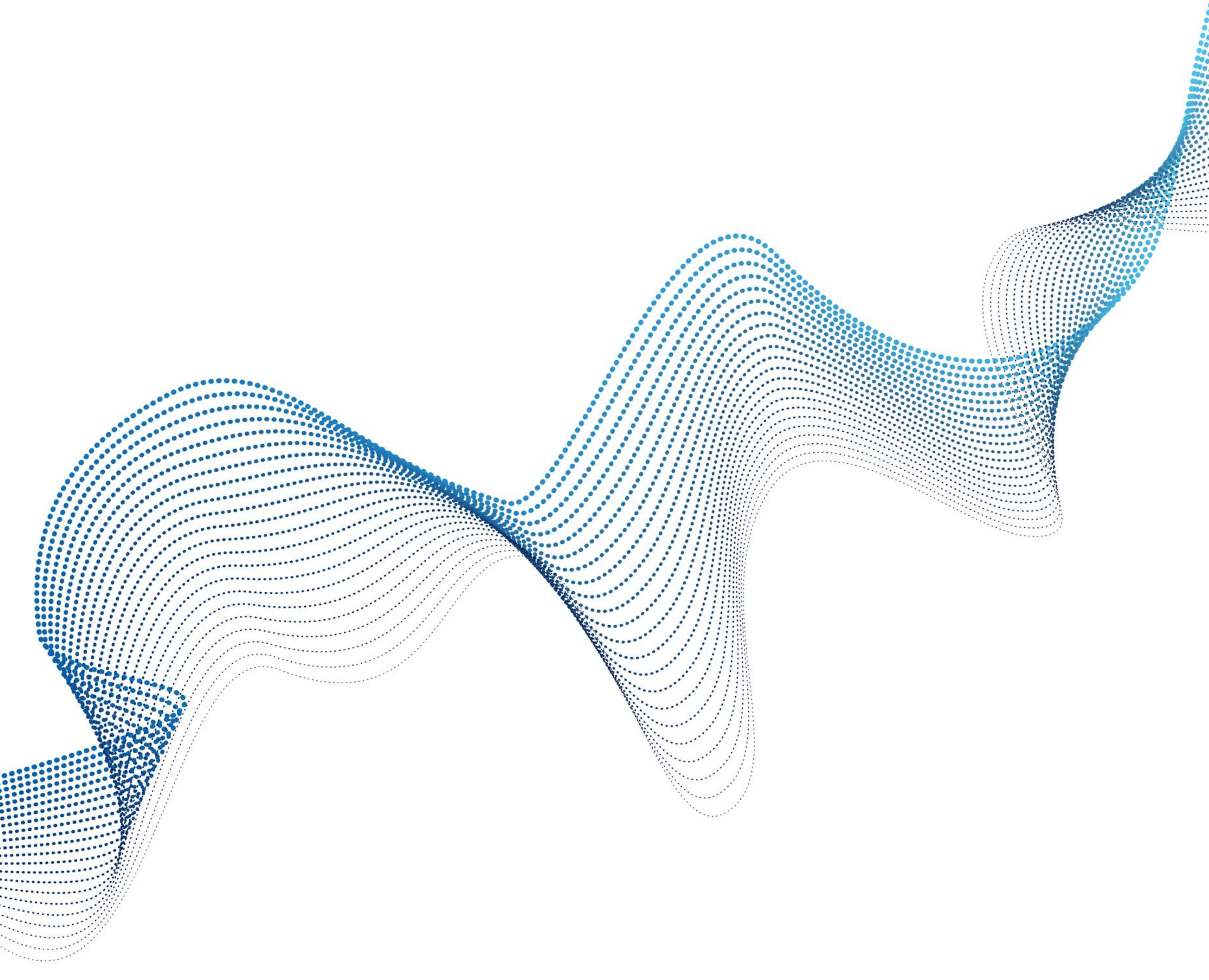
2025-3

A Chemist's Guide to Maestro



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A Chemist’s Guide to Maestro

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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 will explore a selection of tasks in Maestro that could be useful for chemists during the idea generation and lead optimization stages of their projects.

Tutorial Content

1. 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 imported from the PDB directly, or from your Working Directory using **File > Import Structures**, and are added to the Entry List and Project Table. The Entry List is located to the left of the Workspace. The Project Table can be opened 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)

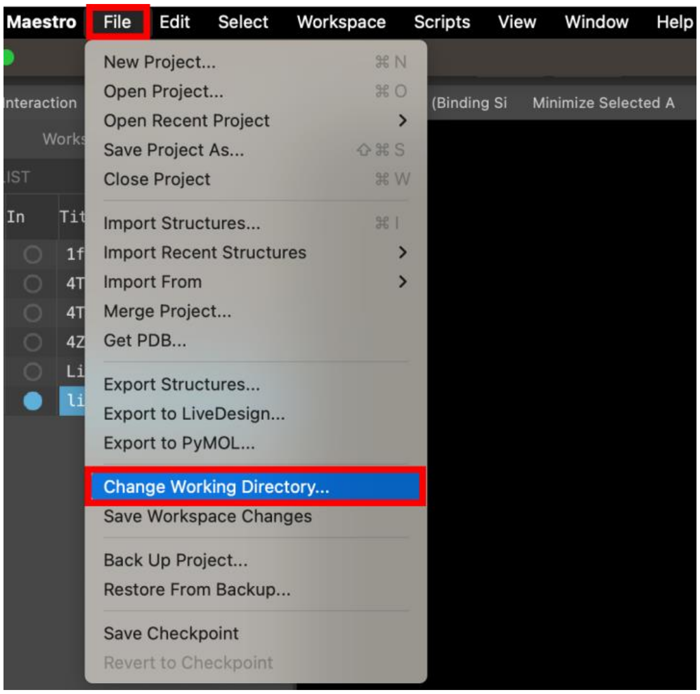
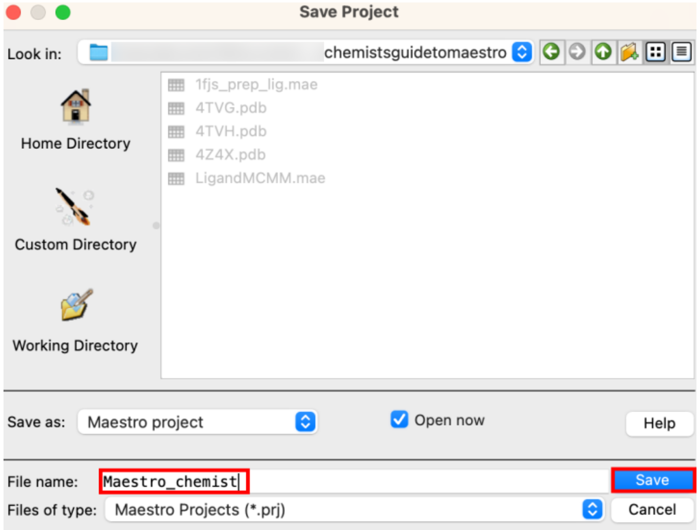


Figure 1-1. Change Working Directory option.

2. Go to **File > Change Working Directory**
3. Find your directory, and click **Choose**.
4. Pre-generated input and results files are included for running jobs or examining output. Download the zip file here: https://www.schrodinger.com/sites/default/files/s3/release/current/Tutorials/zip/achemistsguidetomaestro.zip
5. After downloading the zip file, unzip the contents in your Working Directory for ease of access throughout the tutorial.



Look in: chemistsguidetomaestro

Save as: Maestro project

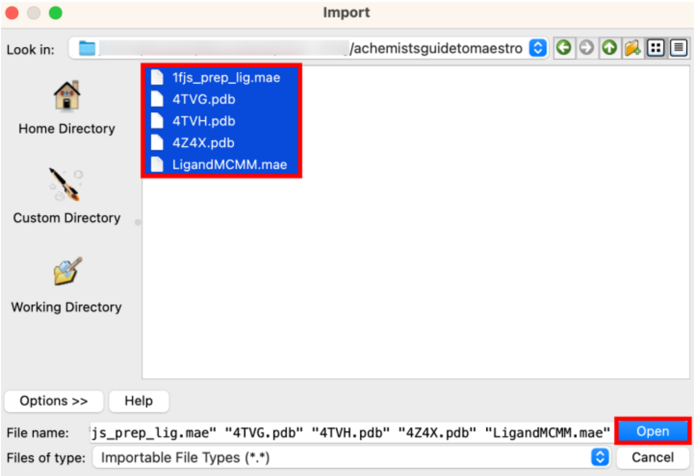
☑ Open now

File name: Maestro_chemist

Files of type: Maestro Projects (*.prj)

Figure 1-2. Save Project panel.

6. Go to **File > Save Project As**
7. Change the File name to **Maestro_chemist.**
8. Click **Save.**
   - The project is now named `Maestro_chemist.prj`.



Look in: /achemistsguidetomaestro

File name: js_prep_lig.mae" "4TVG.pdb" "4TVH.pdb" "4Z4X.pdb" "LigandMCMM.mae"

Files of type: Importable File Types (*.*)

Figure 1-3. Import panel and selection.

9. Go to **File > Import Structures**
10. Select `1fjs_prep_lig.mae`, `4TVG.pdb`, `4TVH.pdb`, `4Z4X.pdb`, `LigandMCMM.mae`
11. Click **Open**.
    - Structures are added in the Entry List.

PDB files can be imported directly into Maestro by navigating to **File > Import PDB** and filling in file information. To save time, we have provided a series of structures.

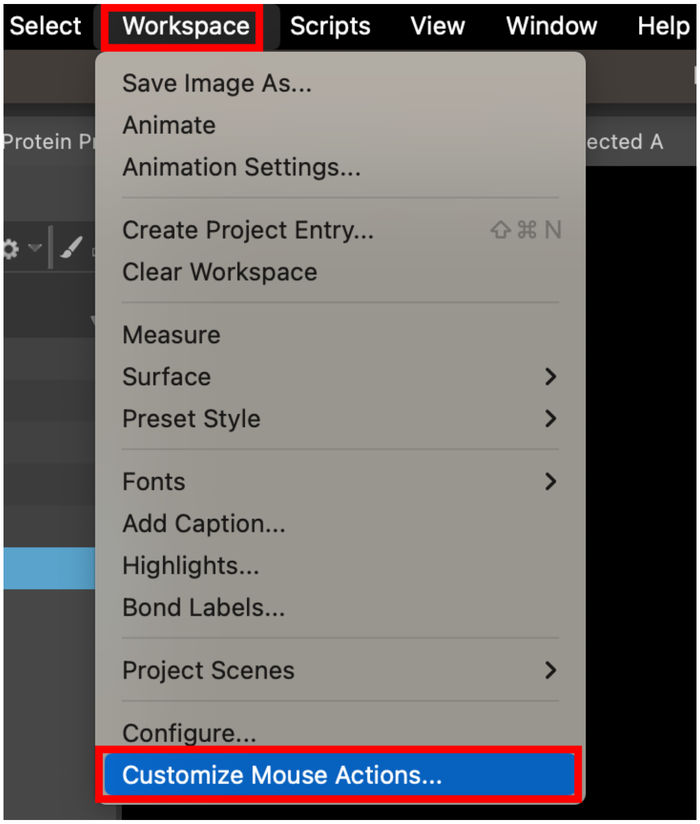


Figure 1-4. Customize Mouse Actions option.

12. Navigate to **Workspace > Customize Mouse Actions**
13. Choose your appropriate mouse or trackpad preferences from the dropdown and read through the corresponding mouse actions.

---

2. Creating and Modifying Molecules

Maestro employs a “select first” paradigm where an object must be selected before an operation is performed on it. In this section, we will cover different methods of copying molecules into and out of the Maestro interface. Then, we will save an image of the Workspace to your Working Directory. Finally, we will make modifications to an existing ligand. Please refer to the Glossary of Terms for the difference between selecting and including structures.

2.1 Copy and paste structures

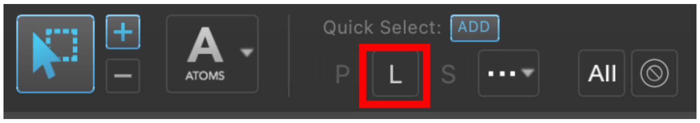


Figure 2-1. The Ligand option in Quick Select.

1. Include **1fjs_prep_lig** in the Workspace.
2. Under Quick Select, click **L.**
    - The ligand is highlighted.

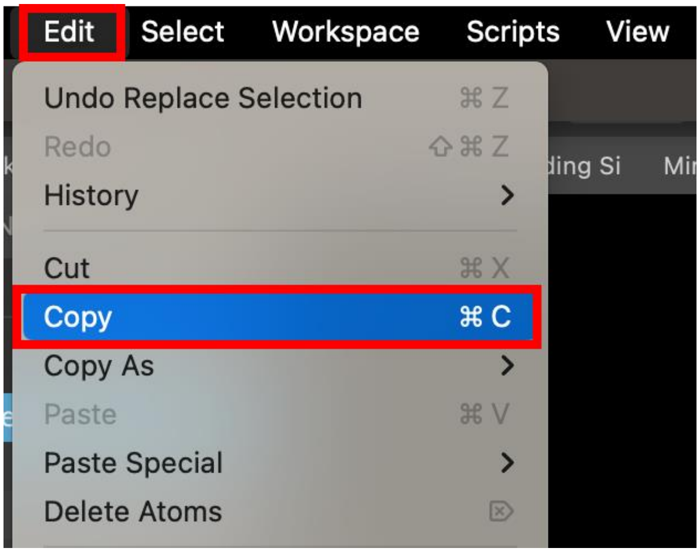


Figure 2-2. Copy option in Edit.

3. Go to **Edit > Copy**
   - ○ The SMILES string of the ligand is ready to be pasted into an external document.
4. Go to **Edit > Paste Special > By Placing**
   - ○ A banner appears.

Paste a structure into the Workspace by picking an empty spot in the Workspace X

Figure 2-3. Exit this mode by clicking the X.

5. Click in the **Workspace**.
   - ○ A copy of the ligand is pasted into the Workspace.
6. Click **X** to close the banner.
   - ○ Pasting function stops.

Banners appear to prompt an action or to provide a notification.

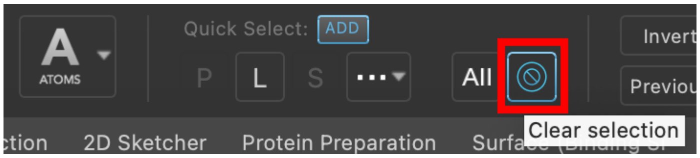
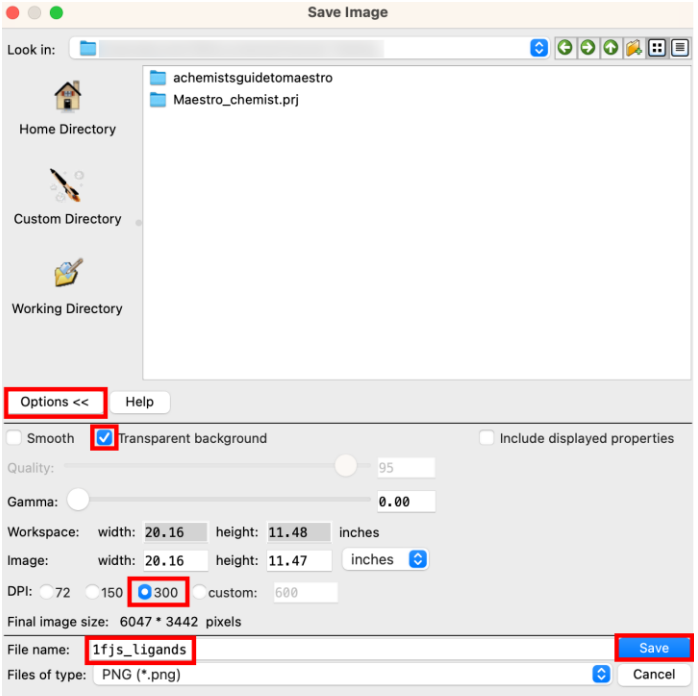


Figure 2-4. Clear selection option in Quick Select.

7. Under Quick Select, click **Clear selection.**
8. Type **Z** to fit structures to the Workspace.



☐ Smooth ☑ Transparent background ☐ Include displayed properties

Quality: 95

Gamma: 0.00

Workspace: width: 20.16 height: 11.48 inches

Image: width: 20.16 height: 11.47 inches

DPI: ○ 72 ○ 150 ● 300 ○ custom: 600

Final image size: 6047 * 3442 pixels

File name: 1fjs_ligands

Files of type: PNG (*.png)

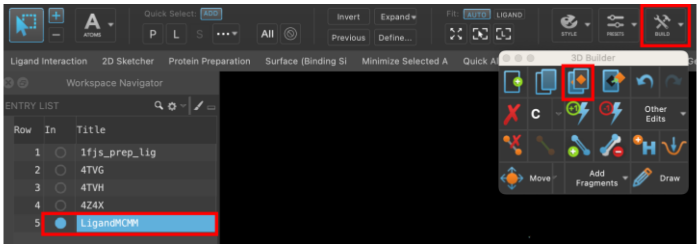
Figure 2-5. Save Image panel with Options open.

9. Click **Workspace > Save Image As**
   - The Save Image panel opens.
10. Click **Options >>** to expand image details.
11. Check **Transparent background** and **300** DPI.
12. Change File name to **1fjs_ligands**.
13. 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 will be saved with the selection highlights.

Go to **Tasks > Browse > Workspace Operations** for more options, such as **Ray Trace** for high-quality images.

2.2 Create a new entry



Row	In	Title
1	○	1fjs_prep_lig
2	○	4TVG
3	○	4TVH
4	○	4Z4X
5	●	LigandMCMM

Figure 2-6. Copy selected atoms to the new entry option in 3D Builder.

1. Include **LigandMCMM** in the Workspace.

Note: Fill in the blue circle to include an Entry in the Workspace. Included entries can be interacted with. Selected Entries have the Title highlighted in blue. Typically this is done when preparing for a computational task.

2. Under Quick Select, click **L**.
    - The ligand is highlighted.
3. Click **Build**.
    - The 3D Builder panel opens.

Note: The 3D Builder panel can be moved by clicking and dragging on the panel name.

Hover over buttons in 3D Builder to see a tooltip about their function. Many tools in Maestro have tooltips and/or links to the help section in the documentation.


New entry created. Entry title: ligand_copy

Figure 2-7. Banner prompt to change Entry title.

4. Choose **Copy selected atoms to new entry**.
    - A banner appears.
5. Change Entry title to **ligand_copy** and click the **check**.
    - A new entry ligand_copy is created in the Entry List.

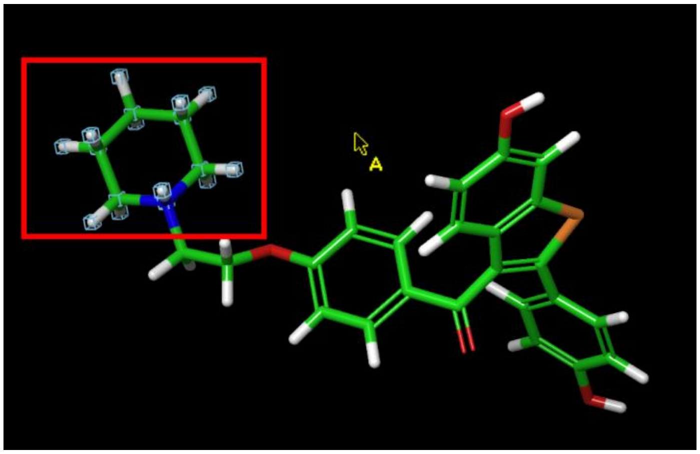


Figure 2-8. Ligand_copy with piperidine

6. Include **ligand_copy** in the Workspace.
7. Use click and drag to select the **piperidine** group.

Note: If in Trackpad/PyMOL mode, use shift-click and drag to select items in the Workspace.

selected.

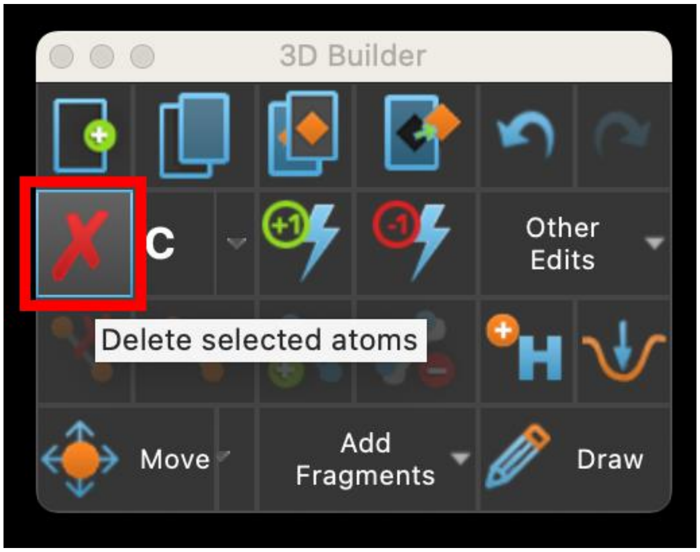


Figure 2-9. Delete selected atoms option.

8. In 3D Builder, click **delete selected atoms**.

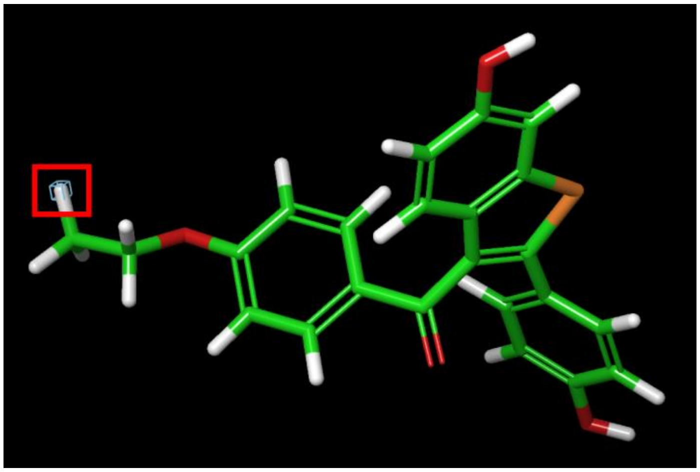


Figure 2-10. Ligand_copy with terminal hydrogen selected.

9. Click a **hydrogen** on the terminal carbon.

Note: Use predictive highlighting to help when selecting items in the Workspace before you commit to the selection by hovering over the atom of interest.

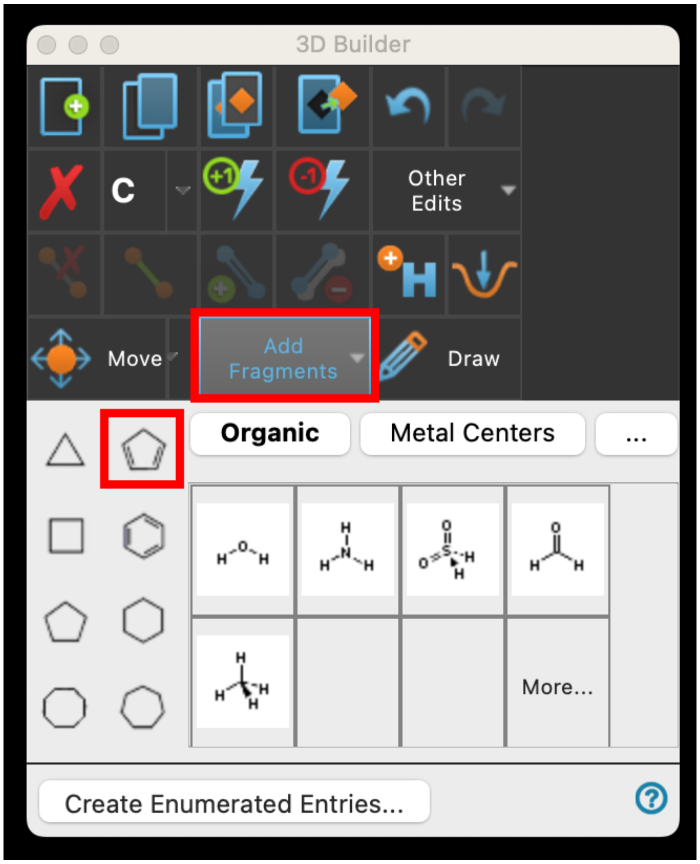


Figure 2-11. Fragments pane in the 3D Builder, with the cyclopentadiene option highlighted.

10. In 3D Builder, click **Add Fragments**.
    - The Fragments pane opens.
11. Choose **cyclopentadiene**.
    - A cyclopentadiene fragment is appended to the molecule in place of the selected hydrogen.

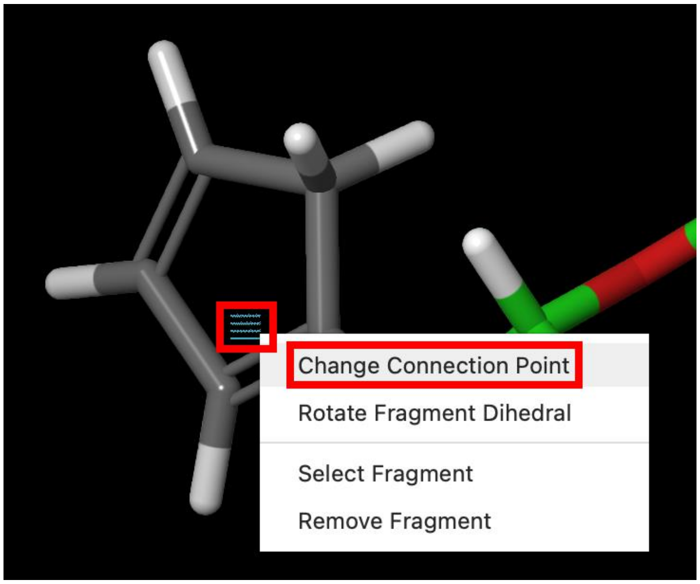


Figure 2-12. Edit Fragment options.

12. Next to the attached fragment, click the **Edit Fragment** four-line icon.
13. Choose **Change Connection Point**.
    - Possible connection points are highlighted.

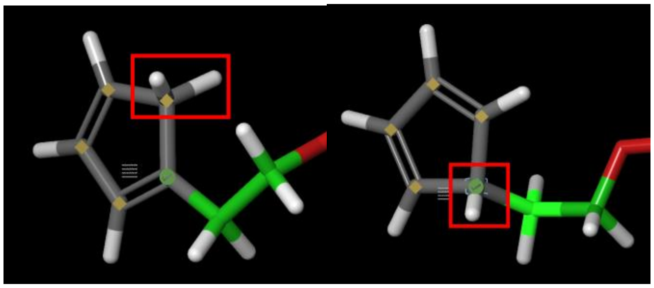


Figure 2-13. Possible sp³ carbon connection point (left). New connection point chosen (right).

14. Click the **sp³ carbon**.
    - The new connection point is marked with a green check.
15. Close the 3D Builder.

Optional: Select **Measure** in the favorites toolbar to measure bond distances and angles.

Optional: Rotate dihedrals using the 3D Builder by selecting from the **Move** dropdown menu.

Hovering over the sp³ carbon will allow you to choose which hydrogen position to set as the connection.

3. Drawing Structures

In this section, we will explore ways to draw molecules in 2D and 3D form. We will use the 2D Sketcher to modify an existing entry and create a new fragment. Then, we will make a copy of an entry to use as a template to create new molecules, using the 3D Builder's Draw function. Finally, a fragment made in the 2D Sketcher will be added to a molecule in the 3D Workspace and the new structure will be minimized.

3.1 Create structures in 2D

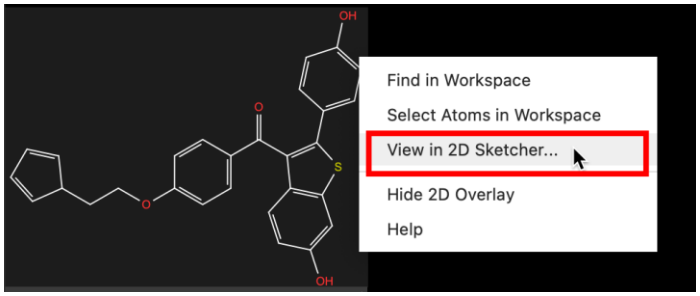
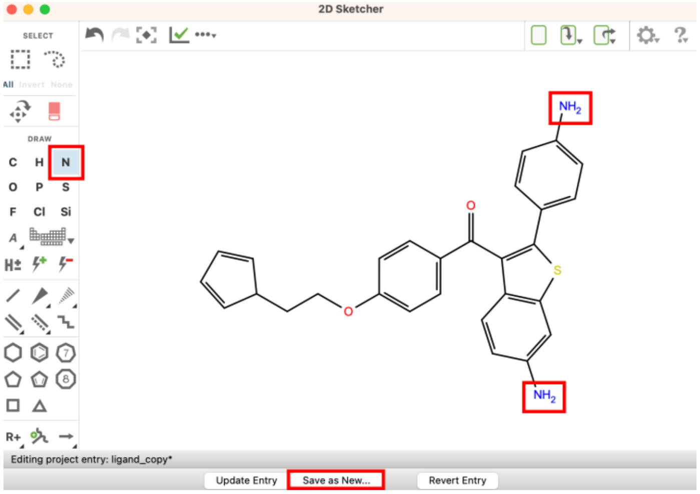


Figure 3-1. View in 2D sketcher option in 2D Overlay.

1. Include **ligand_copy** in the Workspace.
2. Right-click on the **2D Overlay** and choose **View in 2D Sketcher**.
    - The 2D Workspace - 2D Sketcher opens.
    - If the 2D Overlay is not visible in your workspace, turn it on before proceeding with the previous step by clicking **Window > 2D Overlay**.

Note: Right-clicking is a functionality present in many different panels and areas of Maestro.

Optional: Read through the lists of options that appear as they can contain useful shortcuts.



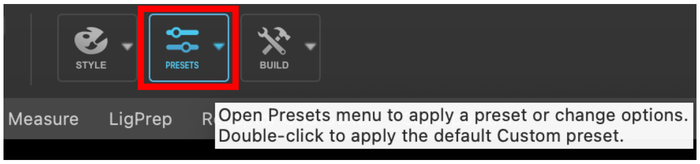
Editing project entry: ligand_copy*

Figure 3-2. Ligand_copy with hydroxyl groups changed to amines.

3. Choose **N** and click both **hydroxyl groups**.
    - The hydroxyl groups are changed to amines.
4. Click **Save as New.**
5. Name the Entry **ligand_2DSketcher**.
    - A new ligand is added to the Entry List with these modifications.
    - The Workspace is rendered in a wire representation.
6. **Close** the 2D Sketcher.

These modifications can also be performed by selecting the connection points and using **Shift+N** with the 3D Builder panel open.

The 2D Sketcher can also be used to update existing entries in the Workspace by selecting Update Entry.



Open Presets menu to apply a preset or change options.
Double-click to apply the default Custom preset.

Figure 3-3. Apply custom Preset style to the Workspace.

7. Double-click **Presets.**
    - The ligand is rendered as ball and stick.

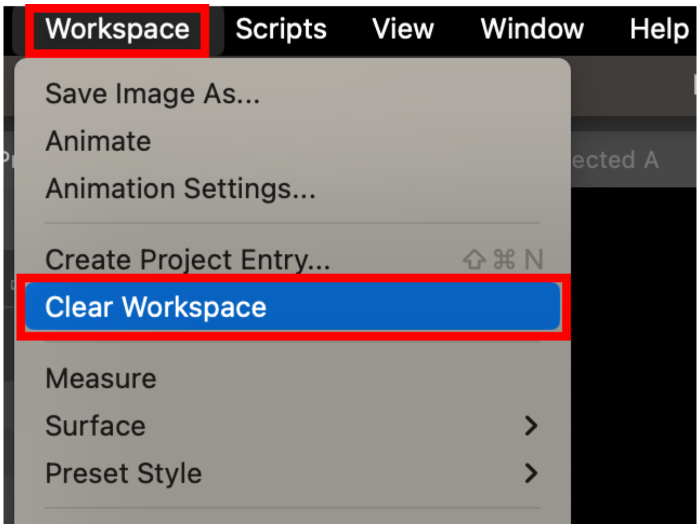


Figure 3-4. Clear Workspace option in the Workspace tab.

8. Go to **Workspace > Clear Workspace**

Note: You can also right-click on empty Workspace and choose **Clear Workspace.**

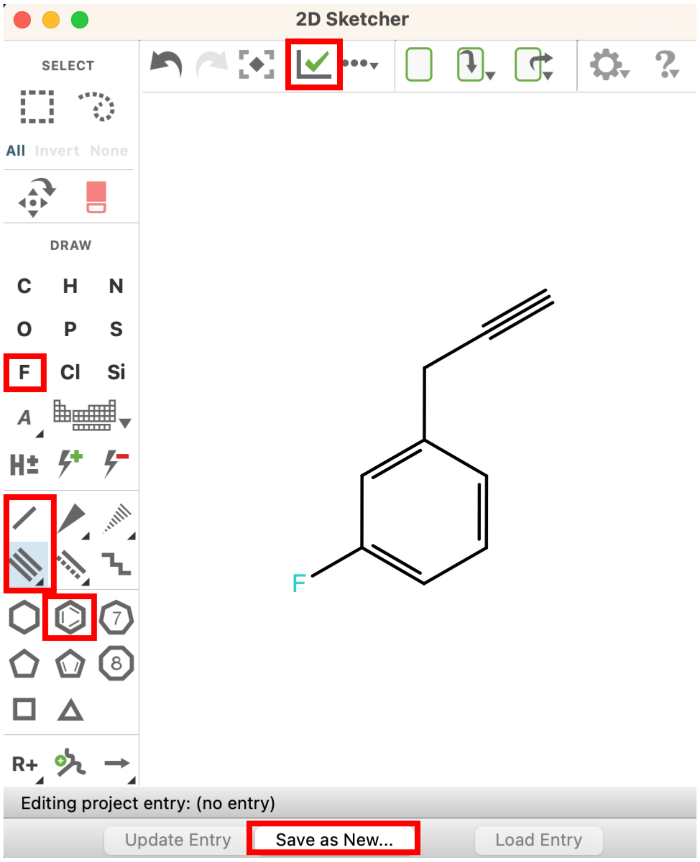


Figure 3-5. Draw and benzene options in the 2D Workspace - 2D Sketcher.

9. Go to **Edit > 2D Sketcher**
    - The 2D Workspace - 2D Sketcher opens.
    - You are still in Draw Mode.
10. In 2D Workspace - 2D Sketcher, click **benzene** and click in the **2D Workspace.**
11. Draw this structure.
    - To add the alkyne, you may type “3” when hovering over an alkane, or use the alkyne drawing tool.
12. Click the green check mark indicating **Cleanup** to fix bond angles.
13. Click **Save as New** and change the Input Entry Title to **fragment**.
14. Click **OK.**
    - A new entry titled fragment is saved to the Entry List.
    - The fragment is shown in 3D in the Workspace.

Ctrl-Z (**Cmd-Z**) will undo previous steps in the 2D Sketcher. This feature is not yet available in all areas of Maestro.

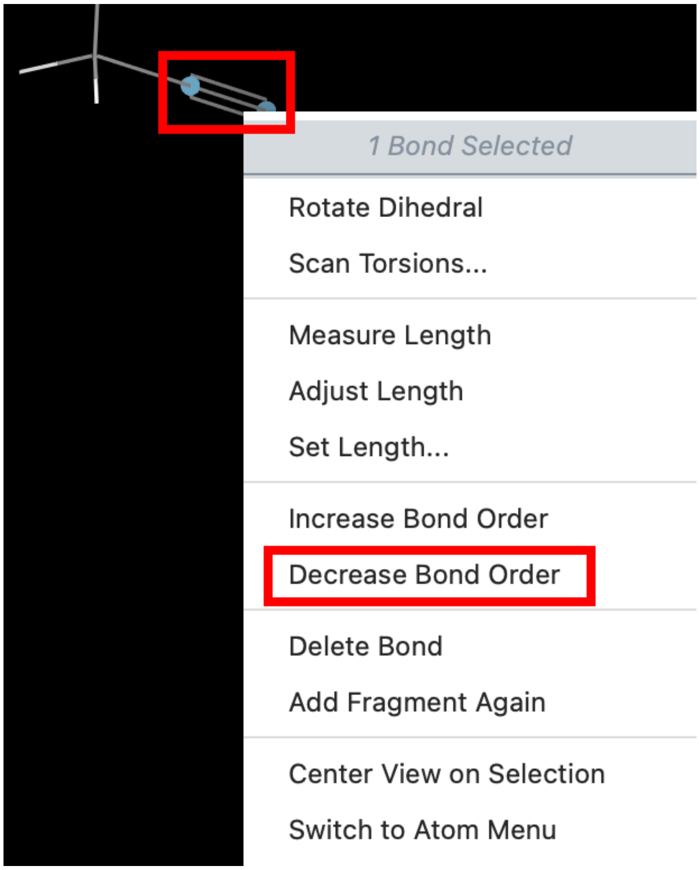


Figure 3-6. Decrease Bond Order option.

15. Right-click on the **alkyne bond**.
16. Choose **Decrease Bond Order**.
    - The alkyne is now an alkene.
17. Under Quick Select, click **All**.
    - The fragment is highlighted.
18. Type **Ctrl+M** (**Cmd-M**) to minimize the selection.
    - The fragment is minimized.

Minimizing selected atoms can also be accessed in the 3D Builder or the Favorites Toolbar or **Edit > Minimize**.

3.2 Create structures in 3D

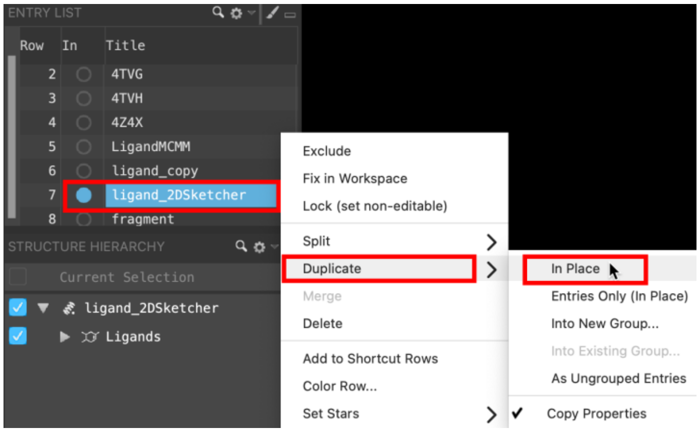


Figure 3-7. Duplicate an entry In Place.

1. Right-click on **ligand_2DSketcher** in the Entry List.
2. Choose **Duplicate > In Place**
3. Double click on the second **ligand_2DSketcher** entry and change the title to **ligand1**.
4. Include **ligand1** in the Workspace.

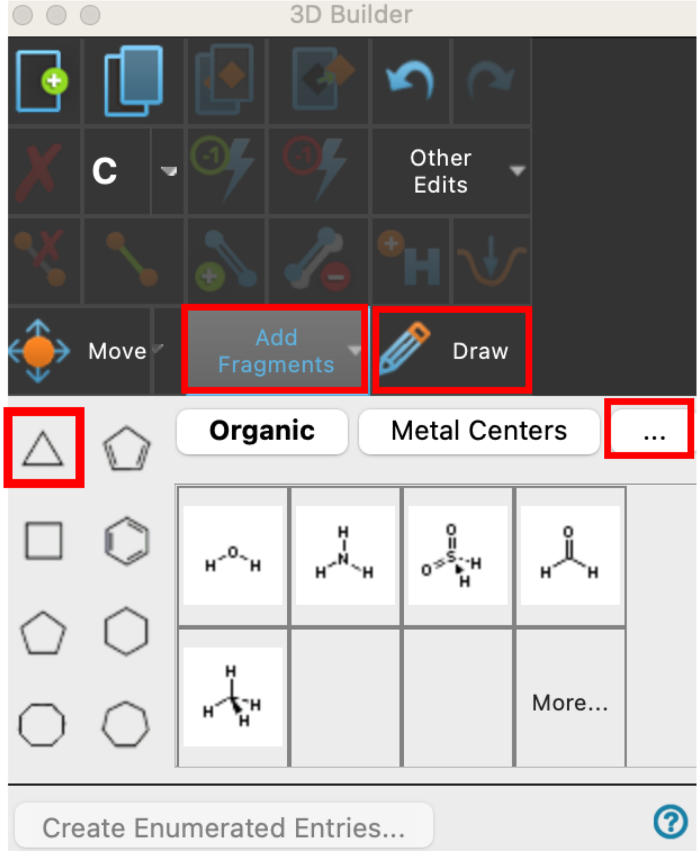


Figure 3-8. Fragment options in the 3D Builder.

5. In 3D Builder, click **Add Fragments** and choose a fragment of your choice.

Note: If you wish to explore all fragments included in the 3D Builder please click the three dots. You may also choose to create your own fragment library or click the pencil icon to draw directly on the 3D structure.

Note: The fragment shown in this example is a cyclopropane.

Using Ctrl-M can perform a quick minimization. It is worth noting that minimizing the structure is less accurate than performing a Jaguar Optimization on your molecule.

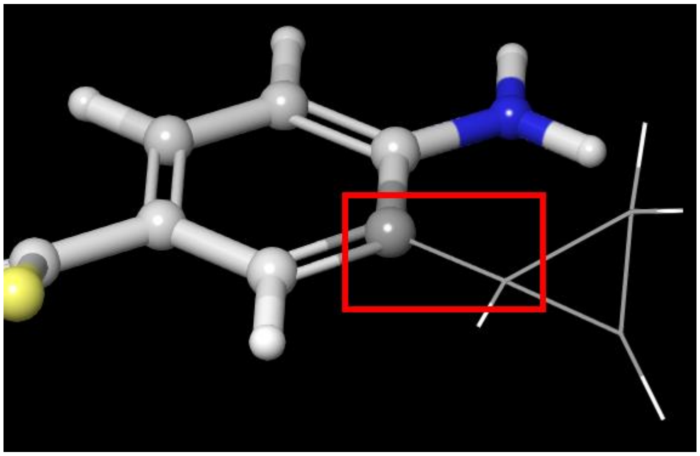


Figure 3-9. A cyclopropane is drawn and connected to the aniline of ligand1.

6. Shift-click to select an aniline carbon on the ligand and a fragment atom.
7. Right-click on one of the selected atoms and choose **Add Bond**.
    - Your fragment is now attached to the ligand.
8. Minimize this structure by selecting the whole molecule and clicking **Minimize Selected Atoms** in the Favorites Toolbar.
9. **Close** the 3D Builder Panel.

Note: If in Trackpad/PyMOL mode, use shift-click and drag to draw bonds.

4. Generating and Visualizing Ligand Conformations

When working with small molecules in Maestro, it is easy to explore the conformational space of a molecule and visualize the results by aligning the conformations to a user-specified 'core.' In this section, we will generate conformations of the small molecule 1FJS_ prep_lig, using MacroModel. We will then use the superposition tool to overlay the conformations to a rigid, common core motif. The core will be defined using a SMARTS pattern generated from the Workspace structure.

4.1 Generate conformations

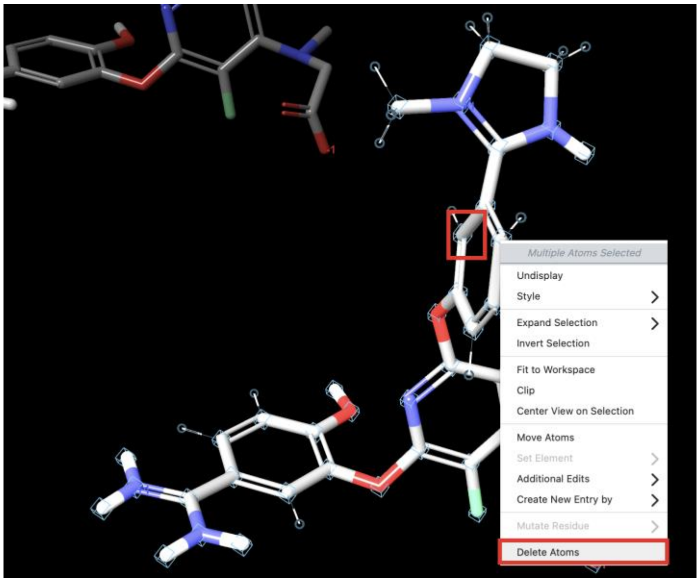
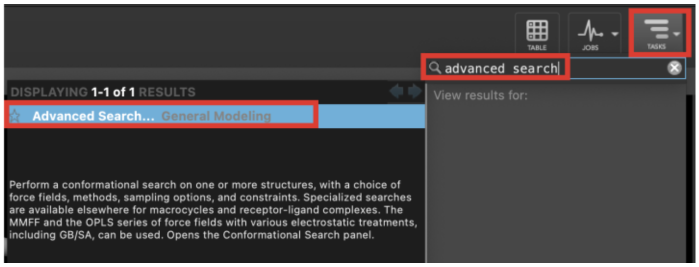


Figure 4-1. Delete Atoms option in Multiple Atoms Selected menu.

1. Include **1fjs_prep_lig** in the Workspace.
2. Click **R** on your keyboard to switch to Residue selection mode.
3. Click on an **atom of a copy** of the ligand to select the structure.

Note: This ligand was copied earlier in Section 2.1. The copied structures need to be removed before the conformational search.

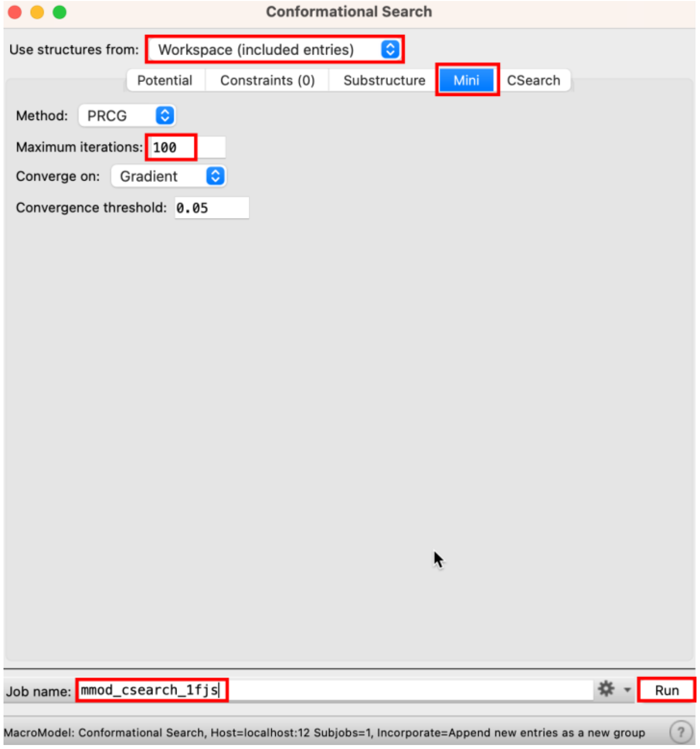
4. Right click on the **selected ligand.**
    - The Multiple Atoms Selected menu opens.
5. Choose **Delete Atoms**.
    - The ligand is deleted from the Workspace.
6. Ensure **only one ligand** structure is included in the Workspace.



Perform a conformational search on one or more structures, with a choice of force fields, methods, sampling options, and constraints. Specialized searches are available elsewhere for macrocycles and receptor-ligand complexes. The MMFF and the OPLS series of force fields with various electrostatic treatments, including GB/SA, can be used. Opens the Conformational Search panel.

Figure 4-2. Advanced Search option in Structure Analysis.

7. Go to **Tasks** and type **Advanced Search.**
8. Click on **Advanced Search** in the displayed results.
    - The Conformational Search panel opens.



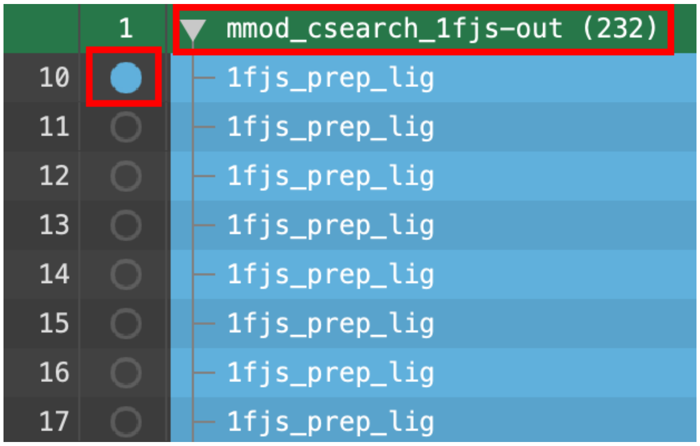
Use structures from: Workspace (included entries)
Method: PRCG
Maximum iterations: 100
Converge on: Gradient
Convergence threshold: 0.05
Job name: mmod_csearch_1fjs

MacroModel: Conformational Search, Host=localhost:12 Subjobs=1, Incorporate=Append new entries as a new group

Figure 4-3. Conformational Search panel with Mini tab selected.

9. For Use structures from, choose **Workspace (included entries)**.
10. Click the **Mini** tab.
11. Set Maximum iterations to **100**.
12. Change Job name to **mmod_csearch_1fjs**.
13. Click **Run**.
    - This job takes ~2 minutes.
    - A banner appears when the job is incorporated.
    - A new group titled **mmod_csearch_1fjs-out** is added to the Entry List.

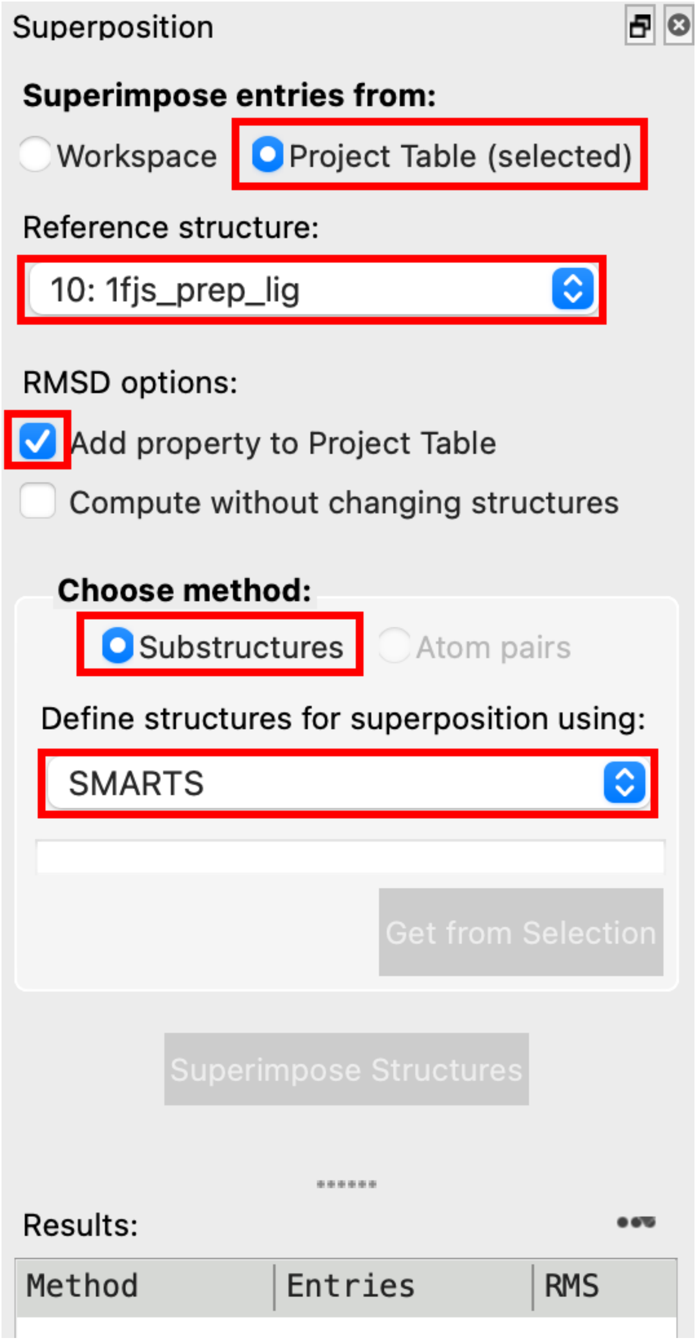
4.2 Align the conformations



1 ▼ mmod_csearch_1fjs-out (232)
10 ● 1fjs_prep_lig
11 ○ 1fjs_prep_lig
12 ○ 1fjs_prep_lig
13 ○ 1fjs_prep_lig
14 ○ 1fjs_prep_lig
15 ○ 1fjs_prep_lig
16 ○ 1fjs_prep_lig
17 ○ 1fjs_prep_lig

Figure 4-4. All entries of the conformational search are selected, the first entry is included.

1. Click the **mmod_csearch_1fjs-out** group.
    - All entries are selected.
2. Include the first **1fjs_prep_lig** entry.
3. Go to **Tasks > Browse > Structure Alignment > Superposition**
    - The Superposition panel opens.



Superposition

Superimpose entries from:

○ Workspace ● Project Table (selected)

Reference structure:

10: 1fjs_prep_lig

RMSD options:

☑ Add property to Project Table

☐ Compute without changing structures

Choose method:

● Substructures ○ Atom pairs

Define structures for superposition using:

SMARTS

Figure 4-5. Selected entries and RMSD properties for Superposition panel.

4. For Entries to superimpose, choose **Project Table (selected)**.
5. For Reference structure, choose **1fjs_prep_lig**.
6. Check **Add property to Project Table**.
7. For Choose method, select **Substructures**.
8. For Define structures for superposition using, choose **SMARTS**.

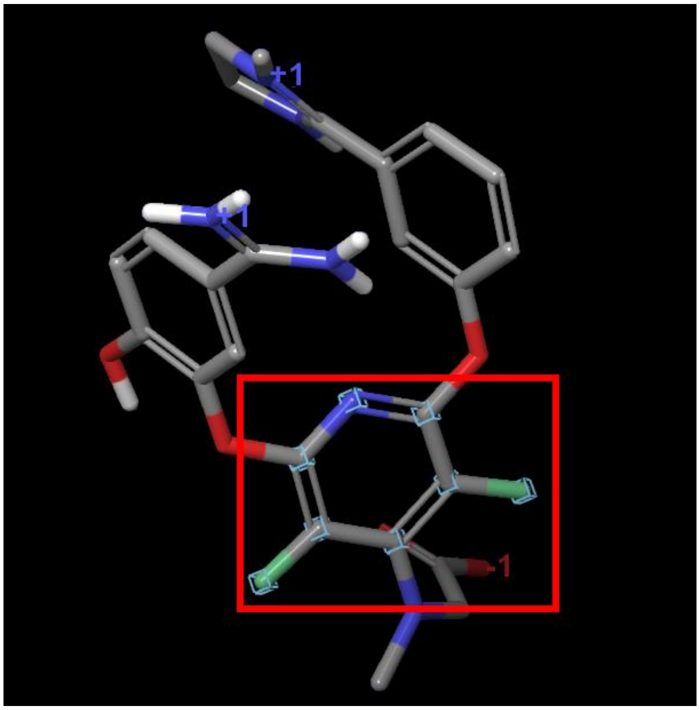
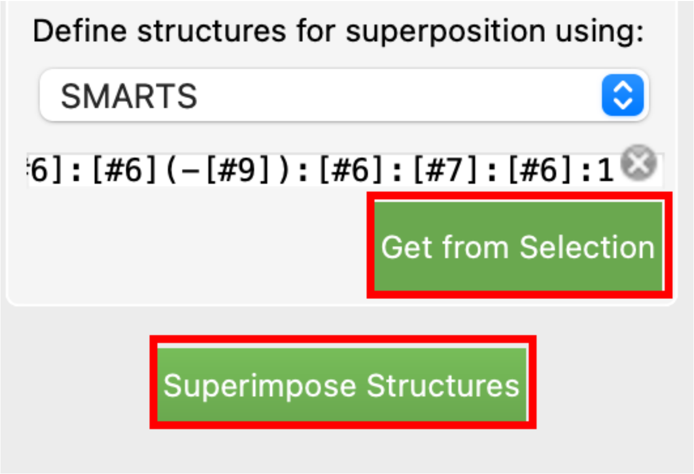


Figure 4-6. Pyridine and two fluorines of 1fjs_prep_lig selected.

Note: In case you are still in Residue selection mode, type **A** on your keyboard to switch to Atom selection mode.

9. In the Workspace, use shift-click to select the atoms of the **pyridine** and the **two fluorines.**



Define structures for superposition using: SMARTS

`6]:[#6](–[#9]):[#6]:[#7]:[#6]:1`

Figure 4-7. The SMARTS tab of the Superposition panel.

10. In the Superposition panel, click **Get from Selection.**
    - The SMARTS pattern is generated.
11. Click **Superimpose Structures.**
    - RMSD properties are calculated.

Note: Click the **Table icon** in the upper right corner of the Workspace to view the RMSD values in the Project Table.

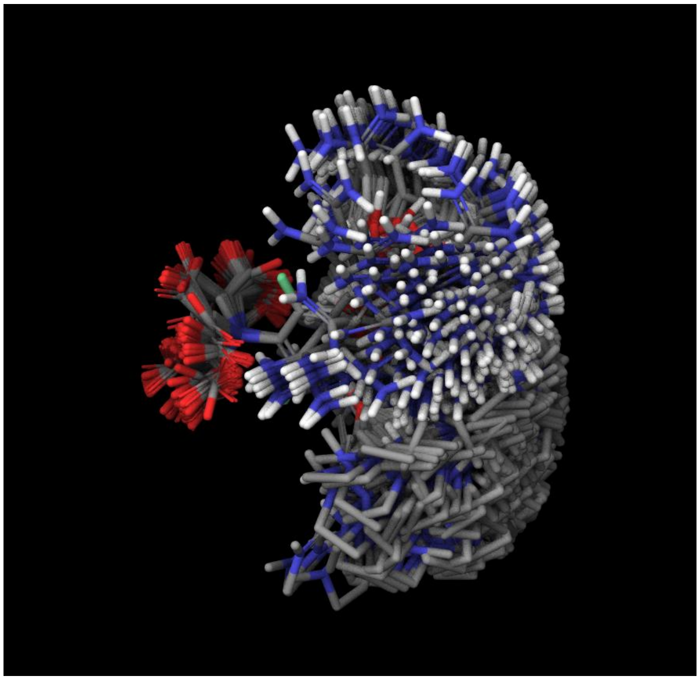


Figure 4-8. Superposition of conformations of 1fjs_prep_lig aligned to the pyridine.

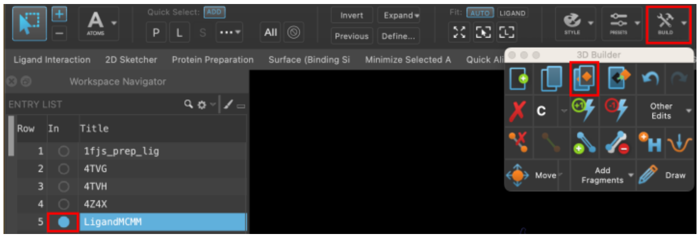
12. Right-click the **mmod_csearch_1fjs-out** group and choose **Include**.
13. Click **Continue** in the Warning window.
    - The conformations are superimposed in the Workspace.
    - Conformations are aligned to the pyridine.
14. **Close** the Superposition panel.

---

5. Small Molecule Alignment

Comparing two structurally similar small molecules can be useful for idea generation. In this section, we will superimpose two ligands, with and without a protein structure. We will begin by aligning two ligands using an automated method, then a manual method, and finally a flexible alignment method. Finally, we will superimpose an unbound ligand onto a ligand bound to a receptor. This is helpful for evaluating how a ligand could fit in a known binding site, without using docking, to assist with lead optimization.

5.1 Superimpose two distinct ligands

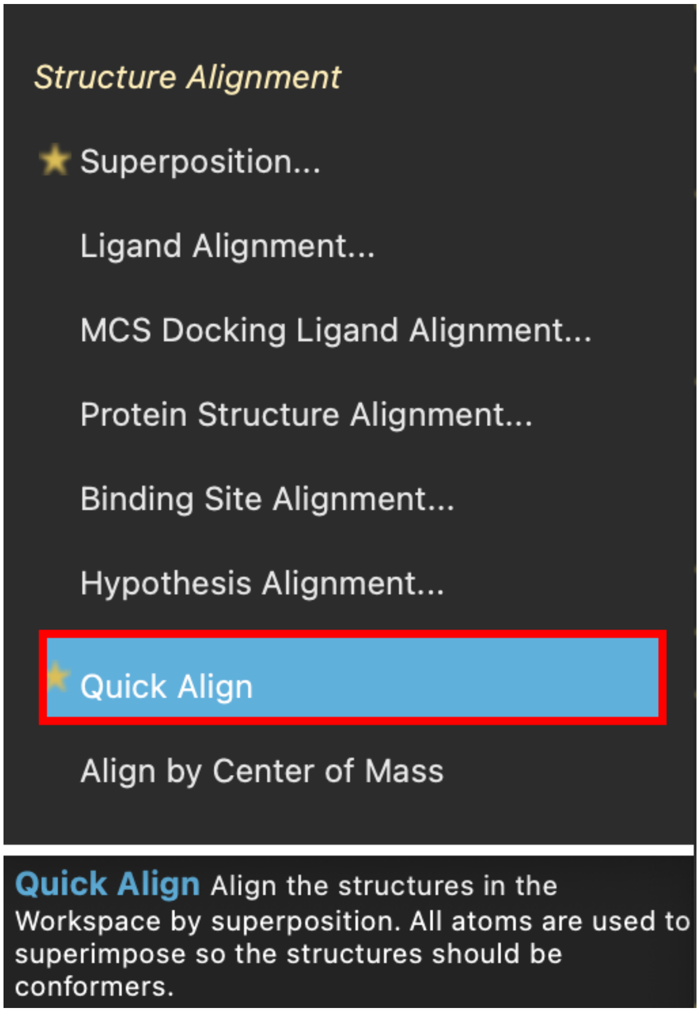


Row	In	Title
1	○	1fjs_prep_lig
2	○	4TVG
3	○	4TVH
4	○	4Z4X
5	●	LigandMCMM

Figure 5-1. Copy selected atoms to new entry option in 3D Builder.

1. Include **LigandMCMM** in the Workspace.
2. Create a new entry of only the ligand titled **ligand_phenol**.

Note: See section 2.2 for details on creating a new entry.



Quick Align Align the structures in the Workspace by superposition. All atoms are used to superimpose so the structures should be conformers.

Figure 5-2. Quick Align option in Tasks.

3. Include and select **ligand_phenol.**
4. Ctrl-click (Cmd-click) to include and select **1fjs_prep_lig.**
5. Go to **Tasks > Browse > Structure Alignment > Quick Align**
   - The ligands are aligned.

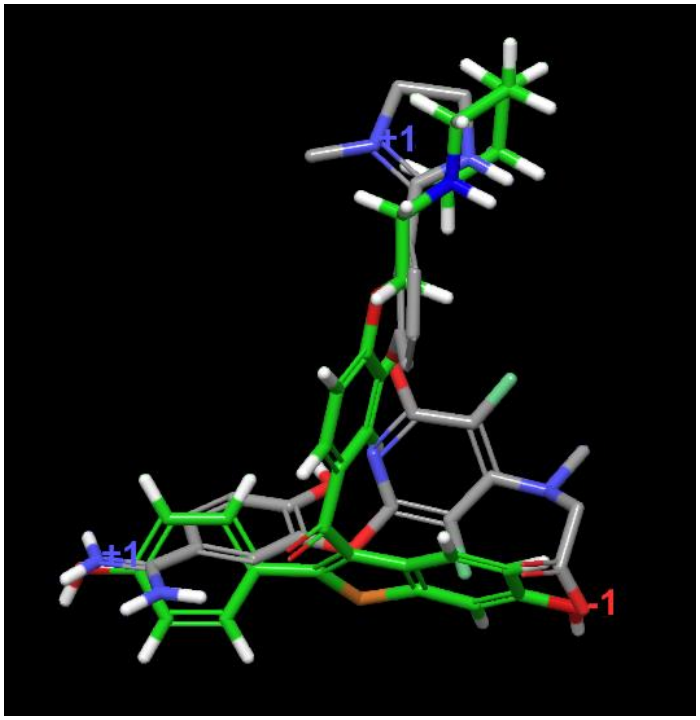
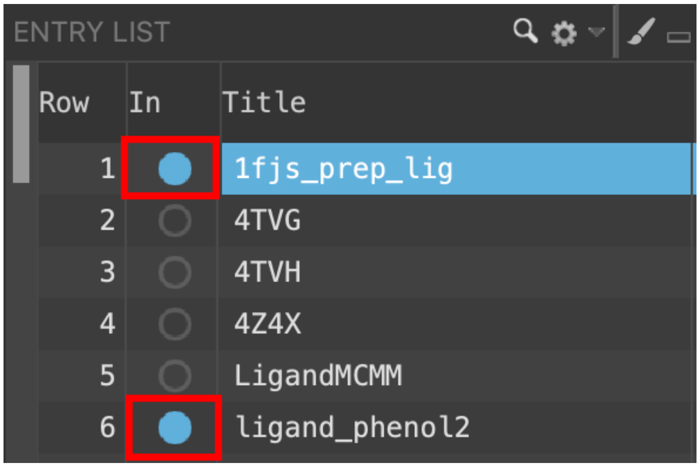


Figure 5-3. Ligand_phenol (green) automatically aligned with 1fjs_prep_lig (gray).

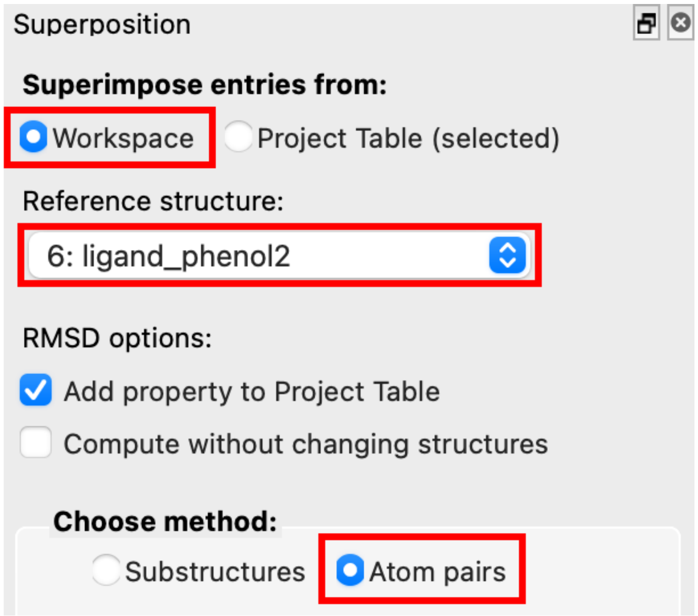
6. Type **Z** to fit ligands to the <u>Workspace</u>.



Row	In	Title
1	●	1fjs_prep_lig
2	○	4TVG
3	○	4TVH
4	○	4Z4X
5	○	LigandMCMM
6	●	ligand_phenol2

Figure 5-4. 1fjs_prep_lig and ligand_phenol2 included in the Workspace.

7. <u>Include</u> **LigandMCMM**.
8. Create a new entry of only the ligand titled **ligand_phenol2**.
9. Ctrl-click (Cmd-click) to <u>include</u> both **1fjs_prep_lig** and **ligand_phenol2**.



Superposition

Superimpose entries from:

● Workspace ○ Project Table (selected)

Reference structure:

6: ligand_phenol2

RMSD options:

☑ Add property to Project Table

☐ Compute without changing structures

Choose method:

○ Substructures ● Atom pairs

Figure 5-5. The Superposition panel using Atom Pairs from Included entries.

Note: Use the **Move Atoms** function to move the ligands closer, if desired. See [Section 3.2](#) for more details.

10. Go to **Tasks > Structure Alignment > Superposition**
    - The Superposition panel opens.
11. For Superimpose entries from, choose **Workspace**.
12. For Reference structure, choose **ligand_phenol2**.
13. Click the **Atom pairs** option.

Note: The order of atom selection is important, always start a pair from the same molecule.

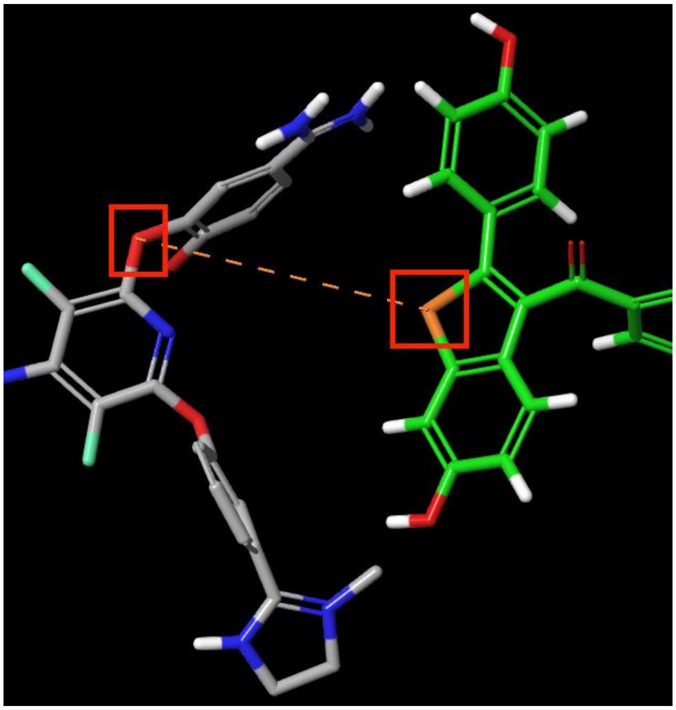


Figure 5-6. First atom pair.

14. Click the **sulfur** of ligand_phenol2.
    - The sulfur is highlighted with a pink box.
15. Click the **oxygen 1** of 1fjs_prep_lig.
    - An orange line connects the atoms.

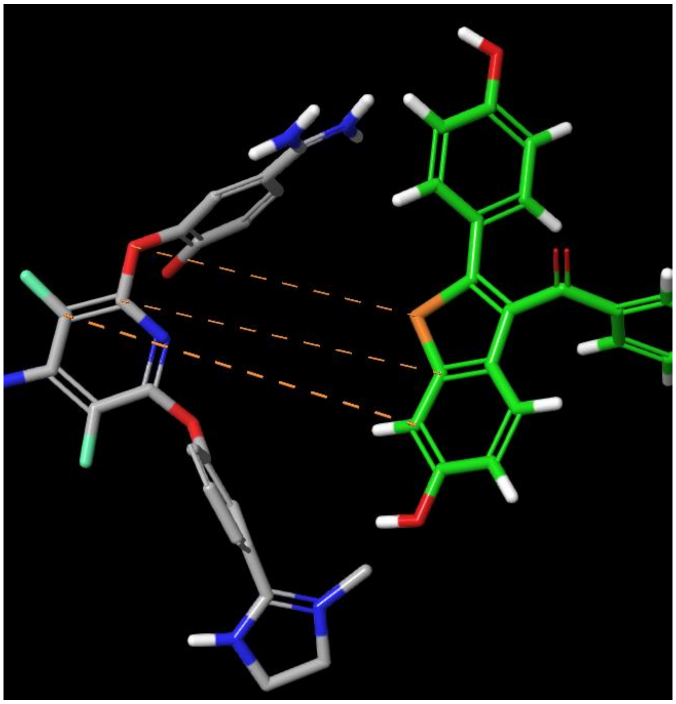


Figure 5-7. Selected atom pairs for superposition. Ligand_phenol2 is shown in green (right) and 1fjs_prep_lig is in gray (left).

16. Click the **carbon 5** of ligand_phenol2.
17. Click the **carbon 6** of 1fjs_prep_lig.
18. Click the **carbon 4** of ligand_phenol2.
19. Click the **carbon 1** of 1fjs_prep_lig.

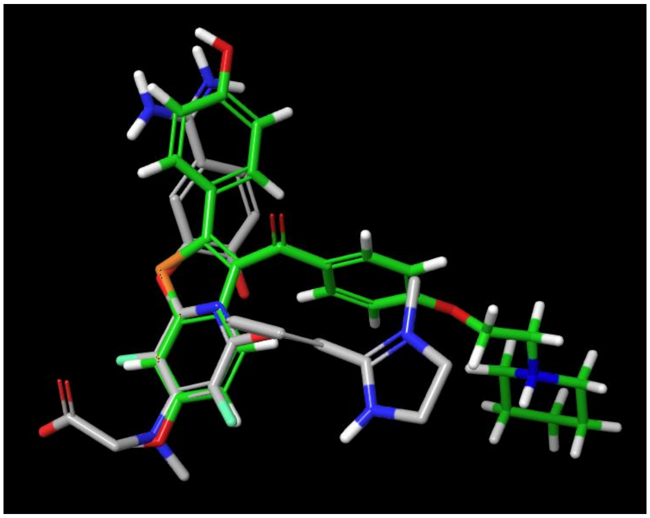
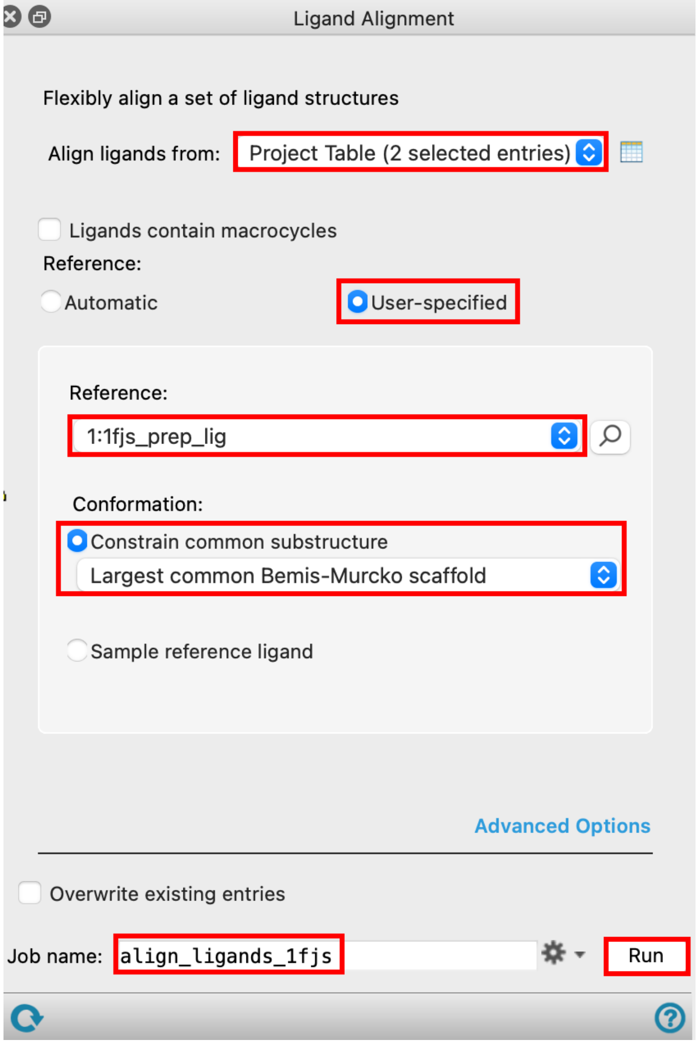


Figure 5-8. Ligands after manual alignment.

20. Click **Superimpose Structures.**
    - The ligands are superimposed.
21. Use **Move** in the 3D Builder to break the alignment.



Ligand Alignment

Flexibly align a set of ligand structures

Align ligands from: Project Table (2 selected entries)

☐ Ligands contain macrocycles

Reference:

○ Automatic ● User-specified

Reference: 1:1fjs_prep_lig

Conformation:

● Constrain common substructure

Largest common Bemis-Murcko scaffold

○ Sample reference ligand

☐ Overwrite existing entries

Job name: align_ligands_1fjs

Figure 5-9. Ligand Alignment panel.

22. Ctrl-click (Cmd-click) to select both **1fjs_prep_lig** and **ligand_phenol2**.
23. Go to **Tasks > Browse > Structure Alignment > Ligand Alignment**
    - The Ligand Alignment panel opens.
24. For Align ligands from, choose **Project Table.**
25. For Reference, choose **User-specified.**
26. Under Reference, choose **1fjs_prep_lig**.
27. Under Conformation, select **Constrain common substructure** and choose **Largest common Bemis-Murcko scaffold.**
28. Set Job name to **align_ligands_1fjs**.
29. Click **Run.**
    - This job takes a few seconds.
    - The **align_ligands_1fjs** group is added to the Entry List.

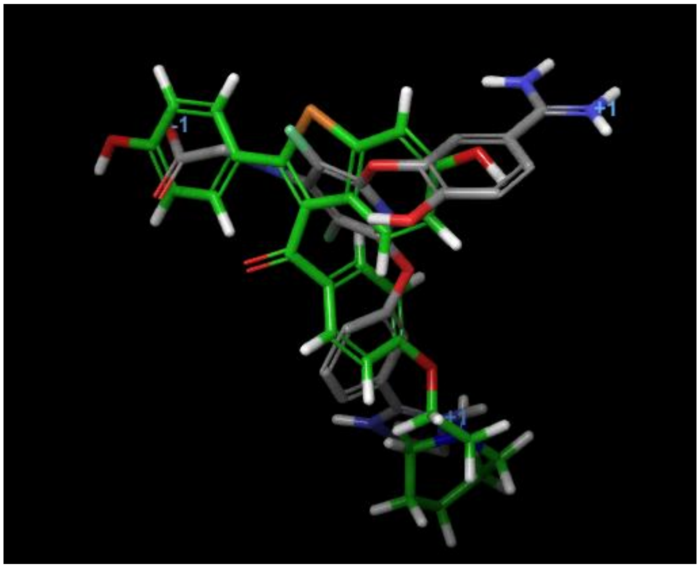
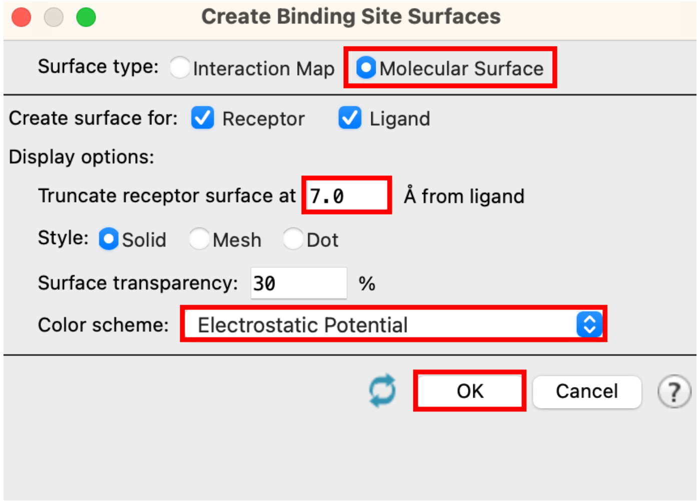


Figure 5-10. Ligands after alignment.

Note: At this point, you can inspect the alignment, and consider altering the parameters until you get something more preferable.

5.2 Superimpose a ligand and a protein-ligand complex



Create Binding Site Surfaces

Surface type: ○ Interaction Map ● Molecular Surface

Create surface for: ☑ Receptor ☑ Ligand

Display options:

Truncate receptor surface at 7.0 Å from ligand

Style: ● Solid ○ Mesh ○ Dot

Surface transparency: 30 %

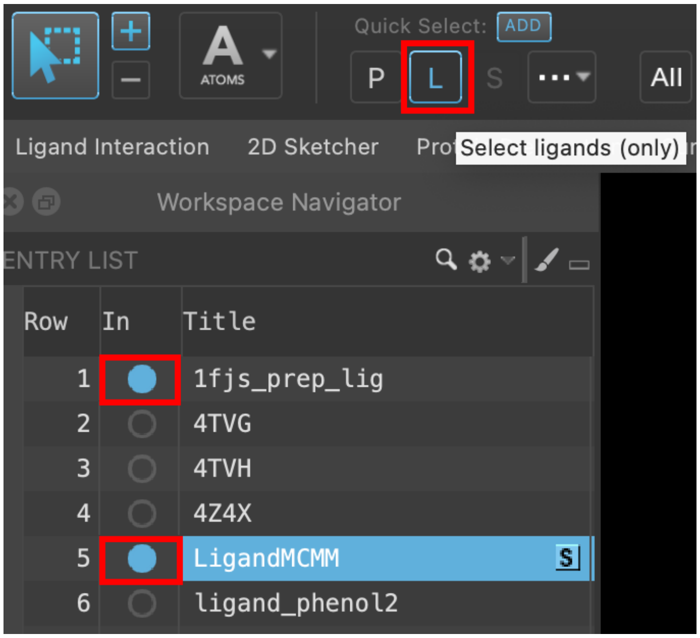
Color scheme: Electrostatic Potential

Figure 5-11. Create Binding Site Surfaces panel.

1. Include **Ligand MCMM**.
2. In the Favorites Toolbar, click **Surface (Binding Site)**.
    - The Create Binding Site Surfaces panel opens.
3. For Surface type, choose **Molecular Surface**.

Note: The Interaction Map allows you to add a SiteMap surface map to your binding site.

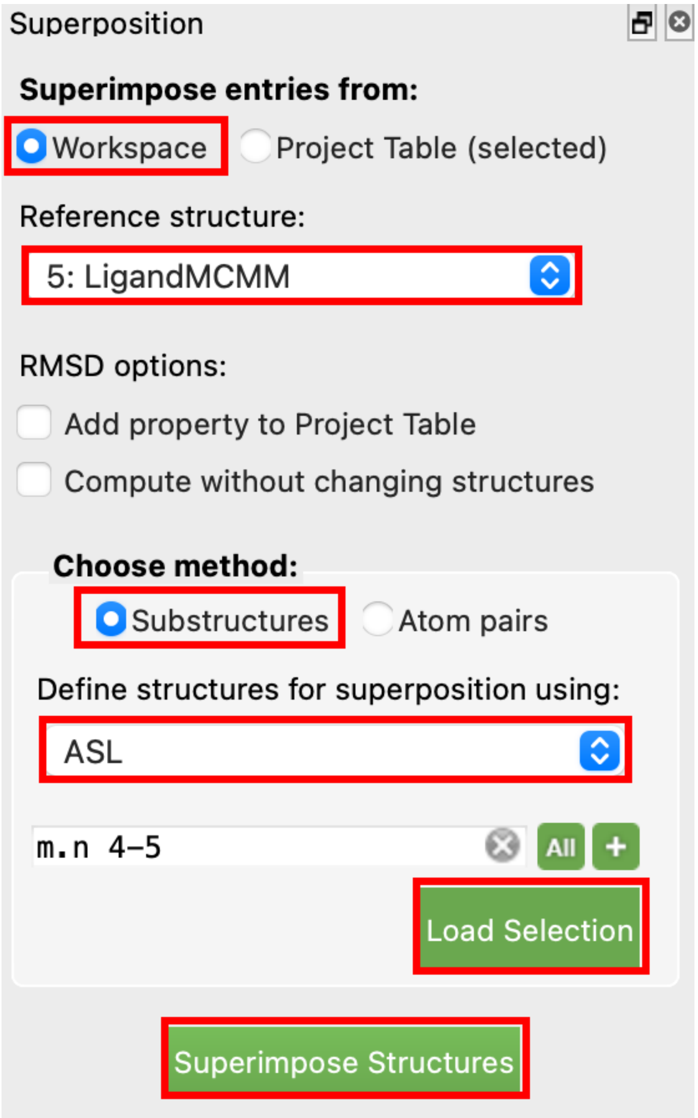
4. For **Truncate receptor surface at**, type **7.0** Å from ligand.
5. For Color scheme, choose **Electrostatic Potential**.
6. Click **OK**.
    - A surface is added to the protein.
    - An 'S' is next to the entry.



Row	In	Title
1	●	1fjs_prep_lig
2	○	4TVG
3	○	4TVH
4	○	4Z4X
5	●	LigandMCMM S
6	○	ligand_phenol2

Figure 5-12. 1fjs_prep_lig and LigandMCMM included in the Workspace.

7. Ctrl-click (Cmd-click) to include **1fjs_prep_lig**.
8. Under Quick Select, click **L**.
    - Both ligands are selected in the Workspace.



Superposition

Superimpose entries from:

● Workspace ○ Project Table (selected)

Reference structure: 5: LigandMCMM

RMSD options:

☐ Add property to Project Table

☐ Compute without changing structures

Choose method: ● Substructures ○ Atom pairs

Define structures for superposition using: ASL

m.n 4-5

Figure 5-13. ASL tab of Superposition.

9. Go to **Tasks > Browse > Structure Alignment > Superposition**
   - ○ The Superposition panel opens.
10. For Superimpose entries from, choose **Workspace.**
11. For Reference structure, choose **LigandMCMM**.
12. For Choose method, choose **Substructures**.
13. For Define structures for superposition using, choose **ASL**.
14. Click **Load Selection.**
15. Click **Superimpose Structures.**
    - ○ 1fjs_prep_lig is aligned to the ligand of LigandMCMM.

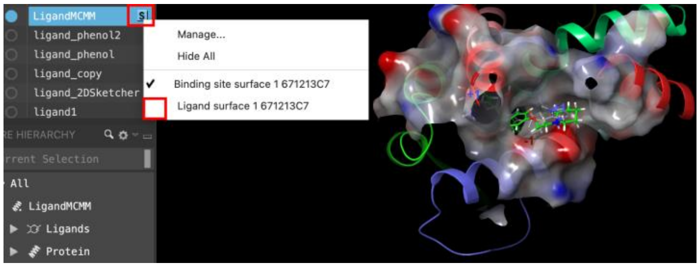


Figure 5-14. 1fjs_prep_lig (gray) is aligned to the receptor-bound ligand (green). Ligand surface is toggled off.

16. Type **L** to zoom to the ligands.

Note: You may choose to toggle off the surface for the Ligand. Click **S** and toggle off the Ligand surface.

This is ***not*** an accurate or rigorous way to evaluate how well a modified ligand will fit into a binding pocket. Visualizing a modified version of a known binder in a binding pocket should be only used to give you a crude sense of the possibility of your design fitting in the binding pocket. The conformational search, scoring function, and post-docking minimization included in the docking protocol lead to predictions that are considerably more accurate than simply overlaying ligands in the binding pocket.

---

6. Protein Alignment

Aligning proteins can give a better understanding of ligand SAR. In this section, we will align three HIV proteases by tertiary structure in order to visualize their similarities and differences. Then, we will align proteins by the binding site in order to compare the area closer to the cognate ligand.

6.1 Align proteins by tertiary structure

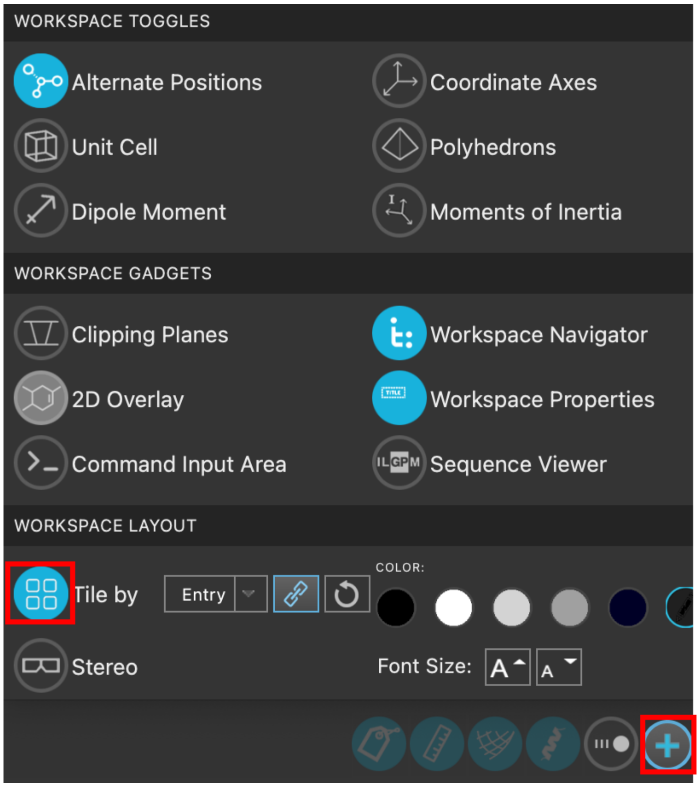
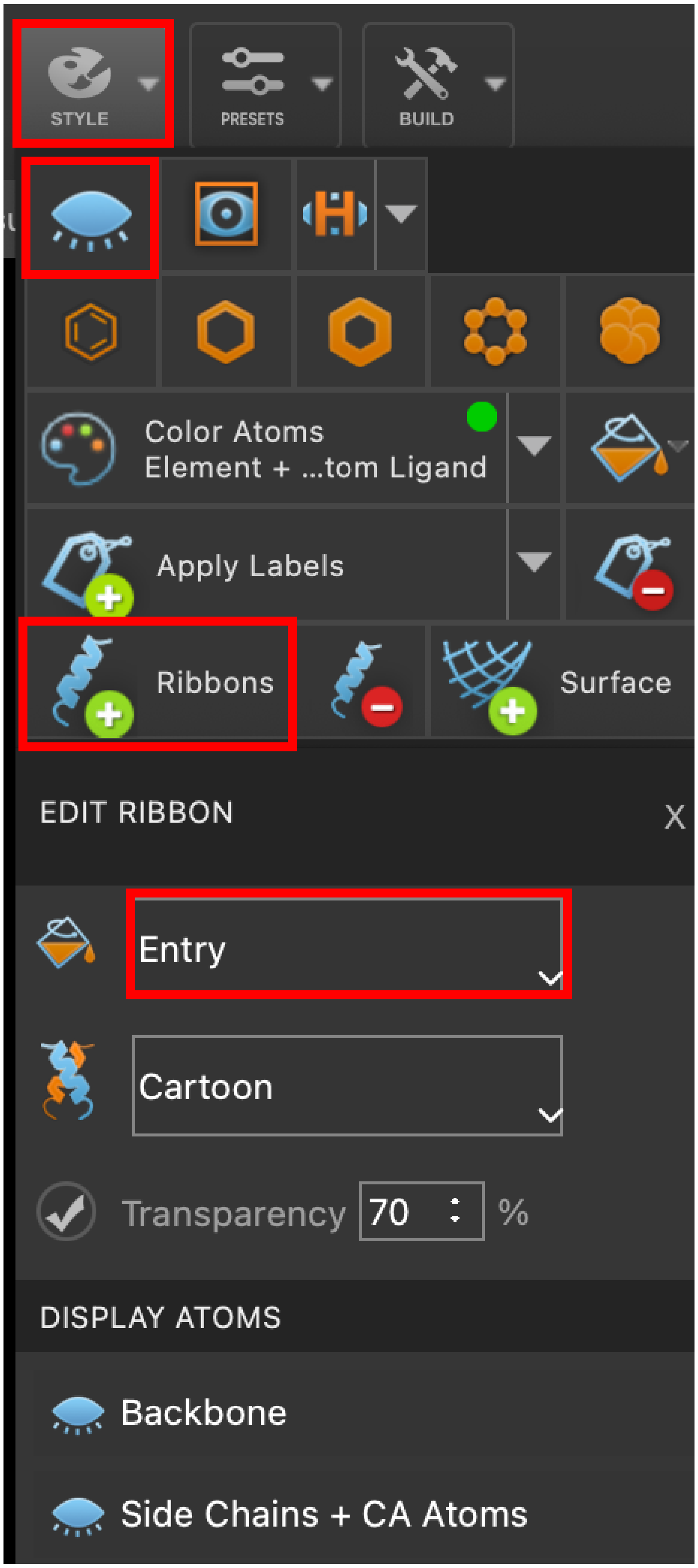


Figure 6-1. Tile option toggled on in the Workspace Configuration panel.

1. Shift-click to include entries **4TVG, 4TVH, 4Z4X** in the Workspace.
2. In the Workspace Configuration Toolbar, click ✚.
3. Click **Tile**.
   - The Workspace is tiled by entry to show all three proteins separately.
4. Under Quick Select, click **P**.
   - Proteins are selected.
5. Type **Z**.



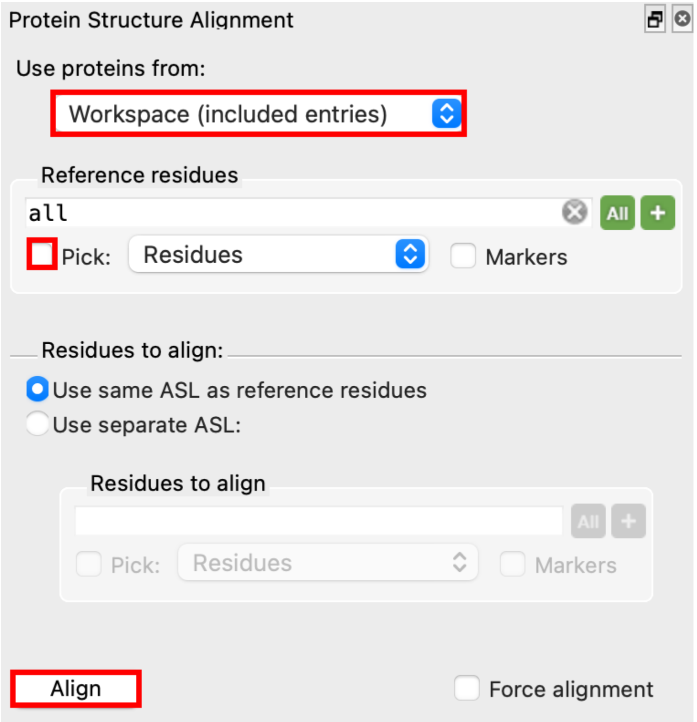
Entry

Cartoon

Transparency 70 %

Figure 6-2. Add ribbons colored by entry.

6. Click the **Style** button.
7. Click **Undisplay selected atoms**.
    - The proteins residues are hidden.
8. Click **Ribbons**.
    - Ribbons are added to the protein structures.
9. Under Edit Ribbon, change the Color Scheme to **Entry**.
10. In the Workspace Configuration Toolbar, click ✛.
11. Click the **Tile by** button to turn off the tiling.
12. Type **Z**.
    - The Workspace is zoomed to the entries.



Protein Structure Alignment

Use proteins from: Workspace (included entries)

Reference residues

all

☐ Pick: Residues ☐ Markers

Residues to align:

● Use same ASL as reference residues

○ Use separate ASL:

Residues to align

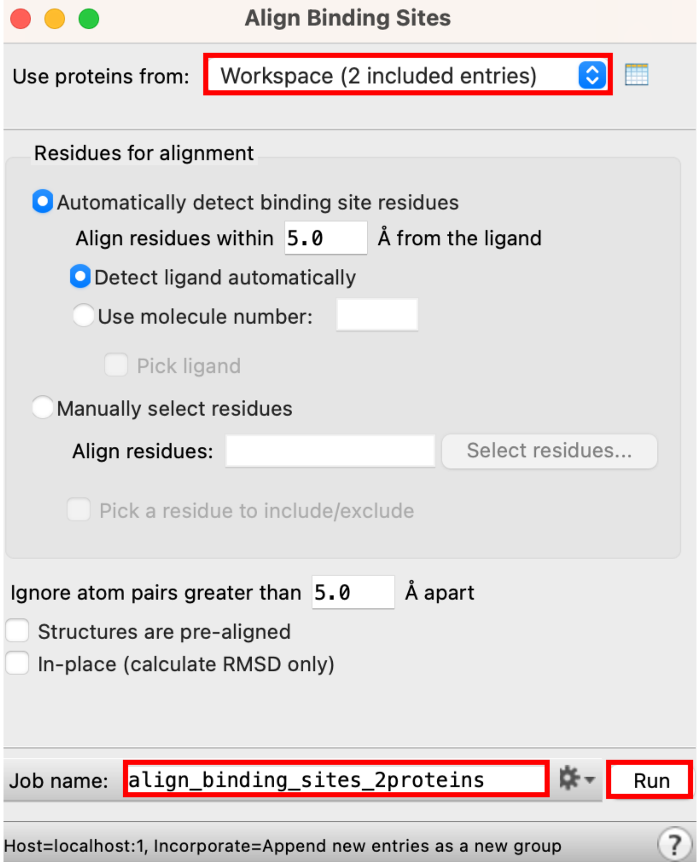
☐ Pick: Residues ☐ Markers

☐ Force alignment

Figure 6-3. Protein Structure Alignment panel.

13. Go to **Tasks > Browse > Structure Alignment > Protein Structure Alignment**
    - The Protein Structure Alignment panel opens.
14. Under Use proteins from, choose **Workspace (included entries)**.
15. Uncheck **Pick**.
16. Click **Align**.
    - The Protein Structure Alignment Results panel opens.
    - Protein sequences, Alignment Scores, and RMSDs are shown.

6.2 Align proteins by binding site



Use proteins from: Workspace (2 included entries)

Residues for alignment

● Automatically detect binding site residues

Align residues within 5.0 Å from the ligand

● Detect ligand automatically

○ Use molecule number:

☐ Pick ligand

○ Manually select residues

Align residues:

☐ Pick a residue to include/exclude

Ignore atom pairs greater than 5.0 Å apart

☐ Structures are pre-aligned

☐ In-place (calculate RMSD only)

Job name: `align_binding_sites_2proteins`

Host=localhost:1, Incorporate=Append new entries as a new group

Figure 6-4. Align Binding Sites panel.

1. Go to **Tasks > Browse > Structure Alignment > Binding Site Alignment**
    - The Align Binding Sites panel opens.
2. In the Entry List, shift-click to select entries **4TVG** and **4TVH**.
3. For Use proteins from, choose **Workspace (2 included entries)**.
4. Change Job name to **align_binding_sites_2proteins**.
5. Click **Run**.
    - This job takes ~1 minute.
    - A new group is appended to the Entry List.

Note: Structure 4Z4X has no ligand bound.

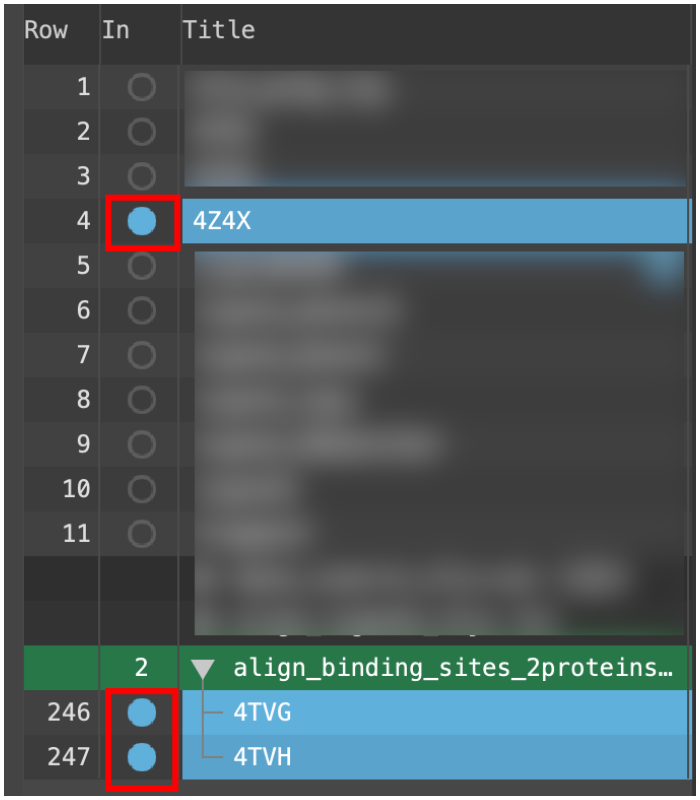


Figure 6-5. Include 4TVG, 4TVH and 4Z4X.

6. Include binding site aligned **4TVG and 4TVH**.
7. Include unaligned **4Z4X**.

Fixing a structure allows other entries to be included without removing the fixed entry from the Workspace.

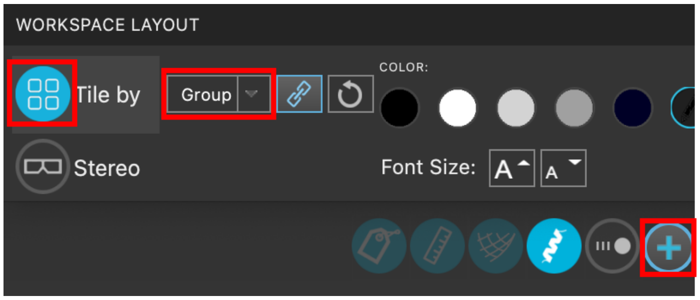


Figure 6-6. Tile the Workspace via the Workspace Configuration panel.

8. Toggle on **Tile** under Workspace Layout in the Workspace Configuration Toolbar.
9. Choose **Group** from the dropdown.

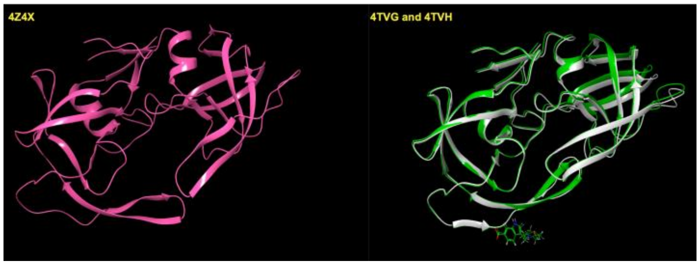


Figure 6-7. Aligned binding sites of proteins

Note: Notice the differences in the binding site area for the two aligned PDB files 4TVG and 4TVH with a ligand and the 4Z4X PDB file without a ligand.

10. Toggle off **Tile by** to return to the default Workspace view.

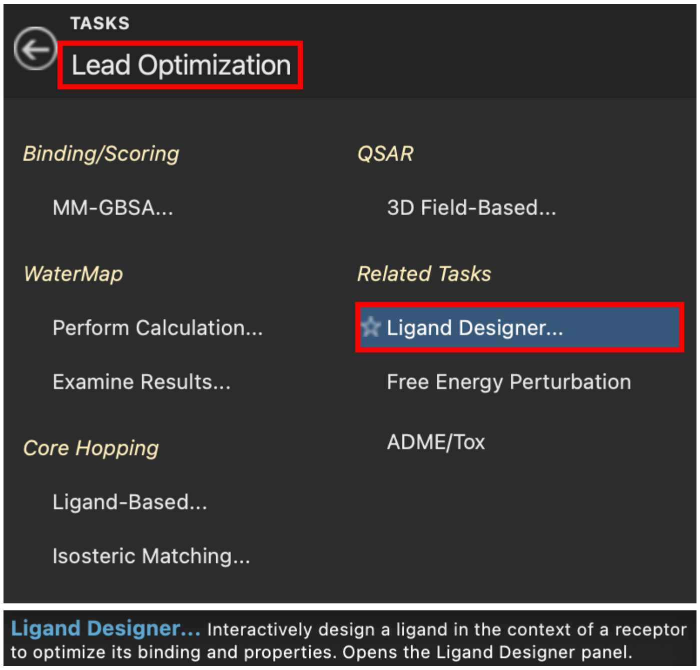
without a ligand (pink) and with a ligand (white and green).	

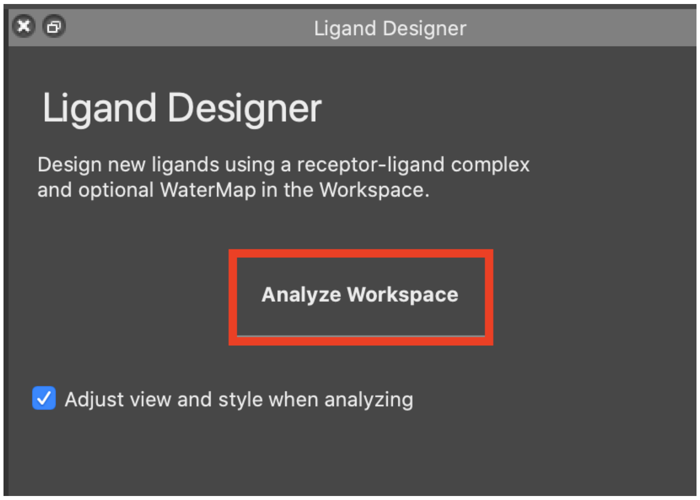
---

7. Introducing the Ligand Designer

After superimposing two molecules, their structures can be further explored by making small adjustments to the rotatable groups of one ligand, or modifying its chemical structure. In this section, we will evaluate chemical modifications to a protein-bound ligand.

7.1 Modify a bound ligand with Ligand Designer

 **Ligand Designer...** Interactively design a ligand in the context of a receptor to optimize its binding and properties. Opens the Ligand Designer panel. *Figure 7-1. Ligand Designer in Lead Optimization.*	1. <u>Include</u> only **LigandMCMM** in the <u>Workspace</u>. 2. Go to **Tasks > Browse > Lead Optimization > Ligand Designer** ○ The Ligand Designer panel opens.



Ligand Designer

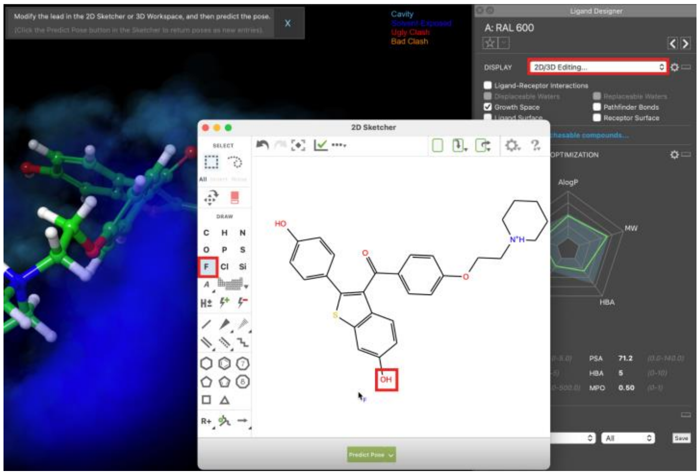
Design new ligands using a receptor-ligand complex and optional WaterMap in the Workspace.

Figure 7-2. Analyze Workspace in Ligand Desinger panel.

5. Click **Analyze Workspace.**
    - New entries are added to the Entry List in the Ligand Designer group.
    - The Workspace now has just a single ligand and a cloud surrounding it to represent the growth space.

Note: You can uncheck **Adjust view and style when analyzing** to retain the view that you currently have when working in the Ligand Designer.

The darker blue in the growth space is the solvent exposed region.



Modify the lead in the 2D Sketcher or 3D Workspace, and then predict the pose.

(Click the Predict Pose button in the Sketcher to return poses as new entries).

Figure 7-3. 2D/3D Editing in Ligand Designer.

6. Under **Display**, select **2D/3D Editing.**

Note: You can make edits either directly in the sketcher or in the Workspace using the 3D Builder. Any change you make in the Workspace with the 3D builder will be mirrored in the 2D sketcher.

7. Change the lower hydroxyl group to a fluorine.

Optional: View Ligand-Receptor Interactions.

The properties under the sketcher are synced with the sketcher and will update automatically before your compound is docked.

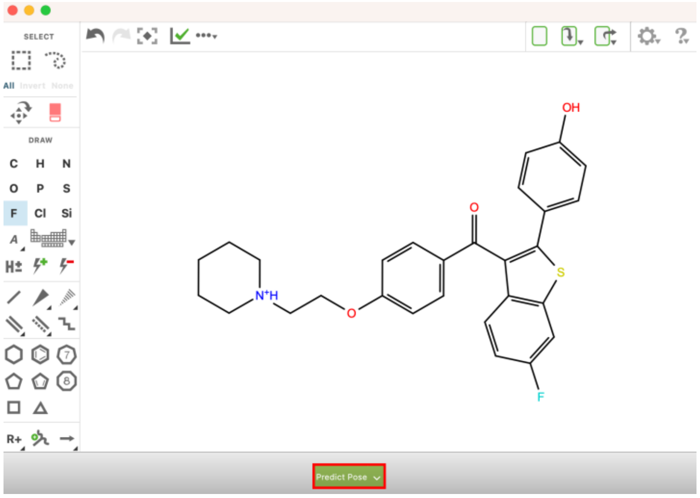


Figure 7-4. Predict pose of the edited ligand.

8. Click **Predict Pose**.
    - A new entry is added to the Entry List.

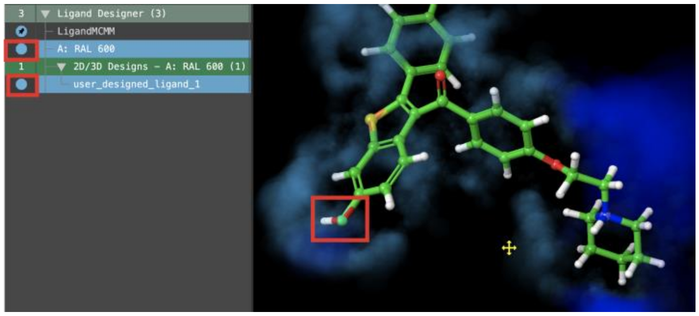


Figure 7-5. View ligand pose in the Workspace.

9. Include **user_designed_ligand_1** and **RAL 600** in the Workspace.

Note: The modification is not predicted to affect the pose of the ligand in the binding pocket.

---

8. Conclusion and References

In this tutorial, we have used Maestro to perform many techniques used by chemists for idea generation and lead optimization. Beginning with copying and creating molecules, we drew molecules in 2D and 3D, then explored the conformational space of a ligand. Several different methods of structure alignment for small molecules, ligands bound to proteins, and proteins were explored. Finally, ligands were further analyzed for lead optimization by redocking a modified ligand using Ligand Designer.

For further learning:

- Introduction to Structure Preparation and Visualization
- Forming Protein-Ligand Interactions with the Ligand Designer
- WaterMap Guided Optimization with the Ligand Designer
- Maestro Training Portal

- Introduction to Molecular Modeling in Drug Discovery Online Course

For further reading:

- Starting Maestro
- Protein Structure Alignment Panel
- Tips for Using Maestro
- Ligand Designer Panel

9. 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

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