CHEM 436 / CHEM 630: Molecular Modelling of Proteins
TUTORIAL #5: Molecular docking

INTRODUCTION

In this tutorial, we will learn the basic procedure for "docking" a flexible ligand into the binding site of a receptor. We will use AutoDockTools (http://mgltools.scripps.edu) to prepare PDBQT files of the receptor and the ligand. The PDBQT format is an extension of the PDB format that contains partial charges ("Q") and atom types ("T"). We will perform the docking calculation using AutoDock Vina (http://vina.scripps.edu), one of the simplest and most efficient docking software currently available.

The docking poses will be visualized using PyMOL and will be compared to those found in the crystal structures of similar proteins.

REQUIRED PRE-LAB READING

AutoDock Vina article:

http://dx.doi.org/10.1002/jcc.21334

PRE-LAB REPORT

- List a few of the residues of your enzyme known to interact with the substrate or to participate directly in the catalysis. Refer to them using the residue numbers from the PDB file "`step3_pbc-setup.pdb`" from Tutorial #4.
- Find an entry in the PDB that contains the ligand you will be using for the docking study (see below) in complex with a protein similar to yours. (You can simply search the PDB website for the three-letter code of the ligand.) Identify which residues of this structure correspond to the active-site residues of your enzyme you have just listed.

READING

Chapter 12 of Leach ("The Use of Molecular Modelling…"): Sections 12.1, 12.6, and 12.7.

REFERENCE MATERIAL

AutoDock Vina video tutorial:
http://vina.scripps.edu/tutorial.html

AutoDock Vina Manual:
http://vina.scripps.edu/manual.html

PROCEDURE

STEP 1: Save one frame of the trajectory into a PDB file

Load the PSF and DCD files from Tutorial #4 into VMD:

```
$ vmd ../step3_pbcsetup.xplor.ext.psf step5_production.dcd
```

Using menu "File > Save Coordinates…" save the last frame of the trajectory (example, 499) as a PDB file. Keep only the protein and its metal ligands (zinc, calcium, magnesium), using the following options:

• Selected atoms: “not water and not resname POT CLA”

• Frames: First = 499, Last = 499, Stride = 0

Save the file in a directory “`tutorial5`”, under the name “`nosolvent.pdb`”.

Note:
This tutorial makes the assumption that the best structure to dock the ligand into is that of the “naked” protein. In general, it might be useful to keep some water molecules at the active site, especially if they are thought to bridge the receptor and the ligand.

Note:
We also make the assumption that the last frame of the simulation represents a conformation of the receptor to which the ligand can bind. This may not necessarily be true, especially if *induced fit* is expected. In practice, one would have to perform flexible docking (which AutoDock Vina can do; see the manual) and possibly repeat the docking procedure on a large number of receptor conformations, sampled from the MD trajectory.

STEP 2: Prepare the PDBQT file for the receptor

Start AutoDockTools:

```
$ adt
```

Load file “`nosolvent.pdb`” (“File > Read Molecule” menu) and remove non-polar hydrogen atoms (“Edit > Hydrogens > Merge Non-Polar” menu).

To create the PDBQT file of the receptor, use menu “Grid > Macromolecule > Choose” and select the only molecule available: “nosolvent”. Save the united-atom structure under the name “`nosolvent.pdbqt`”.

STEP 3: Define the search space

Use menu “Select > Select From String” to highlight the residues identified in the PRE-LAB. For each residue, type its residue number and click on the button “Add”. (You should see yellow crosses on all atoms of the highlighted residues.)

Use the “Grid > Grid Box…” menu and adjust the spacing of the grid points to 1.000 Å (instead of the default 0.375 Å). Use 20 grid points in all three directions — so that your search space will be a cubic volume of 20 Å of side.

Adjust the three “offset” dials such that all highlighted residues are inside the search space. Write down the values of “x center”, “y center”, and “z center”. (They will be used in STEP 5.)

STEP 4: Prepare the PDBQT file for the ligand

Go to the “Ligand Expo” webpage (http://ligand-expo.rcsb.org/ld-search.html) and search for the component identifier (3-letter code) appropriate for your enzyme:

• Glutamate racemase: 003 (“zero, zero, three”)

• Matrix metalloproteinase: RS2

• Carbonic anhydrase II: BZU

• Dihydrofolate reductase: FOL

• NS3 protease: NDL

• Beta-lactamase: TBE

Click “Go” and download the ideal coordinates of the atom in PDB format. This file should be named “`???_ideal.pdb`”. Write down the chemical details of that component: name, structural formula, formal charge, and InChiKey descriptor.

Load the ligand PDB file in your AutoDockTools session using the “Ligand > Input > Open…” menu.

Identify the rotatable bonds using the “Ligand > Torsion Tree > Choose Torsions…” menu. (The rotatable bonds are colored in green.) You could in principle make any “non-rotatable” bond “rotatable” by clicking on it, but we will not add more than what is identified by AutoDockTools.

Click “Done” and save the ligand in PDBQT format using the “Ligand > Output > Save as PDBQT…” menu.

STEP 5: Run AutoDock Vina

Create a file “`conf.txt`” containing the following text, with the question marks (“???”) replaced by the values you chose in STEP 3 and STEP 4.

`conf.txt`

```
receptor = nosolvent.pdbqt
ligand = ???_ideal.pdbqt

out = all.pdbqt
log = all.log

size_x = 20
size_y = 20
size_z = 20
center_x = ???
center_y = ???
center_z = ???

exhaustiveness = 12
```

Run AutoDock Vina:

```
$ vina --config conf.txt
```

This calculation should take less than 5 minutes.

✦ Report the affinity values of all binding modes.

STEP 6: Load the docking results in PyMOL and compare them to the structure of a similar protein-ligand complex

Start PyMOL and open the following files:

- `nosolvent.pdb`
- `all.pdbqt`

✦ Produce a figure showing the best-scoring pose of the ligand and its interaction with the active site residues. Represent the ligand and the interacting side chains using sticks, and highlight any polar contact.

Load the PDB file of the protein-ligand complex you have identified in the pre-lab and align it to your own protein. Align the two protein structures using the residues you have identified in the pre-lab, so that the binding sites are superimposed.

- ✦ Produce a second figure equivalent to the first one, but for the known protein-ligand complex.
- ✦ Produce a third figure in which you superimpose the two proteins structures. For the complex obtained with AutoDock Vina, choose the binding pose that is the most structurally similar to that from the crystal structure. (It may not be the best-scoring pose.)

INSTRUCTIONS FOR THE LAB REPORT

The procedure is fairly straightforward, so keep the report short.

Motivate your choice of the active site using the scientific literature.

Discuss the best docking pose(s) in reference to the scientific literature. As explained above, find published protein structures that contain the ligand you used for the docking study and see how the poses obtained from AutoDock Vina compare to the poses found in those crystal structures. Compare the poses in terms of polar contacts and hydrophobic contacts formed between the ligand and the receptor.

Discuss the non-optimal poses (that have lower affinities) the same way—in terms of the polar and hydrophobic contacts *missing*.