

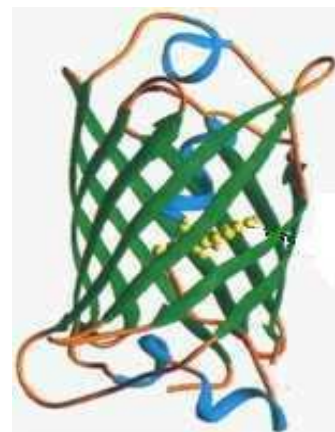
## The Structure of the Green Fluorescent Protein from the Jellyfish, *Aequorea*

**Abstract:** The Green Fluorescent Protein, GFP, from the jellyfish *Aequorea*, has a sequence of 238 amino acids. This protein folds into a structure that resembles a can or barrel. Inside this barrel is the region called the fluorophore, which is responsible for the reaction that results in producing the bright green glow around the outer edge of the bell of *Aequorea*. Scientists have created GFP proteins with changes in its amino acid sequence, which results in new proteins that can emit yellow, blue or cyan light.

### Introduction:

In 1996, Yang and his colleagues elucidated the structure of GFP. They found that GFP was composed of a sequence of 238 amino acids, and forms an 11-stranded beta can structure (Figure 1). This “can or barrel” structure is unique to GFP and has not been observed in other proteins studied.

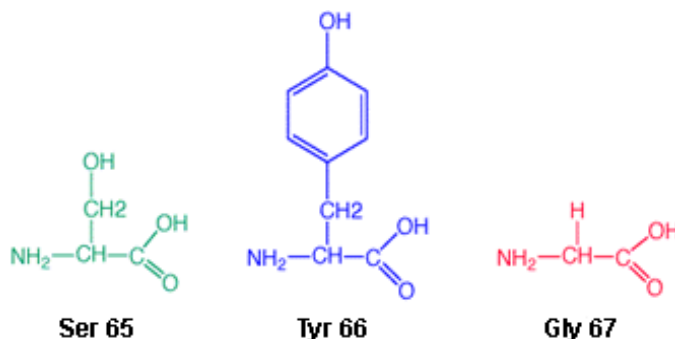
Figure 1: Ribbon diagram of the green fluorescent protein (GFP) drawn from the wild-type crystal structure. The arrows represents the 11 anti-parallel beta strands that form the very compact cylinder. The buried chromophore, which is responsible for GFP's luminescence, is shown in atomic detail in the center.



### The active region, the fluorophore:

In the center of this compact barrel is the region of the protein that is responsible for the chemical reaction that results in the emission of the visible green light. This region is called the chromophore. The chromophore consists of three consecutive amino acids (Figure 2) serine at position 65 (Ser65), tyrosine at position 66 (Tyr66), and glycine at position 67 (Gly67).

Figure 2: Structure of the 3 amino acids that make up the active chromophore in GFP.



After GFP is translated and produced inside the photocells of the jellyfish, it undergoes a spontaneous chemical change (Figure 3), resulting in the formation of alternating double bonds between the three critical amino acids mentioned above (Figure 4). The fluorophore region is the active site of the protein.



Figure 3: The 3 amino acids forming the chromophore in GFP undergoes oxidation and cyclization.

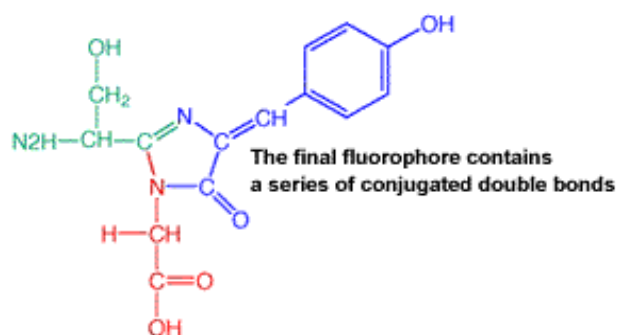


Figure 4: The formed active chromophore contains conjugated double bonds. These double bonds store and release the energy from electrons.

How is green light produced by the active site especially when the jellyfish is in the depth of the ocean? First, blue light is given off by another chemical reaction involving the protein aequorin inside the jellyfish (Figure 5). This blue light is absorbed by the conjugated double bonds of the chromophore resulting in the release of energy in the form of a visible green light.

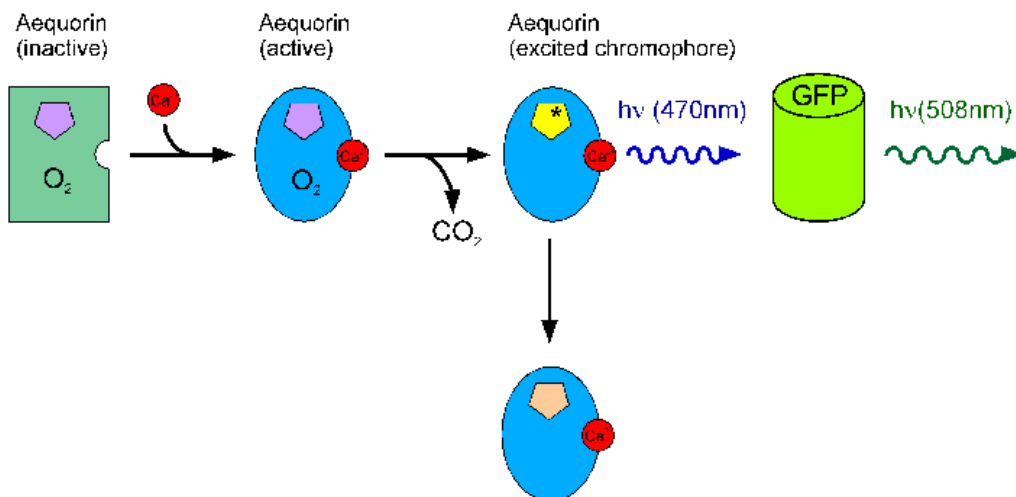


Figure 5: The protein aequorin in an excited state absorbs energy from light of wavelength 470nm. That energy is transferred to GFP and absorbed by the double bonds in GFP's chromophore. GFP releases that energy in the form of green fluorescent light at 508nm.

(<http://public-1.crysl.bbk.ac.uk/PPS2/projects/jonda/intro.htm>)

Since GFP is activated by energy in the form of light at a higher wavelength and produces energy in the form of light at a lower wavelength, it is an example of a fluorescent reaction and technically not a bioluminescent reaction (see glossary).

#### Changes in GFP results in the release of new colors:

Changes in the amino acids in the fluorophore region, as well as in amino acids downstream from the fluorophore, result in changes in the color the fluorophore emits. The biotechnology company, Clontech Inc., has isolated three GFP variants (blue BFP, yellow YFP and cyan CFP).

The amino acid substitutions of the GFP variants are shown below the wildtype (wt) GFP sequence. Numbers in the top line indicate the position of the amino acid in the protein sequence. If no amino acid is listed for the position in the variants, then the amino acid of the wild type is retained in that position.

| Color  | Amino acids | 64  | 65  | 66  | 67    | 68    | 69    | 72    | 145   | 146   | 153   | 163   | 203   |       |       |       |       |       |       |       |
|--------|-------------|-----|-----|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Green  | wtGFP       | --- | Phe | Ser | Tyr   | Gly   | Val   | Gln   | ---   | Ser   | ---   | Tyr   | Asn   | ---   | Met   | ---   | Val   | ---   | Thr   | ---   |
| Green  | EGFP        | --- | Leu | Thr | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| Blue   | EBFP        | --- | Leu | Thr | His   | ----- | ----- | ----- | ----- | ---   | Phe   | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| Yellow | EYFP        | --- | Phe | Gly | ----- | ---   | Leu   | ----- | ---   | Ala   | ----- | ----- | ----- | ----- | ---   | Tyr   | ---   | ----- | ----- | ----- |
| Cyan   | ECFP        | --- | Leu | Thr | Trp   | ----- | ----- | ----- | ----- | ---   | Ile   | ----- | ---   | Thr   | ---   | Ala   | ----- | ----- | ----- | ----- |

EGFP is an enhanced GFP, which results in a more enhanced fluorescent product in mammalian systems. Now several of these GFP colors can be used in combinations as reporter genes to learn about protein interactions, timing of cell cycle events, and much more!

References:

<http://www.npaci.edu/online/v4.14/gfp.html>

Structure of GFP. Note - The image of *A. victoria* is probably a bright field; not bioluminescence.

[http://photoscience.la.asu.edu/photosyn/courses/BIO\\_343/lab/gfp.html](http://photoscience.la.asu.edu/photosyn/courses/BIO_343/lab/gfp.html)

GFP structure image and includes the amino acid structure of the chromophore.

<http://public-1.cryst.bbk.ac.uk/PPS2/projects/jonda/structur.htm>

Short discussion of GFP folding structure and good figures.

<http://public-1.cryst.bbk.ac.uk/PPS2/projects/jonda/intro.htm>

Good figure and explanation of how aequorin and GFP transfer light energy.

<http://www.nsf.gov/od/lpa/news/press/00/pr0034.htm>

The most recent work by Shimomura on aequorin. Very nice summary of work.

[http://pantheon.cis.yale.edu/~wfm5/gfp\\_gateway.html](http://pantheon.cis.yale.edu/~wfm5/gfp_gateway.html)

GFP applications in cell biology, developmental biology and neurobiology.

**aequorin**

A protein found in the jellyfish *Aequorea* that is activated by calcium and emits blue light through a bioluminescent reaction. This blue light is then absorbed by GFP, stimulates a chemical reaction which results in the emission of a visible green light. Both aequorin and GFP are found in high concentrations in the light producing cells on the periphery of the medusa of this jellyfish.

**bioluminescence**

A complex chemical reaction that occurs within a living organism, in which the end product of energy is released in the form of light instead of heat. Some organisms which have this ability are glowworms, fireflies, jellyfish, fungi, and some deep-sea fish.

**chemical reaction**

Any change that alters the chemical properties of a substance, or forms a new substance. During a chemical reaction, reactants are changed into products.

**chemiluminescence**

A general term for the production of light when the excitation energy comes from a chemical reaction. Bioluminescence is a type of chemiluminescence that occurs in a living organism.

**electromagnetic waves or radiation**

Are made up of oscillating electric and magnetic fields that can travel through many substances. Particles that make up the waves are called photons. They consist of many frequencies. At the high frequencies are the gamma rays and X rays, followed by ultraviolet, then the visible light. At the lower frequencies are the infrared radiation waves and microwaves. Within the lower frequencies are the radio waves.

**fluorescence**

A phenomenon shown by certain substances when they are hit by ultraviolet radiation. The substance absorbs high frequency wavelengths and emits it at a lower frequency light. This emission stops as soon as the high frequency radiation is removed. For example, GFP absorbs the higher frequency blue light emitted by aequorin, undergoes a chemical reaction, and emits the lower wavelength green light.

**green fluorescent protein (GFP)**

A protein found in jellyfish that fluoresces, or emits a green visible light when excited by UV light with a wavelength of 395 nanometers. It can function as a biological marker when co-expressed with other proteins. The structure of the protein is cylindrical with the glowing component, an amino acid complex called a *fluorophore*, in the middle of it.

**light**

Consists of electromagnetic waves of specific frequencies and wavelengths. Light is often drawn as a ray. The ray shows the path taken by the light waves, the direction in which the energy is being carried.

**lysis (to lyse)**

The destruction of a cell's membrane or wall, which breaks open the cell, spills its contents, and kills it. The lysate is the contents that are collected from the lysed cell.

**ultraviolet radiation (UV)**

Electromagnetic radiation produced by the sun and or produced when an electrical current passes through ionized gas between two electrodes. It consists of wavelengths between 200 and 400 nanometers. Exposure to excessive amounts of UV radiation damages DNA and can cause health problems such as skin cancer and cataracts in the eyes.

**visible light**

Electromagnetic waves or radiation that is produced by the sun and detectable by our eyes. It consists of wavelengths between 400 and 750 nanometers. Colors depend on the wavelength lengths; a short wavelength (the 400 nm side) looks blue and a long wavelength (the 750 nm side) looks red.