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Contemporary glycomic approaches using ion mobility–mass spectrometry

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Characterization of complex oligosaccharides has historically required extensive sample handling and separations before analysis using nuclear magnetic resonance spectroscopy and electron impact mass spectra following hydrolysis, derivatization, and gas chromatographic separation. Advances in liquid chromatography separations and tandem mass spectrometry have expanded the range of intact glycan analysis, but carbohydrate structure and conformation — integral chemical characteristics — are often difficult to assess with minimal amounts of sample in a rapid fashion. Because ion mobility spectrometry (IMS) separates analytes based upon an effective 'size-to-charge' ratio, IMS is, by extension, highly applicable to glycomics. Furthermore, the speed of IMS, its growing levels of separation efficiency, and direct compatibility with all forms of mass spectrometry, illustrates is core role in the future of glycomics efforts. This review assesses the current state of ion mobility–mass spectrometry applied to glycan, glycoprotein, and glycoconjugate analysis. Currently, assessing optimal ion polarity and adduct type for a glycan class along with the appropriate tandem mass spectrometry technique underpin many of the current glycan analysis efforts using ion mobility–mass spectrometry (IMMS). Once determined, these parameters have enabled a growing and impressive range of glycomics campaigns employing this technique. Additionally, the combination of IMS with tandem mass spectrometry, and even spectroscopic methods, further expands the dimensionality of hybrid instrumentation to provide a more comprehensive assessment of glycan structure across a wide dynamic range. Continued computational efforts to complement experimental and instrumental advancements also serve as a core component of IMMS workflows applied to glycomics and promise to maximize the information gained from mobility separations.

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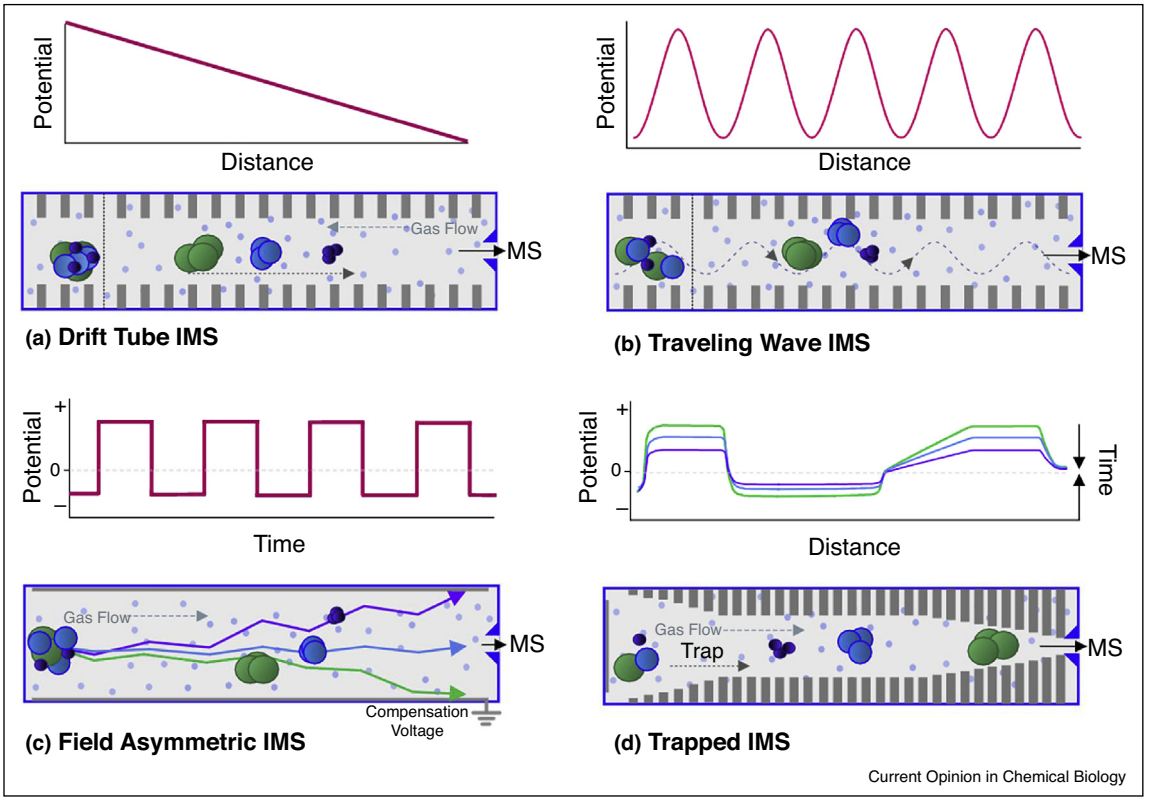
Introduction

When compared to other biopolymers such as proteins and nucleic acids, complex oligosaccharides — otherwise known as glycans — exhibit a comparatively large degree of structural variation. A rudimentary accounting of the range of carbohydrate isomers, even for a simple monosaccharide, illustrates the scope of the challenge facing the analytical research community. Broadly defined as the suite of analytical techniques capable of extracting, separating, and detecting glycans from complex media, glycomics remains central to expanding the present chemical understanding of cell signaling, differentiation, and disease progression. Enzymatic release, chemical cleavage of glycans, and multidimensional separations in both on-line and offline configurations remain at the core of modern glycomics efforts. Although the detectors may differ, the clear majority of complex oligosaccharide separations involve gas chromatography (GC) [1], liquid chromatography (LC) [2], or capillary electrophoresis (CE) [3]. However, recent advances in ion mobility spectrometry (IMS) elevate the utility of this post-ionization mode of separation as a viable complement to existing separation techniques. Compared to its liquid counterparts, gas-phase mobility experiments are often quite rapid, which eases their integration in forming hyphenated techniques using mass spectrometry (MS) [4]. Such hybrid ion mobility mass spectrometers (IMMS) are particularly relevant to glycomics efforts, where stereochemical differences are paramount, as mass spectrometers fundamentally measure m/z ratios and ion mobility provides an additional dimension explicitly related to ion size. When coupled with a detailed understanding of the constraints of ion mobility theory and innovative experimental approaches, ion mobility mass spectrometry continues to demonstrate its utility in answering fundamental questions related to the nature, composition, and range of glycans present in complex matrices. Presently, glycomics efforts using IMMS systems rely upon additional separation domains; however, as the suite of IMS techniques expands, the demands on traditional separation techniques are minimized which further speeds analysis campaigns. In addition to providing a contemporary account of glycomics advances and IMMS instrumentation, the present review also highlights the research opportunities facing the respective instrumental and glycomic research communities.

IMMS instrumentation for glycomics

Conceptually, IMMS instruments are nominally comprised of an ion source, a chamber of constant pressure and temperature for mobility separations, and a vacuum

Figure 1



For visual representation of the four primary types of IMS systems, the use of both instrumental diagrams in addition to the plots of the electric field trends are vital to understanding the distinguishing features of each system. In this figure, each general IMS configuration is illustrated as well as a corresponding electric field plot for **(a)** DT-IMS, **(b)** TWIMS, **(c)** DMS/FAIMS, and **(d)** TIMS. Notice also that the drift gas flow has a specified directionality in all variations except for the TWIMS systems.

stage housing a mass spectrometer. The exact mechanism by which gas-phase mobility of ionized glycans is exploited varies on instrument class with drift tube (DT-IMS) [4], traveling-wave (TWIMS) [5•,6,7,8•], differential/field asymmetric mobility spectrometry (DMS/FAIMS) [9•], and trapped ion mobility spectrometry (TIMS) [10•] finding the widest use for glycan and carbohydrate analysis. Figure 1 includes a brief graphical comparison of the most common ion mobility systems used for glycan analysis. Though all of these systems exploit gas-phase mobility for separation, it should be noted that other instrument configurations are possible but their modes of operation are beyond the scope of this review. Several excellent review articles summarize the operational parameters for different classes of IMS instrumentation, however, it is worth highlighting the relevant aspects of ion mobility, its relationship to kinetic theory, and the implications of the instrumental method imposes on interpreting glycomic information.

Fundamentally, gas-phase ion mobility techniques exploit the magnitude and frequency of interaction between the atomic species found within an analyte ion and surrounding neutrals [11–14]. Originally developed for monoatomic ions (e.g. Ar^+, K^+, Xe^+) in monoatomic gases (e.g. Ar and He) [15,16•], the theory describing these interactions outlines a series of reasonable assumptions that when heeded allow an ion's gas-phase mobility to be determined with a high degree of precision.

$$\Omega_0 = \frac{3}{4\mu v_d v_T N}\frac{eE}{} = \frac{3}{16}\left(\frac{2\pi}{\mu kT}\right)^{1/2}\frac{e}{KN} \quad (1)$$

μ; E is the electric field (V/cm); T is the temperature in Kelvin; N is the gas number density; $v_d v_T$ is the thermal speed; e is the ion charge; k is the Boltzmann's constant and K is ion mobility coefficient.

As a joint property of the target analyte and the colliding neutral, Ω, the ion-neutral collision cross section represents a 'size to charge ratio' that when exploited appropriately yields separations of isomers in the gas-phase, as shown in Eqn. (1) [17•]. It is for this reason that mobility measurements remain attractive to research domains,