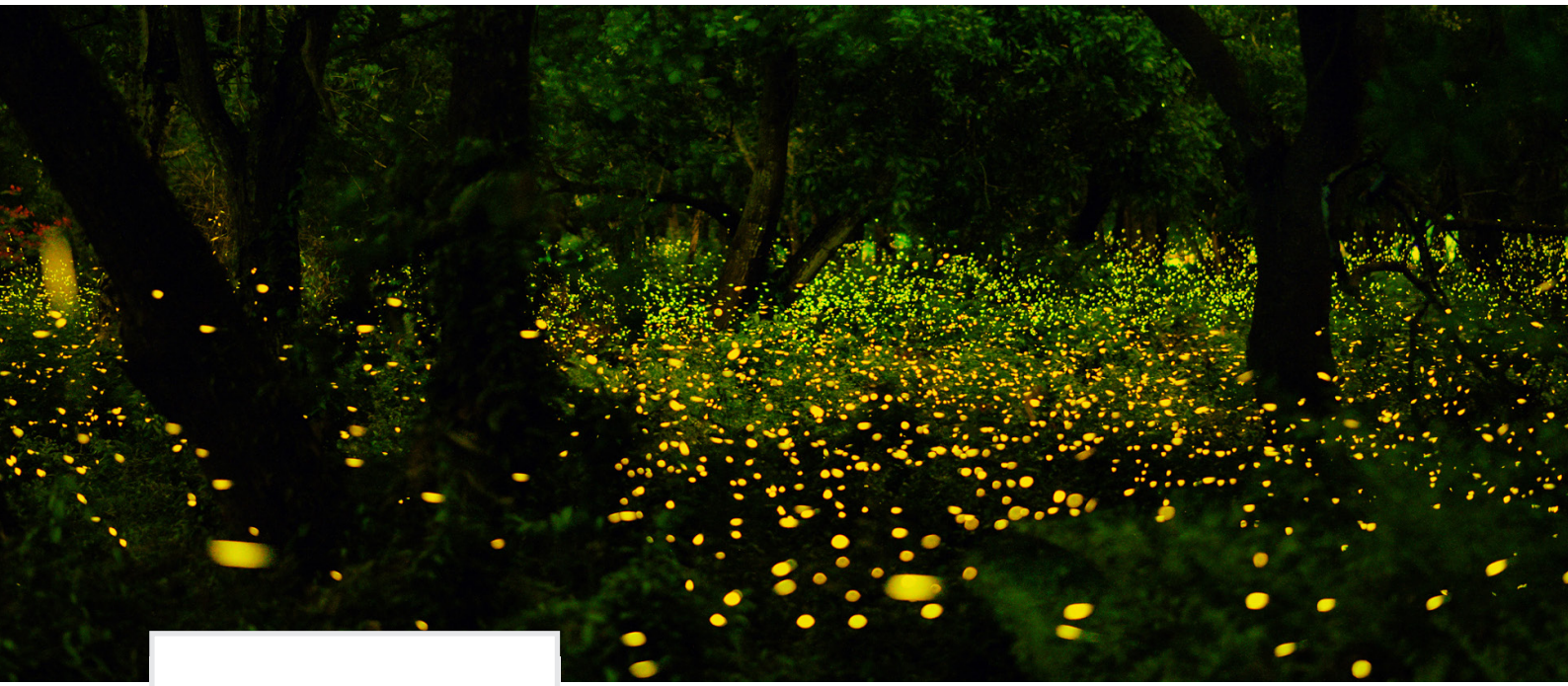

Challenge

Non-invasive imaging of bioluminescent mice

Solution

In vivo imaging with the UVP iBox Scientia

In Vivo Imaging with Bioluminescence: Performing a Luciferin Kinetic Curve in Mice

Introduction

In 1885, French Pharmacologist, Raphael Dubois, produced heat-labile and heat-sensitive extracts from an elaterid beetle—or firefly—of the Pyrophorous genus, which he called luciferin and luciferase, respectively[1]. When combined, these extracts would emit a blue glow. For most of the next century, researchers would discover similar instances of this chemical luminescence from other organisms—in the presence of oxygen and luciferase, luciferin would oxidize to produce blue light (Figure 1).

Isolating biochemically useful quantities of this enzyme for research was laborious. The fact that researchers had to catch fireflies for their research needs, was a major bottleneck to discovery. In 1985, Marlene DeLuca and colleagues from the University of California San Diego set out to resolve this issue. DeLuca's group cloned the luciferase gene from the firefly, Photinus *pyralis*[2], into bacteria. By augmenting extracts from these bacteria with luciferin and ATP, a bright yellow-green flash was observed[2]. Thus, DeLuca's group had successfully engineered a heterologous expression system for firefly luciferase. DeLuca and colleagues expanded on this initial discovery by first generating stable and transient transformants in tobacco plants[3], and subsequently transient and stable transfectants in mammalian cells[4]. Aided by the explosion of recombinant DNA strategies in the 1980s, DeLuca and colleagues could generate *in vivo* luminescence at will.

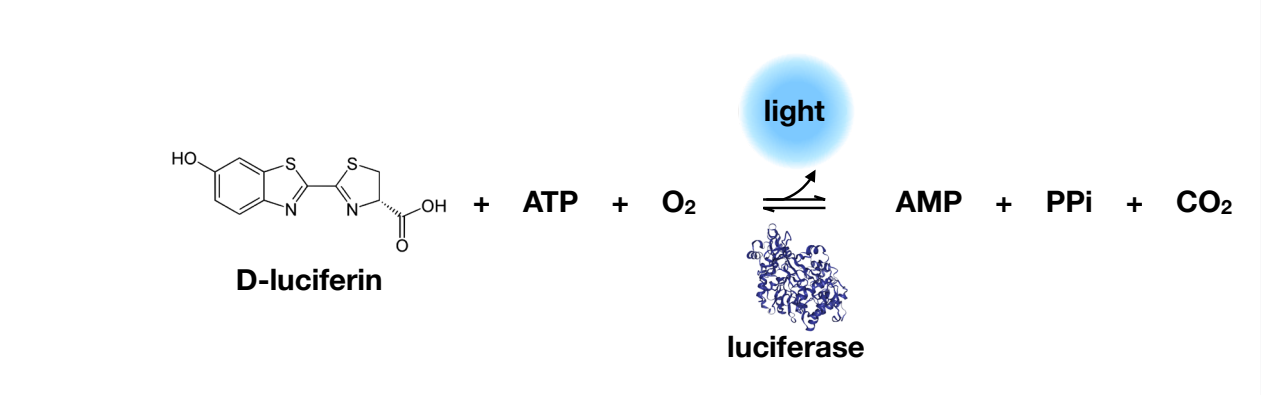

Figure 1: Bioluminescence reactions require ATP, O_2 and the D-luciferin substrate. D-luciferin is oxidized during the enzymatic reaction with luciferase, which produces light. The luciferase protein structure[‡‡] in this image was retrieved from the protein data bank (PDB): 2D1R.

It is naïve to think that DeLuca and her colleagues did not perceive the importance of what they had discovered. It is however, unlikely they could have predicted that nearly 35 years later, heterologous expression of firefly luciferase would be an increasingly popular tool for *in vivo* studies (Figure 2). This is especially true for *in vivo* imaging in animal models where the bioluminescent toolkit continues to expand with the identification of natural occurring variants as well as synthetic analogs produced by various means (reviewed in [5–11]).

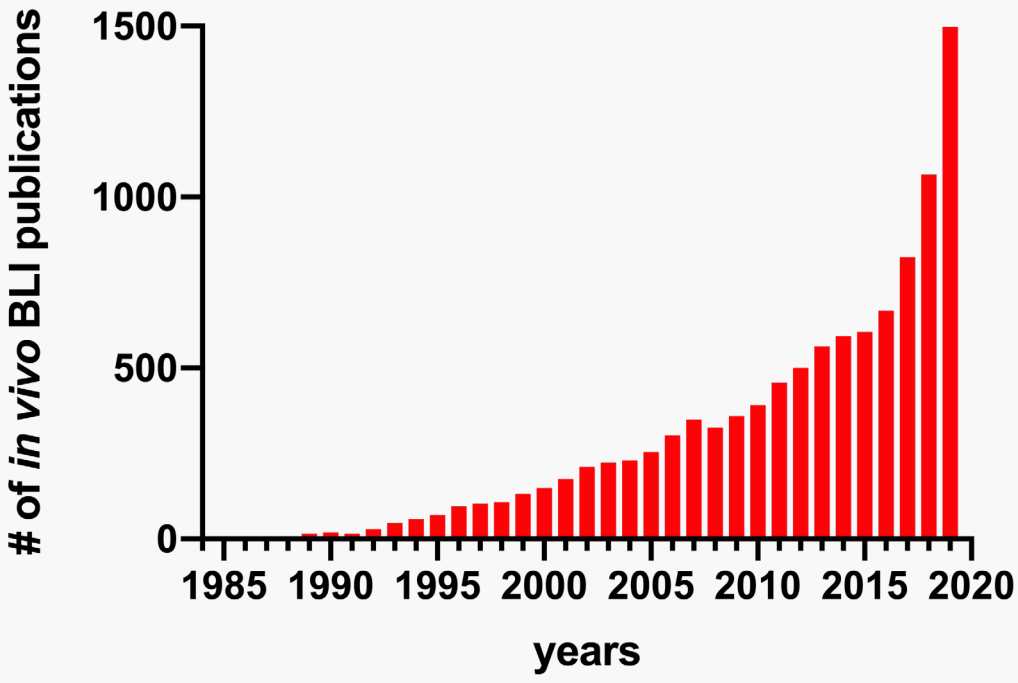

Figure 2: Growing trend of luciferase-based in vivo studies. The co-occurrence of "in vivo" and "luciferase" in the PubMed Central Database were assessed from 1985 to present day.

To meet the needs of our *in vivo* customers and ongoing development in this space, we have developed the UVP iBox Scientia Small Animal Imaging System. This instrument is capable of fluorescence and bioluminescence in vivo imaging of up to 10 mice with a 20 x 29 cm thermal plate, and corresponding field of view. Importantly, each instrument is pre-calibrated to report absolute photon flux (or total flux) to aid researchers in comparing data irrespective of space and time.

An important consideration prior to performing a BLI study is finding the window with which the emission from the luciferase-luciferin reaction peaks. The kinetics of the reaction can vary between substrates as well as tissue types and injection routes. Therefore, researchers should perform a kinetic curve study. Below we provide a protocol and results from a kinetic curve study on nude mice, using the UVP iBox Scientia Small Animal Imaging System.

Protocol

In this protocol we performed an intraperitoneal (IP) luciferin injection in the flank of a nude mouse bearing a subcutaneous (SC) lung cell tumor. Based on previous work, an IP injection with an SC tumor prevents rapid clearing of substrate and underestimation of signal as seen with IV and IP injections, respectively[12]. However, the signal may be as much as half of an SC injection with an SC tumor[12]. It is best practice to determine the route of injection that works best for your system.

Materials

- D-Luciferin
- Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+})
- Syringe and syringe filter

Preparation of Substrate and Substrate Injection into Mice

1. Prepare the substrate fresh for each study at 15mg/ml by suspending salt in sterile DPBS and gently homogenizing the substrate by inverting the tube.

2. Filter-sterilize substrate using a 0.2 micron syringe filter and elute into a sterile tube.

Note: luciferin can oxidize with oxygen and light. Follow the manufacturers protocol for optimal storage.

3. Each mouse should receive 0.15 mg/g body weight. For example, for a 25 g mouse, 3.75 mg or 250 ul of 15mg/ml solution should be used.

4. Sedate animals with preferred anesthesia (gaseous or injectable).

5. Place animal into iBox Scientia and take first timepoint image. Limit your number of replicates to perform a kinetic curve study as you may miss the initial spike in signal, which usually occurs within 5 minutes post-injection[12].

6. Take images every 5-10 minutes for approximately 1 hour, or as needed for your system.

Note: Be sure to check the reproducibility of your injection site/ tumor site combination as partial injection failure may obfuscate your results[12].

Results and Conclusion

In Figure 3 we summarize the results of our kinetic study on mice bearing a subcutaneous lung carcinoma tumor with an intraperitoneal D-luciferin injection. Mice were imaged starting at 5 minutes post-injection and monitored for 70 minutes thereafter. Using our VisionWorks software, a heatmap was applied to each image and normalized to the timepoint with the greatest signal (i.e. 35 min post-injection) to reflect the relative level of BLI signal at each timepoint (Figure 3). Importantly, absolute quantitation is possible using our VisionWorks software, data is reported in photon flux in Figure 3. From this kinetic curve study, we determined that 25-35 minutes post-injection is an optimal window for detecting signal in our subcutaneous lung carcinoma mouse model.

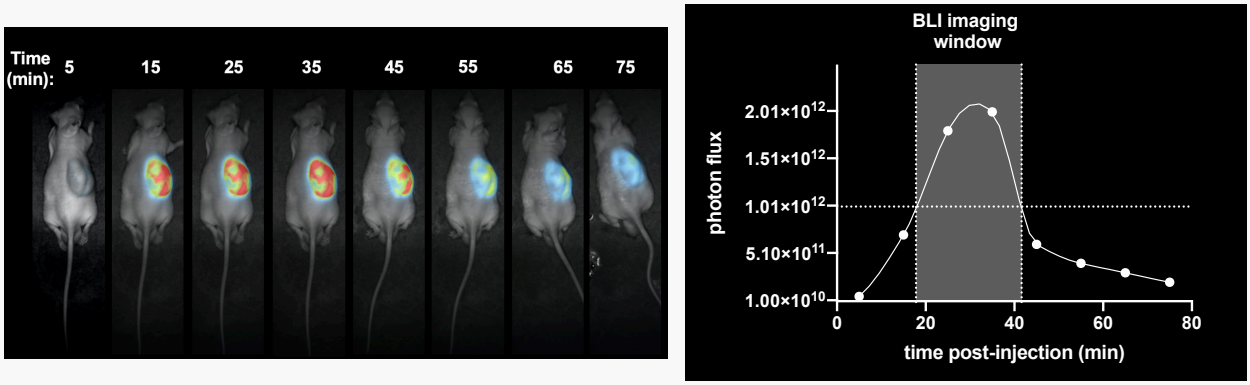

Figure 3: Bioluminescence kinetic curve study. Mice were injected with 15mg/ml solution of D-luciferin prior to anesthetization with ketamine-xylazine. Images were captured 5 minutes post-injection and in intervals of 10 minutes thereafter. A) A white and BLI composite image pseudocolored with a heatmap are shown to demonstrate the relative levels of BLI signal in a mouse bearing a subcutaneous lung carcinoma tumor. B) Area density analysis was performed using the same ROI for all timepoints in our VisionWorks software package. Data is reported as photon flux.

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Headquarters
Analytik Jena US
2066 W. 11th Street
Upland, CA · USA

Phone +1 909 946 3197
Fax +1 909 946 3597

info@us.analytik-jena.com
www.analytik-jena.us

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