# Preface

## Methods in Enzymology (Fluorescence 2008)

Light is a relatively non-invasive probe. As ever more theoretical and experimental details emerge regarding the behavior of molecules and atoms in the excited state, fluorescence spectroscopy continues to be a method of choice to study the behavior of biological macromolecules, membranes and living cells. Some of the topics covered in volume 278 of *Methods in Enzymology* are revisited and new areas of interest are covered.

The chapter by Packard and Komoriya on intracellular protease activity is an example of the application of novel photophysics to studies in the living cell. Merzelyakov and Hristova describe how Förster type resonance energy transfer (FRET) can be utilized to measure dimerization energetics of transmembrane helices. An important feature of fluorescence is that there are several quantities that can be measured. Advantageous applications of the "multiparametric" approach is evident in the chapters by Klymchenko and Demchenko on solvatochromic dyes and Demchenko on site-selective red edge effects and also in the contribution by Periasamy on heterogeneity of fluorescence. During the past few years there has been an explosion of interest in the use of fluorescence spectroscopy to study single molecules. The chapter by Shi, Dertouzos, Gafni, and Steel discusses the application of single molecule spectroscopy in studying enzyme kinetics and mechanism. The single molecule approach is also discussed by Pljevaljcic and Millar in studies of RNA folding and ribonucleoprotein assembly.

Royer and Scarlata describe fluorescence approaches, including fluorescence correlation spectroscopy and fluorescence anisotropy, for quantifying bio-molecular interactions. Hawkins describes several pteridine probes for nucleic acid analysis. Anderson and Schildbach discuss the use of fluophore-labeled oligonucleotides to measure affinities of protein–DNA interactions. Dragan and Privalov describe the use of FRET in studying protein-induced DNA bending.

Two chapters describe biosensors for inorganic ions. Bozym, Hurst, Westerberg, Stoddard, Fierce, and Thompson discuss the determination of zinc using carbonic anhydrase-based fluorescence biosensors. Thompson, Zeng, Ohnemus, and Moffett describe the determination of free metal ions in solution using fiber optic biosensors.

The development of instrumentation and procedures for data analysis involved in fluorescence lifetime measurements had a revolutionary effect on fluorescence spectroscopy, allowing direct studies of a variety of photophysical processes. These studies have been limited by the fact that the time resolution of both pulse and phase instruments are no better than fractions of a nanosecond. Xu and Knutson describe ultrafast florescence spectroscopy via upconversion. This methodology which works well in the picosecond time domain can now be used for tryptophan lifetime studies and is likely to provide new insight in studies of protein fluorescence. Finally Johnson, Fahy, Veldhuis, and Lakowicz describe a fluorescence application of a numerical technique for identifying small pulsatile signals within noisy data.

It is clear that applications of fluorescence spectroscopy to studies of biological macromolecules and cells are proceeding at a rapid rate. Advances are described in this volume.

LUDWIG BRAND
MICHAEL L. JOHNSON