Published in final edited form as:

Analyst. 2010 August ; 135(8): 1830–1838. doi:10.1039/c0an00105h.

Mass-spectrometry-based clinical proteomics – a review and prospective

Carol E. Parker^a, Terry W. Pearson^b, N. Leigh Anderson^c, and Christoph H. Borchers^{a,b}

Christoph H. Borchers: christoph@proteincentre.com

^a University of Victoria-Genome British Columbia Proteomics Centre, Victoria, BC, Canada; Fax: +1-250 483-3238; Tel: +1-250 483-3221

^b Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada

^c Plasma Proteome Institute, Washington, DC, USA

Abstract

This review reports on the current and emerging technologies for the use of mass-spectrometry-based proteomics in clinical applications.

1. Introduction

Mass spectrometry is currently widely used for screening for inborn-errors of metabolism,^{1,2} and for forensic applications,^{3,4} both of which usually involve the analysis of small molecules,⁵ either by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS). Clinical proteomics techniques, as broadly defined, are widely used today to detect a few protein biomarkers such as prostate specific antigen (PSA) as a diagnostic indicator for prostate cancer, cardiac troponin as an indicator of myocardial infarction and glycated hemoglobin⁶ as a measure of glucose control in diabetes. These assays are usually performed by enzyme-linked immunosorbent assay (ELISA) or by high performance liquid chromatography, not by mass spectrometry. Current mass-spectrometry-based proteomics has been focusing on (and doing well at) ‘discovery’ proteomics, with the result that thousands – perhaps tens of thousands – of potential biomarkers for cancer and other diseases have been reported in the literature. A recent search of SciFinder ScholarTM gave over 20 000 ‘hits’ for protein or peptide biomarkers.

2. Biomarker discovery versus biomarker verification and validation

Discovery proteomics, however, usually involves multi-dimensional separation steps and liquid chromatography-tandem mass spectrometry (LC-MS/MS) with long gradients. This means that discovery proteomics is feasible at the level of only a few dozen samples per project, and a single ‘discovery’ experiment on a set of 8 samples can take a minimum of one to two weeks. Only a small number of the reported ‘candidate’ biomarkers have been qualified or verified,^{7–10} and even fewer have been validated, which requires measuring the proposed biomarker in even larger groups of patients.^{11–14} This is because current proteomics methods present several bottlenecks for the analysis of large numbers of samples.^{11,15} In fact, between 2003 and 2008, only 7 protein biomarkers were approved by the US FDA.¹⁶

Biomarker verification (or qualification) can require 100–1000 samples.⁸ However, biomarker validation requires the analysis of even larger numbers of samples (thousands to tens of thousands) in order to be certain that the target molecule is a true biomarker of the disease or condition and to determine the ‘false positive’ rate of the assay. The costs of clinical verification and validation *using the same techniques that were used for biomarker discovery* are so prohibitive, and the time involved is so overwhelming, that very few potential biomarkers have been verified or validated.^{16,17} Current biomarker discovery techniques have sometimes mistakenly been called ‘high-throughput’. They are not ‘high-throughput’ in terms of samples; rather they should be termed ‘high-content’ (although, unfortunately, this term has already been taken and now refers to cellular microscopy¹⁸), or ‘comprehensive’. In fact, a recent journal article, published in 2009, refers to a test on 70 patients as ‘large-scale quantitative clinical proteomics’.¹⁹ For a biomarker discovery project, this is in fact ‘large scale’, but it is a far cry from the scale needed for biomarker validation and true clinical work.

3. Challenges to biomarker validation

Why has validation of a larger number of biomarkers not been achieved? First, most studies are performed on plasma because it is a readily available biofluid. Plasma, rather than serum, is recommended by HUPO (the Human Proteome Organisation)²⁰ because of the variability inherent in the formation of serum. Both plasma and serum, however, are extremely complex mixtures containing a wide dynamic range of components, with a few abundant proteins comprising >90% of the total protein mass.²¹ An excellent review of the challenges in mass-spectrometry-based plasma clinical proteomics is presented by Simpson, *et al.*²² In addition, mass spectrometry (especially the particular ionization techniques used for proteomics, positive-ion ESI and MALDI) suffers from the blessing – or the curse – that it is too ‘universal’. This means that, for peptides, there is very little selectivity to the ionization process – *almost everything* is detected. Thus, finding the peptide of interest on the basis of mass alone, especially if it is derived from a low-abundance protein, has been described as ‘looking for a needle in a needlestack’.²³ Instead, the selectivity must come from fractionation of the sample, often *via* multi-dimensional separation techniques, leading to an enormous number of analyses for the comprehensive analysis of a single original sample.

Digging deeper, one finds additional challenges. Mass spectrometry suffers from suppression effects, *i.e.*, competition for ionization between analytes that are in the source at the same time. Thus separation is important for the reduction of suppression effects, by simplifying the instantaneous mixture of analytes present in the ion source. In ‘shotgun’ discovery proteomics, as described above, the only alternative is sample fractionation. However, once the protein targets are known, as would be the case in clinical proteomics, it becomes possible to selectively enrich the appropriate biomarker targets, either by isolation of the intact protein²⁴ or by enrichment of its proteotypic peptides using antibody-based techniques.^{25–27} In addition, modified peptides can be enriched, for example, by using Immobilized Metal Affinity Chromatography (IMAC) for phosphopeptides.²⁸

Both discovery and quantitative proteomics are usually performed using peptides as surrogates for their parent proteins, because peptides are easier to detect and to sequence than proteins. Quantitative proteomics, however, is even more challenging than qualitative proteomics (for recent reviews, see refs ²⁹ and ³⁰). The digestion process used to produce peptides from proteins is not uniform, but is a function of protein structure. If chemical labeling is used for peptide quantitation, the efficiencies of the labeling techniques must also be taken into account.

4. Emerging technologies

Where do we stand? There are several emerging technologies that show great promise for being able to handle the large numbers of samples required for many proteomics studies. It is obvious

that three key components must be available before real progress can be made. First, automation, especially of the sample preparation steps, must be developed. Current work is already being done on automation and validation of digestion methods (see the recent review by Ahmed for current technologies,³¹ and ref. ³² for a study on comparative digestion efficiencies). There have been recent papers and reports on the automation of solid phase extraction (SPE) for proteomics applications,^{33,34} and descriptions of robotic sample handling devices for use with magnetic beads (including, for example, ThermoFisher's KingFisherTM, which has recently been used to enrich samples in phosphopeptides³⁵). In addition, preparative devices for gel-based protein purification with recovery of intact proteins have been described (for example, Protein Discovery's GelfreeTM system). These systems have the potential for being used for sample depletion or fractionation, and the GelfreeTM system³⁶ has recently been used by the Kelleher group to fractionate³⁷ and purify³⁸ small proteins for 'top-down' proteomics (*i.e.*, the MS-generated cleavage and analysis of intact proteins, rather than the analysis of their proteolytic peptides). Second, the time per analysis and the cost per analysis must be greatly reduced, and, third, more robust and rapid methods of handling the data from large numbers of samples must be developed.

With this in mind, we see three emerging mass spectrometric techniques that have great promise for being able to meet the clinical proteomics challenge. These three techniques are (1) high-throughput multiplexed multiple reaction monitoring (MRM), where dozens of peptides can be quantitated in a single analysis, most likely at higher flow rates than are commonly used today for capillary LC-MS/MS; (2) MALDI-based analysis, where several thousands of sample spots can be analyzed per day, with immuno-purification if necessary (we include both immuno-MALDI (iMALDI),^{39,40} the new improved Surface-Enhanced Laser Desorption Ionization (SELDI),⁴¹ and Nelson's Mass Spectrometric Immuno Assay (MSIA)⁴² in this group); and (3) MALDI-Imaging,^{43,44} which has the potential for cell typing in biopsy samples.

4.1 Electrospray MRM-based analyses

Multiple Reaction Monitoring (MRM) is a mass spectrometric technique involving multiple stages of mass spectrometric separation, usually performed in a triple quadrupole or ion trap mass spectrometer. Each 'reaction' includes a pair of ions: a precursor ion – for peptides, usually the protonated molecular ion – and a product ion – a collision-induced fragment ion of the peptide. The peak heights or areas of the resulting fragment ion can be used for peptide quantitation. Many SRM (selected reaction monitoring) ion pairs can be used in a single experiment to give a MRM method, and most LC-MS/MS data systems allow changing the ions selected as a function of time, thus increasing even further the number of peptides that can be analyzed in each multiplexed analysis.⁴⁵

A large inter-laboratory study sponsored by the US National Cancer Institute (NCI) was conducted in 2009⁴⁶ that demonstrated the reproducibility of MRM-based protein quantitation, thus clearing the way for its use in clinical proteomics. MRM-based approaches, and other mass spectrometric techniques based on peptide quantitation using stable-isotope-labeled standard (SIS) peptides, can be used to correct for suppression effects and to improve the accuracy. Such methods will be greatly facilitated by the availability of SIS peptides, some of which are now being marketed by Thermo Fisher and Sigma-Aldrich (where they are called AQUA (absolute quantification) peptides⁴⁷).

Although capillary LC-MS/MS, with flow rates <200 nL/min is arguably the most sensitive LC-ESI-MS/MS method, it is not a rapid or robust technique, in part due to the very factors which lead to its sensitivity. Because of the low flow rates, there is often a long delay before the appearance of the first peaks in the chromatogram, often 25–40 min. Also, the small i.d. columns (usually 75 μ m) mean that each column contains very little packing material, which is subject to contamination from biological matrices. In addition, these low flow rates mean

that dead-volumes are critical, and reconditioning the column between samples can be time-consuming (we often run 3 blank gradients between each sample to reduce sample carryover). Also, because of this small amount of column packing, a capillary column may be overloaded with an abundant component before enough of a low-abundance component can be loaded for it to be detected. Recent studies in our laboratory⁴⁸ have shown that, for moderate-to-high-abundance peptides, the ability to load more sample on a larger-i.d. column, combined with an ion source designed to handle higher flow rates, can more than compensate for the reduction in sensitivity between capillary ESI and conventional ESI, resulting in a more robust and faster analytical method.

Three examples of cutting-edge high-throughput MRM analyses are described here. The first is described by Kamiie, *et al.*⁴⁹ who reported an MRM method for the analysis of 36 membrane proteins (at the 10 fmol level) in a single 50 min analysis, with 216 MRM channels (one or two target peptides per protein), using ¹³C-labeled SIS peptides as internal standards. A 0.5 mm i.d. column was used, operated at a flow rate of 50 μ L/min. The second example is the study of Barton *et al.*,³⁴ which described a multiplexed MRM assay for 28 proteins in 5 min, using a single internal standard. Validation of the results was performed for IL-2, where the measurements were in agreement with those of standard ELISAs. This truly high-throughput analysis was made possible by the use of ultra-high pressure LC (UPLC) techniques and ‘mini-bore’ (2.1 mm i.d.) columns which allowed the use of higher flow rates (0.5 mL/min). The third example, from our laboratory, is the paper by Kuzyk *et al.*⁵⁰ This paper demonstrates the rigorous MRM quantitation of the 45 most-abundant plasma proteins, all of which are considered to be disease biomarkers, with 31 of these being potential biomarkers for cardiovascular disease. This analysis was done without depletion of the abundant plasma proteins, in a single 45 min scheduled MRM analysis, using 1 peptide per protein and a SIS peptide for each target peptide. This study was performed with capillary columns and nanoscale flow rates and is currently being adapted to higher flow rates for increased speed and ‘robustness’.

4.2 MALDI-based methods

MALDI, with its high acquisition speed, has the capacity for the analysis of thousands of samples per day. This high speed is possible not only because of the ionization process, but because MALDI analysis also reduces the possibility of cross-contamination or the need for column cleanup or re-equilibration between analyses, as is the case for LC/MS-based assays. Our laboratory has recently been developing methods for the low-cost, high-throughput direct analysis of hemoglobin variants without enrichment. These variants include hemoglobin S (sickle-cell) and hemoglobin C (hemoglobin C disease), as well as a method for the detection and monitoring of diabetes by determining the percentage glycation of hemoglobin A1c and albumin.⁵¹

4.3 Less-common ionization techniques

There are several other, less-commonly used, mass-spectrometry-based techniques which appear to have potential for high-throughput clinical proteomics. These are Matrix-free LDI, SIMS (Secondary-Ion Mass Spectrometry), and DESI (Desorption Electrospray Ionization). Each of these techniques has the potential for high-throughput analyses because each can be done in a ‘multiple spots on a target’ format similar to MALDI; each of these has already been used for tissue imaging.

4.3.1 Matrix-free LDI—The original ‘niche’ for Matrix-free LDI (for an excellent review, see Peterson⁵²) was the analysis of small molecules, including small peptides, that are in the mass region that is usually masked by the matrix in conventional MALDI (*i.e.*, ions whose molecular weights are below 800 Da). A variety of surfaces have been used, including DIOS

(desorption/ionization on silica⁵³), carbon-based nanostructures (*e.g.*, nanodiamonds,⁵⁴ and nanotubes⁵⁵) as well as different laser wavelengths. The analyses of insulin⁵⁶ and peptide mixtures⁵⁵ have been reported using IR (infrared) LDI. Conventional 337 nm UV irradiation has been demonstrated at a spatial resolution of 10 μm , allowing the analysis of metabolites within a single plant cell.⁵⁷ It should be noted, however, that these techniques often result in the formation of $(M + \text{Na})^+$ ions which can be difficult to fragment using CID techniques.

4.3.2 SIMS—In SIMS, now usually TOF-SIMS, a pulsed primary ion beam is used to generate secondary ions from a sample on a target.⁵⁸ This technique is traditionally used for the surface analysis of atoms, and (for atoms) SIMS is capable of a spatial resolution of 50 nm.⁵⁹ The development of a Bi_3^+ -cluster ion source induced less fragmentation, and allowed the detection of peptides up to *ca.* 7500 Da,⁶⁰ and allowed imaging of a dot pattern of a protein digest. A recent report by Nygren and Malmberg⁶¹ demonstrated an imaging analysis of a section of a pig thyroid gland using this technique. In this study, a tryptic digest of thyroglobulin was obtained at a spatial resolution of 3 μm , after the tissue sample was sprayed with a solution of trypsin and with TFA as an enhancing matrix. (For comparison, the spatial resolution of current MALDI imaging instruments is 10–100 μm , with 250 amol sensitivities.)

4.3.3 DESI—DESI is an analytical technique that combines features of both ESI and MALDI.⁶² Instead of a laser, a beam of charged ions/droplets, is directed at the surface, and secondary ions are formed and detected. Like ESI, this technique often results in multiply-charged ions. DESI has been used for the analysis of hemoglobin variants in dried blood spots on paper, as well as the analysis of urine samples without the need for removal of salts.⁶³ A spatial resolution of 250 μm has been reported for DESI imaging, with a S/N of 10:1.⁶⁴

Unlike the techniques described above, however, this technique is done under ambient conditions – not in a vacuum –which leads to the exciting prospect of the analysis of living tissues. This has been demonstrated by the Cooks group by the direct analysis of drugs in a human finger held under the ion beam.⁶² DESI is often discussed as a complementary technique to MALDI, in that ‘lipids are more readily ionized by DESI, while peptides and proteins are more readily examined by MALDI (*although in neither case do these represent intrinsic restrictions in the methodology*)’ (from ref. ⁶⁴, our italics).

Although these new ionization techniques are not yet as widely used as MALDI and LC-ESI-MS/MS, their unique features are intriguing and improvements in peptide or protein sensitivities and spatial resolution may lead to their more wide-spread adoption for proteomics studies. Clearly, the advancement in proteomics techniques is not yet over, and these new technologies are definitely worth watching.

5. Affinity enrichment of target analytes

For less-abundant peptides or proteins, MALDI or ESI can be combined with immunocapture of peptides. Immunocapture is a general approach based on the selective enrichment of target peptides using immobilized anti-peptide or anti-protein antibodies. After the target analyte is concentrated on the affinity medium, mass spectrometry is used to provide specific detection and pg/mL sensitivities.

One MALDI-based method and one ESI-based technique have been developed which combine immunocapture of peptides with high-throughput analysis. Both of these techniques are currently being used with the idea of developing clinical assays. The first of these techniques is iMALDI (immuno-MALDI).^{25,26} This technique uses bead-based affinity co-capture of native and SIS peptides, followed by placement of the washed beads on the MALDI target where they are analyzed. Up to 384 (bead-containing) spots can be placed on a standard MALDI

target. Because the binding and washing are done off-target, these steps can be optimized for efficient capture. Elution is not done as a separate step – the application of MALDI matrix solution to the beads while they are on the target releases the analytes. iMALDI can be used for IMAC capture of phosphopeptides as well.⁶⁵ Recent examples of iMALDI include the quantitation of epidermal growth factor receptor (EGFR) in human plasma^{40,66} and the determination of angiotensin I in human plasma.⁶⁷ iMALDI could also be combined with automated spotting robots (for example, Shimadzu's AccuSpotTM, Digilab's Pro-PrepTM digester/spotter, or Leap Technologies' MALDI-spotting robots) to produce high-throughput MALDI methods.

SISCAPA (Stable Isotope Standards and Capture by Anti-Peptide Antibodies),^{27,68} like iMALDI, uses a combination of bead-based affinity co-capture of native and SIS peptides, and typically involves off-line elution of the bound peptides. This technique was originally used with LC-ESI-MRM analysis, but robotic elution (for example, using the ThermoFisher KingFisherTM) could be combined with automated electrospray (for example, by using the Advion NanomateTM) or MALDI-TOF to give a high-throughput analysis. Multiplexing of the SISCAPA method has already been achieved⁶⁹ demonstrating the adaptation of this approach to higher throughput.

6. Methods targeting proteins

While the SISCAPA technique uses anti-peptide antibodies, SIS peptides, and MS detection, the Mass Spectrometric Immuno Assay (MSIA) method, pioneered by Nelson in 1995, uses *anti-protein* antibodies, elution of the bound analytes, followed by MALDI MS-detection.⁷⁰ MSIA was soon developed as a high-throughput automated technique.⁷¹ Like SISCAPA, and in contrast to the SELDI technique described below, the capture medium is in pipette tips, which can automatically be loaded with sample, rigorously washed, and analytes eluted. MSIA (with *anti-peptide* antibodies, MALDI detection and spiked-in *unlabeled* peptides) has been applied to the high-throughput detection of B-type natriuretic peptides (*ca.* 2500–3500 Da) in heart patients.⁷² Meanwhile, the Nelson group recently reported a workflow, still called MSIA, involving ESI analysis of a 51 kDa vitamin-D-binding protein.⁷³

Adding to the confusion – and the probably the unnecessary proliferation of terms – Berna and Ackermann recently reported 'a variation on SISCAPA that uses *anti-protein* antibodies',⁹ which they call Immunoaffinity-LC/MS/MS. In this workflow, the bound proteins are eluted, microwave digested, and analyzed by LC-ESI-MRM-MS (with SIS peptides). The Berna study demonstrated the quantitation of two low-abundance proteins (NTproBNP and Myl3) in human plasma, using 96-well format immunoprecipitation, a 2 mm-i.d. column operated at 0.75 μ L/min, and an MRM-based analysis taking less than 10 min.⁹ In 2010, the Nelson group reported an MSIA study using anti-protein (or anti-large-peptide) antibodies, on parathyroid hormone variants containing up to 84 amino acids. This study used electrospray ionization, with 'top-down' quantitative MRM analysis on these large peptides, using SIS peptides.⁷⁴

Admittedly, part of this confusion in terminology is because of the uncertain definition of what is a large peptide and what is a small protein. Even though many of the intact protein studies were done without internal standards, these studies – whatever the techniques are called – do demonstrate the movement towards 'real' clinical proteomics: automation, high throughput, high sensitivity and high specificity.

Another MALDI-based technique is SELDI^{75–77} which uses a derivatized capture surface (derivatized portions of the MALDI target), and was originally designed to capture proteins, although the capture media should work to capture peptides as well. Four capture chemistries are available: cation exchange, anion exchange, IMAC, and hydrophobic interaction. In

addition, an IgG surface for indirect binding of antibodies, and an amine-capture surface for direct binding of proteins or antibodies are also available. The capture spots are manufactured in sets of 8 spots and 12 sets can be combined to form a MALDI target with a 96-well format for compatibility with robotic systems for sample application and washing. While SELDI has the potential for rapid comparison of the *patterns* of proteins from large numbers of samples, early studies with SELDI for the diagnosis of ovarian cancer were not reproducible between laboratories.⁷⁸ Recently, however, BioRad has partnered with Bruker to form the Lucid Proteomics System,⁴¹ and the use of SELDI with proper internal standards and a MALDI-TOF/TOF instrument with high mass-resolution may still have diagnostic potential. One caveat is that the binding to the medium and the washing of the spots to remove unbound analytes must be done *on the strip*, and both of these steps must be reproducible to produce reproducible results. Another caveat is that the capture of intact proteins, rather than peptides, may be more sensitive to protein degradation, which would lead to changes in the observed 'patterns'. The affinity capture of target analytes is an analytically sound approach, so if the reproducibility problems can be overcome, this technique will certainly have wide-spread applications. However, unless this technique can be combined with 'top-down' sequencing of intact proteins or 'bottom-up' techniques involving peptide sequencing, the identities of the captured proteins cannot be determined. This is an obstacle that SELDI shares with the original MSIA, and with MALDI imaging, as described below – an obstacle that gains increasing importance the closer one comes to clinical use and the need for unambiguous analyte identification.

The final MALDI-based method which we feel has clinical potential is MALDI Imaging,^{79–81} pioneered by Caprioli and his group at Vanderbilt University. This technique has finally 'come of age', and combined with reproducible and reliable sample preparation techniques and matrix deposition instruments (including Shimadzu's ChIP 1000TM, Bruker's ImagePrepTM, and LabCyte's PortraitTM) is now being used by many laboratories around the world. This technique can be used in proteomics research to determine the localization of proteins and peptides in tissue slices, and has also been used for the high-speed differentiation of tumor types in biopsy samples, with 56 samples being processed and analyzed in a single day.⁸² Thus, MALDI Imaging of tissue biopsies has the speed required for clinical applications. MALDI Imaging has even been proposed as a technique to determine tumor margins during surgery.⁸³ It must be kept in mind, however, that tissue imaging is performed basically on an unfractionated MALDI sample, and therefore may be subject to the same non-reproducibility issues as SELDI, including the effects of ion suppression. However, this can be partially overcome by combining in-tissue digestion followed by MALDI imaging with MRM technology.⁴⁴ This approach improves the detection sensitivity and allows accurate and absolute quantitation of proteins in tissues, while maintaining the spatial resolution.

7. Bacterial proteomics

There is another area where mass spectrometry is already making an impact in medicine. This is not generally thought of as 'proteomics', although it certainly is, but the proteomes involved are not human, but bacterial. MALDI has been used for the identification of bacteria since 1996^{84,85} and this technique can be used for determining the causes of infection in patients,⁸⁶ for the detection of bioterrorism agents,⁸⁷ for the detection of toxic molds and bacteria in indoor air⁸⁸ and for the detection of infectious agents in water.⁸⁹ In fact, an entire issue of the 'Chemical Analysis' series (John Wiley & Sons) in 2006 (vol. 169) was devoted to the *Identification of Microorganisms by Mass Spectrometry*. The same mass spectrometry techniques described above are used in this approach to microbial identification because they have the same key characteristics: accuracy and speed. During the first month of 2010, there have already been two review articles on the mass spectrometry of bacteria^{90,91} usually involving MALDI. The combination of MALDI with affinity capture of target peptides (iMALDI) has already been described for *Francisella tularensis*,³⁹ an organism that has the

potential to be 'weaponized'. In a 2009 study using MALDI and Bruker's 'Biotyper'TM software, 95.2% of 1116 strains of bacteria were correctly identified, with a time-to-results of 12 min for direct placement of bacteria on the target and 20 min if an extraction procedure was required.⁹²

8. Current status

Yes, we are definitely getting closer to 'real' clinical proteomics. Two posters presented at the International HUPO Congress in Toronto in September 2009, demonstrated 'brute force' large-scale quantitative proteomics studies. The first, by Qian, *et al.*⁹³ described the quantitative 'discovery proteomics' analysis of 1500 plasma samples from 100 patients, using 2D LC-MS with analysis times of 150 min, using AMT-tags and ¹⁸O labeling for quantitation, the largest such study described to date. The second, by Stephenson, *et al.*,⁹⁴ was a large-scale **D**ifference **G**el **E**lectrophoresis (DIGE) study on typhoid resistance, using >700 patients. Although large scale, these studies were not designed to be high throughput or clinical. In conjunction with this DIGE study, a MS/MS-based study on defence peptides in saliva was performed, using a method developed by Gardner *et al.*⁹⁵ This method was based on LC-ESI-MRM and utilized a 2.1 mm column operated at 300 μ L/min to give a 15 min separation/re-equilibration time. A third study reported at HUPO by Calleson *et al.*⁹⁶ was designed to distinguish between 184 early-stage breast cancer patients and 236 normal patients, with 9 analyses per patient. This study was performed using magnetic IMAC beads, with elution of analytes followed by protein MALDI-MS.

9. National and international proteomics initiatives

The two MRM studies, and the high-speed multiplexed MRM studies described above, exemplify the characteristics that we predict will be important for bringing mass-spectrometry-based proteomics into the clinic: high-throughput clinical analysis on large sample sets achieved by the possibility of automation, combined with high-speed analysis. This need has been recognized at the national and international level, thus there are several concurrent public and private initiatives to create a library of MRM transitions, a repository for selected peptides, and antibodies specific for all human proteins.

With regard to MRMs, one initiative includes the announcement last October of a \$7 million collaboration between Agilent and the Institute for Systems Biology to develop an MRM Atlas for the quantitation of all human proteins.⁹⁷ For the production of antibodies, several projects are underway in both Europe (<http://www.hupo.org/research/hai/>; www.proteomebinders.org) and the USA (<http://antibodies.cancer.gov>) to make antibodies for use in immunohistochemical assays and for the immuno-enrichment of proteins for use in 'top-down' methods where protein targets are enriched, then digested into peptides before analysis by MS. However, anti-peptide antibodies have received much less attention, although one of the most far ranging endeavors is the human protein detection and quantitation project (hPDQ),⁹⁸ proposed by an independent interest group composed of proteomics researchers in academia and industry. This project is designed to enable the development of tools to allow the measurement of all human proteins and includes providing peptide standards and anti-peptide antibodies specific for proteotypic peptides for every human protein. Such reagents will be useful for MRM applications and SISCAPA and iMALDI methods.

It is difficult to put a price tag and time frame on the plans to make anti-protein and anti-peptide antibodies, peptides, and isotopically-labeled peptides available for general use. At the protein level, it should be noted that 8300 polyclonal antibodies (covering 6900 human protein targets) are already available as the Prestige Antibodies line sold by Sigma-Aldrich and Atlas Antibodies (<http://www.atlasantibodies.com>). The antibodies were produced by the Human Proteome Resource which runs the Human Protein Atlas (<http://www.proteinatlas.org>). Atlas

Antibodies expects to commercialize 22 000 anti-human protein Prestige Antibodies by 2015. The production of well-characterized anti-peptide antibodies is much less advanced and requires substantial funding to proceed on any significant scale. Although individual research labs (some in collaboration with companies) are producing anti-peptide reagents for use in proteomics applications (for iMALDI and SISCAPA), there are few well-characterized immunoreagents available today. The hPDQ project estimates that it will take an investment of \$50 million to make such reagents for 2000 human proteins over the next 2 years (*i.e.* 5 standard peptides and 5 monoclonal antibodies against each protein), and another \$250 million to cover the basic human proteome within 5 years. The advantage of this approach, as outlined in the hPDQ proposal, is that such reagents would allow the measurement of any or all human proteins in complex mixtures and that each researcher could tailor the mix for each investigation. In this regard, the invested funds would pay great dividends.

In 2002, HUPO⁹⁹ initiated the Plasma Proteome Project (PPP), which was funded by NIH and administered by NCI, with additional state and corporate sponsors. The PPP has the goal of evaluating a wide variety of 'depletion, fractionation and MS technology platforms', comparing the analysis of plasma *vs.* serum, including sample collection methods, and creating a publicly-accessible database,¹⁰⁰ and advanced search and database curation algorithms.²⁰ Through the years, evaluation of proteomics techniques has been done by large multi-laboratory comparisons of depletion, fractionation, and MS/MS techniques on standard reference samples,²⁰ as well as comparisons of immunoassays and antibody microarrays with MS methods.¹⁰¹ In 2008, phase 2 of the PPP was announced, with the goal of making plasma 'the common pathway for biomarker development and application'.¹⁰² This has involved increasing support for the MRM approach for targeted protein measurement based on the quantitation of proteotypic peptides, including MRM libraries such as PeptideAtlas.^{103,104} Many of HUPO's initiatives are designed to facilitate the transfer of biomarkers from the laboratory to the clinic.^{105–107}

Among the US federal initiatives is the NCI's Clinical Proteomic Technologies for Cancer (CPTC),¹⁰⁸ whose mandate is to accelerate the development, improvement, and standardization of proteomic technologies. One of the key components of this initiative is the Clinical Proteomic Technology Assessment for Cancer (CPTAC) network¹⁰⁹ which has undertaken studies designed to measure the inter-laboratory reproducibility of potential cancer biomarkers and to make available to the public reference data sets, reagents, analytical metrics, and standard operating procedures.^{110–112} CPTAC has been a strong proponent of biomarker verification by MRM-MS, as demonstrated by the inter-laboratory study described above.⁴⁶

CPTC has also funded the development of guidance documents (*e.g.*, mock 510(k) applications) which are designed to assist in understanding the FDA approval process of multiplexed *in vitro* diagnostic tests.^{113,114} More recently, CPTC has entered into a 'memorandum of understanding' with the American Association for Clinical Chemistry (AACC) and the Korea Institute of Science and Technology (KIST) to educate the clinical chemistry community in the area of proteomic standards and technology advances, and to promote the use of technology standards implementation in large-scale national and international clinical proteomic programs.

10. Conclusion

At this point, however, there is still a need for rapid and robust methods which can handle >10 000 patient samples and can be performed routinely, reliably, reproducibly, and at a reasonable cost, either in the clinic or by a contract research laboratory. Once these methods are available, high-throughput assays for quantitative proteomics can be used to help answer today's pressing diagnostic questions, to utilize molecular diagnostics for personalized health management and

to help assess the impact of diet, the environment, and climate change on human health by providing solid data for newly-emerging fields such as nutrigenomics.

Acknowledgments

We thank Genome Canada and Genome BC for platform funding and support. This work was also supported in part by a grant from the National Institutes of Health Grants 1U24 CA126476-04 (PI: Steven A. Carr, Broad Institute of Harvard and MIT) as part of the NCI's Clinical Proteomics Technologies Assessment in Cancer (CPTAC) Program.

References

1. Wilcken B, Wiley V, Hammond J, Carpenter K. N Engl J Med 2003;348:2304–2312. [PubMed: 12788994]
2. Schulze A, Lindner M, Kohlmuller D, Olgemoller K, Mayatepek E, Hoffmann GF. Pediatrics 2003;111:1399–1406. [PubMed: 12777559]
3. Maurer HH. Anal Bioanal Chem 2007;388:1315–1325. [PubMed: 17377775]
4. Maurer HH. J Mass Spectrom 2006;41:1399–1413. [PubMed: 17051522]
5. Chace DH, Kalas TA, Naylor EW. Clin Chem 2003;49:1797–1817. [PubMed: 14578311]
6. Little RR. Clin Chem Lab Med 2003;41:1191–1198. [PubMed: 14598869]
7. AB_biomarker_verification.
http://marketing.appliedbiosystems.com/mk/get/BIOMARKERR_VERIFICATION?_A=94447&_D=60615&_V=0,2010
8. Rifai N, Gillette MA, Carr SA. Nat Biotechnol 2006;24:971–983. [PubMed: 16900146]
9. Berna M, Ackermann B. Anal Chem 2009;81:3950–3956. [PubMed: 19388669]
10. Goodsaid F, Frueh F. AAPS J 2007;9:E105–E108. [PubMed: 17408233]
11. Paulovich AG, Whiteaker JR, Hoofnagle AN, Wang P. Proteomics: Clin Appl 2008;2:1386–1402.
12. Simpson KL, Whetton AD, Dive C. J Chromatogr, B: Anal Technol Biomed Life Sci 2009;877:1240–1249.
13. Palmblad M, Tiss A, Cramer R. Proteomics: Clin Appl 2009;3:6–17.
14. Lin Y, Dynan WS, Lee JR, Zhu ZH, Schade RR. Dig Dis Sci 2009;54:431–457. [PubMed: 19104933]
15. Kim K, Kim Y. Expert Rev Proteomics 2009;6:225–229. [PubMed: 19489693]
16. Anderson NL. Clin Chem 2010;56:177–185. [PubMed: 19884488]
17. Hortin GL, Carr SA, Anderson NL. Clin Chem 2010;56:149–151. [PubMed: 19910505]
18. High-Content_Analysis. 2010.
http://www.biacore.com/high-content-analysis/introduction_hca/what_is_hca/index.html
19. Negishi A, Ono M, Handa Y, Kato H, Yamashita K, Honda K, Shitashige M, Satow R, Sakuma T, Kuwabara H, Omura K, Hirohashi S, Yamada T. Cancer Sci 2009;100:514–519. [PubMed: 19154406]
20. Omenn GS, States DJ, Adamski M, Blackwell TW, Menon R, Hermjakob H, Apweiler R, Haab BB, Simpson RJ, Eddes JS, Kapp EA, Moritz RL, Chan DW, Rai AJ, Admon A, Aebersold R, Eng J, Hancock WS, Hefta SA, Meyer H, Paik YK, Yoo JS, Ping P, Pounds J, Adkins J, Qian X, Wang R, Wasinger V, Wu CY, Zhao X, Zeng R, Archakov A, Tsugita A, Beer I, Pandey A, Pisano M, Andrews P, Tammen H, Speicher DW, Hanash SM. Proteomics 2005;5:3226–3245. [PubMed: 16104056]
21. Anderson L. J Physiol 2005;563:23–60. [PubMed: 15611012]
22. Simpson RJ, Bernhard OK, GDW, Moritz RL. Curr Opin Chem Biol 2008;12:72–77. [PubMed: 18295612]
23. Veenstra TD. J Chromatogr, B: Anal Technol Biomed Life Sci 2007;847:3–11.
24. Wofsy L, Burr BT. J Immunol 1969;103:380–382. [PubMed: 4184826]
25. Jiang J, Parker CE, Robinette D, Borchers CH. BIOforum Eur 2006;10:18–20.
26. Reid JD, Parker CE, Borchers CH. Curr Opin Mol Ther 2007;9:216–221. [PubMed: 17608019]
27. Anderson NL, Anderson NG, Haines LR, Hardie DB, Olafson RW, Pearson TW. J Proteome Res 2004;3:235–244. [PubMed: 15113099]

28. Andersson L, Porath J. *Anal Biochem* 1986;154:250–254. [PubMed: 3085541]
29. Wilm M. *Proteomics* 2009;9:4590–4605. [PubMed: 19743428]
30. Elliott M, Smith D, Kuzyk M, Parker CE, Borchers CH. *J Mass Spectrom* 2009;44:1637–1660. [PubMed: 19957301]
31. Ahmed FE. *J Sep Sci* 2009;32:771–798. [PubMed: 19219839]
32. Proc JL, Kuzyk MA, Hardie DB, Yang J, Smith D, Jackson AM, Anderson NL, Borchers CH. 2010 submitted for publication.
33. Callesen AK, Madsen JS, Vach W, Kruse TA, Mogensen O, Jensen ON. *Proteomics* 2009;9:1428–1441. [PubMed: 19235169]
34. Barton C, Kay RG, Gentzer W, Vitzthum F, Pleasance S. *J Proteome Res* 2010;9:333–340. [PubMed: 19921931]
35. Nilsson CL, Dillon R, Devakumar A, Shi SDH, Greig M, Rogers JC, Krastins B, Rosenblatt M, Kilmer G, Major M, Kaboord BJ, Sarracino D, Rezai T, Prakash A, Lopez M, Ji Y, Priebe W, Lang FF, Colman H, Conrad CA. *J Proteome Res* 2010;9:430–443. [PubMed: 19899826]
36. Tran JC, Doucette AA. *Anal Chem* 2009;81:6201–6209. [PubMed: 19572727]
37. Vellaichamy A, Tran JC, Catherman AD, Lee JE, Kellie JF, Sweet SMM, Zamdborg L, Thomas PM, Ahlf DR, Durbin KR, Valaskovic GA, Kelleher NL. *Anal Chem* 2010;82:1234–1244. [PubMed: 20073486]
38. Lee JE, Kellie JF, Tran JC, Tipton JD, Catherman AD, Thomas HM, Ahlf DR, Durbin KR, Vellaichamy A, Ntai I, Marshall AG, Kelleher NL. *J Am Soc Mass Spectrom* 2009;20:2183–2191. [PubMed: 19747844]
39. Jiang J, Parker CE, Fuller JR, Kawula TH, Borchers CH. *Anal Chim Acta* 2007;605:70–79. [PubMed: 18022413]
40. Jiang J, Parker CE, Hoadley KA, Perou CM, Boysen G, Borchers CH. *Proteomics: Clin Appl* 2007;1:1651–1659.
41. Lucid Proteomics. 2010. <http://www.lucidproteomics.com>
42. Nelson RWK JR, Bieber AL, Williams P. *Anal Chem* 1995;67:1153–1158. [PubMed: 15134097]
43. Cornett DS, Mobley JA, Dias EC, Andersson M, Arteaga CL, Sanders ME, Caprioli RM. *Mol Cell Proteomics* 2006;5:1975–1983. [PubMed: 16849436]
44. Parker, CE.; Smith, D.; Suckau, D.; Borchers, CH. *Advanced Imaging in Biology and Medicine*. Sensen, CW.; Hallgrímsson, B., editors. Springer; Berlin, Heidelberg: 2009. p. 131–146.
45. Kitteringham NR, Jenkins RE, Lane CS, Elliott VL, Park BK. *J Chromatogr, B: Anal Technol Biomed Life Sci* 2009;877:1229–1239.
46. Addona TA, Abbatiello SE, Schilling B, Skates SJ, Mani DR, Bunk DM, Spiegelman CH, Zimmerman LJ, Ham AJL, Keshishian H, Hall SC, Allen S, Blackman RK, Borchers CH, Buck C, Cardasis HL, Cusack MP, Dodder NG, Gibson BW, Held JM, Hiltke T, Jackson A, Johansen EB, Kinsinger CR, Li J, Mesri M, Neubert TA, Niles RK, Pulsipher TC, Ransohoff D, Rodriguez H, Rudnick PA, Smith D, Tabb DL, Tegeler TJ, Variyath AM, Vega-Montoto LJ, Wahlander A, Waldemarson S, Wang M, Whiteaker JR, Zhao L, Anderson NL, Fisher SJ, Liebler DC, Paulovich AG, Regnier FE, Tempst P, Carr SA. *Nat Biotechnol* 2009;27:633–641. [PubMed: 19561596]
47. Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP. *Proc Natl Acad Sci U S A* 2003;100:6940–6945. [PubMed: 12771378]
48. Smith, DS.; Kuzyk, MA.; Cross, TJ.; Proc, JL.; Yang, J.; Dominski, D.; Hunter, CL.; Champion, MM.; Borchers, CH. to be presented at the American Society for Mass Spectrometry and Allied Topics; Salt Lake City, UT. 2010.
49. Kamiie J, Ohtsuki S, Iwase R, Ohmine K, Katsukura Y, Yanai K, Sekine Y, Uchida Y, Ito S, Terasaki T. *Pharm Res* 2008;25:1469–1483. [PubMed: 18219561]
50. Kuzyk M, Smith D, Yang J, Cross T, Jackson A, Hardie D, Anderson L, Borchers C. *Mol Cell Proteomics* 2009;8:1860–1877. [PubMed: 19411661]
51. Petrotchenko, EV.; Borchers, CH. US Provisional Patent application. 61223573. 2009.
52. Peterson DS. *Mass Spectrom Rev* 2007;26:19–34. [PubMed: 16967450]
53. Wei J, Buriak JM, Siuzdak G. *Nature* 1999;399:243–246. [PubMed: 10353246]

54. Najam-ul-Haq M, Rainer M, Huck CW, Hausberger P, Kraushaar H, Bonn GK. *Anal Chem* 2008;80:7467–7472. [PubMed: 18729472]
55. Xu S, Li Y, Zou H, Qiu J, Guo Z, Guo B. *Anal Chem* 2003;75:6191–6195. [PubMed: 14616000]
56. Rousell DJ, Dutta SM, Little MW, Murray KK. *J Mass Spectrom* 2004;39:1182–1189.
57. Holscher D, Shroff R, Knop K, Gottschaldt M, Crecelius A, Schneider B, Heckel DG, Schubert US, Svatoš A. *Plant J* 2009;60:907–918. [PubMed: 19732382]
58. Pachuta SJ, Cooks RG. *Chem Rev* 1987;87:647–669.
59. Johansson B. *Surf Interface Anal* 2006;38:1401–1412.
60. Komatsu M, Murayama Y, Hashimoto H. *Appl Surf Sci* 2008;255:1162–1164.
61. Nygren H, Malmberg P. *Proteomics* 2010;10:1694–1698. [PubMed: 20186756]
62. Takats Z, Wiseman JM, Gologan B, Cooks RG. *Science* 2004;306:471–473. [PubMed: 15486296]
63. Takats Z, Wiseman JM, Cooks RG. *J Mass Spectrom* 2005;40:1261–1275. [PubMed: 16237663]
64. Wiseman JM, Ifa DR, Song Q, Cooks RG. *Angew Chem, Int Ed* 2006;45:7188–7192.
65. Raska CS, Parker CE, Dominski Z, Marzluff WF, Glish GL, Pope RM, Borchers CH. *Anal Chem* 2002;74:3429–3433. [PubMed: 12139050]
66. Shah B, Reid JD, Kuzyk MA, Parker CE, Borchers CH. *Methods in Molecular Biology*. 2010 in press.
67. Reid JD, Holmes DT, Mason DR, Shah B, Borchers CH. *J Am Soc Mass Spectrom*. 2010;10.1016/j.jasms.2010.01.024
68. Anderson NL, Jackson A, Smith D, Hardie D, Borchers C, Pearson TW. *Mol Cell Proteomics* 2009;8:995–1005. [PubMed: 19196707]
69. Whiteaker JR, Zhao L, Anderson L, Paulovich AG. *Mol Cell Proteomics* 2010;9:184–196. [PubMed: 19843560]
70. Kiernan UA, Tubbs KA, Nedelkov D, Niederkofler EE, Nelson RW. *Biochem Biophys Res Commun* 2002;297:401–405. [PubMed: 12237133]
71. Niederkofler EE, Tubbs KA, Gruber K, Nedelkov D, Kiernan UA, Williams P, Nelson RW. *Anal Chem* 2001;73:3294–3299. [PubMed: 11476228]
72. Niederkofler EE, Kiernan UA, O'Rear J, Menon S, Saghir S, Protter AA, Nelson RW, Schellenberger U. *Circ Heart Failure* 2008;1:258–264.
73. Rehder DS, Nelson RW, Borges CR. *Protein Sci* 2009;18:2036–2042. [PubMed: 19642159]
74. Lopez MF, Rezai T, Sarracino DA, Prakash A, Krastins B, Athanas M, Singh RJ, Barnidge DR, Oran P, Borges C, Nelson RW. *Clin Chem* 2010;56:281–290. [PubMed: 20022981]
75. Gast MCW, Schellens JHM, Beijnen JH. *Breast Cancer Res Treat* 2009;116:17–29. [PubMed: 19082706]
76. De Bock M, de Seny D, Meuwis MA, Chapelle JP, Louis E, Malaise M, Merville MP, Fillet M. *J Biomed Biotechnol* 2010;906082. [PubMed: 20029632]
77. Zeidan BA, Townsend PA. *Breast Cancer Res* 2008;10:107. [PubMed: 18644101]
78. Wilkinson, M. 2007.
<http://www.drugresearcher.com/Publications/Pharmaceutical-Science/LabTechnologist.com/Applications/Bio-Rad-tackles-the-SELDI-biomarker-controversy>
79. Burnum KE, Frappier SL, Caprioli RM. *Annu Rev Anal Chem* 2008;1:689–705.
80. Franck J, Arafah K, Elayed M, Bonnel D, Vergara D, Jacquet A, Vinatier D, Wisztorski M, Day R, Fournier I, Salzet M. *Mol Cell Proteomics* 2009;8:2023–2033. [PubMed: 19451175]
81. Fournier I, Wisztorski M, Salzet M. *Expert Rev Proteomics* 2008;5:413–424. [PubMed: 18532909]
82. Groseclose, MR.; Chaurand, P.; Wirth, PS.; Massion, PP.; Caprioli, RM. presented at HUPO 4; Aug. 29–Sept. 1; Munich, Germany. 2005.
83. Gregson C. *Biosci Horiz* 2009;2:134–146.
84. Krishnamurthy T, Ross PL, Rajamani U. *Rapid Commun Mass Spectrom* 1996;10:883–888. [PubMed: 8777320]
85. Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Voorhees KJ, Lay JOJ. *Rapid Commun Mass Spectrom* 1996;10:1227–1232. [PubMed: 8759332]
86. Klouche M, Schroeder U. *Clin Chem Lab Med* 2008;46:888–908. [PubMed: 18624614]

87. Von Seggern, CE.; Halden, RU. *Int J Health Sci.* 2009.
<http://www.thefreelibrary.com/Detection+of+bioterrorism+agents+and+related+public+health+threats...-a0203660877>
88. Larsson L. *J Environ Monit* 2008;10:301–304. [PubMed: 18392271]
89. Pennanec X, Dufour A, Haras D, Rehel K. *Rapid Commun Mass Spectrom* 2010;24:384–392. [PubMed: 20049880]
90. van Veen SQ, Claas ECJ, Kuijper EJ. *J Clin Microbiol* 2010;48:900–907. [PubMed: 20053859]
91. Sauer S, Kliem M. *Nat Rev Microbiol* 2010;8:74–82. [PubMed: 20010952]
92. Eigner U, Holfelder M, Oberdorfer K, Betz-Wild U, Bertsch D, Fahr AM. *Clin Lab* 2009;55:289–296. [PubMed: 19894408]
93. Qian, WJ.; Petritis, BO.; Moore, RJ.; Moldawer, LL.; Maier, RV.; Tompkins, RG.; Camp, DG.; Smith, RD. presented at the the HUPO 8th Annual World Conference; Toronto. 2009.
94. Stephenson, J.; Whisnant, C.; Talley, D.; Garge, N.; Rowland, M.; Gardner, M.; Wagener, D.; Wu, S.; Page, G.; Staats, H.; Majumder, P. presented at the HUPO 8th Annual World Conference; Toronto. 2009.
95. Gardner MS, Rowland MD, Siu AY, Bundy JL, Wagener DK, Stephenson JL. *Anal Chem* 2009;81:557–566. [PubMed: 19072583]
96. Callesen, AK.; Philosof, RS.; Vach, W.; Martinussen, T.; Cold, S.; Mogensen, O.; Kruse, TA.; Jensen, ON.; Madsen, JS. presented at the HUPO 8th Annual World Conference; Toronto. 2009.
97. Langton, T.; Matlow, S. 2009.
<http://www.chem.agilent.com/en-US/PressReleases/Pages/21oct-ca09062.aspx>
98. Anderson NL, Anderson NG, Pearson TW, Borchers CH, Paulovich AG, Patterson SD, Gillette M, Aebersold R, Carr SA. *Mol Cell Proteomics* 2009;8:883–886. [PubMed: 19131327]
99. HUPO. 2010. www.hupo.org
100. PRIDE. 2010. www.bioinformatics.med.umich.edu/hupo/ppp; www.ebi.ac.uk/pride
101. Haab BB, Geierstanger BH, Michailidis G, Vitzthum F, Forrester S, Okon R, Saviranta P, Brinker A, Sorette M, Perlee L, Suresh S, Drwal G, Adkins JN, Omenn GS. *Proteomics* 2005;5:3278–3291. [PubMed: 16038022]
102. Omenn GS, Aebersold R, Paik YK. *Proteomics* 2009;9:4–6. [PubMed: 19053147]
103. PeptideAtlas. 2010.
104. Deutsch EW, Lam H, Aebersold R. *EMBO Rep* 2008;9:429–434. [PubMed: 18451766]
105. Omenn GS. *Dis Markers* 2004;20:131–134. [PubMed: 15502245]
106. Omenn GS. *Clin Chem Lab Med* 2004;42:1–2. [PubMed: 15061371]
107. Omenn, GS.; Ping, P. *Clinical Proteomics: From Diagnosis to Therapy*. Van Eyk, JE.; Dunn, MJ.; Wiley-VCH, Weinheim, editors. Vol. 32. 2008. p. 595-613.
108. CPTC. 2010. <http://proteomics.cancer.gov>
109. CPTAC. 2010. <http://proteomics.cancer.gov/programs/CPTAC/>
110. Rudnick PA, Clauser KR, Kilpatrick LE, Tchekhovskoi DV, Neta P, Blonder N, Billheimer DD, Blackman RK, Bunk DM, Cardasis HL, Ham AJL, Jaffe JD, Kinsinger CR, Mesri M, Neubert TA, Schilling B, Tabb DL, Tegeler TJ, Vega-Montoto L, Variyath AM, Wang M, Wang P, Whiteaker JR, Zimmerman LJ, Carr SA, Fisher SJ, Gibson BW, Paulovich AG, Regnier FE, Rodriguez H, Spiegelman C, Tempst P, Liebler DC, Stein SE. *Mol Cell Proteomics* 2010;9:225–241. [PubMed: 19837981]
111. Paulovich AG, Billheimer DD, Ham AJL, Vega-Montoto L, Rudnick PA, Tabb DL, Wang P, Blackman RK, Bunk DM, Cardasis HL, Clauser KR, Kinsinger CR, Schilling B, Tegeler TJ, Variyath AM, Wang M, Whiteaker JR, Zimmerman LJ, Carr SA, Fisher SJ, Gibson BW, Mesri M, Neubert TA, Regnier FE, Rodriguez H, Spiegelman C, Stein SE, Tempst P, Liebler DC. *Mol Cell Proteomics* 2010;9:242–254. [PubMed: 19858499]
112. Tabb DL, Vega-Montoto L, Rudnick PA, Variyath AM, Ham AJL, Bunk DM, Kilpatrick LE, Billheimer DD, Blackman RK, Cardasis HL, Carr SA, Clauser KR, Jaffe J, Kowalski KA, Neubert TA, Regnier FE, Schilling B, Tegeler TJ, Wang M, Wang P, Whiteaker JR, Zimmerman LJ, Fisher SJ, Gibson BW, Kinsinger CR, Mesri M, Rodriguez H, Stein SE, Tempst P, Paulovich AG, Liebler DC, Spiegelman C. *J Proteome Res* 2010;9:761–776. [PubMed: 19921851]

113. Regnier FE, Skates SJ, Mesri M, Rodriguez H, Težak Z, Kondratovich MV, Alterman MA, Levin JD, Roscoe D, Reilly E, Callaghan J, Kelm K, Brown D, Philip R, Carr SA, Liebler DC, Fisher SJ, Temps P, Hiltke T, Kessler LG, Kinsinger CR, Ransohoff DF, Mansfield E, Anderson NL. Clin Chem 2010;56:165–171. [PubMed: 20007858]
114. Rodriguez H, Težak Z, Mesri M, Carr SA, Liebler DC, Fisher SJ, Tempst P, Hiltke T, Kessler LG, Kinsinger CR, Philip R, Ransohoff DF, Skates SJ, Regnier FE, Anderson NL, Mansfield E. _on_behalf_of_the_Workshop_Participants. Clin Chem 2010;56:237–243. [PubMed: 20007859]

Biographies

Dr Parker received her BA degree from Cornell University and her MS and PhD degrees from the Department of Chemistry at UNC-Chapel Hill, Chapel Hill, NC, USA. She worked in the mass spectrometry group at NIEHS in the Research Triangle Park, NC for nearly 25 years, before leaving to join Dr Borchers at UNC in 2001, where she held the position of Director of the UNC-Duke Proteomics Center. Dr Parker is currently a member of Dr Borchers' group at the University of Victoria, BC, Canada. She has more than 135 publications and more than 40 years of experience in mass spectrometry, including more than 10 years of experience in MS-based proteomics.

Dr Pearson received his BSc and PhD degrees from the University of British Columbia in Vancouver. After post-doctoral training at the Medical Research Council Laboratory in Cambridge, UK, Dr Pearson worked as a staff scientist in its Division of Cell Biology. He then became a staff scientist at the International Laboratory for Research on Animal Diseases in Nairobi, Kenya where he introduced monoclonal antibodies to Africa. He is now a professor of biochemistry at the University of Victoria, Canada, where his research has focused on the biochemical and immunochemical analysis of trypanosomes, the causative agents of African sleeping sickness. His research also focuses on the development of quantitative mass spectrometric methods for application to clinical diagnostics, an approach that involves development of antibody probes for peptide surrogates of biomarker targets.

Dr Leigh Anderson is Founder and CEO of the Plasma Proteome Institute, Washington D.C. (www.plasmaproteome.org). The Institute aims to foster a comprehensive exploration of the plasma proteome and the rapid application of novel protein measurements in clinical diagnostics and clinical trials. Prior to founding PPI, Dr Anderson was Chief Scientific Officer at Large Scale Biology Corporation, which he co-founded in 1985. Dr Anderson obtained his BA in Physics with honors from Yale and a PhD in Molecular Biology from Cambridge University (UK), has published 155 papers and been granted 40 US Patents. He has served on the boards of directors of three public technology companies and consults through Anderson Forschung Group.

Dr Borchers received his BS, MS, and PhD degrees from the University of Konstanz, Germany. After his post-doctoral training and employment as a staff scientist at NIEHS/NIH/ RTP, NC and he became the Faculty Director of the UNC-Duke Proteomics Center and held a faculty position in the Department of Biochemistry at UNC-Chapel Hill School of Medicine, Chapel Hill, NC, USA (2001–2006). Since then, Dr Borchers has held the position of Associate Professor of Biochemistry and Microbiology at University of Victoria (UVic), Canada and the Director of the UVic-Genome Proteomics Centre. His research is centred around the improvement, development, and application of proteomics technologies with major focus on techniques for quantitative targeted proteomics for clinical diagnostics.