
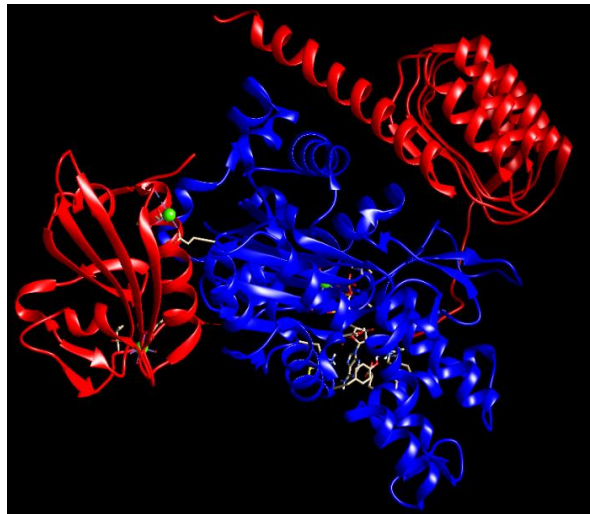
	<p align="center">SKILLS CENTER STANDARD OPERATING PROCEDURE</p>	<p align="center">A BIOFIZZ  PRODUCTON</p>
<p>Chimera Introduction Module Hours:3</p>	<p align="center">Effective Date: 02/05/2021</p>	<p align="center">Revision #1.0 A. Siclair Checked: M. Guzie</p>

BACKGROUND

The Chimera application, established by the Resource for Biocomputing, Visualization and Informatics (RBVI), allows for detailed molecular visualization and interactive assessment of macromolecules (RBVI, 2018). When specifically looking at proteins, this program analyzes characteristics such as chemical makeup, bond formation, and can even be utilized for detailed structure comparison.



The information about specific proteins and their corresponding structures is kept in separate databases and can be converted for use within Chimera. Although the Worldwide Protein Data Bank currently sits at the top of the protein database food-chain, with many smaller databases contributing to it, other reliable sources like the RCSB Protein Data Bank have been around for nearly 50 years and have been accumulating protein information since their creation (Berman et al., 2012). The RCSB Protein Data Bank originally consisted of a mere 7 structures, but now contains documentation about thousands of proteins from numerous different organisms (RCSB PDB).



Actin complex with a chimera of tropomodulin-1 and leiomodulin-1 actin-binding site 2

The most important benefits of protein analysis technology lie in the ability to build upon existing knowledge of structures. Through comparison of various domains, residues, etc., more headway can be made in uncovering homologous regions, functions, and entire proteins between organisms. It is also beneficial for analyzing the role of mutation and protein misfolding and malfunction in disease. Possessing the capacity to magnify such tiny structures and analyze them virtually with a fine-tooth comb is something that could never be feasible without the computing applications of today such as Chimera.

1. PURPOSE

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The purpose of this procedure is to develop an understanding of the UCSF Chimera application and the basic techniques of molecular visualization.

2. SCOPE

This procedure applies to qualified skills center users.

3. RESPONSIBILITY

- 3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
- 3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
- 3.3. It is the responsibility of the user to become trained on the procedure.

4. DEFINITIONS

- 4.1. Protein Data Bank – A database that houses information about 3D macromolecule structures, such as proteins and nucleic acids.
- 4.2. RCSB Protein Data Bank – The first open access digital data resource in all of biology that has been around since 1971.
- 4.3. UCSF Chimera – An application that allows for detailed molecular visualization and interactive assessment of macromolecules.
- 4.4. Chimera X – A condensed version of the UCSF Chimera application that can be used on slower computing devices or with larger molecular structures.



5. MATERIALS/EQUIPMENT

- 5.1. UCSF Chimera Application
- 5.2. RCSB PDB
- 5.3. Worldwide PDB

6. PROCEDURE

6.1. Downloading the Chimera Application

- 6.1.1. Go to the website <https://www.cgl.ucsf.edu/chimera/download.html>

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6.1.2. Go to **Current Production Releases** and download the application that corresponds with the specific computer being used. (Figure 1)

Figure 1

Download Chimera

- [Daily Builds](#)
- [Snapshot Releases](#)
- [Unsupported Releases](#)
- [Old Releases](#)
- [Bug Tracking System](#)
- [Licensing Information](#)
- [Experimental Chimera Features](#)
- [Plug-ins on the Web](#)
- [Graphics Driver Bugs](#)
- [Benchmark Results](#)
- [Chimera Source Code](#)
- [Cygwin Source Code](#)

Tip: Consider trying *ChimeraX*, the successor to Chimera.

Current Production Releases

- See the [release notes](#) for a list of new features and other information.
 - For [more recent changes](#), use the [snapshot](#) and [daily](#) builds; they are less tested but usually reliable.
- **64-bit Releases:**

Platform	Installer, Size, and Checksum	Date	Notes
Microsoft Windows 64-bit	chimera-1.15-win64.exe Size: 152326129 bytes MD5: 95a95db631b7e380a5c29432c1592ba	Dec 18, 2020	Instructions Documentation Runs on Windows 7 or later.
Mac OS X 64-bit	chimera-1.15-mac64.dmg Size: 191085873 bytes MD5: 26c669ae125af17bd89050fb1ae6063c	Dec 18, 2020	Instructions Documentation Runs on Mac OS X 10.12 or later.
Linux 64-bit	chimera-1.15-linux_x86_64.bin Size: 154069682 bytes MD5: 5c8f49ed5d474ed79df944058c78eb39	Dec 18, 2020	Instructions Documentation Compiled on CentOS 5.11.

6.1.3. Follow the directions in the popup window to begin installation.

6.1.4. Make sure to enable desktop icon installation.

6.1.5. Open the application upon installation and allow the final code to load.

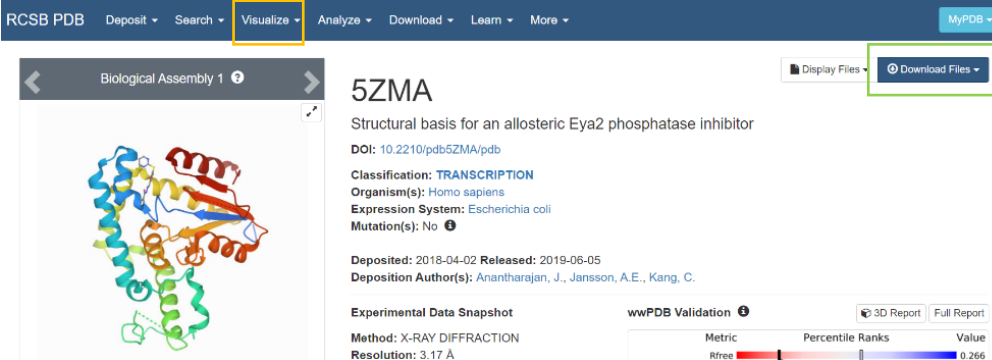
6.2. Finding and Downloading Protein of Interest

6.2.1. Visit the RCSB protein databank website: <https://www.rcsb.org/>

- The Worldwide Protein Data Bank is also an optimal source: <http://www.wwpdb.org/> (But not used in this Procedure).

6.2.2. Search for the protein of interest by clicking **Visualize** and then basic, advanced or sequence search depending on available information. (Figure 2)

Figure 2



RCSB PDB Deposit Search **Visualize** Analyze Download Learn More MyPDB

Biological Assembly 1

5ZMA
Structural basis for an allosteric Eya2 phosphatase inhibitor
DOI: 10.2210/pdb5ZMA/pdb
Classification: TRANSCRIPTION
Organism(s): Homo sapiens
Expression System: Escherichia coli
Mutation(s): No

Deposited: 2018-04-02 Released: 2019-06-05
Deposition Author(s): Anantharajan, J., Jansson, A.E., Kang, C.



Experimental Data Snapshot
Method: X-RAY DIFFRACTION
Resolution: 3.17 Å

wwPDB Validation
Metric: Rfree
Percentile Ranks
Value: 0.266

Display Files **Download Files**

6.2.3. Click on the match that best fits your desired protein (5ZMA as example).

6.2.4. Click the **Download Files** dropdown bar and use PDB format to download into the desired folder. (Figure 2)

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6.3. Importing and Formatting Protein Structure of Interest

6.3.1. Click on the desktop icon for the Chimera application.

6.3.2. Click **File** and **Open**, and then open the downloaded PDB file from your chosen location. (Figure 3)

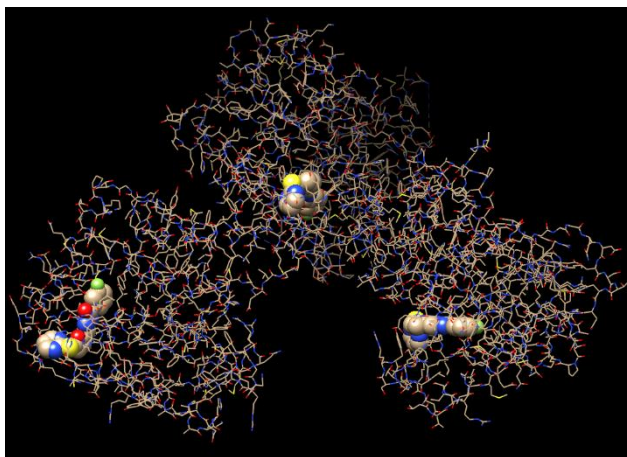
Figure 3





- The structure can be rotated using the left mouse button or touchpad.
- The zoom can be controlled using the right mouse button or touchpad.

6.3.3. Click **Presets** and Interactive Preset 2 in order to color-coordinate all of the non-carbon atoms. (Figure 3)

Figure 4

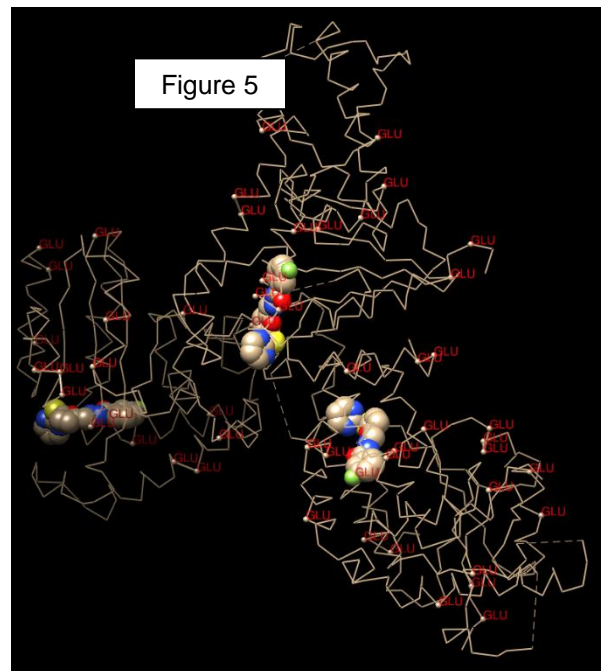


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- The colors are coordinated using the Jmol color system:
<http://www.rbvi.ucsf.edu/chimera/1.10/docs/UsersGuide/colortables.html#byelement>
- 6.3.4. Select specific parts of the macromolecule using the **Select** function. The most basic selector specifications include chain, residue, atom type, chemistry and sequence.
- Selecting specific parts of the protein allows for actions to be applied to only that portion of the molecule instead of the whole structure.
 - Selecting should cause the specified areas to light up in neon green, as well as the magnifying glass in the bottom right corner of the screen.
- 6.3.5. Use the **Action** function to add labels to specific residues, elements, etc. that are selected using the **Select** function.
- 6.3.6. Deselect selected items by clicking **Clear Selection** under the Select tab.



Figure 5 was created using:

1. Clear selection (will apply next steps to whole molecule)
2. Actions
3. Atoms and bonds
4. Backbone only
5. Chain trace
6. Select
7. Residue
8. Glu
9. Actions
10. Label
11. Residue
12. Name
13. Clear selection



6.4. Analyzing the Distance Between Atoms

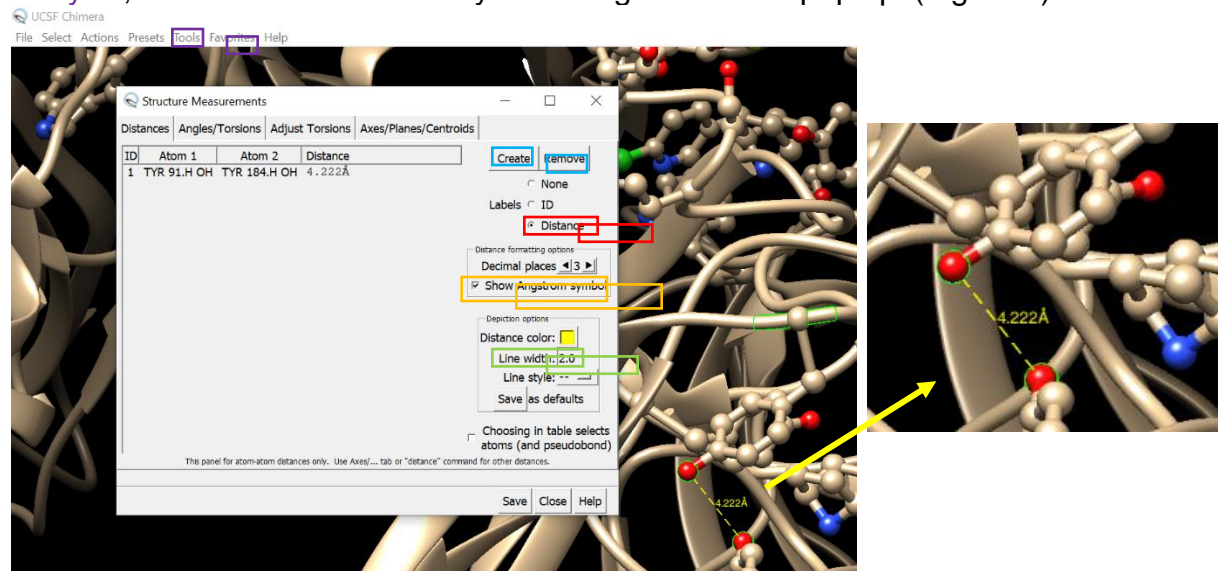
- 6.4.1. Put the molecule in ball-and stick model format using **Actions**, then **Atoms/Bonds**, then **ball and stick**
- 6.4.2. Zoom in and click on a specific atom of interest using Ctrl and the touchpad simultaneously (if on a laptop computer; otherwise, use mouse).
- 6.4.3. Select a second nearby atom of interest using Shift, Ctrl, then the touchpad.

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- Now, both of the atoms of interest should be highlighted in green.

6.4.4. To compare the distance between the two atoms, click **Tools**, then **Structure Analysis**, then **Distances**. An analysis editing box should pop up. (Figure 6)

Figure 6



6.4.5. Ensure that the label value is set to **Distance**, that the **Show Angstrom symbol** box is checked and that the **Line width** is set to a value that is visible. (Figure 6)

6.4.6. Press **Create** to insert the pseudo-bond and distance label onto the image. (Figure 6)

6.5. Saving Final Image



6.5.1. Click **File**, **Save Image**, and pick the appropriate format.

6.5.2. Files may also be saved in PDB format from Chimera, if necessary for use in another application.

7. TROUBLE SHOOTING

7.1. Unable to upload the PDB file into the Chimera application: Re-install the Chimera application and ensure that the file ends with “.pdb” at the end of the name.

7.2. The multimer of interest is only downloading and viewing as a single monomer: Instead of downloading the document directly via PDB format (see step 6.2.4), click one of the **Biological Assembly** tabs. This will require unzipping of the file,



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and potential conversion to PDB format, but will ensure that the entire multimer is retrieved from the site.

- 7.3. The UCSF Chimera application is too slow, or the structure of interest is too large: Although ChimeraX has less tools than UCSF Chimera, it often will run on slower, older computers better than the newer/more detailed application and can be better equipped for larger structures. Try downloading ChimeraX instead.

8. REFERENCES

- Berman H.M., Kleywegt G.J., Nakamura H. & Markley J.L. (2012). The Protein Data Bank at 40: Reflecting on the Past to Prepare for the Future. *Structure*, 20(3), 391-396. <https://doi.org/10.1016/j.str.2012.01.010>
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9. MODULE METHODS TASK

This task will test your knowledge of the basic visualization techniques that can be implemented in the UCSF Chimera application. Choose your own protein from RCSB to complete the following questions.

1. What is the PDB code for your chosen protein?
2. Apply Interactive Preset 2 and submit an image of the protein at this stage.
3. Which color is associated with nitrogen atoms? With oxygen atoms?
4. Label all lysine residues in the protein and submit an image of the resulting labeled structure.
5. What is the distance between the N-terminus and C-terminus of the protein? Submit a picture of the labeled image with the distance in Angstroms and the dotted yellow pseudo-bond.
6. Select all hydrophobic residues and change the color to blue. Select all hydrophilic residues and change the color to red. Submit the resulting image.
7. Select chain A of the protein, and then use the Sequence function under Tools to run the sequence of that specific chain. Submit the result.
8. Select a portion of the protein and apply the Surface setting. Change the color of the protein surface to green.