
Preface

Fluorescent nucleic acid probes, which use energy transfer, include such constructs as molecular beacons, molecular break lights, Scorpion primers, TaqMan probes, and others. These probes signal detection of their targets by changing either the intensity or the color of their fluorescence. Not surprisingly, these luminous, multicolored probes carry more flashy names than their counterparts in the other fields of molecular biology. In recent years, fluorescent probes and assays, which make use of energy transfer, have multiplied at a high rate and have found numerous applications. However, in spite of this explosive growth in the field, there are no manuals summarizing different protocols and fluorescent probe designs. In view of this, the main objective of *Fluorescent Energy Transfer Nucleic Acid Probes: Designs and Protocols* is to provide such a collection.

Oligonucleotides with one or several chromophore tags can form fluorescent probes capable of energy transfer. Energy transport within the probe can occur via the resonance energy transfer mechanism, also called Förster transfer, or by non-Förster transfer mechanisms. Although the probes using Förster transfer were developed and used first, the later non-Förster-based probes, such as molecular beacons, now represent an attractive and widely used option. The term “fluorescent energy transfer probes” in the title of this book covers both Förster-based fluorescence resonance energy transfer (FRET) probes and probes using non-FRET mechanisms.

Energy transfer probes serve as molecule-size sensors, changing their fluorescence upon detection of various DNA reactions. Many types of energy transfer probes can be adapted for homogenous detection formats, i.e., they function autonomously and fluorescently indicate their molecular targets without additional intervention. In this case, the energy transfer phenomenon serves as a “molecular cloaking device,” hiding the probe’s fluorescence until it detects its target. In the nonreactive state, probe fluorescence is quenched as a result of energy transfer to a nonfluorescent acceptor located in close proximity to a fluorophore. After the reaction, relative positions of a fluorophore and a quencher change, resulting in the appearance of fluorescence.

The DNA-based energy transfer constructs can also form “composition fluorophores” in which the emission and absorption properties can be independently tuned, making them attractive for the multiplex detection assays.

Energy transfer probes are especially advantageous when used in multiplex polymerase chain reaction, as parts of biosensor assays, for screening and real-time monitoring of biochemical reactions, and in nanotechnology applications.

Fluorescent Energy Transfer Nucleic Acid Probes: Designs and Protocols presents a wide assortment of methods using both Förster and non-Förster mechanisms of energy transfer in nucleic acids. A broad array of structures and applications of various energy transfer constructs and their optimized design are presented in detail for the first time.

The techniques described include those designed to monitor various types of DNA and RNA reactions including hybridization, amplification, cleavage, folding, and association with proteins, other molecules, and metal ions. This volume also presents techniques for distance determination in protein–DNA complexes and methods to detect topological DNA alterations, mutations, and single nucleotide polymorphisms. It contains the latest cutting-edge nanotechnology applications, such as nanomachines, energy transfer aptazymes, DNAzyme-based biosensors, and logic gates for molecular-scale computation.

Reproduction of technical protocols, readily available from original journal papers, would not warrant an additional publication. *Fluorescent Energy Transfer Nucleic Acid Probes: Designs and Protocols* instead serves as a comprehensive source of information on every method described.

The volume is divided into seven sections consisting of two to five chapters. The first section contains two chapters describing basic principles of selection and optimization of labels for FRET-based (Chapter 1) and non-FRET-based (Chapter 2) probes applicable to all energy transfer constructs. The section provides information necessary for the individualized design of energy transfer probes considering the specific needs of a researcher. Parts II–VI discuss application of energy transfer probes for detection and monitoring of various reactions involving DNA or RNA including: hybridization detection (Chapters 3 and 4), DNA breaks and cleavage monitoring (Chapters 5–7), synthesis and amplification visualization (Chapters 8–12), sequence analysis and mutation detection (Chapters 13–16), and determination of distances and DNA folding (Chapters 17–18). The last section (Chapters 19–22) deals with design and application of molecular devices that use energy transfer, such as biosensors, nanomachines, and logic gates for molecular-scale computation. Chapter 5 describes a molecular machine for DNA breaks detection and therefore belongs to both the nanotechnology and DNA damage fields.

The field of fluorescent probes is constantly evolving and I hope that this volume will not only help its readers use the described techniques, but will prompt them to explore new ways in which energy transfer constructs can facilitate their research.

Researchers using fluorescence in any field of biomedical sciences will benefit from this book. These include molecular and cell biology, embryology, toxicology, radiobiology, experimental and clinical pathology, oncology, experimental pharmacology, drug design, environmental science, and nanotechnology. *Fluorescent Energy Transfer Nucleic Acid Probes: Designs and Protocols* is a helpful resource for both novice investigators and experienced researchers. For a scientist new to the area of fluorescent probes, the book will help to select the suitable probe, to deal with experimental pitfalls and to properly interpret the results. Experienced researchers will find the book useful because it describes the new and unique constructs in detail and can be used as a source of information in development of new energy transfer probes and applications.

I am grateful to all participating authors whose ingenuity made this book possible, and particularly to those of them who submitted their contributions on time. I wish to express my appreciation to Candace Minchew for her expert technical assistance. I also thank Professor John Walker for his generous help with the review process.

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Designs and Protocols

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