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SCHRÖDINGER ACADEMY

VIRTUAL SCREENING

Structure-Based Virtual Screening Using Glide

Structure-Based Virtual Screening Using Glide

Created with Release 14-4

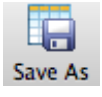
In this tutorial we will use Glide to perform a virtual screen for potential inhibitors of Factor Xa. The tutorial is comprised of five parts.

1. Creating projects and importing structures
2. Preparing the protein structure
3. Generating a Glide grid
4. Executing a Glide screen
5. Analyzing results

Required Files: 1fjs_prep_recep.mae.gz, 1jfs_prep_lig.mae.gz, 50ligs_epik.mae.gz, factorXa_xp_refine_pv.maegz

1: Creating projects and importing structures

Projects (.prj extension) are the main file format for Maestro. A project file may contain hundreds or thousands of entries; these entries may correspond to imported protein and ligand structures and/or to output of modeling-related tasks. Once a project is created, the project file is automatically saved each time a change is made.*



1. *Create a Project*. Open Maestro by double clicking on the desktop icon. A scratch project is created (**Note**: scratch projects are not automatically saved!). Press the **Save As** icon in the **Project** toolbar (or navigate to **Project -> Save As** in the menu bar). In the dialog box that appears, type the name "FXa_glide" in the File Name box; press **Save** (**Figure1**). The name of the project at the top of the Maestro window should now be "FXa_glide.prj."

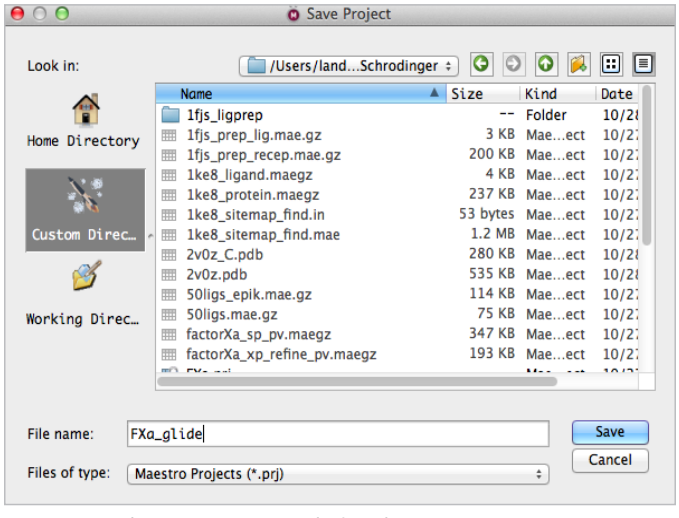


Figure 1. The Save Project dialog box.

2. *Copy Tutorial Files*. In the main menu bar, navigate to **Help -> Tutorials**. In the panel that opens, highlight tutorial 6, corresponding to the Glide Quick Start Guide (**Figure 2**). Press **Copy**. Now all tutorial files will be copied into your working directory.

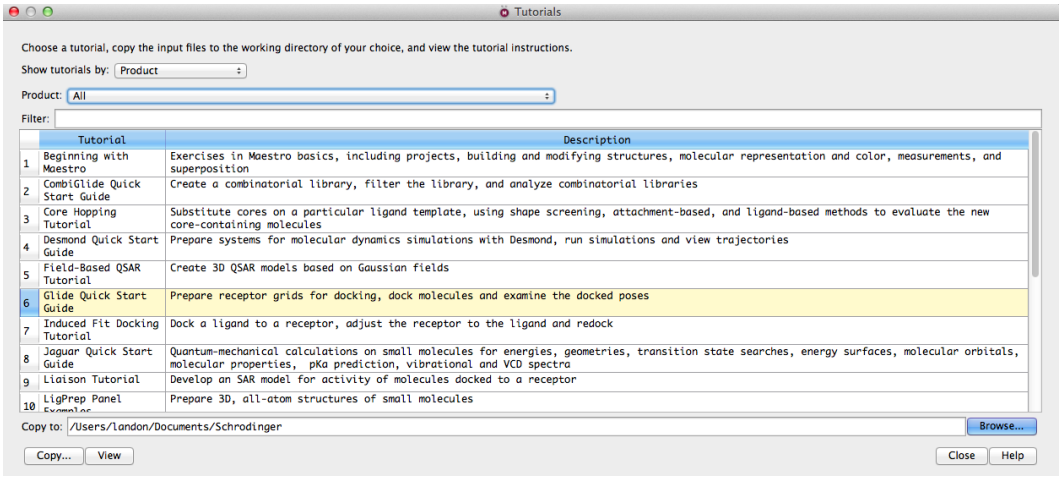
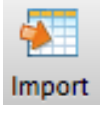


Figure 2. The Tutorials panel.



3. *Import Structures*. Press the **Import** button in the **Project** toolbar. Select files "1fjs_prep_lig.mae.gz," "1fjs_prep_recep.mae.gz," and "50ligs_epik.mae.gz" from your working directory by clicking on one file and then pressing ctrl (or cmd on Mac) then clicking on the second file (both files should be highlighted, **Figure 3**); press **Open**. **Note**: *.mae and *.mae.gz files are the default structure file formats for Maestro. However, all common structure file types are supported.

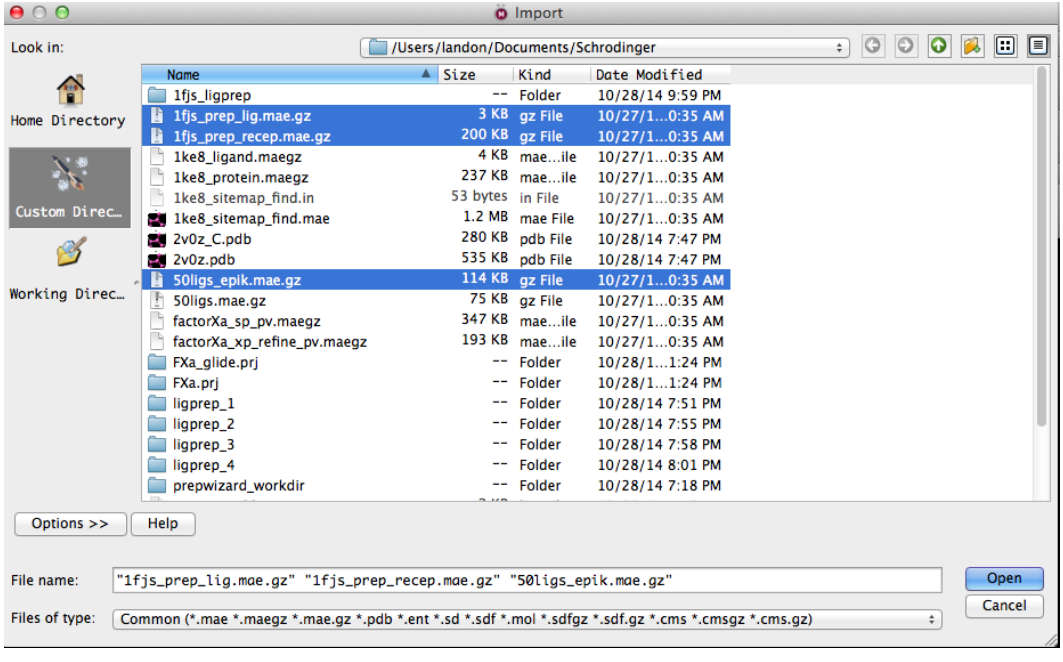
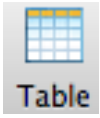


Figure 3. The Import Structure window.

2. Preparing the protein structure

Structure files obtained from the Protein Data Bank often lack necessary information for performing modeling-related tasks. Typically, these are missing hydrogens, partial charges, side chains, and/or whole loop regions. In this section we will use the Protein Preparation Wizard to fix a structure file, rendering it suitable to use for virtual screening.



4. *Merge Structure Entries*. Open the project table by pressing the **Table** icon in the **Project** toolbar. Ensure that the entries "1fjs_prep_lig" and "1fjs_prep_recep" are selected. If you are using a mouse, right click on one of the entries; from the menu that appears, choose "Merge" (**Figure 4**).

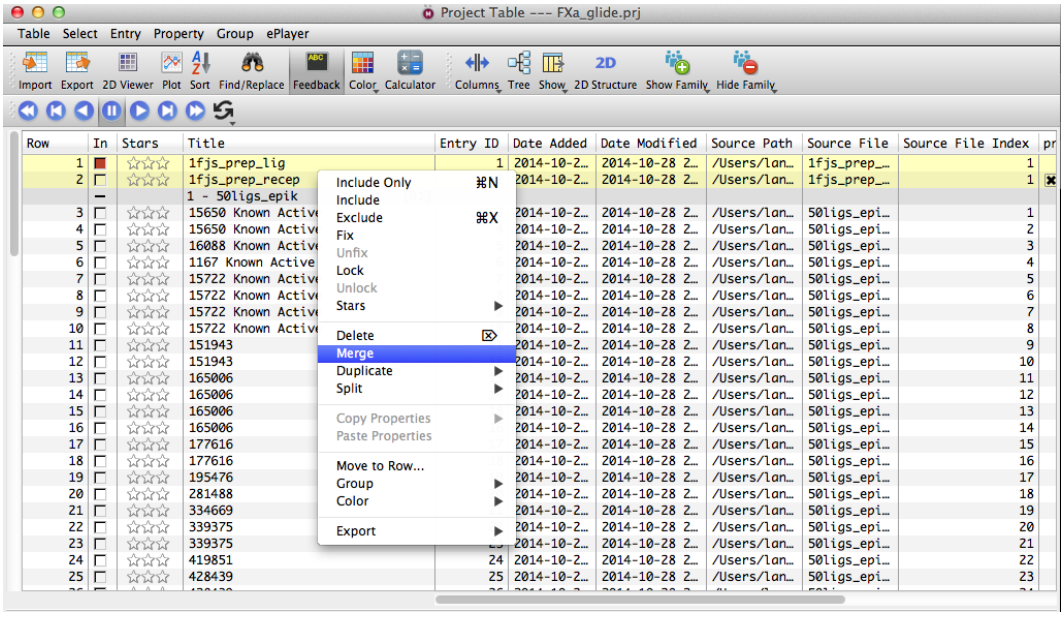


Figure 4. The Import Structures window.

A new merged entry should now appear in the project table. With your mouse or trackpad, double click on the title of the merged entry and change the name to "1fjs_merged"; press Enter to save the change (**Figure 5**). Include **only** the merged entry in the workspace in preparation for the next step, then close the project table.

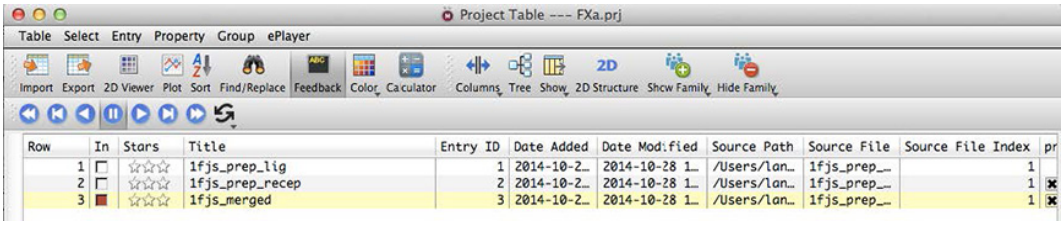


Figure 5. View of the project table after the merged entry has been created and renamed.

5. *Prepare a Protein Structure*. Access the Protein Preparation Wizard by pressing the **Prep Wiz** icon in the **Project** toolbar (**Figure 6**). The preparation wizard consists of a required processing step, followed by optional modification and refinement steps. To run the wizard, a **single** structure must be included in the workspace; this structure can be located in the project table or imported directly from the PDB in the first step. The recommended minimal processing tasks are checked; however, you may also wish to fill in missing side chains and/or loops if they are important for subsequent modeling activities. Press **Preprocess**.

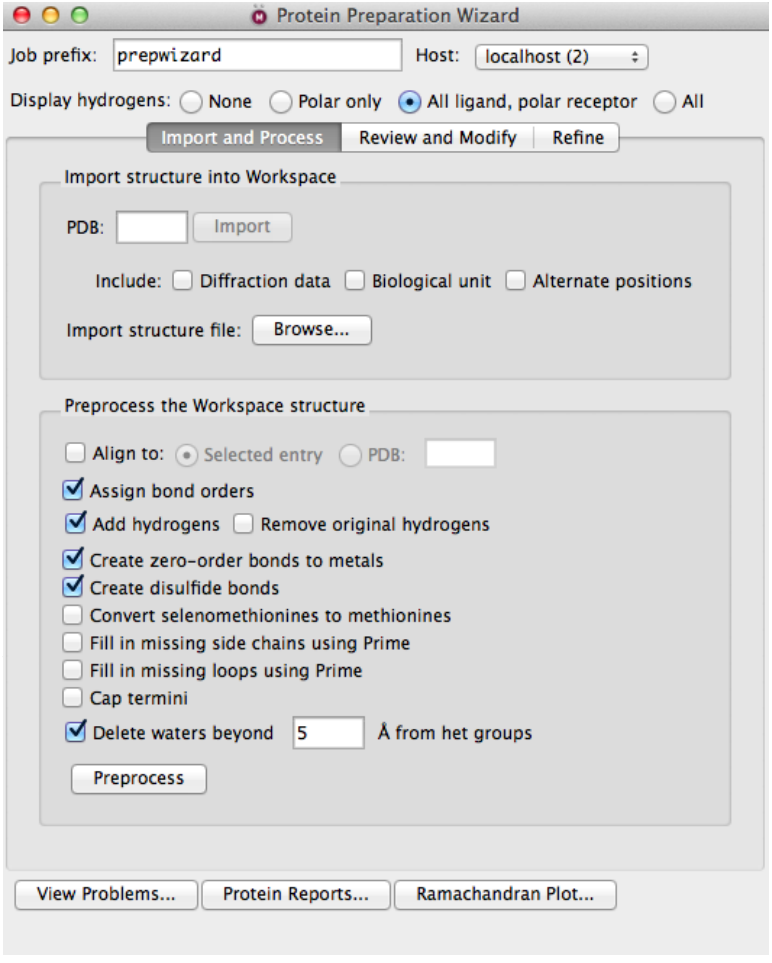


Figure 6. The Protein Preparation Wizard panel.

Once the job is finished, a new, processed entry is added to the project table and replaces the original structure in the workspace.

5B. *Refine the Prepared Structure*. Navigate to the Refine tab in the wizard (**Figure 7**). In this tab the hydrogen bonding network can be optimized by sampling water orientations and flipping Asn, Gln, and/or His side chains. Press **Optimize**. Once the job is finished a new entry will be added to the project table, and the workspace will be updated. Inspect the structure to identify side chains that have been flipped; they will be labeled.

***Note**: So as to accurately reflect experimental conditions, you may also wish to adjust the pH, which will change the protonation states of residues and ligands accordingly.

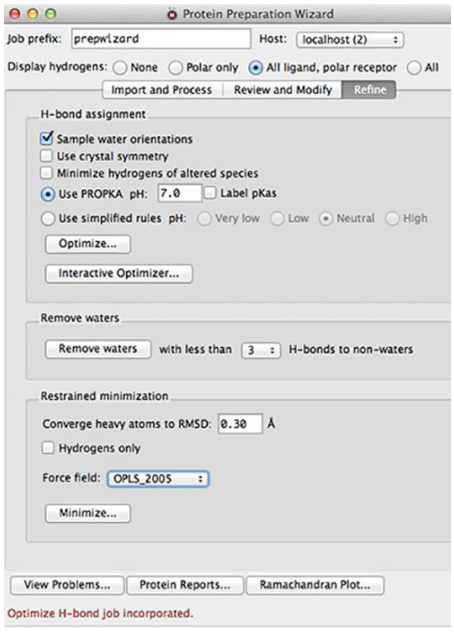
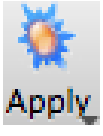


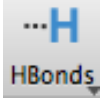
Figure 7. The refinement step of Protein Preparation. In the Refine tab of the wizard, additional optimization steps can be performed.

Additional minimization may be performed on the structure, but it's not necessary.

6. *Rename the Refined Structure*. Double click on the title of the refined structure in the entry list; rename it "1fjs_refined."



7. *Change the Rendering and Visualize Hydrogen Bonds*. Press the **Apply** button in the **Style** toolbar; center the workspace on the ligand by pressing the letter 'L." Visualize hydrogen bond contacts by pressing the **HBonds** button in the **Measurements** toolbar; choose Display. Four hydrogen bonds should be visible (**Figure 8**).



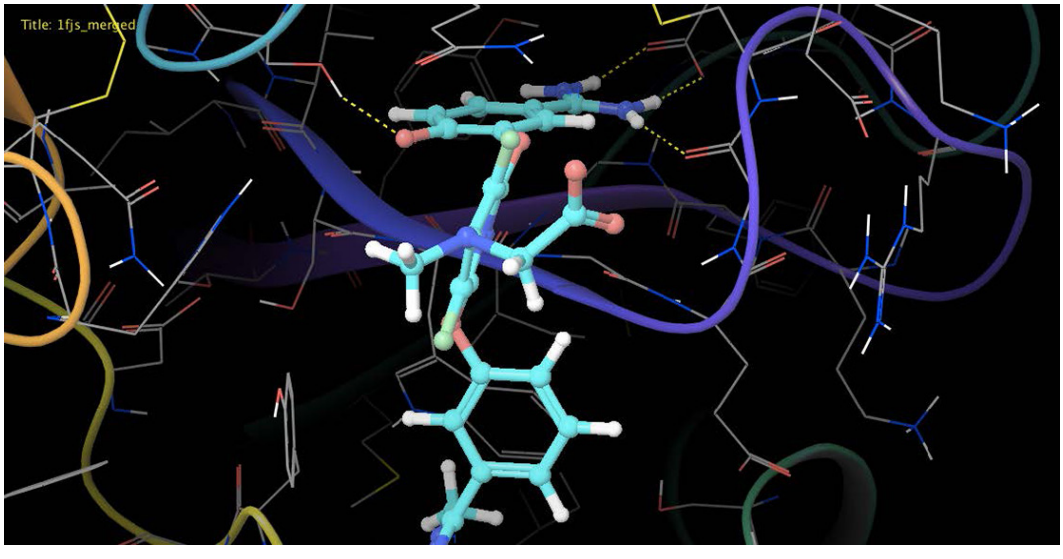


Figure 8. Workspace rendering and hydrogen bond visualization.

3. Generating a Glide grid

*Grid generation must be performed prior to running a Glide screen. The shape and properties of the receptor are represented on a grid by fields that become progressively more discriminating during the docking process. Many different kinds of constraints can be applied during the grid generation stage; for a comprehensive overview of all the options, check out the grid generation video on our website (under **Support -> Videos**) or the Glide User Manual. In this tutorial we will set a hydrogen bond constraint.*

8. *Identify the Binding Site*. Open the receptor grid generation panel (**Tasks -> Docking -> Grid Generation**). In the **Receptor** tab (**Figure 9, right side**) ensure that the following boxes **Pick to identify ligand** and **Show markers** are checked. Then press on the ligand in the workspace; the ligand should now be highlighted in green (**Figure 9, left side**).

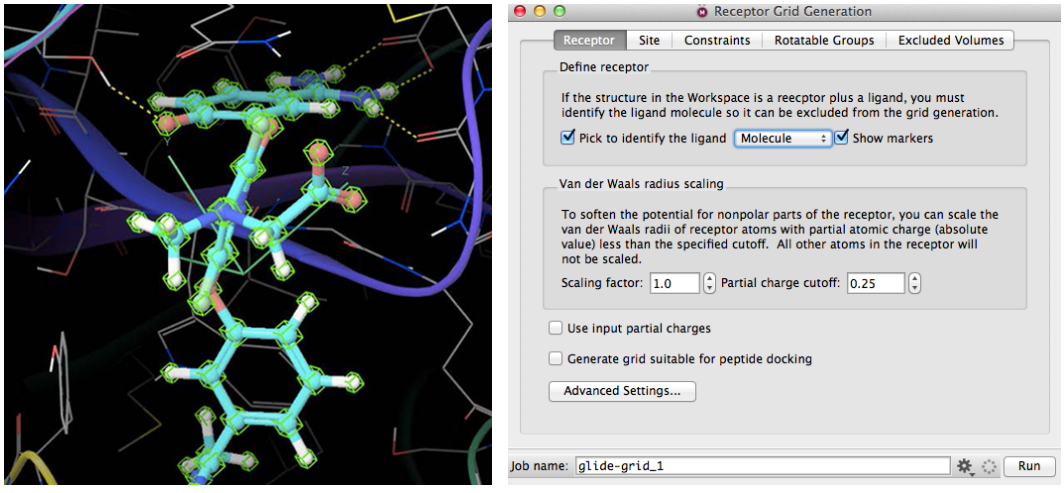


Figure 9. Identifying the binding site for grid generation.

9. *Define the Box Enclosure Dimensions*. Press the **Site** tab. A purple bounding box should now be visible in the workspace; you may need to zoom out to see it (**Figure 10, left side**). The purple bounding box defines the outermost region that docked molecules can occupy. Press the **Advanced Settings** button. A green inner bounding box should now appear in the workspace (**Figure 10, left side**). This box defines the region in which the centroid of the docked ligand can be placed. Adjust the X, Y, and Z sizes to 10, 8, and 6 Angstroms respectively and observe the shape of the green box change. Press **OK**.

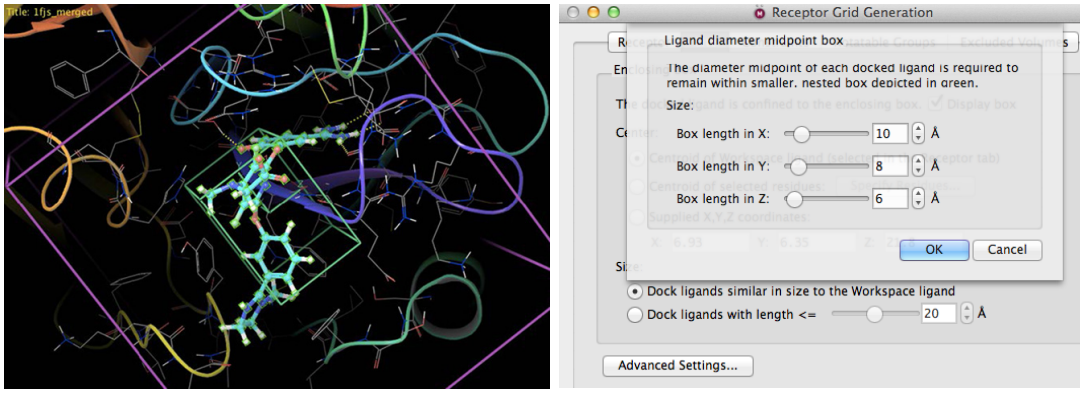


Figure 10. Outer (purple) and inner (green) bounding boxes that define the binding region (**left side**). The inner bounding box dimensions can be manually defined (**right side**).

10. *Set a Hydrogen Bonding Constraint*. Press on the **Constraints** tab followed by the **H-bond/Metal** sub-tab. Zoom into the region of the ligand that is forming hydrogen bonds to Asp189 (**Figure 11, left side**) and press on one of the oxygen atoms of the residue. Crosshatches will appear over these atoms and a new constraint appears in the panel. (**Figure 11, right side**).

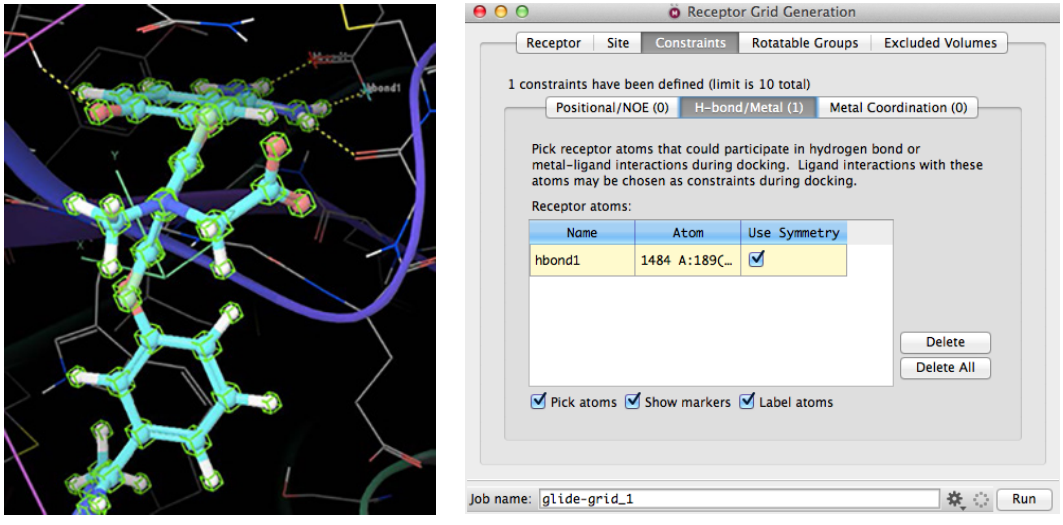


Figure 11. Setting hydrogen bonding constraints (**right side**). After pressing on one of the oxygen residues of Asp189, crosshatches will appear on the atoms that form the hydrogen bonding constraint (**left side**).

11. *Generate the Grid*. At the bottom of the Receptor Grid Generation panel change the job name to "1fjs_grid" and press **Run**. A grid file named "1fjs_grid.zip" will be generated in your working directory (**NOTE**: Your project table will not be updated. To determine whether or not the grid file generated successfully, check the job monitor). Once the job has finished, close the panel.

4. Executing a Glide screen

The minimum requirements for executing a screen include a grid file and a set of ligands. Optionally, you can set additional ligand- and receptor-based constraints as well as incorporate chemical similarity into the final docking score. For a full explanation of these topics, please consult the Glide User Manual. Here we will not set any additional constraints, but we will include the hydrogen bonding constraint that we created in Step 10.

Note: Ligand structures MUST be run through LigPrep (Tasks -> Ligand Preparation) prior to using them for a screen. In this example we are using structures that have already been prepared.

12. *Define the Docking Settings*. Select the group "50ligs_epik" in the project table. Open the **Ligand Docking** panel (**Tasks -> Docking -> Glide Docking**). In the **Settings** tab specify the grid file you generated in Part 3 by pressing **Browse** and then navigate to the "1fjs_grid.zip" file in your working directory. Select **Project Table** (Selected Entries) from the drop down menu for **Use Ligands from** (**Figure 12**).

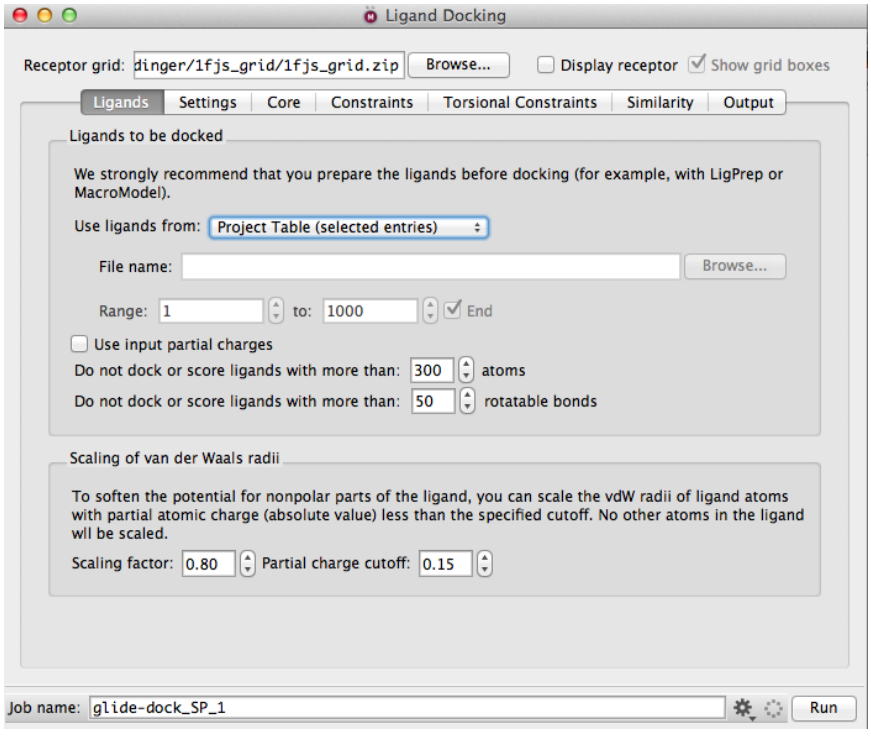


Figure 12. Ligand docking settings.

12B. *Scoring and Sampling Settings*. Press the **Settings** tab to look over the docking settings (**Figure 13**); for the purposes of this tutorial the default settings are adequate. Additional sampling and scoring settings can be accessed from **Advanced Settings**.

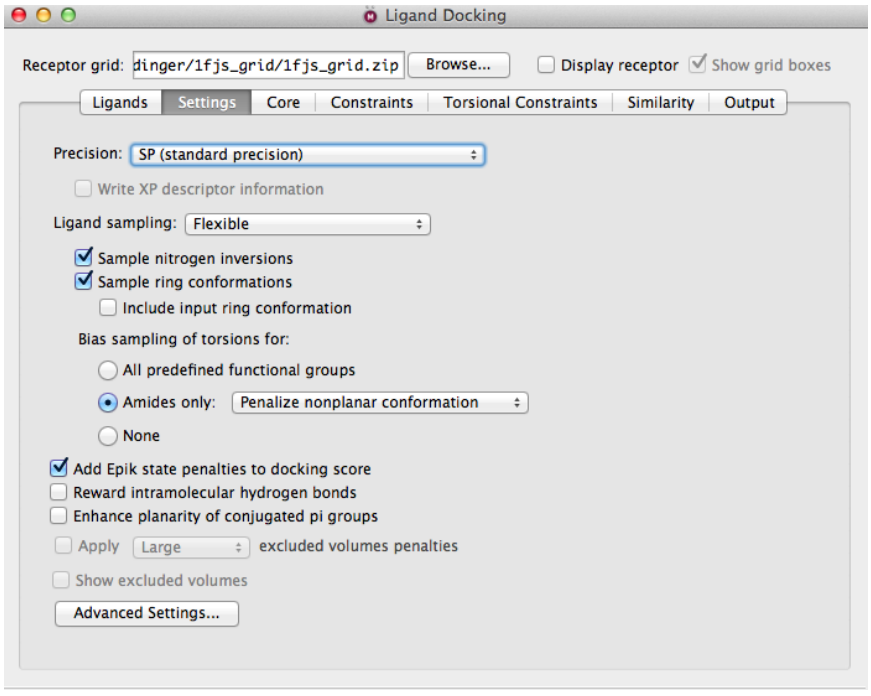


Figure 13. Scoring and sampling settings.

12C. *Include the Hydrogen Bond Constraint*. Press the **Constraints** tab. Check the **Use** box to include the hydrogen bond constraint set during the grid generation step (**Figure 14**).

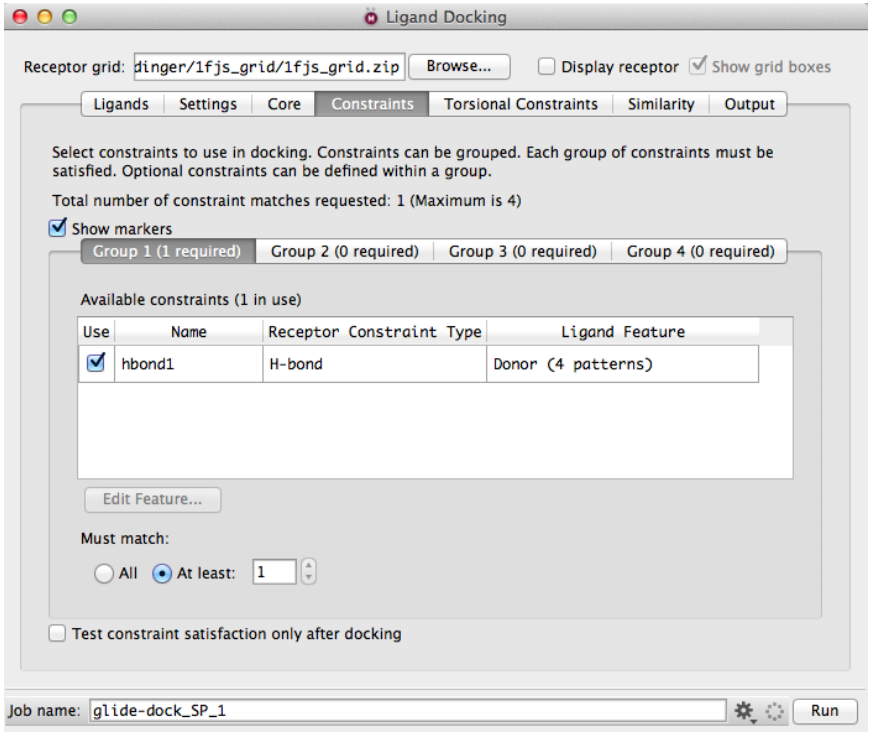


Figure 14. Setting constraints.

12D. *Modify the Output Settings*. Press the **Output** tab. Check the box **Write per-residue interaction scores** (**Figure 15**). Change the job name to "1fjs_glide"; press **Run**.

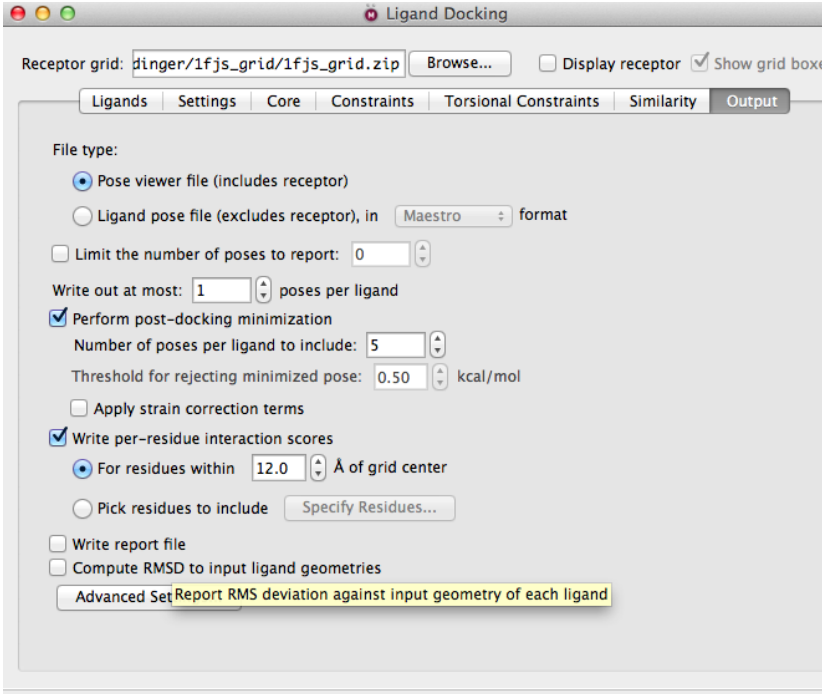


Figure 15. Output settings.

5. Analyzing results

13. *Visualize Results using Pose Viewer*. When the job finishes, a new group with the results will be created in the project table; ensure that the group is selected. Open Pose Viewer (**Tasks -> Docking -> Pose Viewer, Figure 16**). Press **Set Up** and check the box to display **Per Residue Interactions**. Toggle through the results using the right and left arrow keys. Interactions with residues colored in blue contribute negatively (favorably) to the docking score of the molecule, while interactions with residues colored in red contribute positively (unfavorably) to the score.

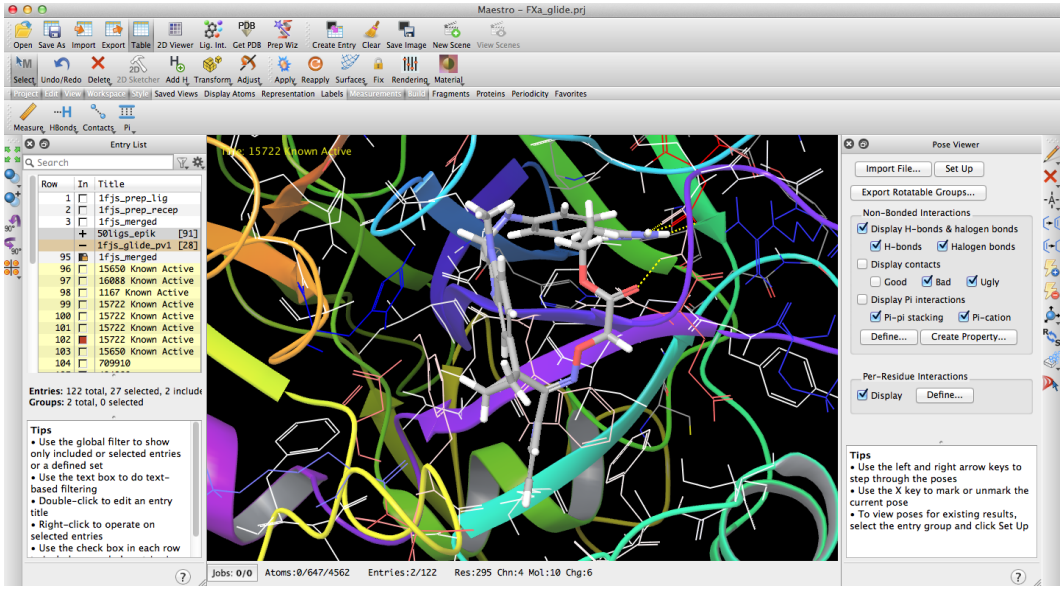


Figure 16. Example docked pose (gray sticks). Residues are colored according to their interaction energies, ranging from red (highly favorable interaction energy) to blue (highly unfavorable interaction energy).

14. *Visualize XP results.* Open the XP visualizer (**Tasks -> Docking -> Visualize XP Interactions**). Press the **Open** button; from your working directory select the file "factorXa_xp_refine_pv.maegz." Choose docking score as the activity property. The table will be populated with results of running XP rescoring on a subset of the original results; individual terms in the scoring function are represented as columns in the table, and are colored according to whether they favorably (blue) or unfavorably (red) affect the docking score. (**Figure 17**). Press **Export Data** to export the spreadsheet as a CSV file.

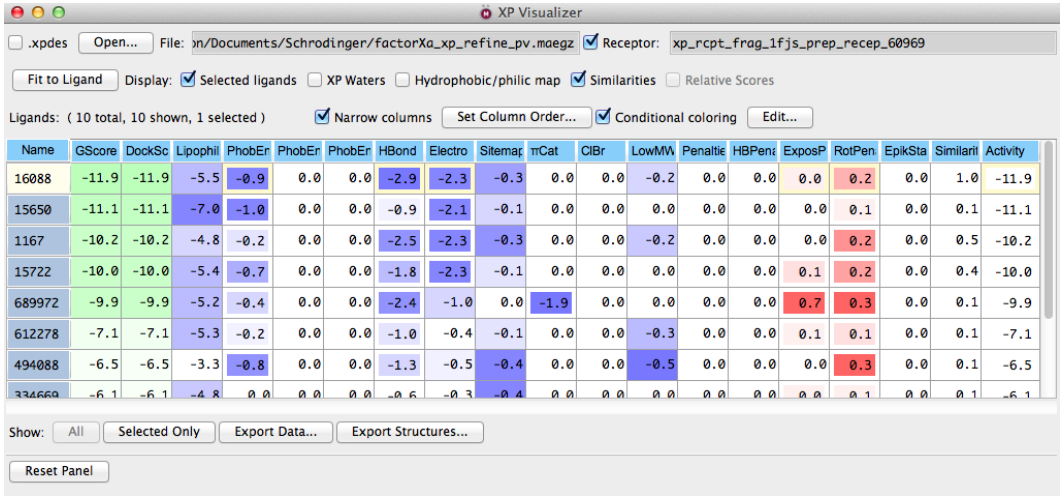


Figure 17. The XP visualizer.