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Editorial: Emerging structural proteomics methodologies

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Editorial on the Research Topic
Emerging structural proteomics methodologies

Proteins are essential molecules for many aspects of biology, and their functions are dictated by their primary structures, post-translational modifications (PTMs), bound ligands, and three-dimensional conformations. Mass spectrometry (MS)-based proteomics has long been utilized to sequence, quantify, and annotate the PTM status of proteins. However, most proteomics applications are unable to assess the higher-order structure (HOS) of these molecules. Additionally, despite the exciting advances in biophysical structural techniques, the high throughput characterization of protein HOS is not trivial due to the complexity and heterogeneity of biological systems; thus, the development of techniques capable of rapid analysis of protein structure is vitally important. In this Research Topic, we and our contributing authors strive to detail the recent MS-based developments that go beyond traditional workflows and seek to assess protein HOS.

One of the earliest developed structural proteomics methods utilizes the spontaneous exchange of heteroatom-bound hydrogen atoms with deuterium. The rate of this hydrogen/deuterium exchange (HDX) depends on the HOS's accessibility to solvent and the strength of any non-covalent bond it is participating in. To localize the exchanged sites, the deuterated proteins are proteolyzed and then subjected to MS analysis. In the comprehensive review by Jethva and Gross, the authors describe how HDX and related label-based MS techniques can be employed to identify antigenic interfaces recognized by immunoglobulins (antibodies).

While these derivatization-driven workflows can generate peptide- and, in some cases, residue-level HOS information, they produce complex data sets and are challenging to apply to complex mixtures of proteins. In contrast, the stability of a protein's three-dimensional structure can indicate a quaternary structure and PTM status and can be interrogated at the proteome level. While multiple workflows exist, this analysis is typically achieved by measuring a protein's resistance to denaturation or proteolysis. In the contributed perspective, Kang et al. detail the breadth of these stability proteomics methods, discuss current applications (including the influence of PTMs and mutations on protein stability), and explore the future of these powerful techniques.

While many structural proteomics methods characterize protein HOS through the detection of proteolytic peptide fragments, unique structural information can be

obtained when analyzing intact proteins. For instance, the stoichiometry and significant heterogeneity arising from multiple co-occurring PTMs are only evident from the localization of these modifications on intact "proteoforms." However, direct detection of intact proteins and protein complexes poses additional challenges for sample preparation and MS instrumentation procedures, requiring development across all stages of the MS methodology.

Sample preparation is critical for a successful proteomics experiment. Takemori and Takemori summarized their innovation in the use of polyacrylamide gels for structural proteomics sample fractionation (known as PEPPI-MS). With the help of extraction enhancers, intact proteins can be efficiently extracted from gel slices under either denaturing or native conditions for downstream structural elucidation by MS. Given the wide acceptance and usage of gels in biochemistry laboratories, PEPPI-MS offers a simple and inexpensive approach with broad applications in structural proteomics.

Matrix-assisted laser desorption/ionization (MALDI)-MS is a widely accessible instrument for detecting large molecules. Through careful optimization of sample preparation, ionization, and fragmentation conditions, Sánchez et al. have developed methods for not only detecting but also successful sequencing of membrane proteins by MALDI-MS. This is significant given the challenges associated with the analysis of this important class of proteins and provides key insights into the rapid characterization of intact proteins by MALDI-MS.

Characterization of intact proteins under non-denaturing conditions (i.e., native MS) enables the detection of many types of non-covalent interactions that are important in coordinating all cellular processes. Gu et al. reviewed recent studies using MS for screening metabolite–protein interactions in drug discovery from natural product libraries, which have tremendous potential but are not well understood scientifically. Direct detection of protein–metabolite complexes in native MS significantly increases the specificity over conventional methods, especially when applying collision-induced affinity selection. This approach has been successfully applied in the development of malaria drugs and cancer drugs involving a proteolysis-targeting chimeric (PROTAC).

In addition to drug screening, the determination of binding affinities is also an essential step for the understanding of biochemical mechanisms. The conventional approach to determining binding affinities is often time and sample consuming. In this perspective, Schulte et al. overviewed native MS-based approaches to determine dissociation constants (K_D) using different ionization techniques. In particular, the recent development of quantitative laser-induced liquid bead ion desorption (qLILBID) MS for RNA as well as RNA–protein complexes was highlighted, expanding the method into new biological applications.

Beyond the *m/z* dimension, coupling of ion mobility with MS (IM/MS) provides an orthogonal dimension of structural information. In their perspective, Cropley et al. show two model cases of using tandem trapped ion mobility spectrometry (tandem TIMS) for multi-stage selection and activation of subpopulations in the overall conformational ensemble. The tandem TIMS configuration was essential to disentangle the extremely similar yet distinct structures of ubiquitin and different glycoforms of the same glycoprotein complex avidin. These examples illustrate the power of tandem TIMS for probing protein and protein complex structures and proteoforms that are practically impossible to isolate and individually characterize in solution.

We would like to acknowledge all authors for their contributions in crafting this Research Topic. We also thank all reviewers for ensuring the quality of the manuscripts. We carefully balanced the opinions from a broad range of geographic regions and career stages. We hope this Research Topic serves as a valuable resource for both newcomers and veterans in the field to learn some of the latest advances in structural proteomics methods. The minimal sample requirements, high speed, and versatility of the methods will undoubtedly offer compelling alternatives to more traditional biophysical approaches for protein characterization.

Author contributions

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Conflict of interest

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