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REVIEW

Advances in quantitative proteomics

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Abstract Large-scale protein quantification has become a major proteomics application in many areas of biological and medical research. During the past years, different techniques have been developed, including gel-based such as differential in-gel electrophoresis (DIGE) and liquid chromatography-based such as isotope labeling and label-free quantification. These quantitative proteomics tools hold significant promise for biomarker discovery, diagnostic and therapeutic applications. They are also important for research in functional genomics and systems biology towards basic understanding of molecular networks and pathway interactions. In this review, we summarize current technologies in quantitative proteomics and discuss recent applications of the technologies.

Keywords two dimensional gel, liquid chromatography-mass spectrometry (LC-MS), stable isotope, quantification, application

1 Introduction

Biological processes are defined by dynamic changes in protein composition, localization, modification and protein–protein interaction. To fully understand these dynamic processes, it is essential to identify and quantify the differences of protein components in a cell, tissue and organism at different metabolic states or in response to different environmental stimuli. Recently, quantitative proteomics has become a powerful tool to directly and accurately detect protein changes in different biological and medical samples. With continuous improvement in mass spectrometry, quantitative proteomics has realized analyzing complex biological and molecular processes in a single experiment (Chen and Harmon, 2006; Cravatt et al., 2007). Now more than ever, different quantitative proteomics strategies can be utilized to study complex functional properties of proteins and their involvement in particular biological processes (Ong and Mann, 2006; Thelen and Peck, 2007; Zhu et al., 2010).

To date, quantitative proteomics approaches can be classified into four general categories (Table 1). The first and most common approach is gel based quantitative proteomics, which includes two dimensional gel electrophoresis (2-DE) and differential in-gel electrophoresis (DIGE). The second approach is based on stable isotope labeling, which includes metabolic labeling and chemical labeling. The third approach is label-free quantitative proteomics, such as spectral counting and liquid chromatography peak alignment. The last approach is targeted quantitative proteomics, including multiple reaction monitoring (MRM) and protein array. The task of quantitative proteomics can be very challenging given the complexity and dynamic range of proteins in biological extracts. Rapid technical advancement in this area is expected to overcome the problems and to drive basic and applied science to a higher level. In this review, we will discuss current quantitative proteomics technologies and summarize recent advances in applications.

2 Current quantitative proteomics technologies

2.1 Gel electrophoresis: 2-DE and DIGE (minimal and saturation)

Although 2-DE has been around for decades, it is still commonly used in modern proteomics (Görg et al., 2004; Chen and Harmon 2006; Fu et al., 2008). In the first dimension, proteins are separated by isoelectric focusing (IEF) according to their different isoelectric points (pIs). In the second dimension, they are separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to their molecular weights (MWs).

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Table 1 Comparison of commonly used quantitative proteomics approaches

approach	gel-based		LC-based					protein array
	2-DE	DIGE	SILAC	cICAT	iTRAQ	spectral counting	MRM	
targeted	no	no	no	no	no	no	yes	yes
labeling	no	Cy2, Cy3 and Cy5	heavy R or K in cell cultures	light and heavy tags	4 or 8 iTRAQ tags	no	no	no
quantification	gel stain	fluorescence	MS	MS	MS/MS	MS/MS	MS/MS	fluorescence
advantages	clear spot pattern, MW and pI info, low cost	high reproducibility and sensitivity	easy sample prep, high accuracy, medium cost	samples simplified, cysteine specific	multiplex with 4/8 tags, high confident ID	No labeling, low cost	high specificity and sensitivity, low cost	clear expression pattern, high sensitivity
disadvantages	limit in reproducibility, membrane proteins and proteins with extreme pI and MW excluded	same as 2-DE, except high cost of Cy dyes	mostly applicable to cell cultures	very few peptides identified per protein, proteins without cysteines missed	not all IDed proteins quantified, expensive in large experiments	fast-scanning MS needed, not good for low abundant proteins	optimization of every transition needed, >2 peptides to be monitored.	only a few arrays available, relatively high cost

LC: liquid chromatography; 2-DE: two dimensional gel electrophoresis; DIGE: differential in-gel electrophoresis; SILAC: stable-isotope labeling with amino acids in cell culture; cICAT: cleavable isotope-coded affinity tags; iTRAQ: isobaric tags for relative and absolute quantification; MRM: multiple reaction monitoring; MS: mass spectrometry; MW: molecular weight; pI: isoelectric point.

Proteins exhibiting differences in abundance between samples are revealed by comparing patterns and densitometry intensities of spots in different gels following Coomassie Blue, silver or Sypro Ruby staining. Protein spots of interest can be excised for mass spectrometry (MS) identification. Although 2-DE is a powerful technique that can simultaneously separate thousands of protein species in complex mixtures, it shows limitation in reproducibility, dynamic range and sensitivity (Gygi et al., 2000). In addition, membrane proteins, very acidic or basic proteins, extremely large or small proteins are often excluded in the 2D gels (Shevchenko et al., 1996).

DIGE was invented to circumvent some of the limitations commonly associated with 2D gels including analytical (gel-to-gel) variation and limited dynamic range (Unlü et al., 1997). This is accomplished by multiplexing equal amounts of different samples that have been pre-labeled with spectrally resolvable fluorescent dyes (Cy2, Cy3 and Cy5), followed by separating the pooled protein samples in the same 2D gel. Protein spots associated with different samples are detected by different fluorescence signals and quantified using spot volumes. Differentially expressed protein spots can be excised for MS analysis. Synthetic N-hydroxysuccinimidyl (NHS) ester derivatives of the cyanine dyes Cy2, Cy3 and Cy5 are commonly used fluorescent protein labeling for DIGE (Unlü et al., 1997). The reagents react with the ε-amine of lysine, which is prevalent in proteins. In minimal DIGE experiments, the fluorescent dyes are used at a low stoichiometry relative to protein quantities. Usually the samples are labeled with Cy3 or Cy5. The Cy2 dye can be used to label an internal standard, which consists of an equal aliquot of each test sample (Alban et al., 2003; Karp et al., 2005). The internal standard not only serves as a loading control, but also allows for normalization of Cy3∶Cy2 and Cy5∶Cy2 ratios for each protein across all the gels in a large experiment. Since minimal dye only labels 1%–3% of the proteins, post-staining of the proteins (e.g., using Sypro Ruby) is required for spot picking. Saturation dye labeling utilizes maleimide reagents for high stoichiometry labeling of cysteine sulfhydyls (Shaw et al., 2003). Reagents for saturation labeling include Cy3 and Cy5 maleimide derivatives (Cy3m and Cy5m). Cy2m was not stable, so it is not commercially available. Saturation dye greatly increases sensitivity due to high stoichiometric labeling. Samples containing as low as 10 μg protein can be separated on a 2D gel to reveal thousands of spots (Fu et al., 2008). Thus, saturation DIGE is useful for profiling rare samples, such as those obtained by laser capture microdissection (Greengauz-Roberts et al., 2005). However, the protein amount in the gel spot is often not adequate for MS analysis, so a preparative gel is needed. Because of simultaneous separation of different samples in one gel, DIGE has greatly enhanced 2D gel reproducibility. In addition, DIGE for expression profiling has been shown to provide superior sensitivity and dynamic range afforded by fluorescence detection (Gharbi et al., 2002; Tonge et al., 2001). Despite the advantages of DIGE, it still suffers from some of the traditional 2D gel problems, such as domination of abundant proteins and underrepresentation of hydrophobic and extreme proteins (Chen and Harmon, 2006).

2.2 Isotope labeling: Metabolic labeling and chemical labeling

Metabolic labeling exploits *in vivo* incorporation of stable isotopes during protein synthesis in cells growing in media

supplemented with the isotopes. Depending on the introduced isotope spacer and the resolving power of mass spectrometer, isotope envelopes of light and heavy-labeled peptides are used for quantification. In the case of N^{15} metabolic labeling, two populations of cells are grown in isotopically distinct media, one with N^{15} and the other with N^{14}. The two samples are then mixed, processed and subjected to MS analysis and comparison of MS peak intensities between the two samples (Oda et al., 1999). However, incomplete incorporation and high cost of pure isotopes have rendered this method less useful (Thelen and Peck, 2007). In addition, each nitrogen atom in each amino acid will be replaced by ^{15}N, resulting in varying mass shifts that depend on the length and amino acid composition of the peptide. This makes result interpretation difficult (Gevaert et al., 2008). An alternative approach that overcomes the problem is known as stable-isotope labeling with amino acids in cell culture (SILAC) (Ong et al., 2002). SILAC is a straightforward procedure in which natural variants of essential amino acids are left out of culture media and replaced by ^{13}C, ^{15}N, or ^{2}H variants. The heavy amino acids are readily incorporated into proteins, and after a few rounds of cell duplication, the proteome can be fully labeled. Several essential amino acids are commonly chosen to achieve efficient labeling, e.g., leucine, lysine, and methionine (Ong et al., 2002; Gu et al., 2003; Ong et al., 2004). The most commonly used amino acids are lysine and arginine. The labeled proteins can be digested with trypsin to generate a single isotopic label in each peptide for MS identification and quantitation (Ong et al., 2003). Thus, SILAC offers simpler analysis than ^{15}N metabolic labeling. Because of near 100% incorporation of the isotope labels, SILAC reduces the number of manipulations and increases the accuracy of quantitation. However, SILAC still displays some shortcomings. First, metabolic conversion of arginine to proline can occur, and the arginine isotope label can thus be diluted into heavy proline. This will complicate data interpretation and quantification. Second, the incorporation level may not be efficient for certain cell lines and tissues (Iliuk et al., 2009). The recent success of generating SILAC mouse has shown the power of SILAC application in large tissues and animals (Krüger et al., 2008).

To overcome the problem of protein quantitation in samples not amenable to metabolic labeling, several chemical labeling approaches have been developed. Chemical labeling makes use of protein functional groups, which can react covalently with chemically identical but mass-differentiated stable isotope tags in different samples. The combined mixtures are then processed and analyzed by MS and/or MS/MS. The isotope peaks corresponding to the same peptide are identified. The peak intensities of the peptides or the isotope tags correlate directly with the relative abundance of the protein in different samples. The prototypical stable-isotope labeling for quantitative proteomics was isotope-coded affinity tag (ICAT) technology (Gygi et al., 1999). The ICAT reagents consist of a cysteine-reactive group, a linker containing eight or zero deuterium atoms and a biotin affinity tag enabling the isolation of tagged cysteine-containing peptides to reduce sample complexity prior to MS analysis. The linker region of heavy ICAT reagent contains eight deuteriums (d_8 ICAT), thus providing sufficient *m/z* spacing in mass spectra. However, it was reported that the d_0 (light) and d_8 (heavy) ICAT tagged peptides exhibited different retention on reverse phase high performance liquid chromatography (HPLC) columns (Zhang and Regnier, 2002). In addition, the retention of the biotin group complicates interpretation of MS/MS spectra. To solve the problems, an advanced version ICAT (cleavable ICAT, cICAT) or solid-phase tags with cleavable linkers was introduced. Carbon-13 instead of deuterium was used for the linker, and an acid cleavable moiety was introduced before the biotin group (Zhou et al., 2002; Hansen et al., 2003). The ICAT method simplifies sample complexity because of biotin affinity purification of cysteine-containing peptides, but is limited to proteins containing cysteine residues. In contrast, quantitative labeling of amine groups allows the quantification of all peptides derived from proteolytic digestion. One of the recently introduced methods is iTRAQ (isobaric tags for relative and absolute quantification) (Ross et al., 2004; Zhu et al., 2010). iTRAQ contains an isobaric tag composing of a balancer group, a reporter group and an amine reactive group. The reporter group is a tag of varying masses, and the balance group also varies in mass to ensure that the total mass of these two groups for different tags is the same. As such, peptides labeled with different iTRAQ tags are isobaric in MS. The reporter groups are released during MS/MS and are detected in the low mass region of peptide MS/MS spectra. They are used to quantify the protein levels in a sample. The iTRAQ kit started with four tags, and now eight tags are commercially available from AB Sciex Inc. So, as many as eight samples can be labeled and analyzed simultaneously (Ow et al., 2008; Zhu et al., 2010). To validate iTRAQ results in a high-throughput manner, mTRAQ reagents were recently introduced by AB Sciex. The mTRAQ tags are chemically identical to those of iTRAQ tags, but differ in their isotopic contents and are not isobaric. The mTRAQ reagent is available in two versions: the “heavy” version has a monoisotopic mass of 145, and the “light” version is 141 (DeSouza et al., 2008). By virtue of this mass difference, MRM transitions can be generated for any given peptide that is labeled with the mTRAQ tags. Therefore, combining with MRM, mTRAQ can be used for absolute quantification of target peptides and validation of iTRAQ discovery results (DeSouza et al., 2008).

Tandem mass tag (TMT) from Thermo Scientific Inc. is another amine-specific chemical tag. Conceptually similar to iTRAQ, TMT tags are also isobaric, and are identified and quantitated by MS/MS. The isobaric TMT reagents contain a TMT fragment, a mass normalization group and a

reactive group. Second generation TMT Tags are designed with a fragmentation-enhancing group, which is proline in some cases. Fragmentation-enhancing group makes that TMT fragment accurately reflects the levels of the tagged peptides (Thompson et al., 2003). This technology was designed to allow multiplexing of up to six samples (Dayon et al., 2008).

2.3 Label free quantification: LC-MS peak alignment, spectral counting and Synapt

Almost all the isotope labeling-based quantitative approaches have limitations: complicated sample preparation procedures, expensive isotope tags, limited number of experiments that can be compared, and variation in labeling efficiency. To overcome these limitations, there has been great advancement in the development of label-free approaches (Patel et al., 2009).

LC-MS peak alignment is based on the observation that MS peak height or area is linearly proportional to the corresponding peptide concentration (America and Cordewener, 2008). In an LC–MS experiment, peaks are determined for quantification using the MS signal summed over the elution time of the peptide. To compare across different samples, highly reproducible LC-MS and careful chromatographic peak alignment are critical in this comparative approach. Some automated methods and software can reduce the data complexity by extracting and comparing spectral or chromatographic peaks from LC-MS data (Li et al., 2005; Letarte et al., 2008). However, some peptides are not equally detectable in different samples with LC-MS methods because of dynamic-range limitation, instrument sensitivity and matrix ion suppression (Zhang et al., 2006). Another label-free method, known as spectral counting, may circumvent the above problems. It is based on the correlation between protein abundance and the number of MS/MS spectra of the peptides derived from the protein (Liu et al., 2004). Fast scanning mass spectrometers such as the Thermo LTQ and LTQ-Orbitrap MS have shown utility and advantage in this application (Zybailov et al., 2009). Spectral counting exhibits good technical reproducibility and dynamic range (Old et al., 2005; Asara et al., 2008). Therefore, it can be used as a simple and reliable way for relative protein quantification. Recently, Waters Company has introduced another label-free quantification system, the Synapt MS. This system is composed of ultra performance LC and quadrupole orthogonal acceleration time-of-flight MS. Protein identification and quantification data were collected in data independent LC-Ion mobility-MS^E acquisition by alternating the energy applied to the gas cell between a low energy state and an elevated energy state. The low energy scan mode is used to obtain accurate precursor ion mass and intensity data for quantification, while the elevated collision energy mode generates multiplex peptide fragments of all peptide precursors for identification (Silva et al., 2006; Cheng et al., 2009). The Synapt system provides unique capabilities to analyze samples differentiated by size, shape and charge, as well as mass, ultimately enabling quantitative analysis of protein changes in complex biologic mixtures.

2.4 Targeted quantitative proteomics

Targeted proteomics represents a different approach from global discovery proteomics, and it is powerful in hypothesis driven research. MRM has been widely used for small molecule quantification in pharmaceutical companies. It is only through the introduction of triple quadrupole (QqQ) and quadrupole ion-trap (QTRAP) instruments with extended mass ranges that MRM has become well suited for proteomic applications, allowing for the detection of target peptides in complex mixtures (Anderson and Hunter, 2006). To carry out MRM experiments, appropriate peptide precursor and its product ion pairs (called transitions) need to be selected, and fragmentation conditions need to be optimized for each transition. Because the tandem mass analyzers are set to monitor unique signals from targets specified, this method offers exquisite selectivity and sensitivity. However, with the increase of sample complexity, the chance of false positive transitions being registered during the analysis becomes high. It is essential to confirm the identity of the precursor ion giving rise to a specific transition. This is accomplished by MRM-initiated detection and sequencing (MIDAS), in which detection of a specific MRM transition automatically triggers a subsequent product ion scan to verify precursor identification (Unwin et al., 2005). Recently, scheduled MRM workflow has been introduced to increase the number of transitions that can be monitored without compromising sensitivity. At least two or more peptides per protein are often monitored so that the peptide quantities can accurately reflect original protein abundance (Kitteringham et al., 2009).

Protein array enables the global observation of biochemical activities on an unprecedented scale, where thousands of proteins can be simultaneously screened for protein–protein interactions, protein–phospholipid interactions and small molecule targets (Bertone and Synder, 2005; Ramachandran et al., 2005). Here is a general procedure of a typical protein array experiment. A large set of capture ligands (proteins or peptides) is arrayed on a solid support. The array is then probed with a protein sample. If an interaction occurs, a signal is revealed on the surface by a variety of detection techniques including fluorescence, chemiluminescence and radioactivity. Three types of protein arrays are available, i.e., analytical array, functional array and reverse phase array. For protein analytical array, a library of antibodies is arrayed on a glass slide. The array is used to monitor protein expression on a large scale (Hamelinck et al., 2005). Functional arrays contain a large set of purified proteins and peptides. This array can be used to study numerous protein interactions (Blackburn and

Hart, 2005). For reverse phase arrays, tissues, cell lysates or serum samples are spotted on the surface and probed with antibodies against the target protein of interest (Cretich et al., 2006). Invitrogen Company has recently commercialized the ProtoArray® technology. The ProtoArray® Human array contains thousands of purified proteins printed in duplicate on a glass slide (http://www.invitrogen.com/protoarray). It enables many high-throughput analyses including protein interaction, kinase substrate identification, small molecule-protein interaction profiling, immune response biomarker profiling, ubiquitin ligase profiling and antibody specificity profiling.

3 Recent application examples

As shown in Table 1, each quantitative method has advantages and disadvantages. There is no one single method that will solve all the analytical challenges associated with large-scale protein quantification. The choice of methods is dependent on research questions to be addressed, experimental materials (e.g., culture cells *versus* tissues), expectation of results (e.g., absolute quantification *versus* relative quantification), and project budget. Because of the complementary nature of most quantitative methods, application of more than one will likely lead to high proteome coverage and accuracy of quantification (Chen and Harmon, 2006).

Since its introduction over a decade ago, DIGE has been widely used to address important questions in many research areas of life sciences and medicine, such as cell signaling, development and disease biomarker discovery (Orenes-Piñero et al., 2007; Lucitt et al., 2008; Tang et al., 2008). Many studies have found the technology highly appropriate for comparative protein profiling of knockout, transgenic or stress/hormone induced responses (Casati et al., 2005; Hjernø et al., 2006; Tang et al., 2008). In addition to large-scale protein expression profiling, saturation DIGE with cysteine-labeling reagents has been used to monitor changes in protein redox status (Hurd et al., 2007, Fu et al., 2008). For example, DIGE was used to discover redox-sensitive proteins in mouse heart tissues (Fu et al., 2008). Cy3m was used to label both the control and H_2O_2 treated heart tissue samples. In parallel, Cy5m was used to label internal standards composed of pooled aliquots of control and treated samples. Each Cy3m-labeled sample was then mixed pairwise with one Cy5m-labeled internal standard and used for 2-DE analysis. Because CyDye labeling was performed directly on protein samples without reduction and alkylation, only free thiols but not oxidized thiols could be labeled. Protein spots with at least a 50% Cy3 signal decrease in H_2O_2 treated samples were subjected to MS identification. In this analysis, NF-κB-repressing protein and epoxide hydrolase were discovered to subject to redox regulation.

SILAC has the advantage of introducing the mass labels early in sample preparation and thus displaying excellent quantification accuracy and reproducibility. Recent studies have shown the efficiency of SILAC method in labeling all proteins without discrimination. Graumann et al. (2008) have quantified 5111 proteins in mouse embryonic stem cells using SILAC. It has also been used to study differential protein phosphorylation in yeast pheromone signaling (Grühler et al., 2005). Kruger et al. (2008) labeled mice with SILAC diet of $^{13}C_6$-substituted version of lysine. The F2 generation was completely labeled in all organs tested. Interestingly, after over four generations of labeling with the heavy diet, mouse development, growth and behavior were not affected. Thus, organs derived from the SILAC mice were served as invaluable materials for a large number of subsequent experiments, in which proteomes of wild-type and knockout mice were compared, and important findings were obtained.

iTRAQ is currently the most widely used chemical labeling method for quantitative proteomics. Shui et al. (2009) employed both iTRAQ and SILAC to study changes in macrophage protein expression in response to exposure to *Mycobacterium tuberculosis* lipids. From a total of 1286 proteins identified, 463 were discovered by both methods, and the rest of proteins were detected by only one of the two methods, highlighting the complementary nature of iTRAQ and SILAC. The differentially expressed macrophage proteins are involved in immune response, oxidation and reduction, vesicle transport, as well as other cellular processes. Using iTRAQ combined with two-dimensional LC and MALDI-TOF/TOF MS, Rajcevic et al. (2009) were able to identify several thousand proteins in membrane-enriched fractions, of which 1460 proteins were quantified and some novel candidate proteins were characterized to involve in the switch from a non-angiogenic to a highly angiogenic phenotype. In a study by Zhou et al. (2007), iTRAQ reagents were also applied to measure quantitative changes in phosphorylation-dependent peptide-protein interactions. Combination of iTRAQ with protein posttranslational modification (PTM) and protein interaction analysis allows quantitative definition of the dynamics in protein PTM and interaction processes.

With the improvement in data analysis workflow, label-free proteomics has shown great potential in large-scale biomarker discovery and validation. Letarte et al. (2008) used a mouse skin cancer model, which has a mutation in the p19ARF gene. LC-MS experiments were performed to identify proteins differentially expressed in control and mutated mice. A significant number of proteins directly associated with cancer development were identified using a peak alignment method. Neubert et al. (2008) demonstrated the utility of LC/MALDI MS and spectral counting in quantification of protein expression differences in *Escherichia coli* growing on different media containing arabinose, fructose or glucose as sole carbon source. As another example, Wang et al. (2008) analyzed breast tumor

cells with distinct malignant phenotypes. Membrane glycoproteins of the tumor cells were identified and quantified by spectral counting. The approach enabled the identification of several distinct glycoproteins with expression indexes correlated with either a precancerous or a malignant, metastatic cellular phenotype. Relative quantification by spectral counting has recently been widely applied in addressing many biological questions. More examples include comparison of protein expression in *Methylobacterium extorquens* AM1 under single carbon (methanol) or multi-carbon (succinate) growth conditions (Bosch et al., 2008), discovery of biomarkers in human saliva proteome in type-2 diabetes (Rao et al., 2009), and large-scale profiling of chloroplast clp protease mutant (Zybailov et al., 2009).

MRM has been used as a quantitative method for analyzing small molecules for decades. It has been increasingly applied to the measurement of targeted peptides in complex samples. Langenfeld et al. (2009) described absolute quantification of a low abundant human cytochrome P450 2D6 in a set of 30 liver samples from different genotypes using MRM LC-MS. Results were validated using quantitative Western blotting and activity assay. The MRM technique turned out to be an efficient and true assay of CYP2D6 in human liver samples. As another example, Whiteaker et al. (2007) have identified up- and down-expressed proteins in cancer tissues. The altered levels of expression of candidate biomarkers were confirmed by MRM-MS in the cancer tissues, followed by detecting the potential biomarkers in serum by affinity enrichment and MRM-MS. This study led to the proposal of fibulin-2 as a true biomarker for breast cancer.

Proteome chips have been used to screen patients' sera for the presence of auto-antibodies. Anderson et al. (2008) selected over 1700 antigen clones from the PlasmID repository to build a custom high density antigen array. To demonstrate that the programmable protein arrays can be used for serologic screening, they provided a direct comparison with a recombinant protein p53 autoantibody ELISA. The array was used for rapid identification of immune response signatures for breast cancer diagnosis and monitoring. Ptacek et al. (2005) studied protein phosphorylation in yeast using protein array. They incubated 87 different protein kinases or kinase complexes separately with radiolabeled ATP on a yeast protein array containing 4400 unique proteins. About 4200 phosphorylation events were identified to affect 1325 different proteins. Clearly, protein array has emerged to follow DNA chips as a powerful tool for miniaturized functional analysis as well as for rapid screening of biomolecules.

4 Future perspectives

Although the quantitative approaches reviewed here provide us with unprecedented opportunities for relative and absolute protein quantification, many challenges still remain. The first challenge is that it is currently very difficult to identify and quantify all protein components in a proteome. This is directly related to protein extraction efficiency, biological variation, proteome complexity and dynamic range limitations of instruments. New methods of sample preparation and introduction of multidimensional LC, high resolution mass spectrometer and MRM in quantitative proteomics will greatly enhance proteome coverage. When genome sequences are available, MRM based targeted proteomics offers a highly sensitive and definitive MS quantification of proteins in a proteome. With scheduled MRM and fast scanning mass spectrometers, high throughput MRM has great potential to achieve full proteome quantification in the foreseeable future. Another challenge for quantitative proteomics is the limitation of standardized software in analyzing large amounts of raw data. Dedicate computational and statistical tools for quantitative data analysis and data sharing are essential. It should be noted that many quantitative proteomics methods are complementary. A good example is iTRAQ MS screening followed by mTRAQ validation of specific iTRAQ data (DeSouza et al., 2008). Therefore, careful and proper experimental design employing complementary approaches to enhance protein quantification coverage, accuracy and fidelity is an important prerequisite for success. Despite the challenges, the recent developments in quantitative proteomic techniques and applications hold big promise for the future. Quantitative proteomics is currently experiencing rapid advancement and has become an indispensable tool in functional genomics, systems biology, biomarker discovery and disease diagnostics.

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