JOURNAL OF CHEMICAL EDUCATION

pubs.acs.org/jchemeduc

Demonstration

Repeatable Chemiluminescence from Luminol without Added Hydrogen Peroxide

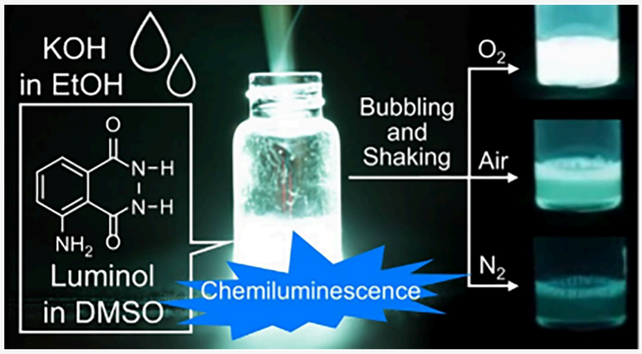
Kanon Ie,* Takahiro Suzuki, and Masayuki Inoue

Cite This: https://doi.org/10.1021/acs.jchemed.5c00155

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Chemiluminescence is the emission of light that results from a chemical reaction, in which part of the energy produced is converted into light energy. These reactions can be difficult to visualize and control. In this article, we describe an experimental approach for demonstrating chemiluminescence to students and researchers. The main purpose of this study is to trigger chemical thinking skills and the spirit of inquiry in students by exploring the roles of oxidants and reaction mechanisms in luminol oxidation reactions. Chemiluminescence was observed when a drop of an ethanol solution containing potassium hydroxide was added into a luminol solution in dimethyl sulfoxide followed by shaking. Using dissolved oxygen as an oxidant, the experimental solution glowed upon shaking and gradually faded when left to stand, allowing repeated observation of chemiluminescence. The experimental demonstration of the proposed luminol oxidation reaction in the classroom helped students to directly experience chemiluminescence and deepen their interest in photochemistry. This exploratory activity of oxidants promoted independent learning among the students. The experimental approach presented in this study is of educational value and can be tailored according to the learning situation and level of understanding of the audience.



KEYWORDS: *High School, Introductory Chemistry, First-Year Undergraduate, General, Demonstrations, Hands-On Learning, Manipulatives, Inquiry-Based, Discovery Learning*

■ INTRODUCTION

Photochemical reactions refer to the process in which light energy induces a chemical reaction by supplying energy to electrons in a substance. Chemiluminescence is considered the reverse process of a photochemical reaction in which light is emitted as a result of a chemical reaction without the need for an external light source. Chemiluminescence is a process wherein chemical energy is directly converted into light energy. Chemiluminescence-based reactions are attractive chemical demonstrations that involve distinct visual changes. Examples of chemiluminescence-based reactions include luminol oxidation reactions,[1−14] plant photosynthesis,[15] and glow sticks.[16] Among the established chemiluminescence-based reactions, the luminol oxidation reaction, which has been known for a long time, is regularly demonstrated in schools and chemistry-related events, such as workshops, seminars, or conferences.[1,7,9,10,12,14] Typically, to demonstrate the luminol oxidation reaction, a hydrogen peroxide solution containing potassium iron(III) hexacyanide is mixed with a sodium hydroxide solution containing luminol in a test tube or triangular flask.[1,10] However, no other innovative methods for demonstrating the luminol oxidation reaction have been found.

In the luminol oxidation reaction, luminol (Figure 1A) is oxidized by an oxidant to an excited 3-aminophthalate ion

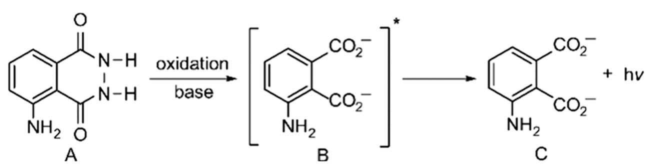


Figure 1. Reaction mechanism of the luminol oxidation reaction.

(Figure 1B), which produces chemiluminescence upon returning to the ground state (Figure 1C), independent of the solvent type.[2−4,12] Schneider reported that the luminol oxidation reaction occurs when 20 μL of saturated sodium hydroxide solution is added dropwise to a solution containing 5 mg of luminol dissolved in 1 mL of dimethyl sulfoxide (DMSO).[3] In addition, Chalmer et al. reported that the addition of a fluorescent substance to a solution containing

Received: February 3, 2025
Revised: June 28, 2025
Accepted: June 30, 2025

luminol results in chemiluminescence in a variety of bright colors.[8]

However, these demonstrations are limited to observing chemiluminescence, and there appear to be no inquiry-based experimental materials that aim to identify the oxidizing agent of luminol. This study proposes an experimental material that enables the repeated observation of chemiluminescence without the need for hydrogen peroxide using DMSO as the solvent for luminol. Chemiluminescence was observed upon adding an ethanol solution of potassium hydroxide dropwise to a solution of luminol in DMSO. The use of ethanol as a solvent for potassium hydroxide instead of sodium hydroxide lowered the base concentration, potentially improving chemical safety. The novelty of this study lies in the use of dissolved oxygen as an oxidizing agent. The dissolved oxygen causes the luminol solution to glow when shaken; when left to stand, the glow fades, allowing the user to experience chemiluminescence repeatedly. The reported study is intended not only to have the viewers experience chemiluminescence but also to trigger their minds to think chemically. The proposed luminol oxidation reaction is expected to trigger curious minds to explore the role of oxidants and reaction mechanisms. A demonstration that allows the determination of the oxidizing agent based on the intensity of light and takes about 20 min to complete is innovative. This study provides a novel way for demonstrating the luminol oxidation reaction, encouraging chemical thinking.

■ MECHANISM OF THE LUMINOL OXIDATION REACTION

The mechanism of the luminol oxidation reaction in polar solvents has been reported by several authors.[2,4,6,13] Luminol deprotonates under basic conditions and oxidizes to form a peroxidized intermediate in the excited state. DMSO is a polar solvent. In a DMSO molecule, the chemical structures of the sulfur and oxygen atoms promote interactions with dissolved oxygen, allowing the dissolved oxygen molecules to disperse easily. Notably, the solubility of oxygen is high in DMSO.[4] The solubility of oxygen in water and DMSO is 8.2 and 26 mg L^{-1}, respectively, indicating that oxygen is three times more soluble in DMSO than in water.[4,5]

Next, luminol oxidation reactions involving the use of DMSO are discussed. Wildes et al. analyzed the reaction mechanism by measuring the fluorescence and absorption of luminol and 3-aminophthalate ions in various solvents.[4] They reported that the transition from the excited state of the carboxylic acid group results in the emission of light at 510 nm, as shown in Figure 2. Although previous studies demonstrated notable differences in the emission wavelengths and pathways, the overall mechanism remained consistent, involving oxygen as an oxidant. For a detailed explanation of the luminol oxidation reaction mechanism based on molecular orbital calculations, please refer to the Supporting Information.

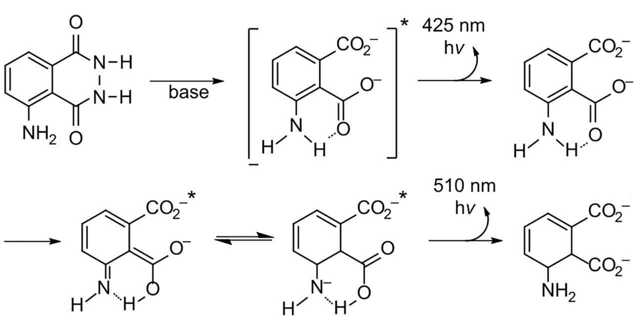


Figure 2. Reaction mechanism of luminol oxidation reaction in DMSO.

■ MATERIALS AND EQUIPMENT

The reagents and equipment required are listed below. All reagents were purchased from Kanto Chemical, Japan, and were used without further purification.

- Luminol (≥95.0%, Kanto Chemical)
- DMSO (≥98%, Kanto Chemical)
- Potassium hydroxide (≥84%, Kanto Chemical)
- Ethanol (94.83−95.85%, Kanto Chemical)
- Test tube (18 mm OD × 180 mm L) and a 13.5 mL screw tube.
- Micropipette or 1 mL Komagome pipet
- 50 mL syringe
- Three-way cock
- Silicone tube
- Oxygen gas canister
- Nitrogen gas canister

■ EXPERIMENTAL HAZARDS

Safety glasses and gloves were worn during the experiments. The possible chemical hazards associated with the reagents used in this study are as follows:

- Luminol may cause skin and eye irritation.
- DMSO is flammable and volatile. DMSO may cause respiratory organ damage if inhaled.
- Potassium hydroxide causes severe skin and eye damage.
- Ethanol is highly flammable and may cause skin and eye irritation.

■ EXPERIMENTS AND OBSERVATIONS

Topic 1. Preparation of Experimental Solutions

For this study, two solutions were prepared. Solution A was prepared by dissolving 0.1 g of luminol in 20 mL of DMSO. Solution B was a saturated potassium hydroxide ethanol solution prepared by dissolving 0.6 g of potassium hydroxide in 1 mL of ethanol. Notably, the dissolution of potassium hydroxide generates a slight heat.

Approximately 3 h after the preparation of Solution B, the acetaldehyde dissolved in ethanol oxidized to enolates, which then underwent aldol reactions, resulting in a brown solution (Figure S2). To avoid this discoloration, solution B was prepared just before starting the experiment; however, even if it turned brown, there was no effect on the luminescence intensity (Figure S3). The prepared solution B was determined to be highly basic and dangerous. Please note that solution B should be prepared under supervision with the utmost care and proper precautions.

Topic 2. Observation of Chemiluminescence

First, 2 mL of solution A was poured into a 13.5 mL screw tube (Figure 3A). Subsequently, 40 μL of solution B was added using a micropipette, and chemiluminescence was observed, as shown in Figure 3B. After 1 s of adding solution B to solution A, luminescence started quenching (Figure 3C). After 3 s of adding solution B to solution A, the liquid surface in contact with air in the screw tube was observed to still glow (Figure 3D). Upon shaking the solution again, the chemiluminescence

https://doi.org/10.1021/acs.jchemed.5c00155
J. Chem. Educ. XXXX, XXX, XXX−XXX

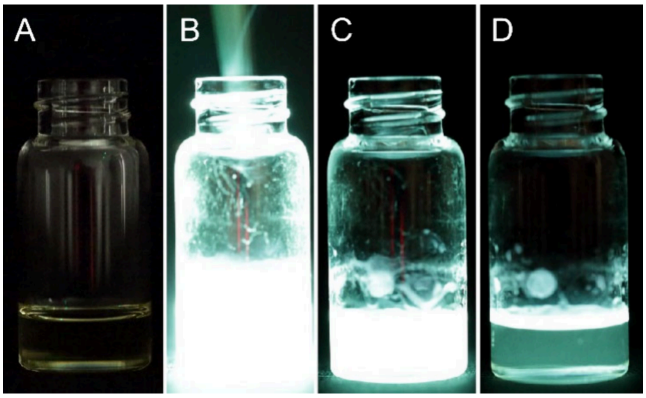


Figure 3. Experimental solution: A) before adding a drop of potassium hydroxide in ethanol, B) immediately after adding a drop of potassium hydroxide in ethanol, C) 1 s after adding a drop of potassium hydroxide in ethanol, and D) 3 s after adding a drop of potassium hydroxide in ethanol.

was regained. Video S1 of this process has been provided in the Supporting Information.

The illuminance of the experimental solution was measured by using an illuminometer (MK Scientific, TES-1336A). The measurement method is described in detail in the Supporting Information. Figure 4 shows that a maximum irradiance of 74.5

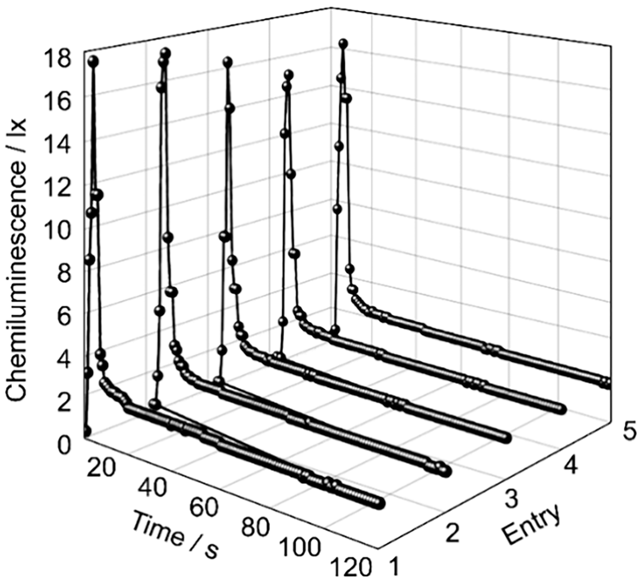


Figure 4. Illuminance vs time. Five measurements were taken under the same measurement conditions.

± 5.8 lx is reached immediately after adding a drop of potassium hydroxide in ethanol to the experimental solution. The irradiance remains at ~2 lx for ~20 s, likely due to the ongoing reaction of luminol at the interface between the solution and oxygen in air within the screw tube. Measurements were conducted using saturated ethanol solutions of sodium hydroxide and rubidium hydroxide (Figure S4), in addition to potassium hydroxide. Based on these results, potassium hydroxide was selected as the base.

Topic 3. Confirming Oxygen as the Oxidizing Agent

Next, three screw tubes were filled with 2 mL of solution A and 40 μL of solution B. A 50 mL syringe was filled with 50 mL of one of the following gases: nitrogen, oxygen, or air. After each gas was blown into the solution, the screw tube was closed with a lid, and the laboratory lights were turned off. Next, the screw tube was shaken up and down to observe the luminescence. When the experimental solution was bubbled with oxygen, very strong luminescence was observed (Figure 5A). When the

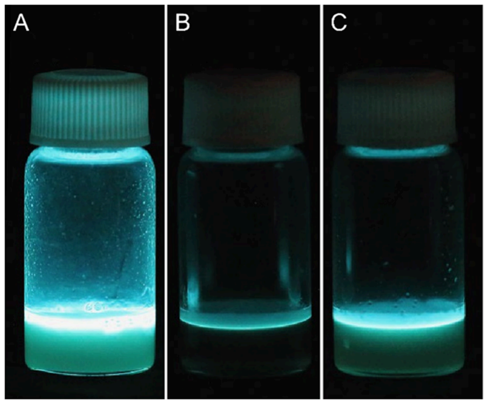


Figure 5. Chemiluminescence after shaking the experimental solution bubbled with (A) oxygen, (B) nitrogen, and (C) air.

experimental solution was bubbled with nitrogen, a faint luminescence was observed (Figure 5B). When the experimental solution was bubbled with air, bright luminescence was observed (Figure 5C). Video S2 shows the chemiluminescence after shaking the experimental solution bubbled with various gases (Supporting Information). The results in Figure 5 suggest that the concentration of oxygen in the experimental solution significantly affects the observed luminescence intensity.

To observe chemiluminescence as a function of time, a drop of potassium hydroxide in ethanol was added to the experimental solution; 2 min later, the timer was set to 0 min. Subsequently, the illuminance was measured after the desired gas was blown through the syringe for 1 s. In Figure 6, the maximum illuminance can be observed to vary depending on the gas blown into the syringe, and the highest illuminance can be observed with oxygen. Comparing the illuminance

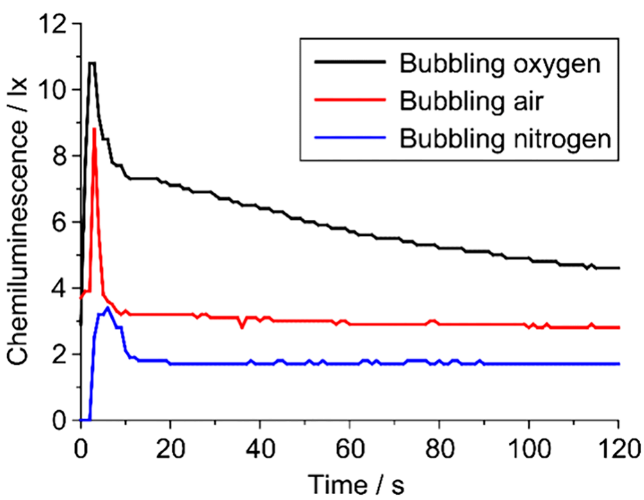


Figure 6. Chemiluminescence as a function of time after blowing the indicated gas into the experimental solution.

https://doi.org/10.1021/acs.jchemed.5c00155
J. Chem. Educ. XXXX, XXX, XXX−XXX

observed after blowing nitrogen or oxygen into the experimental solution, the illuminance observed after blowing oxygen was quenched more slowly, indicating that oxygen is consumed by the experimental solution. The experimental results indicate that when oxygen is blown into the experimental solution, it acts as the oxidizing agent for the luminol oxidation reaction. After sufficiently diluting the alcoholic potassium hydroxide with water, it is collected together with the solution used to observe chemiluminescence as an aqueous organic waste. The collected waste should be disposed of by a waste disposal contractor in accordance with the relevant local, national, and regional laws and regulations.

■ DEMONSTRATION OF THE LUMINOL OXIDATION REACTION IN LEARNING

The experimental demonstrations were conducted in two phases. The first phase took place on October 3, 2024, for 15 min with a class of high school students aged 15−16 years. In this phase, the experiment described in Topic 2 was carried out. During the first 5 min, the process of chemiluminescence and the luminol oxidation reaction were thoroughly explained to the students. Next, the students added three drops of a base using a 1 mL Komagome pipet and observed the chemiluminescence. They then referred to a color chart to record the observed color of the chemiluminescence in the experimental questionnaire (Figure S5). Video S3 shows the students shaking and mixing the luminol solution (Supporting Information). A total of 38 students participated in this round.

The second phase of the experiment was conducted on November 9, 2024, for 50 min with a total of 9 high school students aged 17−18 years. After performing the experiment described in Topic 2 for 10 min, the students connected a 50 mL syringe to a silicone tube and a three-way stopcock and introduced either oxygen, nitrogen, or air into the screw tubes. They then sealed the tubes with caps and observed the luminescence after shaking the tubes. This experiment was based on Topic 3 and was carried out for 15 min. Figure 7 shows a photograph of the students performing the luminol oxidation reaction experiments.

The students who conducted the experiment had little to no prior knowledge of the luminol oxidation reaction; however, they could visually observe the chemiluminescence of the experimental solution when oxygen was blown into it. All students who performed the experiment described in Topic 3 responded that the solution into which oxygen was blown emitted brighter luminescence than the solution into which nitrogen was blown. Based on these results, the students answered the worksheet question, “What is the oxidizing agent of luminol in this system?” Although this question may have been somewhat challenging, it was intended to encourage students to reflect on the role of oxygen in the reaction and to connect their experimental observations with the concept of oxidation. Among the students, 78% correctly answered “oxygen.” In their answers to a questionnaire, the students expressed positive opinions about the experiment; one stated, “It was very beautiful to see the luminescence up close.” Another wrote, “I was impressed to experience the chemiluminescent reaction that I had only heard about in detective dramas, where it is often used for detecting blood traces.” The student worksheet and questionnaire used in this study have been included in the Supporting Information to aid reproducibility and provide further insights into the students’ learning experience. This study involved student participants in classroom settings as part of the general educational activities. According to the institutional policy of Tokyo University of Science, educational research conducted within the scope of standard classroom activities is exempt from formal review by an institutional ethics committee. No personally identifiable information was collected, and informed consent was obtained from all of the student participants prior to data collection. No unexpected or unusually high safety hazards were encountered during the experiments.

Figure 7. Image showing students closing a gas-filled syringe while performing the experiment covered under Topic 3.

■ CONCLUSION

In conclusion, the composition of reaction solutions and experimental conditions can promote inquiry-based learning in chemistry education. By selecting DMSO is selected as the solvent and potassium hydroxide as the base, chemiluminescence can be directly observed. Furthermore, we promoted independent student learning through exploratory questions concerning the oxidant of the luminol oxidation reaction. The demonstration of the luminol oxidation reaction in learning deepened the interest of students in photochemical reactions, encouraging them to understand the roles of oxidants and reaction mechanisms. The flexibility of performing the proposed luminol oxidation reaction facilitates its use in different classroom settings and at different grade levels. The educational value of the proposed luminol oxidation reaction is demonstrated by its ability to promote independent learning in students. The proposed luminol oxidation reaction can be tailored according to the learning situation and the level of audience understanding.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.5c00155.

Although previous studies demonstrated notable differences in the emission wavelengths and pathways, the overall mechanism remained consistent, involving oxygen as an oxidant. For a detailed explanation of the luminol oxidation reaction mechanism based on molecular orbital calculations. Figures S2 and S3 depict the time-dependent changes observed in solution B, including color changes due to aldol reactions involving acetaldehyde and the corresponding decrease in chemiluminescence intensity over 24 h. Figure S4 shows the illuminance measured over time after adding various alkali metal hydroxide solutions (NaOH, KOH, and RbOH) to luminol in DMSO. Potassium hydroxide showed the highest illuminance and was therefore selected as the base for luminescence experiments. Information on how to measure the illuminance and color of potassium hydroxide in ethanol, along with instructions for students. Experimental guide and student questionnaire used in Topic 3. Figure S5 provides a color chart for students to identify the color of chemiluminescence (PDF, DOCX)

Video S1 shows chemiluminescence and quenching of chemiluminescence after adding drops of potassium hydroxide in ethanol to the experimental solution. Video S1 also shows the subsequent emission of light upon shaking the experimental solution containing oxygen (MP4)

Video S2 shows luminescence from the experimental solution after adding a drop of potassium hydroxide in ethanol, followed by shaking in the presence of oxygen, nitrogen, or air (MP4)

Video S3 shows students shaking and mixing the experimental solution in the presence of air (MP4)

■ AUTHOR INFORMATION

Corresponding Author

Kanon Ie − *Graduate School of Science, Tokyo University of Science, Tokyo 162-8601, Japan;* orcid.org/0009-0009-9393-2694; Email: 1724701@ed.tus.ac.jp

Authors

Takahiro Suzuki − *Department of Chemistry, Faculty of Science, Tokyo University of Science, Tokyo 162-8601, Japan;* orcid.org/0000-0001-9512-6383

Masayuki Inoue − *Department of Chemistry, Faculty of Science, Tokyo University of Science, Tokyo 162-8601, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jchemed.5c00155

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We express our sincere gratitude to all students who participated in the experiments for this study and completed the questionnaire.

■ REFERENCES

(1) Huntress, E. H.; Stanley, L. N.; Parker, A. S. The Oxidation of 3-Aminophthalhydrazide ("Luminol") as a Lecture Demonstration of Chemiluminescence. *J. Chem. Educ.* **1934**, *11* (3), 142.

(2) White, E. H.; Bursey, M. M. Chemiluminescence of Luminol and Related Hydrazides: The Light Emission Step. *J. Am. Chem. Soc.* **1964**, *86* (5), 941−942.

(3) Schneider, H. W. A New, Long-Lasting Luminol Chemiluminescent Cold Light. *J. Chem. Educ.* **1970**, *47* (7), 519.

(4) Wildes, P. D.; White, E. H. Differences Between Excited States Produced Chemically and Photochemically. Ion Pairs of Excited States Derived from Luminol. *J. Am. Chem. Soc.* **1973**, *95* (8), 2610−261717.

(5) Fuchsman, W. H.; Young, W. G. A Simplified Chemiluminescence Demonstration Using Luminol and Hypochlorite Bleach. *J. Chem. Educ.* **1976**, *53* (9), 548.

(6) Roswell, D. F.; White, E. H. The Chemiluminescence of Luminol and Related Hydrazides. *Methods Enzymol.* **1978**, *57*, 409.

(7) Bramwell, F. B.; Goodman, S.; Chandross, E. A.; Kaplan, M. A Chemiluminescence Demonstration—Oxalyl Chloride Oxidation. *J. Chem. Educ.* **1979**, *56* (2), 111.

(8) Chalmers, J. H.; Bradbury, M. W.; Fabricant, J. D. A Multicolored Luminol-Based Chemiluminescence Demonstration. *J. Chem. Educ.* **1987**, *64* (11), 969.

(9) Silverman, L. P.; Bunn, B. B. Keeping Chemistry 'Light'. *J. Chem. Educ.* **1993**, *70*, 405.

(10) Phillips, D. B. A. Chemiluminescent Sign. *J. Chem. Educ.* **1993**, *70* (9), 773.

(11) Schechinger, L.; Waldman, A. S. A Convenient Luminol Demonstration. *J. Chem. Educ.* **1995**, *72* (3), 243.

(12) Basso, A.; Chiorri, C.; Bracco, F.; Carnasciali, M. M.; Alloisio, M.; Grotti, M. Improving the Interest of High-School Students Toward Chemistry by Crime Scene Investigation. *Chem. Educ. Res. Pract.* **2018**, *19* (2), 558−566.

(13) Giussani, A.; Farahani, P.; Martínez-Muñoz, D.; Lundberg, M.; Lindh, R.; Roca-Sanjuán, D. Molecular Basis of the Chemiluminescence Mechanism of Luminol. *Chem. Eur. J.* **2019**, *25* (20), 5202−5213.

(14) Korn-Mueller, A. Chemiluminescent Fun with Washing Powder. *J. Chem. Educ.* **2024**, *101* (9), 4064−4069.

(15) Hill, R. Oxygen Evolved by Isolated Chloroplasts. *Nature* **1937**, *139* (3525), 881−882.

(16) Eghlimi, A.; Jubaer, H.; Surmiak, A.; Bach, U. Developing a Safe and Versatile Chemiluminescence Demonstration for Studying Reaction Kinetics. *J. Chem. Educ.* **2019**, *96* (3), 522−527.