Harnessing Nature's Nightlight—
An interview with Dr. Keith Wood

Dr. Keith Wood, Ph.D.
Research Fellow

In 2001, the Society for Biomolecular Screening awarded to Keith Wood the 'Life Sciences Award for Innovation in Automation and High-Throughput Screening.' The award, cosponsored by Perkin Elmer, recognized Dr. Wood's research and development of bioluminescent reporter gene technology. At Promega Keith developed this technology to enable the rapid and reliable quantitation of >100,000 biological samples per day. According to the SBS, "Dr. Wood's work has led to important drug discovery in basic research and has helped propel drug discovery to new frontiers." Keith accepted this award at the annual SBS Conference 2001 in Baltimore, MD, on September 13, 2001

In 1997, Promega received a 'Top 100 Innovative Products' Award for the Dual-Luciferase® Reporter Assay System. Presented annually for more than 35 years, the R&D Magazine 'Top 100 Innovative Products' Awards recognize new products judged to be among the 100 most technologically significant of the year. Previous 'Top 100' winners include anti-lock brakes, the liquid crystal display and the automated teller machine.

Luciferase: From Bugs to Biotech and Beyond

By Kari B. Kenefick, M.S., Promega Corporation

Dr. Keith Wood is the scientist credited with bringing a new light to Promega's technologies—the light of fireflies found on a typical summer's evening. He did so, not by direct assimilation of these charming bugs, but by working with the underlying chemistry and molecular biology responsible for the firefly's glow. The resulting luminescent technologies today constitute one of the most advanced detection capabilities available to life science researchers, with broad applicability to both biochemical and cellular analyses.

We couldn't help but find fascination with a guy who has made a career of manipulating firefly light. Moreover, what has made firefly luciferase so attractive, particularly to the high-tech laboratory?

"It's been an amazing journey..."
Dr. Keith Wood

Keith started firefly luciferase work back in 1985 at the University of California with the first expression of recombinant firefly luciferase. Nearly 20 years later, his team at Promega is still creating innovative luciferase-based products, drawing not just from fireflies but also from click beetles and even some luminous sea creatures, such as sea pansy.

Firefly luciferase is the ultimate in high-sensitivity biodetection: its light-emitting assay is fast with very little interference from the sample matrix, which means that you get the best results possible, quickly. These characteristics have made luciferase a mainstay as a biochemical signal transducer. The enzyme is monomeric and thus can be synthesized using a single open reading frame. Being devoid of post-translational modifications, it is catalytically competent as soon as it leaves the ribosome (Figure 1).

Although best known for its uses as a genetic reporter for monitoring gene expression, as molecular tools have crept into all aspects of bioscience research, so has luciferase. Scientists at Promega have designed luciferase tools for such diverse uses as high-throughput screening, RNAi-mediated gene regulation and cellular metabolism and viability. In fact a combined Highwire/Medline online search of "luciferase and Promega" results in >15,000 hits, returning articles from peer-reviewed science journals that detail experimental uses of luciferase. This begs the question of who uses this twinkle in the dark more, fireflies or scientists?

Although Keith's research generally doesn't involve the fireflies themselves, he has been known to collect these and other assorted luminescent animals while on "vacation." In addition to his innate interest in living lights, he also looks for bioluminescent species that may contain technologically advantageous genes. In fact, Keith has developed new colors of luminescence by using genes from luminous beetles other than fireflies, resulting in a departure from the traditional yellow-green light to include luminescence in colors like orange and red.

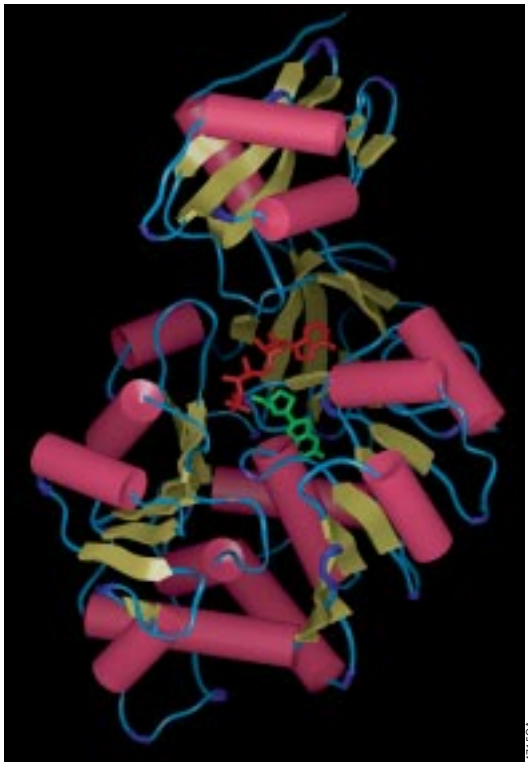

Figure 1. Luciferase model. A molecular model of luciferase shows the overall structure of the enzyme and the hypothesized locations for the reaction substrates, luciferin (green) and ATP (red). The model has provided mechanistic insights into kinetic data from the luminescent reaction and has served as a basis for designing site-directed mutagenesis.

When asked what led him down this lighted path, Keith explains, "When I came to the field of bioluminescence it was really an exciting time in the study of enzyme mechanisms. Biochemistry had previously been dominated by classical techniques of enzyme kinetics using proteins purified from natural sources. Structural studies of enzyme mechanism were done largely by means of cleverly designed organic molecules reacting with essential amino acids."

"I had known from early high school" He continues, "that what I really wanted to do was be able to take the basic building blocks of biological molecules, especially proteins, and rearrange those building blocks into new compositions." Keith explains that in this way he hoped to create new molecules with uniquely useful properties. But he admits that at the time, the early 1970s, these aspirations were based more on science fiction than fact.

It was only when he started graduate school in the 1980s that molecular biology was really taking hold in enzymological research. Tools for rearranging the "building blocks" of proteins were just being developed based on new capabilities for manipulating their cloned genes. Keith says, "Recent advances in molecular biology gave two really important tools to the classical enzymologist."

Sidebar: The Darker Side

One of the more romantic pleasures during a summer evening is the twinkling light displays of the firefly, *Photinus pyralis*. In fact, romance is what the male firefly intends in these displays. He flashes his lantern during flight to signal to females on the ground. When a female flashes her response, the male flies down to meet her. And they live happily ever after, right?

Unfortunately, danger lurks for the Romeo with a lantern. Another species of firefly, *Photuris*, uses a flashing response similar to the female *Photinus sp.* to attract the lighted males. But greed and self preservation, not lust, are the motivating factors for female *Photuris sp.* She is after a chemical compound that *Photinus* fireflies contain, which aids in self-defense against predators such as birds, spiders and other insects. The scheming *Photuris* females eat the *Photinus* males to gain these chemicals (1; Figure 2, Panel A).

Lucibufagins are the protective chemicals found in the blood of *Photinus sp.* fireflies. After eating the male *Photinus sp.*, the female *Photuris* has detectable amounts of lucibufagins in her system and thus reduces her own threat of predation (1).

This defensive compound from fireflies has been characterized by researchers at Cornell University in Ithaca, NY. The compound is chemically similar to bufalin steroids in the venom of poisonous Chinese toads, so researchers combined the Latin for "light" and "toad," hence lucibufagin (1,2).

While lucibufagins normally act to keep away predators that would otherwise eat fireflies, these compounds have proven fatal to some critters that have consumed fireflies.

The bearded dragon lizard, an import from Australia, is becoming increasingly popular in the US, due to its relatively small size and ease of handling (Figure 2, Panel B). While these lizards are insect eaters, several published reports exist of their sudden and violent death soon after ingesting a firefly. Similar reports of firefly toxicosis exist for White's tree frogs, also from Australia, and an African chameleon. Speculation is that because lucibufagin-secreting fireflies do not exist in some countries, there is no chance for these exotic reptiles to know what a danger the beetles can pose (2).

A.

B.

Figure 2. Firefly toxin. Panel A. Female *Photuris sp.* firefly devours a male *Photinus sp.* after preying on his romantic (actually reproductive) intent. Photograph copyright of Dr. Thomas Eisner, Cornell University, reproduced with permission. **Panel B.** *Pogona vitticeps*, the bearded dragon lizard from Australia, has proven extremely susceptible to the lucibufagins contained in certain fireflies. These lizards have no innate fear of fireflies; the beetles in Australia may not contain the poisonous lucibufagins found in some North American beetles.

Facts About Fireflies and Other Luminescent Creatures

Fireflies can be found flying over lawns, meadows and at the edges of streams and woods, beginning at dusk during the summer months. Their lighted displays differ amongst the various firefly species, and a beetle specialist or trained observer can differentiate various species based on the signaling patterns (e.g., number and duration of flashes, as well as time between signals; 3). For instance, the male *Photinus pyralis* flashes a single beam during upward flight, making a "J" shape in the air. The *Photinus* female responds with a single characteristic flash (see sidebar for details on species that imitate *Photinus sp.* females and why they do so). The male *Photinus consumilis* signals with a series of rapid flashes, wherein the female responds with two beams.

Not only are the various light displays characteristic of a species, different species emit different colored signals. *Photinus sp.* have yellow flashes, while *Photuris sp.* flash green and *Pyractomena sp.* have an amber flash (3).

Upon successful mating, firefly eggs are laid on or in the soil. The larvae are carnivorous, consuming snails and small insects. They burrow into the ground and overwinter, emerging in the spring to feed. They then pupate for approximately 2.5 weeks before emerging as adults. The pupae of some fireflies exhibit remarkable whole body luminescence (Figure 3, Panel A).

Female adult *Phengodes sp.*, a larvaform beetle, also exhibit total-body luminescence (Figure 3, Panel B).

In the United States there are more than 170 species of fireflies, most commonly found east of the Mississippi River. The worldwide count is at more than 1,900 species, found on every continent but Antarctica (4).

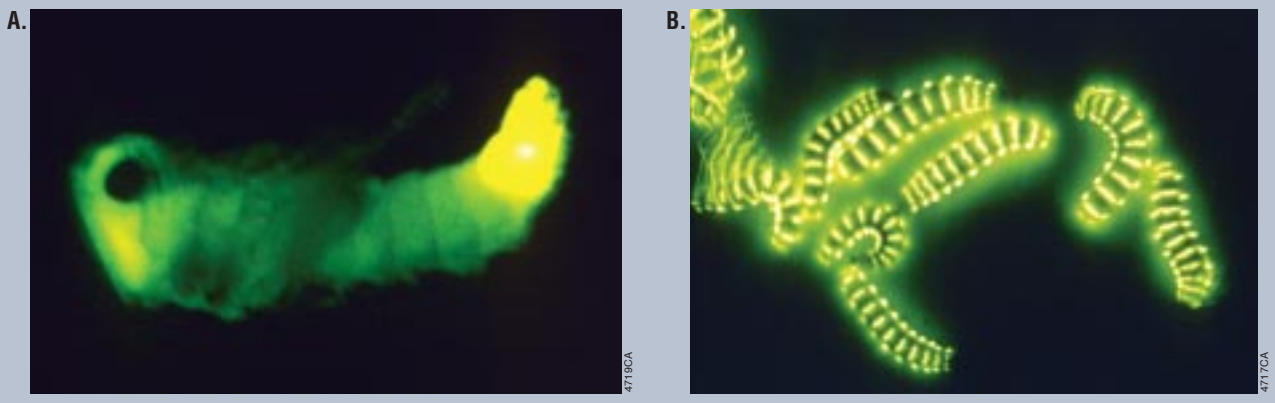

Figure 3. Luminescent organisms. Panel A shows a firefly pupa, which continuously emits green luminescence throughout its body. The pupa also emits a more yellow luminescence in the "tail" region, but this is mostly seen when the pupa is disturbed. The green luminescence will fade in the adult, leaving the more yellow luminescence to serve as a mating signal. **Panel B.** *Phengodes*, a luminous larviform beetle related to fireflies. The female of this beetle remains larviform even in the adult stage and emits a band of continuous luminescence over the length of her body. Both of these luminescent beetles were collected in Madison, WI, where the corporate headquarters of Promega is located.

First, increasingly efficient methods for cloning genes even from complex eukaryotes provided ready access to the amino acid sequences. Traditional methods for determining amino acid sequences were extremely laborious and generally feasible only for small proteins. "From the deduced amino acid sequence of cloned genes, you have the opportunity to look for protein families and determine if what you're studying is related to something else—this was much more difficult using classical enzymology techniques," Keith explains.

Moreover, the techniques being introduced for manipulating nucleic acids provided new tools for studying the relationships between protein structure and function. In particular, site-directed mutagenesis held a tremendous advantage over previously available techniques. Before this technique, a graduate student could spend years developing and characterizing the chemical changes made to an enzyme using custom organic molecules. But with the new methods, Keith says "It was trivial; you could change an amino acid to anything you wanted, very specifically, and see the effect on the protein."

So while he had these exciting new tools with which to make his dreams a reality, Keith needed a protein to manipulate, as well as a laboratory to work in. He had an idea of the kind of laboratory he wanted: one that was highly knowledgeable in traditional enzymology but could benefit from the new tools being developed for molecular biology.

Marlene DeLuca ran such a laboratory in the Chemistry Department at UC–San Diego. Her work was very much in the classical enzymology vein, applying traditional approaches to the study of firefly luciferase. She and her husband Bill McElroy had studied this enzyme since the late 1940's. They knew all that was to be known about firefly bioluminescence, its substrates and all manner of intricacies regarding its catalytic mechanism. Despite this, they knew relatively little about the enzyme's structure. There was even some doubt about whether it was a monomer or heterodimer.

Keith is quick to point out that not only was this a superb laboratory, but luciferase was an ideal molecule with which to work. Luciferase had been well studied classically, and the inherent simplicity of its assay made data collection easy. It held the appeal of allowing rapid characterization of mutant enzymes, thus making it an excellent model for applying molecular biology to investigate the basic principles of enzymology.

Initially, Dr. DeLuca was somewhat reluctant to consider this new approach in her research. Keith admits that to DeLuca and others in the Chemistry Department at the time, these new techniques were viewed as sort of a "black art."

But persistence paid off, and the subsequent cloning, expression and mutagenesis of the luciferase gene revealed a wealth of information on how this intriguing biochemical system worked. It also spawned a hugely important detection technology for the life sciences. Whereas the first applications to come from this work employed the luciferase gene as a genetic reporter, today it is widely recognized as a leading technology.

The first representation to the public of the remarkable abilities of the cloned luciferase gene was a photograph of a luminescent tobacco plant (Figure 4). The photograph was also noteworthy for the sensation it brought as a vivid depiction of the power of cloning.

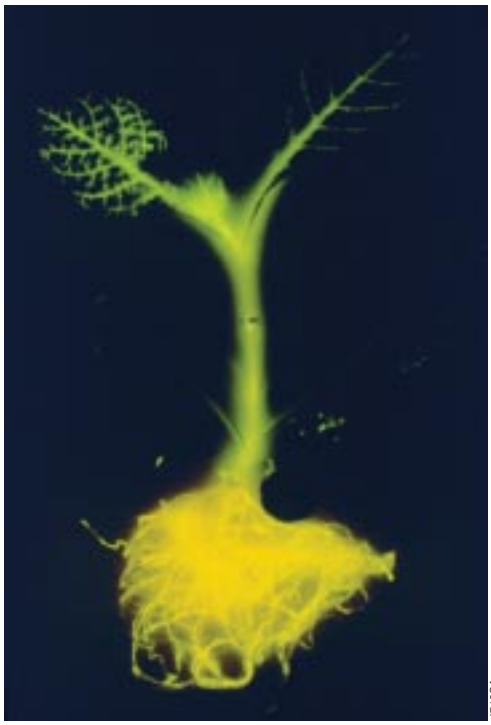

Figure 4. Luminescent tobacco plant. The first representation of a transgenic multicellular organism expressing bioluminescence was a photograph published in 1986 of a tobacco plant containing the firefly luciferase gene. The photograph was also used in the popular media to illustrate the ability to transfer functional genes between organisms and is found in textbooks for high school and college biology. Today, imaging bioluminescence in living plants and animals has become an important tool for many areas of physiology and pathogenesis (5).

While reporter technology is still the most common use for firefly luciferase, its technological advantage is not primarily in the gene itself. Rather it is the sensitivity, reliability and simplicity of the luminescent assay that has made this technology so popular. Building on these benefits, the scientists at Promega have been integrating luminescence into many other assay designs. These are accentuated by the incorporation of novel engineered enzymes and substrates, such as the Ultra-Glo™ Luciferase developed at Promega by use of recursive mutagenesis for greatly increase a physical stability.

In general, luminescent assays provide roughly 100-fold greater sensitivity than analogous fluorescent assays, and thus are increasingly favored for many aspects of life science research. So to answer the question of what makes luciferase so attractive, it is its incredible performance in biological assays. Today this curiosity in a nightlight of nature has grown to become a worldwide technology. "It's been an amazing journey," Keith notes, "from interesting science to a major technology. And we have a lot more still to do."

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