International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

The Emerging Use of Bioluminescence in the Lives of Humans

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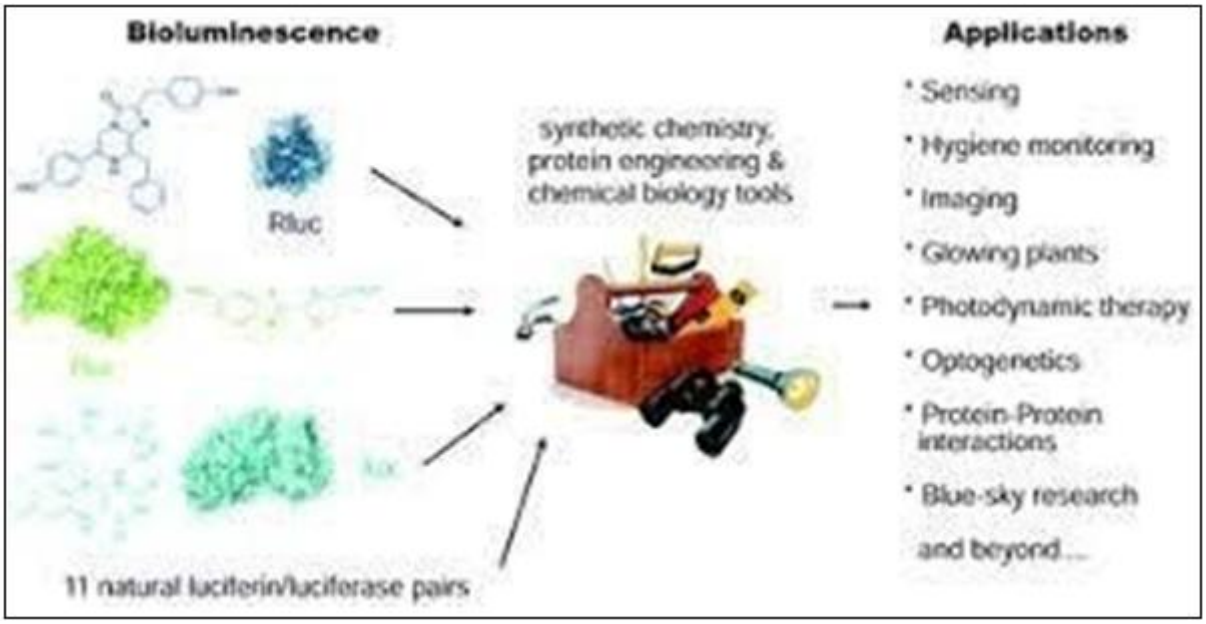
Abstract: ***Bioluminescence is the fascinating natural phenomenon by which light is produced by living organisms. Bioluminescence occurs when the oxidation of a small-molecule luciferin happens in presence of ATP, and which is catalysed by an enzyme known as luciferase. The excited-state species formed during the course of reaction emits light. There are over 30 known bioluminescent systems but the luciferin–luciferase system is the most widely studied one that has been extensively characterised till date, while the other systems are currently under investigation. The different luciferin–luciferase pairs have different light emission wavelengths and hence are suitable for various applications. Recent times have witnessed advances in protein engineering, synthetic chemistry, and medical research which in turn has allowed bioluminescent systems to reach previously uncharted applications. The bioluminescence reaction is now regularly used for gene assays, the detection of protein– protein interactions, drug discovery, hygiene control, analysis of pollution in ecosystems and in vivo imaging in small mammals. Diverging our focus from sensing and imaging; the more recent highlights of the applications of bioluminescence in biomedicine include the bioluminescence- induced photo-uncaging of small-molecules, bioluminescence based photodynamic therapy (PDT) and the use of bioluminescence to control neurons. There has also been an increase in blue-sky research such as the engineering of various light emitting plants. This has led to lots of exciting multidisciplinary works across various disciplines. This review focuses on what in actuality the process of bioluminescence is and how it is slowly becoming an integral part in the lives of humans.***

Keywords: bioluminescence reaction, light, oxidation, luciferin

1. Introduction

Bioluminescence is not at all a common phenomenon, which we come across in our day to day life. When medical researches are taken into the context, bioluminescence has tremendous potential and this rare occurance is just a façade.

Bioluminescence is defined as a phenomenon where light is produced by living organisms and is a special form of chemiluminescence. The word bioluminescence has its roots into both Greek and Latin; where Bios in Greek means life and Luminescence in Latin means light.



What is bioluminescence?

Now, this question has been answered earlier, that it is a phenomenon where visible light is produced by certain living organisms. Now, question arises how do they do so. They have a compound known as luciferin in their body which gets oxidized in presence of molecular oxygen and ATP by a very special enzyme found in their body known as luciferase. This oxyluciferin gets excited and while returning back to the ground state emits visible light.

Where can we find bioluminescence?

Though this phenomenon is not so common to the human eye bioluminescence can be found both in terrestrial ecosystems as well as in the aquatic ecosystems. Some of the terrestrial organisms exhibiting this phenomenon includes fireflies, some varieties of mushrooms while aquatic organisms exhibiting bioluminescence are *noctiluca synctinalis*, algaes etc.

What is the need for exhibiting Bioluminescence?
Bioluminescence is used by different organisms in different ways; some use this to attract their mates, while some use them in order to blind their predators so that they can run away, while some use them to lure their prey. No matter what the purpose is this process has always been fascinating to human and hence a subject of interest.

But before going into the chemistry of bioluminescence it is important to elucidate certain terms;

Chemiluminescence: Chemiluminescence is a broader term and may refer to any chemical reaction that emits visible light on its course. Chemiluminescence was first observed by German chemist H. Brand as he was studying a process, where, waxy white Phosphorous when slowly oxidized at room temperature, gave off a faint greenish glow. The colour was responsible for excited reactive intermediate PO2 and HPO. This reaction was suggestive of the fact that the reaction involved formation of an intermediate, which while returning to the ground state emitted radiation that fell in the visible region and such process appear glowing to the human eye.

One of the very common bioluminescent organism we find in our surroundings is firefly and we will try to understand the chemistry behind bioluminescence by taking this organism into account; the firefly Luciferin-Luciferase system. The pivoital role is played by a compound known as oxyluciferin.

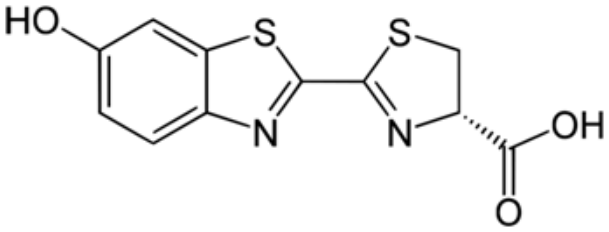


Figure 1: Structure of oxyluciferin

The oxyluciferin is a molecule where a benzothiazolyl moiety and oxythiazolyl moiety is held together by a carbon carbon single bond. The two planar moieties are in a conjugated system, when luciferase enzyme comes and catalyses the reaction with ATP in presence of divalent Magnesium ion which leads to the first excited singlet state of oxyluciferin; upon relaxation to ground state visible light is emitted with great frequency. The mechanistic course of the entire reaction is given below;

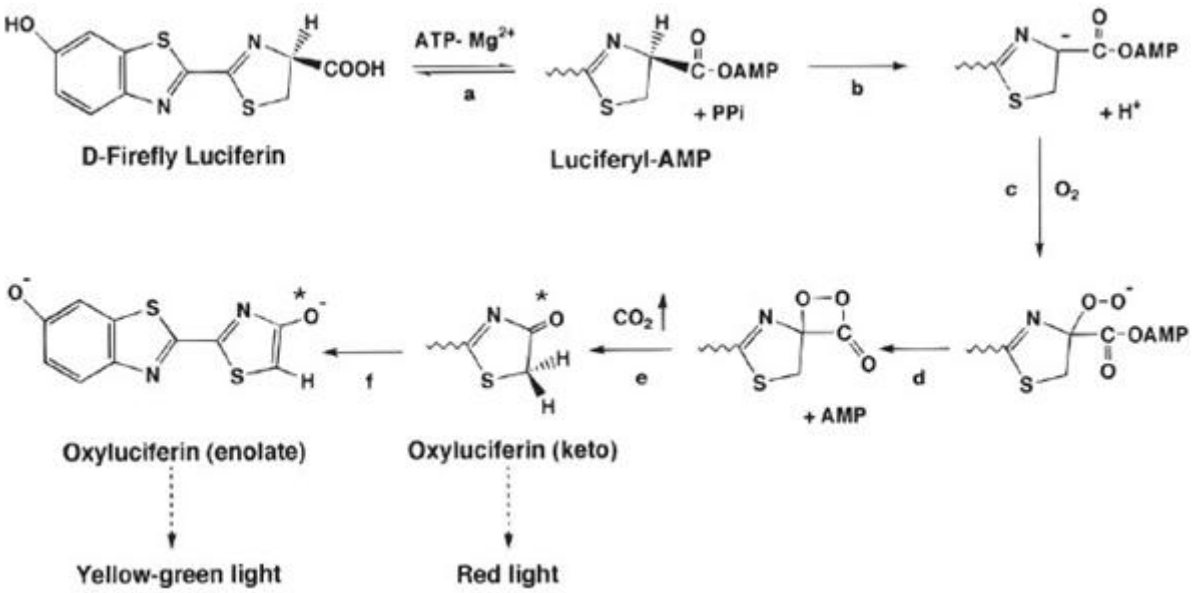


Figure 2: Mechanistic pathway of luciferin reaction

Step 1:
ATP comes and attaches to the carboxyl carbon in the form of AMP with the production of two inorganic phosphates. The product formed is known as AMP-Luciferin.

Step 2:
In the next step first a proton is lost from the ring of oxythiazolyl moiety. In the site from where proton is lost oxygen comes and reacts and forms peroxide linkage with removal of AMP. This molecule formed is known as Fi-dioxetanone.

Step 3:
From the Fi-dioxetanone molecule Carbon dioxide is lost which gives excited oxyluciferin. This excited oxyluciferin while returning to ground state emits visible light.
It maybe noted that, the luciferin-luciferase system is present in other organisms too, but the colour produced by them may be different; this can be accounted from the fact of different orientation of oxyluciferin, caused by the rotation along carbon carbon single bond joining the two planar moiety, and also due to different isomers of the same.

The various aspects of the above mentioned reaction is discussed below:

1) Chemiluminescence by Functionalities
a) The chemiluminophore is a peroxide bond. It is this bond which ruptures during the reaction to give off carbon dioxide and that amount od luminous energy.

b) The aromatic systems are important electron reservoirs as well as CT controlling group. These two characterestics are together known as electron donating fragment.
c) Oxyluciferin's structure has two planar moieties connected together by carbon carbon single bond. Heterojunction means interface between two solids. The electronegative nitrogen of heterocyclic rings and the pi pi conjugation plays an important role in luminescence of luciferin. The term Bio-heterojunction came from Cai who explained the emission of visible light from oxyluciferin with concept of heterojunction, (in analogy to semiconductors).

2) State of substrate

To fully understand the process exact identification and characterization of the light emitter species is necessary. Even though six possible isomers of oxyluciferin is present, the one responsible for light emission in fireflies is the singly deprotonated luciferin in the keto form. Colour modulation can be best explained with the phenolate keto form of oxyluciferin. It depends also on the microenvironment of the cavity.

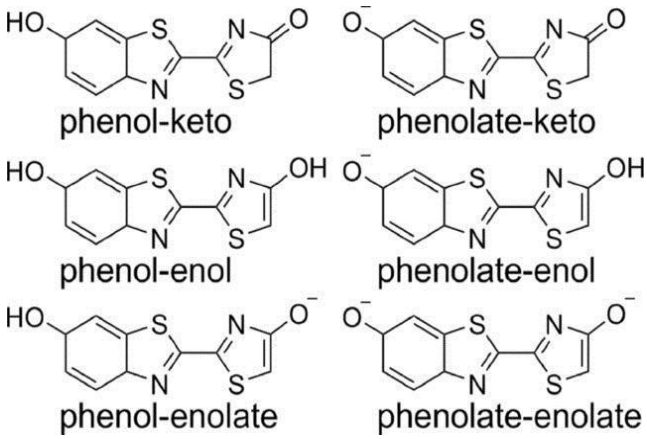


Figure 3: Six possible isomers of oxyluciferin

3) Significance of enzyme

The luciferase enzyme allows-
a) Catalysis of reaction between luciferin and ATP
b) Modulating distance of electron of CT controlling group by orientation of water molecules in the cavity as well as arrangement of residue
c) Applying of an external electrostatic potential on the chromophore.

The energy of emission rather than depending on structure of proteins, depend on the hydrogen bond network connecting the water molecules, residues and substrates in the protein cavity. As a result blue shifted energy is observed with increase in number of hydrogen bonded water molecules attached to the oxygen of benzothiazole moiety. The applied electrostatic potential stabilizes the formal charge carried by benzothiazole moiety of phenolate keto form of oxyluciferin in ground state.

The last two points are important for colour modulation of light emission. As the transition from the state S1 to S0 leads to an internal negative charge transfer to benzothiazole ring from thiazolone, an increase in stabilization of charge on CT controlling group is observed which will further cause higher energy difference between ground state and electronic state and so a strong blue shift is observed.

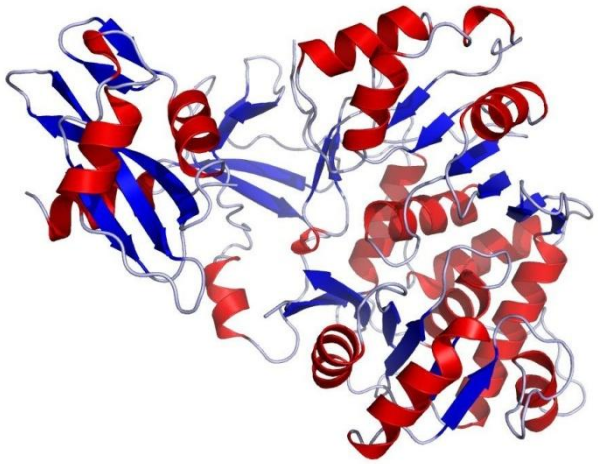


Figure 4: Structure of *Photinus pyralis* firefly luciferase

Applications:

Bioluminescence as an excitation source in Photodynamic Therapy of cancer- Photodynamic therapy on a superficial scale may be defined as process where selective wavelengths of light is used to destroy abnormal cancerous cells. Photodynamic therapy or PDT is a minimally invasive therapeutic modality which is based on production of reactive oxygen species (ROS) that is used in destroying various kinds of cancerous cells. The three fundamental requirements for PDT are; a non toxic photosensitizer, light of specific wavelength and presence of molecular oxygen. Upon excitation of PS through irradiation of target site with cytotoxic effects are triggered. The PS is excited from ground singlet state to excited singlet state from where comparatively longer lived triplet state is achieved which in turn induces formation of ROS.

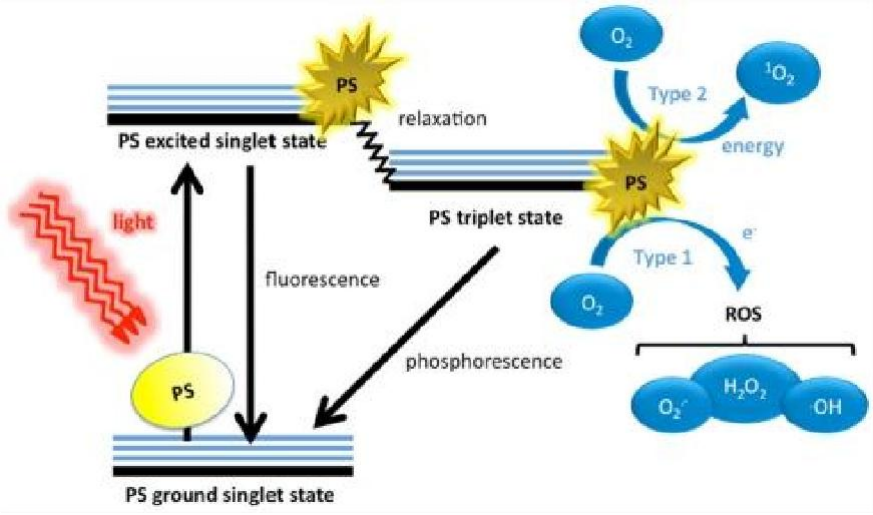


Figure 5: schematic representation of PDT with Jablonskii Diagram

The principal advantage of PT lies in its selectivity which unlike chemotherapy or radiotherapy has very little side effects. But a serious drawback is that PS absorbs wavelength of 600- 800nm weakly and absorbs 400nm or below strongly but radiation of wavelengths of 580nm or below is unfit for biological applications. This is where bioluminescence played its role in making PDT viable. Self illuminating PDT systems based on bioluminescent resonance energy transfer (BRET) were created where non radioactive energy transfer occurred from bioluminescent donor to suitable acceptor molecule. This activates PS intracellularly without the use of any external light source. The firefly BL mediated PDT shows a result of 90% cytotoxicity rate with 100% survival rate of the controlling groups. The singlet oxygen quencher showed that cytotoxicity was due to ROS species produced particularly. Aother advantage of this system was that, there was no need to add ATP to the cell to induce enzyme activity and thus initiate the entire process

In-vivo bioluminescence imaging of transplanted human neural stem cells

Traumatic brain injury is one which is acquired when brain gets damaged by sudden traumas associated with falls, accidents or post operatic results. Treatment of traumatic brain injury has been largely dependent on the use of stem cells to restore lost brain tissue. Stem cells have drawn much attention because of their therapeutic potential for neurological disorders and their ability to differentiate into functional neuronal cell types. Neural stem cells play a vital role in functional recovery that includes Parkinson's disease, Huntington's disease, stroke etc and is shown to restore brain functions in animal models suffering traumatic brain injury. So, a need for non-invasive monitoring system which would be able to evaluate the supportive effect of viable stem cells during a brain injury condition was felt. One such non-invasive monitoring process is bioluminescence imaging. It records observations found due to light emitting firefly luciferase reporter gene, being introduced in those stem cells, that would express the former, and their survival monitored. This method is based on sensitive detection of visible light, produced during enzyme (luciferase) mediated oxidation of molecular substrate

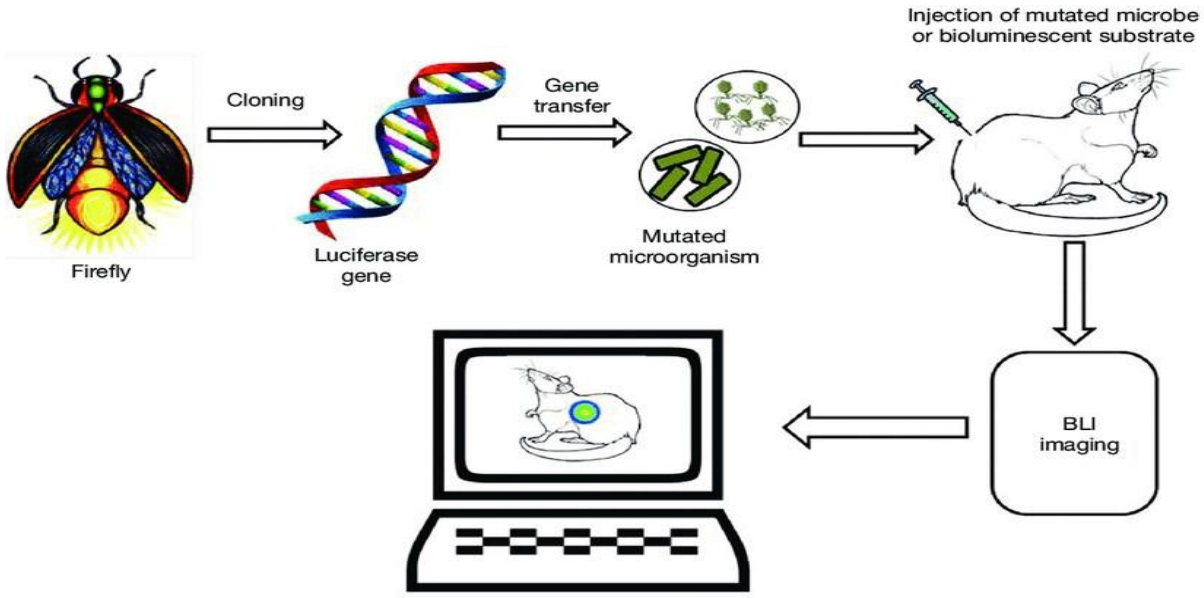


Figure 6: Bioluminescence imaging

Now, keeping aside the scientific concepts if we compare the efficacy of this imaging techniques with the other imaging techniques currently available in the market it will be very clear that bioluminescence imaging is most beneficial for our use. This is further well elucidated with the help of figure 7.

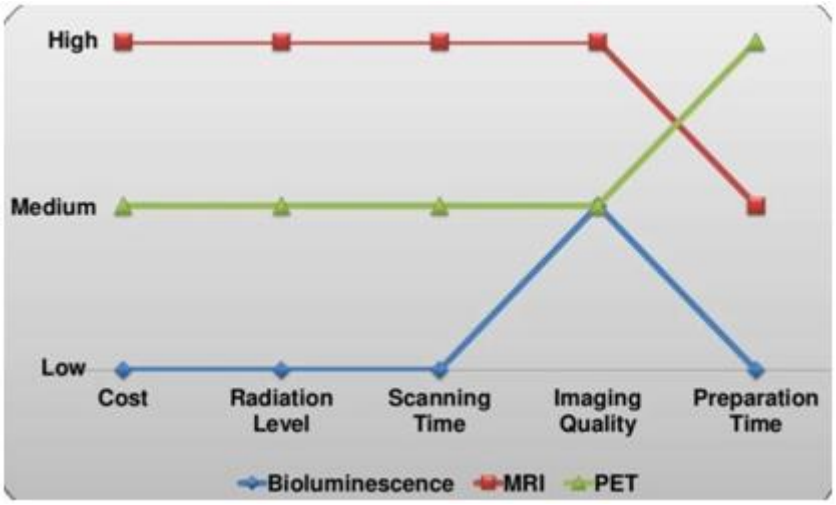


Figure 7: Comparison of bioluminescence with other imaging techniques

Bioluminescence is further studied to use it as an alternative option used for lighting

Various companies have moved their focus on bioluminescence to use it as a source of lighting in near future. Studies have progressed regarding this with much vigour and enthusiasm. The current problems with lighting apparatus includes heavy wastage of energy due to inefficiency of the modalities used, short life span and is susceptible to shocks and vibrations, the electricity used to drive these devices is generated by burning fossil fuels which in turn has a deep negative impact on our environment. So companies are trying to use bioluminescence as a source of lighting. The benefits of bioluminescent lights include almost cent percent efficiency if not 100%, zero effect on environment, unlimited supply once genetically engineered and so on.

Companies like Phillip's and institutions like Cambridge have come forward to prepare their models so that this becomes a viable option for lighting.

The Phillip's concept uses bioluminescent bacteria fed on methane and composted material produce a soft green light, not all that different from the light emitted by fireflies and red tide. The bacteria is housed in a wall of hand-blown glass cells and connected to a food source at the base through thin silicon tubes. The bacteria's food source comes in the form of methane gas, which is converted from solid bathroom waste and vegetable trimmings using the methane digester located in Philips' bio- digester kitchen island--the main hub of the Microbial Home.

The Bio-light could be "powered on" as long as there were a supply of nutrients, but the resulting light isn't bright enough to illuminate an entire room. Instead, Philips sees the Bio-light as more of an ambient light source, as well as a way to power night- time road markings, warning strips for planes and stairs, and more.

2. Conclusion

The emission of visible light by living organisms is an unusual phenomenon, both in terms of its relative rarity and with respect to the biochemical and regulatory mechanisms involved. But where it does occur, bioluminescence is sometimes spectacular and can usually be inferred to have functional importance a consequence of the fact that another organism detects and responds to the light.

The uses of the light may be classified under three headings: defense, offense, and communication. Light may be used defensively to startle or frighten (flashes), to divert predators, as a decoy, or to provide camouflage. Offensively, light may be used as a lure, to attract and convert would-be predators into prey. Communication occurs in courtship and mating displays.

Small models, such as 1,2-dioxetane, dioxetanone, and distinct substituted dioxetanone molecules, ease the understanding of the molecular basis of the reaction and the establishing of the mechanisms. Improvements to the description and the characterization of the light-emitting species can be obtained from studies with the entire molecule and systems that include luciferin and some explicit side molecule or solvent effects. Since the reaction takes place in

a protein, the luciferase–luciferin system needs to be understood by means of effects derived from the protein–substrate interactions. Only then can the reaction mechanism be refined and an explanation be given for the bioluminescent properties of luciferin in its natural environment. Throughout this systematic analysis of the theoretical studies, three moieties of the luciferin–luciferase system were associated with the key components for the bioluminescence process: the chemiluminophore, which opens the path to the excited-state surface, the electron-donating fragment, which lowers the activation energy of the reaction by means of a CT mechanism, and the CT controlling group, which turns the CT mechanism on or off and modulates the color emission, depending on the interactions between this moiety and the protein. The crucial role of these individual parts of the firefly luciferin–luciferase system and their presence in several molecular systems, responsible for light-emission in other organisms, allow us to understand them as the chemical functionalities of the bioluminescence phenomenon. Finally, some effort is spent on discussing the computational challenges one faces in the theoretical studies of the same phenomenon.

Abbreviations

ATP-ADENOSINE TRI-PHOSPHATE BIL-BIOLUMINESCENCE IMAGING PDT-PHOTODYNAMIC THERAPY ROS REACRIVE OXYGEN SPECIES

Acknowledgement

I would like to thank my guide Dr. Nabanita Saha Chowdhury for guiding me all through the course of my work. I'm grateful to the department of chemistry and our principal ma'am for providing us with the opportunity to do such an interesting project work. I would like to further extend my gratefulness to my parents who have provided me with all the resource I needed.
Thank you.

References

[1] E. N. Harvey, "Bioluminescence", Academic Press, New York, N.Y., 1952.
[2] J. R. Badcock and N. R. Merrett, *Nut. Enuiron. Res.* Counc. *News J.*, ***5,***
[3] (3) E. Denton, *Sci. Am.*, 224,64 (1971).
[4] F. H. Johnson, *Compr. Biochem.*, 27,79 (1967); J. **W.** Hastings, *Annu.Reu.* Biochem., 37,597 (1968).
[5] F. H. Johnson and Y. Haneda, Ed., "Bioluminescence in Progress", Princeton University Press, Princeton, N.J., 1966.
[6] M. J. Cormier, D. M. Hercules, and J. Lee, Ed., "Chemiluminescence and Bioluminescence", Plenum Press, New York, N.Y., 1973.
[7] Shimomura, F. H. Johnson, and Y. Saiga, *J. Cell. Comp. Physiol.*, **59,**223 (1962).
[8] W. W. Ward and H. H. Seliger, *Biochemistry*, 13,1500 (1974); *S.* J. Girsch and J. **W.** Hastings, *Am.* SOC*P.h otobiol. Abstr.*, 183 (1973)
[9] M. J. Cormier, J. E. Wampler, and K. Hori, *Fortschr. Chem. Org. Nuturst.*,30,l (1973), and references cited.
[10] K. Hori and M. J. Cormier, ref. 6, **p** 361; J. G. Morin and J. W. Hastings, *J. Cell Physiol.*, **77,305** (1971); 0. Shimomura and F. H. Johnson, Proc. ***Nutl.*** Acud. *Sci.* ***U.S.A.,*** **72,** 1546 (1975);
[11] W. W. Ward and M. J. Cormier, *ibid.*, 72,2530 (1975); 0. Shimomura, S. Inoue, and T. Goto, *Chem. Lett.*, 247 (1975).
[12] W. C. Rhodes and **W.** D. McElroy, *J. Biol. Chem.*, 233,1528 (1956).
[13] E. H. White, F. McCapra, G. F. Field, and W. D. McElroy, *J. Am. Chem.Soc.*, 83, 2402 (1961).
[14] Y. Kishi, T. Goto, Y. Hirata, 0. Shimomura, and F. H. Johnson *Tetrahedron Lett.*, 3427 (1966);
[15] T. Goto, s. Inoue, S. Sugiura, K. Nishikawa, M. Isobe, and Y. Abe, *ibid.*, 4035 (1968).
[16] Shimomura and F. H. Johnson, *Biochemistry*, 7,1734 (1968).
[17] Shimomura, F. H. Johnson, and *Y.* Kohama *Proc. Natl. Acad. Sei.*
[18] F. McCapra and D. W. Hysert, *Biochem. Biophys. Res. Commun.*, 52, D. K. Dunn, G. **A.** Michaliszyn, I. G. Bogacki, and E. **A.** Meighen, *Biochemistry*, 12,4911 (1973).