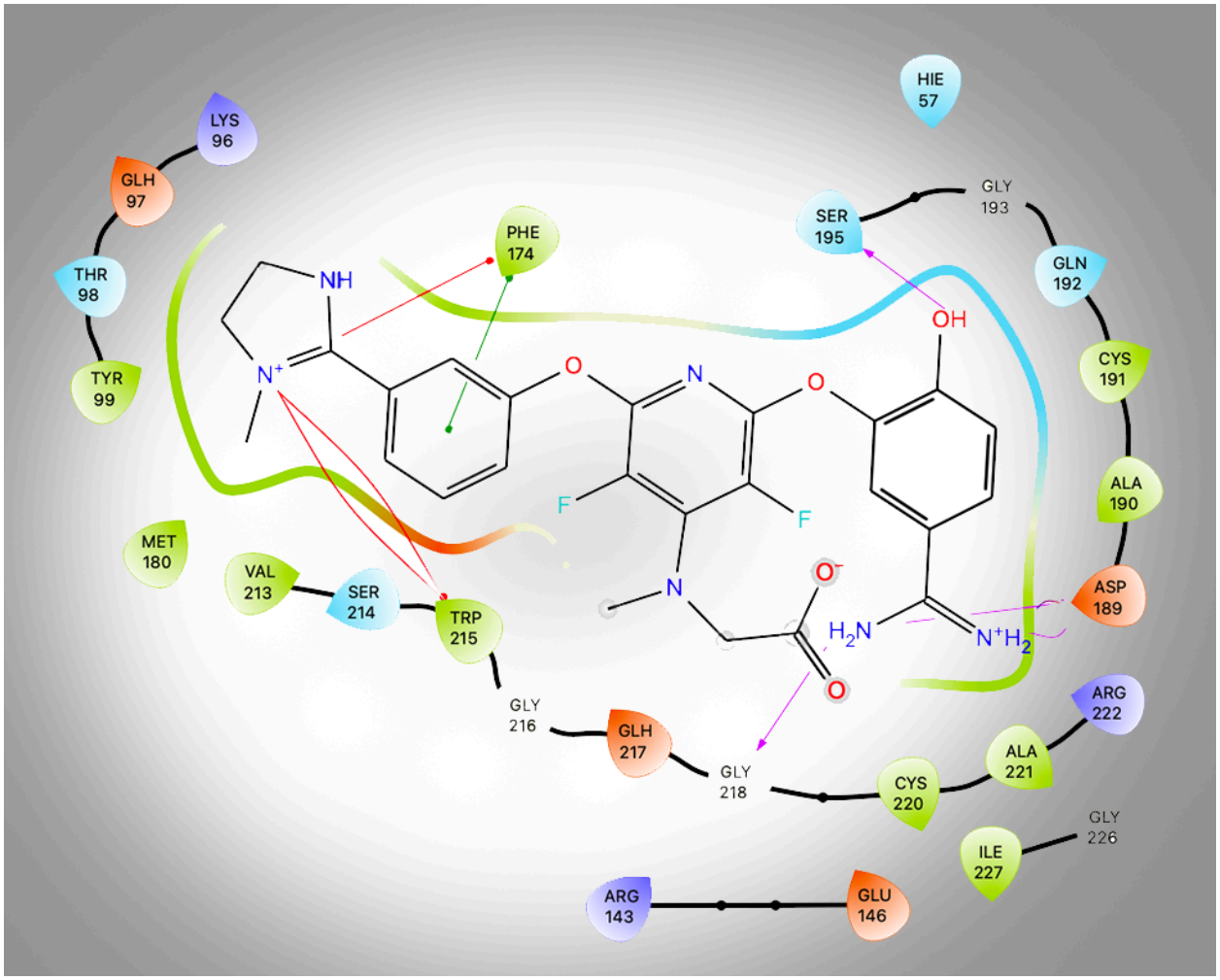
Protein Ligand Docking



Protein Ligand Docking

About this Lesson:

Determining the binding mode of an active molecule to a given protein target is important in the drug discovery pipeline. This lesson will focus on reproducing an experimentally observed binding mode through docking.

Using Maestro, students will learn how to import a .pdb file of a crystal structure, prepare a protein and ligand, as well as dock a ligand into a protein receptor using Schrödinger's Glide. They will also analyze protein-ligand interactions from a Ligand Interaction Diagram.

Learning Objectives:

- Define molecular docking and explain why it is used in drug discovery
- Prepare ligand and protein structures in Maestro
- Learn the steps of a molecular docking workflow using Schrödinger's Glide
- Analyze integral protein-ligand interactions within an active site using the Ligand Interaction Diagram

Lesson Contents:

Standards Alignment:	**Assessments for Understanding:**

• *ACS Guidelines*
  ○ Biological macromolecules (Section 5.1)
• *AAMC MCAT*
  ○ Structure, function, and reactivity of biologically-relevant molecules (5D)
	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 structure-based virtual screen steps
	• Visual assessment of student-generated docking scores from their own set of ligands

Associated Documentation Pages: Glide Documentation

Warm-Up Questions:

Read the article "A systematic analysis of atomic protein-ligand interactions in the PDB" and answer the following questions.

1. What are 7 of the most frequent protein-ligand interactions?

2. What is the most frequent protein family found in the protein data bank?

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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

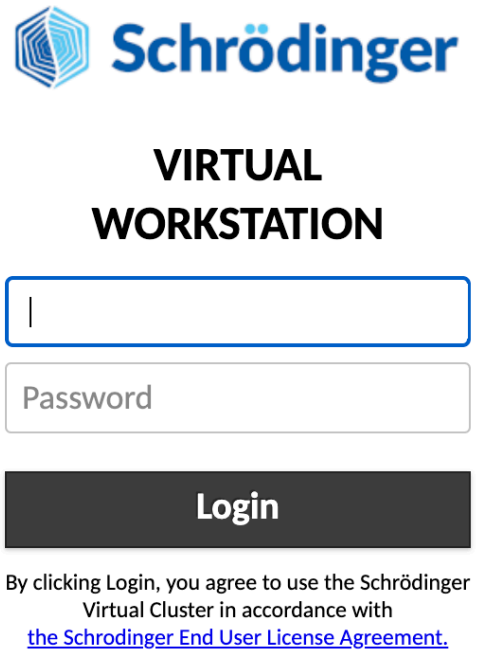


Figure 1-1. Virtual workstation login page.

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

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

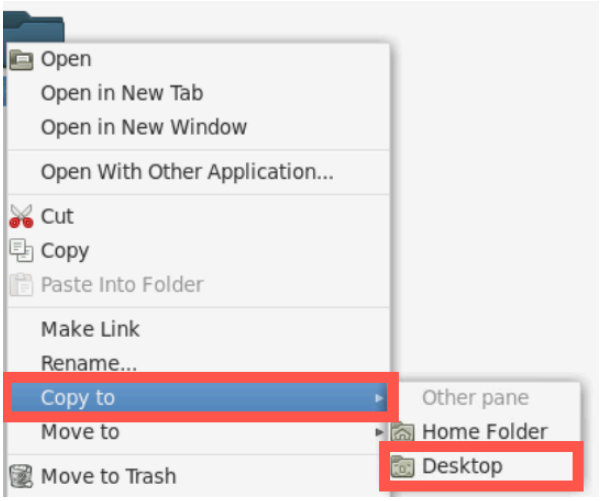


Figure 1-3. Copy the lesson folder to the Desktop.

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

4. Double-click the Maestro icon on the desktop

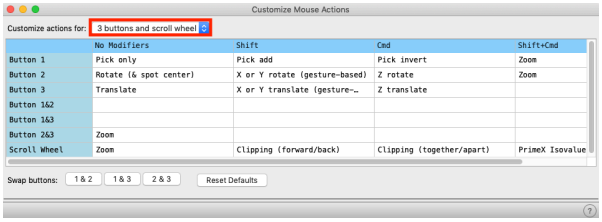


Figure 1-4. Change the mouse actions.

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

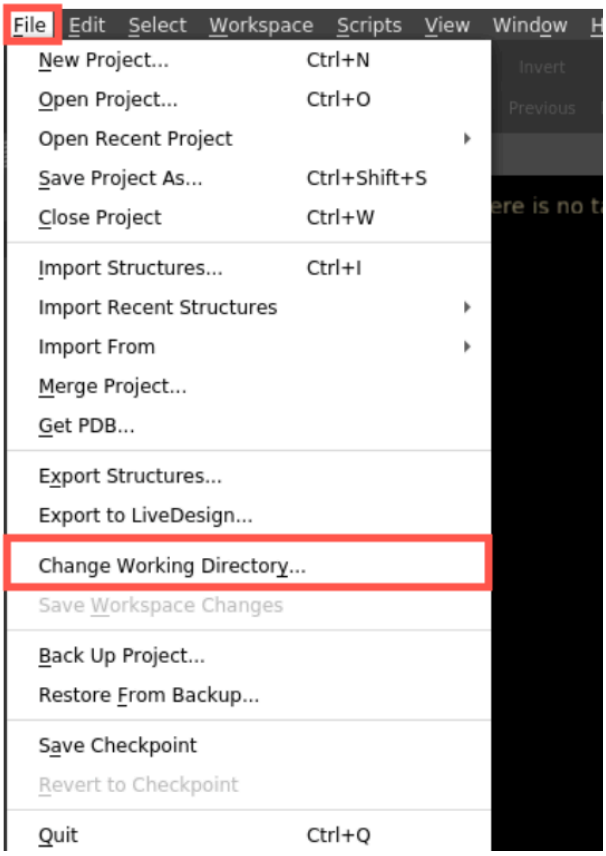


Figure 1-5. Change Working Directory option.

6. Go to File > Change Working Directory

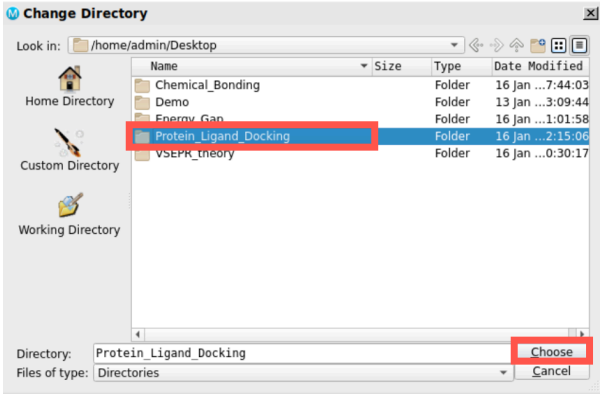


Figure 1-6. Change Working Directory panel.

7. Navigate to Desktop > `protein_ligand_docking` folder and click **Choose**

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

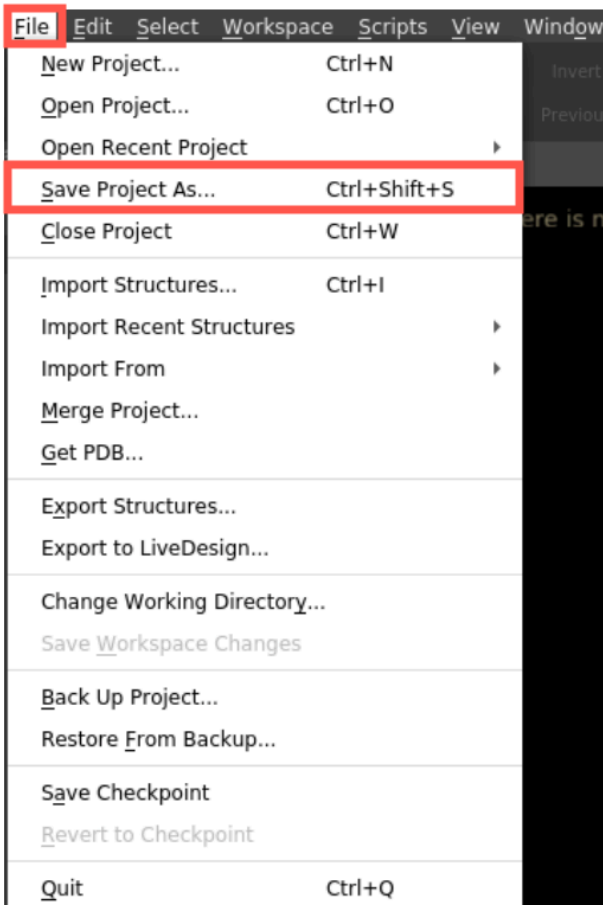


Figure 1-7. Save Project option.

8. Go to File > Save Project As

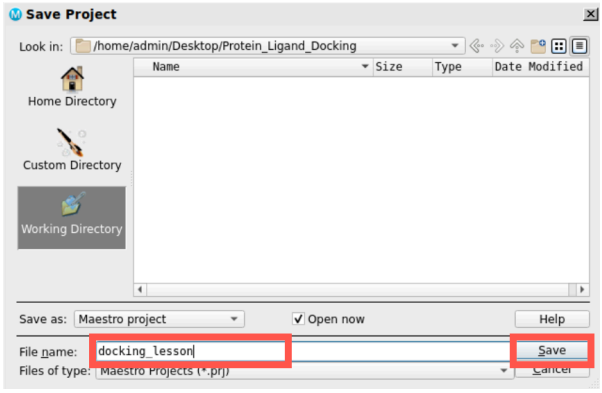


Figure 1-8. Save Project panel.

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

2. Introduction to Protein Ligand Docking

As more protein structures are determined experimentally using X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy, molecular docking is increasingly used as a tool in drug discovery. Molecular docking is often used to predict the binding mode of a small molecule into a protein binding site. Understanding the types of protein-ligand interactions gained from docking poses are fundamental in drug design.

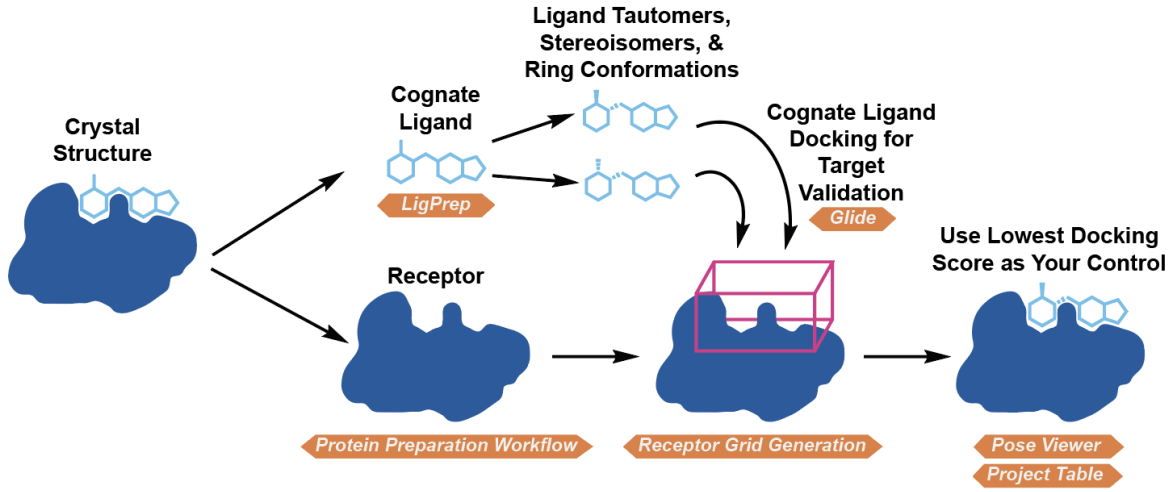


Figure 1. Workflow for Protein Ligand Docking

Figure 1 shows a schematic for how to prepare ligand and protein structures, an essential first step of modeling. Structure files obtained from the PDB, vendors, and other sources often lack necessary information for performing modeling-related tasks. Unfortunately, even when working with a high-resolution x-ray crystallographic structure, researchers can spend considerable time and effort correcting common problems such as missing hydrogen atoms, incomplete side chains and loops, ambiguous protonation states, and flipped residues. Additionally, waters that are crucial for ligand binding may also be missing from apo binding sites. Why would hydrogens, loops and side chains, as well as waters be missing? Let's break it down:

In crystallography, the X-ray beams are scattered off of electrons, and the only electrons around a hydrogen atom are those participating in the covalent bond with another atom. Additionally, the resolution of crystal structures tend to be greater than 1 angstrom. This is

because of inherent dynamic fluctuations of a protein structure within a crystal and small variability between protein structures across different unit cells. However, perhaps this issue will be a thing of the past with the advent of new techniques and advancing of current methodologies.

Loops and side chains of protein residues can often be missing as well from a protein structure – especially crystal and CryoEM structures. There are two reasons for this. The first is that these loops or side chains may access multiple different structures in each of the molecules in the crystal structure. The average over all of these molecules results in no electron density. The second is that even within the crystal structure, these residues or loops may be dynamic within each of the molecules that make up the crystals. For similar reasons, electron density cannot be observed through scattering. To combat this, we will use Prime to construct and add back in missing loops and residues in the lowest energy state. Finally, there may be high energy waters that play an important role in ligand binding that are not resolved in the PDB structure.

What is extra in protein structures? There are often molecules added in to help aid in crystallography that are not biologically relevant. This list of molecules includes small organic stabilizing agents like glycerol and metals like Copper and Zinc. Additionally, sometimes to help with X-ray diffraction, methionines are replaced with selenomethionines since selenomethionines are able to diffract X-rays better with their electron rich outer shells of selenium. Finally, if there are any chains in the structures (other proteins that were added to stabilize the protein of interest), it might be useful to remove these chains before performing docking or structure-based drug discovery.

In order to make these structures suitable for modeling tasks, we use the Protein Preparation Workflow to resolve issues. Similarly, ligand files can be sourced from numerous places, such as vendors or databases, often in the form of 1D or 2D structures with unstandardized chemistry. LigPrep can convert ligand files to 3D structures, with the chemistry properly standardized and extrapolated, ready for use for docking. We will go into more detail on these Maestro panels and workflows throughout the lesson.

Here, we will reproduce an experimentally observed binding mode of 1FJS, which is a serine protease that catalyzes the conversion of prothrombin to thrombin, the first joint step that

links the intrinsic and extrinsic coagulation pathways. In this example, the binding pocket has an ideal protein conformation to dock the ligand. Docking other screening ligands may present itself with more complications since they may prefer binding to a different protein conformation. See the lesson on Structure-Based Virtual Screening to learn more.

3. Preparing Protein Structures for Glide Docking Model

The Protein Preparation Workflow is run within the Preparation Workflow tab. The workflow has processing, modification, and refinement tools that we will use on the 1FJS.pdb structure. This tool is intended to support two main workflows - interactive, single protein preparations and highly-automated bulk protein preparations. Interactive preparations are manually performed in a step-by-step manner, with the opportunity to review the results of each step and easily control the order of modifications. Automatic preparation is pre-set by the user by the use of toggles that control which stages of the workflow are run in a single job. The Automatic workflow allows processing of multiple protein structures in a single job, permitting they maintain the same settings. The recommended minimal processing tasks are checked by default in both workflows but may be modified using the dropdown options. There are also options for filling in missing side chains and/or loops, depending on the needs of your structure. For more information see Protein Preparation Workflow Panel Help.

The Protein Preparation Workflow toggles include Preprocess, Optimize H-Bond Assignments, and Clean up. The Preprocess step fixes structural defects and adds missing information. The Optimize H-bond Assignment section is used for optimizing the hydrogen bonding network – a process that samples water orientations and flips Asn, Gln, and/or His side chains at a specified pH value. Adjusting the pH will change the protonation states of residues and ligands accordingly and is useful if you want to accurately reflect the experimental conditions. The Clean Up section fixes clashes that can occur with adding hydrogens or filling missing sidechains. By default, an RMSD of 0.3 Å is used, minimizing both the hydrogens and heavy atoms via harmonic penalty constraints. Optionally, hydrogen-only minimization can be chosen.

The Preparation Workflow tab may be used in conjunction with the Diagnostics tab and Substructures tab. These tabs are intended for diagnosis and analysis of the protein after preparation steps because automatic procedures cannot cover all possible cases.

3.1. Prepare the Protein using the Protein Preparation Workflow

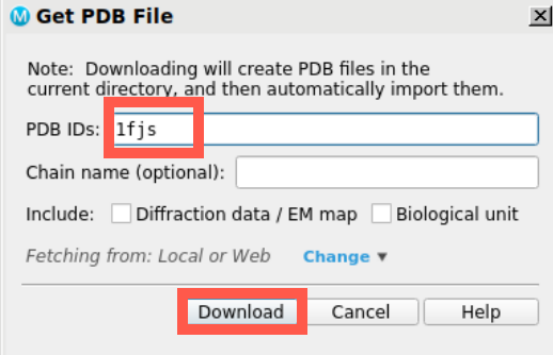


Figure 3-1. Downloading the PDB file into Maestro.

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

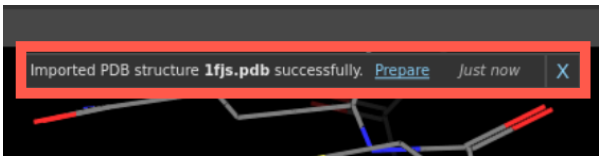


Figure 3-2. Viewing the banner in the workspace.

4. Notice that a banner appears in the top center of your workspace. Banners appear when files have been imported, jobs incorporated into the Entry List, or to prompt a common next step.

By default the structure corresponding to the imported file is both included in the Workspace and selected in the Entry List. Please refer to the Glossary of Terms for the difference between included and selected.

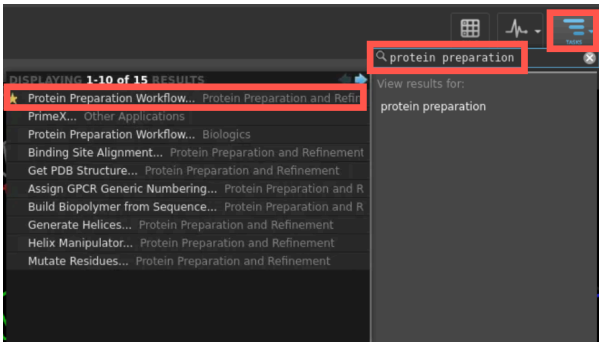


Figure 3-3. Opening Protein Preparation.

5. Go to **Tasks** in the upper righthand corner of the interface
6. Type **protein preparation** in the search bar
7. Select **Protein Preparation Workflow - Protein Preparation and Refinement**

Note: You can also click **Prepare** in the banner. Or, you can click on **Protein Preparation** in your **Favorites Toolbar**. You can add more tools to your Favorites Toolbar by *starring* them in the **Task menu**.

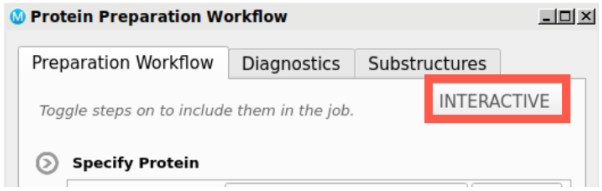


Figure 3-4. The Protein Preparation Workflow in Automatic mode.

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

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

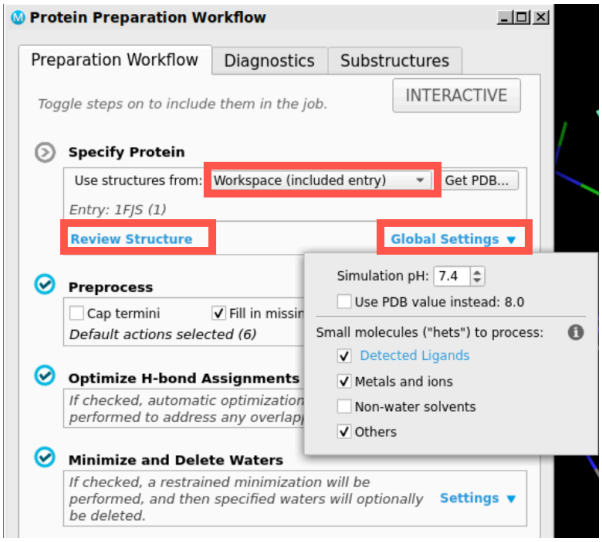


Figure 3-5. Specify the protein from the Workspace.

9. In the Specify Protein section, choose use structures from: **Workspace**
10. Select **Global Settings**
    - A dropdown opens showing the simulation pH and the PDB pH as well as small molecule options
11. Select **Review Structure**
    - The substructures tab opens to show Ligands, Metals, Other, Waters, and Chains

The **Specify Protein** tool provides you with the option to prepare a protein from the Workspace, Project Table, File, or directly from the PDB. In this example, the non-water solvents option is left unchecked. This saves computational resources because the glycerols in our Workspace will not be prepared.

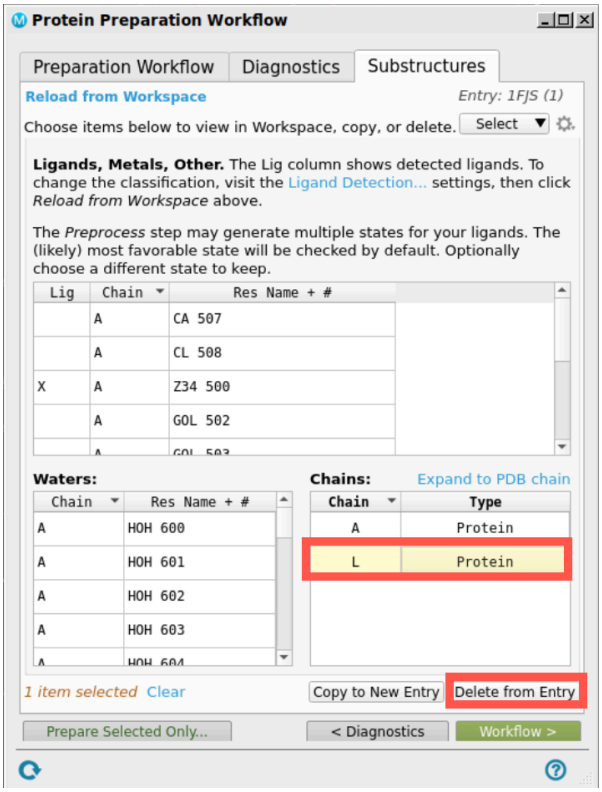


Figure 3-6. Review the structure for preparation in the Substructures tab.

12. Under Chains, click Chain **L**
    - The Workspace zooms to the chain
13. Click **Delete from Entry**
    - The smaller of the two chains is removed, and a new entry named **1FJS - with-deletions** appears in the **Entry List**.

Note: Unless specified, waters and glycerols (GOL) belonging to chain L will not be removed. Glycerols are a crystallographic artifact with no biological relevance. The Select dropdown provides shortcuts for selecting these species based on their proximity to specified chains. Removing waters and glycerols will be available again in the Clean Up and Analysis steps

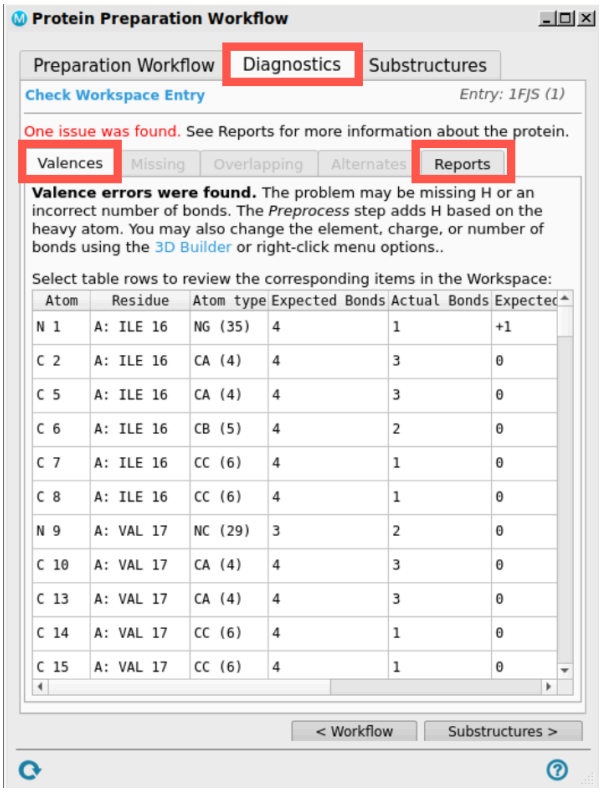


Figure 3-7. View protein issues in the Diagnostics tab.

14. Select the **Diagnostics tab**
    - Valence errors were found, but these bond order issues will be resolved in the protein preparation
    - The Missing tab is empty, indicating there are no missing side chains in the protein structure
15. Select **Reports** to view other issues with the protein that must resolved prior to modeling

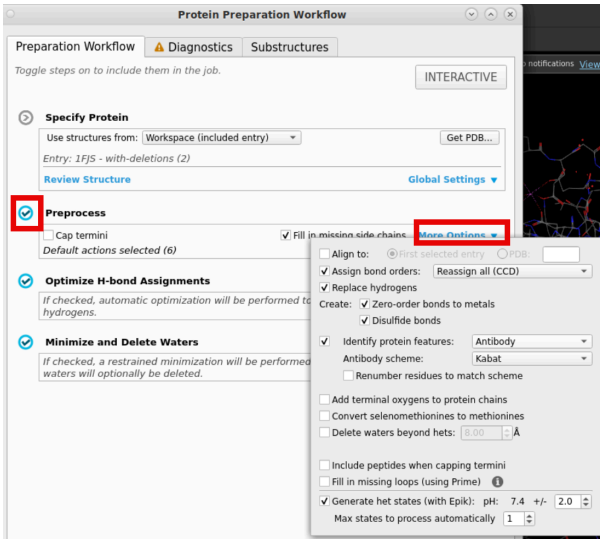


Figure 3-8. Preprocess default settings.

16. Return to the **Preparation Workflow tab**
17. Confirm **Preprocess** is toggled on (colored blue)
    - Notice **Fill in missing side chains** is checked by default. If these were missing in our structure, they would become populated during this step
18. Under Preprocess, select **More Options**

- Among the options provided, notice that missing loops may be filled in using Prime

19. Check the pH range for generating het states with Epik
    - This should align with the physiological or assay pH.

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

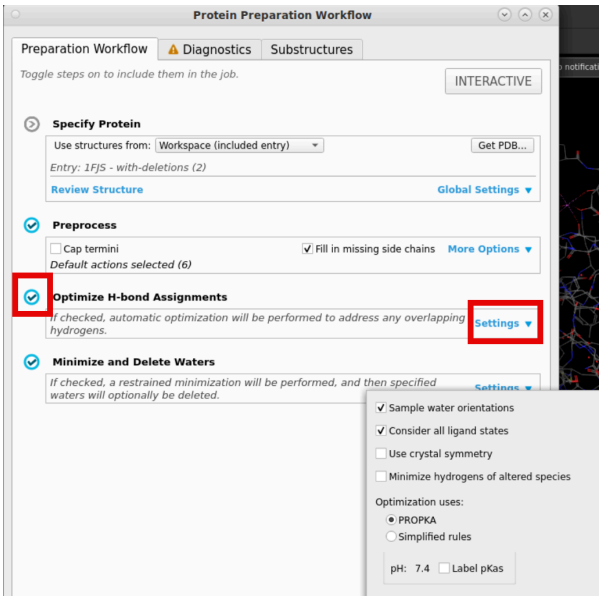


Figure 3-9. Optimize H-Bond Assignment default settings.

20. Confirm **Optimize H-bond Assignment** is toggled on
21. 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
22. Check the pH for Optimization
    - This value should be captured in the pH range chosen during the Preprocess step

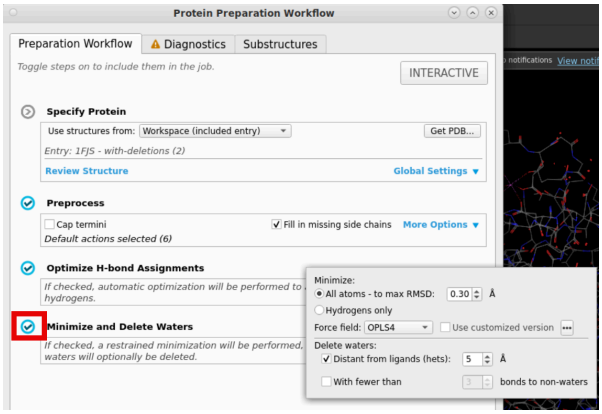


Figure 3-10. Clean Up default Settings.

23. Confirm **Minimize and Delete Waters** is toggled on
24. Click **Settings**
    - Notice that by default distant waters will be removed. The specified distance to ligands can be modified

Figure 3-11. Naming the job.

25. Change the job name to **1FJS_Prepared**

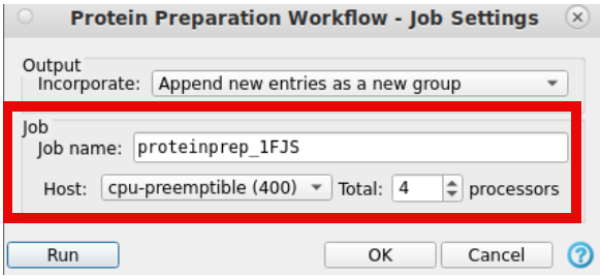


Figure 3-12. Choosing the Job Settings.

26. Adjust the job settings (⚙)
    - Host: **cpu-preemptible (400)**
    - Total: **4 processors**
27. 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 when completed

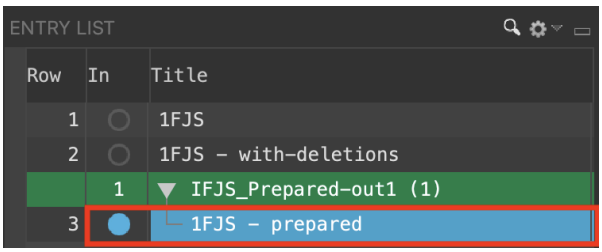


Figure 3-12. Select the protein preparation output.

28. In the Entry List, click on **1FJS – prepared**

In Interactive mode, multiple entries will be added to the Entry List as each individual preparation task will be run as an independent job.

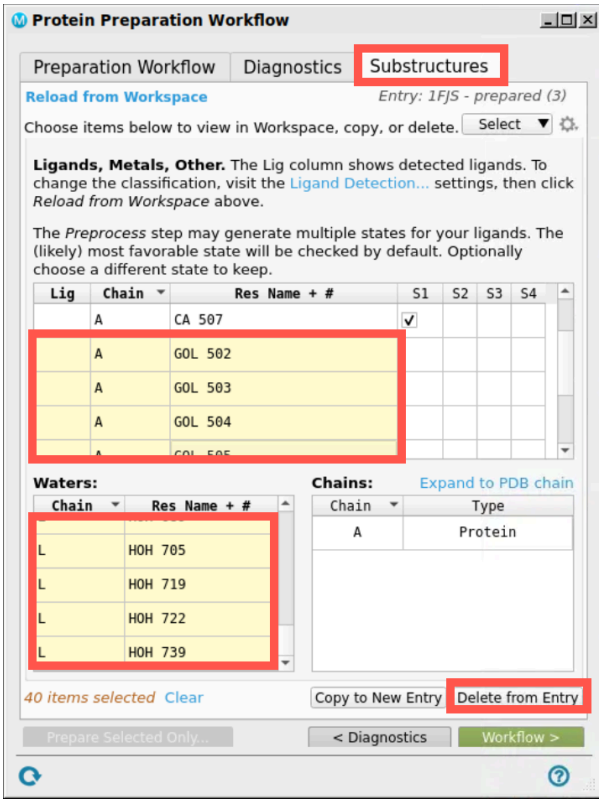


Figure 3-12. Perform Substructure Review.

29. Return to the **Protein Preparation tool** by going to **Tasks > Protein Preparation**, or selecting it from your Favorites Toolbar
30. Click the **Substructures tab**
31. Choose **Load from Workspace**
32. In the Hets table, shift-click to select all **GOL** rows
33. Click **Delete from Entry**
34. In the Waters table, shift-click to select all **waters**
35. Click **Delete from Entry**. This creates a new entry in the prepared entry group called **1FJS - with - deletions**

Note: These waters could have been removed during the **Clean Up** processing step. Depending on your system and research question, you may want to keep certain waters. See the Protein Preparation Workflow Panel Documentation for more details.

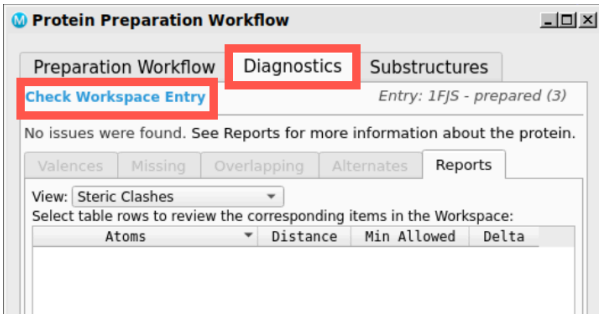


Figure 3-13. Confirm issues have been resolved during preparation.

36. In the 1FJS_Prepared-out1 group, select **1FJS – with-deletions**
37. Return to the Protein Preparation Workflow and click the **Diagnostics** tab
38. Click **Check Workspace Entry** to make sure there are no issues missed during the preparation.
39. **Exit** the Protein Preparation Workflow

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.

Pause & Think #1: List one error that you identified to be a problem prior to running the Protein Preparation Workflow. *Hint:* Select 1FJS prior to preparing the structure in the Entry List and go to the Diagnostics tab in the Protein Preparation workflow panel.

4. Preparing a Ligand Structure

In this section, we will prepare the co-crystallized ligand from the 1FJS structure for use in virtual screening. This is a typical step for **cognate ligand** docking, as it provides important validation prior to screening a larger ligand data set.

The following steps provide an example of how you would prepare a ligand data set using **LigPrep**. Ligand files can be sourced from numerous places, such as vendors or databases, often in the form of 1D or 2D structures with unstandardized chemistry. Before being used in a virtual screen, ligands must be converted to 3D structures, with their chemistry properly standardized and extrapolated.

4.1. Split the prepared structure

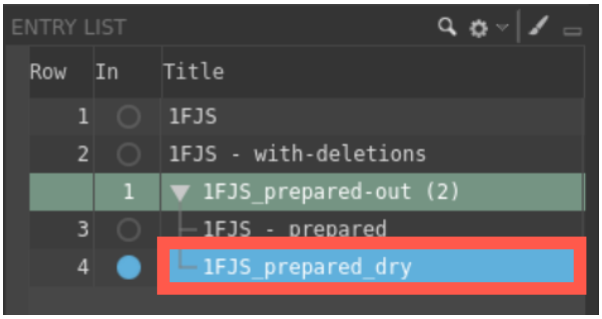


Figure 4-1. Renaming from 1FJS - with-deletions to 1FJS_prepared_dry.

1. In the Entry List, double click 1FJS - with-deletions in the 1FJS_Prepared-out1 group and rename the entry to **1FJS_prepared_dry**

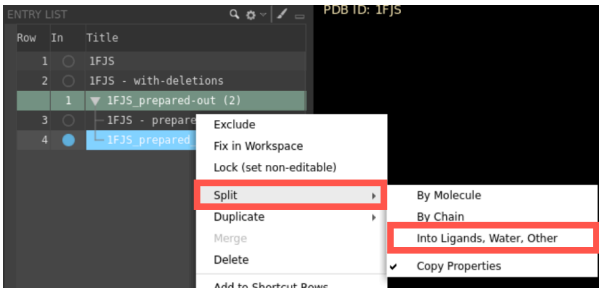


Figure 4-2. Right-click to split an entry into

2. Right-click on **1FJS_prepared_dry**
3. Choose **Split > Into Ligands, Water, Other**
    - Two new entries appear in the Entry List

different components.

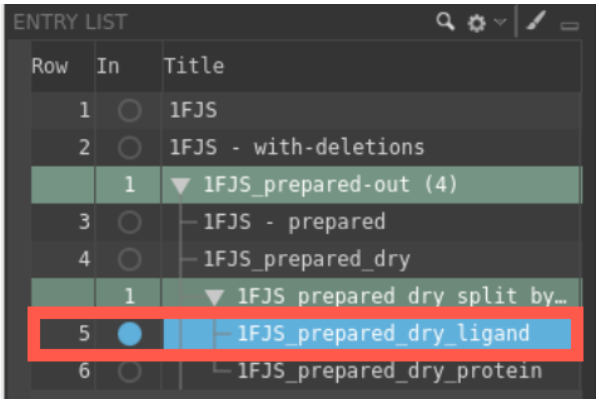


Figure 4-2. Including 1FJS_prepared_dry_ligand

4. Include **1FJS_prepared_dry_ligand**
    - Only the ligand is displayed in the Workspace

4.2. Run LigPrep

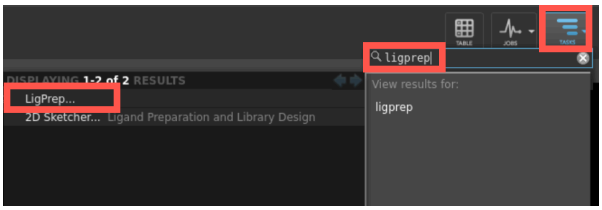


Figure 4-3. LigPrep application in the Task toolbar.

5. Go to **Tasks** and type **LigPrep** in the search bar
6. Select **LigPrep**
    - The LigPrep panel opens

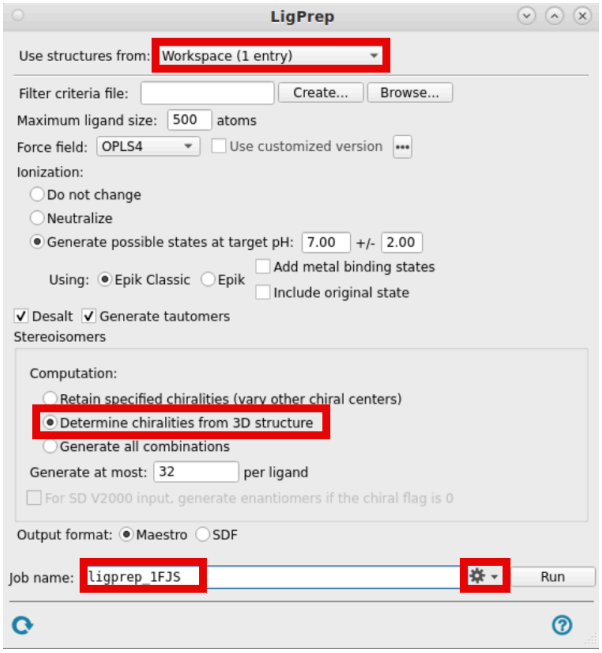


Figure 4-4. The LigPrep panel.

7. For Use structures from, choose **Workspace (1 included entry)**
8. Under Stereoisomers, choose **Determine chiralities from 3D structure**

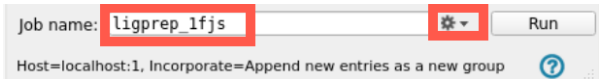


Figure 4-5. Naming the job.

9. Change Job name to **ligprep_1fjs**

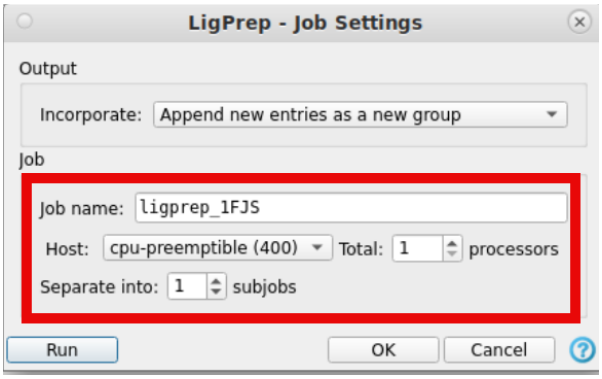


Figure 4-6. Adjusting the Job Settings.

10. Adjust the job settings (⚙)
    - Host: **cpu-preemptible (400)**
    - Total: **1 processors**
11. Click **Run**
    - This job requires a CPU host and should complete in under 3 minutes
    - A new group will be added to the Entry List when completed

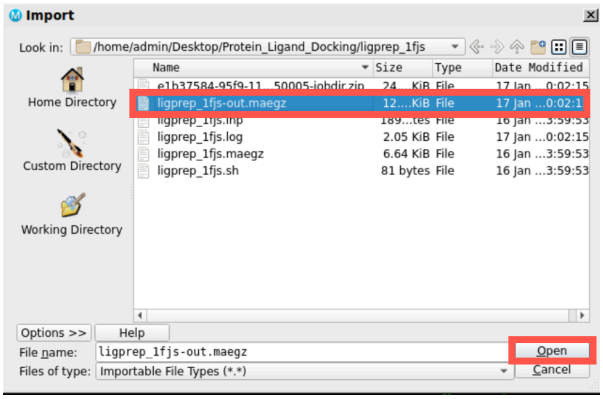


Figure 4-7. Manually importing output files if they did not automatically incorporate.

12. If your entry did not incorporate into your Entry List, go to **File > Import Structures**. If it did, skip steps 12 & 13
13. Go to the folder named **ligprep_1fjs** and open **ligprep_1fjs-out.maegz**

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: Preparing a ligand using LigPrep may produce multiple output structures for each input structure by generating different protonation states, stereochemical outcomes, tautomers, and ring conformations. Why is it important to prepare a ligand before proceeding with docking?

5. Generating a Receptor Grid with Hydrogen-Bond Constraint

Grid generation must be performed prior to docking with Glide. The shape and properties of the receptor are represented in a grid by fields that become progressively more discriminating during the docking process. To add more information to a receptor grid, different kinds of constraints can be applied during the grid generation stage. For a comprehensive overview of constraint options, see the Glide User Manual **(Help > Help > User Manuals > Glide User Manual)**. In this tutorial, we will set a hydrogen bond constraint in our receptor grid.

5.1. Identify the binding site

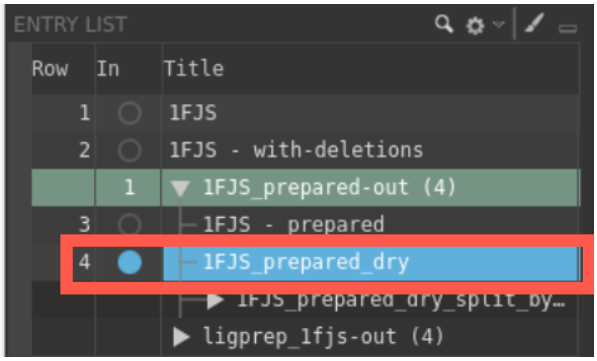


Figure 5-1. Select and include 1FJS_prepared_dry (Entry #4).

1. Click the **In** circle next to **1FJS_prepared_dry** to include it in the Workspace

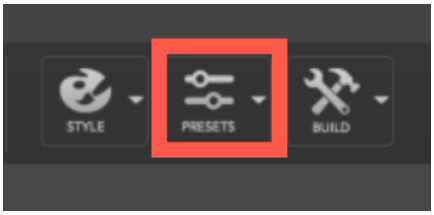


Figure 5-2. Double-clicking Presets to render the protein structure with a Custom Preset.

2. Double-click **Presets**
   - 1FJS_prepared_dry is rendered using the Custom Preset

Figure 5-3. Toggle off the residue labels.

3. You may toggle off the residue labels in the bottom right corner if you wish

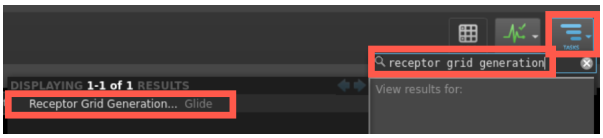


Figure 5-4. Opening up Receptor Grid Generation from the Tasks menu.

4. Go to **Tasks** and type **Receptor Grid Generation** in the search bar
5. Select **Receptor Grid Generation: Glide**
    - The Receptor Grid Generation panel opens

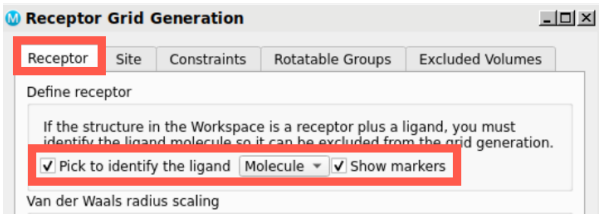
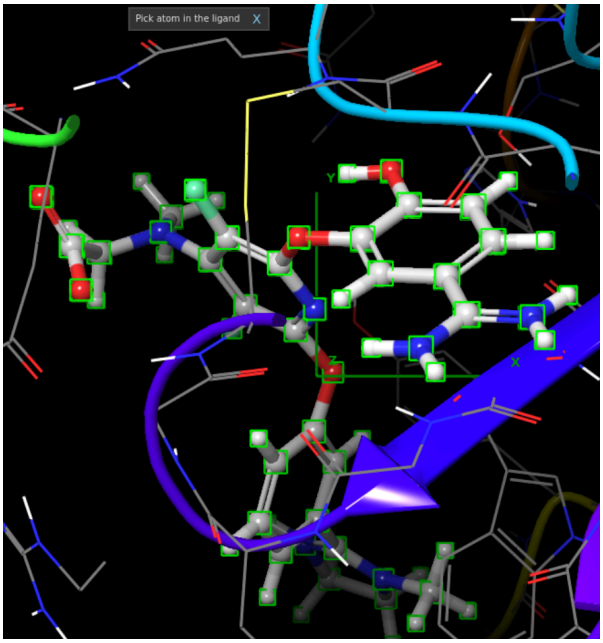


Figure 5-5. The Receptor tab of Receptor Grid Generation.

6. 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



7. Click any atom on the **ligand**. The ligand is the only molecule in the workspace that is in ball-and-stick representation.

Figure 5-6. Selecting any atom part of the ligand highlights the entire molecule with green cubes around each atom.

Figure 5-7. Zooming out to visualize the entire structure.

8. You may **zoom out** to visualize the entire protein structure using the **Fit Tools**

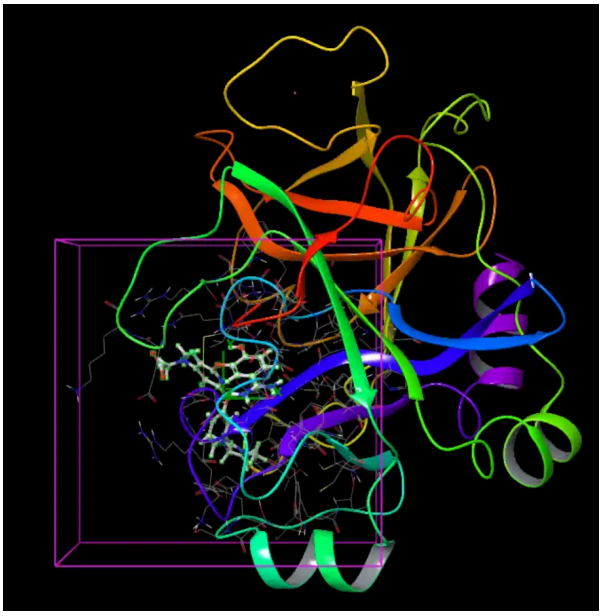


Figure 5-8. The ligand is defined to be excluded from grid generation.

9. The ligand is now highlighted in green with a purple box around it
    - The purpose of this is to exclude the ligand from the grid generation so it may be reused with a variety of other ligands for docking of a larger ligand library

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

5.2. Define the bounding box dimensions

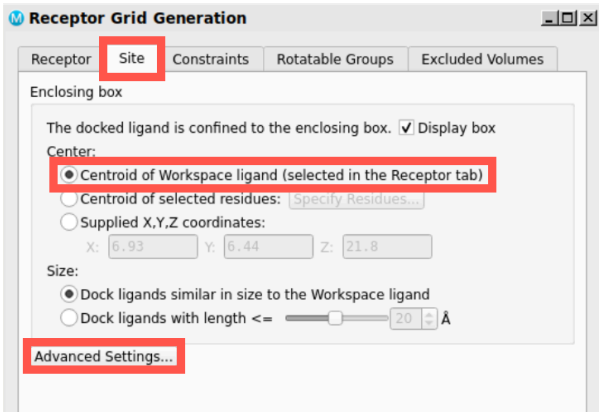


Figure 5-9. The Site tab of Receptor Grid Generation.

1. Click the **Site tab**
2. Select **Centroid of Workspace ligand (selected in the Receptor tab)**
3. Click **Advanced Settings**
    a. A green inner bounding box appears

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

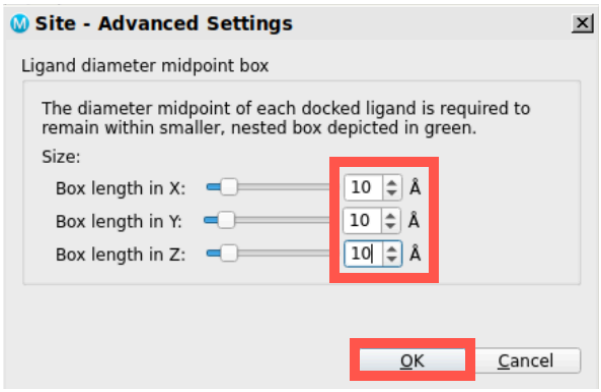


Figure 5-10. Ligand diameter midpoint box panel.

4. Keep the default settings for X, Y, and Z sizes the same at **10, 10, and 10 Å** , respectively.
5. Click **OK**

5.3. Set a hydrogen bonding constraint

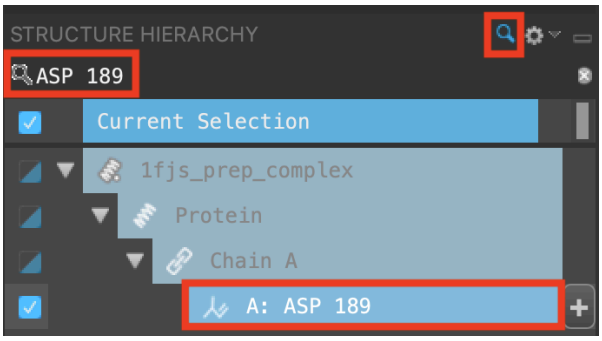


Figure 5-11. Search in the Structure Hierarchy.

1. Minimize the Receptor Grid Generation panel (do not close it)
2. Type **L** to zoom to the ligand
3. In the **Structure Hierarchy** under the Entry List, click the **magnifying glass**
4. In the search field, type **ASP 189**
5. Select **ASP 189**

According to Adler *et al.*, the salt bridge formed between the inhibitor and Asp189 observed in the crystal structure of the complex contributes to the potency of the ligand. For this tutorial, you will set the constraint for this specific hydrogen bond in the receptor grid. Please see the Introduction to Structure Preparation and Visualization tutorial for instructions on how to add residue labels and show H-bonds.

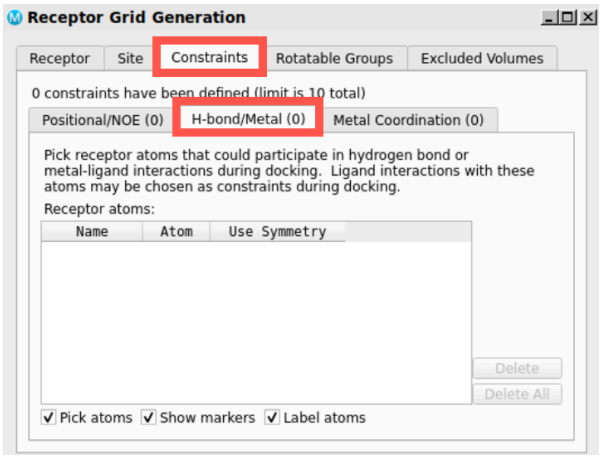


Figure 5-12. The Constraints tab of Receptor Grid Generation.

6. In the Receptor Grid Generation panel, click the **Constraints** tab
7. Click the **H-bond/Metal (0)** tab
    - A banner appears prompting selection of the receptor atom to be the constraint

Figure 5-13. Zoom to selected atoms.

8. Under Fit, click **Fit view to selected atoms**

Figure 5-14. Turn on the Interactions toggle in the workspace toggles.

9. Turn the Interactions toggle on to see the non-covalent interactions between the ligand and active site residues

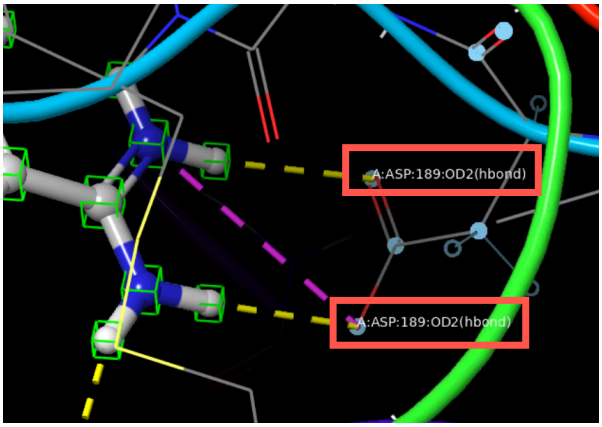


Figure 5-15. Constraint defined on ASP 189.

10. Click an **oxygen atom** of the ASP 189 sidechain
    - Both oxygens are highlighted
    - An H-bond constraint is defined in the Receptor atoms table

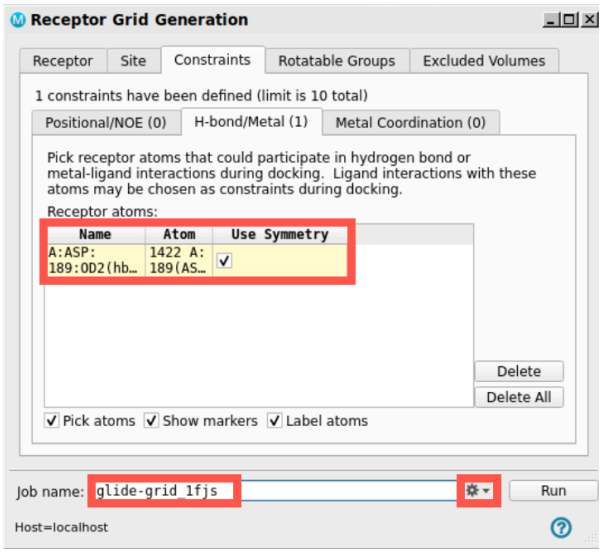


Figure 5-16. Hydrogen-bond constraint is now the receptor grid generation job.

11. Go back to the **Reception Grid Generation** panel and notice that ASP 189 is now listed under the H-bond constraint
12. Change Job name to **glide-grid_1fjs**

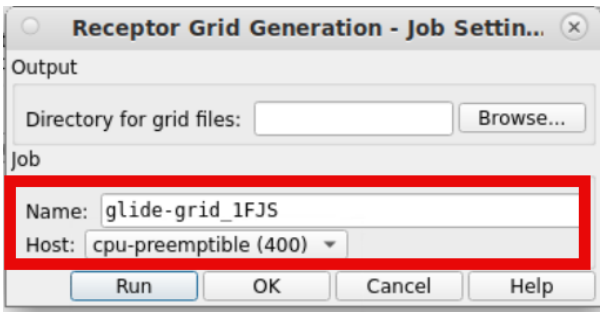


Figure 5-17. Adjusting the job settings

13. Adjust the job settings (⚙)
    - Host: **cpu-preemptible (400)**
14. Click **Run**
    - This job requires a CPU host and should complete in under 2 minutes
    - A folder named glide-grid_1fjs 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.

Pause & Think #3: Why is it necessary to generate a receptor grid? What would happen if you proceeded with docking a ligand without a receptor grid?

6. Docking the Cognate Ligand with Hydrogen-Bond Constraint

The minimum requirements for running a Glide virtual screen are a grid file and a ligand file. It is strongly recommended that the grid file be generated from a protein prepared using the Protein Preparation Workflow and the ligand file be prepared using LigPrep. Additionally, you can choose the scoring function, set ligand- and receptor-based constraints, and define the output. Please see the Glide User Manual for more detail. In this section, we will include the hydrogen bonding constraint that was created in the previous step.

In this section, we will dock the cognate ligand to validate the docking protocol which includes the receptor grid and the necessary constraints. The validated protocol can then be used for virtual screening of a ligand library. The information gained from this step can help with evaluating poses and beneficial interactions, which is useful for hit finding. This job will use the receptor grid file that was generated in the previous section.

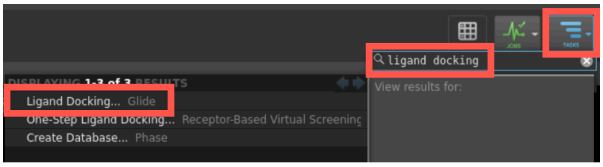


Figure 6-1. Opening Glide Ligand Docking from the Tasks Menu

1. Go to **Tasks** and type **Ligand Docking** in the search bar
2. Select **Ligand Docking: Glide**
   - The Ligand Docking panel will open

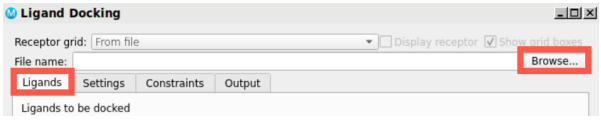


Figure 6-2. The Ligands tab of the Ligand Docking panel.

3. Next to Receptor grid, click **Browse**
4. In the **glide-grid_1fjs folder**, choose **`glide-grid_1fjs.zip`** and click **Open**

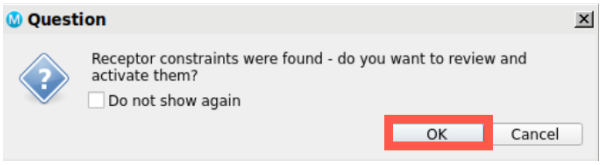


Figure 6-3. A window that verifies receptor constraints.

5. A new window will appear verifying that receptor constraints were found in the receptor grid. Click **OK**.

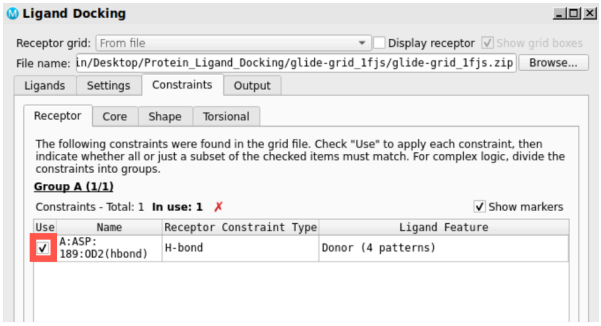


Figure 6-4. The Constraints tab of the Ligand Docking panel.

6. This takes you to the **Constraints** tab. Click on the **Receptor** tab.
7. Under Use, **check** the H-bond constraint for ASP 189

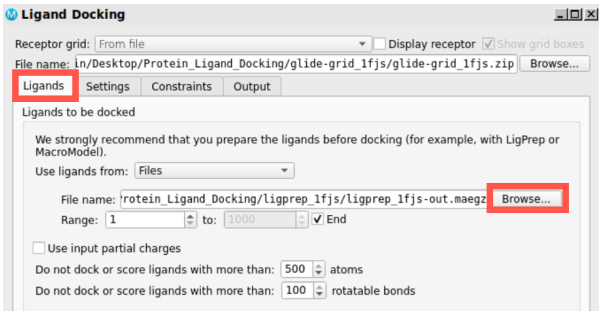


Figure 6-5. The Ligands tab of the Ligand Docking panel.

8. In the Ligands tab, for Use ligands from, choose **Files**
9. Next to File name, click **Browse**
10. Go to the ligprep_1fjs folder and choose **ligprep_1FJS-out.maegz**

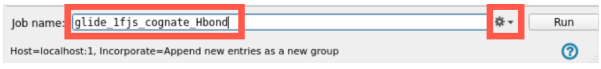


Figure 6-6. Changing the job name.

11. Change Job name to **glide_1FJS_cognate_Hbond**

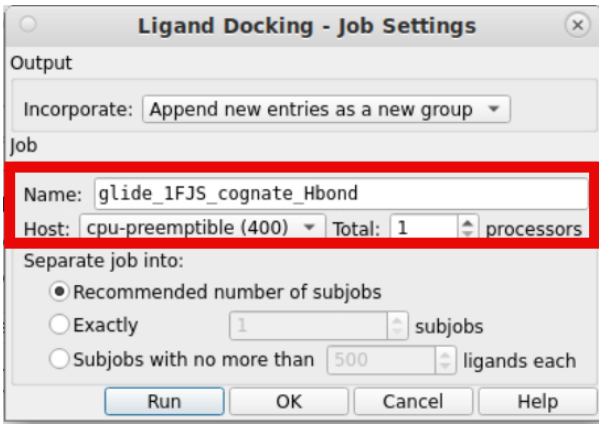


Figure 6-7. Adjusting the Job Settings.

12. Adjust the job settings ( )
    - Host: **cpu-preemptible (400)**
    - Total: **4 processors**
13. Click **Run**
    - This job requires a CPU host and should complete in under 2 minutes
    - A new group will be added to the Entry List when completed

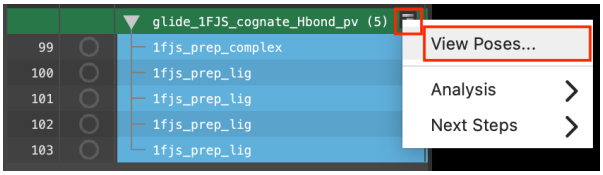


Figure 6-8. View Poses of the cognate ligand. The poses are ranked with the best docking score at the top.

14. Click the **icon** next to the glide-1FJS_cognate_Hbond_pv and select **View Poses**
15. In the Pose Viewer panel, select **Set Up Poses**
16. The 1fjs_prep_complex entry is fixed in the Workspace, the top 1fjs_prep_lig entry is included, and the Pose Viewer panel appears

You may wish to double click the Presets button to have each ligand represented in a unique color.

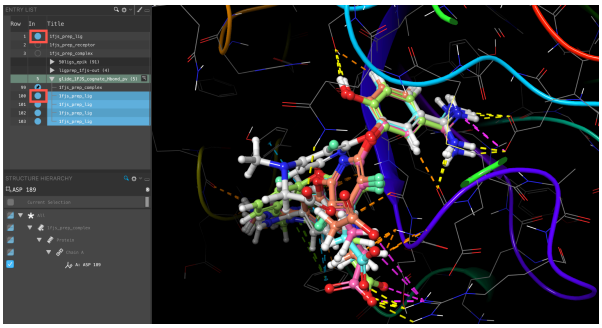


Figure 6-9. Docking result poses shown against the cognate ligand (gray).

17. Include other ligand results
    - H-bonds to ASP 189 are conserved
18. Double-click the **In** circle next to **1fjs_prep_complex**
    - The entry is no longer fixed in the Workspace

Note: To view all docked ligands in their own color, include all ligands to be evaluated and double click the Presets

button, or include all ligands and choose Binding Mode Comparison in the Presets menu.

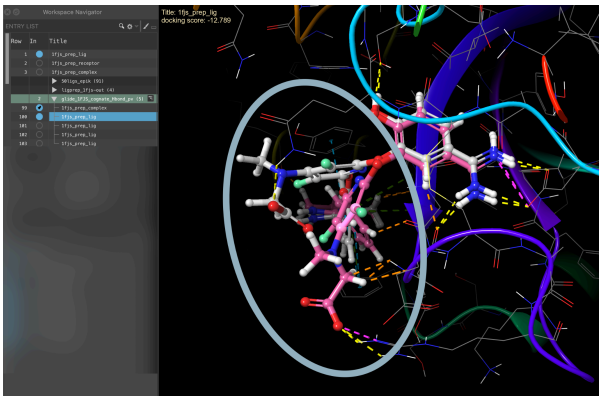


Figure 6-10. An overlay of the best docked pose (pink) with the crystal structure (gray).

19. Include the top docked pose (the pose with the most negative docking score) and **1fjs_prep_lig** to compare the best docked pose to the cognate ligand

As shown in Figure 6-10, the parts of the ligand that form interactions, including the H-Bond at ASP-189, overlap with the cocrystal ligand. However, even in the best docked pose (shown in pink), the solvent-exposed region (circled in gray) does not fully align with that of the cocrystal ligand. It is not surprising given that the binding pocket of 1FJS is solvent exposed and that docking is performed in vacuum. To get a better agreement with the crystal structure pose, there are other constraints that can be considered. Section 7 focuses on core constraints, which can further improve the alignment between the docked poses and the cocrystal ligand. If you would like to go straight to Visualizing Protein-Ligand Complexes, go straight to Section 8.

7. Docking the Cognate Ligand with Hydrogen-Bond and Core Constraints (optional)

You may have noticed that only using a hydrogen-bond constraint does not exactly reproduce the cocrystal pose – the “unmatched” section is in the solvent-exposed region. In this section, you will add another constraint to better align the docked pose to the pose from the crystal structure.

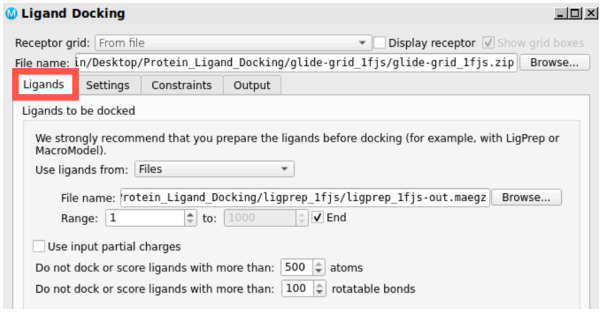


Figure 7-1. The Ligands tab of the Ligand Docking panel remains the same as before.

1. Include and select **1FJS_prepared_dry**
2. Go to **Tasks** and search **Ligand Docking**
3. Select **Ligand Docking: Glide**
4. The Ligand Docking panel opens
5. Keep `glide-grid_1fjs.zip` as the receptor grid
6. Keep `ligprep_1fjs-out.maegz` as the Ligands to be docked file

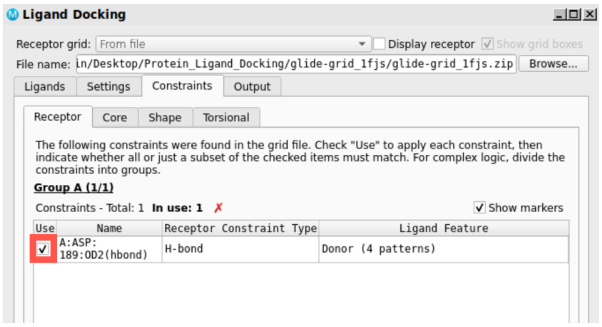


Figure 7-2. The H-bond Constraints tab of the Ligand Docking panel remains the same.

7. Go to the **Constraints** tab. Click on the **Receptor** tab.
8. Under Use, **check** the H-bond constraint for ASP 189

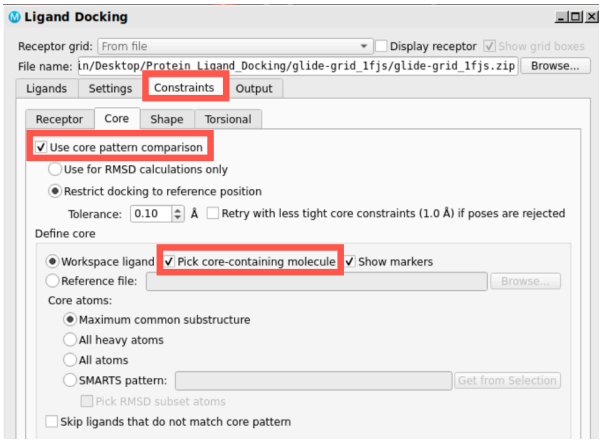


Figure 7-3. The Core Constraints tab of the Ligand Docking panel.

9. Go to the **Core** tab
10. Check mark **Use core pattern comparison**
11. Under Define core, check **Pick core-containing molecule**
12. Minimize the **Ligand Docking panel**

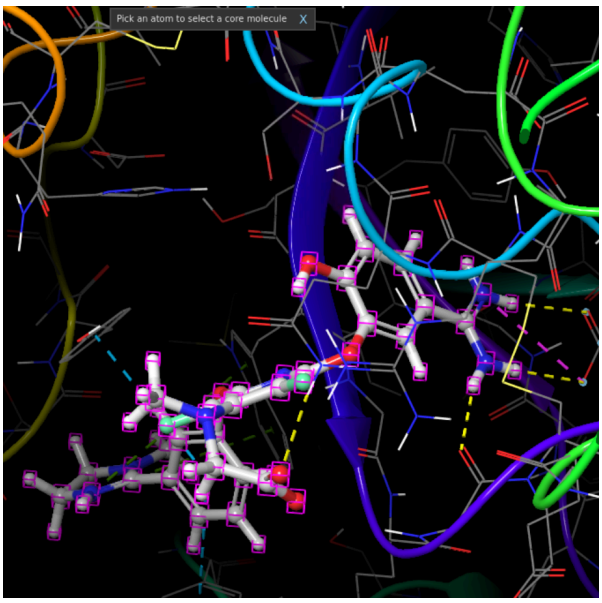


Figure 7-4. Picking an atom part of the ligand for the core constraint.

13. In your Entry List, select and include **1fjs_prepared_dry**
14. Click on the **ligand**

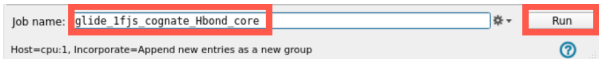


Figure 7-5. Naming and running the job.

15. Go back to the **Ligand Docking panel**
16. Change Job name to **glide_1FJS_cognate_Hbond_core**
17. Click **Run**
    - This job takes about a minute
    - A banner appears to show that files have been incorporated
    - A new group is added to the Entry List

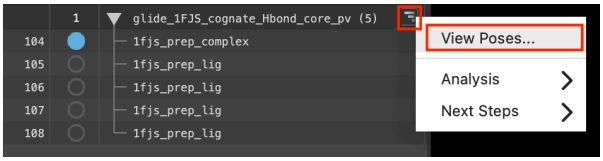


Figure 7-6. View Poses of the cognate ligand with constraints.

18. Click the **icon** next to the glide-1FJS_cognate_Hbond_core_pv and select **View Poses**
19. In the Pose Viewer panel, select **Set Up Poses**
20. The 1fjs_prep_complex entry is fixed in the Workspace, the top

1fjs_prep_lig entry is included, and the Pose Viewer panel appears
    - You may wish to double click the Presets button to have each ligand represented in a unique color.

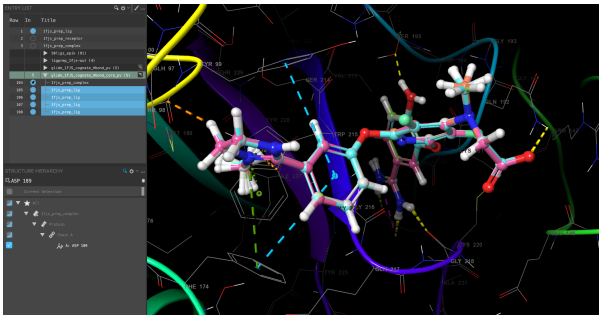


Figure 7-7. Binding poses of the top docked cognate ligands compared to the crystal structure.

21. Include other ligand results
22. Double-click the **In** circle next to 1fjs_prep_complex
    - The entry is no longer fixed in the Workspace

Note: To view all docked ligands in their own color, include all ligands to be evaluated and double click the Presets button, or include all ligands and choose **Binding Mode Comparison** in the Presets menu.

Now all the results from docking perfectly align with the cocrystal pose. You can use core constraints if the screening ligands have the same binding mode and have a similar core constraint to the cocrystal ligand.

8. Visualizing Protein-Ligand Complexes

In this section, we will explore ways to visualize structures in the Workspace. Object representation can be changed in a number of ways using the Style toolbox. Presets offer the ability to quickly render a structure in a number of styles, similar to PyMOL, to facilitate easy visualization. Presets can be used in a variety of ways, from de-cluttering your structure to creating publication-quality images. We will analyze the protein-ligand complex by looking at the interactions, and generate a custom set for some binding residues of interest. Finally, we will visualize the surface of the binding pocket and l save an image of the complex.

8.1. Use the Style toolbox

Figure 8-1. The Style toolbox with CPK representation highlighted.

1. Include entry **1FJS – prepared**
2. Type **L**
    - The Workspace zooms to the ligand
3. Under Quick Select, click **L**
    - The ligand is selected
4. Click **Style**
5. Choose **CPK** representation
    - The ligand is rendered in space-filling (CPK) representation
    - This is only applied to the ligand, since nothing else is selected in the Workspace

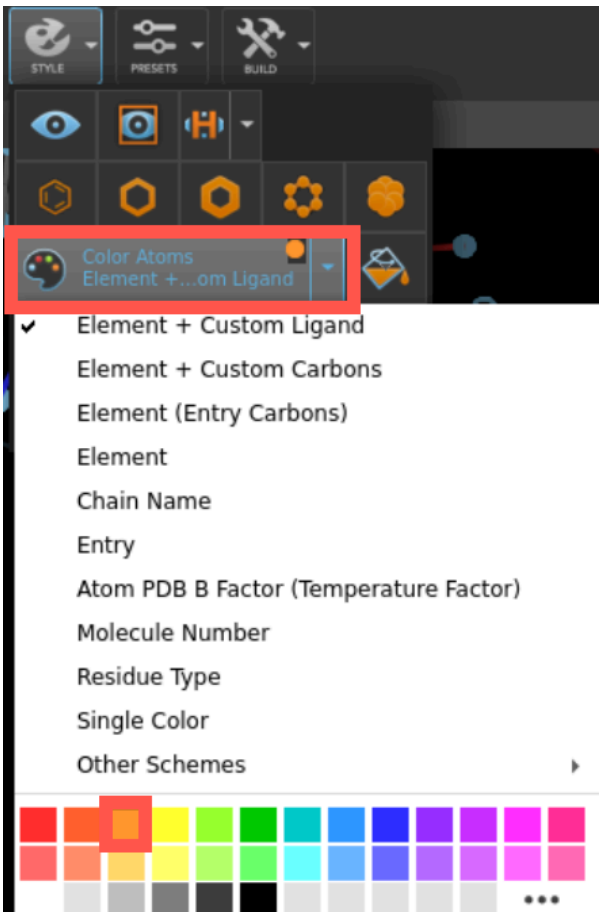


Figure 8-2. The Color Atoms menu.

6. Click the **Color Atoms arrow**
7. Choose **Element (Custom Ligand)**, and pick **orange** from the secondary menu
    - Ligand carbon atoms are orange
8. Under Quick Select, click **P**
    - The protein is selected
9. Type **Z**
    - The Workspace is zoomed to view the selected structure
10. In the Style toolbox, click **Ribbons**
    - Ribbons are added to the protein

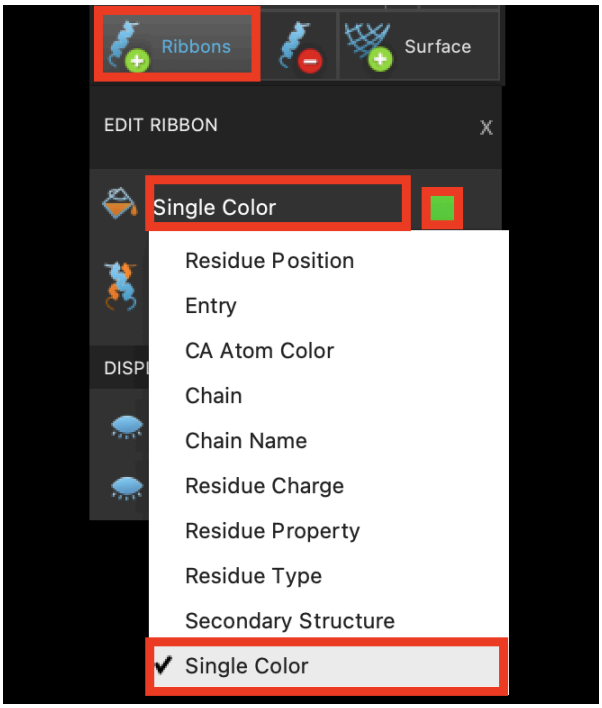


Figure 8-3. The Edit Ribbon panel.

11. Right-click on the **ribbon**
    - The Edit Ribbon panel opens

Note: Use the predictive highlighting to know when you will click on the ribbon.

12. Click **Residue position** in the color scheme
13. Choose **Single Color**

Note: Click the box to the right of the color scheme to choose different colors

8.2. Use the Style toolbox

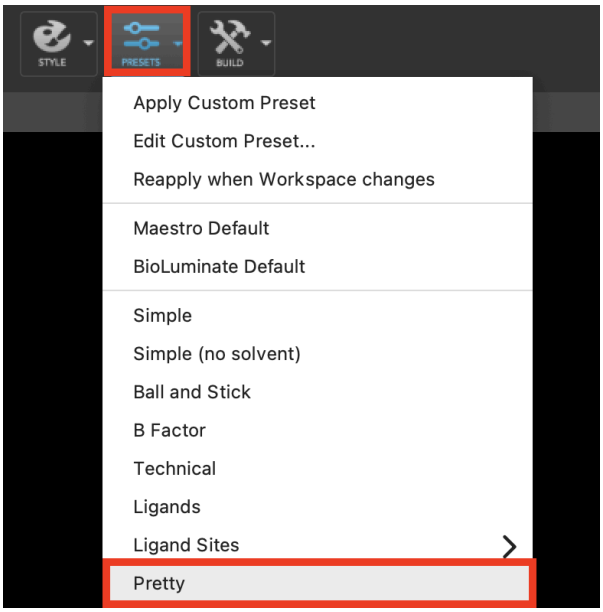


Figure 8-4. The Presets menu.

1. Click **Presets**
2. Choose **Pretty**
    - The Workspace is rendered with ribbons, a green thick-tube ligand, and side chains are hidden
3. Double-click **Presets**
    - The Workspace is redrawn with the Custom Preset
    - The Workspace zooms to the ligand

8.3. Visualize Interactions

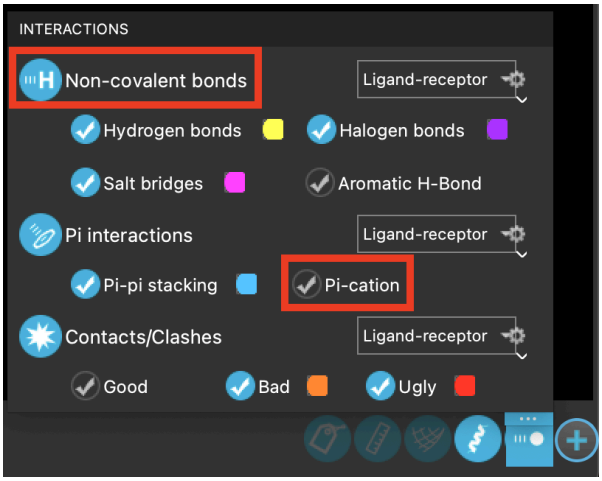


Figure 8-5. The Interactions panel in the Workspace Configuration Toolbar.

1. In the Workspace Configuration Toolbar, click the 3 dots above **Interactions**
    - The Interactions panel opens
2. Turn on **Non-covalent bonds**
3. Turn off **Pi-cation interactions**

Note: Clicking the color to the right of each interaction opens the Preferences panel, where the interaction visualization can be customized

Note: The threshold for Contacts/Clashes is set to 0.89 for bad and 0.75 for ugly. These values correspond to the ratio of the

distance between the two atoms and the sum of their Van der Waals radii.

8.4. Generate and manipulate a surface

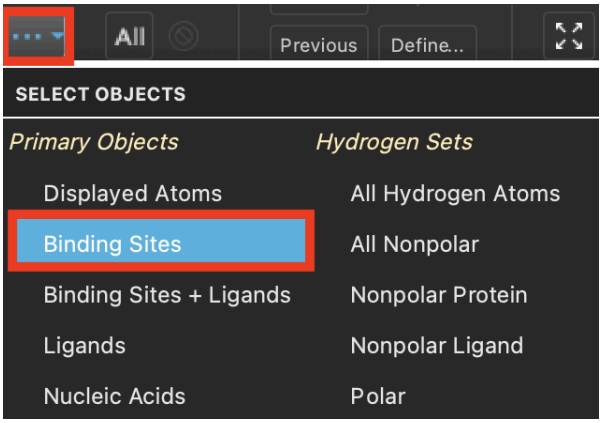


Figure 8-6. More options in Quick Select.

1. Under Quick Select, 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

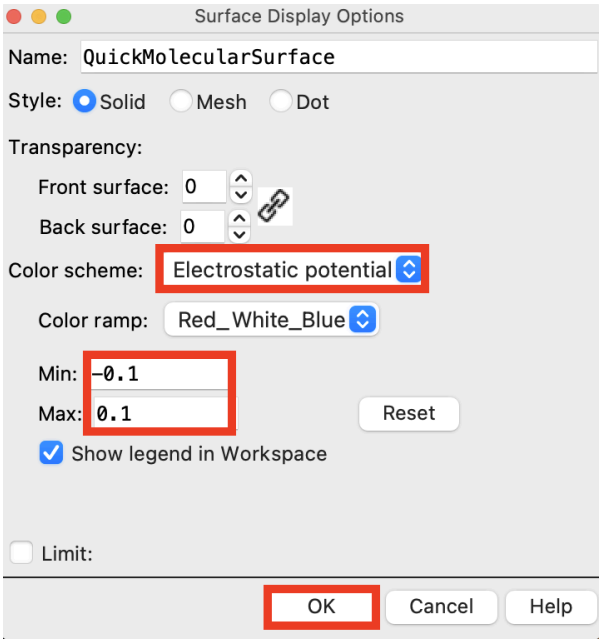


Figure 8-7. The Surface Display Options panel.

3. Right-click the **surface**
4. Choose **Display Options**
    - The Surface Display Options panel opens
5. For Color Scheme, choose **Electrostatic Potential**
6. Change the Min and Max values to **-0.1** and **0.1**, respectively
7. Click **OK**
    - The intensity of the surface colors is increased

8.5. Generate a 2D interaction diagram

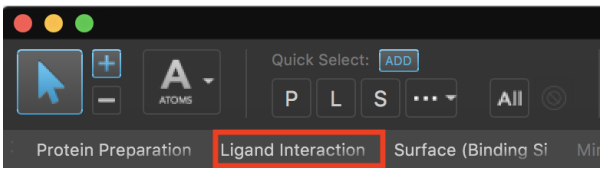


Figure 8-8. Ligand Interaction Diagram in the Favorites toolbar.

1. In the Favorites toolbar, click **Ligand Interaction**
    a. The 2D Workspace - Ligand Interaction Diagram opens

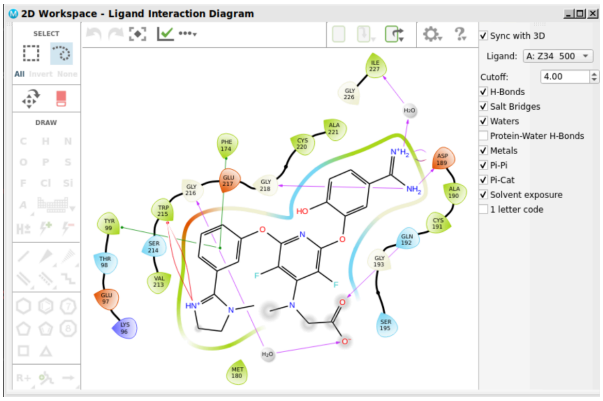


Figure 8-9. The Ligand Interaction Diagram with Sync with 3D turned on and LID legend open.

2. Check mark **Sync with 3D** and rotate the ligand in the Workspace
    a. Ligand orientation is changed in the 2D representation
3. Click the **cog/gear** symbol > **LID Legend**

Note: Images can be saved via File > Save Screenshot

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

Pause & Think #4: What important protein-ligand interactions do you see when the cognate ligand is docked? Take a screenshot of the Ligand Interaction Diagram below. List specific residues and define specific interactions that may play an important role in binding.

9. Individual Exercise

Part A:

Perform the same preparation steps on the 2XIR crystal structure. What were some of the issues with this PDB structure from the first step of protein preparation?

Part B:

Search the Protein Data Bank for another protein-ligand complex and do the following:

A. Import the .pdb file into Maestro
B. Split the structure into Ligands, Waters, and Receptor
C. Prepare the protein using the Protein Preparation Workflow
D. Prepare the ligand using LigPrep
E. Detect the binding site using Receptor Grid Generation
F. Dock all the conformations and tautomers generated from LigPrep back into the prepared protein using Schrödinger's Glide
G. Take a screenshot of your docking results including the docking score and pose of the best docking pose.

10. Summary, Additional Resources, and References

In this lesson, we imported and prepared a protein and ligand file, then visualized and analyzed the protein-ligand complex. A raw PDB file was made suitable for modeling purposes using the Protein Preparation Workflow, and the cognate ligand was extrapolated using LigPrep in the same fashion that would be used for a multi-ligand file. Then the prepared ligand was docked into the prepared protein using Glide. The Workspace Configuration toolbar allowed for toggling various components in the Workspace and the 2D view in the Ligand Interaction Diagram gave another way to analyze information.

For further learning:

- 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

11. 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