Preface

A central challenge of biochemistry and molecular cell biology is to understand how biomolecules interact and react to organize and control cell growth, structure and function. The contributions in this book reveal many ways in which the technologies of specific chemical and genetic labeling can be used in conjunction with optical microscopy to dynamically analyze the spatial and temporal organization of proteins, lipids, and even messenger RNA in single living cells.

More than half a century of progress in biochemistry, genetics, molecular biology and cellular physiology has yielded a rich understanding of the macromolecular basis of structure and information flow in living systems. Complete genomes have been sequenced, tens of thousands of protein structures have been determined, and the activities and functions of thousands of enzymes have been analyzed. Over the past decade, methods that allow for the real-time analysis of molecular function in the environment of the living cell or organism have come to prominence. The ability to dynamically and non-destructively image the translocations, interactions or reactions of one or more chemically unique biomolecules affords a mechanistic understanding of cellular function that is not accessible using traditional biochemical assays.

Selectivity is the unifying idea that runs throughout this book's chapters. *In vivo* studies require non-invasive methods of imparting unique optical or chemical functionalities to particular molecular species. One strategy is to prepare a soluble probe molecule that is fluorescent or otherwise detectable, but which retains the same cellular localization and biological function of the unlabeled species. The other general strategy is to genetically label a protein with a functional tag. This tag can be one of many commonly used autofluorescent proteins, or it can be a receptor protein or polypeptide that binds to a soluble, cell-permeable, small molecule that has the desired functionality.

The first three contributions in this volume describe various strategies for probing the function of the lipid bilayer. The spatiotemporal dynamics of phosphoinositides and their role in activating signaling cascades or membrane trafficking events can be microscopically visualized by expressing fluorescent proteins fused to phosphoinositide-binding motifs. The organization of cholesterol and sphingolipids

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into discrete microdomains within the lipid bilayer can be studied with two approaches described herein. Naturally occurring and synthetic fluorescent analogues of cholesterol can be incorporated directly into the lipid bilayer of living cells, allowing microscopic visualization of cholesterol-rich microdomains. Alternatively, lipid-binding toxins can be used to label and detect sphingolipids, cholesterol and GPI-anchored proteins.

Several contributors offer experimental strategies for appending specific proteins with synthetically optimized small molecules. Proteins of biological interest can be genetically encoded as fusions to receptor proteins or polypeptides. Cell-permeable ligands can be synthesized with a variety of functionalities, including enhanced fluorescence, photoaffinity or analyte sensing capability. Upon addition to culture medium, these ligands can bind specifically and stably to the fusion protein chimeras via high-affinity non-covalent interaction or enzyme-mediated covalent linkage. The smallest possible functional tag for a protein is a single amino acid. A comprehensive description of methods for incorporating unnatural amino acids with specific chemical or optical properties into proteins, both *in vitro* and *in vivo*, is included.

There are comparatively few robust methods for the sequence-specific labeling of nucleic acid species in living cells or organisms. Fusion of bacteriophage MS2 coat protein to fluorescent proteins allows labeling of messenger RNA containing the cognate MS2 hairpin binding site. Tagged mRNAs can be microscopically visualized in living mammalian cells with single transcript resolution. A thorough description of this technique is provided in the final chapter of this volume.

The contributions to *Probes and Tags to Study Biomolecular Function* reflect the efforts of chemists and biologists to bring the study of biochemistry from the test tube to the living cell. Taken together, the experimental tools described in this volume reflect the state-of-the-art in technologies to label biomolecules for *in vivo* studies. The step-by-step protocols and illustrations of typical applications will enable researchers to select the best solution for their experimental problems. Looking ahead, the real-time analysis of molecular function within living cells will continue to be an indispensable approach to studying mechanistic biology. Our contributors have laid a foundation for the future development of more robust and selective labeling technologies that should allow for *in vivo* detection with even greater sensitivity and spatio-temporal resolution.

Chicago, 2008 *Lawrence W. Miller*