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REVIEW ARTICLE

Recent Technological Advances in the Mass Spectrometry-Based Nanomedicine Studies: An Insight from Nanoproteomics

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Abstract: Nanoscience becomes one of the most cutting-edge research directions in recent years since it is gradually matured from basic to applied science. Nanoparticles (NPs) and nanomaterials (NMs) play important roles in various aspects of biomedicine science, and their influences on the environment have caused a whole range of uncertainties which require extensive attention. Due to the quantitative and dynamic information provided for human proteome, mass spectrometry (MS)-based quantitative proteomic technique has been a powerful tool for nanomedicine study. In this article, recent trends of progress and development in the nanomedicine of proteomics were discussed from quantification techniques and publicly available resources or tools. First, a variety of popular protein quantification techniques including labeling and label-free strategies applied to nanomedicine studies are overviewed and systematically discussed. Then, numerous protein profiling tools for data processing and post-biological statistical analysis and publicly available data repositories for providing enrichment MS raw data information sources are also discussed.

Keywords: Nanoproteomics, nanomaterials, nanomedicine, protein quantification, mass spectrometry, biomedicine science.

1. INTRODUCTION

Compared with the bulk phase, nanoparticles (NPs) and nanomaterials (NMs), which are of ultrafine particle size between one nanometer to one hundred nanometers [1, 2], have unique characteristics of electrical, chemical and physical properties (*e.g.* semiconducting-, metallic-, magnetic-, and polymeric-nanosystems) [3], and large specific surface area for guaranteeing their high biological activity [4, 5]. These unique characteristics make NPs play an important role in nanomedicine [6, 7]. Nanomedicine, defined as an application of nanotechnology into the medicine [8], is a newly emerging field and aims at drug delivery and the diagnosis, treatment, and prevention of disease using NPs and NMs [8-10]. Due to the advantages of improving effectiveness by biological targeting of drugs, NPs are widely applied therapeutic and diagnostic approaches in disease [11]. In the field of drug delivery, nanoparticles can reduce the toxicity of the drug and overcome the potential side effects of conventional server therapies. As reported, NP-mediated drug delivery could be used to directly target and deliver the NPs to the given tissue, enhance the bioavailability and maintain the drugs effect in the tissue. For example, gold nanoparticles were observed to be good candidates for improving anticancer targeted drug delivery and drug release [12, 13]. It can carry and selectively release drugs in the desirable tissue, aiming at reducing side effects of drugs on normal cell and simultaneously elevating drug dose in diseased cell [14].

A variety of techniques such as immunoassays [15], mass spectrometry (MS) [16], chromatography [16], protein-microarrays [17] and gel electrophoresis [18] could be applied to the nanomedicine field. Among these techniques, MS is powerful to provide chemical and structural information that is difficult to obtain through other approaches. As reported, MS has become a current major tool due to the desirable throughput, sensitivity, and identification as well as quantitation capability. In particular, it can interpret the encoded information in the genomes, analyze the orientation and conformation of the proteins and prefractionate protein mixtures [19] and small sets of proteins isolated in specific functional contexts. Integrating MS with protein microarrays could provide the novel insight into protein analysis for nanomedicine study.

Owing to offering quantitative and dynamic information on proteins by directly profiling protein expression [20, 21], MS-based proteomics analysis plays an essential role in the realm of biomarkers-identification and characterization [22]. Most diseases' diagnosis, treatment, and prognosis can benefit a lot from it [23]. However, its application may be limited due to such low abundance of most diseases' biomarkers [24]. Thus, in order to rapidly detect low abundance biomarkers, a high-throughput technological platform with the characteristics (ultra-sensitive and robust) is in urgent need. Despite the recent technological progresses in MS, low concentration of biomarkers hinders the discovery of candidate biomarker [24]. Fortunately, the emergence of nanotechnology can make up for this deficiency. For example, some nanoparticles can entrap and concentrate the biomarkers to make them easier for MS to detect [24, 25]. The superiority of integrating nanotechnology into proteomics, including ultralow detection, high-throughput capability, real-time detection, and low sample consumption, can achieve rapid detection on low abundance biomarkers. In sum, nano-assisted MS remarkably enhanced detection efficiency of proteins or peptides and contributed to the precision diagnostics [17]. Till now, in MS-based nanoproteomics studies, diverse quantitative techniques (*e.g.*, labeling, and label-free strategies) have been

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widely applied. Detailed application of liquid chromatography (LC)-MS/MS-based proteome quantification to various aspects of current nanoproteomics is systematically illustrated in Fig. (**1**). In addition, a number of feature selection methods and available public resources have been developed and applied for accelerating nanomedicine science process, especially the nanoparticle-based targeted drug delivery system [26, 27] and nanomaterials-related toxicity [28-30].

Herein, a well-rounded review of current MS-based techniques for nanomedicine was provided. Moreover, the sources of data in nano-based targeted drug delivery and various nanomedicine-related toxicity studies from nanoproteomics perspective have been systematically discussed. First, through the assessments of both challenges and potentials, a variety of quantification techniques and state-of-the-art feature selection methods are overviewed. Second, numerous powerful computational nanoproteomics tools and publicly available data repositories are also discussed. Finally, the prospective orientation for MS-based quantification technique for nanomedicine applications is also proposed.

2. MS-BASED QUANTIFICATION TECHNIQUES FOR NANOPROTEOMIC STUDY

Label-free and labeling MS-based proteomics methods have been widely applied to the various nanomaterials-associated studies [31, 32]. The quantification workflows applied in the proteomic study on nanomaterials are illustrated in Fig. **(2)**, and the advantages and disadvantages of available proteome quantification methods for nanoproteomics studies are provided in Table **1**.

2.1. Labeling Quantification Technique

Labeling quantification involves the addition of certain stable isotope, heavy amino acids or isobaric tags for labeling the sample/cells to detect differences in protein abundance among different samples [31, 32]. A number of labeling approaches were employed for identifying certain changes in protein expression level when studied samples were exposed to NPs [32]. These labeling approaches based on stable isotope labeling mainly include three categories (metabolic labeling, enzymatic labeling, and chemical labeling) for relative and absolute quantitation. As reported, metabolic labeling (*e.g.*, stable-isotope labeling by amino acids in cell culture (SILAC) and N-terminal labeling) could change protein abundances from different samples to the same level, which made the microbial communities not appropriate for this labeling method [33], while chemical and enzymatic labeling based on different isotopic tags could address this issue. In addition, proteome coverage, accuracy, precision and reproducibility of protein quantification were also reported to offer certain differences among the three types of labeling methods. For example, the chemical labeling has unique advantages in both reproducibility and precision compared with the metabolic labeling [34].

In particular, affinity-tag isotope coded, isobaric-tag for relative-absolute quantification (iTRAQ), SILAC, isobaric labeling

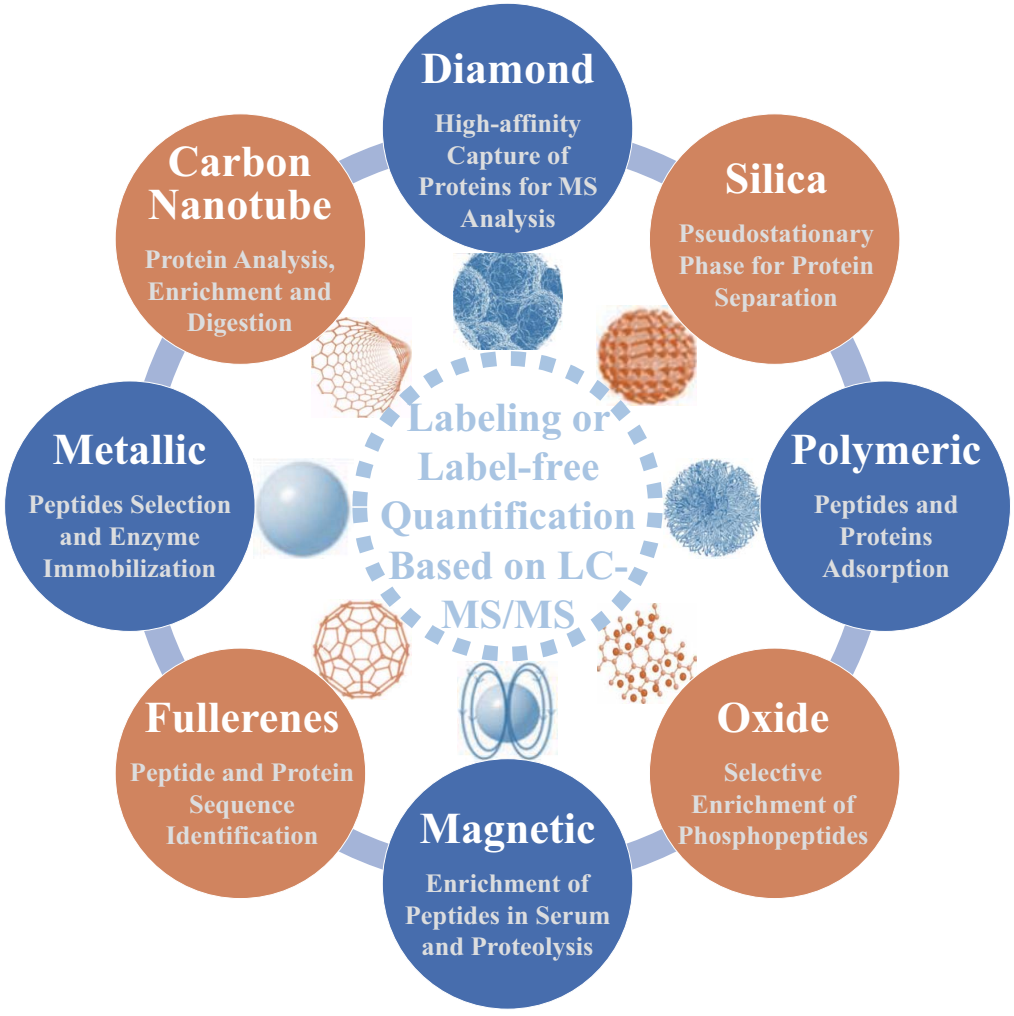

Fig. (1). Application of LC-MS/MS-based proteome quantification to various aspects of current nanoproteomics.

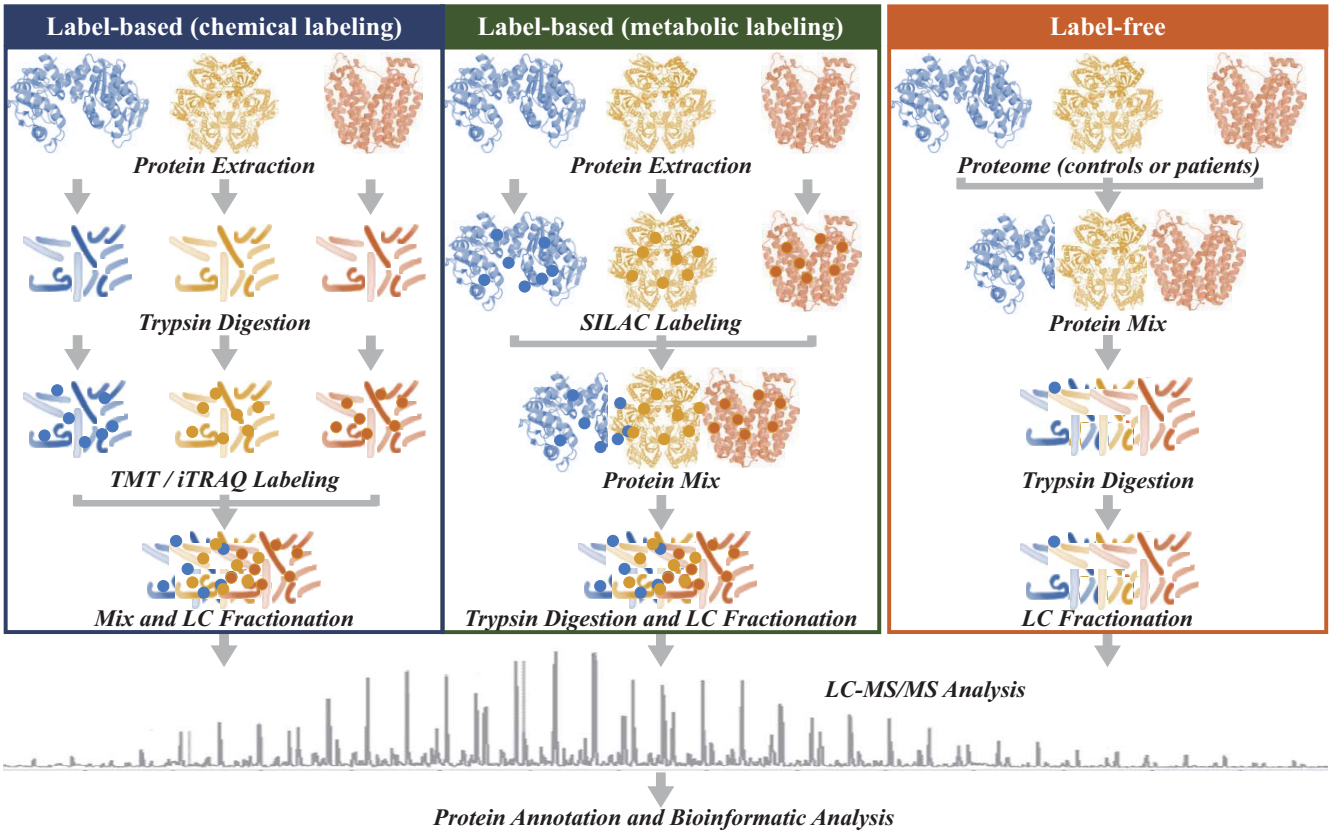

Fig. (2). Schematic illustrations of label-based and label-free proteome quantification workflows applied in current nanoproteomics.

tandem-mass tag, N-terminal labeling, selected reaction monitoring (SRM), terminal amine isotopic labeling of substrates (TAILS), metal-coded affinity tag (MeCAT), isotope dilution, radiolabeled incorporation, and mass-coded abundance tag, chemically synthesized the peptide standards. Almost all the labeling-based quantification methods have the advantages of relative peptide abundances to be directly compared, and reliably detect lower fold changes in protein intensity over a dynamic range from 10- fold to 100-fold. At the same time, this technique has potential disadvantages, such as the preparation of complicated samples, increasing number of sample concentration demand and the incompleteness of labels. For example, the SILAC-based labeling proteomic method has been applied for investigating how the lung cells respond to SILAC-based copper oxide nanoparticles [35], and the stable-isotope dimethyl labeling was utilized for evaluating the toxicity in NRK-52E cells created by Nano Fe_3O_4 that is unmodified [31], and iTRAQ was applied for identifying the effects of zinc oxide nanoparticles on the expression of protein in rats [36]. Detailed descriptions on current labeling proteome quantification methods for nanoproteomics studies are shown in Table **2**.

2.1.1. Isotope-Coded Affinity Tag (ICAT)

The ICAT is the chemical labeling technique for identification and relative or absolute quantification of the proteome. ICAT was described by Steven P. Gygi and his colleague in 1999. It is a gel-free MS based isotopic labeling technique applied in quantitative proteomics to compare up to two samples, based on a class of chemical labeling reagents. The ICAT reagent is composed of a biotin head, a junction and an iodine-activated carbonyl group that specifically reacts with the cysteine side chain [31]. The difference in the heavy isotope atoms is the substitution of eight deuteriums and the light isotopes eight hydrogen atoms in the junction region. Two samples of protein with d0 and d8, ICAT reagent tags, an a pair of different markup peptides eluted at the same time can be identified by the distance of 8kDa as signal [37]. Specifically, 14% of the eukaryotic proteins do not have cysteine residues, so this technique is not suitable for these proteins. There is also some common weakness with 2-d pages, such as not being able to get complete protein information [38].

The ICAT approach is based on two principles and includes four sequential steps [39]. Furthermore, it has been reported that ICAT recognizes the proteins that adsorb to nickel, aluminum and diamond nanoparticles after being exposed to human serum when it is based on dual-label approaches, and quantify the relative affinities with which these proteins bind [40].

2.1.2. Isobaric Labeling Tandem-Mass Tag (TMT)

The TMT is a chemical labeling technique applied for MS/MS-based proteome quantification analysis, which belongs to a family of isobaric mass tags. It allows different samples determined by a relative abundance that is accurate and sensitive [41]. If TMT is combined with other methods, it can offer an alternative to gel-based quantification [41]. In the primary MS, the same peptides in different samples labeled with any TMT reagent showed the same mass-to-charge ratio [42]. In the secondary MS, TMT reported ions were released from the cleavable bond fracture, and 10 TMT reported ion peaks were generated in the low mass areas of the MS [43]. The TMT has been applied in a wide range of area that almost any species of the protein can be separated and identified with low abundance which reflects high sensitivity of this method as well as analyzing 10 samples simultaneously [44]. Weitao Jia *et al*. applied an integrated methodology of TMT method and cerium oxide nanoparticles to evaluate precise stoichiometries in the phosphorylation progress of protein. It was also developed to estimate the level of dephosphorylation of known phosphorylation-sites and then quantify the phosphor-sites [45].

Table 1. The advantages and disadvantages of available proteome quantification methods for nanoproteomics studies.

Quantification Methods		Advantages	Disadvantages	References
Labeling	Chemical Labeling	More precise and more sensitive for detecting protein expression with small fold changes; More accurate quantification; Less instrument time; More simple experimental design.	Identify fewer peptides and fewer proteins; Require advanced MS instruments; Require alternative fragmentation methods.	[175]
	Metabolic Labeling	Allow samples grown in different states; More suitable for samples at the protein level; More suitable for fractionation and enrichment.	Less efficient for many biological systems; Less accurate quantification; Feasible only for selected microorganisms and cell cultures; Limited with respect to the sample type.	[176]
	Enzymatic Labeling	Great versatility; Conveniently handle different samples; No chemical contamination labeling clinical biopsies and animal tissue; Excellent sensitivity;Highly specific isotopic labeling; Complete and stable Labeling.	The label is introduced at the peptide level; The in-spectrum dynamic range is limited; limited to binary comparisons or series thereof; Back exchange is reported.	[177]
Label-free	Peak-instensity	Higher proteome coverage;Higher number of protein identifications;Deeper coverage of the genome;Larger dynamic range for protein identification;Straightforward and convenient;Better capability to detect differentially altered proteins.	Less precise; Depend on the software used for the data analysis.	[178]
	Spectra-counting	Higher proteome coverage;Higher number of protein identifications;Deeper coverage of the genome;Larger dynamic range for protein identification;Quantify more proteins.	Less precise quantification to proteins with low spectral counts; Less sensitive to detect small fold changes; Less accurate.	[34]

Table 2. Currently available labeling proteome quantification methods for nanoproteomics studies.

Type of Labeling	Methods	Type of Quantification	Type of Nanoparticles	Brief Descriptions	References
Chemical labeling	Isotope-coded affinity tags (ICAT)	Relative, Absolute	Diamondnanoparticles; Metal oxide nanoparticles	This method makes denatured and reduced proteins residues labeled with either the 'heavy' or 'light' ICAT reagent.	[179]
Chemical labeling	Isobaric labeling tandem mass tags (TMT)	Relative, Absolute	Metal oxide nanoparticles; Magnetic nanoparticles	The TMT method can modify primary amino groups in proteolytic peptide mixtures based on multiplex TMT reagents.	[180]
Chemical labeling	Isobaric tags for relative and absolute quantification (iTRAQ)	Relative, Absolute	Carbon nanoparticles; Metal oxide nanoparticles	The iTRAQ method modifies primary amino groups in proteolytic peptide mixtures by using multiplex iTRAQ reagents.	[51]
Chemical labeling	Dimethyl labeling	Relative, Absolute	Silica Nanoparticles	This method makes digested and derived sample peptides labeled with isotopomeric dimethyl label.	[181]
Chemical labeling	Terminal amine isotopic labeling of substrates (TAILS)	Relative, Absolute	Gold nanoparticles	TAILS enriches and identifies both natural and proteolysis-generated protein N-termini based on isotopic labeling of amines.	[182]
Metabolic labeling	Stable isotope labeling with amino acids in cell culture (SILAC)	Relative, Absolute	Silver nanoparticles;Metal oxide nanoparticles	SILAC carries heavy stable isotopes and is introduced in all newly synthesized proteins, supplying labeled amino acid.	[183]

(Table 2) Contd....

Type of Labeling	Methods	Type of Quantification	Type of Nanoparticles	Brief Descriptions	References
Enzymatic labeling	Proteolytic ^{18}O Labeling	Relative	Magnetic nanoparticles; Diamond nanoparticles	This relies on class-2 proteases to catalyze the exchange of two ^{16}O atoms for two ^{18}O atoms at the C-terminal carboxyl group of proteolytic peptides.	[184]
Others	Isotope dilution	Absolute	Silver nanoparticles; Gold nanoparticles	The isotope dilution method can make protein quantification based on internal standards (isotope labeled proteins).	[66]
Others	Mass-coded abundance tagging (MCAT)	Relative	Silver nanoparticles	TheMCAT method can relative is based on guanidination of lysine (lysines to homoarginine).	[71]

2.1.3. Isobaric-tag of Relative-Absolute Quantification (iTRAQ)

The iTRAQ is a chemical labeling technique and can conduct relative-absolute quantification for isotopic labeling *in vitro*, which uses isotope reagents to mark 8 polypeptide samples simultaneously [46]. In this method, low abundance protein can be detected, and almost any species of the protein would be isolated and identified rapidly by the high throughput of LC with tandem MS [47]. After the labeled polypeptide samples are mixed equally, the first stage of mass spectrometry (MS1) and the second stage of mass spectrometry (MS2) information of every peptide segment are obtained by separation and MS study [48]. In primary MS, the identical peptides from various datasets exhibit an identical ratio between mass and charge. In 2nd MS, the ratio between mass and charge of peptide and iTRAQ signal are reported, involving relative protein expression. It has been widely used in the research of the mechanism of differentiation and development of animals or plants and the screening of medical biomarkers [49]. In 2004, Darryl Pappinet *et al.* evolved iTRAQ [50], which increases throughput as well as reducing experimental error [51]. Thus, it can cut down the time-length of MS-based study [52].

2.1.4. Stable Isotope-Labeling of Amino-Acids (SILAC)

SILAC is simple, inexpensive, and accurate, based on MS quantitative proteomic method, which uses stable isotope labeling by amino acids in the cell culture and binds specific amino acids to mammalian proteins *in vivo* to detect differences in protein abundance among samples [53]. Multiple proteins can be identified simultaneously by MS and isotope labeling has high efficiency, stability, good repeatability and only a small amount of protein required as specimen, as the results are closer to the true physiological state [54]. Newly synthesized proteins were marked since the cells were prepared with essential amino acids (mostly Arg and Lys) labeled with isotopes (light, medium or heavy). Normally, the cells are labeled with isotopes after being cultured for 5-6 generations. The quantitative and qualitative results of the protein could be obtained *via* LC-MS/MS analysis. This method has been used to quantify the changes in protein production such as the differentiation procedure of muscle cell, investigating the lung cell response to copper oxide nanoparticles SILAC-based [35].

2.1.5. Metal-Coded Tag (MeCAT)

The MeCAT method has been designed as a chemical labeling technology based on MS to investigate the difference of the proteome in the studied organism, allowing to determine protein quantification with low detection limits. It applies a mass shift between various proteins or peptides by encoding them with lanthanide ions trapped in a DOTA -based macrocyclic complex [55, 56]. The MeCAT method produces precise absolute quantitative results on the protein or peptide level regardless of where the workflow is applied [57]. Combining DOTA with different lanthanide ions is the way in which metal-coded affinity labeling is used in metal chelation. Metal complexes are attached to cysteine residues in the protein sample [58]. Proteins were then separated by 2-D electrophoresis and studied by Matrix-assisted laser desorption/ionization (MALDI) or use the electrospray ionization (ESI)-MS to relatively quantify proteins or through the inductively coupled plasma (ICP)-MS for precise absolute quantification [57]. Since the detection limits of rare earth metals are extremely low, they can be accepted for the use of proteomics. Although MeCAT is compatible with relative and absolute quantifications in top-down and bottom-up platforms, but it is laborious [59].

2.1.6. Terminal-Amine Isotopic-Labeling of Substrate (TAILS) and Isotope Dilution

The TAILS is an innovative multiplex quantitative proteomics technique to determine N-terminomes and identify protease substrates and cleavage sites in biological samples. It can discover the proteome of studied targets using proteins' N-terminals and differentiates their concentrations across various targets. Specifically, it adopts the trypsin digestion and breaks the proteins' N-terminal [60]. Moreover, it removes tryptic-protein, which is superior compared with other methods depending on free amino-acids of protein N-termini [61], and makes the assignment of different peptides to their corresponding proteins highly accurate [62]. This method has been applied to associate proteins with diverse sets of signaling-pathways in the catastrophic diseases and gives an improved illustration of the protein molecules related to the disorder [63]. Isotope dilution analysis has been introduced by David Rittenberg in the 1930s, which is popular for measuring the quantity of chemical substances [64]. Isotope dilution has been used to assess a diverse set of analytes, which integrate the IRMS measures, and contain a known level of subjects to the studied target. Due to its advantages, the isotope dilution is known to be of superior metrological standing [65]. Sötebier has separated and quantified the NPs and ions by the HPLC together with both isotope dilution and MS [66].

2.1.7. Stable Isotope-Labeled Proteins

With the wide application of metabolic imaging, radiolabeled amino acids are gaining increased interest among researchers in the field of life science [67]. One or more atoms of a compound molecule can be replaced by radionuclides for identifying as a tracer. The labeled compound can be tracked by radiometric technique. Among radionuclides, carbon14 (barium carbonate), tritium (tritium gas), iodine 125 (sodium iodide solution), iodine 131 (sodium iodide solution), phosphorus 32 (phosphate solution), *etc.* are commonly applied for labeling compounds [68]. Three methods including isotope exchange, chemical synthesis and biochemistry are often applied for radiolabeling [69]. In 1999, Peracchia *et al.* showed that PHDCA-based NP could help enlarge circulation and decrease the accumulation of liver [70].

2.1.8. Mass-Coded Abundance Tagging (MCAT)

Mass-coded abundance tagging (MCAT) is an effective technique for relative quantitation of proteins [71]. It offers a method for *de novo* sequencing of peptides/ proteins present at low levels in complex biological mixtures in a systematic, reproducible, and straightforward manner. The samples can be complex protein mixtures and may be different cell types, such as immune-precipitates or cell lysates [72]. To produce peptides with C-terminal lysine or arginine residues, the samples are extensively digested with trypsin at first. Then, the sample is processed by O-methylisourea, a reagent which modifies the ε-amino group of lysine side-chains, enabling it to convert to homoarginine quantitatively. This increases the mass of the modified peptides by 42 amu per lysine residue, instead of significantly affecting the ionization or fragmentation efficiencies using standard LC-MS conditions. The quantification technique has advantages of operating easily, is cost-effective, and efficacious, compared with other labeling quantitation ones, especially for quantifying complex protein mixtures.

2.2. Label-Free Quantification Technique

The label-free quantification method directly refers to quantifying proteins in tissues or cells or biological fluids and makes a large-scale analysis by linking protein abundance to mass spectrometric signal intensity [73] of the polypeptide and count of MS correlated to proteins when no isotopic or chemically modified proteins or polypeptides are employed [74]. Because label-free quantification can simultaneously identify and quantify proteins without the laborious procedure of isotope-labeling, it is widely used [32, 75], making label-free quantification have unique and advanced advantages, namely simplicity of the experiment and the ability to process a large number of samples [76]. However, the label-free method is highly susceptible to technical differences, while the quantified targets are separately assessed, and the unlabeled method has specific deficiencies in the accuracy, precision, and reproducibility of quantification [34]. The isotope labeling strategy is more accurate and precise than the label-based strategy described previously, but the labeling step is not only complicated but also quite expensive. Moreover, the quantization based on isotope is restricted by the specific number of sample and cannot be applied with every experimental design. In contrast, it is more accessible to apply the label-free method, and there is no limit on the number of samples, but the performance regarding quantization accuracy and accuracy needs to be improved [34]. Detailed descriptions on current label-free proteome quantification methods for nanoproteomics studies was shown in Table **3**.

These superiorities provide great potential to study protein abundance among different samples. Label-free quantitation methods can be classified into two unique groups: (1) the precursor ion spectra-based measurement of signals intensities; (2) MS/MS experiment-based spectral counting, which can be used to count the number of peptides correlated to their corresponding protein target [77]. This process for the area under the curve (AUC)-based proteins quantitation, is not related to the stable isotope standard, including the measuring of ion abundances or signal intensity for the ionized peptides given at a specific retention time, customarily termed as ion counts or peak intensity [78]. Acquisition is optimized in the independent data mode, recently, because it is unworkable for data-dependent MS to analyze the low-abundance proteins, , which exist in the celery secretome, as identified by using peak intensity method [79]. Albeit it's a straightforward strategy, it conceptually guarantees reproducibility and accuracy of detection and quantitation among different samples when analyzing multiple samples, taking considerations like consistency of retention times, repression of noise, choice of the optimum peak, and normalization of peak abundance into account [78, 80]. One crux of the AUC approach is how to balance the survey and fragment scans for the sake of obtaining accurate quantitation as well as the maximum amount of protein identification. Spectral counting is proportionate to the protein amount acquired by the data-dependent method [81]. The strategy of spectral counting is of great diversity because of the normalization factor from summing spectra to modifying counts, and more recently, integrating strategies for the mushroom accuracy. However, there do exist some shortages in spectral counting, one of them being the bias and mutation in the measurement of MS caused by the peptides with different inherency of physicochemical properties.

In label-free quantification, two main quantification approaches (peak intensity and spectral counting) are frequently used to protein relative abundance. Anna Laura Capriotti *et al*. used the label-free method based on peak intensity and spectral counting for devising a gene-delivery system with the study of interactions between NPs and plasma proteins [32]. Moreover, Sabrina Gioria *et al*. studied the impact of silver NPs (AgNPs) on Caco-2 cells at the level of protein using peak intensity-based label-free proteomic technique, and gave an insight into the mechanism of AgNPs [82]. Li Xu *et al*. analyzed the dynamic procedures of hydrogen peroxide-caused protein damage [83]. Ghazala Mustafa *et al*. introduced label-free proteomics based peak intensity to investigate the impact of Al_2O_3 NPs on soybean growth, which showed that under flooding stress, the soybean treated by Al_2O_3 NPs has a lower root-tip cell-death rate [84]. Spectral counting-based LFQ has been applied to analyze the unique corona composition on magnetic NPs, which show the interaction with primary human macrophages [85].

3. NORMALIZATION FOR PROCESSING NANOPROTEOMIC DATASET

Although MS technologies are developing steadily, the data that the MS analysis produced are easily influenced by the systematic biases [86]. However, the bias can not be taken into full consideration by only adjusting the experimental settings because of the exact unknown cause of the deviation [87]. When the peptide signal propagates over a large retention time, leading to overlapping with co-eluting peptides, researchers pay more attention to LC [77]. Moreover, the intensity of MS, the technical variation of retention time, background noise caused by chemical interference and biological variations which lead to multiple signals for the same pep-

Table 3. Currently available label-free proteome quantification methods for nanoproteomics studies.

Methods	Type of Quantification	Type of Nanoparticles	Brief Descriptions	References
Peak intensity	Relative, Absolute	Nanoparticle–protein complex pellet; Iron nanoparticles; Silver nanoparticles; Iodide-modified Ag nanoparticles;Metal oxide nanoparticles.	Peak-instensity is based on the measurement of precursor ion current areas.	[82]
Spectra-counting	Relative, Absolute	Nanoparticle–protein complex pellet; Magnetic nanoparticles.	Spectra-counting is based on counting the number of spectra on tandem MS.	[85]

tide, are other vital concerns that are required to be taken into account [77]. Based on AUC, these aspects of quantitation are required to clean up the original LC-MS data computationally [88]. In order to give an assessment and comparison of peptides in the following aspects, such as experiments, noise repression, optimal peak selection, and peak abundance normalization, the consistency of retention time needs to be taken into account during the process of original LC-MS data by computational techniques [89]. More comparable data are offered and subsequent series of analysis are more reliable through normalization [86]. Normalization of peak intensity values of proteolytic peptides which came from the platform based on LC-MS, plays an important role in the removal of systematic noise caused by sample processing and MS experiment [90]. Actually, in proteomics, all regularly applied normalization methods are of difference. These methods can be divided into scaling methods (such as normalizing by auto, linear regression), use of quality control samples [91]. Specifically, in the label-free proteomics analysis of the Parkinson model, some methods are frequently utilized to normalize the peptide concentration coming from LC-FTICR (Fourier-transform ion cyclotron resonance) MS [92]. Lily Ting *et al*. did their research on the identification of a maximum number of proteins with different richness of the metabolic-labeling proteomics in a marine bacterium by applying median normalization and lowess normalization [93].

4. FEATURE SELECTION TECHNIQUES FOR NANOPROTEOMICS

Feature selection refers to selecting a subset of features which are relevant with the classification of studied samples. Feature selection methods aimed at identifying significant differential features between different sample groups [94-96]. Methods for feature identification or selection are of great importance in nano-medicine applications [97], especially in NPs exposure biomonitoring [98]. The applications of these methods not only facilitate the identification of optimal significant differential physicochemical descriptors but also contribute to toxicity prediction of NMs [99]. For instance, feature selection method analysis of variance (ANOVA) was applied for analyzing and identifying differential proteins by induced silver nanoparticles for exhaustive mechanism of how nanomaterials traversing the lipid membrane of a given cell type [100, 101]. So far, a large number of techniques have already been established and developed to identify the relationship between the cellular toxicity of NMs and their physicochemical descriptors [97]. For example, multivariate feature selection methods are often employed exploring discriminating classification of nanomaterials between toxic and nontoxic [99]. In the area of food and beverage packaging, Carmen Sanchez Reig employed the Multi-Criteria Decision Analysis to select the nanomaterials using in food packaging [102]. Ghalati *et al*. developed a feature selection method so that they could have knowledge about the deposition of nanoparticles in the specific part of the body [103]. A QSTR-perturbation model is developed with the purpose of taking into account the size, chemical composition as well as measurement conditions of nanoparticles, and also predict the cytotoxicity of various nanoparticles against several mammalian cell lines simultaneously [104].

According to the specific theories of screening the variables and classifying the distinct groupings, types of feature selection methods could be grouped to filter, wrapper, and embedded categories. Because of different feature selection methods, it is feasible to select the best descriptor among NMs or the relevant proteins induced by the NMs. After evaluating several NMs, structure-activity relationships building model, Liu R *et al*. found that the support vector machine (SVM) model performed better in knowing the toxicity mechanisms of metal ions as well as metal oxide NPs [105].

5. PROTEOMIC QUANTIFICATION DATABASE

It is well known that it saves a lot of energy and time for science researchers when performing label-free quantification experiments if they are provided with the prepared and validated proteomics data. Currently, there are many databases including multi-organism compendium data based on MS proteomic, such as PRoteomicsIDEntifications database (PRIDE) [106], ProteomicsDB [107], PeptideAtlas [108], and Global Proteome Machine Database (GPMDB) [109]. Moreover, well-established resources aiming at proteomics data based on MRM (multiple reaction monitoring) are accessible, such as Clinical Proteomic Tumor Analysis Consortium (CPTAC) [110], MRMAssayDB [111], PanoramaWeb [112], PeptideAtlas SRM Experiment Library (PASSEL) [113], PeptideTracker [114], and SRMAtlas [115]. In addition, Plasma Proteome Database (PPD) [112] is dedicated to proteins assays reported in the plasma and serum. Detailed information on currently available data repositories for providing the MS-based proteomics data was provided in Table **4**.

As an open-source repository, CPTAC [110] is from the National Cancer Institute (NCI) with well-characterized aiming at proteomic assays. The main purposes of CPTAC are to implement robust quantification of the whole human proteins as well as standardizing the quantification of targeted MS-based assays [116, 117]. Moreover, as the largest public data repository, GPMDB with comprehensive information about proteomic based on tandem MS provided great convenience to the researchers and scientists [118]. In addition, the main purpose of GPMDB was to offer data to the scientists who participated in proteomics with tandem MS [109, 119]. Furthermore, as a web-based tool, MRMAssayDB with the abilities of mapping and linking the targeted assays have integrated many databases into a single repository [111, 120]. MRMAssayDB can annotate the proteins with details provided by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, UniProtKB, and Gene Ontologies, and it can also offer comprehensive searching for the targeted-proteomics assay based on the needs of the users, mainly through the target protein name, accession number, and annotations, for instance, pathways and disease associations. Moreover, MRMAssayDB with the visualization options increased the convenience of the users [121-123].

As an online resource with the abilities to store, share and analyze the targeted proteomic tests based on Skyline [119, 124], PanoramaWeb offers the users comprehensive viewpoint for the peptides, consisting of chromatograms for the precursors of replicates as well as plotting the peak area integrations [112, 125]. Moreover, as a generic data repository, PASSEL makes it easy for the users to submit proteomic datasets obtained from SRM (selected reaction monitoring) [113]. The results of New MRM experiments or the related primary data can also be submitted by the researchers. Moreover, the results of the corresponding internally process can also be accessible by the researchers. Furthermore, after more than ten years' development, PeptideAtlas as a web-available and public database has developed into a multi-organism compendium containing proteomics data based on shotgun MS [126-128]. The results obtained from the analysis of the MS native data files by the standardized ways are returned to the community by such resource. In addition, PeptideAtlas has also gradually developed into a key source, which can be used to design experimental plan, mine data, refine the annotation of the genome, and play a vital role in the development of experiments based on targeted proteomics [108, 129].

As a knowledge repository, the purposes of PeptideTracker are to collect and store information about the concentration variation of proteins in the bio-tissues [114]. The information is provided by PeptideTracker with the capacity of tracing various experiments as well as the acquiring protocols and conditions employed. In

Table 4. Currently available data repositories for nanoproteomics studies.

Database	Login	Raw Data	Type of Download (*File Format*)	Brief Descriptions	References
CPTAC	No	Yes	raw; mzML; PSM; prot; meta	A public repository of well-characterized, MS-based, targeted proteomic assays.	[110]
GPMDB	No	Yes	xml	A publicly available proteomic data repository derived from tandem mass spectrometry.	[109]
MRMAssayDB	No	No	csv	An integrated resource for validated targeted proteomics assays.	[111]
PanoramaWeb	Yes	Yes	sky; skyd; blib	An open-source and freely available targeted proteomics knowledge base.	[112]
PASSEL	No	Yes	xml	A kind of proteomic data repository for the collection and representation of data from SRM.	[113]
PeptideAtlas	No	Yes	raw; wiff; mzML; fasta	A multi-organism, publicly accessible compendium of peptides identified in a large set of tandem mass spectrometry proteomics experiments.	[126]
PeptideTracker	No	No	csv	A knowledge base for collecting and storing information on protein concentrations in biological tissues.	[114]
PPD	No	Yes	xml; xls	This is one of the largest resources on qualitative and quantitative information on proteins reported in plasma and serum.	[112]
PRIDE	No	Yes	raw(Thermo); wiff; xml; mgf; mztab; dat; fasta	It is one of the world-leading data repositories of mass spectrometry (MS)-based proteomics data.	[106]
ProteomicsDB	No	Yes	raw; csv; sif; svg; png	It is a protein-centric in-memory database for the exploration of large collections of quantitative mass spectrometry-based proteomics data	[107]
SRMAtlas	No	Yes	fasta; xml; mysql	A Resource of Targeted Assays to Quantify the Complete Human Proteome.	[115]

addition, according to the submitting histories of the users behind the PeptideTracker, the datasets of the submission are generally from the tissues of mouse and human [130]. Moreover, PPD was primarily described in 2005, which is one of the largest sources of proteins contained in the plasma and serum [112]. Qualitative and quantitative information provided by PPD about the proteins in plasma and serum can be essential for the discovery of biomarkers, and proteins in the extracellular vesicle isolated from plasma can also be obtained.

As one of the most famous and widely accepted data repositories, the PRIDE PRoteomicsIDEntifications (PRIDE) is a database containing the MS-based information, such as the identifications of proteins or peptides, post-translational modifications and supporting spectral data. In addition, as a key member in ProteomeXchange consortium, PRIDE with the ability to provide a single point for the submission of proteomics data based on MS to the common repositories is widely used by the researchers [106, 131, 132]. Moreover, with the capacity of exploring large collections of proteomics data based on MS, ProteomicsDB as a protein-centric in-memory database with a convenient user interface helps researchers to deal with information in the protein-centric style [107]. From the interface, there are some options that can be used to download the data the users are interested in. Furthermore, as a compendium towards targeted proteomics assays, SRMAtlas is capable of detecting and quantifying protein or peptide in complex proteome digests through MS, and the data can be obtained in www.srmatlas.org [115]. Definitive coordinates identifying the peptides from the biological tissues can be provided by human SRMAtlas.

6. TOOLS FOR ANALYZING PROTEOME QUANTIFICATION

The early stage processing and post-biological statistical analysis (aiming at identifying differential expression proteins) is pivotal in the drug discovery against cancer [133]. In proteomics research, a number of resources are freely accessible for processing and visualizing proteomic data, which including web-based software resources and stand-alone resources. As known, compared with stand-alone applications, the web-sever is available as online software regardless of the users' platform, offering advantages over stand-alone applications for accessibility and software updates. Nevertheless, web-servers are slower than the stand-alone software on account of the time cost of web connection and the shared characteristics of the computing element [130, 134]. Table **5** listed recent web servers and stand-alone software or packages and a brief introduction of each. These tools are open source and can be used in theWindows operating system and can be divided into two kinds based on their function: for data preprocessing and for statistical analysis. Among them, some quantification tools are available for both the two functions. As for only data preprocessing tools, nearly all the tools can make normalization and log transformation, but EXIMS, Phosphor-normalizer and QPEP cannot log transform. Among these preprocessing tools, Normalyzer can not only

Table 5. Descriptions of software currently available nanoproteomics studies.

Quantification Tool	Tool Type (Language)	Operating-system	Type of Input (File Format)	Developer	References
(1) Workflows (Both Preprocessing and Statistical Analysis)					
aLFQ	Open Source (*editable R*)	Windows; Linux	Log Transform, NSAF, *etc*.	ETH Zurich	[135]
DAPAR &ProStaR	Open Source (*editable R*)	Windows; Linux; OSX	Quantile, *etc*.	Universite´ Grenoble Alpes&CEA&INSERM	[155]
DanteR	Open Source (*editable R*)	Windows	EigenMS, etc.	Biological Sciences Division, Pacific Northwest National Laboratory	[147]
GiaPronto	Open Source (*editable R*)	Windows	Log Transform, Mean Centering, *etc*.	University of Pennsylvania	[157]
Gmine	Open Source (*editable Java*, *R*, *Perl*)	Windows	EigenMS, *etc.*	QIMR Berghofer Medical Research Institute	[159]
Msstats	Open Source (*editable python*)	Windows	Log Transform	Northeastern University	[143]
Perseus	Open Source (*editable Java*)	Windows	Log Transform, Rank Normalize, *etc*.	Max-Planck Institute for Biochemis-try	[133]
RIPPER	Open Source (*editable Java*)	Windows	Proximity Intensity, *etc*.	University of Minnesota	[154]
mapDIA	Open Source (*editable C++*)	Windows	Log Transform, LOW-ESS, *etc*.	National University of Singapore	[161]
(2) Workflows (Data Preprocessing)					
EXIMS	Open Source (*editable R*)	Windows	Sliding Window, *etc*.	University of Melbourne	[141]
EasyProt	Open Source (*editable Java*)	Windows	Log Transform, Median Centering, *etc.*	Swiss Center for Applied Human Toxicology	[137]
LFQbench	Open Source (*editable R*)	Windows; Linux	Log Transform, Total Intensity	Institute for Immunology, University Medical Center of the Johannes-Gutenberg University Mainz	[169]
NOREVA	Open Source (*editable R*)	Windows	Log Transform, CCMN, *etc.*	Chongqing University	[80]
Normalyzer	Open Source (*editable R*)	Windows; Linux	Log Transform, Total Intensity, *etc*.	Lund University	[86]
Phosphornormalizer	Open Source (*editable R*)	Windows; Linux; OSX	Pairwise, *etc*.	University of Minnesota	[151]
QPEP	Open Source (*editable R*)	Windows	RUV Normalization, *etc*.	University of Sydney	[172]
SPNAS	Open Source (*editable R*)	Windows	Log Transform, Linear Regression, *etc*.	Pacific Northwest National Labora-tory	[90]
(3) Workflows (Statistical Analysis)					
ROTS	Open Source (*editable R*)	Windows; Linux	ROTS	University of Turku	[164]
ProteoSign	Open Source (*editable Java, R, PHP*)	Windows	Linear Models For Mi-croarray Data	University of Crete	[167]

automatically output normalized data based on various normalization methods as in other data preprocessing software, but can also output an evaluation report. As for only statistical analysis tools, there are ROTS and ProteoSign. The former aimed at using reproducibility-optimized test statistic to identify biomarkers between two different groups. The latter mainly applied common linear models to find out differential protein from the output of MaxQuant and Proteome Discoverer quantification. As for tools which are both for preprocessing and statistical analysis, DAPAR &ProStaR, DanteR, Gmine and RIPPER do not include log transformation, but they can normalize the data produced by various protein abundance estimation methods. In addition, DAPAR &ProStaR, GiaPronto, Gmine, and Perseus are free user-friendly online softwares. Others are based on stand-alone software or R packages or framework. Among the above mentioned resources, the aLFQ, Normalyzer, phosphonormalizer, DAPAR &ProStaR, and LFQbench andmsCompare resources were reported to be only available for label-free proteome quantification technique, while the other web tools or packages including EasyProt, MSstats, PIQMIe, Perseus, GiaPronto and ROTS were accessible for processing and analyzing data from both labeling and label-free proteome quantification. Overall, these resources have greatly facilitated the development and advancement of nanoproteomics. The detailed descriptions about these tools are as follows.

It is an open-source bioinformatics tool named aLFQ which supports the calculation of protein quantities by all kinds of methods and offers automatic work process for data analysis. The popular absolute label-free quantification methods for LC-MS/MS proteomics data, such as TopN, APEX, iBAQ, SCAMPI, and NSAF, can also be supported by aLFQ [135]. aLFQ has been used in the identification of prostate cancer biomarkers in urinary exosomes [136]. Moreover, with a modern and actional web server interface, EasyProt also has some exclusive features, for instance, false discovery rate (FDR) estimation and completely combined isobaric labeling and label-free quantification analysis [137]. The essence of EasyProt is that after the identification work, one can adjust the false discovery rate, and not the submission time. The EasyProt platform includes the entire process of a specific data file format generated by the mass spectrometer and the identification and quantification results that are analyzed by specialists with a variety of backgrounds. EasyProt has been applied to identifying potent markers of ovarian cancer [138, 139].

An automated data analysis pipeline called EXIMS (http://exims.sourceforgot.net/), applies a fresh spatial distribution extraction method and sliding window normalization (SWN) to obtain optimal imaging mass spectral data based on peak intensity. By collecting features from molecular [140] image and decreasing medoid distances, EXIMS can calculate the number of possible groups by using a modified version of the subject descriptor and the gray level coexistence matrix. EXIMS has been used in imaging proteome data analysis of rat brains [141, 142]. Moreover, as an open-source package with an unrestricted family of linear mixed models, Msstats (www.msstats.org) provides statistical analysis for relative quantification data for proteins and peptides [143]. With the novel feature of model-based analyses and sample data, MSstats 2.0 can support existing current computational tools and facilitate their use by integrating the methodology across labeling and label-free operational procedure and several data acquisition strategies. Msstats is essential for the diagnosis of colorectal cancer through circulating plasma proteins [144, 145].

A web server named PIQMIe focuses on semi-quantitative proteomics data analysis and visualization for labeling and label-free proteomics experiments, and directly analyzed proteins identification and quantification results from MaxQuant quantification tool. It integrates several experimental peptides and protein recognition and quantification, and then generates a lightweight relational database which contains the linked data available and enables user--driven queries. Moreover, a succinct summary of users' proteomics experiments in visual forms will be provided. PIQMIe has been applied in a lately enunciable SILAC-based proteomics research about bone formation and mineralization [146]. Moreover, a diagrammatic R package named DanteR (http://omics.pnl.gov/ software) has a specialty of comprehensive functions on statistics and diagnosis for quantitative proteomics data processing (including labeling and label-free proteomics experiments), containing imputation, normalization, visualization, hypothesis testing and peptide-to-protein rollup [147]. Moreover, users may conveniently broaden the present function by adding personal algorithms under given Add-On tab. The previous study uses DanteR to analyze protein quantitative expression of ovarian and breast cancer tumor peptidomes [148].

A software platform containing an extensive portfolio of statistical tools, named Perseus, supports biological and biomedical researchers in high-dimensional omics data analysis such as time series analysis, normalization, pattern recognition, multiple hypothesis testing and cross-comparison comparison. With a machine learning model which imports classification and identification for diagnosis and prognosis based on existing patient groups, Perseus probes predictive protein features and supports the downstream analysis for MaxQuant quantification data based on labeling and label-free proteomics experiment. This tool has been applied in cancer-cell proteomes based on quantitative mass spectrometric profiling [149]. Moreover, an open-source software proposed to assess the applicability of all normalization methods, named Normalyzer (http://quantitativeproteomics.org/normalyzer), can automatically output normalized data based on various normalization methods and an evaluation report [86]. The input data includes targeted MS, DNA microarrays, metabolomics or label-free proteomics. The Normalyzer has been applied to the background proteomic and phosphoproteomic datasets for discovering mental uneasiness signal in the mouse model [150].

An R package named phosphonormalizer (https://bioconductor.org/packages/phosphonormalizer) is freely available from Bioconductor [151]. It can normalize label-free MS-based phosphoproteomics data from enriched samples to address bias produced by commonly Global centering-based normalization. The core of Phosphonormalizer is pairwise normalization method, which has predominant manifestation compared to other normalization methods [151, 152]. Moreover, a framework called RIPPER focuses on label-free relative quantification of mass spectrum for multi-omics studies. In particular, it integrates several already published algorithms which are designed for pre-processing, retention time alignment, analyte quantification, and analyte grouping across runs. Moreover, this tool performs based on proximity intensity normalization as the first framework [153]. RIPPER has hope to be used in both label-free quantification proteomics and analyte information extraction for metabolomic studies [154].

A bioinformatics software termed DAPAR &ProStaR can accomplish the label-free based XIC quantitative proteomics data analysis. DAPAR includes workflows for calculating missing values, filtering, normalizing, calculating peptide abundance, achieving zero-hypothesis testing, and seeking out the most likely differentially expressed proteins, and applying false discovery rates. ProStaR's visual user interface makes it easy to access DAPAR functionality through a browser. ProStaR is a visual user interface that gives convenient access to the DAPAR functionalities using a web browser [155, 156]. Moreover, a free dynamic web tool, GiaPronto, could be applied for performing statistical analyses and visualizations of proteomics data with only one click [157]. The software accomplishes normalization and statistics, helps selection of regulated proteins, biomarkers, and Gene Ontology (GO) enrichment, and offers high-resolution pictures and tables for further study. It also supports all kinds of proteomics quantification meth-

ods, such as SILAC isobaric labeling, label-free and process post-translational modifications (PTMs) [158].

A friendly online software named Gmine permits nonspecialists to exploit, cluster and contrast multidimensional biomolecular datasets with a number of help pages and a tutorial. It can also complete the analysis of any dataset (genomic, metagenomic, transcriptomic or proteomic dataset from protein microarrays) which has several hundred to several thousand biological features through various normalization methods and multivariate techniques [159]. It has been applied to differentiate chromophobe renal cell carcinoma from renal oncocytoma [160-163].

Reproducibility-optimized test statistics (ROTS), the *R* package, was a new statistical method for differential abundance analysis in proteomics data, which can be used for identifying differential significant proteins/peptides between two different groups. Specifically, ROTS adjusts a modified t statistics based on the nature of the data, and ranks the features based on the difference between the two groups. This method can accurately process different types of omics data. As reported, the ROTS has been used by a large number of different researches, from transcriptomics to proteomics (including labeling and label-free quantification techniques), performing better than other traditional methods (*e.g.*, SAM, limma, t-test) [164]. Till now, the method was applied, for example, identifying biomarkers of premature carotid atherosclerosis [165, 166]. Moreover, a freely available web-based platform called ProteoSign is used for performing proteomics analysis to find out various protein in a user-friendly and automatically way [167, 168]. Input proteomics quantification data most generated by MaxQuant and Proteome Discoverer is accepted. The software also supports current proteomics data analysis desktop applications. Using the common Linear Models For Microarray Data methodology, ProteoSign statistically assesses the differential abundance proteins between different proteome states.

The R package LFQbench reports the recognition performance, specificity and robustness of different software tools by calculating the accuracy and precision of label-free quantitative MS. Appling software developer-defined filtering criteria, LFQbench imports the results of different label-free quantification software tools which aim at processing SWATH-MS data and calculate protein level quantitative information. Meanwhile, LFQbench can be applied for evaluating the accuracy and precision performance of label-free quantitative experiments using mixed proteome samples [169]. Moreover, an open source modular framework called msCompare, evaluates the overall performance of the freely available label-free data quantification tools based on the original scoring method [170]. It can help scientists select the most accurate combination of modules for analyzing their special LC-MS data sets. This tool has been applied to find prognostic features of proteomics-based breast cancer patients [171].

A web-based software named QPEP can make an all-sided assessment of bias and variance in an accomplished iTRAQ spike-in experiment. Users have more freedom to employ their own method to analyze data set and make the results shared through QPEP comparing other new methodologies. QPEP has been used for normalizing data from labeling proteomic experiment (*e.g.*, iTRAQ) and then evaluate the performance of normalization methods with designed Latin square proteomics data set [172, 173]. Moreover, SPANS focuses on the assessment of normalization methods, including deriving peptide selection components based on normalized values [90]. For determining the most applicable normalization method which can optimize the structure of the data, but not to introduce error into the files that need to be normalized, it assesses the normalized results of the inter-group drift structure. SPANS has been used for statistical analysis of complex omics data [174].

CONCLUSION

Technological advances (especially the computational strategies) have been achieved in the OMIC-based target discovery [185-190], computer-aided drug design [191-196], and so on. In this study, recent trends of progress and development in the nanomedicine of proteomics were discussed. First, a variety of popular proteins quantification techniques including labeling and label-free strategies applied to NPs or NMs studies are overviewed and systematically discussed. Then, numerous protein profiling tools for data processing and post-biological statistical analysis and publicly available data repositories for providing enrichment MS raw data information sources are also discussed.

PROSPECTIVE

Integrating unique nanomaterials with traditional proteomic analysis established a new analysis platform termed 'nanoproteomics'. Compared with the traditional proteomic analysis, its unique advantages of improving the overall sensitivity, proteome coverage, reproducibility, and robustness make it a potentially powerful technique in nano biomedicine. In sum, nanoproteomics has been applied for personalized nanotheranostics for complex diseases. This review could provide invaluable information for nanoproteomics studies.

LIST OF ABBREVIATIONS

ANOVA	=	Analysis of variance
AUC	=	Area under the curve
CPTAC	=	Clinical Proteomic Tumor Analysis Consortium
DB	=	Database
ESI	=	Electrospray ionization
FDR	=	False discovery rate
GO	=	Gene Ontology
GPMDB	=	Global Proteome Machine Database
ICAT	=	Isotope-coded Affinity Tag
KEGG	=	Kyoto Encyclopedia of Genes and Genomes
LC-FTICR	=	Fourier-transform ion cyclotron resonance
LC	=	Liquid chromatography
LFQ	=	Label-free quantitation
MALDI	=	Matrix-assisted laser desorption/ionization
MCAT	=	Mass-coded Abundance Tagging
MRM	=	Multiple reaction monitoring
MS	=	Mass spectrometry
MeCAT	=	Metal-coded affinity tag
NCI	=	National Cancer Institute
NMs	=	Nanomaterials
NPs	=	Nanoparticles
PASSEL	=	PeptideAtlas SRM Experiment Library
PPD	=	Plasma Proteome Database
PRIDE	=	PRoteomicsIDEntifications database
PTM	=	Post-translational modifications
ROTS	=	Reproducibility-optimized test statistic
SILAC culture	=	Stable isotope labeling by amino acids in cell
SRM	=	Selected reaction monitoring
SVM	=	Support vector machine
SWN	=	Sliding window normalization
TAILS	=	Terminal amine isotopic labeling of substrates

TMT = Isobaric Labeling Tandem-mass Tag

iTRAQ = Isobaric-tag for relative-absolute quantification

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Nath Roy D, Goswami R, Pal A. Nanomaterial and toxicity: What can proteomics tell us about the nanotoxicology? Xenobiotica 2017; 47(7): 632-43. [http://dx.doi.org/10.1080/00498254.2016.1205762] [PMID: 27414072]

[2] Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T. Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis* (L.) Schrad. J Nanobiotechnol 2011; 9: 43. [http://dx.doi.org/10.1186/1477-3155-9-43] [PMID: 21943321]

[3] Fathil MF, Md Arshad MK, Ruslinda AR, *et al.* Progression in sensing cardiac troponin biomarker charge transductions on semiconducting nanomaterials. Anal Chim Acta 2016; 935: 30-43. [http://dx.doi.org/10.1016/j.aca.2016.06.012] [PMID: 27543013]

[4] He W, Wamer W, Xia Q, Yin JJ, Fu PP. Enzyme-like activity of nanomaterials. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2014; 32(2): 186-211. [http://dx.doi.org/10.1080/10590501.2014.907462] [PMID: 24875443]

[5] Zhang XQ, Yuan JN, Selvaraj G, Ji GF, Chen XR, Wei DQ. Towards the low-sensitive and high-energetic co-crystal explosive CL-20/TNT: From intermolecular interactions to structures and properties. Phys Chem Chem Phys 2018; 20(25): 17253-61. [http://dx.doi.org/10.1039/C8CP01841C] [PMID: 29901061]

[6] Yang ST, Liu Y, Wang YW, Cao A. Biosafety and bioapplication of nanomaterials by designing protein-nanoparticle interactions. Small 2013; 9(9-10): 1635-53. [http://dx.doi.org/10.1002/smll.201201492] [PMID: 23341247]

[7] Chang XL, Yang ST, Xing G. Molecular toxicity of nanomaterials. J Biomed Nanotechnol 2014; 10(10): 2828-51. [http://dx.doi.org/10.1166/jbn.2014.1936] [PMID: 25992420]

[8] Hua S, Wu SY. Editorial: Advances and Challenges in Nanomedicine. Front Pharmacol 2018; 9: 1397. [http://dx.doi.org/10.3389/fphar.2018.01397] [PMID: 30555328]

[9] Tabassum N, Verma V, Kumar M, Kumar A, Singh B. Nanomedicine in cancer stem cell therapy: From fringe to forefront. Cell Tissue Res 2018; 374(3): 427-38. [http://dx.doi.org/10.1007/s00441-018-2928-5] [PMID: 30302547]

[10] Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T. Toxicity study of silver nanoparticles synthesized from *Suaeda monoica* on Hep-2 cell line. Avicenna J Med Biotechnol 2012; 4(1): 35-9. [PMID: 23407847]

[11] Kawasaki ES, Player A. Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. Nanomedicine 2005; 1(2): 101-9. [http://dx.doi.org/10.1016/j.nano.2005.03.002] [PMID: 17292064]

[12] Arvizo R, Bhattacharya R, Mukherjee P. Gold nanoparticles: Opportunities and challenges in nanomedicine. Expert Opin Drug Deliv 2010; 7(6): 753-63. [http://dx.doi.org/10.1517/17425241003777010] [PMID: 20408736]

[13] Brown SD, Nativo P, Smith JA, *et al.* Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. J Am Chem Soc 2010; 132(13): 4678-84. [http://dx.doi.org/10.1021/ja908117a] [PMID: 20225865]

[14] Aminabad NS, Farshbaf M, Akbarzadeh A. Recent advances of gold nanoparticles in biomedical applications: State of the art. Cell Biochem Biophys 2019; 77(2): 123-37. [http://dx.doi.org/10.1007/s12013-018-0863-4] [PMID: 30570696]

[15] Krasnoslobodtsev AV, Torres MP, Kaur S, *et al.* Nanoimmunoassay with improved performance for detection of cancer biomarkers. Nanomedicine (Lond) 2015; 11(1): 167-73. [http://dx.doi.org/10.1016/j.nano.2014.08.012] [PMID: 25200613]

[16] Proetto MT, Callmann CE, Cliff J, *et al.* Tumor retention of enzyme-responsive Pt(II) drug-loaded nanoparticles imaged by nanoscale secondary ion mass spectrometry and fluorescence microscopy. ACS Cent Sci 2018; 4(11): 1477-84. [http://dx.doi.org/10.1021/acscentsci.8b00444] [PMID: 30555899]

[17] Nicolini C, Bragazzi N, Pechkova E. Nanoproteomics enabling personalized nanomedicine. Adv Drug Deliv Rev 2012; 64(13): 1522-31. [http://dx.doi.org/10.1016/j.addr.2012.06.015] [PMID: 22820526]

[18] Agrawal GK, Timperio AM, Zolla L, Bansal V, Shukla R, Rakwal R. Biomarker discovery and applications for foods and beverages: Proteomics to nanoproteomics. J Proteomics 2013; 93: 74-92. [http://dx.doi.org/10.1016/j.jprot.2013.04.014] [PMID: 23619387]

[19] Riehemann K, Schneider SW, Luger TA, Godin B, Ferrari M, Fuchs H. Nanomedicine-challenge and perspectives. Angew Chem Int Ed Engl 2009; 48(5): 872-97. [http://dx.doi.org/10.1002/anie.200802585] [PMID: 19142939]

[20] Zhang X, Ning Z, Mayne J, *et al. In vitro* metabolic labeling of intestinal microbiota for quantitative metaproteomics. Anal Chem 2016; 88(12): 6120-5. [http://dx.doi.org/10.1021/acs.analchem.6b01412] [PMID: 27248155]

[21] Khan A, Ali A, Junaid M, *et al.* Identification of novel drug targets for diamond-blackfan anemia based on RPS19 gene mutation using protein-protein interaction network. BMC Syst Biol 2018; 12(Suppl. 4): 39. [http://dx.doi.org/10.1186/s12918-018-0563-0] [PMID: 29745857]

[22] Liu H, Webster TJ. Nanomedicine for implants: A review of studies and necessary experimental tools. Biomaterials 2007; 28(2): 354-69. [http://dx.doi.org/10.1016/j.biomaterials.2006.08.049] [PMID: 21898921]

[23] Kaliamurthi S, Selvaraj G, Junaid M, Khan A, Gu K, Wei DQ. Cancer immunoinformatics: A promising era in the development of peptide vaccines for human papillomavirus-induced cervical cancer. Curr Pharm Des 2018; 24(32): 3791-817. [http://dx.doi.org/10.2174/1381612824666181106094133] [PMID: 30398106]

[24] Ray S, Reddy PJ, Choudhary S, Raghu D, Srivastava S. Emerging nanoproteomics approaches for disease biomarker detection: A current perspective. J Proteomics 2011; 74(12): 2660-81. [http://dx.doi.org/10.1016/j.jprot.2011.04.027] [PMID: 21596164]

[25] Fredolini C, Meani F, Luchini A, *et al.* Investigation of the ovarian and prostate cancer peptidome for candidate early detection markers using a novel nanoparticle biomarker capture technology. AAPS J 2010; 12(4): 504-18. [http://dx.doi.org/10.1208/s12248-010-9211-3] [PMID: 20549403]

[26] Wang L, Jia E. Ovarian cancer targeted hyaluronic acid-based nanoparticle system for paclitaxel delivery to overcome drug resistance. Drug Deliv 2016; 23(5): 1810-7. [http://dx.doi.org/10.3109/10717544.2015.1101792] [PMID: 26530693]

[27] Liu W, Yang X, Wang N, *et al.* Multiple immunosuppressive effects of CpG-c41 on intracellular TLR-mediated inflammation. Mediators Inflamm 2017; 2017: 6541729. [http://dx.doi.org/10.1155/2017/6541729] [PMID: 28539706]

[28] Mirzajani F, Askari H, Hamzelou S, *et al.* Proteomics study of silver nanoparticles toxicity on *Oryza sativa* L. Ecotoxicol Environ Saf 2014; 108: 335-9.
[http://dx.doi.org/10.1016/j.ecoenv.2014.07.013] [PMID: 25124680]

[29] Mirzajani F, Askari H, Hamzelou S, *et al.* Proteomics study of silver nanoparticles toxicity on *Bacillus thuringiensis*. Ecotoxicol Environ Saf 2014; 100: 122-30.
[http://dx.doi.org/10.1016/j.ecoenv.2013.10.009] [PMID: 24290895]

[30] Djurišić AB, Leung YH, Ng AM, *et al.* Toxicity of metal oxide nanoparticles: Mechanisms, characterization, and avoiding experimental artefacts. Small 2015; 11(1): 26-44.
[http://dx.doi.org/10.1002/smll.201303947] [PMID: 25303765]

[31] García-Santamarina S, Boronat S, Domènech A, Ayté J, Molina H, Hidalgo E. Monitoring *in vivo* reversible cysteine oxidation in proteins using ICAT and mass spectrometry. Nat Protoc 2014; 9(5): 1131-45.
[http://dx.doi.org/10.1038/nprot.2014.065] [PMID: 24743420]

[32] Yasmeen F, Raja NI, Razzaq A, Komatsu S. Gel-free/label-free proteomic analysis of wheat shoot in stress tolerant varieties under iron nanoparticles exposure. Biochim Biophys Acta 2016; 1864(11): 1586-98.
[http://dx.doi.org/10.1016/j.bbapap.2016.08.009] [PMID: 27530299]

[33] Verberkmoes NC, Russell AL, Shah M, *et al.* Shotgun metaproteomics of the human distal gut microbiota. ISME J 2009; 3(2): 179-89.
[http://dx.doi.org/10.1038/ismej.2008.108] [PMID: 18971961]

[34] Li Z, Adams RM, Chourey K, Hurst GB, Hettich RL, Pan C. Systematic comparison of label-free, metabolic labeling, and isobaric chemical labeling for quantitative proteomics on LTQ Orbitrap Velos. J Proteome Res 2012; 11(3): 1582-90.
[http://dx.doi.org/10.1021/pr200748h] [PMID: 22188275]

[35] Edelmann MJ, Shack LA, Naske CD, Walters KB, Nanduri B. SILAC-based quantitative proteomic analysis of human lung cell response to copper oxide nanoparticles. PLoS One 2014; 9(12): E114390.
[http://dx.doi.org/10.1371/journal.pone.0114390] [PMID: 25470785]

[36] Juang YM, Lai BH, Chien HJ, Ho M, Cheng TJ, Lai CC. Changes in protein expression in rat bronchoalveolar lavage fluid after exposure to zinc oxide nanoparticles: An iTRAQ proteomic approach. Rapid Commun Mass Spectrom 2014; 28(8): 974-80.
[http://dx.doi.org/10.1002/rcm.6866] [PMID: 24623703]

[37] Kumar V, Kleffmann T, Hampton MB, Cannell MB, Winterbourn CC. Redox proteomics of thiol proteins in mouse heart during ischemia/reperfusion using ICAT reagents and mass spectrometry. Free Radic Biol Med 2013; 58: 109-17.
[http://dx.doi.org/10.1016/j.freeradbiomed.2013.01.021] [PMID: 23376233]

[38] Schmidt F, Dahlmann B, Janek K, *et al.* Comprehensive quantitative proteome analysis of 20S proteasome subtypes from rat liver by isotope coded affinity tag and 2-D gel-based approaches. Proteomics 2006; 6(16): 4622-32.
[http://dx.doi.org/10.1002/pmic.200500920] [PMID: 16858736]

[39] Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol 1999; 17(10): 994-9.
[http://dx.doi.org/10.1038/13690] [PMID: 10504701]

[40] Wasdo SC. Differential binding of serum proteins to nanoparticles. Int J Nanotechnol 2008; 5: 92-115.
[http://dx.doi.org/10.1504/IJNT.2008.016550]

[41] Thompson A, Schäfer J, Kuhn K, *et al.* Tandem mass tags: A novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Anal Chem 2003; 75(8): 1895-904.
[http://dx.doi.org/10.1021/ac0262560] [PMID: 12713048]

[42] Hung CW, Tholey A. Tandem mass tag protein labeling for top-down identification and quantification. Anal Chem 2012; 84(1): 161-70.
[http://dx.doi.org/10.1021/ac202243r] [PMID: 22103715]

[43] Hahne H, Neubert P, Kuhn K, *et al.* Carbonyl-reactive tandem mass tags for the proteome-wide quantification of N-linked glycans. Anal Chem 2012; 84(8): 3716-24.
[http://dx.doi.org/10.1021/ac300197c] [PMID: 22455665]

[44] Liu JM, Sweredoski MJ, Hess S. Improved 6-Plex tandem mass tags quantification throughput using a linear ion trap-high-energy collision induced dissociation MS(3) scan. Anal Chem 2016; 88(15): 7471-5.
[http://dx.doi.org/10.1021/acs.analchem.6b01067] [PMID: 27377715]

[45] Jia W, Andaya A, Leary JA. Novel mass spectrometric method for phosphorylation quantification using cerium oxide nanoparticles and tandem mass tags. Anal Chem 2012; 84(5): 2466-73.
[http://dx.doi.org/10.1021/ac203248s] [PMID: 22304650]

[46] Adav SS, Qian J, Ang YL, *et al.* iTRAQ quantitative clinical proteomics revealed role of Na(+)K(+)-ATPase and its correlation with deamidation in vascular dementia. J Proteome Res 2014; 13(11): 4635-46.
[http://dx.doi.org/10.1021/pr500754j] [PMID: 25152327]

[47] Zhang P, Li C, Zhang P, Jin C, Pan D, Bao Y. iTRAQ-based proteomics reveals novel members involved in pathogen challenge in sea cucumber *Apostichopus japonicus*. PLoS One 2014; 9(6): E100492.
[http://dx.doi.org/10.1371/journal.pone.0100492] [PMID: 24949634]

[48] Ikeda D, Ageta H, Tsuchida K, Yamada H. iTRAQ-based proteomics reveals novel biomarkers of osteoarthritis. Biomarkers 2013; 18(7): 565-72.
[http://dx.doi.org/10.3109/1354750X.2013.810667] [PMID: 23937207]

[49] An D, Wei X, Li H, *et al.* Identification of PCSK9 as a novel serum biomarker for the prenatal diagnosis of neural tube defects using iTRAQ quantitative proteomics. Sci Rep 2015; 5: 17559.
[http://dx.doi.org/10.1038/srep17559] [PMID: 26691006]

[50] Zieske LR. A perspective on the use of iTRAQ reagent technology for protein complex and profiling studies. J Exp Bot 2006; 57(7): 1501-8.
[http://dx.doi.org/10.1093/jxb/erj168] [PMID: 16574745]

[51] Pan CH, Chuang KJ, Chen JK, *et al.* Characterization of pulmonary protein profiles in response to zinc oxide nanoparticles in mice: A 24-hour and 28-day follow-up study. Int J Nanomedicine 2015; 10: 4705-16.
[PMID: 26251593]

[52] Shadforth IP, Dunkley TP, Lilley KS, Bessant C. i-Tracker: For quantitative proteomics using iTRAQ. BMC Genomics 2005; 6: 145.
[http://dx.doi.org/10.1186/1471-2164-6-145] [PMID: 16242023]

[53] Ong SE, Blagoev B, Kratchmarova I, *et al.* Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 2002; 1(5): 376-86.
[http://dx.doi.org/10.1074/mcp.M200025-MCP200] [PMID: 12118079]

[54] Hoedt E, Zhang G, Neubert TA. Stable isotope labeling by amino acids in cell culture (SILAC) for quantitative proteomics. Adv Exp Med Biol 2014; 806: 93-106.
[http://dx.doi.org/10.1007/978-3-319-06068-2_5] [PMID: 24952180]

[55] Ahrends R, Pieper S, Kühn A, *et al.* A metal-coded affinity tag approach to quantitative proteomics. Mol Cell Proteomics 2007; 6(11): 1907-16.
[http://dx.doi.org/10.1074/mcp.M700152-MCP200] [PMID: 17627934]

[56] Wang P, Fu T, Zhang X, *et al.* Differentiating physicochemical properties between NDRIs and sNRIs clinically important for the treatment of ADHD. Biochim Biophys Acta, Gen Subj 2017; 1861(11 Pt A): 2766-77.
[http://dx.doi.org/10.1016/j.bbagen.2017.07.022] [PMID: 28757337]

[57] Bergmann U, Ahrends R, Neumann B, Scheler C, Linscheid MW. Application of metal-coded affinity tags (MeCAT): Absolute protein quantification with top-down and bottom-up workflows by metal-coded tagging. Anal Chem 2012; 84(12): 5268-75.
[http://dx.doi.org/10.1021/ac203460b] [PMID: 22659083]

[58] El-Khatib AH, He Y, Esteban-Fernández D, Linscheid MW. Application of higher energy collisional dissociation (HCD) to the fragmentation of new DOTA-based labels and N-termini DOTA-labeled peptides. J Mass Spectrom 2017; 52(8): 543-9.
[http://dx.doi.org/10.1002/jms.3954] [PMID: 28577300]

[59] Messana I, Cabras T, Iavarone F, Vincenzoni F, Urbani A, Castagnola M. Unraveling the different proteomic platforms. J Sep Sci 2013; 36(1): 128-39. [http://dx.doi.org/10.1002/jssc.201200830] [PMID: 23212829]

[60] Prudova A, Gocheva V, Auf dem Keller U, *et al.* TAILS N-terminomics and proteomics show protein degradation dominates over proteolytic processing by cathepsins in pancreatic tumors. Cell Rep 2016; 16(6): 1762-73. [http://dx.doi.org/10.1016/j.celrep.2016.06.086] [PMID: 27477282]

[61] Kleifeld O, Doucet A, Prudova A, *et al.* Identifying and quantifying proteolytic events and the natural N terminome by terminal amine isotopic labeling of substrates. Nat Protoc 2011; 6(10): 1578-611. [http://dx.doi.org/10.1038/nprot.2011.382] [PMID: 21959240]

[62] auf dem Keller U, Overall CM. CLIPPER: An add-on to the TopFIND Proteomic Pipeline for the automated analysis of TAILS N-terminomics data. Biol Chem 2012; 393(12): 1477-83. [PMID: 23667905]

[63] Kleifeld O, Doucet A, auf dem Keller U, *et al.* Isotopic labeling of terminal amines in complex samples identifies protein N-termini and protease cleavage products. Nat Biotechnol 2010; 28(3): 281-8. [http://dx.doi.org/10.1038/nbt.1611] [PMID: 20208520]

[64] Leclercq A, Nonell A, Todolí Torró JL, *et al.* Introduction of organic/hydro-organic matrices in inductively coupled plasma optical emission spectrometry and mass spectrometry: A tutorial review. Part II. Practical considerations. Anal Chim Acta 2015; 885: 57-91. [http://dx.doi.org/10.1016/j.aca.2015.04.039] [PMID: 26231892]

[65] Milton MJT, Wielgosz RI. Uncertainty in SI-traceable measurements of amount of substance by isotope dilution mass spectrometry. Metrologia 2000; 37: 199. [http://dx.doi.org/10.1088/0026-1394/37/3/3]

[66] Sötebier CA, Weidner SM, Jakubowski N, Panne U, Bettmer J. Separation and quantification of silver nanoparticles and silver ions using reversed phase high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry in combination with isotope dilution analysis. J Chromatogr A 2016; 1468: 102-8. [http://dx.doi.org/10.1016/j.chroma.2016.09.028] [PMID: 27663727]

[67] Jager PL, Vaalburg W, Pruim J, de Vries EG, Langen KJ, Piers DA. Radiolabeled amino acids: Basic aspects and clinical applications in oncology. J Nucl Med 2001; 42(3): 432-45. [PMID: 11337520]

[68] Miller PW, Long NJ, Vilar R, Gee AD. Synthesis of 11C, 18F, 15O, and 13N radiolabels for positron emission tomography. Angew Chem Int Ed Engl 2008; 47(47): 8998-9033. [http://dx.doi.org/10.1002/anie.200800222] [PMID: 18988199]

[69] Schirrmacher E, Wängler B, Cypryk M, *et al.* Synthesis of p-(di-tert-butyl[(18)F]fluorosilyl)benzaldehyde ([(18)F]SiFA-A) with high specific activity by isotopic exchange: A convenient labeling synthon for the (18)F-labeling of N-amino-oxy derivatized peptides. Bioconjug Chem 2007; 18(6): 2085-9. [http://dx.doi.org/10.1021/bc700195y] [PMID: 18030993]

[70] Peracchia MT, Fattal E, Desmaële D, *et al.* Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. J Control Release 1999; 60(1): 121-8. [http://dx.doi.org/10.1016/S0168-3659(99)00063-2] [PMID: 10370176]

[71] Cagney G, Emili A. *De novo* peptide sequencing and quantitative profiling of complex protein mixtures using mass-coded abundance tagging. Nat Biotechnol 2002; 20(2): 163-70. [http://dx.doi.org/10.1038/nbt0202-163] [PMID: 11821862]

[72] Tao WA, Aebersold R. Advances in quantitative proteomics *via* stable isotope tagging and mass spectrometry. Curr Opin Biotechnol 2003; 14(1): 110-8. [http://dx.doi.org/10.1016/S0958-1669(02)00018-6] [PMID: 12566010]

[73] Wang W, Zhou H, Lin H, *et al.* Quantification of proteins and metabolites by mass spectrometry without isotopic labeling or spiked standards. Anal Chem 2003; 75(18): 4818-26. [http://dx.doi.org/10.1021/ac026468x] [PMID: 14674459]

[74] Zybailov B, Mosley AL, Sardiu ME, Coleman MK, Florens L, Washburn MP. Statistical analysis of membrane proteome expression changes in *Saccharomyces cerevisiae*. J Proteome Res 2006; 5(9): 2339-47. [http://dx.doi.org/10.1021/pr060161n] [PMID: 16944946]

[75] Tang J, Zhang Y, Fu J, *et al.* Computational advances in the label-free quantification of cancer proteomics data. Curr Pharm Des 2018; 24(32): 3842-58. [http://dx.doi.org/10.2174/1381612824666181102125638] [PMID: 30387388]

[76] Tang J, Fu J, Wang Y, *et al.* ANPELA: Analysis and performance assessment of the label-free quantification workflow for metaproteomic studies. Brief Bioinform 2019. [http://dx.doi.org/10.1093/bib/bby127] [PMID: 30649171]

[77] Neilson KA, Ali NA, Muralidharan S, *et al.* Less label, more free: Approaches in label-free quantitative mass spectrometry. Proteomics 2011; 11(4): 535-53. [http://dx.doi.org/10.1002/pmic.201000553] [PMID: 21243637]

[78] Podwojski K, Eisenacher M, Kohl M, *et al.* Peek a peak: A glance at statistics for quantitative label-free proteomics. Expert Rev Proteomics 2010; 7(2): 249-61. [http://dx.doi.org/10.1586/epr.09.107] [PMID: 20377391]

[79] Blackburn K, Cheng FY, Williamson JD, Goshe MB. Data-independent liquid chromatography/mass spectrometry (LC/MS(E)) detection and quantification of the secreted Apium graveolens pathogen defense protein mannitol dehydrogenase. Rapid Commun Mass Spectrom 2010; 24(7): 1009-16. [http://dx.doi.org/10.1002/rcm.4476] [PMID: 20213632]

[80] Li B, Tang J, Yang Q, *et al.* NOREVA: Normalization and evaluation of MS-based metabolomics data. Nucleic Acids Res 2017; 45(W1): W162-70. [http://dx.doi.org/10.1093/nar/gkx449] [PMID: 28525573]

[81] Liu H, Sadygov RG, Yates JR III. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. Anal Chem 2004; 76(14): 4193-201. [http://dx.doi.org/10.1021/ac0498563] [PMID: 15253663]

[82] Gioria S, Urbán P, Hajduch M, *et al.* Proteomics study of silver nanoparticles on Caco-2 cells. Toxicol *In Vitro* 2018; 50: 347-72. [http://dx.doi.org/10.1016/j.tiv.2018.03.015] [PMID: 29626626]

[83] Xu LJ, Zong C, Zheng XS, Hu P, Feng JM, Ren B. Label-free detection of native proteins by surface-enhanced Raman spectroscopy using iodide-modified nanoparticles. Anal Chem 2014; 86(4): 2238-45. [http://dx.doi.org/10.1021/ac403974n] [PMID: 24460183]

[84] Mustafa G, Sakata K, Komatsu S. Proteomic analysis of flooded soybean root exposed to aluminum oxide nanoparticles. J Proteomics 2015; 128: 280-97. [http://dx.doi.org/10.1016/j.jprot.2015.08.010] [PMID: 26306862]

[85] Vogt C, Pernemalm M, Kohonen P, *et al.* Proteomics analysis reveals distinct corona composition on magnetic nanoparticles with different surface coatings: Implications for interactions with primary human macrophages. PLoS One 2015; 10(10): E0129008. [http://dx.doi.org/10.1371/journal.pone.0129008] [PMID: 26444829]

[86] Chawade A, Alexandersson E, Levander F. Normalyzer: A tool for rapid evaluation of normalization methods for omics data sets. J Proteome Res 2014; 13(6): 3114-20. [http://dx.doi.org/10.1021/pr401264n] [PMID: 24766612]

[87] Karpievitch YV, Dabney AR, Smith RD. Normalization and missing value imputation for label-free LC-MS analysis. BMC Bioinformatics 2012; 13(Suppl. 16): S5. [http://dx.doi.org/10.1186/1471-2105-13-S16-S5] [PMID: 23176322]

[88] Listgarten J, Emili A. Statistical and computational methods for comparative proteomic profiling using liquid chromatography-tandem mass spectrometry. Mol Cell Proteomics 2005; 4(4): 419-34. [http://dx.doi.org/10.1074/mcp.R500005-MCP200] [PMID: 15741312]

[89] Xue W, Wang P, Tu G, *et al.* Computational identification of the binding mechanism of a triple reuptake inhibitor amitifadine for the treatment of major depressive disorder. Phys Chem Chem Phys 2018; 20(9): 6606-16. [http://dx.doi.org/10.1039/C7CP07869B] [PMID: 29451287]

[90] Webb-Robertson BJ, Matzke MM, Jacobs JM, Pounds JG, Waters KM. A statistical selection strategy for normalization procedures in LC-MS proteomics experiments through dataset-dependent ranking of normalization scaling factors. Proteomics 2011; 11(24): 4736-41. [http://dx.doi.org/10.1002/pmic.201100078] [PMID: 22038874]

[91] De Livera AM, Sysi-Aho M, Jacob L, *et al.* Statistical methods for handling unwanted variation in metabolomics data. Anal Chem 2015; 87(7): 3606-15. [http://dx.doi.org/10.1021/ac502439y] [PMID: 25692814]

[92] Callister SJ, Barry RC, Adkins JN, *et al.* Normalization approaches for removing systematic biases associated with mass spectrometry and label-free proteomics. J Proteome Res 2006; 5(2): 277-86. [http://dx.doi.org/10.1021/pr050300l] [PMID: 16457593]

[93] Ting L, Cowley MJ, Hoon SL, Guilhaus M, Raftery MJ, Cavicchioli R. Normalization and statistical analysis of quantitative proteomics data generated by metabolic labeling. Mol Cell Proteomics 2009; 8(10): 2227-42. [http://dx.doi.org/10.1074/mcp.M800462-MCP200] [PMID: 19605365]

[94] Selvaraj G, Kaliamurthi S, Kaushik AC, *et al.* Identification of target gene and prognostic evaluation for lung adenocarcinoma using gene expression meta-analysis, network analysis and neural network algorithms. J Biomed Inform 2018; 86: 120-34. [http://dx.doi.org/10.1016/j.jbi.2018.09.004] [PMID: 30195659]

[95] Selvaraj G, Kaliamurthi S, Lin S, Gu K, Wei DQ. Prognostic impact of tissue inhibitor of metalloproteinase-1 in non-small cell lung cancer: Systematic review and meta-analysis. Curr Med Chem 2018. [http://dx.doi.org/10.2174/0929867325666180904114455] [PMID: 30182835]

[96] Yang Q, Wang Y, Zhang S, *et al.* Biomarker discovery for immunotherapy of pituitary adenomas: Enhanced robustness and prediction ability by modern computational tools. Int J Mol Sci 2019; 20(1): 20. [http://dx.doi.org/10.3390/ijms20010151] [PMID: 30609812]

[97] Jha SK, Yoon TH, Pan Z. Multivariate statistical analysis for selecting optimal descriptors in the toxicity modeling of nanomaterials. Comput Biol Med 2018; 99: 161-72. [http://dx.doi.org/10.1016/j.compbiomed.2018.06.012] [PMID: 29933127]

[98] Marie-Desvergne C, Dubosson M, Mossuz VC. Evaluation of a new method for the collection and measurement of 8-isoprostane in exhaled breath for future application in nanoparticle exposure biomonitoring. J Breath Res 2018; 12(3): 031001. [http://dx.doi.org/10.1088/1752-7163/aabdf2] [PMID: 29651988]

[99] Patel T, Telesca D, George S, Nel AE. Toxicity profiling of engineered nanomaterials *via* multivariate dose-response surface modeling. Ann Appl Stat 2012; 6(4): 1707-29. [http://dx.doi.org/10.1214/12-AOAS563] [PMID: 25191531]

[100] Štefanić PP, Cvjetko P, Biba R, *et al.* Physiological, ultrastructural and proteomic responses of tobacco seedlings exposed to silver nanoparticles and silver nitrate. Chemosphere 2018; 209: 640-53. [http://dx.doi.org/10.1016/j.chemosphere.2018.06.128] [PMID: 29958162]

[101] Selvaraj G, Kaliamurthi S, Cakmak ZE, Cakmak T. *In silico* validation of microalgal metabolites against Diabetes mellitus. Diabetes Mel 2017; 20: 301-7. [http://dx.doi.org/10.14341/DM8212]

[102] Reig CS, Lopez AD, Ramos MH. Nanomaterials: A Map for Their Selection in Food Packaging Applications. Packag Technol Sci 2015; 27: 839-66. [http://dx.doi.org/10.1002/pts.2076]

[103] Farhadi Ghalati P, Keshavarzian E, Abouali O, Faramarzi A, Tu J, Shakibafard A. Numerical analysis of micro- and nano-particle deposition in a realistic human upper airway. Comput Biol Med 2012; 42(1): 39-49. [http://dx.doi.org/10.1016/j.compbiomed.2011.10.005] [PMID: 22061046]

[104] Luan F, Kleandrova VV, González-Díaz H, *et al.* Computer-aided nanotoxicology: Assessing cytotoxicity of nanoparticles under diverse experimental conditions by using a novel QSTR-perturbation approach. Nanoscale 2014; 6(18): 10623-30. [http://dx.doi.org/10.1039/C4NR01285B] [PMID: 25083742]

[105] Liu R, Zhang HY, Ji ZX, *et al.* Development of structure-activity relationship for metal oxide nanoparticles. Nanoscale 2013; 5(12): 5644-53. [http://dx.doi.org/10.1039/c3nr01533e] [PMID: 23689214]

[106] Vizcaíno JA, Csordas A, del-Toro N, *et al.* 2016 update of the PRIDE database and its related tools. Nucleic Acids Res 2016; 44(D1): D447-56. [http://dx.doi.org/10.1093/nar/gkv1145] [PMID: 26527722]

[107] Schmidt T, Samaras P, Frejno M, *et al.* ProteomicsDB. Nucleic Acids Res 2018; 46(D1): D1271-81. [http://dx.doi.org/10.1093/nar/gkx1029] [PMID: 29106664]

[108] Deutsch EW, Lam H, Aebersold R. PeptideAtlas: A resource for target selection for emerging targeted proteomics workflows. EMBO Rep 2008; 9(5): 429-34. [http://dx.doi.org/10.1038/embor.2008.56] [PMID: 18451766]

[109] Craig R, Cortens JP, Beavis RC. Open source system for analyzing, validating, and storing protein identification data. J Proteome Res 2004; 3(6): 1234-42. [http://dx.doi.org/10.1021/pr049882h] [PMID: 15595733]

[110] Whiteaker JR, Halusa GN, Hoofnagle AN, *et al.* CPTAC Assay Portal: A repository of targeted proteomic assays. Nat Methods 2014; 11(7): 703-4. [http://dx.doi.org/10.1038/nmeth.3002] [PMID: 24972168]

[111] Bhowmick P, Mohammed Y, Borchers CH. MRMAssayDB: An integrated resource for validated targeted proteomics assays. Bioinformatics 2018; 34(20): 3566-71. [http://dx.doi.org/10.1093/bioinformatics/bty385] [PMID: 29762640]

[112] Nanjappa V, Thomas JK, Marimuthu A, *et al.* Plasma Proteome Database as a resource for proteomics research: 2014 update. Nucleic Acids Res 2014; 42(Database issue): D959-65. [http://dx.doi.org/10.1093/nar/gkt1251] [PMID: 24304897]

[113] Farrah T, Deutsch EW, Kreisberg R, *et al.* PASSEL: The PeptideAtlas SRMexperiment library. Proteomics 2012; 12(8): 1170-5. [http://dx.doi.org/10.1002/pmic.201100515] [PMID: 22318887]

[114] Mohammed Y, Bhowmick P, Smith DS, *et al.* PeptideTracker: A knowledge base for collecting and storing information on protein concentrations in biological tissues. Proteomics 2017; 17(7): 17. [http://dx.doi.org/10.1002/pmic.201600210] [PMID: 27683069]

[115] Kusebauch U, Campbell DS, Deutsch EW, *et al.* Human srmatlas: A resource of targeted assays to quantify the complete human proteome. Cell 2016; 166(3): 766-78. [http://dx.doi.org/10.1016/j.cell.2016.06.041] [PMID: 27453469]

[116] Whiteaker JR, Halusa GN, Hoofnagle AN, *et al.* Using the CPTAC Assay Portal to Identify and Implement Highly Characterized Targeted Proteomics Assays. Methods Mol Biol 2016; 1410: 223-36. [http://dx.doi.org/10.1007/978-1-4939-3524-6_13] [PMID: 26867747]

[117] Zhu F, Han B, Kumar P, *et al.* Update of TTD: Therapeutic Target Database. Nucleic Acids Res 2010; 38(Database issue): D787-91. [http://dx.doi.org/10.1093/nar/gkp1014] [PMID: 19933260]

[118] Zhang CC, Rogalski JC, Evans DM, Klockenbusch C, Beavis RC, Kast J. *In silico* protein interaction analysis using the global proteome machine database. J Proteome Res 2011; 10(2): 656-68. [http://dx.doi.org/10.1021/pr1008652] [PMID: 21067242]

[119] Jia J, Zhu F, Ma X, *et al.* Mechanisms of drug combinations: Interaction and network perspectives. Nat Rev Drug Discov 2009; 8(2): 111-28. [http://dx.doi.org/10.1038/nrd2683] [PMID: 19180105]

[120] Rao HB, Zhu F, Yang GB, Li ZR, Chen YZ. Update of PROFEAT: A web server for computing structural and physicochemical features of proteins and peptides from amino acid sequence. Nucleic Acids Res 2011; 39(Web Server issue): W385-90. [http://dx.doi.org/10.1093/nar/gkr284] [PMID: 21609959]

[121] Li YH, Yu CY, Li XX, *et al.* Therapeutic target database update 2018: Enriched resource for facilitating bench-to-clinic research of targeted therapeutics. Nucleic Acids Res 2018; 46(D1): D1121-7. [PMID: 29140520]

[122] Zhu F, Qin C, Tao L, *et al.* Clustered patterns of species origins of nature-derived drugs and clues for future bioprospecting. Proc Natl Acad Sci USA 2011; 108(31): 12943-8. [http://dx.doi.org/10.1073/pnas.1107336108] [PMID: 21768386]

[123] Zheng G, Xue W, Yang F, *et al.* Revealing vilazodone's binding mechanism underlying its partial agonism to the 5-HT_{1A} receptor in the treatment of major depressive disorder. Phys Chem Chem Phys 2017; 19(42): 28885-96. [http://dx.doi.org/10.1039/C7CP05688E] [PMID: 29057413]

[124] MacLean B, Tomazela DM, Shulman N, *et al.* Skyline: An open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics 2010; 26(7): 966-8. [http://dx.doi.org/10.1093/bioinformatics/btq054] [PMID: 20147306]

[125] Zhu F, Shi Z, Qin C, *et al.* Therapeutic target database update 2012: A resource for facilitating target-oriented drug discovery. Nucleic Acids Res 2012; 40(Database issue): D1128-36. [http://dx.doi.org/10.1093/nar/gkr797] [PMID: 21948793]

[126] Desiere F, Deutsch EW, King NL, *et al.* The PeptideAtlas project. Nucleic Acids Res 2006; 34(Database issue): D655-8. [http://dx.doi.org/10.1093/nar/gkj040] [PMID: 16381952]

[127] Zhu F, Ma XH, Qin C, *et al.* Drug discovery prospect from untapped species: Indications from approved natural product drugs. PLoS One 2012; 7(7): E39782. [http://dx.doi.org/10.1371/journal.pone.0039782] [PMID: 22808057]

[128] Zhu F, Han LY, Chen X, *et al.* Homology-free prediction of functional class of proteins and peptides by support vector machines. Curr Protein Pept Sci 2008; 9(1): 70-95. [http://dx.doi.org/10.2174/138920308783565697] [PMID: 18336324]

[129] Tao L, Zhu F, Qin C, *et al.* Nature's contribution to today's pharmacopeia. Nat Biotechnol 2014; 32(10): 979-80. [http://dx.doi.org/10.1038/nbt.3034] [PMID: 25299914]

[130] Yang F, Zheng G, Fu T, *et al.* Prediction of the binding mode and resistance profile for a dual-target pyrrolyl diketo acid scaffold against HIV-1 integrase and reverse-transcriptase-associated ribonuclease H. Phys Chem Chem Phys 2018; 20(37): 23873-84. [http://dx.doi.org/10.1039/C8CP01843J] [PMID: 29947629]

[131] Reisinger F, del-Toro N, Ternent T, Hermjakob H, Vizcaíno JA. Introducing the PRIDE Archive RESTful web services. Nucleic Acids Res 2015; 43(W1): W599-604. [http://dx.doi.org/10.1093/nar/gkv382] [PMID: 25904633]

[132] Fu J, Tang J, Wang Y, *et al.* Discovery of the Consistently Well-Performed Analysis Chain for SWATH-MS Based Pharmacoproteomic Quantification. Front Pharmacol 2018; 9: 681. [http://dx.doi.org/10.3389/fphar.2018.00681] [PMID: 29997509]

[133] Tyanova S, Temu T, Sinitcyn P, *et al.* The Perseus computational platform for comprehensive analysis of (prote)omics data. Nat Methods 2016; 13(9): 731-40. [http://dx.doi.org/10.1038/nmeth.3901] [PMID: 27348712]

[134] Lee DY, Saha R, Yusufi FN, Park W, Karimi IA. Web-based applications for building, managing and analysing kinetic models of biological systems. Brief Bioinform 2009; 10(1): 65-74. [http://dx.doi.org/10.1093/bib/bbn039] [PMID: 18805901]

[135] Rosenberger G, Ludwig C, Röst HL, Aebersold R, Malmström L. aLFQ: An R-package for estimating absolute protein quantities from label-free LC-MS/MS proteomics data. Bioinformatics 2014; 30(17): 2511-3. [http://dx.doi.org/10.1093/bioinformatics/btu200] [PMID: 24753486]

[136] Øverbye A, Skotland T, Koehler CJ, *et al.* Identification of prostate cancer biomarkers in urinary exosomes. Oncotarget 2015; 6(30): 30357-76. [http://dx.doi.org/10.18632/oncotarget.4851] [PMID: 26196085]

[137] Gluck F, Hoogland C, Antinori P, *et al.* EasyProt-an easy-to-use graphical platform for proteomics data analysis. J Proteomics 2013; 79: 146-60. [http://dx.doi.org/10.1016/j.jprot.2012.12.012] [PMID: 23277275]

[138] Wang P, Yang F, Yang H, *et al.* Identification of dual active agents targeting 5-HT1A and SERT by combinatorial virtual screening methods. Biomed Mater Eng 2015; 26(Suppl. 1): S2233-9. [http://dx.doi.org/10.3233/BME-151529] [PMID: 26406003]

[139] Zhu F, Zheng CJ, Han LY, *et al.* Trends in the exploration of anticancer targets and strategies in enhancing the efficacy of drug targeting. Curr Mol Pharmacol 2008; 1(3): 213-32. [http://dx.doi.org/10.2174/1874467210801030213] [PMID: 20021435]

[140] Fu T, Zheng G, Tu G, *et al.* Exploring the binding mechanism of metabotropic glutamate receptor 5 negative allosteric modulators in clinical trials by molecular dynamics simulations. ACS Chem Neurosci 2018; 9(6): 1492-502. [http://dx.doi.org/10.1021/acschemneuro.8b00059] [PMID: 29522307]

[141] Wijetunge CD, Saeed I, Boughton BA, *et al.* EXIMS: An improved data analysis pipeline based on a new peak picking method for EXploring Imaging Mass Spectrometry data. Bioinformatics 2015; 31(19): 3198-206. [http://dx.doi.org/10.1093/bioinformatics/btv356] [PMID: 26063840]

[142] Tao L, Zhu F, Xu F, Chen Z, Jiang YY, Chen YZ. Co-targeting cancer drug escape pathways confers clinical advantage for multi-target anticancer drugs. Pharmacol Res 2015; 102: 123-31. [http://dx.doi.org/10.1016/j.phrs.2015.09.019] [PMID: 26438971]

[143] Choi M, Chang CY, Clough T, *et al.* MSstats: An R package for statistical analysis of quantitative mass spectrometry-based proteomic experiments. Bioinformatics 2014; 30(17): 2524-6. [http://dx.doi.org/10.1093/bioinformatics/btu305] [PMID: 24794931]

[144] Surinova S, Choi M, Tao S, *et al.* Prediction of colorectal cancer diagnosis based on circulating plasma proteins. EMBO Mol Med 2015; 7(9): 1166-78. [http://dx.doi.org/10.15252/emmm.201404873] [PMID: 26253081]

[145] Li YH, Xu JY, Tao L, *et al.* SVM-Prot 2016: A web-server for machine learning prediction of protein functional families from sequence irrespective of similarity. PLoS One 2016; 11(8): E0155290. [http://dx.doi.org/10.1371/journal.pone.0155290] [PMID: 27525735]

[146] Kuzniar A, Kanaar R. PIQMIe: A web server for semi-quantitative proteomics data management and analysis. Nucleic Acids Res 2014; 42(Web Server issue): W100-6. [http://dx.doi.org/10.1093/nar/gku478] [PMID: 24861615]

[147] Taverner T, Karpievitch YV, Polpitiya AD, *et al.* DanteR: An extensible R-based tool for quantitative analysis of -omics data. Bioinformatics 2012; 28(18): 2404-6. [http://dx.doi.org/10.1093/bioinformatics/bts449] [PMID: 22815360]

[148] Xu Z, Wu C, Xie F, *et al.* Comprehensive quantitative analysis of ovarian and breast cancer tumor peptidomes. J Proteome Res 2015; 14(1): 422-33. [http://dx.doi.org/10.1021/pr500840w] [PMID: 25350482]

[149] Bohnenberger H, Ströbel P, Mohr S, *et al.* Quantitative mass spectrometric profiling of cancer-cell proteomes derived from liquid and solid tumors. J Vis Exp 2015; (96): E52435. [http://dx.doi.org/10.3791/52435] [PMID: 25867170]

[150] Kuzmanov U, Guo H, Buchsbaum D, *et al.* Global phosphoproteomic profiling reveals perturbed signaling in a mouse model of dilated cardiomyopathy. Proc Natl Acad Sci USA 2016; 113(44): 12592-7. [http://dx.doi.org/10.1073/pnas.1606444113] [PMID: 27742792]

[151] Saraei S, Suomi T, Kauko O, Elo LL, Stegle O. Phosphonormalizer: An R package for normalization of MS-based label-free phosphoproteomics. Bioinformatics 2018; 34(4): 693-4. [http://dx.doi.org/10.1093/bioinformatics/btx573] [PMID: 28968644]

[152] Yang H, Qin C, Li YH, *et al.* Therapeutic target database update 2016: Enriched resource for bench to clinical drug target and targeted pathway information. Nucleic Acids Res 2016; 44(D1): D1069-74. [http://dx.doi.org/10.1093/nar/gkv1230] [PMID: 26578601]

[153] Li B, Tang J, Yang Q, *et al.* Performance evaluation and online realization of data-driven normalization methods used in LC/MS based untargeted metabolomics analysis. Sci Rep 2016; 6: 38881. [http://dx.doi.org/10.1038/srep38881] [PMID: 27958387]

[154] Van Riper SK, Higgins L, Carlis JV, Griffin TJ. RIPPER: A framework for MS1 only metabolomics and proteomics label-free relative quantification. Bioinformatics 2016; 32(13): 2035-7. [http://dx.doi.org/10.1093/bioinformatics/btw091] [PMID: 27153682]

[155] Wieczorek S, Combes F, Lazar C, *et al.* DAPAR & ProStaR: Software to perform statistical analyses in quantitative discovery proteomics. Bioinformatics 2017; 33(1): 135-6. [http://dx.doi.org/10.1093/bioinformatics/btw580] [PMID: 27605098]

[156] Yang FY, Fu TT, Zhang XY, *et al.* Comparison of computational model and X-ray crystal structure of human serotonin transporter: Potential application for the pharmacology of human monoamine transporters. Mol Simul 2017; 43: 1089-98. [http://dx.doi.org/10.1080/08927022.2017.1309653]

[157] Weiner AK, Sidoli S, Diskin SJ, Garcia BA. Graphical interpretation and analysis of proteins and their ontologies (GiaPronto): A one-click graph visualization software for proteomics data sets. Mol Cell Proteomics 2018; 17(7): 1426-31. [http://dx.doi.org/10.1074/mcp.TIR117.000438] [PMID: 29118029]

[158] Chang C, Xu K, Guo C, *et al.* PANDA-view: An easy-to-use tool for statistical analysis and visualization of quantitative proteomics data. Bioinformatics 2018; 34(20): 3594-6. [http://dx.doi.org/10.1093/bioinformatics/bty408] [PMID: 29790911]

[159] Proietti C, Zakrzewski M, Watkins TS, *et al.* Mining, visualizing and comparing multidimensional biomolecular data using the Genomics Data Miner (GMine) Web-Server. Sci Rep 2016; 6: 38178. [http://dx.doi.org/10.1038/srep38178] [PMID: 27922118]

[160] Yu CY, Li XX, Yang H, *et al.* Assessing the performances of protein function prediction algorithms from the perspectives of identification accuracy and false discovery rate. Int J Mol Sci 2018; 19(1): E183. [http://dx.doi.org/10.3390/ijms19010183] [PMID: 29316706]

[161] Teo G, Kim S, Tsou CC, *et al.* mapDIA: Preprocessing and statistical analysis of quantitative proteomics data from data independent acquisition mass spectrometry. J Proteomics 2015; 129: 108-20. [http://dx.doi.org/10.1016/j.jprot.2015.09.013] [PMID: 26381204]

[162] Ebhardt HA, Degen S, Tadini V, *et al.* Comprehensive proteome analysis of human skeletal muscle in cachexia and sarcopenia: A pilot study. J Cachexia Sarcopenia Muscle 2017; 8(4): 567-82. [http://dx.doi.org/10.1002/jcsm.12188] [PMID: 28296247]

[163] Xue W, Wang P, Li B, *et al.* Identification of the inhibitory mechanism of FDA approved selective serotonin reuptake inhibitors: An insight from molecular dynamics simulation study. Phys Chem Chem Phys 2016; 18(4): 3260-71. [http://dx.doi.org/10.1039/C5CP05771J] [PMID: 26745505]

[164] Suomi T, Seyednasrollah F, Jaakkola MK, Faux T, Elo LL. ROTS: An R package for reproducibility-optimized statistical testing. PLOS Comput Biol 2017; 13(5): E1005562. [http://dx.doi.org/10.1371/journal.pcbi.1005562] [PMID: 28542205]

[165] Bhosale SD, Moulder R, Venäläinen MS, *et al.* Serum proteomic profiling to identify biomarkers of premature carotid atherosclerosis. Sci Rep 2018; 8(1): 9209. [http://dx.doi.org/10.1038/s41598-018-27265-9] [PMID: 29907817]

[166] Wang P, Zhang X, Fu T, *et al.* Differentiating physicochemical properties between addictive and nonaddictive ADHD drugs revealed by molecular dynamics simulation studies. ACS Chem Neurosci 2017; 8(6): 1416-28. [http://dx.doi.org/10.1021/acschemneuro.7b00173] [PMID: 28557437]

[167] Efstathiou G, Antonakis AN, Pavlopoulos GA, *et al.* ProteoSign: An end-user online differential proteomics statistical analysis platform. Nucleic Acids Res 2017; 45(W1): W300-6. [http://dx.doi.org/10.1093/nar/gkx444] [PMID: 28520987]

[168] Li YH, Wang PP, Li XX, *et al.* The Human kinome targeted by FDA approved multi-target drugs and combination products: A comparative study from the drug-target interaction network perspective. PLoS One 2016; 11(11): E0165737. [http://dx.doi.org/10.1371/journal.pone.0165737] [PMID: 27828998]

[169] Navarro P, Kuharev J, Gillet LC, *et al.* A multicenter study benchmarks software tools for label-free proteome quantification. Nat Biotechnol 2016; 34(11): 1130-6. [http://dx.doi.org/10.1038/nbt.3685] [PMID: 27701404]

[170] Hoekman B, Breitling R, Suits F, Bischoff R, Horvatovich P. msCompare: A framework for quantitative analysis of label-free LC-MS data for comparative candidate biomarker studies. Mol Cell Proteomics 2012; 11(6): M111 015974.

[171] Pavlou M. Developing a Proteomic Prognostic Signature for Breast Cancer Patients. Doctoral 2014.

[172] Strbenac D, Zhong L, Raftery MJ, *et al.* Quantitative performance evaluator for proteomics (QPEP): Web-based application for reproducible evaluation of proteomics preprocessing methods. J Proteome Res 2017; 16(7): 2359-69. [http://dx.doi.org/10.1021/acs.jproteome.6b00882] [PMID: 28580786]

[173] Xu J, Wang P, Yang H, *et al.* Comparison of FDA approved kinase targets to clinical trial ones: Insights from their system profiles and drug-target interaction networks. BioMed Res Int 2016; 2016: 2509385. [http://dx.doi.org/10.1155/2016/2509385] [PMID: 27547755]

[174] McDermott JE, Wang J, Mitchell H, *et al.* Challenges in Biomarker Discovery: Combining Expert Insights with Statistical Analysis of Complex Omics Data. Expert Opin Med Diagn 2013; 7(1): 37-51. [http://dx.doi.org/10.1517/17530059.2012.718329] [PMID: 23335946]

[175] Latosinska A, Vougas K, Makridakis M, *et al.* Comparative Analysis of Label-Free and 8-Plex iTRAQ Approach for Quantitative Tissue Proteomic Analysis. PLoS One 2015; 10(9): e0137048.

[176] Collier TS, Sarkar P, Franck WL, Rao BM, Dean RA, Muddiman DC. Direct comparison of stable isotope labeling by amino acids in cell culture and spectral counting for quantitative proteomics. Anal Chem 2010; 82(20): 8696-702. [http://dx.doi.org/10.1021/ac101978b] [PMID: 20845935]

[177] Fenselau C, Yao X. 18O2-labeling in quantitative proteomic strategies: A status report. J Proteome Res 2009; 8(5): 2140-3. [http://dx.doi.org/10.1021/pr8009879] [PMID: 19338309]

[178] Megger DA, Bracht T, Meyer HE, Sitek B. Label-free quantification in clinical proteomics. Biochim Biophys Acta 2013; 1834(8): 1581-90. [http://dx.doi.org/10.1016/j.bbapap.2013.04.001] [PMID: 23567906]

[179] Stare SM, Jozefowicz JJ. The effects of environmental factors on cancer prevalence rates and specific cancer mortality rates in a sample of OECD developed countries. IJABE 2008; 5: 24.

[180] Lynn KS, Chen CC, Lih TM, *et al.* MAGIC: An automated N-linked glycoprotein identification tool using a Y1-ion pattern matching algorithm and *in silico* MS² approach. Anal Chem 2015; 87(4): 2466-73. [http://dx.doi.org/10.1021/ac5044829] [PMID: 25629585]

[181] Park KS, Tae J, Choi B, *et al.* Characterization, *in vitro* cytotoxicity assessment, and *in vivo* visualization of multimodal, RITC-labeled, silica-coated magnetic nanoparticles for labeling human cord blood-derived mesenchymal stem cells. Nanomedicine (Lond) 2010; 6(2): 263-76. [http://dx.doi.org/10.1016/j.nano.2009.07.005] [PMID: 19699324]

[182] Cheng PC, Chang HK, Chen SH. Quantitative nanoproteomics for protein complexes (QNanoPX) related to estrogen transcriptional action. Mol Cell Proteomics 2010; 9(2): 209-24. [http://dx.doi.org/10.1074/mcp.M900183-MCP200] [PMID: 19805454]

[183] Hanke S, Besir H, Oesterhelt D, Mann M. Absolute SILAC for accurate quantitation of proteins in complex mixtures down to the attomole level. J Proteome Res 2008; 7(3): 1118-30. [http://dx.doi.org/10.1021/pr7007175] [PMID: 18271523]

[184] Roe MR, McGowan TF, Thompson LV, Griffin TJ. Targeted 18O-labeling for improved proteomic analysis of carbonylated peptides by mass spectrometry. J Am Soc Mass Spectrom 2010; 21(7): 1190-203. [http://dx.doi.org/10.1016/j.jasms.2010.03.029] [PMID: 20434358]

[185] Li XX, Yin J, Tang J, *et al.* Determining the balance between drug efficacy and safety by the network and biological system profile of its therapeutic target. Front Pharmacol 2018; 9: 1245. [http://dx.doi.org/10.3389/fphar.2018.01245] [PMID: 30429792]

[186] Zhu F, Li XX, Yang SY, Chen YZ. Clinical success of drug targets prospectively predicted by *in silico* study. Trends Pharmacol Sci 2018; 39(3): 229-31. [http://dx.doi.org/10.1016/j.tips.2017.12.002] [PMID: 29295742]

[187] Han ZJ, Xue WW, Tao L, Zhu F. Identification of novel immune-relevant drug target genes for Alzheimer's Disease by combining ontology inference with network analysis. CNS Neurosci Ther 2018; 24(12): 1253-63. [http://dx.doi.org/10.1111/cns.13051] [PMID: 30106219]

[188] Zhu F, Han L, Zheng C, *et al.* What are next generation innovative therapeutic targets? Clues from genetic, structural, physicochemical, and systems profiles of successful targets. J Pharmacol Exp Ther 2009; 330(1): 304-15. [http://dx.doi.org/10.1124/jpet.108.149955] [PMID: 19357322]

[189] Li YH, Li XX, Hong JJ, *et al.* Clinical trials, progression-speed differentiating features and swiftness rule of the innovative targets of first-in-class drugs. Brief Bioinform 2019. [http://dx.doi.org/10.1093/bib/bby130] [PMID: 30689717]

[190] Li X, Li X, Li Y, *et al.* What makes species productive of anticancer drugs? Clues from drugs' species origin, druglikeness, target and pathway. Anticancer Agents Med Chem 2018. [http://dx.doi.org/10.2174/1871520618666181029132017] [PMID: 30370862]

[191] Tu G, Fu T, Yang F, Yao L, Xue W, Zhu F. Prediction of GluN2B-$CT_{1290-1310}$/DAPK1 Interaction by Protein–Peptide Docking and Molecular Dynamics Simulation. Molecules 2018; 23(11): E3018. [http://dx.doi.org/10.3390/molecules23113018] [PMID: 30463177]

[192] Zheng G, Yang F, Fu T, *et al.* Computational characterization of the selective inhibition of human norepinephrine and serotonin transporters by an escitalopram scaffold. Phys Chem Chem Phys 2018; 20(46): 29513-27. [http://dx.doi.org/10.1039/C8CP06232C] [PMID: 30457616]

[193] Yang F, Zheng G, Fu T, *et al.* Prediction of the binding mode and resistance profile for a dual-target pyrrolyl diketo acid scaffold against HIV-1 integrase and reverse-transcriptase-associated ribonuclease H. Phys Chem Chem Phys 2018; 20(37): 23873-84. [http://dx.doi.org/10.1039/C8CP01843J] [PMID: 29947629]

[194] Xue W, Yang F, Wang P, *et al.* What contributes to serotonin-norepinephrine reuptake inhibitors' dual-targeting mechanism? The key role of transmembrane domain 6 in human serotonin and norepinephrine transporters revealed by molecular dynamics simulation. ACS Chem Neurosci 2018; 9(5): 1128-40. [http://dx.doi.org/10.1021/acschemneuro.7b00490] [PMID: 29300091]

[195] Xue W, Fu T, Zheng G, *et al.* Recent advances and challenges of the drugs acting on monoamine transporters. Curr Med Chem 2018. [http://dx.doi.org/10.2174/0929867325666181009123218] [PMID: 30306851]

[196] Zheng G, Xue W, Wang P, *et al.* Exploring the inhibitory mechanism of approved selective norepinephrine reuptake inhibitors and reboxetine enantiomers by molecular dynamics study. Sci Rep 2016; 6: 26883. [http://dx.doi.org/10.1038/srep26883] [PMID: 27230580]