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Ion mobility-mass spectrometry of intact protein–ligand complexes for pharmaceutical drug discovery and development

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Mass spectrometry (MS) plays a number of key roles in the discovery and development phases for modern pharmaceutical compounds, ranging from the assessment of protein–ligand binding to biomarker discovery. Historically, however, MS has had a relatively limited role in the drug discovery process in comparison to high-throughput fluorescence and radiometric screens. This picture may be changing, however, as many presumptive protein targets are coupled to human disease pathways through specific protein–protein interactions and protein conformations, rather than enzyme activities. This fact will likely drive the development of high-throughput analytical tools that put a stronger emphasis on the structural information content produced in a screen. Here we summarize recent developments surrounding ion mobility-mass spectrometry (IM-MS), one such MS-based tool that is capable of rapidly measuring changes in protein structure, oligomeric state, and binding stoichiometry from complex mixtures at relatively low concentrations.

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Introduction

The process of introducing a new pharmaceutical compound as an approved therapeutic is a highly integrative endeavor, involving disciplines ranging from computer science to biology [1,2]. Following the identification of viable biological targets, the majority of which are proteins that fall into a few narrow functional classifications, the generation of small molecule drugs can be broken roughly into two equally important phases of work [3]. The first, termed 'discovery', involves the experiments needed to identify molecular scaffolds and structures that alter the function of the desired biological target and terminates with a candidate compound of promising potency. The second, generally referred to as 'development', refers to the experiments and clinical trials that seek to validate the toxicology and safety of the drug for use in patients. In both drug discovery and development, many analytical tools are utilized to assess the binding, structure, stability, and mechanism of action of potential drugs. For example, fluorescence and radiometric assays are routinely used during drug discovery to search vast compound libraries for any that bind to a target protein [4,5]. Mass spectrometry (MS) of large biomolecules is a versatile tool used within both drug discovery and development [3]. Technologies such as electrospray ionization (ESI) [6] enable MS to contribute to proteomic analyses [7], purity assessments of isolated targets, structural determinations of protein–ligand complexes [8,9], and biomarker discoveries [10,11] that link candidate molecules to critical metabolic processes in pre-clinical evaluations.

Over 20 years ago, the first evidence that intact protein–ligand interactions could be retained during ESI-MS was reported [12,13]. Following these key observations, many reports followed that illustrated the power of MS for assessing the stoichiometry and strength of small molecule binding to various protein targets [14]. Subsequent experiments expanded the role ESI-MS to include larger multiprotein targets [15], complex mixtures of small molecule binders [16,17], accurate measurements of dissociation constants [18,19], and ultimately proteome-wide information on direct protein–ligand interactions [20]. In each of these cases, the power of MS for drug discovery and development was clearly illustrated, and its complementarity with other technologies within the pharmaceutical pipeline made clear. The ability of MS to gain a large amount of both qualitative and quantitative information from complex, dynamic biological mixtures is its chief advantage over other analytical tools. When labeling chemistries [21,22], chemical cross-linking [23], hydrogen-deuterium exchange [24–26], and other technologies are combined with MS, it becomes clear that the range of this information content can include significant structural data on the protein–ligand interaction that can rapidly inform the discovery of lead compounds [5].

Ion mobility (IM) spectrometry, a gas-phase separation tool analogous to electrophoresis in solution [27], is often combined with MS to generate tandem, multi-dimensional datasets for complex peptide mixtures [28,29], tissue imaging experiments [30,31], and protein–ligand complexes of pharmaceutical interest [32,33]. IM separates gas-phase

ions based on their differential transport through an environment of inert neutrals in the presence of a relatively weak electric field [34]. Many versions of the technology exist, and in most cases precise measurements of ion transport properties can be used to generate ion size information, in the form of an orientiationally averaged ion-neutral collision cross section (CCS). While IM measurements of protein size take place in the absence of bulk water, and it is clear that surface groups likely rearrange during desolvation [35,36•], many datasets now exist that point to a clear correlation between the CCS values recorded by IM and X-ray or NMR datasets for the same proteins and complexes in solution, thus enabling the utility of IM for structural biology [15,37]. These results, combined with the recent introduction of commercially available technology [38,39] make such IM-MS experiments a potentially powerful addition to the discovery and development process for therapeutic small molecules.

Here, we attempt to summarize recent developments in the field of IM-MS that are of particular relevance for applications in the pharmaceutical sciences. This will include examples, diagrams and data from our laboratory designed to illustrate the potential capabilities of the IM-MS approach. We focus on recent advances in the field, and primarily upon the application of IM-MS to intact protein–ligand complexes. Excellent reviews that engage in broader analyses of IM-MS technology [40], its application to complex mixture analyses [41] and protein structure determination [27,32,42] are available, and we direct interested readers to these works rather than covering such topics in detail here. We conclude by exploring the limitations of the IM-MS experiments discussed in the context of drug discovery and development applications, and estimate the scope of such experiments for the future.

Paradigms for protein–ligand screening by IM-MS

Early IM data for protein ions produced by ESI indicated a strong structural dependence on ion charge [43–46]. Subsequent experiments have refined the ability of IM to separate subtly different conformational forms of proteins over a range of charge states produced during the ESI process [37,47]. Computational methods are typically used in conjunction with IM data to generate atomic models of peptide and protein structure [48], and have advanced significantly over the past few years in their ability to generate such models for larger systems [49]. Smaller protein–ligand systems can be analyzed by IM-MS deduce the binding locations for small molecules within protein targets [50••,51••,52••,53••] and, in some cases, produce atomic models of protein–ligand complexes [54,55]. Larger protein–ligand complexes are currently beyond the scope of such detailed computational methods, and instead often involves the observation of a key protein conformation shift as a function of a known binding event that can be linked directly to compound efficacy. Subsequent experiments can then be constructed to search a broader library of compounds for similar conformation shifts upon binding the same target (Figure 1). This general mode of operation is currently the most-commonly employed approach for IM-MS in the context of protein–ligand analysis and screening, as reflected in the literature surveyed here.

In addition to the above-noted charge state dependence for gas-phase protein CCS, early studies noted other critical variables that affect the gas-phase structure of desolvated protein ions [46,56]. Among these, altering the internal temperature of the ions produced had a dramatic influence on the size of the protein ion recorded by IM, primarily leading to a positive correlation between protein ion CCS and their internal temperature, with protein ions of high internal temperatures adopting large, string-like conformational states [57]. Subsequent data have extended these observations to include protein–protein [32,35,47,58] and protein–ligand [59••,60••] complexes, each of which display similar yet distinct unfolding properties upon gas-phase activation. Most contemporary experiments utilize collisional activation to initiate unfolding [47,59••,60••,61•,62•,63,64], however other activation methodologies have been shown to elicit conformational change [46,48,65–67], although to a lesser extent. Collision induced unfolding (CIU) can be used in two basic modes in the context of protein–ligand screening experiments (Figure 1). Firstly, the surviving population of the most-compact form of the protein, typically that which is most-highly correlated to its solution structure, is tracked as a function of the voltage used to accelerate ions and initiate unfolding. Differences recorded in protein–ligand complex stabilities primarily relate to the stability of the gas-phase complex, and can be compared to both solution measurements and apo-protein CIU data to provide a workable screening methodology [58,59••,61•,62•,63,68]. In addition to measuring the survival of a single conformational form of the protein–ligand complex upon activation, the unfolding pathway of the protein can be followed in detail to generate additional points of comparison between either apo-states or alternate conformational families of the protein. Since many possible tertiary structures project identical ion CCS values, the detection of subtle conformational shifts in protein–ligand complexes is often challenging for IM-MS methods. CIU fingerprints can be a useful tool in circumventing such limitations, as the unfolding intermediates accessed by proteins during CIU can be uniquely related to specific protein–ligand binding modes [58,59••,61•,62•,63].

All of the above modes of operation can be combined into metrics that define the structural stability and conformation changes that occur upon binding an efficacious molecule to a protein target, the properties for which are