Electronegativity, Bonding, and Bioluminescence
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**Purpose**

This lesson is meant as a supplement to a lesson on electronegativity and bonding. It is intended to provide students with an opportunity to analyze how the electronegativity of different atoms can change the properties of bonds and resultant compounds. Through the Bite, students will come to appreciate how their knowledge has applications in the field of cancer research.

**Audience**

This lesson was designed to be used in an introductory high school chemistry course.

**Lesson Objectives**

Upon completion of this lesson, students will be able to:

- compare the electronegativity values of different atoms.
- explain how nonpolar covalent, polar covalent, and ionic bonds can be modeled on a continuum characterized by electronegativity differences between the bonded atoms.
- describe how the electronegativity value of an atom can affect the properties of a bond and the resultant compounds.

**Key Words**

bioluminescence, covalent bond, electronegativity, ionic bond, nonpolar covalent bond, polar covalent bond

**Big Question**

This lesson addresses the Big Question “What does it mean to observe?”

**Standard Alignments**

- **Science and Engineering Practices**
  - SP2. Developing and using models
  - SP6. Constructing explanations and designing solutions

- **MA Science and Technology/Engineering Standards (2016)**

  HS-PS1-1. Use the periodic table as a model to predict the relative properties of main group elements, including ionization energy and relative sizes of atoms and ions, based on the patterns of electrons in the outermost energy level of each element. Use the patterns of valence electron configurations, core charge, and Coulomb’s law to explain and predict general trends in ionization energies, relative sizes of atoms and ions, and reactivity of pure elements.
NGSS Standards (2013)

HS-PS1-1. Use the periodic table as a model to predict the relative properties of elements based on the patterns of electrons in the outermost energy level of atoms.

Common Core Math/Language Arts Standards

CCSS.ELA-LITERACY.RST.9-10.1. Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.

CCSS.ELA-LITERACY.RST.11-12.1. Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.

Misconceptions Addressed

This lesson addresses many common misconceptions about bonding and bonding representations, including:

- Bonds are only represented by lines in models. (Question 4a)
- Covalent bonds only form when atoms have equal or covalent bonds only form when two atoms have very different electronegativities. (Question 4b)
- Only atoms with very different electronegativities share electrons unequally (Questions 4c, 4d, 4e)

Further information about student misconceptions on this topic can be found here.

Primary Sources

Bite “How Do Fireflies Help Scientists to Fight Cancer?” based on:


Chembite write-up


Misconceptions

Materials

- Copies of the Student Handout and Science Bite for each student
- Means to show the image found in the Chembite write-up in color

Time

This lesson should take approximately one 50-minute class period.

Student Prior Knowledge

Students should be familiar with electronegativity trends both across rows and down groups in the periodic table as well as the basic differences between ionic and covalent bonds.

Instructions and Teacher Tips

General Procedure

- Have students read through the worksheet and answer the questions as they go, reading the Science Bite when instructed.
- Check in after most students have finished the first three questions to make sure they have correctly identified the similarities and differences in the three molecules before they begin reading about the research on the three different molecules.
- After students have completed the lesson, consider bringing them together as a class or break into small groups to compare answers and to discuss any remaining questions.

Tips, Extensions, and Variations

- This lesson has two parts. The primary learning objective of the first part, comprised of the first four questions, is to demonstrate student understanding of electronegativity and bonding. The primary learning objective of the second part, in which students read the Bite “How Do Fireflies Help Scientists Fight Cancer?” and answer the last five questions, is focused on the application of knowledge to a real-world problem and on generating excitement about the relevance of basic chemistry knowledge to cutting-edge research.
- At the beginning of the lesson, you may wish to tell students that they don’t need to understand the complexities of line structures in order to complete this lesson. They only need to be able to notice similarities and differences between the structures. In Question 4, students are told that the rings are made up of carbon atoms, but you may find it helpful to explain or review that at the beginning.
- If you have covered the electromagnetic spectrum and visible light, project the figure of the three vials of D-luciferins from the Chembite (https://chembites.org/2012/03/05/shifting-with-selenium/) and ask your students which of the molecules gives off light with the longest wavelength (aminoseleno-D-luciferase; red has a longer wavelength than orange or green). A further connection to light spectra and wavelengths is made in Question 7.
Big Question Discussion

This lesson addresses the Big Question “What does it mean to observe?” Specifically, students should come to understand that observations can be limited, but that these limitations can be overcome with technology. If you choose to delve into the Big Question, consider following these suggested steps:

.movies

As the class comes in, have the figure from the Chembite (https://chembites.org/2012/03/05/shifting-with-selenium/) projected on a board, wall, or screen. Ask students,

“What do you observe about these vials?” Students may comment on their differing colors or that all three seem to contain “glowing” substances.

“How are your observations limited? What are some ways you could learn more about these substances if you had unlimited resources and tools?” Get students thinking about how their observations are limited to just one sense (sight) and by how they are observing a photograph, not the actual substances. Guide students toward thinking about the types of observations they could make using their other senses, analysis tools like microscopes, or by reacting the substances with other substances.

Once students complete the lesson, or if they are struggling with Questions 8 and 9, prompt the class or individuals with questions such as:

“Why do cancer researchers use bioluminescent molecules like D-luciferin?” Consider showing examples of stained cells, from simply dyed onion root tip cells to elaborately stained SEM and TEM images to help draw parallels.

“What are microscopes and telescopes used for?”

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As a wrap-up, have students share their answers to Question 9 and see if you can get student to appreciate how the researchers in this study overcame a limitation in their ability to make observations by inventing a new way to observe cancer cells.

Background Information and Research Details

Bioluminescent imaging (BLI) is a valuable tool for studying cancer because it enables researchers to visualize the activity of genes within the cancer cells as well as cell growth. Using BLI, researchers can monitor how cancer cells respond to drug therapies among other things. Unlike other visualization techniques, BLI can be used in vivo, in living cells.

D-luciferin is a substrate. Bioluminescence is achieved then it interacts with a luciferase enzyme.

Light absorption is the main limiting factor in in vivo BLI studies. Surrounding cells absorb bioluminescence wavelengths below 600 nm, which weakens their signals and therefore limits the potential for researchers to make observations.

To overcome the limitations of D-luciferin, scientists relied on random mutations to produce variants of D-luciferase that resulted in red-shifted emission spectra (a greater proportion of

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light emitted at longer wavelengths) or synthesized variants of D-luciferin in a lab. Prior to the research in the study upon which the Bite is based, however, the synthesized substrate variants weren’t taken up by the cancer cells in the same way as the original D-luciferin, which limited their usefulness. The aminoseleno-D-luciferin the researchers from Stanford synthesized not only resulted in bioluminescence with a longer wavelength, but it maintained the in vivo functions of D-luciferin.

Only 23% of emitted light by D-luciferin is above 600 nm compared with 41% for amino-D-luciferin and 55% for aminoseleno-D-luciferin.

Answers

1. Examine the molecules in Figure 1.
   a. What do all three molecules have in common?

   All of the molecules have the same basic structure: a six-membered ring connected to a five-membered ring, which is in turn bonded to a second five-membered ring. In addition, each has a sulfur atom in the same spot on the first five-membered ring, a —COOH (carboxyl) group in the same spot on the bonded five-membered ring, and nitrogens in the same spots in both five-membered rings.

   b. What is different about the structures for D-luciferin and amino-D-luciferin?

   D-luciferin has an —OH (hydroxy) group on the six-membered ring and amino-D-luciferin has an —NH2 (amino) group in the same position.

   c. What is different about the structures for amino-D-luciferin and aminoseleno-D-luciferin?

   On the bonded five-member ring, amino-D-luciferin has a sulfur atom (S) where aminoseleno-D-luciferin has a selenium atom (Se).

2. Which atom has a higher electronegativity value, sulfur or selenium? Explain how you know.

   In your answer, describe how and explain why electronegativity changes from top to bottom in a group in the periodic table.

   Sulfur has a higher electronegativity than selenium. We know this because both selenium and sulfur are in the same column (group) on the periodic table (6A), but sulfur is the row higher (row 3) than selenium (row 4). This means that sulfur has fewer electrons overall, and the valence electrons are located closer to the nucleus. This in turn means that sulfur has less electron shielding around its nucleus, so it is better able to pull electrons when part of a compound than nitrogen.

3. Which atom has a higher electronegativity value, oxygen or nitrogen? Explain how you know.

   In your answer, describe how and explain why electronegativity changes from left to right across a row in the periodic table.

   Oxygen has a higher electronegativity value than nitrogen. We know this because both oxygen and nitrogen are on the same row in the periodic table (row 2), but oxygen is further to the right (group 6A) than nitrogen (group 5A). This means that oxygen has one more proton in its nucleus than nitrogen has, which in turn means that oxygen atoms have a stronger pull on electrons when part of a compound than nitrogen has.
Each unlabeled point in the six- and five-sided structures in Figure 1 represent a carbon atom. Part of the structures of the D-luciferin and aminoseleno-D-luciferin are modeled as Lewis dot structures below.

![Lewis dot structures of D-luciferin and aminoseleno-D-luciferin](image)

**Figure 2. Two Luciferin Molecules.** D-luciferin and aminoseleno-D-luciferin. Dashed circles represent the part of the molecules shown as Lewis dot structures.

4. Study Figure 2 and answer the following questions.
   a. Circle the electrons that form the covalent bond between oxygen and carbon in D-luciferin and nitrogen and carbon in aminoseleno-D-luciferin.

   ![Circled electrons](image)

   b. Electrons are shared in covalent bonds. Compare how the electrons are shared in the nonpolar covalent bond between two carbon atoms (C–C) to how they are shared in the polar covalent bonds you circled in part a. Are they shared equally or unequally? Explain your answer. *Hint: Think about what periodic trend you could use to help you.*

   Electrons are shared equally in a nonpolar carbon-carbon bond and unequally in polar carbon-nitrogen and carbon-oxygen bonds. This is because two carbon atoms have the same electronegativity, while a carbon and an oxygen or a carbon and a nitrogen have different electronegativities.
c. Compare how the electrons are shared in the polar covalent bond between carbon and oxygen (C–O) in D-luciferin to how they are shared in the polar covalent bond between carbon and nitrogen (C–N) in aminoseleno-D-luciferin. Are they shared equally or unequally? Explain your answer. 

*Hint: Think about what periodic trend you could use to help you.*

Electrons are shared more unequally in a polar carbon-oxygen bond (pulled toward the oxygen) than in a polar carbon-nitrogen bond because oxygen has a higher electronegativity than nitrogen does.

d. It’s helpful to think of bonds on a continuum based on how equally electrons are shared. In a continuum, a property, such as how equally electrons are shared, changes gradually from one extreme to another without clear divisions.

Place the following bonds and labels on the continuum below:

**Bonds:** C–C, C–O, C–N, Na–Cl

**Labels:** ionic bonds, nonpolar covalent bonds, polar covalent bonds

<table>
<thead>
<tr>
<th>Non-Polar Covalent Bonds</th>
<th>Polar Covalent Bonds</th>
<th>Ionic Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>C–C</td>
<td>C–N</td>
<td>Na–Cl</td>
</tr>
</tbody>
</table>

- Electrons shared most equally
- Electrons shared most unequally
- Electrons transferred


e. Does the difference in electronegativity between the two bonded atoms increase or decrease as you move from “electrons shared equally” to “electrons transferred”?

Explain why that makes sense.

It increases; electrons in a nonpolar bond between two identical atoms, such as C–C, would be shared equally as the atoms have equal electronegativity. Polar bonds, on the other hand, form between two atoms with different electronegativities which causes the unequal sharing of electrons. In ionic bonds, the electronegativities are so different that the electrons are completely transferred from one atom to the other.

5. Citing details from the Bite, describe what researchers did to make aminoseleno-D-luciferin and explain why it is a more useful molecule to cancer researchers than D-luciferin is.

The researchers swapped out a couple of atoms in D-luciferin to make aminoseleno-D-luciferin: an —NH₂ (amino) group has been swapped in for the —OH (hydroxy) group, and a selenium atom has been swapped in for a sulfur atom. When they made the swaps, the bioluminescent properties of the substance changed, and less light gets absorbed by surrounding cells. From the Bite: “The changes made to the molecular structures changed the color of the light that is emitted by the molecules. This, in turn, changed how the light is absorbed by surrounding cells in the cancer studies.”
Information about the bioluminescence of D-luciferin, amino-D-luciferin, and aminoseleno-D-luciferin are shown in Figure 3 below.

6. In cancer studies, do surrounding cells mostly absorb wavelengths greater than or less than 600 nm? Explain your answer citing specific information from the Bite and the diagram.

Surrounding cells absorb light with wavelengths shorter than 600 nm. From the Bite, we know that surrounding cells absorb more light emitted from D-luciferin than from aminoseleno-D-luciferin. Looking at the absorption spectra, D-luciferin has has a greater proportion of emission wavelengths shorter than 600 nm than aminoseleno-D-luciferin does.

7. How did decreasing the electronegativity of atoms in D-luciferin affect the properties of the molecule? Discuss both amino-D-luciferin and aminoseleno-D-luciferin in your response.

As scientists decreased the electronegativity of the atoms in D-luciferin, the wavelength of light the molecule produced increased. You can see this in figure one as the peak moves to the right as the electronegativity of the atoms in the molecules decrease. The lowest wavelength of light is produced by D-luciferin, the middle is produced by amino-D-luciferin, and the highest wavelength is produced by aminoseleno-D-luciferin. As the electronegativity of the atoms decreases, the light gets more and more red.

8. Why did the scientists conduct the research described in the Bite? Describe the hypothesis they were testing or the problem they were attempting to solve.

The scientists were trying to improve the usefulness of D-luciferin in cancer studies. The bioluminescence of the molecule helps researchers to visualize cancer cells under a microscope, but its usefulness was limited because the emitted light was being absorbed by surrounding cells.

9. Connect to the Big Question. In the last paragraph of the Bite, the author compares aminoseleno-D-luciferin to a telescope. Thinking about the importance of observation in science, what do you think the author meant by that comparison? How can new observation tools help scientists today like the invention of microscopes and telescopes have helped further scientific research?

The author meant that the research resulted in a tool that can overcome limitations to observation. The microscope and telescope are both tools that once invented, enabled scientists to make new observations that were impossible before. Similarly, the synthesis of aminoseleno-D-luciferin may make it possible for cancer researchers to make observations that were not possible before. By improving researcher’s ability to visualize cancer cells, new observations are possible which may in turn lead to breakthroughs. Similarly, the microscope and telescope enabled scientists to make observations that weren’t before possible.

_v2: revised April 2, 2018; _v3: formatting changes June 22, 2018; _v4: fixed graphic in answer to Q.4d

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