5 Protein Structure and Function: A Molecular Murder Mystery

Introduction

Prior to this lesson, students learned how the **cytochrome c oxidase subunit 1 *(COI)*** gene is used to barcode animals. In this lesson, students learn more about the cytochrome c oxidase protein and its three dimensional structure. In particular, students learn how to identify the active site in cytochrome c oxidase. Once they can find this site, they look at aligned structures (one of which contains a poison) and then determine the identity of a foreign substance that acts as a poison by binding to the active site. This lesson allows students to explore the use of the molecular visualization program Cn3D to learn more about cytochrome c oxidase, a ubiquitous and important protein. In *Lesson Five*, students also learn how ***molecular diagnostics researchers*** might use bioinformatics tools in their career.

Class Time

1 class period of 50 minutes.

Prior Knowledge Needed

- How to open a file in Cn3D.
- Basic knowledge of protein structure, including one-letter abbreviations for amino acids and alpha helices/ beta sheets. [**Note:** One-letter abbreviations can be found in the Circular Codon Table found in *Lesson Four* and in the *Appendix*.]

Learning Objectives

At the end of this lesson, students will know that:

- The **mitochondria** are the site of ATP production in the cell.
- The cytochrome oxidase complex is involved in ATP production, and is located in the **inner mitochondrial membrane**.
- Some poisons, such as carbon monoxide, act by binding to the active site of **enzymes**, preventing them from functioning.

At the end of this lesson, students will be able to:

- View the cytochrome c oxidase protein in Cn3D.
- Identify the active site of the cytochrome c oxidase protein in the three-dimensional structure.
- Characterize a poison (carbon monoxide or cyanide) bound to the active site of the protein.

Key Concepts

- Proteins have multiple levels of structure: primary (the sequence of amino acids), secondary (characteristic structures such as **alpha helices** and **beta sheets** that are formed by interactions between neighboring amino acids), tertiary (includes more distant interactions and domains) and quaternary (interactions between multiple peptide chains). The tertiary structure can be seen as the three-dimensional (3D) structure of a single protein chain, whereas the quaternary structure represents a larger assembly of one or more proteins and is composed of multiple protein chains/subunits.
- The **active site** of a protein is important for its function.
- Substances that can bind to the active site and block access by its normal substrate can interfere with protein function.

Materials

Materials	Quantity
Copies of Student Handout—*Careers in the Spotlight* (handed out in *Lesson One*)	1 per student
Copies of Student Handout—*The Process of Genetic Research* (handed out in *Lesson One*)	1 per student
Class set of Student Handout—*Codons and Amino Acid Chemistry* (from *Lesson Four*, printed in color if possible)	1 per student (class set)
Class set of Student Handout—*Molecular Murder Mystery Instructions*	1 per student (class set)
Teacher Answer Key—*The Process of Genetic Research* (found in *Lesson One*)	1
Teacher Answer Key—*Molecular Murder Mystery*	1
Teacher Resource—*Installing Cn3D* [**Note:** If students will be asked to install Cn3D, make a class set of copies of this handout.]	1 – or – 1 per student (class set)

Computer Equipment, Files, Software, and Media
Computer and projector to display PowerPoint slides. ***Alternative:*** Print PowerPoint slides onto transparencies and display with overhead projector.
Lesson Five PowerPoint Slides—*Molecular Murder Mystery*. Available for download at: http://www.nwabr.org/curriculum/advanced-bioinformatics-genetic-research.
Lesson Five protein structure files for *Part II*. Available for download under the "Resources" tab at: http://www.nwabr.org/curriculum/advanced-bioinformatics-genetic-research.
A student version of lesson materials (minus *Teacher Answer Keys*) is available from NWABR's Student Resource Center at: http://www.nwabr.org/students/student-resource-center/instructional-materials/advanced-bioinformatics-genetic-research.
Online video, "Electron-Transport Chain," produced by Garland Science. The video is 2:17 minutes long. Available at: http://www.youtube.com/watch?v=KXsxJNXaT7w&feature=related.
Computer lab with internet access, the program Cn3D, and a word processing program such as Google® Docs or Microsoft® Word for answering lesson activity and homework questions. [**Note:** Students are asked to insert images of protein structures into their homework document, which cannot be accomplished with a plain text editing program like Notepad.]

Teacher Preparation

- To maximize class time for the lesson activities, it will be useful to install the Cn3D program as well as the structure files for Student Handout—*Molecular Murder Mystery* on classroom computers prior to class. See Teacher Resource—*Installing Cn3D* for complete instructions on installing Cn3D. Alternatively, make a class set of the resource pages and have students install the program. [**Note:** The IT departments of some schools or districts require significant lead time to install or approve installation of programs on school computers.]
- Load the classroom computer with the *Lesson Five* PowerPoint slides.
- Make copies of Student Handout—*Molecular Murder Mystery*, one per student. This handout is designed to be re-used as a class set.

Procedure

Warm Up

1. As students enter the classroom, display the PowerPoint slide for *Lesson Five*, beginning with ***Slide #1***. This slide highlights James Ferrenberg, a molecular diagnostics researcher.

Bioinformatics & Protein Structure: ***Slide #1***

2. Have students retrieve Student Handout—*Careers in the Spotlight*, which they were given during *Lesson One*.
3. Students should think about, and write down, what kind of work a molecular diagnostics researcher might do (*Molecular Diagnostics Researcher Question #1*). This will be revisited at the end of the lesson, including how a molecular diagnostics researcher might use bioinformatics in his or her job.
4. Tell students to keep their *Careers in the Spotlight* Handout available for future lessons.

PART I: Mitochondria – The Powerhouses of the Cell

5. Explain to students the ***aims of this lesson***. Some teachers may find it useful to write the aims on the board.
   a. **Lesson Aim:** Understand that protein structure can impact protein function, using the bioinformatics tool Cn3D to visualize molecules.
   b. **Lesson Aim:** Explore the structure and active site of the COI protein, encoded in part by the barcoding gene.

   Teachers may also wish to discuss the *Learning Objectives* of the lesson, which are listed at the beginning of this lesson plan.
6. Remind students that the cytochrome c oxidase subunit 1 or "***COI***" **gene** used for barcoding is found in the **mitochondria**.

Cytochrome c oxidase subunit 1 *(COI):* *COI* is the cytochrome c oxidase subunit 1 gene. *COI* encodes the COI protein found in the electron transport chain. The entire cytochrome c oxidase protein complex is composed of two copies of 13 different protein chains (subunits 1–13). Subunit 1 contains the enzyme's active site. The sequence of cytochrome C oxidase subunit 1 (*COI*) is similar in all living creatures, and is used for DNA barcoding of animals.

Mitochondria: A part of the cell (organelle) containing enzymes responsible for producing energy in the form of ATP (adenosine triphosphate).

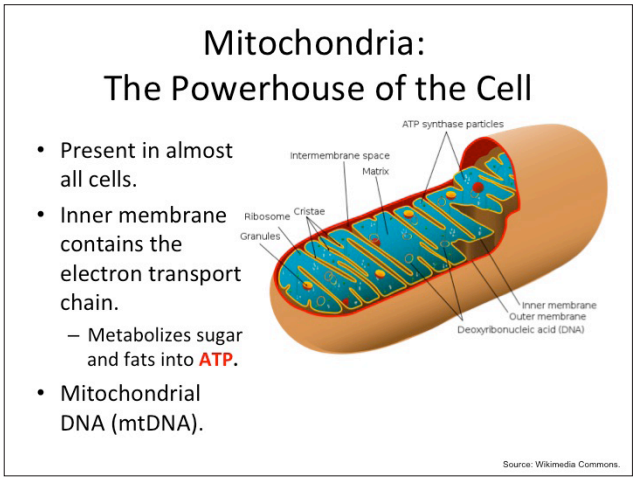
Outer membrane: Phospholipid membrane surrounding and containing the mitochondrion.

Inner membrane: Membrane found inside the mitochondrion that contains the proteins involved in the electron transport chain and ATP synthesis.

Matrix: Space inside the inner membrane of the mitochondrion.

7. Show ***Slide #2***, and convey to students the following information:
   - The function of the mitochondria is to convert food (in the form of sugars and fats) into energy (in the form of ATP) which is used to power almost all of the functions needed by life.
   - Mitochondria are found in almost every cell in the body, because all cells need energy (ATP).
   - Mitochondria contain an **outer membrane** and an **inner membrane** (similar to the membrane that surrounds the whole cell), as well as their own DNA, which encodes genes specific to the mitochondria, including *COI*.
   - The very center of the mitochondrion is called the **matrix** and the space between the two membranes is called the **intermembrane space**.

Bioinformatics & Protein Structure: ***Slide #2***



Intermembrane space: Space between the inner and outer mitochondrial membranes.

Electron transport chain: A series of proteins found in the inner membrane of the mitochondria that couples chemical reactions to produce adenine triphosphate (ATP), which is the energy source used by cells.

Transmembrane proteins: Proteins embedded in and spanning a phospholipid bilayer or membrane.

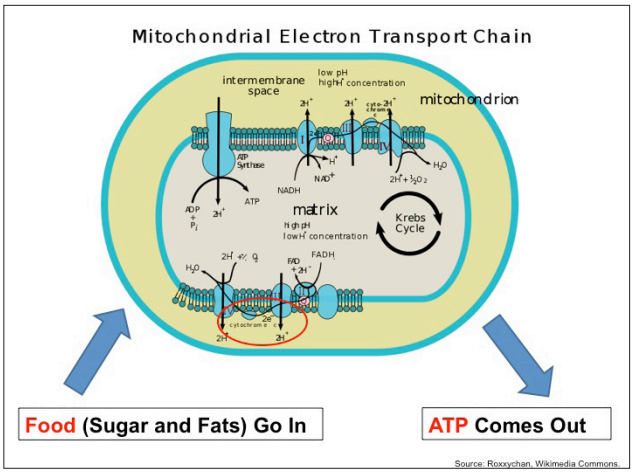
Conserved: DNA or protein sequences are said to be "conserved" if the sequences are the same or very similar.

Proton: Subatomic particle with an electric charge of +1. Also called a hydrogen ion.

Concentration gradient: In biology, a gradient results from an unequal distribution of ions across a cell membrane. When this happens, solutes move along a concentration gradient. This kind of movement is called diffusion.

ATP synthase: Transmembrane protein in the inner mitochondrial membrane that uses the concentration gradient of protons across the membrane to fuel the production of ATP.

8. Show ***Slide #3***, and convey to students the following information:
   - The **electron transport chain** has many different protein components that are found in the inner mitochondrial membrane.
   - These proteins are called **transmembrane proteins** because they are found inside the membrane, with part of the protein sticking out on either side. The cytochrome c oxidase (COI) protein is one of these transmembrane proteins. The vital role COI plays in making ATP helps explain why COI is highly **conserved**, meaning it is found in all species, without many changes or mutations.
   - The proteins of the electron transport chain pump positively-charged **protons** (also called hydrogen ions) from the intermembrane space into the matrix, and create a **concentration gradient**. Like water pumped uphill, when these protons flow back across the inner membrane through channels called **ATP synthase**, the energy of the proton flow is used to make ATP.
   - The portion of the protein where chemical reactions take place is called the **active site**.



Bioinformatics & Protein Structure: ***Slide #3***

Active site: The site in an enzyme where chemical reactions take place. This is often a cleft or pocket on the surface of the enzyme. Also called the functional or catalytic site.

Complex: A group of two or more proteins that associate with one another and work together to accomplish some function. [**Note:** "Complexes" is the plural form of "complex."]

Cytochrome c oxidase complex: One of the protein complexes found in the mitochondria, which contain proteins (cytochromes) involved in the production of ATP (adenosine triphosphate).

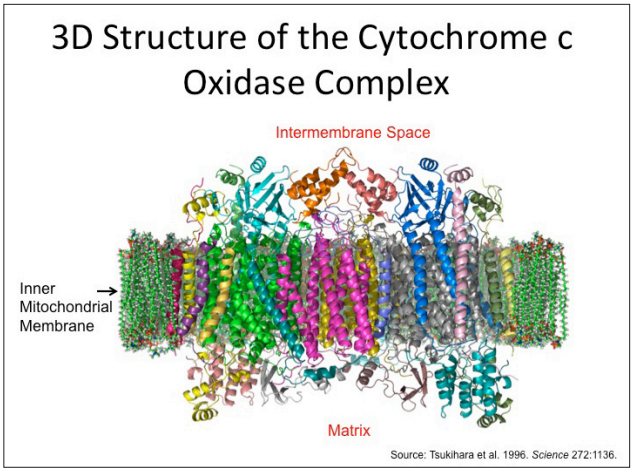
9. Show students the following video, which provides a brief overview of the electron transport chain in action. The video can be found at:

   Electron-Transport Chain Video (2:17 minutes)
   http://www.youtube.com/watch?v=KXsxJNXaT7w&feature=related

10. Tell students that scientists (and textbook authors!) often draw proteins as simple boxes or other two dimensional shapes for simplification. However, each protein is much more complicated, containing hundreds and sometimes thousands of amino acids.

11. Show ***Slide #4***, which shows the three-dimensional crystal structure of the two copies of the 13 subunits of the **cytochrome c oxidase complex** embedded in the inner mitochondrial membrane. ***The COI protein is subunit number 1 of this complex***. Each subunit is shown in a different color. The top of the structure faces into the intermembrane space. The bottom of the structure faces into the mitochondrial matrix. The coils represent **alpha helices** and the flat arrows are **beta sheets**.

[**Note:** These are the same secondary structures that students saw when they viewed the BRCT domain from the BRCA1 protein in *Lesson Five* of the Introductory curriculum, *Using Bioinformatics: Genetic Testing*.]



Bioinformatics & Protein Structure: ***Slide #4***

Alpha helix: A common structure in proteins, characterized by a single, spiral chain of amino acids stabilized by hydrogen bonds between neighboring amino acids. [**Note:** "Alpha helices" is the plural form.]

Beta sheet: A structure that occurs in many proteins and consists of two or more parallel adjacent polypeptide chains arranged so that hydrogen bonds can form between the chains.

12. Ask students why understanding a protein's structure is important. Have them talk to their neighbor for one minute to brainstorm ideas about this question.

13. Lead a discussion on the relationship between structure and function. Key points to cover include:
    - Understanding a protein's structure allows scientists to learn more about how that protein functions.
    - Understanding the structure also reveals how changes to that structure can impact function.
    - When the protein studied is from an infectious disease-causing organism like influenza, or is a protein involved in diseases like cancer, understanding the protein's structure can help scientists develop diagnostic tests and drugs to treat the disease. This is the type of work performed by molecular diagnostics researchers.

PART II: Molecular Murder Mystery—Cytochrome c Oxidase

14. Share with students that they will take a closer look at the three-dimensional structure of cytochrome c oxidase.

15. Distribute copies of Student Handout—*Molecular Murder Mystery*, one per student.

16. Advise students as to whether they will need to download Cn3D and the structure files or whether you have already done so.

17. Briefly review the two main sections of Student Handout—*Molecular Murder Mystery* and what students are expected to do:
    - In the first section, students investigate the overall structure of the protein. In the second section, they examine two overlapping structures – one that contains a poison and one that does not.
    - Their goal is to identify the poison that killed their victim, which can readily be done using their knowledge of the way elements are color coded in Cn3D. Along the way, they will capture images from the computer screen to document their progress.
    - Students will need to create word processing documents using a program such as Google® Docs or Microsoft® Word. Specify how you wish students to turn in their files to you. For example, they may print out the document if a printer is available, or save the file to a shared folder.

18. Assign each student to investigate either Carol Olds or Carl North.

19. You may want the class to stop working on the handout when they have finished *Part I*, in order to review the overall structure of the protein and the characteristics of the active site as a class before allowing students to proceed with *Part II*. If students are struggling with *Part II*, you may wish to demonstrate how to highlight the Fe/heme, Cu, and poisons using a projector, or walk through *Part II* with students as a class.

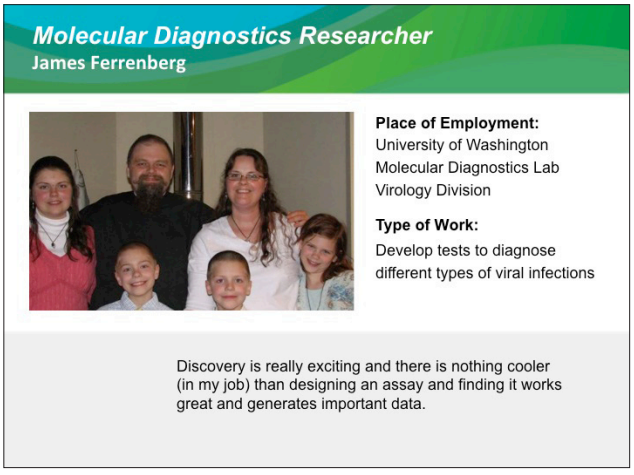
[**Note:** The initials of each victim correspond to the poison used; this has been done for ease of teacher identification. However, do not reveal this to students yet!]

Closure

20. Summarize today's lesson:
    - Students have learned how to use a molecular visualization program, Cn3D, to investigate the structure of the cytochrome c oxidase protein and its active site.
    - They have learned how the structure of a protein can provide insights into how that protein functions.
    - They have also learned how a protein's function can be disrupted by a poison binding to the active site.
21. If your students have participated in the *Using Bioinformatics: Genetic Testing* lessons, you may wish to connect this lesson to their prior knowledge about protein structure. In particular, protein function can be impacted by **mutations**, which change the amino acid sequence and, in some cases, can alter the protein structure. However, in this example, protein function is impacted when other molecules (poisons) block the active site and prevent the normal substrates from binding.

Mutation: A change in a DNA or protein sequence.

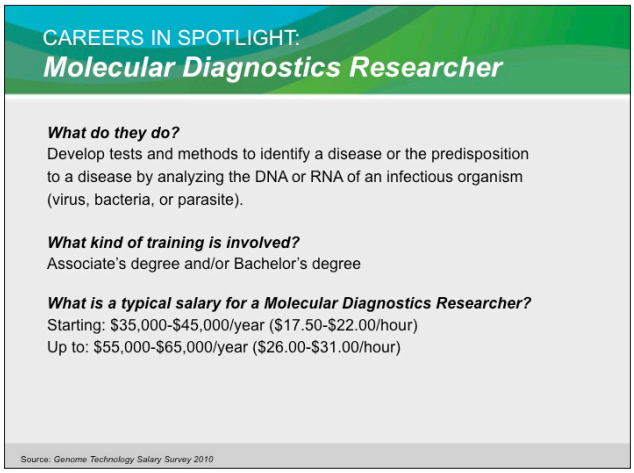
22. You may wish to review the answers to the murder mystery with the class or wait until the beginning of the next class period.
23. Ask students to fill out the section about *Lesson Five* on Student Handout—*The Process of Genetic Research*, which was handed out during *Lesson One*. Students could also answer these questions in their lab notebooks:
    - What ***did you do*** in this lesson?
    - ***Methods:*** What bioinformatics tool(s) and/or database(s) did you use?
    - ***Results & Conclusions:*** What did you find? What could you conclude from your analysis?
    - What ***skills*** did you learn or practice?
24. Lastly, show ***Slide #5***, which returns to the picture of the molecular diagnostics researcher from ***Slide #1***.



Bioinformatics & Protein Structure: ***Slide #5***

25. Show ***Slide #6***, which provides job information for a molecular diagnostics researcher. Review this information with students.

Bioinformatics & Protein Structure: ***Slide #6***



26. Ask students, "What more do we know about molecular diagnostics after today's lesson?" Point out that molecular diagnostic researchers use genetic research and insights into protein structure to study infectious agents like influenza, and to develop laboratory tests to diagnose infectious organisms in human and animal patients. Understanding protein structure can also be instrumental in helping scientists develop drugs to treat infectious diseases. These drugs act in a manner similar to the poisons in this activity. They bind to the active site of an important protein and block the normal chemical reactions that would take place. In the case of carbon monoxide or cyanide binding to COI, carbon monoxide binds to the COI active site, preventing oxygen from binding. The overall effect is that COI can no longer carry out its normal function.

27. Ask students to answer *Molecular Diagnostic Researcher Question #2* on their *Careers in the Spotlight* Handout, which has students explain how this lesson has changed their understanding of what kind of work a molecular diagnostic researcher does.

28. Ask students to also answer *Molecular Diagnostic Researcher Question #3* on their *Careers in the Spotlight* Handout, which has students explain how a molecular diagnostic researcher might use bioinformatics in his or her work.

29. Tell students to keep their *Careers in the Spotlight* Handout available for future lessons.

Homework

A. As homework, ask students to write about the activities they worked on in *Lesson Five* in their Lab Notebooks, on another sheet of paper, or in a word processing program like Notepad or Microsoft Word® which they then provide to the teacher as a printout or via email. This can serve as an entry ticket for the following class. Have them complete these prompts:

   a. Today I learned that…

   b. An important idea to think about is…

   c. Something that I don't completely understand yet is…

   d. Something that I'm really confident that I understand is….

B. The *Lesson Five* Section of Student Handout—*The Process of Genetic Research* could also be assigned as homework.

Extension

For more experiences with Cn3D, including structures with additional poisons, see "A Murder Mystery with Molecular Structures" in the "Tutorials" section at Digital World Biology: http://www.digitalworldbiology.com/dwb/Home.html

Glossary

Active site: The site in an enzyme where chemical reactions take place. This is often a cleft or pocket on the surface of the enzyme. Also called the functional or catalytic site.

Adenosine Triphosphate (ATP): The molecule that is the source of energy for most life processes in living organisms.

Alpha helix: A common structure in proteins, characterized by a single, spiral chain of amino acids stabilized by hydrogen bonds between neighboring amino acids. [**Note:** "Alpha helices" is the plural form.]

ATP synthase: Transmembrane protein in the inner mitochondrial membrane that uses the concentration gradient of protons across the membrane to fuel the production of ATP.

Beta sheet: A structure that occurs in many proteins and consists of two or more parallel adjacent polypeptide chains arranged so that hydrogen bonds can form between the chains.

Complex: A group of two or more proteins that associate with one another and often work together to accomplish some function. [**Note:** "Complexes" is the plural form of "complex."]

Concentration gradient: In biology, a gradient results from an unequal distribution of ions across a cell membrane. When this happens, solutes move along a concentration gradient. This kind of movement is called diffusion.

Conserved: DNA or protein sequences are said to be "conserved" if the sequences are the same or very similar (i.e., if the amino acids have similar chemistries in the case of proteins).

Copper: Copper is a metal element, whose symbol is Cu. The atomic number for copper is 29.

Cytochrome c oxidase subunit 1 (*COI*): *COI* is the cytochrome c oxidase subunit 1 gene. *COI* encodes the COI protein found in the electron transport chain. The entire cytochrome c oxidase protein complex is composed of two copies of 13 different protein chains (subunits 1–13). Subunit 1 contains the enzyme's active site. The sequence of cytochrome C oxidase subunit 1 (*COI*) is similar in all living creatures, and is used for DNA barcoding of animals.

Cytochrome c oxidase complex: One of the protein complexes found in the mitochondria, which contain proteins (cytochromes) involved in the production of ATP (adenosine triphosphate).

Dimer: A structure made of two subunits called monomers.

Electron transport chain: A series of proteins found in the inner membrane of the mitochondria that couples chemical reactions to produce ATP (adenosine triphosphate), which is the energy source used by cells.

Elements: Elements are pure chemical substances consisting of one type of atom. Each element is distinguished by its atomic number. All the elements are listed in the periodic table.

Enzyme: A type of protein that catalyzes (increases the rate of) chemical reactions. For example, ATP synthase is an enzyme that catalyzes or facilitates the creation of ATP (adenosine triphosphate).

Heme: A non-protein compound that contains iron and is vital to the function of the cytochrome c oxidase complex.

Hemoglobin: Iron-containing protein in red blood cells that carries oxygen.

Inner membrane: Membrane found inside the mitochondrion that contains the proteins involved in the electron transport chain and ATP synthesis.

Intermembrane space: Space between the inner and outer mitochondrial membranes.

Iron: Iron is a metal element whose symbol is Fe. The atomic number for iron is 26.

Matrix: Space inside the inner membrane of the mitochondrion.

Medical examiner: Qualified government official whose duty it is to investigate deaths and injuries that occur under unusual or suspicious circumstances.

Mitochondria: A part of the cell (organelle) containing enzymes responsible for producing energy in the form of ATP (adenosine triphosphate).

Monomer: A molecule or subunit that can combine with other monomers to form a polymer. "Mono" means "one" and "poly" means "many."

Mutation: A change in a DNA or protein sequence.

Outer membrane: Phospholipid membrane surrounding and containing the mitochondrion.

Oxidative phosphorylation: The process of using oxidation to provide energy for adding the phosphate groups in ATP. This takes place in the mitochondria.

PBD number: A unique identifier or code assigned to every entry in the Protein Data Bank (PDB). This unique code can be used to search the databases at the National Center for Biotechnology Information (NCBI) to find the structure of interest.

PDB position: "PDB" stands for "Protein Data Bank." The "PDB Position" or "PDB Number" is the position of a particular amino acid in a polypeptide chain.

Proton: Subatomic particle with an electric charge of +1. Also called a hydrogen ion.

Transmembrane proteins: Proteins embedded in and spanning a phospholipid bilayer or membrane.

Resources

Carbon monoxide. Wikipedia. Available at: http://en.wikipedia.org/wiki/Carbon_monoxide

Cyanide. Wikipedia. Available at: http://en.wikipedia.org/wiki/Cyanide

Credit

Ferrenberg, James. Personal Interview. 14 July 2010.

Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R., & Yoshikawa, S. The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 *Å*. *Science*. 1996; 272: 1136–44.

The authors wish to thank Wikimedia Commons for many of the images found in this lesson.

[**Note:** It should be noted that proteins in this activity are from cows, not humans, but the structures would be almost identical.]

5 Molecular Murder Mystery Instructions

Student Medical Examiner Background:

Aim: To understand that protein structure can impact protein function, using the bioinformatics tool Cn3D to visualize molecules.

The Situation: Two individuals were found dead at the scene of a crime. As the **medical examiner**, it is your job to find out how they died.

At 1:05 AM on July 5, 2010, several guests at the Hotel Aurora in Seattle, Washington called 911 to report a loud crash in a nearby room. When paramedics and police arrived 8 minutes later, they found the bodies of two victims: Carol Olds and Carl North, both age 35. They had been visiting Seattle to see the 4th of July fireworks on Lake Union.

While there were no obvious signs of foul play, both victims had apparently eaten a late dinner after the fireworks celebration, as evidenced by the empty dinner plates on the room service cart, which was delivered by hotel staff shortly after midnight. The crash heard by hotel guests was apparently one or more of the victims falling to the floor.

You suspect poison. The question is, which poison or poisons? Some poisons are known to bind to the active site of the cytochrome c oxidase protein complex.

Medical examiner: Qualified government official whose duty it is to investigate deaths and injuries that occur under unusual or suspicious circumstances.

Active site: The site in an enzyme molecule where the substrate binds and where the reaction is facilitated. It is often a cleft or pocket on the surface of the enzyme. Also called a functional or catalytic site.

Enzyme: A type of protein that catalyzes (increases the rate of) chemical reactions. For example, ATP synthase is an enzyme that catalyzes or facilitates the creation of ATP.

Your Challenge: Today, your job as a medical examiner is to:

1. Examine the structure and **active site** of the normal COI protein so that you can recognize it later when you examine samples from the victims.
2. Find the active sites in the protein structures obtained from blood samples taken from the bodies at the crime scene, to determine whether the samples contain a poison.

Instructions: Before you begin, be sure that the program Cn3D is installed on your computer. If the program has not been installed, ask your teacher for directions. Also make sure that you have access to a word processing program like Google® Docs or Microsoft® Word.

PART I: Investigating Cytochrome c Oxidase and its Active Site

1. Open the cytochrome c oxidase structure by following the directions below. Your teacher will let you know whether you should download the structure from NCBI or whether it is already downloaded. If you need to download it, follow these instructions:

   a. Search for "NCBI Structure" in your internet browser.

b. On the ***NCBI Structure Page***, enter "1OCC" into the search bar (use letter O, not zero). This is the **PBD number** (or identifier) for the protein structure for cytochrome c oxidase.

c. Click ***View Structure***. Open the Cn3D structure file when prompted.

PBD number: A unique identifier or code assigned to every entry in the Protein Data Bank (PDB). This unique code can be used to search the databases at the National Center for Biotechnology Information (NCBI) to find the structure of interest.

These commands are for navigating in the Structure Window:

- To ***zoom in***, you can either click the letter z, or hold down the control key, left-click, and move your mouse/fingers. To use the mouse, move the mouse towards the computer screen to make the image larger and away from the screen to make the image smaller. To use the keyboard, click the letter x to ***zoom out***. [**Note:** These commands can also be found under ***View*** in the toolbar.]
- To ***move the structure without turning it*** hold the Shift key, click in the Structure window, and drag the structure with your mouse.

2. Look at the overall structure of this amazing protein. Cytochrome c oxidase (***Figure 1***) spans the inner membrane of the mitochondria. Open the ***Style*** menu, choose ***Coloring Shortcuts***, then ***Secondary Structure***.

   a. Turn the structure around to see the part of the protein that is normally ***inside*** the mitochondrial membrane by looking for the series of green **alpha helices** that are parallel to each other.

   b. Find the parts of the molecule that are located on either side of the membrane by looking for the tan **beta sheets**.

   c. Open the ***Style*** menu; choose ***Coloring Shortcuts***, and then ***Molecule***.

   d. Each of the different protein chains and all the other molecules in the structure will be shown in a different color.

   e. The amino acid sequences of each of the protein chains are shown in the ***Sequence/Alignment Viewer*** window, and are color-coded the same as each protein chain.

3. Create a new word processing document to use as your answer sheet. Give the document a title, such as "Cytochrome c oxidase," and add your name.

4. Within Cn3D, open the ***File*** menu and choose ***Export PNG*** to export an image of the entire cytochrome c oxidase protein, and save this image to your desktop.

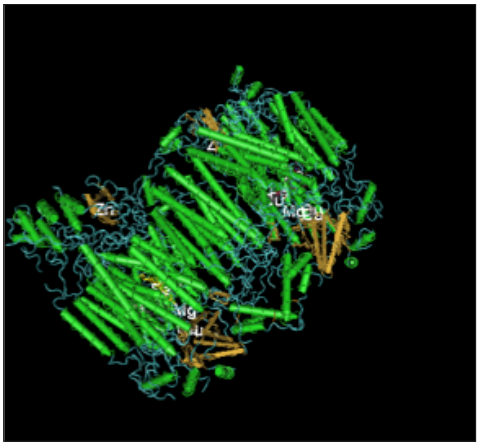


Figure 1: The Cytochrome c Oxidase – Entire Protein. Source: NCBI.

Alpha helix: A common structure of proteins, characterized by a single, spiral chain of amino acids stabilized by hydrogen bonds between neighboring amino acids. [**Note:** "Alpha helices" is the plural form.]

Beta sheet: A structure that occurs in many proteins and consists of two or more parallel adjacent polypeptide chains arranged so that hydrogen bonds can form between the chains.

5. On your answer sheet, insert your protein image. From the ***Insert*** menu, select ***Picture***, and select your protein structure image from your desktop.

6. On your answer sheet, add a title to the image of the entire protein, "Cytochrome c oxidase – entire protein."

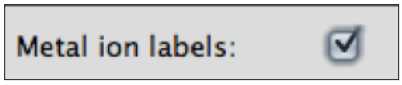
7. On your answer sheet, note how many protein chains are in this structure. (All the chains can be viewed in the ***Sequence/ Alignment Viewer***.)

8. This structure happens to be a **dimer** (a protein made of two subunits called **monomers**). In your document, describe how many protein chains are in each monomer. [**Hint:** The two monomers are identical.]

9. Examine the **iron** and **copper** molecules bound to the protein. These **elements** play an important role in the protein's function:

   a. Notice the ***white*** letters used to label the metals in the molecular structure itself (***not*** in the ***Sequence/ Alignment Viewer***).

   b. If you do not see labels for the metals, open the ***Style*** menu and choose ***Edit Global Style***. Then, click the ***Labels*** button, check the box for ***Metal ion labels***, click ***Apply*** and ***Done***.



   c. To see the heme group and other metals more easily, open the ***Style*** menu and choose ***Rendering Shortcuts → Tubes***.

   d. Open the ***Style*** menu and choose ***Coloring Shortcuts → Element***.

   e. Zoom in and find a copper ion (Cu) located close to an iron ion (Fe).

   f. The iron is part of a structure called a **heme** that is involved in binding oxygen (heme groups are also found in red blood cells in the molecule **hemoglobin**). The heme group looks like a big net with the iron atom in the center.

   g. Double click on the iron (Fe). It will turn yellow – as will the entire heme surrounding it.

   h. Hold the Shift key down and double click the copper ion (Cu). It will also turn yellow. Both ions should now be selected (see ***Figure 2***).

   i. Rotate the protein and notice the space between the Fe and the Cu ions. This space will be important later!

Dimer: A structure made of two subunits called **monomers**.

Monomer: A molecule or subunit that can combine with others of the same kind to form a polymer. "Mono" means "one" and "poly" means "many."

Iron: Iron is a metal element whose symbol is Fe. The atomic number for iron is 26.

Copper: Copper is a metal element, whose symbol is Cu. The atomic number for copper is 29.

Elements: Elements are pure chemical substances consisting of one type of atom. Each element is distinguished by its atomic number. All the elements are listed in the periodic table.

Heme: A non-protein compound that contains iron and is vital to the function of the cytochrome c oxidase complex.

Hemoglobin: Iron-containing protein in red blood cells that carries oxygen.

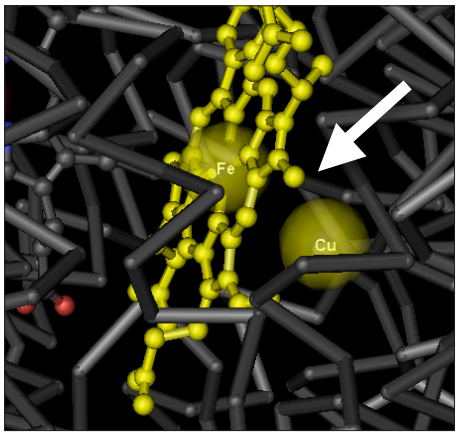


Figure 2: Oxygen Binding Site of Cytochrome c Oxidase. Source: NCBI.

10. Open the ***File*** menu and choose ***Export PNG*** to export an image of the structure, including the area with the Fe and Cu. Insert your image into your "Cytochrome c oxidase" document. Label the image "Oxygen binding site of cytochrome c oxidase."

Optional Extension Activity: Looking at Amino Acids that are Part of the Active Site
If time permits, an optional extension is provided below. Otherwise, proceed to *Part II*.

11. If not already selected, select the heme and the copper in the active site by clicking them with the mouse.

12. Open the ***Select*** menu and choose ***Select by Distance***.

    a. Set the distance at 3 angstroms and click ***OK***.

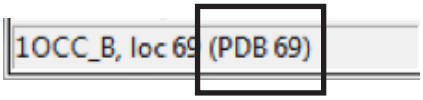
    b. Expand the ***Sequence/Alignment Viewer*** window by clicking in the bottom right corner and dragging it until the window is larger. You should see some of the residues become highlighted in one of the protein chains in the ***Sequence/Alignment Viewer*** window.

    c. Scroll through the ***Sequence/Alignment Viewer*** to see which amino acid residues became highlighted.

13. In your document, describe which chain(s) contains the highlighted residues.

14. In your document, describe which amino acid residue(s) were highlighted. Make a list of these amino acid(s) (by their single-letter code) and their positions (**PDB position**). To determine the position, place your cursor over the amino acid and read the **PDB position** in the bottom left corner of the ***Sequence/Alignment Viewer***.

PDB Position: "PDB" stands for "Protein Data Bank." The "PDB Position" is the position of a particular amino acid in a polypeptide chain.



15. Find out which amino acids are bound to copper.

    a. Open the ***Select*** menu and choose ***Show Selected Residues***. You will see the heme, the copper, and the amino acid residues from the protein chain that binds these substances. [**Note:** You may need to zoom in or out to see these in the protein structure.]

    b. Open the ***Style*** menu and choose ***Rendering Shortcuts***, then ***Ball and Stick***.

    c. Click your mouse inside the ***Sequence/Alignment Viewer*** to deselect the highlighted residues.

    d. You will see metal bonds (white) between the copper or the iron and some of the amino acids.

    e. Select the bound amino acids with your mouse and look for the highlighted amino acids in the ***Sequence/Alignment Viewer*** to identify the names and position numbers for those amino acids.

    f. In your document, record the names and PDB position numbers for any amino acids bound to copper.

Carbon = Black
Nitrogen = Blue
Oxygen = Red
Sulfur = Yellow

16. Open the ***Style*** menu and change the ***Coloring Shortcut*** to ***Element*** (if it is not already selected). In your document, note which element forms an ionic bond to copper.

17. On your answer sheet, explain whether this is the same element for all of the bound amino acids.

PART II: What Killed the Victims?

The copper ion (Cu) and the iron (Fe) that you looked at in *Part I* are both important for cytochrome c oxidase activity. During the process of extracting energy from food (specifically, **oxidative phosphorylation**), molecular oxygen (O_2) binds to the iron. If other substances are bound to the iron, they can block binding by the oxygen, inhibiting the activity of the enzyme. Your job is to determine whether there are other substances bound to the active site in the structures from the crime scene.

Oxidative phosphorylation: The process of using oxidation to provide energy for adding the phosphate groups in ATP. This takes place in the mitochondria.

18. Find a structure file from one of the victims.
    a. Your teacher will assign you one of the victim's structures (Carl North or Carol Olds) to download.
    b. Record the name of your assigned victim on your answer sheet.
    c. Download the appropriate structure from the NWABR Bio-ITEST website (http://www.nwabr.org/curriculum/advanced-bioinformatics-genetic-research) and open it in Cn3D as directed by your teacher.
19. Open the structure in Cn3D. When you first open the file, it will look confusing. That is because there are ***two*** structures layered on top of one another. One of the structures is from a "normal" individual and one is from a victim.
20. Simplify the view by opening the ***Style*** menu and choosing ***Coloring Shortcuts → Element***.
21. To view the structures one at a time, click in the ***Structure window*** and then click the right arrow on your keyboard.
22. Next, click your left arrow.
23. Toggle back and forth (i.e., alternate between the left and right arrows) until you can see the difference between the two structures. There should be something new ***between*** the Fe and the Cu ions in the victim. You can see this even more clearly by doing the following:
    a. Use your mouse to double-click the iron (Fe) and copper (Cu) from one of the structures. Note that the whole heme will be selected when you double click the iron (Fe). You need to be holding down the Control (or Command) key to select both ions.
    b. Toggle to the other structure and double-click the Fe and Cu there, too.
    c. Find the structure that has a molecule between the Fe and Cu. Be sure to ***double-click the molecule between*** the Fe and Cu in that structure.
    d. Open the Select menu and choose ***Show Selected Residues***.
    e. Use your arrow keys to toggle back and forth between the normal version of the protein and the version from the crime scene.
    f. To make the yellow highlighting go away, and to return the elements to their appropriate color, double-click the heme, copper, and additional substance in the ***Structure window***.

24. Export a PNG image of the cytochrome c oxidase active site showing the heme, iron (Fe), and copper (Cu) from the victim and import it into your document. Label the image with the name of the victim (Carl North or Carol Olds).

    a. Use the element color coding to identify the small molecule between the iron (Fe) and copper (Cu). In your document, explain what you found and how you made your conclusions.

    b. Look this molecule up in Wikipedia (http://www.wikipedia.com) to find out whether it could be used as a poison.

Carbon = Black
Nitrogen = Blue
Oxygen = Red
Sulfur = Yellow

25. Add a second label to the image. Label the image in your document "(Name of your poison) bound to the oxygen binding site of cytochrome c oxidase."

26. Underneath the image, write a few sentences about the properties of the poisonous substance and propose a theory to explain how it could have been administered to the victim. Add your name, date, and class period to your answer sheet and save it as directed by your teacher.

5 Molecular Murder Mystery
Teacher Answer Key

PART I: Investigating the Active Site of Cytochrome c Oxidase

5. On your answer sheet, insert your protein image. From the ***Insert*** menu, select ***Picture***, and select your protein structure image from your desktop.

6. Within your document, add a title to the image of the entire protein, "Cytochrome c oxidase – entire protein."

See ***Figure 1***.

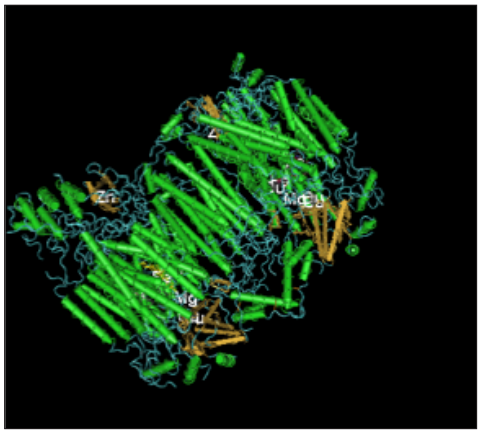


Figure 1: The Cytochrome c Oxidase – Entire Protein. Source: NCBI.

7. On your answer sheet, note how many protein chains are in this structure. (All the chains can be viewed in the ***Sequence/ Alignment Viewer***.)

26 Protein chains.

8. This structure happens to be a **dimer** (a protein made of two **subunits** called **monomers**). In your document, note how many protein chains are in each monomer, if the monomers are identical.

13 Protein chains.

10. Open the ***File*** menu and choose ***Export PNG*** to export an image of the structure, including the area with the Fe and Cu. Insert your image into your "Cytochrome c oxidase" document. Label the image "Oxygen binding site of cytochrome c oxidase."

See ***Figure 2***.

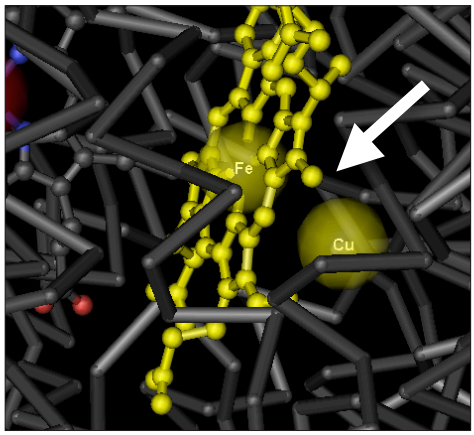


Figure 2: Oxygen Binding Site of Cytochrome c Oxidase. Source: NCBI.

Optional Extension Exercise:

13. In your document, describe which chain(s) contains the highlighted residues.

Chain A (1OCC_A) (or Chain N, if the Fe/Cu pair in the other monomer is selected).

14. In your document, describe which residue(s) were highlighted. Make a list of these amino acid(s) (by their single-letter code) and their positions (**PDB position**). To determine the position, place your cursor over the amino acid and read the PDB position in the bottom left corner of the ***Sequence/Alignment Viewer***.

W126, H240, Y244, H290, H291, D364, H376.

15. In your document, record the names and PDB position numbers for any amino acids bound to copper.

W126, H240, H290, H291.

16. Open the ***Style*** menu and change the ***Coloring Shortcut*** to ***Element***. In your document, note which element forms an ionic bond to copper.

Nitrogen.

17. On your answer sheet, explain whether this is the same element for all the bound amino acids.

Yes.

PART II: What Killed the Victims?

24. Export a PNG image of the cytochrome c oxidase active site showing the heme, iron (Fe), and copper (Cu) from the victim and import it into your document. Label the image with the name of the victim (Carl North or Carol Olds).

See ***Figure 3*** and ***Figure 4***.

25. Add a second label to the image. Label the image in your document "(Name of your poison) bound to the oxygen binding site of cytochrome c oxidase."

See ***Figure 3*** and ***Figure 4***.

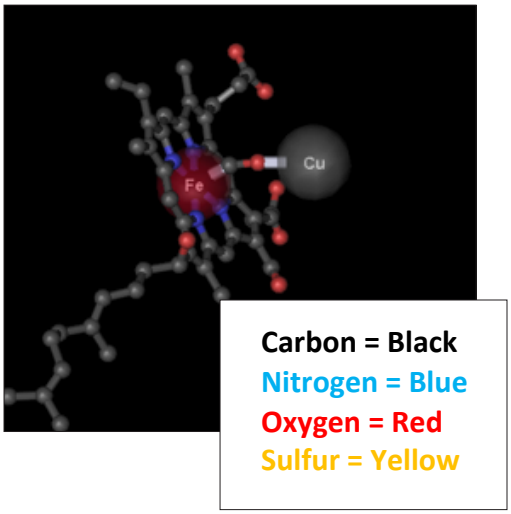


Figure 3: Victim: Carol Olds. Carbon Monoxide (CO) bound to the oxygen binding site of cytochrome c oxidase. Source: Cn3D.

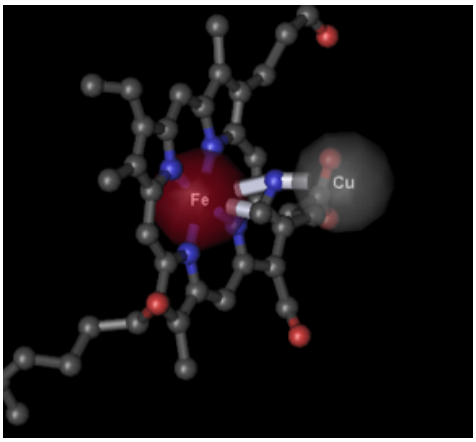


Figure 4: Victim: Carl North. Cyanide (CN) bound to the oxygen binding site of cytochrome c oxidase. Source: Cn3D.

26. Underneath the image, write a few sentences about the properties of the poisonous substance and propose a theory to explain how it could have been administered to the victim. Add your name, date, and class to your answer sheet and save it as directed by your teacher.

Carol Olds: *Carbon monoxide (CO) bound to the oxygen binding site of cytochrome c oxidase.*

Carbon monoxide is a colorless, odorless, and tasteless gas and can cause fatal poisoning by inhalation. It binds to hemoglobin in addition to cytochrome c oxidase.

Carl North: *Cyanide (CN) bound to the oxygen binding site of cytochrome c oxidase.*

The binding of cyanide to cytochrome c oxidase subunit 1 prevents the electron transport chain from working properly, thus preventing the cell from producing ATP. Tissues such as the heart and central nervous system that are strongly dependent on aerobic respiration are greatly impacted by cyanide poisoning. When used as a poison, it is administered as a gas or a salt.

Student answers may vary as to how the poisonous gases were administered to the victims (for example, by exploding canister, delivery of poison-filled balloons, etc.).

5 Installing Cn3D

The Cn3D software allows you to view the three-dimensional structures of macromolecules like proteins and DNA. If Cn3D has not already been downloaded to your computer, follow the instructions below.

1. To download the Cn3D program onto your computer, visit the Cn3D homepage at: http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3dinstall.shtml.

2. Select your Operating System (Windows or Macintosh), as shown in ***Figure 1***.

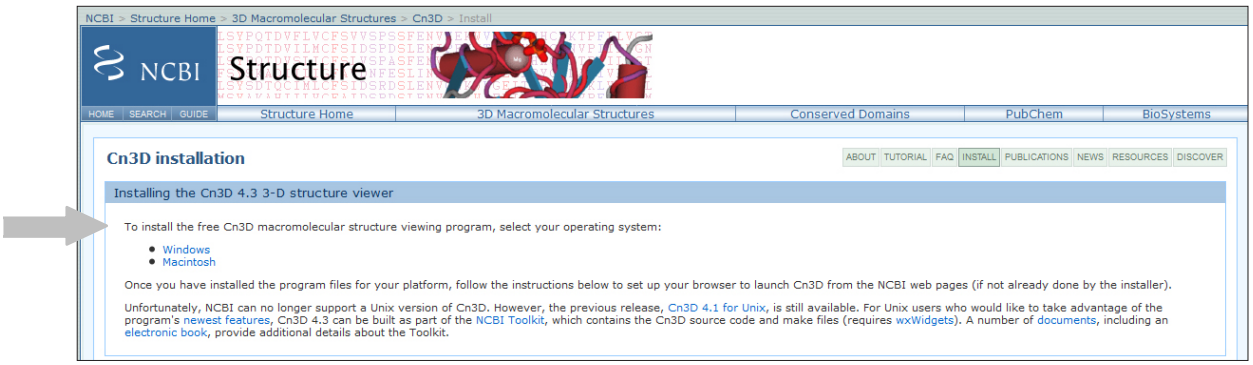


Figure 1: Selecting Your Operating System. Source: Cn3D.

3. Click the link to ***Download the Cn3D 4.3 installer here***.

4. Close all internet browsers and then double-click on the .msi file, or click ***Run*** when prompted. This will launch the Windows Installer application if you have it already. If so, enter the information at the prompts, and then you are done. If not, or if Windows tells you that you need a newer version of the installer software, you can download the latest Windows Installer from Microsoft® by following the appropriate links listed on the download page for your operating system.

5. Once Cn3D is installed, you can click the link ***View Structure in Cn3D*** from any ***Structure*** page at the NCBI. If you are using a Mac, just double click the downloaded file and Cn3D will launch automatically.