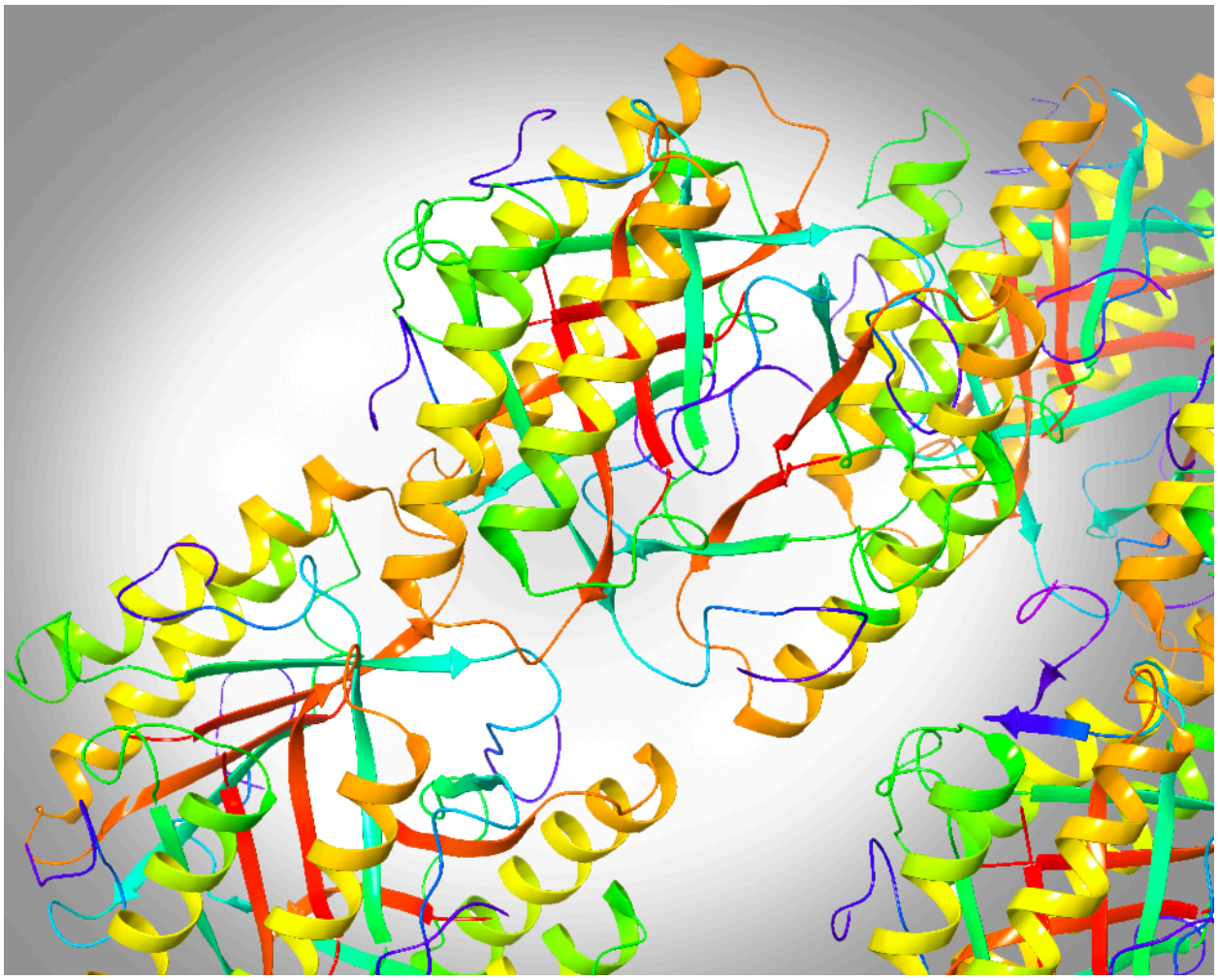
Enzymes



Enzymes

About this Lesson:

Enzymes are biological catalysts, and nearly all of them are proteins. Understanding the structure and function of enzymes is crucial because they accelerate key metabolic processes that are necessary to sustain life.

Using Maestro, students will learn how to use the protein preparation workflow and glide docking panels to prepare a quality enzyme structure, predict substrate binding modes, as well as analyze enzyme-substrate interactions within the binding site.

Learning Objectives:

- Define a catalyst and explain how enzymes work, including inhibitory mechanisms
- Prepare and visualize the enzyme structure using the protein preparation panel
- Assess enzymatic activity by simulating enzyme-substrate binding using Glide
- Analyze enzyme-substrate interactions using the Ligand Interaction panel

Lesson Contents:

Standards Alignment:

- Connections to AP
  - Chemistry of Life (Unit 1)
- AAMC MCAT
  - Biological and Biochemical Foundations of Living Systems (1A)
- IB Diploma Programme
  - Enzymes (Core Topic 2.5)
- ACS Guidelines:
  - Biological Reactions - (Conceptual Topics)

Assessments for Understanding:

The following types of formative assessments are embedded in this lesson:

- Assessment of student understanding through discussion of warm-up questions and filling in any knowledge gaps about the structure and function of enzymes
- Visual assessment of student-prepared enzyme structure, simulated substrate-active site binding, and creation of 2D interaction diagrams

Associated Documentation Pages: Glide Documentation

Warm-Up Questions:

Read the Khan Academy article on Enzymes and answer the following questions:

1) What is a catalyst? How do they work?

2) Give an example of catalysis in your daily life. Identify the catalyst in your example.

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1. Setting Up the Maestro Session

At the start of the Maestro session, it is essential to 1) check your mouse actions, 2) change the file path to the Working Directory for this lesson, and 3) save your project file. The working directory indicated in this section contains the files necessary to complete this lesson. If you do not set the appropriate working directory, you will be unable to run any calculations.

1. Launch the Virtual Cluster

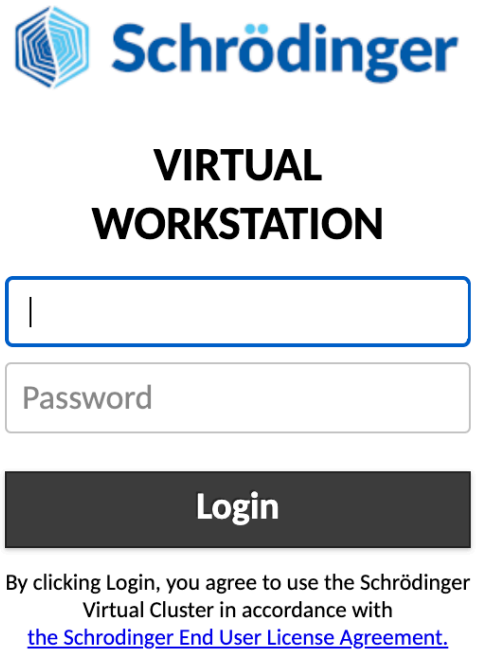

Figure 1-1. Virtual workstation login page.

2. Double-click the **course-data** folder on the desktop

Figure 1-2. Course-data folder on the desktop.

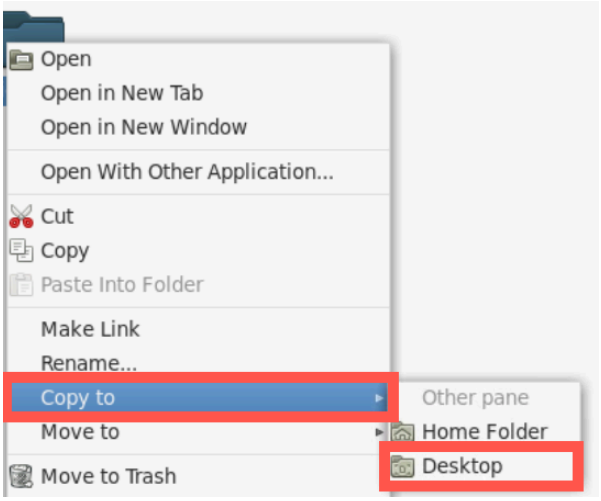

Figure 1-3. Copy the lesson folder to the Desktop.

3. Right-click the Enzymes folder and select **Copy to > Desktop**

4. Double-click the Maestro icon on the desktop

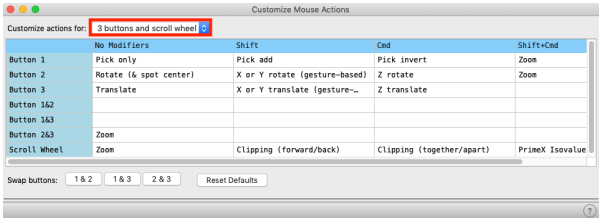

Figure 1-4. Change the mouse actions.

5. Check your mouse actions.
    - Go to **Workspace > Customize Mouse Actions**
    - *Note:* This lesson was made with a three-button mouse with a scroll wheel, but a trackpad can still be used
    - ***Trackpad keys:***
        - **Up/Down trackpad** = Zoom In/Out
        - **Option** = Rotate
        - **Control** = Translate

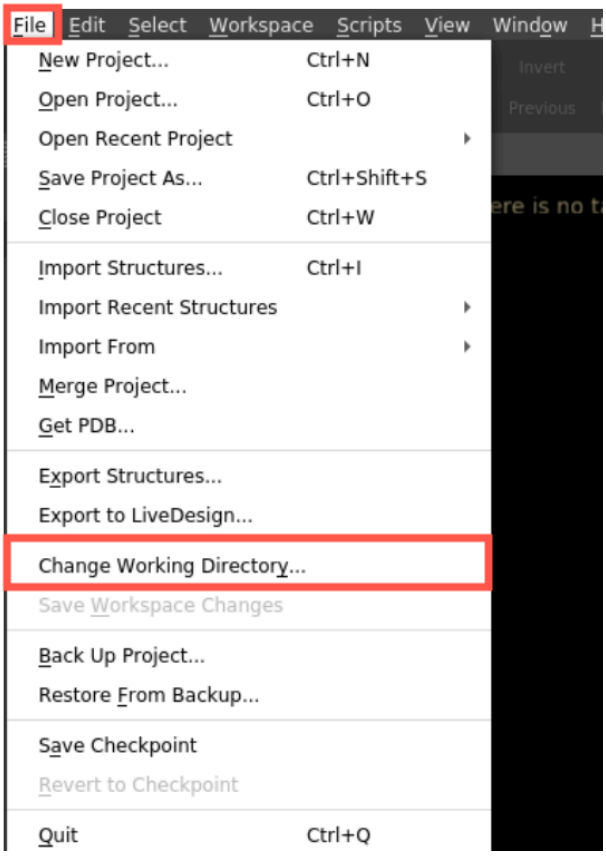

Figure 1-5. Change Working Directory option.

6. Go to File > Change Working Directory

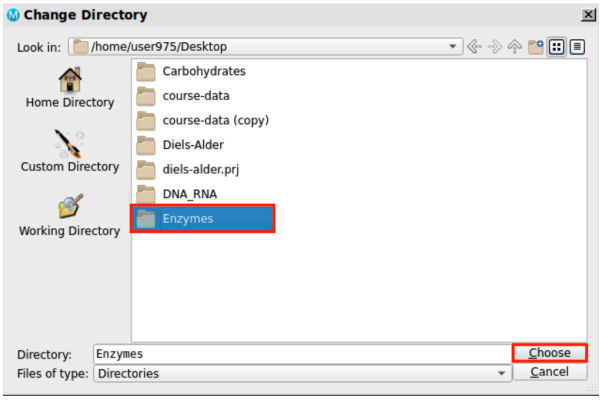

Figure 1-6. Change Working Directory panel.

7. Navigate to Desktop > Enzymes folder and click **Choose**

Pre-generated input and results files are included for running jobs or examining output

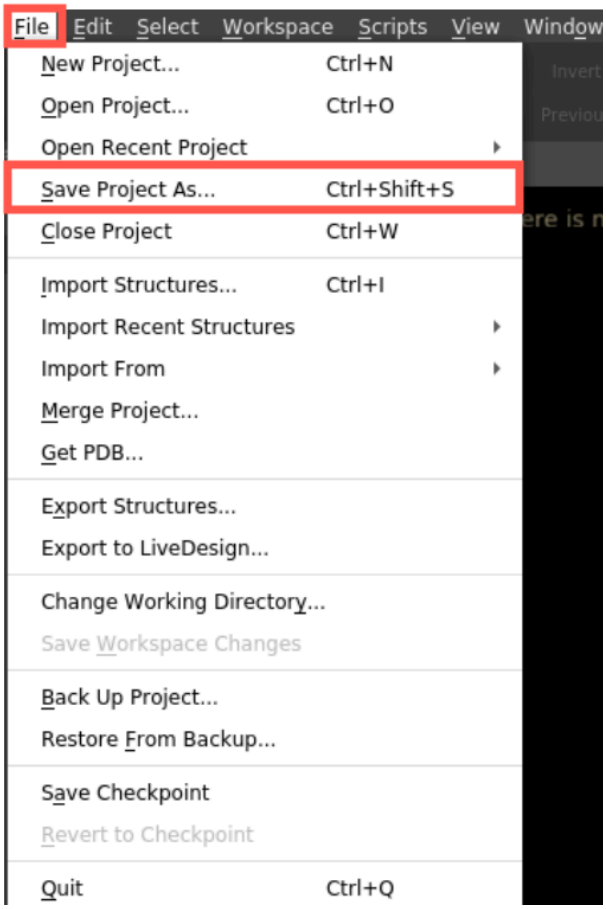

Figure 1-7. Save Project option.

8. Go to File > Save Project As

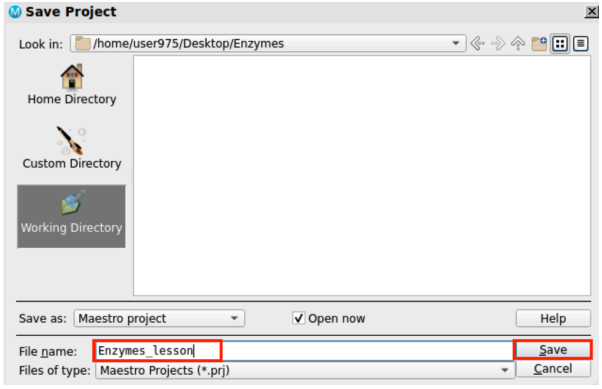

Figure 1-8. Save Project panel.

9. Change the *File name* to `Enzymes_lesson`, click Save
    - The project is now named Enzymes_lesson.prj

2. What are enzymes?

Enzymes are biological polymers that catalyze biochemical reactions in our body. They are crucial players in the human body and are very important for cells to live and function. For example, the food that you eat is broken down by different enzymes known as digestive enzymes, such as amylases, proteases, and lipases. Most enzymes also happen to be proteins and constitute a linear chain of amino acids. The sequence of amino acids in an enzyme specifies its three-dimensional structure, which in turn determines its catalytic activity.

Enzymes are **catalysts**, which means that they speed up the rate of reactions without being consumed by it. The molecules that enzymes act upon are called **substrates** and the part of the enzyme where the substrate binds is called the **active site**. This is where all the catalytic activity takes place, and results in the formation of different molecules known as **products** as can be seen in **Figure 2-1**.

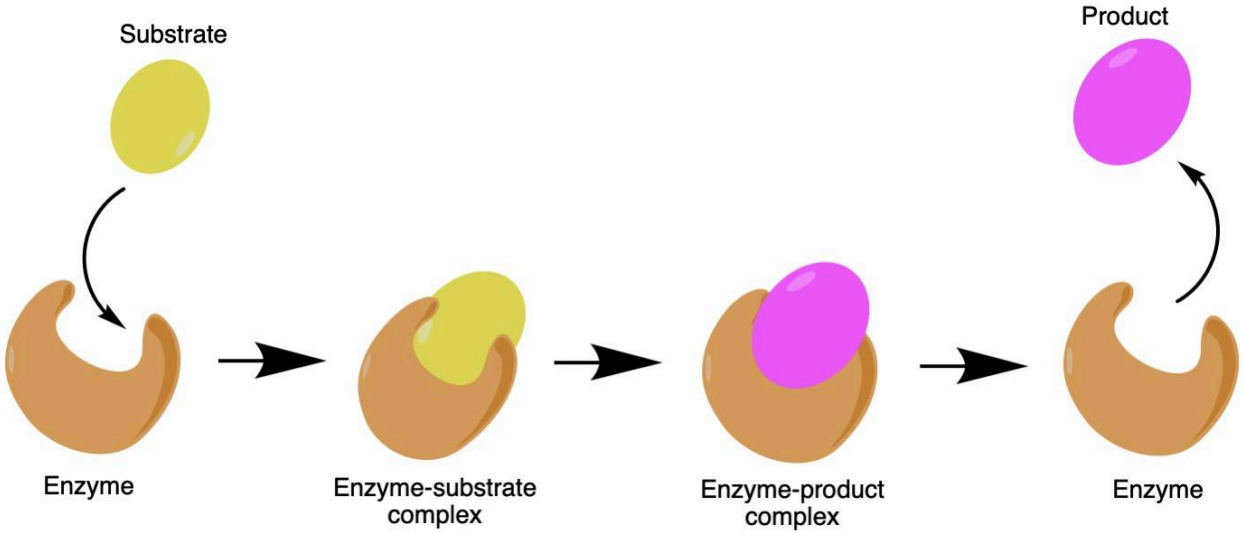

Figure 2-1. Basic stages of an enzymatic reaction

Although enzymes help catalyze important chemical reactions in living organisms, in order to function effectively, biological systems must also be able to regulate the activity of enzymes. This is where special agents called **inhibitors** come into play. Inhibitors are substrates that bind onto enzymes in order to block the enzyme's activity. There are many different types of inhibitors. **Competitive inhibitors** are chemicals that typically resemble the substrate and can therefore directly bind to the active site of the enzyme. Once bound, the competitive inhibitor prevents the substrate from occupying the active site. **Noncompetitive inhibitors**, also known as **allosteric inhibitors**, do not compete with the substrate for the active site. Rather, they bind to a different area on the enzyme known as the **allosteric site**. Both competitive and non-competitive inhibitors can be **reversible** or **irreversible**. Finally, **feedback inhibitors** are the end products of an enzymatic reaction. They inhibit the enzyme that helped produce them by binding to the allosteric site. To visualize the mechanism of inhibition, take a look at **Figure 2-2** below.

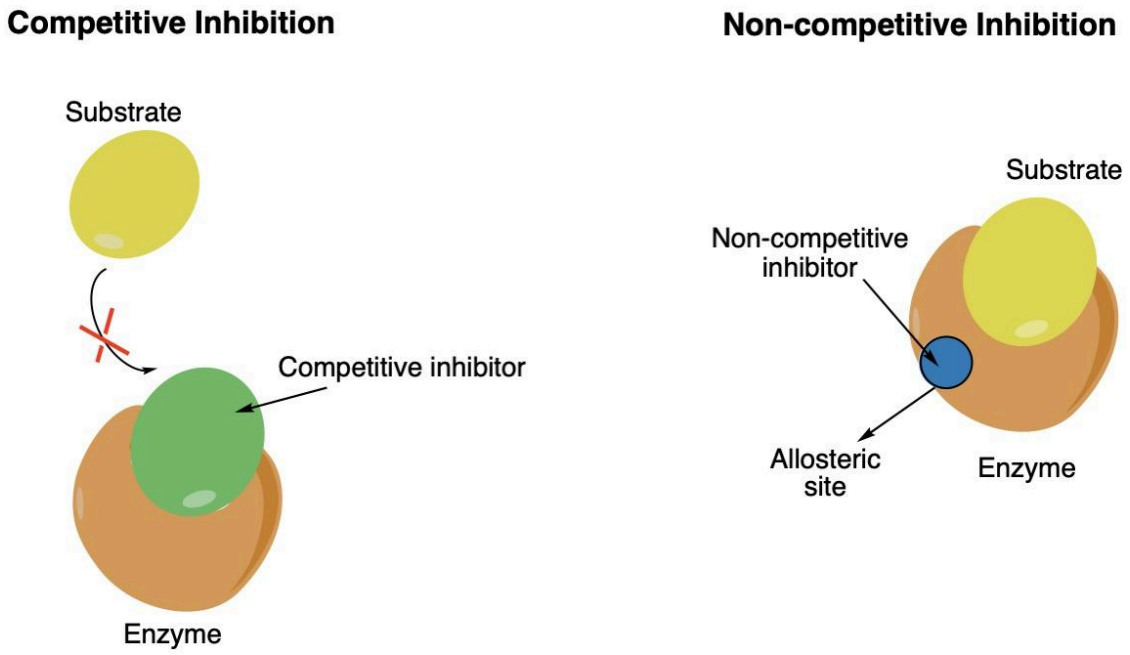

Figure 2-2. Competitive versus non-competitive inhibition

3. Enzyme structure

Enzymes are generally **globular proteins** that react in highly regio- and stereo-specific ways with dissolved solutes. The specificity of enzymatic activity is in turn determined by the enzyme's amino acid sequence. While some amino acids do not play a role in catalytic reactions, the amino acids that do get involved in chemical catalysis are called **catalytic residues**. These residues have side chains that can be large or small, hydrophilic or hydrophobic, and acidic or basic. It is these characteristics that determine the properties of an enzyme's active site. The sequence of amino acids found in the enzyme's active site, along with their positions in 3D space, give the active site a very specific shape and size that makes it suited to bind only to a particular substrate. Enzyme active sites also typically create **non-polar environments** in which bonds can be broken and formed more easily. Unless water participates in the actual reaction, it is usually excluded from the active site. This hydrophobic effect, along with other noncovalent interactions like hydrogen bonds and van der Waals forces, helps the substrate bind to the active site, while simultaneously preventing any unwanted reactions.

Active sites are much smaller than the actual size of an enzyme, and enzymes are much larger than the substrate molecules they bind with. On the smaller end, enzymes can range in size from 62 amino acid residues, such as in 4-oxalocrotonate tautomerase (4-OT), and on the larger end, enzymes can also comprise of over 27,000 amino acid residues, like in the enzyme titin.

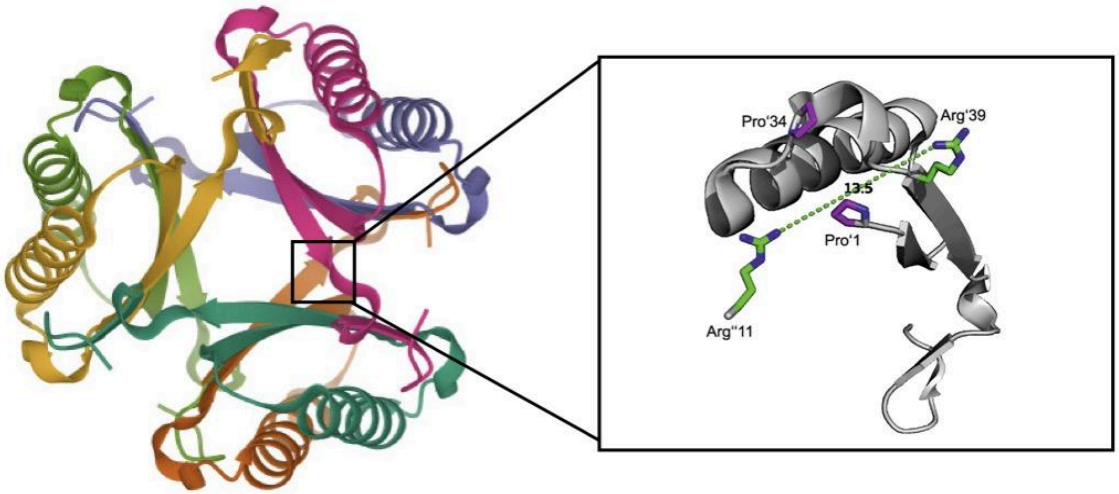

Figure 2-3. Zooming into the active site of enzyme 4-OT (PDB: 4X19)
Lukesch, M.S.; Pavkov-Keller, T.; Gruber, K.; Zangger, K. Substituting the catalytic proline of 4-oxalocrotonate tautomerase with non-canonical analogues reveals a finely tuned catalytic system. *Scientific Reports.* **2019,** 9 (1). DOI:10.1038/s41598-019-39484-9

Practice #1. Name the catalytic residues visible in the active site of 4-OT. Which amino acid residue in **Figure 2-3** contributes to the hydrophobic effects of substrate-active site binding? *Hint: nonpolar residues are often hydrophobic.*

4. Enzyme Classification

According to the International Union of Biochemistry and Molecular Biology (IUBMB), there are six major classes of enzymes. These classifications were formed based on the type of reaction in which the enzymes are used to catalyze and are detailed in **Figure 3-1** below.

Enzyme Type	Reaction Mechanism
Oxidoreductases	Catalyze oxidation and reduction reactions
Transferases	Help transport functional groups among donor and receptor molecules
Hydrolases	Catalyze hydrolysis reactions by adding water to break bonds
Lyases	Catalyze reactions by creating double bonds
Isomerases	Catalyze structural shifts in the substrate molecule
Ligases	Catalyze the binding of two substrate molecules

Figure 3-1. Major classifications of enzymes

Practice #2. Look up the role and function of the following enzymes and classify them according to the IUBMB nomenclature.

1) Pyruvate dehydrogenase

2) DNA ligase

3) Kinase

4) Phosphoglucomutase

5) GTPase

5. Simulating enzyme-substrate binding

In this activity, you will be analyzing the TBK1 (TANK-binding Kinase 1) enzyme bound to an inhibitor compound. TBK1 is an enzyme with **kinase** activity. Specifically, TBK1 is a **protein kinase** that catalyzes the transfer of phosphate groups from high-energy, phosphate-donating molecules to specific substrates.

Why are we examining TBK1? Well, TBK1 happens to play a critical role in inflammatory signaling and is overexpressed and activated in bladder, lung, breast, and colon cancers. TBK1 is also thought to drive oncogenesis by phosphorylating and activating the AKT1 gene, which further promotes cancer growth. TBK1 is thus a potentially interesting target in cancer therapy.

Computational Exercise: Enzyme-substrate binding in Maestro

This exercise involves five parts:

1) Import and prepare the TBK1-inhibitor complex in Maestro using protein preparation workflow
2) Prepare the inhibitor compound using ligprep
3) Generate a receptor grid to prepare for docking
4) Predict and analyze substrate binding poses using Glide Docking
5) Visualize enzyme-substrate interactions after docking

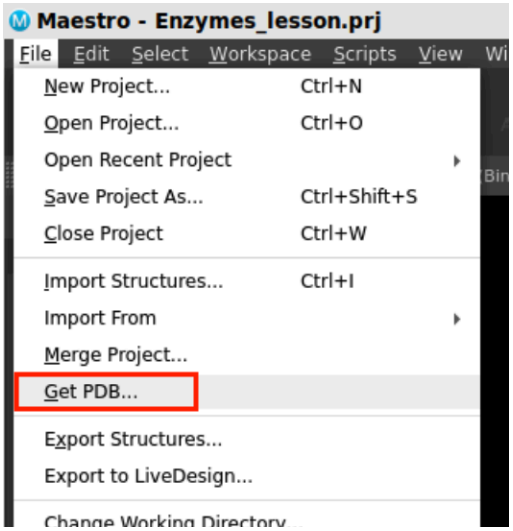

Figure 4-1. Importing structure from PDB database

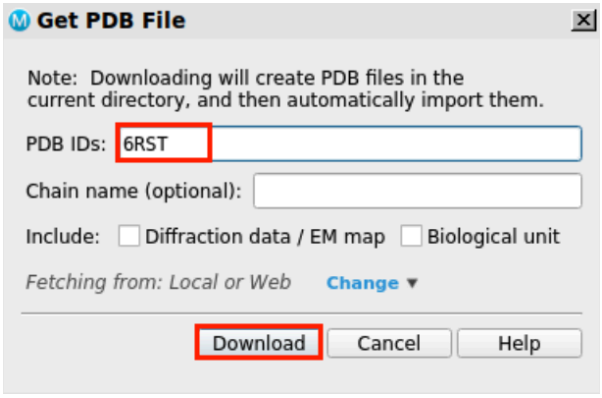

Figure 4-2. Get PDB File panel

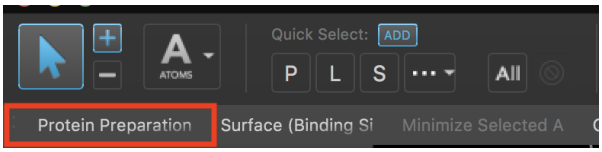

Figure 4-3. The Protein Preparation Workflow in the Favorites toolbar.

Part 1. Import and prepare TBK1-inhibitor complex

When you import a protein directly from a PDB file, it is not a prepared protein, rather it is a protein in its raw state. This means that the structure might have missing hydrogens, partial charges, side chains or whole loop regions. So, you cannot directly run calculations on this structure, such as Glide docking for instance. To resolve these issues, we must first prepare the protein using the Protein Preparation Workflow.

1. Go to > **File** > **Get PDB**
2. For PDB IDs, type "6RST"
3. Click > **Download**
    - 6RST is loaded into the Workspace
    - A banner appears

Note: Banners appear when files have been imported, jobs incorporated into the Entry List, or to prompt a common next step. Here, preparing the enzyme complex will be covered below.

4. In the Favorites toolbar, click Protein Preparation
    - The Protein Preparation Workflow opens

Note: You can also click Prepare in the banner or find the Protein Preparation Workflow in the Task Tool

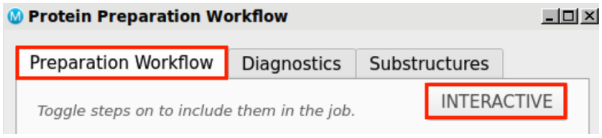

Figure 4-4. The Protein Preparation Workflow in Automatic mode.

5. In the Preparation Workflow tab, confirm the INTERACTIVE button is off
    - When on, the pane will read Protein Preparation Workflow (interactive)

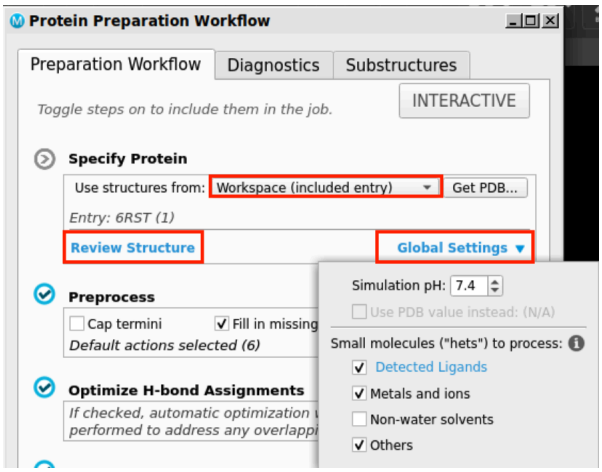

Figure 4-5. Specify the protein from the Workspace.

Note: INTERACTIVE mode can be used for exploring manual options, or to run a single protein in a step-by-step manner. This tutorial will be running an automatic protein preparation.

6. In the Specify Protein section, choose use structures from: **Workspace**
7. Select **Global Settings**
    - A dropdown opens showing the simulation pH and the PDB pH as well as small molecule options
    - The Non-water solvents option is left unchecked. This saves computational resources because the glycerols in our Workspace will not be prepared

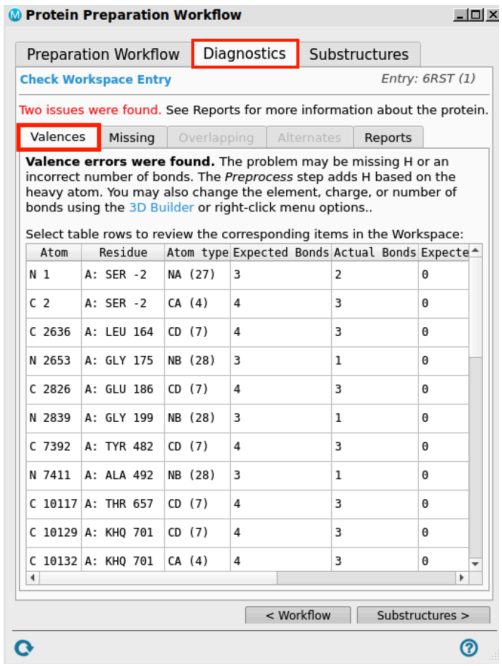

Figure 4-6. View protein issues in the Diagnostics tab.

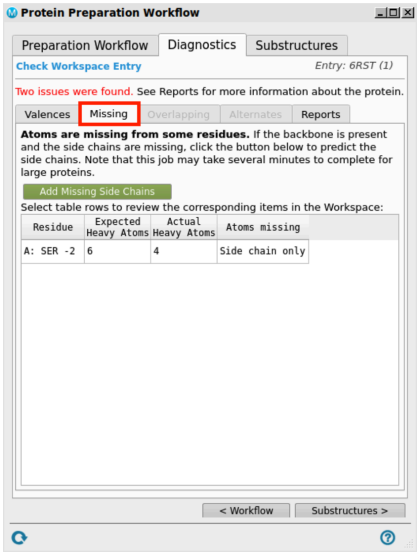

Figure 4-7. View missing side chain in the Diagnostics tab.

Note: The **Specify Protein** tool provides you with the option to prepare a protein from the Workspace, Project Table, File, or directly from the PDB.

8. Select **Review Structure**
    - The substructures tab opens to show Ligands, Metals, Other, Waters, and Chains

Note: our structure only contains one chain.

9. Select the **Diagnostics** tab
    - Valence errors were found, but these bond order issues will be resolved in the protein preparation
    - The Missing tab indicates one missing side chain.
10. Select **Reports** to view other issues with the protein that must resolved prior to modeling

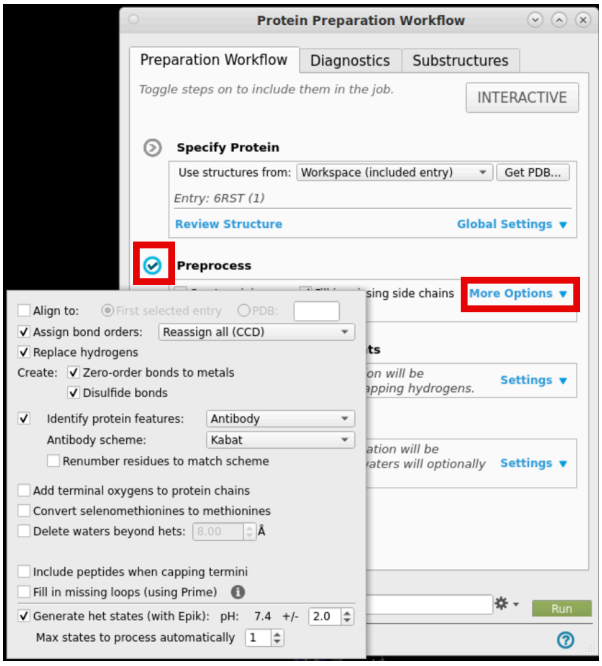

Figure 4-8. Preprocess default settings.

11. Return to the **Preparation Workflow** tab
12. Confirm **Preprocess** is toggled on
    - Notice Fill in missing side chains is checked by default. Since these are missing in our structure, they will become populated during this step.
13. Under Preprocess, select **More Options**
    - Among the options provided, notice that missing loops may be filled in using Prime
14. Check the pH range for generating het states with Epik
    - This should align with the physiological or assay pH.

Note: Depending on your system and research question, you may want to keep certain waters. See Protein Preparation Workflow Panel Help for more details.

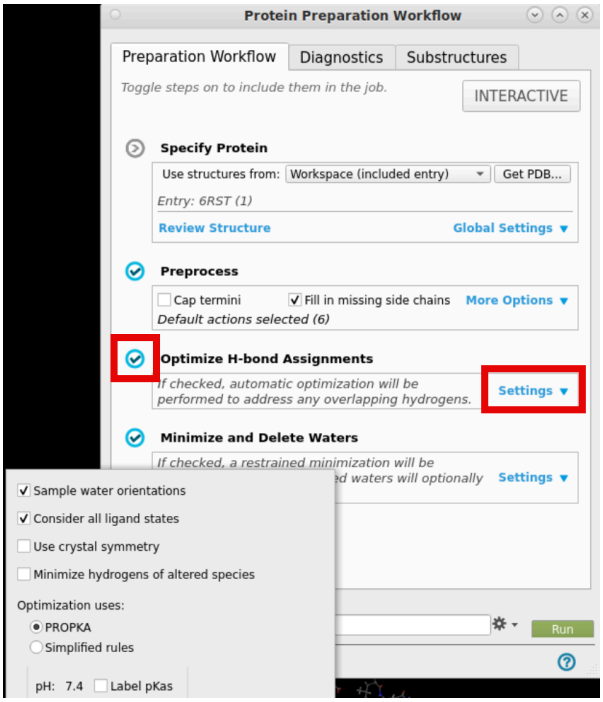

Figure 4-9. Optimize H-bond default settings.

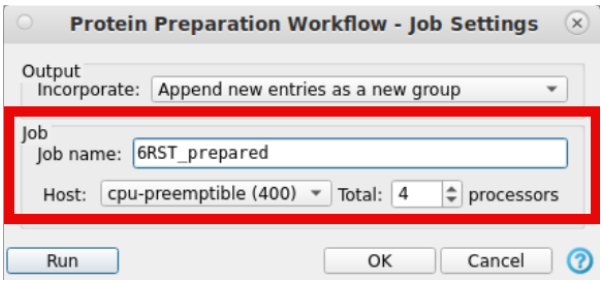

Figure 4-10. Running the protein preparation workflow.

15. Confirm **Optimize H-bond Assignment** is toggled on
16. Click **Settings**
    - Overlapping atoms caused by the addition of hydrogens during the Preprocess step will be corrected, and side chains may be flipped when this job is run
17. Check the pH for Optimization
    - This value should be captured in the pH range chosen during the Preprocess step
18. Change the job name to **6RST_Prepared**
19. Adjust the job settings ( )
    - Host: **cpu-preemptible (400)**
    - Total: **4 processors**
20. Click **Run**
    - This job requires a 4 CPU host and should complete in under 5 minutes. All toggled jobs are running consecutively.
    - A new group will be added to the Entry List
21. In the Entry List, click **6RST_ prepared**

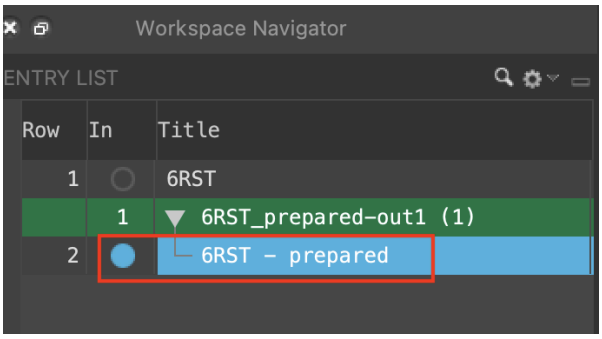

Figure 4-11. Select the protein preparation output

Figure 4-12. Load workspace entry in the substructures tab.

22. Return to the **Protein Preparation tool**
23. Click the **Substructures** tab
24. Choose **Load Workspace Entry**
25. In the Waters table, you can see that all waters have been removed during processing.

Note: Depending on your system and research question, you may want to keep certain waters. See Best Practices for Protein Preparation for more details.

26. Click the **Diagnostics** tab to make sure there are no issues missed during the preparation. You may need to click **Check Workspace Entry**
27. **Exit** the Protein Preparation Workflow

Note: If issues persist after preparation, perform specific interactive protein preparations on the modified protein with adjusted settings. The job type will depend on which problems were found.

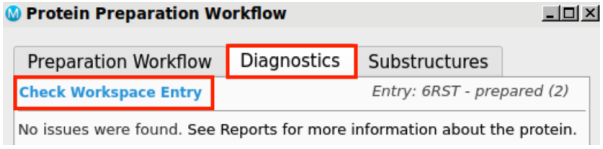

Figure 4-13. Confirm issues have been resolved during preparation.

Pause & Think #1: List one error that you identified to be a problem prior to running the Protein Preparation Workflow. Was it resolved after getting prepared?

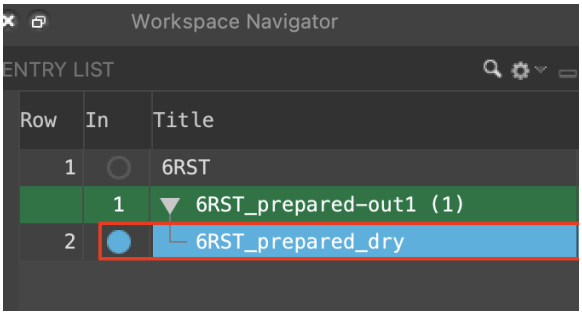

Figure 4-14. Rename prepared enzyme to 6RST_prepared_dry

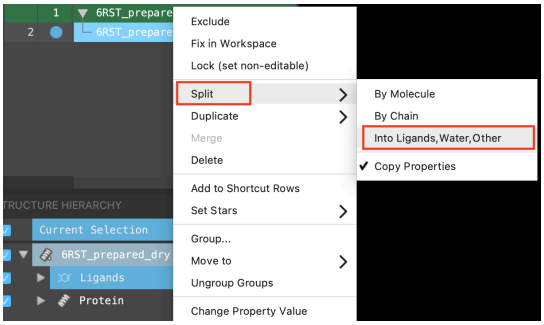

Figure 4-15. Right-click to split an entry into different components.

Part 2. Prepare inhibitor compound

1. In the Entry List, double click 6RST_ prepared in the 6RST_prepared-out1 group and rename the entry to **6RST_prepared_dry**
2. Right-click on **6RST_prepared _dry**
3. Choose **Split > Into Ligands, Water, Other**
    - Two new entries appear in the Entry List
4. Include **6RST_prepared_dry_ligand**
    - Only the ligand is displayed in the Workspace
5. Go to **Tasks > Browse > LigPrep**
    - The LigPrep panel opens
6. For Use structures from, choose **Workspace (1 included entry)**

Figure 4-16. Include 6rst_prepared complex into workspace

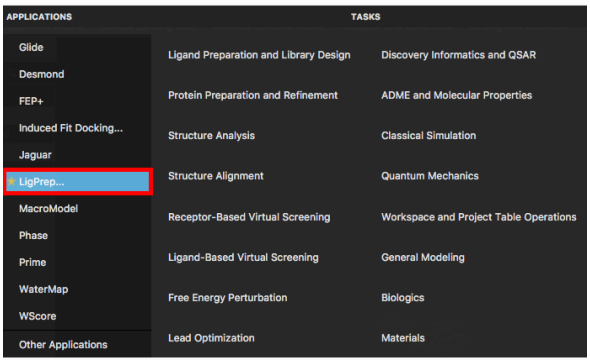

Figure 4-17. LigPrep application in the Task toolbar.

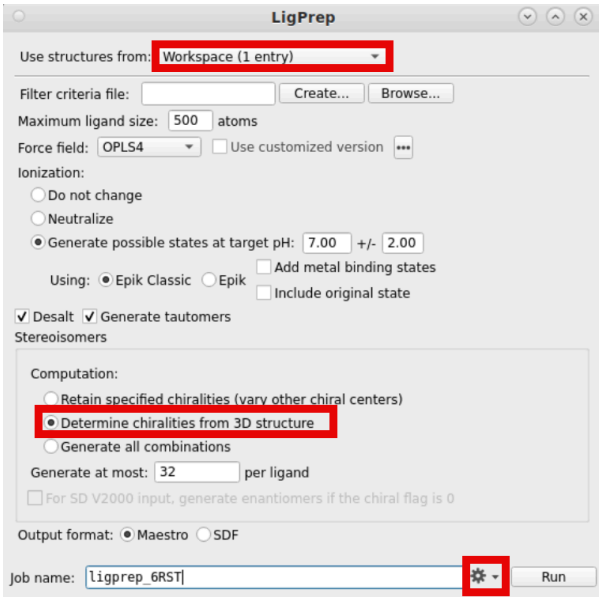

Figure 4-18. The LigPrep panel.

7. Under Stereoisomers, choose **Determine chiralities from 3D structure**
8. Change Job name to **ligprep_6RST**
9. Adjust the job settings
    - Host: **cpu-preemptible (400)**
    - Total: **1 processors**
10. Click **Run**
    - This job requires a CPU host and should complete in under 3 minutes
    - A banner appears when the job has been incorporated
    - A new group is added to the Entry List
    - The number of ligands in this group is shown in parentheses

Note: The Tile functionality is very useful for seeing the slight variations in chemistry for the generated structures. The Tile View can be turned on by clicking the ➕ in the Workspace Configuration Toolbar in the bottom right corner and then clicking the Tile button.

Pause & Think #2. What amino acid residues do you notice in the binding site of TBK1 and what classification of amino acid does it fall under (i.e. (1) non-polar and neutral, (2) polar and neutral, (3) acidic and polar, or (4) basic and polar)? List them and take a screenshot of the binding site and paste it below.

Figure 4-20. Double click presets.

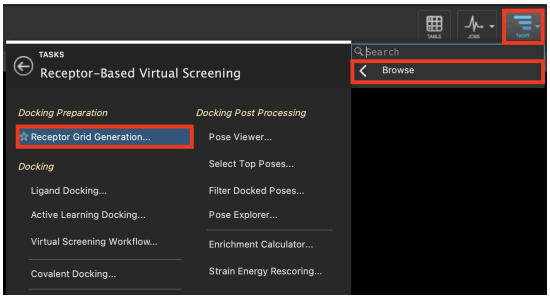

Figure 4-21. Receptor Grid Generation option in Receptor-Based Virtual Screening.

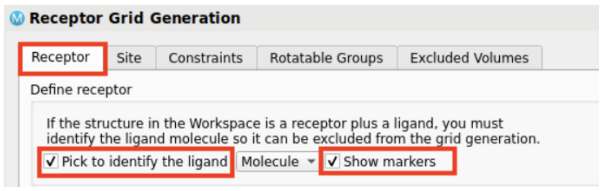

Figure 4-22. The Receptor tab of Receptor

Part 3. Receptor grid generation

Grid generation must be performed prior to docking with Glide. The receptor grid generation panel is used to specify a receptor structure. The shape and properties of a receptor structure are represented in a grid by fields that become progressively more discriminating during the docking process. These grid files represent the active site of the receptor for Glide docking. A receptor grid also ensures that any substrate you plan to dock will stay within the shape and character of the enzyme's active site.

1. Click the **In** circle next to **6RST_prepared_dry** to include it in the Workspace
2. Double-click **Presets**
    - 6RST_prepared_dry is rendered using the Custom Preset

Grid Generation.

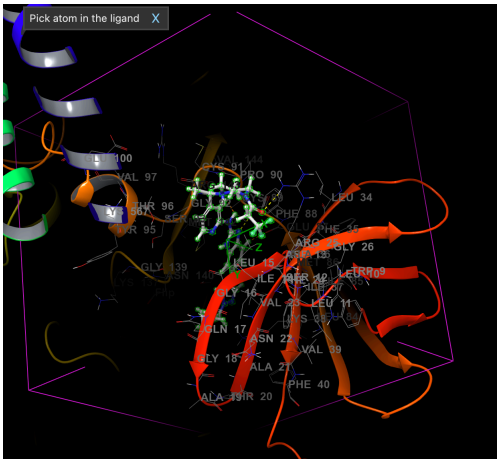

Figure 4-23. The ligand is defined to be excluded from grid generation.

3. Go to **Tasks > Browse > Receptor-Based Virtual Screening > Receptor Grid Generation**
    - The Receptor Grid Generation panel opens
4. Under Define Receptor, check the boxes for **Pick to Identify the ligand (Molecule)** and **Show Markers**
    - A banner in the Workspace will prompt you to click on an atom in the ligand

Note: The purple bounding box defines the region that the docked molecule(s) can occupy to satisfy the initial stages of docking

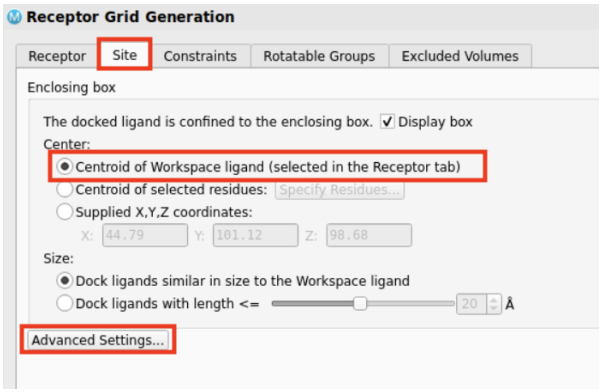

Figure 4-24. The Site tab of Receptor Grid Generation.

5. Click the **Site** tab
6. Select **Centroid of Workspace ligand (selected in the Receptor tab)**
7. Click **Advanced Settings**
    - A green inner bounding box appears in the workspace

Note: The green bounding box defines the region in which the centroid of the docked molecule(s) must occupy to pass the initial stages docking

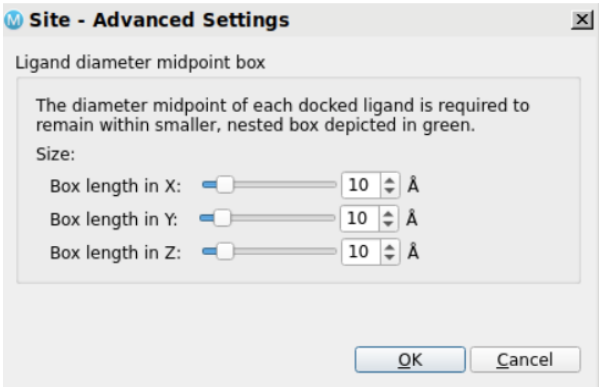

Figure 4-25. Ligand diameter midpoint box panel.

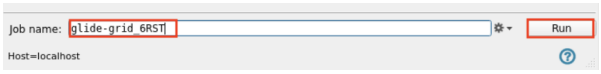

Figure 4-26. Run receptor grid generation job.

8. Leave the settings for **X**, **Y**, and **Z** sizes to the default settings **10**, **10**, and **10** Å, respectively.
9. Click **OK**
10. Change Job name to **glide-grid_6rst**
11. Adjust the job settings ( )
    - Host: **cpu (1600)**
12. Click **Run**
    - This job requires a CPU host and should complete in under 2 minutes
    - A folder named glide-grid_6rst is written to your Working Directory

Note: All Receptor Grid Generations jobs do not incorporate into the Entry List like other jobs have done in the past. Rather, this creates a folder inside of your Working Directory with a .zip file that we will use for docking with Glide in the next section.

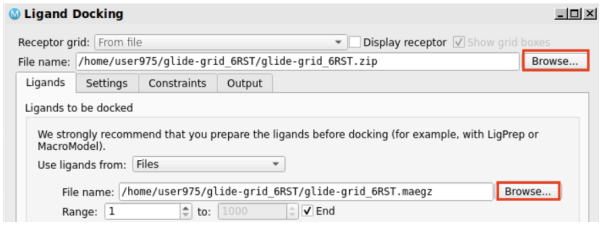

Figure 4-27. The Ligands tab of the Ligand Docking panel.

Figure 4-28. Run glide docking job.

Part 4a. Docking inhibitor into active site for Target Validation

1. Go to **Tasks > Browse > Ligand Docking**
   - The Ligand Docking panel opens
2. Next to Receptor grid, click **Browse**, open the **glide-grid_6RST folder** and choose **glide-grid_6RST.zip**
3. In the Ligands tab, for Use ligands from, choose **Files**

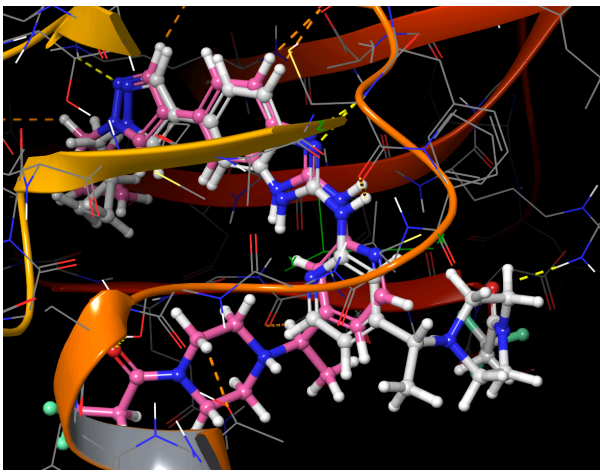

Figure 4-28. Binding pose of the top docked cognate ligand (pink) compared to the crystal structure (gray).

4. Next to File name, click **Browse**, open the **ligprep_6RST folder** and choose **ligprep_6RST-out.maegz**
5. Change Job name to **glide-dock_6RST**
6. Adjust the job settings ( )
    - Host: **cpu-preemptible (400)**
    - Total: **4 processors**
7. Click **Run**
    - This job requires a CPU host and should complete in under 2 minutes
    - A banner appears to show that files have been incorporated

Note: The top 6rst_prep_lig entry is included, and the Pose Viewer panel appears

1. A new group is added to the Entry List
2. Include other **ligand results**
3. Double-click **Presets**

Note: Though only the top ranked result is in strong agreement with the crystallographic pose, all five results accurately capture the pose of the inhibitor compound in the binding site (with varying degrees of success in capturing the solvent exposed region)

Learn more: Multiple Glide docking results can be viewed in the Entry List and be identified by the job name. Docked results will show the receptor in the first row and the docked ligand(s) in the subsequent row(s), where they are ordered by best to worst docking score, or Glide Gscore if Epik state penalties were not applied in LigPrep. The Glide Gscore is broken down by van der Waals electrostatic components and can be seen in the Project Table, using the Property Tree. You can read more about how docking scores/poses are generated here and here and what dependencies they have here and here.

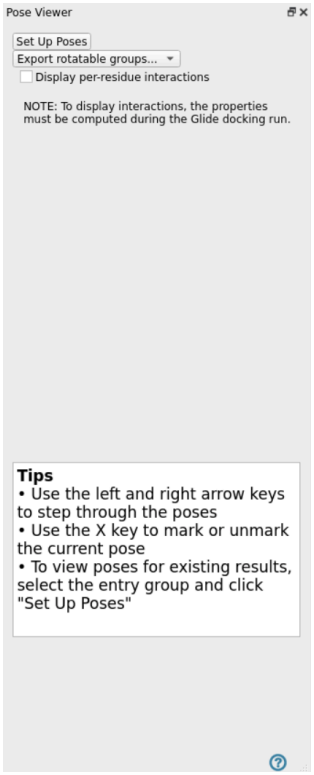

Figure 4-29. Pose Viewer panel.

Part 4b. Analyzing binding poses

1. Open the **Pose Viewer** panel by searching in the Tasks bar
2. Step through the results using the **right** and **left** arrow keys
    - Ligand poses are displayed in the Workspace
    - Residues are colored according to their interaction energies, ranging from green (favorable) to red (unfavorable)
3. Close the **Pose Viewer** panel
4. Type **Ctrl+T (Cmd+T)** to open the Project Table
5. In the Project Table, click the Property **Tree** icon
    - The Property Tree appears on the right of the Project Table
6. Click the **All** box twice
    - All boxes are deselected
7. Click the **Glide** box
8. Click **Primary** deselect **Secondary**

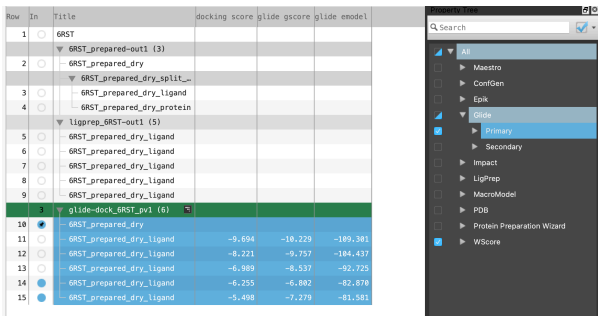

Figure 4-30. Glide Primary properties shown in the Project Table.

- Only the Glide Primary properties are shown

Note: Please see Knowledge Base Article 1027 for more information on the difference between docking score, Glide gscore, and glide model score.

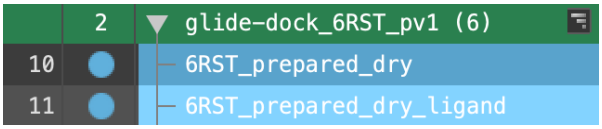

Figure 4-31. Include 6RST_prepared complex and top performing ligand into workspace

Figure 4-32. Ligand Interaction Diagram in the Favorites toolbar.

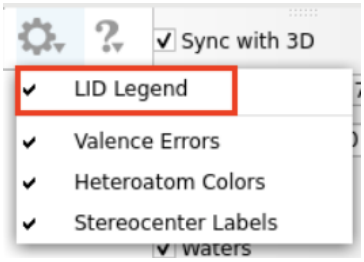

Figure 4-33. Toggle on LID Legend.

Part 5a. Visualizing enzyme-substrate interactions in Maestro

In this section, we will be analyzing 2D interactions between the inhibitor compound and enzyme, and also visualizing the surface of the binding pocket and saving an image of the complex.

1. **Include** only the 6RST_ prepared_dry complex and the top performing ligand in the workspace and **exclude** the other ligand results
2. In the Favorites toolbar, click **Ligand Interaction**
    a. The 2D Workspace - Ligand Interaction Diagram opens
3. Click on the clog and select **> LID Legend**
4. Save an image of the 2D interaction diagram by taking a screenshot

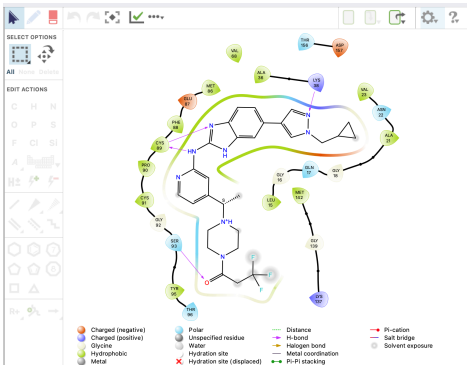

Figure 4-34. The Ligand Interaction Diagram with Sync with 3D turned on and LID legend open.

Note: The residue icon point indicates the direction of the sidechain

Pause & Think #3. What important enzyme-substrate interactions do you see when the inhibitor compound is docked? Take a screenshot of the Ligand Interaction Diagram and paste it below. List specific residues that play an important role in the binding site.

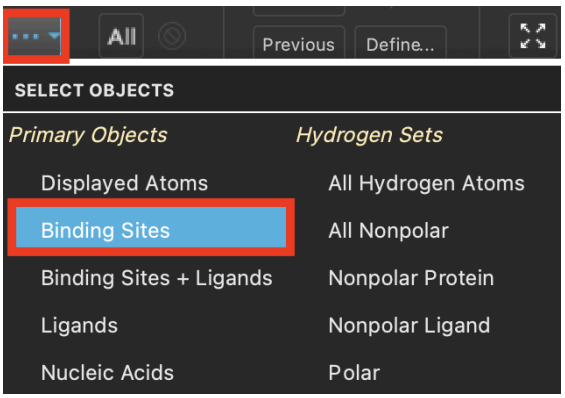

Figure 4-37. More options in Quick Select.

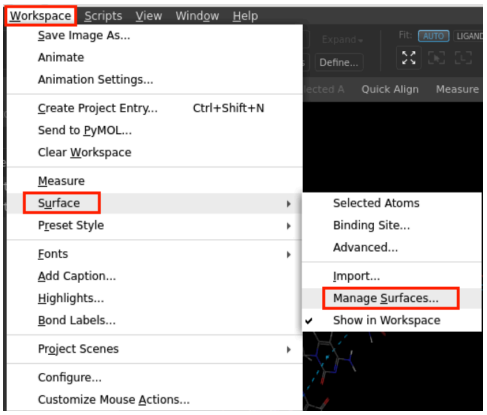

Figure 4-38. Opening the Manage Surfaces panel.

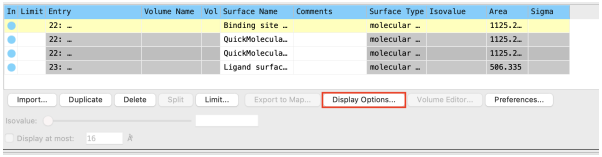

Figure 4-39. Opening Display Options.

Part 5b. Generating a surface for the binding pocket

1. Under the Quick Select panel in the top-left corner of the Maestro interface, click **…** and choose **Binding Sites**
2. Click **Style** and choose **Surface**
    - A solid gray surface is applied
    - An S is next to the title in the Entry List, click to see surface options

Note: Click **Surface (Binding Site)** in the Favorites toolbar to perform the same task

3. Click on > **Workspace** > **Surface** > **Manage Surfaces** in the toolbar
4. Click on > **Display Options**
5. Keep **Style** > as **Solid**
6. Change the **Color Scheme** > **Electrostatic potential**
7. Change the Min and Max values to **-0.1** and **0.1**, respectively
8. Click **OK**
    - The intensity of the surface colors is increased

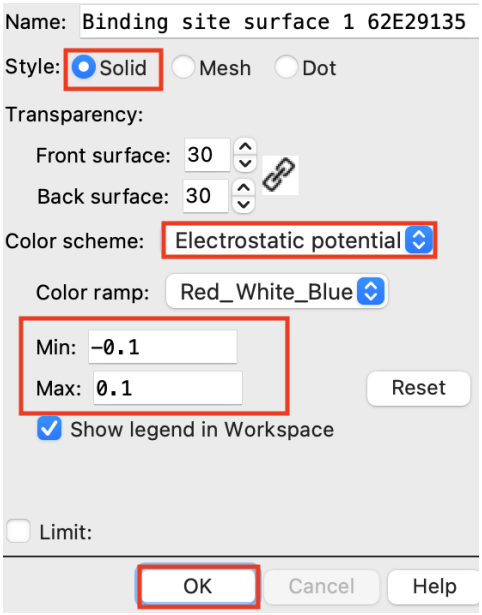

Figure 4-40. Surface Display Options panel.

Pause & Think #4. Take a screenshot of the binding pocket with the surface view toggled on and paste it below. What do you notice about the area surrounding the docked ligand/inhibitor?

6. Individual Exercise

Part A: Enzymes at play in COVID-19

Now that you know what enzymes are, how they work, and how they interact with substrates and inhibitors, it is time to apply our understanding of enzymes to a real-world problem, COVID-19.

SARS-CoV-2 is the virus behind the worldwide outbreak of COVID-19 disease. To understand how SARS-CoV-2 works, as well as how other viruses work, we must first zoom into the **spike proteins** (or S proteins) that cover the surface of SARS-CoV-2. First, a large number of glycosylated S proteins on the surface of SARS-CoV-2 bind to the host cell receptor **angiotensin-converting enzyme 2 (ACE2)**, which helps mediate viral cell entry. When the S protein binds to the receptor, **TM protease serine 2 (TMPRSS2)**, a type 2 TM serine protease located on the host cell membrane, promotes entry of the virus into the cell by **activating** the S protein. Once the virus enters the cell, the viral RNA is released, and replication and translation take place (See our DNA & RNA lesson to learn more!).

Spike proteins thus play a critical role when it comes to eliciting an immune response, particularly in the COVID-19 disease.

1) Based on the description of SARS-CoV-2 above, identify the enzymes involved in the COVID-19 disease.

2) According to IUBMB nomenclature, what classification group(s) do the enzymes from Question (1) fall under?

3) Who/What does “host cell” refer to?

4) Based on what you have learned in this lesson so far, list one possible mechanism by which we can stop the activation of S proteins to prevent virus entry.

Part B: Visualizing interactions in a SARS-CoV-2 enzyme-inhibitor complex

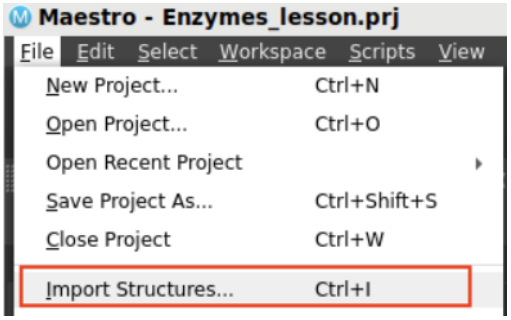

Figure 5-4. Importing prepared 7MEQ structure from the working directory.

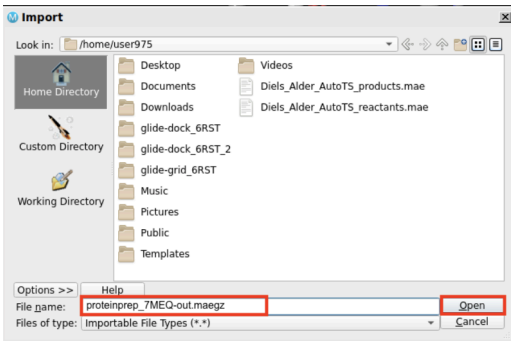

Figure 5-5. Import Structures panel

1. Go to > **File** > **Import Structures**
2. Select the file called “7MEQ_prepared-out.maegz”

Learn More: The raw 7MEQ protein structure contains many valence issues and a missing side loop which have already been resolved for you using PRIME. If you'd like to learn more about refining loops, check out our docs on PRIME.

3. Click > **Open**
   - 7MEQ_prepared is loaded into the workspace
   - A banner appears

Note: Banners appear when files have been imported, jobs incorporated into the Entry List, or to prompt a common next step.

Learn More: 7MEQ is a crystal structure of the enzyme TMPRSS2 in complex with Nafamostat. Nafamostat is a serine

Figure 5-6. Double click presets.

Figure 5-7. Zooming into the ligand

protease inhibitor that is under investigation as a potential COVID-19 therapeutic drug.

4. Double click on Presets
5. Zoom into the inhibitor compound (the ligand) in the workspace
    - Play around with your scroll wheel and look closely at the binding site in the workspace
    - How many interactions can you see in the binding site?

Pause & Think #5: Paste a screenshot of the binding pocket of TMPRSS2 below. What interactions do you see in the binding site of TMPRSS2? *Hint:* use the Ligand Interaction panel to more closely visualize the 2D interactions.

7. Summary, Additional Resources, and References

In this lesson, students learned how to use the Protein Preparation Workflow and Glide Docking panels to import and prepare an enzyme complex, dock an inhibitor compound, analyze enzyme-substrate interactions, as well as calculate binding stability after residue mutations. Using Maestro, students were able to interact with the enzyme-inhibitor complex by using the graphical user interface to zoom in/out, compare various binding poses, and save images of the binding site.

For further learning:

- Enzymes
- Properties of SARS-CoV-2 spike protein
- Target Analysis with SiteMap and WaterMap
- Structure-Based Virtual Screening using Glide
- Ligand-Based Virtual Screening Using Phase
- Introduction to Molecular Modeling in Drug Discovery Online Course
- Target Enablement, Preparation, & Validation Online Course

8. Glossary of Terms

Entry List - a simplified view of the Project Table that allows you to perform basic operations such as selection and inclusion

Included - the entry is represented in the Workspace, the circle in the In column is blue

Project Table - displays the contents of a project and is also an interface for performing operations on selected entries, viewing properties, and organizing structures and data

Recent actions - This is a list of your recent actions, which you can use to reopen a panel, displayed below the Browse row. (Right-click to delete.)

Scratch Project - a temporary project in which work is not saved. Closing a scratch project removes all current work and begins a new scratch project

Selected - (1) the atoms are chosen in the Workspace. These atoms are referred to as "the selection" or "the atom selection". Workspace operations are performed on the selected atoms. (2) The entry is chosen in the Entry List (and Project Table) and the row for the entry is highlighted. Project operations are performed on all selected entries

Working Directory - the location that files are saved

Workspace - the 3D display area in the center of the main window, where molecular structures are displayed

Enzyme - a biological polymer that catalyzes biochemical reactions

Catalyst - a substance that increases the rate of a chemical reaction without itself undergoing any permanent chemical change

Substrate - the molecules that enzymes act upon

Inhibitor - substrates that bind onto enzymes in order to block the enzyme's activity

Catalytic residues - the amino acid residues in the enzyme's binding site that play a role in chemical catalysis