

Autodock – Tutorial

AutoDock4 (<http://autodock.scripps.edu>) is a suite of programs that predict how small molecules bind to a receptor of known 3D structure. We will use **autodock4** to dock a ligand to a set of grids describing the receptor protein; **autogrid4** to calculate these grids; and the graphical interface called **adt** ([AutoDockTools](#)) to set up the files for input to autogrid4 and autodock4. We will use **maestro** to prepare files for AutoDock and to evaluate results.

Summary of tasks:

Structure file preparation – PDB files for the receptor and ligand (maestro)

Receptor – define the active site as a 3D grid, assign atomic charges (adt)

Ligand – define the bonds that can rotate, assign atomic charges (adt)

Receptor – calculate the map files which represent the binding pocket as an energy grid (autogrid4)

Ligand – dock the ligand into the energy grid (autodock4)

Analyze data from docking (maestro, gedit, adt)

The starting point for AutoDock is a clean PDB structure of the receptor. A clean structure will have disorder resolved, disulfide bonds checked and repaired, hydrogens added, waters and any cocrystallized entities removed. This kind of cleanup can be done in a variety of programs including **adt** but we will use **maestro**. In addition, we will use maestro to make a PDB file for the ligand. This example looks at an acetylcholine receptor and various small molecules that act on it.

http://www.pdb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb71_1.html

ACHBP (acetylcholine binding protein) represents the extracellular binding domain of nAChR (nicotinic acetylcholine receptor).

There will be quite a few files created in this exercise so it is a good idea to create a new folder and change your command line prompt so you are working in this new folder. Then use a web browser to go to the PDB (<http://www.pdb.org>) and search for ACHBP. Download 1UW6.pdb into your working folder. Then start **maestro** and import 1UW6. [*Note*: Many PDB files have “alternate locations” reflecting disorder in the structure. The maestro import window allows you to deselect reading those alternate locations. That is not an issue for 1UW6 but it may be for many PDB files.]

We are only interested in Chain A, Chain B and the nicotine in between the 2 chains.

[*Hint*: As you move the cursor over the molecule, information about the chain:residue:residue number: element: atom name: entry name is displayed along the bottom of the workspace. For example, you might see L:HOH 1011:(O)O: X-ray structure..... That means Chain L, water, residue 1011, etc.] To delete by category, click and hold down the **X** icon. Delete waters, then delete chains C through T (you must click on the chains). Then delete the nicotine molecule/residue on the exposed side of Chain B. Duplicate (ungrouped) the trimmed structure twice so you have a total of 3 copies in the project table.

Now delete the nicotine from 1 copy. From the other copy, remove everything except the nicotine. Rename your entries in the project table to reflect the changes. Now there should be 3 entries in the table: the original entry that is 1UW6 A&B + nicotine, one entry that is 1UW6 A&B (no nicotine), and one entry that is just nicotine (no 1UW6). [**** You can rotate and translate the molecules as usual but do not use the tile icon or the global/local transformation icon. We will use the coordinate system from the original PDB file and those icons move molecules out of their original coordinate system.]

The 1UW6 A&B (no nicotine) structure is our receptor. It lacks hydrogens and possibly SS bonds. Display only the Cys residues and check the SS bonds; repair if necessary. [Hint: Display Menu – Display/Undisplay Atoms – Display Only – Select – Residue Tab – Residue Type – Cys – OK.]

Delete any residues that are numbered negative or zero. [Hint: Display – Sequence Viewer – and then move the cursor over the Sequence Viewer to show residue names and numbers. If you find a residue zero or negative, click on it in the Sequence Viewer. It will highlight in yellow in the workspace.]

Add hydrogens and then export this as 1uw6_ab.pdb (on the Export window – make sure you export from the right source – probably Workspace, not Selected entries!). This pdb file is the receptor structure we will use in AutoDock. [Reality Check: there are 6471 atoms in the correct structure.]

Now we need a file for the ligand. Display your nicotine entry in the workspace. The nicotine structure lacks double bonds so you will have to correct that structure and then add hydrogens. Hint: Display – Build Toolbar and then use the single to double bond icon. Use Pubchem if you are not certain what nicotine should look like. When you are done, export this as nicotine.pdb.

One last thing to note before finishing with the nicotine structure is its location. Find the xyz coordinate for one of the atoms on the ring connection bond. [Hint: Display - Atom Labels - xyz coordinate.] This is an estimate for the center of the active site and we will need that for Autodock.

Save the Project if you have not already done so. We are going to move onto AutoDock but we will use Maestro for evaluating the docking results in the context of the original PDB structure. Close Maestro. Make sure all of your docking files are in the current folder.

Autodock4

ADT will prepare 4 separate files which are the inputs for autogrid4 and autodock4. These 4 files are: rec.pdbqt, rec.glg, lig.pdbqt, lig.dlg.

Step 1 – preparation of rec.pdbqt – This is a file with the receptor geometry and charges.

Type **adt** to start Autodock Tools. [Hint: See last page of this document for adt mouse/keyboard tips.]

File – Read Molecule – 1uw6_ab.pdb

Edit – Hydrogens – Merge Nonpolar

Grid – Macromolecule – Choose (1uw6_ab_model1)

When prompted, save as rec.pdbqt

Step 2 – preparation of lig.pdbqt - This is a file with the ligand geometry and charges.

Display- Show/Hide Molecules - uncheck 1uw6_ab_model1. (this will clear the workspace)

Ligand – Input – Open (file type PDB) – nicotine.pdb

Press the N key to normalize the screen view (you wont see the H atoms or double bonds).

Ligand – Torsion Tree – Detect Root

Ligand – Torsion Tree – Choose Torsions

(Nicotine only has one rotatable bond so one can just click “Done”. In many other molecules you have to be very careful and choose only the rotatable bonds you really want.)

Ligand – Output – Save as PDBQT – lig.pdbqt

Step 3 – preparation of rec.gpf – This is a file defining the receptor grid.

Grid – Set Map Types – By Choosing Ligand (nicotine_model1) - Accept

Grid – Grid Box

Set the Center Grid Box to the xyz coordinate value for nicotine that you obtained earlier from Maestro (mine was 84, 69, -10). Change the grid points to 60 in each xyz direction. [*Note:* The spacing between point is 0.375A so the grid is $60 \times 0.375 = 22.5A$ per side. For larger ligands, or to consider a larger region of the receptor, increase the number of points.] In any case, make sure the ligand is fully in the box. You can adjust the x,y,z values to optimize this.

On this Grid Options window, File - Close Saving Current.

Grid – Output -Save GPF – rec.gpf

Step 4 – preparation of lig.dpf – These are the parameters for the ligand docking.

Docking – Macromolecule – Set Rigid Filename – rec.pdbqt

Docking – Ligand – Choose – nicotine_model1 – Accept (default setting are fine)

Docking – Search Parameters – Genetic Algorithm - Change the number of GA runs to 100 - Accept.

Docking – Output – Lamarkian GA – lig.dpf

[*Note:* The settings here instruct autodock4 to use a genetic searching algorithm to try 100 times to optimize the ligand with respect to the receptor grid. It is a statistical sample and the choice of 100 is arbitrary. Larger grids or ligand with many rotatable bond should use more samples.]

File – Exit (we're done with ADT).

Now we'll run autogrid4 and autodock4 on the command line. [*Note:* autogrid takes about 2 minutes; autodock takes about 15 minutes. Wait for autogrid to finish before running autodock.]

autogrid4 -p rec.gpf -l rec.glg

autodock4 -p lig.dpf -l lig.dlg

Type the command: **clean_docking lig.dlg**

[Note: clean_docking was written in the MGCF so tell Kathy Durkin if you would like changes.]

The command takes the lig.dlg file and creates lig_out.pdb. lig_out.pdb contains the clustered hits from the docking calculation and can be read into Maestro. [Note: In our calculation, the raw data has 100 hits. Autodock4 automatically clusters the raw hits, locating duplicates and recategorizing these into a smaller number of distinct hits.]

Both lig.dlg and lig_out.pdb are text files that can be opened with gedit. Doing so will allow you to obtain Binding Energy data and review the clustering.

Read in the lig_out.pdb file into Maestro and compare the output of nicotine docking to the crystal structure. [Note: I found only one hit and it is shifted off a little from the xray. The difference is small compared with the resolution of the structure however there is also some evidence from other sources that there is a water required in the pocket.]

If you want more practice you can rerun the tutorial but this time don't delete all of the waters from 1UW6. Find water number 1090 in the binding pocket. Delete all of the other waters except 1090. Now when you prepare the receptor.pdb file, make it contain Chain A + Chain B + water 1090. You can reuse the lig.pdbqt and lig.dlg files from before and keep the same Maestro Project Table. That way you can compare results with and without the water in the active site.

You can also download other structures from PubChem (SDF – Save). Epibatidine, imidacloprid and cocaine are interesting possibilities. In Maestro, File - Import Structures – SD format. Use MacroModel to minimize the geometry since SDF files are planar. Dock these other structures into 1UW6 and assess their activity as potential ligands for this receptor.

[Hint: A big time saver can be to reuse rec.* files for multiple ligands. In the Step 3 section where you chose Grid – Set Map Types – By Choosing Ligand, instead use Grid – Set Map Types – Directly. Add atom types sufficient to cover a range of ligands so that you only have to do the rec.pdbqt and rec.gpf preparation and autogrid4 steps once. For example, if we had chosen to Set Map Types – Directly and added in Cl, we would be ready to run imidacloprid and epibatidine which have Cl atoms, performing only Step 2 and 4 and the autodock command for each of the new ligands.

The original Autodock papers reported on docking HIV protease inhibitors. This is a good system for study. Morris, G. M., Goodsell, D. S., Halliday, R.S., Huey, R., Hart, W. E., Belew, R. K. and Olson, A. J. (1998), [Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function](#) *J. Computational Chemistry*, **19**: 1639-1662. Huey, R., Morris, G. M., Olson, A. J. and Goodsell, D. S. (2007), [A Semiempirical Free Energy Force Field with Charge-Based Desolvation](#) *J. Computational Chemistry*, **28**: 1145-1152.

You might have a three-button mouse. If so, the mouse buttons can be used alone or with a modifier key to perform different operations. To zoom the molecule (make the molecule look bigger or smaller) in the viewer window, press and hold down the <Shift> key and then click and drag with the middle mouse button. To rotate the molecule, just click and drag with the middle mouse button. To summarize what the mouse buttons do:

Modifier	Left	Middle	Right
None	<u>Pick</u>	<u>Rotate</u>	<u>Translate left/right (X) and up/down (Y)</u>
Shift		<u>Scale or Zoom</u>	<u>Translate in/out (Z)</u>

You can also press the following keys in the viewer window to change the view of the molecule:

Key	Action
R	<u>Reset view</u>
N	<u>Normalize</u> – scale molecule(s) so all visible molecules fit in the viewer
C	<u>Center</u> on the center of gravity of all the molecules
D	Toggle on/off <u>Depth-cueing</u> (blends molecule into background farther away)