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*Commentary*

# The Proteomics: A New Tool for Chinese Medicine Research

Shi-Sheng Li
*The Department of Molecular and Experimental Medicine*
*The Scripps Research Institute*
*10550 North Torrey Pines Road, La Jolla, CA 92037, USA*

Abstract: Proteomics technology is based on the vast analytical power for protein/peptide identification and quantification offered by modern mass spectrometry coupled with hyphenated separation techniques such as two-dimensional gel electrophoresis (2DE) and micro- or nano-scale multidimensional liquid chromatography. The rapid growth of proteomics field provides an array of new tools for the integration of traditional Chinese medicine (TCM) with modern technology and systems biology, and is potentially advancing the progress of modernization and internationalization of TCM. Cho, in this issue of *the American Journal of Chinese Medicine*, highlights the recent application of 2DE-based and bottom-up proteomics in Chinese medicine research, including the exploration of pharmacological mechanisms of the actions of TCM, the facilitation of herb authentication and identification, and the profiling of protein expression following acupuncture treatment in animal models. Recent development in proteomics has provided further refinement on the analysis of proteins posttranslational modifications as well as quantitative comparison of different proteomes, and enabled the study of proteomes of specific diseases or biological processes under clinically relevant conditions. It is conceivable that the application of technologies developed in proteomics, genomics and metabonomics in the clinical practice and basic research of Chinese medicine will eventually lead to the reconciliation and integration of TCM and contemporary medicine. Chinese medicine is fundamentally a highly personalized medicine; perhaps it is time to embrace the arrival of TCM OMICS era in Chinese medicine research.

*Keywords*: Herbal Medicine; Proteomics; Mass Spectrometry; Two-Dimensional Gel Electrophoresis; Bioinformatics; Systems Biology.

Correspondence to: Dr. Shi-Sheng Li, Department of Molecular and Experimental Medicine (MEM-180), The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. Tel: (+1) 858-784-8236, Fax: (+1) 858-784-2243, E-mail: sshli@scripps.edu

## Introduction

Proteome is the protein complement of the genome (Wilkins *et al.*, 1996). The importance of proteome analysis is evident because of the fact that in a majority of cases neither the genomic sequence itself nor the transcriptional profile can be correlated directly with protein expression and gene function although the completion of the Human Genome Project does provide the basis for a better understanding of cellular and molecular mechanisms of human physiology and diseases. Protein levels and functions in biological systems are often regulated by posttranslational modification and protein degradation. More importantly, the protein contents in the biological systems have large dynamic range and change with time and with environment such as stimulus and drug-treatment. Traditional methods used for measuring protein contents such as antibody assays or enzyme activity measurements, have the capacity to detect very limited number of proteins at a time.

Traditional Chinese medicine (TCM) has been used by Oriental people for centuries in the prevention and treatment of human diseases (Wang *et al.*, 2005). Most remedies in TCM are compound formulae consisting of many species of herbs containing complex mixtures of ingredients and chemicals, which act in concert to treat imbalanced body symptoms, likely with the mechanisms of simultaneously treating multiple therapeutic targets (Li *et al.*, 2000; Lao *et al.*, 2006; Zhou, 2007). The potential clinical utilities and adverse effects of these remedies can be further assessed using the methods of systems biology such as profiling the proteome and comparative proteomics in which the entire body's response to the treatment can be measured and quantified at the proteome level. In this issue, the review article by Cho also highlights the recent application of proteomics in facilitating herb authentication and identification, and profiling protein expression following acupuncture treatment in animal models. Recently published studies showed that analgesic effect of acupuncture and its possible mechanisms of action (Lee *et al.*, 2006; Maenaka *et al.*, 2006; Park *et al.*, 2006b). The protein expression profile of the hypothalamus to pain stimulation and acupuncture treatment was analyzed using 2DE-based proteomics. The promise of proteomics is to perform large-scale measurement on the gene expression at the protein level, which could lead to the discovery of novel markers of diseases and new function of proteins, and reveal novel pathophysiological mechanisms and new therapeutic targets for drug development. The development of Nobel prize-winning soft ionization techniques, namely, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), in the late 20th century enables the routine measurement of proteins and peptides by mass spectrometry (MS). The integration of instrument control language (ICL) into MS instruments permits the automatic acquisition of large sets of tandem mass spectra (TMS). The development of computer algorithms allows automated large-scale interpretation of TMS by the correlation of experimental TMS with theoretic TMS deduced from a known genome, identifying the protein or profiling the proteome with high-throughput capacity (Eng *et al.*, 1994; Perkins *et al.*, 1999). There is no doubt that proteomics will redefine biomedical research in the postgenomic era, including basic and clinical research on TCM.

## Top-Down and Bottom-Up Proteomics

The bottom-up and the top-down approaches are currently the two complimentary methods using mass spectrometry to determine protein sequences in proteomics (Chait, 2006). In the bottom-up approach, the proteins of interest are digested with an enzyme such as trypsin, and the resulting peptides are analyzed by a mass spectrometer, i.e., ESI or MALDI, recording the masses of the intact enzymatic peptides and then TMS using low energy collision-induced dissociation (CID) which produces information on their sequence and also posttranslational modifications in some cases. In most cases, the digested peptides are separated or fractionated using liquid chromatographic (LC) methods prior to MS measurement. Protein sequence coverage that the bottom-up proteomics can achieve varies and usually is between 40–90%. In top-down proteomics, intact protein ions typically generated from purified proteins are introduced to the gas phase by ESI and are subsequently fragmented by the methods of electron-capture dissociation (ECD) using ion-electron recombination reactions and/or infrared multiphoton dissociation (IRMPD) in a high performance mass spectrometer, i.e., Fourier transform (FT) instrument. ECD is believed to work in a non-ergodic mechanism with dominant backbone cleavage of proteins and peptides, thus retaining posttranslational modifications of the proteins. The top-down approach has the potential to generate sufficiently informative fragment ions directly from a large intact protein, therefore providing information of its complete amino acid sequence and all of its modifications of the protein. However, there are still many challenges preventing the routine use of top-down approach in most biomedical laboratories. So far, all the proteomics data in TCM research have been generated exclusively by the bottom-up proteomics strategy.

## New Trend in Proteomics: Middle-Down Approach

Top-down proteomics using FT mass spectrometers is considered very powerful but not without limitations. FT mass spectrometers are very expensive and require extensive maintenance. The top-down approach has been dominantly used for studying single purified proteins, and at the present stage is not robust enough to handle protein mixtures requiring online LC separation. In addition, computer algorithms for automatic large-scale analysis of top-down data need further improvement. These limitations have prompted the very recent development of new methods such as electron transfer dissociation (ETD) for middle-down protein sequencing using lower-cost MS instruments (Syka *et al.*, 2004). In middle-down proteomics, large proteins can be digested by limited proteolysis and the resulting peptides or small intact proteins are characterized by sequential ion/ion reactions, which are made possible by the introduction of a second ion source into conventional MS instruments such as linear ion trap. ETD is the ion/ion analog of ECD, and has been increasingly recognized as an important method for the studies of protein posttranslational modifications. It has also been demonstrated that it is feasible to analyze biological mixtures of intact proteins on an LC timescale by ETD MS (Chi *et al.*, 2007).

## Separation Techniques in Proteomics

Large-scale proteome analysis is based on the vast analytical power of modern mass spectrometry coupled online or offline with hyphenated separation techniques. Two major types of separation methods are currently employed in proteomics, namely electrodriven and LC-based approaches. The electrodriven methods primarily used in proteomics studies include isoelectric focusing, one-dimensional polyacrylamide gel electrophoresis (PAGE), 2DE, nondenaturing 2DE, and two-dimensional blue native/sodium dodecyl sulphate PAGE (2D BN/SDS-PAGE). For resolving hydrophobic membrane complexes, 2D BN/SDS-PAGE is very useful, in which native protein complexes are separated in a 1st dimensional non-denaturing PAGE according to their apparent molecular mass and conformation, followed by a denatured 2nd dimensional SDS-PAGE (Reifschneider *et al.*, 2006). Recently, the introduction of "difference gel electrophoresis" (DIGE) has reshaped the areas of 2DE-based quantitative proteomics. In DIGE, proteins from different samples are separately labeled using size- and charge-matched cyanine dyes, which possess different excitation and emission wavelengths. Up to three samples can be applied to the same gel in DIGE, thus eliminating the problem of gel-to-gel variation commonly observed in conventional 2DE approaches and permitting direct comparisons of the samples. Using one of the labels for a pooled mixture of all samples to create an internal standard run in each gel, the patterns of different gels can easily be matched and the spot volume of the samples can be normalized, thus achieving high accuracy for the difference measurement at the protein levels. The LC-based separation techniques in proteomics primarily include ion exchange (IE) chromatography, size exclusion chromatography, affinity chromatography, hydrophilic interaction chromatography, hydrophobic interaction chromatography, and reverse-phased (RP) chromatography. They may have advantages over gel-based techniques in speed, sensitivity, and applicability to different samples and conditions. In addition, many of these approaches can be used in combination for fractionating complex biological samples. For example, in shotgun proteomics, complex protein mixtures are digested enzymatically and the resultant peptide mixtures are loaded to an IE-RP biphasic column, which can be online connected to the MS measurement. This method is often referred to as MudPIT (Multidimensional Protein Identification Technology) (Washburn *et al.*, 2001). The electrodriven and LC-based approaches can also be employed in combination to improve the dynamic range and sensitivity for the detection of low abundant proteins present in mixture samples (Li and Giometti, 2007).

## Proteomics in Chinese Medicine Research

TCM prescriptions have been used for centuries by Oriental people in the disease treatment and health maintenance (Shen *et al.*, 2005; Wong and Woo, 2005). The practice of holistic Chinese medicine shares similarities with many basic concepts of systems biology, the study of the interactions between proteins, genes, and metabolites at the organismal level. In recent years, modern technologies have been used in Chinese medicine research (Huang and Hong, 2005; Park *et al.*, 2006a). New evidence for the efficacy of TCM remedies can

be gathered by employing proteomics technologies. Large-scale analysis of intact proteins has become realistic with the approach of middle-down proteomics. Further bioinformatics development for protein quantification in top-down and middle-down proteomics should facilitate new applications to Chinese medicine research. Enormous challenges remain at the present time, but one can foresee that the application of technologies developed in systems biology in the clinical practice and basic research of Chinese medicine will eventually lead to the reconciliation and integration between TCM and contemporary medicine.

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