

How an enzyme in fireflies, click beetles and glow worms yields different colors

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The glow of fireflies at dusk is a welcome sign of summer. The same enzyme that helps give these familiar bugs their characteristic flash of yellow, yields red light in acidic conditions. Similar enzymes are responsible for red and green lights in other beetles. Despite years of study, however, scientists still don't know the molecular details of how the enzyme works. Now, in the ACS journal *Biochemistry*, one team reports new insights into this mystery.

Most people are familiar with the flash of fireflies. But perhaps less well known, but just as intriguing, are the displays of click beetles and railroad worms, which are a type of beetle larva. At night, two small dots on click beetles' backs glow like a pair of tiny green eyes peering in the dark. Railroad, or glow worms, exhibit a more complex pattern of both green and red lights. Taking advantage of this natural signaling, scientists have harnessed beetles' bioluminescence to help them see when certain genes are expressed, for example. But the structural and mechanistic factors contributing to [beetles'](#) glow have remained elusive. Vadim R. Viviani and colleagues wanted to gain a better understanding of these phenomena.

The researchers used modeling, mutations and spectral and kinetic studies to probe how changes to an [enzyme](#) called luciferase affect what color is emitted. Mutations to two particular parts of the enzyme produced a red glow. These parts display opposite charges, which attract each other and keep a "gate" closed where the light-emitting compound is generated. Under normal conditions, the enzyme active site remains closed restricting the entrance of water, which favors green light emission. But mutations and other alterations that break this attraction open the gate and allow water in. This relaxes the active site, resulting in the emission of light with lower energy in the red region.

More information: Vadim R. Viviani et al. Glu311

and Arg337 stabilize a closed conformation and provide a critical catalytic base and counteraction for green bioluminescence in beetle luciferases, *Biochemistry* (2016). DOI: [10.1021/acs.biochem.6b00260](https://doi.org/10.1021/acs.biochem.6b00260)

Abstract

Beetle luciferases elicit the emission of different bioluminescence colors from green to red. Whereas fireflies luciferases emit yellow-green light and are pH-sensitive, undergoing a typical red shift at acidic pH, higher temperatures and presence of divalent heavy metals, click beetle and railroadworm luciferases, emit a wider range of colors from green to red, but are pH-independent. Despite many decades of studies, the structural determinants and mechanisms of bioluminescence colors and pH-sensitivity remain enigmatic. Here, through modeling studies, site-directed mutagenesis spectral and kinetics studies using a set of recombinant luciferases naturally producing different bioluminescence colors (*Macrolampis firefly*; *Phrixotrix hirtus* railroadworm and *Pyrearinus termitilluminans* click beetle), we investigated the role of E311 and R337 in bioluminescence color determination. All mutants of these residues in firefly luciferase produced red-mutants, indicating that the preservation of charge and length of the side chains of E311 and R337 are essential to keep a salt bridge that stabilizes a closed hydrophobic conformation favorable for green light emission. Kinetic studies indicate that the residue R337 is important for binding luciferin, creating a positively charged environment around excited oxyluciferin phenolate. In *Pyrearinus* green-emitting luciferase, the mutation R334A causes a 40 nm red shift, whereas in *Phrixotrix* red-emitting luciferase the mutation L334R causes a blue-shift which is no longer affected by guanidine. These results indicate that the presence of arginine at position 334 is essential for blue-shifting the emission spectra of most beetle luciferases. Altogether, the results indicate that the residues E311 and R337 play both structural and catalytic roles in bioluminescence

color determination, by stabilizing a closed hydrophobic conformation favorable for green light emission, and also providing a base to accept excited oxyluciferin phenol proton, and a counteraction to stabilize excited phenolate, blue-shifting emission spectra in most beetle luciferases.

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