Molecular Modeling 2012

Exercise 3: Molecular docking to (all) crystal structures of G protein-coupled receptors - Does molecular docking work?
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Task: In this exercise you will use the molecular docking method. Molecular docking algorithms are used to predict the 3D structure of the complex between a protein and a ligand and the affinity between them. Here we focus on structures of G protein-coupled receptors (GPCRs), which are targets of close to 30% of all marketed drugs. You will carry out docking calculations several receptors to test if the molecular docking method works well for these targets. We will do this for (nearly) all GPCR structures solved so far. Select one of the following PDB codes:

4EIY
3RZE
3NYA
3UON
4DAJ
2VT4
4EJ4
4DJH
4EA3
3ODU
3V2W
3PBL

You will first work individually and then share your results with the other students. You will prepare a report in Word individually, which involves answering questions about molecular docking algorithms and the results.
1. Re-docking of a ligand to a GPCR with DOCK Blaster

The aim of the first part is to get familiar with GPCR structures and to start docking calculations for at least one target using an online server, DOCK Blaster. There is an extensive literature on the methods and use of docking in ligand discovery, which you are encouraged to consult.

Pick one of the codes above and start preparing it for molecular docking! Do the following:

1.1. First download the PDB structure from the PDB (www.rcsb.org).

1.2. Inspect the structures in PyMOL. Try to identify the drug binding site and the bound ligand.
   (You can display all non-protein atoms in sticks by typing “show sticks, hetatm” on the command line.)
   • Write down the three-letter abbreviation name for the ligand that is co-crystallized with the receptor:
     PDB code:                                      Ligand:

     At this point I would suggest asking me if you have identified the correct ligands. Otherwise this will cause a lot of problems in the following steps!

1.3. Now go to the online docking server DOCK Blaster: http://blaster.docking.org/
Six steps to start docking for the chosen receptor:
Step A. From the DOCK pull down menu select “Start with a PDB code”.
Step B. In the “PDB code” field type the code for the chosen. In the “Ligand” field type the three letter code for ligand!
Step C. Click “DOCK!”.
Step D. Write down the “job id”:
Step E. If there are no error messages, click “DOCK!”.

   You will receive a detailed message about whether your docking job was submitted, or not. A link at the bottom of the page takes you to the results page. You will notice a yellow highlight move from left to right across the banner of the page. Typically, if your job has started, you will now be at the “Target Prep:Run” stage, which means the automated docking procedure is still running.

Step F. Click the link after “Browse job progress and results here:”. The receptor is now being prepared for docking by the server. After preparing the receptor, the program will attempt to re-dock the co-crystallized ligand in the binding site (“Calibration docking”). You can follow progress in this window. Press “Reload page” now and then to see if it has finished. It should not take more than an hour.

Now repeat steps A to F for another receptor (if there are receptors that have not been submitted yet).

• After submitting the Dockblaster run: Analyze the interactions between the ligand and the receptor for your selected target. Why do these ligands bind to the receptor? What kind of interactions do you see? Between what groups of the ligand and residues of the receptor?

Part 2. Did the docking work? Analysis of the results from automatic docking

To test if molecular docking works for the chosen targets, we will use two criteria: (1) Can molecular docking accurately re-dock the ligand into the binding site? This means that we will test if the program can take the ligand that is co-crystallized with the receptor and put it back where it was. (2) Can molecular docking identify the ligand among a database of decoys? This means that the docking scoring function should give the ligand an energy score that is better than for (presumed) non-ligands. Both these questions are answered by Dockblaster.
If the docking protocol works, we can also request a docking screen of a large chemical library (which you are welcome to do if you want to learn more after the exercise).

Close the browser and return to your job as follows:
1. Point your browser to http://blaster.docking.org
2. In the DOCK menu, pick “Query a job”
3. Enter your job # (that you recorded when the job was submitted, Step D above)
4. If the job has finished, you should see a diagnostic table with color. If not, you will be told the status of your job.
5. The Summary Report of Calibration Docking presents the results of this docking in a compact table. As described above, DOCK Blaster uses two criteria to determine if docking works, which is quantified with two numbers. Each table entry has the two numbers separated by a forward slash (/): the pose fidelity as RMSD difference with the crystallographic ligand and the 0-origin rank compared to decoys (non-ligands). The results are color coded for clarity. Green is good, yellow is borderline, and red is bad.
6. To review the calibration diagnostics, click on “poses and scores”. A table will appear. Each link contains docking results, which you may browse by clicking on them. Look at all results. How do they differ?
7. To analyze a run in more detail, e.g. “Faster/ Scoring #1”: 
   - Left-click on the top left link in the new page (“ligand” under “Faster/ Scoring #1”).
   - Then left-click on “PYMOL” to open the docking result in PyMOL.
   - Compare how well the re-docked ligand (carbons in cyan) compares with the crystallographic one (carbons in magenta). Did the docking work?

When you have analyzed your receptor. Report your results to the other students by answering the results on the whiteboard (Is the best scoring scheme a GOOD, MEDIUM, or BAD result? Also print the jobid for your docking calculation). You can access the results for from other students by using the codes reported on the whiteboard. Analyze two other runs (not only GOOD, but also a BAD one). Save a picture of both ligands docked and put it in your report together with your below conclusions about the binding on the ligands.

**Answer the following questions in the report:**
1. Write and introduction about how molecular docking works (0.5 page) and also include a section about the approximations made, a description of the GPCRs, and the specific proteins that you have analyzed (0.5 page).
2. Include figures of re-docked ligands (and the co-crystallized ligand in the same figure) for three receptors (Figure 1A-C) and the root-mean-square (RMS) distance between them. Analyze the interactions between the receptor and the ligand. Why do these compounds bind (analyze crystal structure)? Does the docking capture these interactions? If not, what other interactions did the docked pose find? Did the program rank the ligand high compared to the decoys?

   **Hint for Figures:**
   Zoom in on the region you want to show and display the ligands in sticks, then write the following on the command line to get a white background and save a high-resolution image:
   ```
   set bg_rgb, (256, 256, 256)
   set ray_shadow,0
   ray
   save FigureX.png
   ```
3. What is the difference between the four different sampling/scoring schemes used by DOCK blaster?
4. We avoided four GPCR PDB codes: 4DKL, 2R4R, 4GRV, 1F88. Why?

**Hand in the report no later than the 20th of Jan (Sun after the exam)! If you pass the first time, you get one bonus point to the exam.**