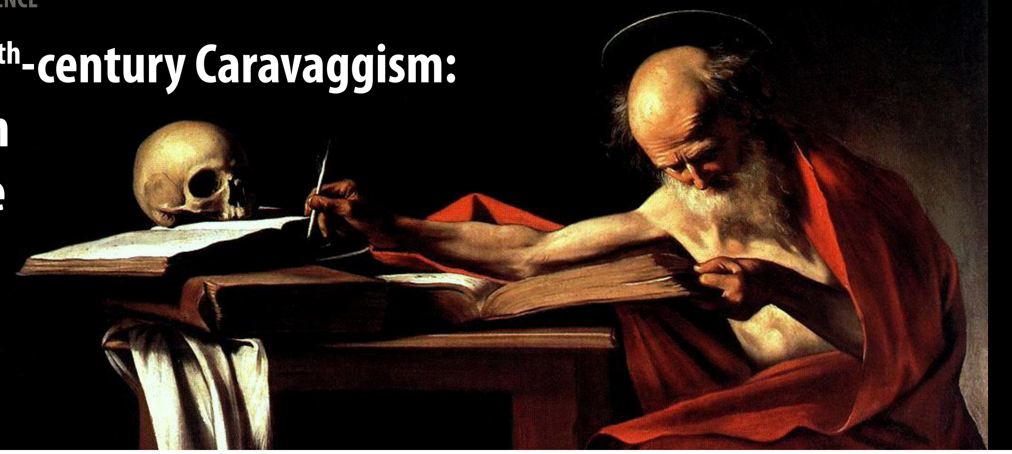
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Bioluminescence and 16th-century Caravaggism: The Glowing Intersection between Art and Science



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Part I – A New Artistic Technique

In this case study, which makes use of several short embedded videos, we will explore one of the oldest fields of scientific study, dating from the first written records of the ancient Greeks, namely, bioluminescence, which is a classic example of the chemistry of life.

Before the mid-1800s, the night brought complete darkness, modulated only by star light or moon light. Occasionally, the darkness of night could be illuminated by a form of light generated by insects or luminous mushrooms. This form of "living light" was later termed *bioluminescence*. Apart from illuminating the darkness of the night, a recent news report has pointed out that this form of "living light" may also have contributed to the success of a famous artistic style developed in the 16th century known as Caravaggism.

> Watch the video below, which defines bioluminescence and provides a brief introduction to the 16th-century artist Michelangelo Merisi da Caravaggio (1571–1610); Caravaggio's chiaroscuro style of painting; and the plausible connection between bioluminescence and Caravaggism.
>
> *Video #1: Bioluminescence and Caravaggism*. Running time: 5:06 min. <https://youtu.be/SVYNzSxFf9c>

In fine art painting, the term *Caravaggism* refers to techniques popularized by the radical Italian mannerist painter Michelangelo Merisi da Caravaggio. Caravaggio's style of painting was based on an early form of photography developed 200 years before the invention of the camera, known as the *camera obscura* technique. At the time, the use of a *camera obscura* to sketch the subject had gained prominence thanks to Leonardo da Vinci's writings on the topic (Anon., n.d.).

The *camera obscura* technique works by projecting reverse images of objects onto the flat wall of a closed box through a lens in an aperture. The objects to be painted are outside of the box. By attaching a mirror to the apparatus, artists are able to trace the exact dimensions of the outside objects and paint them onto a canvas in the closed box. It is reported that Caravaggio worked in a darkroom (the closed box) and illuminated his outside models with candle light. Through a biconvex lens mounted in the hole of the ceiling of the darkroom and a concave mirror, the "shadowy" three dimensional images of the models/objects were projected directly onto the canvas for him to paint. However, by turning his entire room into a *camera obscura*, Caravaggio found himself working in the complete dark. Caravaggio was known never to make preliminary sketches as he painted. How did he manage to create his greatest paintings in complete darkness?

The answer is bioluminescence. Bioluminescence is defined as the emission of light through a chemical reaction of living organisms, such as fireflies, that performs some biological function.

The principal chemical reaction can be described in chemical notation as follows:

$$\text{Luciferin} + O_2 + (\text{ATP}) \xrightarrow[\text{Mg}^{2+}]{\text{Luciferase}} \text{Oxy-Luciferin} + CO_2 + \text{AMP} + \text{PPi} + \text{light}$$

Question

1. In the next section of the case we will explore a source of bioluminescence that occurs in nature which Caravaggio may have employed in his painting. What animal or insect might Caravaggio have used as a source of bioluminescence?

"Saint Jerome Writing," Caravaggio, 1605-1606, <https://upload.wikimedia.org/wikipedia/commons/d/db/Caravaggio_-_San_Gerolamo.jpg>.

Part II – How Fireflies Make Light

Watch the video below, which provides basic facts about fireflies, a type of nocturnal beetle belonging to the family Lampyridae. The video also provides information on the biochemical process and mechanisms of firefly bioluminescence.

Video #2: Fireflies and Firefly Bioluminescence. Running time: 4:11 min.

<https://youtu.be/cNMdgeLGgK4>

Fireflies (or lightning bugs) refer to a group of insects who produce a "cold light" with no infrared or ultraviolet frequencies. This chemically produced light from the lower abdomen of the firefly may be yellow, green, or pale red, with wavelengths from 510 to 670 nanometers. Their display of luminescence at night can range from a continuous steady glow to strong flashes or pulses, lasting less than a second.

Fireflies are not flies. They are beetles. In North America, all members of the beetle family Lampyridae are referred to as fireflies or lightning bugs. In Europe, the word *fireflies* only refers to flying species of the Lampyridae family. There are over 2,000 species of fireflies in the world, with approximately 200 species in North America, and 62 species in Europe in two subfamilies: Lampyrinae and Luciolinae. *Luciola cruciate* is a very common firefly species in Italy and Southern Europe.

Fireflies produce their own light from a chemical reaction involving the oxidation of a compound denominated "luciferine" under the action of an enzyme, or catalyst, "luciferase" in the presence of oxygen (O_2), and two cofactors: magnesium ions and adenosine triphosphate (ATP). ATP is a molecule that provides energy for all cellular activities.

The Biogenesis of Firefly Luciferin

Oba et al. (2014) demonstrated that D- and L-firefly luciferins are biosynthesized in the lantern of the adult firefly from two L-cysteine molecules with p-benzoquinone/1,4-hydroquinone, accompanied by the decarboxylation of L- cysteine (see Figure 1).

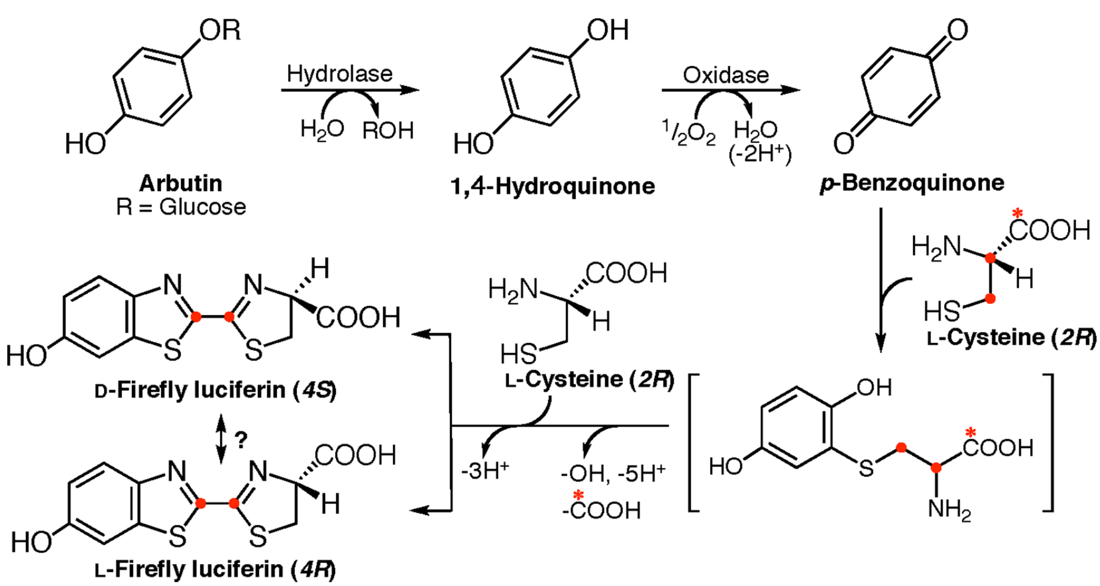

Figure 1. Proposed biosynthetic pathway of firefly luciferin in the lantern of adult firefly; from Oba et al. (2014), Figure 11, CC BY 4.0.

The Firefly Light-Emitting Reaction is Catalyzed by Luciferase

Luciferin (D-LH_2) is activated by luciferase in an ATP-dependent step to form a luciferin-adenylyl intermediate; when oxygen is present this intermediate is rapidly converted to a peroxyluciferin product with a cyclic dioxetatone ring that decays to oxyluciferin with the emission of photons (Figure 2).

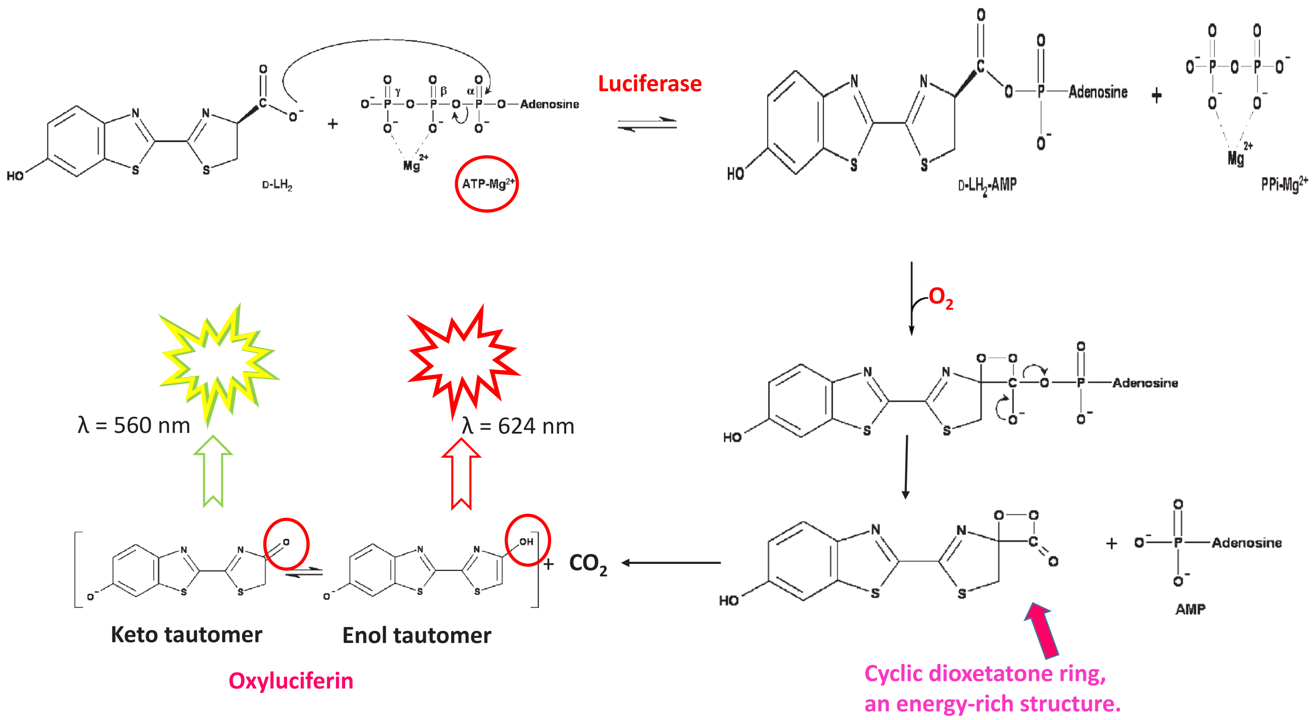

Figure 2. The firefly light–emitting reaction pathway modified from Hopkins et al. (1967); Shimomura et al. (1977), Brachini et al. (2004), and Marques and Esteves da Silva (2009).

Questions

2. Why do you think that fireflies produce light at their different developmental stages?
3. How do you think that fireflies regulate the production of light?
4. Could luciferin be the secret ingredient to Caravaggio's chiaroscuro style of painting 400 years ago? If so, what procedures did Caravaggio have to follow in order to take advantage of bioluminescence in his chiaroscuro style of painting in his time?

Part III – The Function of Bioluminescence

Watch the video below, which explains the biological function and the control mechanism of firefly bioluminescence. It introduces how fireflies have evolved to develop bioluminescence as a communication tool to defend themselves against predators, to lure prey for survival, and to attract mates for reproduction. Natural selection is mentioned in this video as well as the application of firefly bioluminescence in modern medicine.

Video #3: The Biological Function, Control and Application of Bioluminescence. Running time: 9:01 min.

<https://youtu.be/_Fe2_Ct9muI>

The 17th-century German priest, scholar, and scientist, Athanasius Kercher (1602–1680), first pointed out that bioluminescence is used for animal communication (Lee, 2008). The firefly flashes we see today are the result of a still unfolding chain of evolutionary events, all in the service of communication. Ancient adult fireflies did not flash at all. They signaled to mates with chemicals called pheromones, which are still used by some fireflies today in some areas of the world. Not all fireflies flash light. The best known fireflies that flash light are in the family Lampyridae; both males and females can produce light at every stage in their life cycle. Eggs and larvae produce a continuous light emission. Adult fireflies have a much more complex light flashing pattern.

What message does the luminescence of a larva convey? Is it a warning signal? What danger could a larva possibly impose to its predators? The bright and "cold" light can only signal danger if it is associated with a real threat. What real threat could a defenseless firefly larva pose? To find out, Dr. Mark Branham, a professor of entomology at the University of Florida, performed a test on himself. He put the fireflies between his lips. He did not bite or chew. As you saw in the video, he very quickly had a reaction: his lips went numb, his throat constricted, and he tasted something very astringent (Rotunda / Spring 2012 / AMNH.org).

What Dr. Branham experienced was the effect of a strong chemical message, from a chemical called lucibufagin. Lucibufagin is a powerful defensive chemical for fireflies (Eisner et al., 1978). Knight et al. (1999) reported that a seven-month-old male *Pogona sp.* lizard (about 100 gram by weight) died of lucibufagin poisoning within two hours after ingesting one firefly *(Photinus pyralis).* A recent study identified lucibufagin as a cardiac glycoside toxin, which targets the alpha subunit (ATPα) of sodium-potassium protein pumps (Lucy, 2014). It was also reported that firefly females can actually endow their eggs with lucibufagin to protect them and possibly the emergent larvae (Eisner et al., 1997). Many firefly species contain steroidal pyrones which, because of their close relationship to compounds such as bufalin (found in the venom of Chinese toads), are known as lucibufagins (Eisner et al., 1997). The glowing behavior of eggs and larvae of fireflies endowed with lucibufagin are therefore termed as aposematic display. It refers to the bright and conspicuous displays that serve as warning signals of prey, which possess repellent secondary defenses to would-be predators. It was originally recognized by A.R. Wallace (1867), and subsequently termed "aposematism" by E.B. Poulton and Arthur Sidgewick (1890).

Scientists also learned that fireflies of the genus *Photuris* do not produce lucibufagins. But the *Photuris* females hunt species of *Photinus* to acquire defensive lucibufagins. *Photuris* females lure *Photinus* males by flashing with a delay imitative of that of the *Photinus* female. The *Photinus* males are thereby drawn to the female *Photuris*, only to be caught and eaten (Lloyd, 1981, 1984a, 1984b, 1997). The female *Photuris* species in turn are enriched with the defensive lucibufagins. Female *Photuris* species therefore are called firefly "femmes fatales."

Scientists believe firefly bioluminescence began in eggs and larvae and first served as a warning signal. To survive, fireflies exude the defensive chemical lucibufagin from their joints to the outside of their body for immediate tasting by predators. Firefly bioluminescence is to train predators to associate the potent lucibufagin with the glowing signal as an attention-grabbing warning system. With this system, fireflies don't have to die to tell the predators they don't taste good. The clustering of firefly's eggs and larvae makes the warning luminescent signal even stronger to predators. Eventually, firefly larvae's glowing ability carried over to adults and later evolved for courtship displays. The process of firefly bioluminescence being co-opted for another purpose is known as exaptation, and can be seen throughout the animal world.

As to the function of bioluminescence in adult fireflies, many researchers reported that a bioluminescent adult firefly has its own flash fingerprint. Branham and Greenfield (1996) reported male fireflies fly through the air with a species-specific light display. Some flash only once. Some emit "flash trains" of up to nine carefully timed pulses. Others fly in specific aerial patterns, briefly dipping before sharply ascending and forming a "J" of light. A few even shake their abdomens from side to side and appear to be twinkling. Male fireflies of each specific species has its unique light flashing display. As males dart through the air, performing luminous gymnastic displays, female fireflies usually just sit, often immobile. Every now and then, a female firefly will deliver a precisely timed flash response and point her abdomen, or lantern, in the direction of her chosen suitor.

Dr. Branham and coworkers hypothesized the male flash pattern might have to do with female preference. To test this hypothesis, Dr. Branham created a computer-driven robotic firefly that could mimic the flash pattern of male fireflies for every species he studied. When he made the artificial male firefly robot flash, females of the same species sparked like live wires with flashes (Branham and Greenfield, 1996).

Firefly bioluminescence clearly has important biological functions. But how do fireflies control such carefully timed bursts of light flashes? In the video, you saw how research conducted at Tufts University has led to the discovery that fireflies use the gas, nitric oxide, as a biological signal to trigger light production.

According to Trimmer et al. (2001) (see Figure 3):

> *In quiescent mode when no flash is produced, oxygen is consumed by respiration in mitochondria clustered in the peripheral cytoplasm of photocytes (light emitting cells in the firefly abdomen, or lantern). In this state, little oxygen reaches photocyte peroxisomes that contain the light-producing reactions of the luciferin-luciferase pathway. In this quiescent state, ATP produced by oxidative phosphorylation promotes formation and accumulation of the activated luciferin-adenylyl intermediate by luciferase.*
>
> *In flash mode, nerve activity causes octopamine (neurotransmitter) release that transiently activates the production of nitric oxide (NO). Nitric oxide (NO) is a soluble, highly reactive gas formed by natural chemical and physical reactions in the atmosphere. It is also produced by certain animal and plant cells from the amino acid, L-arginine. Because it is so small and diffusible, NO passes through cell membranes and is often used as a biological signal. Once entering the photocyte cell, NO diffuses rapidly and inhibits oxygen use by photocyte mitochondria. Now oxygen delivered by the firefly's tracheal system diffuses through photocytes to the peroxisomes where it triggers the light-producing reaction by firefly luciferase.*

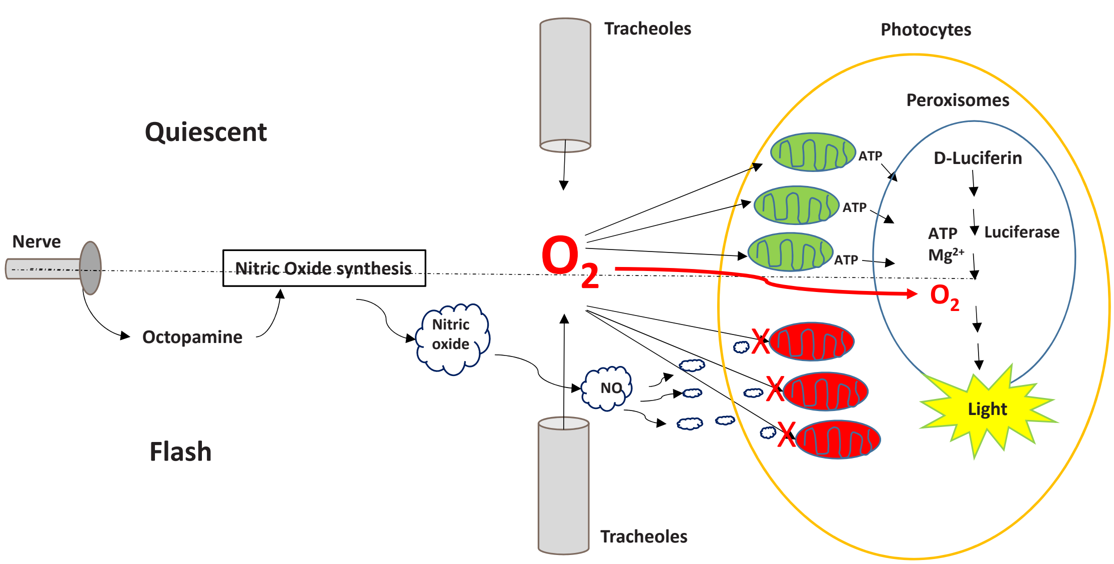

Figure 3. Model for Nitric oxide (NO) control of the firefly lantern flash (based on Trimmer et al., 2001).

Part IV – The Importance of Bioluminescence

Bioluminescence is the result of a chain of evolutionary events centered around animal communication, which plays a critical role in the survival and evolution of luminescence in animal species. Caravaggism is the technique and painting style Caravaggio employed to paint his masterpieces in the 16th century. To test Dr. Roberta Lapucci's hypothesis that Caravaggio used luminescent powder from crushed fireflies to "fix" images in his painting around 415 years ago, it would involve taking samples from some of the world's greatest masterpieces and subjecting them to laboratory testing. This is not ideal and would be very costly. But, it can be done. If it turns out to be true that Caravaggio indeed used crushed fireflies in his painting, it would be a shining example of how the chemistry of life, a naturally evolved biological phenomenon, literally illuminated the world's greatest art.

While the contribution of bioluminescence to Caravaggism remains to be tested, modern scientists have successfully applied the principle of luciferin-luciferase reaction to develop biomedical approaches that are helping to shed light on diseases. Here are two examples:

- Dr. Jenkins and colleagues (2005) used bioluminescent imaging to detect both primary and secondary tumor sites in human breast tumor cells.
- Dr. Leib and colleagues (2013) modified the herpes viral genome with firefly luciferase gene and studied the mice immune response to the herpes viral infection in real time.

Question

5. Can you think of other examples (like the possible use by Caravaggio of bioluminescence in his painting technique) that connect nature and culture?

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