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# Preface

With the onset of full-scale DNA sequencing projects, biologists began to look at organisms in an entirely new way. In contrast to last century's paradigm, genes are not the be-all and end-all of investigations into the workings of life. Sequencing is just the first step in understanding organisms at a molecular level; most of the questions remain unanswered. To really get at the function of genes, biological systems must be analyzed at multiple levels of control—external parameters (environment, developmental stage, molecular signals, etc.) and internal parameters (transcription and mRNA degradation, posttranslational modification, protein dynamics, and metabolite concentrations and fluxes).

Metabolomics, the unbiased identification and quantification of all metabolites in a biological sample, is playing a substantial role in this process. The technology provides high analytical precision, comprehensiveness, and sample throughput. However, the physicochemical diversity of metabolites necessitates the application of different complementary analytical methods. High throughput and unmatched comprehensiveness for different compound classes makes gas chromatography–mass spectrometry (GC–MS) technology a superior technique for metabolomics. Most metabolites can be partitioned into polar and nonpolar fractions, and after specific derivatization to make each fraction volatile, analyzed using GC–MS. A major portion of *Metabolomics* focuses on different GC–MS techniques and their applications to real-world samples, such as analysis of blood samples (Chapter 1), plant metabolite analysis, and mass spectral and retention time index libraries of known and unknown metabolites (Chapter 2), headspace trapping of volatile compounds (Chapter 3), and the integration of GC–MS metabolite profiling with protein and transcript profiling (Chapters 4 and 5) for systems biology approaches. In this context, data integration and biostatistics, especially multivariate data mining, are addressed and fundamental techniques for the analysis of multivariate datasets described (Chapters 6 and 7).

GC–MS, however, has severe limitations. Measurements of higher sugar-phosphates, cofactors, and nucleotides have to be carried out using other techniques. Moreover, the analyses of secondary plant metabolites and metabolites with relative molecular masses exceeding  $m/z$  600–800 (after derivatization) are not reasonable using GC–MS techniques. Sensitive alternative techniques involve the coupling of capillary electrophoresis and mass spectrometry for the analysis of the polar metabolite fraction (Chapter 8) and classical reversed-phase liquid chromatography–mass spectrometry (Chapter 9).

In the future, the structural elucidation of unknowns detected in metabolomic studies and their compilation in mass spectral libraries will be vital. There is no routine procedure, but the methods demonstrated in this book point the way to future efforts in this field, such as LC–MS techniques for polar metabolite fractions (HILIC) and hydrophobic metabolite fractions (C18) (Chapter 9). Liquid chromatography coupled to electrochemical detection and mass spectrometry reveals the redox-active metabolite fraction, which is important in the investigation of diseases in biomedical applications (Chapter 10).

All these techniques lead to measurements of metabolite steady state levels. An important complementary approach is the measurement of metabolic fluxes, which perhaps can be extended to whole heterogeneous pathway networks. Chapter 11 gives some clues about flux measurements in accessible systems like bacteria or cell cultures. In this context, Chapter 12 provides a theoretical framework for the analysis of metabolic pathway networks and relative fluxes between metabolite compartments based on mass conservation and reaction stoichiometries.

Besides the applications of MS in metabolomics, the establishment of unbiased metabolite analysis as a postgenomic top-down analysis tool has been pioneered with nuclear magnetic resonance spectroscopy. These techniques are the focus of three chapters (Chapters 13–15).

I would like to cordially thank all the contributing authors for providing state-of-the-art procedures, detailed protocols, and tips and tricks to avoid pitfalls. I am grateful to series editor, John Walker, for inviting me to edit this volume. I would also like to thank Megan McKenzie for revising parts of the book, and Katja Morgenthal for continuous help. The result is a compendium of analytical technologies with a focus on intelligibility and applicability. My hope is that researchers from all disciplines will find this a useful aid in their metabolomic approaches.

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