Published in final edited form as:

Expert Rev Proteomics. 2009 October ; 6(5): 573–583. doi:10.1586/epr.09.74.

Emerging Affinity-Based Techniques in Proteomics

Shengnan Xie¹, Colby Moya², Betul Bilgin¹, Arul Jayaraman², and S. Patrick Walton^{1,*}

¹Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824-1226, USA

²Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, TX 77843-3122, USA

Summary

Proteomes of interest, such as the human proteome, have such complexity that no single technique is adequate for complete analysis of the constituents. Depending on the goal (e.g., identification of a novel protein vs. measurement of the level of a known protein), the tools required can vary significantly. While existing methods provide valuable information, their limitations drive the development of complementary, innovative methods to achieve greater breadth of coverage, dynamic range, or specificity of analysis. Here, we will discuss affinity-based methods and their applications, focusing on their unique advantages. In addition, we will describe emerging methods with potential value to proteomics as well as the challenges that remain for proteomic studies.

Keywords

Aptamers; Antibodies; Affinity reagents; Bead arrays; DNA barcoding; Protein-protein interactions

Current Proteomics Methods

Proteomics is primarily a technology-driven field, as advances in the ability to separate and detect proteins have led to discoveries related to protein expression, structure, and function. Comprehensive characterization of proteomes is non-trivial and the extent of coverage achieved often depends on the methods available. Parameters such as the complexity of the sample (i.e., the number of proteins), protein modifications (e.g., phosphorylation), and intracellular localization (e.g., nucleus vs. cytosol) all impact the choice of proteomics technology to be applied. In this section, we provide a brief overview of established proteomics techniques and their applicability to different types of protein samples. For a more detailed discussion on these topics, the reader is referred to other reviews [1–3].

Although techniques for separating proteins such as polyacrylamide gel electrophoresis and chromatography have been in practice for a long time, 'large-scale' protein separation was first described by O'Farrell in 1975 [4]. The two-dimensional gel electrophoresis (2-DE) method developed by these authors is a conceptually simple, yet powerful, technique that separates proteins in a polyacrylamide gel in two orthogonal dimensions. Proteins are first separated based on their isoelectric point followed by conventional molecular weight/size-based separation. 2-DE has been applied to a diverse range of samples [5–9]. While simple

*Address correspondence to: Dr. S. Patrick Walton Applied Biomolecular Engineering Laboratory of the Cellular and Biomolecular Laboratory Department of Chemical Engineering and Materials Science 3249 Engineering Building East Lansing, MI 48824-1226 Tel. 517-432-8733 Fax. 517-432-1105 spwalton@egr.msu.edu Web: <http://www.egr.msu.edu/abel/>.

Financial disclosure The authors have no competing financial interests or affiliations with any of the authors or work described.

to use and not requiring expensive instrumentation, 2-DE has some significant drawbacks. For example, not all types of proteins (e.g., membrane proteins, hydrophobic proteins, proteins greater than 150 kDa) are resolved equally well in gel electrophoresis [10]. Second, the loading capacity of gels is limited, which in turn limits the amount of protein that can be separated and the depth of proteome coverage that can be obtained. This is especially important when working with complex biological fluids such as serum where the range of protein concentrations spans at least five orders-of-magnitude [11,12]. Third, the identification and/or characterization of protein modifications (e.g., phosphorylation) in a sample requires the use of additional methods such as immunoblotting [7]. Although approaches such as sample fractionation, use of 'zoom gels' (i.e., narrow pI range isoelectric focusing gels), and more sensitive staining methods (e.g., fluorescence-based stains) have been developed to overcome these drawbacks, today 2-DE is primarily a method for initially characterizing proteomes prior to more thorough analysis by other methods.

The development of mass spectrometry (MS) methods has addressed some of the drawbacks of gel-based proteomics mentioned above, especially in handling complex protein samples and increasing the depth of proteome coverage. The power of MS-based proteomics is evident from studies utilizing it to characterize the proteomes of organelles [13,14], formation and localization of protein complexes [15], and protein modifications [16]. MS can be used for analyzing enzymatically-generated peptides or intact proteins ("bottom-up" and top-down" proteomics, respectively). In bottom-up proteomics [17], proteins are fragmented prior to ionization in the MS [3]. In the top-down format, the masses of intact proteins are measured, followed by fragmentation, separation, and detection in the mass spectrometer [18]. In either case, the ionized protein fragments are separated based on the mass-to-charge ratio of the gas-phase ions. While both approaches have their own unique advantages, bottom-up proteomics is more widely used in protein identification studies because of the ease with which proteins are enzymatically fragmented into peptides and the straightforward coupling of protein identification with powerful conventional protein separation techniques [19].

In bottom-up proteomics, proteins are first separated by gel electrophoresis or by liquid chromatography, prior to proteolytic fragmentation and identification by MS. Alternatively, the entire protein sample can be digested and the generated peptides separated and analyzed [20]. This "shotgun approach" has the advantage of utilizing multiple liquid chromatography techniques (e.g., size exclusion followed by strong-cation exchange chromatography) to separate the complex peptide mixture before MS analysis. However as the number of generated peptides exceeds 2 million [21], the ability to separate ions with very similar mass-to-charge ratios and instrument sensitivity are limiting factors. Moreover, protein identification is based on only a small fraction of the peptides and can lead to loss of information on post-translational modifications.

Thus, while MS has enormous potential for comprehensively characterizing proteomes, it is unlikely that it can be used as a stand-alone, universal proteomic method. The combination of MS with other methods for characterizing protein complexes, function, and localization is expected to generate the greatest depth of proteome coverage. In this review, we will focus primarily on new, complementary technologies currently being developed and applied for proteome characterization.

Affinity-based Methods

Emerging techniques using affinity reagents for the analysis of focused sets of proteins have advantages of being more sensitive, less labor intensive, and more broadly focused than traditional single protein methods. These methods are highly tunable to meet the needs of

the investigator and the system of interest. However, they are only applicable for known protein targets and, furthermore, these targets also need to have well characterized, specific affinity reagents. For genomics techniques, the affinity is based on complementary base-pairing between two nucleic acid strands. For proteomics, more creative approaches are required to identify specific binding partners. To date, the use of antibodies has predominated affinity-based protein analytical methods. Nonetheless, the interactions between proteins and their non-proteinaceous natural binding partners such as nucleic acids (both natural and those derived from *in vitro* selection), lipids, and small molecules have been screened in various array formats (as reviewed recently [22]). Here, we will discuss the state of the art in affinity-based proteomic strategies, focusing primarily on new techniques that are using affinity reagents, principally antibodies and aptamers, in creative ways to circumvent the limitations of existing technologies (see Table 1 for a general comparison of 2-DE/MS-based and affinity-based methods; see Table 2 for a general summary of current antibody-based methods).

Antibody-based proteomic methods

Surface arrays—Planar antibody arrays were the earliest form of proteomic arrays for profiling protein expression levels. Compared to 2-DE/MS methods, antibody arrays can, in some cases, perform more rapid and sensitive multiplex analyses of the non-fractionated proteome [23]. Antibody arrays can generally be classified into three categories: i) immobilized antibodies to capture labeled proteins from the solution [24]; ii) immobilized proteins detected by labeled antibodies [25,26], and iii) dual antibody sandwich assays [27–29]. Protein arrays have been widely used for cytokine profiling in a variety of studies, including providing comprehensive insights for inflammation and aging studies [30,31]. They have also been actively used in biomarker identification for a variety of cancers, including prostate [32], breast [33], hepatic [34], and pancreatic [35]. Discovery of secreted biomarkers continues to be a primary use of protein arrays.

Antibody microarrays have also been applied to study intracellular signaling. Antibodies to epidermal growth factor receptor (EGFR) and ErbB2/Her2 and their tyrosine phosphorylated forms were used to monitor the concentrations and activities of receptor tyrosine kinases in a ratiometric microarray format [36]. A label-free sandwich approach using near infrared spectroscopy was applied to study extracellular regulated kinase (Erk) 1/2 and Stat3 signaling in cell lines and primary cells over time [37].

Unique methods have been explored to improve the sensitivity and performance of antibody-based methods. One such method, rolling circle amplification (RCA) was used to maximize the signal response from protein recognition in a multiplexed assay [38]. After protein capture by monoclonal antibodies on the microarray, secondary, biotinylated polyclonal antibodies for the proteins were added. Finally, the secondary antibodies were bound by anti-biotin antibodies conjugated with DNA primers. Following addition of a circular DNA template, polymerase, and nucleotides, RCA begins and produces long ssDNA strands that were then detected with fluorescently-labeled, complementary oligonucleotides. This technique was used for the simultaneous measurement of 75 cytokines with femto- to picomolar sensitivity. RCA enabled detection of cytokine levels 1000 fold below the sensitivity limit of direct fluorescent detection without amplification. In addition, because of the ligation of the readout to the antibody binding, signal diffusion, often an issue with enzyme catalyzed chemiluminescent readouts, was largely avoided.

Bead-based arrays—Solution-phase, multiplexed immunoassays have been developed on microbeads, enabled by flow cytometric detection of the color-coded beads used to immobilize the antibody [39]. Each bead is then detected by its unique optical signature. The

beads can also be conjugated with other capture molecules such as antigens, receptors, or enzyme substrates. A commercially available platform has been developed using beads labeled with two fluorescent dyes each with 10 different intensities (Luminex Corporation, Austin, TX)[40], which limits analyses to a maximum of 100 unique analytes. However, as the limitation is based on bead encoding, rather than sensitivity or specificity, the potential to expand the technique to greater parallelism, certainly exists.

Compared with planar proteins arrays, bead based assays have advantages such as solution phase processing and detection, increased flexibility, and simplified automation [41]. Unlike planar arrays, which are relatively inflexible after synthesis, bead-based arrays can be readily customized through inclusion of different sets of existing or user-customized beads [40]. Because they are typically based on two-molecule detection (e.g., detection of the bead color and fluorescence from the analyte), bead-based assays often do not require a wash step to reduce non-specific background, which maximizes the sensitivity of the assay while simultaneously simplifying sample processing.

Bead based assays have been applied for a variety of systems, including detection of cytokines [42–47], auto-antibodies [48,49], and biological warfare agents [50]. In one novel application, the bead system was used for fast analysis of binding specificities of monoclonal antibodies [51]. Protein epitope signature tags from which antibodies were generated were immobilized and mixed. The binding specificity of 84 antibodies was assayed by incubating each antibody with the bead mixture followed by detection with fluorescently labeled secondary antibodies. Results from the bead technique were validated with planar microarrays [51]. The authors were also able to use their validated antibodies with the bead system to measure 20 serum proteins from 200 clinical serum samples in under 2 days [52].

Recently, bead-based profiling was applied for measuring the phosphorylation activity of 62 of 90 tyrosine kinases present in various cancer cell lines [53]. Tyrosine kinase specific antibodies were coupled to the beads for immobilization of the enzymes. A biotinylated, antiphosphotyrosine antibody was then used for detection of the phosphorylation status of the enzymes from cell lysates. Kinases with similar phosphorylation patterns across multiple cancer cell lines were identified [53]. Importantly, the frequent activation of SRC in glioblastoma was confirmed in follow up experiments with primary glioblastoma multiform (GBM) patient samples. SRC was also indicated as a potential therapeutic target for GBM. This multiplexed method significantly reduced the required cell number and cost per assay and proved to have specificity and sensitivity equivalent to western blotting.

Quantum dots (QDs) have been explored to address the limit on the number of possible bead encodings. QDs are inorganic crystals composed of a cadmium selenide core and a zinc sulfide shell. Polymeric microbeads coded with QDs were developed by infusing QDs into preformed beads in organic solvents [54]. QDs typically have higher brightness, orders of magnitude higher resistance to photobleaching, and narrower emission spectra, compared to organic fluorophores [55]. Moreover, the properties of QDs result from their chemical composition and physical structure, allowing facile manipulation of their characteristics to avoid spectrum overlap and increase their potential for multiplex applications [55]. QD encoded beads were used in a multiplexed detection of autoantibodies of TopoI and sm-antigen in serum samples of systemic sclerosis patients [56]. QDs were also used directly as fluorescent labels in immunophenotyping [57]. A 17-color staining panel composed of 7 QDs and 10 other fluorophore labeled antibodies and pMHCI antigens was used to probe surface marker expression of antigen specific T-cell populations within an HIV-seropositive individual by polychromatic flow cytometry [57]. It was found that every antigen specific

subset of cells had a unique phenotypic pattern, providing an example of the potential of QDs in proteomic applications.

DNA Barcoding—An alternative to direct readout of protein detection is through the translation of protein signal to the nucleic acid domain through strategies that can collectively be described as DNA barcoding. These methods have the advantage that the terminal readout is the quantity of the nucleic acid, rather than the quantity of protein, therefore the readouts can take advantage of convenient, parallel, sensitive, and relatively ubiquitous methods for nucleic acid detection. One manner by which this is accomplished is to assign antibodies with a unique DNA label. An early application of a barcoding strategy was in the use of gold nanoparticles functionalized with an antibody and multiple copies of a biobarcode oligonucleotide [58]. The particles were then applied in a sandwich-like format with magnetic particles conjugated with another monoclonal antibody for the same protein target. Different oligonucleotides were used as identifiers for different target proteins, and, after magnetic separation, the biobarcodes were eluted from the nanoparticles with dithiothreitol (DTT) and measured using a DNA microarray. This method was used in a multiplexed assay to measure three established cancer markers: prostate specific antigen (PSA, a prostate cancer marker), human chorionic gonadotropin (HCG, a testicular cancer marker), and α -fetoprotein (AFP, a hepatocellular carcinoma marker) at 170 fM concentration in diluted serum. The extraordinary sensitivity resulted from the release of multiple biobarcode oligonucleotides for each protein molecule detected. It is possible that even further sensitivity might be possible using PCR to amplify the released oligonucleotides prior to detection.

Antibodies directly conjugated with DNA tags have also been used to measure six proteins simultaneously in what is termed a proximity ligation assay (PLA) [59]. Each protein analyte was probed by a pair of antibodies each modified with an oligonucleotide probe. Upon binding of both antibodies to the target, the probes were in close proximity and were ligated together in the presence of template strands complementary to the two probes. Ligated products were amplified with specific primers to each probe and detected with real time PCR [59]. This assay can sensitively detect proteins at femtomolar concentrations in 1 μ L samples (roughly 1000–10000 total molecules of protein per sample). The high sensitivity and low background of this assay was due to the amplification capacity of PCR and the dual binding events required for generating a positive signal, similar to the biobarcode assay described above. In a follow up study, PLA was applied for comparing the levels of putative protein markers in plasma samples from pancreatic and ovarian cancer patients [60]. The assay showed cancer biomarkers CA 19–9 and CA-125 were upregulated in respective patient plasma samples, allowing accurate classification of cancers and normals. Moreover, the quantitative protein levels were consistent with measurements using other techniques as published in the literature.

DNA barcoding has been combined with microfluidics for rapid, flexible construction of a tool for plasma protein analysis. Rather than depositing the antibodies directly onto an array, antibodies were conjugated with multiple copies of a ssDNA barcode and were then hybridized to an array functionalized with complementary ssDNA strands [61]. The array could then be applied in a sandwich format with detection antibodies. Coupling the array to a microfluidic system, direct on-chip separation of plasma from blood obviated the need for prior centrifugation, enabling measurement of 12 protein concentrations simultaneously from a 10 μ L sample [61].

In the high-throughput immunophenotyping using transcription (HIT) technique, each DNA tag used to label the antibody contained a T7 promoter and was amplified by the T7 RNA polymerase [62]. The labeled RNA tags were then purified and hybridized to a DNA

microarray for quantification. Using two 48-plex HIT reactions, 90 surface markers on human naïve T helper cells were profiled. Although the assay format was limited to measuring the surface proteins on pure populations of cells, the concept of using existing DNA microarrays for new purposes suggests an efficient means for the rapid development of new proteomics platforms.

Aptamer-based proteomic methods

Antibodies are well-established as affinity reagents for low and high-throughput analytical methods. To circumvent some of the limitations of antibodies (e.g., protein instability), aptamers are being developed as a complementary set of affinity molecules. Aptamers are single-stranded nucleic acids that are selected for their ability to bind specific target molecules with high affinity and specificity [63,64]. In comparison to antibodies, aptamers offer a number of advantages for proteomic applications. First, aptamers can be reliably synthesized from known sequences using chemical or enzymatic procedures [65,66]. Second, because aptamers are generated by *in vitro* selection, aptamers targeting any analyte, including those too toxic to allow generation of antibodies, could potentially be generated. Third, the specificity and affinity of aptamers is tunable through manipulation of the selection conditions, such as by incorporating negative selection against similar analytes that would confound accurate detection of the intended analyte [67]. The high specificity that can be achieved is especially advantageous for multiplex sensing applications. Aptamers can also be generated with chemically-modified nucleotides to enhance their properties for various applications, such as stability to nucleases for measurements in biological fluids [68–70]. Finally, aptamers can be readily engineered for both sensing and readout using designed selection or through *post hoc* manipulations [71–78].

The utility of aptamers for measuring multiple analytes on an array format was first demonstrated in a small molecule array [79]. The array was fabricated by depositing seven allosteric ribozymes (an aptamer with enzymatic functionality that depends on its conformation) onto a gold surface and measuring the fraction of radiolabeled ribozyme that underwent self-cleavage in the presence of different effectors. An aptazyme ligation assay was also developed to detect metabolites, peptides, and proteins on an array format by measuring the fraction of oligonucleotide ligated upon target binding [80]. These studies demonstrated the possibility of utilizing an ensemble of aptamers for multiplexed sensing applications and corroborated that aptamers retain their specificity in complex, mixed analyte environments. Several groups have since developed aptamer arrays to measure multiple proteins simultaneously [81–83].

A four-element RNA aptamer array was developed in which biotinylated RNA aptamers were deposited onto streptavidin coated slides using contact printing. Analyte proteins were labeled with Cy3 or Cy5 fluorescent dyes and detected in a cell lysate background [82]. A flow cell system enabling real time detection of multiple aptamer binding events was also developed on a bead array format [84]. The system contained a silicon chip microfabricated with multiple pyramidal shaped wells where samples could flow through the smaller openings at the bottoms of the wells. Agarose beads loaded with individual aptamers were positioned into each well. Proteins were labeled directly or bound by labeled antibodies and detected by fluorescence microscopy when passing through the wells. This system was used to assess the affinity of *in vitro* selected lysozyme aptamer clones in parallel and also showed specific signal response when cognate protein targets were added to a duplex assay in which ricin and lysozyme aptamers were immobilized on beads and loaded into different wells. Presumably, this assay could be extended to proteomic-scale analyses through a combination of binding, elution, and detection of the individual proteins across a number of wells, each loaded with beads specific for a different protein.

In native aptamer (and antibody) assays for measuring proteins, non-specific interactions are reduced through washes or other means. However, in doing so, specific signal is also reduced. In an effort to maintain sensitivity with minimal background, photoaptamers were developed [85]. Photoaptamers are selected containing bromodeoxyuridine (BrdU) modifications that readily crosslink to bound proteins upon UV exposure. These were applied in the most comprehensive aptamer array described thus far, which measured 17 proteins simultaneously from serum samples [81]. Crosslinked proteins were detected by universal protein stain or specific secondary antibodies. Because of the low background, the detection limits of about one third of the analytes were below picomolar concentration, with several analytes exhibiting an impressive detection limit below 10 fM.

A dual protein detection scheme using an electrochemical biosensor was recently demonstrated [86]. Lysozyme and thrombin were differentially labeled with two different QDs and measured in a competition assay with unlabeled proteins. Picomolar sensitivity was achieved for both proteins. While prior labeling of proteins with QDs (or any other label) is not feasible for most proteomic analyses, this assay demonstrates a new means for reading out multiplexed aptamer-based protein measurements.

Technologies for enabling and enhancing proteomics analyses

Isolation and Characterization of Affinity Reagents

A significant limitation of affinity-based methods is the limited availability of high-quality, validated affinity reagents. Current projects are seeking to address this issue. Antibodypedia provides a database of antibodies targeting human proteins and their utility for a variety of standard laboratory analyses [87]. Broader information on the collection and characterization of affinity molecules for the human proteome can also be obtained from ProteomeBinders [88]. A database for aptamers and their targets has also been compiled (<http://aptamer.icmb.utexas.edu/>).

Affinity Prefractionation

Affinity is not only used as the underlying principle in antibody and aptamer technologies but has also been utilized for pre-fractionating proteins to enhance the depth of proteome coverage. While pre-fractionation techniques such as size exclusion and ion-exchange chromatography have been used in multi-dimensional protein identification to reduce sample complexity, affinity-based separation methods are also useful for enrichment of low abundance proteins in complex samples, decreasing sample complexity, and investigating protein-protein interactions and protein complexes (i.e., interaction proteomics) [89,90]. The high degree of specificity between the bait and the capture molecule minimizes non-specific capture of proteins and improves the efficiency of affinity-based approaches. The most common baits used for affinity-based separation, protein ligands and antibodies, are discussed below in the context of analyzing the human plasma proteome.

Different protein ligand-based sample fractionation and enrichment methods have been used to improve plasma proteome profiling. Since plasma proteins are generally glycosylated, affinity for carbohydrate moieties has been successfully applied for pre-fractionation [91]. In a parallel approach, the binding affinity between hydrazide moieties and glycosylated proteins has also been used to selectively enrich glycoproteins [92]. It was demonstrated that coupling depletion of six high abundance plasma proteins with a multiple lectin affinity column resulted in capture of roughly 50% of the serum glycoproteome [93]. Array-based prefractionation has also been coupled with mass spectrometry for improved proteome resolution [94,95]. Other ligands such as Protein A and Protein G have been used to bind and deplete immunoglobulins [96], which are the second most prevalent class of proteins in human plasma. Antibodies are also used to deplete high abundance plasma proteins.

Polyclonal antibodies against immunoglobulins have been used in affinity columns to remove these high abundance proteins [97]. Immunoaffinity-based depletion is often used in conjunction with other approaches, such as mixed antibody columns, to deplete other high abundance proteins [98].

Transcription Factor Profiling

As with DNA barcoding techniques, using DNA sequences for the readout of protein levels is uniquely feasible for transcription factors (TFs). Their specific DNA recognition provides the natural one-to-one correspondence required for parallel analytical techniques. Multiplex techniques that leverage this binding specificity are often based on the readout of reporter genes. In these methods, an easily assayable reporter construct is placed behind the recognition sequence for the TF of interest, and the expression of the reporter is monitored. Thus, activation of the TF should lead to transcription of the natural targets as well as the construct. This approach was applied recently to the simultaneous measurement of 43 TF activities [99]. In a particularly creative approach, TF activation resulted in the expression of a set of transcripts, all of which contained the same, unique restriction enzyme site. After RNA isolation and RT-PCR, the resulting cDNAs were cleaved with the restriction enzyme resulting in a set of DNAs, each with a unique length specific for a given TF. The products were then assayed by capillary electrophoresis to determine the relative quantities of each length DNA product and, correspondingly, the relative activity of the corresponding TF. Using this approach, TF activities of HepG2 cells before and after induction with biologically active compounds were compared. The value of the parallel TF measurements was further demonstrated by the measurement of unique TF activity profiles from cancer cell lines related to HepG2s, thus suggesting a potential value for TF profiling in the classification of tumors.

A commercial approach also relies on reporter gene constructs for multiplex TF measurements (Panomics/Affymetrix, Fremont, CA). This approach also begins by transfection of cells with reporter plasmids. Reporter RNA is then isolated, reverse transcribed, and assayed by DNA microarray. This technique was applied to analyze trichostatin A (TSA) induction on cardiac myocytes *in vivo* [100]. They monitored 24 TFs simultaneously and determined the interactions among them. Of note, the results showed that the activity of the early growth response gene, EGR-1, increased with TSA induction. While reporter gene-based techniques provide valuable information for *in vitro* systems, they require manipulation of the cells prior to analysis, precluding their use for analysis of samples, for instance, from a clinical setting. Also, transfection of the reporter plasmids may alter the natural cellular signaling and function, resulting in an abnormal response to the stimulus being studied.

Similar commercial approaches that can be applied to isolated protein samples also leverage the Luminex cytometric platform. In these techniques (Panomics/Affymetrix and Marligen, Ijamsville, MD), cellular extracts are incubated with double stranded DNA probes composed of biotinylated TF recognition sites. After removal of unbound probes, bound probes are hybridized with complementary sequences attached to fluorescent beads. Fluorescently-labeled streptavidin is then added to the mixture. In this way, the signals from the streptavidin fluorophore and the bead indicate the presence of a probe and its identity, respectively. These assays required only ~5 µg of cell extract for accurate TF measurements. This technique was used to assess TF activity in untreated HeLa cells and those exposed to phorbol 12-myristate 13-acetate (PMA) and tumor necrosis factor α (TNFα). Based on similarities in their TF activation profiles, it was proposed that their signaling pathways share some common mechanisms [101]. As stated above, these methods have been used to

detect up to 50 TFs in parallel but, again, the number of TFs that can be measured is limited by the number of unique bead spectra.

Another array-based method to profile TF activities was developed by Panomics (Panomics/Affymetrix). In this method, after incubation of biotin labeled TF probes with nuclear extracts, TF bound probes are separated from free probes with spin columns. TF bound DNA complexes are then denatured, and free probes are hybridized to an array of sequences complementary to the TF recognition sequences. With the array, quantitative and qualitative data can be obtained. This method can profile up to 345 TF in parallel. This technique has been applied to the analysis of TF activities in breast cancer lines [102], in analyzing the effects of the extra-cellular matrix on cancer cell phenotypes [103], and in colon tumor cells [104].

Protein-Protein interactions

An emerging area of research that builds on current advances in affinity-based separation is in interaction proteomics – that is the identification of protein complex and protein-protein interactions in the context of cellular function. Most cell functions are not carried out by single proteins; instead, multiple proteins function as a complex to carry out specific functions. Therefore, characterizing the association of a protein with other proteins is of interest. However, proteins are not uniquely associated with a single complex and are often associated with different partners, depending on the cell type and state. Therefore, the ability to isolate binding partners of proteins under physiologically-relevant conditions continues to grow in importance for improving our understanding of protein function. Many array-based approaches using whole proteins and protein domains have been developed with some available commercially (e.g., <http://www.panomics.com/>) [105,106]. In addition, a microfluidic approach was used to exhaustively study the interactions among 43 proteins from *S. pneumoniae* [107].

One unique method for studying these interactions relied on *in situ* expression of proteins, rather than direct printing of the proteins onto an array surface [108]. This approach mitigated issues with the high throughput production and isolation of proteins and protein instability during storage [109–111]. On a nucleic acid programmable protein-protein interaction array, the proteins were translated using rabbit reticulocyte lysate supplemented with T7 polymerase and captured locally with an antibody to the glutathione S-transferase (GST) tag fused at the C-terminal of each protein [109]. Twenty-nine proteins involved in DNA replication initiation were immobilized and expressed on the array and added with the plasmid expressing each component protein. The study detected 28% of all interactions between and among origin replication complexes (ORC) and a minichromosome maintenance complex (MCM2–7) identified by biochemical experiments [112]. Novel interactions were also discovered [109]. The technique was then expanded to over 1000 proteins on the array surface with high fidelity and reproducibility of protein expression [110].

Five-year view

It is still difficult to predict which platform(s) will ultimately make up the core set of tools for proteomics. It can be envisioned that methods will emerge that measure small subsets of proteins with known biological or clinical importance. MS-based methods will likely continue to be used to screen for targets/biomarkers of interest which can then be investigated individually or in parallel using affinity-based methods. For these assays to demonstrate real value in understanding biological phenomena or influencing patient care, the robustness of these methods across laboratories and clinical sites needs to be demonstrated, as reinforced by a recent study [113]. Moreover, once biomarkers are

identified, the roles the proteins play in the disease process must be concretely established so as to best understand how they affect the disease process.

Another area of particular attention should be on the infrastructure requirements for implementation and analysis of proteomic techniques. For broad adoption of any proteomic strategy for a clinical application, the disparities in global infrastructures should also be considered. An emphasis on simple to perform, portable, durable, reusable, minimal reagent assays will lead to improvements in care for far greater numbers of individuals (e.g., [114]).

Expert Commentary

The ultimate utility of a proteomic method is in its flexibility and customizability. The variety of analytes in proteomics studies far exceeds that of genomics studies. Proteins vary in size, charge, structure, localization, function, and modifications. Moreover, proteomic studies can be divided into expression, interaction, functional, and structural, with each requiring specific analytical methods. Because of this complexity and breadth of the analytes being studied, no single proteomic strategy will ever suffice for all studies. Understanding the information that the scientist/clinician desires to get out of the study will likely define the type of technique to be applied.

Affinity methods also have the potential to mitigate the data analysis problem associated with all high-throughput, parallel analytical methods. Well-characterized reagents of known affinity and specificity combined with standardized and validated assays can be used for improved quantitation of parallel protein measurements. In turn, improved quantitation will enhance the characterization of samples and the definition of biomarkers - removing the need for relative quantification and changing analyses from focusing on presence vs. absence of a given protein to assessment of the level of a protein relative to an absolute scale. This sort of quantification will improve the diagnostic and prognostic value of the assays being applied and the targets being studied.

Acknowledgments

Financial support for this work was provided by the National Institutes of Health (#GM079688 and #RR024439 to S.P.W.) and the American Heart Association (0755112Y to A.J.).

References

1. Fey SJ, Larsen PM. 2D or not 2D. Two-dimensional gel electrophoresis. *Current Opinion in Chemical Biology*. 2001; 5:26–33. [PubMed: 11166644]
2. Barnouin K. Two-dimensional gel electrophoresis for analysis of protein complexes. *Methods Mol Biol*. 2004; 261:479–498. [PubMed: 15064477]
3. Han X, Aslanian A, Yates JR. Mass spectrometry for proteomics. *Current Opinion in Chemical Biology*. 2008; 12:483–490. [PubMed: 18718552]
4. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. *Journal of Biological Chemistry*. 1975; 250:4007–4021. [PubMed: 236308]
5. Duan X, Berthiaume F, Yarmush DM, Jayaraman A, Yarmush ML. A mouse serum two-dimensional gel map: Application to profiling burn injury and infection. *Electrophoresis*. 2004; 25:3055–3065. [PubMed: 15349948]
6. Duan X, Yarmush ML, Jayaraman A, L YM. Dispensable role for interferon gamma in the acute phase response: A proteomics analysis. *Proteomics*. 2004; 4:1830–1839. [PubMed: 15174149]
7. Jayaraman A, Roberts KA, Yoon J, et al. Identification of neutrophil gelatinase-associated lipocalin (NGAL) as a discriminant marker of the hepatocyte secreted protein response to IL-1 β : a proteomic analysis. *Biotechnol & Bioeng*. 2005; 91:502–515.

8. Nandakumar R, Nandakumar MP, Marten MR, Ross JM. Proteome analysis of membrane and cell wall associated proteins from *Staphylococcus aureus*. J Proteome Res. 2005; 4:250–257. [PubMed: 15822900]
9. Dooley GP, Reardon KF, Prenni JE, et al. Proteomic analysis of diaminochlorotriazine adducts in wistar rat pituitary glands and LbetaT2 rat pituitary cells. Chem Res Toxicol. 2008; 21:844–851. [PubMed: 18370413]
10. Rabilloud T. Membrane proteins and proteomics: Love is possible, but so difficult. Electrophoresis. 2009; 30:S174–180. [PubMed: 19517508]
11. Miller I, Crawford J, Gianazza E. Protein stains for proteomic applications: which, when, why? Proteomics. 2008; 6:5385–5408. [PubMed: 16991193]
12. Shen Y, Jacobs JM, Camp DG 2nd, et al. Ultra-high-efficiency strong cation exchange LC/RPLC/MS/MS for high dynamic range characterization of the human plasma proteome. Anal Chem. 2004; 76(4):1134–1144. [PubMed: 14961748]
13. Wong DM, Adeli K. Microsomal proteomics. Methods Mol Biol. 2009; 519:273–279. [PubMed: 19381589]
14. Lin RX, Zhao HB, Li CR, Sun YN, Qian XH, Wang SQ. Proteomic analysis of ionizing radiation-induced proteins at the subcellular level. J Proteome Res. 2009; 8:390–399. [PubMed: 19063669]
15. Wepf A, Glatter T, Schmidt A, Aebersold R, Gstaiger M. Quantitative interaction proteomics using mass spectrometry. Nat Methods. 2009; 6:203–205. [PubMed: 19198594]
16. Viner RI, Zhang T, Second T, Zabrouskov V. Quantification of post-translationally modified peptides of bovine alpha-crystallin using tandem mass tags and electron transfer dissociation. J Proteomics. 2009 Epub ahead of print Feb 24, 2009.
17. Dwivedi RC, Spicer V, Harder M, et al. Practical implementation of 2D HPLC scheme with accurate peptide retention prediction in both dimensions for high-throughput bottom-up proteomics. Ana80. 2008:7036–42.
18. Parks BA, Jiang L, Thomas PM, et al. Top-down proteomics on a chromatographic time scale using linear ion trap fourier transform hybrid mass spectrometers. Anal Chem. 2007; 79:7984–7991. [PubMed: 17915963]
19. Yates RJ, Ruse CI, Nakorchevsky A. Proteomics by Mass Spectrometry: Approaches, Advances, and Applications. Annu Rev Biomed Eng. 2009 Epub Apr 13, 2009.
20. Cravatt BF, Simon GM, Yates JR. The biological impact of mass-spectrometry-based proteomics. Nature. 2007; 450:991–1000. [PubMed: 18075578]
21. Ahn NG, Shabb JB, Old WM, Resing KA. Achieving in-depth proteomics profiling by mass spectrometry. ACS Chem Biol. 2007; 2:39–52. [PubMed: 17243782]
22. Stoevesandt O, Taussig MJ, He M. Protein microarrays: high-throughput tools for proteomics. Expert Rev Proteomics. 2009; 6(2):145–157. [PubMed: 19385942]
23. Borrebaeck CA, Wingren C. High-throughput proteomics using antibody microarrays: an update. Expert Rev Mol Diagn. 2007; 7(5):673–686. [PubMed: 17892372]
24. Zhou HP, Bouwman K, Schotanus M, et al. Two-color, rolling-circle amplification on antibody microarrays for sensitive, multiplexed serum-protein measurements. Genome Biology. 2004; 5(4)
25. Wulfeuhle JD, Aquino JA, Calvert VS, et al. Signal pathway profiling of ovarian cancer from human tissue specimens using reverse-phase protein microarrays. Proteomics. 2003; 3(11):2085–2090. [PubMed: 14595806]
26. Paweletz CP, Charboneau L, Bichsel VE, et al. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene. 2001; 20(16):1981–1989. [PubMed: 11360182]
27. Silzel JW, Cercek B, Dodson C, Tsay T, Obremski RJ. Mass-sensing, multianalyte microarray immunoassay with imaging detection. Clinical Chemistry. 1998; 44(9):2036–2043. [PubMed: 9733002]
28. Wiese R, Belosludtsev Y, Powdrill T, Thompson P, Hogan M. Simultaneous multianalyte ELISA performed on a microarray platform. Clinical Chemistry. 2001; 47(8):1451–1457. [PubMed: 11468236]

29. Woodbury RL, Varnum SM, Zangar RC. Elevated HGF levels in sera from breast cancer patients detected using a protein microarray ELISA. *Journal of Proteome Research*. 2002; 1(3):233–237. [PubMed: 12645900]
30. Lin Y, Huang R, Chen LP, et al. Profiling of cytokine expression by biotin-labeled-based protein arrays. *Proteomics*. 2003; 3(9):1750–1757. [PubMed: 12973735]
31. Kverka M, Burianova J, Lodinova-Zadnikova R, et al. Cytokine profiling in human colostrum and milk by protein array. *Clin Chem*. 2007; 53(5):955–962. [PubMed: 17363422]
32. Miller JC, Zhou H, Kwekel J, et al. Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. *Proteomics*. 2003; 3(1):56–63. [PubMed: 12548634]
33. Shin BK, Wang H, Hanash S. Proteomics approaches to uncover the repertoire of circulating biomarkers for breast cancer. *J Mammary Gland Biol Neoplasia*. 2002; 7(4):407–413. [PubMed: 12882525]
34. Melle C, Ernst G, Scheibner O, et al. Identification of specific protein markers in microdissected hepatocellular carcinoma. *J Proteome Res*. 2007; 6(1):306–315. [PubMed: 17203974]
35. Ingvarsson J, Wingren C, Carlsson A, et al. Detection of pancreatic cancer using antibody microarray-based serum protein profiling. *Proteomics*. 2008; 8(11):2211–2219. [PubMed: 18528842]
36. Nielsen UB, Cardone MH, Sinsky AJ, MacBeath G, Sorger PK. Profiling receptor tyrosine kinase activation by using Ab microarrays. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(16):9330–9335. [PubMed: 12876202]
37. Korf U, Derdak S, Tresch A, et al. Quantitative protein microarrays for time-resolved measurements of protein phosphorylation. *Proteomics*. 2008; 8(21):4603–4612. [PubMed: 18972530]
38. Schweitzer B, Roberts S, Grimwade B, et al. Multiplexed protein profiling on microarrays by rolling-circle amplification. *Nat Biotechnol*. 2002; 20(4):359–365. [PubMed: 11923841]
39. Fulton RJ, McDade RL, Smith PL, Kienker LJ, Kettman JR. Advanced multiplexed analysis with the FlowMetrix(TM) system. *Clinical Chemistry*. 1997; 43(9):1749–1756. [PubMed: 9299971]
**Described the first commercially available bead arrays for multiplexed protein analysis.
40. Nolan JP, Sklar LA. Suspension array technology: evolution of the flat-array paradigm. *Trends in Biotechnology*. 2002; 20(1):9–12. [PubMed: 11742671]
41. Templin MF, Stoll D, Bachmann J, Joos TO. Protein microarrays and multiplexed sandwich immunoassays: what beats the beads? *Comb Chem High Throughput Screen*. 2004; 7(3):223–229. [PubMed: 15134528]
42. Oliver, KG.; Kettman, JR.; Fulton, RJ. *Amer Assoc Clinical Chemistry*. 1998. Multiplexed analysis of human cytokines by use of the FlowMetrix system; p. 2057–2060.
43. Carson RT, Vignali DAA. Simultaneous quantitation of 15 cytokines using a multiplexed flow cytometric assay. *Journal of Immunological Methods*. 1999; 227(1–2):41–52. [PubMed: 10485253]
44. Prabhakar U, Eirikis E, Davis HM. Simultaneous quantification of proinflammatory cytokines in human plasma using the LabMAP (TM) assay. *Journal of Immunological Methods*. 2002; 260(1–2):207–218. [PubMed: 11792390]
45. Chen R, Lowe L, Wilson JD, et al. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clinical Chemistry*. 1999; 45(9):1693–1694. [PubMed: 10471693]
46. Cook EB, Stahl JL, Lowe L, et al. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry, allergies vs. non-allergies. *Journal of Immunological Methods*. 2001; 254(1–2):109–118. [PubMed: 11406157]
47. Kellar KL, Kalwar RR, Dubois KA, Crouse D, Chafin WD, Kane BE. Multiplexed fluorescent bead-based immunoassays for quantitation of human cytokines in serum and culture supernatants. *Cytometry*. 2001; 45(1):27–36. [PubMed: 11598944]
48. Bellisario R, Colinas RJ, Pass KA. Simultaneous measurement of antibodies to three HIV-1 antigens in newborn dried blood-spot specimens using a multiplexed microsphere-based immunoassay. *Early Human Development*. 2001; 64(1):21–25. [PubMed: 11408105]

49. Opalka D, Lachman CE, MacMullen SA, et al. Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay 2. *Clinical and Diagnostic Laboratory Immunology*. 2003; 10(1):108–115. [PubMed: 12522048]
50. McBride MT, Gammon S, Pitesky M, et al. Multiplexed liquid arrays for simultaneous detection of simulants of biological warfare agents. *Analytical Chemistry*. 2003; 75(8):1924–1930. [PubMed: 12713052]
51. Schwenk JM, Lindberg J, Sundberg M, Uhlen M, Nilsson P. Determination of binding specificities in highly multiplexed bead-based assays for antibody proteomics. *Mol Cell Proteomics*. 2007; 6(1):125–132. [PubMed: 17060675]
52. Schwenk JM, Gry M, Rimini R, Uhlen M, Nilsson P. Antibody suspension bead arrays within serum proteomics. *J Proteome Res*. 2008; 7(8):3168–3179. [PubMed: 18588325]
53. Du JY, Bernasconi P, Clauser KR, et al. Bead-based profiling of tyrosine kinase phosphorylation identifies SRC as a potential target for glioblastoma therapy. *Nature Biotechnology*. 2009; 27(1):77–83.
54. Han MY, Gao XH, Su JZ, Nie S. Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. *Nature Biotechnology*. 2001; 19(7):631–635.
55. Sukhanova A, Nabiev I. Fluorescent nanocrystal-encoded microbeads for multiplexed cancer imaging and diagnosis. *Crit Rev Oncol Hematol*. 2008; 68(1):39–59. [PubMed: 18621543]
56. Sukhanova A, Susha AS, Bek A, et al. Nanocrystal-encoded fluorescent microbeads for proteomics: Antibody profiling and diagnostics of autoimmune diseases. *Nano Letters*. 2007; 7(8):2322–2327. [PubMed: 17645364]
57. Chattopadhyay PK, Price DA, Harper TF, et al. Quantum dot semiconductor nanocrystals for immunophenotyping by polychromatic flow cytometry. *Nature Medicine*. 2006; 12(8):972–977.
58. Stoeva SI, Lee JS, Smith JE, Rosen ST, Mirkin CA. Multiplexed detection of protein cancer markers with biobarcode nanoparticle probes. *Journal of the American Chemical Society*. 2006; 128(26):8378–8379. [PubMed: 16802785]
59. Fredriksson S, Dixon W, Ji H, Koong AC, Mindrinos M, Davis RW. Multiplexed protein detection by proximity ligation for cancer biomarker validation. *Nature Methods*. 2007; 4(4):327–329. [PubMed: 17369836]
60. Fredriksson S, Horecka J, Brustugun OT, et al. Multiplexed proximity ligation assays to profile putative plasma biomarkers relevant to pancreatic and ovarian cancer. *Clinical Chemistry*. 2008; 54(3):582–589. [PubMed: 18171715]
61. Fan R, Vermesh O, Srivastava A, et al. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. *Nature Biotechnology*. 2008; 26(12):1373–1378.
62. Kattah MG, Collier J, Cheung RK, Oshidary N, Utz PJ. HIT: a versatile proteomics platform for multianalyte phenotyping of cytokines, intracellular proteins and surface molecules. *Nat Med*. 2008; 14(11):1284–1289. [PubMed: 18849997]
63. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. *Nature*. 1990; 346(6287):818–822. [PubMed: 1697402]
64. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*. 1990; 249(4968):505–510. [PubMed: 2200121]
65. Lee JF, Hesselberth JR, Meyers LA, Ellington AD. Aptamer database. *Nucleic Acids Research*. 2004; 32:D95–D100. [PubMed: 14681367]
66. Gold L, Polisky B, Uhlenbeck O, Yarus M. Diversity of oligonucleotide functions. *Annu Rev Biochem*. 1995; 64:763–797. [PubMed: 7574500]
67. Jenison RD, Gill SC, Pardi A, Polisky B. High-resolution molecular discrimination by RNA. *Science*. 1994; 263(5152):1425–1429. [PubMed: 7510417]
68. Lin Y, Qiu Q, Gill SC, Jayasena SD. Modified RNA sequence pools for in vitro selection. *Nucleic Acids Res*. 1994; 22(24):5229–5234. [PubMed: 7529404]
69. Pieken WA, Olsen DB, Benseler F, Aurup H, Eckstein F. Kinetic characterization of ribonuclease-resistant 2'-modified hammerhead ribozymes. *Science*. 1991; 253(5017):314–317. [PubMed: 1857967]

70. Kang J, Lee MS, Copland JA 3rd, Luxon BA, Gorenstein DG. Combinatorial selection of a single stranded DNA thioaptamer targeting TGF-beta1 protein. *Bioorg Med Chem Lett*. 2008; 18(6): 1835–1839. [PubMed: 18294846]
71. Win MN, Smolke CD. A modular and extensible RNA-based gene-regulatory platform for engineering cellular function. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(36):14283–14288. [PubMed: 17709748]
72. Lynch SA, Desai SK, Sajja HK, Gallivan JP. A high-throughput screen for synthetic riboswitches reveals mechanistic insights into their function. *Chemistry & Biology*. 2007; 14(2):173–184. [PubMed: 17317571]
73. Bayer TS, Smolke CD. Programmable ligand-controlled riboregulators of eukaryotic gene expression. *Nature Biotechnology*. 2005; 23(3):337–343.
74. Win MN, Smolke CD. Higher-order cellular information processing with synthetic RNA devices. *Science*. 2008; 322(5900):456–460. [PubMed: 18927397]
75. Xie S, Walton SP. Application and analysis of structure-switching aptamers for small molecule quantification. *Anal Chim Acta*. 2009; 638(2):213–219. [PubMed: 19327463]
76. Lynch SA, Gallivan JP. A flow cytometry-based screen for synthetic riboswitches. *Nucleic Acids Research*. 2009; 37(1):184–192. [PubMed: 19033367]
77. Jhaveri S, Rajendran M, Ellington AD. In vitro selection of signaling aptamers. *Nat Biotechnol*. 2000; 18(12):1293–1297. [PubMed: 11101810]
78. Stojanovic MN, Kolpashchikov DM. Modular aptameric sensors. *J Am Chem Soc*. 2004; 126(30): 9266–9270. [PubMed: 15281816]
79. Seetharaman S, Zivarts M, Sudarsan N, Breaker RR. Immobilized RNA switches for the analysis of complex chemical and biological mixtures. *Nature Biotechnology*. 2001; 19(4):336–341.
80. Hesselberth JR, Robertson MP, Knudsen SM, Ellington AD. Simultaneous detection of diverse analytes with an aptazyme ligase array. *Analytical Biochemistry*. 2003; 312(2):106–112. [PubMed: 12531194]
81. Bock C, Coleman M, Collins B, et al. Photoaptamer arrays applied to multiplexed proteomic analysis. *Proteomics*. 2004; 4(3):609–618. [PubMed: 14997484]
82. Collett JR, Cho EJ, Ellington AD. Production and processing of aptamer microarrays. *Methods*. 2005; 37(1):4–15. [PubMed: 16199170] *Detailed considerations for fabricating RNA aptamer microarrays
83. Stadtherr K, Wolf H, Lindner P. An aptamer-based protein biochip. *Anal Chem*. 2005; 77(11): 3437–3443. [PubMed: 15924373]
84. Kirby R, Cho EJ, Gehrke B, et al. Aptamer-based sensor arrays for the detection and quantitation of proteins. *Analytical Chemistry*. 2004; 76(14):4066–4075. [PubMed: 15253644]
85. Golden MC, Resing KA, Collins BD, Willis MC, Koch TH. Mass spectral characterization of a protein-nucleic acid photocrosslink. *Protein Sci*. 1999; 8(12):2806–2812. [PubMed: 10631998]
86. Hansen JA, Wang J, Kawde AN, Xiang Y, Gothelf KV, Collins G. Quantum-dot/aptamer-based ultrasensitive multi-analyte electrochemical biosensor. *Journal of the American Chemical Society*. 2006; 128(7):2228–2229. [PubMed: 16478173]
87. Bjorling E, Uhlen M. Antibodypedia, a portal for sharing antibody and antigen validation data. *Mol Cell Proteomics*. 2008; 7(10):2028–2037. [PubMed: 18667413]
88. Taussig MJ, Stoevesandt O, Borrebaeck CA, et al. ProteomeBinders: planning a European resource of affinity reagents for analysis of the human proteome. *Nat Methods*. 2007; 4(1):13–17. [PubMed: 17195019] **Valuable resource for information about affinity reagents for protein analyses
89. Pernemalm M, Lewensohn R, Lehtiö J. Affinity prefractionation for MS-based plasma proteomics. *Proteomics*. 2009; 9:1420–1427. [PubMed: 19235168]
90. Oeljeklaus S, Meyer HE, Warscheid B. New dimensions in the study of protein complexes using quantitative mass spectrometry. *FEBS Lett*. 2009; 583:1674–1683. [PubMed: 19376113]
91. Yang Z, Hancock WS. Monitoring glycosylation pattern changes of glycoproteins using multi-lectin affinity chromatography. *J Chromatogr A*. 2005; 1070:57–64. [PubMed: 15861788]

92. Stahl-Zeng J, Lange V, Ossola R, et al. High sensitivity detection of plasma proteins by multiple reaction monitoring of N-glycosites. *Mol Cell Proteomics*. 2007; 6:1809–1817. [PubMed: 17644760]
93. Plavina T, Wakshull E, Hancock WS, Hincapie M. Combination of abundant protein depletion and multi-lectin affinity chromatography (M-LAC) for plasma protein biomarker discovery. *J Proteome Res*. 2007; 6:662–671. [PubMed: 17269723]
94. Darii E, Lebeau D, Papin N, et al. Quantification of target proteins using hydrogel antibody arrays and MALDI time-of-flight mass spectrometry (A2M2S). *N Biotechnol*. 2009*Interesting coupling of antibody array with mass spectrometry
95. Ekstrom S, Wallman L, Hok D, Marko-Varga G, Laurell T. Miniaturized solid-phase extraction and sample preparation for MALDI MS using a microfabricated integrated selective enrichment target. *J Proteome Res*. 2006; 5(5):1071–1081. [PubMed: 16674096]
96. Liu T, Qian WJ, Mottaz HM, et al. Evaluation of multiprotein immunoaffinity subtraction for plasma proteomics and candidate biomarker discovery using mass spectrometry. *Mol Cell Proteomics*. 2006; 5:2167–2174. [PubMed: 16854842]
97. Kojima K. High-performance immunoaffinity chromatography, an immunoaffinity membrane for selective removal of plasma components, and safety evaluation of the latter system. *J Biochem Biophys Methods*. 2001; 49:241–251. [PubMed: 11694282]
98. Yang Z, Hancock WS. Approach to the comprehensive analysis of glycoproteins isolated from human serum using a multi-lectin affinity column. *J Chromatogr A*. 2004; 1053:79–88. [PubMed: 15543974]
99. Romanov S, Medvedev A, Gambarian M, et al. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. *Nat Methods*. 2008; 5(3):253–260. [PubMed: 18297081] **Measurement of transcription factor levels in parallel using reporter genes of unique lengths
100. Davis FJ, Pillai JB, Gupta M, Gupta MP. Concurrent opposite effects of trichostatin A, an inhibitor of histone deacetylases, on expression of alpha-MHC and cardiac tubulins: implication for gain in cardiac muscle contractility. *Am J Physiol Heart Circ Physiol*. 2005; 288(3):H1477–1490. [PubMed: 15388503]
101. Yaoi T, Jiang X, Li X. Development of a fluorescent microsphere-based multiplexed high-throughput assay system for profiling of transcription factor activation. *Assay Drug Dev Technol*. 2006; 4(3):285–292. [PubMed: 16834534] *Bead-based technique for transcription factor profiling
102. Jiang X, Roth L, Lai C, Li X. Profiling activities of transcription factors in breast cancer cell lines. *Assay Drug Dev Technol*. 2006; 4(3):293–305. [PubMed: 16834535]
103. Dozmorov MG, Kyker KD, Hauser PJ, et al. From microarray to biology: an integrated experimental, statistical and in silico analysis of how the extracellular matrix modulates the phenotype of cancer cells. *BMC Bioinformatics*. 2008; 9(Suppl 9):S4. [PubMed: 18793468]
104. Li Q, Dashwood WM, Zhong X, Nakagama H, Dashwood RH. Bcl-2 overexpression in PhIP-induced colon tumors: cloning of the rat Bcl-2 promoter and characterization of a pathway involving beta-catenin, c-Myc and E2F1. *Oncogene*. 2007; 26(42):6194–6202. [PubMed: 17404573]
105. Jones RB, Gordus A, Krall JA, MacBeath G. A quantitative protein interaction network for the ErbB receptors using protein microarrays. *Nature*. 2006; 439(7073):168–174. [PubMed: 16273093]
106. Stoevesandt O, Kohler K, Wolf S, Andre T, Hummel W, Brock R. A network analysis of changes in molecular interactions in cellular signaling. *Mol Cell Proteomics*. 2007; 6(3):503–513. [PubMed: 17190788]
107. Gerber D, Maerkl SJ, Quake SR. An in vitro microfluidic approach to generating protein-interaction networks. *Nat Methods*. 2009; 6(1):71–74. [PubMed: 19098921]
108. MacBeath G, Schreiber SL. Printing proteins as microarrays for high-throughput function determination. *Science*. 2000; 289(5485):1760–1763. [PubMed: 10976071] **First proof-of-principle protein array for measuring protein-protein interactions

109. Ramachandran N, Hainsworth E, Bhullar B, et al. Self-assembling protein microarrays. *Science*. 2004; 305(5680):86–90. [PubMed: 15232106]
110. Ramachandran N, Raphael JV, Hainsworth E, et al. Next-generation high-density self-assembling functional protein arrays. *Nat Methods*. 2008; 5(6):535–538. [PubMed: 18469824]
111. He M, Stoevesandt O, Palmer EA, Khan F, Ericsson O, Taussig MJ. Printing protein arrays from DNA arrays. *Nature Methods*. 2008; 5(2):175–177. [PubMed: 18204456]
112. Bell SP, Dutta A. DNA replication in eukaryotic cells. *Annual Review of Biochemistry*. 2002; 71:333–374.
113. Addona TA, Abbatiello SE, Schilling B, et al. Multi-site assessment of the precision and reproducibility of multiple reaction monitoring-based measurements of proteins in plasma. *Nat Biotechnol*. 2009
114. Martinez AW, Phillips ST, Butte MJ, Whitesides GM. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angew Chem Int Ed Engl*. 2007; 46(8):1318–1320. [PubMed: 17211899]

Key issues

- Multiple strategies will be required for thorough and deep analysis of the proteomes of important organisms, e.g., humans.
- Affinity-based proteomics techniques enable flexible, sensitive, and convenient proteomic measurements.
- Various solid and solution phase proteomics platforms with unique advantages have been established and applied in a broad range of studies.
- High-quality, affinity reagents are crucial to the success of affinity-based methods.

Table 1

Comparison of conventional and affinity-based proteomics techniques

Property	2DE/MS-based	Affinity-based
Applications	Identification, expression, post translational modifications	Expression, interactions, function, post translational modifications
Specificity	Tunable based on separation method used	Depends on specificity of affinity reagents
Sensitivity	Difficulty with low abundance proteins in mixtures	Nano- to femtomolar protein concentration
Breadth of coverage	Can approach all proteins in a sample	Focused sets of proteins defined by researcher
Instrumentation Requirements	Significant	Varies broadly depending on technique
Pretreatment	Typically requires removal of high abundance proteins	Often no pretreatment required

Table 2

General specifications of antibody-based techniques*

Techniques	Detection limits	Current multiplexing capacity (# of analytes)	Advantages	Limitations	References
Surface arrays	Nano- to femtomolar	10 to ~1000	High throughput, existing infrastructure	Cross reactivity on surface	[24]–[38]
Encoded bead arrays	Nano- to picomolar	<100	Easy processing, flexible, customizable	Limit on multiplexing capacity	[39], [42]–[53]
DNA barcoding, proximity ligation	Pico- to femtomolar	<50	Flexibility to use nucleic acid analytical methods	Requires DNA-antibody conjugation	[58]–[62]

* Aptamers, when available, can be used instead of antibodies in many applications.