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# Proteomics in 2002: A Year of Technical Development and Wide-Ranging Applications

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**Review Contents**



The term *proteome* was coined in the mid 1990s (*1*). A proteome *i*s the ensemble of proteins related to a genome. Proteomics is the study of the proteome. Proteomics has seen a tremendous growth in popularity over the last 9 years, as illustrated by the number of publications related to proteomics. We thought that it would be warranted to include "proteomics" as part of the annual review of *Analytical Chemistry*. This is the first annual review on proteomics, and we hope to see many more.

In the early days, proteomics was founded by the combination of two-dimensional gel electrophoresis (2DE) and mass spectrometry (MS). Over the years, proteomics has expanded to include profiling, functional, and structural proteomics based on a broad range of technologies. *Profiling proteomics* consists of identifying the protein present in a biological sample or the proteins that are differentially expressed between different samples. This approach is often used to make a list of proteins present in a sample and to discover proteins that are differentially expressed between different samples.

*Functional proteomics* attempts to discover protein functions based on the presence of specific functional groups or based on their involvement in protein−ligand interactions, such as protein complexes. Proteins need to interact with other molecules to perform their roles. Therefore, knowing the interactors of a protein can help to discover its role in the cell. Similarly, pathways can be defined as a cascade of specific protein interactions required to activate cellular functions. Posttranslational modifications, such as phosphorylation, define the activity, localization, and degradation of proteins and are key in understanding the functions of proteins. In some ways, functional proteomics focuses on understanding part of the wiring diagram of a cell.

*Structural proteomics* attempts to determine the tertiary structure of proteins, the structure of protein complexes and small-molecule protein complexes. X-ray crystallography and prediction of protein structures by computational biology are its main methods.

In this review, we will cover the main technical developments as well as the applications that were published in 2002. Over 1300 papers with the word proteome or proteomic(s) are reported in Pubmed for 2002. The key characteristic of 2002 was the number of applications that were reported. This review does not attempt to be exhaustive. Instead, it focuses on 200 publications that illustrate the breath of proteomic development and applications.

## TECHNOLOGY DEVELOPMENT IN PROTEOMICS

2002 was a year of continuous technology developments. Key developments in 2DE, protein processing and production, protein phosphorylation mapping, protein/peptide arrays, gel-free methods, and analytical informatics are reported.

**2DE Development.** Guttman et al. (*2*) undertook the miniaturization of 2DE using ultra-thin-layer gel electrophoresis. They utilized short immobilized pH gradient (IPG) strips for the first dimension followed by ultrathin SDS polyacrylamide gel electrophoresis for the second dimension. Detection of proteins was achieved by laser-induced fluorescence detection through incorporation of the Sypro Red dye prior to the second dimension.

IPG is the first dimension typically used in 2DE. It can also be used on its own for the analysis of proteins. Poland et al. (*3*) developed a new protocol for the electroblotting of IPG. This would be of particular interest to researchers interested in the separation of protein isoforms due to posttranslational modifications. They

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illustrated their approach using seven isoforms of recombinant erythropoietin beta.

Gorg et al. (*4*) reported the prefractionation of proteins prior to narrow pH 2DE. In their approach, they fractionated the proteins by flat-bed isoelectric focusing in Sephadex granular gels. The pH fractions were removed from the flat bed and directly installed on IPG strips to begin narrow pH 2DE. They demonstrated this approach using mouse liver lysates.

Klein et al. (*5*) proposed the utilization of microporous hollow fiber membranes for isoelectric focusing in gel. In this approach, an immobilized pH gradient is cast in the hollow fiber. The separation of proteins is performed as usual. However, there was no need to extrude the IPG after the run. Instead, the hollow fiber is wetted and the proteins are transferred to the second dimension through the wall of the fiber.

Separating basic proteins in an IPG strip is often problematic. This is often explained by the electroosmotic pumping that occurs at extreme pH in IPG strips and by migration of DTT in high pH. Hoving et al. (*6*) introduced a modified IPG protocol that improves the separation of basic proteins. They first reduced the electroosmotic pumping effect at extreme pH by adding glycerol and 2-propanol to the focusing medium. They also introduced excess DTT at the cathode to improve the separation. Using this approach, they were able to get marked improvement in the pH 6.2−8.2 range and provided some improvement up to pH 9.5.

The yield of the in-gel digestion of proteins is often an issue. Ross et al. (*7*) modified the buffer for the second dimension of 2DE to include an acid-labile surfactant (ALS) instead of the conventional SDS. They reported an increased number of peptides observed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS of a Coomassie-stained 2DE. They attributed this improvement to the decomposition of ALS due to the acidic conditions during the staining and destaining of the gel.

Ros et al. (*8*) developed an approach to selectively extract proteins from a flowing stream based on their p*I*. This is achieved by flowing the protein mixture in a flow chamber located underneath an IPG strip. The proteins that are at equal p*I* to the gel do not enter the IPG gel, while the other proteins enter the IPG gel. Therefore, the flow through from the chamber is enriched in proteins that are selected according to the p*I* above the chamber.

The solubilization of hydrophobic proteins is often a challenge for 2DE. Luche et al. (*9*) studied the effects that different detergents have on the 2DE profiles of human red blood cells membrane and *Arabidopsis thaliana* leaf membrane. They presented dodecyl maltoside, Triton X-100, and Brij 56 as good alternatives to sulfobetaine detergents.

Differential protein analysis by 2DE has always been a complex process involving 2DE alignments. Alternatively, differential in-gel electrophoresis (DIGE) (*10*) allows two different samples to be run on the same 2DE by providing a different fluorescent tag to the two original protein mixtures. Fluorescent dyes (Cy3 and Cy5) are used to label different protein mixtures that can be mixed prior to separation by 2DE. Because the two fluorescent dyes do not emit at the same wavelength, the expression levels from the two protein mixtures can be deconvoluted even though they are on the same gel. Yan et al. (*11*) coupled this approach with MALDI-TOF MS identification of proteins. They used *Escherichia coli* to demonstrate the performance of this approach and the identification of the proteins contained in 179 spots that showed different expression by fluorescence. A different approach was developed by Jiang and English (*12*) based on metabolic labeling of proteins using Leu and Leu-$d_{10}$. The cell lysates were mixed and separated by 2DE followed by protein identification based on MS analysis of their tryptic peptides. Peptides of identical sequences from the two different samples can be distinguished by MS as they differ in mass due to the presence of Leu or Leu-$d_{10}$. The observed intensities can be used to quantify the changes in protein expression between different samples. Smolka et al. (*13*) combined the isotope-coded affinity tag (ICAT) reagent with 2DE for the differential analysis of proteins. In this approach, a first protein sample is labeled with the light ICAT reagent and a second protein sample is labeled with the heavy ICAT reagent. The samples are separated by 2DE or mixed and separated by 2DE followed by silver staining. They utilized yeast grown in glucose and galactose to demonstrate this approach.

Malmstrom et al. (*14*) presented a database focused on 2DE and MALDI TOF MS (http://2ddb.org/). This database allows the rapid view of gel images and protein identification. Cho et al. (*15*) reported the development of a LIMS system for 2DE and spot information. Randic et al. published a series of papers (*16*−*19*) on the graph representation of proteomic maps. The main focus of the papers is to demonstrate that graph theory can be used to characterize 2DE maps. In particular, they demonstrated the potential of this approach using data previously published on the effects of peroxisome proliferators on protein abundance in mouse liver (*16*).

**Protein Processing and Protein Production.** Recently, Russell et al. (*20*) demonstrated that the rate of protein digestion using trypsin can be improved using digestion conditions that incorporate organic solvent. They demonstrated that the rate of protein digestion in organic solvents, such as methanol, acetone, 2-propanol, and acetonitrile, is improved over the conventional aqueous digestion of proteins. Furthermore, it appeared that the protein sequence coverage increased when organic solvents were present during the enzymatic digestion. Craft et al. (*21*) developed a hydrophobic based microcolumn for the capture and digestion of proteins. In this approach, the protein mixture of interest is flown through the microcolumn, where the proteins are adsorbed. Trypsin is then introduced for the digestion to proceed, and the resulting peptides are analyzed by MS. Peterson et al. (*22*, *23*) reported the development of a microdevice that contains immobilized trypsin on porous polymer monoliths to form an enzymatic microreactor. They reported digestion times as low as 1 min (*22*).

The purification of significant quantities of proteins is important for functional and structural proteomic studies. Sawasaki et al. (*24*) reported a cell-free system for the high-throughput synthesis of gene products. This approach is based on the translation apparatus of wheat seeds and is performed in vitro. They reported that one person could produce up to 50 proteins (0.1−2.3 mg) over a 2-day period. Lamla et al. (*25*) also reported the use of a cell-free system for the production of Strep-tag labeled proteins. The tag is used to enrich the protein of interest. Liu et al. (*26*) reported a phage display approach for the production of mono-

clonal antibodies against proteins on a large scale. Braun et al. (*27*) shuffled 32 cDNAs encoding human proteins into 4 expression vectors compatible with bacterial expression. They used an automated protein purification procedure based on the presence of affinity tags to purify the 128 protein products. Doyle et al. (*28*) utilized the ligation-independent cloning approach with expression in *E. coli*. Proteins from *Xylella fastidiosa* and *Ciona instestinalis* were expressed using this approach. They reported protein yields ranging from 10 to 100 $\mu$g.

Lo et al. (*29*) developed a set of probes that can be used to selectively recognize active protein tyrosine phosphatases in gel. The probes consisted of a recognition head (phosphate group), a trapping group to retain the attachment, a linker, and a reporter group. They demonstrated the detection of protein tyrosine phosphatases in the presence of standard proteins.

**Protein Phosphorylation Mapping.** The global mapping of protein phosphorylation became increasingly feasible in 2002 due to the introduction of significant new methods. Stancato et al. (*30*) developed a new method for the purification of phosphorylated proteins. They used a mixture of anti-phosphotyrosine antibodies to extract phosphorylated proteins from cell pellets lysed under either native conditions or denaturing conditions. They demonstrated using IFNa- or IL2-treated primary human lymphocyte extracts, 2DE, and western blotting that different profiles of proteins are obtained from the native and denaturing conditions.

Ficarro et al. (*31*) reported a MS approach that rapidly analyzed the phosphoproteome. They combined the esterification of the acidic amino acid residues with immobilized metal affinity chromatography (IMAC) purification of phosphorylated peptides, followed by MS identification and mapping of phosphorylated peptides. The esterification of the acidic residues reduces the binding of acid peptides to the IMAC material. Using yeast, they demonstrated that 383 phosphorylation sites could be rapidly mapped. They achieved a sufficient level of detection to identify rare protein phosphorylations, such as tyrosine phosphorylation in yeast and the phosphorylation of proteins with a low codon bias (i.e., low abundance). The method described by Ficarro was also used to identify phosphorylation sites in whole protein digests from capacitated human sperm (*32*). They were able to map over 60 phosphorylation sites using this approach. Alternative approaches, based on the chemical derivatization of the phosphorylated proteins, have been proposed by Zhou (*33*) and Oda (*34*).

Goshe et al. (*35*) reported the application of phosphoprotein isotope-coded affinity tags to enrich low-abundance phosphoproteins. This approach consists of performing $\beta$-elimination of phosphate from phosphorylated amino acid residues in proteins followed by a labeled EDT addition and by biotinylation of the previously phosphorylated serine and threonine. The labeled peptides are then purified through an avidin column and analyzed by MS. Li et al. (*36*) reported a method to identify phosphoserine- and phosphothreonine-containing peptides by tandem mass spectrometry (MS/MS). $\beta$-Elimination followed by sulfite addition was used to detect the phosphorylation sites as cysteic acid and $\beta$-methylcysteic acid residues. Ruse et al. (*37*) developed a method based on liquid chromatography coupled to MS for the quantification of protein phosphorylations using selected ion monitoring. Chen et al. (*38*) used a variety of MS-based approaches to study hyperphosphorylated Net1 involved in regulating mitotic exits in yeast. They applied a $Ga^{3+}$ IMAC purification of Net1 phosphopeptides coupled to MS, an alkaline phosphatase treatment of Net1 followed by peptide identification by MS, MALDI-PSD on the Net1-derived peptides and nanospray MS/MS on the Net1-derived peptides. They concluded that none of these methods were able to identify all of the phosphorylation sites of Net1. It is worth noting that the IMAC-based approach they used was not the novel approach published by Ficarro (*31*).

Maguire et al (*39*) developed an approach for studying the phosphotyrosine proteome derived from thrombin-activated platelets. In this paper, they immunopurified phosphotyrosine-containing proteins using the phosphotyrosine antibody 4G10 from resting and thrombin-activated human platelets. The two phosphotyrosine proteomes were separated by 2DE and analyzed by MS. They found that 67 proteins were only in the activated form of the platelets, and they managed to identify 10 phosphoproteins by MALDI-TOF MS. Imam-Sghiouar et al. (*40*) utilized a similar approach to study the phosphoproteome differences between control B-lymphoblasts and B-lymphoblasts from a patient suffering from Scott syndrome, an inherited hemorrhagic disorder. They focused on the phosphotyrosine proteome using an anti-phosphotyrosine antibody, performed 2DE on the immunoprecipitates and utilized MALDI-TOF MS for protein identification. They identified through their method 23 proteins in the immunoprecipitate in the control $\beta$-lymphoblast and identified 4 proteins that are different between the Scott syndrome and the control. A series of anti-phosphoserine and anti-phosphothreonine antibodies were used by Grønborg et al. (*41*) for the immunopurification of serine/threonine-phosphorylated proteins followed by gel separation and analysis by MS.

Zappacosta et al. (*42*) reported an approach that uses different MS-based analysis methods for phosphopeptide mapping. An HPLC-(electrospray ionization) ESI-MS system that monitors specific marker ions for phosphopeptides and manual fraction collections were used to collect fractions that specifically contained phosphopeptides. The fractions were then subjected to a MALDI-PSD or nanoflow-ESI-MS/MS analysis with a precursor ion scan. They were able to map phosphorylation sites of gel-separated proteins at <200 fmol. They also mapped 11 in vivo phosphorylation sites on the yeast protein kinase YAK1.

Fenaille et al. (*43*) recently described a method for the immunopurification of protein modified by malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). These modified proteins are commonly produced by the oxidation of $n - 6$ fatty acids in foods and potentially in certain diseases. Immobilized anti-HNE and -MDA antibodies were used to purify HNE- and MDA-derivatized standard peptides and HNE- and MDA-derivatized peptides from standard proteins. The purified peptides were then analyzed by MS.

MacDonald et al. (*44*) combined classical Edman degradation using $^{32}P$ labeling as well as a cleavage of radiolabeled protein program (CRP) for the rapid mapping of sites of phosphorylations on proteins.

Raska et al. (*45*) introduced a MALDI-QqTOF method to directly sequence (MS/MS) phosphopeptides from IMAC beads. In this approach, an aliquot of IMAC beads is placed on a MALDI target followed by an aliquot of matrix solution. MALDI-Qqtof is then used to measure the mass of the phosphopeptides bonded

to the IMAC as well as sequencing them through MS/MS.

Ruse et al. (*46*) presented a method for quantitating specific protein phosphorylation using HPLC-ESI-MS. This method uses selected ion monitoring to determine the peak areas of certain tryptic peptides from a protein of interest. The quantitafication is achieved by comparing the peak areas for the phosphopeptide of interest to a reference peptide obtained from an unmodified region of the protein of interest. They were able to quantify the amount of phosphorylation with a 10−15% RSD. A method for the absolute determination of the stoichiometry of protein phosphorylation was developed by Zhang et al. (*47*). They used a chemical approach to label the N-terminus of peptides with either a D(5)- or D(0)-propionyl group. MALDI-TOF MS was used to demonstrate that the stoichiometry of phosphorylation can be determined within 10%.

MacCoss et al. (*48*) proposed an approach that combined multienzyme digestion of proteins and nanoflow HPLC-ESI-MS/MS for the mapping of phosphorylation sites. They reported that an increase in protein coverage is achieved using a combination of trypsin, subtilisin, and elastase to digest proteins. They demonstrated that the determination of phosphorylation sites using a pull-down in *S. pombe* of tap tagged CDC2. They determined that 200 proteins were present in the pull-down, and they obtained greater than a 40% sequence coverage for 40% of these proteins. Phosphorylation sites were mapped on two of the interactors of CDC2. Eight liters of *S. pombe* was used to achieve these results.

Steen et al. (*49*) used immonium ion scanning for the selective detection of phosphotyrosine-containing peptides in complex mixtures. Gronborg et al. (*50*) utilized phosphoserine- and phosphothreonine-specific antibodies to enrich phosphorylated proteins, followed by analysis by MS. They applied this approach to the measurement of the differences between normal cells and cells treated with a phosphatase inhibitor.

Some nucleic acids, called allostetic ribozymes, are able to catalyze reactions when activated by small molecules or macromolecules. Vaish et al. (*51*) used modified ribozymes to pull out ERK 1/2 proteins, as well as specifically pulling out their phosphorylated forms. This could provide a novel approach to generate reagent that specifically recognizes phosphorylated proteins. Furthermore, these reagents are produced by in vitro combinatorial synthesis allowing large libraries to be generated.

**Protein/Peptide Arrays.** Schweitzer et al. (*52*) combined rolling-circle amplification (RCA) for the amplification of signal with antibody pull-down for the expression mapping of proteins. In this system, antibodies specific to some proteins are immobilized on an array. The array is incubated with a lysate, washed, and then probed with secondary antibodies specific for the proteins of interest. Then, a universal antiantibody coupled to RCA is used to detect and quantify the pull-down proteins. Using this approach, they developed a microarray specific for 51 cytokines.

Pellois et al. (*53*) developed a microchip with individually addressable peptide/peptidomimetics to perform epitope mapping of antibodies. Woodbury et al. (*54*) developed a protein array on slides to perform a sandwich assay using antibodies for the detection of human growth factor (HGF) in serum. They were able to detect HGF at a concentration of 0.5 pg/mL (6 fM) while preserving the linearity over 3 orders of magnitude. This could potentially lead to the development of a proteomic array for the detection of serum proteins. Wang et al. (*55*) reported the development of an antibody array on hydrogel pads for the detection of 43 cytokines and chemokines in human plasma. Zhu et al. (*56*) overexpressed and purified 5800 yeast open reading frames. They printed protein microarrays using the expressed proteins. These protein microarrays were used to screen interactions with proteins and phospholipids. Pavlickova et al. (*57*) presented an array for the detection of antibodies in human serum. They developed a surface chemistry consisting of a gold layer, a biotinylated self-assembled architecture, and a streptavidin probe. IgM was detected in human serum using this array in a sandwich assay.

**Gel-Free Methods.** Zhou et al. (*58*) introduced a novel solid-phase-based ICAT reagent. They developed an ICAT reagent that is linked to beads through a photocleavable linker. Briefly, in this approach two protein mixtures of interest are digested. The cysteine-containing peptides are reduced and captured on beads with either the leucine-$d_0$ or leucine-$d_7$ solid-phase ICAT. The beads are then combined, and washed, and the tagged peptides are released by photocleaving the linker. They compared the solid-phase approach to the conventional ICAT approach using yeast cell lysates and identified more proteins with the solid-phase approach. Borisov et al (*59*) studied the fragmentation of peptides labeled with the ICAT reagent and peptides labeled with iodoacetyl-PEO-biotin. They concluded that the presence of the labels on cysteine provide a useful identifier of the cysteine-containing peptides.

The isotopic labeling approaches often rely on deuterium/hydrogen to provide the mass shift observable by MS. However, the change from hydrogen to deuterium labels affects the separation of peptides by HPLC. Zhang and Regnier (*60*) clearly demonstrated that deuterium/hydrogen labeling reagents change the mobility of the labeled peptides, whereas $^{13}C/^{12}C$ labeling reagents do not significantly change the elution profiles of peptides. In another paper, Zhang et al. (*61*) demonstrated that the position of the deuterium/hydrogen within the tag itself, the proximity of hydrophilic group, and the size of the tag affect the magnitude of the elution changes that the isotopic labels cause.

Qiu et al. (*62*) reported the development of a novel class of chemically modified resins called acid-labile isotope-coded extractants (ALICE). This reagent consists of a cysteine reactive group attached to a linker and to an acid-labile group attached to a resin. The resin is available with a light and heavy linker. In this approach, the heavy/light resins are incubated with the individual tryptic digest from the protein mixtures. Once the capture of cysteine-containing peptides is achieved, the resins are mixed, and heavy/light labeled peptides are cleaved off the resins and analyzed by MS.

Gu et al. (*63*) introduced an in vivo labeling strategy with deuterium-labeled lysine residue to introduce a 4-Da mass tag at the carboxyl terminus of peptides derived using certain proteases. Differential analysis can thus be achieved by growing cells with the normal and the heavier lysine. Berger et al. (*64*) proposed a differential analysis approach based on the in vivo labeling of proteins using $^{13}C$-labeled and unlabeled lysine. They also used endoproteinase LysC digestion to produce proteomic samples

consisting of heavy/light peptide pairs and ESI-MS/MS for the identification of peptide provenance. They tested their method using yeast.

Zhu et al. (*65*) proposed a method to insert mass signatures in peptides to detect protein modifications from peptide mass maps. They used light and heavy forms of methionine-*S*-methyl-$d_3$ and serine-2,3,3-$d_3$ as mass tags for methione (oxidation) and serine(phosphorylation) modifications.

Cagney and Emili (*66*) introduced a method for the differential quantitation of proteins using guanidination of C-terminal lysine on tryptic peptides. This method, termed mass coded abundance tagging (MCAT), was coupled to ESI-MS/MS for proteomic studies. Ong et al. (*67*) proposed a method called SILAC for stable isotope labeling by amino acids in cell culture for the quantitative analysis of proteins. In particular, Leu-$d_0$ and Leu-$d_3$ were applied in the growth media to obtain differential labeling of proteins.

Isotopic labeling of proteins during their trypsinization using $^{18}O$ and $^{16}O$ water was recently introduced (*68*, *69*). In this approach, two different samples are respectively digested in $^{18}O$ and $^{16}O$ water, mixed, and analyzed by MS. Recently, Reynolds et al. (*70*) extended this method using endoprotease Glu-C as the catalytic agent with a particular interest in the study of N-linked glycopeptides. Liu and Regnier (*71*) combined $^{18}O$ labeling and primary amines labeling using [$^1H_3$]- and [$^2H_3$]-*N*-acetoxysuccinamide for the coding/decoding of C-terminal peptides and acylated N-terminal peptides.

Tags specific for the amidination of N-termini and lysine residues were developed by Beardsley and Reilly (*72*). In this approach, *S*-methylthioacetimidate and *S*-methyl thiopropionimidate are used as a labeling pairs. They proposed that this pair of tags increases the basicity of lysine residues and therefore improves the MALDI ionization.

Chelius and Bondarenko (*73*) reminded us that a no-label strategy can be used for the gel-free differential quantitation of proteins. They used the peak area observed for peptides to determine the differential quantitation of proteins.

Pratt et al. (*74*) developed a method to follow the dynamics of protein turnover in proteomics. They used deuterated Leu to label in vivo proteins in yeast. They performed the labeling over seven doubling times ensuring over 99% incorporation of deuterated Leu. They then added an excess of unlabeled Leu and sampled the yeast culture over time. The proteins were then digested and analyzed by MS. The ratio of labeled versus unlabeled peptides was used to measure the dynamics of protein turnover.

Wilkins et al. (*75*) applied displacement chromatography coupled to ESI-MS to selectively enrich low-abundance peptides in complex mixtures. They utilized a modified displacement chromatography mode that included selected concentrations of eluent in the displacer solution. They have obtained promising results with the human growth hormone.

Wagner et al. (*76*) developed an on-line 2D HPLC system with sample preparation to analyze proteins and peptides of molecular weights below 20 kDa. The low molecular weight proteins/peptides were separated from the matrix using restricted-access material followed by ion exchange chromatography and by reversed-phase chromatography. They were able to resolve about 1000 peaks within 96 min on this system with no off-line sample handling.

Kachman et al. (*77*, *78*) presented an MS-based alternative approach to 2DE by combining protein separation by liquid isoelectric focusing as the first phase with nonporous silica reversed-phase HPLC as the second phase. An ESI-TOF MS was then used to accurately measure the mass of the proteins. As well, the system was coupled to a fraction collector, and the proteins in the fractions were identified by peptide mass fingerprinting. The end results were a virtual 2D image as well as the identification of the proteins. This approach was used for comparing ovarian cancer and epithelial cell lines. Michels (*79*) presented a two-dimensional gel-free approach for the separation of proteins. They used submicellar capillary electrophoresis to separate proteins in a first dimension followed by capillary electrophoretic separation at pH 11.1. Laser-induced fluorescence detection was used for the detection of proteins.

Gevaert et al. (*80*) developed a novel gel-free approach for the chromatographic isolation of methionine-containing peptides. In this approach, complex protein mixtures were digested with trypsin and the resulting peptides were separated by reversed-phase chromatography followed by fraction collection. Next, the fractions were dried and reacted with hydrogen peroxide to turn methionine into methionine sulfoxide. The samples were then separated by reversed-phase HPLC. The experimental conditions were tuned so that methionine sulfoxide-containing peptides eluted 7 to 1 min before the bulk of the unmodified peptides. MALDI-TOF-MS and LC-ESI-MS/MS were used to analyze the resulting peptides. They identified 754 different proteins using this approach on *E. coli* lysate.

Lee et al. (*81*) developed a four-column reversed-phase chromatography system that can be run from a single HPLC pump. They demonstrated the potential of this system for high-throughput proteome analyses of yeast grown on two different carbon sources and using the ICAT quantitation approach.

A method, termed mass western experiment, was developed by Arnott et al. (*82*) to detect specific proteins in complex mixtures without the need for antibodies. In this approach, membrane proteins are labeled with the ICAT reagent. Instead of trying to identify as many proteins as possible, the experiment focuses on the peptides predicted to be present in the mixture for a few specific proteins. This is performed by HPLC-ESI-MS/MS and a MS spectrum, followed by a few CID spectra for the specific peptides, is continuously generated. This approach was demonstrated using ErbB2 present in SKBR-3 and MCF-7 cell lines by focusing on one specific peptide to prove the presence of ErbB2.

Hydrophobic proteins are a challenge for 2DE as well as for gel-free approaches. In particular, for gel-free approaches, solubilization strategies are often not compatible with reversed-phase HPLC-ESI-MS/MS analyses of peptides. Blonder et al. (*83*) developed a sample preparation method that combines carbonate extraction of hydrophobic proteins with organic solvent solubilization followed by proteolysis. They tested this protocol using membranes from *Deinococcus radiodurans* and were able to identify roughly 15% of the predicted hydrophobic proteome based on the GRAVY index.

Gilligan et al. (*84*) developed a gel-free method for the study of protein−protein interactions. They combined surface plasmon resonance (SPR) for the capture and detection of interactions. They then eluted off the proteins from the SPR and performed

protein analysis and protein identification by MS. They illustrated this approach using an antibody on the SPR to pullout lysozyme from a mixture of lysozymes spiked into cytosolic proteins from kidney cells.

**MS Bioinformatics.** Liebler et al (*85*) developed a pattern recognition algorithm (SALSA) that detects specific features in MS/MS spectra. This algorithm scores MS/MS spectra for the presence of product ions, neutral losses, charged losses, and ion pairs in order to detect peptides and modified peptides. Eddes et al. (*86*) developed an algorithm called CHOMPER to rapidly validate the MS/MS search results from SEQUEST. Sadygov et al. (*87*) presented a version of Sequest that survives node failures on a computer cluster as well as being able to determine the charge states of peptides. Mackey et al. (*88*) developed an algorithm for rapid protein identification using short peptide sequences. They based their approach on the FASTA algorithm and modified it to handle MS and Edman degradation-type sequences. Tabb et al. (*89*) reported the development of tools for comparing protein identifications from large data sets. Rusconi and Belghazi (*90*) presented a software called massXpert used to predict and analyze mass spectra (see http://frl.lptc.u-bordeaux.fr/website-frl/massxpert/massxpert-main.html). Field et al. (*91*) developed a software called RADARS, for the automated archiving and processing of mass spectral data.

## PROFILING PROTEOMICS

Profiling proteomics is the large-scale study of the proteome and the comparative changes in the proteome. Gene expression analysis is routinely applied to measure the level of mRNA expressions—so why measure protein expression levels? The question that has always been an issue is, "How predictive is the mRNA expression of the protein expression levels?". There has been widespread disagreement on this subject. Recently, Lian et al. (*92*) studied the temporal patterns of protein and mRNA expressions during myeloid development in the MPRO murine cell line. They identified 123 known proteins and 29 new proteins. They obtained a strong correlation between mRNA and protein expression over multiple points during myeloid development. Griffin et al. (*93*) performed a similar study on yeast grown on two different carbon sources. They combined mRNA expression measured by cDNA array, and proteomic expression measured by the ICAT approach. They concluded that in many cases there is a correlation between the mRNA and the protein expression, such as the genes involved in glycolysis. In other cases, the correlation is poor, for example, in protein synthesis genes. Interestingly, a systematic study of mRNA and protein expression was performed on lung adenocarcinomas by Chen et al. (*94*). A total of 165 protein spots and their corresponding gene expressions were measured on 76 lung carcinomas and 9 none-neoplastic lung tissues. A poor correlation was obtained between protein and gene expressions. Ørntoft et al. (*95*) studied gene expressions and protein expressions in pairs of noninvasive and invasive human transitional cell carcinomas. They obtained a good correlation between protein expressions and gene expressions by focusing on high-abundance proteins. The overall conclusion of these papers suggests that the best approach for understanding cellular processes is to combine as many tools as possible, such as gene expression and protein expression.

**Animal Proteome.** Wait et al. (*96*) reported the analysis of the bovine serum. In particular, they compared serum obtained from a normal cow to the serum obtained from a cow suffering from udder edema, a metabolic problem of early lactation. 2DE of the serum proteins were performed and protein identification was done by MALDI-TOF MS and HPLC-ESI-MS/MS. The overloading of 2DE clearly illustrated the limitation of the approach for the analysis of serum proteins. They, however, managed to identify a series of bovine-related proteins.

Benvenuti et al. (*97*) worked on the mechanisms that limit the mitotic ability of somatic cells. They utilized 2DE coupled to MS to discover the proteins differentially expressed upon a replicative senescence using rat embryo fibroblast cell lines. They found 43 spots differentially expressed.

The Myc oncoprotein is frequently altered in human cancer. Shiio et al. (*98*) have performed a proteomic study to determine the effect of Myc on the protein expression profile of cells. They used ICAT and tandem MS to study the global protein expression profile in rat *myc*-null cells compared to *myc*-plus cells(null cell to which *myc* was introduced). They identified and quantified 528 proteins of which 177 had at least 2-fold changes in expression. Interestingly, a significant portion of the proteins for which they detected changes in expression were not predicted by RNA expression studies.

**Bacterial Proteome.** Tan et al. (*99*) performed a comparative study of the proteome of extracellular proteins in *Edwardsiella tarda*, a Gram-negative enteric bacterial pathogen. They studied three avirulent and four virulent forms of the bacterial by 1D gel electrophoresis and were able to find three virulent forms displaying two major protein bands compared to the avirulent forms. 2DE were further run on two of these virulent forms and the spots at the previously observed molecular weights were excised, digested with trypsin, and analyzed by MS. As well, Edman degradation was used for the spots that could not be identified by MS. Flagellin and SseB were the main proteins observed.

Trémoulet et al. (*100*) performed a proteomic study of *E. coli* to compare the effects of biofilm versus planktonic growth modes. 2DE was performed on the protein extracted from *E. coli* growth under the two different conditions. The different spots were excised, in-gel digested, and analyzed by MS. Seventeen proteins were observed to be up- or downregulated between the two 2DE.

Duché et al. (*101*) performed 2DE analysis of *Listeria monocytogenes* under different salt concentrations to see the effects of stress on protein expression. The differentially expressed spots were identified by MALDI-TOF MS, and 42 proteins were differentially expressed between low- and high-salt conditions.

The recent completion and annotation of the Gram-positive bacterium *Streptomyces coelicolor* has opened the door to its study using proteomic methods. Hesketh et al. (*102*) recently studied the proteins expressed by this bacterium. They applied 2DE to separate the proteins contained in the bacterium and MALDI-TOF MS to identify the proteins. They managed to identify 770 different proteins representing roughly 10% of the 7825 theoretical proteins.

*Chlomydia pneumoniae* is a human pathogen that causes respiratory infections. Montigiani et al. (*103*) combined genomics and proteomics to study *C. pneumoniae*. A total of 141 selected clones from *C. pneumoniae* were used to generate recombinant

fusion proteins. Mouse sera against these proteins were produced and used to detect FACS proteins present on the surface of *C. pneumoniae*. A total of 53 of the 141 selected genes were positive for membrane expression. As well, protein isolated from *C. pneumoniae* were separated by 2DE and systematically identified by MS. A total of 21 out of the 53 proteins identified by FACS were also observed on the 2DE.

**Brain Proteome.** The brain is a very complex organ. Klose et al. (*104*) performed a detailed analysis of the mouse brain proteome to establish a baseline of the proteins present in the brain. A total of 8767 proteins were resolved using 2DE, and 466 proteins were identified using MS. The study of the fetal brain proteome will help us to understand early neuronal development. Fountoulakis et al. (*105*) studied the human fetal brain by 2DE and identified 1700 proteins produced from 437 genes. Seven gene products were identified for the first time in fetal brain. Gozal et al. (*106*) analyzed the CA1 and CA3 regions of rat hippocampus following exposure to intermittent hypoxia (sleep apnea). Fifteen proteins were differentially expressed between the CA1 and CA3 regions of the brain. Similarly, Chen et al. (*107*) studied the proteome of human temporal cortex lobes. Seven spots were obviously different between the left and right lobes. Huntington's disease is an inherited disease that starts in midlife and leads to death. Zabel et al. (*108*) studied the proteome of the Huntington's disease using a mouse model by 2DE. The pituitary gland is important for the regulation of physiologic and metabolic processes. Beranova-Giorgianni et al. (*109*) studied the proteome of the normal pituitary gland by 2DE followed by protein identification by MS. A total of 38 proteins were identified. Carboni et al. (*110*) worked on optimizing the preparation protocol for the 2DE analysis of rat brains.

Yu et al. (*111*) used the ICAT approach to study the changes in protein expression between the ± treatments of mouse's cortical neurons with camptothecin. The protein extracts from the ± experiments were labeled with the light and heavy forms of the ICAT reagent, respectively. The labeled protein extracts were then mixed, digested, and analyzed by HPLC-ESI-MS/MS on an ion trap MS while Fourier transform mass spectrometry (FTMS) was used to quantify the peptide abundances. A total of 125 proteins were differentially expressed due to the treatment with camptothecin. Che and Fricker (*112*) reported the analysis of neuropeptides in mice using differential isotopic tags and MS. In their approach, they used the $H_6$ and the $D_6$ form of acetic anhydride to label the C-terminal basic amino acids of neuropeptides. These labeled peptides are then purified by affinity chromatography on anhydrotrypsin agarose and analyzed by MS. They applied this method for the differential analysis of neuropeptides between a $Cpe^{fat}$/$Cpe^{fat}$ mouse (lacking carboxypeptidase E activity) pituitary extract and a wild-type mouse pituitary extract, resulting in an observation of 23 different neuropeptides.

**Breast Proteome.** Wulfkuhle et al. (*113*) reported the first proteomic analysis of human breast ductal carcinoma using 2DE followed by the identification of proteins by MS. They ran 10 sets of 2DE of matched normal ductal/lobular units and ductal carcinoma in situ from whole tissue sections. They also ran 2DE using up to 100 000 epithelial cells obtained by laser capture microdissection. They analyzed 315 spots by MS and found that 57 proteins were differentially expressed between normal ductal/lobular units and ductal carcinoma in situ. The expression trends of some of the proteins were confirmed by immunohistochemical analysis. Chen et al. (*114*) studied the effects of doxorubicin on a breast cancer cell line by 2DE and focused on a few proteins. Hathout et al. (*115*) studied the resistance of MCF-7 toward melphalan by 2DE. Cytosolic proteins from resistant and nonresistant MCF-7 cell lines were separated by 2DE. The proteins that fluctuated on the 2DE were analyzed by MALDI-TOF MS and ESI-MS/MS. Five proteins were observed to be differentially expressed in the cytosol fraction of the melphalan resistant MCF-7 cell line.

The regulation of proteins is key to maintaining balance in cells. In some instances, the regulation of proteins might be obtained by forming disulfides between glutathione and the cysteine under oxidative stress. Fratelliet al. (*116*) studied the human T lymphocytes to discover proteins that are subject to glutathionylation under oxidative stress with hydrogen peroxide and diamide. Proteins extracted from the stressed T lymphocytes were separated by 2DE and compared to a control gel. A total of 38 proteins were identified between the oxidative stress and the control. A major group of these proteins are involved in carbohydrate metabolism.

**Cerebrospinal Fluid Proteome.** Reports on proteomic analyses of cerebrospinal fluid (CSF) have been sparse. Yuan et al. (*117*) performed a cataloging exercise of the protein present in CSF. They identified 22 proteins present in CSF using 2DE coupled to MS. A detailed cataloging of CSF proteins was undertaken by Sickmann et al. (*118*). They reported the identification of more than 480 spots. Recently, Davidsson et al. (*119*) performed a differential proteomic analysis using CSF from patients afflicted with frontotemporal dementia compared to the control patients. 2DE separated the proteins present in CSF from the 15 affected patients versus the 12 controls. The expression of six proteins was found to be significantly altered. The discovery of protein patterns in CSF might help in the treatment of diseases. Choe et al. (*120*) used 2DE to study the proteins present in CSF of normal volunteers versus Alzheimer-affected patients. Nine molecular markers were identified to segregate Alzheimer patients versus normal volunteers. A similar study by Davidsson et al. (*121*) reported six proteins to be significantly altered between controls and Alzheimer patients. Uchida et al. (*122*) performed a SELDI-MS detection of biomarkers in CSF from patients affected by rheumatoid arthritis. They detected a few mass peaks that were correlating with the disease. They then fractionated the proteins and analyzed each fraction by surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF MS). The fractions that contained key markers were further analyzed by 1D gel followed by protein identification using MALDI-TOF MS. They reported that MRP8 might be a biomarker in CSF for rheumatoid arthritis.

**Esophage Proteome.** Xia et al. (*123*) utilized 2DE coupled to MALDI-TOF MS to study esophageal squamous cell carcinomas. They performed the study using 24 pairs of matched carcinomas/matched adjacent normal epithelium cells. They reported that three isoforms of annexin I were downregulated in the cancer tissue. Zhou et al. (*124*) applied the DIGE method followed by MS to identify protein markers for esophageal cancer. Using this approach, they were able to visualize 1038 spots in cancer cell lysates versus 1088 protein spots in normal lysates.

**Eye Proteome.** Crabb et al. (*125*) studied age-related macular degeneration (AMD) by focusing on Drusen and Bruch's membranes. They obtained samples from 18 normal volunteers and 5 patients afflicted with AMD and performed HPLC-ESI-MS/MS analysis of the proteins. A total of 129 proteins were identified and 16% of them were demonstrated to be localized to the Drusen.

**Fungal Proteome.** Pitarch et al. (*126*) presented a method for the proteomic analysis of the cell wall proteins of *Candida albicans*. They performed membrane protein solubilization using hot SDS and DTT followed by extraction by mild alkali conditions or by glucanases and chitinases treatments. Nandakumar and Marten (*127*) worked on the solubilization of intracellular proteins from *Aspergillus oryzae*. They concluded that the best way of extracting the soluble proteins was to use mechanical lysis with glass beads and a bead beater. 2DE was performed on these fractions followed by MS analysis. Melin et al. (*128*) studied the effects of the antibiotic produced by *Streptomyces halstedii* on the proteome of *Aspergillus nidulans* (fungus). They discovered by 2DE that 20 proteins change expression with/without treatments with the antibiotic. MS was used to identify five proteins. Thomas-Hall et al. (*129*) studied novel strains of yeast obtained from Antarctic soils. They obtained a proteome fingerprint for these novel strains.

Washburn et al. (*130*) performed multidimensional protein separation for differential quantitation of yeast grown either under $^{14}N$ minimal media or $^{15}N$-enriched minimal media. These yeast samples were mixed, digested, and analyzed by multidimensional chromatography consisting of step elution ion exchange chromatography coupled to reversed-phase chromatography. The multidimensional approach identified over 800 proteins while the single dimension approach only identified 103 proteins.

**Heart Proteome.** Myocardial ischemia is a combined effect of oxygen deprivation to the heart muscle and the poor removal of metabolites due to a reduced blood flow. For example, this can be caused by a combination of coronary obstruction and excessive exercises. Schwertz et al. (*131*) studied the myocardial protein expression following myocardial ischemia and reperfusion with and without a serine protease inhibitor (reduces necrosis). A total of 509 spots were found by 2DE separation of the material obtained from a rabbit model. Ten spots differentially expressed were analyzed by MS. Superoxide dismutase precursor and $\alpha$,$\beta$-crystallin were identified in these spots. McDonough et al. (*132*) developed a protocol for analyzing myocardial biopsies (20−50 $\mu$g) by 2DE. This technique uses a combination of Coomassie and silver staining of the proteins, MS protein identification was by peptide mass fingerprinting and confirmation by western blotting. Labugger et al. (*133*) optimized protein solubilization from human cardiac tissues for 2DE analysis. They solubilized the proteins using 19 different conditions and focused on the detection of the myofilament protein troponin T. The best results were obtained by homogenization in 6 M urea, 2.5 M thiourea, 4% 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate, and 2.5 M sodium chloride.

Neverova and Van Eyk (*134*) developed a "gel-free" protein fraction approach based on reversed-phase chromatography for the study of cardiac muscle. They compared the changes in chromatographic profiles obtained for muscles from a coronary artery ligation animal and from a sham-operated animal. Fractions obtained from the changing regions of the profiles were digested and analyzed by MALDI-TOF-MS. Troponin T and myosin heavy chain were found to be differentially expressed.

**Kidney Proteome.** Toxic effects of drugs can often be observed in organs such as the liver and the kidney. In fact, a drug called gentamicin causes toxicity in the kidney by reducing the capacity of the tubules in the kidney to reabsorb proteins. Charlwood et al. (*135*) performed a proteomic analysis of a rat kidney cortex treated with gentamicin. The proteins extracted from the cortex were separated by 2DE. The 2DE of cortex with/without treatment with gentamicin were compared and the different spots analyzed by MS. Over 20 proteins were found to be different between the treated and nontreated rat kidney.

**Liver Proteome.** Liver diseases, such as carcinoma, are a leading cause of death worldwide. Kim et al. (*136*) studied the difference in proteomes between normal tissues and hepatocellular carcinoma tissues using 2DE coupled with MALDI-TOF MS. Nine spots were overexpressed and 28 underexpressed. Eleven proteins were identified in these spots. Fountoulakis and Suter (*137*) worked to establish a catalog of the proteins present in the rat liver by 2DE followed by protein identification by MS. They identified 273 proteins present in the liver. The level of toxicity that a drug has on the liver is an important factor in the development of drugs. Man et al. (*138*) demonstrated that toxic effects on the liver of animal models (rat) from drugs can be monitored by 2DE coupled to MS.

**Lung/Respiratory Tract Proteome.** Proteomics was also used to study the difference in protein patterns between bronchoalveolar lavage fluids obtained from 7 normal children and 10 children with malignancies and affected by respiratory infections (*139*). Predic et al. (*140*) followed the changes in protein expression over the first 4 h after stimulation of human lung fibroblasts with endothelin-1. They were able to detect 70 proteins with changes in expression after stimulation using pulsed [$^{35}S$]-methionine labeling. Of these, 35 were identified by MS. Some of the proteins were functionally linked. Malmstrom et al. (*141*) isolated fibrotic cells from human lung biopsies from 12 patients. These cells were separated by 2DE followed by protein identification by MALDI-TOF MS. Cytoskeletal, scavenger, metabolic, and adhesion proteins were observed in these 2DE. Kanamoto et al. (*142*) described the proteomics analysis of Mv1Lu lung epithelial cells treated with TGF$\beta$1. 2DE were run from lysates obtained from the ± TGF$\beta$1 experiments, and 38 low-abundant spots were analyzed by peptide mass fingerprinting. A total of 28 of the 38 proteins identified are potential new targets of TGF$\beta$1.

Cilia are important in the protection of the respiratory tract for mucociliary clearance. Ostrowski et al. (*143*) cultured human bronchial epithelial cells obtained from excess surgical tissues. Cilia were isolated from the culture using a deciliation buffer. The proteins present in the cilia were then separated by 1DE and 2DE and analyzed by MS. As well, lysates from cilia were digested and analyzed by HPLC-ESI-MS/MS and multidimensional HPLC-ESI-MS/MS. A total of 214 proteins were identified through the combination of all these techniques.

**Muscle Proteome.** Linnane et al. (*144*) reported proteomic results from their continuing clinical trial on the effect of coenzyme $Q_{10}$ on human quadriceps muscles. They obtained muscle samples from aged individuals and treated them with placebo or coenzyme

$Q_{10}$. 2DE of these samples were obtained. Over 2000 spots were observed while 174 spots were induced and 77 spots were repressed by coenzyme $Q_{10}$ treatment. Taurin et al. (*145*) studied the effect of apoptosis of smooth muscle cells on the remodeling of vessel walls. In particular, they focused their study on the inhibition of apoptosis in cultured vascular smooth muscle cells. 2DE of the protein extracts from normal versus inhibited muscle cells were performed. Mortalin, a member of the HSP70 protein family, was observed to be highly overexpressed when apoptosis was inhibited. Isfort et al. (*146*, *147*) studied the proteomes of rat soleus and tibialis anterior muscle following immobilization. 2DE was performed on the tissues at different times postimmobilization. A total of 17 and 45 spots were observed to significantly change for respective pairs of normal/atrophic soleus and normal/atrophic tibialis muscles.

**Ovarian Proteome.** Jones et al. (*148*) used 2DE to display the proteins present in invasive epithelial ovarian cancer versus low malignant potential tumors. In the first instance, they performed 2DE on 50 000 cells obtained by laser capture microdissection and found spots that were differentially expressed. They then selected these spots from the same position on 2DE of whole undissected cryostat section lysates (larger amount of cells) and performed in-gel digestion followed by identification by MS. They found the FK506 binding protein, the Rho G-protein dissociation inhibitor, and the glyoxalase 1 to be uniquely overexpressed in invasive ovarian cancer.

**Parasite Proteome.** Parasites are one of the leading causes of mortality in developing countries. The development of efficient treatments requires a strong understanding of their proteomes.

The malaria parasite (*Plasmodium falciparum*) resides in the host erythrocyte within a vacuole. Lasonder et al. (*149*) performed a proteomic study of *P. falciparum* and identified 1289 proteins. Florens et al. (*150*) studied the effect of the *P. falciparum* life cycle on its proteome using the mudPIT protein identification approach. They followed over 2400 proteins in the physiological stages. Greenbaum et al. (*151*) studied the role of the protease facipain 1 in host cell invasion in human malaria parasites. For more details, see the Functional Proteomics section.

Cohen et al. (*152*) studied the proteome of toxoplasma gondii by 2DE followed by protein identification by MS. They separated over a 1000 proteins from *Toxoplasma gondii*. Unfortunately, the identification of the proteins was more limited due to the incomplete genome sequence.

**Plant Proteome.** The analysis of the plant proteomes is a challenge. The genomes of the majority of plants are not sequenced, thus limiting the identification of proteins by database searching. The application of proteomics on plants is at a very early stage of development, and the number of publications related to plant proteomics is relatively low. Salekdeh et al. (*153*), however, analyzed the proteome of the rice leaves during drought stress and their recovery. Drought was simulated on potted plants over a 23-day period. The proteins were extracted from the leaves, separated by 2DE, and analyzed by MS. Forty-two proteins out of over 1000 proteins examined were found to have a change in their level of expression in response to gradual drought stress. Furthermore, the change in expression was reversed when the plants under stress were watered.

Koller et al. (*154*) also performed a proteomic study of rice. They focused their study on the protein extracted from the leaf, root, and seed tissue of rice. Two proteomic methods were used to study the plant: 2DE followed by protein identification by MS as well as a "gel-free" multidimensional peptide separation method coupled to the MS (MudPIT approach (*155*)). Using these two approaches, they reported the cataloging of 2528 unique proteins from rice; 33% of these proteins had unidentified functions by homology searching. As well, different protein patterns were observed in the leaf, root, and seed tissue. Bardel et al. (*156*) reported a similar study on the pea mitochondria purified from leaves, roots, and seeds. The analysis was performed by 2DE followed by Edman degradation, MALDI-TOF MS, and ESI-MS/MS. Differences in the mitochondria proteomes were observed between the leaves, roots, and seeds. Mikami et al. (*157*) reported that rice membrane-associated proteins are better separated using nonequilibrium pH gradient electrophoresis/SDS−PAGE instead of 2DE.

The amyloplasts contained in the endosperm of seeds control the process of grain filling (starch production). Therefore, understanding the changes in protein content in the amyloplasts could help in understanding the process of grain filling. Andon et al. (*158*) presented the proteomic analysis of wheat amyloplasts using 1D and 2D gel electrophoresis, followed by in-gel digestion and protein analysis by HPLC-ESI-MS/MS. They identified 171 proteins from the amyloplasts, 63 of which were from the membrane. The majority of protein identification was achieved by finding homologous proteins in other crop species. Maltman et al. (*159*) also performed a study on the endosperm of *Ricinid communis*. They focused on the proteins contained in the endoplasmic reticulum during the germination and development of the bean.

Ferro et al. (*160*) developed a subcellular fractionation approach for a proteome study of a plant's chloroplast membrane. Their objectives were to identify membrane proteins involved in the transport activities. The chloroplasts were obtained from spinach leaves and purified by centrifugation on Percoll gradients, an established approach for membrane fractionation. An organic extraction step was used to obtain the hydrophobic proteins from the chloroplast membrane fraction. The proteins were separated by gel electrophoresis, in-gel digested, and analyzed by ESI-MS/MS using a Q-TOF mass spectrometer. A total of 27 new chloroplast envelope proteins were discovered by this approach.

Chivasa et al. (*161*) studied the proteins extracted from the cell wall of *A. thaliana* with 2DE and peptide mass fingerprinting (MALDI-TOF MS) as part of the effort to establish the *Arabidopsis secretome* database. Fukao et al. (*162*) performed a proteomic analysis of the proteins contained in leaf peroxisome in cotyledons of *Arabidopsis*. They developed a method to isolate leaf peroxisomes and were able to identify 20 novel proteins contained in the leaf peroxisome of *Arabidopsis*.

**Prostate Proteome.** Ahram et al. (*163*) performed a study of human prostate cancer with 2DE coupled to MS identification of the differentially expressed proteins. They used 12 matched normal prostate cells and high-grade tumor cells samples that were microdissected or captured by laser capture microdissection. The study revealed 40 proteins that were differentially expressed in the tumor versus normal prostate cells. However, only 6 of the

40 proteins were reproduced in more than one pair of samples. Only one of the six proteins was seen in more than two sample pairs. Similarly, Meehan et al. (*164*) performed a proteomic study by 2DE with normal versus malignant prostate tissues obtained from 34 radical prostatectomies. A total of 20 proteins observed on the 2DE appeared to be significantly reduced in malignant transformation.

**Subcellular Proteome.** The complexity of cellular proteome can often be reduced by subcellular fractionations. Rabilloud et al. (*165*) performed a comparative study of human mitochondrial tRNA disorders. They used 2DE followed by MS to study the effect on mitochrondial proteins of single-point mutations that cause different types of mitochondrial encephalopathy. They discovered that many spots were up- and downregulated between wild-type cell lines and the mitochondrial encephalopathy representative cells. Pflieger et al. (*166*) studied the yeast mitochondrial proteome by combining 1D gel electrophoresis and protein identification by HPLC-ESI-MS/MS. They identified 179 proteins of which 29 had previously unknown subcellular localization. Zhou et al. (*167*) analyzed the spliceosome proteome using HPLC-ESI-MS/MS. They purified the spliceosome proteins, digested them with trypsin, and performed protein identification by MS. They identified 58 novel proteins that are part of the spliceosome. Similarly, Rappsilber et al. (*168*) studied the human spliceosome. They identified 55 novel proteins associated with splicing complexes using MS. Pizzaro-Cerda et al. (*169*) performed a proteomic study of the phagosomes of human epithelial cell line LoVo. They utilized latex beads coated with internalin A and internalin B. 2DE of the phagosome fractions were obtained, and differential spots were analyzed by MS. One major protein (MSF) was found to be specifically recruited on the internalin B phagosomes. Galeva and Altermann (*170*) studied the rat liver microsome by proteomics. They performed 1D and 2D gel electrophoresis of proteins isolated from microsomal vesicles followed by protein identification by MALDI-TOF MS. Low et al. (*171*) established a list of the proteins present in human erythrocyte membranes using 1D and 2D gel electrophoresis coupled to MS.

**Serum and Blood Diseases Proteome.** SELDI-TOF MS was increasingly used in 2002 for the discovery of biomarkers. Petricoin et al. (*172*) applied SELDI-TOF to the diagnosis of prostate cancer by profiling the serum from patients. Known sets of sera from patients with prostate cancer and sera from normal patients were used to train bioinformatic tools that relate the SELDI-TOF patterns to the diagnosis. Once trained, the bioinformatic tools successfully diagnosed 95% of the patients with prostate cancer and 78% of the normal patients. They perfomed a similar study on ovarian cancer using serum from patients (*173*). SELDI-TOF with a pattern-matching algorithm was used by Adam et al. (*174*) to classify normal men and men afflicted with prostate cancer based on their serum profiles. A total of 96% of the patients were properly classified between prostate cancer and normal patients. Li et al. (*175*) performed a similar study to predict breast cancer based on serum profiles obtained by SELDI-TOF MS. They screened serum samples from 103 breast cancer patients at different stages of the disease, 41 healthy patients, and 25 patients with benign breast diseases. They discovered that the combination of three protein biomarkers (masses) provided a sensitivity of 93% for cancer patients and a specificity of 91% for control patients. Paweletz et al. (*176*) also used SELDI-TOF MS to identify protein patterns that might be indicative of breast cancer. They perform their analysis on nipple aspirate fluid. They performed a study involving 12 women with breast cancer and 15 healthy controls. Rai et al. (*177*) also reported a protein marker discovery program for ovarian cancer by proteomics of plasma from patients at multiple stages of the disease. They discovered that the combination of a novel set of protein biomarkers performed better than the known ovarian markers. Adkins et al. (*178*) utilized a protein A/G column to reduce the amount of albumin present in serum. The remaining proteins were digested with trypsin and the resulting peptides separated by ion exchange chromatography coupled to fraction collection. The fractions were then analyzed by HPLC coupled to an ESI- ion trap mass spectrometer. A total of 490 proteins were observed in the serum. Lee et al. (*179*) used proteomics to study the proteins present in serum from normal mice versus mice knockout for the mannose receptor. Four out of 52 proteins identified had higher levels of expression in the knockout.

Chronic myeloid leukemia (CML) is a stem cell disease that can rapidly progress from a benign chronic state to a fatal state. Smith et al. (*180*) studied the molecular mechanisms underlying the progression of the disease. In particular, they focused on a model cell line that has conditional expression of Bcr-Abl PTK. Long-term exposure of this cell line to Bcr-Abl simulates the progression of CML. They performed 2DE of short- and long-term exposures followed by MALDI-TOF MS analysis of the differentially expressed proteins. Four major differences were observed: leukotriene A4 hydrolase, annexin VI, vacuolar ATP synthase catalytic subunit A, and mortalin. Juan et al. (*181*) also studied human myeloid leukemia by proteomics. In particular, they studied the differentiation of the cell line HL-60 into macrophages by induction using 12-*O*-tetradecanoylphorbol-13-acetate. A total of 682 proteins spots were observed on the 2DE of these cells, of which 136 were differentially expressed. MS was used to identify the differentially expressed proteins. Han et al. (*182*) published a method to analyze protein contained in microsomal fractions. They used ICAT to label the protein contained in the microsomal fraction of naïve and in vitro differentiated human myeloid leukemia cells. The purified proteins were identified and quantified by MS. They determined the relative abundance for 491 proteins contained in the microsomal preparations.

**Synovial Proteome.** The synovial fluid is a viscous straw-colored substance found in joints, tendon sheaths, and other areas of the body. Sinz et al. (*183*) studied the changes in synovial and plasma fluid proteomes obtained from patients suffering from different forms of arthritis. Fibrin $\beta$-chain, calgranulin B and C, and serum amyloid were selectively observed in some forms of arthritis.

**Urine Proteome.** Urine is potentially another source of protein biomarkers related to a disease or related to drug treatments. The use of urine as a source of potential biomarkers for proteomic studies has been limited in the literature. Pang et al. (*184*) performed a systematic study of the proteins contained in urine at different stages of inflammation. A healthy patient provided urine before and after acute inflammation and again after full recovery. Three different approaches were used to analyze the proteins: 2DE coupled to MS, "gel free" performed by HPLC-

ESI-MS/MS, and 2D "gel free" performed by SCX-HPLC-ESI-MS/MS. A total of 36 proteins were identified to be different by 2DE, 16 proteins were identified by 1D "gel free", and 90 proteins were identified by the 2D "gel-free" approach. Thongboonkerd et al. (*185*) established a catalog of the proteins present in normal human urine.

## FUNCTIONAL PROTEOMICS

Functional proteomics is the study of protein function at the proteome level. The mapping of protein−ligand interactions is key in understanding protein functions, discovering protein complexes, and deciphering cellular pathways. 2002 also saw the publications of key papers related to the large-scale mapping of protein−protein interactions.

**Protein−Protein Interactions.** Recently, Ho et al. (*186*) as well as Gavin et al. (*187*) introduced high-throughput approaches based on MS for the rapid mapping of protein interactions, complexes, and pathways in yeast. Both papers combined molecular biology, protein tagging, immunopurification, and MS for the analysis of protein complexes. Ho et al. (*186*) discovered interactions for about 70% of the genes they looked at for a total of 1578 different interacting proteins, representing 25% of the yeast genome. Gavin et al. (*187*) discovered interactions for roughly 26% of the gene they attempted to analyze for a total of 1440 distinct yeast protein interactions representing approximately 25% of the yeast genome. Epitope tagging, followed by a two-step purification approach, was utilized by Shevchenko et al. (*188*) for the study of protein interactions on 53 genes in budding yeast. A total of 38 of these gene products pulled out 220 interacting partners. The agreement with published yeast-two hybrid data was only 14%, thus reflecting the different nature of the two approaches.

Walsh et al. (*189*) reported the discovery of novel interactions in yeast type 1 protein phosphatase by affinity purification coupled to MS. In their approach, they performed the in vivo expression of protein phosphatase coupled to protein A through a TEV linker. Immobilized immunoglobulin was used for the affinity purification of protein A linked to protein phosphatase and its interactors. Protein phosphatase and its interactors were released from the affinity beads by treatment with TEV protease. The released proteins were separated by gel electrophoresis and identified by peptide mass fingerprinting on a MALDI-TOF MS.

Bécamel et al. (*190*) developed a proteomic approach to study the protein associated with G-protein-coupled receptors. The C-terminal tail of the receptor was fused to glutathione S-transferase to form a bait. This bait was immobilized on glutathione-Sepharose beads and exposed to whole-brain extracts from mice. A control system was developed using a mutated version of the C-terminal tail of the receptor. The extracted proteins were separated by 2DE. The spots observed to be different between the normal versus the mutant pull-downs were identified by MS. Fifteen proteins that interacted with the C-terminal tail of the receptor were observed. A method, based on cyanogens bromide-activated Sepharose matrix, was developed by Nelson et al. (*191*) for isolating interacting proteins. In this method, activated Sepharose is added to bind 50% of the proteins present in a mixture. The matrix is then washed until little proteins are eluted. The proteins that are interacting with the proteins covalently attached to the matrix are eluted using EGTA and separated by 2DE followed by MS identification of the proteins. The expectation is that this method could be used for differential analyses of the interacting proteins following cell treatments.

Molecular biology-based methods have been reported for mapping protein−protein interactions. Jorgensen et al. (*192*) used a set of 6000 yeast gene deletion strains to screen for size homeostasis. A total of 500 abnormal mutants were detected. A genetic interaction network based on synthetic lethal interaction for the 249 large mutants was constructed with 3 other mutants. Two methods were reported for mapping protein−protein interactions by directly or indirectly activating enzymes. The first method, by Wehrman et al. (*193*), monitors protein−protein interactions using the $\beta$-lactamase enzyme in mammalian cells. The complementarily nature of the $\alpha$- and $\omega$-fragments of $\beta$-lactamase is used to build a protein interaction reporting assay. Fusion proteins are made with the $\alpha$- and $\omega$-fragments, and the interaction can be tested by cotransfection in mammalian cells. The second method introduced by Eyckerman et al. (*194*) is called mammalian protein−protein interactions trap (MAPPIT). This method takes advantage of the mutation of critical tyrosine residues on the cytosolic domains of a receptor that results in the loss of STAT activation while JAK proteins are still activated. This group demonstrated that the recruitment of any other proteins containing a STAT binding site would reactivate the signaling.

Mellor et al. (*195*) presented a database of putative functional links between proteins called Predictome. This database does not necessarily indicate interactions between proteins but does indicate that proteins are linked through their participation in the same pathway or the same cellular process. The Predictome database is mainly based on yeast-two hybrid data, phylogenetic links, chromosomal proximity links, and fusion links. They reported a 30% false positive rate in their prediction of links between proteins.

The two-hybrid approach allows the determination of protein−protein interactions in a reporter-based assay. Walhout et al. (*196*) reported the combination of two-hybrid analysis, phenotypic analysis, and gene expression mapping in *Caernorhabditis elegans* germ line. They generated an interaction map for 600 transcripts, which was compared to a phenotypical map obtained by RNA interference as well as to a data set of 553 gene expressions.

Bader and Hogue (*197*) recently compiled and analyzed 15 143 interactions for 4825 proteins in yeast. They compared the two MS-based interaction data sets and the two-hybrid data sets. They concluded that current approaches are not saturating the interactome and that the combination of different approaches would lead to a clearer biological picture. Grindrod (*198*) presented a range-dependent graphs method for the modeling of large proteome data sets such as the ones obtained in protein−protein interactions.

**Protein−Small-Molecule Interactions.** Drug discovery is often achieved by screening compound libraries while looking for phenotypical changes. However, the targets of many drugs still remain unknown. The ability to map the interactions of small molecules (drugs) and biomolecules (proteins) would facilitate the discovery of drug targets and possibility the label extension of drugs. As well, the quantitative mapping of the small-molecule−protein interactions could be used to follow enzyme activity. The

improvement over the past decade of protein identification by MS is rejuvenating the idea of fishing potential protein targets using small-molecule probes.

Winssinger et al. (*199*) recently described small-molecule microarrays that were used to monitor changes in active enzyme amounts. They focused their initial study on cysteine proteases. They designed acrylated versions of the known cathepsins and caspase-3 inhibitors tethered to encoded fluorescent PNA. The probes were incubated with crude cell lysates. The unbonded probes were removed using a low molecular weight cutoff membrane. A microarray that specifically recognized the encoded PNA was then exposed to the remaining lysate. The fluorescent readout from the microarray was used to quantify the amount of probes attached to proteins. They used a LC-biotin-labeled caspase-3 affinity reagent to extract caspase-3 from crude lysate and identified it with MS. Adam et al. (*200*) introduced trifunctional chemical probes to detect and to identify enzyme activities in complex biological samples. They developed a trifunctional probe that consists of a fluorescent group for detection, a biotin group for purification, and a sulfonate ester reactive group. Greenbaum et al. (*151*) studied the role of the protease facipain 1 in host cell invasion in human malaria parasites. They developed an approach that utilizes a small-molecule probe to fish out the cysteine proteases that are present during the life cycle of the parasite. Briefly, a lysate from cultured parasites was reacted with a biotinylated small molecule. Then, the probe−protease complexes were affinity purified, and the proteins were identified by MS. They isolated, calpain 1 and falcipains 1, 2, and 3.

## STRUCTURAL PROTEOMICS

*Structural proteomics* studies the three-dimensional structure of protein, protein complexes, and small-molecule−protein complexes. There were relatively few publications related to structural proteomics in 2002. It seems that majority of advances in structural biology was reported in meetings and other formats (*201*). Of interest, Müller et al. (*202*) performed a bioinformatic analysis of the structure of the proteins in the human proteome. They used the information already available for human proteins and proteins from other species to look at the level of structural similarity in the human proteome. They discovered that 39% of the human proteome can be assigned to known structure and that some level of structural information is available for 77% of the proteome.

Ge et al. (*203*) utilized the "top-down" FTMS base approach for the structural characterization of proteins. In particular, they utilized electron capture dissociation MS to study proteins involved in the biosynthesis of thiamine and coenzyme A and the hydroxylation of proline residues in proteins. VerBerkmoes et al. (*204*) combined the "top-down" protein FTMS-based approach with the "bottom-up" peptide identification-based approach for the characterization of proteins on a large-scale. In particular, they focused their efforts on the proteome of *Shewanella oneidensis.*

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