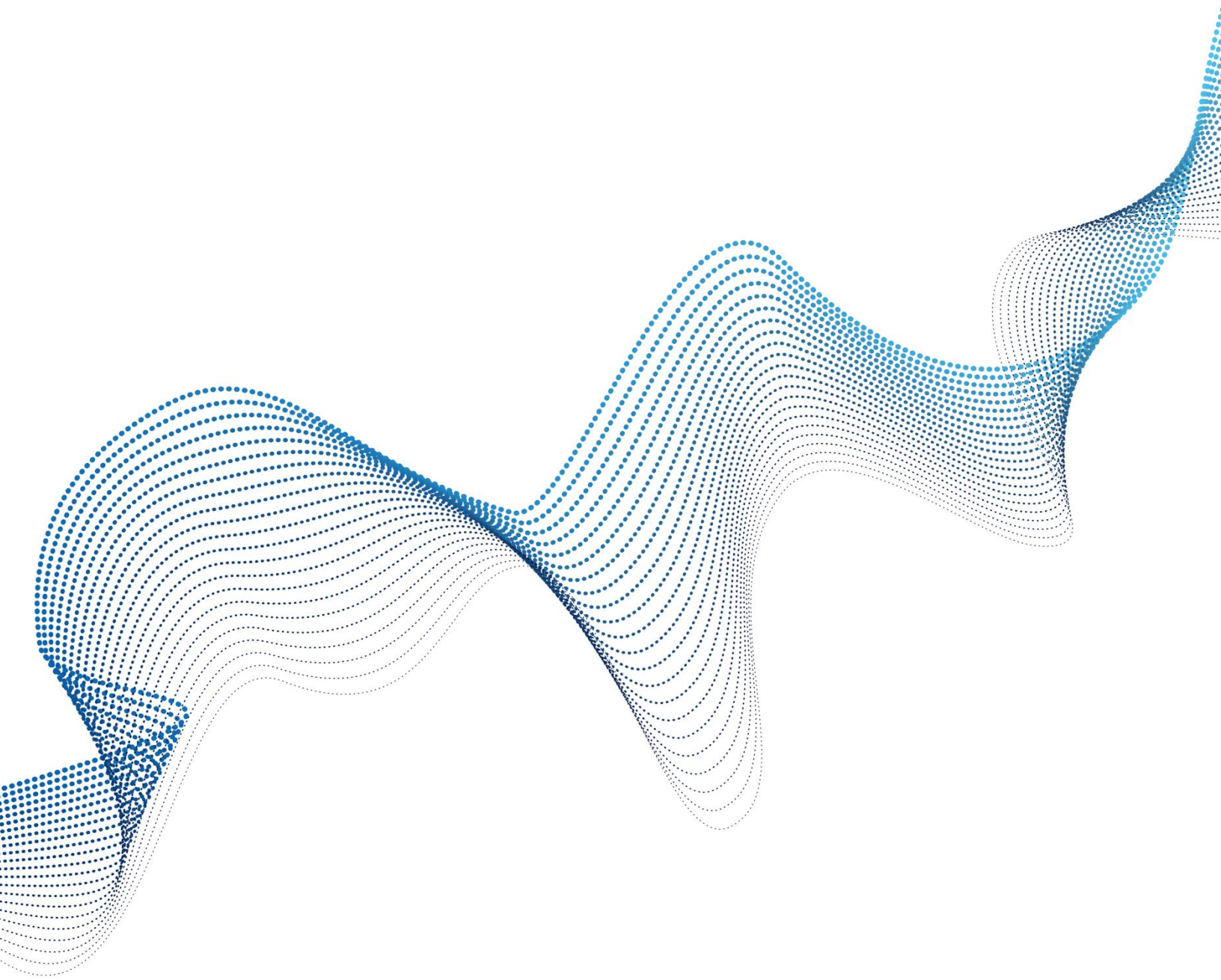
2025-3

Introduction to Structure Preparation and Visualization



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Introduction to Structure Preparation and Visualization

[DOWNLOAD TUTORIAL FILES]

This tutorial is written for use with a 3-button mouse with a scroll wheel.

Words found in the Glossary of Terms are shown like this: Workspace

Abstract:

This tutorial provides an introduction to the fundamental principles and techniques of structure visualization and preparation in Maestro. You will learn the essential steps to visualize the target structure and to address structural issues for modeling applications.
This tutorial however does not cover the details of protein structure refinement. Further, preparation of lipids, membrane proteins, DNA, RNA, etc. is not covered.

Tutorial Content

1. Introduction to Structure Visualization and Preparation

1.1 Structure Visualization

Biomolecules are highly complex and diverse, and are composed of thousands of atoms held together via covalent and non-covalent interactions. Visualization plays a very important role in the study of biomolecules, helping to identify and understand how the properties of biomolecules are related to their structure. Visualization techniques can address some simple yet important questions:

a) How flexible is the target?
b) Does the target have a ligand bound to it?
c) Which residues are playing a crucial role in the binding site if the target has a bound ligand?
d) What are the various interactions present between the ligand and the receptor?

1.2 Structure Preparation

Structure files obtained from the PDB, vendors, and other sources often lack necessary information for performing modeling-related tasks mostly due to experimental limitations of structural biology techniques and insufficient or ambiguous data. Typically, these files are missing hydrogens, partial charges, side chains, and/or whole loop regions. Proteins in their raw state may also have incorrect bond order assignments and side chain orientations. To make these structures suitable for modeling tasks, you will use the Protein Preparation Workflow to find and resolve common structural issues.

The Protein Preparation Workflow in Maestro involves a series of structural and functional checks designed to prepare protein and nucleic acid (DNA/RNA) structures for accurate molecular modeling and simulations. First, the structure is assessed for missing atoms, residues, or side chains, which are then added or corrected. Hydrogen atoms are added to ensure proper protonation states, and the protein is optimized for correct bond lengths, angles, and torsions. The overall geometry is checked for any steric clashes or unusual bond geometries. Functional checks include evaluating the protonation states of ionizable residues at the relevant pH, identifying potential disulfide bonds, and ensuring proper orientation of active site residues. The final protein structure is minimized to relieve any unfavorable interactions and prepare it for further computational analysis, such as docking or molecular dynamics simulations.

In this tutorial, you will learn how to import structures, visualize protein structures, ligand binding sites, ligand-receptor interactions and molecular surfaces. You will also learn how to prepare protein and ligand structures, an essential first step for modeling projects.

Although this tutorial guides you to prepare protein and ligand structures for Glide docking, these steps would be the starting point for many computational experiments, including molecular dynamics simulations (Desmond), and lead optimization (FEP+).

If you prefer to watch video tutorials, see the Protein Preparation and Ligand Preparation videos from the Getting Going with Maestro video series.

2. Creating Projects and Importing Structures

At the start of the session, change the file path to your chosen Working Directory in Maestro to make file navigation easier. Each session in Maestro begins with a default Scratch Project, which is not saved. A Maestro project stores all your data and has a `.prj` extension. A project may contain numerous entries corresponding to imported structures, as well as the output of modeling-related tasks. Once a project is created, the project is automatically saved each time a change is made.

Structures can be built in Maestro or can be imported directly from the PDB or from your Working Directory using **File > Import Structures** (or drag-and-dropped), and are added to the Entries and Project Table. The Entries is located to the left of the Workspace. The Project Table can be accessed by **Ctrl+T (Cmd+T)** or **Window > Project Table** if you would like to see an expanded view of your project data.

1. Double-click the **Maestro** icon.
    - (No icon? See Starting Maestro)

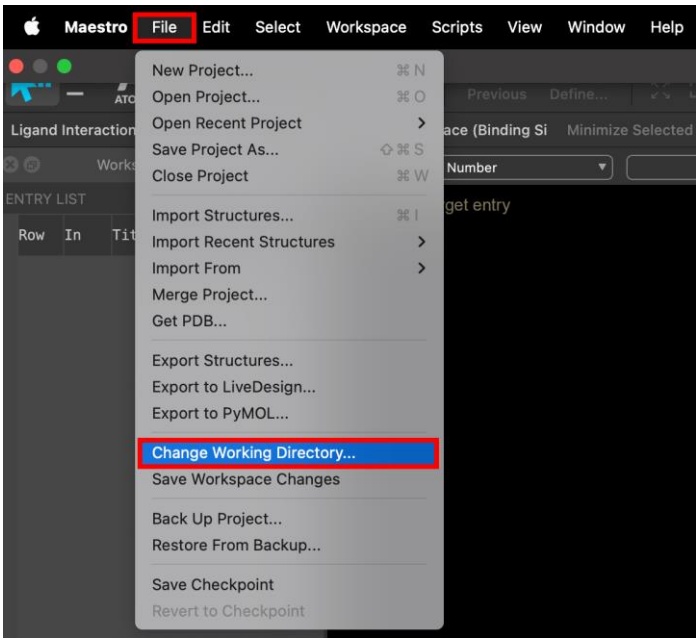


Figure 2-1. Change Working Directory option.

2. Go to **File > Change Working Directory**.
3. Find your directory, and click **Choose**.
4. Pre-generated results files are included for examining the output. Download the zip file here: link
5. After downloading the zip file, unzip the contents into your Working Directory for ease of access throughout the tutorial.

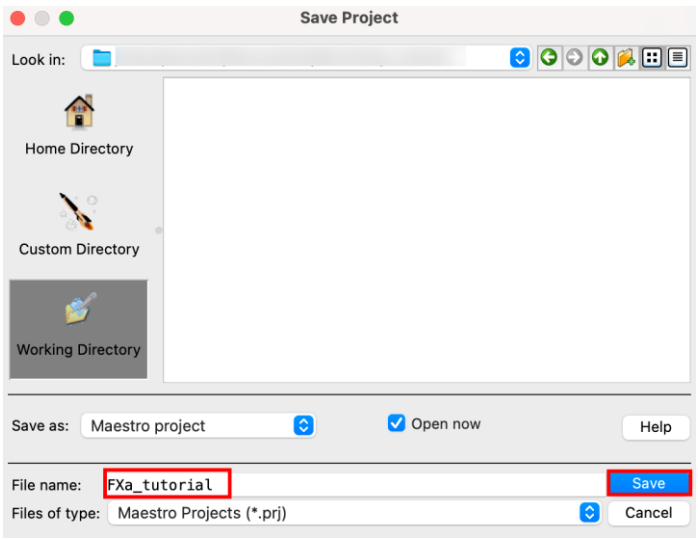


Figure 2-2. The Save Project panel.

6. Go to **File > Save Project As**.
7. Change the File name to **FXa_tutorial**.
8. Click **Save**.
    - The project is now named `FXa_tutorial.prj` and is saved in your Working Directory.

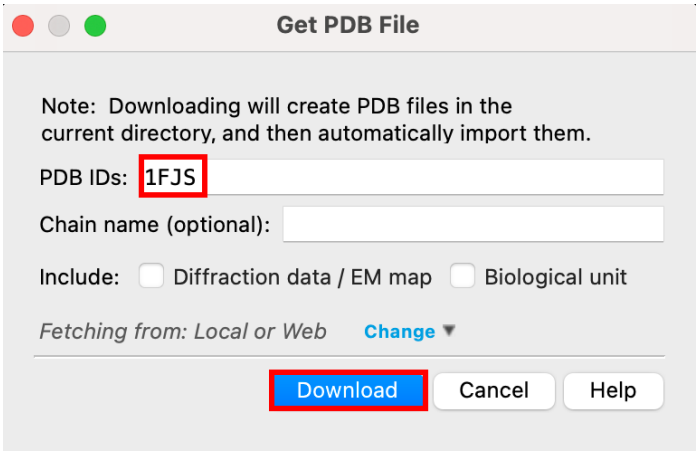


Figure 2-3. The Get PDB File dialog box.

9. Go to **File > Get PDB**.
10. For **PDB IDs**, type **1FJS**.
11. Click **Download**.
    - A banner appears and 1FJS is loaded into the Workspace.

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

Most of the entries in the PDB contain the diffraction data that can be used together with the deposited model to create and view the electron densities. Downloading diffraction data is usually unnecessary but if you wish to perform advanced structure refinement and detailed electron density map analysis, check the **Diffraction data / EM map** option. Further, if you want to download the entire biological unit rather than the asymmetric unit, check the **Biological unit** option.
For more information on biological assembly and asymmetric unit, click here.

3. Visualizing the Target Structure

In this section, you will explore ways to visualize structures in the Workspace. To demonstrate the three-dimensional configuration and spatial arrangement of atoms in biomolecules, different representation models can be used. Object representation can be changed in a number of ways using the Style Toolbox. Presets offer the ability to quickly render a structure in preset styles, similar to

PyMOL, to facilitate easy visualization. Presets can be used in a variety of ways, from decluttering your structure to creating publication-quality images.

3.1 Use the Style Toolbox

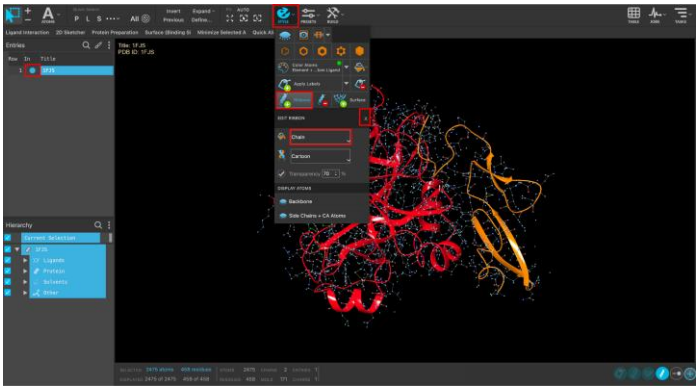


Figure 3-1. Using the Style Toolbox.

Imported structures in Maestro are included in the Workspace and selected in the Entries by default. Please refer to the Glossary of Terms for the difference between included and selected.

1. Go to the **Style** Toolbox.
    - All the atoms in the structure are selected.
2. Click on **Ribbons**.
    - The structure in the Workspace appears in cartoon styled ribbons.
    - Edit Ribbon menu appears.
3. For the Color scheme, choose **Chain**.
    - The structure is rendered in two different colors (red and orange), indicating there are two distinct chains in 1FJS.
4. **Close** the **Edit Ribbon** menu.

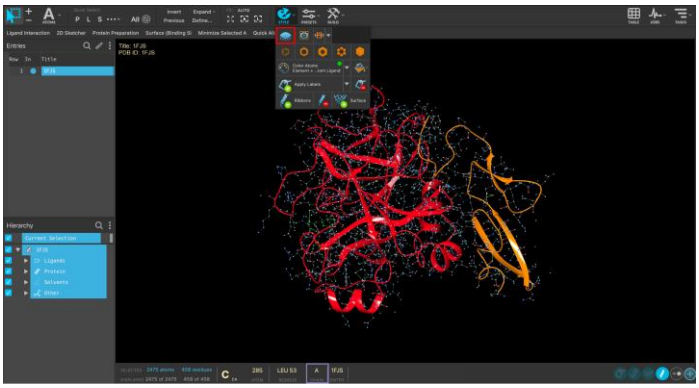


Figure 3-2. Hiding the selected atoms and identifying the chains in 1FJS structure.

5. Click on the **hide icon (closed-eye button**  **)** to undisplay all the selected atoms.
6. Hover over the chains to identify their names in the **Status Bar**.
    - The red colored larger chain is Chain A while the orange colored smaller chain is chain L.

Figure 3-3. Showing the ligand in the Workspace via the Hierarchy.

7. Check the **Ligands** box in the **Hierarchy** to display the ligand in the Workspace.
    - Notice that the ligand interacts only with chain **A**.

Figure 3-4. Exploring representation models in the Style Toolbox.

8. Under **Quick Select**, click **L**.
    - The ligand is selected.
9. Go to **Style** Toolbox and choose **ball-and-stick** representation.
    - The ligand is rendered in ball-and-stick representation.
    - This is only applied to the ligand since nothing else is selected in the Workspace.
10. Type **Z**.
    - The Workspace is zoomed to the selected structure.

3.2 Apply a Preset Style

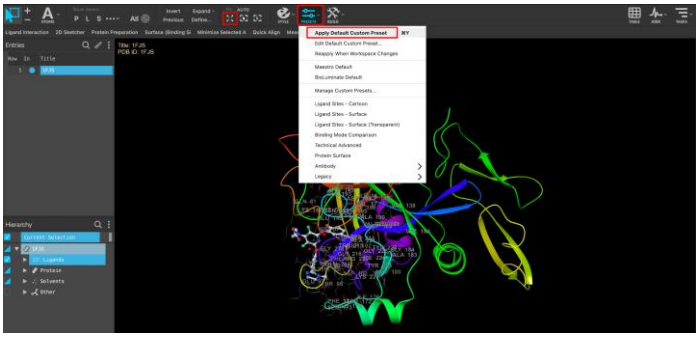


Figure 3-5. Applying default custom preset to the structure via Presets menu.

11. Click on **Presets**.
12. Choose **Apply Default Custom Preset** in the Presets menu.

Note: You can also double-click Presets to apply the default custom preset.

13. Click the **Fit-all** button to view the entire structure in the Workspace.
14. Rotate the structure and try identifying the N- and C-terminal ends.

By default, residues within 8 Å of the ligand and waters and ions within 3 Å of the ligand are displayed. The structure is colored according to the residue position. The N-terminus is red colored while the C-terminus is purple colored. There is a rainbow color ramp between these ends.
You can change these default settings via the **Edit Default Custom Preset** option in the Presets menu as per your interest. Try changing the color scheme to **Secondary Structure** to identify the secondary structure elements in the structure.

4. Preparing Protein Structures for Glide Docking

In this section, you will learn how to prepare the protein structure for docking in Glide. 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. These tools support two main workflows - **Interactive**, single protein preparation and **Automatic**, bulk protein preparations. The 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. The 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 and 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.

The **Preparation Workflow** tab may be used in conjunction with the **Diagnostics** tab and **Substructures** tab for the diagnosis and analysis of the protein structure. The Diagnostics tab lists the issues present in the protein structure. The Substructures tab provides options to view, copy or delete ligands, water, chains, etc. in the Workspace. The Protein Preparation Workflow toggles include **Preprocess**, **Optimize H-Bond Assignments**, and **Minimize and Delete Waters**.

For more information on the Protein Preparation Workflow panel, see the Protein Preparation Workflow Panel Documentation. For best practices to follow while preparing protein structures for modeling tasks, click here.

4.1 Prepare the Protein using the Protein Preparation Workflow

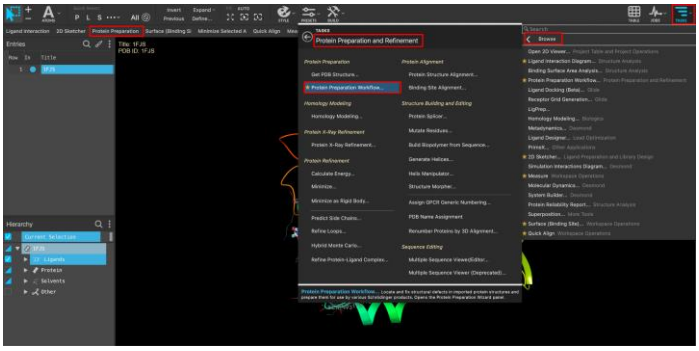


Figure 4-1. The Protein Preparation Workflow.

1. Go to **Tasks > Browse > Protein Preparation and Refinement > Protein Preparation Workflow**.
    - The Protein Preparation Workflow panel opens in Preparation Workflow tab.

Note: You can also click Protein Preparation in the Favorites toolbar.

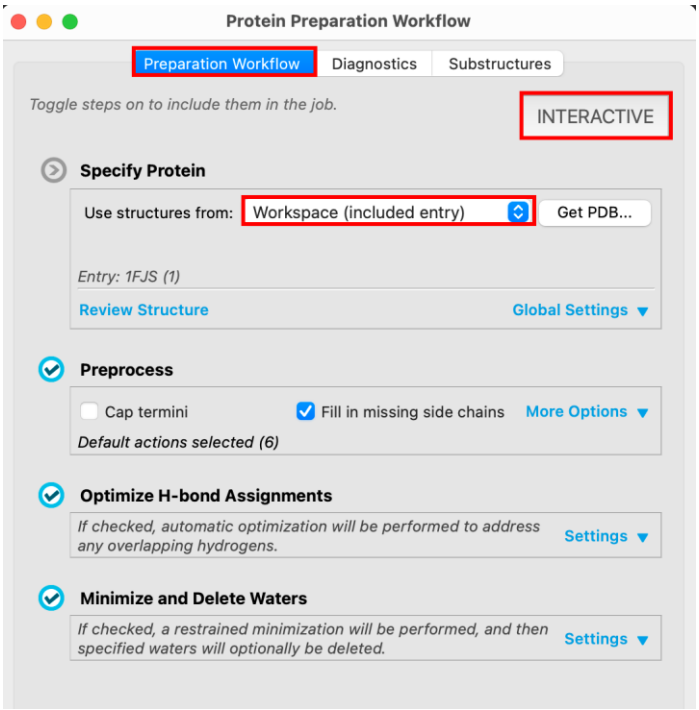


Figure 4-2. Specifying the protein from the Workspace.

2. In the **Preparation Workflow** tab, confirm the INTERACTIVE button is off.
    - When on, the panel will be titled Protein Preparation Workflow (Interactive).

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

3. For **Use structures from** under Specify Protein section, choose **Workspace (included entry)**.

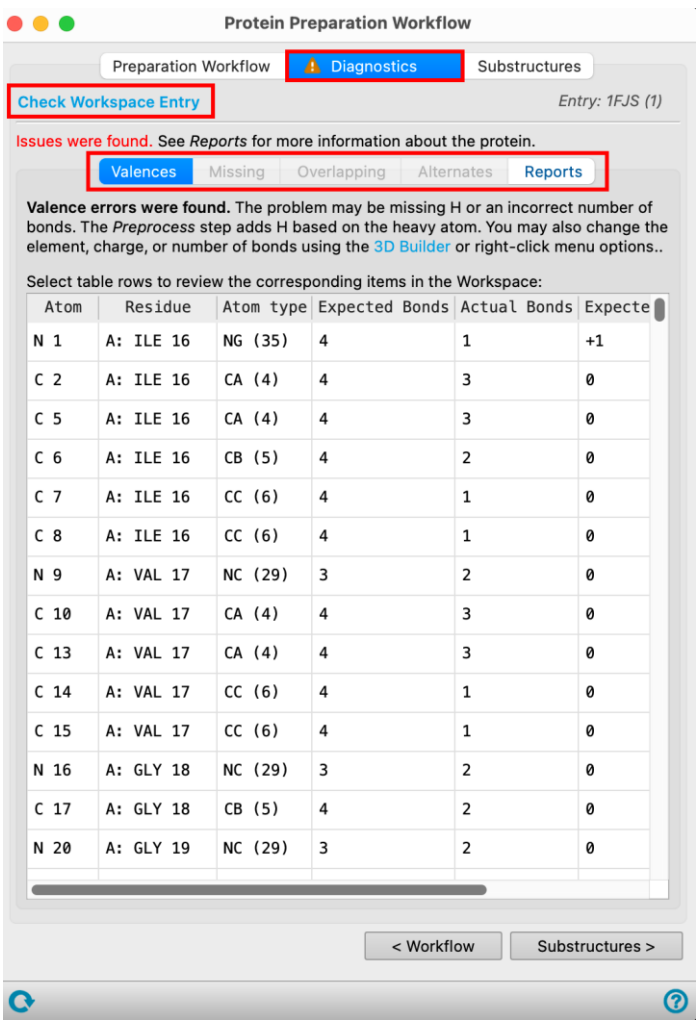


Figure 4-3. Viewing issues present in the protein in the Diagnostics tab.

4. Go to the **Diagnostics** tab to view the issues present in the structure.
5. Click **Check Workspace Entry**.
    - Valence errors are present indicating missing H-atoms or incorrect bond orders.These will be resolved during the protein preparation.
    - Notice that the **Missing** and **Overlapping** tabs are empty. This indicates that the structure does not have residues with missing atoms and pairs of atoms having bad contacts (too close).
    - Further, the **Alternates** tab is empty which implies there are no residues with alternate positions in the input structure.

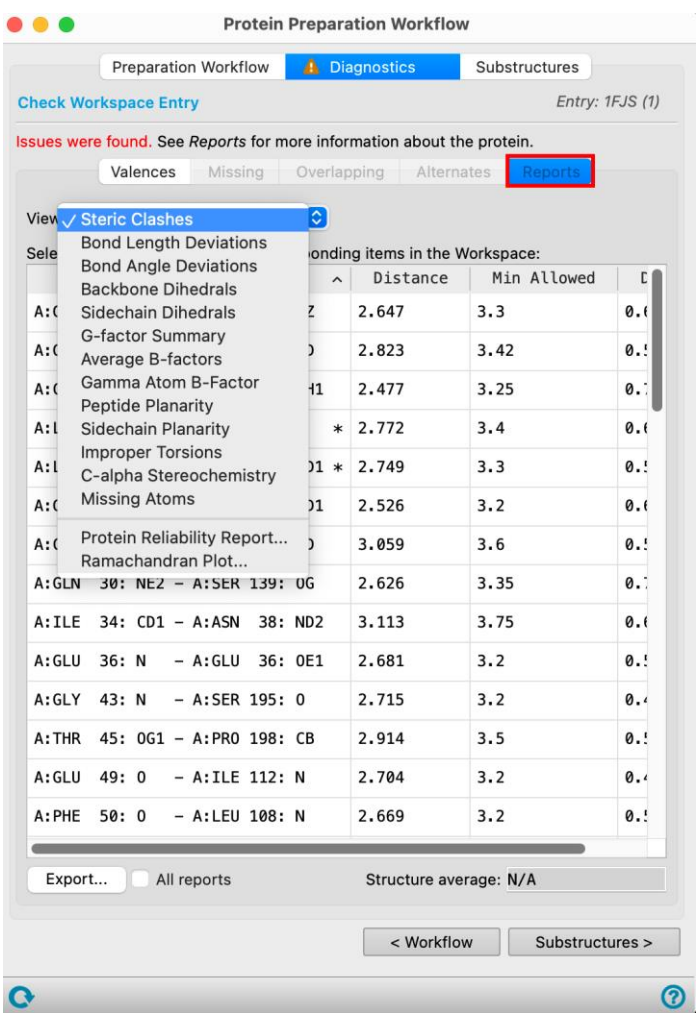


Figure 4-4. The Reports tab.

Optional: Select **Reports** to view common structure and quality metrics which can help you prepare your structure.

Figure 4-5. The Protein Reliability Report for 1FJS.

Optional: You can assess the quality of your target structure via the **Protein Reliability Report**. Output files for this job are in the tutorial zip archive. Navigate to the **prot_rel_1FJS** folder. Import `prot_rel_1FJS-reliability-out.maegz` and click **Protein Reliability Report** in the View option menu of the **Reports** tab to see the report. For more details on each metric, see the Protein Reliability Report Panel Documentation.

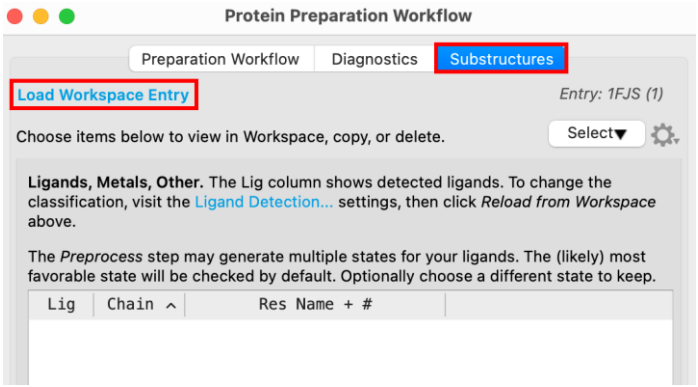


Figure 4-6. Reviewing the structure before preparation.

You will now review your structure before submitting the preparation job.

6. Go to the **Substructures** tab and click **Load Workspace Entry**.
    - The Substructures tab opens to show Ligands, Metals, Waters and Chains.

Note: You can also go to the Substructures tab by clicking **Review Structure** under Specify Protein section in Preparation Workflow tab.

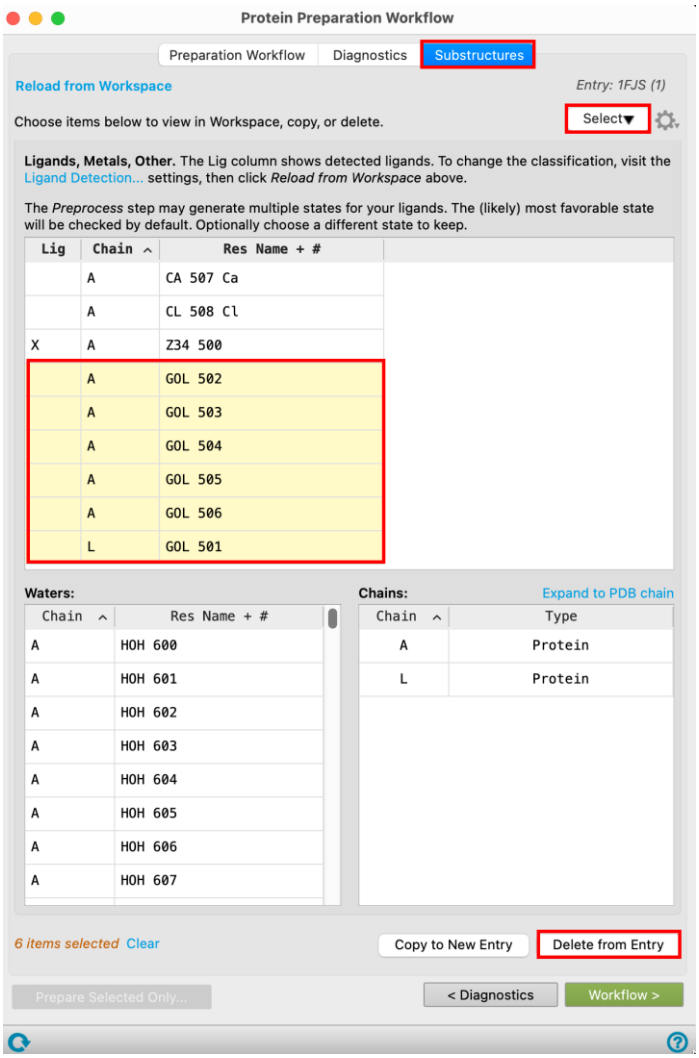


Figure 4-7. Deleting glycerols in the Hets table.

Glycerols are a crystallographic artifact with no biological relevance, so you will delete them.

7. In the Hets table, shift-click to select all the glycerol molecules (GOL XXX).
8. Click **Delete from Entry**.
    - A new entry 1FJS-with-deletions is added to the Entries and automatically included.

Note: The **Select** dropdown provides shortcuts for selecting these species based on their proximity to a specified chain.

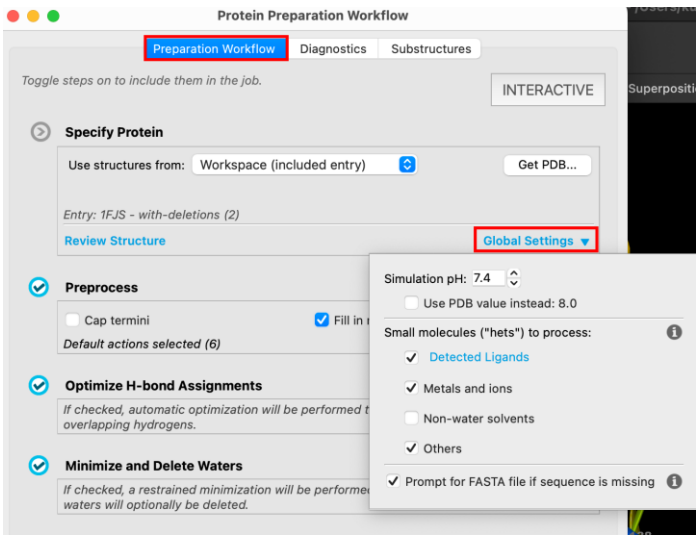


Figure 4-8. The Global Settings dialog box.

9. Return to the **Preparation Workflow** tab.
10. Click **Global Settings**.
    - A dropdown opens showing the Simulation pH and the PDB pH as well as Small molecules options.

Note: pH plays an important role in a protein's function. The simulation pH has a default value of 7.4, which is the physiological pH in the cytosol. Adjusting the pH will change the protonation states of residues and ligands and is necessary if you want to accurately reflect the experimental conditions.

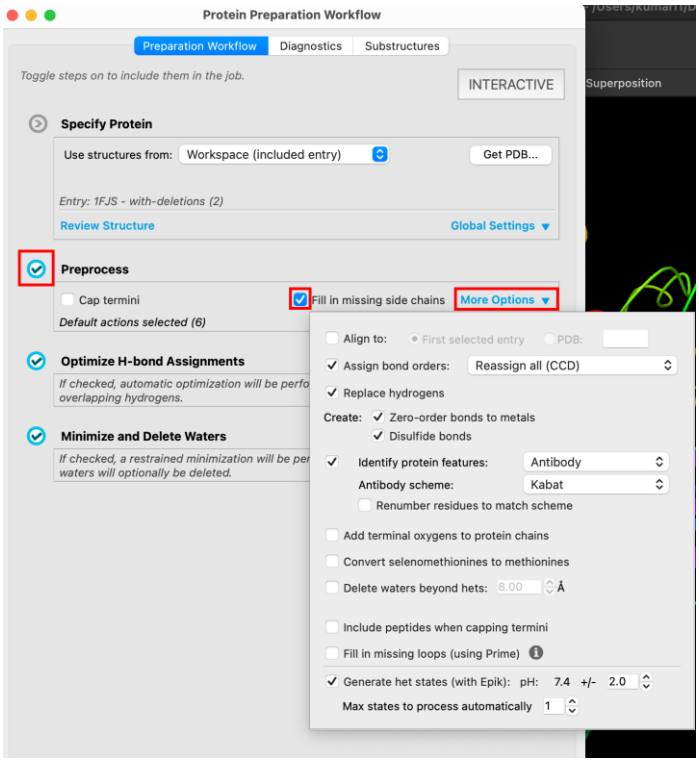


Figure 4-9. Preprocess default settings.

The **Preprocess** step fixes structural defects and adds missing information such as hydrogen atoms.

11. Confirm **Preprocess** is toggled on, indicated by a checkmark beside it.
    - Notice that **Fill in missing side chains** is checked by default. If these were missing in your structure, they would become populated during this step.

Optional: Click **More Options** if your research problem demands any modifications in the default settings.

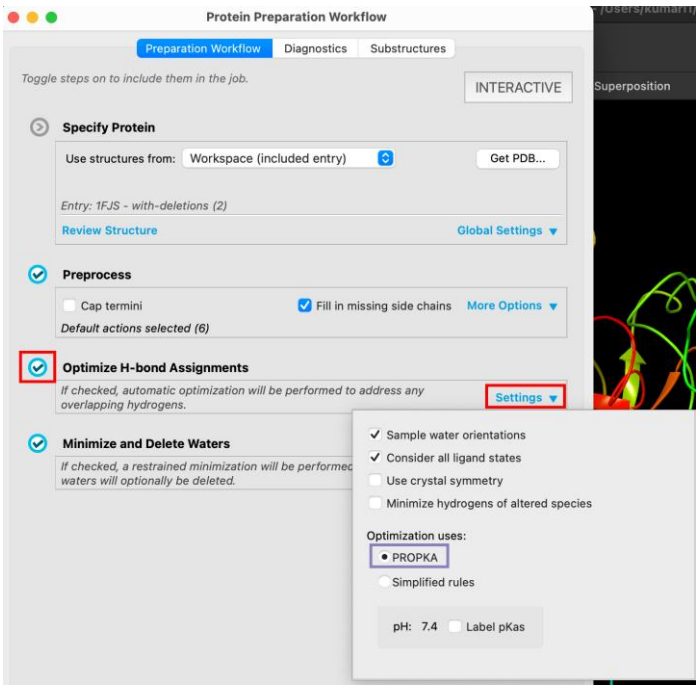


Figure 4-10. Optimize H-bond Assignments default settings.

The **Optimize H-bond Assignments** section is used for optimizing the hydrogen bond network – a process that samples water orientations and flips ASN, GLN, and/or HIS side chains at a specified pH value. Overlapping atoms caused by the addition of hydrogens during the Preprocess step will be corrected in this step.

12. Confirm **Optimize H-bond Assignments** is toggled on.

Note: This step uses PROPKA to optimize the H-bond network. If your system contains atypical protonation states, you should carefully inspect the results or use the interactive mode to manually assign states for particular residues.

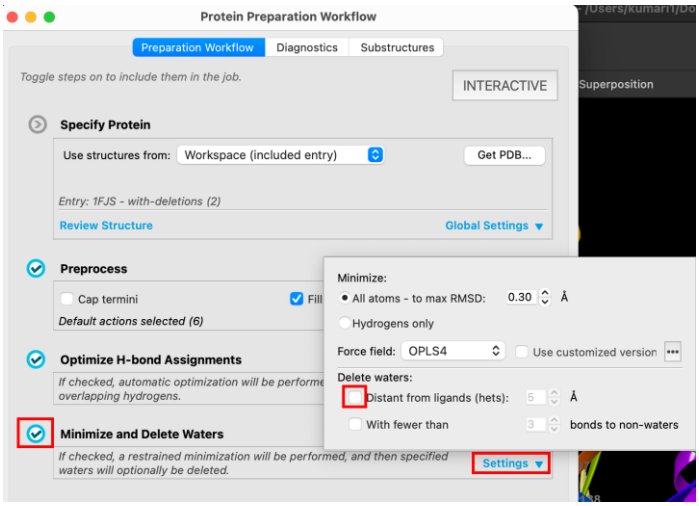


Figure 4-11. Minimize and Delete Waters settings.

The **Minimize and Delete Waters** section performs an all atom restrained minimization on the structure and by default deletes waters that are far from the Het groups. We strongly recommend leaving the waters in place during the preparation.

13. Confirm **Minimize and Delete Waters** is toggled on.
14. Click **Settings**.
15. For Delete Waters, uncheck **Distant from ligands (hets)**.

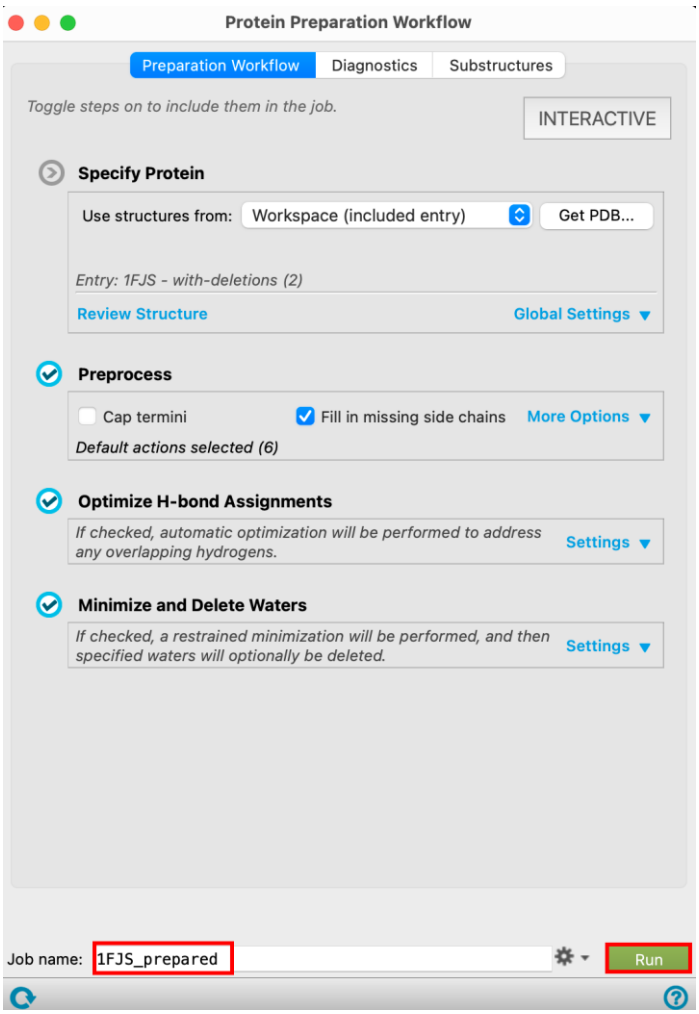


Figure 4-12. Running the preparation job.

16. Change the job name to **1FJS_prepared**.
17. Click **Run**.
    - This job takes ~ 2 minutes and a banner appears on completion.
    - A new group 1FJS_prepared-out is added to the Entries.
18. **Close** the Protein Preparation Workflow panel.

Note: After the preparation is complete, you should review your prepared structure once more to check if all issues were resolved and whether manual adjustments (e.g. of the hydrogen bond network) are needed.

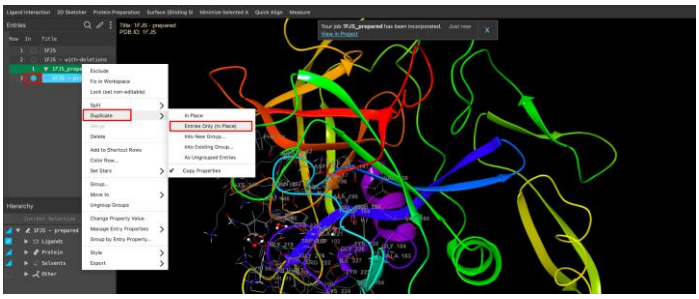
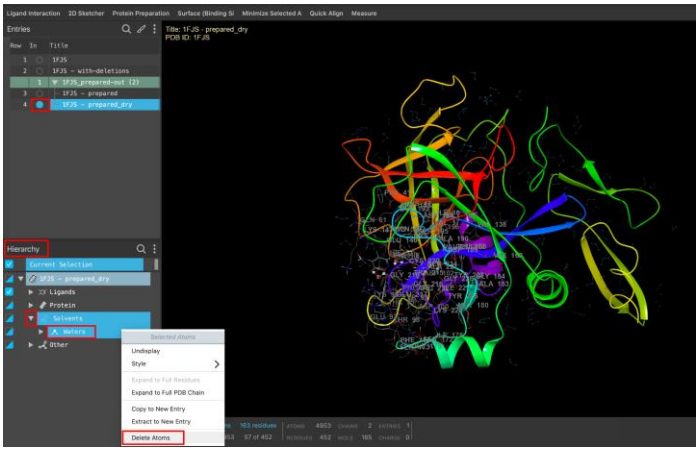


Figure 4-13. Duplicating the prepared protein structure.

When preparing the structure for docking, you will need to make an informed decision about which water molecules to retain in the active site. Any water left in the structure will be considered an immutable part of the receptor (see this article). For this example, none of the waters are considered structural, so you will create a fully dry structure.

19. Include **1FJS - prepared** entry.
20. Right-click the 1FJS - prepared and choose **Duplicate** > **Entries Only** (**In Place**).

	21. Double-click the duplicated 1FJS - prepared entry and rename it to **1FJS - prepared_dry**.
 *Figure 4-14. Deleting water molecules from the duplicated structure.*	22. In the Hierarchy, expand **Solvents**. 23. Right-click the **Waters** and choose **Delete Atoms**. *Note*: By deleting the waters in the duplicated entry, the original structure is preserved in case you need it for other applications.

5. Preparing Ligands for Glide Docking

In this section, you 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.

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. These ligand structures may contain missing hydrogens, incorrect geometries, inappropriate protonation states, or undefined chiral centers, which can lead to erroneous docking results if not addressed. Further, Glide requires ligands in specific formats (e.g., 3D structures with filled valences and optimized geometries) to evaluate binding interactions effectively. So, before being used in a virtual screen, ligands must be converted to 3D structures, with their chemistry properly standardized. To know about all the necessary requirements a ligand structure must meet for Glide docking, read the Ligand Preparation for Glide Documentation. The following steps provide an example of how you would prepare a ligand structure using LigPrep.

5.1 Extract the ligand from the structure

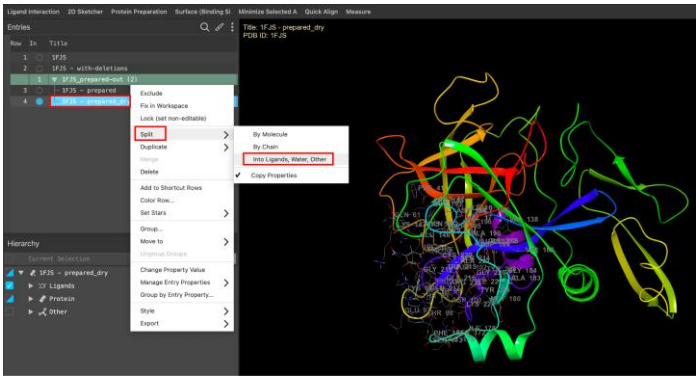


Figure 5-1. Splitting the prepared protein structure into different components.

1. Right-click on **1FJS_prepared_dry**.
2. Choose **Split** > **Into Ligands, Water, Other**.
    - A new group is added to the Entries.

5.2 Run LigPrep

Here, we show how to run LigPrep for the cognate ligand which is already imported in the Workspace. For larger ligand libraries, you can directly provide a file containing the ligands to LigPrep. For more information and supported formats, see the LigPrep panel documentation.

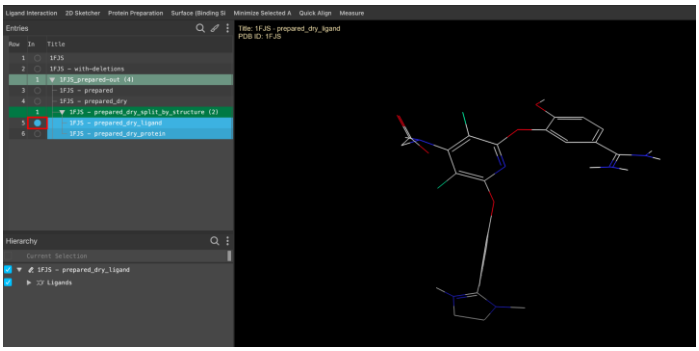


Figure 5-2. Including the ligand to display in the Workspace.

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

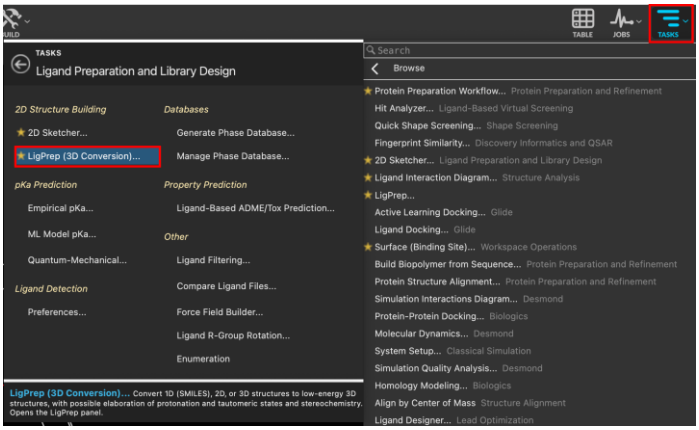


Figure 5-3. Opening the LigPrep application.

4. Go to **Tasks > Browse > Ligand Preparation and Library Design > LigPrep (3D Conversion)**.
    - The LigPrep panel opens.

Figure 5-4. The LigPrep panel.

5. For Use structures from, choose **Workspace (1 entry)**.

You now need to specify which protonation and tautomer states LigPrep should consider and how it generates these.

6. For Generate possible states at target pH Using, choose **Epik**.

The pKa is a key property to consider given its importance in determining the ionization state of a molecule at physiological pH. To know about how **Epik Classic** and **Epik** are used for calculating pKa values, generating protonation states, and creating tautomeric forms of small molecules, read this paper. For workflows requiring high precision like docking, we recommend using Epik.

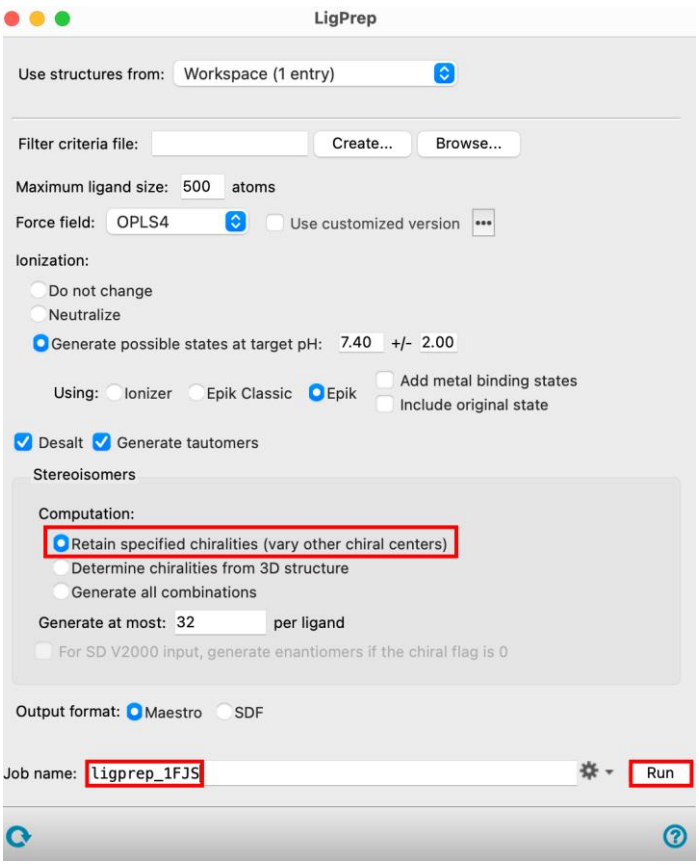


Figure 5-5. Running the LigPrep job.

The stereochemistry of ligand is crucial as it influences how the ligand fits into the receptor's binding site. Notice that under Stereoisomers, the option to retain specified chiralities (vary other chiral centers) is selected by default for Computation.

7. Change the Job name to **ligprep_1FJS**.
8. Click **Run**.
    - This job takes a few seconds to complete.
    - Once the job is completed, a new group ligprep_1FJS-out is added to the Entries.
    - The output group contains two protomer/tautomer states of the input ligand present in the specified pH range.
9. **Close** the LigPrep panel.

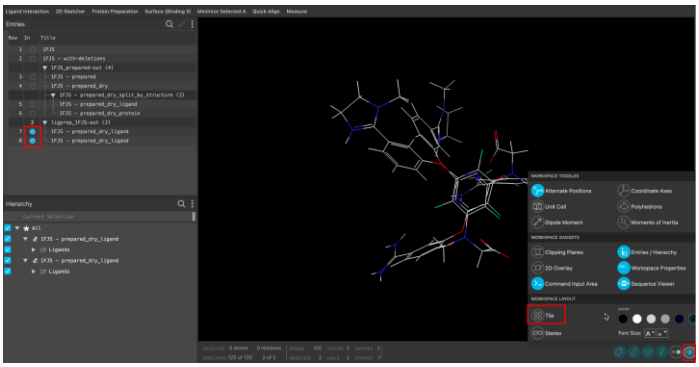


Figure 5-6. Tiling the generated ligand structures.

The **Tile** functionality is very useful for seeing the slight variations in chemistry for the generated structures.

10. Shift-click to include all the generated ligand structures.
11. Click the in the Workspace Configuration Toolbar in the bottom right corner and then click the **Tile** button under Workspace Layout.
    - The ligand structures are tiled by entries in the Workspace.

Figure 5-7. Tiled ligand structures in the Workspace.

You can now compare the different states generated for the cognate ligand.

12. Click on **Tile by** or press Ctrl+L (Cmd+L) to toggle off the Workspace tiling.

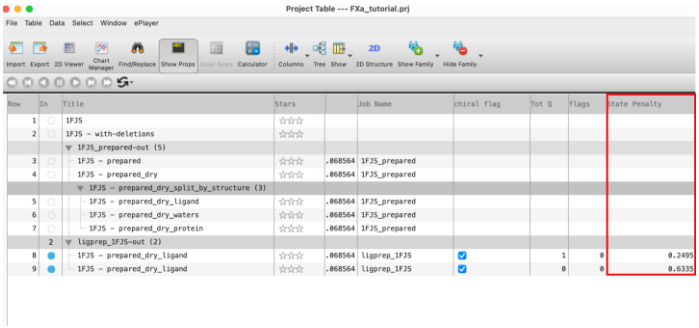


Figure 5-8. The Project Table.

Optional: Try navigating to the **Project Table** in the upper right corner to view properties calculated by LigPrep, such as the Epik state penalty.

6. Analyzing the Structure After Preparation

In this section, you will analyze the protein-ligand complex by looking at the interactions in the 2D Ligand Interactions Diagram and 3D Workspace. Then you will generate a Custom Set for some binding residues of interest. Finally, you will visualize the surface of the binding pocket, and save an image of the complex.

6.1 Visualize Interactions in 2D and 3D

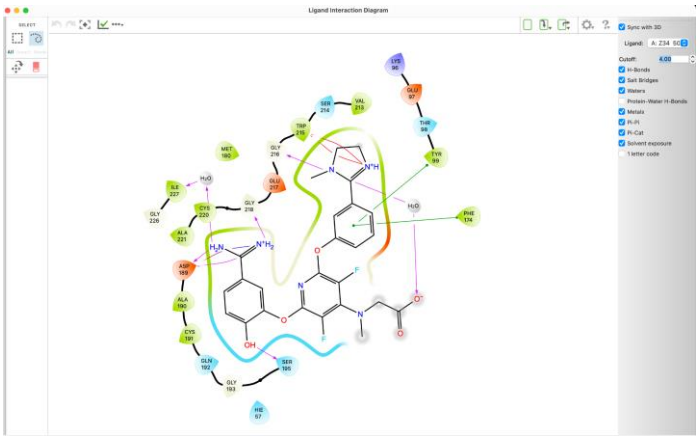


Figure 6-1. The Ligand Interaction Diagram.

You will now use the Ligand Interaction Diagram to visualize the residues in the binding site interacting with and stabilizing the ligand. You may adjust the cutoff distance to see only the relevant residues.

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

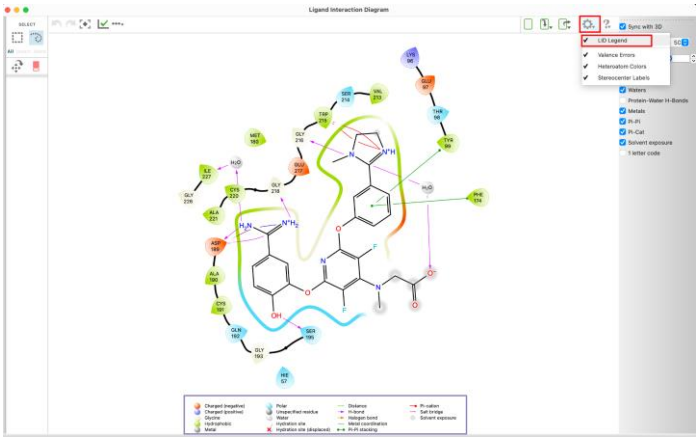


Figure 6-2. *Viewing ligand-receptor interactions in the 2D Ligand Interaction Diagram.*

3. Click the **Configure View (settings)** icon and choose **LID Legend**.

Note: The Select, Move and Erase tools are available in the Ligand Interaction Diagram for modifying residues and interactions. Please navigate to **Edit > 2D Sketcher** to modify your ligand structure.

Optional: Click the **Export** icon and choose **Save Image** to export the Ligand Interaction Diagram as an image. For visual clarity, we recommend modifying the resolution to 2000 x 2000 px while saving the image.

4. **Close** the Ligand Interaction Diagram.

In the Ligand Interaction Diagram, the ligand is displayed as a 2D structure. Residues are represented as colored teardrop shapes, labeled with the residue name and residue number, and colored according to their properties. The chain is represented as a black line connecting residues. Interactions between the residues and the ligand are drawn as lines, colored by interaction type (see the LID Legend). For detailed explanation, please refer to the Ligand Interaction Diagram panel features.

For the 1FJS structure, you can see that **ASP 189**, **SER 195** and **GLY 218** are involved in H-bonding with the ligand. **ILE 227** is involved in water mediated H-bonding with the ligand. Further, **TYR 99**, **PHE 174** and **TRP 215** are involved in pi interactions with the ligand.

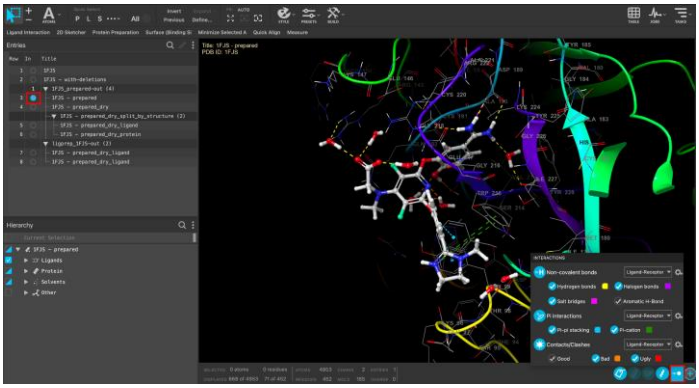


Figure 6-3. Visualizing ligand-receptor interactions in the 3D Workspace.

5. Right-click the **Interactions Toggle** in the bottom right corner of the Workspace.
    - The Interactions panel opens.

Note: You can toggle on/off different interactions as per your interest. You can also customize the visualization by clicking the color to the right of each interaction.

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.

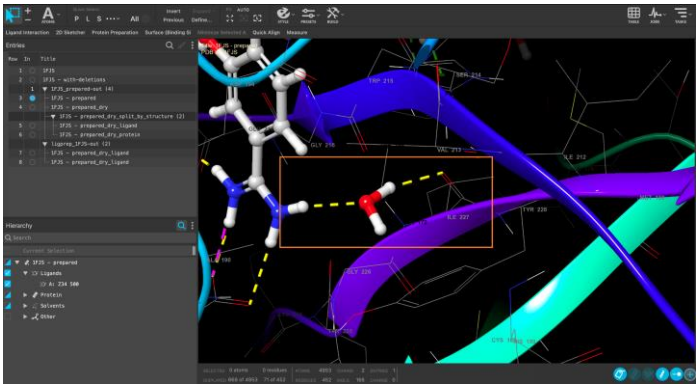


Figure 6-4. Visualizing the water mediated H-bond formed by ILE 227 residue.

6. Rotate the structure in the Workspace and try visualizing the water mediated H-bonds and other interactions you identified in the 2D Ligand Interaction Diagram.

6.2 Create a Custom Set

Custom Sets in Maestro offer several advantages for managing and working with atom/residue selections within your project. Once you save your selections as a Custom Set, these are readily available. This is particularly helpful for complex selections you frequently need. You will now create a Custom Set of the residues that form key interactions with the ligand.

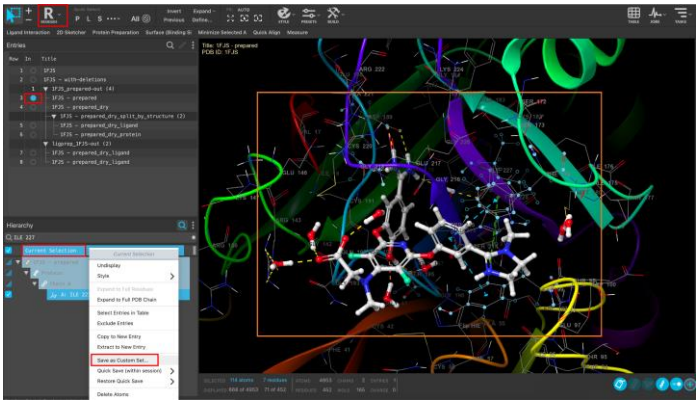


Figure 6-5. Selecting the interacting residues via Hierarchy to create a Custom Set.

By default, you are in the atom selection mode in Maestro, indicated by the letter **A** near the cursor pointer. You can change the selection scope from the Selection Toolbar or alternatively you can type the initial letter of the desired mode (**A**toms, **R**esidues, **C**hains, **M**olecules) as a shortcut.

7. Type **R** to switch to Residue selection mode.
8. Locate the binding site residues by number and type in the Hierarchy and Ctrl+Click (Cmd+Click) to select the residues **TYR 99**, **PHE 174**, **ASP 189**, **SER 195**, **TRP 215**, **GLY 218**, and **ILE 227** in the Workspace.
9. In the Hierarchy, right-click the **Current Selection** and choose **Save as Custom Set**.
    - The Save Selection as Custom Set panel opens.

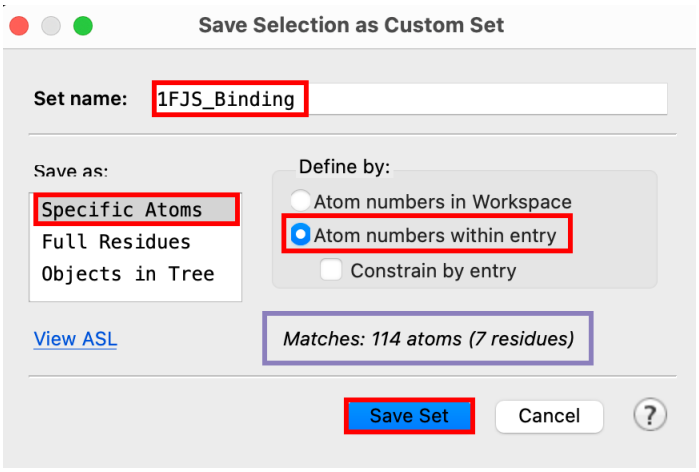


Figure 6-6. Saving the selection as Custom Set.

10. For Set Name, type **1FJS_Binding**.
11. For Save as, choose **Specific Atoms**.
12. For Define by, choose **Atom numbers within entry**.
    - This will ensure that the selection will remain consistent when multiple entries are in the Workspace.
13. Click **Save Set**.

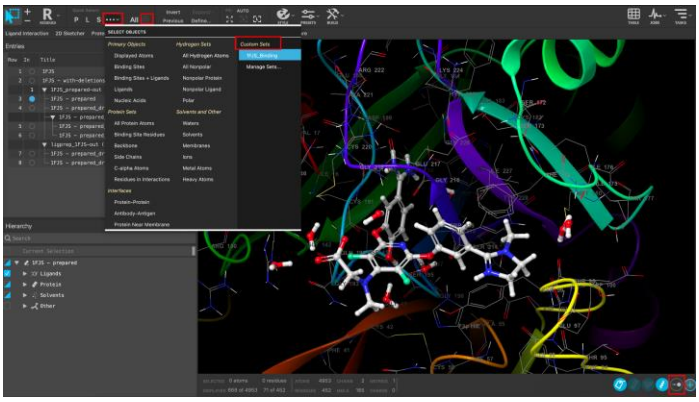


Figure 6-7. Custom Sets section in the Selection Toolbar dropdown.

Custom Sets can be accessed and edited through the Custom Sets section in the dropdown in the Selection Toolbar.

14. Click the **Clear selection** button.
15. Turn off the **Interactions Toggle**.

6.3 Generate and Manipulate a Surface

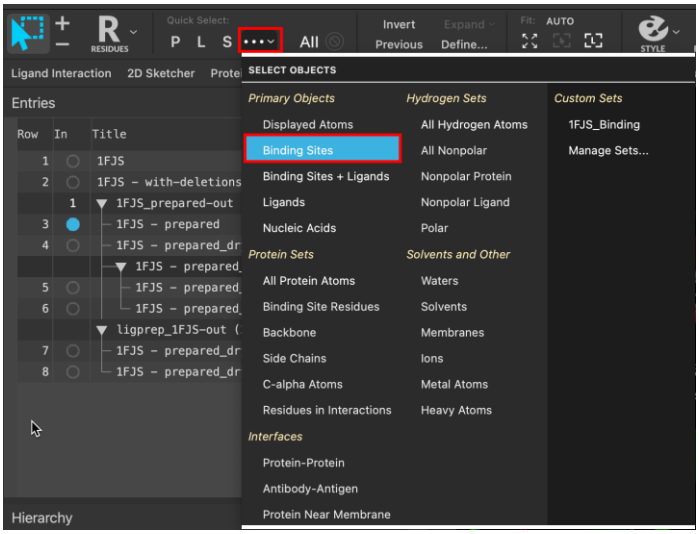


Figure 6-8. Exploring more options in Quick Select.

16. Under Quick Select, click the **Choose item** button and choose **Binding Sites**.

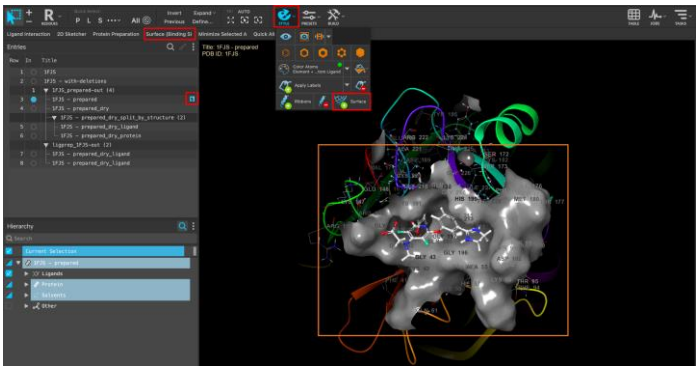


Figure 6-9. Visualizing the binding site surface.

17. Go to the Style **Toolbox** and choose **Surface**.
    - A solid gray surface is applied.
    - The surface menu appears next to the title in the Entries.

Note: You can also click **Surface (Binding Site)** in the Favorites toolbar to reproduce the same action.

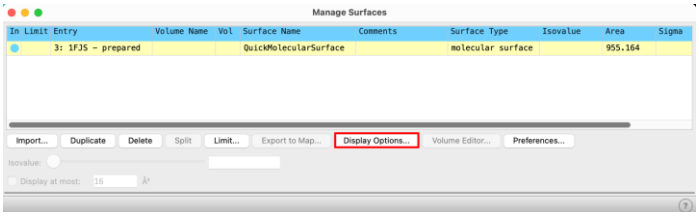


Figure 6-10. The Manage Surfaces panel.

18. Right-click and choose **Manage**.
    - The Manage Surfaces panel opens.
19. Choose **Display Options**.
    - The Surface Display Options panel opens.

Note: You can also hover over the surface in the Workspace and right-click (when you see a mesh adjacent to the cursor).

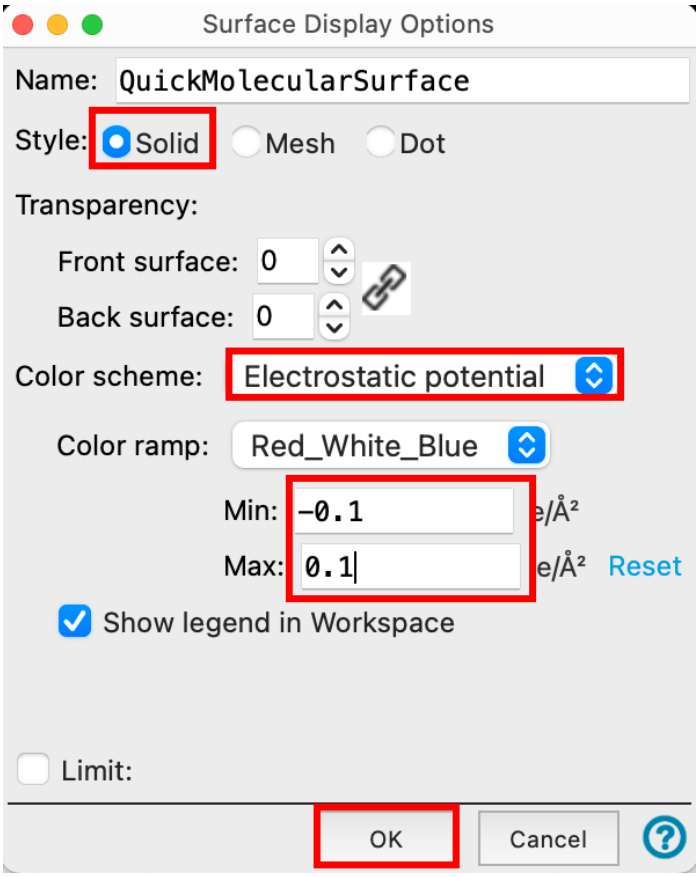


Figure 6-11. The Surface Display Options panel.

Coloring the surface by electrostatic potential can help you understand the character of the binding pocket. Adjusting the Min and Max values allows you to emphasize contrasts.

20. For Color Scheme, choose **Electrostatic potential**.
21. Change the Min and Max values to **-0.1** and **0.1**, respectively.
22. Click **OK**.
23. **Close** the **Manage Surfaces** panel.

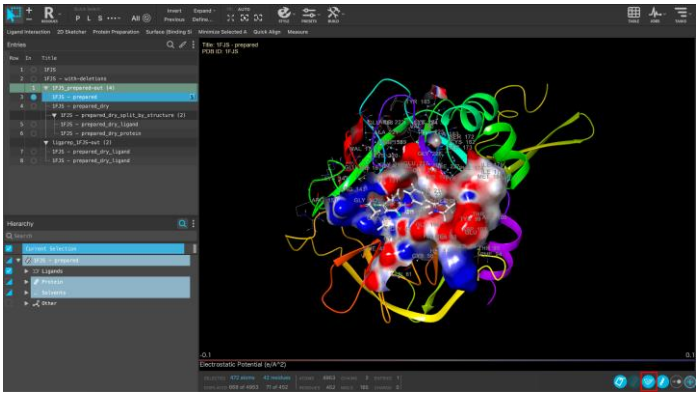


Figure 6-12. Visualizing the ESP map.

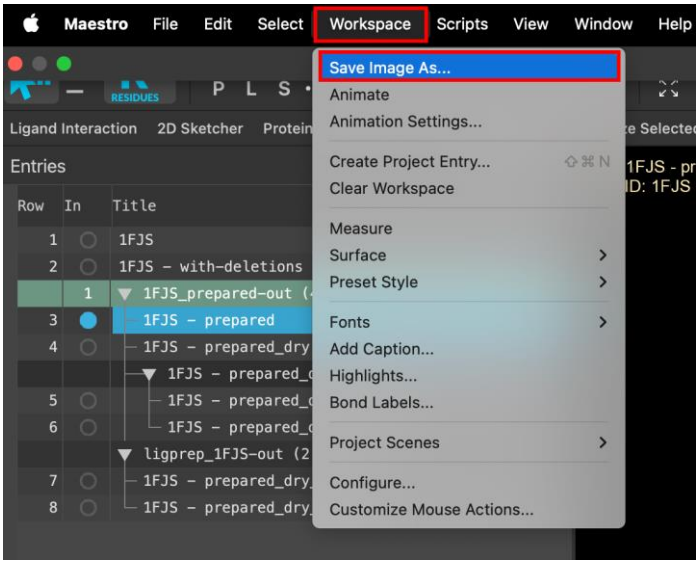
Take your time to visualize and interpret the ESP map.

Note: You can hide the generated surface by turning off the **Surfaces Toggle** in the Workspace Configuration Toolbar in the bottom right corner.

Visualizing molecular surfaces, particularly binding site surfaces of proteins aids in identifying critical structural features such as cavities, grooves, and pockets that may serve as binding sites. By overlaying the ESP map onto this surface, you can visualize the distribution of positive and negative charges, offering insights into electrostatic complementarity between the protein and the ligand. For example, positively charged regions on the surface may attract negatively charged ligands or functional groups, facilitating specific interactions critical for binding. The regions in shades of blue are associated with positively charged residues while those in shades of red are associated with negatively charged residues. The white colored regions are those with neutral electrostatic potential and often have non-polar or hydrophobic residues.

To locate and characterize potential binding pockets or cavities on the protein's surface that are likely to accommodate ligands, you can use **SiteMap**. For more details, please refer to the Target Analysis with SiteMap and WaterMap tutorial.

6.4 Save an image of the Workspace



24. Go to **Workspace** > **Save Image As**.
    - The Save Image panel opens.

Note: You can also right-click anywhere in the Workspace and choose the **Save Image** option.

Figure 6-13. Save Image As option in the Workspace menu.

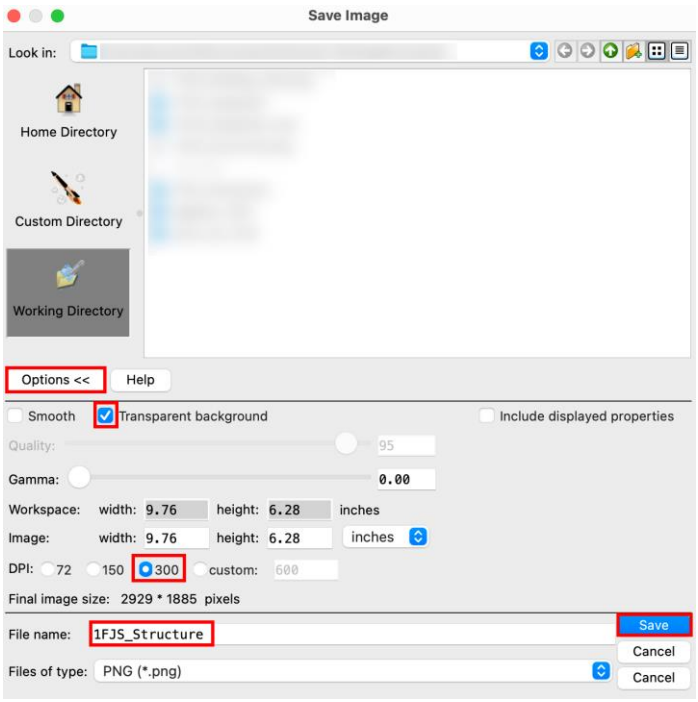


Figure 6-14. Saving the image of the Workspace via the Save Image panel.

You can adjust the size and resolution of the image to meet quality requirements, e.g. for printing.

25. Click **Options >>**.
26. Check **Transparent background** and select **300 DPI**.
27. Change the File name to **1FJS_Structure**.
28. Click **Save**.
    - The image is saved in the Working Directory.

Note: You can also export the structure to PyMOL from the **File** menu and **Ray Trace** the structure to get high quality images for publications. See the free Visualizing Science with PyMOL 3 online course for detailed instructions.

7. Conclusion and References

In this tutorial, you imported and visualized a protein structure. You adjusted structure visualization options both manually and with one click using Presets. Then you prepared the protein and ligand structure for Glide Docking. A raw PDB file was made suitable for modeling purposes using the Protein Preparation Workflow, and the cognate ligand was prepared using LigPrep in the same fashion that would be used for a multi-ligand file. Finally, you visualized key interactions in the 2D Ligand Interaction Diagram and in the 3D Workspace to analyze the protein-ligand complex. The Workspace Configuration toolbar allowed for toggling various components in the Workspace. Visualizing the surfaces gave another way to analyze the protein-ligand complex.

For further learning:

- Getting Going with Maestro Video Series: Preparing Protein Structures
- Getting Going with Maestro Video Series: Ligand Preparation
- Target Analysis with SiteMap and WaterMap

- Structure-Based Virtual Screening using Glide
- Ligand-Based Virtual Screening Using Phase
- Refining Crystallographic Protein-Ligand structures using GlideXtal and Phenix/OPLS
- Homology Modeling of Protein-Ligand Binding Sites with IFD-MD
- Introduction to Molecular Modeling in Drug Discovery Online Course
- Understanding and Visualizing Target Flexibility
- Visualizing Science with PyMOL 3

For further reading:

- Protein Preparation Workflow Panel Documentation
- Best Practices for protein preparation
- How to prepare a large ligand library using LigPrep
- Docking performance of the glide program as evaluated on the Astex and DUD datasets: a complete set of glide SP results and selected results for a new scoring function integrating WaterMap and glide - 2012 paper from Schrödinger evaluating the performance of Glide on the Astex and DUD datasets. The paper highlighted both the performance of Glide SP, as well as the sizable impact careful protein preparation can have on docking performance. The authors found that the "average AUC was greater than 0.7 for all best-practices protein families demonstrating consistent enrichment performance across a broad range of proteins and ligand chemotypes." This paper is also the first to introduce the WScore, which is described more in reference 18.
- Effects of histidine protonation and rotameric states on virtual screening of M. tuberculosis RmlC - An open access publication which indicates the importance of accurate protonation during protein preparation, and indicates that for binding pockets that contain residues that can be protonated, deprotonated, or adopt various rotameric states, it may be worth taking preparing all combinations for a virtual screening campaign.
- Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments - 2013 paper from Schrödinger introducing the Protein Preparation Wizard, and demonstrating the importance of properly prepared structures for virtual screening applications.
- Protein Reliability Report Help page - A section in the Maestro user manual that explains the 21 metrics that make-up the Protein Reliability Report, and notes their

cutoffs.

- Can the Protein Preparation workflow be run from the command line?

8. Glossary of Terms

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

Entries - 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 Entries (and Project Table) and the row for the entry is highlighted. Project operations are performed on all selected entries

Working Directory - the location where files are saved

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