

Enzyme/Substrate Reactions



Teacher Guide



ScienceBridge/UC San Diego

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Content written/prepared by the following:

UCSD - ScienceBridge

Jeremy Babendure

Alegra Bartzat

Maggie DeAngelis

Heather Gastil

Shelley Glenn Lee

Heather Liwanag

Johnnie Lyman

James Short

Cover Image: Portabella mushrooms, from which the tyrosinase enzyme is extracted for use in this lab.

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1 Program and Lab Overview

ScienceBridge Program

About ScienceBridge

ScienceBridge is a Science Outreach Initiative based at University of California, San Diego (UCSD) that serves secondary school teachers and students by connecting students to current and relevant scientific research through classroom activities, university experiences and community events. The foundation of the ScienceBridge program is our Teacher Professional Development program, from which the following activity was developed at UCSD in collaboration with local science teachers and is now offered as a training and implementation package for the high school classroom.

One primary goal of ScienceBridge is to create very affordable and accessible labs that engage students with authentic science experiences. We work to optimize each activity to minimize the dependency on expensive equipment and other resources sometimes lacking at a school site. In doing so, we have created activities that can be implemented in virtually ANY classroom, but are also able to be “ramped up” or have added complexity to challenge more advanced students or to utilize available classroom resources. ScienceBridge also supports and is helping to optimize student-run biotechnology sites within specific school districts that will allow materials to be available and sustainable over time, eliminating dependency on external resources.

Professional development & curricula

ScienceBridge’s Teacher Professional Development strives to create connections between teachers and scientists, increase teachers’ and students’ access to current scientific information and resources, and encourage the engagement of students as leaders in the classroom. Each ScienceBridge teacher is trained to use the materials and lab protocol created at UCSD and brings a handful of students from his or her science classroom. These student leaders will learn to use the resources and serve as teaching assistants and resident “experts” in the classroom during activity implementation. All student and teacher input is encouraged and considered at all times, such that our training sessions, curriculum, and resources are the most effective and useful to the audience.

We are very pleased to offer these resources to you and hope you have a great experience with this lab activity!

For more information and program updates, visit:

sciencebridge.ucsd.edu

Lab Description

Enzymatic reactions

Enzymes are critical to biochemical reactions inside every living thing on Earth, from the tiniest viruses and simplest bacteria to the most complex of mammals. Enzymes are proteins that speed up specific reactions within cells. For instance, our food is broken down by enzymes (e.g. milk protein by the enzyme **lactase**, starch by **amylase**), our DNA is “unzipped” during transcription by the enzyme **helicase**, and our body defends itself from bacterial infection by destroying the bacterial cell wall with **lysozyme**. Like all biological proteins, enzymes are difficult to see — their interactions with substrates and the products they help create are not easily observed. This ScienceBridge lab allows students to visually observe enzymatic reactions using a common enzyme that is easily obtained, manipulated and observed in the classroom.

The enzyme **tyrosinase** is widely distributed throughout the living world — it is present in bacteria, plants, animals, and fungi — and is responsible for the browning of fruit, the fur color of certain animals, and skin pigmentation in human beings. It is easily extracted from mushrooms and, when reacted with the substrate L-DOPA, will create a red/brownish product that can be seen within a test tube and measured against a colorimetric chart. From this reaction, students can measure reaction rate and compare enzymatic rates under different conditions such as a change in pH, increased temperature, and in the presence of an inhibitor.

After completing the lab, students will have a better understanding of enzyme structure and function and will have gained experience with both quantitative and qualitative methods of data collection and analysis, including graphing. Students learn what factors (pH, temperature, inhibitors) alter protein structure, and understand that protein structure is critical to function.

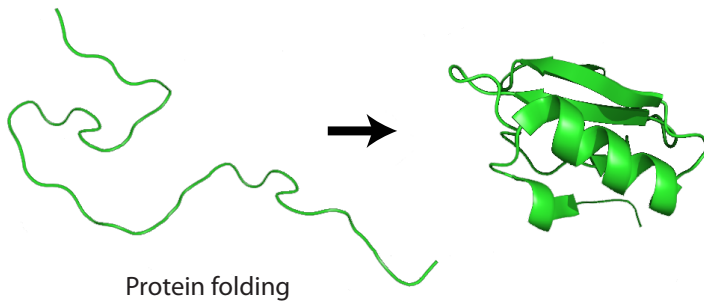
The ScienceBridge Enzymatic Reactions lab is useful to both Biology and Chemistry teachers, as it covers the biological function of enzymes, protein structure and function, and reaction rates. It is an affordable and highly visual lab that will engage any level of student and can be adapted to allow for extended investigation and experimentation.

Appendix 1 – Enzymes are special proteins

All living things are constructed of proteins that are produced by cells following the DNA-RNA-protein pathway. Proteins make up our flesh and blood, carry oxygen to our lungs, and regulate our bodily functions. Enzymes are proteins, too. They aid in digesting our food, creating pigment in our skin, and are required for DNA replication, elimination of toxins, and other vital bodily functions. From viruses to mammals, enzymes are required by every living thing to exist and survive.

Enzymes are macromolecules created from a chain of amino acids folded into a unique 3-dimensional shape. Enzymes differ from other proteins due to the presence of an “active site”—the location where interactions and reactions between molecules takes place. This unique characteristic allows enzymes to aid in biochemical reactions by speeding up the process of building or breaking down substances. The enzyme acts on molecules by providing the conditions for chemical bond creation or breakage. **See Appendix 2**

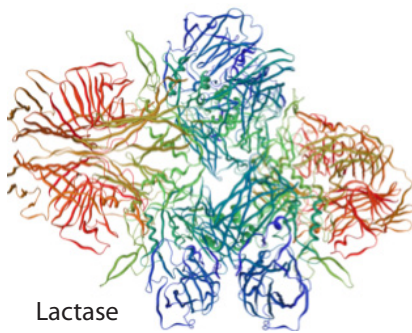
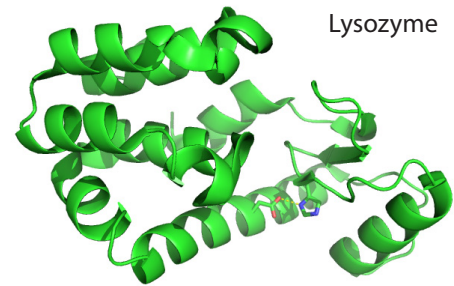
Often, enzymes are named for the substrate they interact with (i.e. protein) and adding the suffix –ase (“prote-ase”).



A few examples of enzymes are:

Lysozyme

A naturally occurring enzyme (part of the innate immune system), found in our saliva and nasal drippings, lysozyme acts like scissors to cut peptide bonds that make up the cell wall of bacteria, thus destroying and killing potentially harmful organisms. Viruses also create and use lysozyme to break into and infect bacterial cells.



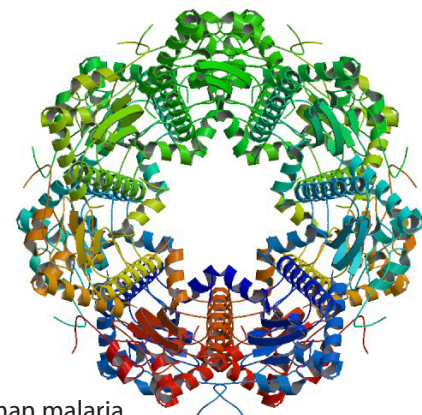
Lactase

The enzyme found in the digestive system of mammals, including humans that drink milk and/or eat milk products. It helps to break down milk proteins into simple sugars that can be utilized by the body. **See Appendix 3**

Protease

A group of enzymes that break down proteins in the body into smaller molecules through the hydrolysis of peptide bonds (belonging to the larger enzyme group **hydrolases**). For this reason, proteases are utilized in detergents and soaps.

Similar to protease, there are many digestive enzymes which break down larger molecules (e.g. **amylase** breaks starch into sugars, **lipase** breaks down lipids into fatty acids) into smaller counterparts through hydrolysis.

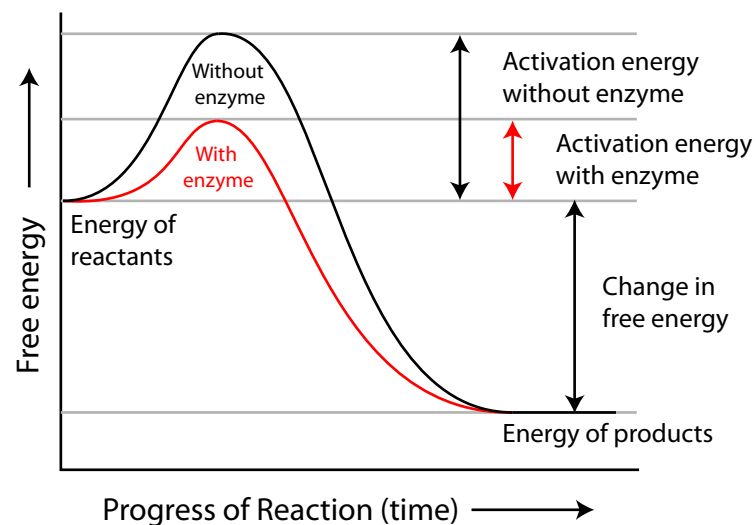


Appendix 2 – Enzymes act as a Biological Catalyst to assist and speed up biochemical reactions.

A **catalyst** speeds up a chemical reaction without being consumed in the reaction. Enzymes are biological catalysts—they are required for specific biochemical reactions to occur within living organisms. Enzymes help to create **products** by either breaking down pre-existing chemical bonds and molecules or building new ones. When products are created, the substrate is used up, but the enzyme is still present.



Enzymes lower the **activation energy** of a reaction by creating an environment that allows for reactions to occur faster and with less required energy. They sometimes achieve this state by altering the shape of the substrate to allow for easier breakage or connection of chemical bonds.



Some chemical reactions will occur without enzymes, but benefit from the catalytic nature of the molecule. An analogy would be a human being attempting to go up and over a mountain pass. Walking may take days, but the journey could be made. By using a bicycle, the time it takes to complete the journey is reduced significantly. A stronger catalyst, such as a car, can speed the process even further. Some enzymes are weak catalysts (like the bicycle) and some are strong (such as the car).

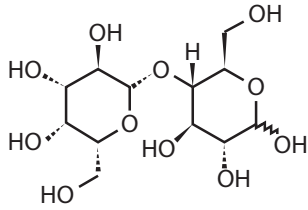
Another analogy could be made regarding the building of a product from two or more existing molecules (**anabolic** reaction) and the breaking down of a molecule into two or more sub-units (**catabolic** reaction). If there are two people who would be a great couple, but they do not know each other, the chances of them meeting are low and the time it may take for that meeting could be long. A matchmaker acts as a catalyst to bring two people together faster and more efficiently than if the match was made on its own. Along the same line, a relationship could be broken up by a rumor or event that was presented to the couple by an outside entity. This person or entity is a catalyst for a breakup, as it weakens the bond between the two people.

An example of an **anabolic** biochemical reaction is the creation of granulated sugar from fruit sugar through bond formation. The enzyme **sucrose synthetase** helps to form sucrose sugar (white granular sugar) from glucose and fructose (fruit sugars).

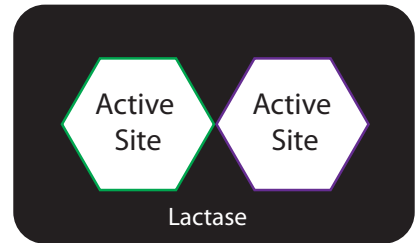
See Appendix 3

Appendix 3 – Enzymes act as biological catalysts to assist and speed up biochemical reactions.

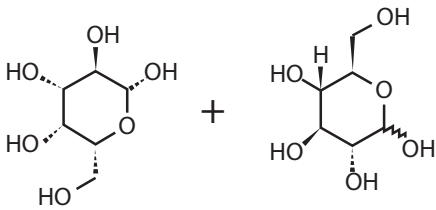
Enzymes catalyze particular reactions and only work with specific substrates. For example, lactase is an enzyme that breaks down milk protein. Lactase does not react with any other protein or substance other than milk protein. (and, vice versa-- lactose, the milk protein, is unable to be broken down/digested without the enzyme lactase being present).



SUBSTRATE



A common catabolic reaction is the digestion or breakdown of milk protein (lactose) by the enzyme lactase into simple sugars (glucose and galactose).



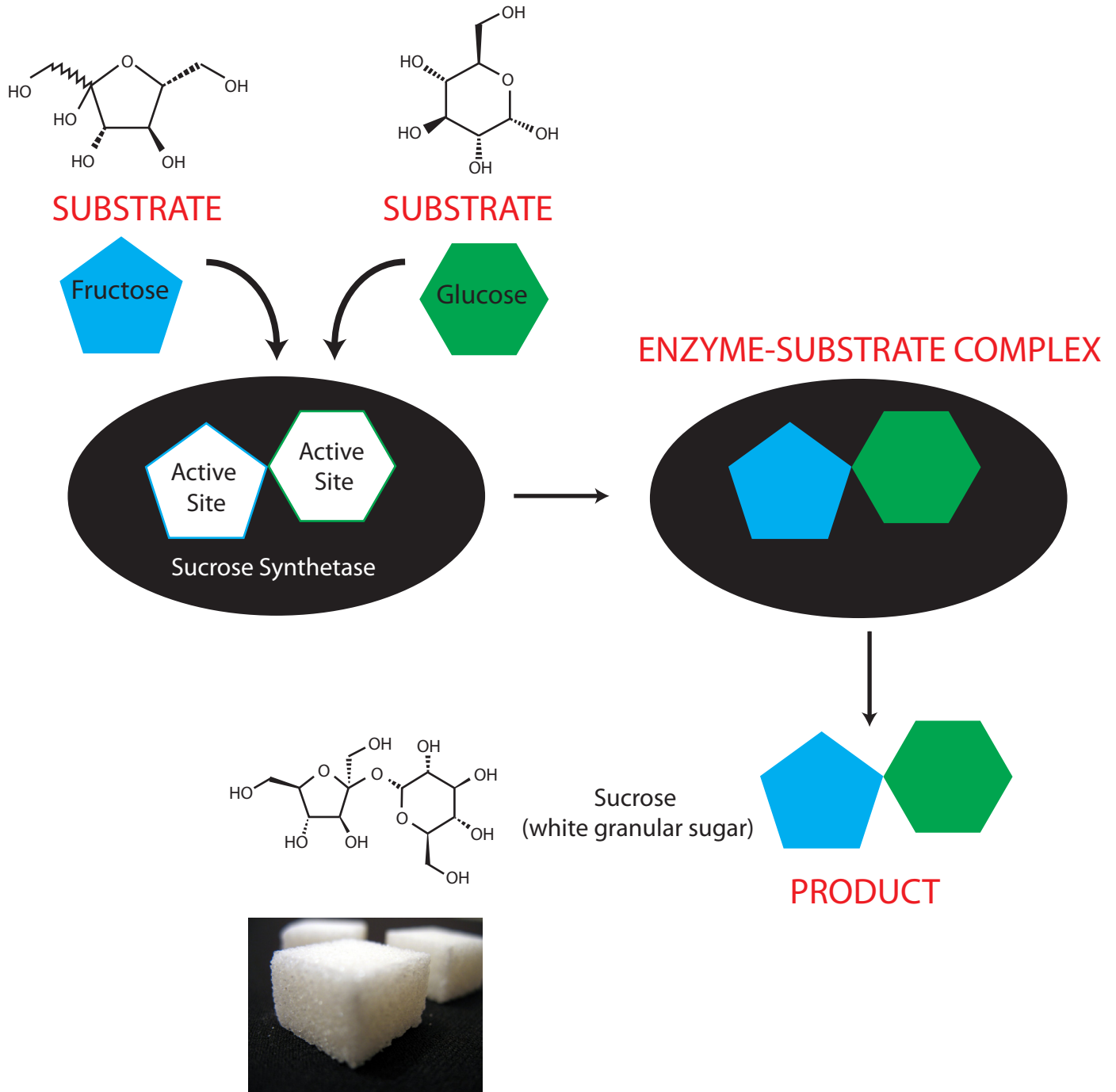
PRODUCTS



The specificity of enzymes is due to different characteristics of the enzymes and substrates including shape, charge, and hydrophobic/phyllic properties.

The shape of the active site and the fit of the substrate has been referred to as a **lock and key model**, where, like a jigsaw puzzle, the substrate and enzyme connect. However, many scientists believe that the enzyme will change shape to fit the substrate into the active site. This is known as the **induced fit model**.

The enzyme sucrose synthetase combines two separate substrates (glucose and fructose) into a single molecule (sucrose). This type of reaction is representative of an anabolic reaction. Glucose and fructose are both simple sugars (monosaccharides) and sucrose synthetase combines them into a polysaccharide (sucrose).



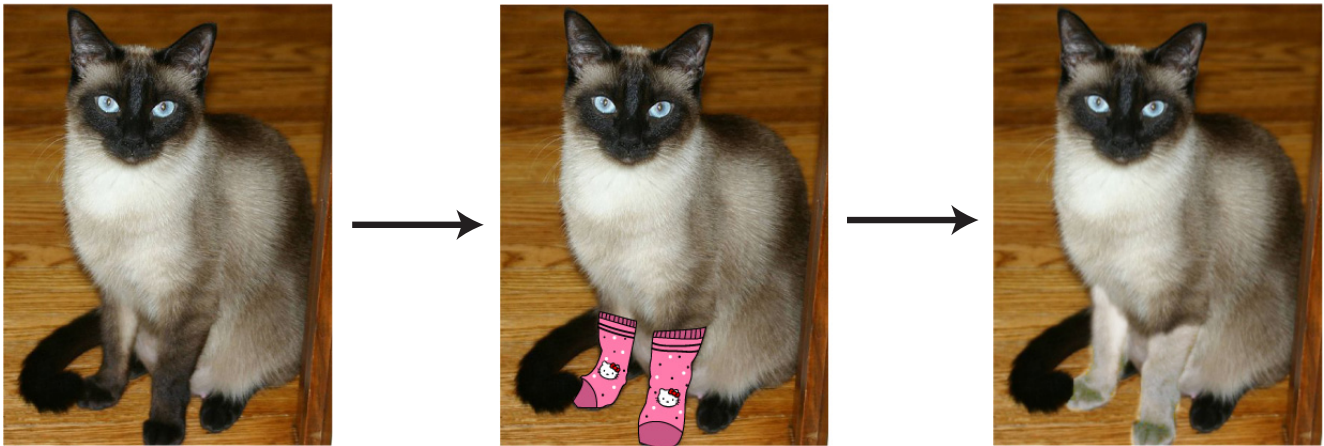
Appendix 4 – Enzymes are sensitive to changes in pH and temperature.

Most enzymes are only functional within a specific, narrow range of pH and temperature. Enzymes in humans and other mammals will not function if the body temperature rises or drops too much. Digestive enzymes in our stomachs function in a highly acidic environment, and will not work outside of the stomach where the pH is higher and less acidic.

Enzymes can be denatured (rendered useless) by heating or other factors that change the shape, thus the specificity and function, of the enzyme. They can be “unfolded” and can sometimes re-fold to function properly under the right conditions.

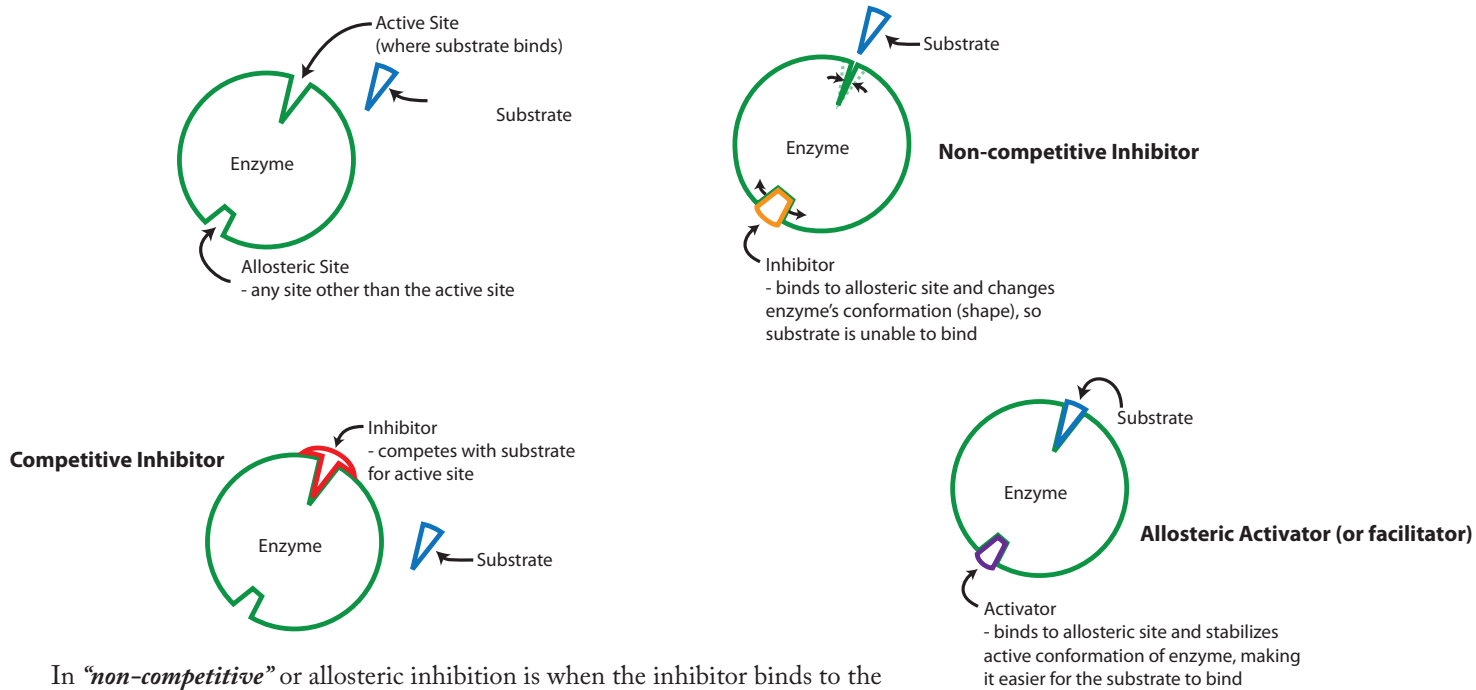
Tyrosinase, the enzyme used in the lab activity, is an example of an enzyme that is temperature sensitive. It is optimally functional within a specific temperature range in most organisms (e.g. humans at body temperature), but is uniquely dependant on temperature in Siamese cats. For example, the color pattern observed most frequently is black face, black tail, black paws, with a brown or blonde mid-section (like the cat on the left).

The black pigment is a result of tyrosinase activity at the extremities, where temperatures are significantly cooler than the rest of the body. At these locations, tyrosinase is actively working to produce melanin, resulting in dark fur. Theoretically, if socks were to be applied to the cat for a long period of time (enough time for the previous fur to fall out and new fur to grow in), the now warmer front legs would reduce the activity of tyrosinase in that area, resulting in lighter fur (like the cat on the right).



Appendix 5 – Enzymes activity can be slowed or prevented by the presence of inhibitors.

Inhibitors are natural or synthetic molecules that do not allow the enzyme to react with the substrate. In “*competitive*” inhibition, the inhibitor and the substrate compete for the same enzyme. If the inhibitor binds to the active site, the enzyme cannot function properly and cannot react with the substrate.



In “*non-competitive*” or allosteric inhibition is when the inhibitor binds to the enzymes at another location other than the active site, thus changing the shape of the protein structure and altering the active site enough that the substrate cannot bind. An allosteric activator creates the correct shape for the substrate to bind to the enzyme.

Inhibitors may slow down or completely prevent enzyme function. By understanding inhibitors, scientists can apply what they know to creating and utilizing inhibitors for the benefit of health and medicine, economic growth, and other human concerns. Both naturally occurring and synthetically produced inhibitors are utilized in our everyday lives.

Examples of inhibitors

Common preservatives used in food products are actually enzyme inhibitors. **Sodium benzoate** is a common preservative used to preserve flavor, prevent browning, and to give a longer shelf life to the product.

Enzyme inhibitors are commonly used as insecticides and chemical warfare agents—regular enzyme function within the nervous system can be blocked or slowed, resulting in death. Acetylcholinesterase (AChE) is the enzyme targeted by inhibitors found in insecticides and chemicals such as serum (nerve) gas. AChE is found in many animals and it functions to break down the neurotransmitter acetylcholine in the synaptic cleft (for more info on cholinesterases, see **Appendix 6**). Reversible competitive inhibitors have been identified and manufactured into antidotes used to treat people exposed to nerve gas or other similar agents.

Some common drugs that act as enzyme inhibitors are Viagra, HIV protease inhibitors, and some cancer drug treatments (chemotherapy).

Tyrosinase, the enzyme used in this lab, has many inhibitors, both natural and synthetic.

Appendix 6 – Cholinesterases

Cholinesterase is an enzyme found in the circulatory and nervous systems of many animals, including humans. This enzyme acts to metabolize a neurotransmitter, acetylcholine, that functions in both the peripheral nervous system and central nervous system. Acetylcholine activates muscles, stimulates the body (when excited) to release substances such as sweat and epinephrine (adrenaline), and plays a significant role in addiction.

Two similar yet distinct cholinesterases exist: “acetylcholinesterase” and “butyrylcholinesterase”. Both are equally effective, but work on different sizes of choline substrates. Acetylcholinesterase converts acetylcholine into the inactive metabolites choline and acetate. This enzyme is abundant in the synaptic cleft, and its role in rapidly clearing free acetylcholine from the synapse is essential for proper muscle function. Certain neurotoxins work by inhibiting acetylcholinesterase, thus leading to excess acetylcholine at the neuromuscular junction, causing paralysis of the muscles needed for breathing and stopping the beating of the heart. The buildup of acetylcholine allows for an increase in the level and duration of activity in the junction.

The cholinesterase active site is buried deep in the globular protein, and the molecular construct of the enzyme creates a streamlined gorge straight to the active site, allowing for very quick in-and-out action on the substrate.

Inhibitors of acetylcholinesterase occur naturally as venom and poisons, and are used as nerve agents in terrorism attacks and warfare (e.g. nerve gas or serum gas). Humans have also taken advantage of the work of cholinesterase inhibitors by creating pesticides and insecticides that act on the nervous system of ants, ticks, aphids, and other invertebrates.

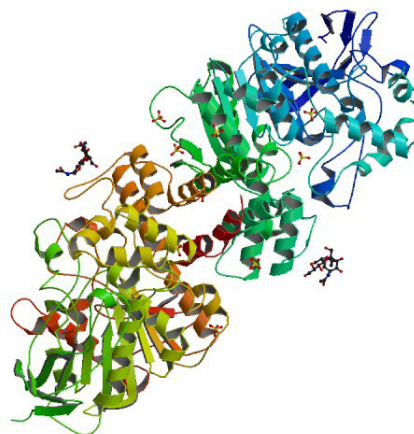
Read more about cholinesterases and the research being conducted at UCSD’s School of Pharmacy

<http://pharmacy.ucsd.edu/faculty/TaylorLab/>

1906?	Discovery of cholinesterases
1960’s-1970’s	Inhibitors and substrates found that bind and interfere with catalytic reaction of cholinesterase
1980’s	Protein Purification techniques allowed for identification of cholinesterase active site and gene sequence to allow for cloning of gene that encode for cholinesterase
1990’s	Three-dimensional structure of a cholinesterase was solved
2000’s	Determination of >125 primary structures and >60 three-dimensional structures of cholinesterase

Fun fact

- Acetylcholine was the first neurotransmitter to be identified.



Human recombinant acetylcholinesterase

Appendix 7 – Albinism and Tyrosinase

Albinism is an autosomal, recessive genetic condition which is characterized by a lack of pigmentation (melanin) in fish, amphibians, reptiles, birds, and mammals — including human beings. The lack of pigmentation is due to a mutation in the gene that codes for tyrosinase. Without tyrosinase, the products needed to form melanin cannot be produced.

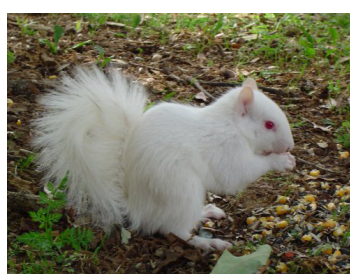
Because melanin creates color in skin, eyes, and hair, any albinistic organism will appear white or very pale with light blue eyes (or eyes appearing reddish due to the retinal blood vessels shining through).

Approximately 1 in 70 people is a carrier of the albinism gene, leading to an incidence of 1 in 17,000 among human beings. While there are different forms of albinism with various causes, one type with a frequency of 1: 40,000 persons is oculocutaneous Type 1 (OCA1) which is caused by a mutation in the tyrosinase gene, TYR. Albinistic persons with OCA1 produce little or no melanin leading to white, almost translucent hair and skin. They tend to have poor eyesight.

Since albinistic persons produce no melanin as a result of the tyrosinase mutation, they cannot tan and must be careful about long exposure to sunlight. They are particularly susceptible to melanoma, a very deadly form of skin cancer.



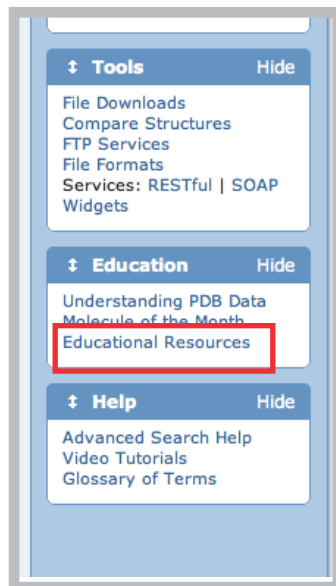
Two heterozygotes for albinism will have a 1:4 chance of having albino offspring.



Appendix 8 – Protein Data Bank

Enzymes and other proteins are complex structures which can be difficult to visualize. The Protein Data Bank contains images of many proteins to help illustrate the structure and function of individual proteins. Programs for visualizing these proteins are free and available to teachers. The following guide shows how to download a protein and some ideas for use in the classroom. This process takes you through the protein data bank, but if you just wish to download the visualization software, go to www.pymol.org and follow the instructions on pg. 122 of this guide. If you just want a quick view of proteins without downloading software, simply go to www.rcsb.org and type in a protein type in the top search bar. See instructions for this on pg. 123 of this guide.

To access the Protein Data Bank




Go online to www.rcsb.org and this will take you to the homepage for the protein data bank. This page contains a wealth of information on molecular structures.

Scroll down the page, looking at the left side menu and click on **Educational Resources** (pictured above). This takes you to a page of educational resources based on molecules in the PDB. In the first paragraph on this page, you will see a link to **Looking at Structures**. Clicking on this link takes you to information about the PDB and how it can be used to visualize a variety of structures, along with providing a brief introduction.

Educational Resources

[Kiosk Viewer](#) | [Posters](#) | [Website Tutorials](#) | [Animations](#)

These Educational Resources are aimed at teachers and students
 For researchers and educators interested in how to understand PDB
 please see the [Looking at Structures](#) feature.



Molecules in Motion Kiosk Vi

The **Molecules in Motion Kiosk Viewer** is a full
 components within the structure.

On the Understanding PDB page, there are several sections to help you make sense of this database. Downloading a visualization program to your computer allows you to bypass any internet issues you may have at your school during class time. Under the Visualizing Structures heading, you will see a blue link to **Visualization Programs**. Click on this link to see what visualization programs can help you illustrate about various proteins, including options for shapes, colors, and structures.

header section of text that summarizes the protein sequence and a long list of the atoms and their coordinates determine these atomic coordinates.

- **Visualizing Structures**
While you can view PDB files directly using a text editor, tools, such as the ones on the RCSB PDB website, allow on **experimental methods** and the chemistry and bio may use **visualization programs** to allow you to see pictures of it. These programs also often include analyzing structural features.
- **Reading Coordinate Files**

Toward the top of the new page you will see **Molecular Graphics Software Links**. This will take you to a large list of molecular graphics software options. Depending on your potential level of use, this page contains some excellent links to additional materials available online. During the training, you were shown proteins in a program called PyMOL. Follow these directions to download and use this program.

try each one to find which one fills your needs the best. The MBT

ed methods to create high-quality images and images of complex

Molecular Graphics Software Links if you are interested in

- **Mole**
- **Progr**
- **Reso**
- **R-val**
- **Struc**
- **Elect**

r in specific arrangements. Since they are far too small to see with developed several ways to do this, each way highlighting different

Click the link for **PyMOL** on the top, right-hand side of the page. This is the visualization software you will need to download to look at proteins.

links

- **PyMOL**
A free and open-source molecular graphics system and publication-quality imagery. PyMOL is scriptable Python language. Supports Windows, Mac OSX, U
- **QuteMol**
An open source (GPL), interactive, high quality m exploits the current GPU capabilities through Ober

fit; displays multiple
i-bond identification,
d-screening interface
create custom

Download the PyMOL program. This can run on Linux, Mac OSX or on Windows. When you click this link it will take you to a registration page. On this page, there is a link for students and instructors. Click **pymol.org/educational** to go to the free education version.



please contact licensing@schrodinger.com for assistance.

Open-Source and Educational Users: Please consult & participate in the [PyMOL Community Wiki](#) in [Official PyMOL Downloads and Documentation site](#) is for [PyMOL Sponsors](#) only.

Students and Instructors

Please visit <http://pymol.org/educational> for educational versions of PyMOL.

Fill in the necessary information in the registration. *Warning* The registration letters the form asks you to fill in to confirm your registration can be very hard to read! You will receive your download credentials via your e-mail address, so make sure this website will not be blocked by your e-mail. Click on the link sent in the e-mail to download PyMOL. A username and password will be provided in the e-mail and will be asked for when you click on the download link.

Registration For Educational-Use-Only PyMOL Builds

I am a:

Your First Name:

Your Last Name:

Your Email Address:

Your Telephone Number:

Institution:

Comments (optional):

Class Name:

Class Number:

Class Duration (through date):

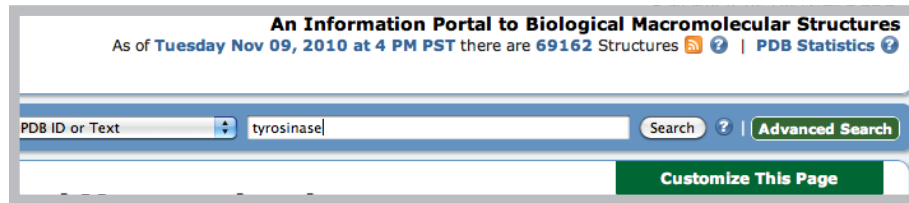
Once you have entered your username and password, download information will come up. Right click on the download information for your appropriate system and download the program. Once you have the program downloaded and opened, you will need some proteins to look at! To do this, return to www.rcsb.org to find some proteins of interest.

To download, right-click for "Save Target As...", "Save Link As...", or "Download Linked File".

Current Release

- **PyMOL 1.3r1 edu** (Sept 2010)
 - Windows: [pymol-v1.3r1-edu-Win32.msi](#)
MD5SUM: 265fa652aa4880304f4a59e1af653a1b
 - Macintosh: [MacPyMOL-v1.3r1-edu.tar.bz2](#)
MD5SUM: 260793a054ef791987af9e6300ed1ce0
 - Linux: [pymol-v1.3r1-edu-Linux-x86-TclTk8.5.tar.bz2](#)
MD5SUM: 96da619d58ec137bbf05769e9f59ce4b

To start viewing proteins, there are two approaches. The simplest approach is to type in a protein in the search bar on rcsb.org to view that protein. In this example, we have typed in the enzyme tyrosinase.



A wide variety of articles on the protein you chose may appear. Some will be more relevant than others, so you may need to scroll down and find the particular protein you are looking for. In this example, we will look at the first hit for tyrosinase. Click on the picture of the tyrosinase protein to choose this model.

2ZWD Crystal structure of the copper-bound tyrosinase in the deoxy-form obtained by soaking the deoxy-form crystal in dioxygen

Authors: Matoba, Y., Sugiyama, M.

Release Date: 2009-12-15

Experiment: X-RAY DIFFRACTION with resolution of 1.35 Å

Compound: 2 Polymers [[Display Full Polymer Details](#) | [Display for All](#)]
2 Ligands [[Display Full Ligand Details](#) | [Display for All](#)]

Citation: Not Available.

A new page will come up with a large version of the protein, as pictured below. Below that picture there is a link which says **View in Jmol**. Click this link to view the protein. The protein can be rolled around via mouse click to be viewed in any orientation.

Biological Assembly

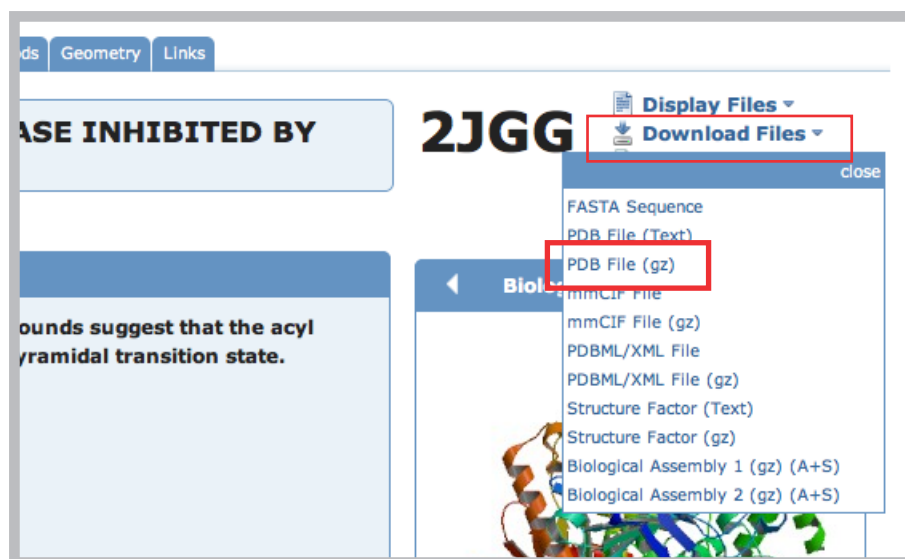
More Images...

View in Jmol SimpleViewer
Other Viewers Protein Workshop

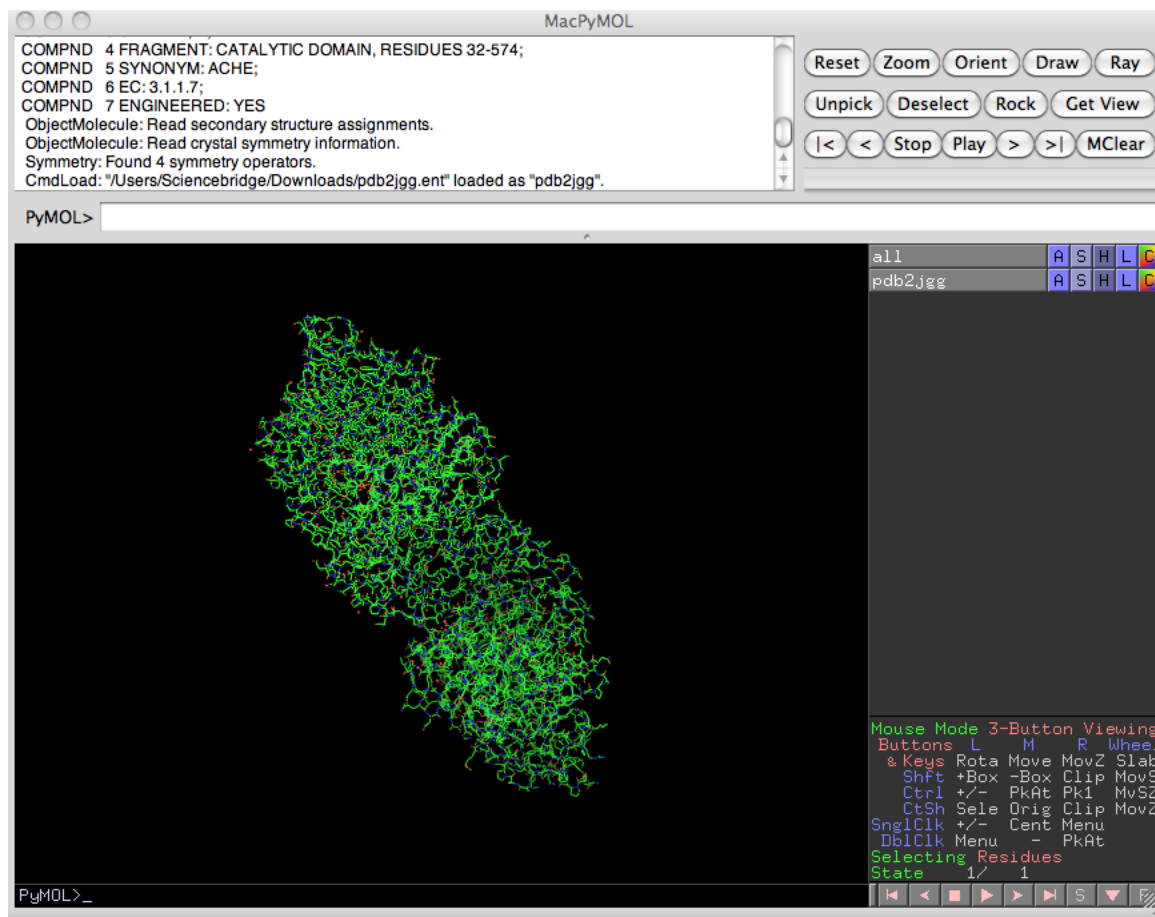
Biological assembly assigned by authors and generated by PISA (software)

PyMOL

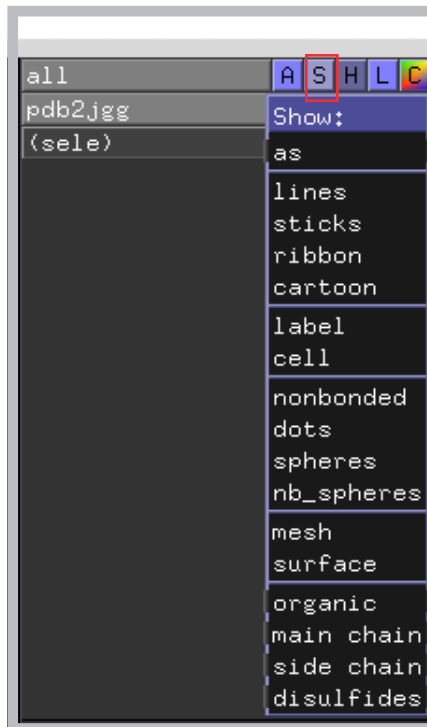
To download the protein you want to view in PyMOL, look for the 4 digit alphanumeric code in the top right corner of the page. Click on **Download Files** and the following menu will appear. Click on **PDB file (gz)** to download the correct file type to be run in PyMOL. When this file is finished downloading, open it using PyMOL to view your chosen protein.



Once you have opened your molecule in PyMOL, it should resemble the screen below. Click on the molecule and move your mouse around to change the orientation of the molecule.



To change the appearance of your protein, click on the letter “S” (for Show) beside your molecule’s ID. The menu picture below will appear. Click on the different appearances to see which version of the protein you want to use. To remove these options, click on the “H” button (for Hide) and choose which options you wish to hide. Show and hide options until you have reached the desired appearance of your protein.



To customize your protein

If you click on “C” for Color you can choose Color by Chain and choose a color scheme to add a color to each subunit if the protein consists of more than one subunit. You can also select the “S” in the bottom right corner for sequence. This will show you the sequence of the protein at the top. You can click on any single amino acid or many amino acids and choose “S” for show and choose sticks to show the amino acids on the structure.

If you want to create hotkeys for certain snapshots of the protein, place the protein in the desired orientation, go to the top menu and select “Scene” --> Store --> F1. Now F1 will always take you to this specific snapshot. You can do this for other snapshots and select F2, F3, etc. to store multiple snapshots.

A two-button mouse with a wheel will allow for the easiest movement in PyMOL. Left click allows you to select amino acids and holding left click will rotate the protein. Holding right click will zoom in and out, while holding scroll will allow you to move the protein on the screen.

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