Preface

Single-cell analysis is a rapidly expanding area of research, and a growing number of analytes ranging from nucleic acid material to proteins to small molecules can now be characterized at the single-cell level. Interest in single-cell measurements has surged as the importance of cellular heterogeneity in both normal and pathological biological processes has become clear. While variation between cells arises at multiple points in cellular physiology, differences in enzymatic activity are a key driver of nongenetic cellular heterogeneity. Understanding the causes and effects of this heterogeneity is an important problem for basic science and clinical medicine. For example, cell-to-cell variation in enzyme activity has important implications for druggable targets, since variation in enzyme activity likely suggests variation in drug response between cells. As a result, a variety of methods have recently been developed to address the challenges of single-cell enzyme assays.

Single-cell measurements are challenging particularly for mammalian or microbial cells since these entities are small, complex, dynamic systems, and each method presented in this volume addresses these challenges in unique ways. The first section of this volume presents techniques based on microscopy and spectroscopy. The second section details a variety of methods that involve physical containment of cells or cell contents in small volumes, using traditional microfluidics, droplet-based microfluidics, or other microscale structures to facilitate analysis. Finally, the third section describes mass spectrometric measurements of single cells with an emphasis on both instrumentation and chemical tools to assist these analyses.

Microscopy is the original single-cell analysis method, and modern instrumentation and spectroscopic methods have enhanced the information content of these measurements. Microscopy provides both spatial and temporal information about cellular biochemistry, and studies of single-cell enzyme activity rely on both. In Chapter 1, An and colleagues present a method to examine spatial and temporal organization of multienzyme complexes in cells. Another advantage of microscopy is the ability of the microscope to act as a detector for other technologies. Chapter 2 by Landry and coworkers presents methods to image single secreted molecules by reading out fluorescence emitted from functionalized carbon nanotubes.

In Chapter 3, Knudsen et al. take advantage of single-molecule detection to examine topoisomerase activity in immobilized cells.

The second section is focused on methods that address the limited sample volume of a single-cell, which makes effective fluid control and containment critical. Microscale structures, including droplets, compartments, and channels or capillaries, therefore play an important role in many single-cell techniques. When single cells are encapsulated in droplets, their secreted enzymes are readily assayed without dilution, as illustrated in Chapter 4 by Chen and coworkers. Similarly, droplets can contain cell lysates for further analysis or for directed evolution, as described by Hollfelder and colleagues in Chapter 5. In Chapters 6 and 7, Li et al. and Chen and Yoon describe how individual cells can also be isolated by microfluidic structures for analysis of drug resistance and proteolysis, respectively. Besides isolating individual cells, microscale fluid control benefits single-cell enzyme measurements in other ways. In Chapter 8, Jesorka and colleagues review advances in microfluidic manipulation of cells and their environments for single-cell protein analysis, with an emphasis on the utility of open-space systems. Jiang and coworkers describe a "nanokit" in Chapter 9 that mixes cell components with small volumes of reactants and then electrochemically measures the response. Finally, microscale systems are beneficial for electrophoretic separations since they confine cell contents to small volumes while providing high efficiency separations, providing potential for highly multiplexed measurements. In Chapter 10, Allbritton et al. detail automated capillary electrophoresis measurements of enzyme activity in single cells, while Chapter 11 by Culbertson and colleagues describes similar measurements made using microfluidic systems.

The third section highlights mass spectrometric methods for single-cell analysis. Modern mass spectrometers are sufficiently sensitive to detect molecules from individual cells, and high mass resolution and tandem MS experiments provide unprecedented chemical information about the complex biochemical mixture inside cells. Ongoing research is improving the spatial and temporal resolution of these measurements. In Chapter 12, Moellering and coworkers describe the use of multipart, targeted chemical probes to measure enzyme activity with single-cell resolution. Spatial resolution and detailed chemical information are also important in studies of enzyme networks and metabolomics. Mass spectrometry is particularly well suited to these studies in its ability to identify dozens to hundreds of molecular components, which can then be mapped onto specific biochemical pathways. In Chapter 13, Nemes and coworkers describe the use of a microprobe for spatially selective sampling of single cells in *Xenopus* and zebrafish

embryos or in neural tissue, elucidating both the temporal and spatial variation in enzyme networks during development. In Chapter 14, Laskin et al. demonstrate the use of electroosmosis into a nanopipette for sampling of contents from individual cells prior to MS analysis.

This volume is a comprehensive overview of the leading techniques in single-cell enzyme measurements, including instrumentation, molecular tools, and integrated devices. We hope that the detailed outlines of cutting-edge procedures will make single-cell measurements of enzyme activity more accessible and more widely used, advancing both our biological understanding of cellular heterogeneity and leading to the development of even more advanced techniques and tools.

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