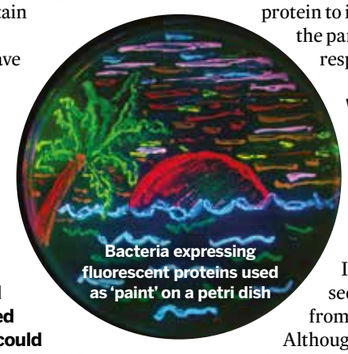
The light fantastic

The remarkable American biochemist Roger Y Tsien tells us how he made his most famous discovery

In 2008 Roger Y Tsien shared the Nobel Prize in Chemistry for the discovery and development of the green fluorescent protein (GFP). This glowing molecular tool has revolutionised many areas of biochemistry research, allowing researchers to visualise the expression of certain genes or certain molecules within cells. Molecular biologists have since found countless uses for GFP and similar molecules, and fluorescent proteins are now an essential part of biochemists' molecular toolkit.


Bacteria expressing fluorescent proteins used as 'paint' on a petri dish

Before you discovered GFP, your work involved looking for dyes that could help image neuronal activity. What inspired you to work in this field?
The visual system is the only sensory system with the ability to display lots of events in spatiotemporal detail, so one has to use one's own visual system to investigate another creature's nervous system. From very early on in graduate school, I was attracted to developing techniques for visualising neuronal activity as the best way to resolve many neurons firing simultaneously.

What led you to look at fluorescent proteins and their related genes?
My colleagues and I had painstakingly built dyes such as Fura-2 and Indo-1 – with molecular weights near 840 – for recognising and visualising small calcium ions, whose molecular weight was only 40. So it seemed that for the more general problem of recognising biochemical messengers such as cyclic AMP (molecular weight 329), let alone full sized proteins, we would have to adopt the techniques of molecular biology rather than synthetic chemistry.

I started in around 1988 by discussing a collaboration with Alexander Glazer on phycobiliproteins, a family of fluorescent proteins from blue-green algae. However, these needed a separate partner protein to insert the chromophore, the part of the molecule responsible for its colour.

Why was the fluorescent protein of the jellyfish *Aequorea victoria* so useful?
In 1992, Douglas Prasher at the Woods Hole Oceanographic Institution cloned and sequenced the gene for GFP from *Aequorea victoria*.

Although he was unable to work on GFP any further himself, he was willing to give samples of its DNA to requestors, of which there were two: Martin Chalfie and me. Marty's lab discovered that GFP didn't need help from any other protein in the jellyfish, so GFP had both availability and autonomy. It has taken us almost 30 more years to engineer an easily expressible phycobiliprotein.

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Left to right: Nobel Prize winners Paul Krugman, Martin Chalfie and Roger Tsien with the then US president George W Bush

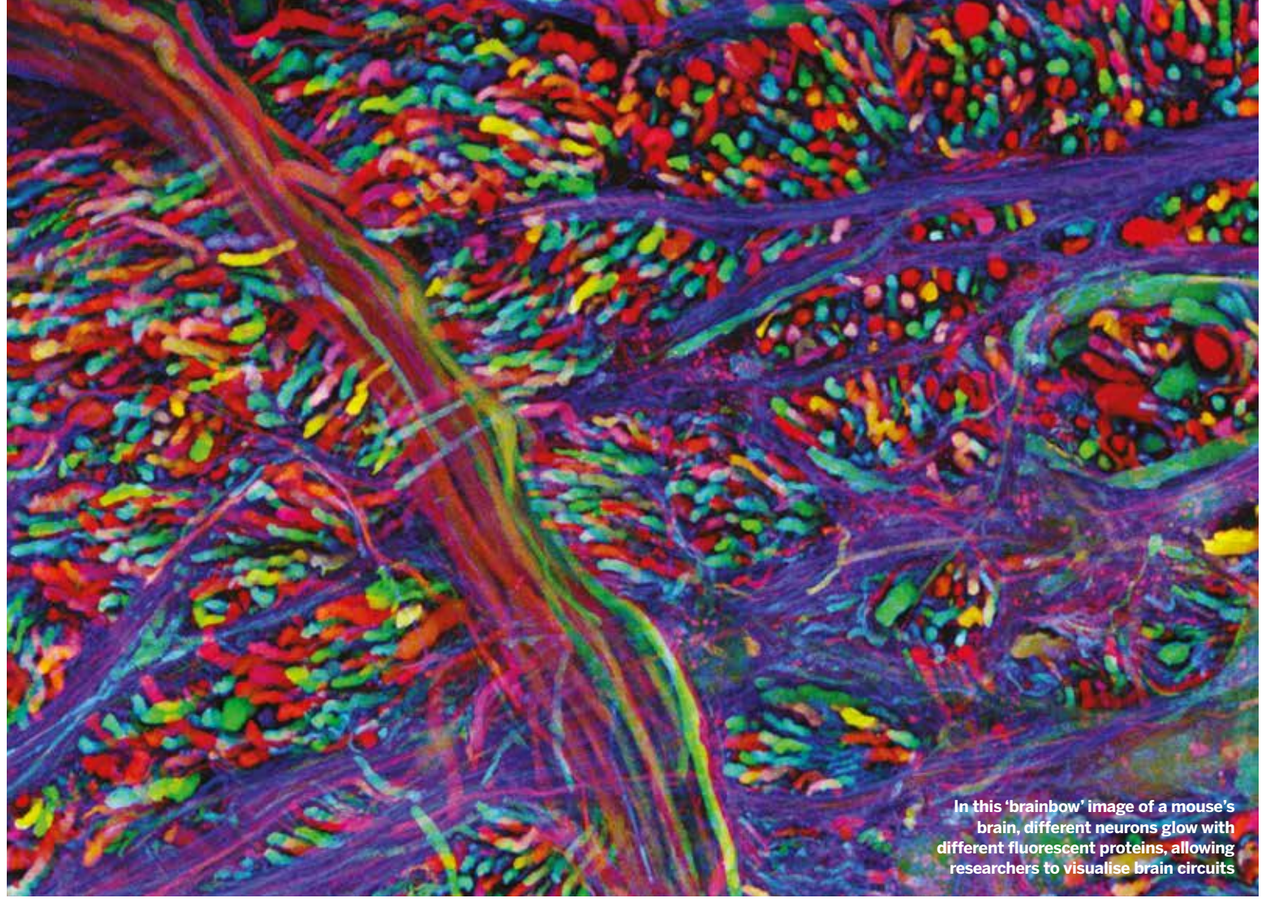
Did you ever imagine that GFP and its derivatives would be used by so many researchers in so many different ways?
I knew that an autonomously fluorescent protein module would be of immense value, but I didn't anticipate it would have quite so many uses.

Do you have a favourite way in which GFP has been used?
It was satisfying when we got a phenomenon called fluorescence resonance energy transfer (FRET) working between mutants of GFP. FRET senses the proximity of two fluorophores of different colours and had been a major goal when we set out. But that's now long in the past.

How else do you think fluorescence might be used in the future?
I can't foresee a limit to future applications of fluorescence. After all, fluorescence is an unusual and very useful property of a small proportion of molecules. Under the right circumstances it can be observed in anything from single molecules to oceans, over nanoseconds to many days, using the naked eye to the most sophisticated instruments.

Can you tell us a little about fluorescence assisted cancer surgery?
In cancer surgery, fluorescence guidance would be helpful because tumour tissue doesn't look any different from normal tissue under ordinary white light illumination. We want to use biochemical differences between the tumour and normal tissue to make the tumour fluorescent, so that the surgeon can decide where to cut with realtime guidance.

Unfortunately, one cannot use GFP or its homologues, because they can be linked to malignancy only by sophisticated gene therapy that is not practical yet or ethical in human patients. Instead, we are exploiting extracellular enzymes that are turned on in practically all solid malignant tumours. We have engineered fluorescent substrates that are triggered by these enzymes to enter cells and become trapped, and also to change colour by modulating FRET (the same phenomenon mentioned above). A small biotech company partly founded by me has just started a clinical trial with such molecules, together with the instrumentation for surgeons to see the fluorescence as they operate.



In this 'brainbow' image of a mouse's brain, different neurons glow with different fluorescent proteins, allowing researchers to visualise brain circuits

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What is GFP?

GFP stands for green fluorescent protein. It is a protein that glows green in the presence of UV or blue light, originally found in the bioluminescent and fluorescent jellyfish *Aequorea victoria*.

In 1992, the gene for GFP was sequenced by American biologist Douglas Prasher. The first to express the gene in another organism was Martin Chalfie, an American biochemist who shared the Nobel Prize with Tsien. He inserted the gene for GFP into the bacteria *E. coli* and nematode worm *C. elegans*. The resulting organisms then glowed green in the presence of UV or blue light.

The protein itself is a barrel shaped molecule with a colour producing 'chromophore' in the centre – it is thought just three amino acids in the protein chain create the fluorescent 'chromophore'. It is stable, non-toxic to most organisms when expressed in cells, and requires only UV/blue light and oxygen to emit its eerie glow, making it perfect for *in vivo* applications.

Green fluorescent protein has since been used in thousands of different ways. Replacing a gene with the gene for GFP can result in GFP being expressed in the organism only in the places where the original gene would have been expressed, creating a bright visual pattern of expression. By selectively labelling specific proteins, we can create images to see exactly where those proteins are present.

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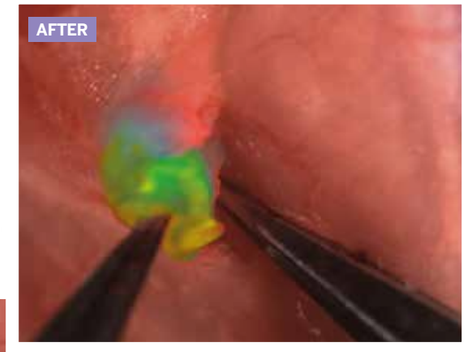

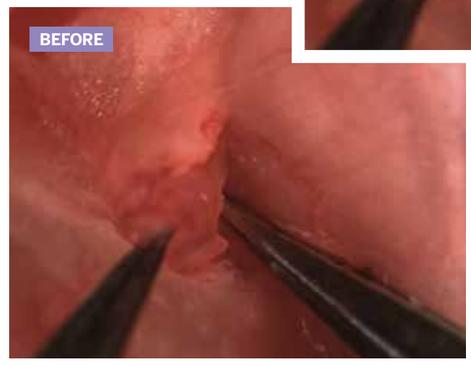

LIGHTING THE WAY
A matched pair of photographs showing a tumour about to be excised, viewed without and with the aid of tumour imaging peptides

DR JEAN LIVET / WWW.OLYMPUSBIOSCAPES.COM

What else is your lab working on at the moment?
We are trying to gather evidence for a new hypothesis for how and where the brain might store permanent memories at the molecular level[1].

Previous hypotheses have assigned the site of memory storage to be various proteins within synapses, the places at which neurons communicate with each other. The difficulty with these hypotheses is that proteins inside synapses undergo continuous rapid turnover and replacement, so that memories would require recopying very many times over an animal or person's lifetime.

Instead, we are looking at the glycoproteins (proteins plus carbohydrates) known to form a coating just outside synapses. We are accumulating evidence that this coating, once formed, is basically stable but can be locally remodelled to strengthen individual synapses – and thus serve as molecular substrates for memory.

You hold around 100 or so patents for various other biotechnology tools. Which are you most proud of?
In 1994, we started a biotech company called Aurora Biosciences to use new fluorescence assays to speed up drug screening in the pharmaceutical industry. One of the projects Aurora took on was to find drugs to help cystic fibrosis. Most experts thought Aurora's chances were negligible, as the market for such rare disease remedies was thought to be too small, and gene therapy was considered a much more promising approach. However, the Cystic Fibrosis Foundation backed Aurora's efforts, and fluorescence screening found the drug that was recently lauded by President Obama as an example of 'precision medicine'. Such a long time is required before one knows whether one has success or not.

Were you interested in science as a child?
I was always obsessed by pretty colours and by technologies that seem useful. One of my earliest memories is of a beach that had a zone of coarse pebbles surrounded by two zones of sand. I tried to lay down a bridge of sand across the pebbles to make crossing more comfortable for my tiny bare feet. Of course, the bridge would have been washed away by the next big wave or high tide. Perhaps that's a metaphor for much of my career.

You have a long tradition of engineering in the family. Do you consider yourself a chemist, biologist, bioengineer or what?
I'm a muddled mix. When I was applying for my first faculty position, several biology departments rejected me on the grounds that I was a chemist, and at least one chemistry department turned me down as too much of a biologist. Almost all my work has been involved with tool building, but I have never had a formal engineering course or appointment. Fortunately, most forward looking departments have now adopted a more flexible and interdisciplinary viewpoint. Personally, I don't care much for labels.

References
1) Tsien, R. Y. Very long-term memories may be stored in the pattern of holes in the perineuronal net. *Proc. Natl. Acad. Sci. USA* **110**(30), 12456–12461 (2013).

Roger Y Tsien is professor of pharmacology and professor of chemistry and biochemistry at the University of California, San Diego. After graduating from Harvard, Tsien also held posts at Cambridge and Berkeley. He is also a noted biochemical inventor who holds more than 100 patents. He shared the Nobel Prize in Chemistry in 2008 for his development of GFP.