Investigating Drug-Target Interactions of Fluoroquinolone Antibiotics – Molecular Modeling Guidelines

Project Goal: To prepare and examine a virtual protein-ligand docking model for fluoroquinolone/quinolone antibiotics and their two known biological targets – bacterial DNA gyrase and topoisomerase IV.

Objectives

This will be accomplished by using two drug-bound protein structures – (1) ***moxifloxacin*** bound to ***DNA gyrase*** (PDB 5cdq); and (2) moxifloxacin bound to ***topoisomerase IV*** (PDB 2xkk). You will need to apply the workflow you learned in the Protein-Ligand Docking Lesson[1] and complete the following objectives in Maestro:

1. Import the two PDB files– 5cdq and 2xkk – and prepare the protein structures using the **Protein Preparation Workflow**.

2. Prepare the co-crystallized cognate ligand, moxifloxacin, using **LigPrep**.

3. Validate each protein-ligand docking model by generating a **Receptor Grid** then docking the prepared moxifloxacin to the defined grid using **Ligand Docking** in *Glide*.

4. Identify the non-covalent interactions taking place between moxifloxacin and the binding site of each target using the **Ligand Interaction Diagram**.

5. Using your validated DNA gyrase and topoisomerase IV docking models, dock the structure of the quinolone analog you are preparing in the laboratory (**6e**, **6f**, or **6h**). You will do this by creating a new structure in the **2D Sketcher**, preparing it with **LigPrep**, then docking the structure using **Ligand Docking**.

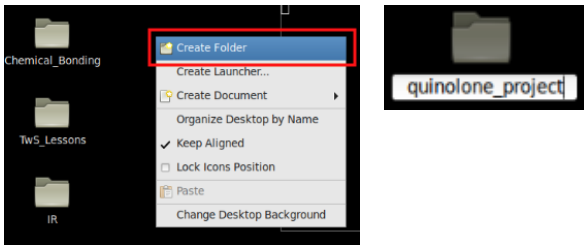
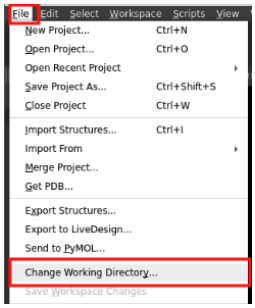
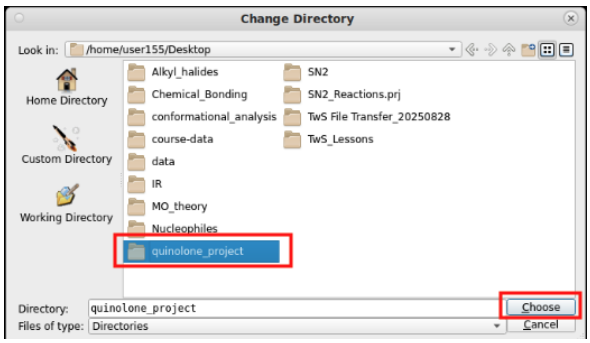
Graded Assessment

As a group, you will present your results and analysis at the final lab meeting presentation (see Lab Syllabus for exact date). In your presentation you should be prepared to present and discuss the results from your completed objectives 1-5 above. You will be provided with a slide deck template that includes an outline of your presentation and specific data and discussions you should include.

[1] Technical instructions for this lesson has been adapted from the Protein-Ligand Docking Lesson, which can be found at https://www.schrodinger.com/teaching-with-schrodinger/

Instructions

Before you start working on our objectives, you will need to **create a new folder** in the working directory and **save a new project file**.

Schrödinger Virtual Workstation Login: https://teaching2025-east.gcp.tsg.schrodinger.com/workstation/#/	1. Log into the virtual workstation and wait for your Desktop to be set up.
 *Figure 0-1. **Create** a new **folder** on your Desktop*	2. On your Desktop, right-click and select **Create Folder**. Name this folder 'quinolone_project.'
 *Figure 0-2. Open a **Maestro** session*	3. Double click on the **Maestro** icon to open a session.
  *Figure 0-3. **Change** the **Working Directory***	4. Go to **File → Change Working Directory** 5. Find the 'quinolone_project' folder you created on your Desktop and click **Choose**. *You will need to repeat steps 4 & 5 EACH TIME you log in to work on this assignment in Maestro.

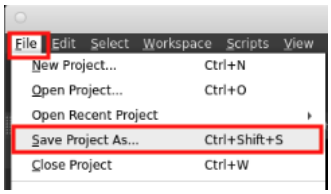
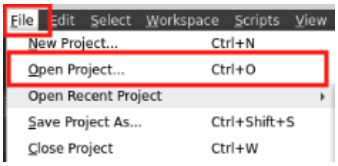
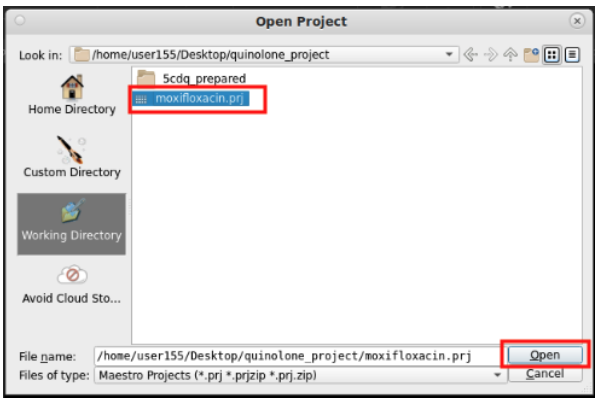


*Figure 0-4. **Save** a new **project***

6. Go to **File → Save Project As**
7. Name this project '`moxifloxacin`' and click **Save**.

(a)





(b)

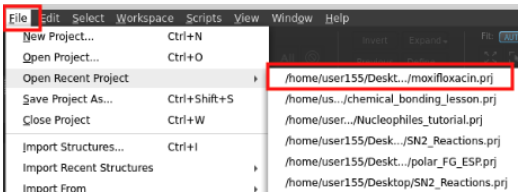


*Figure 0-5. (a) **Open** a saved **project**; (b) **Open** a saved **recent project***

To open this project in a new session:

(a) Go to **File → Open Project →** select '`moxifloxacin.prj`' and click **Open**.

(b) Alternatively, you can go to **File → Open Recent Project →** select '`moxifloxacin.prj`' in the list

*You will need to open this project 'moxifloxacin.prj' EACH TIME you log in to work on this assignment in addition to changing the Working Directory. **These two steps ensure that your progress is saved.***

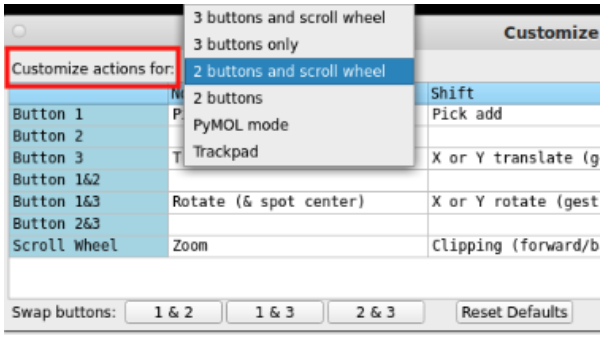


Figure 0-6. Select the best mouse option for your set up.

8. Check your **Mouse Actions** and select the option based on your set- up.
   a. Mac: **Workspace → Customize Mouse Actions → Customize actions for:**
   b. PC: **Edit → Customize Mouse Actions → Customize actions for:**

Note: An external mouse with a 2- or 3-button + scroll wheel is recommended.

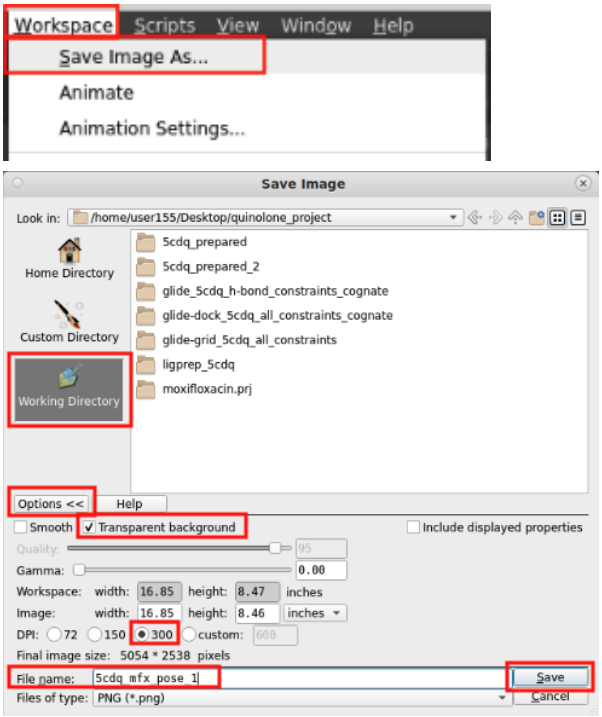


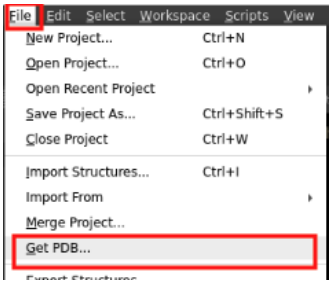
Figure 0-7. Saving an image from the Workspace

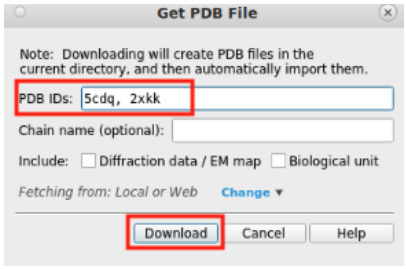
Throughout this project, you will need to save images of the 3D structures in your Workspace to use in your presentation. To save an image:

- Go to **Workspace → Save Image As**
- Click **Options>>** if it is not already shown
- Check **Transparent background**
- Change the **DPI** to **300**
- Change the **File name** (no spaces, please)
- Click **Save**

Your image will be saved as a .png file in your Working Directory. To access it outside of the Virtual Cluster Desktop, open a Chrome Browser, log into your Google Drive and upload a copy there.

Objective 1: *Import the two PDB files– 5cdq and 2xkk – and prepare the protein structures using the* ***Protein Preparation Workflow****.*





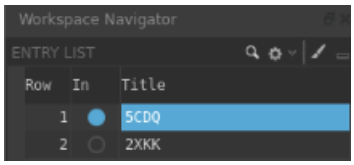


Figure 1-1. Import PDB files

1. Go to **File → Get PDB**
2. In the **PDB IDs** field, type '5cdq, 2xkk' then click on **Download**.
   - The two PDB files are loaded into the Workspace, and you should see the two files in the Entry List.
3. Make sure that 5cdq is both Included and Selected as shown. We will repeat this preparation for 2xkk later.

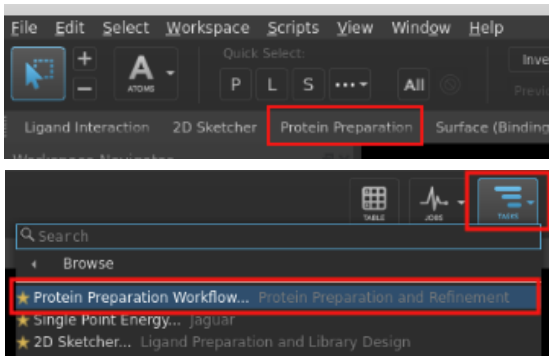


Figure 1-2. Open ***Protein Preparation Workflow*** *via the* ***Favorites*** *tool bar or the* ***Task*** *finder*

4. In the **Favorites** toolbar at the top, click **Protein Preparation** to open the **Protein Preparation Workflow** panel (alternatively, go to **Task → Protein Preparation Workflow**)

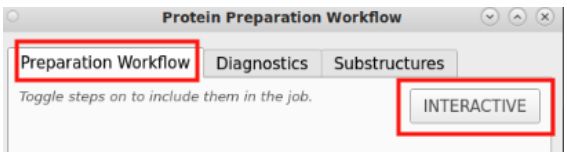


Figure 1-3. Preparation in NON-interactive mode

5. In the **Preparation Workflow** tab, confirm that the **INTERACTIVE** mode is OFF (should be gray).

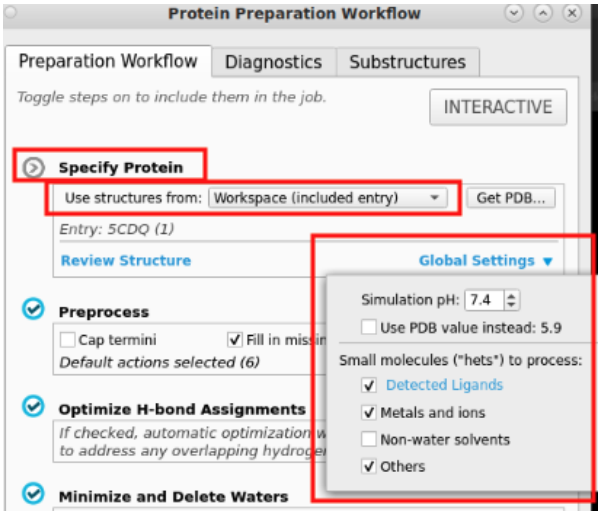
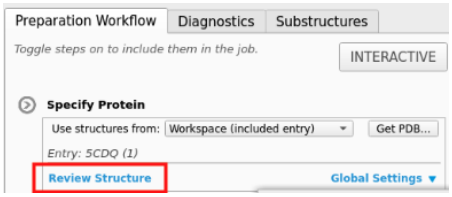
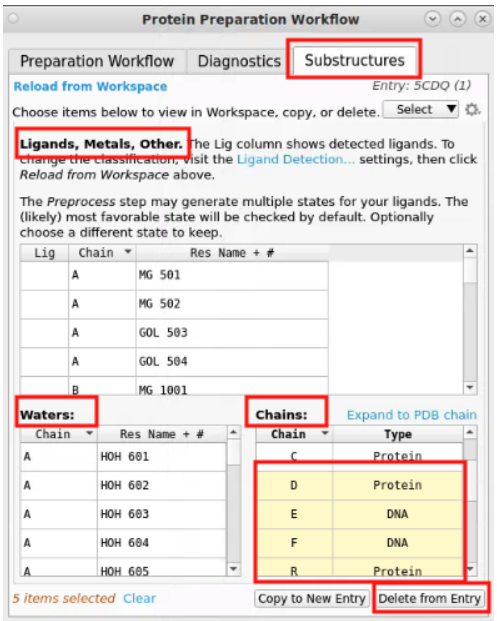


*Figure 1-4. **Specify Protein** settings*

6. In the **Specify Protein** section, next to Use structures from: choose **Workspace (included entry)**
7. Click on **Global Settings** and confirm the following: Simulation pH 7.4; check Detected Ligands, Metals and ions, Others; leave non-water solvents unchecked.





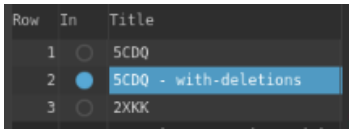


Figure 1-5. 5cdq with chain deletions

8. Select **Review Structure**
   - The **Substructure** tab opens and shows the 3 substructure categories– **Ligands/Metals/Other**; **Waters**; and **Chains**.
9. Under **Chains**, control-click the following rows:
   - **Protein** chains D, R, S, T, U
   - All **DNA** chains E, F, V, and W.

   You will need to scroll to view all the chains listed here.
10. Click **Delete from Entry**.
    - You should now only have **Protein** chains **A**, **B**, and **C** remaining in this list
    - A new entry appears in the Entry List ('5cdq – with-deletions')

    This step removes all protein and DNA fragments from the crystal structure that are not needed for our docking model.

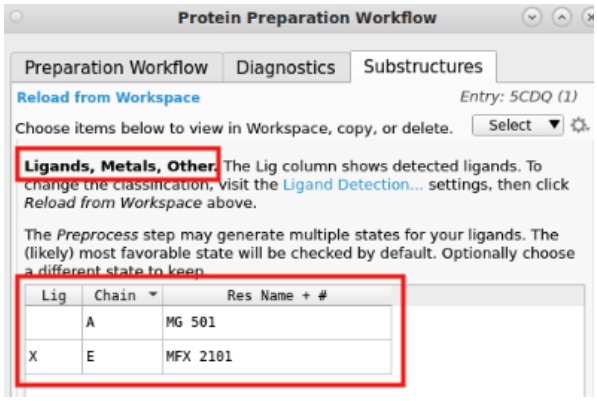


Figure 1-6. 5cdq with extraneous MFX, MG, and GOL deleted

11. Under **Ligands, Metals, Other**, control-click all EXCEPT for the following rows:
    - Chain A MG501
    - Chain E MFX_2101

    This step will remove all extraneous moxifloxacin ligands, magnesium ions, and glycerol molecules that not needed for our docking model.

12. Click **Delete from Entry**.
    - You should now only have moxifloxacin **MFX_2101 (chain E)** and magnesium **MG501 (chain A)** remaining in this list

13. We will leave the **Waters** table for now as we can remove them later.

Note: The decision to delete certain substructures requires good understanding of what elements may or may not affect your ligand docking model, as well as the specific research question you are trying to address. This is something we delve deeper into in Medicinal Chemistry (CH335). For now, all deletions have been specified for you.

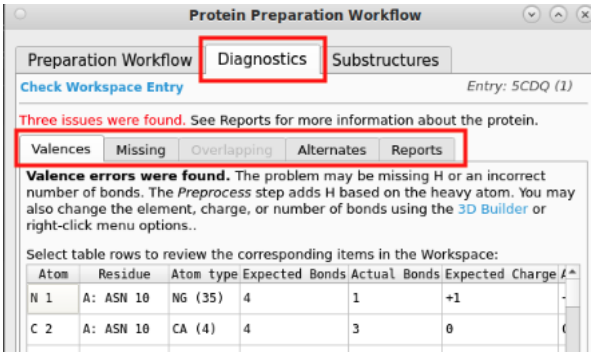


Figure 1-7. Issues identified prior to protein preparation

14. Select the **Diagnostics** tab to review issues identified with the protein structure prior to preparation.
    - Note that there are valence errors, missing sidechains/loops, and alternate positions for side chains. The Reports tab will indicate other issues that may need to be resolved before docking.
    - No action is required at this point.

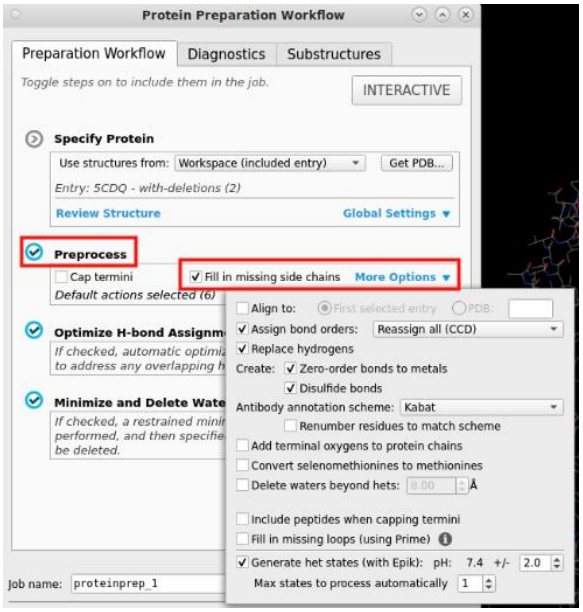


*Figure 1-8. **Preprocess** settings*

15. Return to the **Preparation Workflow** tab.
16. Confirm that **Preprocess** is toggled on and that **Fill in missing side chains** is checked.
17. Click on **More Options** and verify that the settings match those that are shown in the Figure. Note that the pH should match that which you specified in step 7 (**Specify Protein**).

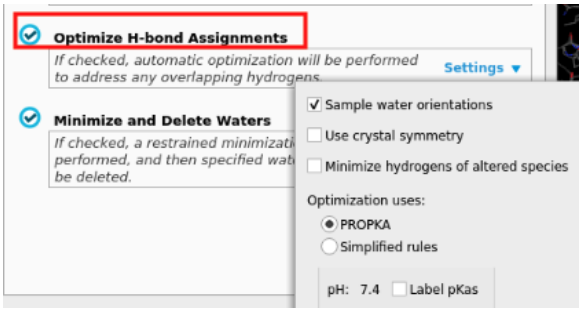


*Figure 1-9. **Optimize Hydrogen-Bond Assignments** settings*

18. Confirm that **Optimize H-Bond Assignments** is toggled on.
19. Click on **Settings** and verify that they match those shown in the Figure. Again, the pH for optimization should also match those specified in the **Preprocess** settings (Step 17).

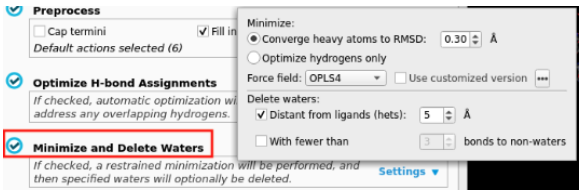


*Figure 1-10. **Minimize and Delete Waters** settings*

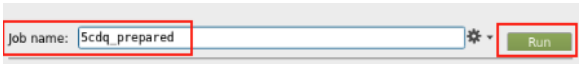
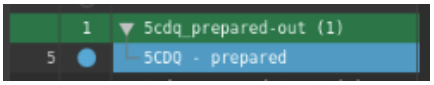


*Figure 1-11. Run **Protein Preparation** job*

20. Confirm that **Minimize and Delete Waters** is toggled on.
21. Click on **Settings** and verify that they match those shown in the Figure. Distant waters will be removed in this step.

22. Change the **Job name** to `5cdq_prepared`
23. Click **Run**
    - The job will take a few minutes to run. Once it is complete, a new group will be added to the Entry List.



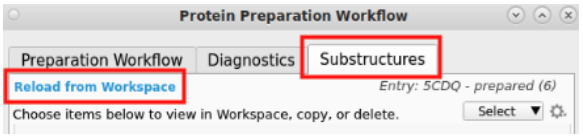


*Figure 1-12. Review **Substructures** after protein preparation*

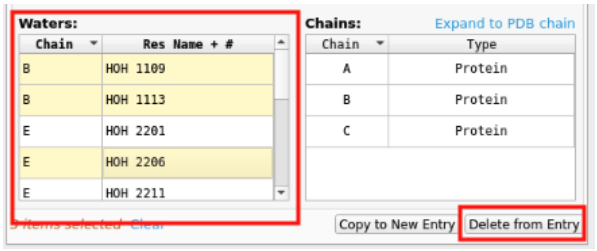


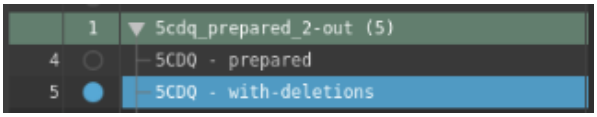
Figure 1-13. Delete any unnecessary remaining waters after protein preparation

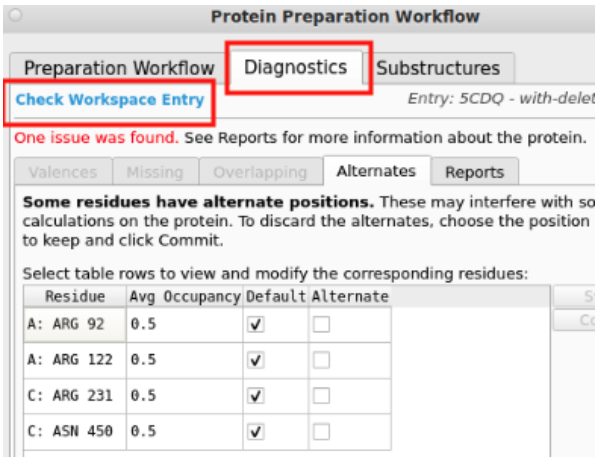
*Figure 1-14. The **interactions** toggle*

24. In the Entry List, click '5cdq_prepared-out (1)' and make sure that the '5cdq – prepared' entry is both included and selected.
25. Return to the **Protein Preparation Workflow** panel and click the **Substructures** tab.
26. On the top left corner, click **Reload from Workspace**.

27. In the **Waters** table, shift-click to select the following waters:
    - Chain B: HOH1109, HOH1113
    - Chain E: HOH2206, 2216
    - Chain F: HOH2211, 2216, 2217
28. Click **Delete from Entry**.
29. You should only have the following waters remaining in your table:
    - Chain E: HOH2201, 2211, 2213
    - Chain F: HOH2214

Note: Turn on the interaction toggle in the lower right-hand corner to view the interactions in the binding site.



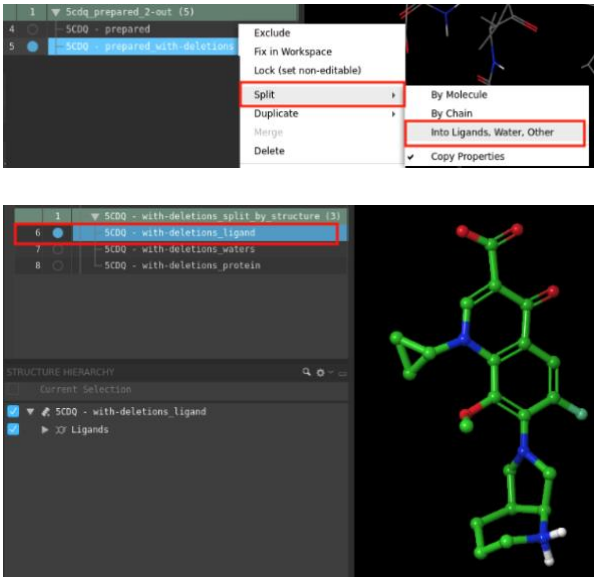
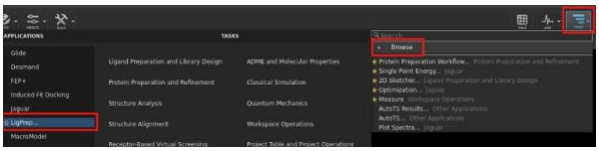


30. In the '5cdq_prepared-out' group, select and include '5cdq - with-deletions' entry.
31. Return to the **Protein Preparation Workflow** panel and click the **Diagnostics** tab to see if there are remaining issues after the preparation. Click on **Check Workspace Entry** if the panel is not automatically updated.

Note: For now, you may ignore the issues in the Alternates tab. These side chains are far from the binding site and should not interfere with our docking experiment.

*Figure 1-15. Review remaining issues in the **Diagnostics** tab*	32. **Exit** the **Protein Preparation Workflow**

Objective 2: *<u>Prepare</u> the co-crystallized cognate ligand, moxifloxacin, using **LigPrep***

 *Figure 2-1. **Split** the prepared structure to prepare the ligand*	1. In the Entry List, right-click on '5cdq – with-deletions' in the '5cdq_prepared-out' group 2. Choose **Split → Into Ligands, Water, Other** ○ A new group with 3 new entries is added to the <u>Entry List</u> 3. <u>Include</u> '5cdq – with-deletions_ligand' ○ Only the ligand (moxifloxacin) should be displayed in the <u>Workspace</u>
 *Figure 2-2. Open **LigPrep** using the **Task** toolbar*	4. Go to **Tasks → Browse → LigPrep** to open the **LigPrep** panel.

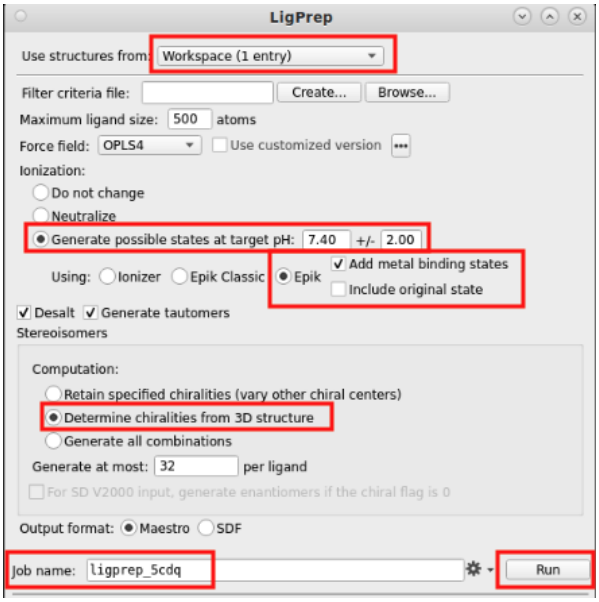
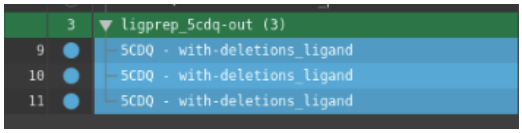


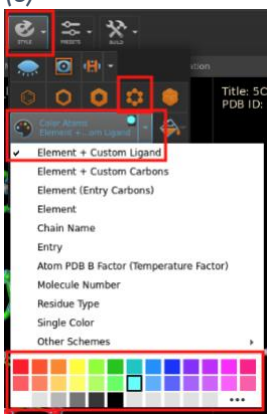
*Figure 2-3. **LigPrep** settings*

(a)



(b)

(c)



(d)

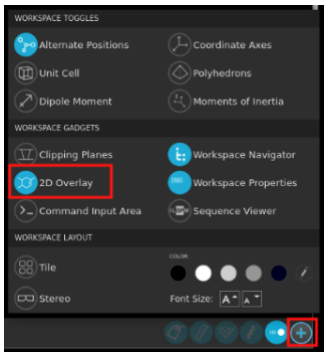


*Figure 2-4. (a) **LigPrep** output; (b) Using the **Tile** function; (c) change 3D renderings using the **Style** toolbox; (d) **2D** structure **overlay***

5. For Use structures from, choose **Workspace (1 included entry)**
6. Under Ionization, choose **Generate possible states at target pH** using **Epik** and **Add metal binding sites**
7. Under Stereoisomers, choose **Determine chiralities from 3D structure**.
8. Change the Job name to `ligprep_5cdq` and click **Run**.
   - The job will only take a minute to run.

9. A new group will be added to the Entry List.
   - The number of ligands in this new group is shown in parentheses.
   - You can view these prepared structures simultaneously by including the ones you would like to view and using the **Tile** functionality from the **Workspace Configuration Toolbar**.
   - Use the **Style** toolbox to change each ligand output to a ball-and-stick model and change its color.
   - It is also useful to overlay the 2D structure of each ligand in the Workspace

Note*: Multiple ligands generated from **LigPrep indicates that there is more than one reasonable 3D structure for the ligand.

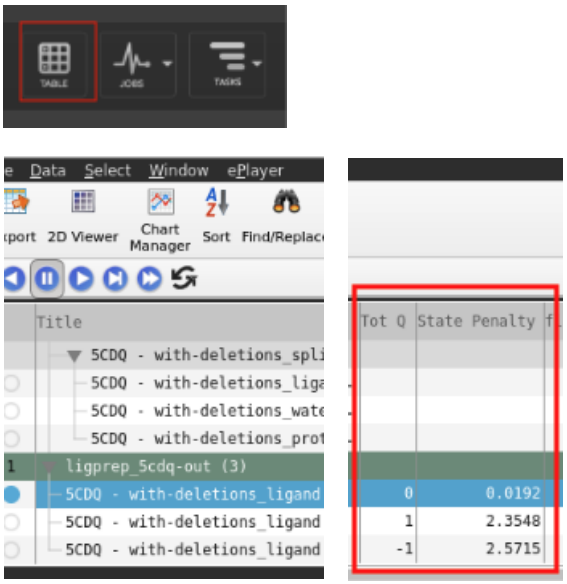


Figure 2-5. ***Project Table*** *can be used to compare calculated properties between ligand output structures*

10. Type Ctrl+T (or Cmd+T for PC) to open the **Project Table**. Alternatively, click on the **Table** button on the upper right corner.
    - Various calculated properties can be viewed, added/removed from this table.

11. Review the following:
    - **Total Q** represents the net charge of the structure. Notice that each structure has different charge states. Use the 2D structure overlay to verify that this makes sense. (You will need to discuss this in your presentation)
    - **State Penalty** is associated with the relative free energy of the output structure and smaller values correlate to better structures. Note that the three ligands are ranked accordingly.
12. **Close** the **Project Table**.
    - No other actions are required at this point.

Objective 3: *<u>Validate</u> each protein-ligand docking model by generating a* ***Receptor Grid*** *then docking the prepared moxifloxacin to the defined grid using* ***Ligand Docking*** *in* ***Glide****.*

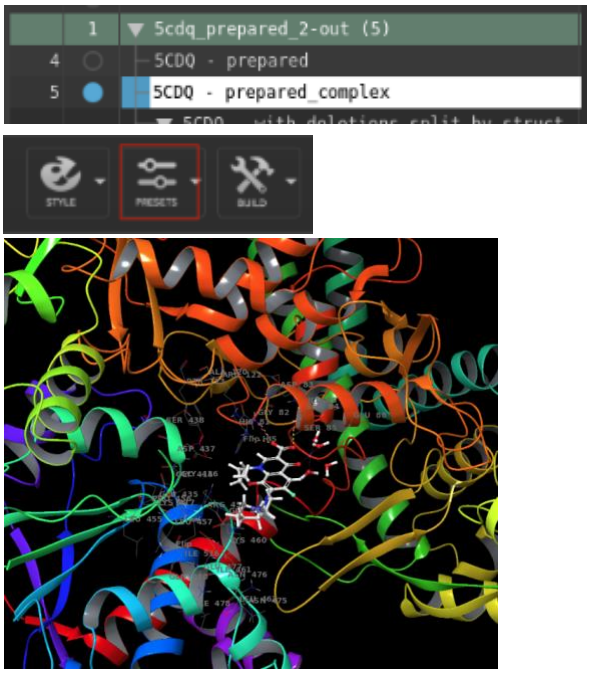


Figure 3-1. Change the name of the prepared protein complex and apply ***Preset*** *renderings*

1. Click on the '5cdq –with-deletions' entry and rename it '5cdq – prepared_complex' (This is the prepared protein complex with ligand intact and waters deleted)
2. <u>Include</u> the '5cdq – prepared_complex' entry.
3. Double-click **Presets**.
   - The 5cdq prepared complex should be rendered based on a Custom Preset. You will see ribbon representations for the protein backbone.

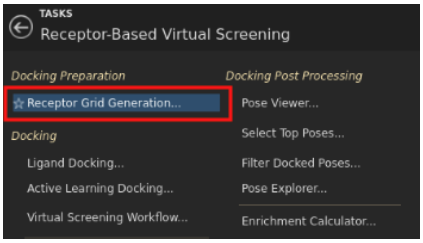
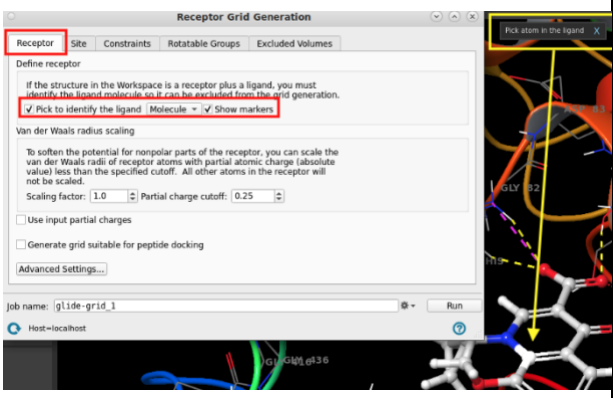


Figure 3-2. Open the ***Receptor Grid Generation*** *panel*

4. Go to **Tasks → Browse → Receptor-Based Virtual Screening → Receptor Grid Generation**



5. In the **Receptor Grid Generation** panel under the **Receptor** tab, check the box next to **Pick to identify the ligand (Molecule)** and **Show markers**.
6. The **Pick atom in the ligand** banner should appear. Carefully hover over any atom in your moxifloxacin ligand and click once. This should place green boxes around all atoms in the ligand and a purple box

Figure 3-3. ***Receptor Grid*** *settings and picking an atom in the ligand to identify*

around the binding site of the ligand.

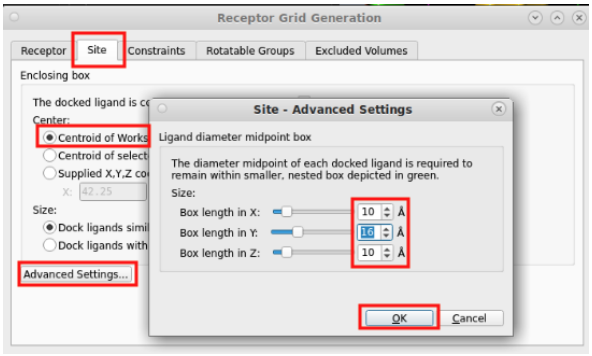
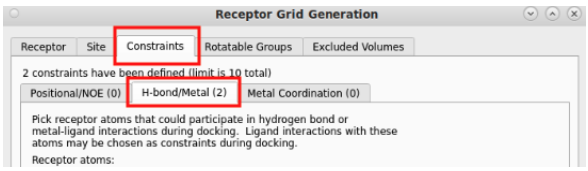


Figure 3-4. ***Receptor Grid Site*** *settings*

7. Go to the **Site** tab and select **Centroid of Workspace ligand** and click on **Advanced Settings**. This should place a smaller green box inside of the purple box from step 6. Adjust the **Box length** for **X, Y, Z** to **10, 16,** and **10** Å, respectively as shown. Notice that the green box dimensions adjust to enclose the ligand. Click **OK**.



8. Go to the **Constraints** tab and click the **H-bond/Metal** tab.

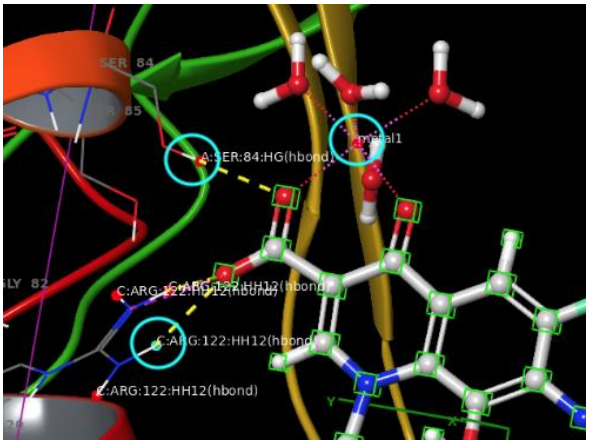


Figure 3-5. Setting ***H-bond/Metal Constraints*** *in the* ***Receptor Grid****.*

9. The **Pick an atom in the receptor to be treated as a constraint atom** banner appears. Carefully hover over and click on the following atoms as shown in *Figure 3-5*:
   - Chain A Ser84 OH hydrogen
   - Chain A Mg501 ion
   - Chain C Arg122 NH hydrogen

Note: If you have difficulty locating these side chains, you can use the Structure Hierarchy to find them in the Workspace.

10. The three constraints should be displayed in the receptor atoms table. Change the **job name** to `glide-grid_5cdq_all_constraints` and click **Run**.
11. When the grid generation is complete a banner will appear that says, 'Your job glide-grid_all_

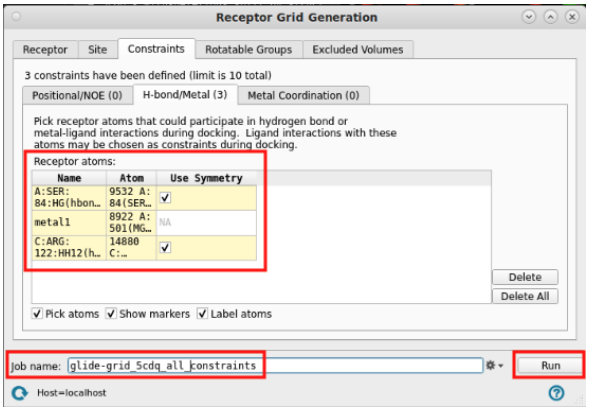
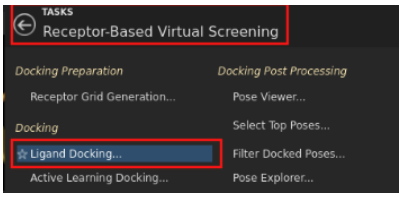
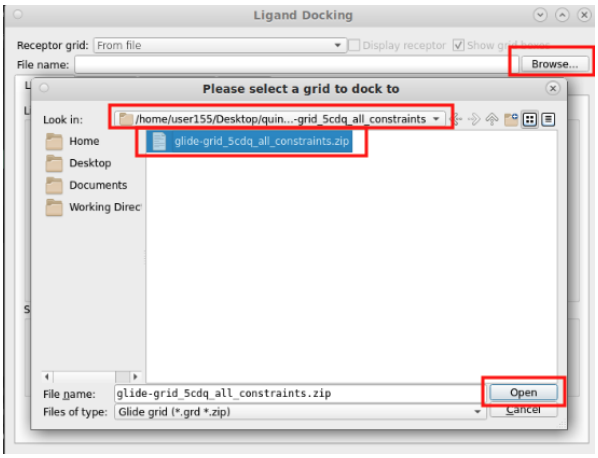


*Figure 3-6. Run a **glide-grid** job.*

constraints has completed.' This is automatically saved in your Working Directory.





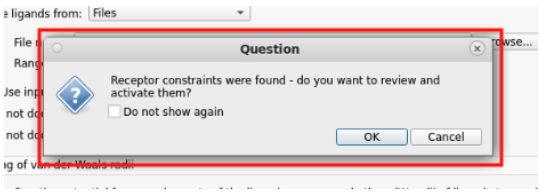


*Figure 3-7. **Ligand Docking** settings – selecting the grid*

12. Go to **Tasks → Browse → Receptor-Based Virtual Screening → Ligand Docking**

13. In the **Ligand Docking** panel, next to next to **File name** for the **Receptor Grid**, click **Browse** and double-click on the 'glide-grid-5cdq_all_constraints folder' and choose `glide-grid_5cdq_all_constraints.zip` and click **Open**.

14. You may see a pop-up that asks you to review and activate constraints. Click **OK** to review the Constraints.

15. In the **Constraints** tab select the **Receptor** tab to see the constraint options.
16. Under **Use**, check only the two H-bond constraints we set earlier during protein preparation.

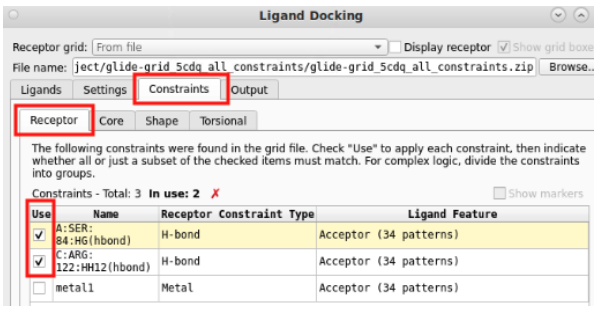


Figure 3-8. ***Ligand Docking*** *settings – choosing* ***Constraints***

Note: We will attempt our first docking with just the hydrogen bond constraints – later we will have the option to run additional docking jobs different number of constraints or none at all.

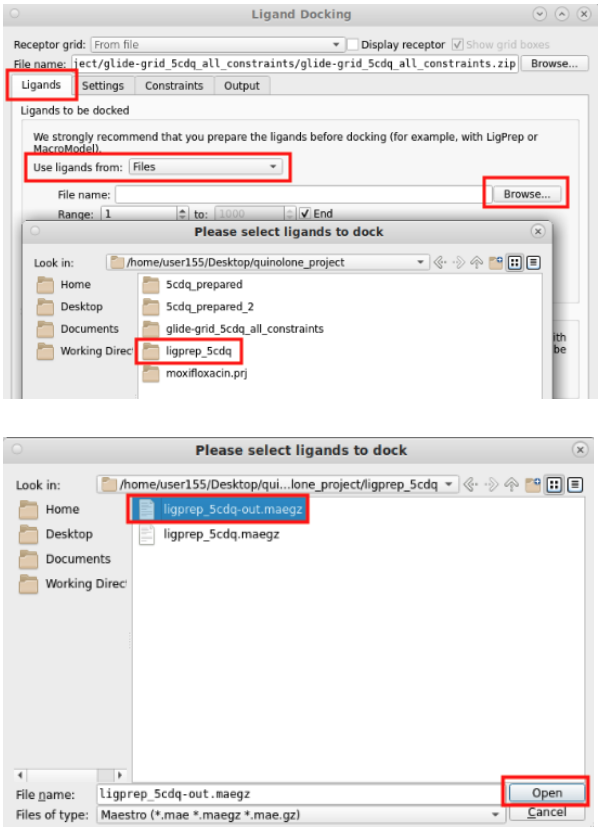


Figure 3-9. ***Ligand Docking*** *settings – choosing the* ***Ligand***

17. Go to the **Ligands** tab and for **Use ligands from**, choose **Files**

18. Next to **File name**, click **Browse** and go through the following folders 'quinolone_project' → 'ligprep_5cdq' to find `ligprep_5cdq-out.maegz` and click **Open**. These include the three **LigPrep** output structures of the cognate ligand, moxifloxacin, generated previously.

Figure 3-10. Run ***Ligand Docking*** *in* ***Glide***

19. Change the **Job name** to `glide_5cdq_all_constraints_cognate` and click **Run**.
    - This job will take a few minutes, and when it is complete a banner will show that files have been incorporated into the Entry List.

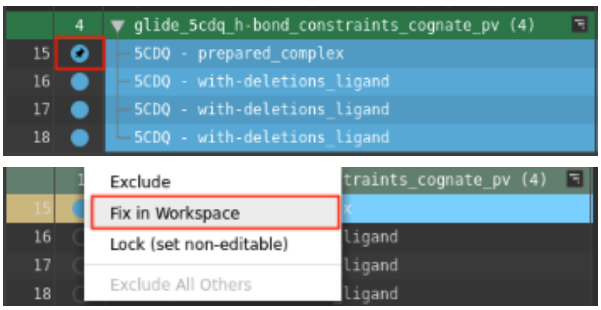


Figure 3-11. Include and Fix the 5cdq – prepared_complex

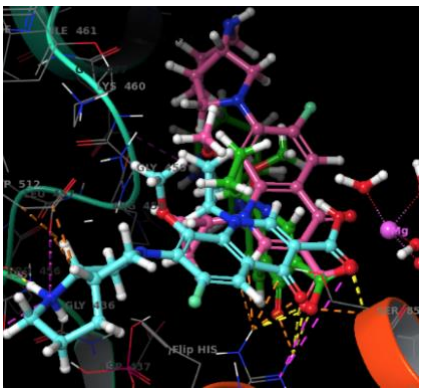


*Figure 3-12. Include and apply **Presets** to view the docked ligands in different colors*

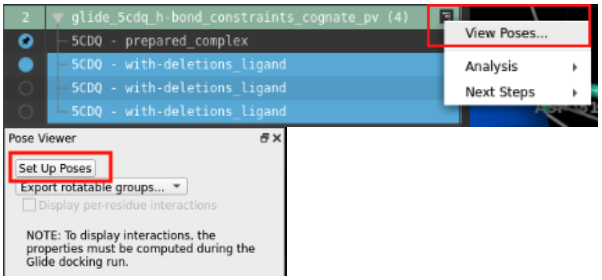


*Figure 3-13. Use the **Pose Viewer** to view each docked pose individually.*

20. In the newly incorporated 'glide_5cdq_h-bond_constraints_cognate_pv (4)' group, include and fix the '5cdq – prepared_complex' entry if it is not already. (Include the 5cdq – prepared_complex' entry and right-click to select **Fix in Workspace**.)

21. With all entries included, double-click on **Preset**. Each docked ligand should now be a different color for easy comparison.

22. You can go through each docked ligand using the **Pose Viewer** panel on the right. Use the right and left arrow keys to go through each ligand pose that docked.

23. If you do not see the **Pose Viewer** panel or wish to open it again later, right-click on the **Workflow Action Menu** icon to the right of the group entry and select **View Poses**. You can click **Set Up Poses** to view through the docking results of the selected group.

Note: You should be able to see whether the docked ligand poses maintain hydrogen boding with our constraints – SER84 and ARG122.

Which docked ligand pose is the closest in alignment with the moxifloxacin bound to the crystal structure? [You can try including the '5cdq – with-deletions_ligand' entry you generated when you split the complex into Ligands, Water, and Other prior to running LigPrep.

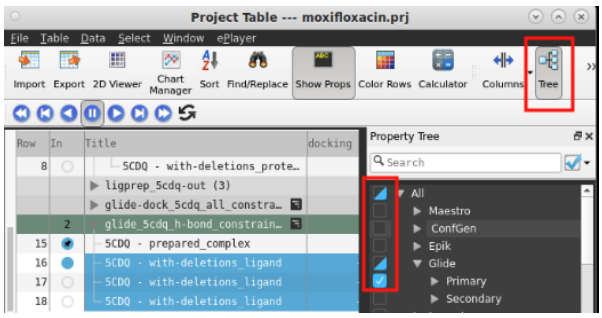


*Figure 3-14. View calculated properties from **Glide** docking using the **Property Tree** in the **Project Table**.*

24. Open the **Project Table** if it is not already.
25. Click on the **Property Tree** icon
26. In the **Property Tree** section click the **All** box *twice to deselect all* properties listed.
27. Click on the **Glide** box then the **Primary** box.
    - The **Project Table** should now only show **Glide Primary** properties (docking score, glide score, glide emodel).

Note: In general, the more negative scores indicate better docking. If you are interested in learning more about the differences between these docking scoring types, see Schrödinger's Knowledge Base Article 1027.

28. Double-click the **In** circle next to '5cdq – prepared_complex' in the Entry List to *remove* the **Fix** in the Workspace.

Objective 4: *Identify the non-covalent interactions taking place between moxifloxacin and the binding site of each target using the* ***Ligand Interaction Diagram****.*

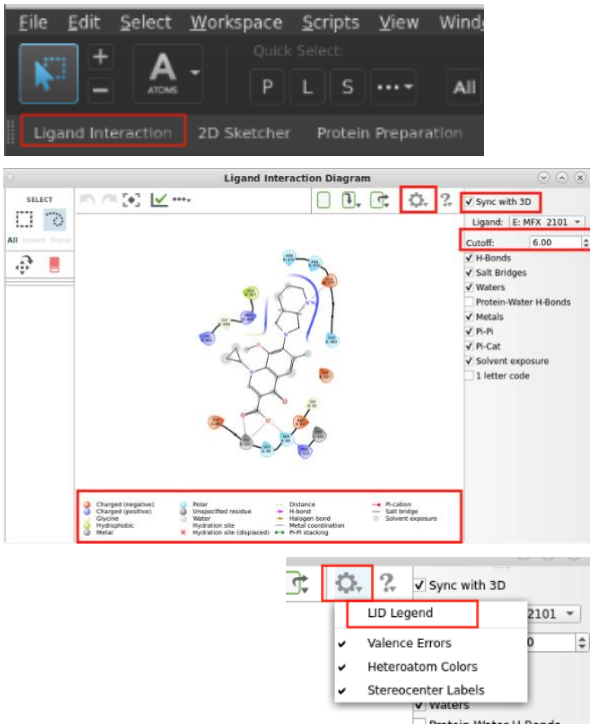


Figure 4-1. Generate a ***Ligand Interaction Diagram*** *that shows key non-covalent interactions between the ligand and the binding site.*

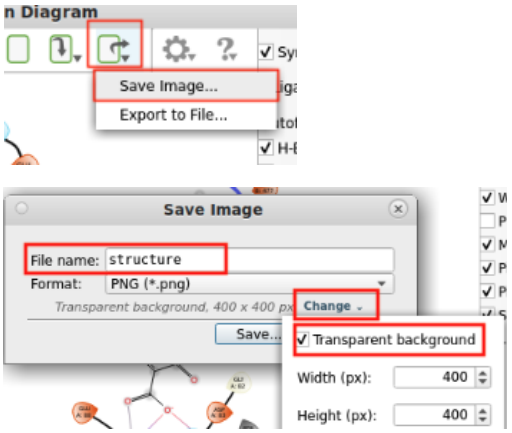


Figure 4-2. Saving an image of the ***Ligand Interaction Diagram***

1. Click the **Ligand Interaction** button in the **Favorites** toolbar.
2. In the **Ligand Interaction Diagram** panel, make sure that **Sync with 3D** is checked. When you rotate the ligand in the Workspace, the ligand orientation should automatically update in the **2D Workspace**.

Note: You may need to check/uncheck the **Sync with 3D** box if auto-update is slow.

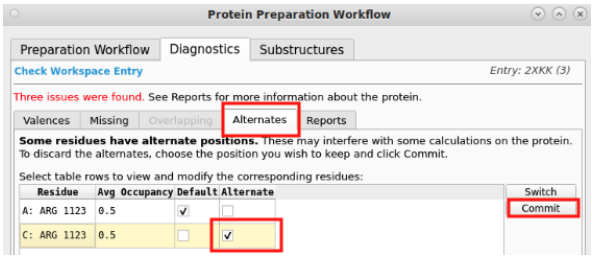
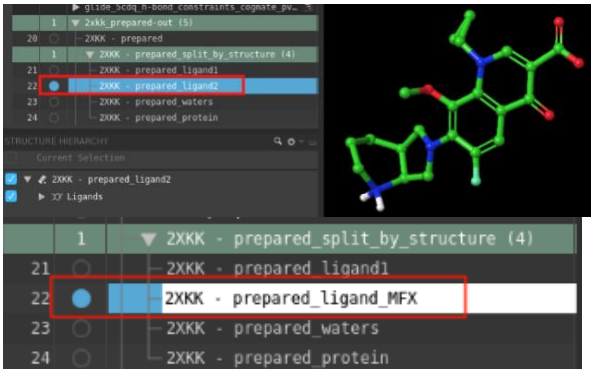
3. Change the interaction **Cutoff** to **6.00**. This will increase the sidechain residue cutoff distance.
4. Click the **cog** icon and check **LID Legend** to view the legend and learn what specific colors and symbols mean in the **Ligand Interaction Diagram**.

Note: You will need to rotate the structure as well as zoom in/out to obtain the best renderings of the **Ligand Interaction Diagram** and to properly identify key interactions taking place at the binding site. You will also need to save these images for your presentation.

5. To save an image in the LID, click the **Export** icon → choose **Save Image**
6. Next to **File name**, use an informative name for the image (no spaces) and if you would like, **Change** the background to **Transparent background**.

Once you have completed *Objectives 1-4* for PDB 5cdq (moxifloxacin bound to DNA gyrase), you will need to repeat the workflow for **PDB 2xkk** (moxifloxacin bound to

topoisomerase IV). Return to the **Instructions** for these steps and make the following adjustments specific for PDB 2xkk.

Protein Preparation Workflow • Preparation Workflow tab → Specify Protein/Review Structure	
○ Ligands, Metal, Other	• Keep: ○ Chain A MG1504 ○ Chain F MFX1100 • Delete all other rows
○ Chains	• Keep: ○ Chain A ○ Chain C • Delete all DNA Chains E, F
• Diagnostics tab → Alternates tab  *Figure 1-16. 2xkk **Alternates** settings*	• Change the **C:ARG1123** residue to the **Alternate** position and click **Commit** *Note*: If you have the **Interactions** toggle on, you will notice that the alternate position for ARG1123 in Chain C makes better contact with the ligand when the alternate position is selected.
• Save protein preparation job as:	• `2xkk_prepared`
LigPrep • Split → Into Ligands, Water, Other  *Figure 2-6. Identify and rename MFX ligand from '2xkk – prepared_split_by_structure'*	• You will notice that there are two ligand entries here. **LigPrep** should be done with the moxifloxacin ligand, '2xkk – prepared_ligand2'. The other identified ligand, '2xkk – prepared_ligand1' is a co-crystallized peptide that needs to be deleted from the Workspace before proceeding with grid generation. • Click on the '2xkk - prepared_ligand2' entry and rename it to '**2xkk – prepared_ligand_MFX**' for clarity

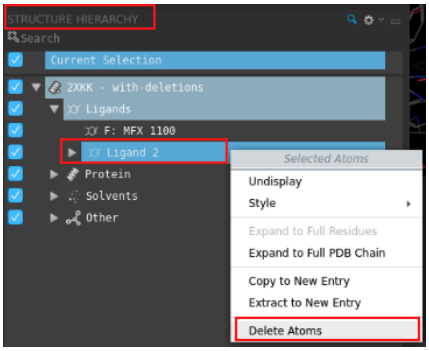


Figure 2-7. Delete Ligand 2 from Workspace

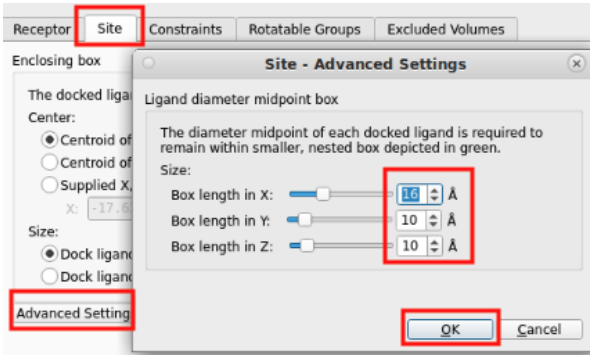
- To delete the other ligand: Include and select the '2xkk – with-deletions' entry and Use the Structure Hierarchy to find 'Ligand 2,' right-click on the entry and select **Delete Atoms**. Confirm Deletion when prompted.

Save LigPrep job as:

- `Ligprep_2xkk`

Receptor Grid Generation

- Site → Advanced Settings



- Change box dimensions to fit the MFX ligand X=16Å, Y=Z=10Å

- Constraints

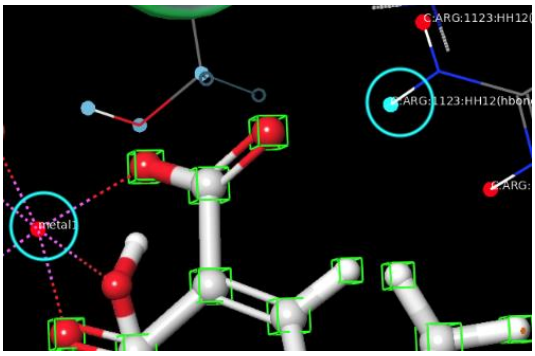


*Figure 3-15. **Receptor Grid** settings for 2xkk*

- When the **Pick an atom in the receptor to be treated as a constraint atom** banner appears, carefully hover over and click on the following atoms as shown:
  - Chain A Mg1504 ion
  - Chain C Arg1123 NH hydrogen

Save Glide-grid job as:

- `glide-grid_2xkk_all_constraints`

Ligand Docking

- Receptor grid file

- 'quinolone_project' → 'glide-grid-2xkk_all_constraints folder' → `glide-grid_2xkk_all_constraints.zip`

• Ligand file • Job name	• 'quinolone_project' → 'ligprep_2xkk' → `ligprep_2xkk-out.maegz` • `glide_2xkk_cognate`

Objective 5: *Using your validated DNA gyrase and topoisomerase IV docking models, dock the structure of the quinolone analog you are preparing in the laboratory (6e, 6f, or 6h). You will do this by creating a new structure in the **2D Sketcher**, preparing it with **LigPrep**, then docking the structure using **Ligand Docking**.*

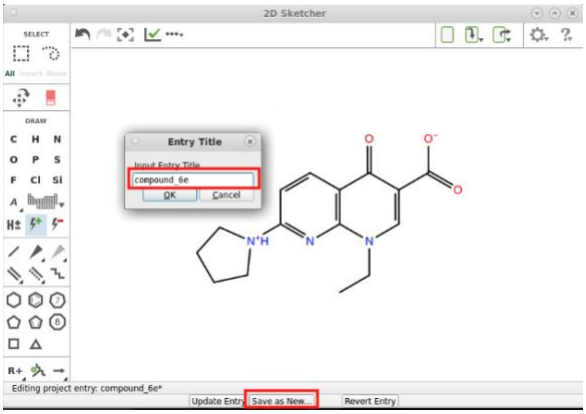


*Figure 5-1. Create a new entry for compounds 6e/6f/6h using the **2D Sketcher***

1. (Optional) Include one of the MFX ligand entries if you would like to modify an existing ligand in the 2D sketcher. You can always start with a blank sketcher.
2. Open the **2D sketcher** and modify/create one of the synthesized quinolones (6e is shown in *Figure 1-5* as an example.)
3. **Save as New** and name it appropriately (e.g. compound_6x)

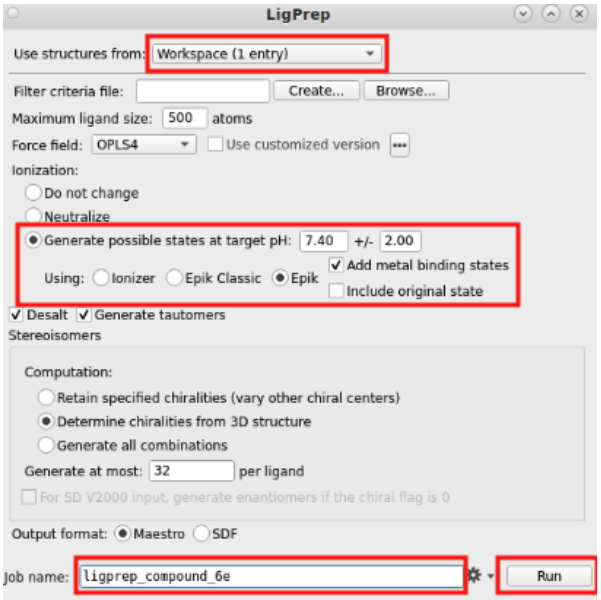


*Figure 5-2. **LigPrep** settings for compounds 6e/6f/6h*

4. Include 'compound_6x'
5. Go to **Tasks → Browse → LigPrep** to open the **LigPrep** panel.
6. For Use structures from: choose **Workspace (1 included entry)**
7. Under Ionization, choose **Generate possible states at target pH** using **Epik** and **Add metal binding sites**
8. Under Stereoisomers, choose **Determine chiralities from 3D structure**.
9. Change the **Job name** to '`ligprep_compound_6x`' and click **Run**.
10. The job will only take a minute to run.

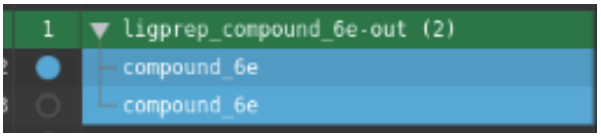
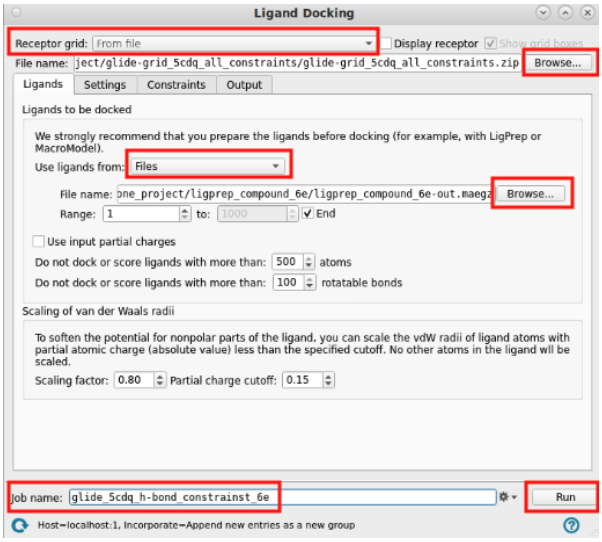


*Figure 5-3. **LigPrep** output entries for compounds 6e/6f/6h*

11. A new group will be added to the Entry List.
12. Open the **Project Table** to review calculated properties for **Total Q** and **State Penalty** as previously done for the cognate ligand.
13. **Close** the **Project Table**.



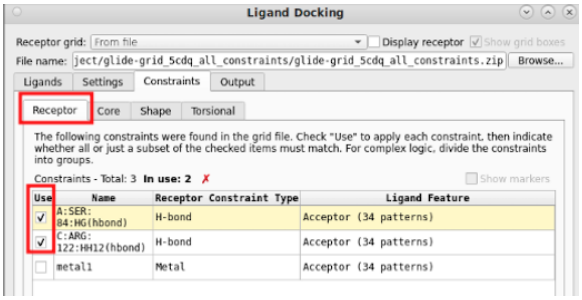


*Figure 5-4. **Ligand Docking** settings – choosing **Constraints***

14. Go to **Tasks** → **Browse** → **Receptor-Based Virtual Screening** → **Ligand Docking**
15. In the **Ligand Docking** panel, next to next to **File name** for the **Receptor Grid**, click **Browse** and double-click on the 'glide-grid-5cdq_all_constraints folder' and choose `glide-grid_5cdq_all_constraints.zip` and click **Open**.
16. You may see a pop-up that asks you to review and activate constraints. Click **OK** to review the Constraints.
17. In the **Constraints** tab select the **Receptor** tab to see the constraint options.
18. Under **Use**, check only the two H-bond constraints we set earlier during protein preparation.

Note: We will attempt our first docking with the two hydrogen bond constraints on – later you will repeat this ligand dock *without* any constraints.

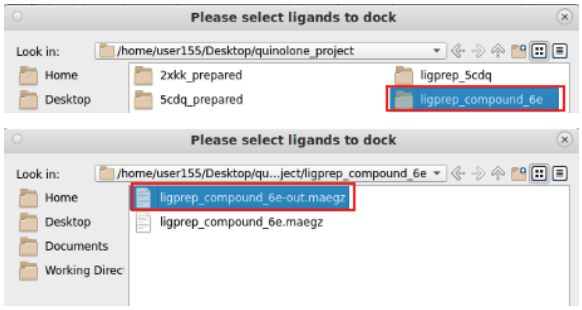


*Figure 5-5. **Ligand Docking** settings – choosing the **Ligand***

19. Go to the **Ligands** tab and for **Use ligands from**, choose **Files**
20. Next to **File name**, click **Browse** and go through the following folders 'quinolone_project' → 'ligprep_5cdq' to find `ligprep_compound_6x-out.maegz` and click **Open**. These

	include the two **LigPrep** output structures generated previously.
 *Figure 5-6. **Run Ligand Docking** job*	21. Change the **Job name** to `glide_5cdq_h-bond_constraints_6x` and click **Run**. o This job will take a few minutes, and when it is complete a banner will show that files have been incorporated into the Entry List. 22. Repeat steps 14-20 for two additional docking jobs with the following changes and corresponding job names: • Dock the prepared compound 6x using the 5cdq receptor grid with no constraints – name the job `glide_5cdq_no_constraints_6x` • Dock the prepared compound 6x using the 2xkk receptor grid – name the job `glide_2xkk_6x`

References

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