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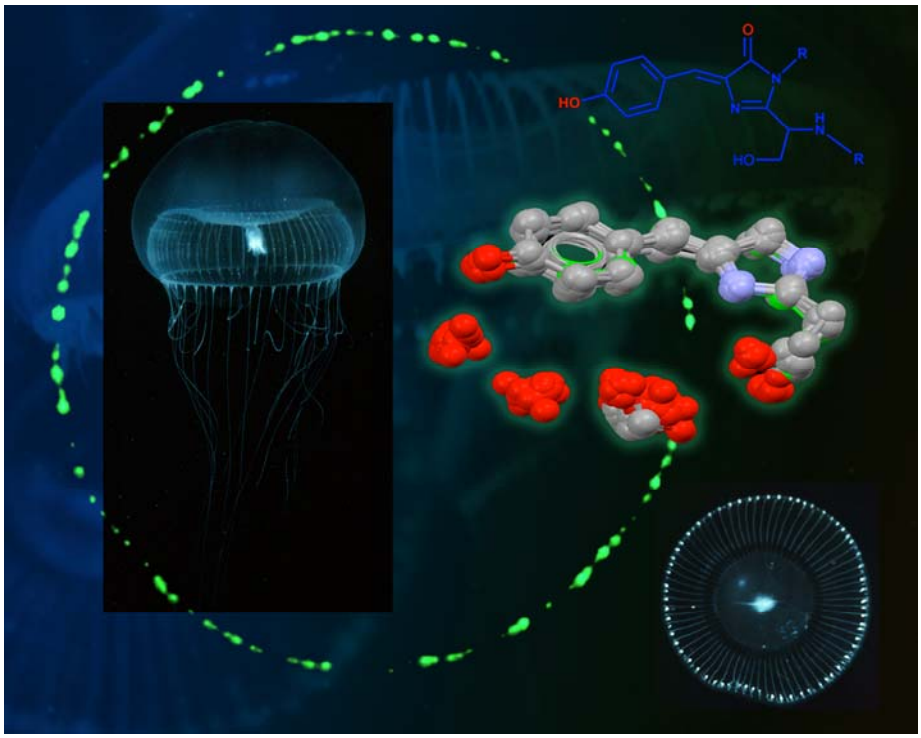
2009 Green Fluorescent Protein issue

Reviewing the latest developments in the science of green fluorescent protein

Guest Editors Dr Sophie Jackson and Professor Jeremy Sanders

All authors contributed to this issue in honour of the 2008 Nobel Prize winners in Chemistry, Professors Osamu Shimomura, Martin Chalfie and Roger Y. Tsien

Please take a look at the issue 10 table of contents to access the other reviews



The discovery and development of the green fluorescent protein, GFP

Jeremy K. M. Sanders and Sophie E. Jackson

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This themed issue is dedicated to Osamu Shimomura, Martin Chalfie and Roger Tsien, who shared the Nobel Prize in Chemistry in 2008 for their pioneering work which led to "*the discovery and development of the green fluorescent protein, GFP*." Professors Shimomura, Chalfie and Tsien all played vital roles in the different steps that have led from the first isolation and characterisation of GFP from jellyfish to its now widespread use throughout the Life Sciences. This single protein (or now family of related proteins) has become the most important tool in contemporary bioscience. In his announcement of the Nobel Prize on the 8th October 2008, Professor Gunnar Öquist, Secretary General of the Royal Swedish Academy of Sciences, spoke about "*the illuminating biochemical discovery that has revolutionised the Life Sciences*." Illuminating indeed, as the importance of GFP lies in its remarkable, brightly glowing green fluorescence.

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The story starts back in the early 1960s when Osamu Shimomura, who had an interest in bioluminescence, first isolated a bioluminescent protein (aequorin) from the jellyfish *Aequorea victorea*. Shortly afterwards, he identified another protein which had bright green fluorescence when illuminated with UV light. This was the aptly named green fluorescent protein (GFP). Collecting and isolating sufficient material to undertake a detailed characterisation of GFP proved to be a laborious and painstaking job. Over the summers of 19 years, Shimomura and co-workers collected 85 000 jellyfish off Friday Harbor in Washington on the West Coast of North America, an undertaking it is difficult to contemplate in modern day science. By 1979, Shimomura had sufficient GFP to determine the structure of the chromophore which lies at the heart of the molecule and is responsible for the protein's unique properties. The work is a beautiful example of classical biochemistry, an area of science which has largely been lost from today's scientific communities. In one interview Shimomura admits that "*I didn't know any use of that protein, of that fluorescent protein, at that time*," but this piece of blue sky research has ultimately produced the most widely used tool in all of the Life Sciences. Without Shimomura's work, GFP may well have never been discovered.

The next significant step in the story came with the cloning of the gene for GFP, along with aequorin, from *Aequorea victorea* which was accomplished by Ward and Prasher in Cormier's laboratory, and by 1992 there was a full sequence of the protein. Despite this progress, the full potential of GFP was yet to be recognised as it was assumed that the formation of the green fluorescent chromophore was a post-translational event that was likely to require enzymatic catalysis.

Prasher passed on the GFP gene to Martin Chalfie, and the next stage was begun. Chalfie's group were the first to express GFP in *Escherichia coli* and produced glowing green cultures establishing that this protein with its

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Jeremy Sanders is Professor of Chemistry and Head of the School of Physical Sciences in the University of Cambridge. His current research interests in supramolecular chemistry are a long way from GFP, but during the 1970s he was a junior lecturer when Roger Tsien was a PhD student in the Cambridge Department of Physiology. Roger had invented and designed a new intracellular indicator for Ca ions, and needed the facilities of the Chemical Laboratory in order to prepare and characterize his new molecules; JKMS (among several others) was happy to help.

Sophie E. Jackson

Dr Sophie Jackson is a Reader in Biophysical Chemistry at Cambridge University. She completed a B.A. in Chemistry at Oxford University before starting a PhD at Imperial College, University of London which was completed in Cambridge. She was awarded William Stone Research Fellowship (Peterhouse, Cambridge), and a Human Frontiers Science Program Organisation Postdoctoral Fellowship in the Chemistry Department at Harvard University. She returned to Cambridge in 1995 as a Royal Society University Research Fellow. Her research focuses on different aspects of protein folding and assembly processes, and her current interests include understanding how large complex protein structures form.

unique properties could be used as a fluorescent marker *in vivo*. The work in *E. coli* suggested that it might be possible to use GFP in the cells of all living organisms and by 1994 Chalfie and co-workers had shown that it was possible to express GFP in the touch neurons of *Caenorhabditis elegans*. These experiments established the potential of GFP to act as a fluorescent marker *in vivo* and opened the doors for research in this area. This was one of those rare breakthrough moments in science. In the same year, Tulle Hazelrigg established that GFP could be fused to a protein without affecting the latter's function and that the cellular location of the fusion protein could be mapped from its green fluorescent signal.

Roger Tsien also received the gene for GFP from Prasher and collaborated with him to express the protein in *E. coli*. His early work shed light on the mechanism by which the chromophore forms. But more importantly, he began engineering the protein to systematically change and enhance its spectral properties. By 1994 his group had produced a blue-fluorescent variant of GFP. This was the start of an enormous programme of work which has resulted in the development of a vast array of fluorescent proteins that span the visible spectrum, and which recently have also made inroads into the near-red region. Tsien has also pioneered the development of fluorescent proteins that act as biosensors, including the development of intracellular calcium and pH sensors in addition to redox-sensitive GFP indicators. It is through his work, as well as that of many others, that fluorescent proteins, including many engineered variants of GFP, have such a widespread use throughout the Life Sciences.

This themed issue enables us to honour the contribution that Shimomura, Chalfie and Tsien have made, and to illustrate the tremendous impact that their work has had in many fields. The issue is a collection of review articles that collectively covers a spectrum range of science—from the molecular biophysics that is essential in determining the spectral properties of the chromophore, through the extensive engineering of the systems and development of novel FPs and new methods, to the myriad of biological applications in which fluorescent proteins are used. We warmly thank all the authors for participating in this endeavour, and for their efforts and contributions to this themed issue, culminating in the production of a selection of excellent tutorial and critical reviews over a wide range of topics. We are also grateful to the editorial and production staff at the RSC for all their support, attention to detail and above all patience. We hope that this issue will provide a valuable resource for the scientific community; that the tutorial reviews offer an excellent introduction into the field of fluorescent proteins for those yet to work with these fascinating molecules, whilst the critical reviews provide in depth analyses of these complex proteins for the rest. We hope that biophysicists can learn from the more biological reviews and that the biological readers can gain important insight into the molecules which they use routinely from the biophysical studies.

Most of all we are all indebted to Shimomura, Chalfie and Tsien, without whom many of us would still be working in the dark!

