Virtual Screening With GLIDE

More and more GPCR's structures have been published recently and docking is becoming an attractive tool to apply to this target family. In this training we will screen the file P29274_docking-random.mol2 with Glide docking tool to see the compounds that could potentially be binders of A2A adrenergic receptor.

In general GPCR's and particularly A2A adrenergic receptor are particularly difficult targets for docking because of the large cavity, induced fit effects, influence of certain water molecules and the tendency of certain compounds with pseudo-symmetric structures to flip during the docking. In that case we will not use the IFD procedure nor XP scoring function because of time constraints but we will use some constraints during the docking to improve the docking efficiency.

To perform the docking with Glide, you need to perform:

1) Protein Preparation
2) Grid Generation
3) Ligand Prepapration
4) Ligand Docking (Screening)

All files will be provided so that each step can be performed independently and a project with all results for the different steps is provided: Strasbourg-`Chemoinformatics-Docking-Training.prjzip`.

1. Protein Preparation

In this part we will prepare the protein by cleaning up the XRay structure, adding protons, fixing bond orders, optimizing protonation states and hydrogen bond networks and performing a minimization under restraints.

- Start Maestro
- Save your project: `Project -> Save as ->` `Chemoinformatics-Docking-Training.prj`
- `Project -> Import Structures` and navigate to the `Strasbourg-Chemoinformatics-Schrodinger-Training` and choose the `3eml.pdb` file.
- Click `OK` on the information pop-up windows
- Go to `Workflows -> Protein Preparation Wizard…`

• Bond order assignment, hydrogen addition and water treatment

- In the first Tab, uncheck the checkbox "delete waters beyond…" (we will keep all the waters during the preparation)
- Click on preprocess: Bond orders will be assigned and hydrogen atoms will be added
- Review the overlapping issues in the panel that pops up at the end. Since we only have overlap issues with H atoms, we will ignore them because they will disappear when the structure will be minimized later. Click OK.

• System and ligand state review and selection

- Click on the Tab "Review and Modify"
- We will choose the system we want to keep for the study. In that case we will only remove the SO4 and STE co-crystallized molecules. Select those molecules in the HET part of the panel (using mouse left-click and Shift-left-click or CTRL-left-click). Then click on delete to delete your selection (colored in yellow).
- Click on Generate States to generate possible protonation states of your co-crystallized ligand (ZMA) and review the structure of your ligand to check if everything is correct.

• Hydrogen bonds, protonation states and coordinates refinement

- Go to the Refine Tab. To optimize the HBond network in the protein (flip ASN/GLN/HIS etc… and generate better protonation states for HIS, GLU etc…) click on Optimize. You can review the results with the Interactive Optimizer. But we will keep the default results of the automatic optimization.
- Click on Minimize

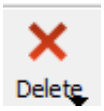
Your protein is now prepared.

We will now remove all water molecules before performing the docking. To organize our project we will first move all the structures generate during the preparation to a group and keep it as a record and then work on duplicated prepared structures for the docking itself. We will thus use the grouping and duplicate functions in Maestro.

In the project Table (Project -> Show Table) select all the entries (left-click and SHIFT-left-click) and then move those entries into a group (right-click and in the context menu choose Group -> New and type Preparation). All the structure generated during the preparation are now kept for the record in the Preparation group. We will need to work more on the prepared structure and remove water from this structure. To do that, first duplicate the last entry of the group (right-click and in the context menu choose Duplicate -> Ungroup). Do the same

thing again to have a second copy. Then change the Title of the first entry to 3EML-Prepared and the second to 3EML-Prepared-No-Waters

But we still have all water molecules that are in the binding site and that will prevent the docking of some ligands. We need to remove them or at least some of them. In that case we will remove all of them.

Include the second entry in the workspace (click on the "In" box of that entry in the project Table). Then in the Workspace click and hold on the Button 

and in the menu choose: Waters. All Water molecules will be removed from the structure.

2. Grid Generation

To perform more efficiently the docking calculations Glide does not work with the structure itself but with a grid representing the properties of the structure (i.e. electrostatic potential generated on each grid points, van der Waals etc…). We will thus generate such a grid from the prepared structure.

Include in your workspace the second entry called 3EML-Prepared-No-Waters (you can find it by opening Strasbourg-`Chemoinformatics-Docking-Training.prjzip` if you have not prepared the protein).

- In Applications -> Glide -> Receptor Grid Generation
- When you are in the first tab of the panel, click on one atom of the ligand. The ligan will be used to define the grid position and size. Since we will dock compounds of the same size, we will keep the box as it is defined by default (you can see the position of the Grid when you are in the second Tab). For other case if you want to explore the pocket more in details you can extend the size of the grid.
- As you can see in the co-crystallized structure, there is a hydrogen bond motif between the ligand and the receptor. This motif is known to be important for ligand binding. We will thus define hydrogen bond constraints that we will use during the screening. To do so go to Constraints Tab and choose the H-bond/Metal sub-Tab. Then in the workspace click on HD22 of ASN 253, on OD1 of ASN 253 and OE2 of GLU 169. Three symmetry constraints will thus be defined and appear in the Panel.
- We will leave all the other settings to their default values.
- Click on start and call your job `glide-grid_2Asn253_Glu169_Hbonds_Constraints`. The job will take3-4 minutes to complete. Nothing will be incorporated in the project Table at the end. You can monitor the status of your job by looking at `Applications -> Monitor Jobs`…

3. Ligands Preparation

During this step we will prepare the ligands by generating possible tautomers and different protonation states as far as minimizing the structures themselves.

- Import the file `P29274_docking-random.mol2` (`Project -> Import Structures -> Change the filter to .mol2 files`)
- Select the entire group (`P29274_docking-random, all entries should be in yellow`)
- Go to `Applications -> Ligprep`.. and change:
    - `File` to `Project Table (Selected Entries)` to use the selected entries as input structures
    - `Retain specified Chiralities` to `Determine Chirality from 3D structures` (Since lack of chirality specifications has already been taken into account in the input file)
    - `Start` the calculation with the name `ligprep_Defaults_From3D`

At the end of the job, you should get the prepared ligands incorporated in your project.

4. Ligands Virtual Screening

In this step we will screen the set of prepared ligands using the grid that was previously generated based on the 3EML pdb structure. For the screening you need to define only the grid (not the receptor itself) and the set of ligands you want to screen (in our case the ones that we just prepared). As a reminder we will use the constraints defined previously with the specific H bonds in our docking.

- Select the group of prepared ligands by clicking on the group header (all ligands under the group `ligprep_Defaults_From3D-out1` in the project table should be in yellow)
- `Applications -> Glide -> Ligand Docking…`
- In the Settings Tab click on `Browser` to specify the receptor grid file and select the file `glide-grid_2Asn253_Glu169_Hbonds_Constraints.zip` that should have been generated during the grid generation (Otherwise it is accessible in the `Strasbourg-Chemoinformatics-Training` directory)
- We will keep all the other settings as they are (`SP precision, flexible ligand sampling and add Epik state penalties`)
- In the Ligands Tab, switch `Use Ligands` from file to `Project Table (Selected Entries)`
- In the `constraint` Tab, check the boxes for all 3 constraints and choose to `match at least 2` of those constraints.
- In the output tab, since the pocket is significantly bigger than the ligand size, we will increase the number of poses subject to post docking minimization. Change the `Perform Post-docking minimization/number of poses per ligand to include from 5 to 10`.
- We will keep the number of poses per ligand to 1 since we will stick to the conditions of large virtual screening (if you are more interested in poses, then you may choose to increase this value)
- Start the job with the name `glide-dock_SP_10PDM_2_out3_HBonds_constraints` with the option Incorporate set to `Append new entries as a new group`.
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5. Analysis of the results

At the end, the results are incorporated in the project table as a pose viewer file (first entry of the group is the receptor the subsequent entries are dock poses of the ligands).

- Visualize the results in the group glide-`dock_SP_10PDM_2_out3_HBonds_constraints_pv1` with the pose viewer function of Maestro.
- You can look at the enrichment curve in `Scripts -> Docking Post-Processing -> Enrichment Calculator`. Select the docking results in the group `glide-dock_SP_10PDM_2_out3_HBonds_constraints_pv1` and in the script panel use structures from Project Table (Selected entries) and for the active file choose `Actives.txt` available in the `Strasbourg-Chemoinformatics-Training` directory)
- Click on start and look at the results and the different plots.