# Preface

The aim of this book is to be a useful resource for experienced proteomics practitioners as well as an aid to newcomers in order to become acquainted with the theory and practice of a wide array of mass spectrometric techniques for proteome research. The role of mass spectrometry (MS) in proteomics has gradually evolved from being a technique used to identify proteins in gels to be the preferred method for protein detection and quantification. Seminal work at the beginning of this century demonstrated that the hyphenation of liquid chromatography with tandem mass spectrometry (LC-MS/MS) allowed the protein biochemist to quantify thousands of proteins within time frames that make these techniques powerful readouts for biological experiments. Consequently, LC-MS/MS has found a niche in many different areas of biological and biomedical research, mainly due to the introduction of user-friendly and high-performance instrumentation and because of the development of new quantitative strategies and of powerful bioinformatics tools to cope with the analysis of the large amounts of data generated in proteomics experiments. These advances are making possible the analysis of proteins on a global scale, meaning that proteomics can now compete with cDNA microarrays for the analysis of whole genomes.

In this volume of *Methods in Molecular Biology*, we provide protocols and up-to-date reviews of the applications of LC-MS/MS, with a particular focus on MS-based methods of protein and peptide quantification and the analysis of post-translational modifications. **Section I** presents overviews of the use of LC-M/MS in protein analysis. Quantifying protein expression changes, sites of modification, enzymatic activities and metabolites can be used in combination as a measure of pathway activity and implies that it may be possible to quantify pathway fluxes at a depth of analysis and scale which has previously not been possible. **Chapter 1** discusses such systems biology approaches, which will undoubtedly provide a better understanding of normal biological processes and insight into the molecular aetiology of disease.

Choosing the most appropriate methods should be based on the question that needs to be addressed and the nature of the samples. The pros and cons of the different quantitative techniques, reviewed in great depth in **Chapter 2**, will assist in the most appropriate use of quantitative LC-MS/MS for biomedical research. **Section I** of this volume also reviews the instrumentation available for LC-MS/MS (**Chapter 3**) and the various bioinformatics tools (**Chapter 4**) used for the analysis of different and often very complex data sets.

LC-MS/MS can also be used to detect and quantify post-translational modifications, the proteomes of cellular organelles, protein–protein interactions and to profile protein abundance in biological fluids – applications that are outside the scope of genomics (these applications are reviewed in **Chapters 1**, **2**, and **5**). However, in contrast with genomics, for which the technology is well developed, off-the-shelf protocols for MS-based proteomics do not yet exist. This volume meets this demand and describes step-by-step protocols for the main applications of LC-MS/MS in protein analysis and some more novel applications.

**Section II** details protocols for the analysis of post-translational modifications, with particular focus on phosphorylation (**Chapters 6** and **7**) and glycosylation (**Chapter 8**). The most popular techniques for quantitative proteomics, including those based on multiple reaction monitoring (**Chapters 9** and **10**), metabolic labelling (**Chapter 11**), chemical tagging (**Chapter 12**) and label-free (**Chapter 13**) approaches are covered in **Section III**. **Section IV** then describes how these quantitative proteomic techniques can be used to investigate cell biochemistry by comparative assessment of membrane proteomes between two or more cell populations (**Chapter 14**), characterizing the proteomes of organelles (**Chapter 15**) and by more accurately and specifically mapping protein–protein interactions (**Chapter 16**). Another popular application of LC-MS/MS is for biomarker discovery in biological fluids and **Section V** gives protocols for such workflows; **Chapter 17** focuses on the analysis of serum proteins, whilst **Chapters 18** and **19** deal with the analysis of proteins and peptides in urine, respectively. Finally, **Section VI** describes relatively novel applications of LC-MS/MS in proteomics; **Chapter 20** shows how LC-MS/MS is not only useful to quantify protein expression, identify (and quantify) their post-translational modifications and map their interactions but also to quantify their enzymatic activity. Although this book focuses on protocols that analyse proteins at the peptide level (the so-called bottom-up approach), the final chapter of the volume, **Chapter 21**, describes a protocol for the analysis of full-length proteins (i.e. for top-down proteomics). This bias is not intentioned, but simply reflects the current relative popularity of the two approaches.

Collectively, these protocols and review chapters illustrate the formidable power and versatility of LC-MS/MS in biological and biomedical research. Although the large number of techniques and applications available means that no single volume can be exhaustive, virtually all the main analytical concepts and applications are discussed throughout the chapters. Indeed, the techniques and concepts learned throughout this volume should allow proteomic practitioners to apply LC-MS/MS to tackle essentially any biological problem, the only limitation perhaps just being our own imagination and creativity.

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