
Preface

This book provides an overview of the current applications of SELDI-TOF-MS (surface-enhanced laser desorption/ionization time-of-flight mass spectrometry), with an emphasis on study and experimental design, data analysis and interpretation, and assay development. SELDI is distinct from other time-of-flight mass spectrometer (TOF-MS) technologies in that it couples features of chromatography and mass spectrometry, facilitating analyte enrichment, and sample cleanup on an array surface. For the analyses of crude biological samples, all mass spectrometric techniques must eliminate substances found in the sample (i.e., buffer salts, detergents) which will interfere with MS detection and use fractionation or enrichment techniques to simplify the complex mixture. SELDI significantly reduces the need for off-line sample preparation by derivatized array surfaces to specifically capture and enrich proteins with specific biochemical properties. Washing of the arrays removes nonspecifically bound proteins and interfering compounds, greatly reducing signal suppression in the mass spectrometer caused by salts and detergents. For complex sample types, such as serum or plasma, SELDI analysis is often combined with upstream fractionation and enrichment techniques to maximize the number of unique proteins detected. After capture of the sample on the array, a matrix molecule, which enhances laser energy transfer and analyte ionization, is added to promote laser-based desorption and ionization. This part of the process is similar to MALDI-TOF-MS, but with a distinct advantage provided by the chemistry of the array. Proteins and peptides that have been enriched and retained on the array are then detected by the ProteinChip® SELDI System, a TOF-MS equipped with a laser desorption ion source.

In the growing field of proteomics, SELDI technology has been widely used for biomarker discovery and characterization in diverse applications including diagnostics, drug development, and basic research. Because chromatographic array surfaces capture proteins based on general chemistry rather than specific molecular affinity (as with antibody recognition), “undirected” studies can be performed to reveal novel biomarkers.

SELDI-based biomarker studies can typically be divided into four phases: discovery, validation, purification and identification, and assay development. In the discovery and validation phases, it is especially important to optimize study design and statistical methods to avoid preanalytical bias and yield robust markers. Identification can be performed at the end of the discovery phase or at any point during the validation phase, and generally requires standard protein purification procedures (column chromatography, size filtration, SDS-PAGE, etc.), followed by protease digestion and sequence analysis on a tandem mass spectrometer. Identification of the biomarkers provides insight into the disease biology and facilitates the development of analyte specific assays. Once the biomarkers have been positively identified, assays can be developed for routine testing on the most appropriate platform such as a quantitative immunoassay. SELDI-based immunoassays are particularly useful for detecting biomarkers with posttranslational modifications. This book provides information on optimizing study design, experimental protocols, and data analysis and interpretation to yield robust biomarkers and biomarker assays, using examples from different disease areas.

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SELDI-TOF Mass Spectrometry

Methods and Protocols

Clarke, C.H.; McCarthy, D.L.B. (Eds.)

2012, X, 239 p. 54 illus., 4 illus. in color.,

ISBN: 978-1-61779-418-6

A product of Humana Press