

Advances in quantitative proteomics using stable isotope tags

Mark R. Flory, Timothy J. Griffin, Daniel Martin and Ruedi Aebersold

A great deal of current biological and clinical research is directed at the interpretation of the information contained in the human genome sequence in terms of the structure, function and control of biological systems and processes. Proteomics, the systematic analysis of proteins, is becoming a critical component in this endeavor because proteomic measurements are carried out directly on proteins – the catalysts and effectors of essentially all biological functions. To detect changes in protein profiles that might provide important diagnostic or functional insights, proteomic analyses necessarily have to be quantitative. This article summarizes recent technological advances in quantitative proteomics.

The complete genome sequence produced by the Human Genome Project (HGP) [1,2] contains, in principle, the information required for the development and the physiology of a human being. However, much of the knowledge about how the sequence information relates to physiology in health and disease is currently lacking. The genome project has also catalyzed the emergence of new approaches and technologies that attempt to generate and interpret the data required for a comprehensive understanding of biological processes. Among these, systematic and quantitative profiling technologies have been broadly applied to identify diagnostic expression patterns specific to pathological cellular states, such as cancer [3], and to provide clues to the genes and gene products that are responsible for, or correlate with, a specific state of a system. This might, in turn, reveal fundamental mechanisms of biological control that are potential therapeutic targets in the case of pathologically perturbed systems.

The most mature of these profiling technologies are those that measure expression changes at the level of mature mRNA products [4–6]. However, despite their widespread use, mRNA-based analyses of gene expression have shortcomings. Their use as tools for the comprehensive investigation of biological processes is limited by the observation that quantitative measurements at the mRNA level cannot provide accurate reflections of either the absolute amount or the induced changes in abundance at the protein level [7–9]. Measurements made at the mRNA level also fail to detect the post-translational modifications, cellular localizations and activities of proteins or the macromolecular interactions that cooperatively affect their function. The use of mRNA profiling techniques as tools for the detection of prognostic or diagnostic patterns is limited by the frequently difficult

access to the target tissue or the inability to identify the target tissue in the first place. Quantitative proteomics has recently emerged as a complementary technology to mRNA profiling with the ability to characterize comprehensively the structural and functional aspects of protein products [10].

Quantitative proteomics based on stable isotope tagging

A key aspect to the comprehensive characterization of protein products is the quantitative analysis of protein profiles. For this, two alternative approaches have been developed. The first and most widely used method is based on high resolution two-dimensional electrophoresis (2DE) and mass spectrometry, the second on quantitative mass spectrometry via stable isotope tagging of proteins and peptides. In the 2DE-based method, complex protein mixtures are initially separated electrophoretically and stained. Specific proteins are then selected for mass spectrometric identification based on quantitative comparison of the 2DE staining patterns of suitable experimental and control protein samples. Whereas the technique is mature and robust, several conceptual and technical considerations limit its general utility [11]. Most significantly, a study using unfractionated soluble proteins from a whole-cell yeast (*Saccharomyces cerevisiae*) lysate demonstrated that even with maximal sample loading and extended electrophoretic separation, low-abundance proteins, which constitute nearly half the yeast proteome, were systematically excluded [12].

The most significant recent advances in quantitative proteomics have been catalyzed by quantitative mass spectrometry, the subject of this review. This method consists of the following four steps: (1) differential isotopic labeling of separate protein mixtures; (2) digestion of the

Mark R. Flory
Timothy J. Griffin
Daniel Martin
Ruedi Aebersold*

Institute for Systems
Biology, 1441 North
34th Street, Seattle,
WA 98103-8904, USA.

*e-mail: raebersold@
systemsbiology.org

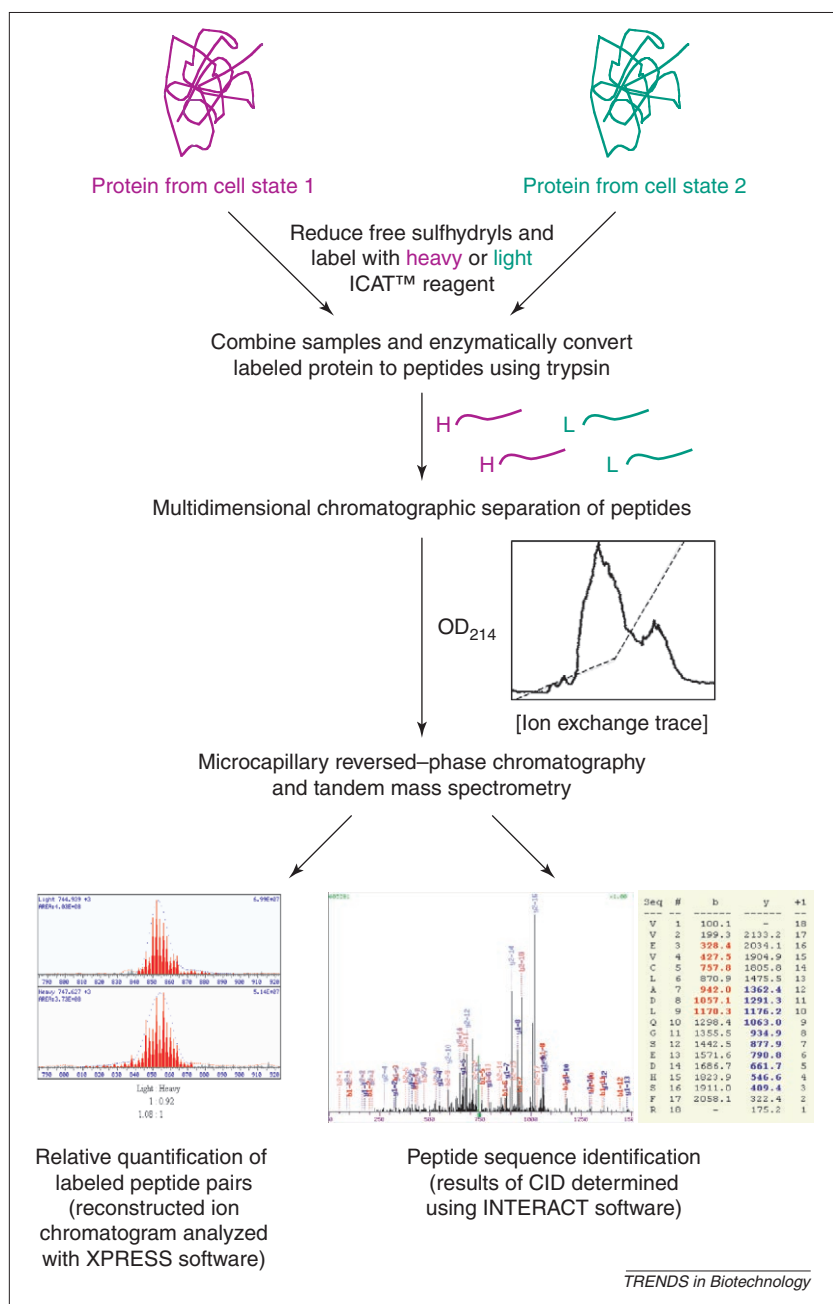


Figure 1. Strategy for isotope-coded affinity tag (ICAT™) reagent-based analysis of protein targets

Proteins from two cell states are denatured, reduced and labeled with either isotopically heavy or isotopically light ICAT™ reagent. Following digestion with trypsin, ICAT™-reagent-labeled peptides are separated by multidimensional liquid chromatography before analysis by reversed-phase tandem mass spectrometry. Both the identity and relative abundances of peptide peak pairs are determined.

combined, labeled protein mixtures followed by separation of the resulting peptides by multidimensional liquid chromatography (LC); (3) analysis of the separated peptides by automated tandem mass spectrometry (MS–MS); and (4) automated database searching to identify the peptide sequences (and hence the proteins from which they were derived) and determination of relative protein abundance from the mass spectral data.

The most commonly used and now commercially supported isotope labeling method for quantitative proteomics has been the isotope-coded affinity tag (ICAT™) reagent methodology [13]. It is based upon the covalent labeling of cysteine residues in separate polypeptide isolates using chemically identical but isotopically different reagents, followed by proteolysis, multidimensional LC and mass spectral analysis to quantify and determine the sequence of the isolated peptides. The approach is shown in Fig. 1. The multidimensional LC method that has seen the most widespread use couples strong-cation exchange (SCX) LC with reversed-phase (RP) microcapillary LC (μLC) [14–17]. Peptides are fractionated by electrostatic charge by SCX LC and the generated fractions are then sequentially further separated by RP–μLC either with the two columns linked on-line [14,16] or applied sequentially in an off-line configuration [15,17].

Multidimensional LC has been shown to overcome the sensitivity limitations of commonly employed 2DE methods to separate complex mixtures before mass spectral analysis. This was illustrated by a study in which tryptic peptides generated from a yeast lysate were extensively fractionated and analyzed by LC–MS–MS. Proteins with codon-bias values indicating very low protein abundance were detected conclusively if three dimensions of chromatographic separation were combined with MS sequencing protocols that maximized the number of sequencing operations [15]. The identification in that study of ICAT™ reagent-labeled low-abundance proteins also indicates that the labeling reaction proceeds significantly toward completion for protein mixtures with a predicted dynamic range of five to six orders of magnitude.

The sequence of the isotopically labeled (i.e. ICAT™-reagent-labeled) peptides eluting from the RP–μLC column are identified by a highly automated process most commonly using on-line electrospray ionization (ESI) operated in data-dependent MS–MS mode. The acquired MS–MS spectra are then searched against a peptide sequence database using SEQUEST [18] or other analogous software routines such as MASCOT (<http://www.matrixscience.com/cgi/index.pl?page=../home.html>) and ProFound (<http://prowl.rockefeller.edu/cgi-bin/ProFound?INTRO>) to determine the sequence of the detected peptide. The mass spectral signals of the identified, differentially isotopically labeled peptide sequences are then reconstructed and the relative intensities of these signals are measured to determine the relative abundance of the peptide (and hence the protein from which it is derived) between the two starting mixtures [13,17]. Although the majority of applications of the ICAT™ reagent have been carried out in this ‘gel free’ format using multidimensional LC, the approach is versatile and has also been shown to be

effective in conjunction with initial separation of protein mixtures by 2DE followed by mass spectral analysis [19]. The separation by 2DE and mass spectrometric analysis of differentially isotopically labeled proteins provides accurate quantification of each protein contained in protein spots of comigrating proteins and thus addresses a common and important problem in protein profiling based on gel electrophoresis patterns.

Recent advances of isotope-tagging based proteomics technology

Since the description of the principles of the method, variations and improvements in isotope incorporation, mass spectrometry and data processing and analysis have been reported. These advances demonstrate that the combination of selective chemistries, tandem mass spectrometry and data processing algorithms provides a generic and increasingly useful approach to quantitative proteomics.

Advances in tagging chemistries and isotope incorporation

The general approach to quantitative proteomics involving selective tagging, stable isotope dilution and mass spectrometry has catalyzed the proliferation of a variety of different isotopic labeling methods. Stable isotope incorporation has been achieved in a variety of ways, including metabolic labeling [20,21,30], enzymatically directed methods [22–24] and chemical labeling using externally introduced tags such as that facilitated by the ICAT™ reagent [13]. More recently, a solid-phase method for the isolation and isotopic labeling of cysteine-containing peptides in complex mixtures has been developed that involves tag addition at the end of the labeling procedure after trypsinization and sample preparation [25]. Despite the potential for biases resulting from differences in sample digestion and manipulation that might occur before addition of the tag, the solid-phase method is simpler, more efficient and more sensitive in side-by-side comparisons with the solution-based ICAT™ labeling protocol [25]. Another technological advance is a second-generation ICAT™ reagent containing a cleavable linker (Applied Biosystems, Foster City, CA, USA) that promises to increase further the sensitivity and versatility of quantitative proteomic studies. Alternative strategies for the specific isotopic labeling of amino-acid residues other than cysteine have also been developed, such as per-methyl esterification of carboxylic acid groups [26], specific labeling of lysine residues [27] and peptide N-termini [28]. In addition, an alternative strategy employing differential chemical labeling of lysine residues that does not require stable-isotope incorporation has also

been described [29]. The combined use of a variety of labeling reagents reactive with different functionalities should increase sample coverage and should also help to reduce the confound of non-unique peptides when quantitating proteins on the peptide level isolated from complex mammalian samples.

The use of chemical reactions to introduce tags detectable by MS into specific sites in proteins and peptides has also been extended to probe functional aspects of proteins contained in complex mixtures. Several methods that enable differential isotopic labeling and isolation of phosphorylated peptides have been described [30–33] as demonstrating or suggesting the potential for quantitative profiling by MS of protein phosphorylation on a proteome-wide scale. Selective chemistries have also been used to investigate the activity of specific classes of enzymes. This was achieved by the synthesis of reagents that bind selectively to proteins in an activity-dependent manner [34]. Incorporation of stable isotopes into such reagents holds great potential for the profiling of protein activities by MS. The ability to quantify relative abundances, post-translational modifications and activities of proteins across whole proteomes and in purified biological complexes will undoubtedly revolutionize our understanding of biological mechanisms acting in both normal and diseased cellular states.

Advances in MS instrumentation and methods for quantitative proteome analysis

Although it is robust, sensitive and automated, the analysis of complex, isotope-tagged peptide mixtures by on-line LC–ESI MS–MS suffers from the demand for continual sample consumption and the requirement for untargeted, or ‘on the fly’, selection of precursor ions for sequencing. The coupling of matrix-assisted laser desorption ionization (MALDI) to a tandem mass spectrometer promises to overcome these limitations and greatly increase the efficiency of global proteomic comparisons of biological cell states. In this instance, peptides are prepared as described above except that they are eluted and spotted directly from a microcapillary reversed-phase liquid chromatography column on a MALDI sample plate. A mass spectrum is first obtained for each spot to quantify heavy and light ICAT™ reagent-labeled pairs of proteins. Tandem mass spectrometry can then be focused directly on those spots containing ICAT™ reagent-labeled peptide pairs showing interesting changes in relative abundance (Fig. 2). Because the samples are physically separated into spots on the sample plate, this method avoids needless collision-induced dissociation (CID) sequence analysis of ICAT™ reagent-labeled peptide pairs showing no change in abundance. Therefore, the method significantly reduces

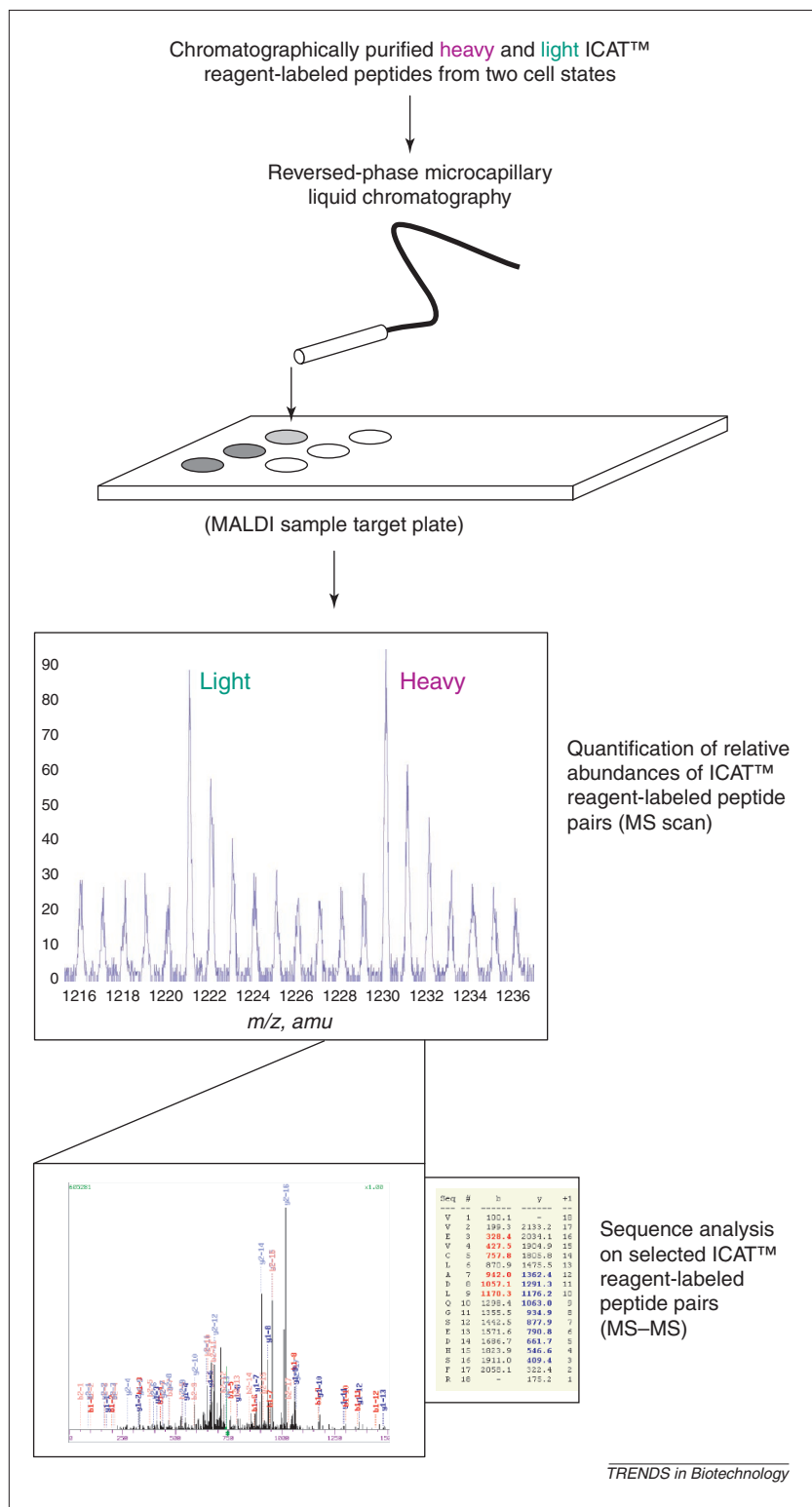


Figure 2. Implementation of matrix-assisted laser desorption ionization (MALDI) for global proteomic comparisons

Purified isotope-coded affinity tag (ICAT™)-reagent-labeled peptides are eluted and spotted directly from a microcapillary reversed-phase liquid chromatography column onto a MALDI sample plate. A mass spectrum is obtained for each spot to quantify relative levels of heavy and light ICAT™-reagent-labeled peptide pairs. Tandem mass spectrometry can then be targeted to spots containing ICAT™-reagent-labeled peptide pairs exhibiting interesting changes in relative abundance.

instrumentation time during data collection and subsequent data analysis by the researcher, making it extremely attractive for analysis of global proteome states. This method has been pioneered [35] using a tandem quadrupole time-of-flight (QTOF) mass spectrometer [36] but could conceivably also be implemented on MALDI ion trap [37] and time-of-flight (TOF) instruments [38].

Advances in data processing and management

The automation of proteomic data collection has created an imbalance between the ability to generate proteomic data and the capacity to interpret these data. The analysis of a single experiment requires an assessment of each selected or 'identified' sequence, the culling of incorrect and redundant assignments, the analysis of single-ion chromatograms to quantify each peptide and the integration of all of these into a multidimensional database linking all peptides to the proteins from which they originated. Subsequently, proteomic data are related to other types of data, such as those determined using gene expression arrays. Each one of these tasks demands the development and refinement of open source software tools to prevent bottlenecks during data analysis.

Several software solutions have been implemented to reduce the amount of spectra that require manual curation. The first of these approaches removes spectra below preset quality thresholds before database searching, saving both curation and computational time. A second approach integrates the scoring features of SEQUEST and the properties of the identified peptide into a single probability score of a correct identification. This function has excellent operating characteristics and allows the elimination of the bulk of the false identifications with only minimal loss of correct identifications, thereby vastly reducing the number of individual spectra that must be examined [39].

Quantification software such as XPRESS [17] currently provides a sophisticated level of automation to the process of quantitatively analyzing the elution profile of all identified peptides. Analogous commercially available software packages, such as ProICAT™ (Applied Biosystems, Foster City, CA, USA), have also been developed to provide analytical tools specifically tailored to specific brands of chromatographic and mass spectrometric instruments. The XPRESS software (described in more detail online at: <http://www.systemsbio.org/research/software/proteomics/>) fits a curve to an elution profile based on a reconstructed single-ion trace chromatogram and subsequently calculates a ratio between a pair of peptides. The calculated expression ratio from all peptides from a single protein can be assembled into a composite ratio for that protein, which includes mean and error measurements.

Whereas the current generation of software requires manual curation of each peptide elution, future generations will be able to assess the fit of the curve assigned to a chromatographic peak, calling the attention of the user to only those proteins that either demonstrate a significant change in abundance between the experimental and control samples or that contain outliers that require human attention. In this way, the quantification process and subsequent data analysis are targeted to only the relevant, 'biologically interesting', proteins in the experiment. Finally, once the data are culled using quantitative software such as XPRESS or ProICAT™, composite data from multiple experiments – those collected from a time-course analysis, for example – can be analyzed collectively using INTERACT software. INTERACT permits multiple datasets to be analyzed using a form-based internet-accessible interface. INTERACT allows the researcher to quickly filter and sort selected MS–MS database search results according to various parameters as dictated by the user. Internet links are included for each peptide to allow the researcher to view the mass spectrum and the reconstructed chromatographic peaks, thus facilitating analysis of even the extremely large datasets that arise from proteomic profiling studies [17].

Collectively, these advances in stable isotope incorporation, mass spectrometry and data processing contribute to the further development of a robust and automated platform for quantitative proteomic studies.

Representative applications of stable isotope tagging

Proteomic profiling cellular extracts

One of the earliest descriptions of quantitative proteomic profiling involved the detection of protein abundance changes in two different yeast cultures (e.g. mutant and wild-type) grown on either ¹⁴N- or ¹⁵N-enriched cell growth medium. Following an appropriate growth interval, the two cell cultures were combined and isolated proteins were separated by 2DE. Gel spots were excised and analyzed by MS, revealing the identity of the protein and the relative abundance of the protein in the two cultures based on the measured nitrogen isotope ratio. These measurements allowed not only determination of protein abundance differences between the two yeast cultures but also revealed *in vivo* phosphorylation sites in a biologically interesting target protein [30]. Pioneering proteomic profiling methods such as these have been improved upon by implementing post-isolation isotopic labeling methods that can be applied to virtually any protein sample and, as discussed below, by adding multidimensional chromatographic separation steps before mass spectral analysis.

The multidimensional protein identification technology (MudPIT) method, which involves two liquid chromatographic separation steps, tandem mass spectrometry and database searching, was used to detect and identify 1484 proteins in whole-cell protein extracts from the budding yeast proteome [16]. This protein set included regulatory proteins, such as members of the MAP kinase pathway; transcription factors, including those in the SWI–SNF cell cycle regulatory complex; and proteins associated with the membranes of various organelles, including the plasma membrane (e.g. Pma1p), endoplasmic reticulum, the Golgi apparatus, the nucleus, and other membrane-bound structures. Proteins with pIs outside the narrow range afforded by 2DE were detected, including 12 proteins with pIs <4.3 and 29 proteins with pIs >11. A large range of protein masses was seen, including proteins with masses <10 000 and >190 000 Da. Importantly, more than 50% of the predicted low-abundance proteins with a codon index below 0.2 were also represented [16].

Although successful for analysis of yeast proteins, improvements in chromatographic separation and mass spectrometric instrumentation will undoubtedly be needed for complete coverage of complex eukaryotic protein samples that are predicted to span greater than six orders of magnitude in abundance. However, preliminary studies indicate that multidimensional LC- and affinity-based chromatographic separation steps before mass spectrometry allow for a more complete description of a cellular proteome, including low-abundance and recalcitrant proteins [15,16]. This method will undoubtedly provide a platform upon which even more robust strategies applicable to the analysis of higher eukaryotes that demonstrate extremely large dynamic ranges in protein abundance.

Proteomic profiling organelles and cellular fractions

The implementation of multiple dimensions of chromatographic separation and isotopic labeling strategies has enabled the proteomic profiling of complex cellular mixtures such as those isolated from cellular organelles and cellular fractions. Extended proteome coverage has also been facilitated through the use of gas-phase fractionation (GPF), the iterative mass spectral analysis of a sample over multiple unique *m/z* ranges, or 'windows', within a larger mass spectrum survey scan *m/z* range (e.g. 400–2000 *m/z*) [40]. Implementation of GPF for proteins enriched in yeast peroxisomal membranes demonstrated significant increases in both sensitivity and reproducibility in identifying proteins known to be associated with this organelle [41].

A combination of multidimensional LC separation and ICAT™ tagging has also been used to characterize proteins from higher eukaryotes, including humans. Specifically, the method has been successfully used to determine the ratios of abundance of nearly 500 proteins associated with the microsomal fractions of control and *in vitro*-differentiated human myeloid leukemia (HL-60) cells [17]. This study represents the most comprehensive analysis to date of membrane-associated proteins and the first study in which the relative abundance of identified proteins was determined directly and systematically. This study indicates this approach and the supporting software tools are broadly applicable to the analysis of large sets of proteins, notably including those refractory proteins that have previously not been accessible using standard proteomic technologies.

Conclusions

The technical advances and applications described here indicate that quantitative proteomics based on stable isotope tagging and automated mass spectrometry is developing into a mature technology that can interface with a multitude of biological and clinical research questions. We expect that the performance, sample throughput, precision of measurement and level of automation will continue to increase. We furthermore expect that the basis of the method, the combination of selective chemical reactions and mass spectrometry will be applied to probe proteomic properties that indicate, directly or indirectly, the function and activity of proteins. Such measurements include the systematic analysis of protein phosphorylation, the analysis of protein linkage maps [42,43] and enzyme activity profiles. Improved performance and utility of proteomics technology will therefore increasingly impact biology and medicine.

However, it is possible that lack of access to suitable research infrastructure and trained personnel will limit the penetrance of quantitative proteomics as a general research tool. Technologic complexity and the high costs of required equipment and trained personnel make proteomics prohibitively expensive for many research groups. A suitable solution might be the establishment of high-throughput proteomic centers, analogous to the successful structural genomics data collections centers, the national beamlines. These publicly funded facilities would allow for the assembly of a critical mass of experts in protein biochemistry, separation sciences, mass spectrometry, computation and informatics, making proteomics accessible to other publicly funded academic researchers. In addition, such centers would pioneer the development of the technologies and software necessary to propel the field of global quantitative proteomics forward and train

scientists in the current technologies. Realization of the full potential of this promising technology, therefore, requires coordinated plans for technology development, dissemination and application.

Acknowledgements

This work was supported by NIH Grants No. P41-RR11823-03, 1-R33-CA89807, 1-R33-CA84698, and 1-R33-CA93302 to R.A., and NIH Genome Postdoctoral Genome Training Grant fellowships to M.R.F. and T.J.G. We also acknowledge generous support from Merck Genome Research Institute and Oxford Glycosciences.

References

- Lander, E.S. et al. (2001) Initial sequencing and analysis of the human genome. *Nature* 409, 860–921
- Venter, J.C. et al. (2001) The sequence of the human genome. *Science* 291, 1304–1351
- Shipp, M.A. et al. (2002) Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat. Med.* 8, 68–74
- Diehn, M. et al. (2000) Examining the living genome in health and disease with DNA microarrays. *JAMA* 283, 2298–2299
- Hughes, T.R. et al. (2001) Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nat. Biotechnol.* 19, 342–347
- Lockhart, D.J. and Winzler, E.A. (2000) Genomics, gene expression and DNA arrays. *Nature* 405, 827–836
- Gygi, S.P. et al. (1999) Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.* 19, 1720–1730
- Ideker, T. et al. (2001) Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* 292, 929–934
- Griffin, T.J. et al. (2002) Complementary profiling of gene expression at the transcriptome and proteome levels in *S. cerevisiae*. *Mol. Cell. Proteomics* 1, 323–333
- Fields, S. (2001) Proteomics in genomeland. *Science* 291, 1221–1224
- Griffin, T.J. and Aebersold, R. (2001) Advances in proteome analysis by mass spectrometry. *J. Biol. Chem.* 276, 45497–45500
- Gygi, S.P. et al. (2000) Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. *Proc. Natl. Acad. Sci. U.S.A.* 97, 9390–9395
- Gygi, S.P. et al. (1999) Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat. Biotechnol.* 17, 994–999
- Link, A.J. et al. (1999) Direct analysis of protein complexes using mass spectrometry. *Nat. Biotechnol.* 17, 676–682
- Gygi, S.P. et al. (2002) Proteome analysis of low-abundance proteins using multidimensional chromatography and isotope-coded affinity tags. *J. Proteome Res.* 1, 47–54
- Washburn, M.P. et al. (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat. Biotechnol.* 19, 242–247
- Han, D.K. et al. (2001) Quantitative profiling of differentiation-induced microsomal proteins using isotope-coded affinity tags and mass spectrometry. *Nat. Biotechnol.* 19, 946–951
- Eng, J. et al. (1994) An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc. Mass Spectrom.* 5, 976–989
- Smolka, M. et al. (2002) Quantitative protein profiling using two-dimensional gel electrophoresis, isotope-coded affinity tag labeling, and mass spectrometry. *Mol. Cell. Proteomics* 1, 19–29
- Washburn, M.P. et al. (2002) Analysis of quantitative proteomic data generated via multidimensional protein identification technology. *Anal. Chem.* 74, 1650–1657
- Ong, S.E. et al. (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol. Cell. Proteomics* 1, 376–386

- 22 Shevchenko, A. et al. (1997) Rapid 'de novo' peptide sequencing by a combination of nano-electrospray, isotopic labeling and a quadrupole/time-of-flight mass spectrometer. *Rapid Commun. Mass Spectrom.* 11, 1015–1024
- 23 Mirgorodskaya, O.A. et al. (2000) Quantitation of peptides and proteins by matrix-assisted laser desorption/ionization mass spectrometry using (18)O-labeled internal standards. *Rapid Commun. Mass Spectrom.* 14, 1226–1232
- 24 Yao, X. et al. (2001) Proteolytic 18O labeling for comparative proteomics: model studies with two serotypes of adenovirus. *Anal. Chem.* 73, 2836–2842
- 25 Zhou, H. et al. (2002) Quantitative proteome analysis by solid phase isotope tagging and mass spectrometry. *Nat. Biotechnol.* 20, 512–515
- 26 Goodlett, D.R. et al. (2001) Differential stable isotope labeling of peptides for quantitation and de novo sequence derivation. *Rapid Commun. Mass Spectrom.* 15, 1214–1221
- 27 Peters, E.C. et al. (2001) A novel multifunctional labeling reagent for enhanced protein characterization with mass spectrometry. *Rapid Commun. Mass Spectrom.* 15, 2387–2392
- 28 Munchbach, M. et al. (2000) Quantitation and facilitated de novo sequencing of proteins by isotopic N-terminal labeling of peptides with a fragmentation-directing moiety. *Anal. Chem.* 72, 4047–4057
- 29 Cagney, G. and Emili, A. (2002) De novo peptide sequencing and quantitative profiling of complex protein mixtures using mass-coded abundance tagging. *Nat. Biotechnol.* 20, 163–170
- 30 Oda, Y. et al. (1999) Accurate quantitation of protein expression and site-specific phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6591–6596
- 31 Zhou, H. et al. (2001) A systematic approach to the analysis of protein phosphorylation. *Nat. Biotechnol.* 19, 375–378
- 32 Goshe, M.B. et al. (2001) Phosphoprotein isotope-coded affinity tag approach for isolating and quantitating phosphopeptides in proteome-wide analyses. *Anal. Chem.* 73, 2578–2586
- 33 Ficarro, S.B. et al. (2002) Phosphoproteome analysis by mass spectrometry and its application to *Saccharomyces cerevisiae*. *Nat. Biotechnol.* 20, 301–305
- 34 Cravatt, B.F. and Sorensen, E.J. (2000) Chemical strategies for the global analysis of protein function. *Curr. Opin. Chem. Biol.* 4, 663–668
- 35 Griffin, T.J. et al. (2001) Quantitative proteomic analysis using a MALDI quadrupole time-of-flight mass spectrometer. *Anal. Chem.* 73, 978–986
- 36 Loboda, A.V. et al. (2000) A tandem quadrupole/time-of-flight mass spectrometer with a matrix-assisted laser desorption/ionization source: design and performance. *Rapid Commun. Mass Spectrom.* 14, 1047–1057
- 37 Krutchinsky, A.N. et al. (2001) Automatic identification of proteins with a MALDI-quadrupole ion trap mass spectrometer. *Anal. Chem.* 73, 5066–5077
- 38 Bienvenu, W.V. et al. (2002) Matrix-assisted laser desorption/ionization-tandem mass spectrometry with high resolution and sensitivity for identification and characterization of proteins. *Proteomics* 2, 868–876
- 39 Keller, A. et al. Empirical statistical method to estimate the accuracy of peptide identifications made by MS-MS and database search. *Anal. Chem.* (in press)
- 40 Spahr, C.S. et al. (2001) Towards defining the urinary proteome using liquid chromatography-tandem mass spectrometry. I. Profiling an unfractionated tryptic digest. *Proteomics* 1, 93–107
- 41 Yi, E.C. et al. (2002) Approaching complete peroxisome characterization by gas-phase fractionation. *Electrophoresis* 18, 3205–3216
- 42 Gavin, A.C. et al. (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415, 141–147
- 43 Ho, Y. et al. (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* 415, 180–183

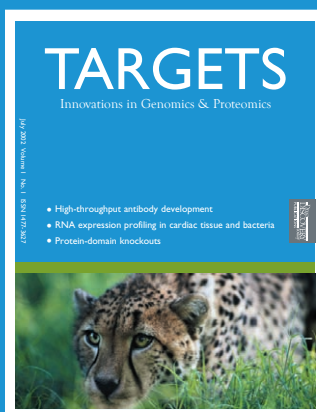
Drug DISCOVERY
PUBLICATIONS today

- Peer reviewed for authority
- Reaching the key decision makers
- Meeting your needs across the drug discovery business

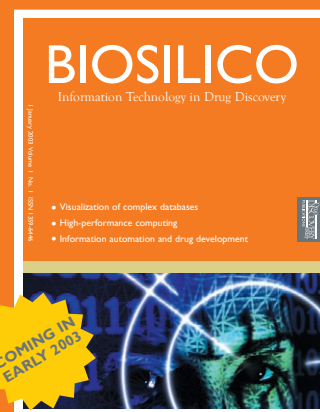
FREE copies available at www.drugdiscoverytoday.com



- From lead identification to clinical trials
- Most highly cited journal in drug discovery
- Your strategic overview
- 24 issues per year



- Innovations in genomics & proteomics
- New high-quality review journal
- Tactical issues that impact your work
- 6 issues per year



COMING IN
EARLY 2003

- Information technology in drug discovery
- New high-quality review journal
- Covers key tactical issues
- 6 issues per year