PREFACE

Proteomics is the large-scale study of proteins, particularly their structures and functions. Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells. The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system. This book presents and discusses various topical data on proteomics, including: proteomic technologies used in celiac disease, protein location in plant proteomics, the prediction of protein-protein interactions (PPIs) using proteomics, proteomic studies of mild cognitive impairment, among others.

Chapter 1 - Proteomic technologies are used with increasing frequency in the scientific community. In this review the authors would like to highlight their use in celiac disease. The available techniques that include two-dimensional gel electrophoresis, mass spectrometry, antibody and tissue arrays, have been used to identify proteins or protein expression changes specific of gut tissue from patients with celiac disease. A number of studies have employed proteomic methodologies to look for diagnostic biomarkers in body fluids or to examine protein expression changes and posttranslational modifications during signaling. The fast technological development of technologies, along with the combination of classic techniques with proteomics, will lead to new discoveries which will consent a better understanding of the pathogenesis of celiac disease and its complications (i.e. refractory CD and cancer), and to possibily indicate targets for an early diagnosis of CD complications and for specific terapeutic approaches.

Chapter 2 - Organelle proteomics allows the characterization of complex proteomes to understand the protein networks which regulate growth and development, as well as adaptation and evolution. Purification of organelles is

of paramount importance and diverse protocols are published. Some organelles such as chloroplasts, mitochondria, and the nucleus are surrounded by membranes which facilitate their purification. Others have membranes easily disrupted (vacuoles and peroxisomes), or are complex systems for protein trafficking (endoplasmic reticulum, Golgi, and secretory vesicles). The cell walls present different difficulties since they have no physical limits allowing purification. The purity of the targeted cell compartment is usually evaluated by biochemical and/or immunological methods. Nevertheless, in any sub-cellular proteomic analysis, proteins from a different compartment can be detected and the difficulty is to decide whether it is a contamination, or the unexpected location is real and has a functional significance. Software to predict sub-cellular location of proteins is available. However, since not all the targeting signals are known at present, carefulness in the use of such tools is recommended. Different tactics to solve this puzzle are discussed in this commentary.

Chapter 3 - The simultaneous analysis of all proteins expressed by a cell, tissue or organism in a specific physiological condition is the main goal of proteomic studies. Gel-based proteomic is the most popular and versatile method of global protein separation and quantification. This is a mature approach to screen the protein expression at the large scale. Based on two independent biochemical characteristics of proteins, two-dimensional electrophoresis combines isoelectric focusing, which separates proteins according to their isoelectric point, and SDS-PAGE, which separates them further according to their molecular mass. The next typical steps of the flow of gel-based proteomics are spots visualization and evaluation, expression analysis and finally protein identification by mass spectrometry. At present, two-dimensional electrophoresis allows simultaneously to detect and quantify up to thousand protein spots in the same gel in a wide range of biological systems for the study of differentially expressed proteins. However, gel-based proteomic has a number of inherent drawbacks. In this review article, the benefits, difficulties, limits and perspectives of gel-based proteomic approaches are discussed.

Chapter 4 - In this commentary, the authors review algorithms for the analysis of liquid chromatography-mass spectrometry (LC-MS) data. Mass spectrometry is a technology that can be used to determine the identities and abundances of the compounds in complex samples. In combination with liquid chromatography, it has become a popular method in the field of proteomics, the large-scale study of proteins and peptides in living systems.

The data sets obtained from an LC-MS experiment are large and highly complex. The outcome of such an experiment is called an LC-MS map and is a collection of mass spectra. They contain, among the signals of interest, a high amount of noise and other disturbances. That is why algorithms for the low-level processing of LC-MS data are becoming increasingly important.

In this commentary, the authors revied the state-of-the-art of quantification algorithms, their capabilities and also limitations and outline avenues for future research.

Chapter 5 - Nowadays, one of the most important goals of Proteomics is the prediction of protein-protein interactions (PPIs), whose knowledge is vital for all biological processes. In the present paper the authors propose an approach to the prediction of protein-protein interactions in yeast based on the well-known paradigm of Support Vector Machines (SVM) for classification and feature selection methods using Genomics/Proteomics information from the main databases. In order to obtain higher values of specificity and sensitivity for their predictions, the authors took a high reliable set of positive and negative examples for the construction of the SVM model. The authors then extracted a set of proteomic/genomic features from these examples and also introduced a similarity measure in the calculation of the features that allow us to improve the prediction capability of their model. In the analysis of the results, the authors also applied their approach to in vitro datasets, obtaining high accuracy classifications. Their final SVM classifiers obtain a low error rate in the prediction for each pair of proteins of several datasets for both in vitro and in silico methodologies.

Chapter 6 - Recent innovations in liquid chromatography-mass spectrometry (LC-MS) based methods have facilitated comparative and functional proteomic analyses of large numbers of proteins derived from complex samples without any need for protein or peptide labelling. Here the authors discuss the features of label-free LC-based proteomics techniques. The authors first summarize recent methods used for quantitative protein analyses by MS techniques. The major challenges faced by label-free LC-MS based approaches are discussed; these include sample preparation, peptide separation, data mining and quantification. Absolute quantification, kinetic approaches and database search algorithms are also addressed. The authors focus on the Expression^{E} System^{TM} (Waters, Manchester, UK), a relatively new platform allowing label-free quantification of peptides for which mass and retention time have been accurately measured. Enhancing the power of this method will require developments in both separation technology and bioinformatics/statistical analysis.

Chapter 7 - Mild cognitive impairment (MCI) is arguably the earliest form of Alzheimer's disease (AD). Better understanding of brain changes in MCI may lead to the identification of therapeutic targets to slow the progression of AD. Oxidative stress has been implicated as a mechanism associated with the pathogenesis of both MCI and AD. In particular, among other markers, there is evidence for an increase in the levels of protein oxidation and lipid peroxidation in the brains of subjects with MCI. Several proteins are oxidatively modified in MCI brain, and as a result individual protein dysfunction may be directly linked to these modifications (e.g., carbonylation, nitration, modification by HNE) and may be involved in MCI pathogenesis. Additionally, Concanavalin-A-mediated separation of brain proteins has recently led to the identification of key proteins in MCI and AD using proteomics methods. This chapter will summarize important findings from proteomics studies of MCI, which have provided insights into this cognitive disorder and have led to further understanding of potential mechanisms involved in the progression of AD.

Chapter 8 - The general strategy in proteomic research consists in sample preparation, protein or peptide separation, their identification, and data interpretation. A critical step is certainly protein or peptide separation. Since increasingly complicated biological structures are studied by mass spectrometry (MS), the need for more powerful and highly resolving separation methods is growing. Consequently, multidimensional separation techniques in combination with MS have emerged as a powerful tool for the large-scale proteomic analysis. Until recently, two dimensional gel electrophoresis (2-DE) was the technique most often used for protein separation. The limitations of 2-DE in detecting low abundance, very small or large proteins, basic and membrane/hydrophobic ones, as well as difficulties with process automation, have forced researchers to look for other methods of protein separation, such as multidimensional liquid chromatography coupled to MS (MDLC-MS) or tandem MS (MDLC-MS/MS). MDLC combines two or more forms of LC to increase the peak capacity, and thus the resolving power of separation, to better fractionate peptides prior to entering the mass spectrometer. In this chapter, the authors analyze status and recent developments of the MDLC experiments in their fundamental components. It describes a variety of separation modes that have been employed to achieve protein-level or peptide-level separation, including size exclusion chromatography, ion exchange chromatography, and reversed-phase chromatography. The authors also discuss the advantages and disadvantages of

two different approaches that can be followed for the studies of proteomics: protein-level separation or peptide-level separation.

Commentary - It is well established that high doses of ionising radiation, such as used in radiotherapy, increase risk of cardiovascular diseases (CVD). Observed effects include direct damage to the coronary arteries, marked diffuse fibrotic damage of the pericardium and myocardium, pericardial adhesions, stenosis of the valves and microvascular damage. In contrast, there are considerable uncertainties concerning health effects of low doses of ionising radiation on heart. The need to explore potential biological and physiological effects at low doses is being increasingly acknowledged as the plans for new nuclear power plants and novel medical applications using low-dose radiation are emerging.