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## Preface

Protein microarrays are a rapidly growing segment of proteomics that enable high-throughput discovery-driven research through direct measurement of the molecular endpoints of various physiological and pathological states. The human genome has some 30,000 protein-coding genes, while the human proteome is estimated to have at least 90,000 proteins. By now, protein microarrays have been used for identifying protein–protein interactions, discovering disease biomarkers, identifying DNA-binding specificity by protein variants, and for characterization of the humoral immune response. In this volume, we provide concise descriptions of the methodologies to fabricate microarrays for comprehensive analysis of proteins or the response to proteins that can be used to dissect human disease. These methodologies are the toolbox for revolutionizing drug development and cell-level biochemical understanding of human disease processes.

Three general categories of arrays have been developed, which we describe in detail in this volume. The first and most commonly used are the *protein-detecting analytical microarrays*, described in Part I. Conventionally, the design of these arrays is based on the principle of a sandwich immunoassay. Thus, these capture protein on an array surface from biologic samples and quantify presence of those specific analytes using a detection reagent. Arrays may be coated with antigen-specific antibodies to detect specific proteins from body fluids (Chap. 1), whose identity can be confirmed using label-free detection based on mass spectrometry (Chap. 2). An alternative to detection on solid phase uses newly available bead-based strategies (Chap. 3). Antibody-based detection can be also implemented in a high-throughput fashion on reverse-phase protein arrays. Here, cell lysates are printed to a solid support, followed by quantitative immunodetection, as described in Chap. 4. These general designs have been further modified by other investigators to optimize exploration of specific biologic problems. For example, aptamer (Chap. 5) and recombinant lectin (Chap. 6) arrays have been successfully developed.

A second category of protein microarray is *antigen microarrays* that seek to detect antigen-specific antibody from biologic samples (primarily serum and plasma), covered in Part II. Here, arrays are coated with tens to thousands of proteins in order to detect specific reactive antibodies. These have proven valuable for biomarker discovery and detection. Many possible formats of antigen expression on microarrays are now available. Both commercial high-density protein microarrays that express recombinant protein for serum profiling, as well as technology for custom production of arrays to express a tailored collection of proteins, are now available (Chap. 7). Technology to synthesize comprehensive arrays of peptides has also been established (Chap. 8). Finally, high-throughput protein fractionation strategies have been developed that enable array spotting of antigens in their native format (Chap. 9). Production and isolation of proteins can be cost- and labor-intensive. As an alternative, programmable arrays, in which cDNA-containing plasmids are spotted on solid support and protein is freshly translated in situ, offer a versatile solution to the problem of recombinant protein production (Chap. 10).

The final category of protein microarray is *protein function microarrays* to interrogate direct biochemical and physical interactions among biomolecules (Part III). These include profiling of protein–protein, protein–lipid, protein–DNA/RNA, and small molecule interactions. In Chap. 11, we provide protocols for high-throughput mammalian-based detection of protein–protein interactions, operating on the principle of two-hybrid screening techniques. Programmable arrays have been also developed for this purpose (Chap. 12). Among the many specific applications of protein function arrays are the detection of kinase–substrates interactions (Chap. 13) and the characterization of posttranslational modifications that can serve important regulatory functions in eukaryotic cells (Chap. 14).

In most cases, discovery by protein microarray screening requires *validation* of candidate targets, in order to focus subsequent biologic studies. Part IV of this volume offers two separate approaches to candidate target validation. Both require independent production of the protein analyte to confirm specific reactivity.

Both the generation of protein microarrays and the implementation of validation steps have been greatly accelerated by the recent availability of large insect and mammalian proteome libraries. Within these libraries, numerous open reading frames have been cloned and deposited in vector formats that are amenable to protein expression (Part V).

The two final sections of the volume are devoted to signal detection strategies (Part VI) as well as data analysis techniques (Part VII). The most conventional and widely used methods are based on fluorometric or colorimetric methods (Chap. 18), while newer label-free detection systems, such as using FRET (Chap. 19) or surface plasmon resonance (SPR) (Chap. 20), will likely be increasingly employed in the future. Validated software for analysis of protein microarrays is only developing now and is obviously critically important for data analysis (Chap. 21). Finally, knowledge of the publicly available databases that are relevant to proteomics studies can enable more efficient data analysis (Chap. 22).

We hope that this volume provides a solid framework for understanding how protein microarray technology is developing and how it can be applied to transform our analysis of human disease. I am grateful to all the authors for their outstanding contributions to this edition.

*Boston, MA*

*Catherine J. Wu*

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