

BASIC BIOLUMINESCENCE

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Imagine that you are walking along the Selangor River in Malaysia. As you emerge into a clearing in the evening twilight, you are startled to see a tree on the opposite bank burst into light as if lit by hundreds of tiny Christmas tree lights. Then, just as suddenly as it ignited, the entire tree goes dark, only to light up again a few seconds later. As you move closer while the blinking continues, you realize that the lights aren't artificial lights, but rather hundreds of fireflies; fireflies that are blinking in perfect synchrony as if governed by a single controller. This phenomenon is one of the most impressive of instances of "Living Light", i.e., from organisms that produce light. (see also the Historical Vignette on [Bioluminescence](#))

In this module we'll briefly explore the processes that create this *Bioluminescence*, and the function that this light performs for the creatures that produce it, as well as the many applications of bioluminescence in scientific research and commerce.



Figure 1. The firefly "Christmas Tree" as observed in regions of S.E Asia, from Malaysia to Papua-New Guinea. It is due to the synchronous flashing of fireflies.

What Is Bioluminescence?

Bioluminescence is defined as the emission of light from a living organism that functions for its survival or propagation. It is a "cold" light, resulting from a specific biochemical mechanism involving chemical processes, often specific for that organism. Bioluminescent organisms occur mostly in the marine environment, and bioluminescence is one of the major communication mechanisms in the deep sea (1). Although less common terrestrially, observations are naturally more frequent there.

Bioluminescence can be thought of as a chemiluminescence that is catalyzed by an enzyme. This light emission from an organism needs to be distinguished from other forms of luminescence, many also having biological function, fluorescence, iridescence, diffraction, etc. (2).

The Wide Distribution Of Bioluminescence

Bioluminescent organisms are found throughout the biosphere, but only at levels below the mammals and plants. The occurrence appears randomly distributed among genera, and sometimes is found in some species within a genus but not others, without evident reason. Some 17 phyla and at least 700 genera contain luminous species. Bioluminescence has been demonstrated in cephalopods, copepods, ostracods, amphipods, euphausiids, and many fish, annelids and jellies, to name but a few marine species. On land there are many types of bioluminescent insects, fireflies, glow-worms, click beetles, and some diptera, and there are many types of luminescent fungi responsible for glowing wood. The bioluminescent bacteria occur both terrestrially as well as marine. In only a few cases have the bioluminescence components from the various systems been characterized, and the overall chemistry established. Many, if not most luminous organisms in the deep sea, still remain to be investigated.

Over the past several hundred years, many scientists have been busy with the collection and classification of bioluminescent organisms. In fact even earlier, Aristotle (350 BCE) was probably the first to make systematic observations of luminescent species and later, complete and extensive descriptions of luminous organisms were

published by Pliny the Elder (23-79 CE) (3). One example he described was the luminous mollusc, (Figure 2) a Roman delicacy, the bioluminescence mechanism of which is still not completely solved.



Figure 2. *Left:* The bioluminescent mollusc *Pholas dactylus*, in the U.K. commonly known as a "Piddock". It has worldwide distribution. *Right:* Purple jellyfish, common in the Mediterranean and described by Pliny the Elder.

The discovery of bioluminescent organisms has been the goal of many expeditions of ocean-going research vessels. Marine submersibles are also deployed for the study of bioluminescence in the deep ocean. Luminous coastal organisms are more accessible, and can be usually be collected by inexpensive methods.



Figure 3. *Left:* The soft coral, *Renilla reniformis*, the "Sea Pansy" (about 30 mm across), found in intertidal coastal areas. *Right:* Bioluminescent mushrooms in the light (*top*) and dark (*lower*); in the USA the bioluminescent fungus is called "Foxfire".

Anatomic Distribution

The tissue distribution of the components of the bioluminescent system within organisms, is quite varied. The anatomic location of bioluminescence gives clues as to the source of component synthesis, storage, transport, and the functional role of the luminescence. One key organ is the "photophore" or the light producing organ, quite evidently seen in many luminous fish and very vividly in cephalopods. Photophores are normally made up of complex photogenic (light emitting) cells.

Bioluminescent reaction components have also been detected in the stomach, secretory organs and liver of some organisms (mostly believed to be there as a result of synthesis or storage).



Figure 4. A bioluminescent squid from the deep ocean. Some squid can project luminous clouds from their mouths, and also have spectacular photophores (light emitting tissue). From the [Bioluminescence Web Page](#).

Geographic Distribution

Bioluminescent organisms are found world-wide, for example the so-called "phosphorescence" in sea-water is observed in all oceans, particularly densely in bays and coral reefs, where high concentrations of nutrients promote blooms of the responsible organisms. One location in Puerto Rico named the [Bioluminescent Bay](#), is well known for spectacular displays of this dinoflagellate luminescence. "Red Tides" are often blooms of luminescent phytoplankton.

A great variety of firefly species are found in the temperate to tropical regions of the Americas and parts of S.E. Asia. Several types of glow-worm have been identified in North America, Europe, and Australasia. Interestingly, some species of bioluminescent organisms are luminous in one location and not in another, e.g., the "Midshipman Fish", *Porichthys notatus*. The luminescence of one population of this species has been postulated to relate to the availability in that particular area of a dietary source required for the light reaction. Around Japan, the firefly squid (*Watasenia*) displays spectacular luminescence, and is found in large numbers in restricted localities. Small crustaceans such as ostracods, are also found in abundance in Japanese coastal waters.

How Does Bioluminescence Work? All bioluminescence reactions involve an oxygen oxidation of an organic molecule (called the luciferin). The reaction is catalyzed by an enzyme called a luciferase and in many cases, the bioluminescence intensity is assumed to reflect the velocity of the enzyme-substrate reaction, and this intensity is used to analyze the kinetics on the Michaelis-Menten model (Figure 5A). It was first a puzzle that the bioluminescence of aequorin and subsequently of several other like organisms, was found not to involve oxygen kinetically, and these proteins were labeled "photoproteins" (Figure 5B). It was eventually established that the oxygen had already bound to the luciferin, and the photoprotein therefore could be more accurately thought of as a luciferase binding a stabilized reaction intermediate, a peroxy-luciferin.

Many bioluminescent reactions *in vitro* require cofactors in addition to oxygen, e.g., ATP and Mg²⁺ for the firefly, Ca²⁺ for photoproteins (1, 2, 4). In the animal itself (*in vivo*), there are additional proteins involved for production and regulation, some called "accessory proteins", examples being the fatty acid reductase group of enzymes that produce the bacterial luciferin, a long-chain aldehyde, and there are luciferin-binding proteins in the dinoflagellate and Sea Pansy bioluminescence systems. Also, there are "antenna proteins" that act to modulate the color of bioluminescence, the famous Green-fluorescent protein (GFP) in the jellyfish, and the Lumazine Protein family in bacteria (4). These are named "antenna proteins" by analogy to proteins of similar function in photosynthesis, except that they act in a reverse sense.

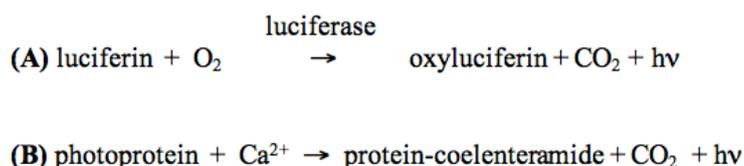


Figure 5. Reaction Schemes for a luciferin/luciferase reaction **(A)**, and for a typical photoprotein reaction triggered by calcium **(B)**. The reaction product is the light ($h\nu$) emitting species, the protein-bound oxyluciferin or protein-bound coelenteramide.

To date, there are five known distinct chemical classes of luciferins, namely, aldehydes, benzothiazoles, imidazolopyrazines, tetrapyrroles and flavins. An imidazolopyrazine derivative, aptly named "coelenterazine", is the luciferin found in coelenterates and many other marine bioluminescence systems (5, 6).

Physics: Characteristics Of The Light Emission

Bioluminescence results from a chemical reaction that releases a large amount of energy which, instead of being dissipated as heat as in a normal chemical reaction, is channeled to populate the product molecule in its excited electronic state. This excited state is the same one produced in that molecule by the absorption of radiation, so that the spectral distribution of the bioluminescence is often the same as that of the product fluorescence. The color of the bioluminescence however, is sometimes "tuned" by the protein environment of the product excited state, a property evolved to suit the function of the light emission, that is for communication, defense against predation, etc.

Visible radiation corresponds to light in the wavelength range of 400-700 nm (Figure 6). Bioluminescence spectra are broad bands with widths at half-height around 50 nm. The bioluminescence maximum of most marine species falls within the range of 450-510 nm (7), whereas terrestrial organisms have predominantly a yellow-green bioluminescence color. In ocean water, blue to green (400-500 nm) luminescence achieves maximum transmission, whereas terrestrial species have their maximum visual sensitivity for yellow light. Visual pigments of most marine organisms are correspondingly most sensitive in the blue-green region.

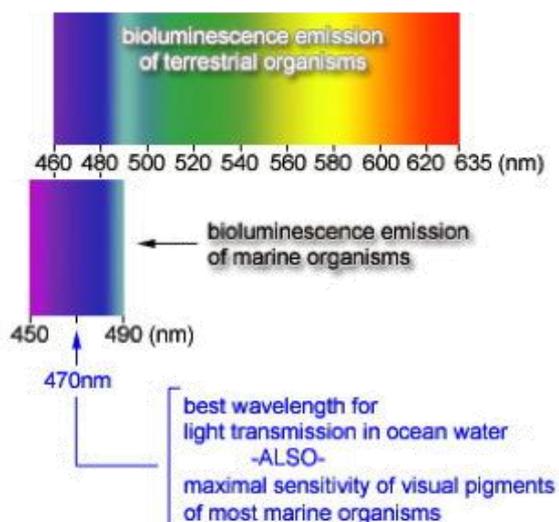


Figure 6. The end-product of bioluminescence is visible light. The visible part of the spectrum is 400-700 nm, and the emission maxima of most luminous marine organisms falls within the range of 450-490 nm.

What Are Some Of The Functions Of Bioluminescence?

As a result of its prevalence, bioluminescence plays an important role in the ecology of the ocean. The function of bioluminescence in the oceans is more clearly understood in the context of the essentially dark environment below about 200 m. The functions of bioluminescence are for:

- Defense
- Schooling of fish
- Luminous lure
- Feeding
- Communication (in the dark)
- Mating
- Camouflage

Impact Of Molecular Biology And Bioluminescence

The cloning of various components of bioluminescent systems has heralded major advances in biological research. The calcium-dependent photoprotein aequorin from the jellyfish *Aequorea victoria* was cloned in 1985 (8, 9). Because the intensity of its luminescence varies with calcium concentration, aequorin has been used for advantage in the monitoring of cell calcium. In 1985, firefly luciferase was cloned (10). As an extremely sensitive method for the assay of ATP, this bioluminescence system has found wide application, e.g., to detect microbial contamination in foodstuffs, water systems, etc. Living organisms contain ATP so its assay detects the presence organisms that might lead to spoilage or toxicity. Many other luciferases have been cloned, including bacterial luciferase from *Vibrio harveyi*, sea pansy *Renilla reniformis* luciferase (11), and the South American click beetle luciferase (12).

Current Applications Of Bioluminescence

The "Green-Fluorescent Protein" or GFP, is probably the most famous protein in Biology (Nobel Prize in

Chemistry, 2008). GFP was cloned in 1992 (13), and expressed in various organisms in 1994 (14). Since that time the number of literature citations has risen into many thousands, as applications of GFP have increased. In particular, GFP is now well established as an excellent gene tag or protein tag. GFP can be fused to a protein of interest, and fluorescence (and therefore the protein of interest) can be tracked within a cell to study its localization and behavior. GFP has outstanding structural stability, and with the property of being able to form the fluorescence *in situ* without the external addition of substrate, GFP becomes an excellent tool for studying cell and sub-cellular processes (15).

Rapid and effective diagnostic tests based on bioluminescence are constantly evolving in the marketplace. For example, "Microtox" for water quality/toxicity testing employs the bioluminescent marine bacteria *Vibrio fischeri*. When this organism is challenged by a toxin, the respiration pathway is disrupted, resulting in a decrease in bioluminescent intensity.

Some "fun" applications and ideas exist such as the prospect of luminous Christmas Trees and walkways in addition to luminescent beer and champagne. The use of light sticks for night concerts and guiding aircraft to airport gate positions are but a few every day applications of this widespread phenomenon: luminescence.

Questions That Remain

Although bioluminescence is a spectacular phenomenon in biology with a long history of investigation, questions abound ranging from the biological advantage of light emission to the animal, to the molecular mechanism of efficient excited state population. Some fish possess a photophore containing a culture of bioluminescent bacteria, and use this to lure prey. The firefly flash is for sexual communication, but why the synchrony of the firefly "Christmas Tree", and what is the advantage of luminosity to earthworms? How did the efficient generation of biological light originate in various organisms, and what is the evolutionary history? What is the metabolic or dietary source of the luciferins, and what are the control mechanisms for light flashing? At the molecular level, the chemical-physical pathway that generates the reaction products in its excited state, is an outstanding current problem. Together with this, the shifting of bioluminescence color via the luciferase binding site environment perturbing the excited state energy level, or by coupling to the excited state of antenna proteins by some process, continue to be challenging areas of current research (16).

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