Reviews in Fluorescence 2006

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(Eds.)

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PREFACE

This is the third volume in the Reviews in Fluorescence series. To date, two volumes have been both published and well received by the scientific community. Several book reviews have also favorably described the series as an "excellent compilation of material which is well balanced from authors in both the US and Europe". Of particular mention we note the recent book review in JACS by Gary Baker, Los Alamos.

In this 3rd volume we continue the tradition of publishing leading edge and timely articles from authors around the world. We hope you find this volume as useful as past volumes, which promises to be just as diverse with regard to content.

Finally, in closing, we would like to thank Dr Kadir Aslan for the typesetting of the entire volume and our counterparts at Springer, New York, for its timely publication.

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CONTENTS

THE HISTORY OF FRET:
From conception through the labors of birth

Robert M. Clegg[1]

1.1. INTRODUCTION

This chapter is an excursion into the historical development of energy transfer. This chapter is not concerned with a detailed review of applications, or a review of modern theoretical developments; this is available elsewhere (Van Der Meer *et al.*, 1994; Wu and Brand, 1994; Clegg, 1996). The topic is the emergence of Förster resonance energy transfer FRET. I also examine the ideas, experiments and theories that formed the scientific backdrop that preceded and led up to FRET.

FRET is a physical process whereby the excited state energy of one chromophore molecule, the "donor", can be transferred to a neighboring chromophore, the acceptor, in the ground state. This can take place whenever the two molecules are close enough, usually separated by less that 7 nm provided certain other conditions are met.

FRET is one of the major experimental methods for discovering whether two molecules are in close proximity, or for determining the distance between two specific locations on macromolecules and in molecular complexes. Energy transfer is used to follow conformational changes of macromolecules, either statically or in real time. It has recently become a major experimental technique in the field of single molecules. Since the "efficiency" of energy transfer (that is, the fraction of energy absorbed by the donor that is transferred to the acceptor) is usually measured with fluorescence tools, and fluorescence is sensitive, specific and widely available, FRET has become very popular. The chromophores (donors and acceptors) that are used for accomplishing this measurement are usually attached (often covalently) to other macromolecules, such as proteins, nucleic acids, and lipids. The energy transfer can be detected relatively easily and it is often used qualitatively to signify intimate interaction

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between two "labeled" biomolecules. Sometimes one or both of the participating chromophores occur naturally in biological macromolecules, such as tryptophan or chlorophyll. However, the number and variety of synthetic fluorescence probes available for labeling has expanded tremendously in the last several years. Several readable reviews of FRET for a general audience are readily available (Clegg, 1992; Van Der Meer *et al.*, 1994; Clegg, 1996; Clegg, 2004a).

The FRET measurement is now applied routinely with a wide variety of samples: micro structures (such as DNA and protein chips and micro/nano assay arrays), living biological cells, and even whole organisms. It is a very powerful technique, fairly simple, and can be carried out in most laboratories with their existing spectrometers and microscopes. Although the technique has been readily available and applied since the early 1950s, the use of FRET has literally exploded in the last few years, in academic research as well as industrial applications, especially in biotechnology and bioengineering. This flurry of activity has many reasons. First, FRET measures interactions and dynamics on a spatial scale that is unique. Also, our ability to produce well defined and pure macromolecules in the laboratory has increased dramatically in the last few years, and it is relatively easy to label them specifically with fluorophores. In the last several years we have developed the ability to produce hybrids of specific proteins with fluorescent proteins (for instance, GFP, YFP, CFP and RFP, respectively green-, yellow-, cyan-, and red-fluorescence proteins) that can be produced *in vivo* under genetic control in the living cell (and in tissue); certain pairs of these proteins can undergo FRET. These fluorescence proteins have revolutionized the field of biological fluorescence, especially the measurement of FRET, in the fluorescence microscope. A great number of excellent synthetic fluorophores are available commercially, with the required chemical groups attached for specific labeling to biomolecules. In addition there have been many instrumentation improvements and innovations that make the FRET measurement much more sensitive and convenient. These chemical, biological, and instrumentation advances have expanded tremendously the range of applications, and the ease of carrying out the experiments.

In spite of the wide spread use of such a well known and useful technique, and the availability of several excellent treatise and reviews of the underlying theory, not to mention the hundreds of experimental applications published every year, little is published about the historical development of the major concepts. The historical events are not only interesting in themselves, but understanding and appreciating the major theoretical insights realized by the pioneers of energy transfer, and the scientific context in which they worked, provides insight into the mechanism, and leads to a better appreciation of the original contributions. A short history of the contributions of the Perrins and Foerster to FRET has been published recently (Clegg, 2004b). This chapter is a more extensive examination of the state of affairs and the general state of knowledge that was prevalent in physics at the time, leading up to the first observations and theoretical explanations of energy transfer.