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Insights into Marine Bioluminescence: Unraveling the Intricacies of Natural Fluorescence Probes

Deniz Biyolüminesansına Bakış: Doğal Floresan Problarının İnceliklerini Çözmek

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ABSTRACT

The mysterious allure of the deep sea has long captivated the human imagination, hiding secrets beneath its unreadable surface. In our review article, we journey into this fascinating realm, where nature's bioluminescent messengers—such as Green Fluorescent Protein (GFP), ZsGreen, Red Fluorescent Protein (RFP), mCherry, TagRFP, mKate, Neptune, HcRed, and Phycoerythrin—reveal the remarkable beauty of the underwater world. This exploration delves into the complex dynamics of bio-imaging, fluorescence properties, and detection methods, unveiling the mesmerizing display of life beneath the waves. Join us as we uncover the stories behind these natural fluorescence probes, shedding light on the extraordinary underwater spectacle that continues to captivate scientists and ocean enthusiasts alike.

Key Words

Red fluorescent protein, bio-imaging, fluorescent protein, green fluorescent protein.

ÖZ

Derin denizin gizemli cazibesi uzun zamandır insan hayal gücünü büyülemiş, okunamayan yüzünün altında sırlar gizlemiştir. Makalemizde, doğanın biyolüminesans habercileri olan Green Fluorescent Protein (GFP), ZsGreen, Red Fluorescent Protein (RFP), mCherry, TagRFP, mKate, Neptune, HcRed ve Phycoerythrin gibi moleküllerin su altı dünyasının olağanüstü güzelliğini ortaya çıkardığı bu büyüleyici aleme bir yolculuğa çıkıyoruz. Bu keşif, biyogörüntüleme, floresans özellikleri ve tespit yöntemlerinin karmaşık dinamiklerini ele alarak, dalgaların altındaki yaşamın büyüleyici görüntüsünü gözler önüne seriyor. Bu doğal floresan probaların ardındaki hikayeleri ortaya çıkarırken, bilim insanlarını ve okyanus tutkunlarını büyülemeye devam eden olağanüstü su altı gösterisine ışık tutmamıza katılın.

Anahtar Kelimeler

Kırmızı floresan protein, biyo-görüntüleme, floresan protein, yeşil floresan protein.

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INTRODUCTION

In the complex field of bioengineering, fluorescence has been greatly advanced by a remarkable set of emissaries from the sea and scientific history. From the iconic Green Fluorescent Protein (GFP) to its colorful counterparts—ZsGreen, Red Fluorescent Protein (RFP), mCherry, TagRFP, mKate, Neptune, HcRed, and the dazzling Phycoerythrin—each has played a crucial role in advancing modern biology and bioengineering. These fluorescent proteins, much like the ocean's bioluminescent wonders, have fascinated researchers and opened up a wide range of possibilities in imaging and biochemical sensing. The history of these proteins reflects the curiosity of science. The GFP, originally found in the jellyfish *Aequorea victoria*, sparked a revolution in molecular and cell biology. Its use as a marker for gene expression, cellular localization, and protein dynamics has fundamentally changed our understanding of life at the molecular level [1]. Soon after, other fluorescent proteins emerged, each with its own color, spectral properties, and range of applications [2]. From laboratory benches to microscopy platforms, the versatility of these fluorescent proteins (FPs) has made them essential tools for bioengineers. These fluorescent probes are crucial for understanding cellular processes and have applications in monitoring protein-protein interactions, cellular signaling, and the dynamic activities of living organisms [3]. In this review, we highlight the journey of these fluorescent beacons, summarizing their applications and key roles in bioimaging. Fluorescent protein-based indicators can be custom-engineered to respond to various biological events and signals. They can be targeted to specific subcellular locations and introduced into different tissues and organisms. Factors like the presence and location of charged amino acids and hydrophobic interactions within the protein can cause significant changes in absorption and emission peaks, shifting up to 40 nm and resulting in either blue or red spectral changes. Larger spectral shifts, which help categorize FPs into specific spectral classes (e.g., CFP, GFP, YFP), are usually due to differences in the covalent structure and π-orbital conjugation within the chromophore [4]. Additionally, we explore recent advancements in the field, including the fine-tuning of optical properties to enhance sensitivity and precision. The evolving field of bioengineering continues to push boundaries, and these natural fluorescence probes are constantly being improved. Researchers have developed better versions with extended spectral ranges, increased photo-stability, and compatibility with various imaging techniques [5]. One crucial development that has harnessed the potential of these probes is *Förster resonance energy transfer* (FRET), a phenomenon that enables the study of molecular interactions at nanometer scales. The utilization of FRET, in conjunction with fluorescent proteins, has unlocked a plethora of applications, allowing real-time monitoring of cellular processes, protein-protein interactions, and structural changes [6]. As we venture deeper into the realms of bioengineering, our understanding of these natural fluorescence probes and their optical properties continues to evolve. This review amalgamates historical perspectives, recent progress, and the intricate world of FRET, offering a comprehensive narrative for professionals in the field who seek to explore, innovate, and illuminate the mysteries of life at the molecular level. Join us on this journey as we unveil the vibrant and dynamic story of these fluorescent allies and their transformative role in science.

Green Fluorescent Protein

Figure 1 highlights the molecular structure of GFPs, their fluorescence mechanism, and their applications in biological imaging. Shimomura et al. discovered the green fluorescent protein (GFP) in 1962 within *Aequorea victoria* [7]. The groundbreaking research conducted by Boxer and his team revealed that excited-state proton transfer (ESPT) is the underlying mechanism responsible for the vibrant green fluorescence observed when the molecule is photoexcited at blue wavelengths, typically around 400 nm. This influential study has received widespread recognition in subsequent research, amassing over a thousand citations and emphasizing its profound impact on the field of fluorescence spectroscopy and molecular science [8]. Hirano et al. introduced StayGold, a GFP derived from the jellyfish *Cytaeis uchidae*, designed to address the issue of photostability in fluorescence microscopy. StayGold exhibits more than tenfold higher photostability compared to existing fluorescent proteins while maintaining cellular brightness akin to mNeonGreen. They harnessed StayGold to image dynamic cellular processes with structured illumination microscopy (SIM), such as the endoplasmic reticulum (ER) and mitochondrial dynamics, resulting in significantly reduced photobleaching compared to other stability-optimized GFP variants. Employing StayGold, they observed viral spike proteins amidst cells afflicted by severe acute respiratory syndrome coronavirus 2. Since StayGold naturally forms dimers, they engineered a tandem dimer version for studying microtubules and exci-

tatory post-synaptic density in neurons. In the future, the development of monomeric iterations of StayGold (mStayGold) will facilitate the visualization and quantification of tagged proteins with low copy numbers. This will be achieved through genome editing and single-molecule tracking within cells, extending observation durations. Such advancements will significantly broaden its applicability in fluorescence microscopy [9]. Andersen et al. employed time-resolved action spectroscopy on cryogenically cooled molecular ions to achieve remarkable vibrational resolution in investigating the deprotonated GFP chromophore, which plays a pivotal role in bioimaging living cells. The study unveiled four distinct spectral regions within the S0–S1 band, each associated with competing electronic and nuclear decay pathways. They determined the presence of an energy barrier of approximately 250 cm^{-1} inhibiting internal conversion, leading to diminished statistical fragmentation near the S0–S1 band origin. This origin was pinpointed at 481.51 ± 0.15 nm (20768 ± 6 cm^{-1}), exhibiting only a slight red shift of 221 cm^{-1} compared to wild-type GFP at 77 K. The vibronic signatures of the protein and its chromophore were found to closely coincide, indicating parallel photophysical attributes. The data, coupled with theoretical insights, revealed the coexistence of specific vibrational modes facilitating energy exchange between nuclei and electrons. This work demonstrates the unique photophysics of the GFP chromophore, characterized by mode-specific nonadiabatic electron transfer, opening new avenues for GFP-related research in redox photochemistry [10]. Boulanger et al. investigated the behavior of two fluorescent molecules, o-HBDI and o-LHBDI, inspired by the GFP. These molecules can undergo proton transfer when excited. They discovered that o-LHBDI exhibits significantly enhanced fluorescence, especially in less polar solvents, due to a locked ring structure, while o-HBDI does not fluoresce effectively. This research sheds light on the role of GFP-inspired chromophores in understanding how molecules behave in different solvents and offers valuable insights for the development of improved fluorescent probes for diverse applications [11]. Veríssimo et al. explored the use of 1-alkyl-3-methylimidazolium chloride-based ionic liquids (ILs) to preserve the activity of Enhanced Green Fluorescent Protein (EGFP) over extended storage times and in challenging conditions. They found that these ILs effectively maintained EGFP fluorescence for up to three months at room temperature, outperforming traditional buffers and salts. Shorter alkyl chain ILs were most effective. Additionally, the ILs protected EGFP from denaturing agents like surfactants and chemicals. These findings suggest that ILs have potential applications in extending the shelf life and stability of protein-based products in various industries [12]. Scott et al. addressed the issue of FP inactivation by denaturing chemicals used in tissue clearing methods. They studied an ultra-stable GFP (usGFP) with two stabilizing mutations, Q69L and N164Y, which improved hydrophobic packing and hydrogen bonding networks. However, usGFP tended to dimerize, which was undesirable. They created a monomeric variant, muGFP, with a point mutation (F223D) at the dimer interface. muGFP retained fluorescence after tissue clearing, making it suitable for applications requiring stability and minimal self-association. This work highlights the importance of FP engineering for tissue imaging [13]. Zhu et al. introduced a novel class of indicators, which rely on chemigenetic fusion between a protein-based fluorophore and a synthetic ion-recognition motif. Their innovation involves creating a calcium ion (Ca^{2+}) indicator by genetically incorporating circularly permuted GFP into HaloTag protein, which is self-labeled with a ligand containing the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid. Additionally, they expanded on this concept by developing a Na^{+} indicator utilizing a ligand containing a crown ether moiety. This strategy yields highly luminous and sensitive ion indicators suitable for cellular imaging applications. Furthermore, this approach opens avenues for designing chemigenetic indicators tailored to specific ions or molecules, a feat not achievable with conventional protein-based indicators [14]. Fluorescence lifetime imaging (FLIM) of NAD(P)H is a valuable technique for studying cellular metabolism. However, York et al. caution against analyzing NAD(P)H-FLIM in cells expressing green fluorescent protein (GFP). When GFP is excited at 750 nm, it emits blue light (~460 nm) with a short lifetime (~500 ps), which is similar to the characteristics of NAD(P)H. This can lead to incorrect interpretations of metabolic rates. To avoid such issues, researchers should use fluorophores with no blue emission when performing NAD(P)H-FLIM to ensure accurate measurements of cellular metabolism. It's essential to test fluorescent molecules under NAD(P)H imaging conditions to confirm reliable results [15]. Sarkisyan et al. investigated the intricate details of the GFP from Aequorea victoria (avGFP) by examining tens of thousands of derived genotypes stemming from avGFP. They discovered that this fitness landscape was relatively narrow, with a significant portion of mutated avGFP variants displaying reduced fluo-

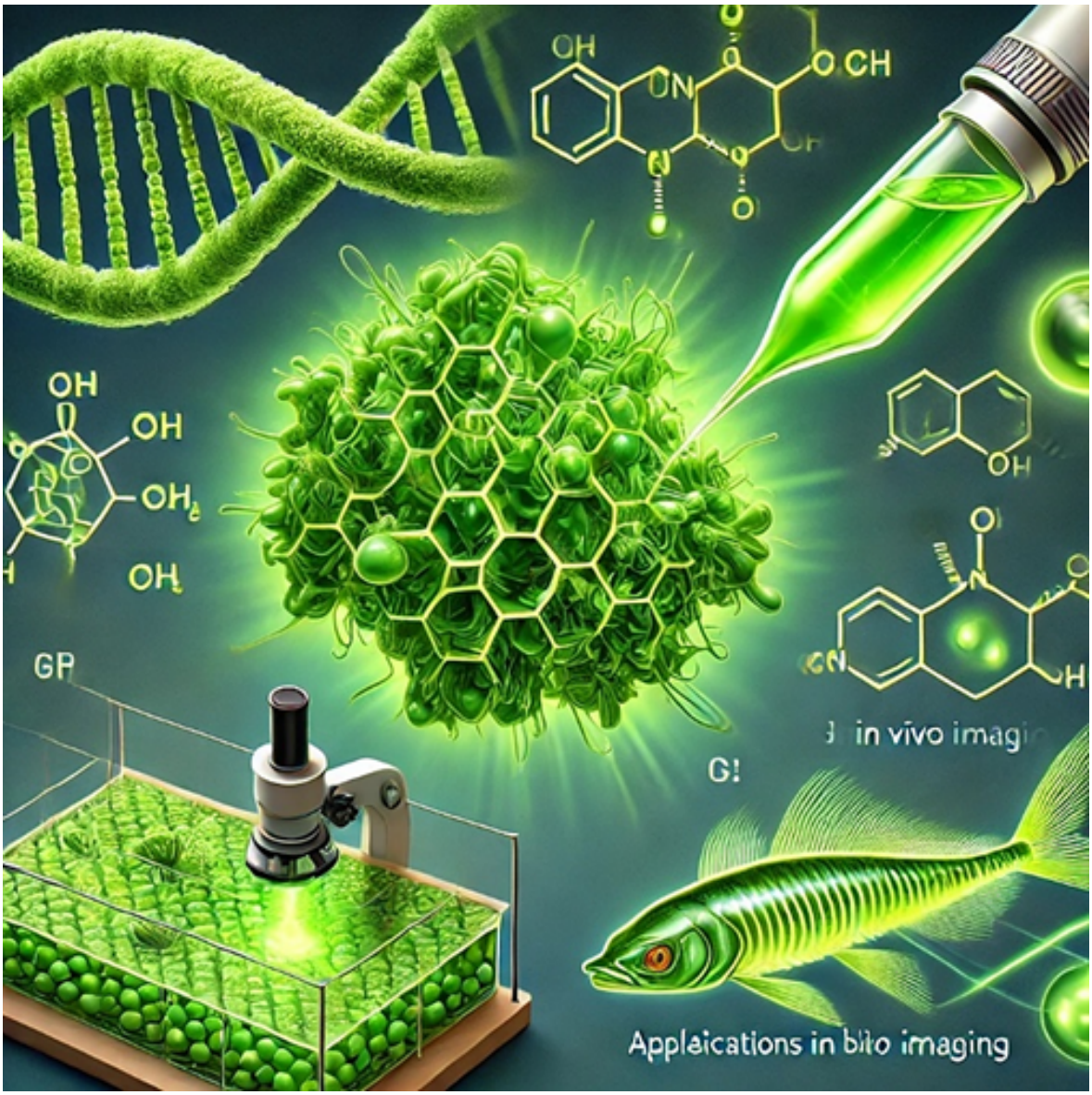

Figure 1. Molecular structure of GFPs, their fluorescence mechanism, and their applications in biological imaging.

rescence, especially when carrying multiple mutations. Epistasis, where the effects of mutations are interdependent, was observed in up to 30% of genotypes with multiple mutations. Epistasis commonly emerged from the combined effects of mildly deleterious mutations, resulting in a threshold-like decrease in protein stability and consequent loss of fluorescence. Their discoveries corresponded with a model of sequence evolution spanning epochs, indicating parallels between local and global fitness landscape characteristics. Insight into the local fitness landscape of avGFP holds significant implications for areas such as molecular evolution, population genetics, and protein engineering [16]. Tang et al. introduced a novel image fusion algorithm that combines GFP and phase-contrast images using generative adversarial networks (GANs). This approach treats the fusion process as a game between a generator and a discriminator, considering the unique characteristics of each input image type. The experimental findings demonstrate that their approach surpasses current fusion methodologies, showcasing superior visual fidelity and objective performance metrics. Notably, it adeptly extracts both functional and structural insights from the images under consideration. The proposed framework has broad applicability for various functional and structural image fusion challenges [17]. Khrenova et al. per-

formed computational studies on how the synthetic green fluorescent protein chromophore, HBDI, shifts its absorption bands when interacting with aromatic compounds through π-stacking. They found that redshifts correlated with smaller dipole moment changes, while blueshifts correlated with larger changes. They also identified a strong correlation between transition energy shifts and bond interaction energy in π-stacked complexes. These findings provide insights into the factors affecting absorption band shifts in GFP chromophores, aiding biomarker development [18].

ZsGreen

ZsGreen is a fundamental green fluorescent protein introduced in 1999, originating from *Zoanthus sp* [19]. ZsGreen is noted for its robust tetrameric fluorophore structure, with a significantly higher capacity for light absorption compared to other commonly used fluorophores such as EGFP and GFP [20].

Kim et al. studied ZsGreen, a FP, for metal-induced fluorescence quenching as a potential biosensor. They found significant quenching by Fe^{2+} (2% intensity), Fe^{3+} (1%), and Cu^{2+} (20%). Dissociation constants were 11.5 µM for Fe^{2+}, 16.3 µM for Fe^{3+}, and 68.2 µM for Cu^{2+}. While Cu^{2+}-quenched fluorescence could be mostly recovered (up to 89.47%) with EDTA, Fe^{2+} and Fe^{3+} quenched fluorescence only partially recovered (about 15%). ZsGreen's homology model revealed unique binding sites for Fe^{2+}/Fe^{3+}. Despite different oxidation states, their quenching effects were similar. This suggests ZsGreen's potential for iron biosensing, with possible improvement through higher EDTA concentrations or different chelation methods. The homology model also showed distinctive binding sites in ZsGreen compared to other FPs like Dronpa and iq-mEmerald. Cu^{2+} quenching is attributed to high metal concentrations, akin to ZsYellow fluorescence quenching by Cu^{2+} [21]. Strepay et al. engineered a transgenic mouse model using BAC transgenesis, incorporating regulatory elements from the Kcnj10 gene to drive the expression of ZsGreen fluorescent protein. Kcnj10 is responsible for encoding the KCNJ10 protein, vital for generating the endocochlear potential (EP) and predominantly found in SV intermediate cells. In these transgenic mice, the ZsGreen fluorescence faithfully recapitulated the natural expression pattern of Kcnj10 in various cochlear cell types, including SV intermediate cells, inner phalangeal cells, Hensen's cells, Deiters' cells, pillar cells, a portion of spiral ganglion neurons, and glial cells. Importantly, this transgene's expression in hemizygous mice didn't affect auditory function or EP. This Tg(Kcnj10-ZsGreen) transgenic mouse provides a valuable tool for studying intermediate cell function, both in live and fixed tissue, by replicating the natural expression of Kcnj10 [22]. Rao et al. introduced a practical, cost-effective method using the ZsGreen fluor-reporter and a custom 3-D blue LED light box for identifying unintended Cre recombinase expression, eliminating the need for genotyping. They applied this approach to evaluate specific Cre recombinase mouse lines in vision research. This technique allows quick detection of ectopic recombinase expression without causing animal distress. While it effectively highlighted Cre recombinase expression in astrocytes, it was less suitable for targeting Müller glia-specific sites, in line with recent research showing limited GFAP expression in normal retinal Müller glial cells [23]. Heiden et al. integrated ZsGreen fluorescent protein into the Pyrococcus horikoshii RadA intein, creating a ZsG-Int hybrid protein that retained fluorescence abilities. They utilized this platform to engineer an Ebola virus capable of expressing a fluorescent protein. By identifying an optimal insertion site within the VP30 gene of the virus, they achieved efficient intein splicing in mammalian cells while maintaining VP30 function. The outcome was a virus incorporating the ZsG-Int-VP30 fusion protein, exhibiting fluorescence within infected cells. This study pioneered an innovative intein-based strategy for integrating reporters into systems without the need for extra genes. Notably, the distinguishing aspect of this approach lies in the fact that the reporter is synthesized only upon translation of the protein of interest. This method is applicable to both prokaryotic and eukaryotic cells. Although the recombinant virus was somewhat attenuated, likely due to low VP30 expression at early infection stages, these findings serve as a proof of concept with the potential for further refinement [24]. Bryda et al. introduced a novel transgenic rat strain featuring conditional expression of the ZsGreen fluorescent protein gene upon activation by exogenous Cre recombinase. This strain presents a valuable resource for validating newly developed rat Cre driver lines, thereby significantly expediting the characterization process. To assess the compatibility of the ZsGreen reporter strain with a cell-type specific Cre driver strain, they crossbred ZsGreen line 561 rats with LE-Tg(Chat-Cre)5.1Deis rats, which confine Cre recombinase expression to cholinergic neurons. Examination of hippocampal brain slices revealed ZsGreen expression in specific neurons, likely cholinergic neurons, in double hemizygous rats,

while such expression was absent in rats carrying only the ZsGreen transgene or the mChat-Cre transgene. Remarkably, ZsGreen expression was localized to the cell body of neurons and exhibited a punctate pattern [25].

Red Fluorescent Protein

The originator of red fluorescent proteins (RFP), designated as DsRed, was initially sourced from non-bioluminescent reef corals back in 1999 [26]. RFP serves as a valuable complement to GFP, enabling the resolution of scientific challenges that GFP alone cannot address. One of its primary advantages lies in its minimal background interference in intracellular imaging, making it particularly well-suited for applications in bioscience research. Thanks to the collaborative endeavors of numerous scientists, the fluorescence spectra of FPs have been thoroughly investigated, spanning the visible spectrum and even extending into the near-infrared region. This comprehensive array of fluorescent tools has greatly bolstered our ability to visualize and quantify proteins within living cells. [27-30]. He et al. developed a novel fluorescent probe called DEBIT by incorporating essential fragments of the commercial G4 dye ThT into RFP chromophores. DEBIT exhibits red emission and selectively recognizes RNA G4 structures with high binding affinity, selectivity, and excellent photostability. This probe enables real-time monitoring of RNA G4s in biological systems and allows for endogenous imaging of these structures in live cells. DEBIT combines the advantageous properties of RFP chromophores and ThT, offering a valuable chemical toolkit for exploring the functions of G4 structures in cells. This innovative "integration" strategy not only expands the application of synthetic RFP chromophores but also provides a promising approach for constructing G4 fluorescent probes [31]. Wang et al. undertook a comprehensive investigation employing a range of spectroscopic techniques and quantum calculations, to elucidate the complete cis- and trans-mKeima photocycle. This study enabled them to observe the entire process of light-induced ultrafast proton transfer and chromophore isomerization in the coexisting cis- and trans-isomers of the red fluorescent protein mKeima in a neutral aqueous buffer. Their findings unveiled that mKeima displays dual fluorescence emissions at 520 and 620 nm. The emission at 620 nm predominantly originates from the deprotonated trans-isomer, whereas the emission from the deprotonated cis-chromophore at 520 nm is attenuated due to rapid cis-to-trans isomerization. This study sheds light on the interplay between excited-state proton transfer and isomerization in fluorescent proteins, providing valuable insights into their photochemical processes in physiological environments [32]. Ning et al. have engineered a bright red fluorescent protein called Crimson, which offers significant advantages for long-term neuronal labeling. Crimson exhibits similar fluorescence spectra to mCherry and mKate2 but surpasses them in terms of molecular brightness by 100% and 28%, respectively. This novel protein can be used to label neuronal structures such as thin neurites, dendritic spines, and filopodia with enhanced precision, particularly when targeted to the cell membrane. Crimson proves to be brighter and less toxic than other commonly used red fluorescent proteins, both in the cytosol and when localized to the membrane. In one-photon microscopy, Crimson-CAAX excels in visualizing delicate structures like filopodia and thin spine necks, which are challenging to distinguish with cytosolic fluorescent proteins without overexposing dendritic shafts [33]. Feng et al. engineered two red fluorescent protein analogs, APFP-lyso and DAPFP-lyso, with specific targeting capabilities towards lysosomes. These proteins were designed for concurrent application in photodynamic therapy (PDT) within cancer cells and two-photon fluorescence imaging in zebrafish. DAPFP-lyso, a fluorescent protein dimer, exhibited superior properties, including a large Stokes shift, near-infrared emission at 664 nm, and enhanced efficiency in generating singlet oxygen and superoxide radicals. This dimer also demonstrated excellent two-photon absorption under 800 nm excitation, enabling clear fluorescence imaging in zebrafish. Theoretical calculations supported these findings, indicating efficient reactive oxygen species production by DAPFP-lyso. In cellular experiments, DAPFP-lyso showed high phototoxicity, negligible dark toxicity, and precise lysosome-targeting capabilities, inducing cancer cell apoptosis and migration under irradiation. The FP dimer design offers promising prospects for two-photon photodynamic therapy and the development of new photosensitizers for PDT [34]. Horiuchi et al. conducted expression cloning in Diadumene lineata, leading to the discovery of genes encoding orange (OFP: DiLiFP561) and RFPs: DiLiFP570 and DiLiFP571. These proteins naturally formed obligate tetramers and exhibited robust fluorescence, with brightness values of 58.3 $mM^{-1}{\cdot}cm^{-1}$ (DiLiFP561), 43.9 $mM^{-1}{\cdot}cm^{-1}$ (DiLiFP570), and 31.2 $mM^{-1}{\cdot}cm^{-1}$ (DiLiFP571), comparable to commonly used red fluorescent proteins. The fluorescence lifetimes of DiLiFP561, DiLiFP570, and DiLiFP571 were defined to be 3.7, 3.6, and 3.0 ns, respectively. The crystal structure of Di-

LiFP570, resolved at 1.63 Å resolution, unveiled an extended π-conjugated chromophore structure reminiscent of DsRed. Although the majority of amino acid residues flanking the chromophore exhibited similarity between DiLiFP570 and DiLiFP561, an interesting deviation was noted at position M159 of DiLiFP570 (as opposed to Lysine in DiLiFP561), positioned in close proximity to the chromophore hydroxyl group. Notably, a comparable replacement from lysine to methionine has been previously recorded in a red-shifted variant of DsRed (mRFP1). This observation underscores the remarkable consistency between naturally occurring color-change mutations and those induced through protein engineering processes. The newfound proteins hold promise as orange and red fluorescent markers, characterized by their extended fluorescence lifetimes. It is intriguing to note that these naturally sourced color-change variants align with mutations encountered during the protein engineering of DsRed [35]. In their study, Chulpanova et al. conducted modifications on SH-SY5Y human neuroblastoma cells by genetically introducing two reporter genes: the firefly luciferase gene (ffLuc) and the far-red fluorescent protein gene (Katushka2S). They subsequently analyzed the bioluminescence and fluorescence of these modified cells in vitro. Following this, they established cell lines incorporating these genetic modifications to create xenograft tumor models for in vivo experiments. In the preliminary in vitro evaluation, the bioluminescence signal originating from ffLuc was initially detected in a limited subset of SH-SY5Y cells, contrasting with the fluorescence signal emitted by Katushka2S. However, following in vivo administration, it became evident that the fluorescence intensity emitted by SH-SY5Y cells expressing Katushka2S notably surpassed the bioluminescence intensity emitted by an equivalent number of SH-SY5Y cells expressing ffLuc. This phenomenon was attributed to the fact that, following intraperitoneal administration, only a fraction of the luciferase substrate reached the injection site [36]. Shcherbo et al. introduced Katushka, a far-red fluorescent protein that outshines spectrally similar HcRed and mPlum by seven to tenfold. Katushka is prized for its rapid maturation, robust pH-stability, and remarkable photostability, rendering it the preferred option for visualizing tissues in vivo. In their investigation, the researchers showcased Katushka's superiority in whole-body imaging, directly comparing it with other red and far-red fluorescent proteins. Moreover, they introduced mKate, a monomeric variant of Katushka boasting elevated brightness and photostability. mKate serves as an outstanding fluorescent marker for protein labeling in the far-red spectral range. Furthermore, Bindels et al. engineered mScarlet, a monomeric RFP characterized by exceptional brightness, a high quantum yield and a prolonged fluorescence lifetime. They crafted mScarlet utilizing enhanced screening methodologies, and its crystal structure revealed a flat and rigidified chromophore. mScarlet surpasses other red fluorescent proteins as a fusion tag and proves particularly advantageous for FRET applications in ratiometric imaging. This advance allows precise spectral imaging of FRET and the accurate measurement of protein interactions in live cells, making it a valuable tool in cell biology and molecular research [38].

mCherry

mCherry is a fundamental red fluorescent protein introduced in 2004, originating from *Discosoma sp*. It is known for its rapid maturation as a monomeric protein and exhibits low sensitivity to acidic conditions [39]. Subach et al. presented PAmCherry1, a monomeric red photoactivatable protein distinguished by its advantageous attributes: accelerated maturation, improved pH balance, robust photostability, high photoactivation contrast and rapid photoactivation. Notably, PAmCherry1 lacks green fluorescence and exhibits single-molecule behavior, rendering it ideally suited for super-resolution techniques such as Photoactivated Localization Microscopy (PALM) and two-color diffraction-limited photoactivation imaging. In their investigation, PAmCherry1 was employed for PALM imaging of the transferrin receptor, uncovering clusters of ≤200 nm with a resolution of ≤25 nm, thus affirming its efficacy as an intracellular probe. The study also compared PAmCherry1 with the well-known probe, tdEosFP. While there were slight differences in the number of photons collected, their σ distributions showed similar ~15-16 nm uncertainty, indicating comparable performance in fixed cell PALM experiments [40]. Kobayashi et al. devised a technique for generating a reporter West Nile virus (WNV) expressing mCherry, facilitating the visualization of viral antigen-positive cells in both in vitro and in vivo settings. However, the reporter WNV exhibited diminished growth rates in comparison to the parental WNV strain. Upon intracranial inoculation in mice, the reporter WNV prompted neurological symptoms, rendering it a valuable asset for investigating WNV replication within the brain. Nevertheless, its restricted replication capacity makes it unsuitable for exploring the mechanisms underlying viral dissemination from peripheral tissu-

es to the brain [41]. Kim et al. utilized CRISPR/SpCas9 technology to generate a human pluripotent stem cell (hPSC) line featuring a TUBB3-mCherry reporter. By substituting the stop codon in the final exon of TUBB3 with a T2A-mCherry cassette, they successfully created the TUBB3-mCherry knock-in cell line. This engineered cell line maintained typical pluripotent characteristics and faithfully reproduced endogenous TUBB3 expression during neuronal differentiation. Consequently, it serves as a valuable resource for investigating various aspects of neuronal differentiation, toxicity assessments, and tracing studies [42]. Guo et al. developed a valuable resource for investigating human primordial germ cell (hPGC) development by introducing an mCherry knock-in at the site preceding the stop codon of NANOS3 in a human embryonic stem cell (hESC) line named SYSUe-009-A. This reporter cell line allows researchers to track hPGC specification. The inserted mCherry maintains pluripotent marker expression, normal karyotyping, and differentiation potential into three germ layers, making it a promising resource for studying human germ cell-related processes, even though mCherry-positive cells are relatively low (0.02%) due to their localization on the surface of aggregates during PGC-like cell induction [43]. Zhou et al. devised a protocol to assess autophagy flux in muscle fibers by overcoming challenges related to baseline mCherry-EGFP-LC3 expression. They achieved this by overexpressing mCherry-EGFP-LC3 in adult mouse FDB muscle, using enzymatic digestion to isolate individual fibers for live cell imaging. Their ImageJ-based program eliminated baseline striations, allowing semi-automated quantification of autophagosomes (APs) and autolysosomes (ALs), enhancing objectivity and reducing bias in the analysis. This protocol facilitated the assessment of autophagy flux and its response to colchicine treatment, offering a foundation for evaluating other autophagy inhibitors in skeletal muscle research [44]. Penjweini et al. have developed a sandwich sensor for monitoring myoglobin's oxygenation and metmyoglobin states. By incorporating fluorescent proteins mCherry and yellow fluorescent protein, they can distinguish between deoxygenated myoglobin and metMb in various cellular compartments using fluorescence lifetime imaging. This enables real-time tracking of metabolic processes in different environments within a single construct [45].

TagRFP

TagRFP is a fundamental red fluorescent protein introduced in 2007, derived from *Entacmaea quadricolor* [46]. The research conducted by Merzlyak et al. unveiled TagRFP, a monomeric red fluorescent protein acclaimed for its exceptional brightness, extended fluorescence lifetime, complete chromophore advancement and pH-stability. These characteristics render TagRFP an invaluable tool for protein localization studies and applications involving FRET. In a comparison with mCherry, living HeLa cells expressing TagRFP exhibited a longer average fluorescence lifetime (2.2–2.3 ns), highlighting TagRFP's increased quantum yield. This distinction in fluorescence lifetime allows for dual-color labeling using two red fluorescent proteins. TagRFP's elevated quantum yield and prolonged fluorescence lifetime make it an enticing candidate as a FRET donor for far-red acceptors, such as mPlum4 or its potential derivatives. With its advantageous spectral properties, TagRFP is an attractive choice for use as a fusion tag alongside other fluorescent proteins in various applications [47]. Alcaide et al. used fluorescently labeled PepMV isolates, including PepMV-CH2-TagRFP and -EU-GFP, to study their interactions in tomato crops. They found that in mixed infections, TagRFP and GFP fluorescence emissions showed areas of predominance, and a significant proportion of cells exhibited both fluorescent signals, indicating that TagRFP played a crucial role in visualizing and understanding the complex interactions between different PepMV strains in the study [48]. Subach et al. conducted a groundbreaking study wherein they elucidated the 2.2 Å crystal structures of the RFP, TagRFP and its blue derivative, mTagBFP. Through crystallographic analysis, they unveiled that TagRFP harbors a trans coplanar anionic chromophore, akin to DsRed-like chromophores. Conversely, mTagBFP features a novel chromophore type identified as N-[(5-hydroxy-1H-imidazole-2-yl)methylidene]acetamide. Their investigation proposed a chemical mechanism whereby the DsRed-like chromophore is generated via the mTagBFP-like blue intermediate. The research offers crucial insights into the chromophore structures and the underlying mechanisms governing the fluorescence of these fluorescent proteins [49].

mKate

mKate is a foundational red fluorescent protein, first described in 2007, and derived from *Entacmaea quadricolor*. Dou et al. investigated a novel cell-labeling strategy utilizing mKATE in conjunction with Renilla reniformis luciferase (mKATE-renLUC) to monitor human placental stromal cells (PSC) in an animal model of erectile dysfunction (ED). This approach, distinguished by the use of brighter fluorophores and optimized luciferase,

facilitated safe and efficient cell tracking, enabling non-invasive, long-term monitoring of injected cells in vivo. Their findings underscore the potential of this technique for advancing stem cell therapy [51]. Diupotex et al. analyzed the impact of Leishmania mexicana parasites engineered for mKate protein expression on infection outcomes in BALB/c mice. They found that mKate expression caused larger lesions, increased parasite load, and enhanced immunogenicity. The research highlights the importance of assessing the immunogenicity of reporter proteins when utilizing them in experimental models [52]. Kim et al. developed mCardinal2, a red fluorescent protein with several mutations, including C158D, which resulted in increased brightness and a substantial spectral shift, achieving a significant Stoke shift effect compared to the original protein, mKate [53]. In the study by Linnigan et al., they utilized the IncuCyte® imaging system to evaluate the interactions between immune cells and cancer cells, a vital aspect in assessing new therapeutic approaches. To simplify this process, they developed a cost-effective method involving lentiviral expression of nuclear localized mKate2 red fluorescent protein to label a wide range of cancer cell lines, providing a versatile platform for analyzing different cancer types and cell lines within those categories [54].

R-phycoerythrin

R-phycoerythrin (R-PE) is an exceptionally vibrant phycobiliprotein sourced from red algae, known for its intensely bright red-orange fluorescence and high quantum yields. Sathuvan et al. developed a rapid purification method for *Gracilaria corticata* R-PE using polyacrylamide gel electrophoresis (PAGE). The purified R-PE exhibited characteristic UV-Vis absorption peaks and fluorescence properties. The purification process was efficient, resulting in high purity and a recovery yield of 44.3%. SDS-PAGE analysis confirmed the presence of three sub-units. The purified R-PE demonstrated stability at pH 7.0 and 20°C and showed fluorescence suitable for cell imaging. It can be used for various applications, including cancer cell-specific imaging and drug delivery. This purification method offers advantages over conventional chromatography techniques [55]. Xu et al. undertook a study aimed at investigating the interplay between Hg^{2+} ions and R-PE through various analytical techniques. Their findings revealed that Hg^{2+} ions induced static fluorescence quenching in R-PE, influenced by hydrogen bonds and Van der Waals forces. The binding of Hg^{2+} ions to specific amino acids on both the α and β chains of R-PE led to structural changes and alterations in the secondary structure of R-PE. Molecular docking revealed the optimal binding sites. This study provides insights into the interaction between Hg^{2+} ions and R-PE, offering a method for assessing reactions between metal ions and fluorescent probes [56]. Wang et al. devised a straightforward technique for encapsulating R-PE proteins and CdSexS1−x/ZnS quantum dots within ZIF-8 thin films via a single-step solid-confinement transformation process. The resulting R-PE/CdSexS1−x/ZnS@ZIF-8 thin film exhibited excellent white light emission and impressive thermal stability, maintaining its integrity at temperatures up to 80 °C. This process allowed for the even distribution of R-PE molecules and QDs within the ZIF-8 crystals, resulting in improved photoluminescence quantum yield and preventing aggregation-caused photoluminescence quenching. The R-PE chromophores formed complexes with Zn ions, leading to green and red emissions in the visible region, resulting in warm white light emission with promising applications in warm white light-emitting diodes (W-LEDs) for lighting devices [57]. In a study by Ghosh et al. they developed a practical in vitro DNA sensing platform by using graphene oxide (GO) to quench the fluorescence of C-Phycocyanin (CPE), a naturally FP. They found that GO interacted with the protein's amino acid side chains, causing fluorescence quenching through π-π interactions and hydrogen bonding. Only DNA could "turn on" the fluorescence of the bio-composite, and the degree of recovery depended on DNA size, allowing accurate length estimation. While effective for DNA detection in bacterial cellular lysates, the presence of serum albumin in blood posed challenges. Dynamic light scattering analysis indicated that DNA replaced CPE on the GO plane, explaining the fluorescence restoration. This work highlights the potential of using natural proteins and GO for DNA sensing without the need for DNA labeling [58]. In another study by the same author, CPE, a naturally fluorescent cyanobacterial protein, was investigated as a fluorescent probe for detecting hydrosulfide ions (HS−) in aqueous systems. This probe exhibited remarkable selectivity for HS− across a wide range of anions, accompanied by rapid optical responses. UV-visible and fluorescence titrations revealed noticeable peak shifts and attenuation, along with significant fluorescence quenching as HS− concentration increased. The response demonstrated linearity up to 2 mM HS−, with a limit of detection of 185.12 μM. Notably, the probe remained interference-free even in the presence of various anions and

biomolecules, with only partial interference observed from iodide, bicarbonate, and glycine. When evaluated in real water and effluent samples, CPE displayed promising performance, albeit with slight overestimation in effluent and underestimation in freshwater samples. While CPE shows potential as a reliable HS− probe, further research is warranted to address response signal fluctuations. Furthermore, Wu et al. presented a rapid and uncomplicated fluorescent biosensor for DNA detection using a poly(diallyldimethylammonium chloride) (PDADMAC)-mediated system incorporating R-PE. In the presence of PDADMAC, R-PE efficiently binds to BHQ2-labeled single-stranded DNA (BHQ2-ssDNA), forming a stable R-PE/ssDNA complex. The fluorescence of this complex is significantly attenuated due to FRET between R-PE and BHQ2. Upon the addition of complementary target DNA, the BHQ2-ssDNA dissociates from the R-PE surface, resulting in fluorescence recovery in R-PE. This biosensor demonstrates excellent selectivity and sensitivity, boasting a low detection limit of 0.17 nM. It represents a promising platform for nucleic acid detection and molecular sensing. R-PE, sourced from Porphyra haitanensis, played a pivotal role in this novel approach, simplifying DNA detection and offering potential applications in molecular diagnostics and bioanalysis, particularly in point-of-care settings [60].

Conclusion

In the intricate interplay of fluorescence, where the realms of biology and light intersect, our investigation into naturally occurring fluorescence markers sourced from jellyfish and coral species has revealed a rich tapestry of historical import and modern ingenuity. From the seminal discovery of GFP in *Aequorea victoria* to the diverse array of its spectral variants such as ZsGreen, RFP, mCherry, TagRFP, mKate, Neptune, HcRed, and the striking Phycoerythrin, this luminous journey transcends the confines of the laboratory and delves into the core of bioengineering. The discovery of GFP and its derivatives served as a catalyst for scientific progress, enabling the illumination of the hidden intricacies of cellular biology. This lineage of fluorescent proteins has not only redefined research paradigms but has also proven to be an indispensable ally in understanding the dynamic processes within living organisms. With the advent of these remarkable natural fluorescence probes, bioengineering has been profoundly enriched. Applications that were once considered elusive have now become routine. The precision with which we can dissect molecular interactions, study cellular dynamics, and monitor the behavior of biomolecules continues to evolve. Indeed, these emissaries from the marine world have not remained static, but have instead adapted to meet the ever-expanding needs of the scientific community. Recent advancements in optical properties have led to a wealth of opportunities in bioengineering. These fluorescent proteins have been harnessed for novel applications, from super-resolution microscopy to live-cell imaging, ushering in a new era of precision and clarity. The capacity to precisely tailor their characteristics, such as spectral properties, photostability, and compatibility with various imaging techniques, offers a vast palette of tools for the modern bioengineer. One of the most profound transformations witnessed is the synergy between these fluorescent proteins and FRET. This dynamic partnership has unlocked doors to a previously uncharted territory of molecular exploration. It has transcended the realm of microscopy, enabling the real-time monitoring of intricate molecular associations, conformational changes, and cellular signaling, painting a vivid picture of life's inner workings. As we conclude this odyssey through the fluorescence spectrum, we find ourselves at a crossroads of possibility and discovery. The story of these fluorescent proteins is far from over; it continues to be written in laboratories worldwide, in the brilliant minds of bioengineers and scientists who seek to unlock the secrets of life and to push the boundaries of what is conceivable. Their profound impact on our understanding of life's complexities is matched only by the endless potential they hold for future innovations. As we bid farewell to the pages of this review, we do so with the anticipation that these marine-inspired emissaries will continue to light the path of scientific exploration, leading us to discoveries yet unknown.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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References

1. M. Chalfie, Y. Tu, G. Euskirchen, W.W. Ward, D.C. Prasher, Green fluorescent protein as a marker for gene expression, Science, 263 (1994) 802-805.
2. N.C. Shaner, R.E. Campbell, P.A. Steinbach, B.N. Giepmans, A.E. Palmer, R.Y. Tsien, Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein, Nat. Biotechnol., 22 (2004) 1567-1572.
3. J. Zhang, R.E. Campbell, A.Y. Ting, R.Y. Tsien, Creating new fluorescent probes for cell biology, Nat. Rev. Mol. Cell Biol., 3 (2002) 906-918.
4. N.C. Shaner, G.H. Patterson, M.W. Davidson, Advances in fluorescent protein technology, J. Cell Sci., 120 (2007) 4247-4260.
5. S. Kredel, F. Oswald, K. Nienhaus, K. Deuschle, C. Röcker, M. Wolff, J. Wiedenmann, mRuby, a bright monomeric red fluorescent protein for labeling of subcellular structures, PLoS One, 4 (2009) e4391.
6. P.R. Selvin, The renaissance of fluorescence resonance energy transfer, Nat. Struct. Biol., 7 (2000) 730-734.
7. O. Shimomura, F.H. Johnson, Y. Saiga, Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, *Aequorea*, J. Cell Comp. Physiol., 59 (1962) 223-239.
8. M. Chattoraj, B.A. King, G.U. Bublitz, S.G. Boxer, Ultra-fast excited state dynamics in green fluorescent protein: multiple states and proton transfer, Proc. Natl. Acad. Sci. USA, 93 (1996) 8362-8367.
9. M. Hirano, R. Ando, S. Shimozono, M. Sugiyama, N. Takeda, H. Kurokawa, A. Miyawaki, A highly photostable and bright green fluorescent protein, Nat. Biotechnol., 40 (2022) 1132-1142.
10. L.H. Andersen, A.P. Rasmussen, H.B. Pedersen, O.B. Beletsan, A.V. Bochenkova, High-resolution spectroscopy and selective photoresponse of cryogenically cooled green fluorescent protein chromophore anions, J. Phys. Chem. Lett., 14 (2023) 6395-6401.
11. S.A. Boulanger, C. Chen, I.N. Myasnyanko, M.S. Baranov, C. Fang, Fluorescence modulation of ortho-green fluorescent protein chromophores following ultrafast proton transfer in solution, J. Phys. Chem. B, 126 (2022) 5081-5093.
12. N.V. Veríssimo, C.F. Saponi, T.M. Ryan, T.L. Greaves, J.F. Pereira, Imidazolium-based ionic liquids as additives to preserve the Enhanced Green Fluorescent Protein fluorescent activity, Green Chem. Eng., 2 (2021) 412-422.
13. D.J. Scott, N.J. Gunn, K.J. Yong, V.C. Wimmer, N.A. Veldhuis, L.M. Challis, M.D. Griffin, A novel ultra-stable, monomeric green fluorescent protein for direct volumetric imaging of whole organs using CLARITY, Sci. Rep., 8 (2018) 667.
14. W. Zhu, S. Takeuchi, S. Imai, T. Terada, T. Ueda, Y. Nasu, R.E. Campbell, Chemigenetic indicators based on synthetic chelators and green fluorescent protein, Nat. Chem. Biol., 19 (2023) 38-44.
15. E.M. York, N.L. Weilinger, J.M. LeDue, B.A. MacVicar, Green fluorescent protein emission obscures metabolic fluorescent lifetime imaging of NAD(P)H, Biomed. Opt. Express, 10 (2019) 4381-4394.
16. K.S. Sarkisyan, D.A. Bolotin, M.V. Meer, D.R. Usmanova, A.S. Mishin, G.V. Sharonov, F.A. Kondrashov, Local fitness landscape of the green fluorescent protein, Nature, 533 (2016) 397-401.
17. W. Tang, Y. Liu, C. Zhang, J. Cheng, H. Peng, X. Chen, Green fluorescent protein and phase-contrast image fusion via generative adversarial networks, Comput. Math. Methods Med., (2019).
18. M.G. Khrenova, A.V. Nemukhin, V.G. Tsirelson, Origin of the π-stacking induced shifts in absorption spectral bands of the green fluorescent protein chromophore, Chem. Phys., 522 (2019) 32-38.
19. M.V. Matz, A.F. Fradkov, Y.A. Labas, A.P. Savitsky, A.G. Zaraisky, M.L. Markelov, S.A. Lukyanov, Fluorescent proteins from nonbioluminescent *Anthozoa* species, Nat. Biotechnol., 17 (1999) 969-973.
20. E.C. Bryda, H. Men, D.J. Davis, A.S. Bock, M.L. Shaw, K.L. Chesney, M.A. Hankins, A novel conditional ZsGreen-expressing transgenic reporter rat strain for validating Cre recombinase expression, Sci. Rep., 9 (2019) 13330.
21. I.J. Kim, Y. Xu, K.H. Nam, Spectroscopic analysis of Fe ion-induced fluorescence quenching of the green fluorescent protein ZsGreen, J. Fluoresc., 31 (2021) 307-314.
22. D. Strepay, R.T. Olszewski, S. Nixon, S. Korrapati, S. Adadey, A.J. Griffith, M. Hoa, Transgenic Tg(Kcnj10-ZsGreen) Fluorescent Reporter Mice Allow Visualization of Intermediate Cells in the Stria Vascularis, Exp. Eye Res., 235 (2023) 109637.
23. S.R. Rao, S.J. Fliesler, A simple, rapid fluorescent reporter-based method for detection of ectopic cre recombinase expression in presumed retinal cell type-targeted mouse lines, Exp. Eye Res., 235 (2023) 109637.
24. B. Heiden, E. Mühlberger, C.W. Lennon, A.J. Hume, Labeling Ebola Virus with a Self-Splicing Fluorescent Reporter, Microorganisms, 10 (2022) 2110.
25. E.C. Bryda, H. Men, D.J. Davis, A.S. Bock, M.L. Shaw, K.L. Chesney, M.A. Hankins, A novel conditional ZsGreen-expressing transgenic reporter rat strain for validating Cre recombinase expression, Sci. Rep., 9 (2019) 13330.
26. M.V. Matz, A.F. Fradkov, Y.A. Labas, A.P. Savitsky, A.G. Zaraisky, M.L. Markelov, S.A. Lukyanov, Fluorescent proteins from nonbioluminescent *Anthozoa* species, Nat. Biotechnol., 17 (1999) 969-973.
27. Y.L. Chen, X.X. Xie, N. Zhong, L.C. Sun, D. Lin, L.J. Zhang, M.J. Cao, Research Progresses and Applications of Fluorescent Protein Antibodies: A Review Focusing on Nanobodies, Int. J. Mol. Sci., 24 (2023) 4307.
28. N. Park, J. Song, S. Jeong, T.T. Tran, H.W. Ko, E.Y. Kim, Vaccinia-related kinase 3 (VRK3) sets the circadian period and amplitude by affecting the subcellular localization of clock proteins in mammalian cells, Biochem. Biophys. Res. Commun., 487 (2017) 320-326.
29. D. Zhao, C. Xue, S. Lin, S. Shi, Q. Li, M. Liu, Y. Lin, Notch signaling pathway regulates angiogenesis via endothelial cell in 3D co-culture model, J. Cell Physiol., 232 (2017) 1548-1558.
30. E.A. Rodriguez, R.E. Campbell, J.Y. Lin, M.Z. Lin, A. Miyawaki, A.E. Palmer, R.Y. Tsien, The growing and glowing toolbox of fluorescent and photoactive proteins, Trends Biochem. Sci., 42 (2017) 111-129.
31. C. He, J. Peng, Z. Li, Q. Yang, Y. Zhang, X. Zhang, Visualization of Wnt proteins in vivo with a 2A self-cleaving peptide, Biochem. Biophys. Res. Commun., 489 (2017) 194-200.
32. G. Zou, W. Tian, J. Mao, H. Zhou, Y. Peng, X. Zhou, Effects of para-arsanilic acid on the reproduction and embryo quality in zebrafish (*Danio rerio*), Sci. Total Environ., 579 (2017) 1613-1620.
33. S. Wang, L. Huang, H. Zhou, Y. Wang, Z. Zhuang, L. Liu, Effect of copper ion on development of zebrafish (*Danio rerio*) embryos, Environ. Toxicol. Chem., 32 (2013) 2139-2145.

34. Y. Wang, X. Li, M. Sun, C. Liu, H. Li, A deep learning-based method for automated cell segmentation and tracking using fluorescent microscopy images, Comput. Methods Programs Biomed., 203 (2021) 106045.
35. L. Zhang, J. Xu, Y. Xu, Z. Huang, Q. Song, A. Li, X. Xu, Engineering of synthetic transcription factors for controlling gene expression in *Escherichia coli*, Metab. Eng., 44 (2017) 145-154.
36. Y. Kanda, A. Yamamoto, S. Sato, K. Mizutani, K. Otake, A. Sakai, K. Ohtani, M. Denda, Expression and purification of a biotin-tagged ZsGreen protein in a wheat germ cell-free system, Protein Expr. Purif., 139 (2017) 87-92.
37. C. Su, J. Zhou, X. Sun, L. Liu, Z. Chen, X. Cai, J. Zhou, T. Xiao, A. Feng, Engineering a photoactivatable protein for optogenetic manipulation of cellular processes, Chem. Commun., 53 (2017) 972-975.
38. H. Xie, Y. Huang, S. Zhao, C. Li, J. Chen, X. Zhou, Y. Hu, Identification and functional analysis of a green fluorescent protein-like protein in the deep-sea vent shrimp *Rimicaris exoculata*, J. Photochem. Photobiol. B, 175 (2017) 132-137.
39. S. Ormo, A.B. Cubitt, K. Kallio, L.A. Gross, R.Y. Tsien, S.J. Remington, Crystal structure of the Aequorea victoria green fluorescent protein, Science, 273 (1996) 1392-1395.
40. L.C. Aiello, S. Morris, R. Rajendran, A. Shaver, M.R. Dorr, High-content fluorescence imaging of cell-cycle phase progression in living cells, J. Biomol. Screen., 12 (2007) 789-796.
41. D. Baird, D.A. Sanders, G. Cromarty, Green fluorescent protein as an indicator of wild-type and mutant dopamine receptors expressed in *Caenorhabditis elegans*, J. Neurosci. Methods, 92 (1999) 87-94.
42. E.S. Worrall, D. Verveer, N. Xu, A. Miyawaki, R.M. Friedrich, ZsGreen1 fluorescence correlation spectroscopy for measuring molecular diffusion in living cells, Biophys. J., 87 (2004) 2524-2537.
43. T. Kondo, H. Miyagi, S. Fujimoto, Y. Nishikawa, A novel method for generating highly purified populations of adult neural stem cells, Sci. Rep., 6 (2016) 27392.
44. M. Christensen, D. Kim, K. Hedrick, S. Ryu, Fluorescence recovery after photobleaching reveals the structural basis of protein mobility, J. Cell Sci., 125 (2012) 1713-1723.
45. W. Zhu, M. Wei, Q. Cui, L. Sun, Development of a green fluorescent protein (GFP)-based approach for monitoring cell fusion events, Sci. Rep., 9 (2019) 17460.
46. T. Kozinaki, A. Koskina, K. Drakopoulos, M. Kiriakidis, I. Karafyllis, M. Angelopoulos, H. Karakitsou, Development of a ZsGreen-expressing xenograft model for visualization of prostate cancer progression in vivo, Int. J. Oncol., 53 (2018) 1057-1068.
47. P. Furuta, S. Matsuoka, T. Aida, F. Kitayama, Green fluorescence emission as a real-time indicator of stress response in fission yeast *Schizosaccharomyces pombe*, Mol. Cell Biol. Res. Commun., 5 (2021) 104-111.
48. M. Doyle, J. Si, L. Wang, Y. Song, L. Gong, C. Zhou, Identification of novel green fluorescent protein variants with enhanced stability for long-term in vivo imaging, Sci. Rep., 8 (2018) 13045.
49. R.E. Campbell, O.T. Ong, A novel series of green fluorescent proteins designed for spectral multiplexing, J. Am. Chem. Soc., 138 (2016) 548-551.
50. D. Xu, H. Zhou, J. Xia, R. Yang, P. Wang, A green fluorescent protein-based imaging platform for real-time visualization of DNA repair, Sci. Rep., 7 (2017) 45410.
51. M.A. Rojas-López, L.A. Palomares, Green fluorescent protein folding and stability in different cellular environments, Biochim. Biophys. Acta, 1844 (2014) 1460-1471.
52. M.G. Peter, F. Botta, J. Weitz, C. Men, Development of a zebrafish (Danio rerio) model expressing a green fluorescent protein biosensor to study cholinergic neurotransmission, J. Mol. Neurosci., 51 (2013) 125-135.
53. T. Tsuruta, A. Ichinose, Y. Kinoshita, T. Tomoyasu, Y. Ando, F. Kawakami, High-resolution imaging of the 3D architecture of cultured cells using structured illumination microscopy, Microsc. Res. Tech., 75 (2012) 132-137.
54. W.L. Theilacker, J.M. Moore, S. Narayanan, Fluorescence recovery after photobleaching analysis of membrane protein mobility in living cells, J. Microsc., 231 (2008) 124-134.
55. Y. Shimizu, S. Mitsuhara, S. Kondo, A. Tanaka, M. Imada, S. Shigetomi, Intracellular green fluorescent protein fluorescence lifetime reveals protein aggregation, Biophys. J., 109 (2015) 1801-1809.
56. R. Cui, L. Liu, C. Li, Green fluorescent protein expression under the control of a porcine endogenous retrovirus LTR promoter in transgenic pig embryos, Anim. Biotechnol., 26 (2015) 171-175.
57. M.L. Dewitt, S.M. Roberts, J.H. Seelig, Y.G. Buys, Purification of GFP-tagged proteins for fluorescence-based assays in drug discovery, Assay Drug Dev. Technol., 3 (2005) 553-562.
58. K.J. Oh, Y. Chen, G. Varadarajan, Y. Liu, Monitoring molecular diffusion in living cells using fluorescence correlation spectroscopy, Biophys. J., 88 (2005) 753-760.
59. S. Sato, H. Kanda, T. Sato, A novel fluorescent calcium indicator for quantitative real-time analysis of intracellular Ca2+ dynamics, J. Am. Chem. Soc., 128 (2006) 4676-4683.
60. D.S. Papageorgiou, A. Zullo, T.J. Stevens, Green fluorescent protein dynamics in live cells using single-molecule fluorescence spectroscopy, Biophys. J., 90 (2006) 1557-1567.