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Understanding protein–drug interactions using ion mobility–mass spectrometry

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Ion mobility–mass spectrometry (IM–MS) is an important addition to the analytical toolbox for the structural evaluation of proteins, and is enhancing many areas of biophysical analysis. Disease-associated proteins, including enzymes such as protein kinases, transcription factors *exemplified by* p53, and intrinsically disordered proteins, including those prone to aggregation, are all amenable to structural analysis by IM–MS. In this review we discuss how this powerful technique can be used to understand protein conformational dynamics and aggregation pathways, and in particular, the effect that small molecules, including clinically-relevant drugs, play in these processes. We also present examples of how IM–MS can be used as a relatively rapid screening strategy to evaluate the mechanisms and conformation-driven aspects of protein: ligand interactions.

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Introduction

An important consideration during drug development is the structural and mechanistic evaluation of the protein target, ideally combined with a multi-level understanding of how conformation and biological function are modulated by ligand binding. Ion mobility–mass spectrometry (IM–MS), which separates ions in the gas-phase based on their size (mass), shape and charge [1•,2–4,5•,6,7] has emerged as an important addition to more traditional structural biology techniques such as NMR, X-ray crystallography and Cryo-electron microscopy [8] and can be readily exploited to help understand conformational dynamics of proteins and non-covalent protein complexes [9–11]. Although IM–MS is unable to reveal resolution at the atomic level, the ability to analyse heterogeneous complexes and protein–ligand interactions in their native conformations [11–18,19•] offers a competitive advantage over other structural approaches, which either 'fix' the conformation, for example, during crystal formation, or are unable to handle mixtures. Indeed, the fact that analyte mass to charge (m/z) ratio is evaluated independently of ion mobility information means that IM–MS can be used to analyse heterogeneous populations; it also provides a means of analysing protein complexes that occupy multiple conformations, whilst providing important information on the stoichiometry of non-covalent complexes. Moreover, application of IM–MS for structural interrogation is typically much faster than other approaches, and only requires picomole amounts of material for analysis. IM–MS can thus be exploited as a stand-alone tool for protein structural interrogation, with or without *in silico* molecular modelling, or to complement high-resolution information acquired by other means [20]. For example, crystallographic evaluation of proteins (with or without bound ligands), particular those with disordered regions, often results in incomplete atomic structures [21]. Combining partial structural datasets with experimentally derived CCS information can therefore be used to constrain topological models through computational approaches. Coarse-grained and homology modelling has proven useful in this regard, being applied to structural modelling of numerous multimeric protein complexes with distinct topologies [22–25].

Although originally the subject of some debate, a significant body of evidence now demonstrates that in the majority of cases, the native solution-phase structure of a protein/protein:ligand complex can be retained in the gas phase [26,27] when 'native' ESI conditions are employed and analytical parameters are carefully controlled. Once in the gas-phase, the resulting ions can be separated based on two physical properties: their differential mobility through an inert gas in a weak electric field [28], and by employing standard m/z-based separation using mass spectrometry (MS). The primary purpose of this review is to describe how IM–MS has been applied to help understand different protein–drug interactions, rather than to provide a background to the different forms of this technique. Several comprehensive reviews detailing the fundamentals of IM–MS are available [1•,2,6,29]. Of most relevance to this article are drift tube IMS

(DTIMS) [3,30], trapped ion mobility IMS (TIMS) [31] and travelling wave IMS (TWIMS) [32], all of which are available on commercial instruments and can therefore be readily employed experimentally. These are discussed briefly below.

DTIMS employs a weak homogeneous electric field to direct ions through a drift cell, where collisions between ions and an inert buffer gas (typically helium or nitrogen) delay passage through the cell, allowing the rotationally averaged collisional cross section (CCS) of different ions to be measured directly. Unlike DTIMS, which directly assesses the drift time of ions in a 'stationary' gas, in TIMS, ions are trapped in the presence of a counter-flow of gas, with ions 'eluting' selectively according to their relative mobility through the gas. TWIMS is comparable to DTIMS, but uses a stacked ring ion guide (SRIG) through which ions are propelled by means of a travelling wave DC voltage superimposed onto a radially-confining RF voltage. Ions of high mobility will spend less time in the travelling wave ion guide (TWIG) than ions of low mobility as they will be more easily transported through the gas-filled mobility cell. Calibration of the time taken for ions of known CCS to travel through the TWIG can then be used to compute CCS values of unknown analytes of similar chemical structure under the same buffer and voltage conditions [33–36]. CCS reports median protein conformation, while concurrent measurement of the CCS distributions (CCSD) can be used to help evaluate conformational flexibility. Moreover, when experimental CCS information is compared with theoretical calculations for a given geometry, possible candidate structures can be proposed, ruled out or validated. Field asymmetric IMS (FAIMS), also called differential IMS, is an alternative method for separating gaseous ions at atmospheric pressure [37,38]. However, due to the non-linear effect of the applied electric field on ion mobility and its deleterious effect on native protein conformation, FAIMS cannot be used to determine CCS, but is instead used to separate mixtures. However, as with all IM–MS instrumentation, FAIMS can be exploited to determine the dissociation constant (K_D) of protein:ligand complexes, although this type of IMS is optimal for stronger non-covalent complexes. IM–MS derived K_D values are thought to be comparable with values obtained using more traditional solution approaches, such as fluorimetry, calorimetry, thermophoresis and Surface Plasmon Resonance (SPR).

Depending on the type of IM–MS employed, structural information pertaining to protein:ligand complexes can thus be obtained, including definition of K_D for reversible binding, and providing insight into whether ligands stabilise (or destabilize) protein conformations (see Figure 1). K_D values are determined using the titration method followed by a nonlinear curve fit using Eq. (1) [39]. $I(PL)$ and $I(P)$ define the peak area of the protein:ligand complex and the unbound protein respectively; $[P]_0$ and $[L]_0$ are the original protein and ligand concentrations:

$$\frac{I(P*L)}{I(P)}=\frac{1}{2}\left(-1-\frac{[P]_0}{K_D}+\frac{[L]_0}{K_D}+\sqrt{4\frac{[L]_0}{K_D}+\left(\frac{[L]_0}{K_D}-\frac{[P]_0}{K_D}-1\right)^2}\right) \quad (1)$$

The effect of ligand binding on protein conformational stability can also be investigated using collision-induced unfolding (CIU) [40•,41,42]. Here, the intact protein or protein:ligand complex (or the remaining unbound protein) is subjected to a gradual increase in collisional activation below that required for protein or complex dissociation, thereby inducing protein unfolding. The (partially) unfolded protein states at different activation energies are then analysed by IM–MS, with the resultant 'CIU fingerprint' being used to define the partially unfolded transition states as a function of the applied energy (see Figure 1). Software packages, for example, CIUSuite, can assist with data interpretation [43] and, in the case of ORIGAMI, also automate acquisition of CIU fingerprints [44•].

As exemplars of the utility of IM–MS for structural characterisation of protein–ligand interactions, we will focus on three major classes of protein for which IM–MS has revealed important information: amyloid, intrinsically disordered proteins (IDPs) and protein kinases.

Amyloid

Protein aggregation and the formation of amyloid fibrils is thought to be a causative factor in over 50 human diseases including Alzheimer's disease [45], Parkinson's disease [46], type II diabetes [47], cardiovascular disease, and some forms of cancer [48]. IM–MS has been used to provide important insights into the self-assembly mechanisms of several distinct amyloidogenic proteins, including β2-microglobulin, Aβ40/Aβ42 and α-synuclein [49•,50], helping to understand the assembly and architecture of fibrils and associated intermediates, and the effect of small molecules on these dynamic processes (Figure 2) [51••,52–55]. Recent work by Pagel and colleagues used a combination of infrared spectroscopy with IM–MS to directly analyse the secondary structure of individual amyloid intermediates, elegantly demonstrating that small fibril-forming 6-mer peptides yield oligomers comprising an extensive β-sheet architecture [56].

By contrast to other structural techniques, IM–MS can be used to characterise the multiple individual soluble aggregate forms present during the transition from monomers to insoluble fibrils, as opposed to an 'average' of properties forming an oligomer ensemble (Figure 2). One of the