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> ONE MONTH, ONE PROTEIN <

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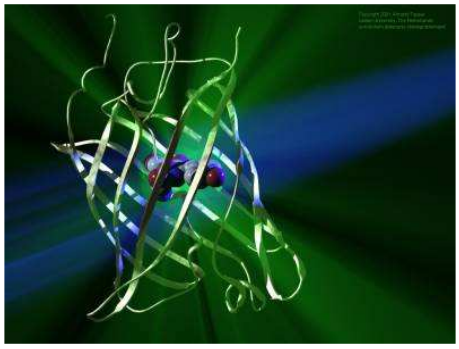
The greenest of us all

Vivienne Baillie Gerritsen

A bulb emitting light seems quite natural but…a jellyfish? As early as the first century, Pliny described the light of *Pulmo marinus*, now known as *Pelagia noctiluca*, a purple jellyfish. Bioluminescence is a spectacular phenomenon which predates by far the electric bulb and on which much research has been done since the 18th century. Jellyfish have been squeezed through cheesecloth, rubbed onto countless surfaces and submitted to electrical stimuli; all for the sake of a green glow. Jellyfish are not the only species to luminesce; corals, sea gooseberries, fish, bacteria, toadstools, plankton, fungus, glow-worms and many more also do. And the glow is not only green but for some, yellow, red, cyan or blue.



Green fluorescent protein

Courtesy of phantatomix.com

The Pacific Northwest jellyfish *Aequoria victoria* glows bright green thanks to a now highly popular protein: the green fluorescent protein or GFP. GFP is a spontaneously fluorescent protein, discovered quite by chance in the 1960s by Shimomura and his team. Prior to GFP, they had in fact extracted another luminescent protein – aequorin – which gives off a blue light in the presence of calcium. Yet the jellyfish give off a green light. How? In a subsequent step, GFP transduces, via energy transfer, the blue chemiluminescence of aequorin into green fluorescent light. And this is the glow that can be seen on the surface of seawater.

The structure of GFP was solved in 1996. It has a novel three-dimensional structure: the β-can. The overall shape is that of a barrel, with a diameter of 30Å and a length of 40Å. Eleven tightly fitted staves of β-sheet form the body of the barrel (hence its name) and an α-helix runs up the axis. The latter forms a scaffolding onto which the chromophore of the protein is held. Besides the beauty of its 3D structure, GFP protects the chromophore against photochemical damage and the passage of unwanted, diffusible ligands.

GFP makes green light out of aequorin's blue light. How does it produce its own green glow in the first place? The chromophore is a modified Ser-Tyr-Gly sequence. GFP can actually glow once this short tripeptide has adopted a cyclic conformation with the help of a little oxygen. And it does this with neither cofactors nor enzymatic components.

This is what tickled so many biologists' fancy: a molecule that can glow on its own. GFP has been expressed as a functional transgene and its use in cell, developmental and molecular biology is invaluable. Fluorescent GFP has been expressed in bacteria, yeast, slime mould, plants, Drosophila, zebrafish and mammalian cells. It can be tagged on to numerous other sequences without its own function being modified or that of the sequences. It has been used as a non-invasive marker in living cells, and can be used as a cell lineage tracer, a reporter of gene expression as well as a measure of protein-protein interactions. Numerous GFP mutants have been created: those that can tolerate more heat, those that give off a brighter glow or glow at a specific stage of cell development, those that are more photostable and so on.

Tobacco plants have been engineered in order to track introduced genes, and the recombinant tobacco plants glow green under ultraviolet

light. This kind of engineering could prove to be very useful to know whether a gene which has been inserted into a crop – say a gene which makes a plant resistant to a certain herbicide – stays within the crop and does not spread outside.

And with the green glow came 'Transgenic Art'. Early on this year, an American artist, Eduardo Kac, displayed the first transgenic rabbit: Alba. When Alba is exposed to blue light, she glows green. Kac argues that the rabbit is his way of getting involved in the debate on the ethics of gene technology. Though one could wonder what Alba thinks.

Why do animals and plants glow in the first place? Bioluminescence can take up to several percent of a cell's energy, so it must have an important role. Some believe it is used as a means of communication and could serve to attract prey, for example, or could even be used as a means of protection where a flash of light could scare off a predator. Another interesting hypothesis is DNA repair. In some bacteria, damaged DNA is repaired by an enzyme – a photolyase – which needs light to function. In the ocean depths, there is no light. And it may be that bacteria create their own light in order to repair their DNA.

Bioluminescence is also a military affair. In the First World War, soldiers used to smear their helmets with rotting wood – which is far better than wearing a jellyfish – to avoid running into each other. At sea, when plankton is disturbed, it glows, and satellites, ships or aircraft are used to detect the telltale luminescent trails of submarines. This is how the German submarine U-34 was destroyed on November 9th, 1918.

The uses of green fluorescent protein are many and varied. From a natural glow to molecular biology, from warfare to transgenic art…and even poetry:

The green gene
Christopher Dinesh Raj

Oh, fluorescent one!
From the time they cloned you
and the time, I first set my eyes upon you,
something drives us
to do something new with you.
You have been excited and emitted,
mutated to yellow, cyan and blue,
broken and permutated.
You have been driven with a promoter,
be it viral T7 or the brain's own L7,
been expressed in every cell type,
fused to the you-name-it gene at the N or C terminal.
Confused, I am.
The cells don't mind you,
and the Ni-Nta columns will bind you,
provided you have been His-tagged,
only to make you pure.
You have been glorified here,
and stolen there,
still you shine like an emerald in mother nature's crown.
Allow me to make a new construct out of you,
mutate and transform you,
shine some blue light upon you,
and I am certain my peers will turn green.
Yes, green with envy,
because My GFP is brighter.

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