Science in School
The European journal for science teachers

Issue 53 - 15/06/2021

Bioluminescence: combining biology, chemistry, and bionics

Claas Wegner, Marcel Hammann, Carolin Zehne

Engaging with biomimetic design encourages students to explore the principles of form and function in relation to evolutionary adaptation.

Introduction and overview

This teaching unit uses the bioluminescence of fireflies to demonstrate concepts related to enzymatic and chemical reactions. The teaching unit is composed of two parts:

- An introduction to the biological and chemical basics of bioluminescence.
- Experiments demonstrating enzymatic and ion-based luminescence reactions and their dependence on temperature.

An extension activity is also provided to explore the bionic aspects of the topic by comparing fireflies and light-emitting diodes (LEDs).

These activities are aimed at students aged 16 or older, who should be familiar with the basic properties of a cell, how to conduct experiments and note down observations, and the properties and processes of chemical reactions. The unit can also be used for interdisciplinary teaching, e.g., dealing with the properties of light in physics. After completing the unit, students should be able to:

- Describe the structural properties of a photophore in Lampyridae
- Relate these properties to light-generating processes taking place in photophores
- Explain the chemical processes involved in luminescence
- Compare and contrast the processes and properties of bioluminescence and chemiluminescence
- Explain the role of enzymes in enzymatically catalysed reactions
- Formulate hypotheses and test them in experiments

An overview of the relevant theoretical background necessary to understand the tasks and experiments, and to help students with potential questions, is provided in the attached PDF.

Teaching unit

The teaching unit on fireflies and LEDs is composed of two parts and should take two to three lessons. In the first part, students uncover the basic biological and chemical aspects of the topic. In the second part, they conduct experiments to explore the catalytic acceleration of a reaction, temperature-dependent enzymes, and ion-based luminescence reactions. Based on their results, students then propose explanations for discussion in the group.

The time devoted to the students' independent work on worksheets 1–3 should be no longer than half of the first lesson, to ensure that there is enough time to generate hypotheses for the second activity in subsequent lessons.

In the second and third lessons, students conduct experiments in their research teams. Worksheets 4–6 provide relevant background information and procedures for the two experiments, along with questions to help the students interpret the results. After each experiment, results are collected and discussed in class and compared with the hypotheses generated in part one. This way, experience-based hypotheses are compared with empirical evidence.

Activity 1

In the first lesson, students are introduced to the topic. This first activity is theory-based. Worksheets 1–3 introduce facts about Lampyridae, and students are encouraged to think about how bioluminescence works and generate hypotheses.

1. Students are first provided with the unit's structure to ensure transparency and a video[1] may be shown to introduce the topic and spark interest.
2. They are then divided into groups (research teams) to work through Worksheets 1–3, which introduce three species of Lampyridae (*Lampyris noctiluca*, *Lamprohiza splendidula*, and *Phosphaenus hemipterus*, Worksheet 1), the photophore and its structural properties (Worksheet 2), as well as bioluminescence reactions (Worksheet 3).
3. After that, the students should consider the following questions:
   a. Would the bioluminescence reaction work in a test tube?
   b. How would heating to above 50°C affect this reaction?
   c. Would you expect a different result for non-enzymatic chemiluminescence?
4. Students should then generate hypotheses based on these questions, which they can test in subsequent experiments. The students' hypotheses are collected in class.

Activity 2

This activity is carried out using dried *Vargula hilgendorfii* samples.[2] These contain a luciferase and the luciferin substrate, which can react to produce light when the sample is pulverized and rehydrated. Students should follow the steps in Worksheet 4 to carry out and interpret the experiment. Students should be given a whole lesson for this activity.

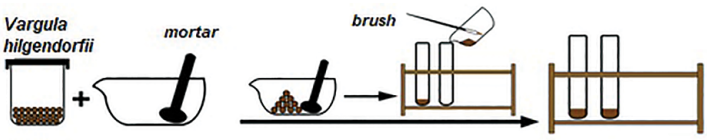
Materials

- Worksheet 4
- A pipette
- Two test tubes
- A bristle brush
- Use of a kettle
- A small pestle and mortar
- 30 dried *Vargula hilgendorfii*

Important: all materials must be completely dry! The required substances are not harmful, according to the GHS/CLP.

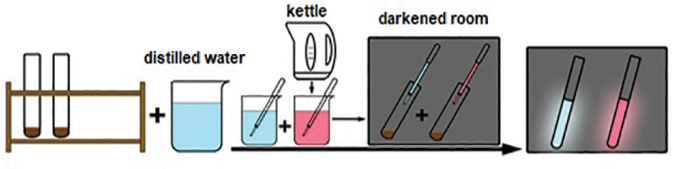
Procedure

1. Grind two batches of 15 *Vargula hilgendorfii* using the small mortar. The resulting powders are swept into two dry test tubes using the bristle brush.



Setup for activity 2 Image courtesy of Marcel Hammann

2. Once all teams have prepared their materials, the teacher closes the blinds. In the darkened room, 2 ml cold water (20°C) are pipetted into one of the test tubes with 2 ml warm water (ca. 80°C) in the other one.



Final steps: triggering the luminescence reaction
Image courtesy of Marcel Hammann

Activity 3

This activity in the third lesson helps students to gain a deeper understanding of chemical processes involved in chemiluminescence. In the experiment, they work with luminol based on Worksheet 5.

Name	Amount	GHS/CLP hazard symbol
luminol (3-aminophthalhydrazide)	~ 0.02 g	N/A
ammonium chloride (NH_4Cl)	~ 0.4 g	(GHS07 attention: harmful to health)
sodium carbonate (Na_2CO_3)	~ 0.4 g	(GHS07 attention: irritant)
hydrogen peroxide (3 %) (H_2O_2)	~ 6 ml	(GHS05 corrosive: slightly) (GHS07 attention: irritant)

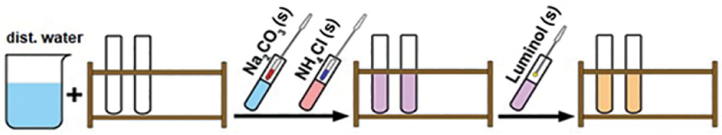
Materials

- Worksheets 5 and 6
- A powder spatula (ca. 17 cm)
- A pipette (3 ml)
- Two copper wires
- A thermometer
- Two test tubes
- Use of a kettle
- A microspoon spatula
- A test-tube rack
- A tall beaker (150 ml)

Procedure

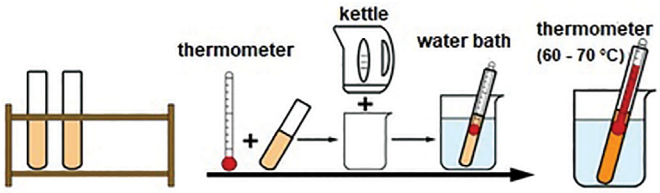
1. Two test tubes are filled (one-third each) with distilled water. Ammonium chloride (ca. 0.2 g) and sodium carbonate (ca. 0.2 g) are added to each test tube using the tip of a powder spatula. Luminol (ca. 0.02 g) is added with the help of a microspoon spatula. Both solutions are mixed thoroughly through gently shaking.

Powder spatula (left) and microspoon spatula (right)
Image courtesy of Marcel Hammann



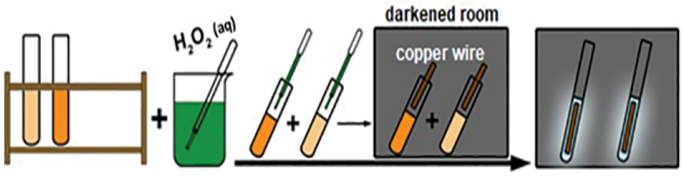
Setup for activity 3 Image courtesy of Marcel Hammann

2. Water is heated in a kettle and poured into the beaker, as a water bath. A thermometer is added to one of the test tubes and the solution is heated to 60–70°C in the water bath. If necessary, the water in the beaker is replaced with hot water from the kettle.



Heating of the second tube Image courtesy of Marcel Hammann

3. After one of the test tubes has been heated, 3 ml of hydrogen peroxide (3%) are added with a pipette to both test tubes. Copper wire is then held into the solution of each test tube in a darkened room. The luminosity of the two solutions is compared.



Final steps: triggering of the luminescence reaction
Image courtesy of Marcel Hammann

4. After students have completed the experiment, Worksheet 6 should be handed out during the last 20 minutes of the lesson. They should now compare their findings from Activities 2 and 3 and note down the differences and similarities between bio- and chemiluminescence.

Optional extension activity

In an optional extension activity, LEDs are introduced and bionic aspects of the topic can be explored, along with their relevance in the real world. Worksheet 7 guides students through the extension activity. After completing this activity, students should be able to:

- Compare and contrast the structural properties of photophores and LEDs
- Infer bionic applications based on the structural properties of photophores

In the first half of this lesson, some background information is

provided about how LEDs function and the similarities between firefly luminescence and LEDs. Students should note down some initial assumptions about how an LED's luminous efficiency could be increased and then explore the effects of putting a lens in front of an LED torch in the second half of the lesson.

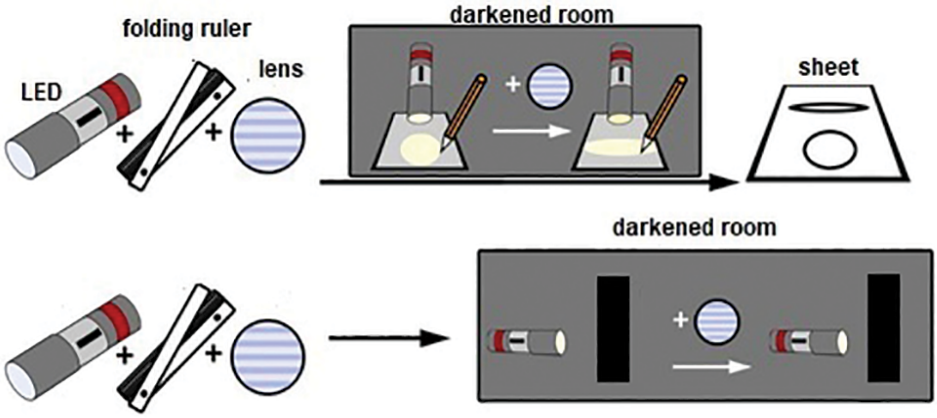
Materials

- Worksheet 7
- An LED torch
- A lens
- Two sheets of paper
- A folding ruler
- A pen

Procedure

1. An LED torch is pointed at a sheet of paper (30 cm distance) in a darkened room. The illuminated area is then circled with a pen.
2. A lens is put in front of the LED torch and the illuminated area is circled again on the paper.
3. The LED torch is then pointed at a wall with and without the lens (3 m distance).

Students should note down and discuss their observations of how the lens changes the appearance of light from the LED torch. They should then further compare the structural properties of the LED torch and a photophore, with the help of a schematic overview, followed by a class discussion of the results.



Workflow for Activity 4 Image courtesy of Marcel Hammann

References

1. For example: a video showing the life cycle of British glow-worms: https://vimeo.com/31952006.
2. These samples can be ordered from online science supply shops, e.g., https://www.carolina.com/ or https://www.der-hedinger.de/.

Resources

- Read about further teaching activities on the chemistry of bioluminescence: Farusi G, Watt S (2016) Living light: the chemistry of bioluminescence. *Science in School* **35**:30–36.
- Watch a video on the mechanism of bioluminescence.
- Read about chemiluminescence: Welsh E (2011) What is chemiluminescence? *Science in School* **19**:62–68.

Authors biography

Prof. Dr Claas Wegner is professor of biology didactics at Bielefeld University and professor of psychology at the University of Applied Sciences for Small and Medium-Sized Enterprises (Fachhochschule des Mittelstands – FHM), as well as founder and head of the Osthushenrich-Center for research into intellectual giftedness (Osthushenrich-Zentrum für Hochbegabungsforschung – OZHB) at the Department of Biology at Bielefeld University.

Marcel Hammann (M.Ed.) is a former student of the Department of Biology, Bielefeld University, and a secondary school teacher.

Carolin Zehne (M.Ed.) is a lecturer at Bielefeld University (teaching English as a foreign language).