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Bioluminescence: A Boon in Medical Technology

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Abstract -*The process through which living things emit visible light is referred to as bioluminescence. Small laboratory animals can now be molecularly imaged using bioluminescence imaging, a potent methodology that has been developed over the past ten years. This allows for the in vivo examination of ongoing biological processes. The in vivo BLI toolbox consists of a list of luciferase compounds that may produce bioluminescent light signals as well as sophisticated and potent instrumentation made to non-invasively detect and measure these light signals as they are emitted from the living subject. Numerous useful uses of novel instruments created by deriving them from the chemistry of naturally occurring light-producing molecules have lately resulted in a related Nobel Prize. Marine species use bioluminescence for a variety of essential processes, including reproduction and defence. To understand these interactions and the distributions of luminous organisms, new instruments and platforms allow observations on individual to oceanographic scales. This technology provides valuable means for monitoring of different biological processes for immunology, oncology, virology and neuroscience. Here, we go through the fundamentals of bioluminescence imaging as well as several applications that are pertinent to the field of medicinal chemistry.*

***Key Words*: Bioluminescence, luciferase, optical-imaging, Pyrearinus termitilluminans (ELuc), immunohistochemical labelling, luminometers.**

1.INTRODUCTION

Bio- stands for life or the living, while lum or lumen is derived from the Latin lumen or lux, which stands for light. One of nature's most astounding phenomena is bioluminescence, or light created by a living thing. It can appear to be science fiction rather than science. When the reaction takes place in living things, scientists refer to the phenomenon as "bioluminescence". Typically, bioluminescence is blue or blue-green. However, it can also be almost violet (bright purple), greenish-yellow, and less frequently red. [1] Beginning in the early 20th century, imaging technologies were developed to supplement morphological findings. A living organism's molecular, cellular, biochemical, and physiological processes can be visually (and frequently quantitatively) studied in relation to time and space using molecular imaging (MI). Three categories can be used to categorise imaging systems: the energy source (X-rays, positrons, photons, or sound waves), the spatial resolution (macroscopic, mesoscopic, or microscopic), or the sort of information acquired (anatomical, physiological, cellular, or molecular). [2-4]

In theory, the four main biological purposes of bioluminescence are defence, counter-illumination, prey luring, and intraspecific communication. [5-7] Biologic processes can be seen as they take place inside a living subject thanks to in vivo bioluminescent imaging (BLI). The information acquired is unparalleled in its capacity to shed light on biology outside the realm of the typical in vitro experiment, which largely ignores the intricate interactions of a live system. In vivo BLI generates distinctive bioluminescent outputs that are subsequently caught externally by cutting-edge cameras using the luciferase family of proteins[8].The most widely used reporters are Herpes Simplex Virus-1 thymidine kinase (positron

emission tomography), firefly luciferase (bioluminescence imaging), green fluorescence protein (fluorescence imaging), transferrin receptor (magnetic resonance imaging), and variants with improved spectral and kinetic properties tailored for use in vivo[9,10].An overview of bioluminescence technology and its applications for tracking a variety of biological properties in challenging conditions are given in this article[11].

2.Bioluminescence and its system.

2.1 Bioluminescence imaging:

Mammalian tissues exhibit low intrinsic bioluminescence, making bioluminescence an interesting method for in vivo optical imaging in these tissues. As a result, pictures can be produced with astonishingly high signal-to-noise ratios. There are numerous distinct bioluminescent systems in nature, each needing a particular enzyme and substrate [12,13]. Luciferase, an enzyme, oxidises luciferin, a substrate, in a chemical reaction that produces luminescence and photon emission. For some luciferases to function, cofactors like ATP and Mg^{2+} are necessary. Another popular optical imaging technique that produces light through a chemical reaction is fluorescence. This light creation, in contrast to BL, is initiated by an outside light source. Additionally, there are differences between these two imaging modalities in terms of signal strength and signal-to-noise (S/N) ratio. Although fluorescent signals are often brighter than bioluminescence, auto-fluorescence also contributes to a higher background noise level. The high sensitivity of bioluminescence is mostly a result of the background being nearly gone, which results in higher S/N ratios. [2,14]

Firefly luciferase and its substrate D-luciferin are the most popular options of luciferase and luciferin for in vivo BLI. The substrate is nontoxic and stable in cells and live animals, and this combination emits photons at tissue-penetrating red and near-infrared wavelengths. Bioluminescence may be easily seen several minutes after standard intra-peritoneal (IP) injection of the substrate. [15,16]. In vivo bioluminescence imaging involves tagging biological entities or process elements with a reporter gene that encodes one of several light-generating enzymes, such as bacteria, tumour cells, immune cells, or genes (luciferases). Low quantities of light produced by luciferases in a living animal can be seen outside of the mammalian tissues' absorbing and scattering environment. Haemoglobin, which mostly absorbs in the blue and green area of the spectrum, or up to around 600 nm, is the main absorbing molecule in mammalian tissues. [17-21]

2.2 Light produced by Bioluminescence:

Bioluminescence is a result of an oxygen-based chemical reaction, just like fire. The reaction nonetheless takes place without producing a lot of heat. Similar to this, while many luciferase-luciferin combinations have been found in nature, only a small number have been refined for use in bio-imaging applications. The size and structure of the bioluminescent pairs vary, but they all have the same mechanism for producing light: the luciferase catalyses the oxidation of the complimentary luciferin. An electrically excited intermediate is created during the enzymatic reaction, and when this molecule relaxes to the ground state, a photon of light is released. Light's colour (wavelength) is mostly determined by the small molecule emitter's structure, while enzyme residues also have an impact [11,22,23].

An enzymatic process causes bioluminescence. By assisting a substrate in reacting, an enzyme quickens the chemical reaction. Instead of being converted into another molecule, the enzyme is just employed again in the reaction. The reaction occurs outside of the organism in shrimp that emit light due to their bioluminescence. The reaction takes place in the cells of some animals. In some cases, it is created by organism-dwelling bacteria. However, the light is produced by the same fundamental reaction between an enzyme and a substrate. Different luciferin molecules are used by various species of organisms. This shows that different organisms' capacity to create light underwent different evolutionary processes.

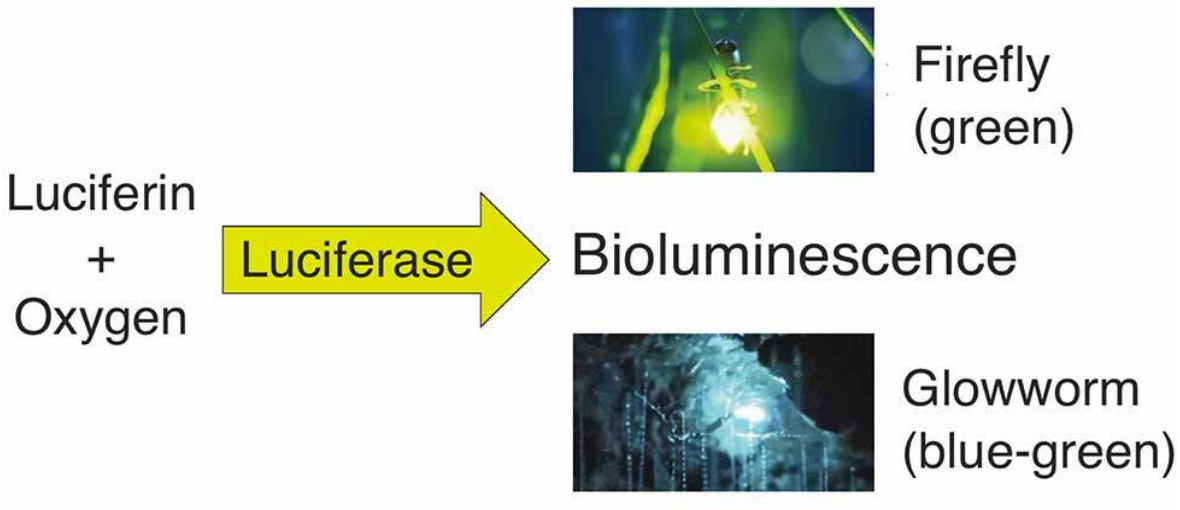

Fig 1: Light produced by bioluminescence**.**

The substrate, luciferin, and oxygen are joined by the enzyme luciferase. Oxyluciferin and light are the by-products of the process. After the reaction, the enzyme is recycled and can be utilised once more. The firefly's luciferase and luciferin (Fluc and D-luciferin, respectively) are the two bioluminescent pairs that are most frequently employed for in vivo imaging. Following delivery via conventional i.p. injection, D-luciferin is comparatively stable and can penetrate most cell and tissue types.[11] Since decreased FMN, which is required for the reaction to continue and must be provided by an enzyme other than the luciferase itself, is required, the bacterial bioluminescent reaction falls under the category of two component systems. It was hypothesised that the protein LuxG serves a similar purpose in bioluminescent bacteria because of LuxG's significant resemblance to Fre, a flavin reductase in E. coli. [5,24-27]

2.3 Variety of Bioluminescence Organisms:

In order to produce bacterial luminescence, $FMNH_2$ is oxidised alongside a long-chain aldehyde and a two-subunit luciferase.[28] Bioluminescence occurs frequently in the sea environment but is uncommon in terrestrial habitats. Some bioluminescent organisms are found in many different types of marine life, including bacteria, squid, and fish. From the ocean's surface to its deep-sea floor, researchers have discovered species that emit bioluminescence. The shimmering brightness of protozoans in tropical seas, the flashing signals of fireflies, or the eerie glow of germs on decomposing flesh or fish. A vast variety of protists and animals, including bacteria, fungus, insects, marine invertebrates, and fish, exhibit the phenomena sporadically, but genuine plants, amphibians, reptiles, birds, and mammals are not known to naturally exhibit it.[29] A better comprehension of the conditions required for luminescence and the genetic lux cassettes that create light have led to several biotechnological applications (Meighen 1991). (Dunlap & Kita-Tsukamoto 2006) [28]. Three different species of crustaceans with particularly eye-catching luminous traits include copepods, shrimp, and ostracods. There are many luminous copepods in the oceans of the world. Some people live on land, while others are subaquatic. Pleuromma and Metridia are two well-known families of luminous copepods.[29]

2.4 Bioluminescence Mechanism:

• Bacterial bioluminescence is based on a traditional two-component system that consists of a tiny chemical that serves as the light-emitting species during the reaction and an enzyme (referred to as luciferase) that catalyses the bioluminescent reaction. When it comes to bacterial bioluminescence, flavin mononucleotide (FMN), which goes through a series of processes, is the source of the luciferin.[5]

• FMN is a versatile cofactor that is frequently utilised in nature in a variety of biological processes that handle electrons, such as oxidation-reduction reactions. The oxidation of a long-chain fatty aldehyde, which is produced in bioluminescent bacteria from myristoyl-ACP, to the matching long-chain fatty acid provides the energy needed to occupy the excited state of the luciferin.[5]

• The luciferin-luciferase system is a metabolic process that is present in many species that produce light through bioluminescence. The route generates light using the tiny chemical luciferin and the enzyme luciferase.[30]

• When luciferin is changed into oxyluciferin by luciferase, the luciferin-luciferase system is triggered. The light is then produced by the oxyluciferin's reaction with oxygen. The light is created by a chemiluminescent process, which is a chemical reaction that results in the production of light.[30]

$$Luc + D\text{-}LH_2 + ATP \xrightarrow{Mg2+} Luc + LH_2\text{-}AMP + PPi$$

$$Luc + LH_2\text{-}AMP + O_2 \longrightarrow Luc + AMP + oxyluciferin^* + CO_2$$

$$Luc + AMP + Oxyluciferin^* \xrightarrow{Mg2+} Luc + Oxyluciferin + AMP + h\upsilon$$

[15,31,32]

• The L (+) luciferin will react with firefly luciferase and adenosine triphosphate (ATP) in the presence of Mg2+ ions to form the intermediate E. LH-AMP and to release inorganic pyrophosphate, but the subsequent reaction with molecular oxygen does not occur. The D (-) isomer of firefly luciferin is biologically active in the production of light. Dehydroluciferin is not a by-product of the light reaction steps, despite the fact that some of it seems to be produced during the enzymatic oxidation of luciferin. It is also found in the light organs of fireflies, and as we'll explore later, we think it plays a crucial role in the firefly's system for controlling flashes. A thorough explanation of the organic process by which D (-) luciferyl-adenylate combines with molecule oxygen to create a product molecule with excited electrons.[32,33]

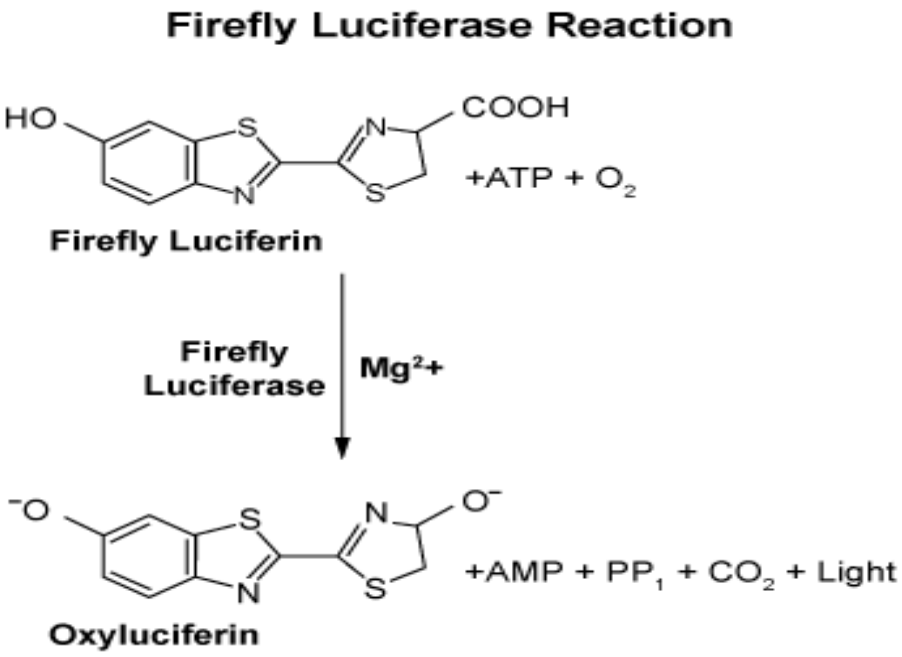

Fig 2: Firefly luciferase reaction.

2.5 Glowing Adoption:

Scientists are still learning more about the evolution of this particular adaption as well as its entire function and purpose. But organisms can benefit from bioluminescence:

- Locate food,
- Hide from predators,
- Find mates,
- Protect themselves from or warn off predators,
- Find or detect prey and
- Communicate.[1]

2.6 Uses of Bioluminescence:

Bioluminescence has a wide range of applications. Some examples include;

- Attracting prey or mates;
- Protecting against predators;
- Communicating with others;
- Illuminating the way in the dark;
- Designating territory and
- Digesting food. [30]

3.APPLICATION OF BIOLUMINESCENCE

- **In vivo detection of Bioluminescence:**

The creation of the enhanced green-emitting beetle luciferase from Pyrearinus termitilluminans (ELuc), whose light signal in mammalian cells is more than ten times larger than that of firefly luciferase, improved the spatiotemporal resolution of bioluminescence imaging. Although a luciferase reporter is currently used for bioluminescence imaging, including at the single-cell level, it can be challenging to perform subcellular or organelle imaging due to insufficient signal intensity in viable cells and because subcellular imaging with a higher-magnification lens requires a greater luminescence intensity.
Figure 2 depicts the higher resolution subcellular localization of ELuc in the cytosol, peroxisome, and nucleus of mammalian cells.

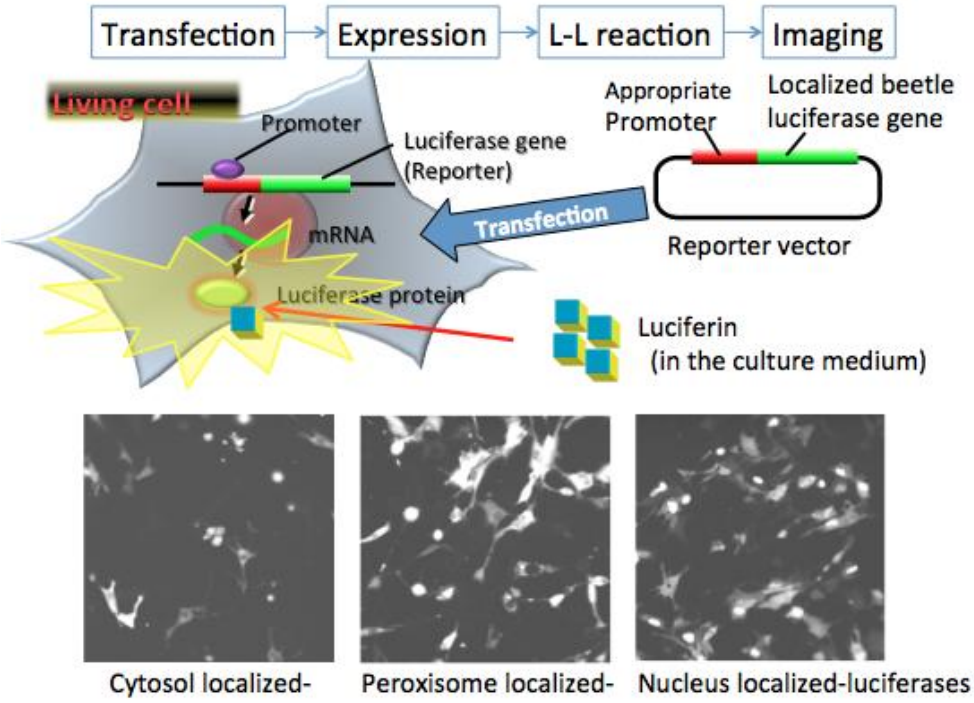

Fig 3: Detection of in-vivo bioluminescence.

Organelles in living cells can be imaged using in vitro bioluminescence. The organelle-targeting luciferase gene sequence for the cytosol, peroxisome, and nucleus in mammalian cells is part of the reporter plasmid vectors. The promoter region controls the expression of the luciferase genes in living cells once the plasmid has been transfected into the target cells.

Firefly luciferin, which is introduced to the media for the imaging experiment, penetrates the organelle and is catalysed to produce light by the expressed firefly luciferase there. The light signal, which denotes the location or mobility of organelles in living cells, can be used to visualise the localisation of the expressed luciferase protein. In this instance, specialised equipment using a CCD photon imaging system is used to measure the bioluminescence imaging. [33-35]

- **Ex vivo detection of Bioluminescence:**

The tumour tissue had fibrous stroma and tubular glands that were moderately differentiated. The tumour cells had an intense immunoreactive response to the antigen, according to immunohistochemical labelling. The method of immunohistochemical imaging employing direct luciferase labelling and indirect peroxidase labelling is shown in Figure 3. Three of the serial paraffin slices were initially prepared by being heated in a microwave. After the first antibody has been incubated for the immunohistochemical detection, the section is twice rinsed with PBS buffer before being treated with the second antibody-conjugated peroxidase. The segment for malignancy was cleaned twice with buffer and then appropriately processed for immunohistochemistry staining. The material is directly visible by the luciferin-luciferase response after being incubated with the anti-cancer antibody-luciferase conjugate for 30–60 min. A cypridinid luciferin solution is added to the section after incubation and washing, and under dim lighting, photos of the tumour cells' bioluminescence are promptly captured using a cooled CCD camera system run at a high sensitivity range. Strong bioluminescence images that matched those from immunohistochemistry staining were produced. [33,36]

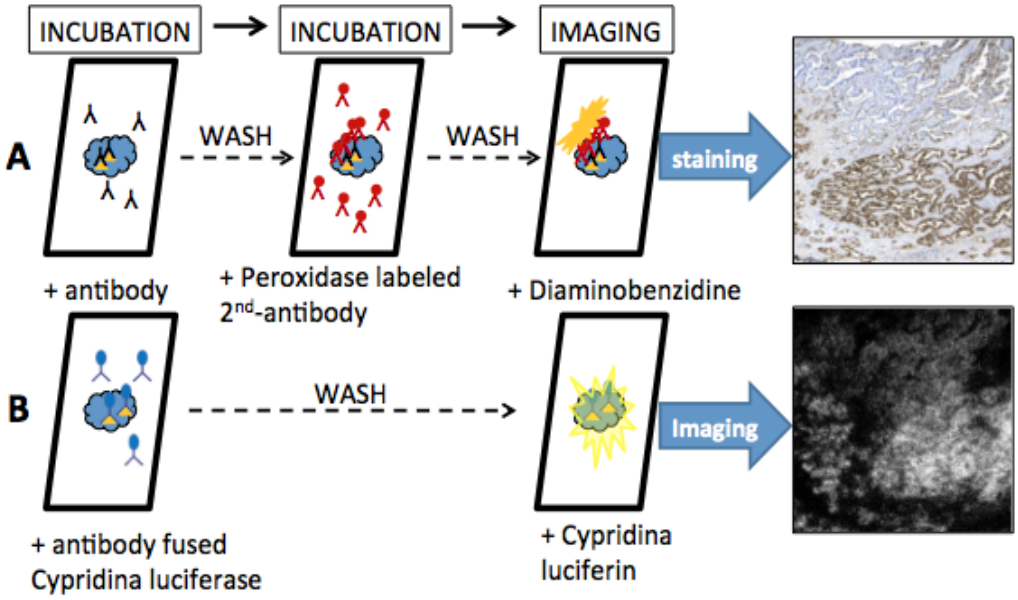

Fig 4: Detection of ex-vivo bioluminescence.

- **Cell tracking**

Live animals can be repeatedly scanned for luciferase-expressing cells, with the signal's strength corresponding to the density of cells in a particular location. As a result, bioluminescence imaging is highly adapted for following cells over time in vivo, and several investigations from other fields have benefited from this property. Additionally, it was mentioned that effective immune cell expansion could occur after the implantation of just one HSC. In a more recent instance, bioluminescence technology was employed to quickly assess the proliferative potential of cancer stem cells in vivo and investigate their functions in metastases. From patient breast tumour biopsies, the authors separated cancer stem cells and transfected them with genes encoding Fluc or Rluc. Immunocompromised mice were given the luciferase-expressing cells orthotopically, and their progress was watched over time. [11,37,38]

- **Infectious disease imaging**

Different strains of Salmonella typhimurium were engineered to express a bacterial luciferase and infected into test animals in a ground breaking report studying pathogens in living organisms. The effectiveness of an antibiotic was observed non-invasively in this model, along with the visualisation of the infection site and infected tissues. The infectious protein prions, which cause deadly neuro degenerative disorders, can be found using the sensitive marker glial fibrillary acidic protein (GFAP). [39,40,41]

- **Common uses include:**

• Researchers utilize bioluminescence markers to monitor the movement of particular cells or species.
• One method for measuring environmental contaminants is bioluminescence.
• Pipeline leaks and other underwater infrastructure problems can occasionally be found using bioluminescent bacteria.
• Traditional Chinese medicine makes use of specific species of firefly.
In some nations, bioluminescent microbes are also employed as food additives.[30]

4.BIOLUMINESCENCE IMAGING TECHNOLOGY ADVANCEMENTS:

- **Developments in reagents and detector technologies:**

A first and promising step toward obtaining three-dimensional in vivo images of biological processes marked with optical reporters is computerized fluorescence tomography. Farkas and colleagues used near-infrared fluorescence-labelled beads and antibodies to demonstrate in vivo fluorescence tomographic imaging in live, naked mice. The resolution of the images and the capacity to produce data in three dimensions are addressed by improvements in the BLI instrumentation. The scattering of light by tissue structures will always limit the resolution of diffuse optical imaging, but collecting bioluminescence data from various angles of the animal subject and relating the data from each angle to the others may make it possible to calculate the depth of origin in three-dimensional space. [17,42,43]

- **Tracking protein function and abundance:**

It is necessary to use techniques to explore individual biomolecules in addition to cells and gene transcription to fully understand how biological systems work mechanistically. One of the main types of biological macromolecules, proteins perform a variety of tasks. Major signalling networks can be upset, biosynthetic pathways can be changed, and membrane integrity can be compromised, all of which can exacerbate disease. Engineered luciferases and luciferins have been developed over the past ten years to provide information on a variety of aspects of protein biology, such as their localization and stability, interactions with other proteins, and enzymatic activity. To track the position and amount of the biomolecules, for instance, direct luciferase attachment to proteins of interest might be utilised. In fact, luciferase fusions have been utilised to view a wide range of proteins, including proteins destined for proteasomal degradation and the signalling chemicals HIF-1 and catenin. However, luciferase fusions by themselves are unable to give readings on specific protein activities, such as their interactions with other proteins. [11,44-46]

- **Monitoring enzymatic activity under challenging conditions:**

In addition to their interactions with other proteins, proteins also control cellular activities through enzymatic processes. Enzymes have been classified into many different classes, and many of them, such as kinases and proteases, are essential for cell signalling. Engineered luciferins and luciferases have been created to provide real-time reports on enzymatic activities. These imaging technologies can facilitate screenings for therapies intended to either inhibit or boost enzymatic activity in addition to providing a dynamic readout on protein function in complicated contexts. "Designer" luciferases, in addition to altered luciferins, can offer immediate readouts on enzyme activity. The majority of these probes contain cyclic luciferase variants that have been linked into inactive conformations. A functioning luciferase is created after the connection is severed ("activation"). A number of these "activatable" luciferases with protease-specific linkers were created by Wood and colleagues. In the presence of specific protease activity, the linkers are cleaved, releasing Fluc, which emits light and ultimately serves as a readout for protease activity. Recently, caspase-7 activation was observed using one of these "activatable" luciferases in both live cells and in vivo tumour models.[11,47-49]

- **Drug development and testing:**

Bioluminescence is a straightforward and direct diagnostic for cell survival and proliferation since fluc light emission necessitates the generation of ATP and new proteins. In heterogeneous models, bioluminescence is a desirable option for tracking cell viability and potential treatments. A more accurate representation of human tumours, the McMillin group has shown the value of bioluminescence for drug screening using stroma and tumour cell mixes. These problems might be resolved by creating novel luciferases and luciferins, and research in this field is continuing. Bioluminescence has benefited drug development efforts in domains other than direct measurements of cell death. This imaging method, for instance, can be applied to the in vivo direct tracking of treatments. Both protein and small molecule biologics can be seen, which offers new perspectives on distribution and targeting.[11,50-52]

5. DRAWBACKS AND RESTRICTIONS:

BLI offers benefits and drawbacks, much like any other imaging method. The luciferase activity is frequently assumed to be closely associated with the reporter gene's transcriptional activity, leading to the assumption that the BL signal is linear with respect to the number of cells when data interpretation is

carried out. When planning a BLI experiment, these issues should be taken into consideration since they may affect the BL signal generated.

• ***Signal quantification***: Since the relative light units (RLUs) obtained from a luciferase reaction are arbitrary units, it is challenging to standardise in vitro BL experiments. The RLU differs significantly between luminometers or photon detectors.

• ***Stability and the enzyme's half-life*****:** It can vary widely different luciferases and can last anywhere from a few hours to many days. The half-life of your luciferase should be predetermined based on the test parameters. The stability of the luciferase can also be impacted by external and endogenous variables.

• ***Cellular environment*****:** Both secreted and non-secreted luciferases may be exposed to various intra- or extracellular circumstances that could have an immediate impact on their activity. The enzyme's proteolytic degradation, pH, temperature, and H_2O_2 levels are only a few of the many variables that could affect the BL signal.

• ***Promoter activity*****:** Transcriptional activity is frequently investigated using bioluminescent reporters. Cis-transcriptional reporter systems make it possible to examine how genes are regulated and expressed. Either creating point mutations or deletions in the promoter region of a gene of interest or using various transcription factor binding sites connected to a minimum promoter to drive the expression of luciferase are used to accomplish this. This method complements traditional in vitro molecular biology and biochemistry approaches by being useful for reporting several processes that affect transgene expression, including as signal transduction, receptor activation, and transcription factor activity.

• ***Light quenching and scattering*****:** Because pigmented molecules like haemoglobin and melanin absorb light and mammalian tissues scatter it, bioluminescence imaging in deep tissues is difficult. Compared to skin or muscle, highly vascularized organs release fewer light signals. Due to melanin's ability to absorb light, highly pigmented mice likewise produce less light than their white or buff counter parts. [2,53,54]

6. CONCLUSION:

Numerous scientists from many different domains have been drawn to the fascinating phenomena of light emission by living things for many years. BLI offers insightful information regarding biological processes in both whole cells and small animal models. By putting target genes and processes in the proper physiological environment, animal models of human diseases considerably advance our understanding of the mechanisms behind pathogenesis. It combines the flexibility of a tiny molecule with genetically programmed specificity, requiring both an enzyme (luciferase) and a substrate (luciferin). Bioluminescence is typically used for assessing tumour burden and gene expression. This makes BLI a potent tool that can be used to explore a growing number of biomedical issues that were previously unreachable for analysis. Real-time monitoring of promoter activity and regulation is possible using BLI, whereas in vivo investigations of protein function and interactions are feasible. It is possible to foresee advancements that increase the usefulness of the imaging technologies now in use, such as multimodality imaging.

The advancement made over the past few decades and the interest with bioluminescence will undoubtedly spur numerous other research initiatives to push the boundaries of human understanding.

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