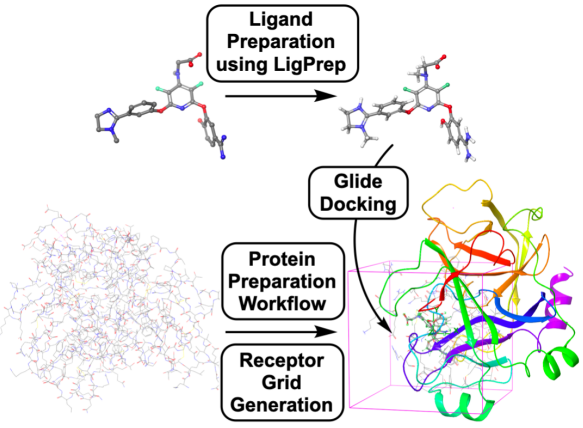
Protein Ligand Docking

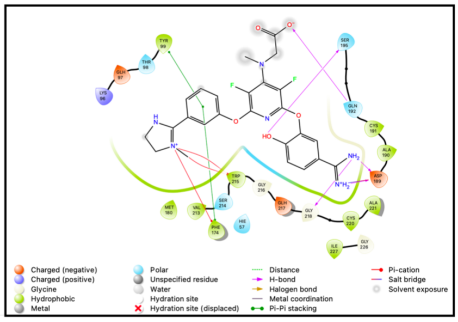
Created with: Release 2021-3
Prerequisites: working knowledge of Maestro
Files Supplied: Protein_ligand_docking_worksheet
Categories: biochemistry, medicinal chemistry

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

- 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

Standards

- *ACS Guidelines*
  - Biological macromolecules (Section 5.1)
- *ETS Chemistry GRE*
  - Organic Chemistry – Amino acids, Peptides (3F)
- *AAMC MCAT*
  - Structure, function, and reactivity of biologically-relevant molecules (5D)

Assessments

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

Warm-Up Questions: To be done on their own or at the beginning of class

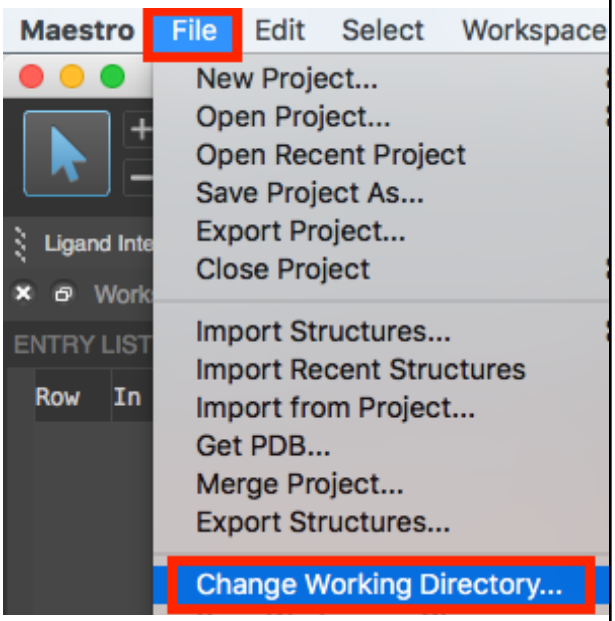
Read the article "A systematic analysis of atomic protein-ligand interactions in the PDB" and answer the following questions.

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?

Lesson Outline

1. What you will need for this lesson

	1. Go to the 'Data' folder and open your Class Folder found on the virtual cluster's desktop. 2. Right-click on the folder called "Protein_Ligand_Docking" and copy folder to Desktop ● Here, you will find the lesson plan, worksheet, and any additional resources
 *Figure 1-1. Open Maestro.*	3. Open **Maestro** a. See Starting Maestro if you need help
 *Figure 1-2. Change Working Directory option.*	4. Go to **File > Change Working Directory** 5. Find your "Protein_Ligand_Docking" folder that you duplicated to your Desktop, and click **Choose**

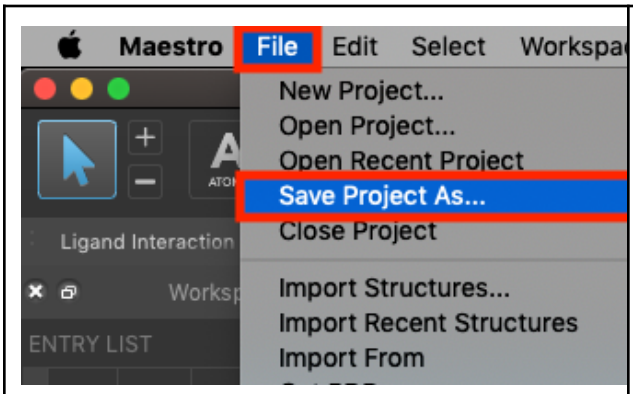


Figure 1-3. Save Project panel.

6. Next, go to **File > Save Project As**
7. Type "**Docking_tutorial**" and click **Save**
    a. The project will be titled **Docking_tutorial.prj**

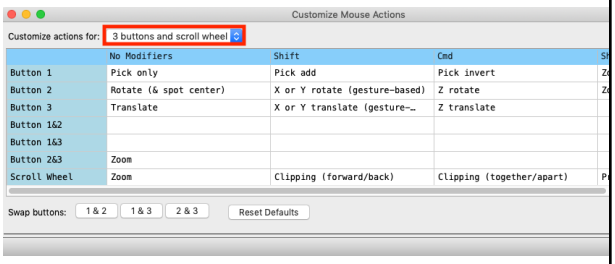


Figure 1-4. Choose the best mouse option for your set up.

8. Finally, check your **Mouse Actions**
    a. PC : **Edit > Customize Mouse Actions**
    b. Mac : **Workspace > Customize Mouse Actions**
9. Make sure you have **the best option chosen for your set up**. This lesson was written with a three-button mouse with a scroll wheel, meaning the scroll wheel is a button as well as a wheel. If you do not have a mouse, choose **Trackpad**.

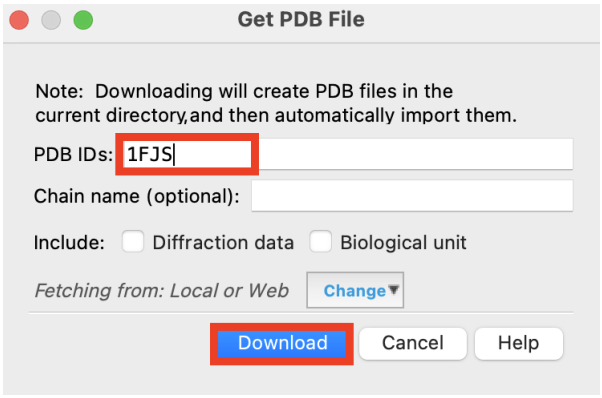


Figure 1-5. Get PDB File panel.

10. Go to **File > Get PDB**
11. For PDB IDs, type **1fjs**
12. Click **Download**
    - 1FJS 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 protein will be covered in the following section.

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.

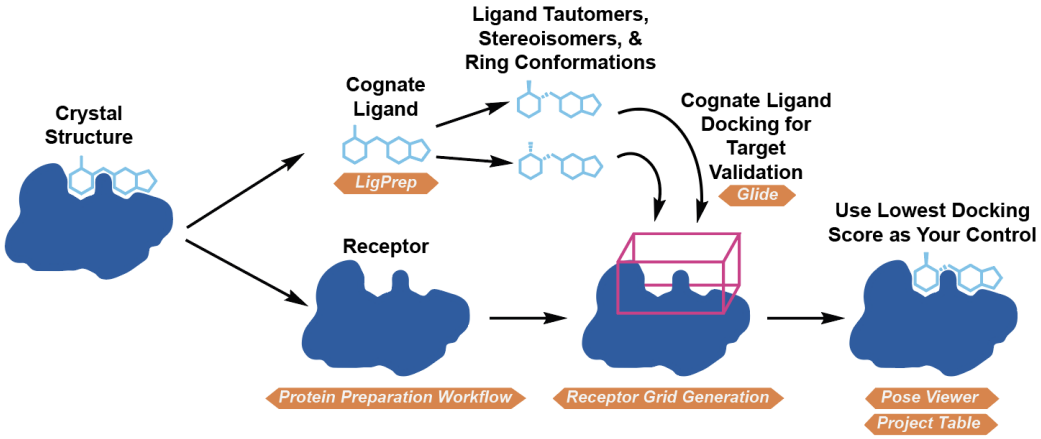


Figure 1. Workflow for Protein Ligand Docking

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. The Protein Preparation Workflow in the Favorites toolbar.

1. 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

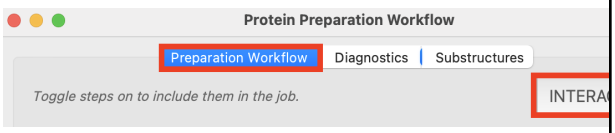


Figure 3-2. The Protein Preparation Workflow in Automatic mode.

2. 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 tutorial will be running an automatic protein preparation.

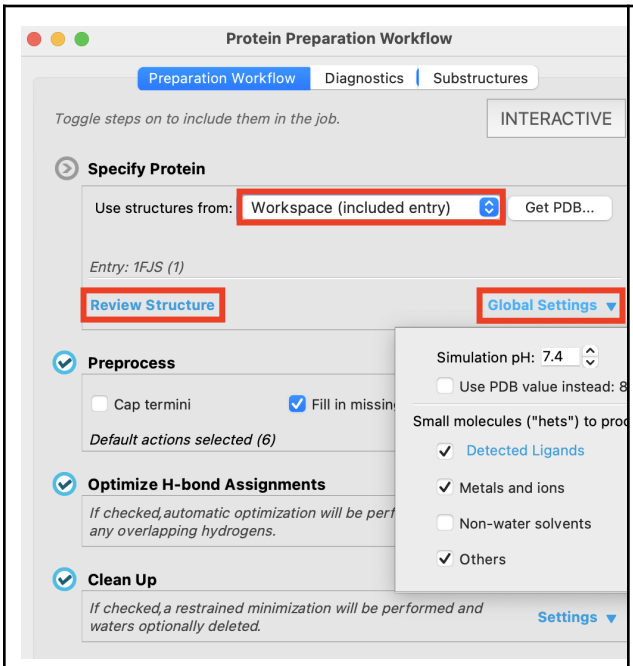


Figure 3-3. Specify the protein from the Workspace.

3. In the Specify Protein section, choose use structures from: **Workspace**
4. 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

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.

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

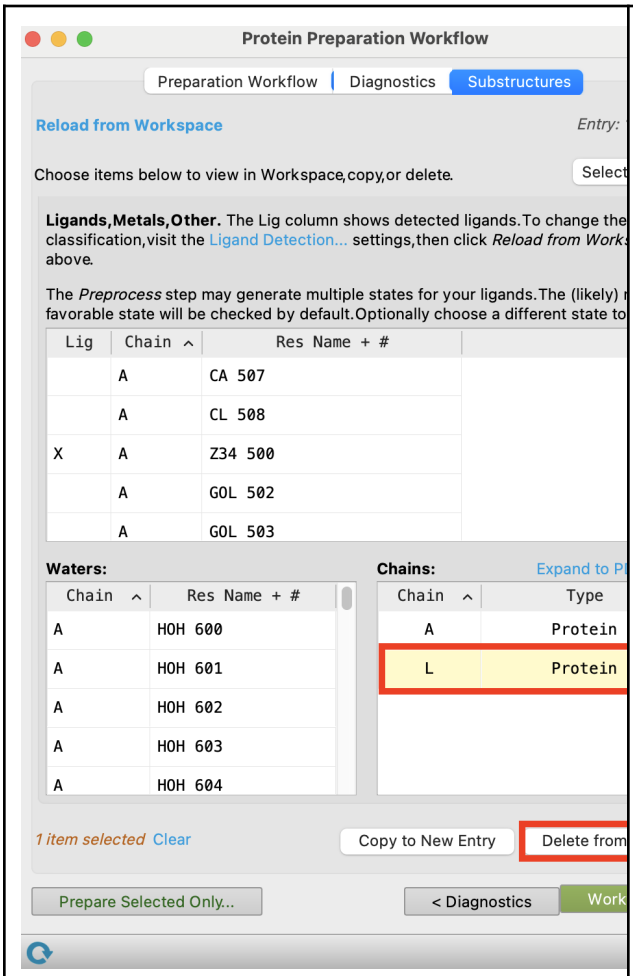


Figure 3-4. Review the structure for preparation in the Substructures tab.

6. Under Chains, click Chain **L**
    - The Workspace zooms to the chain
7. Click **Delete from Entry**
    - The smaller of the two chains is removed, and a new entry 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

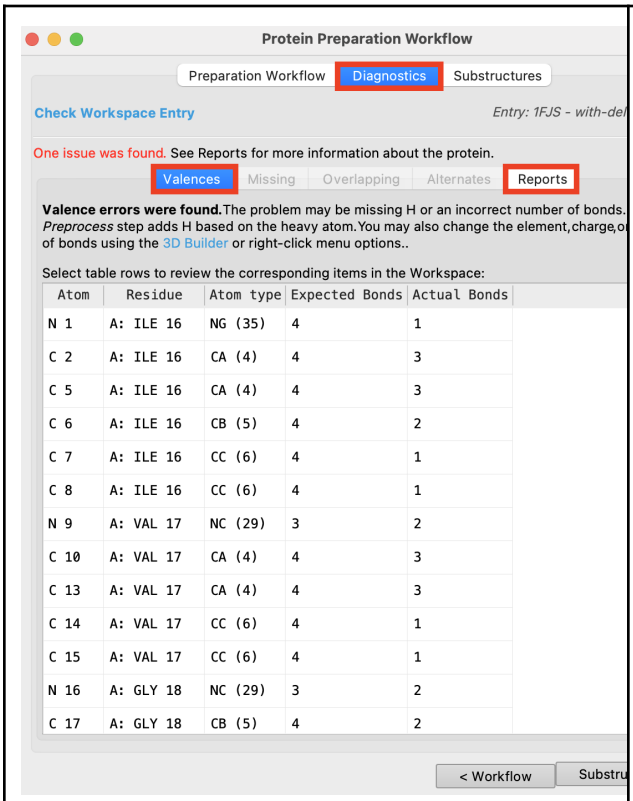
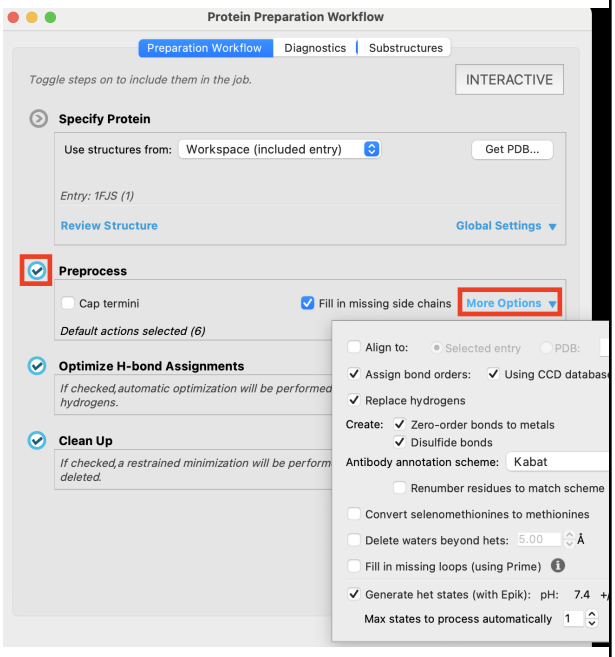


Figure 3-5. View protein issues in the Diagnostics tab

8. 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
9. Select **Reports** to view other issues with the protein that must resolved prior to modeling



10. Return to the **Preparation Workflow** tab
11. Confirm **Preprocess** is toggled on
    - Notice Fill in missing side chains is checked by default. If these were missing in our structure they would become populated during this step
12. Under Preprocess, select **More Options**
    - Among the options provided, notice that

Figure 3-6. Preprocess default settings

missing loops may be filled in using Prime

13. 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.

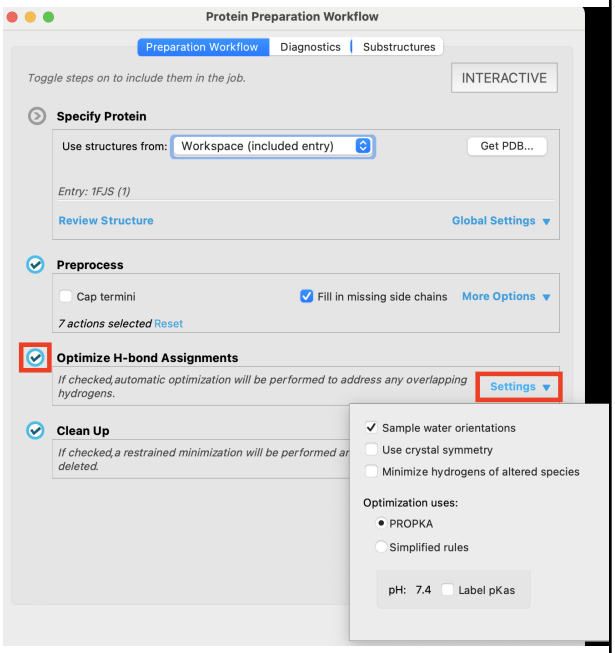


Figure 3-7. Optimize H-Bond Assignment default settings

14. Confirm **Optimize H-bond Assignment** is toggled on
15. 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
16. Check the pH for Optimization
    - This value should be captured in the pH range chosen during the Preprocess step

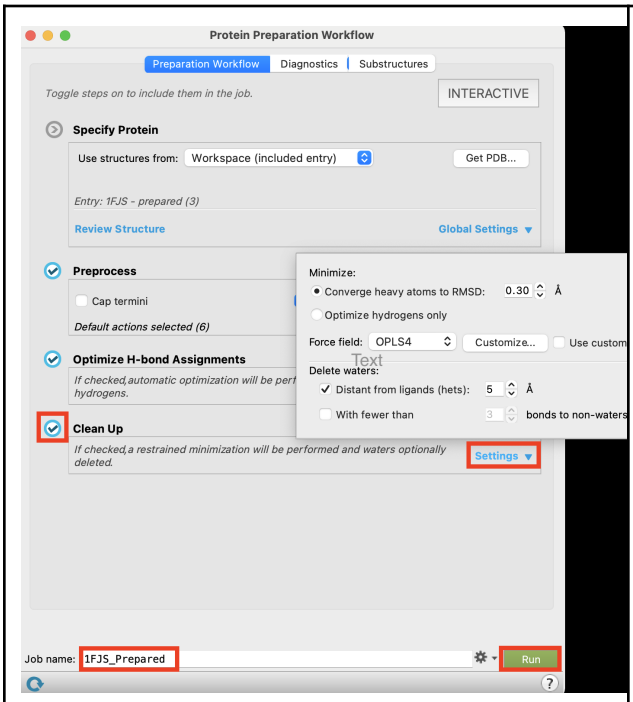


Figure 3-8. Clean Up default Settings

17. Confirm **Clean Up** is toggled on
18. Click **Settings**
    - Notice that by default distant waters will be removed. The specified distance to ligands can be modified
19. Change the job name to **1FJS_Prepared**
20. Click **Run**
    - This job will take a few minutes as all toggled jobs are running consecutively
    - A new group will be added to the Entry List

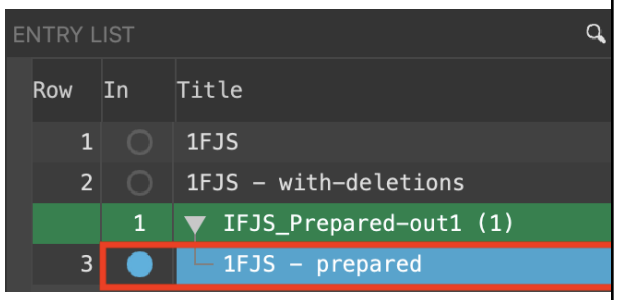


Figure 3-9. Select the protein preparation output

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

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

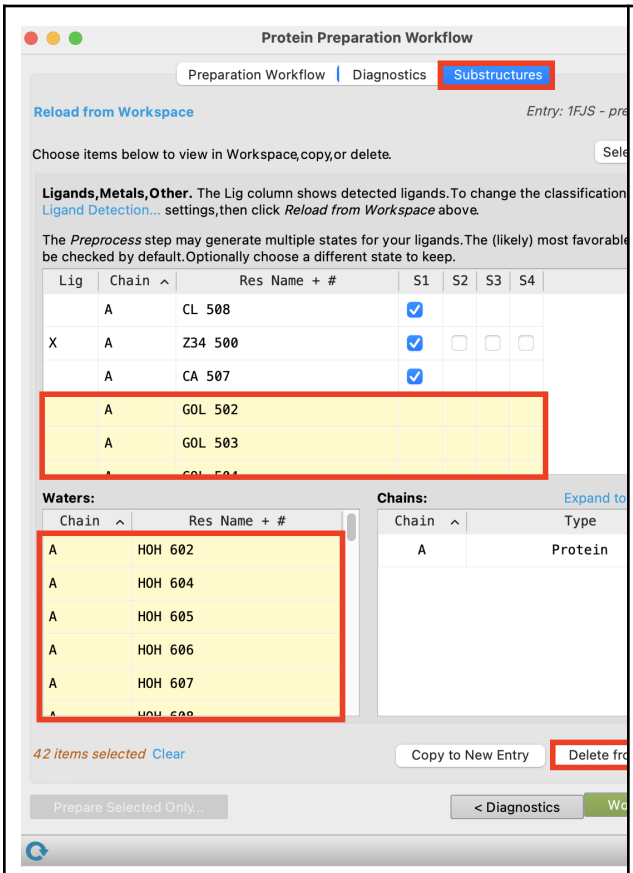


Figure 3-10. Perform Substructure Review

22. Return to the **Protein Preparation tool**
23. Click the **Substructures** tab
24. Choose **Reload from Workspace**
25. In the Hets table, shift-click to select all **GOL** rows
26. Click **Delete from Entry**
27. In the Waters table, shift-click to select all **waters**
28. Click **Delete from Entry**

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 Protein Structure Preparation using the Protein Preparation Workflow or Protein Preparation Workflow Panel Help for more details.

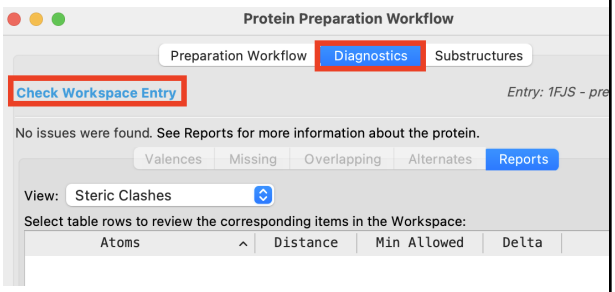


Figure 3-11. Confirm issues have been resolved during preparation

1. In the 1FJS_Prepared-out1 group, select **1FJS – with-deletions**
2. Return to the Protein Preparation Workflow and click the **Diagnostics** tab to make sure there are no issues missed during the preparation. You may need to click **Check Workspace Entry**
3. **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.

Question #1:

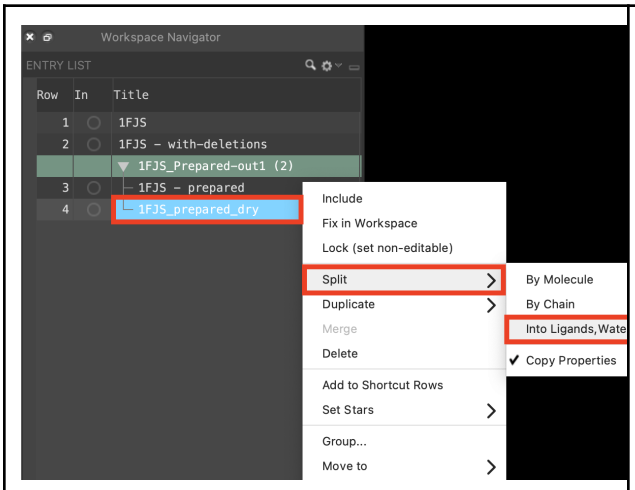
List one error that you identified to be a problem prior to running the Protein Preparation Workflow.

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.

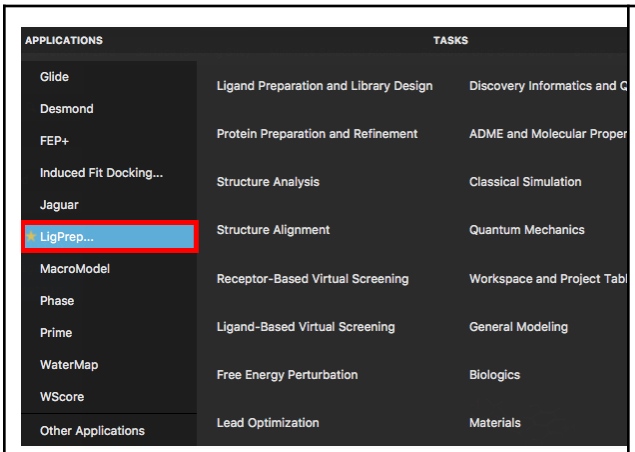
4.1 Split the prepared structure



1. In the Entry List, double click 1FJS - with-deletions in the 1FJS_Prepared-out1 group and rename the entry to **1FJS_prepared_dry**
2. right-click on **1FJS_prepared _dry**
3. Choose **Split > Into Ligands, Water, Other**
    - Two new entries appear in the Entry List

Figure 4-1. Right-click to split an entry into different components.	4. Include **1FJS – prepared_dry_ligand** ○ Only the ligand is displayed in the Workspace

4.2 Run LigPrep

 *Figure 4-2. LigPrep application in the Task toolbar.*	1. Go to **Tasks > Browse > LigPrep** ○ The LigPrep panel opens

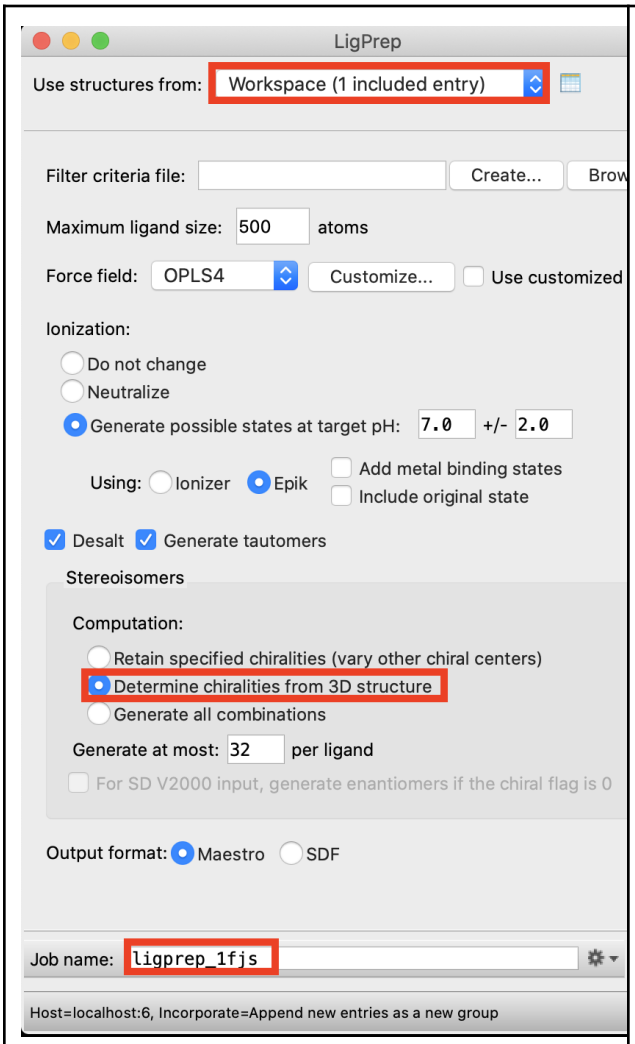


Figure 4-3. The LigPrep panel.

2. For Use structures from, choose **Workspace (1 included entry)**
3. Under Stereoisomers, choose **Determine chiralities from 3D structure**
4. Change Job name to **ligprep_1fjs**
5. Click **Run**
    - 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.

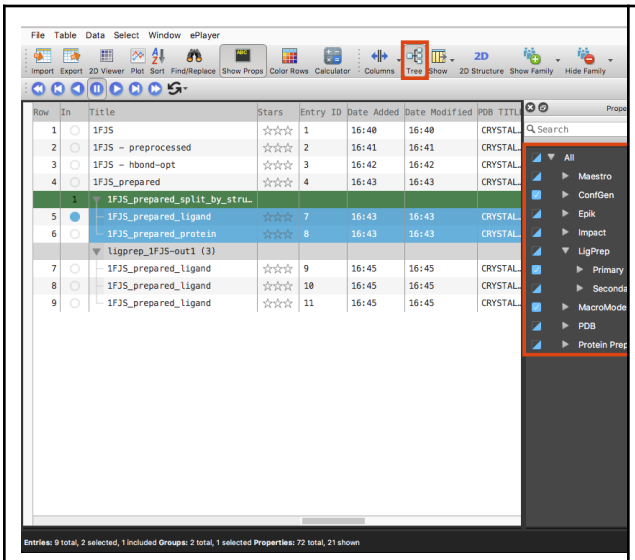


Figure 4-4. The Project Table with the Property Tree open.

6. Type **Ctrl+T (Cmd+T)** to open the Project Table
7. Click **Tree** to open the Property Tree
    - Different calculated properties can be toggled on and off
    - Click the **arrow** next to each application to view more properties

Question #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

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 grid generation videos on our website or 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

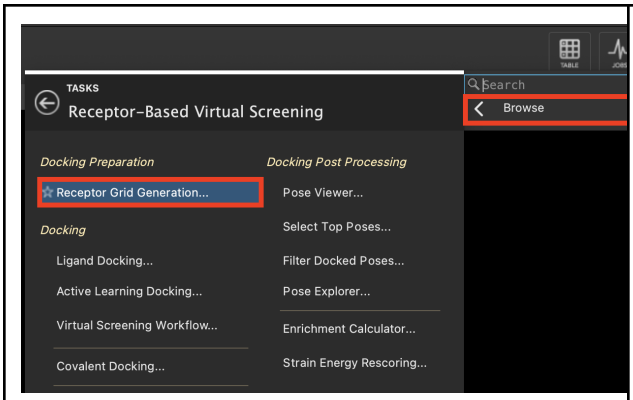


Figure 5-1. Receptor Grid Generation option in Receptor-Based Virtual Screening.

1. Click the **In** circle next to **1fjs_prep_complex** to include it in the Workspace
2. Double-click **Presets**
   - 1fjs_prep_complex is rendered using the Custom Preset
3. Go to **Tasks > Browse > Receptor-Based Virtual Screening > Receptor Grid Generation**
   - The Receptor Grid Generation panel opens

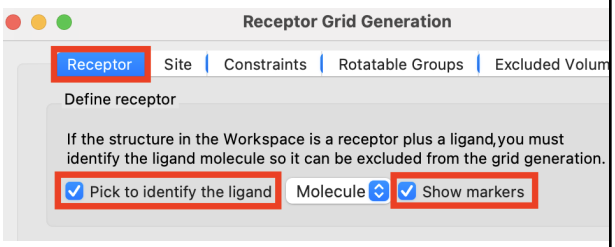


Figure 5-2. The Receptor tab of Receptor Grid Generation.

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

Figure 5-3. The ligand is defined to be excluded from grid generation.

5. Click on the **ligand**
    - The ligand is now highlighted with a purple box around it
    - The ligand will be excluded from the grid generation

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

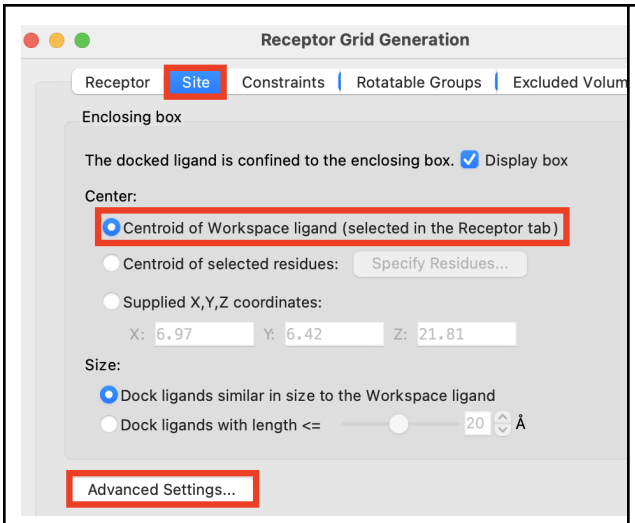


Figure 5-4. The Site tab of Receptor Grid Generation.

1. Click the **Site** tab
2. Select **Centroid of Workspace ligand (selected in the Receptor tab)**
3. Click **Advanced Settings**
    - A 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

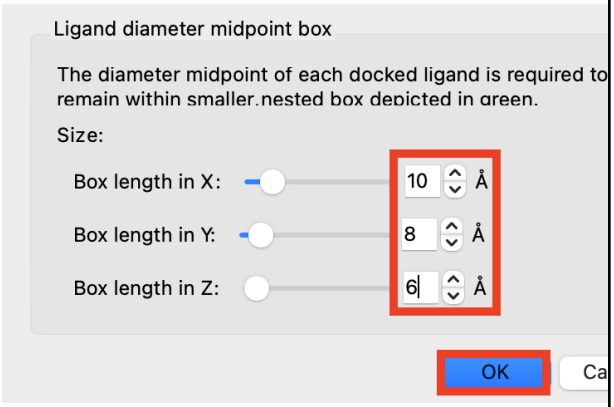


Figure 5-5. Ligand diameter midpoint box panel.

4. Adjust the settings for **X**, **Y**, and **Z** sizes to **10**, **8**, and **6** Å , respectively.
    - The shape of the green box is changed
5. Click **OK**

5.3 Set a hydrogen bonding constraint

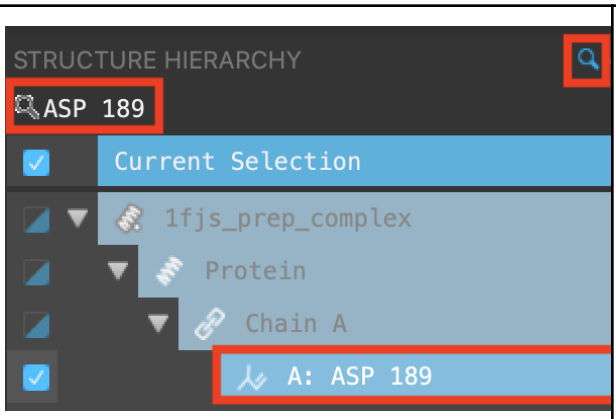


Figure 5-6. Search in the Structure Hierarchy.

1. Type **L t**o zoom to the ligand
2. In the Structure Hierarchy, click the **magnifying glass**
3. In the search field, type **ASP 189**
4. Select **ASP 189**

Note: Please see the Introduction to Structure Preparation and Visualization tutorial for instructions on how to add residue labels and show H-bonds

Figure 5-7. Zoom to selected atoms.

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

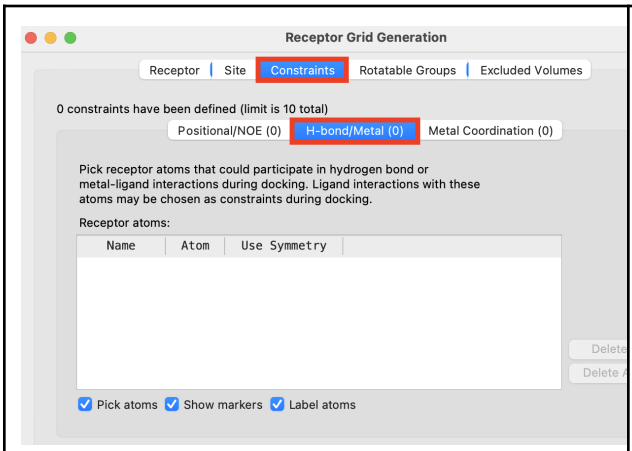


Figure 5-8. The Constraints tab of Receptor Grid Generation.

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

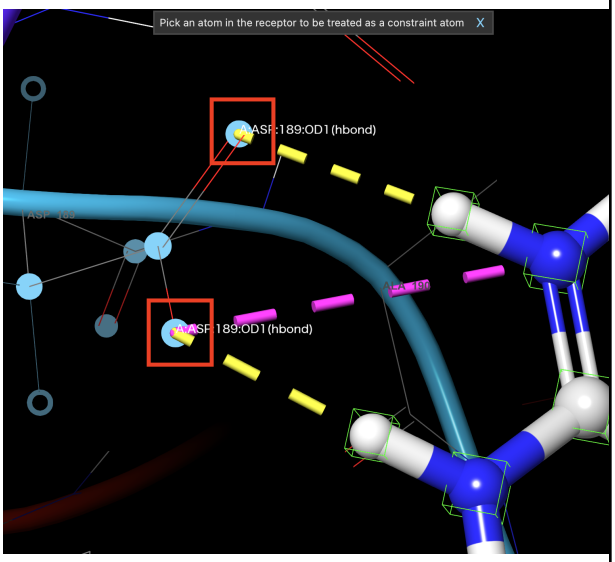


Figure 5-9. Constraint defined on ASP 189.

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

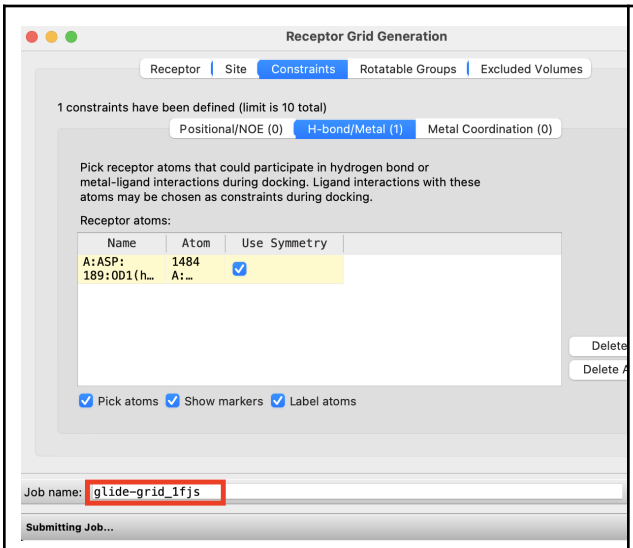


Figure 5-10. Run receptor grid generation job.

9. Change Job name to **glide-grid_1fjs**
10. Click **Run**
    - This job will take about a minute
    - A folder named glide-grid_1fjs is written to your Working Directory

Question #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 a Ligand

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. The information gained from this step can help with evaluating poses and beneficial interactions, which is useful for future hit finding. This job will use the receptor grid file that was generated in the previous step.

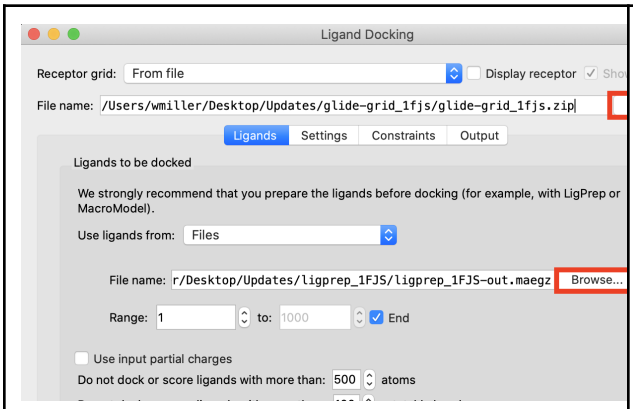


Figure 6-3. The Ligands tab of the Ligand Docking panel.

1. Go to **Tasks > Browse > Receptor-Based Virtual Screening > Ligand Docking**
    - The Ligand Docking panel opens
2. Next to Receptor grid, click **Browse** and choose **`glide-grid_1fjs.zip`**
3. In the Ligands tab, for Use ligands from, choose **Files**
4. Next to File name, click **Browse** and choose **`ligprep_1FJS-out.maegz`**

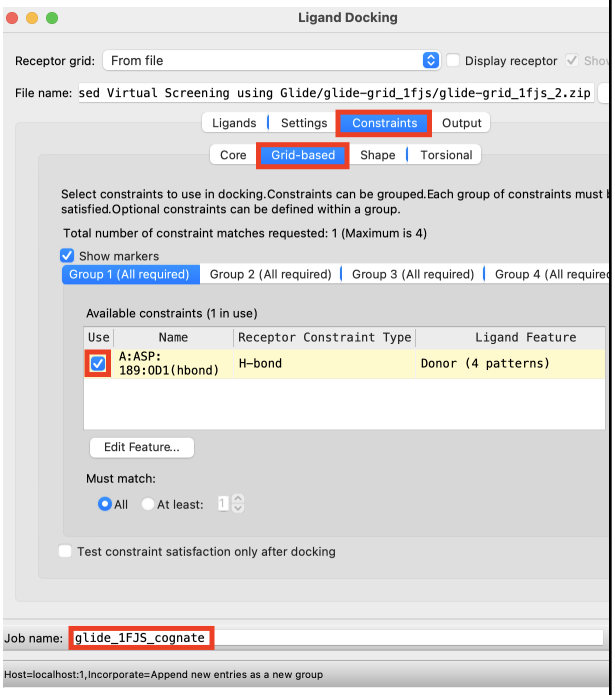
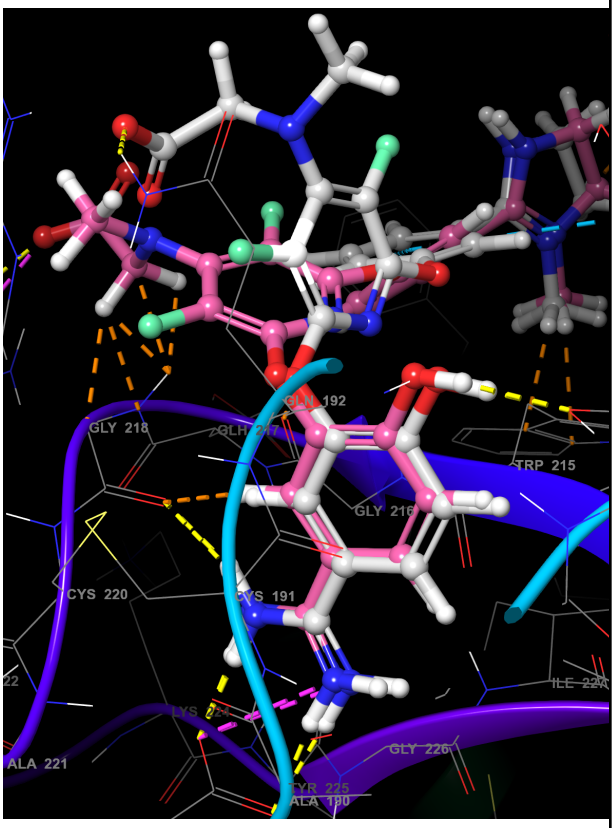


Figure 6-4. The Constraints tab of the

5. Click the **Constraints** tab
6. Click on the **Grid-based** tab
7. Under Use, **check** the H-bond constraint for ASP 189
8. Change Job name to **glide_1FJS_cognate**
9. 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

Ligand Docking panel.	
 *Figure 6-5. Binding pose of the top docked cognate ligand (pink) compared to the crystal structure (gray).*	*Note*: The 1fjs_prep_complex entry is fixed in the Workspace, the top 1fjs_prep_lig entry is included, and the Pose Viewer panel appears 10. Include other **ligand results** ○ H-bonds to ASP 189 are conserved 11. Double-click **Presets** 12. Double-click the **In** circle next to **1fjs_prep_complex** ○ The entry is no longer fixed in the Workspace *Note*: Though only the top ranked result is in strong agreement with the crystallographic pose, all three results accurately capture the pose of the ligand in the binding site (with varying degrees of success in capturing the solvent exposed region)

7. Analyzing Docked Poses

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.

7.1 Visualize the results using Pose Viewer

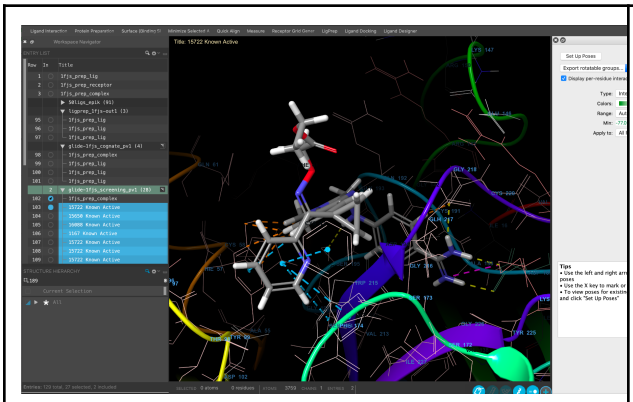


Figure 7-1. Pose Viewer panel.

1. 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)
2. Close the **Pose Viewer** panel

7.2 Analyze the results

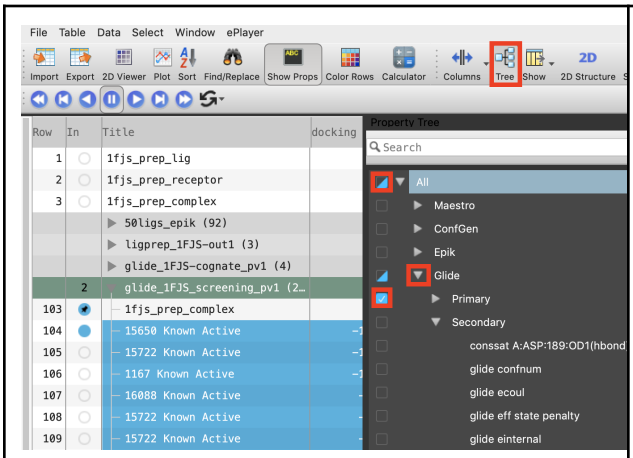


Figure 7-2. Glide Primary properties shown in the Project Table.

1. In the Project Table, click the Property **Tree** icon
    - The Property Tree appears on the right of the Project Table
2. Click the **All** box twice
    - All boxes are deselected
3. Click the **Glide** box
4. Click **Primary**
    - 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 emodel score.

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

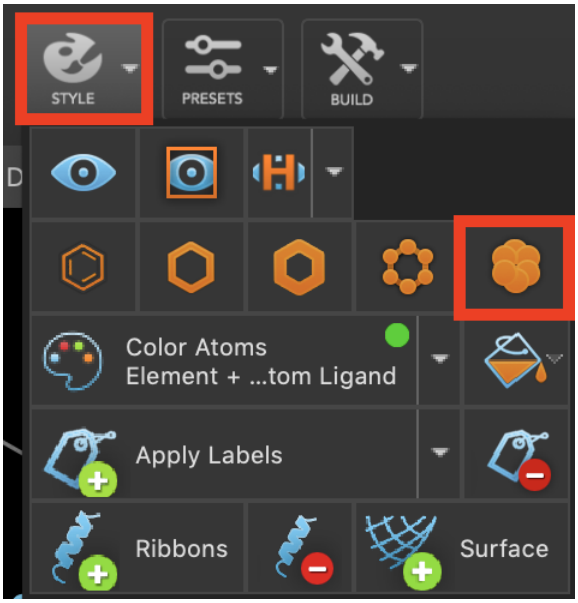


Figure 8-1. The Style toolbox with CPK representation highlighted.

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

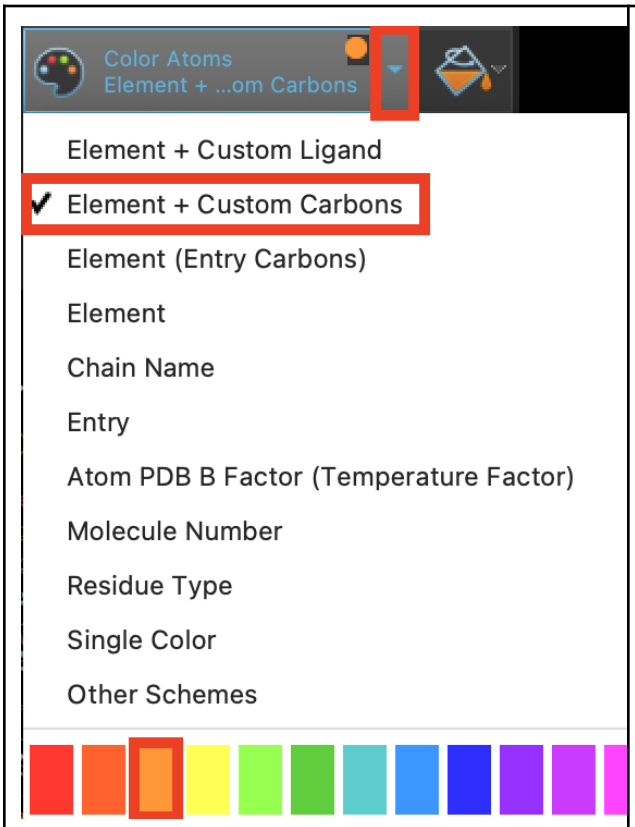


Figure 8-2. The Color Atoms menu.

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

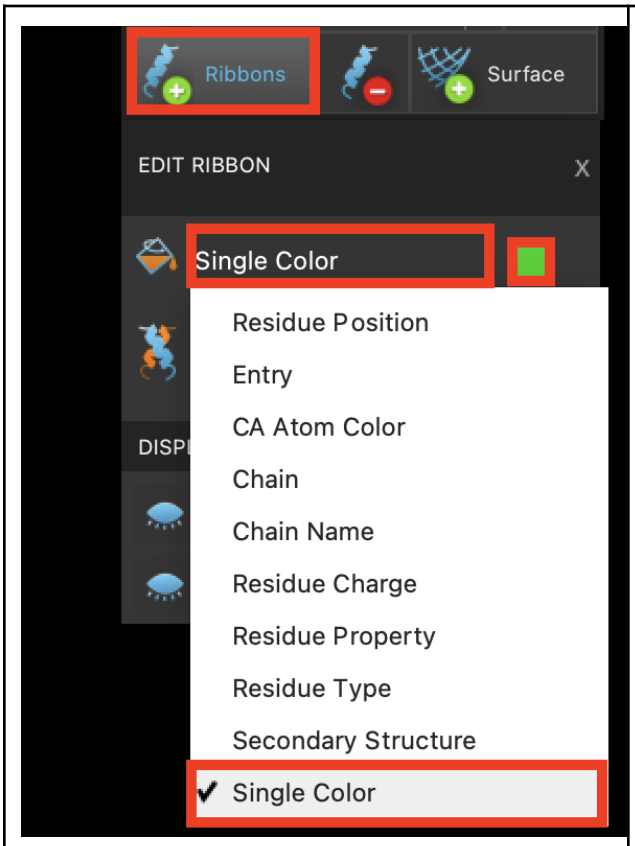


Figure 8-3. The Edit Ribbon panel.

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 Apply a Preset style

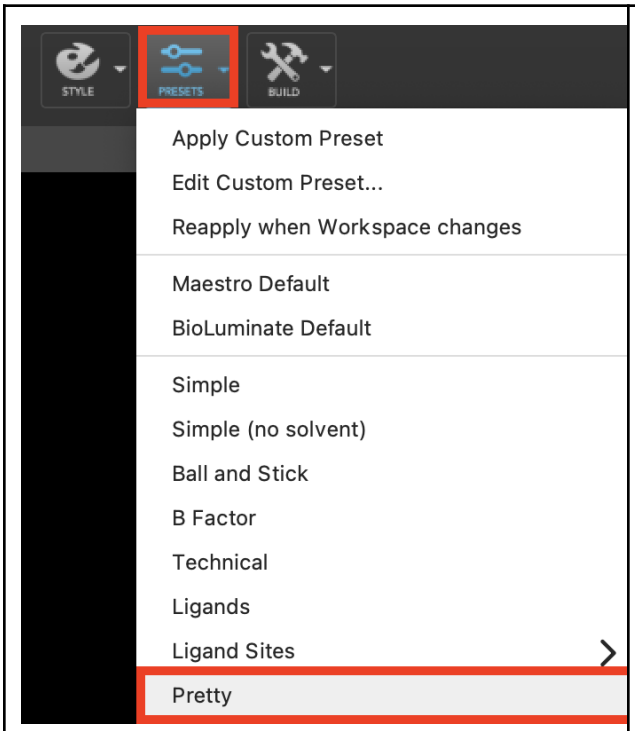
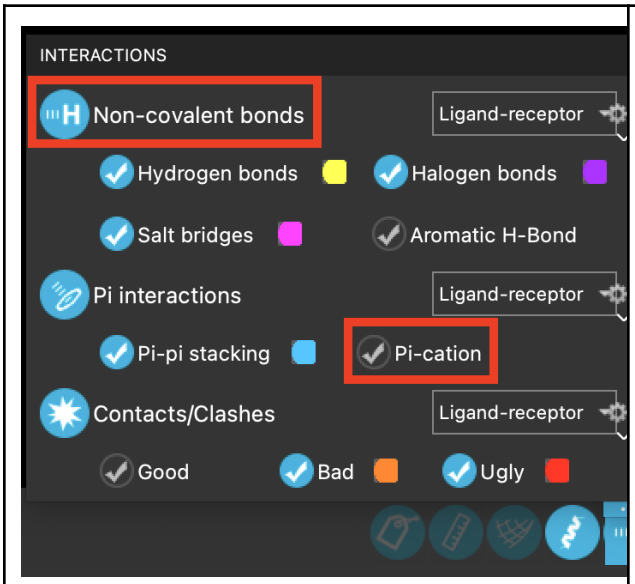


Figure 8-4. The Presets menu.

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



1. In the Workspace Configuration Toolbar, right-click **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.

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

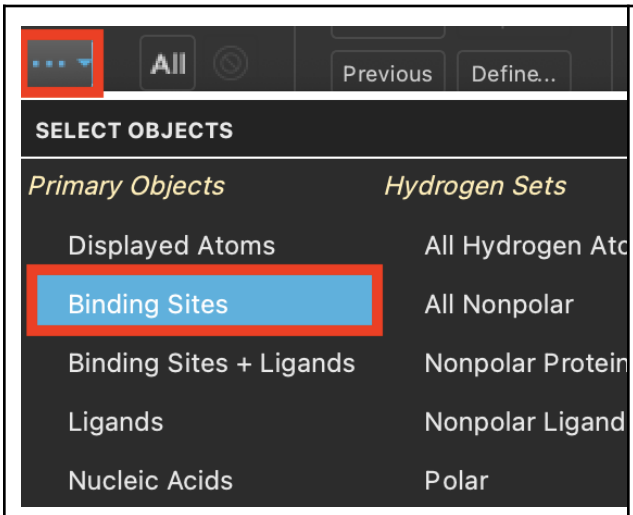


Figure 8-6. More options in Quick Select.

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

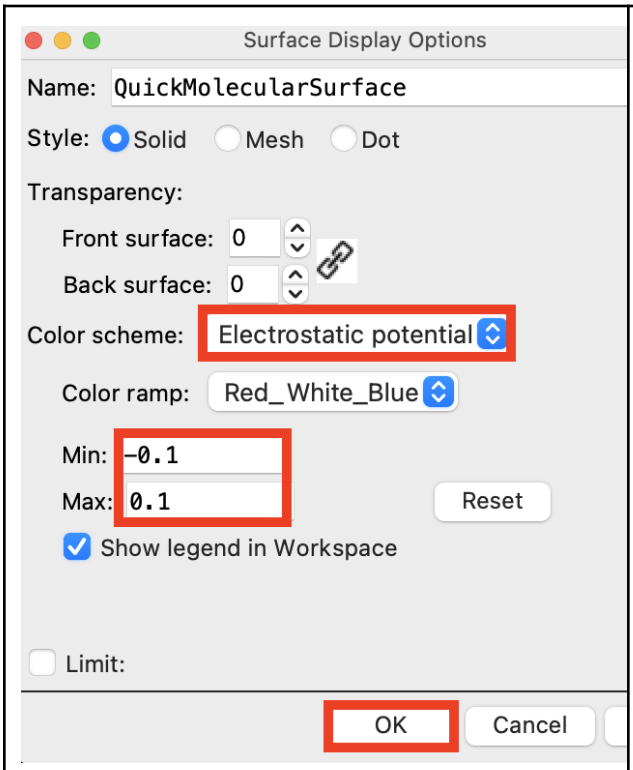


Figure 8-7. The Surface Display Options panel.

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

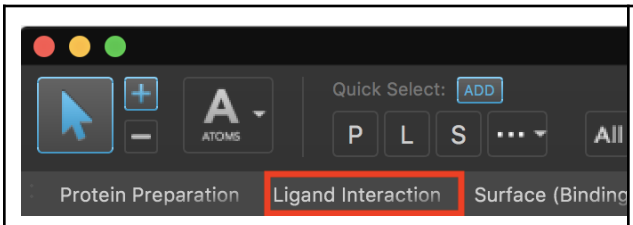


Figure 8-8. Ligand Interaction Diagram in the Favorites toolbar.

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

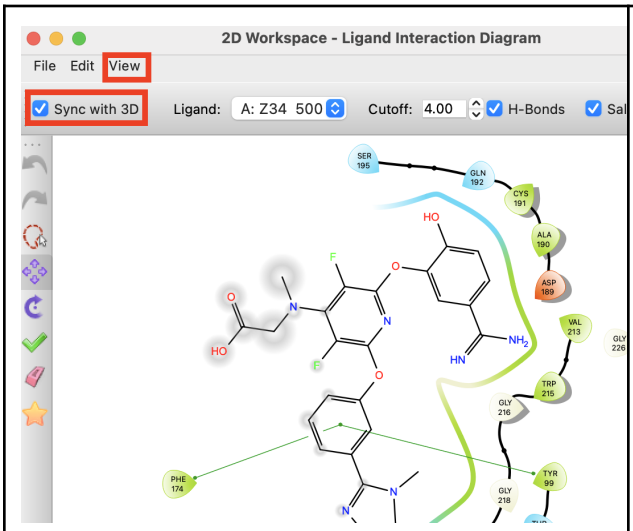


Figure 8-9. The Ligand Interaction Diagram with Sync with 3D turned on and LID legend open.

2. Check **Sync with 3D** and rotate the ligand in the Workspace
    - Ligand orientation is changed in the 2D representation
3. Choose **View > LID Legend**

Note: Images can be saved via **File > Save Screenshot**

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

8.6 Save an image of the Workspace

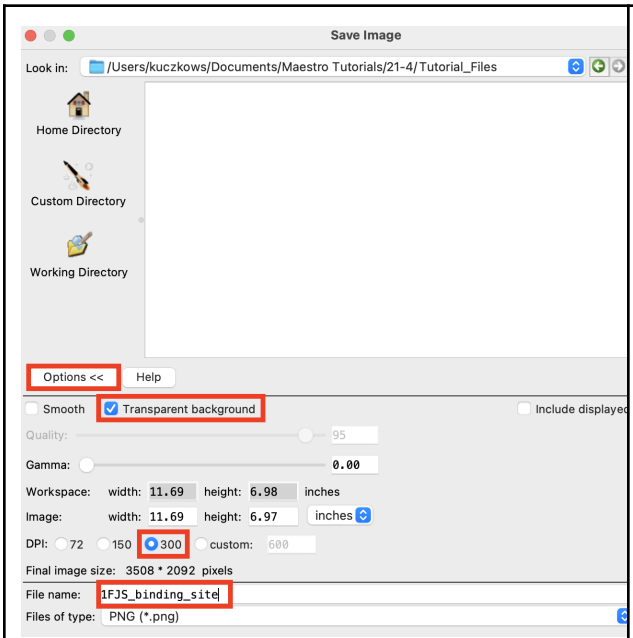


Figure 8-10. Save Image panel with expanded Options shown.

1. Go to **Workspace > Save Image As**
    - The Save Image panel opens
2. Click **Options >>**
3. Check **Transparent background** and select **300** DPI
4. Change File name to **1FJS_binding_site**
5. Click **Save**
    - A .png image of the Workspace is saved to your Working Directory

Note: If an item is highlighted in the Workspace, the image is saved with the selection highlights

	Note: Go to **Tasks > Browse > Workspace and Project Table Operations** for more image options, including **Ray Trace**

Question #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 information, please see:
Maestro 11 Training Portal
Protein Preparation Workflow Panel Help

Glossary of Terms:

cognate ligand - a ligand that is bound to its protein target

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

incorporated - once a job is finished, output files from the Working Directory are added to the project and shown in the Entry List and Project Table

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

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