Trends in Analytical Chemistry 124 (2020) 115533

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Native IM-Orbitrap MS: Resolving what was hidden

Michael L. Poltash, Jacob W. McCabe, Mehdi Shirzadeh, Arthur Laganowsky, David H. Russell*

Department of Chemistry, Texas A & M University, 3255 TAMU, College Station, TX, 77843, USA

ARTICLE INFO

Article history:
Available online 31 May 2019

Keywords:
Ion mobility-mass spectrometry
Native mass spectrometry
Intact protein complexes
Protein-ligand interactions
Fourier transform-ion mobility
High resolution mass spectrometry
Periodic focusing drift tube ion mobility
Orbitrap mass spectrometer
Ion mobility
Membrane protein

ABSTRACT

Native ion mobility-mass spectrometry (IM-MS) is an emerging biophysical approach to probe the intricate details of protein structure and function. The instrument design enables measurements of accurate first-principle determinations of rotationally-averaged ion-neutral collision cross sections coupled with high-mass, high-resolution mass measurement capabilities of Orbitrap MS. The inherent duty-cycle mismatch between drift tube IM and Orbitrap MS is alleviated by operating the drift tube in a frequency modulated mode while continuously acquiring mass spectra with the Orbitrap MS. Fourier transform of the resulting time-domain signal, i.e., ion abundances as a function of the modulation frequency, yields a frequency domain spectrum that is then converted (s^{-1} to s) to IM drift time. This multiplexed approach allows for a duty-cycle of 25% compared to <1% for traditional "pulse-and-wait" IM-ToF-MS. Improvements in mobility and mass resolution of the IM-Orbitrap allows for accurate analysis of intact protein complexes and the possibility of capturing protein dynamics.

1. Introduction

Over the past two decades ion mobility (IM)-mass spectrometry (MS) has spawned new approaches for analytical separations of small molecules [1], including challenging separations problems in the areas of lipidomics [2,3], metabolomics [4], and proteomics [5,6]. Recent improvements in sample preparation, native electrospray ionization (ESI), and IM-MS technologies are expanding the utility of gas-phase measurements for studies of solution-phase structure(s) as well as how noncovalent protein-ligand interactions affect structure-function relationships [7,8]. IM-MS provides information that is complementary to other biomolecule structural techniques, such as X-ray crystallography, nuclear magnetic resonance, and cryo-electron microscopy. Unlike traditional techniques that report the population-averaged signal, IM-MS can interrogate heterogeneous populations of conformers and probe individual structure(s) [9]. Such measurements are especially important for biophysical characterization of noncovalent protein-ligand interactions by determination of equilibrium binding constants and thermodynamics of the individual binding events [10–12]. These new biophysical capabilities mark an important step in understanding the dynamical effects of protein-ligand interactions, such as deciphering the role of protein conformation and molecular interactions with target molecules.

The rapidly growing field of native IM-MS and structural biology has been frustrated by the inadequate analytical figures-of-merit, *viz.* resolution in both ion mobility (R_{IM}) and mass ($R_{m/z}$) dimensions, of commercially available IM-MS instruments [13]. Another, often overlooked, limitation of IM-MS studies of large proteins is the requirement of calibrant protein complexes for the determination of rotationally-averaged ion-neutral collision cross sections (CCS) for commercially available IM-MS instruments, such as the Synapt G1/2 [14,15]. Moreover, calibrant libraries remain sparse for large proteins, protein complexes and membrane protein complexes [15–17]. In addition, the poor mass resolution has hindered the application of native IM-MS to probe interactions of intact protein complexes with small molecules, such as drugs.

An often underappreciated fact is that many commercial MS and IM-MS platforms were developed for the "omics" era where the key analytical figures-of-merit are high throughput, dynamic range, and limits-of-detection [18]. However, in most cases instrumentation developed for "omic" applications are not optimal for native IM-MS studies [19]. This includes the ability to retain solution-phase conformations during the transition from solution to the gas phase that represents a requisite of native IM-MS [20]. Thus, it is imperative that ESI conditions as well as IM-MS instrument operating parameters be optimized for preserving both non-

* Corresponding author.
E-mail address: russell@chem.tamu.edu (D.H. Russell).

https://doi.org/10.1016/j.trac.2019.05.035
0165-9936/

covalent interactions and solution-phase conformations. The use of static spray, nanoflow ESI (nano-ESI) achieved by small bore capillaries (ranging from a few 100 nm to 10 μm) provides significant improvements in native MS by allowing for the analyses of proteins at lower concentrations (high nM to low μM) and mass spectra that contain fewer background ions [21,22]. Additionally, nano-ESI accelerates droplet evaporation thereby allowing for more efficient desolvation of protein complexes [23]. Taken together, the quality of results from native IM-MS studies require careful attention to sample preparation, optimized ESI conditions and proper tuning of the IM-MS.

2. Discussion

2.1. Limitations of ion mobility and mass resolution

As noted above, native IM-MS has challenged the analytical figures-of-merit deemed suitable for traditional "omics" studies. The characteristics for first-generation (Synapt G1) IM-MS and next-generation drift tube (DT)-IM-MS instruments (IM-Exactive Plus EMR Orbitrap) are compared in Fig. 1. R_{IM} and $R_{m/z}$ are defined (Fig. 1A) in terms of peak width (full width at half maximum, FWHM), but a more important consideration is resolving power (RP_{IM}), i.e., CCS/ΔCCS, where ΔCCS corresponds to the difference in CCS of two closely spaced peaks of two separable conformers. For example, a RP_{IM} of ≥10 is needed to resolve ΔCCS of 1000 Å^2 for an ion having a CCS of 10,000 Å^2, whereas a RP_{IM} of 100 can resolve ΔCCS as small as 100 Å^2. Similar observations can be made for $\Delta m/z$ by comparison of the mass resolution for the Synapt G1 travelling wave (TW) IMS and the Orbitrap Exactive Plus EMR instruments (Fig. 1C). For example, resolving individual lipid bound states of the 127 kDa trimeric ammonia transport channel (AmtB), an integral membrane protein, mixed with three different lipids represents a modern challenge [24]. Signals for different lipid bound states to AmtB are not resolved by the G1 mass analyzer, whereas these same signals are baseline resolved in the mass spectrum acquired using the Orbitrap mass analyzer. The various bound states are masked by poor $R_{m/z}$ of the G1 mass analyzer that impedes the ability to interrogate complex lipid binding to membrane protein complexes [24]. However, it is worth noting that the commercial Orbitrap platform has obligatory data pre-processing that is partially responsible for the increased m/z resolution [25].

Although the $RP_{m/z}$ of high magnetic field Fourier transform ion cyclotron resonance (FTICR) instruments exceeds that of the Orbitrap, the analysis of intact protein complexes is limited [26] and coupling of DT-IMS to FTICR instruments is challenging owing to the duty-cycle mismatch. In fact to our knowledge the only attempt to develop DT-IM using FT-ICR utilized a design that is not compatible with native MS studies [27,28]. Several groups have made considerable progress in this area using trapped ion mobility spectrometers (TIMS), and they have also made considerable progress in determinations of CCS for soluble proteins [29,30].

2.2. IMS analyzers for large biomolecules

There are a number of IMS techniques capable of analyzing large biomolecules, *viz.* DT-IMS, TWIMS, TIMS, differential ion mobility spectrometry (DIMS). Of these techniques DT-IMS currently meets the necessary criteria for native IM-MS studies of protein complexes, especially for membrane proteins and their complexes. The main requisites for native IM-MS are the following:

(i) ESI conditions that are optimized for retaining solution-phase structure during the ion's transition from solution to the gas phase. For the analysis of membrane proteins, they are often encapsulated within detergent micelles and the

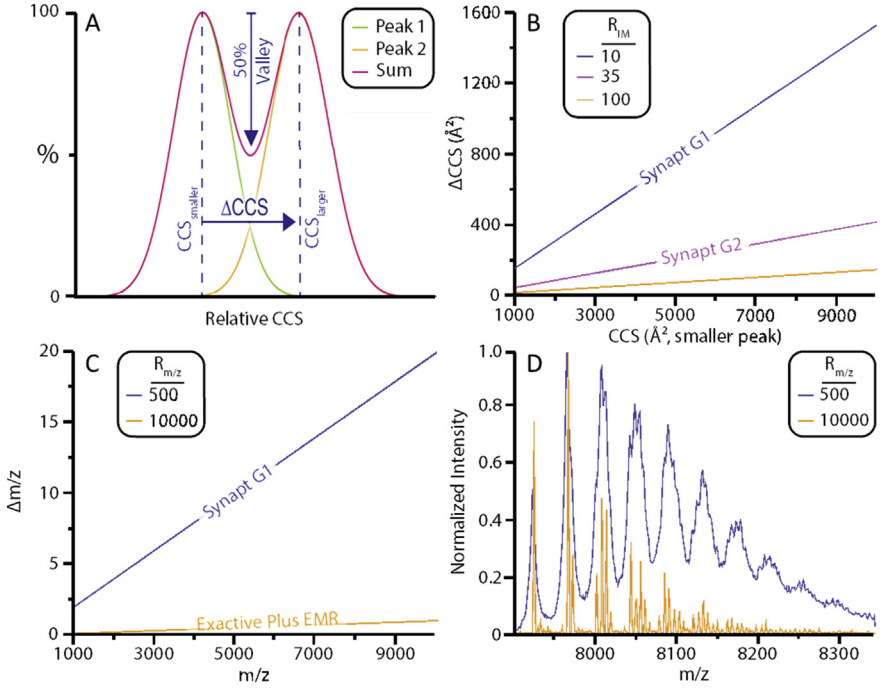

Fig. 1. Comparisons of R_{IM} and $R_{m/z}$ for the Synapt G1 and Exactive Plus EMR Orbitrap for the analysis of large biomolecules. (A) Defines R_{IM} in terms of CCS/ΔCCS with a 50% valley between peaks and similarly for $R_{m/z}$ where $m/z/\Delta m/z$. (B) and (C) contain plots of resolving power (RP) for both R_{IM} and $R_{m/z}$, respectively. Improved resolution has much more dramatic differences for larger CCS and higher m/z values clearly illustrating the need for increased resolution for large biomolecules. Any point one or above these trend lines indicates combinations that will be resolved. (D) Experimental data of a 127 kDa trimeric membrane protein AmtB mixed with three heterolipids (POPG, POPC, and TMCDL). The ToF $R_{m/z}$ is ~500 at m/z 7900 as compared to ~10,000 at m/z 7900 for the Orbitrap [24].

detergents must be stripped prior to IMS and MS analysis [31]. Native IM-MS studies using a low R_{IM} instrument has demonstrated that after detergent removal the membrane protein retain "native-like" structure with measured CCS in agreement with those calculated from atomic structures [17]. However, higher R_{IM} instrumentation is needed to elucidate subtle changes in protein structure, either as a result of activation or by changes in the local environment.

(ii) Determination of accurate CCS for large biomolecules must be determined by first-principles using the Mason-Schamp equation [32]:

$$\Omega = \left(\frac{3ze}{16N_0}\right)\left(\frac{2\pi}{\mu k_b T}\right)^{1/2}\left(\frac{t_d E_z}{L}\right)\left(\frac{760}{P}\right)\left(\frac{T}{273.15}\right)$$

Where Ω is CCS, z is the charge of the ion, e is the elementary charge, N_0 is the number density of the drift gas, μ is the reduced mass of the ion and drift gas, k_b is the Boltzmann constant, T is temperature in Kelvin, t_d is the drift time of the ion, E_z is the applied electric field, P is pressure in Torr, and L is the length of the drift tube.

The use of internal calibrations for determining CCS of proteins/protein complexes is limited by the number of accurate protein CCS determined using first-principles [16], and even fewer for membrane protein complexes [15,17]. Importantly, the majority of CCS determined using first-principles used an ion mobility device with low R_{IM} (~25) [16].

(iii) IMS must maintain excellent ion transmission of large biomolecules. Native IM-MS often requires low sample concentrations and volumes generating lower signals than smaller biomolecules necessitating more sensitive instrumentation. Travelling wave and periodic focusing (PF) described below efficiently transmits large biomolecules for such measurements.

The DT used for this instrument, a PF-DT, was first introduced in 2004 [33], to provide radial confinement of ions thereby minimizing ion losses as they traverse the DT (Fig. 2B) [34–36]. Recent studies of SIMION trajectories also revealed that ion transmission for the PF-DT increases with increasing ion charge as illustrated in Fig. 2C revealing that PF-DT is optimized for studies of large, multiply-charged ions [37]. Importantly, first-principles CCS determination by the Mason-Schamp equation is maintained in PF-DT by incorporating an α-factor to account for the increased path length that arises because the ions trajectories are modulated by the non-linear E-fields. It is important to note, however, that the α–factor approaches unity as the charge on the ion increases [38].

Ultimately, a high-resolution IM-MS platform engineered for studies of intact protein complexes was constructed by coupling a homebuilt PF-DT-IM to an Orbitrap mass analyzer (see Fig. 2A) [39]. Briefly, ions generated by a static nano-ESI source pass through a heated capillary where excess solvent can evaporate prior to entering an RF ion funnel that focuses ions prior to entering the PF-DT-IM analyzer. Ions exiting the mobility PF-DT enter an octupole ion guide en route to the HCD cell of the Orbitrap where they are subsequently transferred to the C-Trap, then injected into the Orbitrap for mass analysis.

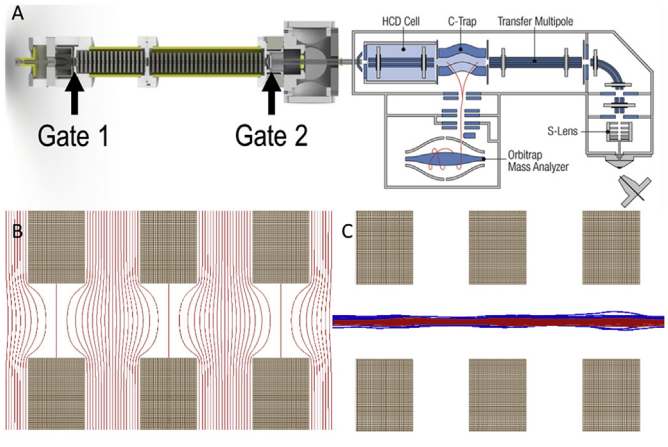

Fig. 2. (A) Solidworks rendering of the IM-Orbitrap platform utilizing a nano-ESI source, heated capillary vacuum interface, RF ion funnel, PF DT, octupole ion guide, and an Orbitrap mass analyzer. Dual gates are required to overcome the inherent duty cycle mismatch between DT IMS separation and Orbitrap mass analysis. A complete instrument description is contained in Ref. [39]. (B) The equipotential lines derived from a PF DT electrode design. As ions traverse electrodes, they experience a distant-dependent effective potential giving rise to periodic focusing between each electrode. (C) Ion trajectory simulations of groups of ubiquitin (8.7 kDa, 5+, blue) and AmtB (126 kDa, 15+, red) in a PF DT. Note that the larger AmtB protein complex has narrower radial distributions. This characteristic makes PF DT excellent for its use in analyzing large biomolecules.

2.3. Overcoming the duty-cycle mismatch for DT-IMS and Orbitrap MS

Throughout the evolutionary development of IM-MS the "pulse-and-wait" data acquisition scheme has been widely used, especially for IM-TOF MS instruments. "Pulse-and-wait" describes an acquisition method in which a small packet of ions is pulsed into the DTIMS for temporal separation and the ions are detected by using high-speed mass analyzers, i.e. a ToF. As early as the 1970s Karasek recognized limits associated with operating IMS instruments in the "pulse-and-wait" mode. To circumvent this problem, they developed the first dual-gate IMS techniques as a means to monitor analyte specific ion arrival times [40–42]. In fact, the dual gate mode is analogous to selected-ion monitoring MS analysis [43]. Hill et al. later implemented a similar approach whereby the dual gate is employed for achieving frequency modulated DT-IM operation, i.e., the potential applied to IM entrance (Gate 1) and exit (Gate 2) aperture plates are synchronously pulsed with a digital waveform [44,45]. There are two major advantages for frequency modulated DT-IMS: (i) the duty-cycle using frequency modulated dual gate mode is 25% comparted to 1% for "pulse-and-wait" devices, and (ii) Fourier transform of the frequency modulated signal, i.e., ion abundances detected by the Orbitrap as a function of the modulation frequency, yields a frequency domain spectrum that is then converted to ion mobility arrival-time distributions (see Fig. 3).

2.4. Preliminary studies of protein complexes and ligand binding

The performance of the native ESI-FT-IM-Orbitrap instrument was initially evaluated using small soluble proteins (ubiquitin, cytochrome C, and lysozyme) as well as several relatively small protein complexes (streptavidin-biotin complex, GlnK-ADP, transthyretin-thyroxine, and transthyretin-Zn(II)) [39]. Here, we revisit the latter two examples in order to highlight two very important features of the instrument: (i) acquisition of ion mobility data for both apo and holo-forms of protein complexes, and (ii) the importance of high resolving power for mass analysis for studies of such complexes. Fig. 4A illustrates the importance of $R_{m/z}$ to distinguish the tetrameric complex from its nucleotide bound forms. Importantly, Orbitraps possess the $RP_{m/z}$ to resolve not only ADP binding, but also individually bound sodium adducts, a phenomenon that is generally not illuminated by instruments with lower $RP_{m/z}$. Notably, the resolution of these ADP and sodiated species enables more accurate CCS determinations of defined

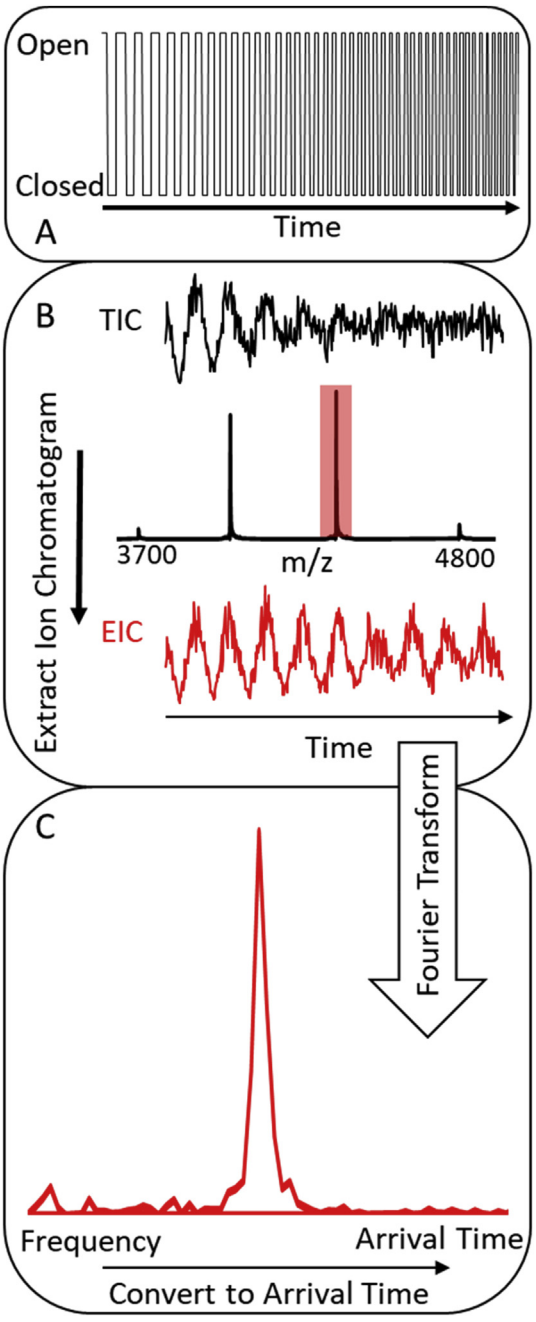

Fig. 3. (A) A linear chirp digital wave form (radio frequency) signal applied to Gate 1 and Gate 2 is scanned across a range of frequencies that correspond to the frequencies (ATD^{-1}) of the analyte ions of interest. The open/closed times of this signal are equal, which provides a 50% duty-cycle at each gate, thus the overall duty-cycle is 25%. (B) The resultant total ion chromatograms (TIC) are obtained by continuously acquiring the mass spectrum from which the extracted ion chromatogram (EIC) can be extracted over the *m/z* range scanned. (C) IM profiles can be obtained from a Fourier transform of the EIC where the resultant frequency is converted to arrival time. An exemplative spectrum of streptavidin (~53 kDa) is shown to illustrate FTIMS deconvolution.

chemical compositions whereas lower $R_{m/z}$ instruments would report on the average (Fig. 4B).

The data for transthyretin (TTR) in complex with thyroxine (T_4) and Zn(II) further illustrates the importance of high resolution IM-MS instrumentation (Fig. 4D–G). The high-resolution IM-Orbitrap MS was able to baseline resolve TTR-T_4 interactions for the first time and also provides structural insight into this molecular interaction. That is, a reduction in peak width of the protein when bound to T_4, showing greater structural homogeneity is revealed by the IMS data. Surprisingly, the improved $R_{m/z}$ revealed a phenomenon that has not been reported by IM-MS measurements. Zn(II) bound to the protein was hidden by poor $R_{m/z}$, and therefore had been completely ignored in previous TTR studies using IM-MS [50]. The TTR-Zn(II) complex, however, shows a notable increase in IMS peak width, a phenomenon that is opposite of the structural effects seen be TTR-T_4 complexes. These high-resolution IM-Orbitrap MS measurements illustrate the power of data with higher precision and accuracy than ever before. Most importantly, high resolution data as acquired on our native IM-orbitrap platform will undoubtedly have important biological implications by resolving the unresolvable on low-resolution IM-MS instruments.

High-resolution IM-Orbitrap MS platforms can lend insight into systems that have thus far been difficult or impossible to explore. The newly afforded resolution is set to tease out structural changes associated with post-translational modifications, ligand binding, metal binding, and even drug binding, leading to greater insight into protein structure-function relationship. The advancements in resolution are now challenging the biological questions that can be addressed, including those that remain intractable using other biophysical techniques.

2.5. Future challenges: sample preparation, greater instrument versatility

Here, we have described an instrument that utilizes a number of cutting-edge technologies to improve the measurements made using IM-MS. However, the instrument performance for intact protein complexes is largely set by the quality of the biological samples. For example, small adducts can easily complicate ion mobility and mass spectra owing to overlapping peaks, peak broadening, and overall chemical heterogeneity. This in turn would result in mass spectra that are below the analytical-figures-of-merit of the instrument. While such phenomena are often hard to decipher with poor RP, these issues in sample preparation are now amplified by high RP instruments. Alarmingly, these small perturbations can have impactful, deleterious effects when exploring protein structure or significant biological consequences. Methods for improving sample purity and homogeneity are critical when it comes to utilizing the technology available today. In fact, high $R_{m/z}$ afforded by these platforms can be used to gain insight into unknown chemical species that are present in biological samples. Such information can then lead to improved purification and protein expression workflows. It is clear that IM-MS can significantly improve not only final biophysical analyses, but also improve the means by which proteins are produced. For example, in Fig. 4, TTR was observed in the presence of Zn for the first time. With this new information, we can better understand the role that Zn(II) plays in altering protein structure, but we can also develop efficient purification methods for metal removal, such as treating the sample with metal chelators [51]. Improved protein purity lends itself to more accurate and reproducible results with fewer independent variables but substantially raises the bar for protein standards.

Moreover, improved R_{IM} and $R_{m/z}$ can facilitate analysis of new proteins and the observation of new phenomena that have not yet been revealed by IM-MS. For example, Fig. 5 illustrates three membrane protein channels, LeuT [52,53], Glt_{PH} [54,55], and Kir2.2 [56]. For these structures, two crystallographic conformations are overlaid along with their root mean square deviation (RMSD). Interestingly, the minimum required resolution to observe these structural changes, for example upon channel gating for Kir2.2 [57], is not correlated with RMSD. Disappointingly, commercial IM-MS instruments are currently unable to resolve such differences.

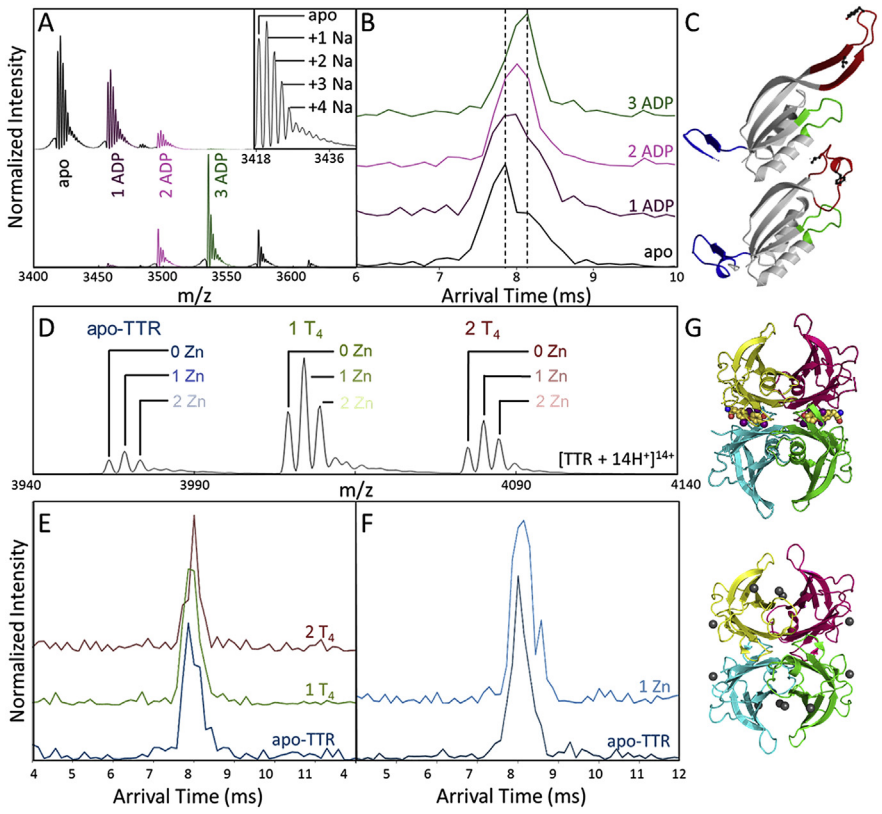

Fig. 4. (A) Mass spectrum showing the region of the $[M + 12H^+]^{12+}$ trimeric GlnK complex and the product ions formed upon binding of ADP. (Inset) Magnified mass spectrum of the "apo" peak showing up to ten Na^+ adducts on GlnK. (B) The extracted ATDs of GlnK-ADP complexes excluding any sodiated ions. (C) XRD structures of GlnK (Top: PDB 1QY7 [46], Bottom: PDB 1HWU [47]) showing the two conformers observed by IMS denoting an open (top) and closed (bottom) "T-loop" upon the binding of ADP. (D) Partial mass spectrum showing the region of the $[M + 14H^+]^{14+}$ TTR complex and the product ions formed upon binding two T_4 and Zn(II). A resolving power ($RP_{m/z}$; FWHM) of 840 is required to separate the apo and Zn-containing ions. (E) The extracted ATDs of TTR containing one and two T_4's. (F) The extracted ATDs of TTR bound to Zn(II). (G) XRD structures of TTR bound to (Top: PDB 2ROX [48]) T_4 and TTR bound to (Bottom: PDB 3GRG [49]) Zn(II).

These examples highlight the necessity for high-resolution IM measurement capabilities.

Several challenges still limit the capabilities of native IM-MS, and many new methods were not incorporated into the platform described herein. Many tools can further improve the utility of this instrument such as the inclusion of novel dissociation techniques, especially surface-induced dissociation for studies of the topology of protein complexes [58,59], ultraviolet photodissociation [60], and electron transfer dissociation [61]. Moreover, the addition of mass filters and the ability to mobility select ions could provide even greater biophysical analyses.

3. Conclusions

The development of a native-IM-Orbitrap MS has been made possible by the coalescence of many different methods developed over the last few decades coming together into a single platform. Improved ionization techniques through static spray nano-ESI has enabled the analyses of large biomolecules with retention of native structure and noncovalent interactions. Periodic focusing DT afford high R_{IM} needed for the analysis of protein complexes while providing excellent ion transmission. Orbitrap MS provides the necessary improvements in $R_{m/z}$ and *m/z* detection range to open the door for complex IM-MS measurements. Multiplexing techniques, specifically FT-IM, enable the technique to remain highly sensitive with reasonable acquisition times for biophysical analyses. Lastly, improvements in sample preparation and purification lend insights into structural biology that have never been possible before. Continued

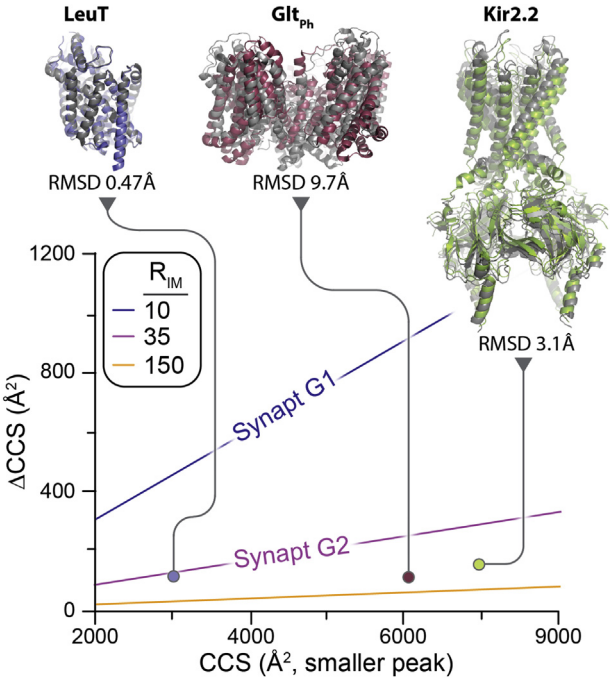

Fig. 5. Plot of the minimum ΔCCS from the smaller peak yielding resolved peaks given a defined mobility resolving power. Shown are inward-outward and open-to-out states of LeuT [52,53], outward- and inward-facing states of Glt_{PH} [54,55], and closed and open states of Kir2.2 [56,57].

improvements in both instrumentation and biological sample preparation will only continue to drive the technology forward.

Acknowledgements

The authors wish to thank Dr. Brian Clowers and Dr. Alexander Makarov for their helpful discussion regarding this work, as well as Greg Matthijetz and Will Seward for their help with electronics and machining, respectively. This work was supported by the National Science Foundation (CHE-1707675 (DHR)), National Institutes of Health (DP2GM123486 (AL), R01GM121751 (DHR and AL), P41GM128577 (DHR)), and endowment funds provided by the MDS Sciex Professorship (DHR).

References

[1] C. Lapthorn, F. Pullen, B.Z. Chowdhry, Mass Spectrom. Rev. 32 (2013) 43. https://doi.org/10.1002/mas.21349.
[2] G. Paglia, P. Angel, J.P. Williams, K. Richardson, H.J. Olivos, J.W. Thompson, L. Menikarachchi, S. Lai, C. Walsh, A. Moseley, R.S. Plumb, D.F. Grant, B.O. Palsson, J. Langridge, S. Geromanos, G. Astarita, Anal. Chem. 87 (2015) 1137. https://doi.org/10.1021/ac503715v.
[3] J.E. Kyle, X. Zhang, K.K. Weitz, M.E. Monroe, Y.M. Ibrahim, R.J. Moore, J. Cha, X. Sun, E.S. Lovelace, J. Wagoner, S.J. Polyak, T.O. Metz, S.K. Dey, R.D. Smith, K.E. Burnum-Johnson, E.S. Baker, Analyst 141 (2016) 1649. https://doi.org/10.1039/C5AN02062J.
[4] J.C. May, C.B. Morris, J.A. McLean, Anal. Chem. 89 (2017) 1032. https://doi.org/10.1021/acs.analchem.6b04905.
[5] J. McLean, B.T. Ruotolo, K. Gillig, D.H. Russell, Int. J. Mass Spectrom. 240 (2005) 301. https://doi.org/10.1016/j.ijms.2004.10.003.
[6] Y. Zhong, S.-J. Hyung, B.T. Ruotolo, Expert Rev. Proteom 9 (2012) 47. https://doi.org/10.1586/epr.11.75.
[7] A.J.R. Heck, Nat. Methods 5 (2008) 927. https://doi.org/10.1038/nmeth.1265.
[8] A.C. Leney, A.J.R. Heck, J. Am. Soc. Mass Spectrom. 28 (2017) 5. https://doi.org/10.1007/s13361-016-1545-3.
[9] L. M Young, J. Saunders, R. Mahood, C. H Revill, R.J. Foster, L.-H. Tu, D. P Raleigh, S. Radford, A. Ashcroft, Nat. Chem. 7 (2015) 73. https://doi.org/10.1038/nchem.2129.
[10] X. Cong, Y. Liu, W. Liu, X. Liang, D.H. Russell, A. Laganowsky, J. Am. Chem. Soc. 138 (2016) 4346. https://doi.org/10.1021/jacs.6b01771.
[11] J.W. Patrick, C.D. Boone, W. Liu, G.M. Conover, Y. Liu, X. Cong, A. Laganowsky, Proc. Natl. Acad. Sci. U.S.A. (2018).
[12] R. Daneshfar, E.N. Kitova, J.S. Klassen, J. Am. Chem. Soc. 126 (2004) 4786. https://doi.org/10.1021/ja0316972.
[13] D.E. Clemmer, D.H. Russell, E.R. Williams, Accounts Chem. Res. 50 (2017) 556. https://doi.org/10.1021/acs.accounts.6b00548.
[14] B.T. Ruotolo, J.L. Benesch, A.M. Sandercock, S.J. Hyung, C.V. Robinson, Nat. Protoc. 3 (2008) 1139. https://doi.org/10.1038/nprot.2008.78.
[15] T.M. Allison, M. Landreh, J.L.P. Benesch, C.V. Robinson, Anal. Chem. 88 (2016) 5879. https://doi.org/10.1021/acs.analchem.6b00691.
[16] M.F. Bush, Z. Hall, K. Giles, J. Hoyes, C.V. Robinson, B.T. Ruotolo, Anal. Chem. 82 (2010) 9557. https://doi.org/10.1021/ac1022953.
[17] A. Laganowsky, E. Reading, T.M. Allison, M.B. Ulmschneider, M.T. Degiacomi, A.J. Baldwin, C.V. Robinson, Nature 510 (2014) 172. https://doi.org/10.1038/nature13419.
[18] K. Giles, S.D. Pringle, K.R. Worthington, D. Little, J.L. Wildgoose, R.H. Bateman, Rapid Commun. Mass Spectrom. 18 (2004) 2401. https://doi.org/10.1002/rcm.1641.
[19] S.-H. Chen, D.H. Russell, J. Am. Soc. Mass Spectrom. 26 (2015) 1433. https://doi.org/10.1007/s13361-015-1191-1.
[20] J.A. Loo, D.E. DeJohn, P. Du, T.I. Stevenson, R.R. Ogorzalek Loo, Med. Res. Rev. 19 (1999) 307. https://doi.org/10.1002/(SICI)1098-1128(199907)19:4<307. AID-MED4>3.0.CO;2-2.
[21] D.N. Mortensen, E.R. Williams, Anal. Chem. 87 (2015) 1281. https://doi.org/10.1021/ac503981c.
[22] D.N. Mortensen, E.R. Williams, J. Am. Chem. Soc. 138 (2016) 3453. https://doi.org/10.1021/jacs.5b13081.
[23] Z. Xia, E.R. Williams, Analyst 144 (2019) 237. https://doi.org/10.1039/C8AN01824C.
[24] M.L. Poltash, J.W. McCabe, J.W. Patrick, A. Laganowsky, D.H. Russell, J. Am. Soc. Mass Spectrom. (2018). https://doi.org/10.1007/s13361-018-1976-0.
[25] O. Lange, E. Damoc, A. Wieghaus, A. Makarov, Int. J. Mass Spectrom. 369 (2014) 16. https://doi.org/10.1016/j.ijms.2014.05.019.
[26] J.L. Lippens, M. Nshanian, C. Spahr, P.F. Egea, J.A. Loo, I.D.G. Campuzano, J. Am. Soc. Mass Spectrom. 29 (2018) 183. https://doi.org/10.1007/s13361-017-1799-4.
[27] B.K. Bluhm, K.J. Gillig, D.H. Russell, Rev. Sci. Instrum. 71 (2000) 4078. https://doi.org/10.1063/1.1288235.
[28] B.K. Bluhm, S.W. North, D.H. Russell, J. Chem. Phys. 114 (2001) 1709. https://doi.org/10.1063/1.1334896.
[29] M.E. Ridgeway, M. Lubeck, J. Jordens, M. Mann, M.A. Park, Int. J. Mass Spectrom. 425 (2018) 22. https://doi.org/10.1016/j.ijms.2018.01.006.
[30] K.J. Dit Fouque, J. Moreno, J.D. Hegemann, S. Zirah, S. Rebuffat, F. Fernandez-Lima, Anal. Chem. 90 (2018) 5139. https://doi.org/10.1021/acs.analchem.7b05230.
[31] N.P. Barrera, N. Di Bartolo, P.J. Booth, C.V. Robinson, Science 321 (2008) 243.
[32] H.E. Revercomb, E.A. Mason, Anal. Chem. 47 (1975) 970. https://doi.org/10.1021/ac60357a043.
[33] K.J. Gillig, B.T. Ruotolo, E.G. Stone, D.H. Russell, Int. J. Mass Spectrom. 239 (2004) 43. http://doi.org/10.1016/j.ijms.2004.09.005.
[34] R.C. Blase, J.A. Silveira, K.J. Gillig, C.M. Gamage, D.H. Russell, Int. J. Mass Spectrom. 301 (2011) 166. http://doi.org/10.1016/j.ijms.2010.08.016.
[35] C.M. Gamage, J.A. Silveira, R.C. Blase, D.H. Russell, Int. J. Mass Spectrom. 303 (2011) 154. http://doi.org/10.1016/j.ijms.2011.01.025.
[36] J.A. Silveira, C.M. Gamage, R.C. Blase, D.H. Russell, Int. J. Mass Spectrom. 296 (2010) 36. http://doi.org/10.1016/j.ijms.2010.07.019.
[37] D. Manura, D. Dahl, SIMION (R) 8.0 User Manual, 2008.
[38] J.A. Silveira, J. Jeon, C.M. Gamage, P.-J. Pai, K.L. Fort, D.H. Russell, Anal. Chem. 84 (2012) 2818. https://doi.org/10.1021/ac203294q.
[39] M.L. Poltash, J.W. McCabe, M. Shirzadeh, A. Laganowsky, B.H. Clowers, D.H. Russell, Anal. Chem. 90 (2018) 10472. https://doi.org/10.1021/acs.analchem.8b02463.
[40] F.W. Karasek, Anal. Chem. 43 (1971) 1982. https://doi.org/10.1021/ac60308a024.
[41] F.W. Karasek, W.D. Kilpatrick, M.J. Cohen, Anal. Chem. 43 (1971) 1441. https://doi.org/10.1021/ac60305a025.
[42] F.W. Karasek, O.S. Tatone, Anal. Chem. 44 (1972) 1758. https://doi.org/10.1021/ac60319a009.
[43] I.A. Low, R.H. Liu, S.A. Barker, F. Fish, R.L. Settine, E.G. Piotrowski, W.C. Damert, J.Y. Liu, Biomed. Mass Spectrom. 12 (1985) 633. https://doi.org/10.1002/bms.1200121102.
[44] F.J. Knorr, R.L. Eatherton, W.F. Siems, H.H. Hill, Anal. Chem. 57 (1985) 402. https://doi.org/10.1021/ac50001a018.
[45] K.A. Morrison, W.F. Siems, B.H. Clowers, Anal. Chem. 88 (2016) 3121. https://doi.org/10.1021/acs.analchem.5b04223.
[46] Y. Xu, P.D. Carr, P. Clancy, M. Garcia-Dominguez, K. Forchhammer, F. Florencio, N. Tandeau de Marsac, S.G. Vasudevan, D.L. Ollis, Acta Crystallogr. D 59 (2003) 2183. https://doi.org/10.1107/S0907444903019589.
[47] E. Machado Benelli, M. Buck, I. Polikarpov, E. Maltempi de Souza, L.M. Cruz, F.O. Pedrosa, Eur. J. Biochem. 269 (2002) 3296. https://doi.org/10.1046/j.1432-1033.2002.03011.x.
[48] A. Wojtczak, V. Cody, J.R. Luft, W. Pangborn, Acta Crystallogr. D 52 (1996) 758. https://doi.org/10.1107/S0907444996003046.
[49] L.d.C. Palmieri, L.M.T.R. Lima, J.B.B. Freire, L. Bleicher, I. Polikarpov, F.C.L. Almeida, D. Foguel, J. Biol. Chem. 285 (2010) 31731. https://doi.org/10.1074/jbc.M110.157206.
[50] S.-J. Hyung, C.V. Robinson, B.T. Ruotolo, Chem. Biol. 16 (2009) 382. https://doi.org/10.1016/j.chembiol.2009.02.008.
[51] M.L. Poltash, M. Shirzadeh, J.W. McCabe, Z. Moghadamchargari, A. Laganowsky, D.H. Russell, Chem. Commun. 55 (2019) 4091. https://doi.org/10.1039/C9CC00682F.
[52] H. Krishnamurthy, E. Gouaux, Nature 481 (2012) 469. https://doi.org/10.1038/nature10737.
[53] S.K. Singh, C.L. Piscitelli, A. Yamashita, E. Gouaux, Science 322 (2008) 1655. https://doi.org/10.1126/science.1166777.
[54] D. Yernool, O. Boudker, Y. Jin, E. Gouaux, Nature 431 (2004) 811. https://doi.org/10.1038/nature03018.
[55] N. Reyes, C. Ginter, O. Boudker, Nature 462 (2009) 880. https://doi.org/10.1038/nature08616.
[56] X. Tao, J.L. Avalos, J. Chen, R. MacKinnon, Science 326 (2009) 1668. https://doi.org/10.1126/science.1180310.
[57] S.B. Hansen, X. Tao, R. MacKinnon, Nature 477 (2011) 495. https://doi.org/10.1038/nature10370.
[58] A.S. Galhena, S. Dagan, C.M. Jones, R.L. Beardsley, V.H. Wysocki, Anal. Chem. 80 (2008) 1425. https://doi.org/10.1021/ac701782q.
[59] M. Shirzadeh, C.D. Boone, A. Laganowsky, D.H. Russell, Anal. Chem. 91 (2019) 2345. https://doi.org/10.1021/acs.analchem.8b05066.
[60] B.J. Goolsby, J.S. Brodbelt, J. Mass Spectrom. 33 (1998) 705. https://doi.org/10.1002/(SICI)1096-9888(199808)33:8<705. AID-JMS679>3.0.CO;2-2.
[61] J.E.P. Syka, J.J. Coon, M.J. Schroeder, J. Shabanowitz, D.F. Hunt, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 9528. https://doi.org/10.1073/pnas.0402700101.