

Scientific Inquiry Mini-Lesson Toolkit: Protein Stability and Temperature

Part 1: Learner Activity

Background

Proteins perform many essential jobs in living organisms, but they must maintain a particular three-dimensional shape to function properly. Changes in environmental conditions can disrupt the interactions that help maintain this shape. When a protein loses its normal structure, the process is called denaturation (OpenStax, 2018). Temperature can cause denaturation, but it does not always happen at the same temperature for every protein.

Seelig and Seelig (2023) investigated protein unfolding using differential scanning calorimetry (DSC), a technique that measures how a sample's heat capacity changes with temperature. Their research examines both heat denaturation and cold denaturation. This challenges the assumption that proteins only unfold when they become too hot.

Observe or Read: Imagine you are a scientist studying how temperature influences protein stability. You have been given findings from published research. Your task is to identify patterns, develop an evidence-based explanation, and consider what the data cannot tell you.

Inquiry Question: How does temperature affect protein stability, and what evidence shows that a protein has unfolded?

Make a Prediction: Before examining the data, predict how increasing or decreasing temperature might affect a protein's structure. Do you think all proteins will respond in the same way? Explain your reasoning in two or three sentences.

Examine the evidence: Review the simplified findings below, adapted from Seelig and Seelig (2023).

Protein and experimental condition	Approximate unfolding midpoint
Lysozyme, heat denaturation	62°C
β -lactoglobulin, heat denaturation	58°C
β -lactoglobulin, cold denaturation in a urea-containing solution	6°C

Note: These are approximate reported unfolding midpoints, not a complete experimental dataset. The cold-denaturation result was obtained under a specific condition involving urea, which affects protein stability. These values should not be treated as universal limits for all proteins.

Explain: Answer the following questions using the data and background information.

- ❖ Which protein has the higher reported heat-unfolding midpoint: lysozyme or β -lactoglobulin? What is the temperature difference?
- ❖ What does the β -lactoglobulin result suggest about the relationship between temperature and protein stability?
- ❖ Why is it important to consider the presence of urea when interpreting the cold-denaturation result?
- ❖ What additional measurements would help you determine whether a protein had unfolded? Explain why measuring temperature alone would not be enough.
- ❖ What can you conclude from these findings, and what questions remain unanswered?

Draw a Conclusion to Reflect or Extend: Write a short paragraph answering the inquiry question. Use at least two specific data values as evidence, explain what the evidence suggests, and identify one limitation of your conclusion.

Part 2: Instructor Guide

Purpose of the Activity: By the end of this activity, learners should be able to explain protein denaturation, interpret published scientific findings, distinguish evidence from assumptions, and construct a conclusion supported by data.

Recommended Audience: Introductory biology or biochemistry students.

Estimated Time: 15–20 minutes

Facilitation and Expected Responses: Begin by asking students what they already know about proteins and why their shapes matter. Invite them to make predictions before presenting the data, so they have an opportunity to compare their original ideas with the evidence. Allow them to review the background information listed under the learner activity for additional information.

During the analysis, students should recognize that lysozyme's reported heat-unfolding midpoint is approximately 4°C higher than β -lactoglobulin's. They should also identify that β -lactoglobulin can undergo unfolding under both high- and low-temperature conditions. The cold-denaturation finding is specific to a urea-containing solution; students should not assume that the same midpoint applies to the protein in every environment.

For question four, students may suggest monitoring structural changes with a suitable spectroscopic technique or examining heat-capacity changes using DSC. Explain that DSC measures heat capacity as a function of temperature and can provide evidence about unfolding transitions. Temperature readings by themselves do not demonstrate that a protein's structure has changed.

Encourage students to distinguish a result from a general rule. The data support the idea that protein stability depends on both temperature and experimental conditions. They do not establish a single temperature at which all proteins unfold.

Safety and Accessibility: This is a paper-based data-analysis activity and does not require heating samples, handling chemicals, or operating laboratory equipment. Provide the data in a screen-reader-compatible table or as a downloadable text document. Read the instructions aloud when helpful, allow written or oral responses, define scientific vocabulary, and give learners time to make predictions before discussion. Students can work independently, in pairs, or in small groups.

Connection to Sustainable Development Goal 4: This activity supports United Nations Sustainable Development Goal 4 (Quality Education) by encouraging evidence-based reasoning and making scientific research accessible to introductory learners. Its use of published findings, plain-language explanations, and flexible response options supports inclusive participation and meaningful science learning (United Nations, n.d.).

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References

OpenStax. (2018). Biology 2e (Section 3.4, Proteins). Rice University.

<https://openstax.org/books/biology-2e/pages/3-4-proteins>

Seelig, J., & Seelig, A. (2023). Protein stability—Analysis of heat and cold denaturation without and with unfolding models. *The Journal of Physical Chemistry B*, 127(15), 3352–3363.

<https://doi.org/10.1021/acs.jpcb.3c00882>

United Nations. (n.d.). Goal 4: Quality education.

<https://www.un.org/sustainabledevelopment/education/>