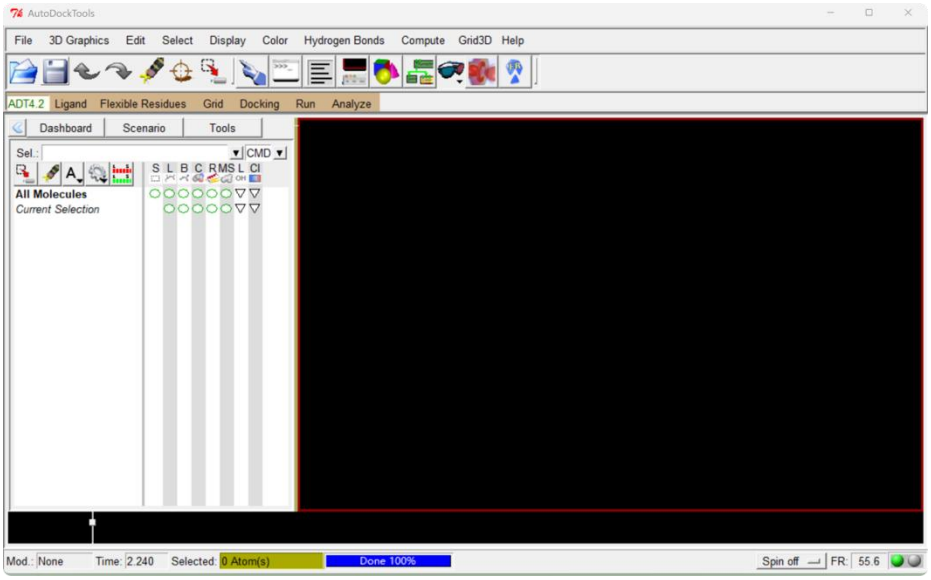
Case study for EPSDock

1、Experimental Environment:

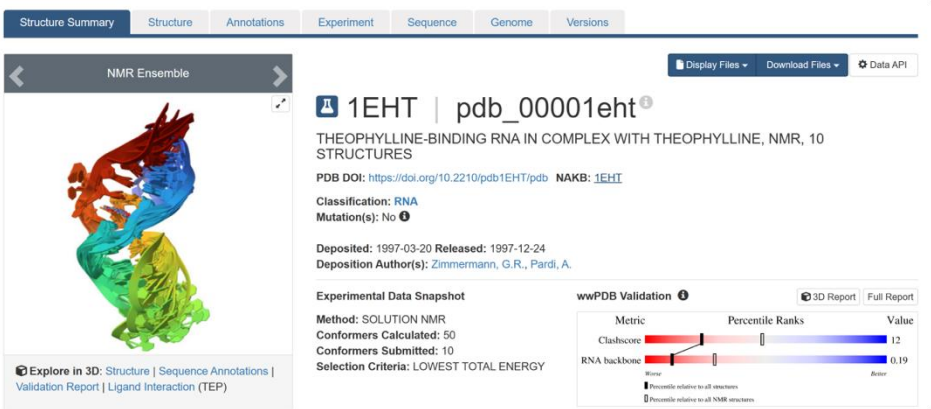
Windows 11 and Ubuntu 18.04

Install MGL Tools in the Ubuntu 18.04 environment:

Download link: https://ccsb.scripps.edu/mgltools/downloads/

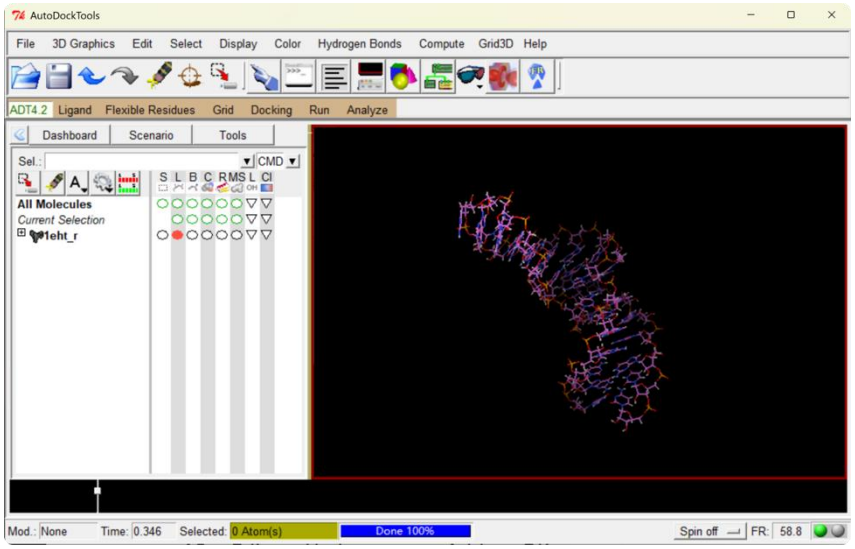


2、Download PDB file for RNA target (use PDB ID: 1EHT as an example).



3、Prepare the RNA receptor PDB file.

In Windows, use PyMOL to extract the receptor macromolecule (1eht_r) and the ligand small molecule (1eht_l) from the 1eht co-crystallized compound, remove water molecules from the environment, and save the files in .pdb format as 1eht_r.pdb and 1eht_l.pdb. Open the receptor PDB file using the ADT (AutoDock Tools) software.



4、Use ADT to preprocess the RNA receptor file

1） Preparing receptor and ligand files using AutoDock Tools (ADT) software:
Go to **File** → **Preferences** → **Set** → **Startup Directory** → Set it to the folder containing the receptor and ligand files → Click **Make Default** → Close the window.

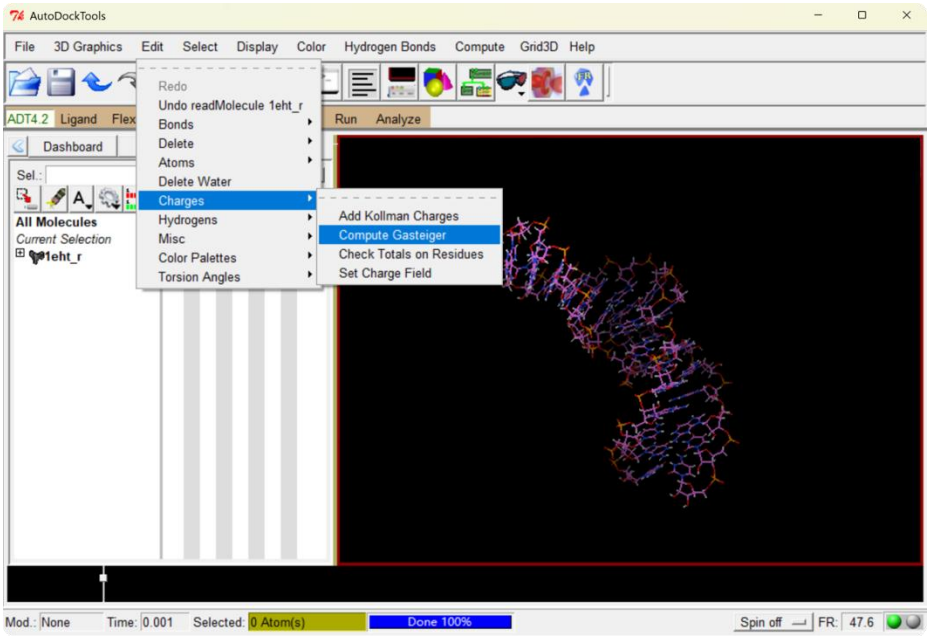
2) Go to **File** → **Preferences** → **Set** → **Startup Directory** → Set it to the folder containing the receptor and ligand files → Click **Make Default** → Close the window.

3) Go to **File** → **Read Molecule** → Select the receptor macromolecule .pdb file → Click **Open**.

4) Go to **Edit** → **Hydrogens** → **Add** → Click **OK**.

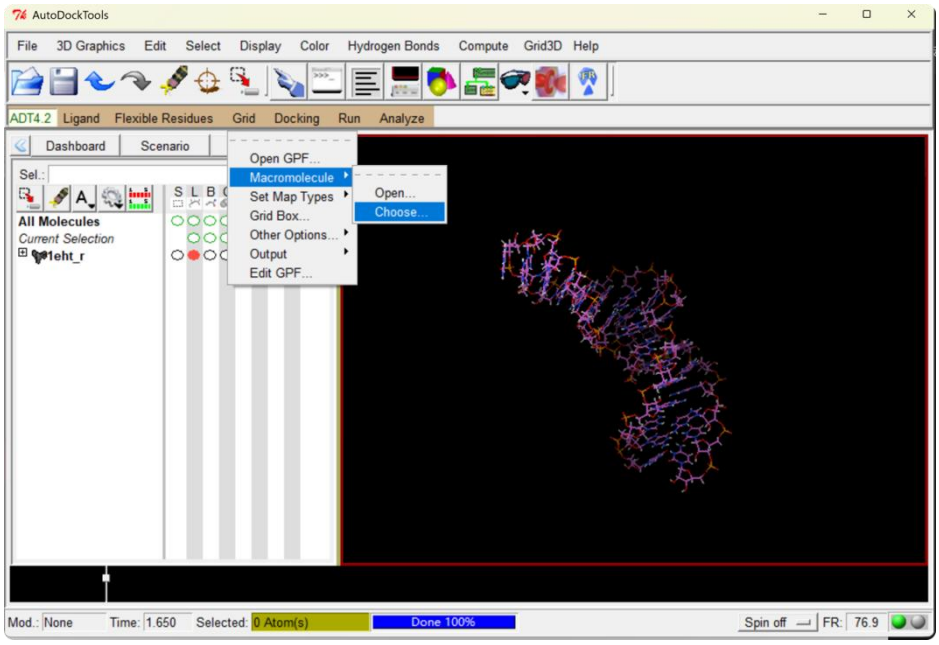
5) Go to **Edit** → **Charges** → **Compute Gasteiger** → Click **OK**.

6)Navigate to **Edit** → **Charges** → **Compute Gasteiger** → Click **OK**

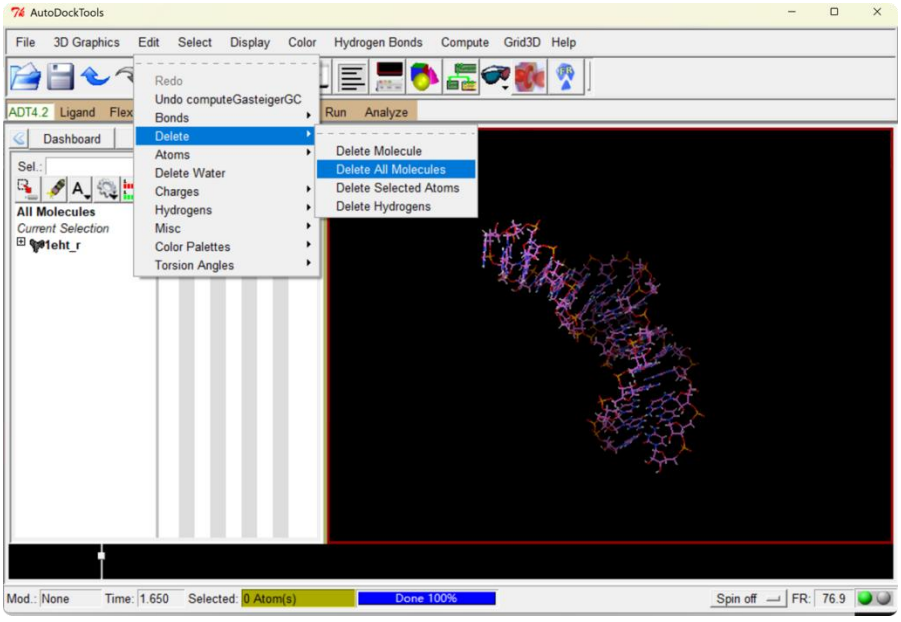


5、Use Grid to prepare the RNA receptor and ligand file

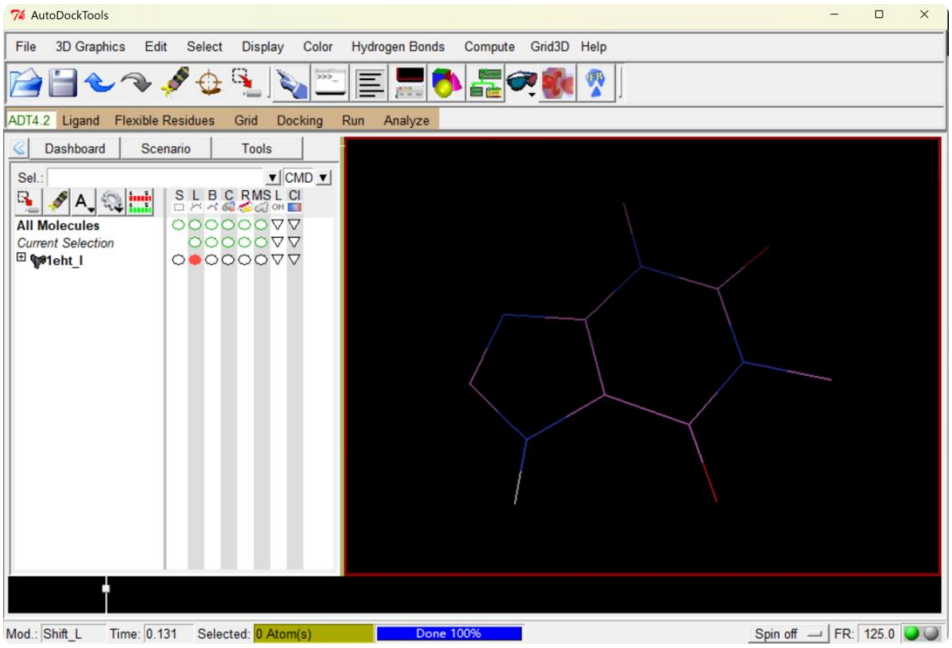
1) Go to **Grid** → **Macromolecule** → **Choose** → Select the receptor molecule → **Select Molecule** → Click **OK** → Save to the working folder → **Save**.



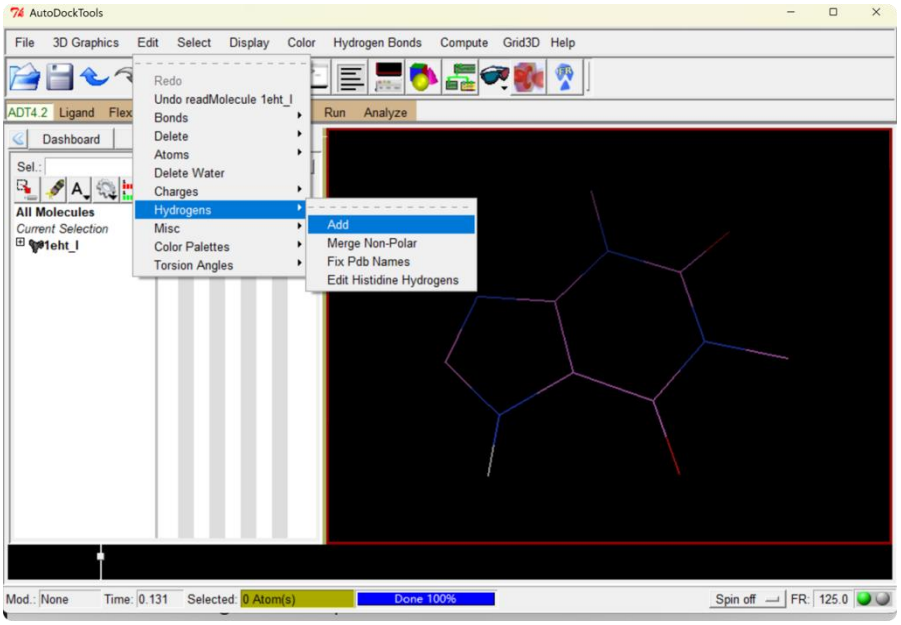
2) Edit—>Delete All Molecules—>CONTINUE;



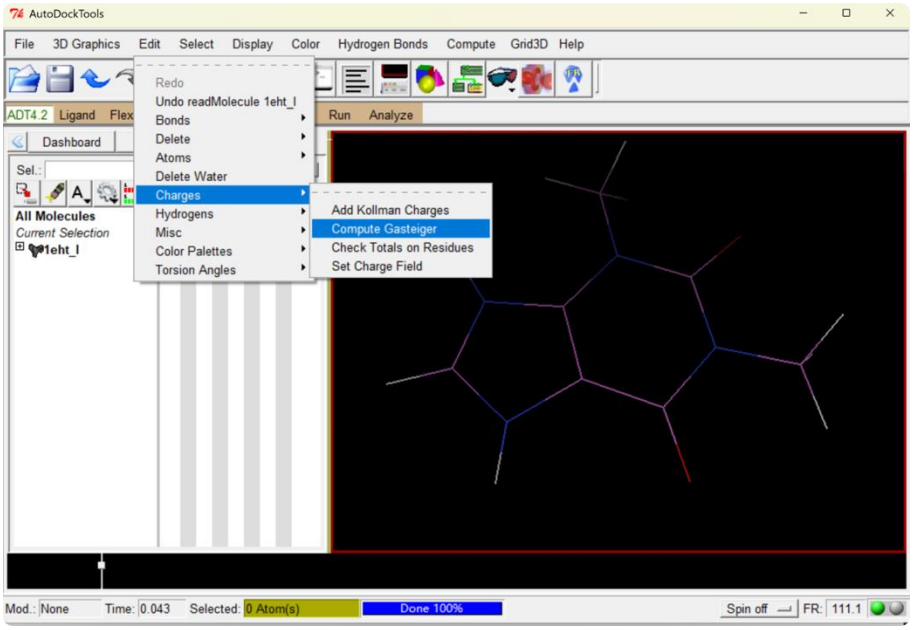
3) Go to **File** → **Read Molecule** → Select the ligand molecule **1eht_l.pdb** → Click **Open**.



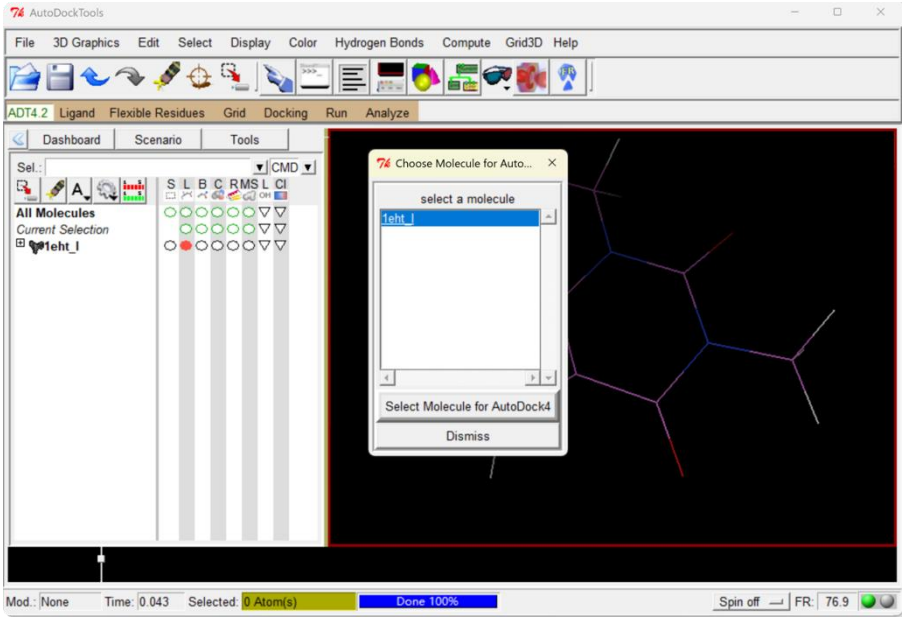
4) Edit—>Hydrogens—>Add—>OK;



5) Go to **Edit** → **Charges** → **Compute Gasteiger** → Click **OK**

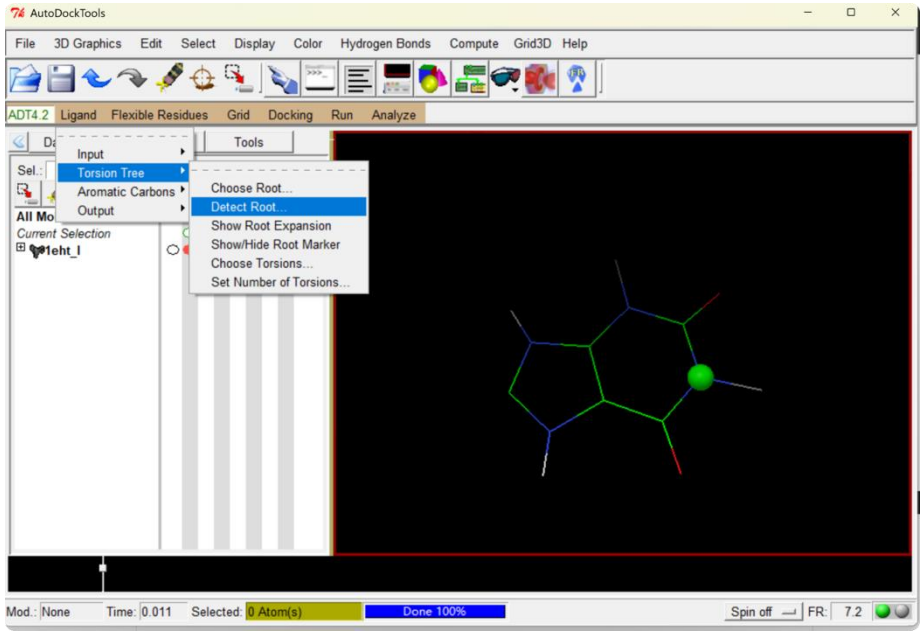


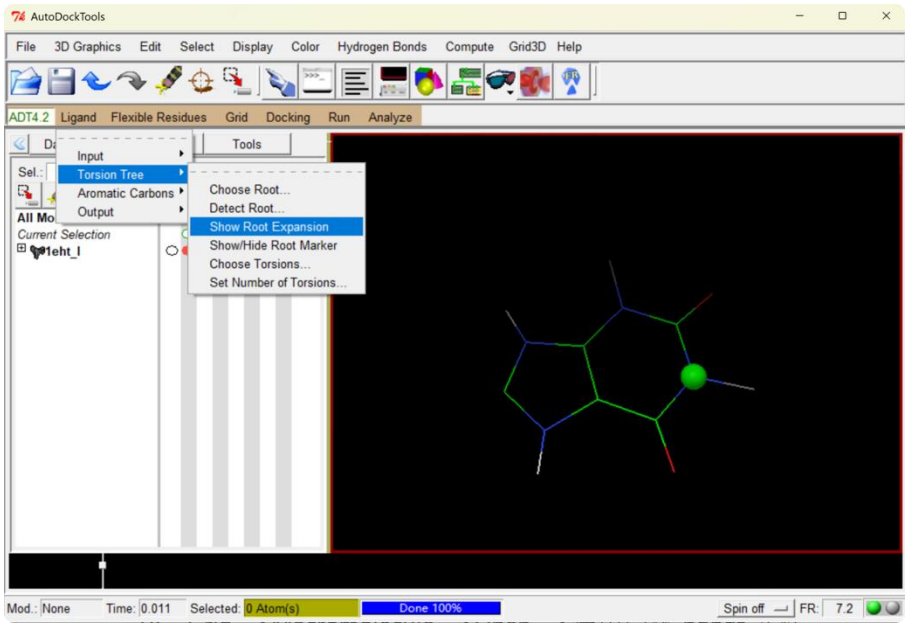
6) Go to **Ligand** → **Input** → **Choose** → Select the ligand molecule → **Select Molecule for AutoDock4** → Click **OK**



7) Ligand—>Torsion Tree—>Detect Root；

8)

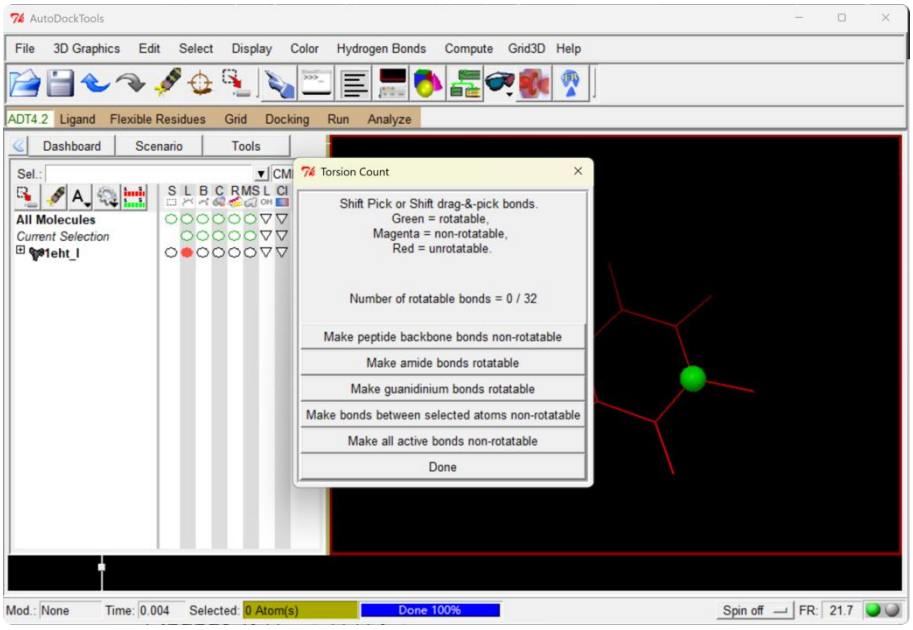




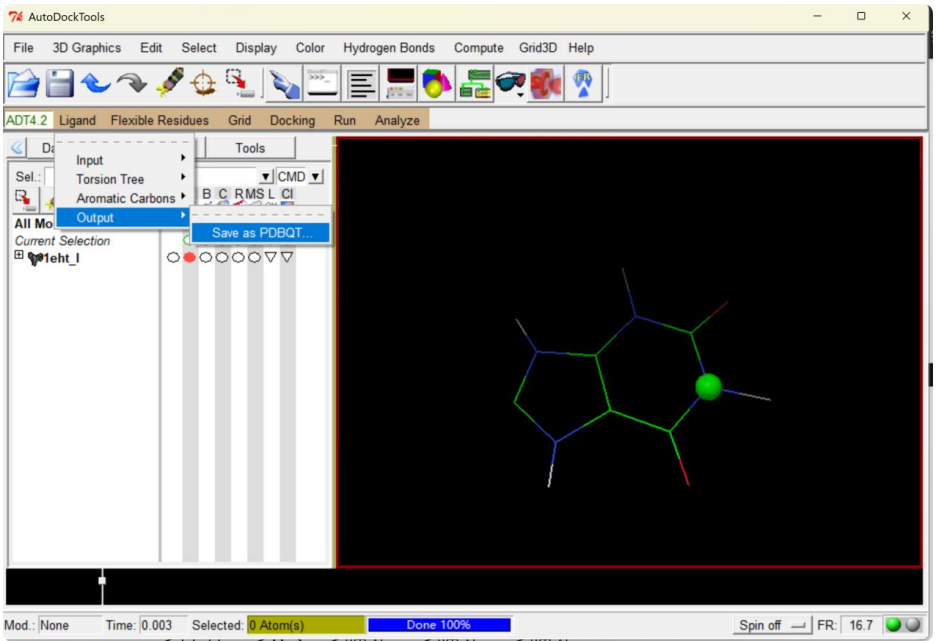
Ligand—>Torsion Tree—>Show Root Expansion；

9) Ligand—>Torsion Tree—>ShowHide Root Marker；

10) Ligand—>Torsion Tree—>Choose Torsions—>Done；

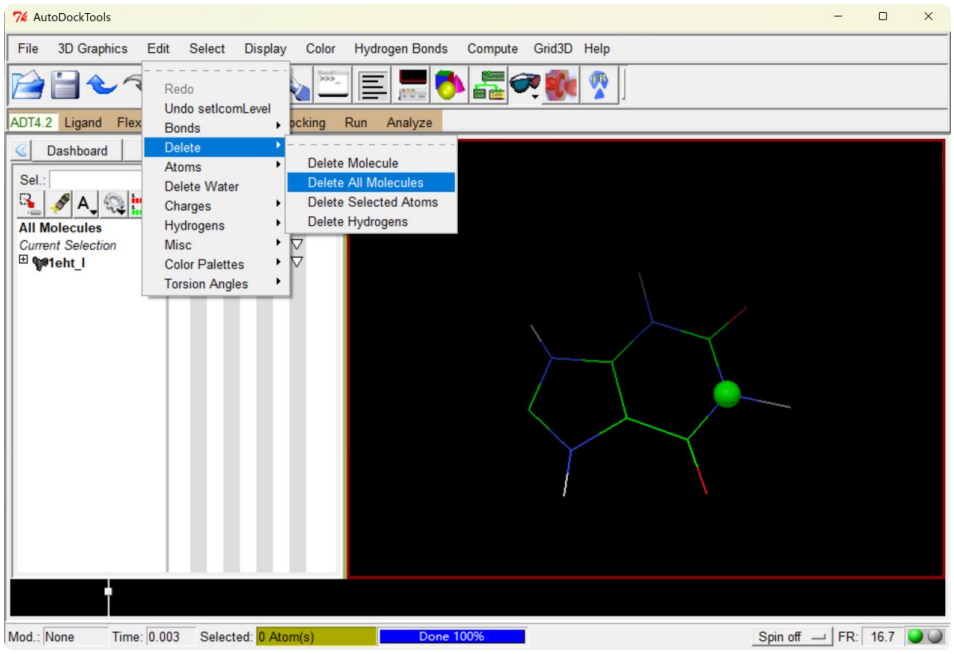


11）

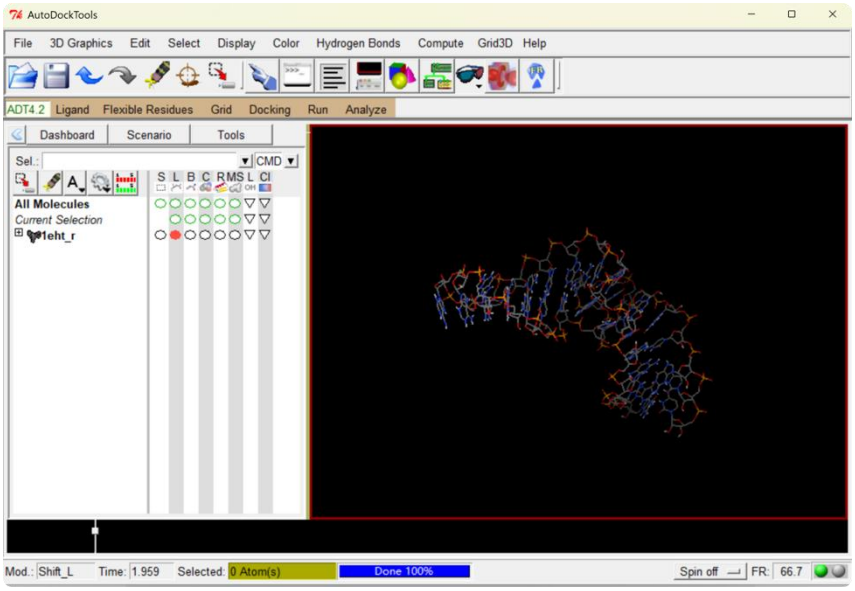


Ligand—>Output—>Save as PDBQT;

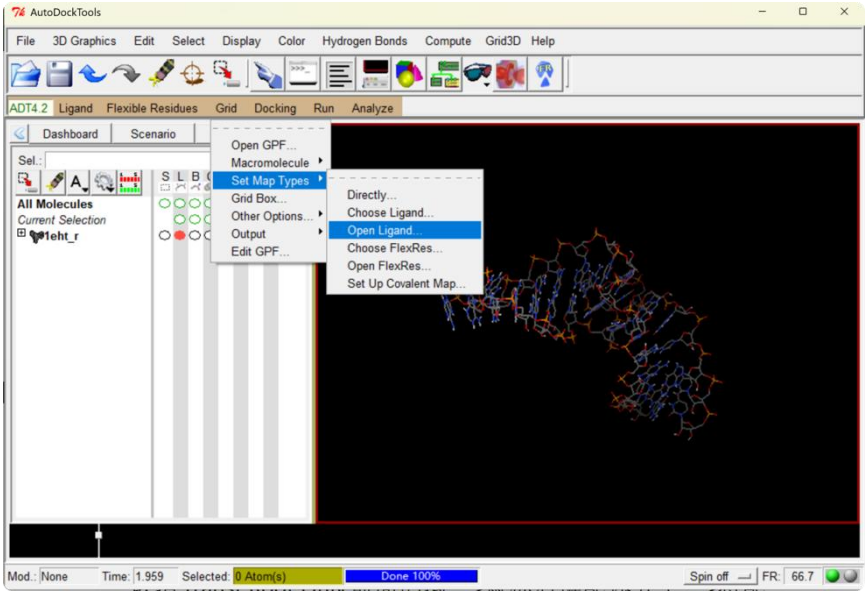
12）Edit—>Delete All Molecules—>CONTINUE;



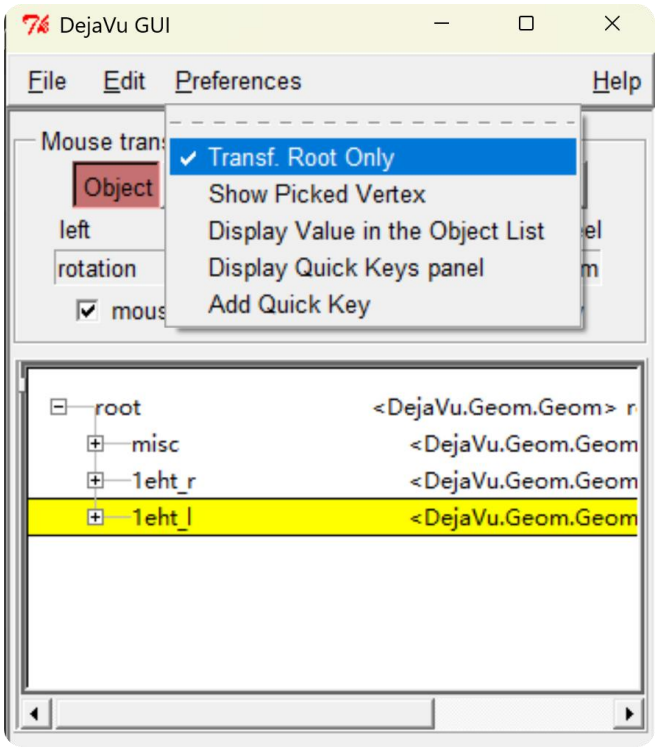
13）Go to **Grid** → **Macromolecule** → **Open** → Select the receptor .pdbqt file → Click **Open** → **Yes** → **OK** → **OK** → **OK**.



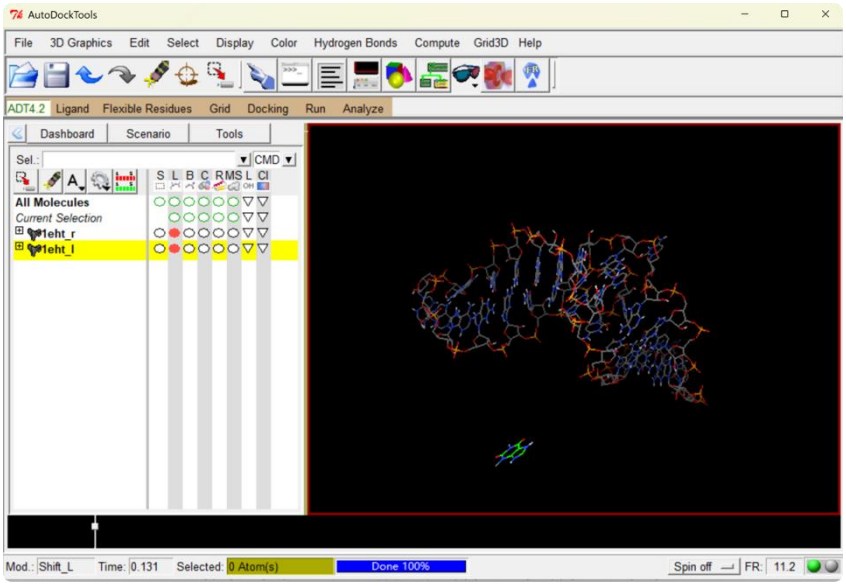
14) Go to Grid → Set Map Types → Open Ligand → Select the ligand molecule .pdbqt file → Click Open.



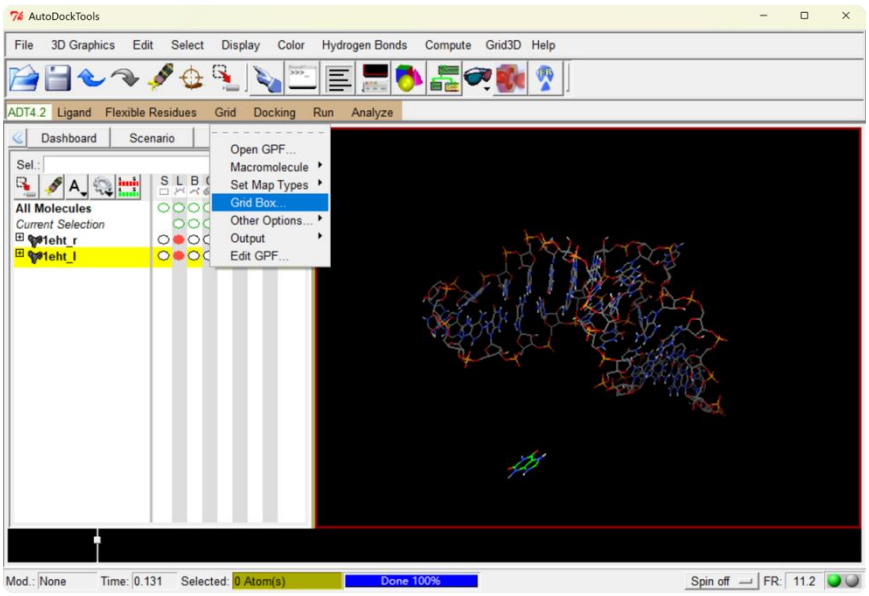
15） In the DejaVu GUI, navigate to the root object → Select the ligand molecule → Open Preferences → Uncheck the box next to "Transt Root Only" → Right-click the ligand molecule → Move the ligand molecule outside of the receptor molecule → Open Preferences again → Check the box next to "Transt Root Only".

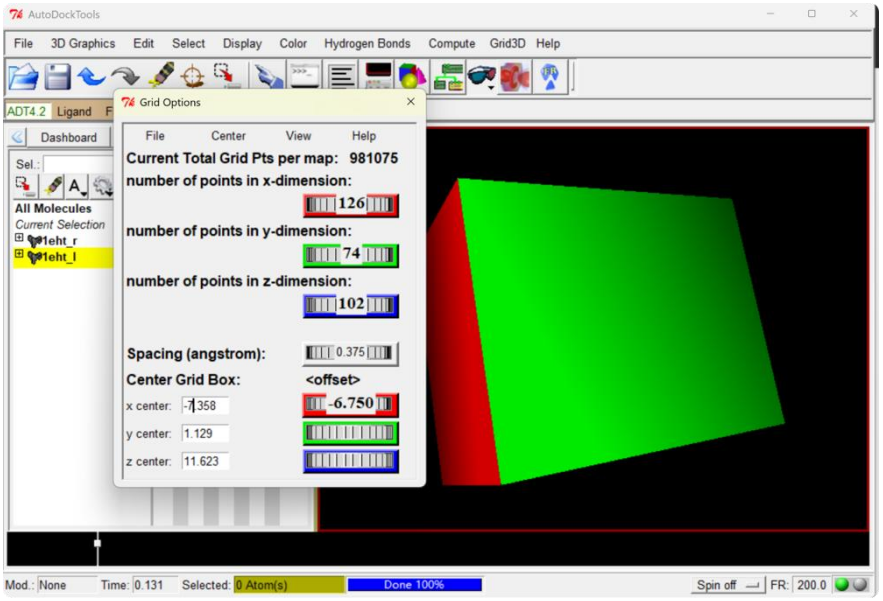


16） Go to Grid → Grid Box → Adjust the box size to enclose the entire protein → File → Close Saving Current.

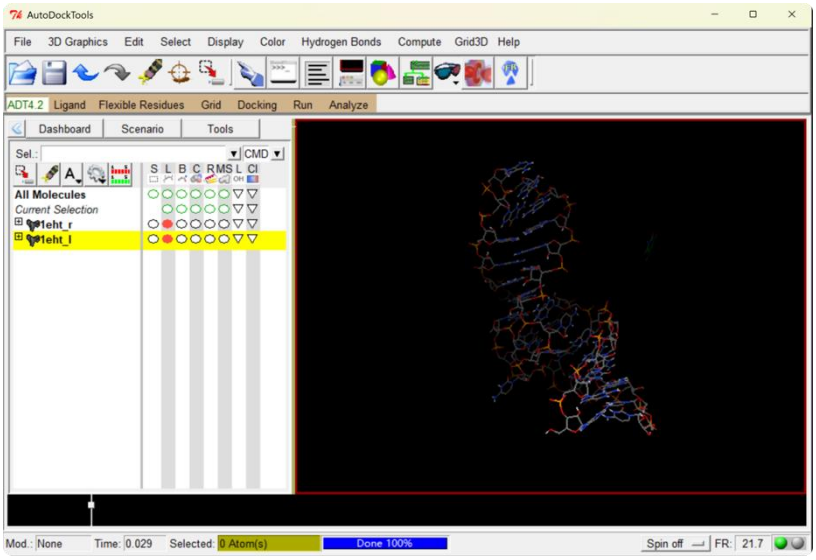


17） Navigate to Grid → Grid Box → Adjust the box size to ensure it encloses the entire protein → Go to File → Select Close Saving Current.



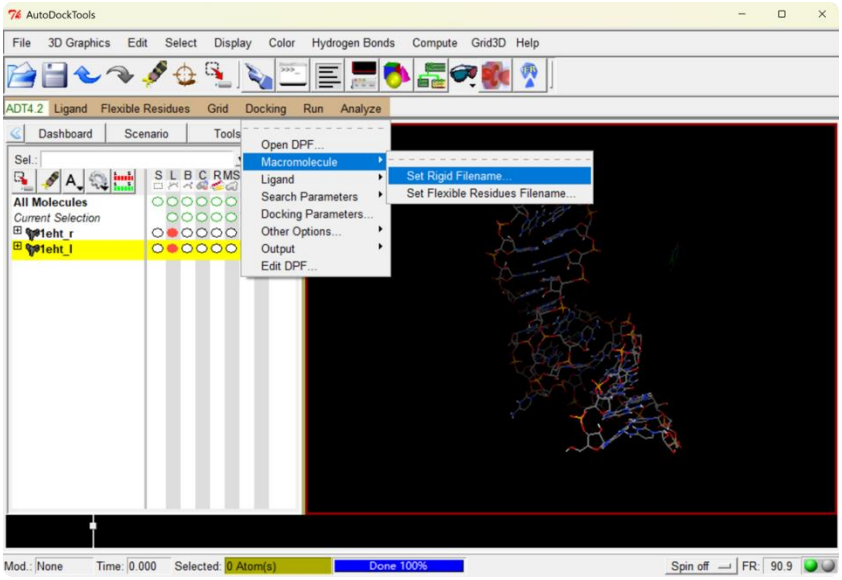


18) Go to Grid → Output → Save GPF → Save the .gpf file



19) Navigate to the folder containing the protein receptor and ligand files using the cd command, then execute the following command:

// autogrid4 -p 1eht.gpf



20） After the Grid calculation completes, proceed to:

Docking → Macromolecule → Set Rigid Filename → Select the RNA molecule (to set the RNA molecule as rigid)

6、Perform docking

1) After the Grid process is completed, go to Docking → Macromolecule → Set Rigid Filename → Select the RNA molecule (to set the RNA molecule as rigid)

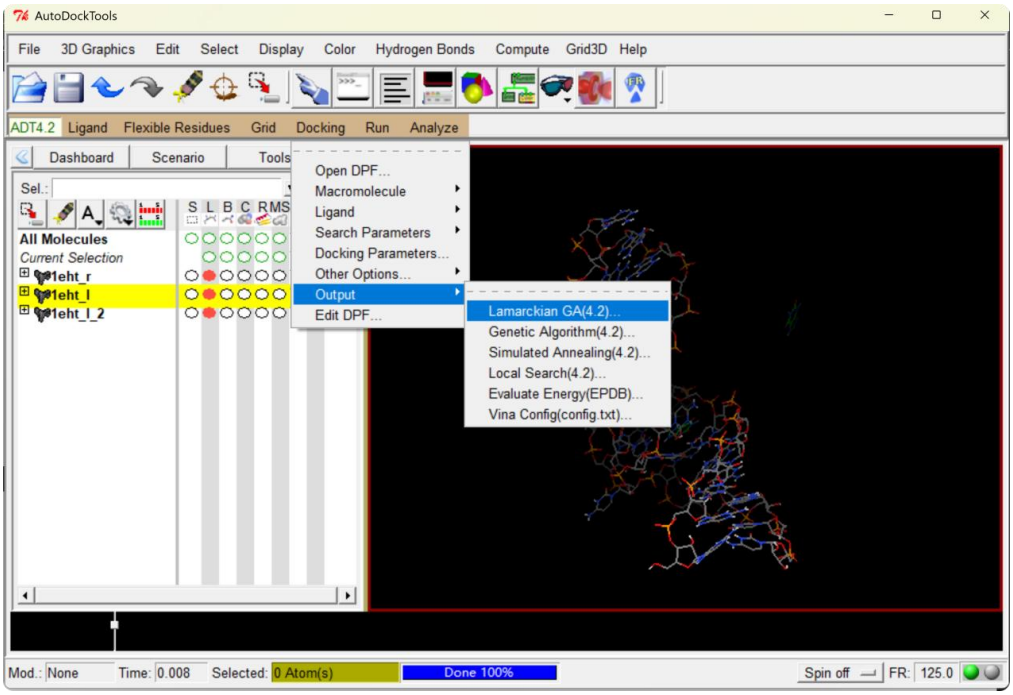
2)Docking → Ligand → Open → Select the ligand → Accept.

3) Docking → Ligand → Choose → Select 1eht_l_2 → Select Ligand → Accept.

4) Docking → Search Parameters → Genetic Algorithm (GA) → Accept

5)Docking—>Docking Parameters—>Accept;

6) Go to Docking → Output → Lamarkian GA → Save the file in .dpf format



7) Modify the run parameters of the search algorithm in 1eht.dpf,

Original search algorithm parameter settings:

```
ga_pop_size 150                 # number of individuals in population
ga_num_evals 2500000              # maximum number of energy evaluations
ga_num_generations 27000          # maximum number of generations
ga_elitism 1                  # number of top individuals to survive to next generation
ga_mutation_rate 0.02           # rate of gene mutation
ga_crossover_rate 0.8           # rate of crossover
ga_window_size 10               #
ga_cauchy_alpha 0.0             # Alpha parameter of Cauchy distribution
ga_cauchy_beta 1.0              # Beta parameter Cauchy distribution
set_ga                        # set the above parameters for GA or LGA
sw_max_its 300                  # iterations of Solis & Wets local search
sw_max_succ 4                   # consecutive successes before changing rho
sw_max_fail 4                 # consecutive failures before changing rho
sw_rho 1.0                    # size of local search space to sample
sw_lb_rho 0.01                  # lower bound on rho
ls_search_freq 0.06             # probability of performing local search on individual
set_psw1                      # set the above pseudo-Solis & Wets parameters
unbound_model bound               # state of unbound ligand
ga_run 10                     # do this many hybrid GA-LS runs
analysis                      # perform a ranked cluster analysis
```

8) Change to the parameters for the EPSDock search algorithm

```
pso_c1 2.05                    # the First Confidence Coefficient
pso_c2 2.05                          # the Second Confidence Coefficient
pso_k 50                     # Max number of particles informed by a given one
pso_swarm_moves 2500000            # total swarm Moves
pso_size 50                    #swarm size
pso_n_exec 100                  #Number of requested PSO runs or the total running times
sw_max_its 50                  # iterations of Solis & Wets local search
sw_max_succ 2                   # consecutive successes before changing rho
sw_max_fail 2                  # consecutive failures before changing rho
sw_rho 1.0                     # size of local search space to sample
sw_lb_rho 0.01                 # lower bound on rhon
ls_search_freq 0.06             # probability of performing local search on individual
set_psw1                       # set the above pseudo-Solis & Wets parameters
unbound_model bound              # state of unbound ligand
do_cpso
analysis                       # perform a ranked cluster analysis
```

9) Navigate to the directory containing the receptor and ligand files, then execute the following command to run the docking experiment:

//epsdock -p 1eht.dpf

10) Extracting docking results:

Open the docking log file 1eht.dlg to view the results, including:

The lowest energy conformation of the docked structure.

The docking coordinates of the ligand.

```
USER    RMSD from reference structure       = 2.689 A
USER
USER    Estimated Free Energy of Binding    =   -5.78 kcal/mol  [=(1)+(2)+(3)-(4)]
USER    Estimated Inhibition Constant, Ki   =   58.27 uM (micromolar)  [Temperature = 298.15 K]
USER
USER    (1) Final Intermolecular Energy     =   -5.78 kcal/mol
USER        vdW + Hbond + desolv Energy     =   -5.68 kcal/mol
USER        Electrostatic Energy            =   -0.09 kcal/mol
USER    (2) Final Total Internal Energy     =   +0.00 kcal/mol
USER    (3) Torsional Free Energy           =   +0.00 kcal/mol
USER    (4) Unbound System's Energy  [=(2)] =   +0.00 kcal/mol
```

As a reference, EPSDock Experimental Setup:

Software Environment:

OS: Ubuntu Desktop 18.04 LTS

Docking Tools: EPSDock, FIPSDock, AutoDock, AutoDock Vina, rDock, Glide

Visualization/Analysis: PyMOL, ADT (AutoDock Tools 4.6)

Hardware Environment:

CPU: Intel i9-9900 @ 3.1 GHz

GPU: NVIDIA GTX 1060

(Note: This configuration assumes all software is properly installed and configured for GPU acceleration where applicable.)