# Using AutoLigand with AutoDockTools

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## *Introduction*

**AutoLigand** identifies and characterizes ligand binding sites in a macromolecule, using the grid-based energy evaluation method in **AutoGrid**. This tutorial will show you how to use AutoLigand with the command line interface and interactively with **AutoDockTools**, or **ADT**. Previous experience with ADT and AutoGrid is recommended.

**You can run AutoLigand two ways.** (1) The AutoLigand GUI generates a single fill starting at the user-specified point. (2) When AutoLigand is run from the command line, the code runs a single fill if input coordinates are given or scans the entire grid box and outputs the ten best fills if no input starting point is given.

**AutoLigand uses special AutoGrid grids.** AutoLigand is designed to use three atom type grids (carbon, oxygen and hydrogen) along with the electrostatic (e) and desolvation (d) grids, all calculated with a 1 Å grid spacing. Note that since docking is normally run using a 0.375 Å grid, it is best to set up a subdirectory for the AutoLigand map files and results, to keep them separate from docking studies. It is often easiest to put the receptor PDB file, the grid maps, and AutoLigand results all in the same subdirectory, to simplify the visualization of results.

## Exercise One: Preparing the Grid Maps.

The grid parameter file tells AutoGrid which receptor to compute the potentials around, the types of maps to compute and the location and extent of those maps. For AutoLigand only the C, OA, and HD maps are used plus an electrostatics map and a separate desolvation map, all calculated with a 1 Å grid spacing.

1. Start **AutoDockTools** by clicking on the ADT icon.

2. **Grid** → **Macromolecular** → **Open**

> Select **1hsg.pdbqt** and click **Open**. This will read coordinates that were previously prepared in ADT to include hydrogen atoms and charges.

> It will ask you if you want to keep the charges. Select the **Yes** button, and then **OK** on the warning popup.

3. **Grid** → **Set Map Types** → **Directly...**

> Edit the selections in the **AutoGpf Ligand** widget to include only C, HD, and OA.

> Close this widget with the **Accept** button.

4. **Grid** → **Grid Box...**

> Opens the **Grid Options Widget**. The goal is to generate a grid box that encompasses the entire receptor molecule at a 1 Å spacing.

> Set the **Spacing** to 1 Å. You can use the thumbwheel, or hover over the thumbwheel and type in the value.

> Adjust the number of points of the x y z wheels to cover the entire molecule: **60**, **60**, **60** should be enough in this case.

> Close this widget by clicking **File** → **Close saving current**.

5. **Grid** → **Output** → **Save GPF...**

Opens a file browser allowing the user to specify the name of the grid parameter file. The convention is to use '.gpf' as the extension.

Write the GPF as: `1hsg.gpf`

6. `Run` → `Run AutoGrid`

Click the `Launch` button to start the grid calculation.

Starts the AutoGrid 4 job. On most platforms, this opens a Process Manager that allows you to see specifics about current AutoGrid and AutoDock jobs. It is a limited process manager that you can use to terminate an AutoGrid or AutoDock process by selecting its entry. You are asked if you really want to kill it.

(The AutoGrid 4 calculation takes about 2 minutes on a Mac.)

Please note that you can easily start a job from the command line:

`./autogrid4 —p 1hsg.gpf —l 1hsg.glg &`

7. When the grids are finished, stop ADT: `File` → `Exit`

## *Exercise Two: Running AutoLigand via Command Line.*

To run AutoLigand and find the ten best binding sites, type the following in the terminal window:

```
./pythonsh AutoLigand.py 1hsg 100 > 1hsg100.log
```

where the 1hsg is the base name of the map files and 100 is the number of fill points to use (for this exercise).

The command takes several minutes to run depending on the number of fill points you have requested (500 points can take hours).

The code generates up to ten FILL files and one Results file. Some of the top ten fill results may collapse to the same answer, thus reducing the number of fills to less than ten. The results are listed in rank order from best Total Energy per Volume of each of the fills generated.

Each fill file is output as a PDB file:

```
FILL_100out#.pdb
```

where 100 is the number of points in the fill and out# is the rank of that fill.

When the calculation has finished, look at the log file by typing

```
more 1hsg100.log
```

The log file includes values for the volume of each point, and the energy per volume (EPV) for each point. The volumes will probably be slightly different, since the volume depends on the shape of the fill points (long, thin fills will have larger volumes than compact fills).

The results may be visualized in ADT:

1. Start ADT by clicking on the icon.

2. `File` → `Read Molecule`

Read all of the `FILL_100out*.pdbqt` files by clicking on the first one, then shift-click on the last one.

`File` → `Read Molecule`

Choose `1hsg.pdbqt` to read the protein coordinates.


3. Use the Dashboard to change the display

> If you prefer, you can undock the Dashboard into a separate window by clicking on the Dashboard icon, third from the right.

> *Each FILL file and the protein coordinates are included as a line in the dashboard. The circles change the representation, the diamonds change the color, and the show/hide buttons may be used to turn each one on and off.*

> For **All molecules** (the top line), click on **Chain** coloring.

> For **1hsg** (at the bottom), click on the **Lines** circle to turn off lines, and then on the **Ribbons** circle to turn on ribbons.

4. Notice that fills are found in the active site, in two exosites on either side, and in several grooves on the surface. You can toggle the **CPK** button on and off to identify each fill.

5. When you are finished, stop ADT: `File` → `Exit`

## *Exercise Three:  Running AutoLigand in ADT.*

In this exercise we will use the Graphical User Interface to run several fills in the active site, finding the optimal volume for the ligand.

1. Start ADT by clicking on the icon. Then, set the default directory:

> File → Preferences → Set → StartupDirectory

> Change to: /home/user#/Desktop/AL_tutorial

> And click: Set

2. File → Read Molecule → 1hsg.pdbqt

> You can use the dashboard to change to a ribbon representation if desired.

3. File → Browse Commands

> Under Select a Package, click AutoDockTools

> Under Select a Module, click AutoLigand Command

> click Load select module and then OK

> This will load the AutoLigand commands into ADT

4. Compute → AutoLigand → Run AutoLigand...

> This opens the widgit and shows a box around the space which can be searched.  The File Base Name is pre-filled with the name of the map files (1hsg in this case) and set to 100 points.

> The start location is defaulted to the center of the grid box or the last start point used.  There are three ways to change the starting point:

> 1.  Shift-click on an atom next to where you wish to start filling.

> 2.  Enter the X Y Z coordinates

> 3.  Adjust the slider bars

> Make sure the crosshair is in the middle of the active site.

> Then, make sure the number of points is 100 and click OK

*[Important Note: if other fills are run at the same size, the fills will be overwritten. Use different number of points, or rename the FILL file if you wish to run the GUI again and keep your first results.]*

5. `Compute` → `AutoLigand` → `Run AutoLigand...`

Repeat the calculation using the same start position as the last run, for 200, 300, and 400 points. Write down the energy per volume (EPV) values for each size, and determine the optimal size:

100 points _____

200 points _____

300 points _____

400 points _____

6. Use the Dashboard to display only the best fill

7. `File` → `Read Molecule` → `indinavir.pdb`

This reads in the structure of the drug indinavir bound in the active site. You can use the dashboard to change the color and representation if desired.

8. Compare the shape of the fill and the shape of the drug. This may be used to suggest modifications of existing inhibitors to improve binding.