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Fluorescent Proteins I

From Understanding to Design

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Aims and Scope

Fluorescence spectroscopy, fluorescence imaging and fluorescent probes are indispensible tools in numerous fields of modern medicine and science, including molecular biology, biophysics, biochemistry, clinical diagnosis and analytical and environmental chemistry. Applications stretch from spectroscopy and sensor technology to microscopy and imaging, to single molecule detection, to the development of novel fluorescent probes, and to proteomics and genomics. The *Springer Series on Fluorescence* aims at publishing state-of-the-art articles that can serve as invaluable tools for both practitioners and researchers being active in this highly interdisciplinary field. The carefully edited collection of papers in each volume will give continuous inspiration for new research and will point to exciting new trends.

Preface

A plethora of reviews, popular science books, and scientific textbooks have been written on the significance of fluorescent proteins in the life sciences. More than 30,000 references can be found in bibliographic databases which refer to at least one among the members of this protein family (see Fig. 1). Most of these narrate on how fluorescent proteins may be used to label gene products, how they may be visualized in cellular compartments by fluorescence microscopy, or how they may be expressed in individual cells, thus provoking novel findings in ontogenesis. In most of the experiments described, fluorescent proteins are being exploited as miniaturized light bulbs, the length scale is that of microns, and the time scale is that of seconds or longer. There is no doubt that fluorescent protein technology has revolutionized life sciences in that proteins have become universal and standard tools in molecular biology laboratories.

A minor fraction of roughly 5% of all publications deals with the *nanoscopic* properties of fluorescent proteins (FPs) acting as light bulbs. Early achievements include the crystallographic analysis of their molecular structure [1, 2], the discovery of excited-state proton transfer in the naturally occurring FP [3, 4], and the erratic light emission of individual members of FPs [5, 6]. Especially the last experiments, along with low temperature studies [7, 8], have revealed that FPs exhibit a tremendous heterogeneity in terms of structure and dynamics.

It is therefore not astonishing that FPs have had a large impact on other areas of biophysical research, e.g., in studies on protein folding [9–11]. However, the irregular emission of light by FPs also has impacted experiments in the life sciences: most operators of fluorescent protein technology, whom I was talking to, were concerned about weird experimental features like rapid initial fading in time-lapse microscopy, sometimes with sudden fluorescence recovery, or changing FRET-ratios upon continuous illumination. Such annoying findings can be traced back to the wealth of light-driven processes in the proteins, and I am quite sure that more surprises of that kind have been experienced by others. It should be emphasized here that such “strange” photodynamics have initiated seminal studies on protein diffusion and high-resolution microscopy [12–14].

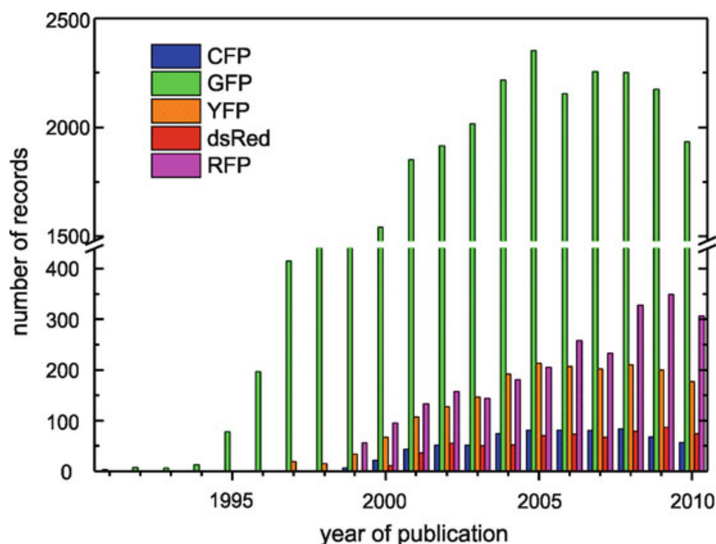

Fig. 1 Number of references related to fluorescent proteins (database: web-of-science). The number of articles dealing with Green Fluorescent Protein has reached saturation now at a level of typically 2,000 articles per year for almost a decade. Those on the Red Fluorescent Proteins are still increasing

Volumes 11 and 12 of the *Springer Series on Fluorescence* deal with various aspects of fluorescent proteins. The first volume (*Fluorescent Proteins I*) is devoted to the molecular, i.e., mainly optical, properties of fluorescent proteins. In the first part, the primary processes leading to fluorescence are discussed: excitation, relaxation, and other processes in the excited state and in emission. Fluorescence proteins are treated as "ordinary" fluorophores, and one article is highlighting our opportunities to circumvent the synthetic limitations given by nature. The second part focuses on the mechanisms that make the difference to conventional fluorophores: isomerization, protonation, as well as reversible and irreversible photochemical reactions. The knowledge on how these processes are affected by the surrounding of the FP allows for tailoring it with respect to spectacular applications, applications that are not conceivable with "ordinary" fluorophores.

In the second volume (*Fluorescent Proteins II*), the key aspect is on applications. Its first part is giving an overview on how many unconventional photophysical properties latently exist in naturally occurring and how double-resonance experiments enable the information to be extracted from microscopy data in an unprecedented way. More on high-resolution microscopy will be found in forthcoming volumes of this series. Quantitation, a central objective of analysis, is the comprehensive caption of the articles in the next part. We may state, justifiably, that researchers have reliable tools at hand to quantify some of the most abundant ions after more than a decade of development. Other physiological parameters of

overwhelming importance like the transmembrane potential still need to experience this development. The last part reports on three examples of utmost biological relevance and how ultrasensitivity in bioanalysis, i.e., single-molecule technology, is merged with FP technology. This combination has resulted in an understanding of processes on a molecular level and in detection limits that were not even thought of some 15 years ago.

A preface is also always the occasion to deeply acknowledge the support by others. First of all, I have to thank my family who tolerated my commitment to this experience. I also would like to express my thanks to my coworkers, to my colleagues, and to the representatives of Saarland University for their understanding. In times of growing competitiveness in many academic areas, it is not self-evident to dedicate a substantial amount of time to such a book project. For the same reason, I especially appreciate the immense work of all authors of these two volumes who are all passionate, but busy scientists and who (more or less) voluntarily spared no pains to complete their manuscripts in a wonderful and highly professional way. By now, it also may be appropriate to apologize for my e-mail bombardments!

Saarbrücken, Germany Gregor Jung

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