print

Automated Liquid Chromatography MALDI FT-ICR MS Platform for Proteomics: Rationale for an Off-Line Approach and Optimized Implementation

Eric C. Peters*, Ansgar Brock, Qui Phung, Jonathan Fitchett, David M. Horn, Christer Ericson, Scott B. Ficarro, and Arthur R. Salomon, *Genomics Institute of the Novartis Research Foundation

A number of sophisticated approaches have been developed to study the structure and function of genes, including the whole-scale sequencing of entire organisms (1), global transcriptional profiling (2), and forward genetic studies (3). However, these techniques are ultimately limited by the fact that they only assess intermediates on the way to the protein products of genes that ultimately regulate biological processes (4, 5). Processes such as RNA processing, proteolytic activation, and hundreds of possible post-translational modifications (PTMs) can result in the production of numerous proteins of unique structure and function from a limited number of genes. Additionally, biological activity often results from the assembly of numerous proteins into an active complex, the nature and composition of which can only be explored at the protein level. Therefore, proteomic studies should be able to answer many questions about cellular processes and diseases that cannot be answered by genomic methods alone (6). However, such studies are far harder to perform than their genomic counterparts, and any general analysis platform must possess high sensitivity, be tolerant of a wide range of experimental and analytical conditions, and be able to process and display massive amounts of information. More importantly, these analysis systems must be able to perform extremely high-throughput measurements, since unlike the relatively fixed nature of the genome, the expression and interactions of proteins are in a constant state of flux, varying over time, tissue type, and in response to environmental changes.

Historically, two-dimensional gel electrophoresis (2DE) has been the dominant technique for assessing large-scale changes in protein expression patterns (7). The development (8, 9) and emergence of biological mass spectrometry (MS) in the early 1990's greatly increased the amount of information obtained using this technique, enabling the identification of thousands of encoded proteins by peptide mapping and/or tandem MS experiments (10). Although powerful, the technique as practiced remains laborious, and possesses several widely recognized limitations, including the difficulty of comparing results between laboratories (11), operational difficulty in handling certain classes of proteins, and potential unwanted chemical modifications (12, 13). More importantly, an even greater shortcoming of the classic 2DE technique is its inability to accommodate the extreme range of protein expression levels inherent in complex living organisms due to sample loading restrictions imposed by the gel-based separation technology employed (14). This limitation is of particular concern in that most interesting classes of regulatory proteins are often expressed at low copy numbers per cell. Extensive protein prefractionation schemes based on differing solubility (15, 16), isoelectric point (17), or subcellular location (18) have been proposed to address the problem of analyzing low abundance proteins. However, questions remain as to whether the integrity of the original protein mixture can be maintained, and all of these approaches greatly increase the number of relatively slow 2DE experiments that need to be performed.

These concerns have prompted a number of groups to explore alternative techniques to 2DE. For example, recognition of the fact that the full potential of MS methodologies is greatly underutilized in 2DE systems has led multiple groups to explore analysis platforms based solely on multi-dimensional chromatography combined with MS and/or tandem MS methods (19, 20). The chromatographic separations serve to disperse the complexity of the initial sample, and can be performed at both the peptide (21) as well as at the protein level (22), although protein identification is primarily performed using peptides (23). Using these methodologies, the identification of encoded proteins present in affinity complexes, organelles, and even whole organisms has been performed (24, 25). Additionally, novel methodologies for reducing the complexity of the analyte mixture based on the presence of particular amino acids (26-28) or specific PTMs (27, 29) have been employed to analyze even low abundance regulatory proteins or PTMs (30, 31, 32). Similarly, techniques for assessing quantitative differential display of base proteins (27, 28) or PTMs (30, 33) between two samples have also been described.

A Potentially Powerful Paradigm: LC MALDI MS

Although these liquid chromatography/MS-based approaches for the wide-scale analysis of protein expression patterns exhibit tremendous potential for improved analyses, they have to date overwhelmingly employed electrospray ionization

(ESI) methods due to the simplicity of their implementation. However, the operational parameters of ESI coupling methods also impose several limitations. Specifically, the separation system and mass spectrometer employed are coupled directly in real time, making the construction of parallel analysis systems difficult (or at least extremely costly), and often preventing the mass spectrometer from continually collecting useful data due to the equilibration and washing periods typical of separation techniques. More importantly, current instrument control and data analysis software is not nearly fast enough to allow real time data-dependent processing during the course of a chromatographic separation except when employing simple selection criteria such as peak intensity. This necessitates that upon the completion of a separation and subsequent analysis of the resulting data, the same sample must often be rerun to focus on those species that exhibited the desired selection criteria (34). Additionally, targeted experiments monitoring the levels of several particular species over time still requires the active engagement of the mass spectrometer over the whole course of the chromatographic run, even though the species of interest themselves elute only in specific narrow time windows during the gradient profile. Ultimately, these and other limitations result in dramatic reductions in overall platform throughput.

In light of these considerations, several groups have begun to explore MALDI-based analysis platforms that involve the creation of a “permanent record” of the multidimensional separation by depositing the effluents of the final separation columns directly onto MALDI target plates. Decoupling the separation step from the mass spectrometer in this manner guarantees that the chromatography can be performed free of artificially imposed restrictions, while the mass spectrometer can operate at maximum throughput. The resulting plates can also be reanalyzed as required without the need to repeat the separation step, thus decreasing sample requirements while simultaneously greatly increasing the overall throughput of the system.

Clearly, the sample deposition method employed is critical to the success of creating high fidelity, reproducible “permanent records” of the liquid chromatographic separations. “Heart fractionation” methods (35) are relatively simple to implement, but potentially sacrifice a significant part of the chromatographic resolution obtained during the separation. By contrast, sophisticated piezo-actuated microdispenser systems fabricated by the anisotropic wet etching of monocrystalline silicon have been described for the deposition of chromatographic effluents directly onto MALDI target plates (36, 37). Similarly, specialized liquid junction-coupled sub-atmospheric pressure deposition chambers for the off-line coupling of capillary electrophoresis (CE) with MALDI MS have also been described (38, 39). In light of the potential power of this off-line approach, our group has developed a simple but robust fully automated system for the real time deposition of the effluents of mHPLC columns and matrix onto MALDI target plates.

An Automated Deposition System for LC MALDI MS Approaches

Samples are first fractionated using such chromatographic modes as strong cation exchange, immobilized metal affinity, or amino acid specific enrichment methods based on the purpose of the overall experiment (27). Regardless of the initial separation performed, the resulting column effluent is loaded directly onto a reversed-phase mHPLC column. The outlets of a series of these columns are held in parallel, and MALDI target plates clamped to an x,y translational stage are physically moved automatically underneath the columns during the course of the separation. Figure 1 shows the concomitant deposition of a chromatographic run and matrix onto a MALDI target plate. It is important to note that the outlets of the columns and the target plates never come into direct physical contact. Instead, the effluents of the columns are transferred to the plates using an electrically mediated mechanism by applying a constant or intermittent negative potential to the target plates. This results in either a continuous stream or a series of droplets of precisely controlled volume respectively. In theory, the deposition of a continuous stream should more accurately maintain the fidelity of the separation process (38, 39). However, such an approach would also require the MALDI system to query the entire length of the trace, since eluting samples would not be localized to any particular position.

Figure 1

Automated Deposition of a Reversed-Phase mHPLC Chromatography Separation with Concomitant Matrix Addition.

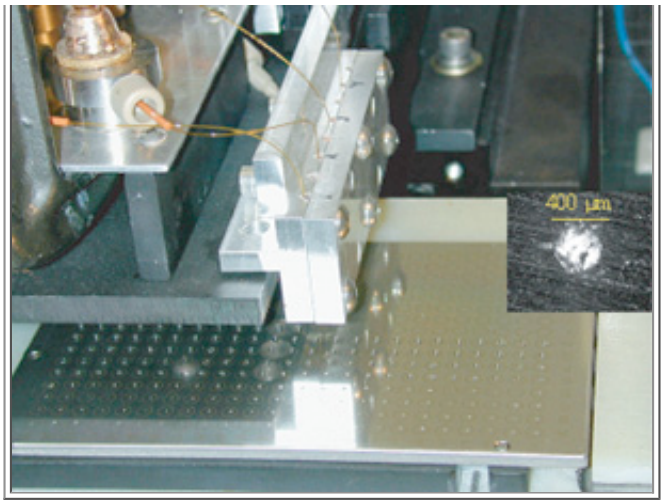
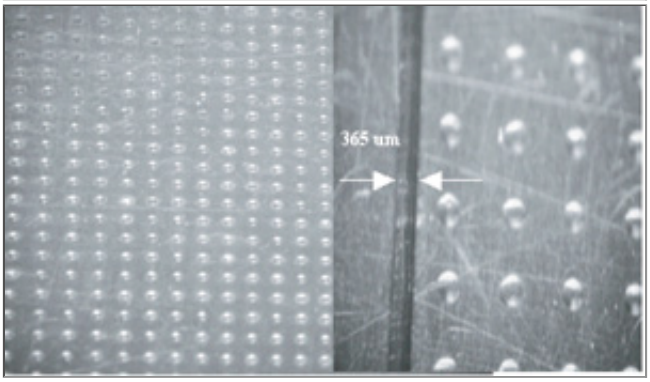


Figure 2 shows an example of the capabilities of our deposition system. Specifically, roughly 150 nL aqueous droplets were precisely arrayed on a three by five square inch stainless steel plate in a 6144 microtiter array format, with each spot clearly distinguished from its nearest neighbors. Using this deposition system, matrix can also automatically be applied to the target plate before, during, or after the chromatographic process. Additionally, the deposition system works equally well with aqueous or numerous organic solvents, enabling the possibility of novel, automated on-plate recrystallization processes no longer limited to solvent mixtures of acetonitrile and water that often further increase analyte sensitivity. This unique combination of automation and operational flexibility enables facile optimization of the many variables known to effect MALDI crystallization processes.

Figure 2

6144 Microtiter Format Array of 150 nL Aqueous Droplets Deposited Onto a Stainless Steel Plate. A 365 mm Diameter Capillary Has Been Placed Across the Plate for Scale Perspective.



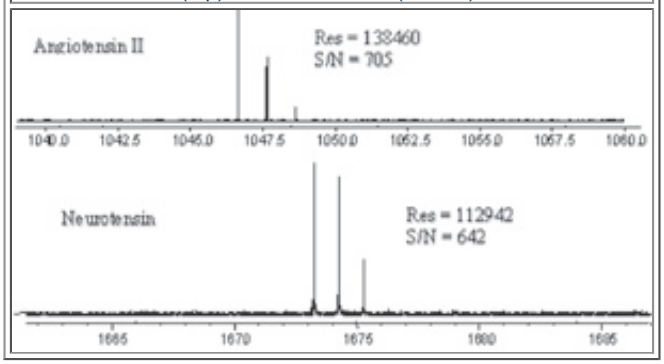
In addition to the technical capabilities of the deposition system, our platform also makes use of specially patterned target plates consisting of hydrophilic regions arrayed on an otherwise hydrophobic surface (40). After deposition of a sample onto a target region, both the analyte and matrix localize into an area far smaller than that occupied by the originally deposited droplet as the sample solvent evaporates, resulting in the effective concentration of the analyte on the hydrophilic regions. The use of such target plates provides considerable operational advantages in our analysis platform. For example, the sensitivities of ESI methods are known to be highly concentration dependent, often necessitating the use of nanochromatography to achieve maximum sensitivity (19). Although effective, such nanoscale chromatography systems present numerous operational problems and often involve the manual loading of samples directly onto the separation column before it is connected to the HPLC system. By contrast, the surface-patterned target plates further concentrate the samples after the chromatographic process is complete, enabling the use of 300 mm id capillary columns and commercially available autosamplers. In addition, the localization of analytes to precisely defined locations approximately 400 mm in diameter on the target plates prevents the MALDI stage from querying areas that contain no analytes (39). More importantly, increasing the size of the MALDI laser spot to approximately 400

micrometers allows the entire sample to be queried simultaneously, thus greatly reducing the problem of searching for “sweet spots” often encountered when using the dried droplet method of sample preparation. Together, these factors greatly increase the sample throughput of the overall platform.

The unique capabilities of our deposition system have enabled the development of robust, optimized spotting methodologies for a variety of different matrices, including alpha-cyano-4-hydroxycinnamic acid (HCCA). Figure 3 shows the quality of the signals obtained after the injection of 500 total atomoles of each analyte peptide, automated reversed-phase mHPLC and sample plate deposition, and automated mass spectrometric analysis using a custom-built MALDI source and a highly modified commercial FT-ICR MS. Although the “permanent” nature of the deposited MALDI sample enables the extended accumulation of signal for low concentration species, these signals were obtained from only 100 laser shots in a single accumulation event. Such excellent signals are routinely obtained in less than 7 seconds after a fully automated sample preparation and analysis process, demonstrating the system’s capability for rapid, high-sensitivity sample throughput.

Figure 3

MALDI Mass Spectra After Injection, Chromatographic Separation, and Automated Deposition of 500 Total Atomoles of Angiotensin II (top) and Neurotensin (bottom).



Novel Chemical Labels for Increasing the MALDI Ionization Efficiencies of Peptides

Even after the issues involving the reproducible deposition of mHPLC column effluents with matrix onto target plates have been addressed, a MALDI-based analysis platform still presents several other distinct challenges. For example, the performance of MALDI mass spectrometry can greatly be affected by competitive ionization effects, which are especially prevalent in complex mixtures (41). The automated nature of our deposition system eliminates some of these problems by providing a reproducible environment for the recrystallization of analyte and matrix molecules. In addition, MALDI has been shown to preferentially promote the ionization of more hydrophobic peptides in complicated mixtures due to their enhanced co-crystallization with the matrix (42), thus providing an incomplete representation of a sample’s composition. However, since the peptide mixtures in our platform are subjected to reversed-phase mHPLC rather than CE immediately before deposition, all of the peptides deposited in an individual spot should exhibit nearly identical hydrophobicities, and therefore co-crystallize more similarly without resorting to complicated recrystallization methods (38, 39). Nevertheless, there are other sources of ion suppression in MALDI-based methods that cannot be rectified solely by the deposition system.

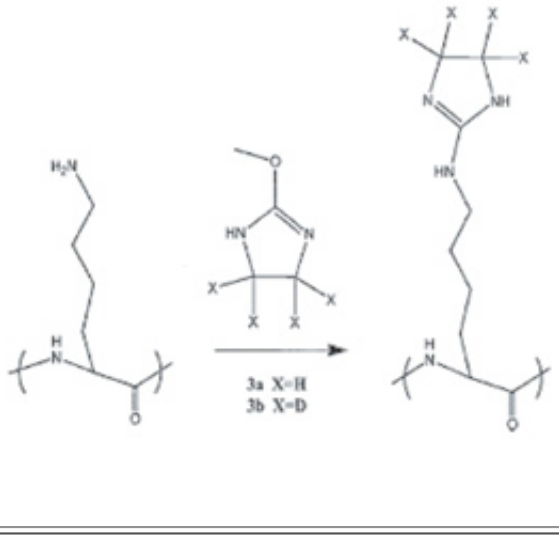
Krause and coworkers investigated the dominance of arginine-containing peptides in the MALDI MS analysis of tryptic digests, and found that peptides containing an arginine residue exhibit a four- to eighteen-fold increase in signal intensity compared to those peptides containing a lysine (43). This bias is thought to result from the higher basicity of the arginine residue compared to lysine. Greater sequence coverages could in theory be obtained using a labeling methodology that either increases the basicity of lysine residues (44-48) or reduces the basicity of arginine residues (49). However, the former method has attracted more attention, because reducing the basicity of arginine is considered detrimental to the overall sensitivity. Based on earlier work done with proteins (50), several groups (45-48) have recently reported the use of O-methylisourea to convert C-terminal lysine residues in tryptic digests to more basic homoarginine residues. In all cases, this treatment resulted in higher sequence coverages in peptide mapping experiments compared to the underivatized sample. Although effective in increasing ionization efficiencies, this label is

not amenable to the facile incorporation of inexpensive stable isotopic species for differential quantitation studies.

Therefore, our group developed a labeling reagent that increases the ionization efficiencies of lysine-containing peptides while simultaneously enabling differential quantitation measurements (51). Figure 4 shows the reaction of 2-methoxy-4,5-dihydro-1H-imidazole with the e-amino group of a lysine residue to form its 4,5-dihydro-1H-imidazol-2-yl derivative. Peptide mapping experiments of tryptic protein digests after functionalization with the above reagent showed total amino acid sequence coverages nearly double those of their unlabeled counterparts (51). It is important to note that although the term high-throughput has traditionally been equated with faster measurements, this labeling scheme also increases the overall throughput of the system, as it enables the collection of greater amounts of data in the same unit time.

Figure 4

Reaction of a Lysine Residue with 2-Methoxy-4,5-Dihydro-1H-Imidazole to Form a 4,5- Dihydro-1H-Imidazol-2-Yl Derivative.



In addition to its ability to affect increased MALDI ionization efficiencies, this chemical label can further increase the efficiency of the analysis platform due to the isotopic substitutions that can easily be made at the two methylene ring carbons. The combination of a 4 Da mass difference per differentially labeled moiety in conjunction with the unique chemical selectivity of the label for lysine residues rather than the free a-amino group at a peptide's N-terminus enables the user to accurately determine the number of lysine groups contained within a given peptide, and thus further limit the number of search possibilities when attempting to determine the identity of the protein from which it arose.

Conclusion

The sheer complexity and ever changing nature of the proteome necessitates the development of more powerful, higher-throughput analysis platforms. In theory, a MALDI-based LC/MS platform could favorably address many of the operational disadvantages inherent in the real time coupling of separation systems and mass spectrometers using ESI ionization. However, in order to realize these potential advantages, numerous aspects of the MALDI process such as instrumentational issues, sample deposition, and chemical derivatization need to thoroughly be investigated and optimized. The sample processing methods described above have been designed to maximize the throughput of any MALDI-based analytical platform, and are therefore compatible with a wide range of commercially available instrumentation. However, as the second part of this article ("Automated Liquid Chromotagraphy MALDI FT-ICR MS Platform for Proteomics: Automated High Performance Mass Spectrometry and Data Analysis" which will be published in the Winter 2002 issue) demonstrates, further significant increases in overall system throughput can also be obtained by interfacing the techniques described above with specific MS detector systems that provide extremely high mass accuracy and high resolution measurements.

References

1. S. Broder and J.C. Venter, “Sequencing the entire genomes of free-living organisms: the foundation of pharmacology in the new millennium.” Annu Rev Pharmacol Toxicol, 40, 97-132 (2000).
2. D.J. Lockhart and E.A. Winzeler, “Genomics, gene expression and DNA arrays.” Nature, 405, 827-836. (2000).
3. J. Rossant and C. McKerlie, “Mouse-based phenogenomics for modelling human disease.” Trends Mol Med, 7, 502-507. (2001).
4. S.P. Gygi, Y. Rochon, B.R. Franza, and R. Aebersold, “Correlation between protein and mRNA abundance in yeast.” Mol Cell Biol, 19, 1720-1730. (1999).
5. B. Futcher, G.I. Latter, P. Monardo, C.S. McLaughlin, and J.I. Garrels, “A sampling of the yeast proteome.” Mol Cell Biol, 19, 7357-7368. (1999).
6. A. Pandey and M. Mann, “Proteomics to study genes and genomes.” Nature, 405, 837-846. (2000).
7. G.L. Corthals, V.C. Wasinger, D.F. Hochstrasser, and J.C. Sanchez, “The dynamic range of protein expression: a challenge for proteomic research.” Electrophoresis, 21, 1104-1115. [pii] (2000).
8. M. Karas and F. Hillenkamp, “Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons.” Anal Chem, 60, 2299-2301. (1988).
9. J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, and C.M. Whitehouse, “Electrospray ionization for mass spectrometry of large biomolecules.” Science, 246, 64-71. (1989).
10. S.D. Patterson and R. Aebersold, “Mass spectrometric approaches for the identification of gel-separated proteins.” Electrophoresis, 16, 1791-1814. (1995).
11. T. Voss and P. Haberl, “Observations on the reproducibility and matching efficiency of two- dimensional electrophoresis gels: consequences for comprehensive data analysis.” Electrophoresis, 21, 3345-3350. (2000).
12. E. Bordini, M. Hamdan, and P.G. Righetti, “Alkylation power of free Immobiline chemicals towards proteins in isoelectric focusing and two-dimensional maps, as explored by matrix assisted laser desorption/ionization-time of flight-mass spectrometry.” Electrophoresis, 21, 2911-2918. (2000).
13. M. Galvani, E. Bordini, C. Piubelli, and M. Hamdan, “Effect of experimental conditions on the analysis of sodium dodecyl sulphate polyacrylamide gel electrophoresis separated proteins by matrix-assisted laser desorption/ ionisation mass spectrometry.” Rapid Commun Mass Spectrom, 14, 18-25 (2000).
14. S.P. Gygi, G.L. Corthals, Y. Zhang, Y. Rochon, and R. Aebersold, “Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology.” Proc Natl Acad Sci U S A, 97, 9390-9395. (2000).
15. R.S. Taylor, et al., “Proteomics of rat liver Golgi complex: minor proteins are identified through sequential fractionation.” Electrophoresis, 21, 3441-3459. (2000).
16. A.S. Nouwens, et al., “Complementing genomics with proteomics: the membrane subproteome of Pseudomonas aeruginosa PAO1.” Electrophoresis, 21, 3797-3809. (2000).
17. B. Herbert and P.G. Righetti, “A turning point in proteome analysis: sample prefractionation via multicompartment electrolyzers with isoelectric membranes.” Electrophoresis, 21, 3639-3648. (2000).
18. E. Jung, M. Heller, J.C. Sanchez, and D.F. Hochstrasser, “Proteomics meets cell biology: the establishment of subcellular proteomes.” Electrophoresis, 21, 3369-3377. (2000).
19. J.R. Yates, 3rd, “Mass spectrometry. From genomics to proteomics.” Trends Genet, 16, 5-8. (2000).
20. R. Aebersold and D.R. Goodlett, “Mass spectrometry in proteomics.” Chem Rev, 101, 269-295. (2001).
21. S.E. Martin, J. Shabanowitz, D.F. Hunt, and J.A. Marto, “Subfemtomole MS and MS/MS peptide sequence analysis using nano-HPLC micro-ESI fourier transform ion cyclotron resonance mass spectrometry.” Anal Chem, 72, 4266-4274. (2000).
22. D.B. Wall, et al., “Isoelectric focusing nonporous RP HPLC: a two-dimensional liquid-phase separation method for mapping of cellular proteins with identification using MALDI-TOF mass spectrometry.” Anal Chem, 72, 1099-1111. (2000).
23. J. Peng and S.P. Gygi, “Proteomics: the move to mixtures.” J Mass Spectrom, 36, 1083-1091. (2001).
24. A.J. Link, et al., “Direct analysis of protein complexes using mass spectrometry.” Nat Biotechnol, 17, 676-682. (1999).
25. A.C. Gavin, et al., “Functional organization of the yeast proteome by systematic analysis of protein complexes.” Nature, 415, 141-147. (2002).
26. S. Wang and F.E. Regnier, “Proteomics based on selecting and quantifying cysteine containing peptides by covalent chromatography.” J Chromatogr A, 924, 345-357. (2001).
27. J. Ji, et al., “Strategy for qualitative and quantitative analysis in proteomics based on signature peptides.” J Chromatogr B Biomed Sci Appl, 745, 197-210. (2000).
28. S.P. Gygi, et al., “Quantitative analysis of complex protein mixtures using isotope-coded affinity tags.” Nat Biotechnol, 17, 994-999. (1999).
29. P. Cao and J.T. Stults, “Phosphopeptide analysis by on-line immobilized metal-ion affinity chromatography-capillary electrophoresis-electrospray ionization mass spectrometry.” J Chromatogr A, 853, 225-235. (1999).
30. M.B. Goshe, et al., “Phosphoprotein isotope-coded affinity tag approach for isolating and quantitating phosphopeptides in proteome-wide analyses.” Anal Chem, 73, 2578-2586. (2001).
31. L. Riggs, C. Sioma, and F.E. Regnier, “Automated signature peptide approach for proteomics.” J Chromatogr A, 924, 359-368. (2001).
32. P. Blume-Jensen and T. Hunter, “Oncogenic kinase signalling.” Nature, 411, 355-365. (2001).
33. Y. Oda, T. Nagasu, and B.T. Chait, “Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome.” Nat Biotechnol, 19, 379-382. (2001).
34. R. Pieper, et al., “Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T cells.” J Exp Med, 189, 757-766. (1999).
35. T.J. Griffin, et al., “Quantitative proteomic analysis using a MALDI quadrupole time-of-flight mass spectrometer.” Anal Chem, 73, 978-986. (2001).
36. S. Ekstrom, et al., “Integrated microanalytical technology enabling rapid and automated protein identification.” Anal Chem, 72, 286-293. (2000).
37. S. Ekstrom, et al., “Signal amplification using "spot-on-a-chip" technology for the identification of proteins via MALDI-TOF MS.” Anal Chem, 73, 214-219. (2001).
38. J. Preisler, F. Foret, and B.L. Karger, “On-line MALDI-TOF MS using a continuous vacuum deposition interface.” Anal Chem, 70, 5278-5287. (1998).
39. J. Preisler, P. Hu, T. Rejtar, and B.L. Karger, “Capillary electrophoresis--matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a vacuum deposition interface.” Anal Chem, 72, 4785-4795. (2000).
40. M. Schuerenberg, et al., “Prestructured MALDI-MS sample supports.” Anal Chem, 72, 3436-3442. (2000).
41. S.L. Cohen and B.T. Chait, “Influence of matrix solution conditions on the MALDI-MS analysis of peptides and proteins.” Anal Chem, 68, 31-37. (1996).
42. R. Kratzer, C. Eckerskorn, M. Karas, and F. Lottspeich, “Suppression effects in enzymatic peptide ladder sequencing using ultraviolet - matrix assisted laser desorption/ionization - mass spectormetry.” Electrophoresis, 19, 1910-1919. (1998).
43. E. Krause, H. Wenschuh, and P.R. Jungblut, “The dominance of arginine-containing peptides in MALDI-derived tryptic mass fingerprints of proteins.” Anal Chem, 71, 4160-4165. (1999).References
44. V. Bonetto, A.C. Bergman, H. Jornvall, and R. Sillard, “C-terminal sequence analysis of peptides and proteins using carboxypeptidases and mass spectrometry after derivatization of Lys and Cys residues.” Anal Chem, 69, 1315-1319. (1997).
45. F.L. Brancia, S.G. Oliver, and S.J. Gaskell, “Improved matrix-assisted laser desorption/ionization mass spectrometric analysis of tryptic hydrolysates of proteins following guanidination of lysine-containing peptides.” Rapid Commun Mass Spectrom, 14, 2070-2073 (2000).
46. J.E. Hale, J.P. Butler, M.D. Knierman, and G.W. Becker, “Increased sensitivity of tryptic peptide detection by MALDI-TOF mass spectrometry is achieved by conversion of lysine to homoarginine.” Anal Biochem, 287, 110-117. (2000).
47. T. Keough, M.P. Lacey, and R.S. Youngquist, “Derivatization procedures to facilitate de novo sequencing of lysine- terminated tryptic peptides using

postsource decay matrix-assisted laser desorption/ionization mass spectrometry." Rapid Commun Mass Spectrom, 14, 2348-2356 (2000).
48. R.L. Beardsley, J.A. Karty, and J.P. Reilly, "Enhancing the intensities of lysine-terminated tryptic peptide ions in matrix-assisted laser desorption/ionization mass spectrometry." Rapid Commun Mass Spectrom, 14, 2147-2153 (2000).
49. H. Cui, J. Leon, E. Reusaet, and A. Bult, "Selective detection of peptides containing specific amino acids residues." J. Chromatogr. A, 704, 27-36 (1995).
50. J.R. Kimmel, Methods Enzymol., 11, 584 (1967).
51. E.C. Peters, D.M. Horn, D.C. Tully, and A. Brock, "A novel multifunctional labeling reagent for enhanced protein characterization with mass spectrometry." Rapid Commun Mass Spectrom, 15, 2387-2392 (2001).

Dr. Eric C. Peters *received his B.S. (Chemistry) from Georgetown University in 1989, his M.S. (Polymer Chemistry) from Cornell University in 1996, and a Ph.D. (Chemistry) from the University of California, Berkeley in 1999. At Berkeley, Dr. Peters developed a number of novel monolithic polymer supports for use in chromatography, fluidics, catalysis, and combinatorial chemistry applications. Since 1999, Dr. Peters has been at the Genomics Institute of the Novartis Research Foundation in San Diego, where his research has focused on the development of novel integrated LC/MS platforms for proteomics applications.*

Dr. Peters can be contacted at peters@gnf.org

*This article was published in the Fall 2002 issue of APR, Volume 5, Issue 3, on pages 72-81.