Ligand Pose Inspection Recommendations

***Important*:** There is no single correct workflow for pose inspection. If you have colleagues who are already doing these types of analyses, we recommend reaching out to them to ensure that you are all on the same page.

***Note*:** The order of these sections does not fully reflect a particular workflow. While some sections provide step-by-step instructions, others just suggest things to consider. We would recommend that you read through the whole document.

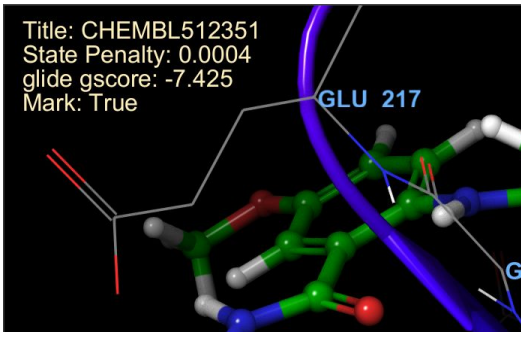
General Notes on Docking

1. Just because a compound docked doesn't mean it will bind
2. Docking scores can be used/interpreted categorically
    - Look for trends between docked/active compounds (from the SAR) and docked/inactive compounds. A cutoff can be selected in the docking score between active and inactive compounds. Ideally, this cutoff will be based on a ROC plot constructed using docking scores for known actives and inactives (decoys). This will help to evaluate larger collections of compounds to dock.

Workspace Properties

Easily view relevant properties associated with your ligand in the workspace

1. Hover over the top left corner of the Workspace until you see the entry name (or "There is no target entry) and double-click to open the Change Workspace Properties panel. Click Add and select:
    - Epik State Penalty
    - Glide Score
    - Stars or Mark (see #8 in the General Inspection Workflow section)
    - Anything else that you would want to see when looking at poses



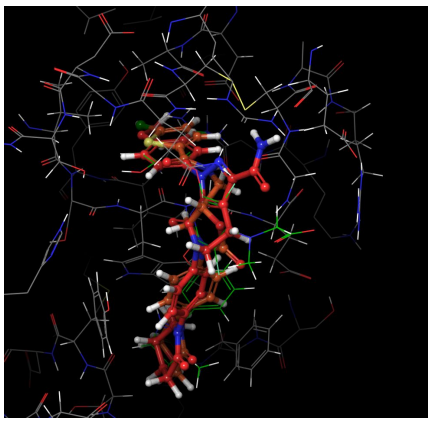
2. Click close if you would just like these to appear in your current project, click Save as Defaults to edit your global Maestro preferences

***Note*:** When editing your Workspace Properties (either in the project or globally), you can only add properties that are *present in your current project*. You may want to

load a project in which you have run a LigPrep and docking job so they will appear as options. Once you make a global edit, it will be applied toward all of your Maestro projects.

***Note*:** Workspace Properties can be displayed by clicking the **+** icon to open the Workspace configuration panel and clicking Workspace Properties. They will only show when there is either one entry in the Workspace, only one non-fixed entry in the Workspace, or only one select entry in the Workspace. For pose inspection purposes, it would therefore be best to make sure your receptor is fixed in the Workspace.

General Inspection Workflow



1. Fix the receptor in the Workspace
2. Display only residues within 5-7 Å of the ligand
3. Render all docked ligands in a tube representation and in a similar color that contrasts with the protein (leave the reference ligand in the wire representation)
4. Display all hydrogens on the ligands and nearby residues
    - Are any too close? Use measurements to find out.

***Note*:** Most of steps 1-3 can be set as a Custom Preset which will automate the process for you. The only thing you will need to do manually after you have the Preset set-up is differentiate the rendering style of the reference ligand and docked ligands

5. Center on the ligand and turn off Auto fit
6. Do the hydrogens bonds being shown make sense?
    - Hydrogen bonds that reach toward to the surface should be de-emphasized as waters will generally make those interactions
7. Rotate to look down bonds to make a Neuman projection - look for eclipsed atoms
8. Add the Stars or Mark columns to the Entry List to mark preferred compounds
    - If using the Mark column, you can type X to mark or unmark an entry

***Note*:** For a quick way to go through many docked ligands, consider using the Pose Viewer panel. It is particularly useful if you checked Write per-residue interaction scores when setting up the Glide docking job.

***Note*:** When looking into selectivities between two proteins, looking at the docking poses in a surface representation of each protein (in a different color) can be a nice visual

Inspection Tip and Tricks

***Note*:** If available, 3D stereo could be useful.

1. Look for ligand-protein lone pair-lone pair clashes, which would be unfavorable
2. N-H N-H pointed toward each other would be unfavorable
3. Planar, trans amides are preferred to non-planar or cis amides
4. Look for aminopyridines/aminopyirimidines to have sp2 amines (especially if H is on the exocyclic N)
5. Sulfonamides should be kinked (not linear like other amides)
6. Use CSD to analyze the torsion range for a particular motif (**Note**: This requires a CSD Mogul installation which is separate from Maestro)
    - We would recommend reading this paper on "Small Molecules Conformational Preferences Derived from Crystal Structure Data"
7. Flag if the Epik state penalty is very high
    - If there is a big discrepancy, run Jaguar pKa
    - Note that the pKa could be an ensemble of microstates, and Glide may find the best binder but not know the distribution of states

***Note*:** Ideally you would have addressed (removed or annotated) exotic Epik states while preparing the library.

What About Water?

***Note*:** Water should have been already considered during the preparation of the system for docking

1. Check that the waters are making a full complement of hydrogen bonds
2. Check that the water has no contacts with hydrophobic heavy atoms
3. Look for potential hydrophobic enclosure of waters, since it is highly unfavorable (especially if it is on more than two sides of the water).

What About Other Grids?

***Note*:** Docking is often done with an ensemble of grids.

1. How consistent is a compound's protonation/tautomeric variant pose score?
2. How consistent is the compound's protonation/tautomeric variant pose geometry?

Clustering and Sorting Suggestions

***Note*:** Ideally the compounds would have been annotated by Vendor ID, amount, availability, and price before docking (if not, and you are looking into purchasing compounds, this would be a good time to do so). This can be particularly helpful for voting, since it's very valuable to have a sense of how attainable a compound is.

1. Turn on Ugly Contacts in the interactions panel and calculate the number of contacts in the Project Table (these are stored as standard properties)
    i. Sort by Intra-Ligand Ugly Contacts
    ii. Sort by Protein-Ligand Ugly Contacts
2. Sort by the number of heavy atoms

***Note*:** There are also several ligand efficiency scores in the docking results that can be used here as well.

3. Cluster by spectral clustering using the Spectral Clustering panel
4. Cluster by substructure
5. Sort by 'attainability' (see the note in the beginning of this section)

Other Things to Consider

1. The Site Map Phobic and Philic Surfaces around the binding site
2. The Connolly Surface of the receptor binding site
3. Relevant WaterMap sites (if you have a WaterMap for the system)

Please see Decision Making in Structure-Based Drug Discovery: Visual Inspection of Docking Results for some more suggestions, and an analysis of how computational chemists and medicinal chemists generally inspect binding poses.