROTAC Efficacy, *ACS Cent. Sci.*, 2020, **6**(7), 1223–1230, DOI: [10.1021/acscentsci.0c00049](https://doi.org/10.1021/acscentsci.0c00049).
- 29 L. M. Sternicki, J. Nonomiya, M. Liu, M. M. Mulvihill and R. J. Quinn, Native Mass Spectrometry for the Study of PROTAC GNE-987-Containing Ternary Complexes, *ChemMedChem*, 2021, **16**(14), 2206–2210, DOI: [10.1002/cmdc.202100113](https://doi.org/10.1002/cmdc.202100113).
- 30 J. H. Song, N. D. Wagner, J. Yan, J. Li, R. Y. C. Huang, A. J. Balog, J. A. Newitt, G. Chen and M. L. Gross, Native mass spectrometry and gas-phase fragmentation provide rapid and in-depth topological characterization of a PROTAC ternary complex, *Cell Chem. Biol.*, 2021, **28**(10), 1528–1538, e1524, DOI: [10.1016/j.chembiol.2021.05.005](https://doi.org/10.1016/j.chembiol.2021.05.005).
- 31 X. Huang, H. Kamadurai, P. Siuti, E. Ahmed, J. L. Bennett and W. A. Donald, Oligomeric Remodeling by Molecular Glues Revealed Using Native Mass Spectrometry and Mass Photometry, *J. Am. Chem. Soc.*, 2023, **145**(27), 14716–14726, DOI: [10.1021/jacs.3c02655](https://doi.org/10.1021/jacs.3c02655).
- 32 C. Jackson and R. Beveridge, Native mass spectrometry of complexes formed by molecular glues reveals stoichiometric rearrangement of E3 ligases, *Analyst*, 2024, **149**(11), 3178–3185, DOI: [10.1039/d4an00110a](https://doi.org/10.1039/d4an00110a).
- 33 R. C. E. Deutscher, C. Meyners, M. L. Repity, W. O. Sugiarto, J. M. Kolos, E. V. S. Maciel, T. Heymann, T. M. Geiger, S. Knapp, F. Lermyte and F. Hausch, Discovery of fully synthetic FKBP12-mTOR molecular glues, *Chem. Sci.*, 2025, **16**(10), 4256–4263, DOI: [10.1039/D4SC06917J](https://doi.org/10.1039/D4SC06917J).
- 34 E. V. S. Maciel, J. Eisert, J. Müller, T. Habeck and F. Lermyte, Mass Spectrometry Analysis of Chemically and Collisionally Dissociated Molecular Glue- and PROTAC-Mediated Protein Complexes Informs on Disassembly Pathways, *J. Am. Soc. Mass Spectrom.*, 2025, **36**(2), 355–367, DOI: [10.1021/jasms.4c00429](https://doi.org/10.1021/jasms.4c00429).
- 35 J. K. Dreizler, C. Meyners, W. O. Sugiarto, M. L. Repity, E. V. S. Maciel, P. L. Purder, F. Lermyte, S. Knapp and F. Hausch, Broad Target Screening Reveals Abundance of FKBP12-Based Molecular Glues in Focused Libraries, *J. Med. Chem.*, 2025, **68**(9), 9525–9536, DOI: [10.1021/acs.jmedchem.5c00220](https://doi.org/10.1021/acs.jmedchem.5c00220).
- 36 T. M. Geiger, M. Walz, C. Meyners, A. Kuehn, J. K. Dreizler, W. O. Sugiarto, E. V. S. Maciel, M. Zheng, F. Lermyte and F. Hausch, Discovery of a Potent Proteolysis Targeting Chimera Enables Targeting the Scaffolding Functions of FK506-Binding Protein 51 (FKBP51), *Angew. Chem.*, 2024, **63**(3), e202309706, DOI: [10.1002/anie.202309706](https://doi.org/10.1002/anie.202309706).
- 37 E. Fabbri, F. Fiorentino, F. Casano, A. Mai and D. Rotili, Native mass spectrometry for proximity-inducing compounds: a new opportunity for studying chemical-induced protein modulation, *Expert Opin. Drug Discovery*, 2025, **20**(5), 643–657, DOI: [10.1080/17460441.2025.2486146](https://doi.org/10.1080/17460441.2025.2486146).
- 38 Y. Hao, B. Zhang and R. Chen, Application of mass spectrometry for the advancement of PROTACs, *J. Pharm. Biomed. Anal.*, 2025, **261**, 116829, DOI: [10.1016/j.jpba.2025.116829](https://doi.org/10.1016/j.jpba.2025.116829).
- 39 M. S. Gadd, A. Testa, X. Lucas, K. H. Chan, W. Chen, D. J. Lamont, M. Zengerle and A. Ciulli, Structural basis of PROTAC cooperative recognition for selective protein degradation, *Nat. Chem. Biol.*, 2017, **13**(5), 514–521, DOI: [10.1038/nchembio.2329](https://doi.org/10.1038/nchembio.2329).
- 40 M. F. Jarrold, Applications of Charge Detection Mass Spectrometry in Molecular Biology and Biotechnology, *Chem. Rev.*, 2022, **122**(8), 7415–7441, DOI: [10.1021/acs.chemrev.1c00377](https://doi.org/10.1021/acs.chemrev.1c00377).



- 41 E. Deslignière, A. Rolland, E. H. T. M. Ebberink, V. Yin and A. J. R. Heck, Orbitrap-Based Mass and Charge Analysis of Single Molecules, *Acc. Chem. Res.*, 2023, **56**(12), 1458–1468, DOI: [10.1021/acs.accounts.3c00079](https://doi.org/10.1021/acs.accounts.3c00079).
- 42 T. P. Wörner, J. Snijder, A. Bennett, M. Agbandje-McKenna, A. A. Makarov and A. J. R. Heck, Resolving heterogeneous macromolecular assemblies by Orbitrap-based single-particle charge detection mass spectrometry, *Nat. Methods*, 2020, **17**(4), 395–398, DOI: [10.1038/s41592-020-0770-7](https://doi.org/10.1038/s41592-020-0770-7).
- 43 J. Bellamy-Carter, M. Mohata, M. Falcicchio, J. Basran, Y. Higuchi, R. G. Doveston and A. C. Leney, Discovering protein-protein interaction stabilisers by native mass spectrometry, *Chem. Sci.*, 2021, **12**(32), 10724–10731, DOI: [10.1039/d1sc01450a](https://doi.org/10.1039/d1sc01450a).
- 44 P. Kirsch, A. M. Hartman, A. K. H. Hirsch and M. Empting, Concepts and Core Principles of Fragment-Based Drug Design, *Molecules*, 2019, **24**, 4309, DOI: [10.3390/molecules24234309](https://doi.org/10.3390/molecules24234309).
- 45 C. W. Murray and D. C. Rees, The rise of fragment-based drug discovery, *Nat. Chem.*, 2009, **1**(3), 187–192, DOI: [10.1038/nchem.217](https://doi.org/10.1038/nchem.217).
- 46 J. Osborne, S. Panova, M. Rapti, T. Urushima and H. Jhoti, Fragments: where are we now?, *Biochem. Soc. Trans.*, 2020, **48**(1), 271–280, DOI: [10.1042/bst20190694](https://doi.org/10.1042/bst20190694).
- 47 M. Bon, A. Bilslund, J. Bower and K. McAulay, Fragment-based drug discovery—the importance of high-quality molecule libraries, *Mol. Oncol.*, 2022, **16**(21), 3761–3777, DOI: [10.1002/1878-0261.13277](https://doi.org/10.1002/1878-0261.13277).
- 48 D. A. Erlanson, S. W. Fesik, R. E. Hubbard, W. Jahnke and H. Jhoti, Twenty years on: the impact of fragments on drug discovery, *Nat. Rev. Drug Discovery*, 2016, **15**(9), 605–619, DOI: [10.1038/nrd.2016.109](https://doi.org/10.1038/nrd.2016.109).
- 49 S.-A. Poulsen, Fragment Screening by Native State Mass Spectrometry, *Aust. J. Chem.*, 2013, **66**(12), 1495–1501, DOI: [10.1071/CH13190](https://doi.org/10.1071/CH13190).
- 50 L. M. Sternicki and S.-A. Poulsen, Fragment-based drug discovery campaigns guided by native mass spectrometry, *RSC Med. Chem.*, 2024, **15**(7), 2270–2285, DOI: [10.1039/D4MD00273C](https://doi.org/10.1039/D4MD00273C).
- 51 H. J. Maple, R. A. Garlish, L. Rigau-Roca, J. Porter, I. Whitcombe, C. E. Prosser, J. Kennedy, A. J. Henry, R. J. Taylor, M. P. Crump and J. Crosby, Automated Protein–Ligand Interaction Screening by Mass Spectrometry, *J. Med. Chem.*, 2012, **55**(2), 837–851, DOI: [10.1021/jm201347k](https://doi.org/10.1021/jm201347k).
- 52 N. Drinkwater, H. Vu, K. M. Lovell, K. R. Criscione, B. M. Collins, T. E. Prisinzano, S.-A. Poulsen, M. J. McLeish, G. L. Grunewald and J. L. Martin, Fragment-based screening by X-ray crystallography, MS and isothermal titration calorimetry to identify PNMT (phenylethanolamine N-methyltransferase) inhibitors, *Biochem. J.*, 2010, **431**(1), 51–61, DOI: [10.1042/bj20100651](https://doi.org/10.1042/bj20100651), (accessed 9/3/2024).
- 53 V. Vivat-Hannah, C. Atmanene, D. Zeyer, A. Van Dorsselaer and S. Sanglier-Cianfèrani, Native Ms: An ‘Esi’ Way to Support Structure- and Fragment-Based Drug Discovery, *Future Med. Chem.*, 2010, **2**(1), 35–50, DOI: [10.4155/fmc.09.141](https://doi.org/10.4155/fmc.09.141).
- 54 C. D. Moore, H. Wu, B. Bolaños, S. Bergqvist, A. Brooun, T. Pauly and D. Nowlin, Structural and Biophysical Characterization of XIAP BIR3 G306E Mutant: Insights in Protein Dynamics and Application for Fragment-Based Drug Design, *Chem. Biol. Drug Des.*, 2009, **74**(3), 212–223, DOI: [10.1111/j.1747-0285.2009.00862.x](https://doi.org/10.1111/j.1747-0285.2009.00862.x).
- 55 L. A. Woods, O. Dolezal, B. Ren, J. H. Ryan, T. S. Peat and S.-A. Poulsen, Native State Mass Spectrometry, Surface Plasmon Resonance, and X-ray Crystallography Correlate Strongly as a Fragment Screening Combination, *J. Med. Chem.*, 2016, **59**(5), 2192–2204, DOI: [10.1021/acs.jmedchem.5b01940](https://doi.org/10.1021/acs.jmedchem.5b01940).
- 56 P. K. Chrysanthopoulos, P. Mujumdar, L. A. Woods, O. Dolezal, B. Ren, T. S. Peat and S.-A. Poulsen, Identification of a New Zinc Binding Chemotype by Fragment Screening, *J. Med. Chem.*, 2017, **60**(17), 7333–7349, DOI: [10.1021/acs.jmedchem.7b00606](https://doi.org/10.1021/acs.jmedchem.7b00606).
- 57 J. Schiebel, N. Radeva, H. Köster, A. Metz, T. Krotzky, M. Kuhnert, W. E. Diederich, A. Heine, L. Neumann, C. Atmanene, D. Roecklin, V. Vivat-Hannah, J.-P. Renaud, R. Meinecke, N. Schlinck, A. Sitte, F. Popp, M. Zeeb and G. Klebe, One Question, Multiple Answers: Biochemical and Biophysical Screening Methods Retrieve Deviating Fragment Hit Lists, *ChemMedChem*, 2015, **10**(9), 1511–1521, DOI: [10.1002/cmdc.201500267](https://doi.org/10.1002/cmdc.201500267).
- 58 M. Göth, V. Badock, J. Weiske, K. Pagel and B. Kuropka, Critical Evaluation of Native Electrospray Ionization Mass Spectrometry for Fragment-Based Screening, *ChemMedChem*, 2017, **12**(15), 1201–1211, DOI: [10.1002/cmdc.201700177](https://doi.org/10.1002/cmdc.201700177).
- 59 T. J. El-Baba, C. A. Lutomski, A. L. Kantsadi, T. R. Malla, T. John, V. Mikhailov, J. R. Bolla, C. J. Schofield, N. Zitzmann, I. Vakonakis and C. V. Robinson, Allosteric Inhibition of the SARS-CoV-2 Main Protease: Insights from Mass Spectrometry Based Assays, *Angew. Chem.*, 2020, **59**(52), 23544–23548, DOI: [10.1002/anie.202010316](https://doi.org/10.1002/anie.202010316).
- 60 D. Erlanson, *Poll results: fragment finding methods and structural information needed for fragment-to-lead efforts*, 2024, <https://practicalfragments.blogspot.com/2024/11/poll-results-fragment-finding-methods.html> (accessed July 2025).
- 61 D. C. Rees, A. K. H. Hirsch and D. A. Erlanson, Introduction to the themed collection on fragment-based drug discovery, *RSC Med. Chem.*, 2022, **13**(12), 1439, DOI: [10.1039/d2md90037h](https://doi.org/10.1039/d2md90037h).
- 62 I. J. P. de Esch, D. A. Erlanson, W. Jahnke, C. N. Johnson and L. Walsh, Fragment-to-Lead Medicinal Chemistry Publications in 2020, *J. Med. Chem.*, 2022, **65**(1), 84–99, DOI: [10.1021/acs.jmedchem.1c01803](https://doi.org/10.1021/acs.jmedchem.1c01803).
- 63 D. Erlanson, Capivasertib: the seventh approved fragment-derived drug. Practical Fragments, 2023, <http://practicalfragments.blogspot.com/#:~:text=Capivasertib%3A%20the%20seventh%20approved%20fragment%2Dderived%20drug> (accessed 2023 23/11/2023).
- 64 A. Douangamath, D. Fearon, P. Gehrtz, T. Krojer, P. Lukacik, C. D. Owen, E. Resnick, C. Strain-Damerell, A. Aimon, P. Ábrányi-Balogh, J. Brandão-Neto, A. Carbery, G.



- Davison, A. Dias, T. D. Downes, L. Dunnett, M. Fairhead, J. D. Firth, S. P. Jones, A. Keeley, G. M. Keserü, H. F. Klein, M. P. Martin, M. E. M. Noble, P. O'Brien, A. Powell, R. N. Reddi, R. Skyner, M. Snee, M. J. Waring, C. Wild, N. London, F. von Delft and M. A. Walsh, Crystallographic and electrophilic fragment screening of the SARS-CoV-2 main protease, *Nat. Commun.*, 2020, **11**(1), 5047, DOI: [10.1038/s41467-020-18709-w](https://doi.org/10.1038/s41467-020-18709-w).
- 65 E. Resnick, A. Bradley, J. Gan, A. Douangamath, T. Krojer, R. Sethi, P. P. Geurink, A. Aimon, G. Amitai, D. Bellini, J. Bennett, M. Fairhead, O. Fedorov, R. Gabizon, J. Gan, J. Guo, A. Plotnikov, N. Reznik, G. F. Ruda, L. Díaz-Sáez, V. M. Straub, T. Szommer, S. Velupillai, D. Zaidman, Y. Zhang, A. R. Coker, C. G. Dowson, H. M. Barr, C. Wang, K. V. M. Huber, P. E. Brennan, H. Ova, F. von Delft and N. London, Rapid Covalent-Probe Discovery by Electrophile-Fragment Screening, *J. Am. Chem. Soc.*, 2019, **141**(22), 8951–8968, DOI: [10.1021/jacs.9b02822](https://doi.org/10.1021/jacs.9b02822).
- 66 J. Klose, Y. Yu, G. Di Trapani, K. F. Tonissen, L. M. Sternicki and S.-A. Poulsen, Electrophilic fragment screening using native mass spectrometry to identify covalent probes for surface cysteines, *Aust. J. Chem.*, 2025, CH25081, accepted manuscript July 2025.
- 67 Analytical Procedures for Quality of mRNA Vaccines and Therapeutics (Draft Guidelines: 3rd Edition); United States Pharmacopeia–National Formulary (USP–NF), 2024, <https://www.uspnf.com/notices/analytical-procedures-mrna-vaccines-20240802> (accessed July 2025).
- 68 Draft concept paper for the development of a guideline on quality aspects of mRNA vaccines for veterinary use; European Medicines Agency, 2025, <https://www.ema.europa.eu/en/guideline-quality-aspects-mrna-vaccines-veterinary-use> (accessed July 2025).
- 69 J. Camperi, S. Lippold, L. Ayalew, B. Roper, S. Shao, E. Freund, A. Nissenbaum, C. Galan, Q. Cao, F. Yang, C. Yu and A. Guilbaud, Comprehensive Impurity Profiling of mRNA: Evaluating Current Technologies and Advanced Analytical Techniques, *Anal. Chem.*, 2024, **96**(9), 3886–3897, DOI: [10.1021/acs.analchem.3c05539](https://doi.org/10.1021/acs.analchem.3c05539).
- 70 G. J. Guimaraes, J. Kim and M. G. Bartlett, Characterization of mRNA therapeutics, *Mass Spectrom. Rev.*, 2024, **43**(5), 1066–1090, DOI: [10.1002/mas.21856](https://doi.org/10.1002/mas.21856).
- 71 I. D. G. Campuzano, A Research Journey: Over a Decade of Denaturing and Native-MS Analyses of Hydrophobic and Membrane Proteins in Amgen Therapeutic Discovery, *J. Am. Soc. Mass Spectrom.*, 2023, **34**(10), 2413–2431, DOI: [10.1021/jasms.3c00175](https://doi.org/10.1021/jasms.3c00175).
- 72 J. T. S. Hopper, Y. T.-C. Yu, D. Li, A. Raymond, M. Bostock, I. Liko, V. Mikhailov, A. Laganowsky, J. L. P. Benesch, M. Caffrey, D. Nietlispach and C. V. Robinson, Detergent-free mass spectrometry of membrane protein complexes, *Nat. Methods*, 2013, **10**(12), 1206–1208, DOI: [10.1038/nmeth.2691](https://doi.org/10.1038/nmeth.2691).
- 73 A. Laganowsky, E. Reading, J. T. S. Hopper and C. V. Robinson, Mass spectrometry of intact membrane protein complexes, *Nat. Protoc.*, 2013, **8**(4), 639–651, DOI: [10.1038/nprot.2013.024](https://doi.org/10.1038/nprot.2013.024).
- 74 M. T. Marty, H. Zhang, W. Cui, R. E. Blankenship, M. L. Gross and S. G. Sligar, Native Mass Spectrometry Characterization of Intact Nanodisc Lipoprotein Complexes, *Anal. Chem.*, 2012, **84**(21), 8957–8960, DOI: [10.1021/ac302663f](https://doi.org/10.1021/ac302663f).
- 75 M. T. Marty, H. Zhang, W. Cui, M. L. Gross and S. G. Sligar, Interpretation and Deconvolution of Nanodisc Native Mass Spectra, *J. Am. Soc. Mass Spectrom.*, 2014, **25**(2), 269–277, DOI: [10.1007/s13361-013-0782-y](https://doi.org/10.1007/s13361-013-0782-y).
- 76 K. K. Hoi, C. V. Robinson and M. T. Marty, Unraveling the Composition and Behavior of Heterogeneous Lipid Nanodiscs by Mass Spectrometry, *Anal. Chem.*, 2016, **88**(12), 6199–6204, DOI: [10.1021/acs.analchem.6b00851](https://doi.org/10.1021/acs.analchem.6b00851).
- 77 R. Rogawski and M. Sharon, Characterizing Endogenous Protein Complexes with Biological Mass Spectrometry, *Chem. Rev.*, 2022, **122**(8), 7386–7414, DOI: [10.1021/acs.chemrev.1c00217](https://doi.org/10.1021/acs.chemrev.1c00217).
- 78 J. Gan, G. Ben-Nissan, G. Arkind, M. Tarnavsky, D. Trudeau, L. Noda Garcia, D. S. Tawfik and M. Sharon, Native Mass Spectrometry of Recombinant Proteins from Crude Cell Lysates, *Anal. Chem.*, 2017, **89**(8), 4398–4404, DOI: [10.1021/acs.analchem.7b00398](https://doi.org/10.1021/acs.analchem.7b00398).
- 79 S. Niu, B. C. Kim, C. A. Fierke and B. T. Ruotolo, Ion mobility-mass spectrometry reveals evidence of specific complex formation between human histone deacetylase 8 and poly-r(C)-binding protein 1, *Int. J. Mass Spectrom.*, 2017, **420**, 9–15, DOI: [10.1016/j.ijms.2016.12.017](https://doi.org/10.1016/j.ijms.2016.12.017).
- 80 K. Ishii, M. Zhou and S. Uchiyama, Native mass spectrometry for understanding dynamic protein complex, *Biochim. Biophys. Acta, Gen. Subj.*, 2018, **1862**(2), 275–286, DOI: [10.1016/j.bbagen.2017.09.019](https://doi.org/10.1016/j.bbagen.2017.09.019).
- 81 A. Politis, C. Schmidt, E. Tjioe, A. M. Sandercock, K. Lasker, Y. Gordiyenko, D. Russel, A. Sali and C. V. Robinson, Topological models of heteromeric protein assemblies from mass spectrometry: application to the yeast eIF3:eIF5 complex, *Chem. Biol.*, 2015, **22**(1), 117–128, DOI: [10.1016/j.chembiol.2014.11.010](https://doi.org/10.1016/j.chembiol.2014.11.010).
- 82 A. Sinz, C. Arlt, D. Chorev and M. Sharon, Chemical cross-linking and native mass spectrometry: A fruitful combination for structural biology, *Protein Sci.*, 2015, **24**(8), 1193–1209, DOI: [10.1002/pro.2696](https://doi.org/10.1002/pro.2696).
- 83 N. J. Thompson, S. Rosati and A. J. Heck, Performing native mass spectrometry analysis on therapeutic antibodies, *Methods*, 2014, **65**(1), 11–17, DOI: [10.1016/j.ymeth.2013.05.003](https://doi.org/10.1016/j.ymeth.2013.05.003).
- 84 J. Adhikari, J. Heffernan, M. Edeling, E. Fernandez, P. N. Jethva, M. S. Diamond, D. H. Fremont and M. L. Gross, Epitope Mapping of Japanese Encephalitis Virus Neutralizing Antibodies by Native Mass Spectrometry and Hydrogen/Deuterium Exchange, *Biomolecules*, 2024, **14**, 374, DOI: [10.3390/biom14030374](https://doi.org/10.3390/biom14030374).
- 85 A. P. Liu, Y. Yan, S. Wang and N. Li, Coupling Anion Exchange Chromatography with Native Mass Spectrometry for Charge Heterogeneity Characterization of Monoclonal Antibodies, *Anal. Chem.*, 2022, **94**(16), 6355–6362, DOI: [10.1021/acs.analchem.2c00707](https://doi.org/10.1021/acs.analchem.2c00707).



- 86 M. A. den Boer, S. H. Lai, X. Xue, M. D. van Kampen, B. Bleijlevens and A. J. R. Heck, Comparative Analysis of Antibodies and Heavily Glycosylated Macromolecular Immune Complexes by Size-Exclusion Chromatography Multi-Angle Light Scattering, Native Charge Detection Mass Spectrometry, and Mass Photometry, *Anal. Chem.*, 2022, **94**(2), 892–900, DOI: [10.1021/acs.analchem.1c03656](https://doi.org/10.1021/acs.analchem.1c03656).
- 87 T. P. Wörner, J. Snijder, O. Friese, T. Powers and A. J. R. Heck, Assessment of genome packaging in AAVs using Orbitrap-based charge-detection mass spectrometry, *Mol. Ther. - Methods Clin. Dev.*, 2022, **24**, 40–47, DOI: [10.1016/j.omtm.2021.11.013](https://doi.org/10.1016/j.omtm.2021.11.013).
- 88 L. F. Barnes, B. E. Draper, Y.-T. Chen, T. W. Powers and M. F. Jarrold, Quantitative analysis of genome packaging in recombinant AAV vectors by charge detection mass spectrometry, *Mol. Ther. - Methods Clin. Dev.*, 2021, **23**, 87–97, DOI: [10.1016/j.omtm.2021.08.002](https://doi.org/10.1016/j.omtm.2021.08.002).
- 89 E. H. T. M. Ebberink, A. Ruisinger, M. Nuebel, M. Thomann and A. J. R. Heck, Assessing production variability in empty and filled adeno-associated viruses by single molecule mass analyses, *Mol. Ther. - Methods Clin. Dev.*, 2022, **27**, 491–501, DOI: [10.1016/j.omtm.2022.11.003](https://doi.org/10.1016/j.omtm.2022.11.003), (accessed 2025/07/23).
- 90 K. W. Lee, A. Z. Salome, M. S. Westphall, T. Grant and J. J. Coon, Onto Grid Purification and 3D Reconstruction of Protein Complexes Using Matrix-Landing Native Mass Spectrometry, *J. Proteome Res.*, 2023, **22**(3), 851–856, DOI: [10.1021/acs.jproteome.2c00595](https://doi.org/10.1021/acs.jproteome.2c00595).
- 91 A. Z. Salome, K. W. Lee, T. Grant, M. S. Westphall and J. J. Coon, Matrix-Landing Mass Spectrometry for Electron Microscopy Imaging of Native Protein Complexes, *Anal. Chem.*, 2022, **94**(50), 17616–17624, DOI: [10.1021/acs.analchem.2c04263](https://doi.org/10.1021/acs.analchem.2c04263).
- 92 T. K. Esser, J. Böhning, P. Fremdling, M. T. Agasid, A. Costin, K. Fort, A. Konijnenberg, J. D. Gilbert, A. Bahm, A. Makarov, C. V. Robinson, J. L. P. Benesch, L. Baker, T. A. M. Bharat, J. Gault and S. Rauschenbach, Mass-selective and ice-free electron cryomicroscopy protein sample preparation via native electrospray ion-beam deposition, *PNAS Nexus*, 2022, **1**(4), 153, DOI: [10.1093/pnasnexus/pgac153](https://doi.org/10.1093/pnasnexus/pgac153).
- 93 T. K. Esser, J. Böhning, P. Fremdling, T. Bharat, J. Gault and S. Rauschenbach, Cryo-EM samples of gas-phase purified protein assemblies using native electrospray ion-beam deposition, *Faraday Discuss.*, 2022, **240**(0), 67–80, DOI: [10.1039/D2FD00065B](https://doi.org/10.1039/D2FD00065B).
- 94 P. Fremdling, T. K. Esser, B. Saha, A. A. Makarov, K. L. Fort, M. Reinhardt-Szyba, J. Gault and S. Rauschenbach, A Preparative Mass Spectrometer to Deposit Intact Large Native Protein Complexes, *ACS Nano*, 2022, **16**(9), 14443–14455, DOI: [10.1021/acsnano.2c04831](https://doi.org/10.1021/acsnano.2c04831).
- 95 A. Oluwole, D. Shutin and J. R. Bolla, Mass spectrometry of intact membrane proteins: shifting towards a more native-like context, *Essays Biochem.*, 2023, **67**(2), 201–213, DOI: [10.1042/ebc20220169](https://doi.org/10.1042/ebc20220169), (accessed 7/11/2025).
- 96 D. S. Chorev, L. A. Baker, D. Wu, V. Beilsten-Edmands, S. L. Rouse, T. Zeev-Ben-Mordehai, C. Jiko, F. Samsudin, C. Gerle, S. Khalid, A. G. Stewart, S. J. Matthews, K. Grünwald and C. V. Robinson, Protein assemblies ejected directly from native membranes yield complexes for mass spectrometry, *Science*, 2018, **362**(6416), 829–834, DOI: [10.1126/science.aau0976](https://doi.org/10.1126/science.aau0976).
- 97 D. S. Chorev, H. Tang, S. L. Rouse, J. R. Bolla, A. von Kügelgen, L. A. Baker, D. Wu, J. Gault, K. Grünwald, T. A. M. Bharat, S. J. Matthews and C. V. Robinson, The use of sonicated lipid vesicles for mass spectrometry of membrane protein complexes, *Nat. Protoc.*, 2020, **15**(5), 1690–1706, DOI: [10.1038/s41596-020-0303-y](https://doi.org/10.1038/s41596-020-0303-y).
- 98 W. Sakamoto, N. Azegami, T. Konuma and S. Akashi, Single-Cell Native Mass Spectrometry of Human Erythrocytes, *Anal. Chem.*, 2021, **93**(17), 6583–6588, DOI: [10.1021/acs.analchem.1c00588](https://doi.org/10.1021/acs.analchem.1c00588).
- 99 M. S. Fischer, H. T. Rogers, E. A. Chapman, S. Jin and Y. Ge, Native Top-Down Proteomics of Endogenous Protein Complexes Enabled by Online Two-Dimensional Liquid Chromatography, *Anal. Chem.*, 2025, **97**(25), 13663–13671, DOI: [10.1021/acs.analchem.5c02341](https://doi.org/10.1021/acs.analchem.5c02341).
- 100 Z. Hou, M. Luan, L. Zhan, X. Wang, S. Yuan, K. Cao, Y. Sheng, H. Yin, Y. Liu and G. Huang, Native Mass Spectrometry for Peptide–Metal Interaction in Picoliter Cell Lysate, *Anal. Chem.*, 2022, **94**(40), 13829–13833, DOI: [10.1021/acs.analchem.2c02390](https://doi.org/10.1021/acs.analchem.2c02390).
- 101 S. Chen, T. Getter, D. Salom, D. Wu, D. Quetschlich, D. S. Chorev, K. Palczewski and C. V. Robinson, Capturing a rhodopsin receptor signalling cascade across a native membrane, *Nature*, 2022, **604**(7905), 384–390, DOI: [10.1038/s41586-022-04547-x](https://doi.org/10.1038/s41586-022-04547-x).

