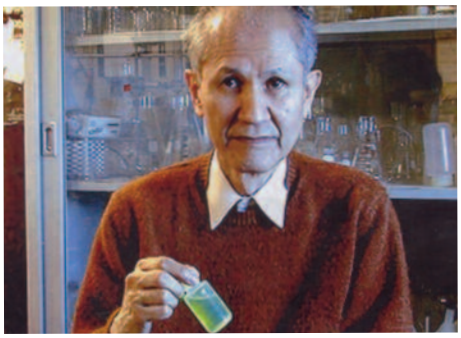

# Curious people

**Professor Osamu Shimomura**

**Nobel Laureate**

**Curious researcher in bioluminescence**

Age 89

Osamu Shimomura has dedicated his life to the study of bioluminescence, and in particular, one luminous jelly fish, *Aequorea victoria* (Figure 1)[1]. He discovered how this jellyfish produces green light, through two proteins, aequorin and the green fluorescent protein (GFP)[2,3,4]. His pioneering work, quite surprisingly, has revolutionised cell biology, biomedical research, and drug discovery, and created at least one billion dollar market. Aequorin is triggered to produce light when it binds calcium ($Ca^{2+}$). This means that by expressing its DNA in live cells, and even intact organisms, the concentration of free $Ca^{2+}$ can be monitored and imaged. Since $Ca^{2+}$ is a universal chemical switch in all animal, plant, and many microbial cells[5,6], this means that a fundamental property of all life can be studied while the cell or organism remains alive. In contrast, whenever the DNA coding for GFP, or a range of mutants or its relatives, genetically engineered, are expressed in a cell, the cell becomes fluorescent. So a cancer cell can be seen sloughing off a tumour and relocating to form a metastasis. Even organelles, such as mitochondria and the endoplasmic reticulum, and molecules, can be tagged with GFP, to be seen moving about in the cell. In 2008, Shimomura was awarded the Nobel Prize for Chemistry[7], with two other American scientists, Roger Tsien and Martin Chalfie.

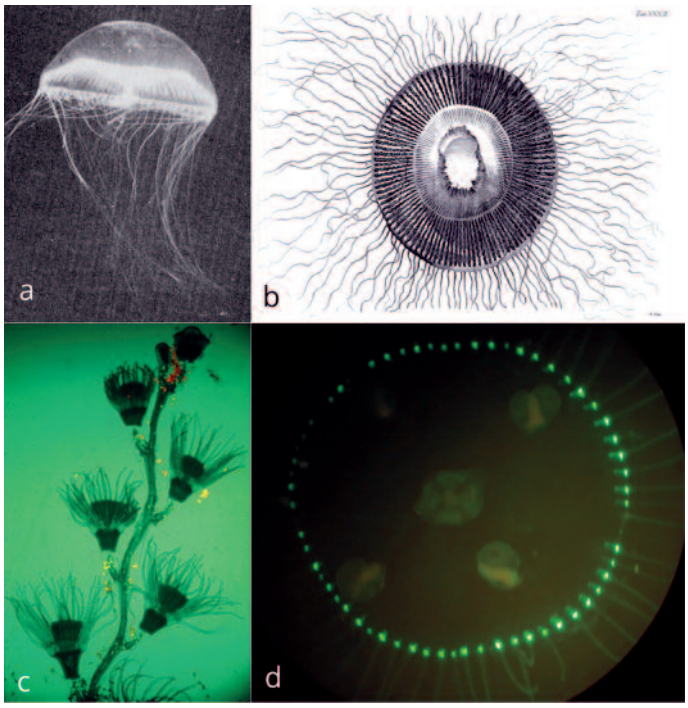


**Figure 1** *The luminous jelly fish and hydroid* Aequorea *and* Obelia

*(a)* Aequorea victoria *courtesy of Professor Steve Haddock; (b)* Medusa aequorea, *first described by Peter Forskål in 1775/1776, a pupil of Linnaeus; (c) The hydroid* Obelia geniculata, *courtesy of Welston Court Science Centre; (d) The jelly fish Obelia showing the photocytes through their GFP.*

Shimomura was born in Japan in 1928. He recounts an extraordinary story from his teens. He lived with his family in the town of Isahaya, where he went to school. But, he also had to work in a factory that repaired aeroplane engines. On 9th August, 1945, while sitting at his work stool, there was a flash of light, which temporarily blinded him and his colleagues, followed by a huge explosion. The atomic bomb had been dropped on the neighbouring town of Nagasaki! Amazingly he survived.

After graduating at Nagasaki Pharmacy School in 1951, in 1955 he began his first experiments with bioluminescence, in the laboratory of Professor Yoshimasa Hirata (1915 – 2000) at Nagoya University. The aim was to isolate the luciferin from the sea firefly, the marine ostracod *Cypridina,* now called *Vargula.* Then, after an invitation from Frank Johnson, another bioluminescence enthusiast in the USA, Shimomura moved to Princeton, USA. Johnson introduced him to the bioluminescent jellyfish *Aequorea,* explaining that the chemical reaction causing the light had yet to be revealed. So, in June 1961, they both headed for Friday Harbor, the famous marine laboratory on the west coast of the United States, where *Aequorea* were plentiful. At that time the dogma on bioluminescence, discovered by Dubois in 1887, and propagated by Newton Harvey at Princeton, was that it was caused by the oxidation of a small molecule, generically known as a luciferin, the reaction being catalysed by a protein known generically as luciferase. This had turned out to be the case with fireflies and the ostracod *Cypridina.* But, once Shimomura tried to extract a luciferin and luciferase from the jellyfish, it did not work! He tried extracting these in various ways, including different pH's. Something seemed to work a bit, but it was not convincing. Ready to finish for the day, and meet up with Johnson in the bar, he threw the remains of his jellyfish extracts into the sink. To his surprise, and delight, they resulted in a bright blue flash of light! Realising there was seawater in the sink, he then tried the various salts that make up sea water. He soon discovered that is was calcium that triggered the light emission.

Taking a large amount of crude *Aequorea* extract back to Princeton, in September 1962, he succeeded in purifying the protein that caused the light emission. He called this aequorin, naming it a photoprotein, as he discovered that the 'luciferin' was tightly bound to the protein, with oxygen. All you had to do was add calcium, and this triggered the chemiluminescent reaction at the protein's active centre. But, it only happened once, unlike the turnover of a luciferase, which produces a photon every time the luciferin is oxidised. During the purification of aequorin, he found that there was a green protein that came with it, perhaps a bit of a nuisance at first. He managed, eventually, to separate the two. The green protein turned out to be fluorescent, and was found in several luminous jellyfish and hydroids. Thus, Jim Morin, in his study of *Obelia,* named it the green fluorescent protein[8]. All it

does is shift the light emission from blue to green. Yet, this apparently obscure effect has revolutionised biomedical research and drug discovery.

During the years that followed, Shimomura studied many other bioluminescent animals[1], such as the freshwater limpet *Latia* and the fireflies, really flies, in New Zealand, the marine worm *Chaetopterus,* the decapod shrimp *Meganyctiphanes,* that forms the krill the some whales eat, the firefly squid *Watasenia,* the scale worm *Acholöe* with Marie-Tèrése Nicolas and Jean Marie Bassot, the luminous millipede *Luminodesmus,* and the brittle star *Ophiopsila,* and the puzzle of luminous mushrooms and other fungi.

In 1979, he, with others, finally discovered the precise structure of the chromophore in GFP, formed by cyclisation of three of its amino acids - serine, tyrosine and glycine at positions 65, 66, and 67 in the protein. This was similar to the cyclisation of phenylalanine, tyrosine and tyrosine in the formation of coelenterazine, the 'luciferin' bound tightly in the photoproteins aequorin and obelin. Shimomura named the lumiphore in these proteins coelenterazine, having discovered it in coelenterates (Phyla Cnidaria and Ctenophora). But, ironically, these organisms do not make coelenterazine. *Aequorea* at the Monterey aquarium, and *Obelia* or *Clytia* cultures, are not luminous unless fed coelenterazine. Only two groups of organisms have so far been discovered that synthesise coelenterazine de novo – decapod shrimp and copepods. In fact, it turns out that coelenterazine is responsible for the majority of bioluminescence in the sea, being found in at least eight phyla[9,10,11]. Interestingly, many non-luminous organisms also contain coelenterazine. As we all know fish do not eat apples! Vitamin C from fruit, and other sources, is required to protect us against oxygen toxicity. Coelenterazine is an excellent scavenger of toxic oxygen species, and so appears to be the vitamin C of many marine species.

In 1981, Shimomura moved to the marine laboratory at Wood's Hole, at Cape Cod, Massachusetts, on the east coast of the USA, where he worked until his formal retirement in 2002. He and his wife Akemi live in Falmouth, not far from Wood's Hole, still carrying out some chemistry in his garage.

This inspiring story is a wonderful example of how curiosity, asking the key questions, and carrying out the key experiments, can, quite surprisingly lead to major scientific breakthroughs. Long may this continue. Darwin was one of the first to describe the green bioluminescence of a jelly fish *Clytia,* in his Beagle zoology notebook. In fact, the first two entries are dinoflagellates off Tenerife. But he had a problem, highlighted in Chapter 6 of 'On the Origin of Species – Difficulties on Theory'[12]. He could not see how small change by small change could lead to a completely new phenomenon. In fact, all you need is 3-4 amino acids to create the necessary solvent cage for a new enzymatic reaction[12,13].

Was Shimomura's ground breaking work serendipitous? Good luck favours the brave, but also the observant. Shimomura tells us that his ground breaking work depended on guidance from his three mentors, some accidental happenings, the assistance of many people, the incredible development of science and techniques over his 60 year career, and humbly, his own efforts. As Bertram Russell (1872–1970), once wrote: 'In art nothing worth doing can be done without genius; in science even a very moderate capacity can contribute to a supreme achievement.' I would prefer to change 'very moderate' to 'collaborative. The story of GFP involves seminal contributions from many others, not the least from Milt Cormier's lab in Athens, Georgia, famous for its work on bioluminescence, and where Bill Ward carried out crucial work on GFP purification from *Aequorea* and the sea pansy *Renilla,* defining carefully many of GFP's key properties[14,15]. And it was Doug Prasher, from Milt Cormier's lab, who actually cloned the DNA coding for GFP[16]. Also Cormier's lab was one of the first to clone the DNA coding for aequorin. While I was on a lecture tour in the US in 1985, Milt showed me the aequorin protein sequence, not yet published, telling me 'its only biochemistry Tony'. This stimulated me to start work on genetic engineering of bioluminescence.

Osamu Shimomura's story has much to inspire the next generation. Bioluminescence and fluorescence has proved to be a wonderful educational tool[17]. Something we have exploited in the Darwin Centre in Wales (www.darwincentre.com and see article by Valerie Morse in this issue). As the sixteenth century pioneer of the Renaissance, Albrecht Dürer (1471–1528), wrote:

*'Be guided by Nature, and do not depart from it thinking you can do better yourself. You will be misguided, for truly art is hidden in Nature, and he who can draw it out possesses it.'*


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