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In silico Analysis of *Renilla Muelleri*, *Photinus Pyralis* and *Metridia Longa* Luciferase

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Abstract

Luciferase derived from different organism is widely used for various biological process in the cells or tissues. Current *in silico* study was done to investigate the protein-substrate interactions of *Renilla muelleri, Metridia longa* and *Photinus pyralis* luciferase with their respective substrates coelenterazine, Luciferin, and ATP. RaptorX which is an online tool was used for luciferase modeling. PyRx v9.0 was used for protein-substrate binding. Online server Cluspro was used for protein-protein docking. CASTp was used for protein active site pocket prediction. *Photinus pyralis* luciferase was bonded with ATP molecules through Glu83, Asp153, Asp44, Ser85, Gln87 and His171, while *Photinus Pyralis* luciferase was bonded with luciferin molecules through five different residues i.e. His171, Arg62, Met90, Leu63 and Val168. *Photinus pyralis* residues that were docked with ATP and luciferin molecules were present in N terminal domain of *Photinus pyralis* luciferase. In case of *Renilla muelleri, catalytic residues,* His285 was present in its all the docking complex. *Renilla muelleri* and *Metridia longa* luciferase were also docked with different substrate and found that efficiency of *Renilla muelleri* and *Metridia longa* luciferase was lower towards Photinus pyralis substrates as compared to their own substrate coelentereazine. According to the findings, it has been concluded that luciferase of every light emitting organism required specific (its own) substrate for proper light reaction.

Keywords: *Renilla muelleri*; *Metridia longa*; *Photinus pyralis*; luciferase

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1. INTRODUCTION

In bioluminescent organisms, enzyme Luciferases catalyze the light-producing chemical reactions. Mainly insect like fireflies (*Photinus pyralis*) luciferases need oxygen ATP, D-luciferin (organic molecule) and Adenosine-tri-Phosphate (ATP) molecule as substrates[1]. Magnesium (Mg^{+2}) is used as cofactor in the light

emitting process. As a result, green lights having peak emission at 566 nm is emitted. Contradictory to the above phenomenon *Metridia Longa*[3] and *Renilla*[4] luciferase have no need of cofactors to start the light emitting process. These two organisms perform oxidative decarboxylation of coelenterazine substrate to start light emission and this light have peak emission of 482 nm. Structure of coelenterazine has been known as representative luciferin[4,5].

Photinus pyralis luciferase has been extensively studied organism for more than 5 decades and has served as one of the best described bioluminescence[1,2]. Bioluminiscense catalysis by luciferase is two-step process;[6] (a) D-luciferin adenylation by ATP & Mg^{+2} and (b) luciferyl adenylate oxidation. When excited oxyluceferin comes to ground state from high-energy state, energy is emitted in the form of green-yellow light of 566 nm[7].

Beside this reaction many other factors effects bioluminescence reaction, such as solvent, cytidine nucleotide and coenzyme A. *Photinus pyralis* has been reported to be used for the measurement of ATP produced by microorganisms[8,9]. The gene responsible for bioluminescence has been used as gene expression and regulation reporter[10,11] (Figure 1).

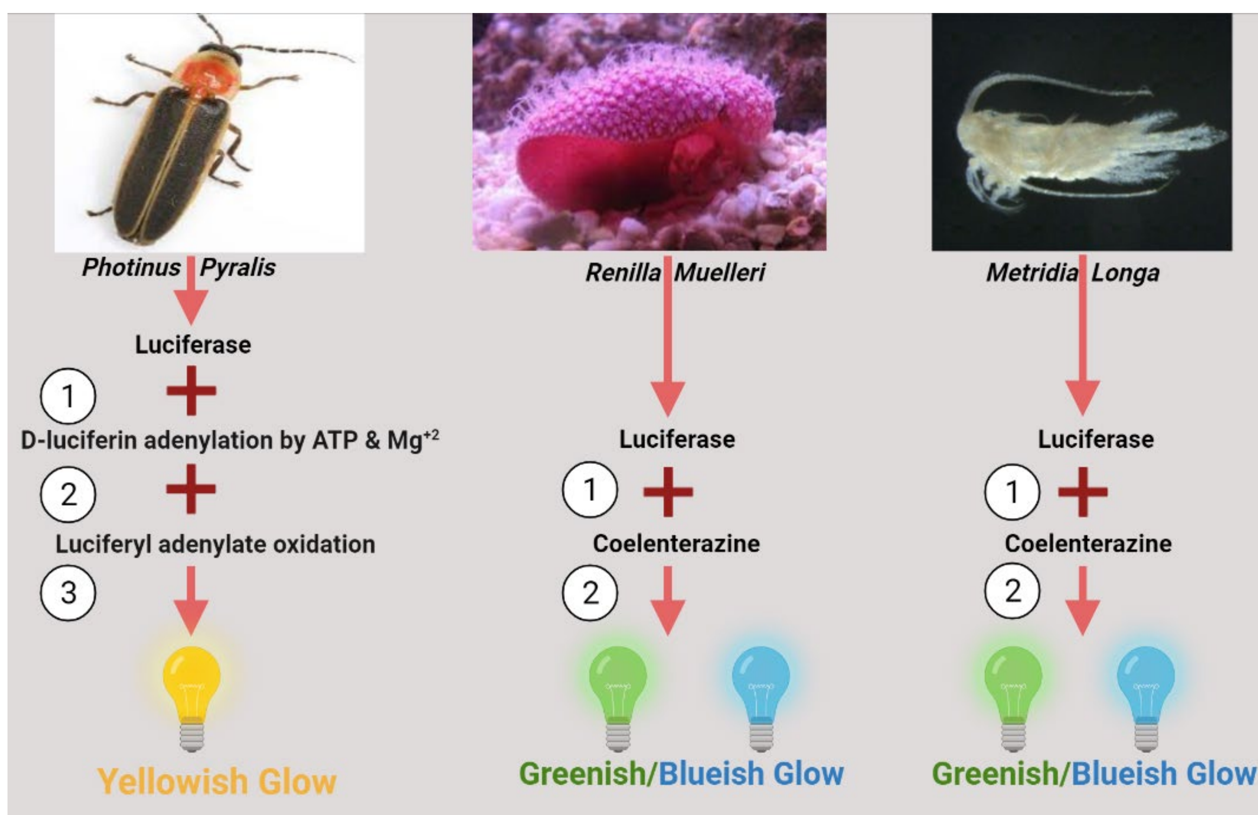


Figure 1. Limit emitting reaction process of Photinus Pyralis, *Renilla Muelleri* and *Medridia Longa*.

The enzyme present in *Photinus pyralis* i-e *Photinus pyralis* luciferase has been extensively studied over the last 4-5 decades and has played a vital role in analysis of many biological pathways [12,13]. 3D structure of luciferase from *Photinus pyralis* in absence of substrate has already resolved[14]. *Photinus Pyralis* also called North American firefly has three domains (i) a large N-terminal domain having 1-436 residues (ii) 2nd domain is 4 residue domain from 436-40 known as flexible linker domain and (iii) C-terminal domain of 10 residues from 440-450. During reaction, substrate comes in the middle of N and C terminal and forms a sandwich type complex. N-Terminal has been reported to contain the active site where substrate will bind while C-terminal domain is thought to perform adenylation and oxidation[15,16,17].

Main purpose of current *in silico* analysis was to analyze protein-substrate inter-action of *Renilla Muelleri*, *Metridia Longa and Photinus Pyralis* luciferase with substrate coelenterazine, Luciferin and ATP respectively. In addition, to check the efficiency of *Renilla Muelleri* and Metridia Longa substrate, coelenterazine molecule as compared to *Photinus Pyralis* luciferase substrate luciferin and ATP molecules.

2. MATERIALS AND METHODS

In current *in silico* study, protein 3D structure of *Renilla Muelleri, Photinus pyralis* and *Metridia Longa* luciferase was obtained using RaptorX online tools[18]. Protein sequences were obtained from UniProt database[19]. RaptorX is a template-based protein structure-modeling server. It requires protein sequence in FASTA format and provide 3D structure in PDB format. Models with high confidence score (CS score) were selected.

Predicted 3D models were verified using PROCHEK tool[20], which provide results in the form of Ramachandran plot. Results of PROCHEK tool will be available in supplementary file S1. 3D structures were visualized using Chimera1.13.1 [21].

CASTP 3.0 tool was used to predict the active site pockets of *Renilla Muelleri, Photinus pyralis* and *Metridia Longa* luciferase[22]. *Renilla Muelleri, Photinus pyralis* and *Metridia Longa* luciferase active-site pockets were noted.

For protein-protein interaction, another online tool Cluspro[23] and offline java based software LigPlot+ v2.1[24] were used. Cluspro performs protein structure prediction of both 'protein–protein' as well as 'protein–small molecule' docking. The inputs to Cluspro servers are protein structures in PDB format. The methods behind the server are very effective and allows large-scale docking experiments. Among all the predicted docked complexes, complex with low binding energy was elected.

For protein-substrate docking of *Renilla Muelleri, Photinus pyralis and Metridia Longa* luciferase with ATP, luciferin and coelenterazine molecules, offline tool PyRx was used. PyRxv0.9 is an offline multiple ligand binding tool[25] and used for virtual molecular docking of compounds of desired biological function and this can be achieved through docking of small-molecule libraries to a macromolecular libraries[25]. All the values were kept default.

3. RESULTS AND DISCUSSIONS

3D models and active site pockets of *Photinus pyralis (*Uniprot ID# Q27758, 550 amino acid protein*) Renilla Muelleri* (Uniprot ID#Q9BLZ3, 311 amino acid protein) and *Metridia Longa* Luciferase (Uniprot ID# A0A1L6CBM1, 190 amino acid protein) were determined (Figure 2).

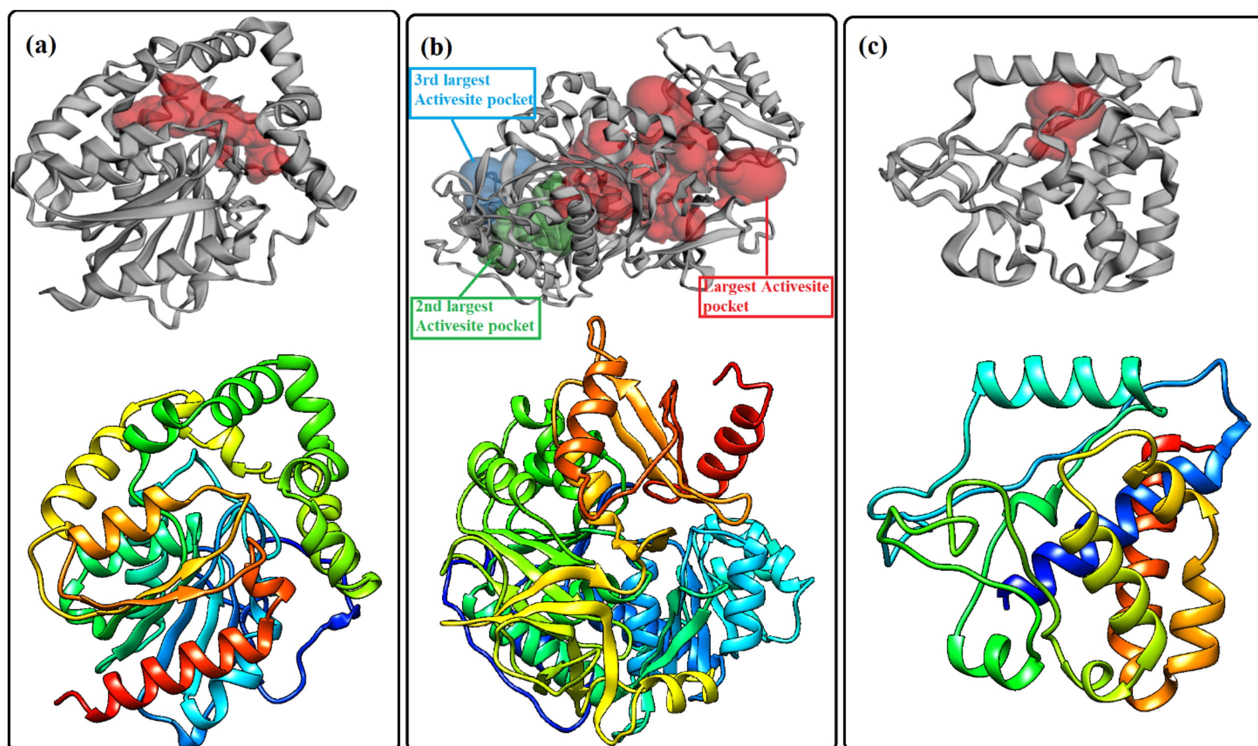


Figure 2. *Renilla Muelleri* luciferase 3D structure showing active site pockets (b) Photinus pyralis luciferase 3D structure showing active site pockets (c) *Metridia Longa* luciferase 3D structure showing active site pockets.

Results found that *Photinus Pyralis* binding residues were present in 2nd largest pocket, which consists of 40 amino acids, while only residue (83Glu; ATP binding residue) was present in 1st largest active site pocket (supplementary Table 1). *Photinus pyralis* luciferase was bonded with ATP molecules through seven different residues i.e., Glu83, Asp153, Asp44 (Bonded through Attractive charge), Ser85, Gln87 (Bonded through Carbon Hydrogen Bond) and His171 (Bonded through Pi-Sigma Bond).

While *Photinus Pyralis* luciferase was bonded with luciferin molecules through five different residues i.e. His171 (Pi-Pi T-shaped bond), Arg62, Met90, Leu63 andVal168 (bonded through Pi-Alkyl bond). His171 was common residues between ATP and Luciferin but bonded through different bond (Figure 3).

Renilla Muelleri and *Metridia Longa* Luciferase were docked with their substrate coelenterazine molecule as well as the ATP and Luciferin molecules (*Photoinus pyralis* luciferase substrates). Results were as expected that the interaction of *Renilla Muelleri,* and *Metridia Longa* Luciferase were higher with coelenterazine molecule as compared to *Photoinus pyralis* luciferase substrates.

Renilla Muelleri luciferase was interacting with its substrate coelenterazine molecule through eight different residues i.e., Lys189 (Bonded through unfavorable donor-donor interaction), Pro187, Met185 (Bonded through Pi-Pi Stacked and Pi-Alkyl interaction), Pro259, Phe261 (Bonded through Hydrogen Bond), Phe262, Trp156 and His285 (Bonded through Pi-Pi T-shaped interaction) (Figure 4). While in case of ATP and Luciferin, *Renilla Muelleri* luciferase was interacting through only five and six residues respectively (Figure 5).

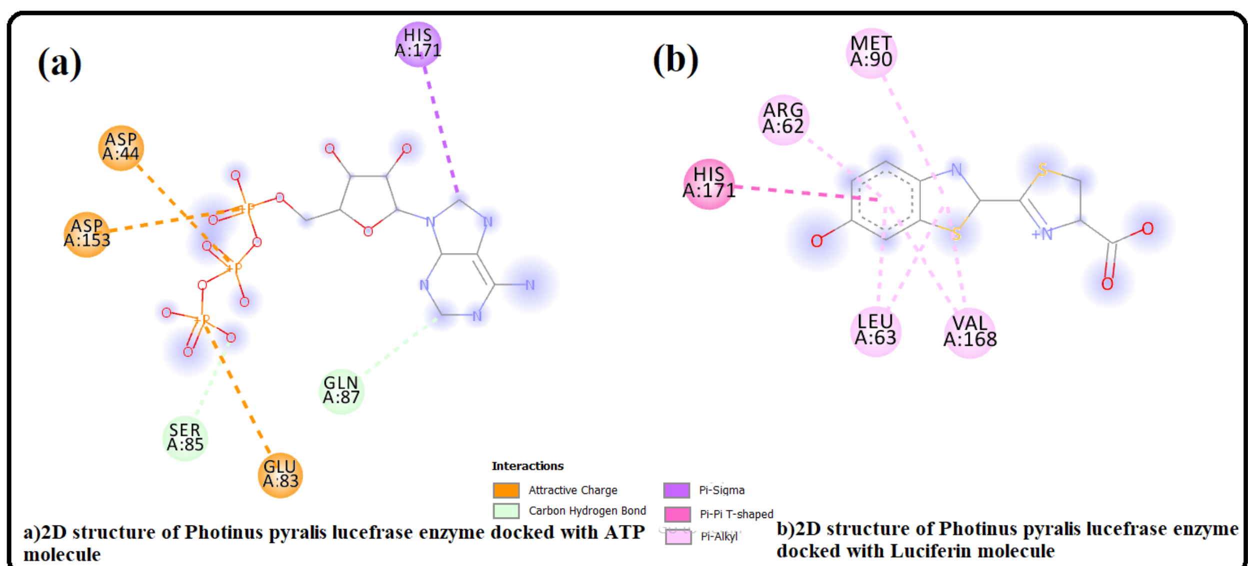
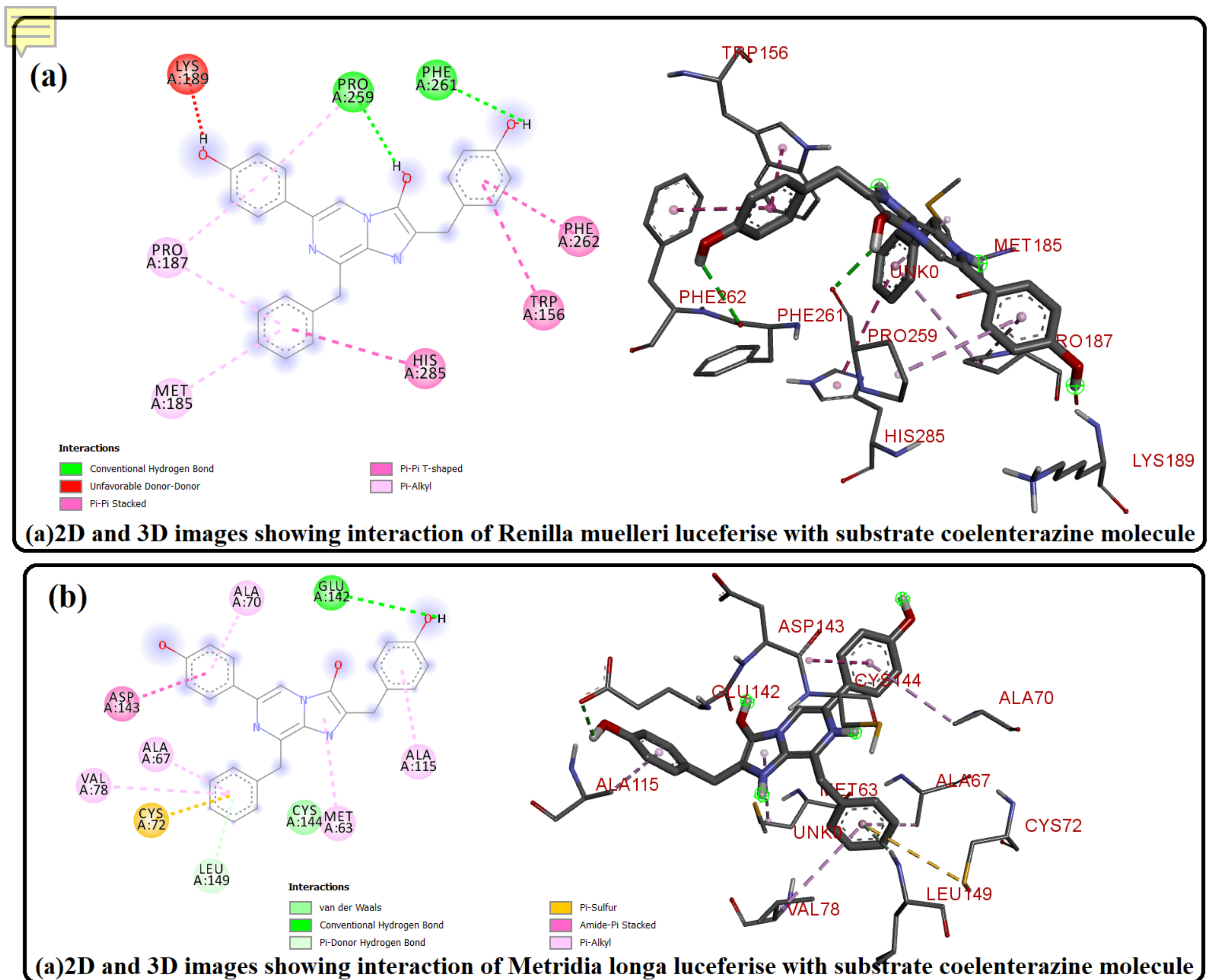


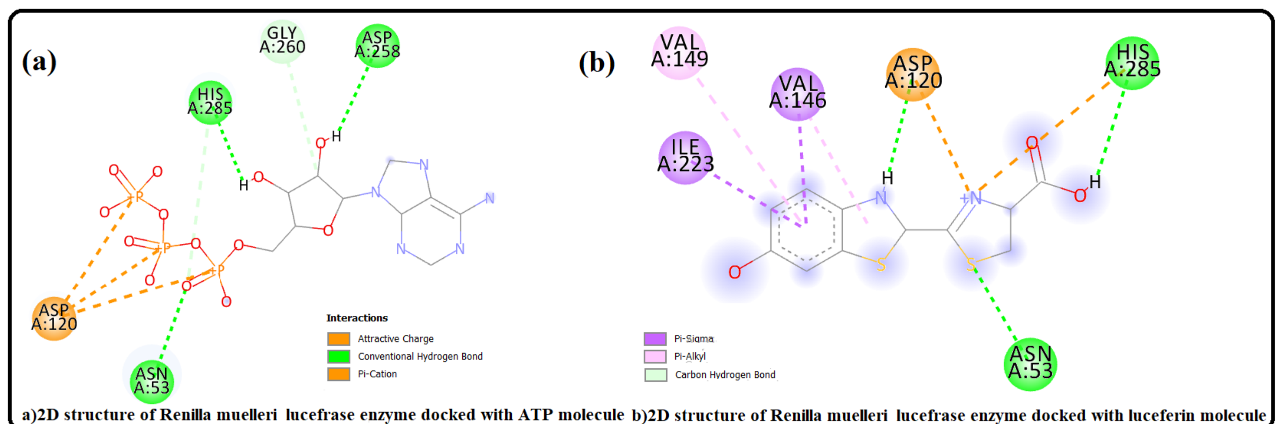
Figure 3. (a) 2D structure of *Photinus pyralis* luciferase enzyme docked with ATP molecule (b) 2D structure of *Photinus pyralis* luciferase enzyme docked with luciferin molecule visualize through Discovery studio 2020.



(a)2D and 3D images showing interaction of Renilla muelleri luceferise with substrate coelenterazine molecule

(a)2D and 3D images showing interaction of Metridia longa luceferise with substrate coelenterazine molecule

Figure 4. (a) 2D and 3D images showing interaction of *Renilla Muelleri* luciferase with substrate coelenterazine molecule (b) 2D and 3D images showing interaction of *Metridia Longa* luciferase with substrate coelenterazine molecule visualize through Discovery studio 2020.



a)2D structure of Renilla muelleri lucefrase enzyme docked with ATP molecule b)2D structure of Renilla muelleri lucefrase enzyme docked with luceferin molecule

Figure 5. (a) 2D structure of *Renilla Muelleri* luciferase enzyme docked with ATP molecule (b) 2D structure of *Renilla Muelleri* luciferase enzyme docked with luciferin molecule.

When *Metridia Longa* Luciferase was docked with coelenterazine molecule, it was interacting with its substrate coelenterazine molecule through 10 different residues i.e. Cys144 (Bonded through Van der Waals interaction), Leu149 (Bonded through Pi-Donar hydrogen bond), Cys72 (bonded through Pi-Sulpher bond), Asp143 (Bonded though Amide-Pi stacked interaction), Val78, Ala67, Ala70, Met63, Ala115 (bonded through pi-Alky bond) and Glu142 (Bonded through hydrogen bond) (Figure 4b).

While in case of ATP and Luciferin molecule the interaction of *Metridia Longa* was through seven, seven residues respectively (figure 6). All the active site interacting residues of *Renilla Muelleri and Metridia Longa* luciferase were present in its 1st largest pockets (supplementary Table 1).

Interaction of *Renilla Muelleri, Metridia Longa and Photinus pyralis* luciferase were also determined and found that highest interaction were observed between *Renilla Muelleri* and *Metridia Longa* luciferase i.e. through 9 bonds, while interaction was noted between *Renilla Muelleri* and *Photinus Pyralis* luciferase was much lower i.e. only through 2 bonds (figure 7).Complete details of residues of *Renilla Muelleri. Metridia Longa* and *Photinus Pyralis* luciferase proteins that were involved in making interactions are summarized in table 1.

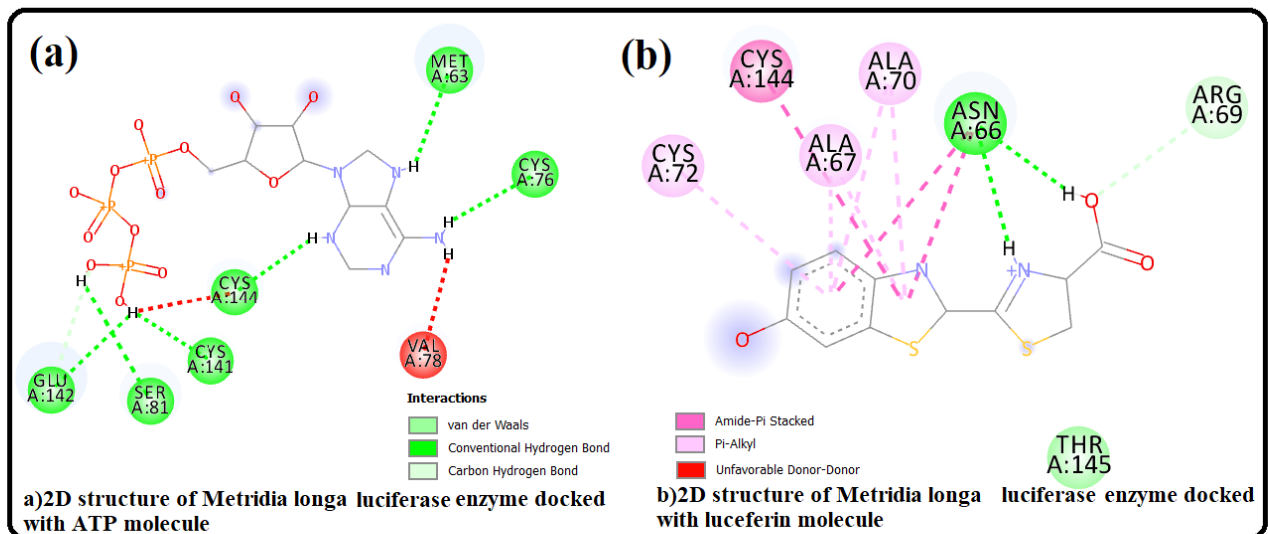


Figure 6. (a) 2D structure of *Metridia Longa* luciferase enzyme docked with ATP molecule (b) 2D structure of *Metridia Longa* luciferase enzyme docked with luciferin molecule

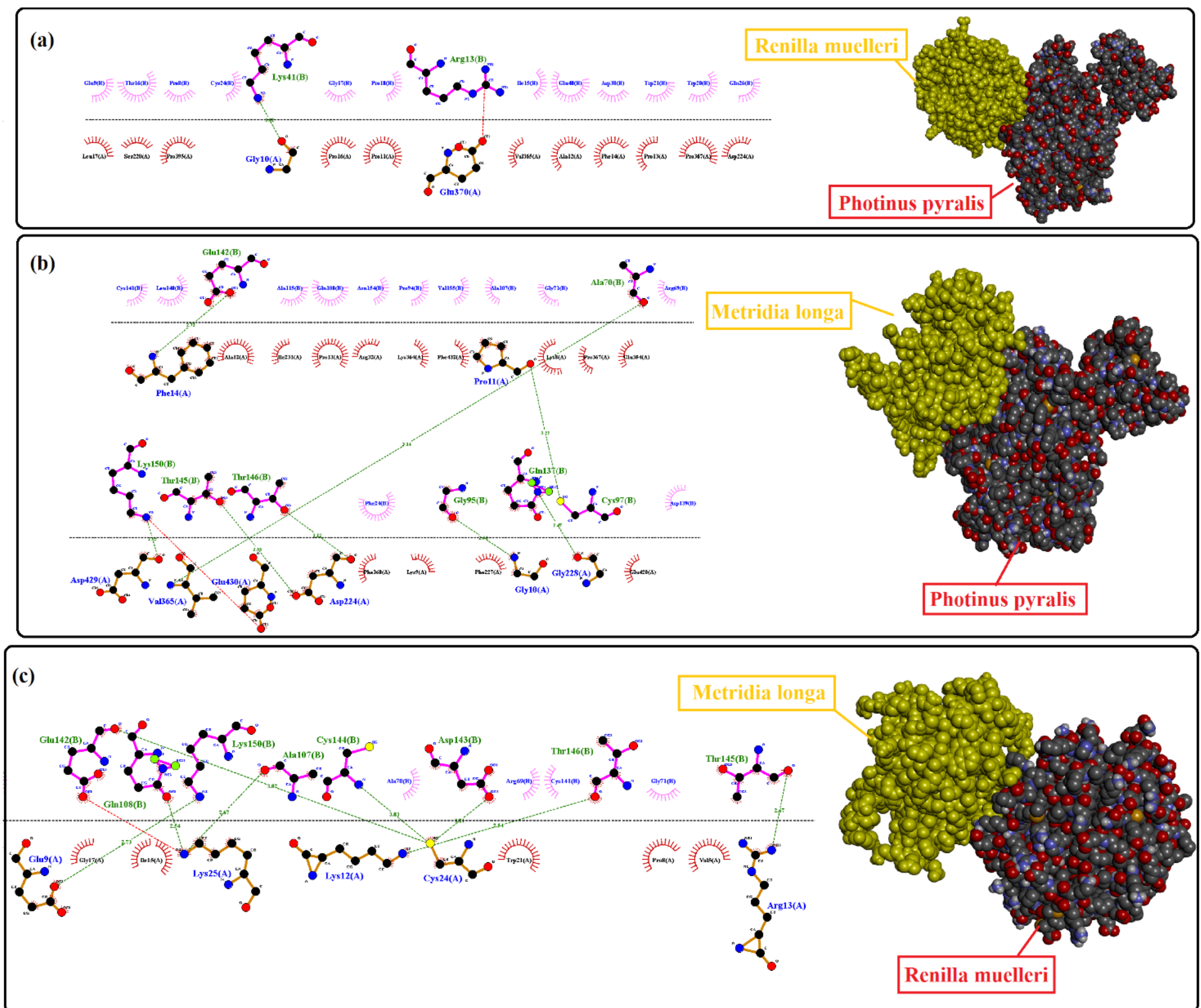


Figure 7. (a) Protein-Protein interaction between *Photinus pyralis* and *Renilla Muelleri* luciferase (b) Protein-Protein interaction between *Photinus pyralis* and *Metridia Longa* luciferase

Table 1. Interacting residues of *Renilla Muelleri, Metridia Longa* and *Photinus Pyralis* luciferase that were involved in making interaction.

Interacting proteins	Interacting residues of *Renilla Muelleri* lucifierase	Interacting residues of Metridia Longa luciferase	Interacting residues of *Photinus Pyralis* luciferase	No of bonds
Renilla Muelleri and Metridia Longa luciferase interaction	Glu9, Lys25, Lys12, Cys24, Arg13	Glu142,Gln108,Lys150, Ala107,Cys144,Asp143, Thr146, Thr145	____	9
Renilla Muelleri and *Pyralis* luciferase interaction	Lys41,Arg13	____	Gly10,Glu370	2
Metridia Longa and *Photinus Pyralis* luciferase interaction	___	Glu142,Ala70, Lys150, Thr145,Thr146,Gly95, Gln137,Cys97	Phe14,Pro11, Asp429,Val365, Glu430,Asp224, Gly10,Gly228	8

Photinus pyralis

Photinus pyralis luciferases have been widely documented for more than decades and are some of the best described bioluminescence systems[1,2]. Luciferase catalyzes bioluminescence in two steps [6] : (a) D-luciferin

adenylation by Mg and ATP, (b) luciferyl adenylate oxidation. Lastly, the excited oxyluciferin falloffs to the ground state and emitts green-yellow light with very high efficacy[3]. Several factors i.e. solvent, coenzyme-A[26,27], and nucleotide cytidine[28] effect bioluminescence reaction. *Photinus pyralis* luciferase is useful to determine ATP derived from microorganisms[29] and the luciferase gene is used as a reporter of gene expression and regulation[30]. *Photinus pyralis* luciferase has a large 550 amino acid long protein, among which N-terminaldomain (1–436 amino acid) is linked by a flexible linker (436–440 amino acid) to a C-terminal domain (440–550 amino acid).

The active site pocket is located in the N-terminal domain[31], and some researchers suggest that the C-terminal domain is also very important for the adenylation and oxidation steps [16-17]. In our study both the substrate, i.e., Luciferin and ATP molecule were binded with *Photinus pyralis* on its N terminal domain (1-436 residues). This supported the results of previous studies that active site binding residues were present in N-terminal domain of *Photinus Pyralis* that is present between 1-436 residues[16,17] .

Renilla mulleri

Renilla luciferase (RLUC) is a blue/greenish light emitting single subunit luciferase. It is isolated from the marine anthozoan *Renilla mulleri* [32]. Apart from its efficacy as a reporter for gene expression assays, *Renilla* luciferase (RLUC) has also known in assays for protein interaction based on fragment complementation[33] and transfer of bioluminescence resonance energy[34]. Coelenterazine is the RLUC substrate, which consists of a central aromatic imidazopyrazinone. Imidazopyrazinone is derivatives of phydroxyphenyl, benzyl and phydroxybenzyl moieties[35, 36] .

RLUC has a large hydrophobic active site in its structure. In addition, within this active site, Asp120, His285, and Glu144 are supposed to be the putative catalytic trio. Mutagenesis data and inactivation with diethylpyrocarbamate specify that among these trio, His285 is important for catalysis, presumably as a general base[37,38]. In our study, we docked *Renilla* luciferase with ATP, luciferin and Coelenterazine molecules and found that in all the docking complexes His285 was common residues that was involved in making bonds with all the substrates individually (figure 4 and 5). Moreover, we also found that these trio residues Asp120, His285, and Glu144 that are previously reported to have putative catalytic were present in the largest active site pocket of RLUC (supplementary Table 1). The Renilla bioluminescent system in-vivo is comprised of three proteins—the luciferase, green-fluorescent protein, and coelenterazine-binding protein (CBP). *Renilla* luciferase has an advantage over firefly luciferase as it has single chain polypeptide and have cell permeable and nontoxic substrate i-e coelenterazine. However, bioluminescence quantum yield of *Renilla mulleri* is much less than that of firefly luciferase. *Renilla mulleri* luciferase has effective bioluminescence when substrate coelenterazine is bound with the CBP domain [39,40,41].

Metridia longa

At the start of the 2000s, using the functional screening the first copepod luciferases were cloned from the *Metridia Longa species and Gaussia* princeps[42,12]. Later, the same approach was applied to isolate three additional isoforms of the *M. longa* luciferase[14,43]. Based on the comparison of amino acid sequences of *Metridia* isoforms with each other and with those of the other copepod species, these isoforms were suggested to be the encodes by the four groups of non-allelic paralogous genes[43] . All copepod luciferases are single-chain proteins with the molecular mass of approximetly 18.4–24.3 kDa. The luciferases comprise a natural signal peptide for secretion, variable N-terminus constituting up to one-third of the amino-acid sequence, which does not considerably affect their light producing purpose[14], and a conserved C-terminal domain, where the enzyme active site is present[44]. This conserved region is formed by two similar repetitive region of about 70 a.acids which, in turn, comprise 32 highly conserved a.acids, each containing five conserved Cys residues[12,45] . The presence of these cysteines suggests the presence of up to 5 S-S bonds per luciferase molecule[14], which are most likely responsible for the extreme stability of these luciferases[43,44]. In our study when we dock *Metridia Longa* with its substrate Coelenterazine molecule. We found that among all the substrate bonding resides, cysteine was bonded to the substrate molecule through sulphide bond. This shows the stability of the complex, while no sulphide bond was absorbed, when we docked *Metridia Longa* with the substrate of *Photinus pyralis* i.e., ATP and Luciferin molecule (Figure 4 and 6). Although in all the *Metridia Longa* and substrate docked complexes, sulphide bond was

only found in *Metridia longa*-Coelenterazine docked complex showing the stability of the complex and confirm the finding of previous studies [14,43,44].

4. CONCLUSIONS

Present *in silico* study was done to investigate the protein-substrate interaction of *Renilla Muelleri*, *Metridia Longa* and *Photinus Pyralis* luciferase with substrate Coelenterazine, Luciferin and ATP respectively. Efficiency of Renilla Muelleri and *Metridia Longa* were higher towards coelenterazine as compared to ATP and Luciferin molecules (Photinus pyralis substrate). Main conclusion from the results of the current study is that for luminescing of any luciferase form any organisms must requires their respective substrate for proper light emitting reaction, while their interaction with substrate of other species is much lesser as compared to their own substrate. This may affect the quantity and efficiency of light emitted by the organism. According to our knowledge, this is the first *in silico* study of this type, it will further explore the spectrum of luciferase from different species, and their respective subtract interaction.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOURS CONTRIBUTION

Conceptualization, M.M., M.H. and A.N.; data curation, M.H and A.U investigation, K.B., and M.M.; methodology, A.U.K. and M.U.F.Q.; project administration, S.F.; software, M.M.; supervision, M.M.; validation, S.A.R.; writing—original draft, W.U.K.; writing—review and editing, M.M., M.A.K and A.N. All authors have read and agreed to the published version of the manuscript.

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