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Status of Mass Spectrometry-Based Proteomics and Metabolomics in Basic and Translational Research

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For this issue of *Biochemistry*, a collection of timely topics is presented under the overarching theme of modern molecular measurement in mass spectrometry (MS). This perspective discusses those contributions and offers some general thoughts on this changing field, increasingly driven by what was once considered "ultra-high" mass accuracy. Four papers form the core of the collection covering both protein and metabolite levels in the depiction of integrated "omics" applied in biological research (Figure 1). One sees evidence in these submissions of several trends and a steady shift in protein mass spectrometry toward a "top down" philosophy of molecular analysis (i.e., no degradation prior to MS) that has been used so effectively for small molecules over the past 30 years.

Basic Concepts in MS. From left to right, Figure 2a shows how the distribution of isotope peaks changes as the molecular weight increases in moving from a small molecule to a small peptide to even a small intact protein. Figure 2b shows the process of targeting and fragmenting specific molecular species by tandem mass spectrometry (MS/MS for short). The way in which MS/MS is applied to whole proteins, peptides (usually from a tryptic digest), or small molecules in complex mixtures is another main theme of this collection. MSI, or mass spectral imaging, is another significant thread through the articles. MSI seeks spatial resolution and is a distinct departure from the "grind-n-find" approach that typifies most protein and molecular biochemistry using mass spectrometry. This timely collection describes a major shift toward routine acquisition of spectra with high mass accuracy (e.g., <10 ppm), and the steadily improving sensitivity of MS (already capable of accessing picomole to attomole sample amounts).

Hundreds of "Western Blots" in <1 h. Significant scientific resources are directed at obtaining protein measurements based on antibodies. Uneven supply chains and instability of quantitation over the months and years of projects have created a need for improved stability, robustness, and multiplexing of targeted molecular species in mixtures. D. Liebler reports from the forefront of a large wave of activity in this regard, with F. White, S. Carr, and others also early to realize the limitations of stochastic data acquisition in bottom up proteomics. Therefore, developing targeted but multiplexing assays allows a new tier in assay stability and efficiency. Here a resurgence of the triple-quadrupole mass spectrometer (where much of peptide MS first began in the early 1980s) is in full swing, thus breathing new life into this venerable type of MS instrument. By highlighting key advantages of these so-called "MRM" or "SRM" type assays (e.g., targeting dozens to hundreds of peptide sequences, including those of variants and modified forms all in the same assay), the piece by Liebler and Zimmerman captures the state of affairs in grand style (DOI: 10.1021/bi400110b).

Trends in Ionization: Let the Big Molecules Fly. Despite the fact that membrane proteins are major targets for drug development, Schey, Gray, and Nicklay (DOI: 10.1021/bi301604j) point out that determination of their modifications and protein interaction partners has lagged behind the analogous studies of soluble proteins. Recent advances in the analysis of membrane protein posttranslational modification are highlighted, and the authors focus their attention on MS-based analysis of aquaporins (AQPs) as a prototypical integral membrane protein. Several new studies mapping phosphorylation, glycosylation, and fatty acid acylation on AQPs isolated from a variety of tissues and cells are discussed. Biological insights arising from representative studies offer readers context (including the structural biology of the protein) in the language of specifics for this highly modified class of proteins. Also introduced are the "bottom up" and "top down" strategies of protein analysis that are based on MS of (tryptic) peptides and whole proteins, respectively. As a segway to the next article on MS imaging, tissue distributions by imaging mass spectrometry are also highlighted in the piece.

MALDI Imaging Mass Spectrometry: Molecular Mapping *in Situ*. The notion of using a mass spectrometer as a microscope is not new. The use of it for proteomics of soft samples is. Angel and Caprioli (DOI: 10.1021/bi301519p) discuss the surging use of matrix-assisted laser desorption ionization (MALDI) imaging mass spectrometry (IMS) as a relatively new imaging modality that allows mapping of a wide range of biomolecules within a thin tissue section. The technology uses a laser beam to directly desorb and ionize molecules from discrete locations on the tissue that are subsequently recorded. IMS (or MSI is another acronym used in the field) can directly measure molecules from small metabolites to proteins *in situ*. The contributed review outlines the use of MSI for the discovery and understanding of proteins and small molecules in basic and clinical research.

Obtaining the Sum of the Parts: Small Molecule Mass Spectrometry. The piece by Brown et al. and colleagues at Vanderbilt University (like the many authors in this collection) captures the modern trends of MS and MS/MS with ≪20 ppm mass accuracy in grand fashion (DOI: 10.1021/bi400060e). With the 2002 Nobel Prize in Chemistry going to soft ionization methods for MS, there has been renewed interest in molecular ionization arcing from fundamentals to new technologies. In this spirit, Brown et al. capture the modern options for metabolomics, also known as "small molecule mass spectrometry". In this realm,

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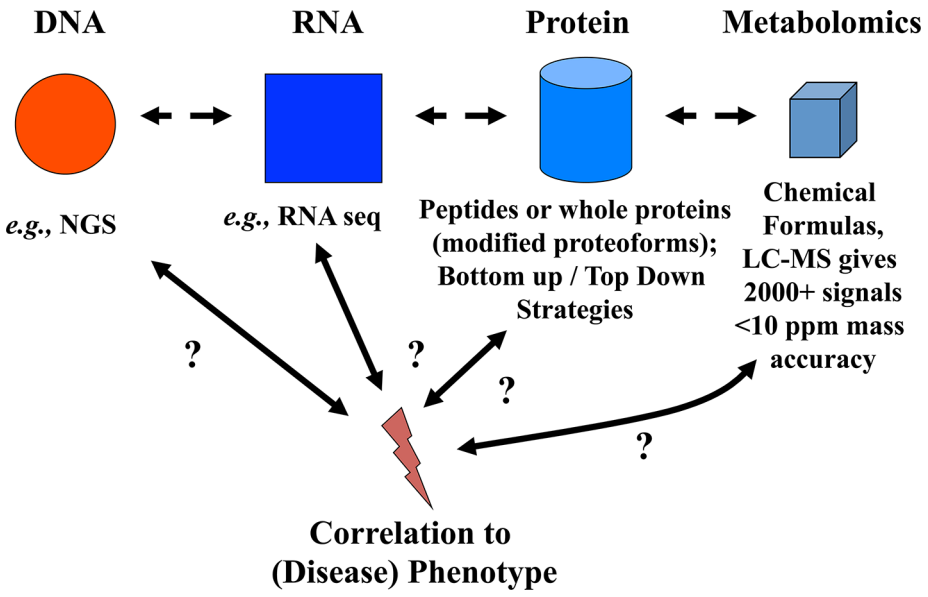

Figure 1. Depiction of applying several omics and the concept of statistical correlation between molecular signals and complex (disease) phenotypes in the cellular or organismal populations.

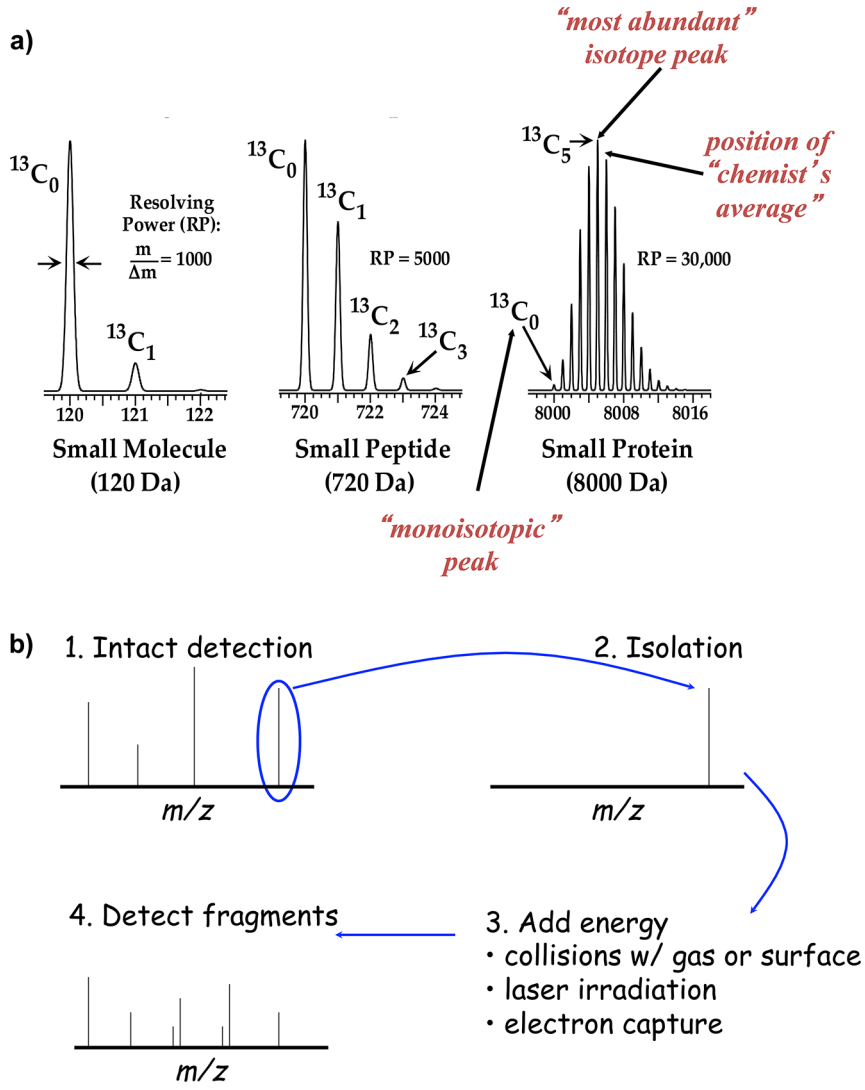

Figure 2. (a) Small and large molecule isotope distributions when resolved by mass spectrometry. The resolving power (RP) is the mass/Δmass ratio. (b) Process of MS/MS.

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the top down philosophy of molecular measurement pervades today. In fact, back in 1901 when J. J. Thomson first dispersed "positive rays" with a magnetic field, through the petroleum analysis of the 1960s, until the 1980s brought intact compound analysis to MS, today we routinely keep molecules intact for molecular weight measurement. Then, when ready, one selects a mixture component by mass (or more precisely, its m/z ratio) and fragments those precursor ions into a collection of fragment ions during MS/MS (Figure 2b). As resonates through the other articles in this collection, the top down approach is spreading nicely in the field generally.

As pointed out by Brown, metabolomics is a rapidly growing field of research used in the identification and quantification of the small molecule metabolites. This provides insights into cellular and organismal metabolism and is gaining importance in clinical medicine (e.g., localizing pharmaceutical compounds *in vivo*). New MS technologies offer extensive information about thousands of compounds below the ∼1500 Da soft limit that informally divides small molecule MS from that focused on "large molecules". A wide range of small peptides, lipids, and sugars along with their biosynthetic intermediates can be accessed, as metabolomics assumes its place in the pantheon of main omic approaches. The article also touches upon MS-based imaging for a "workup free" analysis option.

Departing Thoughts. One would be remiss in not drawing a correlation among the four contributions and describing a bit about the genesis of this collection. Over the past two decades of strong support, Vanderbilt University has built up a juggernaut in the area of metabolomics and proteomics. Through a strong institutional commitment to basic and translational research, the team there has extended the frontiers of MS technology and improved our understanding of the elusive molecular mechanisms underlying complex systems and disease. No wonder, then, why this group has reached critical mass. Please enjoy the contributions in this "one-stop shopping" for the state-of-the-art in protein and small molecule mass spectrometry.

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Notes

The authors declare no competing financial interest.