

# Luciferase: A Shining Mystery

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FUNCTION - “*La Luciferase, ça mange quoi en hiver?*”<sup>1</sup>

You are at dinner in the backyard of your friend’s house. The weather is exquisite: it is a warm summer night. Suddenly, you see all these tiny yellow lights dancing at the back, and they slowly assemble into a glowing swarm. How did these lights appear? What is the cause of this luminous energy? The answer: Luciferase! More specifically, it is the protein that makes the bottom of fireflies emit light.

The process of life forms to produce light is called bioluminescence. It is a beautiful phenomenon to observe, but it also serves a purpose. In certain fish species, it allows them to be able to see in the darkness of ocean abysses. For others, it helps them escape from predators, as the light they emit sends a signal that can be interpreted as “do not eat me, I am toxic!”. In the case of the firefly, it allows them to communicate with each other, particularly when the time comes for them to find a mate.

Then, luciferase is the protein behind this amazing phenomenon in fireflies. The process requires a lot of energy: approximately 8 ATP molecules are required to emit a single photon of green light. The ATPs helps the oxygen required for the reaction to bind with luciferase’s

cofactor, luciferin. When luciferase breaks the highly strained oxygen-luciferase complex to break, carbon dioxide is emitted, and the complex is highly electronically excited from the proceeding. As the complex goes back to its basal level of excitement, green photons are emitted, and the process can start all over again.[1]

Since the reaction does not require many reactants (only ATPs and oxygen) and is self-contained, the process can be tweaked for other purposes. Luciferase can be attached to proteins so that scientists can see where these proteins go into cells. Luciferase can also be used to see if there is ATP in a certain environment since light will be emitted. Luciferase can also be attached to multiple cancer cells to find out how collections of cancer cells are moving and developing. The possibilities are endless!

## A LOOK AT THE STRUCTURE - FROM ZERO TO GLOW

Proteins are primarily composed of amino acids, which in their turn are formed of an amino group (nitrogen and 2 hydrogen atoms covalently bound), a carboxylic group (a carbon atom bound to a group of oxygen and hydrogen and with an oxygen atom in a double bond) and a side chain all linked to the same carbon atom (the alpha-carbon). The amino acid has different properties depending on the side chain bound to its alpha-carbon. Amino acids are bound with each

<sup>1</sup>Québécois French expression meaning “Luciferase, what is it and what does it do?”

other by a peptide bond, a covalent bond linking the carbon atom of the carboxylic group of the 1st amino acid to the nitrogen atom of the amino group of the 2nd amino acid. Polypeptides (amino acids bound together) can adopt different conformations by rotating about the carbon-alpha-carbon bond and the nitrogen-alpha carbon bond. Steric hindrance due to their side chains prevents certain conformations for certain amino acids. 3 different secondary structures derive from these conformations:

- 1) The  $\alpha$ -helice, which is maintained in place by hydrogen bonds linking the nth and (n+4)th amino acids of a chain. The side chains extend outwards of the helices and the latter exhibit a net dipole, which influences its behaviour within the protein.
- 2) The  $\beta$ -sheet, which looks like a pleated sheet of amino acid. The side chains extend above or below the sheets and the latter can either be parallel or anti-parallel.
- 3) The coils, which bind the helices and sheets of a chain.

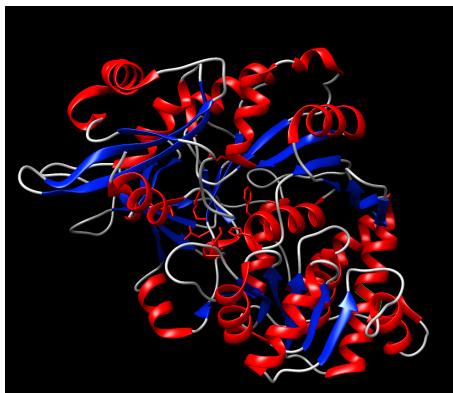


Figure 1: Luciferase and its secondary structures: its  $\alpha$ -helices in red, its  $\beta$ -sheets in blue, and its coils in grey

Luciferase is formed by a single chain of amino acids. It contains 24  $\beta$ -sheets and 19  $\alpha$ -helices. Another level of structure is formed beyond the  $\alpha$ -helices and  $\beta$ -sheets

by different types of interactions: the tertiary structure. Hydrogen bonds can link oxygen atoms from certain amino acids, like the bond between the nitrogen atom of serine 300 with an oxygen atom of isoleucine 296. Covalent disulphide bonds can also link side chains of amino acids to form the tertiary structure, like the bond between methionine 198 and methionine 251. Ionic bonds linking amino groups and oxygen atoms can also be formed, like the bond linking AMP to glycine 341. The last interaction is the weakest of them: the Van der Waals forces between induced dipoles of secondary carbons. There are numerous examples of this in luciferase.

#### STRUCTURE-FUNCTION RELATIONSHIP - WHAT'S GLOWING ON

The structure of the surface of luciferase enables it to perform its function in a quite elegant and clever way. The entire surface of the protein does not allow for any molecule to get inside, except for a small tunnel, where the reactants and products of the bioluminescent reaction (oxygen and phosphate groups among others) can get to and from the reaction site. (Figure 2 and 3) The interior of the protein contains  $\beta$ -sheets that

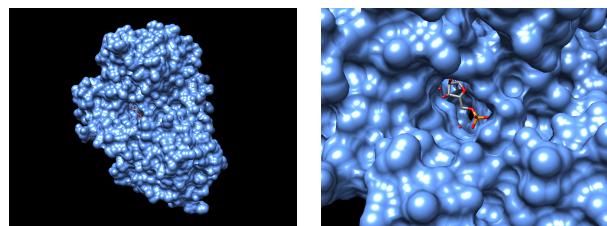


Figure 2: Representation of the surface of luciferase and the tunnel leading to the reaction site.

are arranged in a way that resembles  $\beta$ -barrels, which are formed by anti-parallel  $\beta$ -sheets. These structures are typically water-soluble and are capable of holding

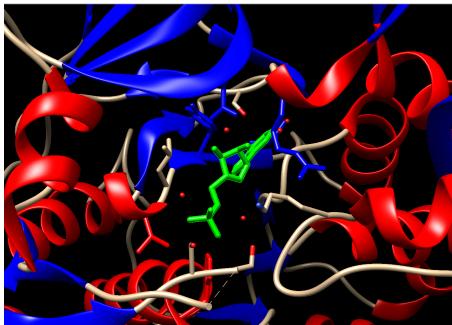


Figure 3: Reaction site of luciferase pre-reaction where the Mg-ATP is in green.

a hydrophobic molecule at their center. In the case of luciferase, the structure brings the water molecule to the reaction site, since they will be useful during the reaction. The hydrophobic Mg-ATP complex can then be placed at the center of this structure. (Figure4) To

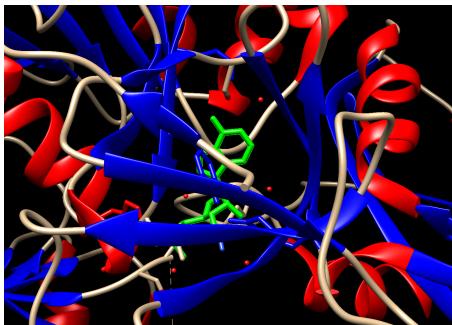


Figure 4: The barrel structure formed by the  $\beta$ -sheets at the reaction site. The water molecules are the red balls and in green is the Mg-ATP.

carry out the reaction, water molecules enter the tunnel to reach the reaction site. Oxygen can then bind to luciferin and ATP to form a high-energy intermediate analogue (SLU (luciferyl-sulphamoyl-group complexed to adenosine). The oxygen in the water molecules, when bound to this high-energy intermediate, helps keep the latter momentarily bound to luciferase, building potential energy across the bonds through steric hindrance. (Figure5) When the bonds and atoms are not able to carry this high amount of energy any longer, oxy-

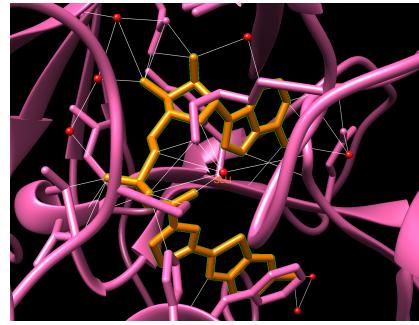


Figure 5: Reaction site of luciferase complexed to high-energy intermediate analogue (SLU (luciferyl-sulphamoyl-group complexed to adenosine) in orange). Water molecules (in red) help the SLU complex to stay momentarily bound to luciferase.

ciferin lets go of an oxygen atom and ATP (which becomes AMP). The resulting luciferin (SLU) becomes once again available to receive another oxygen and ATP to start the reaction all over again and produce more light! (Figure6) How does this reaction produce light?

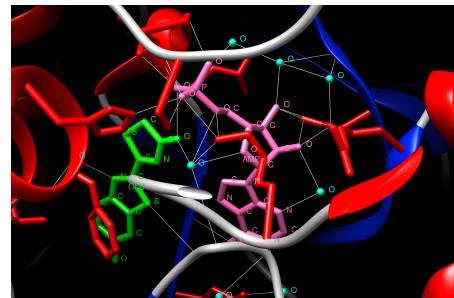


Figure 6: Reaction site of luciferase post-reaction where the AMP is in green and the CoA (oxyluciferin) is in pink.

Well, to answer this question, more nuclear chemistry explanations are required. In a nutshell, the electrons circulate around their respective atoms on a specific shell which can be identified with a certain energy level. An electron with high energy will navigate on a higher-energy level than an electron with less energy. In our case, when luciferin (SLU) and ATP became the higher-energy intermediate, their electrons were raised from their initial level to a higher-energy one. When the high-energy intermediate breaks down to AMP and OLU, the

electrons go back to their initial level and emit this extra energy to be able to do so. This extra energy is emitted as photons travelling as a wave with a wavelength of about 550 nanometers which, you guessed it, appears to the eye as green light!

### CONCLUSION

You now know more about this wonderful process that fireflies use to make a warm summer night more magical. This hopefully gave you a nice story to tell your family and friends at your next backyard BBQ. Here is another fun fact for you: if luciferase is mutated and we change the 286th amino acid from serine to asparagine, the light emitted by the reaction becomes red! It is unknown why a single amino acid change can cause a change of emitted energy, especially since the amino acid change did not even occur at the reaction site, but it is thought that slight changes in the packing of amino acids in the enzyme

and a less pronounced conformational change during the reaction could be the cause. Goodsell [2][3]

### REFERENCES

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