Proteins Under Pressure
STEM Learning Module

Module Theme | Science
Pacing | 1 Block class period (~90 minutes)

Background Needed | Protein Synthesis and molecular bonds, basic Boyle's Law
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Materials/Resources

- Organism Cards (10 copies)
- Student worksheet
- Regular marshmallows (optional for demo)
- Large Syringes (optional for demo)

Objectives & Learning Outcomes

- Students will be able to describe how pressure changes with ocean depth.
- Students will be able to explain how increasing pressure affects gas volume (Boyle's Law)
- Students will be able to explain how high pressure can disrupt protein structure.
- Students will be able to analyze organism data to identify the relationship between ocean depths and TMAO concentrations.
- Construct a model that explains how TMAO helps deep-sea organisms survive extreme pressure.

Activity Summary

In this activity, students will model the impact of pressure on gas molecules to understand the importance of Trimethylamine N-oxide (TMAO) as a protein stabilizer in deep-sea organisms.

Introduction

Nearly half of Earth's ocean exists in a realm humans will never naturally visit. Approximately 49% of the global ocean lies within the Abyssal Plain, between 4,000 and 6,000 meters below the surface. Just above it sits the Midnight Zone (Bathypelagic Zone), spanning 1,000–4,000 meters and encompassing another 36.3% of the ocean. Together, these two zones make up the vast majority of the ocean's habitable space. And yet — these environments are defined by extreme pressure.
For every 10 meters (33 feet) of depth, ocean pressure increases by 14.5 pounds per square inch (psi) — roughly one additional atmosphere of pressure. By the time you reach the depths where ROV *Hercules* routinely dives during expeditions aboard E/V *Nautilus*, organisms are experiencing pressures 100 to 600 times greater than the atmospheric pressure at sea level.

At the deepest end of that range, the force pressing down on an organism is equivalent to the weight of up to 15 jumbo jet aircraft. Under those conditions, materials we are familiar with — plastic foam, air-filled objects, and even rigid containers — compress dramatically. Proteins, the molecular machines that allow cells to function, are especially vulnerable. High pressure can force proteins to unfold, a process known as denaturation, causing them to lose their shape and function.

Yet life thrives there. Fish swim. Crustaceans crawl. Microbes metabolize. Entire ecosystems persist in darkness under crushing force.

So what makes it possible for organisms to survive — and function — at these depths?

Lesson Overview

One key molecule that helps make life under this pressure possible is Trimethylamine N-oxide (TMAO). TMAO is found in organisms ranging from bacteria to humans, but it is especially important in animals that experience extreme environmental stress, like deep-sea fish and migrating salmon. TMAO acts as a chemical stabilizer inside cells. It helps:

- Protect proteins from unfolding under high pressure
- Maintain proper protein function across temperature changes
- Reduce the toxic effects of ammonia
- Support fluid balance when salt concentrations change

Proteins are delicate, three-dimensional structures. If they lose their shape — a process called denaturation — they stop working. High pressure can force proteins to unfold, just like heat can. TMAO helps prevent that from happening.
Salmon provide an incredible example of TMAO in action. Because salmon are anadromous (migrating from freshwater to saltwater and back again), they experience major physiological stress.

Moving from rivers to the ocean means:

- Increased salt concentration
- Osmotic dehydration
- Pressure changes as they move vertically in the marine water column

Research shows that salmon adjust their TMAO levels during these transitions. The molecule helps:

- Protect cellular proteins
- Maintain fluid balance
- Stabilize tissues under changing environmental pressure.

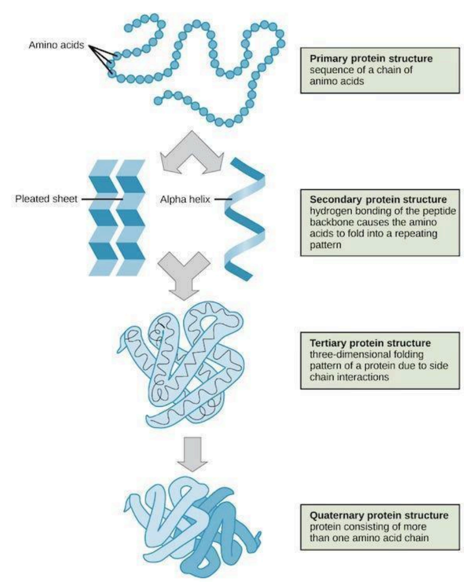
So whether it's a salmon transitioning to the ocean or a deep-sea fish living 3,000 meters down, TMAO plays a powerful protective role.

Scientists aboard E/V *Nautilus* collect data on the location, depth, and pressure of organisms observed during ROV dives. They've also demonstrated what pressure does to materials that don't have molecular stabilizers like TMAO.

When [Styrofoam cups are attached to ROV *Hercules*] and sent to the seafloor, they return dramatically compressed — crushed by pressure.

In this lesson, students are going to model the impact of pressure on objects as well as compare the concentration of the TMAO compound in order to synthesize their own ideas about the significance of the TMAO compound.

- What happens to a material that doesn't have a protective molecule?
- And what does that tell us about how important TMAO is for life in the deep ocean?



Remember what you learned about proteins in biology; their structure and shape are what determine their ability to function and the type of job they do for the cell. We know that proteins fold based on the molecular interactions between the amino acids of the polypeptide chain. We learned that when a mutation in the mRNA sequence occurs, there can be a change of amino acid, influencing the final folding of the protein.
We also know that proteins are very sensitive to cellular stressors; higher temperatures can influence protein folding, changing their structure and ultimately their function. But what would happen to a protein under pressure?

Driving Question: How do organisms survive crushing pressure in the deep ocean?

Links to Next Generation Science Standards

- **MS-LS1-2: Structure Function:** Develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function.
- **MS-LS2-1: Interactions in Ecosystems:** Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.

- **HS-LS1-1: Structure and Function:** Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.
- **HS-LS1-3: Feedback Mechanisms:** Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.

Lesson Procedure

1. **Boyle's Law Demo** (5-10 minutes)**.** Either have students do this themselves or watch the video that is in the slides.
    a. Hand each pair of two students a marshmallow and a plastic syringe, as well as the student document. Tell them to place the marshmallow into the syringe and then follow the instructions on their document.
    a. Place the marshmallow into the syringe and put the plunger back.
    b. Place your thumb over the end of the syringe where the needle is usually located. Holding your thumb in place, pull the plunger out. Observe what happens to the marshmallow as you do so.
    c. With your thumb still in place, push the plunger in and observe what happens.
    d. Draw what you observe in the boxes on your worksheet.

2. **Review Protein Synthesis** (10 minutes)
    a. **Ask:** What determines the shape of a protein?
        Guide toward
        - Amino acid sequence
        - Folding
        - Weak bonds help hold the structure together
    b. Remind the class about how the shape of the hemoglobin protein allows oxygen to bind. This allows for the function of carrying oxygen within the red blood cells.

3. **Show the hemoglobin slide and review that the structure of a protein determines its function.**
    a. The structure of the hemoglobin protein allows oxygen molecules to bind.
    b. Then ask: What holds that shape together?

Show them the protein synthesis image and point out that the amino acid chain (primary structure) results in a folded structure (tertiary)

Explain: Proteins are held together by **weak molecular bonds**, including:

- Hydrogen bonds
- Hydrophobic interactions
- Ionic interactions

Key idea: These are NOT strong covalent bonds. They are delicate molecular attractions.

Tell Students: “Proteins are stable — but only under the right conditions.”

4. **Connection to Pressure** (10 minutes)

Transition back to the marshmallow and ask students to answer, “What did pressure do to the marshmallow?”

    a. The pressure compressed the marshmallow.
    This shows that pressure pushes molecules closer together.
    b. Have students turn to their partner to discuss: If we imagine the marshmallows as a protein inside a cell, what would happen if the cell were put under extreme pressure?

    c. **Tell Class:** So if high pressure destabilizes proteins… How can deep-sea organisms survive?
        i. Pause and give students time to think.
        ii. Show the short [OET video on deep-sea pressure]

5. **TMAO, the mystery compound -** There is one important compound that helps them do this.
    a. Today, we are going to try to use some clues to think about why this compound is so important for deep-sea ocean creatures.
    b. **Show the picture of** Trimethylamine N-oxide (TMAO)
        i. Have students discuss with their neighbor: "What do you notice about this compound?"
            - You can scaffold by asking students to look for repeating aspects of the molecule.
        ii. Remind the class that we name compounds based on their components.
            - What do we see in this compound?
            - Three CH3's (methyl groups)
            - A positive Nitrogen
            - And a negative Oxygen
        iii. Show the name of the molecule and share that the name is not as important as the structure.
        iv. Before we talk more about the structure of our mystery molecule, let's take a look at where we find it in the ocean.
        v. We are going to try to use data about the concentrations of TMAO in deep-sea organisms to try to understand what this compound might be helping with.

6. **Organism Sorting (**15 minutes)
    a. **Tell students:** We are going to look at different organisms within the Mariana Trench Each creature will have:
        i. A picture
        ii. They live in the ocean
        iii. Pressure of the water at that depth
        iv. Concentrations of TMAO in their bodies
    b. **Tell students:** The goal is to determine the relationship between ocean depth and TMAO for different organisms to try to understand the importance of TMAO.
    c. Give them 5-10 minutes to work in pairs, sorting the organisms, recording their data, and making a graph.
    d. Discuss as a class what they noticed about the relationship between ocean depth and TMAO concentrations in organisms. Make sure to connect to pressure and ask them to describe the relationship between pressure and depth as well.

7. **Transition to TMAO** (15 Minutes)
    a. **Tell students, "**Deep-sea organisms don't ignore physics. They adapt chemically."
    b. Show the next picture of the compound and **ask students** to describe the structure of the Trimethylamine N-oxide compound. They will draw and describe it in their worksheet under #5.
    c. Ask the class, where else do we see pyramids and tetrahedrons?
        i. Show the slide with the Hawthorn Bride and the Oregon Convention Center.
    d. Why might the structure of this compound be important? (Remind them what we know about what structure equals)
    e. TMAO stabilizes proteins under pressure.

8. **Share an analogy**
    a. Protein = folded origami structure
       Weak bonds = tiny pieces of tape
       Pressure = squeezing the paper
    b. Without reinforcement → structure crumples
       With TMAO → extra stabilization keeps shape intact

9. Ask the class to **discuss with their partner** what would happen to proteins and cells if deep-sea organisms didn't have Trimethylamine N-oxide to stabilize them?
    c. Show the class the OET styrofoam cup video.

Extensions & Adaptations

You can expand this lesson by delving deeper into the effects of water molecules under pressure on cells and molecules. For AP classes, have students read this article and discuss the chemical properties that TMAO provides to hydrogen bonds within water molecules. For younger students or to extend this lesson over multiple days, you can have students build a three-dimensional model of Trimethylamine N-oxide using mini marshmallows and toothpicks.

References

Emily Harwitz, special to C&EN. “How a Chemical Protects Fish from the Extreme Pressures of the Deep.” Chemical & Engineering News, American Chemical Society, 25 Oct. 2022, cen.acs.org/physical-chemistry/modeling/chemical-protects-fish-extreme-pressures/100/web/2022/10.

Liu, Qi, et al. “Trimethylamine N-oxide (TMAO) and trimethylamine (TMA) determinations of two Hadal Amphipods.” Journal of Marine Science and Engineering, vol. 10, no. 4, 23 Mar. 2022, p. 454, https://doi.org/10.3390/jmse10040454.

Samerotte, Athena L., et al. “Correlation of trimethylamine oxide and habitat depth within and among species of teleost fish: An analysis of causation.” Physiological and Biochemical Zoology, vol. 80, no. 2, Mar. 2007, pp. 197–208, https://doi.org/10.1086/510566.

Webb, Thomas J., et al. “Biodiversity’s big wet secret: The global distribution of marine biological records reveals chronic under-exploration of the deep pelagic ocean.” PLoS ONE, vol. 5, no. 8, 2 Aug. 2010, https://doi.org/10.1371/journal.pone.0010223.

Marianne Nergaringrad, Veterinary. “The Potential Role of TMAO in Mitigating Fish Health Challenges during Winter.” Main, The QRILL Company AS, 12 Feb. 2025

Student Worksheet **Name: ________________________**

When the syringe is pulled out:	When the syringe is pushed in:

1. What's inside the marshmallow?

2. What is pressure doing to the gas molecules?

3. Organize the deep-sea creatures from shallowest to deepest, then record the information in the table below.

Depth (m)	Pressure (atm)	TMAO (mmol/kg)	Organism

4. Graph the relationship between depth and TMAO for each organism, then explain the relationship seen in the graph in the space below.

5. Draw a trimethylamine N-oxide molecule (TMAO) below and describe its shape.

Pacific Rock Fish	Pacific Rock Fish
 Photo: Ocean Exploration Trust	Depth: 0–200 m Pressure: ~1–20 atm TMAO: 20 mmol/kg Ocean Zone: Sunlight (Epipelagic)
Sablefish  Photo: Ocean Exploration Trust	**Sablefish** Depth: 300–900 m Pressure: ~30–90 atm TMAO: 75 mmol/kg Ocean Zone: Twilight Zone (Mesopelagic)
Rattail Fish (Grenadier)  Photo: Ocean Exploration Trust	**Rattail Fish (Grenadier)** Depth: 1,000–3,000 m Pressure: ~100–300 atm TMAO: 150 mmol/kg Ocean Zone: Midnight Zone (Bathypelagic)

Abyssal Snailfish  Photo: Sleek Snailfish Monterey Bay Aquarium	**Abyssal Snailfish** Depth: 3,000–6,000 m Pressure: 300–600 atm TMAO: ~200 mmol/kg Ocean Zone: Abyssal Plain
Mariana Snailfish  Photo: SOI/HADES/University of Aberdeen (Dr. Alan Jamieson)	**Mariana Snailfish** Depth: 6,000–8,000 m Pressure: ~600–800 atm TMAO: 250+ mmol/kg Ocean Zone: Hadal Zone