PREFACE

Mass spectrometry is a major analytical tool in many fields of the natural sciences extending its use to clinical research applications. With constantly improved sensitivity, the mass spectrometrist increasingly encounters sample-related difficulties in the course of work and needs to collaborate tightly with the scientists of the laboratory which submitted the sample. Since mass spectrometry is more or less indifferent to the origin of the analyte, scientists running the analyses are forced to become knowledgeable in many different fields from plants and bacteria to insects and mammalia. Each sample type is accompanied by specific matrix problems which often require method development. Scientists can become quite distracted from the original task due to the need to identify and remove contaminants and to test separation products and analyte recovery.

For this book, I have asked researchers to share protocols, handling tips and words of caution with their fellow scientists. Often, small tricks make a difference for the success of an experiment and those are mostly not published. The book focuses on just these details from work areas in proteomics and small molecule analysis. This could be, e.g., short-cuts to achieve specific sample purity, comparisons of methods and tools, time-saving technical advances or sensitivity-increasing methodology. Authors contributed an impressive collection of manuscripts which reflect the everyday struggle for best experimental results in protein preparation and detection (Chapters 1-9), tissue imaging (Chapter 10) and methodologies for the investigation of organic molecules and metabolites (Chapters 11-12).

Chapter 1 starts with a practical guideline in the combination of chemical cross-linking and high-resolution mass spectrometry for beginners as well as for those who are already working in the field of protein mass spectrometry.

The chapter was supplied by the specialists in the area– the research group of A. Sinz. The purpose of Chapter 2 is to focus on topics that are often underestimated in their importance for optimized results in mass spectrometric analyses, namely sample preparation. Hess et al. discuss common contaminants such as buffers, solubilization reagents and those contaminants that are introduced through the use of specific biological protocols. The chapter outlines how best to deal with these common problems in order to maximize the returns from a proteomics experiment. In phosphoproteome analysis, major difficulties are the effective isolation of phosphoproteins from whole cell lysates, recovery and detection of phosphopeptides. Chapter 3 is concerned with various approaches and methods used for effective phosphoprotein isolation and analysis including practical information, which can be transferred directly to the bench. Another protein modification, spontaneous deamidation of asparagine to (iso)aspartate in a non-enzymatic reaction is the topic of Chapter 4. Both gel electrophoresis and mass spectrometry may serve as a sensitive experimental "clock" to monitor this process as shown be Schwöppe and colleagues.

Chapter 5 discusses aspects of electron transfer dissociation (ETD) in the field of peptide and protein identification. The technique is sensitive to labile protein modifications and therefore of high interest in the life sciences. Suder et al. describe principles of the methodology, necessary equipment, basic settings of the instruments, and selected applications. Cleavage and oxidation of peptides containing tyrosine or tryptophan can be achieved by electrochemical conversion and an on-line electrochemistry–mass spectrometry (EC-MS) methodology to that end is described in Chapter 6.

The isolation of proteins from their biological background can be a challenging task. Poor preparation may result in smears and streaks on two-dimensional polyacrylamide gels or lack of separation in chromatography-based experiments. Both the choice of buffers and methods of physical treatment (sonification, ultracentrifugation) make a difference, in particular for lipid-rich sample types. A step-by-step protocol is presented in Chapter 7 for the preparation of brain proteins. Similarly difficult to obtain are low-abundant serum proteins due to the need for removal of major proteins of less interest. Chapter 8 describes strategies that have been developed for that purpose.

Comparative fluorescence gel electrophoresis (CoFGE) was recently introduced to improve comparability of two-dimensional gel electrophoresis experiments. The technology is a combination of one- and two-dimensional gel electrophoresis termed gel-strip-sandwich and is based on fluorescent labeling of proteins to allow multiple samples to be separated on a single gel.

Marker proteins form a spot grid overlaying the separated proteome sample. The anchor spots allow reproducible assignment of protein spot coordinates. A step-by-step protocol for this methodology is presented in Chapter 9.

Chapter 10 switches gears and deals with the increasingly popular method of mass spectrometry-based tissue imaging. Heeren and colleagues present a strategy that determines the molecular distribution of cholesterol on the surface of a tissue section. Different sample coating methods and other technical obstacles are discussed. Mass spectrometry is also one of the main investtigative tools in metabolomics. Simulation of drug metabolites generated by photoexcitation of TiO2 by UV light in aqueous solution was performed in Chapter 11 as an important element in metabolism studies, in particular for monitoring illicit drugs and to support legal actions. The last chapter is devoted to a novel atmospheric pressure glow discharge plasma source. The source can be used for analysis of organic compounds and can be considered as a soft ionization technique as no or little fragmentation is observed.

For this book, I intentionally did not restrict authors to a specific format. However, I asked for illustrative figures and detailed recipes so that manuscripts would really be useful to the practitioner. Each article can be obtained separately in electronic format to provide researchers with exactly the information they need. Following the great response to the book idea, a next volume is planned and I hereby invite every reader to send us his/her tips from the bench. Finally, I would like to thank NOVA for its enthusiastic support of the book project.

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