Teacher Resource for:

Ligand binding to the FeMo-cofactor: Structures of CO-bound and reactivated nitrogenase

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GENERAL USE OF *Science* in the Classroom

Student Learning Goals:

"One fundamental goal for K-12 science education is a scientifically literate person who can understand the nature of scientific knowledge."[1]

The U.S. National Academy of Sciences defines science as: "Any new finding requires independent testing before it is accepted as scientific knowledge; a scientist is therefore required to honestly and openly report results so that they can readily be repeated, challenged, and built upon by other scientists. Proceeding in this way over centuries, the community effort that we call science has developed an increasingly accurate understanding of how the world works. To do so, it has had to reject all dogmatic claims based on authority, insisting instead that there be reproducible evidence for any scientific claim."

An important student learning goal, central to any understanding of "the nature of scientific knowledge," is to give each student an appreciation of how science is done.

This includes knowing why:

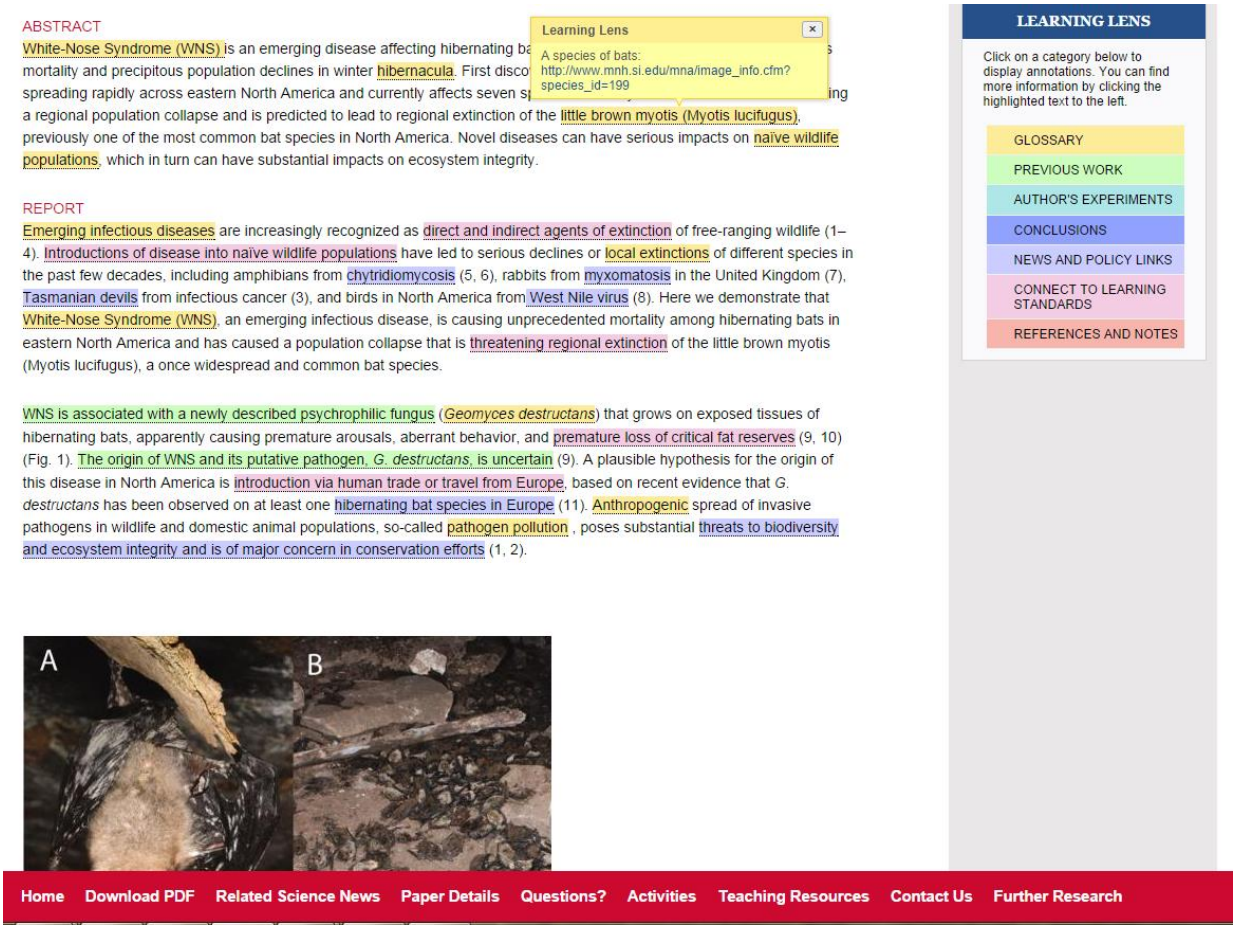
- Scientists must be independent thinkers, who are free to dissent from what the majority believes.
- Science can deal only with issues for which testable evidence can be obtained.
- All scientific understandings are built on previous work
- It is to be expected that one scientist's conclusions will sometimes contradict the conclusions of other scientists.
- Science is a never-ending venture, as the results from one study always lead to more questions to investigate.

[1] *A Framework for K-12 Science Education*, National Research Council, 2012

Using This Resource

Learning Lens:

The Learning Lens tool can be found on the right sidebar of each resource and is the source of annotations. Click on the headings to highlight portions of the text of the corresponding research article. A subsequent click on the highlighted text will produce a text box containing more information about that particular piece of text. Below is an example of the Glossary function of the Learning Lens.



An example of the resource with the Glossary, Previous Work, Author's Experiments, News and Policy Links, and References and Notes tools turned on. The Glossary tool is in use.

Learning Notes:

Learning Notes accompany each figure and are designed to help students deconstruct the methods and data analysis contained within each figure.

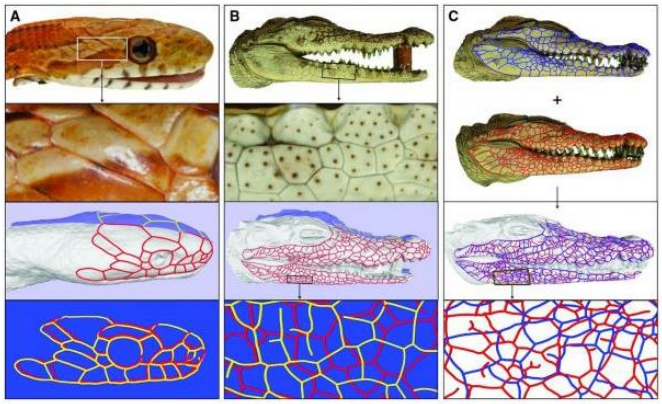


Fig. 1. Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). (B) Polygonal head scales in crocodiles have a largely random spatial distribution without symmetrical correspondence between left and right. (C) Head scales from different individuals have different distributions of scales' sizes and localizations (blue and red edges from top and bottom crocodiles, respectively).

Method: 3D geometry and color-texture reconstruction | Panel A | Panel B | Panel C

The authors took 120 color pictures of each animal to create detailed, three-dimensional models of reptile heads. Watch this video in which the authors further explain their modeling methods:

http://www.sciencemag.org/content/suppl/2012/11/29/science.1226265.DC1/1...

LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

- GLOSSARY
- PREVIOUS WORK
- AUTHOR'S EXPERIMENTS
- CONCLUSIONS
- NEWS AND POLICY LINKS
- CONNECT TO LEARNING STANDARDS
- REFERENCES AND NOTES

References:

The Reference section of each resource is annotated with a short statement about how or why each reference relates to the current research study.

6. L. Lewejohann, B. Zipser, N. Sachser, "Personality" in laboratory mice used for biomedical research: A way of understanding variability? Dev. Psychobiol. 53, 624 (2011).
7. G. Kempermann, The neurogenic reserve hypothesis: What is adult hippocampal neurogenesis good for? Trends Neurosci. 31, 163 (2008).
8. Lewejohann et al., Behavioral phenotyping of a murine model of Alzheimer's disease in a seminaturalistic environment using RFID tracking. Behav. Res. Methods 41, 850 (2009).
9. B. Steineret al., Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46, 41 (2004).
10. Garthe, J. Behr, G. Kempermann, Ad… flexible use of spatially precise learning strategies. PLoS ONE 4, e54…
11. G. Kempermann, New neurons for 'su… 27 (2012).
12. G. Kempermannet al., Why and how … brain plasticity. Front Neurosci 4, 189 (2010).
13. K. Fabelet al., Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. Front. Neurosci. 3, 50 (2009).
14. Amrein, H. P. Lipp, Adult hippocampal neurogenesis of mammals: Evolution and life history. Biol. Lett. 5, 141 (2009).
15. G. Kempermann, Why new neurons? Possible functions for adult hippocampal neurogenesis. J. Neurosci. 22, 635 (2002).
16. C. Crabbe, D. Wahlsten, B. C. Dudek, Genetics of mouse behavior: Interactions with laboratory environment. Science 284, 1670 (1999).
17. Lewejohann et al., Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. Genes Brain Behav. 5, 64 (2006).
18. D. W. Bailey, How pure are inbred strains of mice? Immunol. Today 3, 210 (1982).
19. R. Lathe, The individuality of mice. Genes Brain Behav. 3, 317 (2004).
20. C. Julieret al., Minisatellite linkage maps in the mouse by cross-hybridization with human probes containing tandem repeats. Proc. Natl. Acad. Sci. U.S.A. 87, 4585 (1990).
21. R. P. Talenset al., Epigenetic variation during the adult lifespan: Cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11, 694 (2012).
22. P. B. Baltes, J. R. Nesselroade, S. W. Cornelius, Multivariate antecedents of structural change in development: A simulation of cumulative environmental patterns. Multivariate Behav. Res. 13, 127 (1978).
23. M.E. Raijmakers, P. C. Molenaar, Modeling developmental transitions in adaptive resonance theory. Dev. Sci. 7, 149 (2004).
24. K. Friston, M. Breakspear, G. Deco, Perception and self-organized instability. Front. Comput. Neurosci. 6, 44 (2012).
25. Van de Weerd et al., Effects of environmental enrichment for mice: Variation in experimental results. J. Appl. Anim. Welf. Sci. 5, 87 (2002).
26. D. P. Wolferet al., Laboratory animal welfare: Cage enrichment and mouse behaviour. Nature 432, 821 (2004).
27. K. Lewin, Dynamic Theory of Personality (McGraw-Hill, New York, 1935).
28. K. L. Jang, R. R. McCrae, A. Angleitner, R. Riemann, W. J. Livesley, Heritability of facet-level traits in a cross-cultural twin

Learning Lens

This paper showed that while both physical activity, like running, and living in an enriched environment can result in the generation of new hippocampal neurons in mice, a combination of the two activities leads to even greater rates of neurogenesis.

LEARNING LENS

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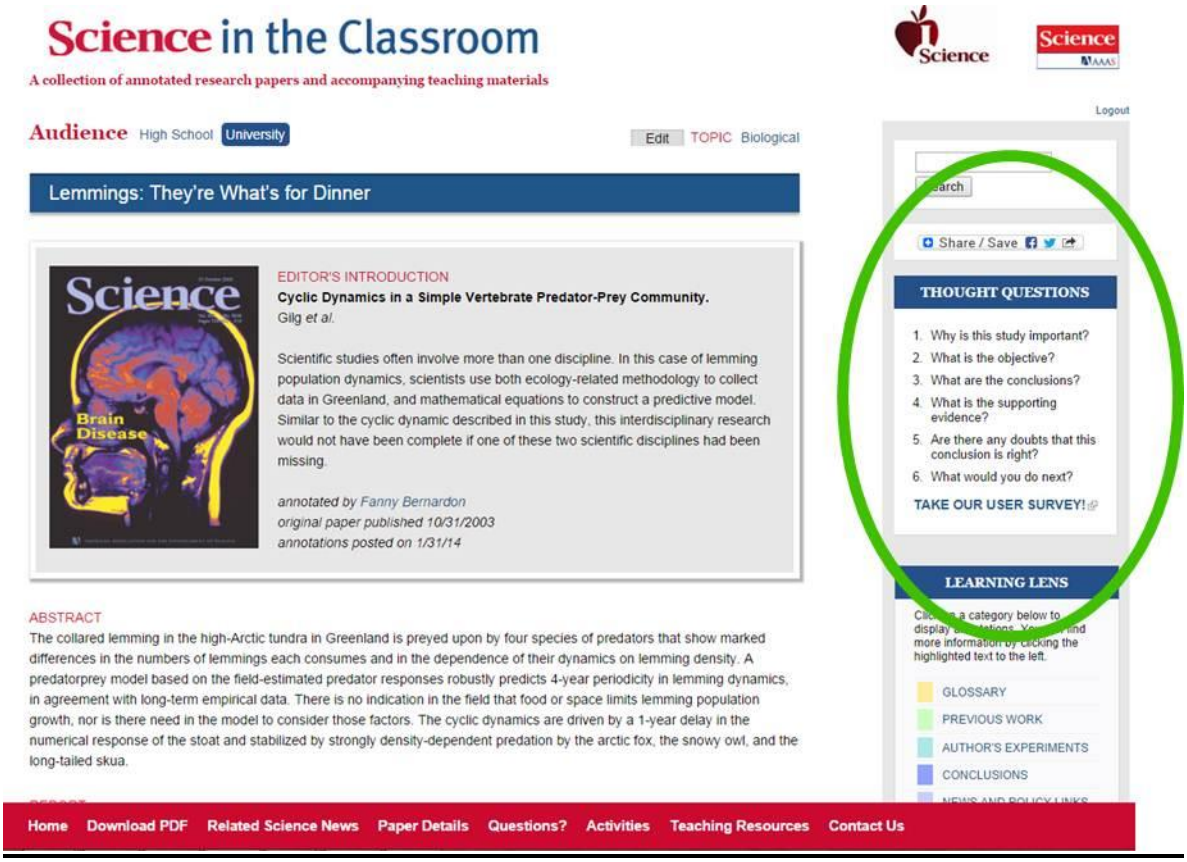
Thought Questions

Thought Questions are located above the Learning Lens in the right sidebar of each resource. These questions were written to be universal and applicable to any primary research paper. Thought questions do not have a single answer, or a correct answer for that matter, and can be used to stimulate discussion among students.



Suggestions for Classroom Use:

In addition to the thought questions discussed above, other resources are provided for use in the classroom. These can be found toward the end of the teacher guides associated with each specific article and include:

1. Discussion questions specific to the article, related to the standards, and/or associated with the figures.

2. Activities tied to the articles.

Some ways to use the *Science* in the Classroom articles:

1. Assign to student groups to read and discuss during class.

2. Assign small sections of the article to student groups to read and discuss during class, with the expectation that they will present or use jigsaw to teach the entire class what is in their part of the article.

3. Assign to individual students to complete during class or as homework.

4. Assign reading as an extra credit project.

Some ideas for interactive student engagement after reading the article:

1. Students write answers to discussion questions (for example, those linked to the standards or those linked to the diagrams).

2. Go over the abstract, as well as information about the purpose and structure of an abstract, and have students write their own abstracts for the articles in language that could be understood by their peers.

3. Have students edit the article, or parts of the article, to a simpler reading level.

4. Have students, alone or in small groups, use the annotated list of references to explain how the scientists who wrote this article built on the published work of at least one independent group of scientists in making their discoveries. In the process, did they produce data that supports the findings of the earlier publication that they have cited in the text? In what way does this article support the statement that scientific knowledge is built up as a “community effort”?

5. Use the article and discussion questions linked to the standards and the diagrams for a teacher-led classroom discussion. The discussion can focus on the nature of science and scientific research, as well as on the science in the article itself.

6. Have students give a classroom presentation about the article, parts of the article, or their answers to discussion questions.

ARTICLE-SPECIFIC MATERIALS

Connections to the nature of science from the article

- Why do chemists want to investigate dinitrogen reduction by nitrogenase?
- What does the crystal structure of CO-inhibited nitrogenase contribute to this goal?

The importance of this scientific research

- Deeper understanding of the nitrogenase enzyme's reactivity with varied inhibitors and substrates
- More refined model of the structure of nitrogenase during inhibition, comparable to during substrate reduction

The actual science involved

- Protein crystallography
- Enzyme activity and inhibition

Connect to Learning Standards:

Next Generation Science Standards

- Science and Engineering Practice 1: Asking questions (for science) and defining problems (for engineering).

  The authors make sense of two possible problems for the experiment: the necessary constant presence of CO and the requirement of crystallization during enzyme turnover. In order to study the binding of the inhibitor to the active site, the experimental setup had to be able to yield crystals for observation while the MoFe-cofactor was engaged in inhibition with CO, not in the unbound resting state.

  This is challenging because crystals are typically grown from stationary, long-lived complexes. Procedures are further complicated by the continued need of CO and other chemicals for turnover conditions.

  Ultimately, rapid crystallization protocols were used under CO to obtain the crystals for analysis, thus overcoming the identified obstacles.

- Science and Engineering Practice 2: Developing and using models and Practice 5: Using mathematics and computational thinking. This surface representation in Figure 3 is a mathematical model of the binding cavity for the potential sulfur binding site (SBS) based on and incorporating the electron density and surface charge data acquired from diffraction of the CO-inhibited nitrogenase crystals.

- Science and Engineering Practice 6: Constructing explanations (for science) and designing solutions (for engineering). Here variable arguments are constructed, critiquing the reasoning by which residue is identified as the displaced S2B and acknowledging that other possible conclusions can be drawn from the same data, which should be taken into account.

Summary of the Article for the Teacher:

It is recommended that this not be used by students in place of reading the article.

General Overview:

The reduction of dinitrogen (N2) by the enzyme nitrogenase is one of the most widely applicable transformations in chemistry, yet its mechanism is still very poorly understood. By replacing the substrate N2 with the comparable inhibitor CO, scientists can slow and stop the enzyme's reaction, allowing them to study the structure of nitrogenase midreaction. X-ray crystallography is used to produce a well-resolved, groundbreaking crystal structure of nitrogenase during and after inhibition by CO, suggesting opportunities for similar studies with myriad substrates and inhibitors that could shed light on the mechanism of nitrogenase's capacity to reduce N2, eventually allowing scientists to mimic the enzyme's unique activity.

Topics Covered:

- Biological nitrogen fixation by nitrogenase
- Crystal structures and electron density maps
- Enzyme reactivity with substrates and inhibitors

Why this research is important:

Atmospheric dinitrogen (N2) makes up 78% of Earth's air, but although nitrogen is an essential component of many chemicals, it is notoriously difficult to cleave the bond of the N2 molecule in order to incorporate nitrogen atoms into other chemicals, a process known nitrogen fixation. Industrial processes, such as the Haber-Bosch process, account for much of the fixed nitrogen the world uses today, but in turn consumes an estimated 1% to 3% of the world's annual energy output. On the other hand, biological nitrogen fixation is catalyzed by nitrogenase at nearly ambient conditions, making it a subject of continuing research by chemists who wish to emulate the mild conditions of the natural reaction. Isolating a crystal structure of nitrogenase midreaction provides a glimpse into the mechanism of this enigmatic reduction, suggesting both possible mechanistic pathways and paving the way for further studies with other substrates and inhibitors.

Methods used in the Research:

- Anaerobic protein assays
- Rapid crystal growth
- X-ray crystallography
- Electron density maps
- Anomalous density maps
- Enzyme reduction activity and inhibition

Conclusions:

- Inhibition of nitrogenase by CO completely halts enzymatic activity, but quantitative enzymatic activity is regained upon the removal of the inhibitor.
- The structure of CO-inhibited nitrogenase is obtained, clearly demonstrating the displacement of a belt sulfur atom by CO during inhibition, suggesting the displacement of the sulfur atom may be integral to the enzyme's activity and that the interstitial carbon may be essential to the stability of the complex during rearrangement.
- The displaced sulfur atom is proposed to relocate to the subunit interface during inhibition, but return to its original position between two Fe atoms upon reactivation as demonstrated by the crystal structure of the reactivated cofactor.

Areas of Further Study:

- What other species exist along the mechanistic pathway N_2 fixation by nitrogenase?
- What other crystal structures can potentially be obtained by binding substrates and inhibitors to nitrogenase?
- Is further refinement of the nitrogenase structure achievable? Is it necessary?

Resources for Interactive Engagement:

Discussion Questions

1. What is the goal of this study?

2. Why is this study important?

3. Are there any other explanations for the observed results?

4. The authors assert that CO acts as a reversible inhibitor to nitrogenase, meaning that while present it halts the enzyme's activity, but when removed, the enzyme regains its ability to facilitate reactions. What method to the authors use to measure nitrogenase's enzymatic activity in order to draw this conclusion? Do you think their method provides an accurate representation of the inhibition and reactivation of the enzyme that can be compared with its N_2 reduction? Why or why not?

5. How do the electron density maps that the authors obtain through x-ray crystallography allow them to identify the structure of a molecule on the atomic scale? What role do anomalous electron density maps play in helping to define the structural changes of CO inhibition?

6. Through crystallographic data, the authors identify a potential sulfur binding site (SBS) to which the displaced belt sulfur atom (S2B) could theoretically relocate during inhibition by CO. What evidence supports the likelihood that the S2B atom relocates to the SBS? What other explanations for these data do the authors note are also important possibilities to consider? Could this site be relevant during nitrogenase's reactions with substrates as well as inhibitors?

7. In the context of determining the mechanism of biological N_2 fixation by nitrogenase, why is it important that the authors have been able to obtain a crystal structure of CO-inhibited MoFe protein?