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## Green Fluorescent Protein: discover, structure, purification, applications, and future prospects

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### Abstract

Green fluorescent protein (GFP) Firstly discovered from the jellyfish *Aequorea victoria* and it was widely used in biological science as a universal fluorescent tag. Many scientists have focused their efforts on the identification and development of fluorescent proteins with novel characteristics and enhanced properties, to be used for visualization of structural organization and dynamic processes in living cells. The discovery of green fluorescent protein (GFP) followed by purification and elucidation of its molecular structure introduced a new and promising marker for biological studies. GFP is intrinsically fluorescent, has low toxicity, pH and heat stability and is easily imaged and quantified. This review article focuses on the discovery, diversity of fluorescent patterns and colors, other fluorescent protein, purification, structure, properties, and numerous applications of GFP in biological research such as *in vivo* imaging of cancer, promoter and protein fusion constructs, biosensors, *Ex vivo* imaging, and imaging the efficacy of therapeutics.

**Keywords:** Green Fluorescent Protein, Aqueous two-phase system, Hydrophobic Interaction Chromatography, cancer imaging, biosensor.

### 1. Introduction

Fluorescence is the occurrence when a substance emits light after having previously absorbed (excited by) light or other electromagnetic radiation. It is a dazzling interplay between light and matter and a particular type of luminescence. Typically, the light that is released longer wavelength and lower energy than the light that was absorbed. However, it is also conceivable for emission to occur at a wavelength that is shorter (as in the case of two-photon absorption) or that is the same as the irradiation (resonance fluorescence). There are three main steps in the fluorescence generating process: (a) the incoming photon excites a susceptible molecule (a process that takes femtoseconds to complete); (b) the vibrational relaxation of

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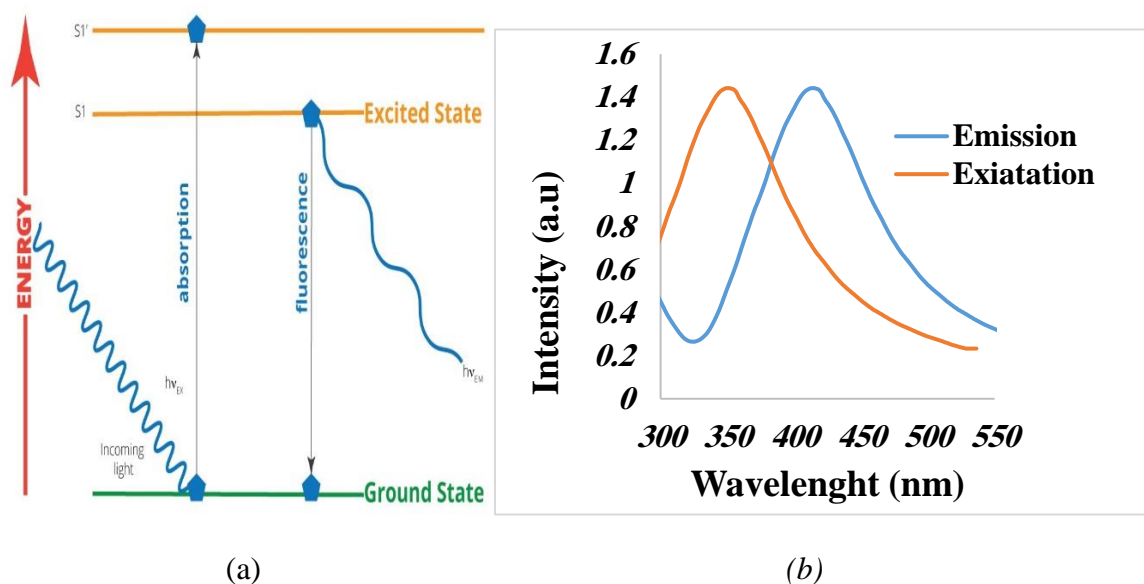
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excited state electrons to their lowest energy level (a process that takes picoseconds to complete); and(c) the release of a photon with a larger wavelength as the molecule enters its ground state (occurring) based on nanoseconds [1].

Fluorescence can be observed when the emitted light is in the visible spectrum and the absorbed radiation is in the ultraviolet (UV) region of the electromagnetic spectrum, which is invisible to the human eye. This gives the fluorescent substance a distinctive color that can only be seen when exposed to UV light. Although fluorescence is invisible to the human eye, it can be detected using a UV with a wavelength between 320 and 390 nm, 365 nm being the most popular [2].

Various fluorophore compounds, as shown in Fig. 1, absorb light at a specific wavelength and then re-emit it at longer wavelengths.



**Fig.1.** (a) The fundamental idea of fluorescence is represented by a Jablonski diagram, which shows the changes in energy state that cause "fluorescence" and non-radiative decay to the ground state. (b) Alexa 488's red-shifted emission profile and excitation.

Biofluorescence incident when short wavelengths of light (ultra-violet or blue light) are absorbed and then reemitted as higher wavelengths in living organisms, upshot in fluorescent colors including blues, greens, and reds. This phenomenon is prevalent among animals, being the most within the cnidarians, arthropods, and cartilaginous and ray-finned fishes. Biofluorescence is common and phenotypically diverse in marine lineages, notably cryptically patterned, well-camouflaged coral-reef lineages, and that it is widespread throughout the fish family tree [3]. Biofluorescence can begin as a result of either biotic (such as sunlight) or

abiotic sources of light. A family of natural small molecule fluorophores that significantly contribute to shark skin biofluorescence, these small molecule metabolites represent a parallel bromo-tryptophan-kynurenine biosynthetic pathway to that of the established tryptophan-kynurenine pathway widely found in vertebrates. The specific mechanisms causing biofluorescent light to be produced in most animal lineages are unknown but can include proteins like green fluorescent protein, pigments, metabolites, or mineralization. Numerous studies have demonstrated that biofluorescence occurs in response to ultraviolet and visible blue light excitation [4]. The observed fluorescence is an intrinsic, the *Glaucomys* flying squirrels have a feature that could be important to the environment. To explain the existence of fluorescence in *Glaucomys*, put up four theories: Fluorescence is particularly significant on landscapes covered in snow, 2) the characteristic is adaptive in the squirrels' peculiar nocturnal-crepuscular light environment, 3) fluorescence is used in intraspecific communication, and 4) fluorescence plays a role in antipredator behavior. These explanations do not always conflict with one another [5].

### 1. Discovery of Green Fluorescent Protein

Shimomura's task was to identify the bioluminescent system in *Aequorea victoria*[6]. The first protein in charge of the fluorescence was isolated and purified from *A. victoria* and given the name aequorin. Contrary to the greenish luminescence seen in the light-emitting tissues of *A. victoria*, the purified aequorin emits bluish light. Shimomura subsequently proposed that *A. victoria* contains yet another "green protein. Aequorin, the light-emitting tissue of *A. victoria*, and this new "green protein's" respective emission spectra were then acquired by his team after they had successfully extracted and purified the "green protein" [7].

The luminescence spectrum of aequorin was broad, with a peak at 460 nm. The fluorescence spectrum of the GFP was sharp, with a peak at 508 nm. Apparently, the light organs of the jellyfish contain these two proteins, aequorin and the green protein, of which the aequorin emits blue light in the presence of  $\text{Ca}^{+2}$  and the GFP emits green fluorescence when excited. The green protein was later called green fluorescent protein, GFP [8].

Finally, they determined that the fluorescence was produced by this "green protein" as a result of aequorin absorbing bluish light. At the time, it was unknown what mechanism mediated the energy transfer from aequorin to GFP. This was clarified in 1974 and indicated that the energy transfer from aequorin to GFP was mediated by Förster resonance energy transfer (FRET). The blue light from the aequorin was quickly absorbed by GFP, resulting in

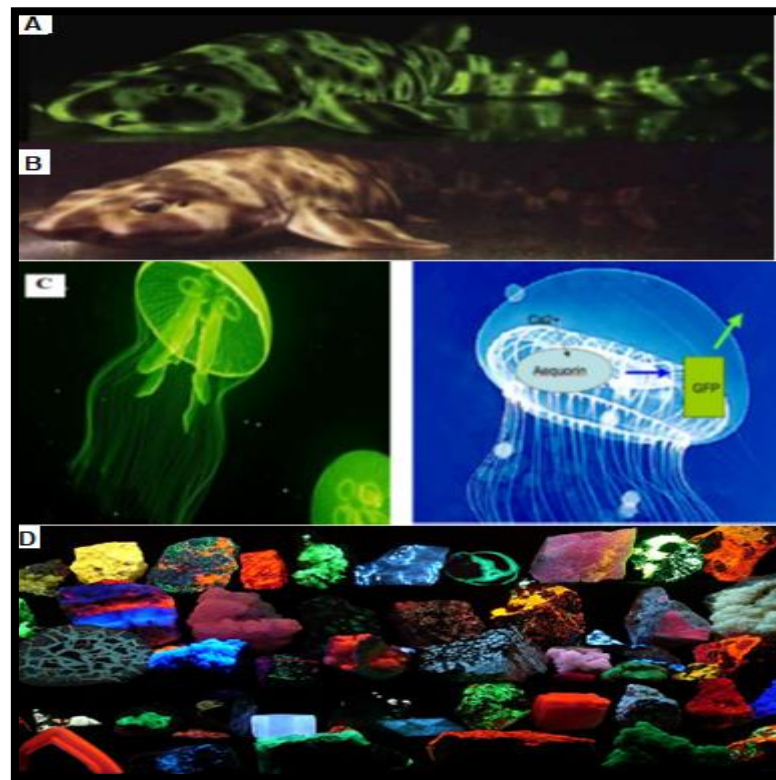
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its distinctive fluorescence [9]. In the early decades following its discovery, GFP did not garner much attention. Not until Douglas Prasher and his team used GFP as a fluorescent tag to report gene expression in 1994 [10], did GFP evolve into the crucial research tool it is today.

GFP's function in nature is to change the color of aequorin's blue bioluminescence from blue to green. Such a shift decreases light scattering, increases light throughput through water, and improves quantum efficiency. Later, Shimomura and his team was able to isolate and purify the green protein and then obtain the emission spectra of both the novel "green protein" and aequorin from the light-emitting tissue of *A. Victoria* [6].

After several years of strenuous efforts, Shimomura and Johnson had able to uncover a large part of the intramolecular chemistry involved in the luminescence of aequorin, including the chemical structure of the functional moiety "coelenterazine" in the protein and also the means to regenerate spent aequorin into the original, active aequorin [11, 12, 13]. During the same period, green fluorescent proteins similar to Aequorea GFP were found in a number of other bioluminescent coelenterates [9, 14, 15, 16].

Other creatures such as corals, sea anemones, zoanithids, copepods, and lancelets have been reported to express GFP [17]. The majority of lancelet species have been shown to produce GFP in various parts of their bodies. Numerous species of marine copepods, particularly those from the Pontellidae and Aetideidae families, have been shown to contain GFP-like proteins [18]. Sharks produce biofluorescence, a green glow that results from the water's blue light being absorbed and converted into green light. These sharks are lazing on a seafloor covered in a dense population of microorganisms [19].



**Fig.2.** Diversity of fluorescent patterns and colors; Female swell shark (*Cephaloscyllium ventriosum*) imaged under: A;fluorescent lighting; B; white light,C;Bioluminescent jellyfish andD;Fluorescent minerals and rocks under UV light.

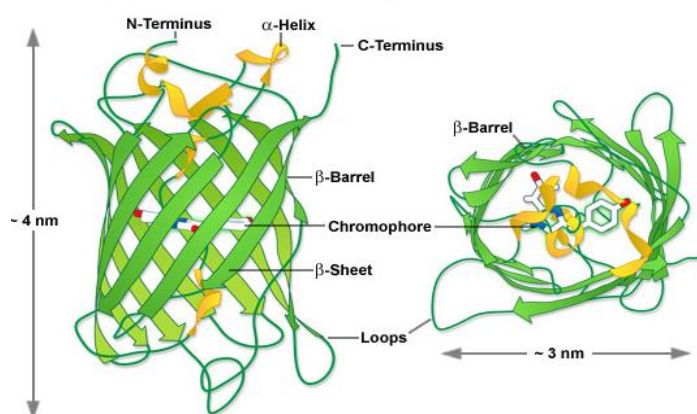
## 2. Structure of GFP:

GFP in its wild-type form has a molecular weight of 26.9 KDa and 238 amino acids. It is made up of 11  $\beta$ -sheets that form a barrel-like structure and an  $\alpha$  helix that runs diagonally through the barrel. The  $\alpha$ -helix contains the chromophore (color carrier) of GFP, which is formed from the side chains of three amino acids as the protein folds. Core motif of the GFP is a *p*-hydroxybenzylidene-2,3-dimethylimidazolinone (*p*-HOBDI) chromophore, that formed from Ser-65, Tyr-66 and Gly-67 react and link via covalent bonds and by a network of hydrogen bonds between the side chains to form the chromophore (**Fig. 3**). The GFP beta barrel's interior exhibits unique polarity. On one side of the central helix, there is an internal cavity containing four water molecules, whereas the other side has a collection of hydrophobic side chains, which is more characteristic of a protein core. The GFP chromophore is stabilized by interactions with a number of polar side chains. Three of them (His 148, Thr203, and Ser205) create hydrogen bonds with the chromophore's phenolic hydroxyl group. Arg96 and Gln94

interact with the carbonyl group of the imidazolidone ring, these stabilizing hydrogen bonding interactions with the chromophore [20].

GFP has two absorption bands, centered around 395 nm and 480 nm, that are associated with the neutral and deprotonated anionic forms of the chromophore, respectively. The anionic chromophore (p-HBDI) is what emits the characteristic green light at 509 nm. It has the largest absorbance peak at 395 nm, followed by a lesser peak at 475 nm. When stimulated at 395 nm, the maximum emission is at 508 nm [21].

GFP is membrane permeant, can make any protein as a fusion protein and generally does not obstruct the functionality of the molecule to which it is bound. GFP is relatively nontoxic to the cells as the same way as synthetic fluorophores and dyes because the fluorophore is buried with the  $\beta$ -barrel of the GFP. It is stable in environments of pH 5-12, in the presence of proteases and oxidants, and at temperatures as high as 65° C [22].

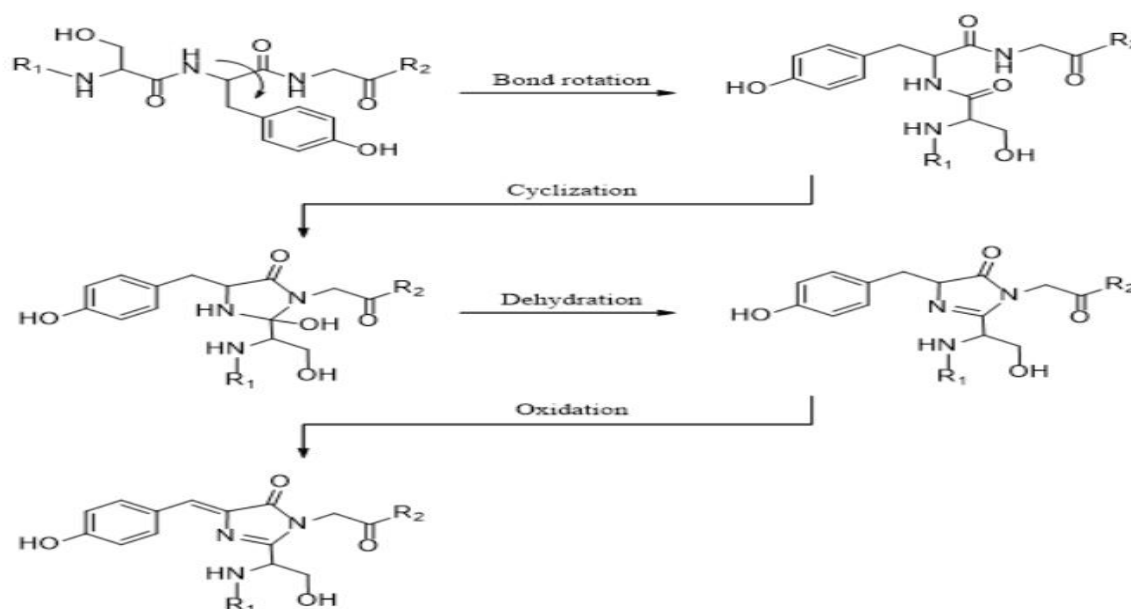


**Fig. 3.** GFP's crystal structure as seen from the side (left) and axially (right). A ball-and-stick representation of the chromophore is displayed.

### 3.1. Maturation of GFP

GFP undergoes maturation, a post-translational modification (PTM), of its chromophore to acquire fluorescence rather than relying on external cofactors. The formation of Chromophore maturation is the result of a sequence of chemical processes starting with nucleophilic cyclization, followed by dehydration and atmospheric oxygen oxidation, and is completed by dehydration of the S65 peptide bond, leaving a conjugated p-hydroxybenzylidene imidazolidone ring system that fluoresces green in blue light as stated by Banerjee *et al.*, (2017)[22].

Figure 4 illustrates the mechanism, the original triplet of amino acids first. The migration of molecular oxygen into the active site of the closed beta barrel is the gradual process of chromophore development. Particularly Arg96, which stabilizes the enolate form of intermediate 1 by creating a salt bridge with the negatively charged oxygen atom, and Glu222, which accepts protons from the water molecules to cycle between the protonated and deprotonated states, the side chains surrounding the chromophore are essential for stabilizing the intermediates in the chromophore maturation process [23]. The Tyr66 alpha-beta carbon double bond causes the chromophore's two coplanar aromatic rings to adopt the cis configuration. Short wavelength light causes the chromophore to isomerize to the trans form, along with distortion of its planar geometry and surrounding side chain packing, which results in photobleaching, the light-induced loss of fluorescence. For GFP and other fluorescent proteins, this kind of photobleaching appears to be a slowly reversible process [20].



**Fig. 4.** Maturation mechanism of GFP chromophore.

### 3.2. Fluorescent protein mutants

Although wild Green fluorescent protein (wGFP) is one of the most popular fluorescent proteins because of its brightness and high photostability, it has two main disadvantages; pH sensitivity and a slight tendency to dimerize at concentrations above 5 mg/ml. Mutations at critical positions where non-polar amino acids are replaced by hydrophilic ones can reduce this dimerization, so GFP mutants were developed with increased brightness, stability, and emission spectra. GFP variants including EGFP (enhanced green fluorescent protein), BFP (blue fluorescent protein) YFP (yellow fluorescent protein), CFP (cyan fluorescent protein) (Table



1). The first mutated version of wGFP is the S65T mutant (EGFP) developed by Roger Tsien et al. was five times brighter than the original and showed a shorter maturation time. Enhanced Blue Fluorescent Protein was constructed by several rounds of mutating wtGFP. Y66H skipped the emission peak from the green to the blue spectrum [24].

The critical cyan derivatives mutation is the Y66W substitution, which causes the chromophore to form with an indole rather than phenol component. The red-shifted wavelength of the YFP derivatives is accomplished by the T203Y mutation and is due to  $\pi$ -electron stacking interactions between the chromophore the substituted tyrosine residue [2]. These two classes of spectral variants are used for Förster resonance energy transfer (FRET) experiments.

**Table (1):** characteristics of several GFP mutants. EGFP (enhanced green fluorescent protein), BFP (blue fluorescent protein) YFP (yellow fluorescent protein), CFP (cyan fluorescent protein), and are examples of fluorescent proteins.

Mutant	Mutations	Excitation wavelength (nm)	Emission wavelength (nm)	Quantum yield
0Wild type	-	395 (470)	509	0.77
EGFP	S65T	484	510	0.70
BFP	Y66H Y145F	380	440	0.20
YFP	S65G, V68L, S72A, T203Y	512 (498)	529	0.63
CFP	T66W	439 (453)	476 (501)	0.24

### Nomenclature of modified GFPs

The nomenclature of modified GFPs is often confounding due to the overlapping mapping of several GFP versions onto a single name. For example, mGFP often refers to a GFP with N-terminal palmitoylation that causes the GFP to bind to cell membranes. While, the same name is used for monomeric GFP, which is often achieved by the dimer interface breaking A206K mutation [25].

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## New class of GFPs

In 2016, a phycobiliprotein called allophycocyanin from the cyanobacterium *Trichodesmium erythraeum* gave rise to a novel family of fluorescent proteins known as small ultra red fluorescent protein (smURFP). Without the use of an external protein called a lyase, smURFP integrates the chromophore biliverdin autocatalytically. The diminutive ultra-red fluorescent protein, which is extraordinarily photostable, is utilized to construct biosensors and is visible in living mice. It is biophysically as bright as enhanced green fluorescent protein. *In vitro* protein diagnostics with attomole (10<sup>-18</sup> M) sensitivity, viral particle encapsulation, and fluorescent protein nanoparticles are a few novel uses for smURFP. However, the availability of biliverdin restricts the fluorescence of fluorescent proteins that bind to biliverdin; hence, additional biliverdin is required to increase brightness [26].

### 4. Purification of green fluorescent protein:

The GFP has been purified using a variety of chromatographic processes to reach a high level of purity. These chromatographic techniques typically require multiple steps, lengthy processes, and complex operations, which raises the cost of purification.

#### 4.1. Aqueous two-phase system (ATPS)

Because of the necessity for a low-cost GFP purification approach is critical, the aqueous two-phase system (ATPS) has received a lot of attention. One of the ATPS family's most promising members is the alcohol/salt ATPS [27]. Alcohol/salt ATPS has a number of benefits, including affordability, rapid phase separation, straightforward operational processes, and simple scale-up. Additionally, this type of ATPS is environmentally friendly because salt and ethanol may be recycled using regular procedures [28].

The purification of GFP is a two-step process; ethanol-based protein extraction into the organic phase, followed by a second hydrophobic interaction chromatography (HIC) step to recover the protein into the aqueous phase. Ammonium sulphate and tert-butanol are combined in the three phases partitioning method to precipitate proteins from their crude extracts. Protein precipitates into the interphase between the organic and aqueous phases because of t-butanol's binding to hydrophobic GFP regions in the presence of a lot of ammonium sulphate. However, in this instance, HIC-affinity chromatography is required to further fractionate the protein. The second method is a two-step process that involves precipitating the proteins from the polyethylene glycols (PEG) phase using free Zn<sup>2+</sup> after a two-phase aqueous extraction

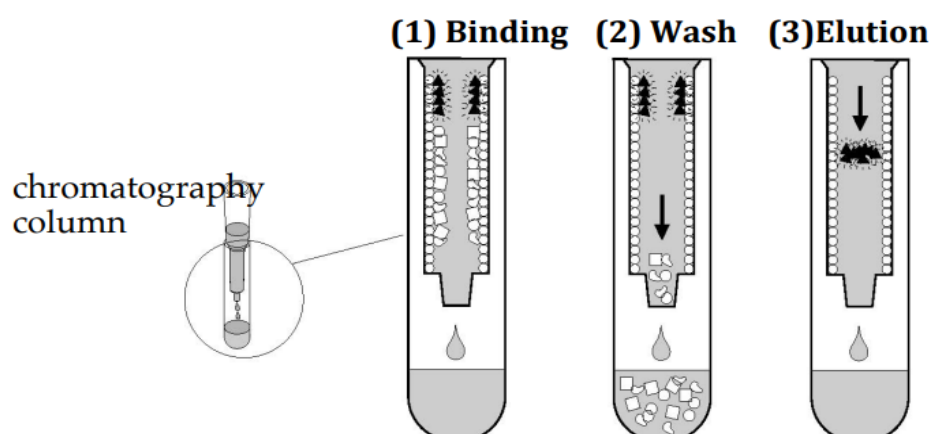
using PEG/salt. The method's drawback is how challenging it is to remove PEG from the final protein formulations [29].

The major characteristics shared by all members of the fluorescent protein family, such as their compact structure and moderately hydrophobic protein surface, which ensures their stability in solutions of organic solvents, form the foundation of this technique's adaptability. In each step GFP extraction efficiencies should be identified, and their purification capabilities should also assess. To characterize the fluorescence of GFP, both gel-based imaging and the spectrofluorometric approach can be used [30].

#### 4.2. Hydrophobic Interaction Chromatography (HIC)

The GFP has a surface that is noticeably higher in hydrophobic amino acids than that of most proteins. As a result, it will bond to a solid that likewise possesses a hydrophobic surface more firmly than most other proteins do thanks to the hydrophobic effect. Selectively bind and elute GFP using the hydrophobic effect's additional significant characteristic. The hydrophobic effect is amplified in solutions with a high salt content, while it is diminished in solutions with a low salt concentration. Use ammonium sulphate  $((\text{NH}_4)_2\text{SO}_4)$  instead of other salts because it is highly soluble in water and won't affect most proteins, it turns out [31].

The solid substance known as the "resin" will be tightly packed inside a tube known as a chromatography column to simplify the procedure' execution. In Fig. 5, circles represent the hydrophobic solid resin, black triangles represent GFP, and other forms represent different molecules:



**Fig. 5.** Hydrophobic interaction chromatography column.

Equilibration: To get the column ready for chromatography, a solution with the same buffer as Step (1) is passed across it.

1) Binding: A high salt buffered solution containing the cell's contents, including GFP, is passed through the column. Most other molecules do not bind via the hydrophobic effect, but GFP does. Those that pass through the column and don't stick are discarded.

2) Wash: The column is run through a low-salt solution. Because GFP contains more hydrophobic amino acids on its surface than any other molecule, it stays bound. Other molecules wash away. The washing machine discards the molecules.

Elution: running a solution devoid of salt through the column. GFP falls off the column in reasonably pure form for examination since the hydrophobic contact between the column and GFP is broken down by this.

### **5. The multiple applications of GFPs:**

GFP has been regarded as a valuable tool in the field of biology and biotechnology [32]. GFP's distinctive spectral and physiological characteristics results from changes to amino acids. Heat, photobleaching, an alkaline pH, organic salts, and a variety of proteases cannot harm GFP. Autofluorescence, no fixation required, no requirement for a co-factor non-invasive, frequently noticeable, simple to measure, and capable of in vivo expression. These characteristics have significantly influenced biochemical applications, transcription studies, and cell biology by making it possible to image practically any protein, as well as by acting as a reporter gene. In intact cells, GFP is a recognized marker for protein targeting and gene expression. It is widely utilized in biological research as genetically encoded fluorescent markers that produce high fluorescence when the protein structure is properly altered [33,34].

### **Fluorescence microscopy**

Fluorescence microscopy has been completely redesigned as a result of the accessibility of GFP and its derivatives in cell biology and other scientific sciences. Fluorescent proteins like GFP are often far less damaging when lit in living cells, whereas the majority of tiny fluorescent compounds, such as FITC (fluorescein isothiocyanate), are extremely phototoxic when utilized in live cells. This led to the creation of highly automated live-cell fluorescence microscopy systems that may be used to track the expression of one or more fluorescently tagged proteins in cells throughout time [35]. GFP can be used in the imaging experiments of the living cells in a variety of ways. The directly fixation of GFP to a target protein is the most straightforward way to use it. To demonstrate a successful transfection of a particular gene of interest, GFP, for instance, can be included into a plasmid expressing other genes. Another approach is to utilize a mutant form of GFP known as a fluorescent timer, whose fluorescence

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gradually shifts from green to yellow over time. Based on the color provided by the fluorescent protein, researchers can use the fluorescent timer to examine the condition of protein production, such as recently active, continually activated, or recently deactivated [36].

### **5.1. GFP *in vivo* imaging of cancer**

Any type of cancer process, including primary tumor growth, tumor cell motility and invasion, seeding and colonization of metastases, angiogenesis, and the interaction between the tumor and its microenvironment (tumor-host interaction), can be seen using fluorescent proteins. Chishima *et al.* (1997) used GFP for the first time to see cancer cells *in vivo* in their study [37].

Thanks to fluorescent proteins, which are available in a variety of colors, it is now possible to color-code cancer cells according to their genotype or phenotype. Real-time imaging can be done without damaging the tissues of the animals since some fluorescent proteins have lengthy excitation wavelengths. Longer wavelength light rarely harms proteins and DNA due to its lower energy. Fluorescent proteins' long wavelength excitation lowers the amount of photobleaching as compared to dyes with a shorter wavelength excitation. As a result, it is possible to track the development and spread of cancer across an entire animal in real time [38].

### **5.2. *Ex vivo* imaging using fluorescent proteins:**

- Fluorescent protein-expressing tumors and metastases can be observed non-invasively in intact animals. Fluorescent proteins can be successfully transfected into tumor cells.
- Depending on the fluorescent protein's linkage, transgenic mice can express a fluorescent protein in either all cells or just a subset of cells. Tumors expressing various fluorescent proteins can be implanted into these animals to produce a dual-color view of the tumor-host interaction.
- Tumor cells can express two or more fluorescent proteins of various colors. Red fluorescent protein can be used to label the cytoplasm, while green fluorescent protein can be used to label the nucleus. This makes it possible to see nuclear-cytoplasmic dynamics in action. Studying cancer cell invasion, seeding in distant tissues, and dormancy can be done via single-cell imaging.

- Compared to luciferase imaging, fluorescent protein imaging offers several benefits, such as stronger signals, substrate independence, availability in many colors, and simpler and less expensive equipment needs .

Fluorescent proteins can be employed in molecular imaging' to see how single-gene alterations affect things like cancer spread or treatment susceptibility [38].

### **5.3. GFP is in promoter and protein fusion constructs.**

GFP and its variations have been utilized to detect gene expression in a wide range of species, including mice, yeast, bacteria, and human cells. Gene expression patterns can be observed by using GFP promoter fusions. It is unable to conduct in-depth examinations of the beginning and ending of gene expression (with a resolution of minutes) because of the dynamics of GFP creation (the fluorophore takes some time to develop) and stability (the protein looks to be long-lived). Protein fusions are helpful for figuring out a protein's subcellular localization whether that localization varies throughout development, under various growth conditions, or in diverse genetic backgrounds. These fusion constructs can occasionally be utilized to examine a protein or promoter of interest. GFP has been used to pinpoint the nucleus, endoplasmic reticulum, Golgi, mitochondria, peroxisomes, and synaptic terminals. Organisms that have been tagged and subjected to different environments can be manipulated to produce mutants with altered or absent expressions. GFP is a marker for the presence of microbes and viruses. Labelling of viral proteins makes GFP a helpful transfection marker in molecular biology studies. Infectious processes in both plants and animals can be observed using GFP [39].

### **5.4. Imaging the efficacy of therapeutics:**

It is also possible to directly see the efficacy of therapeutic anticancer treatments in tumor-bearing mice without the need for any invasive procedures because of the properties of fluorescent proteins, which enable whole-body imaging in real time. Rapid investigation of the impact of single or many gene alterations on cancer aggressiveness and therapeutic sensitivity is made possible by fluorescent protein imaging, particularly whole-body imaging. Fluorescence stereomicroscopy was then used to find GFP-fluorescent cancer nodules. Only when GFP-gene conjugates were systemically delivered through the tail vein to nude mice that had been subcutaneously infected with cancer cells were GFP expression seen in the target cells [40].

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### 5.5. GFP-biosensors:

GFP has special physical characteristics that have made it a treasure trove for the creation of biosensors. In contrast to biomarkers, which are correlated with the expression of a particular gene product, biosensors are not. Biosensors can work *in vivo* or in a lab setting. GFP variants with analyte-sensitive characteristics are genetically encoded *in vivo* biosensors. There have been reports of GFP biosensors that can detect changes in pH, certain ions (such as Cl<sup>-</sup> or Ca<sup>2+</sup>), reactive oxygen species, redox status, and particular peptides [41, 42]. Additionally, changes that permit the fluorescence to be selectively activated (irreversible or reversible) have been observed [43]. Single GFP domain or Fluorescence resonance energy transfer (FRET) pair genetically encoded GFP biosensors are also possible. Ratiometric and nonratiometric GFP pH indicators have both been described as A ratiometric indicator is a type of dual-wavelength fluorescent dye that allows researchers to accurately measure intracellular calcium concentrations. Ratiometric indicators offer several advantages over single-wavelength indicators because of their unique spectral properties that deliver more robust and reproducible results. A ratiometric readout reduces the problems associated with dye leakage, photobleaching, uneven dye loading and measuring Ca<sup>2+</sup> in cells of varying thicknesses, non-ratiometric dyes are Ca<sup>2+</sup> indicators that show a large increase in intensity upon binding to Ca<sup>2+</sup>, which means the relative change in fluorescence intensity is indicative of a change in [Ca<sup>2+</sup>].[44].

Green fluorescent proteins can be used as Ca<sup>+2</sup> indicators. However, unlike aequorin or obelin, these photosensitive proteins do not exhibit changes in the spectral quality following calcium binding. Various GFP versions are tuned to Ca<sup>+2</sup> sensitive proteins that are naturally present in cells [45].

It is not surprising that many different types of biosensors have been developed using GFP and its homolog fluorescent proteins as there are numerous ways to generate a signal. Future GFP-biosensor microarrays with the potential to detect a multitude of analytes in a single assay are anticipated as a result of the ability of GFP and its variations to be immobilized and even dried with keeping structure and biosensor function. In addition to being widely applicable, such content should be very affordable to generate and would be simple to use, store, and read. One day, GFP-based biosensor arrays on paper or film could be used in homes to quickly diagnose infections without a trip to the hospital or they could be an essential component of devices that continuously monitor the water and air, making the world a healthier and safer place [20].

## 6. Conclusion

GFP is one of the most popular *in vivo* fluorescent tags, it can visualize all cell types including organs, tissues and cell cultures in whole animals that can be applied in different fields including Immunology, neurobiology, development, and carcinogenesis. It has numerous advantage that has transformed the life sciences.; it does not require the addition of cofactors or substrate, is relatively less toxic to cells, has easy quantitation, stable to pH change, proteases, and temperature upto 65°C and it does not alter the function and localization of the fusion partner. All these features put the scientists under the challenge of discovering of additional GFP in other bioluminous species than *Aequorea Victoria*. The application of GFP in cellular, molecular, and discovery studies appears to have a better future.

**Conflicts of Interest:** The authors declare no conflict of interest.

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## المُلخَص العَرَبِي

البروتين الفلوريسنت الأخضر : الإكتشاف ، التركيب ، والتنقية ، التطبيقات والآفاق المستقبلية

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## المُلخَص

تم اكتشاف البروتين الفلوريسنت الأخضر أولاً من قنديل البحر وكان يستخدم علي نطاق واسع في العلوم البيولوجية كعلامة فلورية عالمية. ركز العديد من العلماء جهودهم علي تحديد وتطوير البروتينات الفلورية ذات الخصائص الجديدة والخصائص المحسنة , لإستخدامها في تصور التنظيم الهيكلي والعمليات الديناميكية في الخلايا الحية . قدم إكتشاف البروتين الفلورى الاخضرمتبوعاً بتنقية وتوضيح هيكله الجزيئى علامة جديدة وواعدة للدراسات البيولوجية .

البروتين الاخضر الفلوريسنت هو في جوهره الفلوريسنت , لدية سمية منخفضة , ودرجه حموضة واستقرار حراريا ويمكن تصويره بسهولة وتحديد كميته .

تركز مقالة المراجعة هذه علي اكتشاف وتنوع انماط وألوان الفلوريسنت , والبروتينات الفلورية الاخرى , والتنقية , والهيكل , والخصائص , والتطبيقات العديده للبروتين الفلوريسنت الاخضر في العلوم البيولوجية مثل التصوير الحي للسرطان , كمحفز ومدمج للبروتينات , والاستشعار الحيوية , تصوير الجسم , فاعلية العلاجات.