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The application of ion-mobility mass spectrometry for structure/function investigation of protein complexes

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Ion-mobility mass spectrometry (IM-MS) is an approach that can provide information on the stoichiometry, composition, protein contacts and topology of protein complexes. The power of this approach lies not only in its sensitivity and speed of analysis, but also in the fact that it is a technique that can capture the repertoire of conformational states adopted by protein assemblies. Here, we describe the array of available IM-MS based tools, and demonstrate their application to the structural characterization of various protein complexes, including challenging systems as amyloid aggregates and membrane proteins. We also discuss recent studies in which IM-MS was applied towards investigations of conformational transitions and stabilization effects induced by protein interactions.

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Introduction

Proteins are inherently dynamic entities that sample multiple conformational states for their functional activities [1,2]. Therefore, a complete understanding of the structure–function relationships of proteins requires experimental methods that can capture the spread of the conformational states they adopt. However, this complexity can present a significant challenge to many of the 'classical' high-resolution structural biology tools as X-ray crystallography [3], Nuclear Magnetic Resonance (NMR) [4] and Electron Microscopy (EM) [5]. Here, we will focus on a structural technique that can capture the conformational dynamics of a system, Ion-Mobility Mass Spectrometry (IM-MS). While IM-MS does not provide atomic resolution structures, it has the advantage that co-existing populations of a given assembly can be detected within a single spectrum.

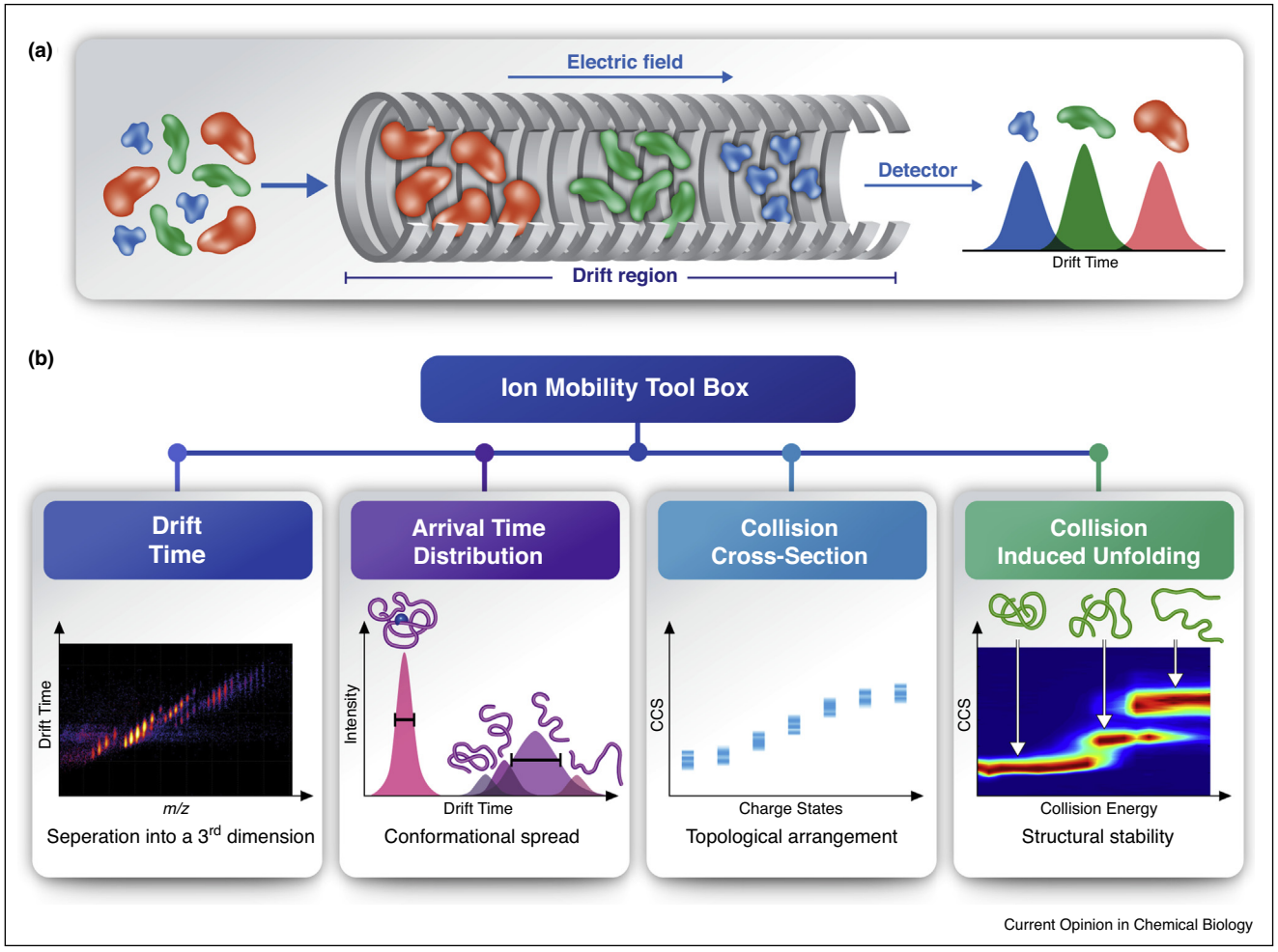
IM-MS is a method that couples MS measurements with IM separation (recent reviews include [6–13]). By means of this method, the time it takes for a protein (or its various populated structural states) to transverse a weak electrical gradient in a gas-filled chamber is measured. The drift time depends not only on the mass and charge, but also on the shape of the analyzed protein complex. Larger ions collide more frequently with the neutral gas, hindering their progress and therefore increasing their drift time relative to more compact ions [14] (Figure 1a). This offers a way to distinguish between conformational states of the same protein. As the number of collisions is proportional to the surface area of the protein, the drift time measurement can be used to determine the rotationally averaged collision cross-section (CCS) value, indicating the three-dimensional shape of the protein. For intact proteins and protein complexes introduced from non-denaturing solutions (native IM-MS), this approach provides insights into the stoichiometry, composition, connectivity, topology and conformational heterogeneity of the analyzed biomolecules.

Different methods for applying the electric field and introducing the buffer gas gave rise to various IM-MS platforms, such as drift tube (DT) [11], traveling wave (TW) [10], differential mobility [15], transversal modulation [16], overtone [17], field asymmetric [18] and trapped IM-MS [19]. While each method has proven its worth, here we will focus on the two most common types of mobility techniques DT and TW. In DT, a homogeneous, linear electric field is used, and CCS values are determined directly from the measured drift time and the experimental conditions applied [11]. In TW, on the other hand, potential waves continually propagate through the drift tube [10]. Manipulation of the travelling wave frequency and height enables ion separation. Although this method enhances the duty cycle, absolute CCS values cannot be determined, and an indirect calibration procedure is required [20,21]. To date, both DT and TW IM-MS instruments utilizing a quadrupole time-of-flight platform (QTOF) are commercially available, and the application of the technique to structural studies is rapidly increasing [22,23].

Overall, when applying IM-MS, multiple features can be extracted, enabling in-depth analysis (Figure 1b), among them:

i. *Drift Time* — Separating ions according to their drift time adds an extra dimension to conventional MS measurements, yielding a three-dimensional

Figure 1



Structural characterization of protein complexes by IM-MS. **(a)** Ion mobility is a method that measures the time it takes ions to travel through a gas-filled chamber, across which a weak electric field is applied. In the drift tube, ions experience two counter forces of electrostatic pulling through the cell and collisions with the buffer gas, which delay their movement. Ions with a larger surface area will experience more collisions with the buffer gas and as a result, will take longer to traverse the drift tube, in comparison to smaller, more compact ions of the same molecular mass and charge, which will undergo fewer collisions with the buffer gas, and hence will display greater mobility and a shorter drift time. **(b)** IM-MS measurements enable the extraction of various structural properties. For example, mobility-based separation adds a third dimension to native MS analyses, and resolves heterogeneous samples that contain closely related species. Measurements of arrival time distributions reflect the conformational range of the examined species, and can be converted to CCS values. When measured over a range of collision energies, as is characteristic of CIU protocols, CCS can reflect the stabilities and intermediate folding states of the different protein species while they undergo *in vacuo* unfolding.

spectrum containing information regarding the mass-to-charge (m/z) ratio, abundance of ions, and drift time. Consequently, the ion mobility capability not only provides structural information, but also enables to separate overlapping peaks by distributing the data into a third dimension, thereby allowing analysis of heterogeneous or polydisperse complexes of markedly similar composition.

ii. *Arrival Time Distribution* — The structural heterogeneity of proteins and complexes is reflected in the full width at half maximum of the arrival time distributions (ATDs). Sharp peaks indicate a single conformation, while broader peaks are consistent with multiple states, indicating conformational flexibility of the protein assembly in solution. Thus, the impact of protein interactions and/or substrate binding on the conformational spread of the analyzed protein species may be determined.

iii. *Collision Cross-Section* — By measuring the ATD of each charge state, it is possible to calculate, either directly (DT) or indirectly (TW), CCS values [11,20,21]. The derived CCS represents the effective area of the gas-phase ion that can interact with the drift gas, averaged over all orientations. Thus, it can