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Proteomics beyond large-scale protein expression analysis

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Proteomics is commonly referred to as the application of high-throughput approaches to protein expression analysis. Typical results of proteomics studies are inventories of the protein content of a sample or lists of differentially expressed proteins across multiple conditions. Recently, however, an explosion of novel proteomics workflows has significantly expanded proteomics beyond the analysis of protein expression. Targeted proteomics methods, for example, enable the analysis of the fine dynamics of protein systems, such as a specific pathway or a network of interacting proteins, and the determination of protein complex stoichiometries. Structural proteomics tools allow extraction of restraints for structural modeling and identification of structurally altered proteins on a proteome-wide scale. Other variations of the proteomic workflow can be applied to the large-scale analysis of protein activity, location, degradation and turnover. These exciting developments provide new tools for multi-level 'omics' analysis and for the modeling of biological networks in the context of systems biology studies.

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Introduction

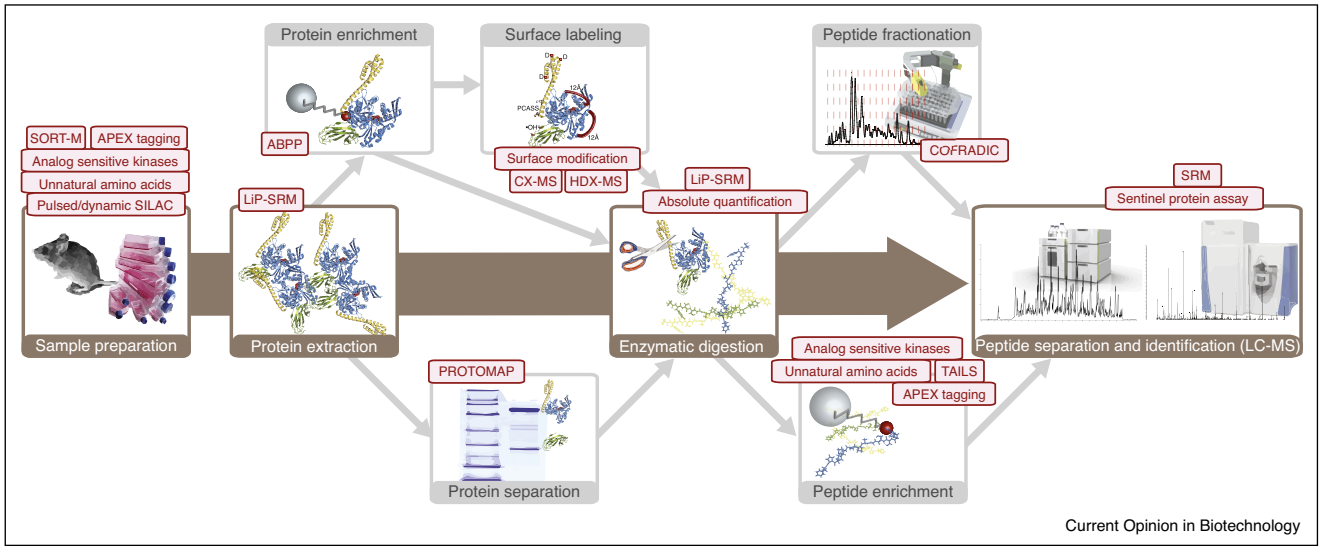
In the two decades of its existence, the field of proteomics has developed at astounding speed. Technical advances, especially in liquid chromatography (LC) and mass spectrometry (MS) have improved sensitivity, coverage, reliability, and through-put of proteomic analyses.

The oldest and most popular application of proteomics involves characterization of the protein content of a biological sample. In a shotgun proteomics workflow, this is typically achieved by tryptic digestion of a protein sample, optional fractionation of the resulting peptides and their analysis by LC–MS (Figure 1). Peptide identifications from MS spectra are validated by statistical models and translated into protein identifications using protein inference tools. Such workflows have been used to generate an inventory of the protein content of a sample, resulting in the cataloguing of the proteomes from a variety of tissues and cell types and culminated recently in the drafts of the complete human proteome inventory [1,2]. Coupled to suitable sample enrichment and fractionation steps, these strategies have also been used to catalogue various post-translational modifications (PTMs) [3] or the protein content of specific subcellular organelles [4] and to identify the interactors of proteins of interest [5].

With the development of quantitative shotgun proteomics strategies, the qualitative information contained in proteome inventories has been complemented with information on protein abundance differences in proteomes from cells and tissues at different biological states. These studies typically rely on the differential isotopic labeling of proteomes from different samples, which are then combined before MS analysis. With the improvement of LC–MS robustness and reproducibility, label-free methods involving the sequential analysis of different samples have also recently gained momentum. While comparative proteome quantification studies have been successfully applied to a wide range of questions, the endpoint of such experiments is still a long list of proteins with their relative abundance values. Such lists are used to derive biological hypotheses, which are then tested by orthogonal techniques.

Besides these classical and widespread applications, recently, alongside the technical improvements of LC–MS instrumentation, an explosion of novel peptide-based (bottom-up) proteomics strategies and applications beyond mere expression analyses have arisen. In this review, we will focus on such advances where the specificity, resolving power and sequencing capacities of LC–MS systems are cleverly exploited to expand proteomic analyses to the measurement of protein activity, structure, turnover, degradation, complex stoichiometry, localization and of the dynamics and activation state of cellular pathways (Figure 2). Furthermore, we analyze the applicability of these tools in systems biology projects by comparing performance features such as degree of multiplexing and compatibility with analysis of complex biological extracts. The examples we report were chosen to

Figure 1



Workflow of LC–MS based proteomics experiments. Steps of a typical shotgun proteomics experiment are connected by the brown arrow; proteins are extracted from a sample and enzymatically digested, followed by LC–MS analysis of the resulting peptides. Optional steps are indicated by grey arrows. Red boxes highlight the approaches that are presented in this review and are placed at the level of steps that are specifically modified or introduced by each approach. SORT-M, APEX tagging, pulsed and dynamic SILAC and approaches based on analog sensitive kinases or unnatural amino acids require genetic manipulation of the organism under study and/or metabolic incorporation of modified amino acids. LiP-SRM requires specific protein extraction conditions to preserve native structures and use of broad-specificity proteases. PROTOMAP relies on separation of proteins by SDS-PAGE. In ABPP, proteins are enriched based on their activity. CX-MS, surface modification and HDX-MS are preferably performed after purification of proteins or protein complexes. Analysis of protein complex stoichiometry by subunit absolute quantification requires addition of peptide or protein-based internal standards to the samples, before enzymatic digestion. COFRADIC relies on pre-fractionation of peptide mixtures to isolate N-terminal peptides. In the APEX tagging, analog sensitive kinase and TAILS approaches and in some approaches relying on the incorporation of unnatural amino acids, modified peptides are specifically enriched from the peptide pool. Finally, in SRM and in the sentinel protein assay, the LC–MS step is adjusted to target the analysis to peptides of interest.

give a broad overview of non-conventional proteomics applications, which is obviously far from comprehensive.

Targeted protein network analyses

Selected reaction monitoring (SRM) MS has recently emerged as an alternative, targeted approach to shotgun proteomics. It relies on the generation of specific and sensitive mass spectrometric assays for sets of proteins of interest, and their application to the quantification of the proteins across multiple samples at high throughput. SRM has been increasingly exploited for the targeted analysis of (all) the components of a protein network, such as a pathway or a system of interacting proteins. For example, SRM has been applied to study the dynamics of central carbon metabolism in yeast cells grown in different nutrients [6] and to differentially analyze the responses of proteins with high sequence overlap, such as isoenzymes or products of mutated genes [7,8]. Similar strategies were used to analyze the dynamics of phosphorylation events in the EGFR signaling network upon EGF stimulation [9], the cross-talk between 20 different PTMs of core histones [10] and the dynamics of protein interactomes [11,12]. Coupled to the use of heavy-labeled, accurately quantified internal standards, SRM-based approaches have also been used to calculate absolute copy numbers and stoichiometry of protein complex subunits, such as the 13 proteins that constitute the human adenovirus vector [13], and the 32 nucleoporins from the human nuclear pore complex [14•].

Degradomics: large-scale analysis of proteolytic events

Proteolysis plays an important role in cellular processes such as protein turnover, protein sorting, enzyme activation and apoptosis. More than 550 proteases are known in humans, but their roles and substrates are poorly characterized. As proteolytic events occur post-translationally, the activity of proteases is invisible to gene expression analysis. Proteomics techniques for identifying protease substrates on a large scale rely either on the different migration of proteins and their fragments through SDS-PAGE or on the capture of new protein N-termini or C-termini generated upon proteolysis. For example, in the PRotein TOpography and Migration Analysis Platform (PROTOMAP) approach [15], control and protease-treated samples are analyzed in different SDS-PAGE lanes, or in the same lane after differential isotope labeling of the two samples [16]. Protein bands are in-gel digested and the resulting peptides analyzed by LC–MS. The

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