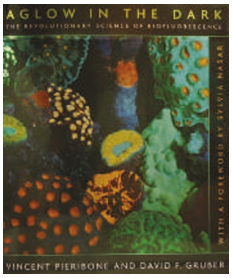
Biofluorescence: the making of a new technology



Aglow in the dark: The revolutionary science of biofluorescence

By Vincent Pieribone and David Gruber

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An extraordinary number of living creatures, from bacteria to insects to sharks, are capable of 'lighting up'. Known as bioluminescence, this 'living light' has long fascinated humans. In 79 AD, Pliny recorded the first human use of bioluminescence as a kind of torch, by rubbing a stick against jellyfish slime. However, only recently has bioluminescence become a science, evolving from description and speculation to explanation — and a revolutionary new approach to biology.

Aglow in the dark, by Vincent Pieribone and David Gruber, chronicles this saga of discovery of bioluminescence as it entertainingly traces the history of human interaction with bioluminescence, and charts the development of green fluorescent protein (GFP) as one of the groundbreaking discoveries of the 20^{th} century. The text is superbly written and gripping throughout. As an authoritative introduction to the science of fluorescent proteins, the book should be obligatory reading for every newcomer to biology.

In their opening chapters on bioluminescence, Pieribone and Gruber describe surprising features of fluorescence, such as its role in allowing forensic technicians to find fingerprints at crime scenes. Woven into this account are other fascinating facts and anecdotes on bioluminescence — including how the swarms of plankton that float on waves glitter when disturbed, turning a submarine or boat into a highly visible target. These chapters also chronicle the challenges faced by early researchers, such as Edmund Harvey who first popularized the scientific study of living light, and Osamu Shimomura who unraveled how the palm-sized jellyfish *Aequoria victoria* glowed.

The story of Osamu Shimomura is particularly moving: briefly blinded by the atomic bomb blast over Nagasaki as a sixteen year-old, Shimomura took on the monumental task of determining the chemical nature of bioluminescence as a self-taught graduate student. With inexhaustible energy and skilled efforts, he clarified the mechanism of bioluminescence by painstakingly purifying luciferin — the fuel component of bioluminescence that combines with luciferase (the igniter or catalyst) to generate light. Shimomura's discoveries extended to a second fluorescent protein, GFP, which converts one colour of light to another, but it would take another 30 years before GFP was transformed into the tool so widely used in biomedical research today.

The chapters that chronicle the path of GFP development will appeal to anyone interested in this scientific revolution. Pieribone and Gruber bring to life the cast of interesting and quirky characters who worked against the backdrop of the explosion in information technology and the genome revolution of the 90's. These breakthroughs, in turn, sparked a search for fluorescent proteins that could be re-engineered and mass produced.

At first, molecular cloning techniques were slow to be adopted. The consensus among scientists studying bioluminescent and fluorescent proteins was that fluorescent proteins like GFP would not fluoresce on their own. So research on GFP progressed slowly, with only structural researchers like William Ward dedicated to its study.

This quickly changed when Doug Prasher decided to try to isolate the gene for GFP. Although the National Institutes of Health denied him funding, Prasher purified the gene regardless. He published his findings, but then left the field. Fortunately, two scientists, Martin Chalfie and Roger Tsien, read Prasher's paper and sensed the dramatic potential of GFP. Chalfie asked whether the cloned gene could be expressed in another organism, while Tsien saw long-term potential in making GFP glow brighter so it could be used as an all-purpose biological reporter. In the end, GFP proved to be a stand-alone structure, tailor-made for the new molecular revolution. Within a few years, GFP had been expressed in cells from virtually all organisms under biological study.

The focus soon turned to making GFP brighter and capable of emitting different colours. The crystal structure of GFP, solved by James Remington and Roger Tsien, greatly helped this endeavour as it revealed the exact arrangement of the fluorophore. Mutagenesis of amino acids bound to the fluorophore was key to changing the excitation–emission spectrum of GFP, and it produced GFP variants with different colours and capacities for changing colour in response to environmental stimuli.

With the cloning of new fluorescent proteins from species other than jellyfish came the discovery of a red fluorescent protein — the holy grail of fluorescence due to red light's ability to penetrate deep into tissues, thus providing a better picture of how biological systems operate. An enterprising young Russian researcher named Sergey Lukyanov examined whether the brilliant fluorescent colours exhibited by some corals resulted from fluorescent proteins like GFP. No one had previously thought of this because fluorescence had been linked to bioluminescence, which corals lack. Obtaining coral from a friend's 100-gallon aquarium in a small apartment in Moscow, Lukyanov purified and then cloned the fluorescent protein from a species of *Discosoma* coral, the first red fluorescent protein, now called dsRed.

In portraying the activities of a unique creative community in a clear and animated fashion, Pieribone and Gruber convey the spirit of scientific endeavor perfectly: discovery is not just a goal, but an unpredictable process as dependent on intellectual genius as on creative inspiration and pure luck. The pivotal figures traced in the book are shown to have all worked in an uncertain, rapidly changing scientific terrain, building on the work of others in ways they could hardly have anticipated. Pieribone and Gruber, however, leave no question of what the future of fluorescent protein technology holds. One has only to look at the current biological journals to realize that biofluorescence is revolutionary: use of fluorescent protein technology is now the order of the day.

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