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Ion mobility-mass spectrometry strategies for untargeted systems, synthetic, and chemical biology

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Abstract

Contemporary strategies that concentrate on only one or a handful of molecular targets limits the utility of the information gained for diagnostic and predictive purposes. Recent advances in the sensitivity, speed, and precision of measurements obtained from ion mobility coupled to mass spectrometry (IM-MS) have accelerated the utility of IM-MS in untargeted, discovery-driven studies in biology. Perhaps most evident is the impact that such wide-scale discovery capabilities have yielded in the areas of systems, synthetic, and chemical biology, where the need for comprehensive, hypothesis-driving studies from multidimensional and unbiased data is required.

Keywords

Ion mobility; Mass spectrometry; Ion mobility-mass spectrometry; Omics; Systems biology

Introduction

One of the clear paradigms from genomics and genome medicine is the potential of broad scale genome-wide association studies (GWAS) to correlate genetic alterations with phenotype. In tandem with advances in molecular characterization approaches with nuclear magnetic resonance (NMR) and mass spectrometry (MS), these broad scale concepts are more recently utilized in molecular or metabolome-wide association studies (MWAS) to correlate the dynamic metabolite complement in tissues or bodily fluids with phenotypic diversity [1,2]. The MWAS strategy is highly complementary with many systems biology strategies that entail characterizing, quantifying, and cataloging the biomolecular inventory of a sample at specific dimensions of space (e.g., cellular, tissue, or organism levels) and time (e.g., point in the life cycle, healthy vs. diseased state, longitudinal exposure). These largely hypothesis-independent data are then integrated with bioinformatics strategies to derive significant biomolecular signatures that describe the phenotype. Importantly, the

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generalized workflow of these strategies is well suited for many studies in systems, synthetic, and chemical biology in that although the specific query must be tailored to the question at hand, the prevailing analytics are one that conceptually requires the rapid generation of untargeted and rich datasets that are then interrogated to reveal those molecules most salient to the query based upon the systems-wide analysis.

Biological systems-wide analyses necessitate the acquisition of multi-dimensional datasets where individual dimensions represents molecular separations distinguishing different physical characteristics for orthogonal molecular selectivity. Oftentimes such datasets are challenging to obtain, in particular for limited or large numbers of samples, because of sacrifices in either molecular breadth, or sampling rate. While quantitation of gene transcription is dominated by array technology, many omics endeavors, such as metabolomics, proteomics, lipidomics, and glycomics are most commonly performed using MS or LC-MS [3]. In large part, this is attributed to the necessity of requiring massive numbers of experiments to understand metabolic and molecular networks under different conditions and the high throughput afforded by contemporary MS instrumentation that makes satisfying this requirement feasible [4,5]. Nevertheless, in many contemporary LC-MS or GC-MS omics studies, typically the class of molecule (e.g., proteomics, lipidomics, glycomics, etc.) is purified prior to analysis which simplifies the scope of the study, but restricts the molecular breadth in untargeted approaches. Clearly, large-scale systems-wide experiments motivate the development of measurement strategies that incorporate higher throughput, higher selectivity, are comprehensive, and require minimal sample manipulation.

Recently, approaches using gas-phase electrophoresis, namely ion mobility spectrometry integrated with mass spectrometry (IM-MS), have been demonstrated to provide additional analyte selectivity without significantly compromising the speed of MS-based measurements. In these arrangements, the IM dimension provides molecular structural information, while the MS dimension affords accurate mass information. Importantly, the correlations of molecular density and mass obtained by the combination of IM-MS also permits the integration of omics measurements (Figure 1A), where very little sample pretreatment is necessary as the data is organized into well discerned patterns corresponding to the class of molecule to which a particular signal corresponds [6,7]. The structural and mass information afforded by IM-MS has found widespread utility in two primary areas: (i) elucidation of biomolecular tertiary and quaternary structure in structural biology [8–10], and (ii) rapid characterization of complex samples on the basis of structure and mass. Recent aspects of the latter, specifically for systems-wide analyses in systems, synthetic, and chemical biology is the focus of this report.

Data dimensionality and information content

There exist a multitude of arrangements for performing IM, many of which parallel strategies for MS mass-to-charge selectivity. In the context of structural biology and untargeted analyses using structure and mass correlations, IM-MS is commonly accepted to correspond to time-dispersive IM coupled with time-of-flight MS. Although a variety of implementations of this combination have been described since the 1960s, in recent years,

the commercialization of IM-MS platforms based-on electrodynamic IM fields [11], and electrostatic IM fields [12] has fostered a considerable increase in IM-MS related publications and, congruently, advancement and expansion in IM-MS applications. This is especially true for applications centered on the analysis of complex biological samples.

One of the primary reasons time-dispersive IM-MS has been widely adopted is because the drift time across the ion mobility cell, analogous to LC retention time, can predictably be correlated to an observed collision cross section (Ω , Å²), which is a rotationally averaged apparent surface area of the ion. This is achieved through ion-neutral collisions with an inert background buffer gas as ions traverse a drift region under the influence of the defined electric field. Conformationally diffuse molecules experience a larger number of collisions relative to a conformationally dense molecule of the same mass, which results in a longer time spent in the mobility drift cell. Importantly for untargeted analyses, biomolecular classes distribute into unique regions of IM-MS separations space, or conformational space (Figure 1A). These mobility-mass correlations emerge as a result of the polymeric properties of biomolecules (*e.g.*, amino acids comprising peptides, monosaccharides forming glycans) and the prevailing intramolecular and intermolecular forces for each biomolecular class [6,7]. These correlations have been expanded to finer-grain analysis within biomolecular classes, and for predictive purposes [12,13]. For example, Figure 1A (right) is an expanded region centered on lipid species by selection of the collision cross section and *m/z* region highlighted. The fine structure shows that for a large cohort of sphingolipids and glycerophospholipids that these two major classes of lipids separate into distinct regions within the coarse lipid region. Much recent attention has focused on the fine structure information that can be obtained for a wide variety of molecular classes, including those for peptides/proteins [14–16], lipids [17–19], and carbohydrates [20,21]. A sense of reproducibility for these collision cross section measurements for a recent interlaboratory study suggest that for 125 metabolite species, the precision of the collision cross sections measured to be better than 5% for the relative standard deviation [22].

There are many recent directions being pursued to advance the structural measurements afforded by IM-MS. These include efforts to improve the instrumental figures of merit such as enhanced IM resolution [23], modular IM components for tailorable IM-MS platforms [24], and interfacing ion activation methods such as surface induced dissociation (SID) complementary to conventional CID [25]. Additional attractive strategies are actively pursued to modify the IM separation itself to provide additional structural characterization and quantification capabilities including the use of alternate IM drift gases and/or the effects of solvation on structure [26,27], energy resolved separations [28], and isotopic labeling strategies in the IM-MS [29], among others.

Unraveling untargeted data to targeted identifications in systems-wide analyses

One of the key advantages of IM-MS is the throughput of the analyses. The timescales of separation are uniquely suited for integrating LC (min separations) with IM (ms separations) with MS (us separations). However, in untargeted strategies, this results in a deluge of data. A single LC run of 10s of minutes easily results in the generation of $>10^4$ IM separations

with $>10^6$ corresponding MS spectra. Thus, strategies such as that depicted in Figure 1B have been developed, whereby multidimensional feature extraction can be performed [30,31], followed by one of several strategies for self-organization of the data, with the goal of the latter to project high-dimensional data in a visually interpretive scheme in order to highlight the molecules of interest which are then targeted for identification and validation [32–35]. Such approaches have been demonstrated in a wide array of emerging applications ranging from systems diagnosis of drug addiction [32], to wound healing [36], to cancer [37,38], to drug discovery efforts [39–41]. Based on the success of these untargeted IM-MS approaches, new frontiers in synthetic biology using 3D organotypic cell culture to emulate human constructs on a chip based format have facilitated moving the boundaries of molecular breadth and throughput required for rapid analysis in these human-on-a-chip constructs [42–44].

Concluding remarks

The molecular breadth and throughput of IM-MS separations provides a means for integrating untargeted omics measurements without sample pretreatment to isolate classes of molecules of interest. Importantly, these analytical advances permit rapid and unbiased characterization of extremely complex samples, which is opening new avenues of inquiry in biology using systems-wide analyses.

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Highlights

- Ion mobility-mass spectrometry for untargeted omics studies.
- Coarse and fine-grained IM-MS correlations reveal molecular class and sub-class.
- Molecular breadth is enhanced through integrated omics strategies.
- Bioinformatic strategies refine untargeted data for identification and validation.

Figure 1.

Untargeted workflows for ion mobility-mass spectrometry analysis. (a) In 2-dimensional IM-MS analysis, biochemical classes partition in predictable regions, while outside of these regions contain structurally unique molecules such as conjugates of multiple classes. The right panel illustrates the capability for obtaining finer structural detail within the IM-MS measurement illustrated for lipids. (b) In one example of a discovery-driven IM-MS workflow, complimentary samples are subjected to LC-IM-MS analysis and molecular features representing scalar values of retention time, mass, collision cross section, and signal intensity are subsequently extracted from the 3-dimensional datasets. These tabulated features are subjected to one of several unsupervised statistical methods which seeks to reduce the dimensionality of the dataset in order to identify the most significant molecular features. Shown are two such methods: (1) clustering maps of self-organized data which groups related features based on correlations across individual scalar components, and (2)

multivariate statistical analysis which reduces a highly-dimensional dataset into a binary comparison based on the two most descriptive components of the data. (c) From these statistical methods, the most descriptive molecular features are highlighted, and these features can be targeted for identification. Molecular identification proceeds by putative matching based on exact mass measurement which are then validated through other orthogonal pieces of information, such as retention time, cross section, and mass fragmentation data.