PREFACE

The Editors are proud to present this volume of *Methods in Enzymology*, focused largely on Enzymes as Sensors. We are fortunate to have gathered a constellation of star investigators to describe their current work in this field. The editors (and many of the authors) have felt for some years that for sensing and many other tasks, biomolecules, such as enzymes, have outstanding attributes. Indeed, biomolecules now represent the method of choice for recognition of many analytes and transducing their presence or level as a signal one can interpret. In this case, necessity has been the mother of invention because answering the essentially analytical questions in biology of how much of an analyte is present, how fast is that amount changing, and how is it distributed in the matrix (often of a living cell), and collecting this information in real time on a submicron scale is a challenge beyond most traditional analytical methods. The proportion of the chapters herein describing methods focused on answering precisely such difficult questions is an index of the inherent power of enzymes and other biological molecules to address such questions.

The volume begins with a chapter by Drs. Sanford and Palmer reviewing one of the central developments of recent years, the use of expressible fluorescent proteins in sensors of many kinds. The second chapter, by Dr. Wynn and colleagues, details a novel development for increasing the ruggedness of biologically derived sensors, namely, encoding them in a very robust form, the bacterial spore. The chapter by Dr. Arts et al. covers a rapidly growing sensing approach with exciting new applications called bioluminescence resonance energy transfer, or BRET. Dr. Staiano and colleagues follow with a chapter on using enzymes as sensors, which focuses on apoenzymes as nonconsuming sensors. Drs. Newman and Zhang take a much broader view in the fifth chapter, using diverse approaches to understand signaling networks well above the cellular scale. Conversely, Dr. Okada et al. in their chapter zoom in on a GTPase component of the signaling cascade that controls the polarity of yeast budding. Drs. Niu and Guo describe a powerful approach, incorporating a variety of different, unnatural amino acids into protein sensors for diverse sensing applications. The eighth chapter by Dr. Lai is an outstanding review of electrochemically based nucleic acid sensors and their unique advantages. In the next chapter, Dr. Yamaura et al. describe a very subtle approach for adapting the recognition capabilities of cell surface

receptors such as the $GABA_A$ receptor to turn on sensors. The Editors next discuss numerous issues in the sensing of metal ions, with zinc as an archetype. Dr. Dumitrescu and colleagues describe another group of electrochemically transduced sensors, emphasizing determination of key analytes such as neurotransmitters and reactive oxygen species. Drs. Branchini and Southworth in the twelfth chapter also focus on bioluminescent sensing in describing their engineered luciferases for ATP detection. Dr. Johnsson and his colleagues describe a flexible, modular approach to bioluminescent sensing that is well suited for point-of-care applications. In the 14th chapter, Dr. Anderson and her colleagues describe a fascinating approach to sensing in austere environments using paper-based sensing. Dr. Cass and colleagues take us farther afield in describing sensors that incorporate microneedles specifically to gently pierce the skin to provide access to interstitial fluid and its analytes. In the final chapter, Dr. Kerppola and colleagues describe an exciting approach for sensing called bimolecular fluorescence complementation, and its application to studying multiprotein complexes with fly polytene chromosomes.

After more than 60 years, *Methods in Enzymology* as a series, and developing new methods in experimental science in general, is still relevant and worthwhile. Technology development remains a fruitful part of the scientific enterprise, at least as judged by citations: In January, 2017, 13 of the 15 most cited papers in the *Proceedings of the National Academy of Sciences* (http://www.pnas.org/reports/most-cited) were overtly methodological in nature albeit from many fields, and all of the top 10 most cited papers overall are methodological (Van Noorden et al., *Nature* 514, 550 (2014)). Moreover, the highly cited nature and consequent impact of methodological papers has been maintained in the biological and chemical sciences for at least 40 years. In the current "business model" of government/nonprofit funding of academic research, a case could be made that (for instance) Sanger et al.'s invention of dideoxy sequencing (cited 68,000 times and counting) represents a good return on investment.

We hope you enjoy this collection.

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