
Preface

An Overview of Quantitative Proteomics by Mass Spectrometry

The dynamic nature of the proteome and its complexity undoubtedly present huge technological challenges. Recent developments in mass spectrometry (MS) dramatically improved the throughput in protein identification and quantification. Although major advances have been made and these technologies already offer a great opportunity for better understanding human diseases and for identifying biomarkers, it is apparent that we still need to put major efforts to improving the correct methods. For example, the coverage of the proteome for all organisms is still very limited and our understanding of the dynamic processes of posttranslational modifications, at the most, is still rudimentary. *Quantitative Proteomics by Mass Spectrometry* describes in detail the methods and protocols used for many of the most significant recent developments in this field. MS has played a major role in proteomics and it is becoming an essential tool for studying complex biological systems and diseases. Some instruments have reached attomole and even zeptomole sensitivity. Newborns today can be screened for almost 50 diseases, mainly using MS, for less than \$2 per disease.

Most of the quantitative proteomics approaches by MS utilize isotopic labels as a reference for either relative or absolute quantitation. These labels can be introduced *in vivo*, for example, growing an organism in a media enriched with specific isotopes. Several chapters in this book describe this approach. Ong and Mann describe the stable isotope labeling by amino acids in cell culture approach where cells are labeled by growing them in the presence of isotopically labeled amino acids. Wu and MacCoss describe a procedure for labeling mammalian organisms and use the tissues as standard reference in Multidimensional Protein Identification Technology (MudPIT) analysis. Sato et al. describe a procedure where isotopically labeled cells are used as a standard in the analysis of various tissues.

An isotopic label can also be introduced by proteolyzing a protein with trypsin in the presence of ^{18}O -water. Fenselau and Yao describe this approach and Liu et al. couple the labeling with ^{18}O -water to cysteine containing peptide enrichment for high-throughput quantitative proteomics.

Another way to introduce an isotopic label is to react the protein or the protein mixture with a reagent that contains isotopes. Excellent targets for this reaction are cysteines because they are a very reactive nucleophile. Ranish et

al. describe the isotope coded affinity tags (ICAT) approach for quantifying protein complexes, and Turko and Sechi describe the use of acrylamide as a simple tool in quantitative proteomics. Several other amino acids can be the targets of specific reactions with isotopically labeled reagents. For example, Regnier describes a coding strategy involving the labeling of both amine and carboxyl groups.

Substantial effort is ongoing in the characterization of posttranslational modifications and perhaps the major advancements have been in the characterization of the phosphoproteome. The procedure described by Zhang et al. couples affinity enrichment of phosphopeptides to stable-isotope labeling and perhaps this is one of the most comprehensive approaches to characterize the phosphoproteome that has been developed. Labeling with isotopes has been used mostly for determining the relative quantities of proteins, isotopically labeled reagents and peptides can also be used for determining the absolute quantities of specific peptides and proteins. For example, Gerber describes how to determine the absolute quantity of a specific protein and its phosphorylation state and Lu et al. describe the use of an isotopically labeled reagent that targets cysteines and that can be used for absolute quantitation.

Tandem MS has been widely used for the detection of inborn errors of metabolism. This is perhaps one of the most apparent applications of MS to disease detection and Turecek et al. describe a procedure for the determinations of enzyme activities that could potentially be used for large-scale screening of newborns. Quantitation can also be achieved without labeling with isotopes and Roy and Becker describe this methodology. The challenge here is to be able to have a highly reproducible system and excellent software for correcting experimental variations that are usually intrinsic in a proteomic experiment.

The methodologies described here are among the leading technologies in quantitative proteomics used today. Their application to complex biological systems and human diseases is becoming a reality. Although we are a long way from a comprehensive understanding of the proteome, considering the pace of recent developments we can be optimistic that MS will indeed play a key role in deciphering the complexity of cellular networks and in the development of patient-tailored medicine.

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