Molecular Docking Tutorial

The use of VMD, Autodock Tools 1.4.4 and Autodock 4.0

Khaled H. Barakat

Jack Tuszynski Group

PharmaMatrix workshop in Computational Biophysics

February 17- 20 2009

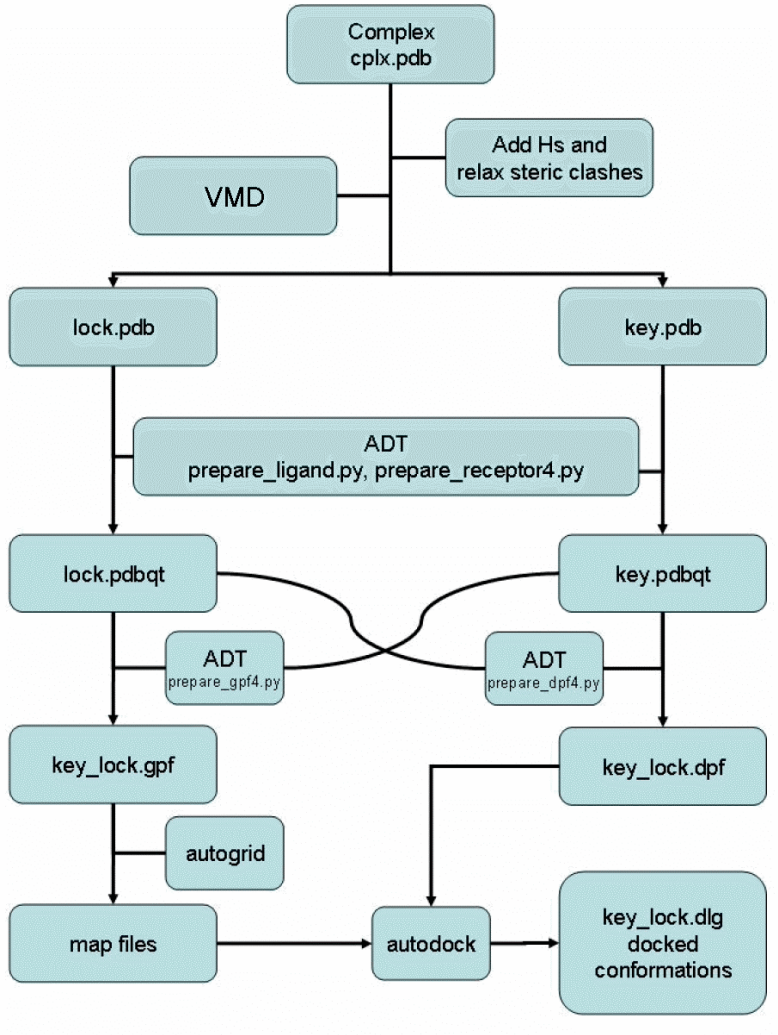
As in every science field any experimental methodology needs to be validate prior any production work!

Therefore in the very first use of the autodock program you will be trained to see if a docking program (Autodock 4.0) could be suitable to study the binding mode of a certain ligands (docking assessment). Of course for any docking program the goal should be the reproduction of the experimental bound conformation of a ligand into its target macromolecule (docking assessment).

We will use the (Histone deacetylase 8 / Trichostatin A) HDAC8/TSA complex (PDB entry code 1t64) for the docking assessment and to learn how to use Autodock in its rigid and flexible modes

NOTE. THIS TUTORIAL IS INTENDED TO BE AN INTRODUCTION FOR DOCKING AND HOW TO USE DOCKING PROGRAMS. SOME PARTS OF THE TUTORIAL ARE TAKEN FROM AUTODOCK TUTORIALS.

Docking Flowchart



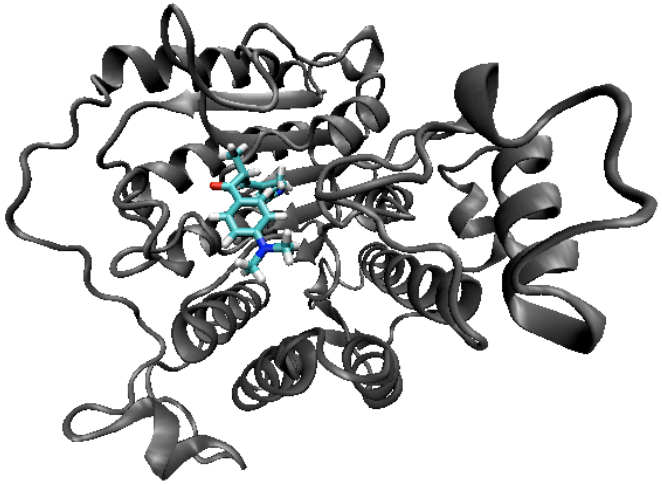
Overall Steps:

1. Get the complex (CPLX) coordinates (i.e. from the PDB).
2. Clean the complex (delete all the water and the solvent molecules and all non-interacting ions).
3. Add the missing hydrogens/side chain atoms and minimized the complex (AMBER Program).
4. Clean the minimized complex (delete all the water and the solvent molecules and all non-interacting ions).
5. Separate the minimized CPLX in macromolecule (LOCK) and ligand (KEY).
6. Prepare the docking suitable files for LOCK and KEY (pdbqt files).
7. Prepare all the needing files for docking (grid parameter file, map files, docking parameter files).
8. Run the docking.
9. Analyze the docking results.

NOTE. In your home folder you will find the DOCKING folder under which are saved all the calculations already done for you. A second folder (MD_files) has been made in which you find only the initial files to run all over the tutorial.

1. Docking Assessment using the bound ligand conformation:

In this tutorial you will be guided in running docking experiments from the AMBER optimized complex. The program VMD will be used to prepare the macromolecule (lock) and the inhibitor (key) files.
Next the program AutoDockTools 1.4.4 (ADT) will be used to prepare the needed files and parameters to run the dockings and to analyze the results.
In the first step we will see if the docking program will be successful in reproducing the experimental complex using as starting point the experimental ligand binding conformation as found in experimental complex (1t64).



PDB: 1T64

1.1. Preparing the pdb file from geometry optimized complex.

1.1.1 Open the 1T64-A_Min.pdb file and read it carefully.
Use the command **less 1T64-A_Min.pdb**

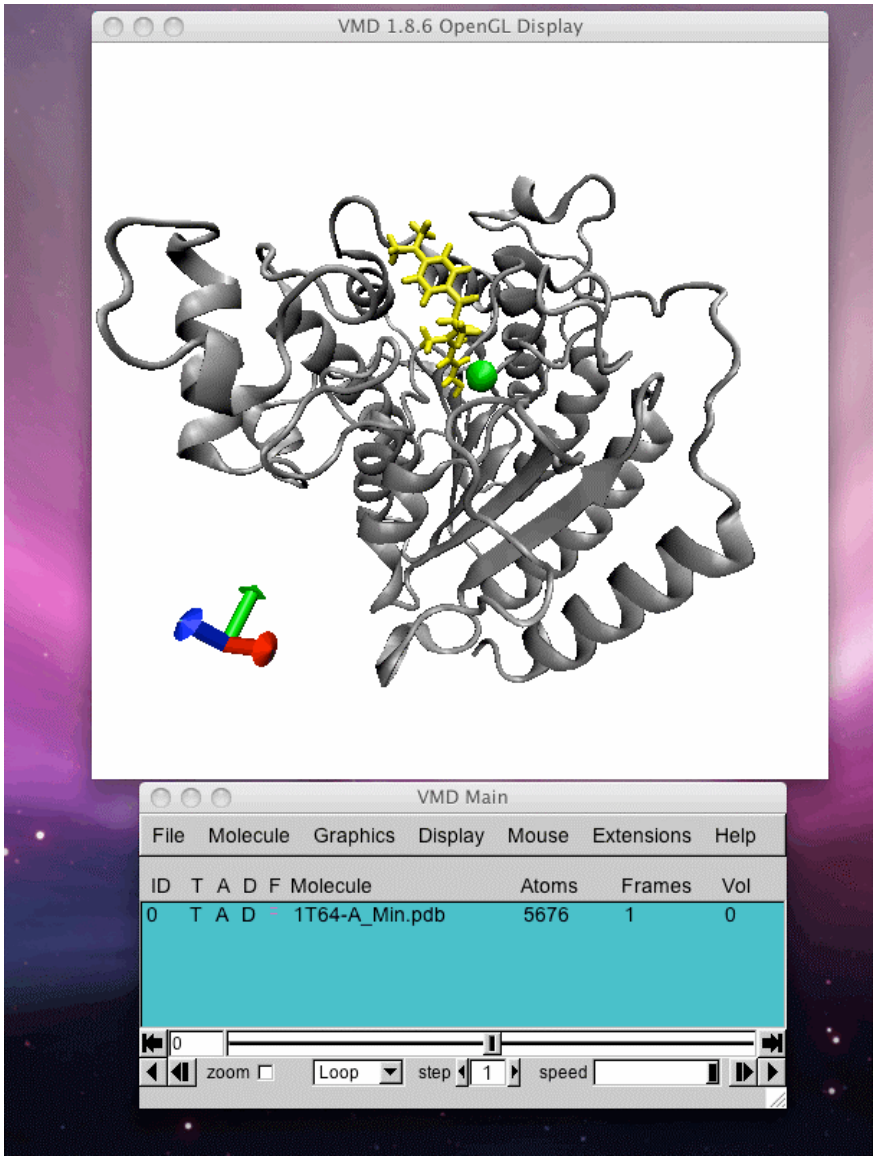
1.1.2 Open VMD by **typing VMD and hitting the enter key**. Browse to the MD_files Directory and load the 1T64-A_Min.pdb file:

```
ATOM   5618  HA  VAL   364      37.437  18.403  22.556
ATOM   5619  CB  VAL   364      36.275  20.062  21.837
ATOM   5620  HB  VAL   364      36.625  20.606  22.716
ATOM   5621  CG1 VAL   364      36.231  21.058  20.665
ATOM   5622 1HG1 VAL   364      35.992  20.532  19.740
ATOM   5623 2HG1 VAL   364      35.500  21.833  20.851
ATOM   5624 3HG1 VAL   364      37.200  21.549  20.552
ATOM   5625  CG2 VAL   364      34.840  19.593  22.120
ATOM   5626 1HG2 VAL   364      34.854  18.655  22.667
ATOM   5627 2HG2 VAL   364      34.338  20.309  22.757
ATOM   5628 3HG2 VAL   364      34.296  19.430  21.190
ATOM   5629  C   VAL   364      36.670  17.797  20.722
ATOM   5630  O   VAL   364      36.842  17.826  19.483
ATOM   5631  OXT VAL   364      36.009  16.893  21.286
TER
ATOM   5632 ZN   ZN    365      44.358  35.239  45.997
TER
ATOM      1  C16 INH     1      43.676  38.384  58.878
ATOM      2  H16 INH     1      44.530  38.142  59.520
ATOM      3  H19 INH     1      44.003  39.056  58.080
ATOM      4  H20 INH     1      42.930  38.912  59.477
ATOM      5  N2  INH     1      43.118  37.155  58.304
ATOM      6  C17 INH     1      41.859  36.666  58.893
ATOM      7  H17 INH     1      41.086  36.596  58.121
ATOM      8 1H2  INH     1      41.501  37.337  59.678
ATOM      9 2H2  INH     1      42.010  35.676  59.333
ATOM     10  C4  INH     1      43.851  36.370  57.429
ATOM     11  C5  INH     1      43.293  35.242  56.850
ATOM     12  C6  INH     1      44.037  34.473  55.967
ATOM     13  H6  INH     1      43.539  33.602  55.562
ATOM     14  H5  INH     1      42.274  34.940  57.064
ATOM     15  C3  INH     1      45.155  36.725  57.098
ATOM     16  H3  INH     1      45.632  37.599  57.522
```

1.1.3 Visualize the Protein and it's inhibitor:

Open graphics – representation
Click on the selected atoms field
Type: "protein" thin hit the "Enter Key"
Click on Draw Style
Select New Cartoon as the Drawing method
Select COLORID 6 as the coloring method
Click on the Create Representation Button
Type: "resname INH" in the selected atom field
Click on Draw Style
Select LICORICE as the Drawing method
Select COLORID 4 as the coloring method
Click on the Create Representation Button
Type: "resname ZN" in the selected atom field
Click on Draw Style
Select VDW as the Drawing method
Select COLORID 7 as the coloring method

You should get the following representation for the complex:


VMD 1.8.6 OpenGL Display
VMD Main
File Molecule Graphics Display Mouse Extensions Help
ID T A D F Molecule Atoms Frames Vol
0 T A D 1T64-A_Min.pdb 5676 1 0
0
zoom
Loop
step 1
speed

IMPORTANT!!
If the complex comes directly from the AMBER program also the HIE and HID residue have to be fixed into HIS, otherwise ADT (next section) will not recognize them correctly.

The lock and key files can be prepare directly in a UNIX shell using some simple UNIX commands:

Once checked out the inhibitor residue name (see 1.1.1) the lock and key file can be prepared using the cat and grep UNIX commands as following:

```
Prompt> cat cplx_filename.pdb | grep INH > key_filename.pdb

Prompt> cat cplx_filename.pdb | grep -v INH | sed 's/HIE/HIS/' | sed 's/HID/HIS/' > lock_filename.pdb
```

And use VMD to check them all.

1.2. Preparing the file for docking using ADT and run the docking.

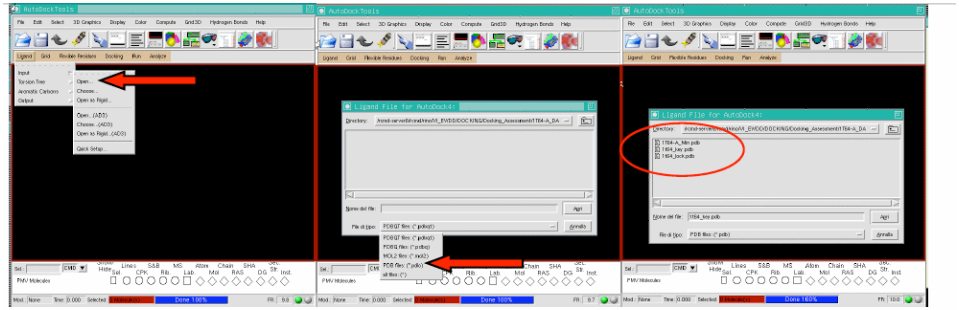
1.2.1. Some rules from the ADT online tutorial:
(http://autodock.scripps.edu/faqs-help/tutorial/using-autodock-4-with-autodocktools)

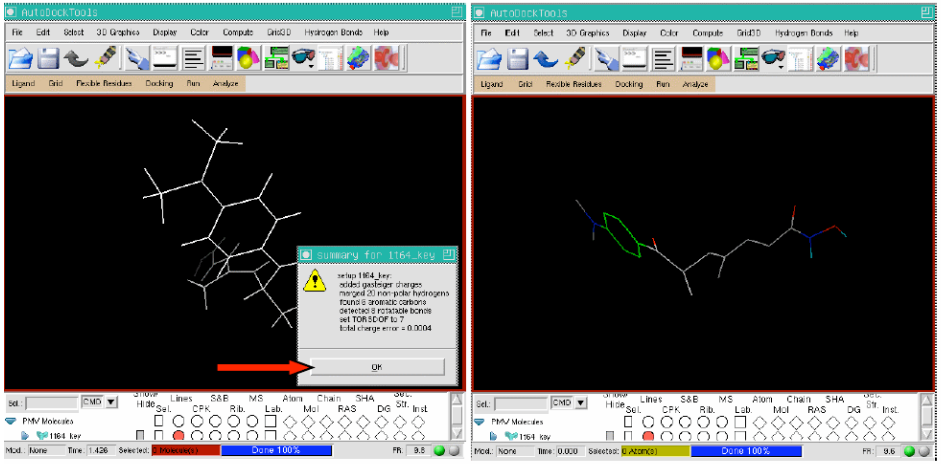
A) You should always start **ADT** in the same directory as the macromolecule and ligand files. You can start **ADT** from the command line in a Terminal by typing **"adt"** and pressing <Return> or <Enter>.
B) For both the macromolecule and the ligand, you should always add polar hydrogens, compute Gasteiger charges and then you must merge the non-polar hydrogens. Polar hydrogens are hydrogens that are bonded to electronegative atoms like oxygen and nitrogen. Non-polar hydrogens are hydrogens bonded to carbon atoms.
C) You need one **AutoGrid** map for every atom type in the ligand plus an electrostatics map. *E.g.:* for ethanol, C2H5OH, you would need C, OA and HD maps plus an electrostatics 'e' map plus a desolvation 'd' map.
D) The grid volume should be large enough to at least allow the ligand to rotate freely, even when the ligand is in its most fully extended conformation.
1.2.2. Preparing a ligand file for Autodock.

(Taken from Autodock Tutorials)

Start ADT from a UNIX shell and open a ligand file using the **Ligand - Input - Open** … sequence.

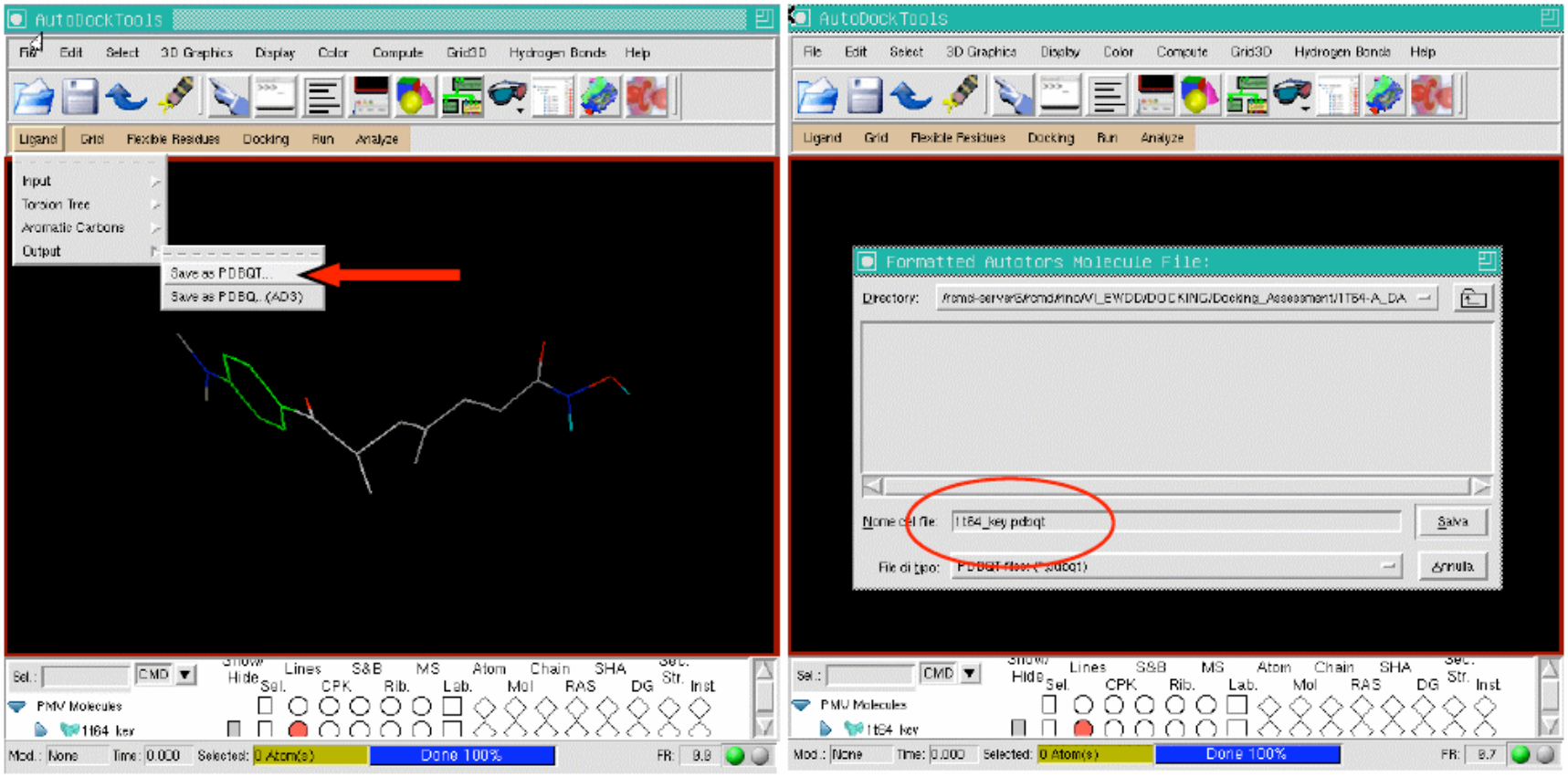
Set the file type to *.pdb and choose the key file (1t64_key.pdb). Click OK in the upcoming window.





Save the file as pdbqt (Ligand, Output, Save as PDBQT…) giving a proper name

(1t64_key.pdbqt) and check the written file.

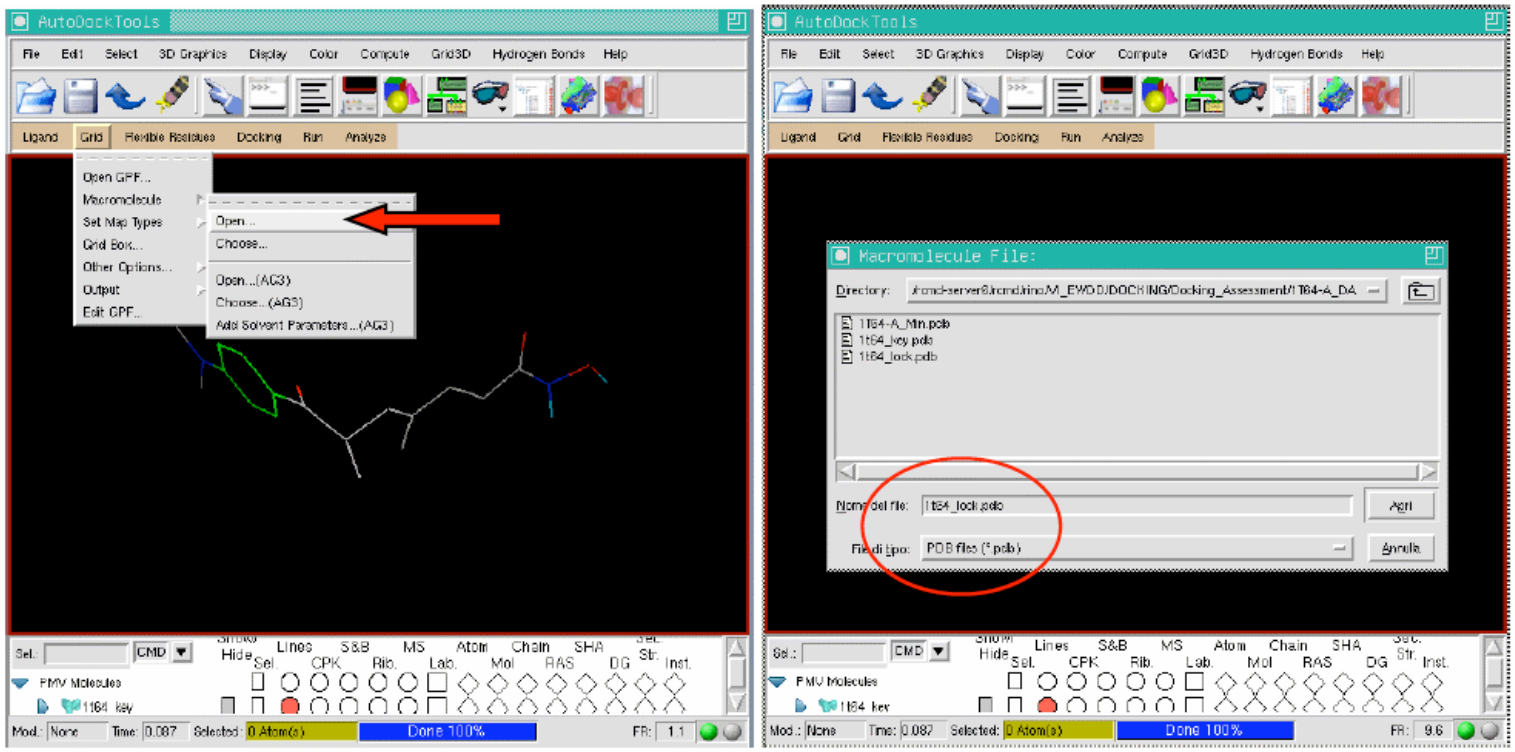


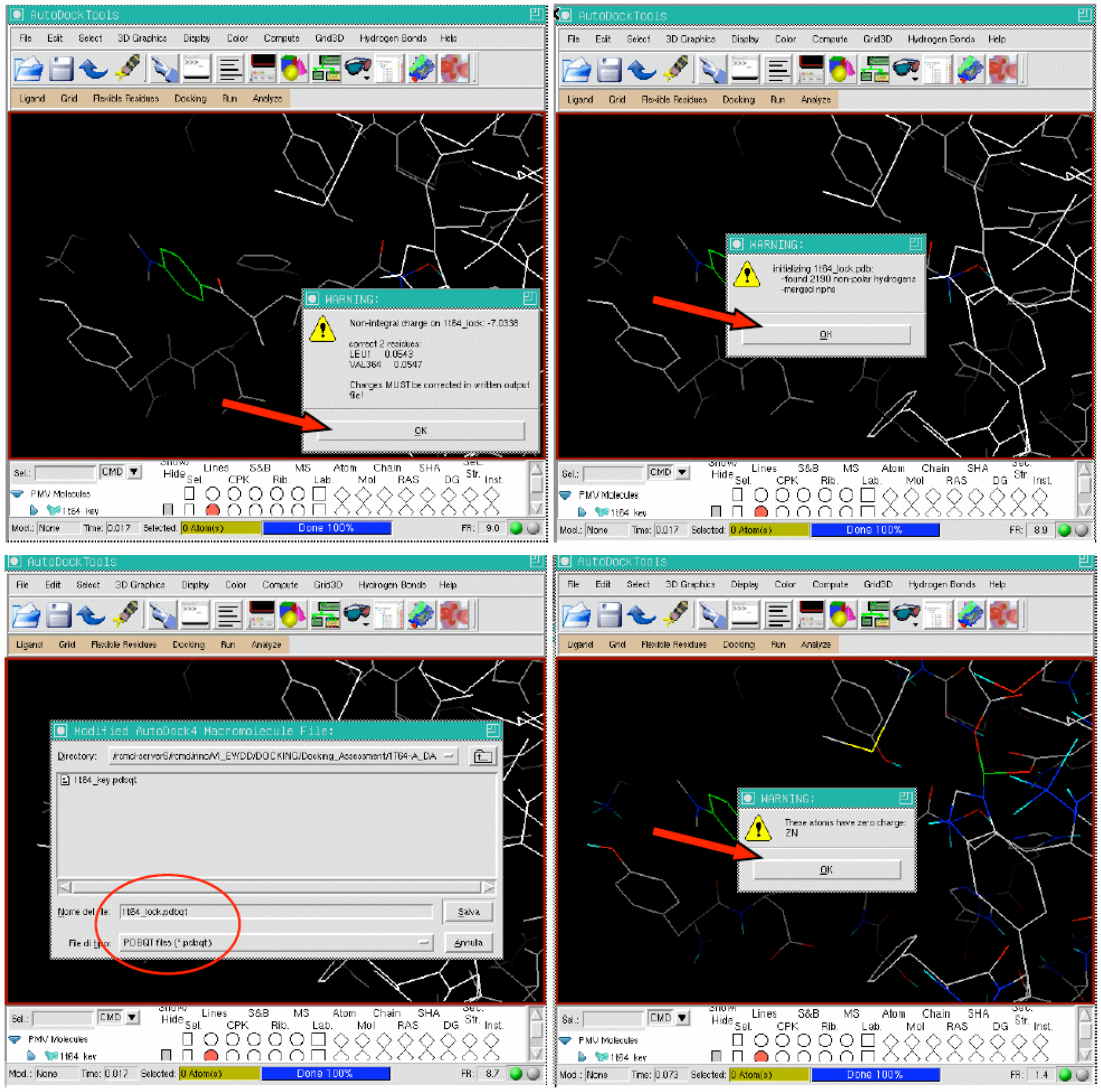
Example of a pdbqt file for a ligand (1t64_key.pdbqt).

```
REMARK  7 active torsions:
REMARK  status: ('A' for Active; 'I' for Inactive)
REMARK    1  A    between atoms: C1_2  and  C7_16
REMARK    2  A    between atoms: C10_3  and  C11_4
REMARK    3  A    between atoms: C12_5  and  C13_6
REMARK       I    between atoms: C13_6  and  N1_20
REMARK    4  A    between atoms: C4_13  and  N2_21
REMARK    5  A    between atoms: C7_16  and  C8_17
REMARK    6  A    between atoms: C8_17  and  C9_18
REMARK    7  A    between atoms: N1_20  and  O1_22
ROOT
HETATM    1  H11 INH     1      41.930  33.744  45.539  0.00  0.00     0.244 HD
HETATM    2  O1  INH     1      42.652  34.244  46.108  0.00  0.00    -0.287 OA
ENDROOT
BRANCH   2   3
HETATM    3  N1  INH     1      42.264  33.997  47.418  0.00  0.00    -0.234 N
HETATM    4  C13 INH     1      43.022  34.527  48.351  0.00  0.00     0.264 C
HETATM    5  H1  INH     1      41.843  33.072  47.563  0.00  0.00     0.197 HD
HETATM    6  O2  INH     1      43.738  35.462  48.003  0.00  0.00    -0.266 OA
BRANCH   4   7
HETATM    7  C12 INH     1      43.072  33.844  49.643  0.00  0.00     0.076 C
HETATM    8  C11 INH     1      43.861  34.212  50.643  0.00  0.00     0.012 C
BRANCH   8   9
HETATM    9  C10 INH     1      44.016  33.580  51.936  0.00  0.00    -0.065 C
HETATM   10  C9  INH     1      45.218  33.640  52.523  0.00  0.00    -0.008 C
HETATM   11  C15 INH     1      42.809  32.883  52.515  0.00  0.00     0.048 C
BRANCH  10  12
HETATM   12  C8  INH     1      45.544  32.977  53.846  0.00  0.00     0.079 C
HETATM   13  C14 INH     1      46.492  31.785  53.580  0.00  0.00     0.022 C
BRANCH  12  14
HETATM   14  C7  INH     1      46.207  34.026  54.716  0.00  0.00     0.166 C
HETATM   15  O3  INH     1      47.394  34.257  54.604  0.00  0.00    -0.292 OA
BRANCH  14  16
HETATM   16  C1  INH     1      45.362  34.812  55.667  0.00  0.00     0.011 A
HETATM   17  C6  INH     1      44.037  34.473  55.967  0.00  0.00     0.017 A
HETATM   18  C2  INH     1      45.914  35.955  56.225  0.00  0.00     0.017 A
HETATM   19  C5  INH     1      43.293  35.242  56.850  0.00  0.00     0.030 A
HETATM   20  C4  INH     1      43.851  36.370  57.429  0.00  0.00     0.029 A
HETATM   21  C3  INH     1      45.155  36.725  57.098  0.00  0.00     0.030 A
BRANCH  20  22
HETATM   22  N2  INH     1      43.118  37.155  58.304  0.00  0.00    -0.390 N
HETATM   23  C16 INH     1      43.676  38.384  58.878  0.00  0.00     0.149 C
HETATM   24  C17 INH     1      41.859  36.666  58.893  0.00  0.00     0.149 C
ENDBRANCH  20  22
ENDBRANCH  14  16
ENDBRANCH  12  14
ENDBRANCH  10  12
ENDBRANCH   8   9
ENDBRANCH   4   7
ENDBRANCH   2   3
TORSDOF 7
```

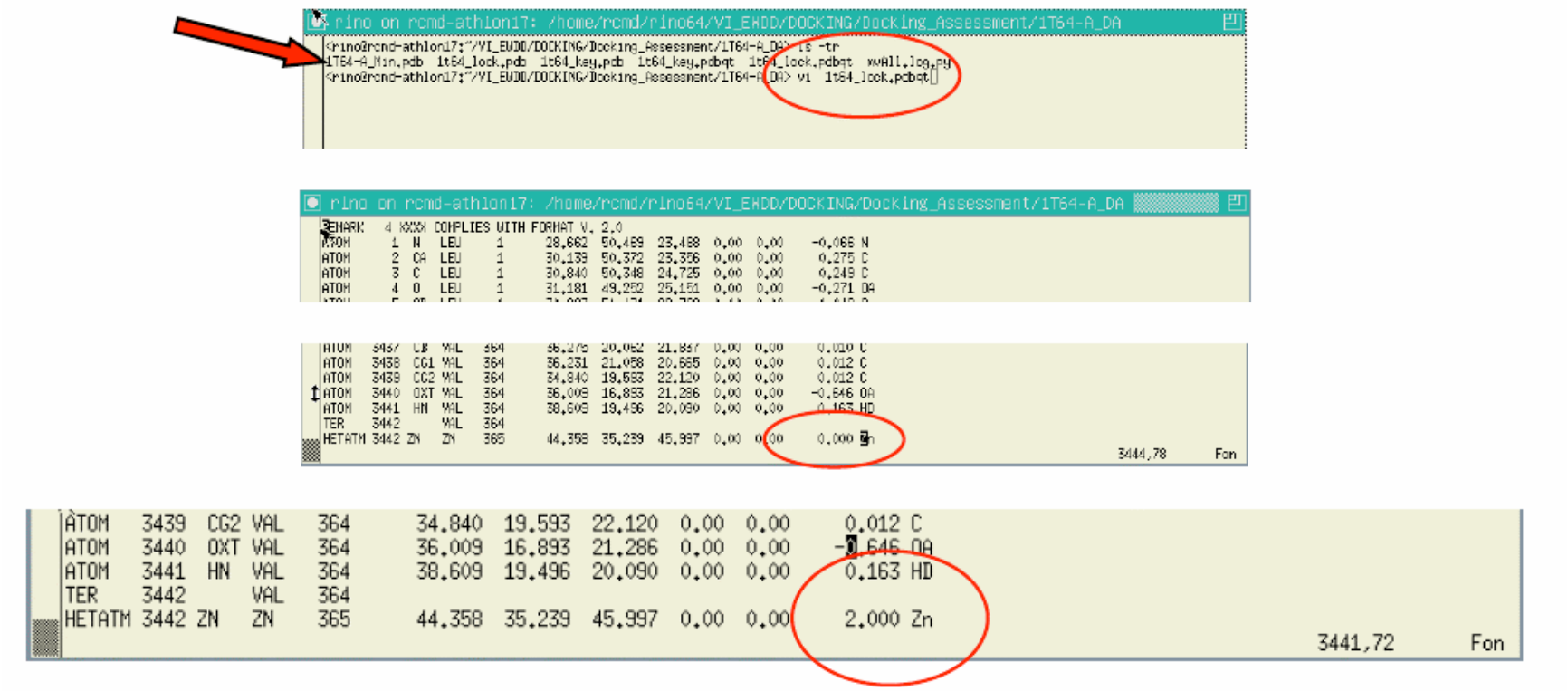
1.2.3. Preparing the macromolecule file.

Open a macromolecule file using the Grid - Macromolecule - Open … sequence. Set the file type to *.pdb and choose the lock file (1t64_lock.pdb). Click OK in the upcoming window. Ignore the warning about the charge and save the file with a proper name (1t64_lock.pdbqt), ignore the Zn zero charge warning and click OK.




AutoDockTools
WARNING:
Non-integral charge on 1t64_lock: -7.0338
correct 2 residues:
LEU1 0.0543
VAL364 0.0547
Charges MUST be corrected in written output file!
OK
AutoDockTools
WARNING:
OK
AutoDockTools
Modified AutoDock4 Macromolecule File:
Directory:
1t64_key.pdbqt
None del file:
1t64_lock.pdbqt
Salva
File di tipo:
PDBQT files (*.pdbqt)
Annulla
AutoDockTools
WARNING:
These atoms have zero charge:
ZN
OK

In a UNIX shell edit the 1t64_lock.pdbq file and correct the Zn charge into +2.0.



ATOM 3439 CG2 VAL 364 34.840 19.593 22.120 0.00 0.00 0.012 C
ATOM 3440 OXT VAL 364 36.009 16.893 21.286 0.00 0.00 -0.646 OA
ATOM 3441 HN VAL 364 38.609 19.496 20.090 0.00 0.00 0.163 HD
TER 3442 VAL 364
HETATM 3442 ZN ZN 365 44.358 35.239 45.997 0.00 0.00 2.000 Zn
3441,72 Fon

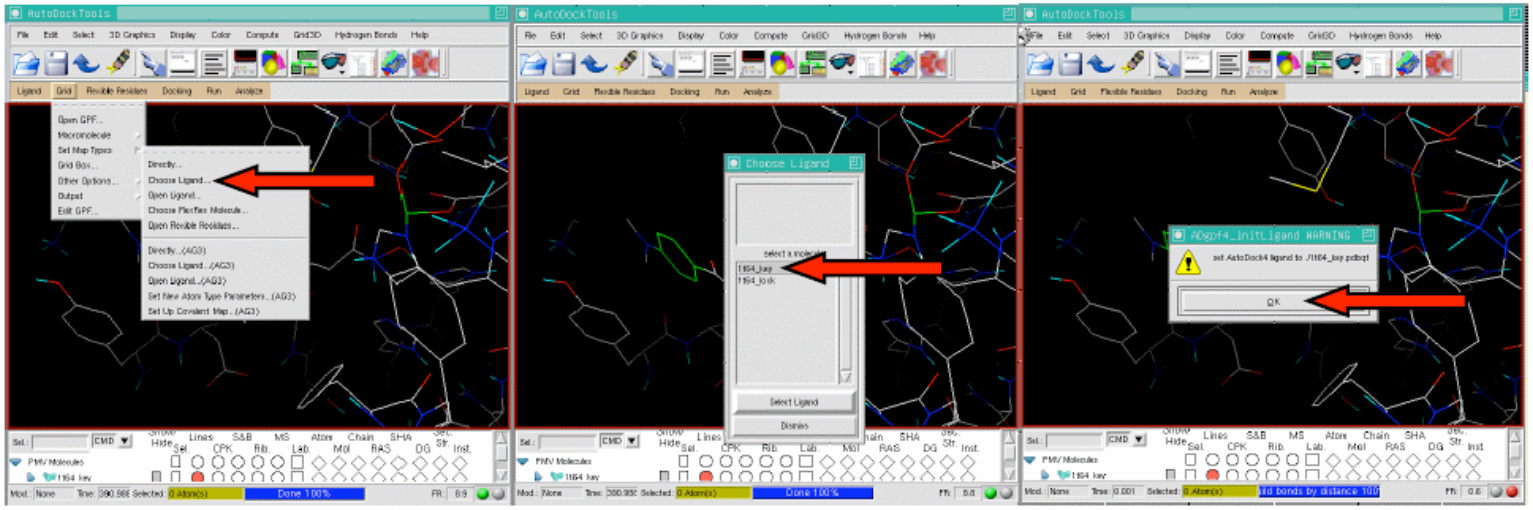
1.2.4. Preparing the GRID parameter file and running Autogrid4.

The grid parameter file tells **Autogrid4** which receptor to compute the potentials around, the types of maps to compute and the location and extent of those maps.

1.2.4.1. Selecting the map types.

In general, one map is calculated for each atom type in the ligand plus an electrostatics map and a separate desolvation map. The types of maps depend on the types of atoms in the ligand. Thus one way to specify the types of maps is by choosing a ligand. If the ligand you formatted in 1.2.2 is still in the Viewer use this procedure:

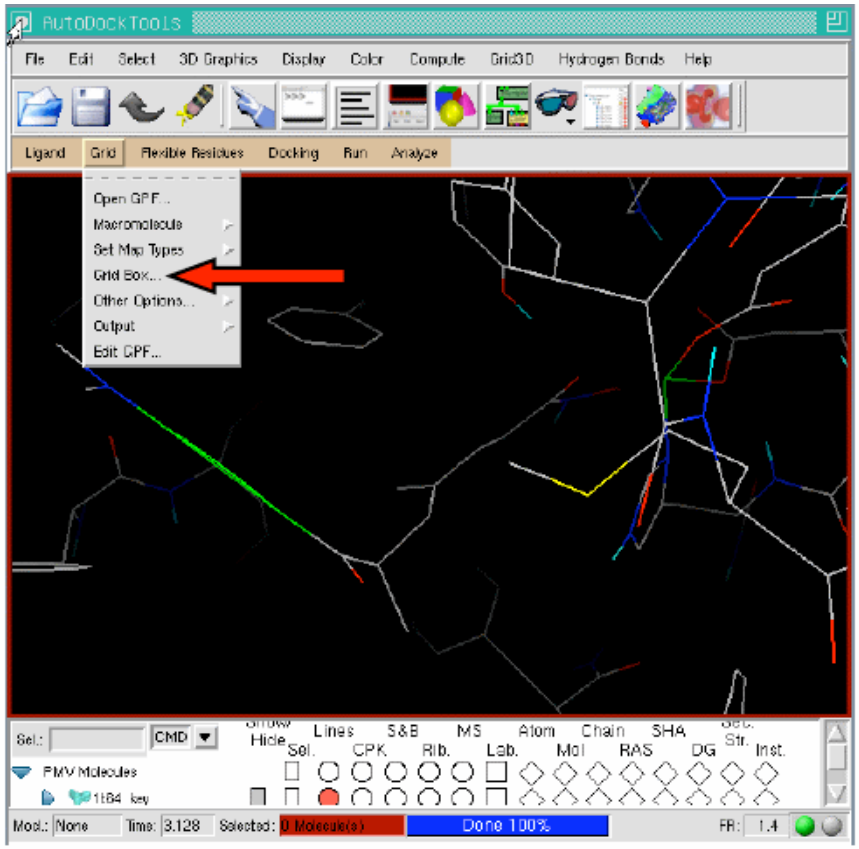
Grid - Set Map Types - Choose Ligand … - 1t64_key - Select Ligand.

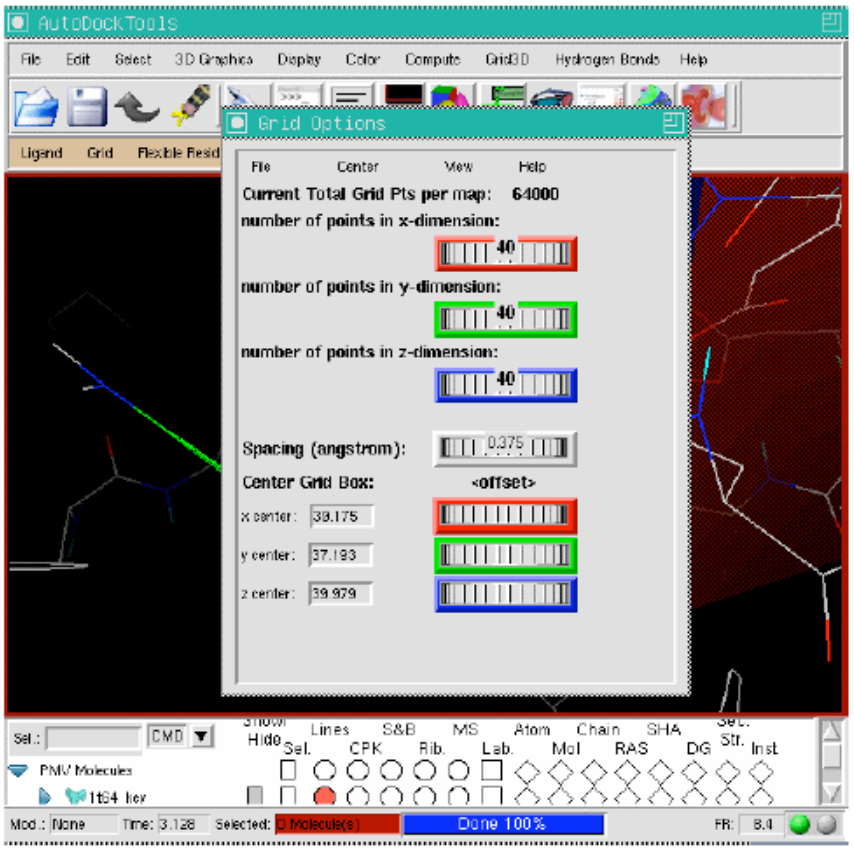


1.2.4.2. Setting the grid box.

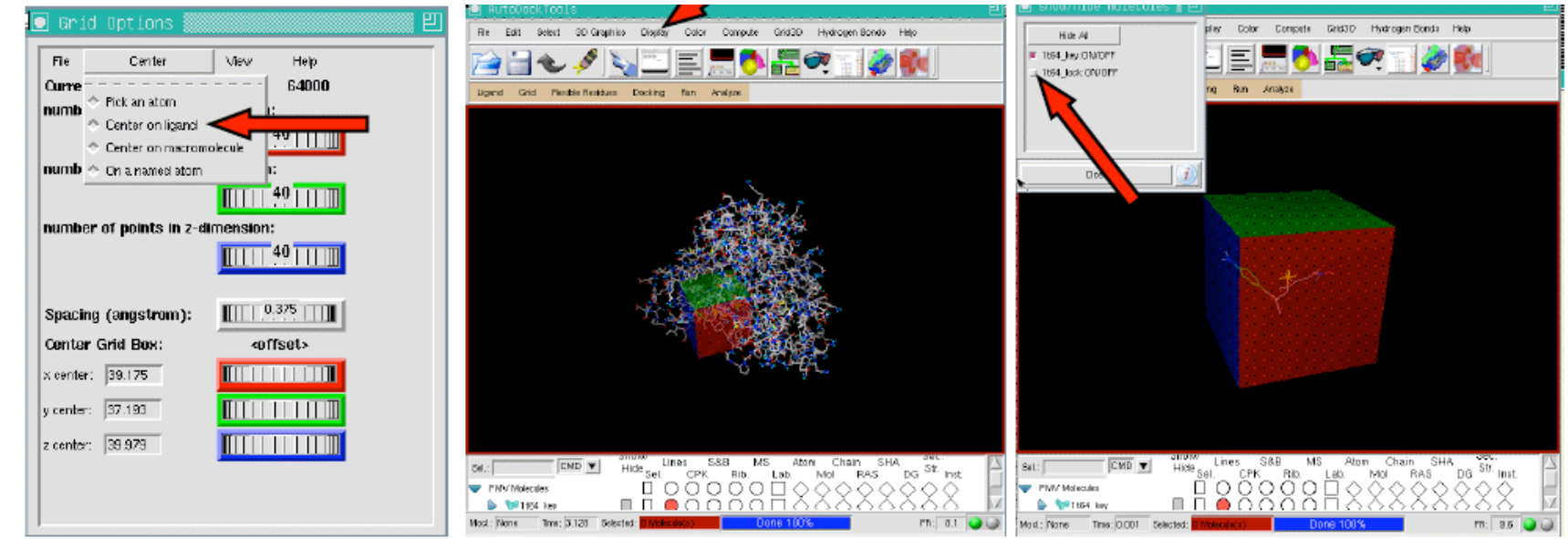
The central position and size of the grid docking box is set using the following procedure:

Grid - Grid Box …





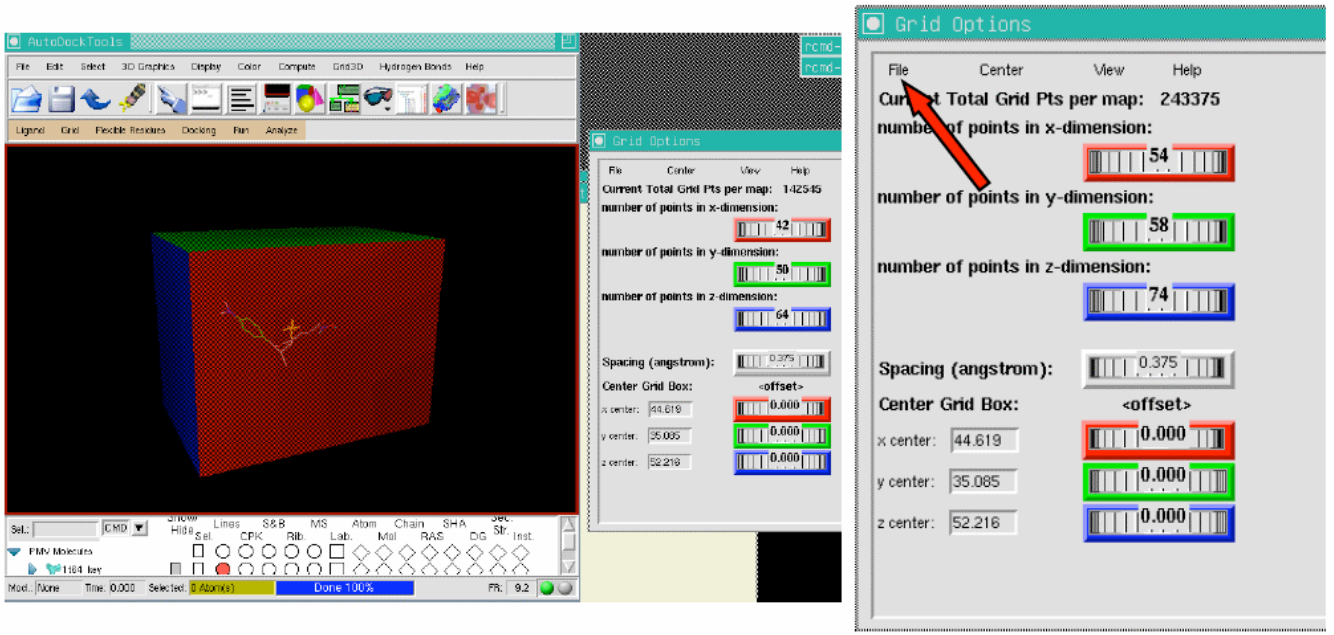
Set the center of the grid in the center of the ligand (key) and zoom out in the ADT main window (to zoom out **ctrl+middle mouse button** or **ctrl+c** and **n** keys for the full view) to check the size of the grid you are making. Turn off the lock molecule display (**Display - Show/Hide molecule…)** and re-center the view (**ctrl+c** and **n** keys).



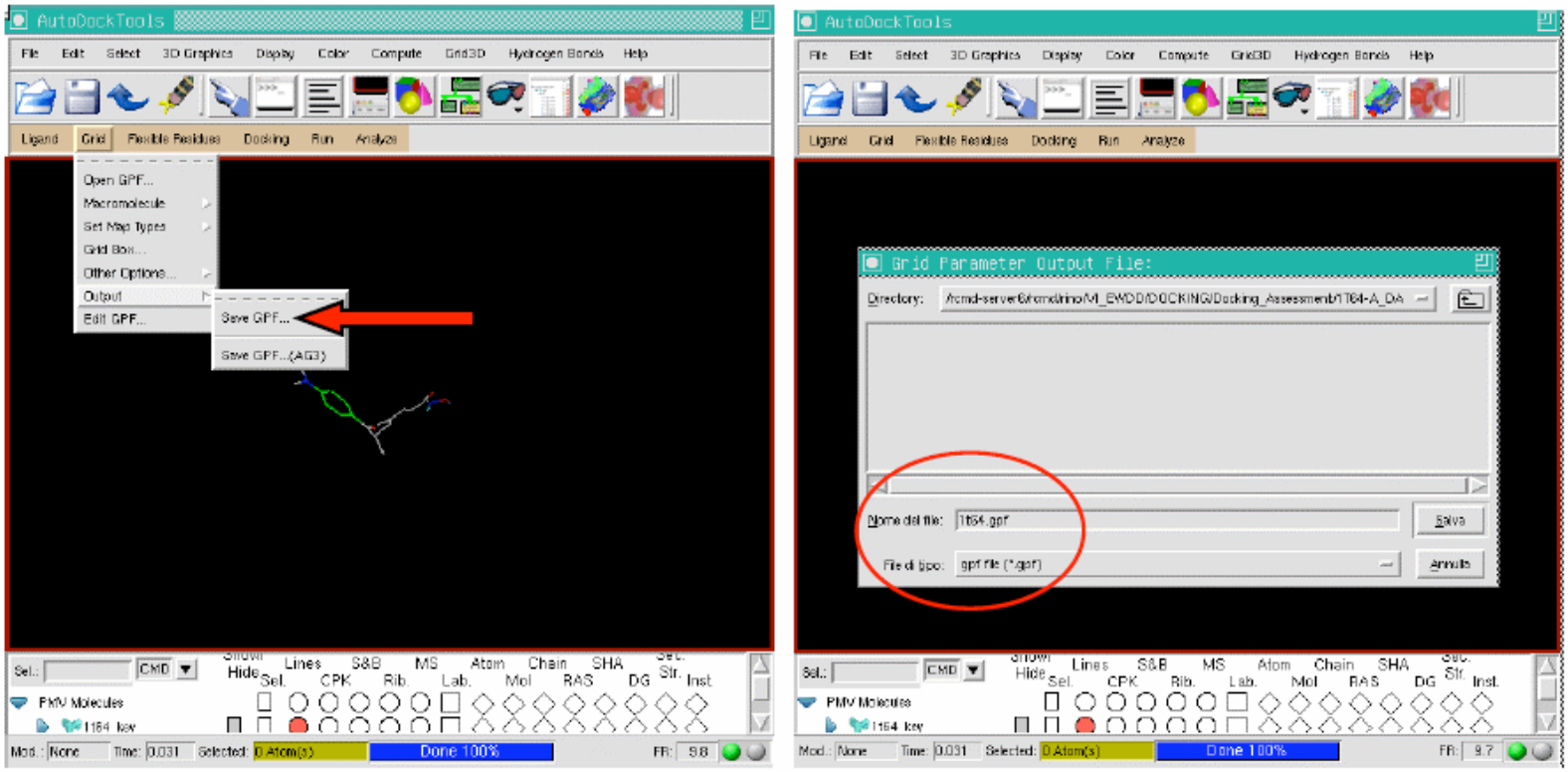
As you can see the TSA is almost fully embedded in the grid, but the size is not big enough to allow a free rotation of the molecule. As a rule of thumb the grid size should be as large as at least twice the double of the maximum distance you can measure between any two atoms of the co-crystallized ligand.

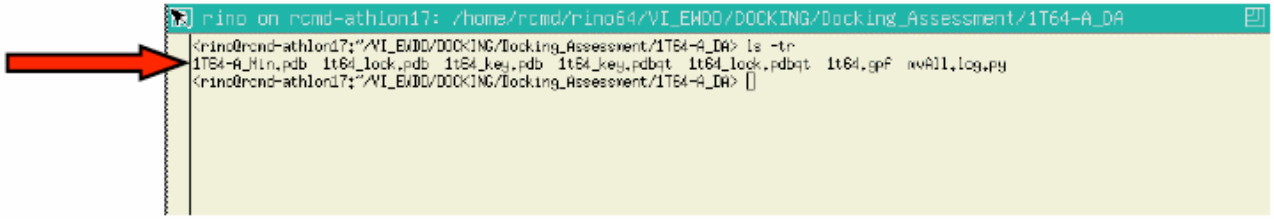
In the case you do not have any ligand position information the grid should be centered in the putative binding site and sized to embrace all the residue making the binding pocket. To set up the grid size you can inspect it visually and adjust the dimension by using the 3 thumbwheel widgets. So for instance the suitable grid size should be 54 x 58 x 74 points using the default grid spacing of 0.375. Adjust all the values and save the information, save the gpf file and check the written file.

(Grid option): File - Close saving current



(Autodock Tools): Grid - Output - Save GPF…





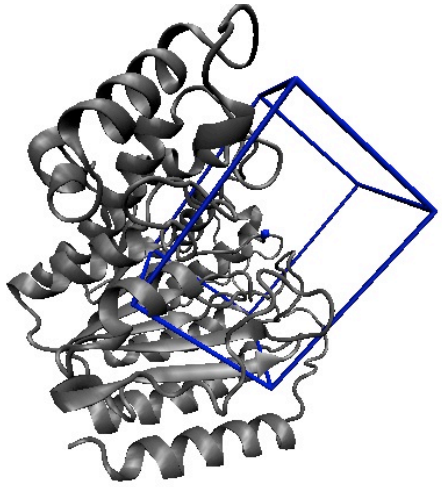
Example of a grid parameter file (1t64.gpf)

```
npts 53 58 74                           # num.grid points in xyz
gridfld 1t64_lock.maps.fld              # grid_data_file
spacing 0.375                           # spacing(A)
receptor_types A C HD N NA OA SA Zn     # receptor atom types
ligand_types A C HD N OA                # ligand atom types
receptor 1t64_lock.pdbqt                # macromolecule
gridcenter 44.619 35.085 52.216         # xyz-coordinates or auto
smooth 0.5                              # store minimum energy w/in rad(A)
map 1t64_lock.A.map                     # atom-specific affinity map
map 1t64_lock.C.map                     # atom-specific affinity map
map 1t64_lock.HD.map                    # atom-specific affinity map
map 1t64_lock.N.map                     # atom-specific affinity map
map 1t64_lock.OA.map                    # atom-specific affinity map
elecmap 1t64_lock.e.map                 # electrostatic potential map
dsolvmap 1t64_lock.d.map                 # desolvation potential map
dielectric -0.1465                      # <0, AD4 distance-dep.diel;>0, constant
```

NOTE. How to determine grid center and number of points?
In the utilities folder you will find a python script called **box.py** that helps in calculating the grid center and the number of points. This utility also helps in the building of a PDB file that can be visualized in any molecular visualizer (i.e. VMD). The output of the box.py utility has to be saved to a *filename.pdb* file as described in the output itself.
In a UNIX shell type:

Python box.py 1T64.gpf > box.pdb

You can visualize the output file using VMD



1.2.4.3 Run autogrid4 to make the grid maps. You can easily start a job from the command line

Giving the following:

```
Prompt> autogrid4 –p 1t64.gpf –l 1t64.glg &
```

After a few minutes the job will stop and a “done” message will return. Check the list of the file and you will notice that a number of new files have been created; those are the grid map files that autodock4 will use for the docking.

1.2.5. Preparing the docking parameter file and running Autodock4.

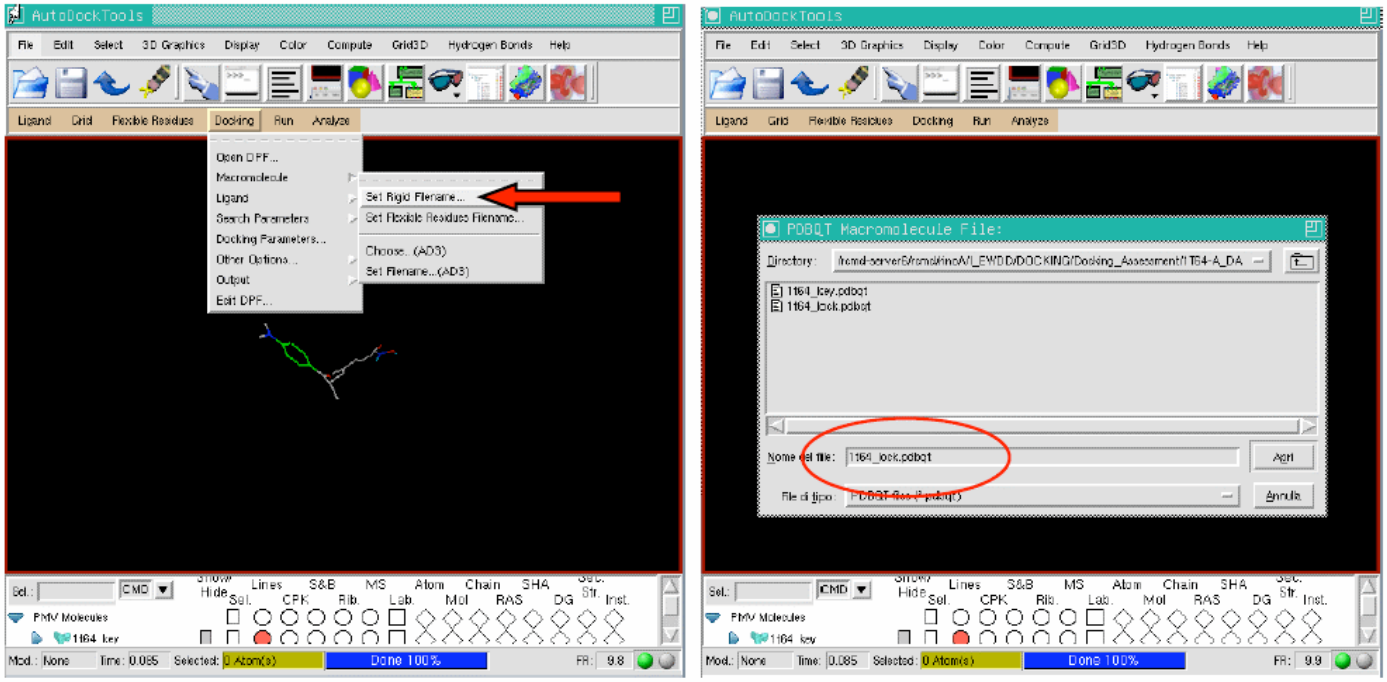
The docking parameter file tells AutoDock which map files to use, the ligand molecule to move, what its center and number of torsions are, where to start the ligand, the flexible residues to move if sidechain motion in the receptor is to be modeled, which docking algorithm to use and how many runs to do. It usually has the file extension, ".dpf". Four different docking algorithms are currently available in AutoDock: SA, the original Monte Carlo simulated annealing; GA, a traditional Darwinian genetic algorithm; LS, local search; and GALS, which is a hybrid genetic algorithm with local search. The GALS is also known as a Larmarckian genetic algorithm, or LGA, because children are allowed to inherit the local search adaptations of their parents.

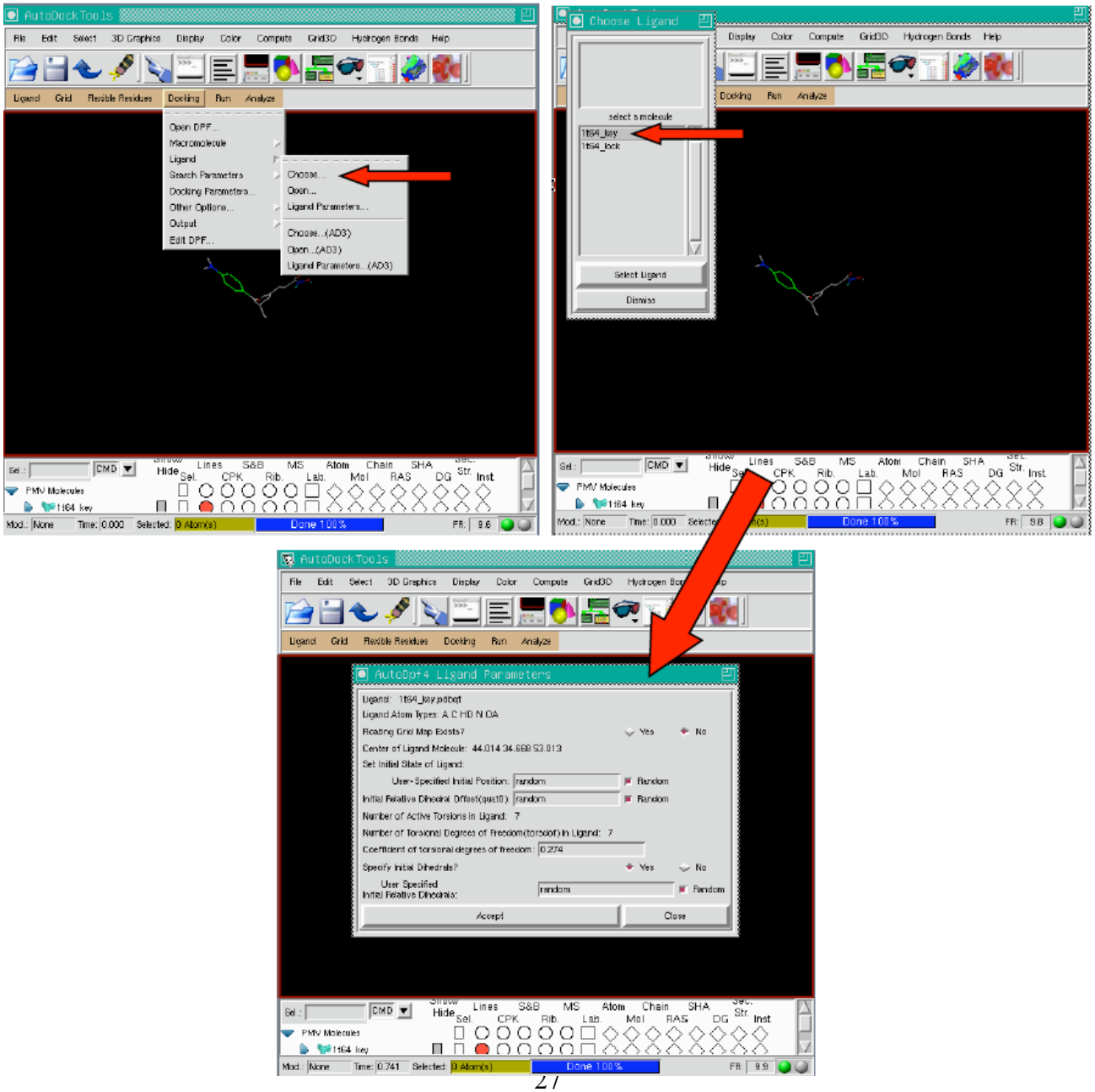
Each search method has its own set of parameters, and these must be set before running the docking experiment itself. These parameters include what kind of random number generator to use, step sizes, etc. ADT lets you change all of these parameters, and others not mentioned here.

(Taken from the online ADT tutorial).

1.2.5.1. Preparing the dpf file.

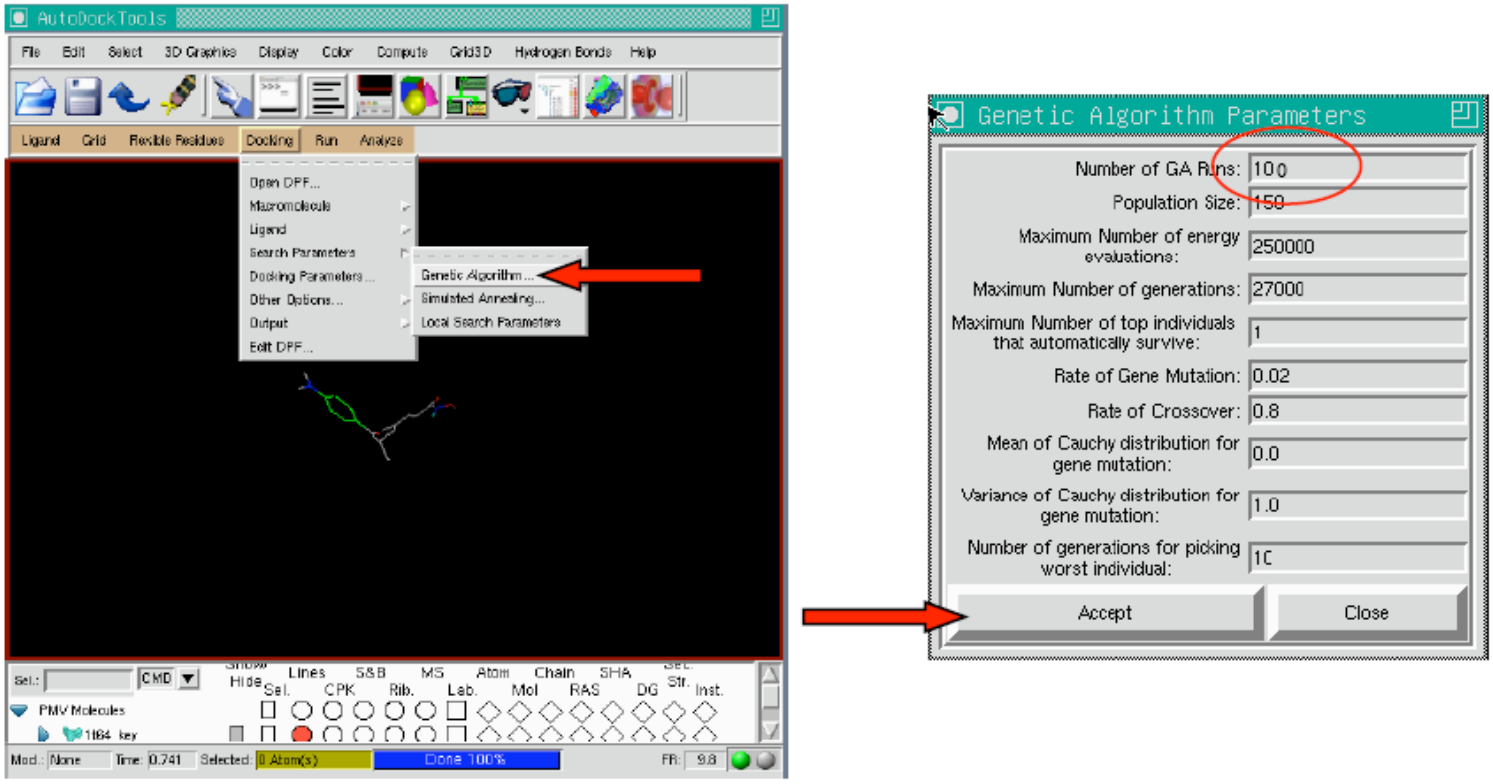
To set-up the dpf file follow this procedure:
Docking - Macromolecule - Set Rigid Filename…
Docking - Ligand -Choose…
And select 1t64_lock.pdbqt and 1t64_key as the macromolecule and ligand filenames, respectively.
Accept all the suggested setting in the AutoDpf4 Ligand Parameters window



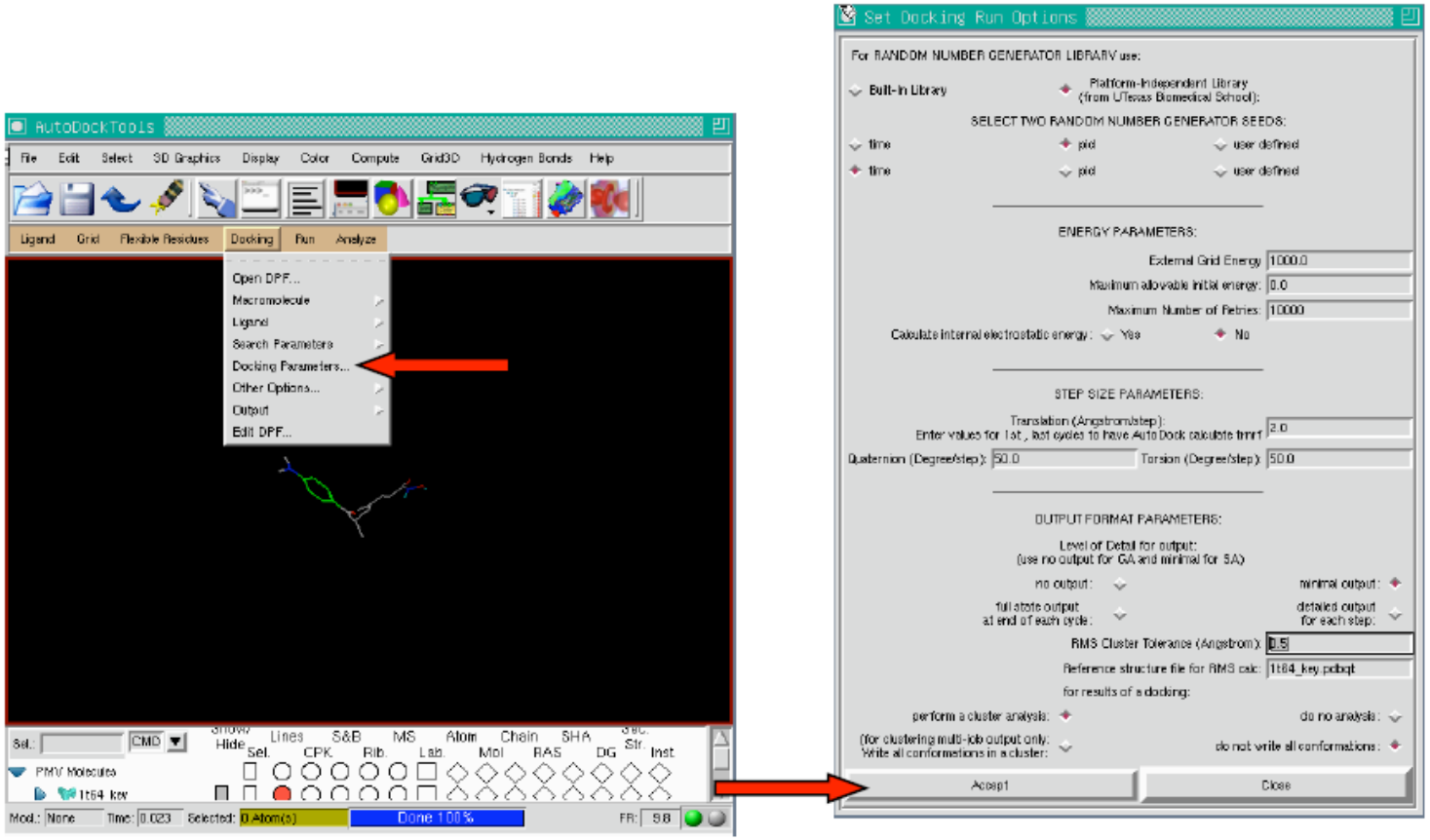

AutoDockTools
File Edit Select 3D Graphics Display Color Compute Grid3D Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyze
Open DPF...
Macromolecule
Ligand
Search Parameters
Docking Parameters...
Other Options...
Output
Edit DPF...
Choose...
Open...
Ligand Parameters...
Choose...(AD3)
Open...(AD3)
Ligand Parameters...(AD3)
Choose Ligand
select a molecule
1t64_lay
1t64_lock
Select Ligand
Dismiss
AutoDpf4 Ligand Parameters
Ligand: 1t64_lay.pdbqt
Ligand Atom Types: A C HD N OA
Floating Grid Map Exists?
Center of Ligand Molecule: 44.014 34.668 53.013
Set Initial State of Ligand:
User-Specified Initial Position: random
Initial Relative Dihedral Offset(qua10): random
Number of Active Torsions in Ligand: 7
Number of Torsional Degrees of Freedom(torsdof)in Ligand: 7
Coefficient of torsional degrees of freedom: 0.274
Specify Initial Dihedrals?
User Specified Initial Relative Dihedrals:
Accept
Close
PMV Molecules
1t64 key

Now let's set the searching and docking parameters; we will use GALS and for this exercises all the default value will be let unchanged (only the ga run will be increase to 100). Save the file as 1t64.dpf and check the written files.

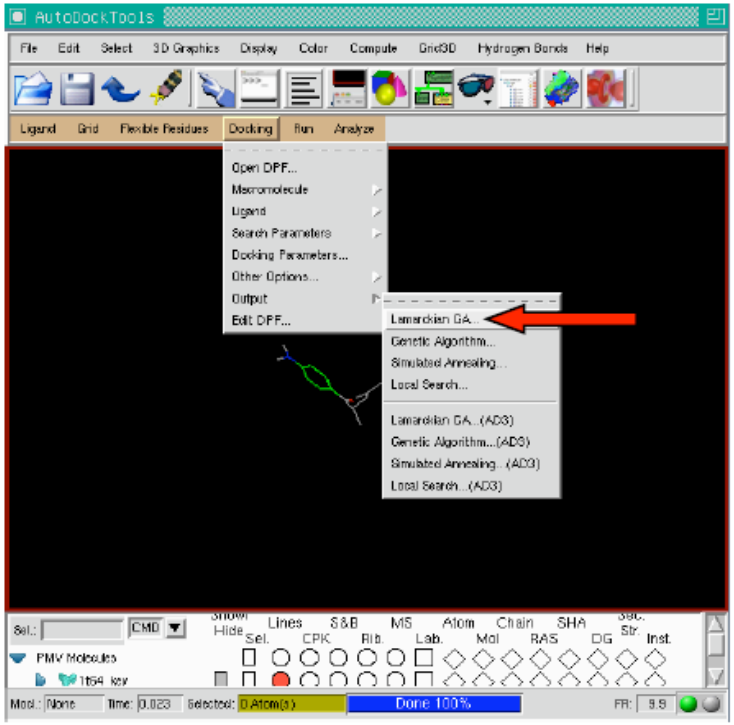
Docking → Search Parameters → Genetic Algorithm…

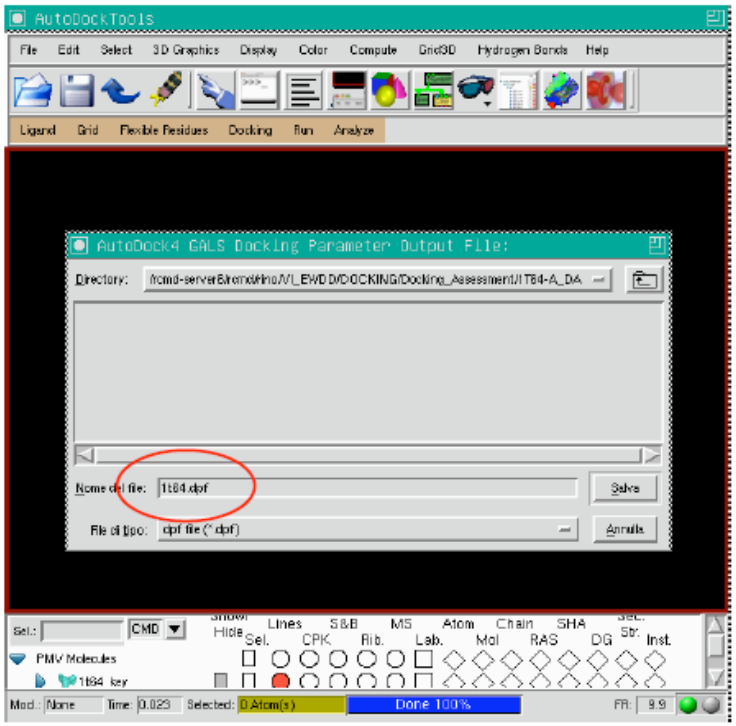


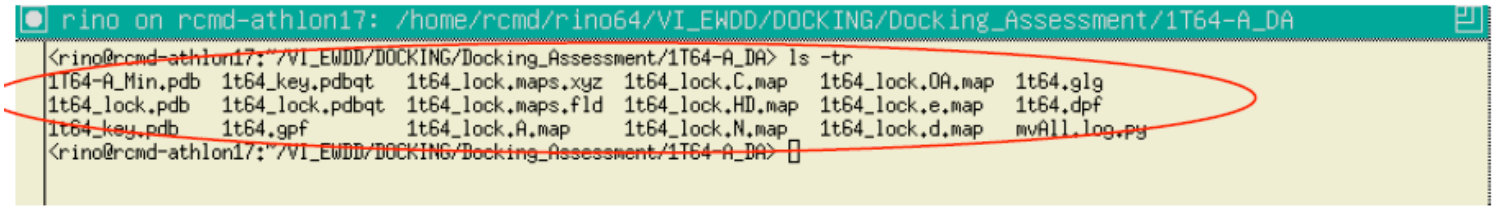
Docking → Docking Parameters…



Docking → Output → Lamarckian GA…







Example of a docking parameter file (1t64.dpf)

```
outlev 1                                # diagnostic output level
intelec                                 # calculate internal electrostatics
seed pid time                           # seeds for random generator
ligand_types A C HD N OA                # atoms types in ligand
fld 1t64_lock.maps.fld                  # grid_data_file
map 1t64_lock.A.map                     # atom-specific affinity map
map 1t64_lock.C.map                     # atom-specific affinity map
map 1t64_lock.HD.map                    # atom-specific affinity map
map 1t64_lock.N.map                     # atom-specific affinity map
map 1t64_lock.OA.map                    # atom-specific affinity map
elecmap 1t64_lock.e.map                 # electrostatics map
desolvmap 1t64_lock.d.map               # desolvation map
move 1t64_key.pdbqt                     # small molecule
about 44.0136 34.668 53.0125            # small molecule center
reorient random                         # initial orientation of ligand
tran0 random                            # initial coordinates/A or random
quat0 random                            # initial quaternion
ndihe 7                                 # number of active torsions
dihe0 random                            # initial dihedrals (relative) or random
tstep 2.0                               # translation step/A
qstep 50.0                              # quaternion step/deg
dstep 50.0                              # torsion step/deg
torsdof 7 0.274000                      # torsional degrees of freedom and
coefficient
rmstol 0.5                              # cluster_tolerance/A
extnrg 1000.0                           # external grid energy
e0max 0.0 10000                         # max initial energy; max number of retries
ga_pop_size 150                         # number of individuals in population
ga_num_evals 250000                     # 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 100                      #
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_sw1                                 # set the above Solis & Wets parameters
compute_unbound_extended                # compute extended ligand energy
```

1.2.5.2 Run autodock4 to launch the docking. You can easily start a job from the command line giving the following:

```
Prompt> autodock4 –p 1t64.dpf –l 1t64.dlg &
```

After some minutes with the set docking parameters the job is over and a “done” message appears. Using a modern CPU the job will take about 10-15 minutes to stop.

1.3. Analyzing the docking results.

Reading a docking log or a set of docking logs is the first step in analyzing the results of docking experiments. (By convention, these results files have the extension ".dlg".)

During its automated docking procedure, AutoDock outputs a detailed record to the file specified after the -1 parameter. In our example, this log was written to the file 'ind.dlg'.

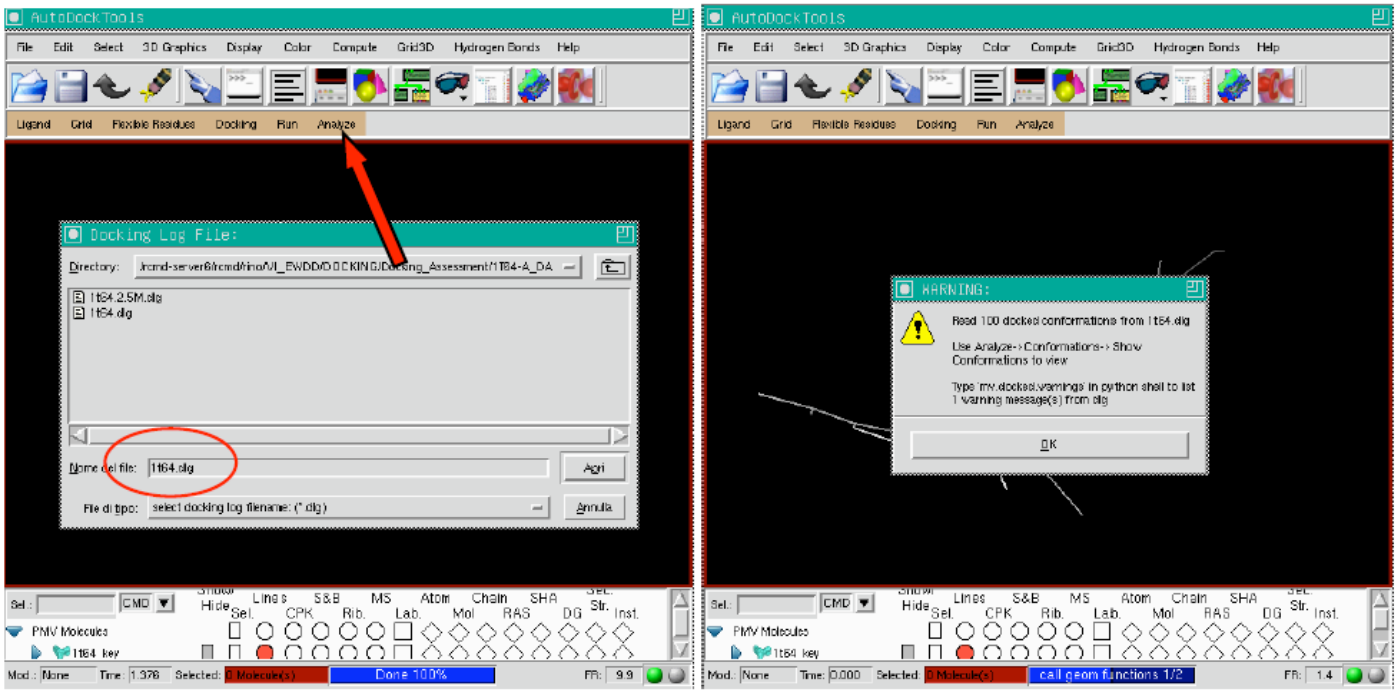
The output includes many details about the docking which are output as AutoDock parses the input files and reports what it finds. For example, for each AutoGrid map, it reports opening the map file and how many data points it read in. When it parses the input ligand file, it reports building various initial data structures. After the input phase, AutoDock begins the specified number of runs. It reports which run number it is starting; it may report specifics about each generation. After completing the runs, AutoDock begins an analysis phase and records details of that process. At the very end, it reports a summary of the amount of time taken and the words 'Successful Completion'.

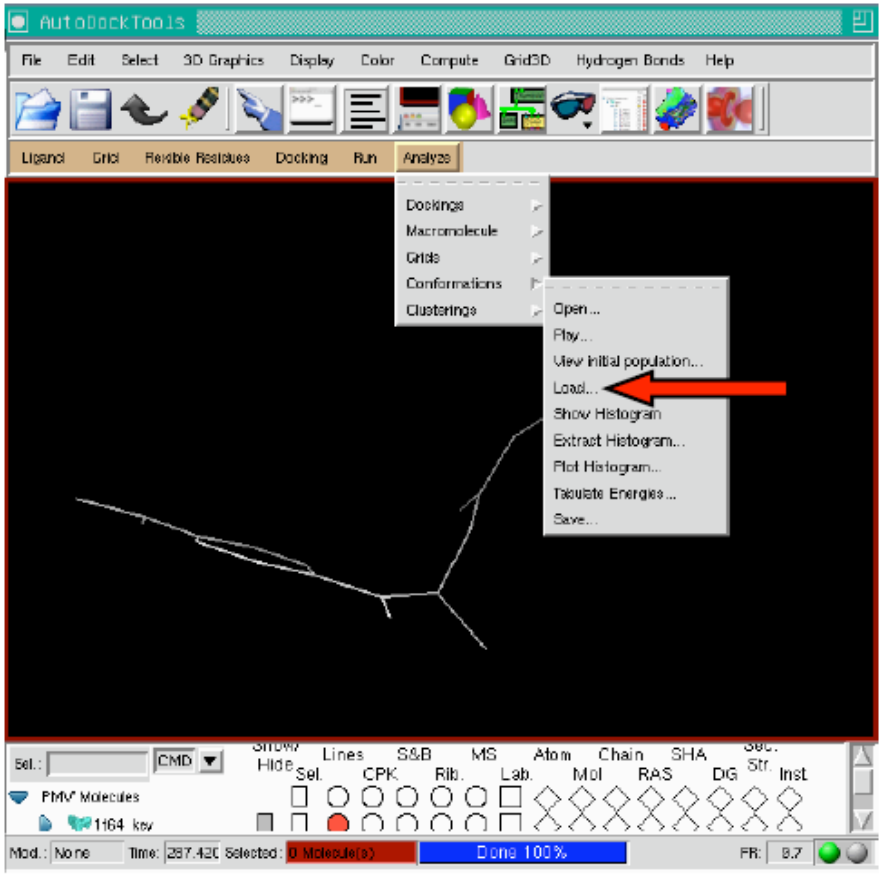
The level of output detail is controlled by the parameter "outlev" in the docking parameter file. For dockings using the GA-LS algorithm, outlev 0 is recommended.

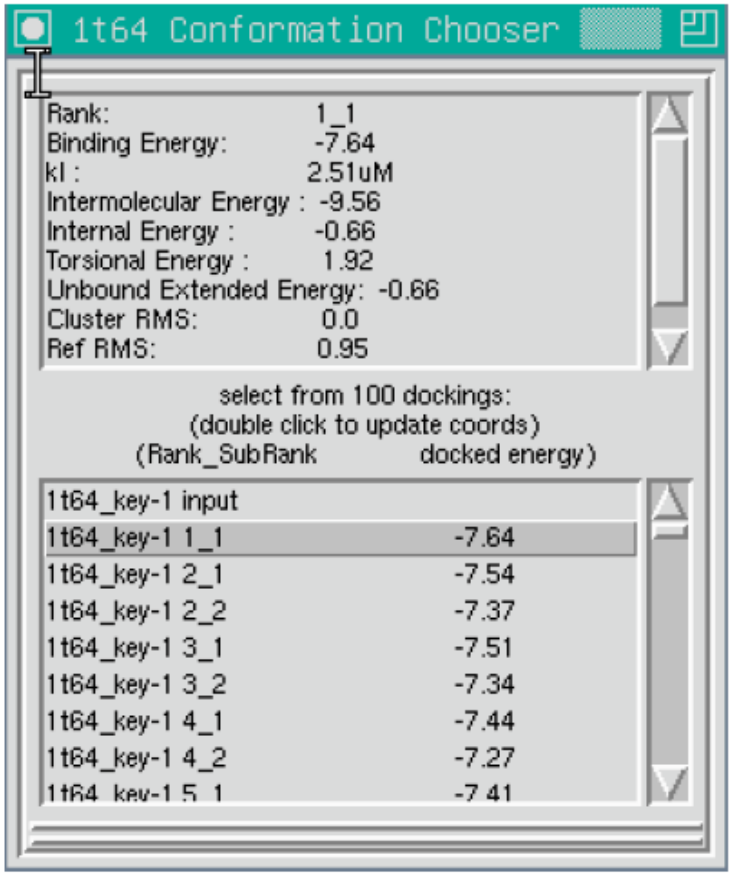
The key results in a docking log are the docked structures found at the end of each run, the energies of these docked structures and their similarities to each other. The similarity of docked structures is measured by computing the root-mean-square-deviation, rmsd, between the coordinates of the atoms. The docking results consist of the PDBQT of the Cartesian coordinates of the atoms in the docked molecule, along with the state variables that describe this docked conformation and position.

1.3.1. Load the results

Before starting this section, you should undisplay any molecules in the Viewer using the **Display → Show/Hide Molecule**
And follow this procedure:
Analyze → Docking → Open…
Analyze → Conformations → Load…



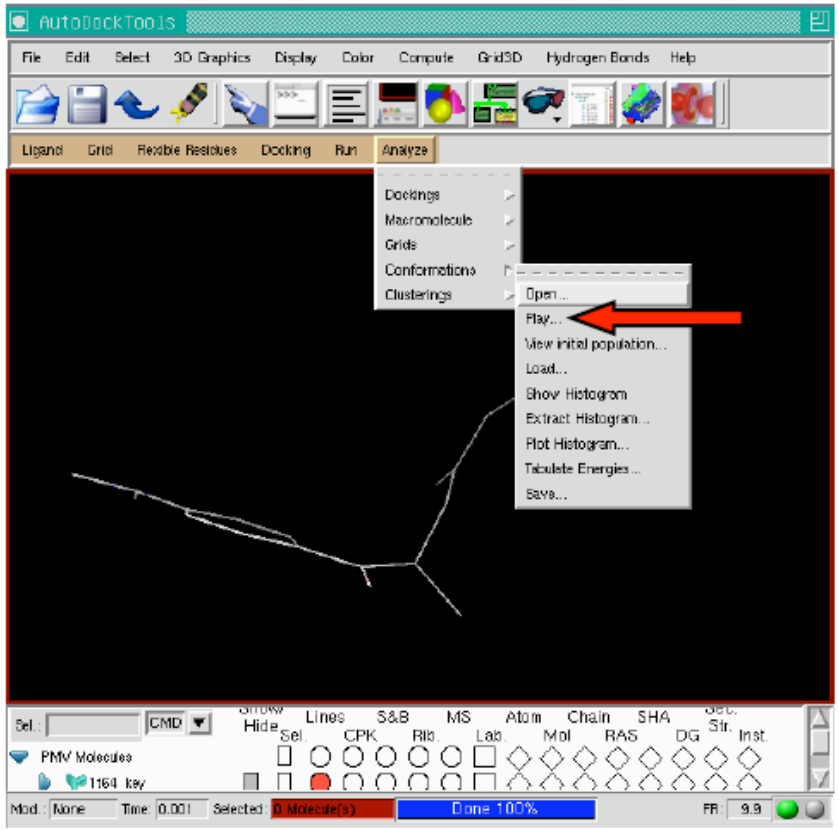

AutoDockTools
File Edit Select 3D Graphics Display Color Compute GridSD Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyze
Dockings
Macromolecule
Grids
Conformations
Clusterings
Open...
Play...
View Initial population...
Load...
Show Histogram
Extract Histogram...
Plot Histogram...
Tabulate Energies...
Save...
Sel.: CMD
Show/ Hide Lines S&B MS Atom Chain SHA Sec. Str.
Sel. CPK Rib. Lab. MOL RAS DG Inst.
PMV Molecules
1t64_key
Mol.: None Time: 287.420 Selected: 0 Molecule(s) Done 100% FR: 8.7

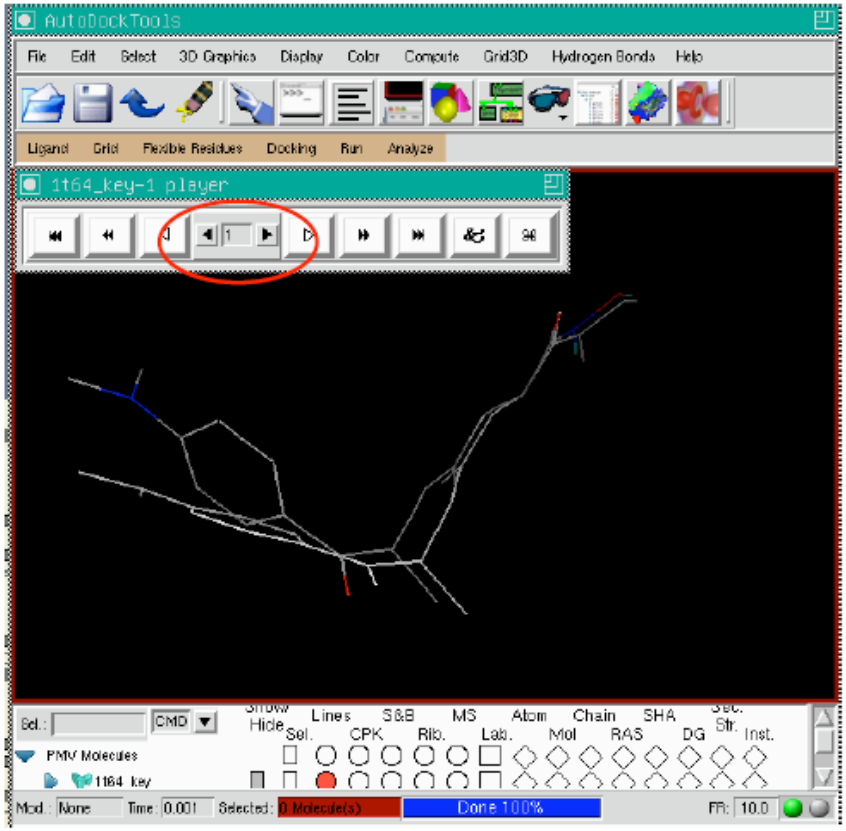

1t64 Conformation Chooser
Rank: 1_1
Binding Energy: -7.64
kI : 2.51uM
Intermolecular Energy : -9.56
Internal Energy : -0.66
Torsional Energy : 1.92
Unbound Extended Energy: -0.66
Cluster RMS: 0.0
Ref RMS: 0.95
select from 100 dockings:
(double click to update coords)
(Rank_SubRank docked energy)
1t64_key-1 input
1t64_key-1 1_1 -7.64
1t64_key-1 2_1 -7.54
1t64_key-1 2_2 -7.37
1t64_key-1 3_1 -7.51
1t64_key-1 3_2 -7.34
1t64_key-1 4_1 -7.44
1t64_key-1 4_2 -7.27
1t64_key-1 5_1 -7.41

1.3.2. Visualize the results.

Follow this procedure:

Analyze →Conformations → Play





1.3.3. Clustering the results.

An Autodock docking experiment usually has several solutions. The reliability of a docking result depends on the similarity of its final docked conformations. One way to measure the reliability of a result is to compare the rmsd of the lowest energy conformations and their rmsd to one another, to group them into families of similar conformations or "clusters".

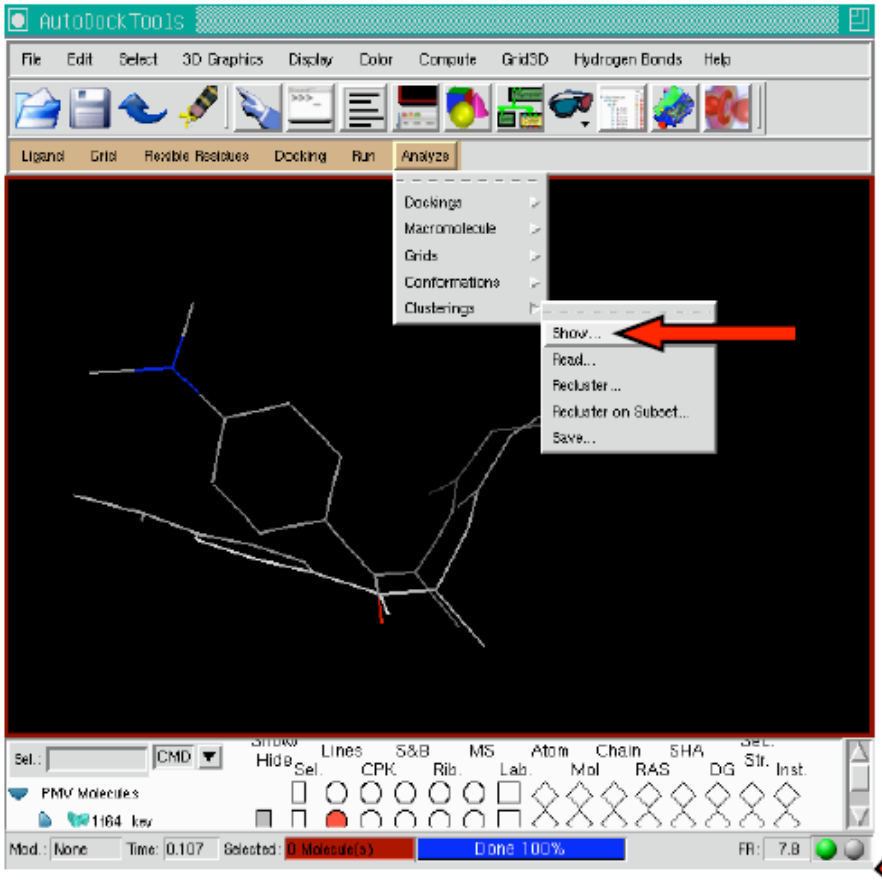
The dpf keyword, **analysis,** determines whether clustering is done by AutoDock. As you will see below, it is also possible to cluster conformations with ADT. By default, AutoDock clusters docked results at 0.5Å rmsd. This process involves ordering all of the conformations by docked energy, from lowest to highest.

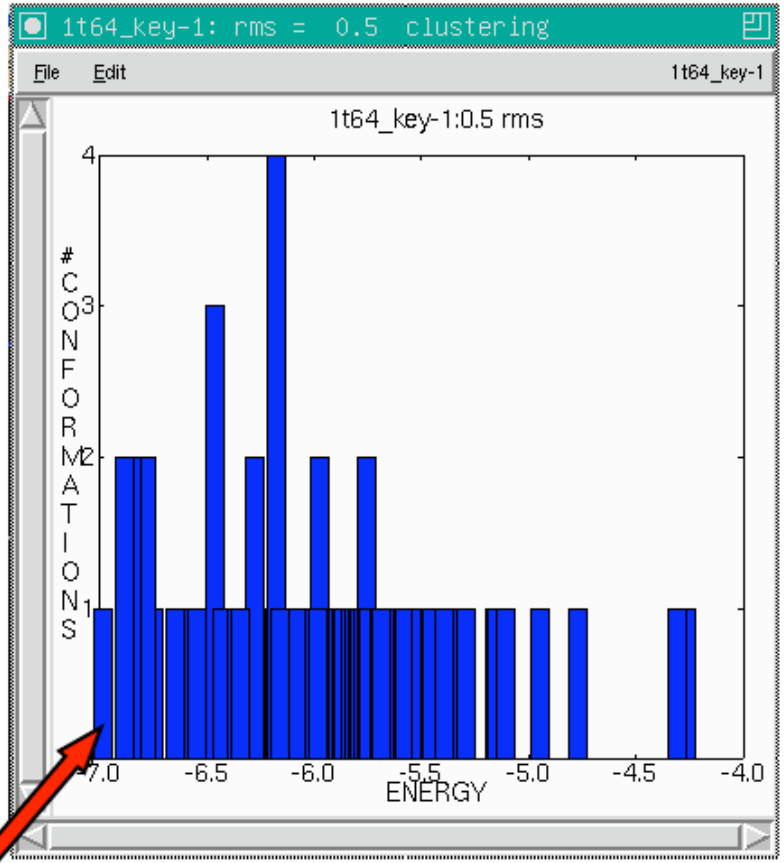
The lowest energy conformation is used as the seed for the first cluster. Next, the second conformation is compared to the first. If it is within the rmsd tolerance, it is added to the first cluster. If not, it becomes the first member of a new cluster. This process is repeated with the rest of the docked results, grouping them into families of similar conformations.

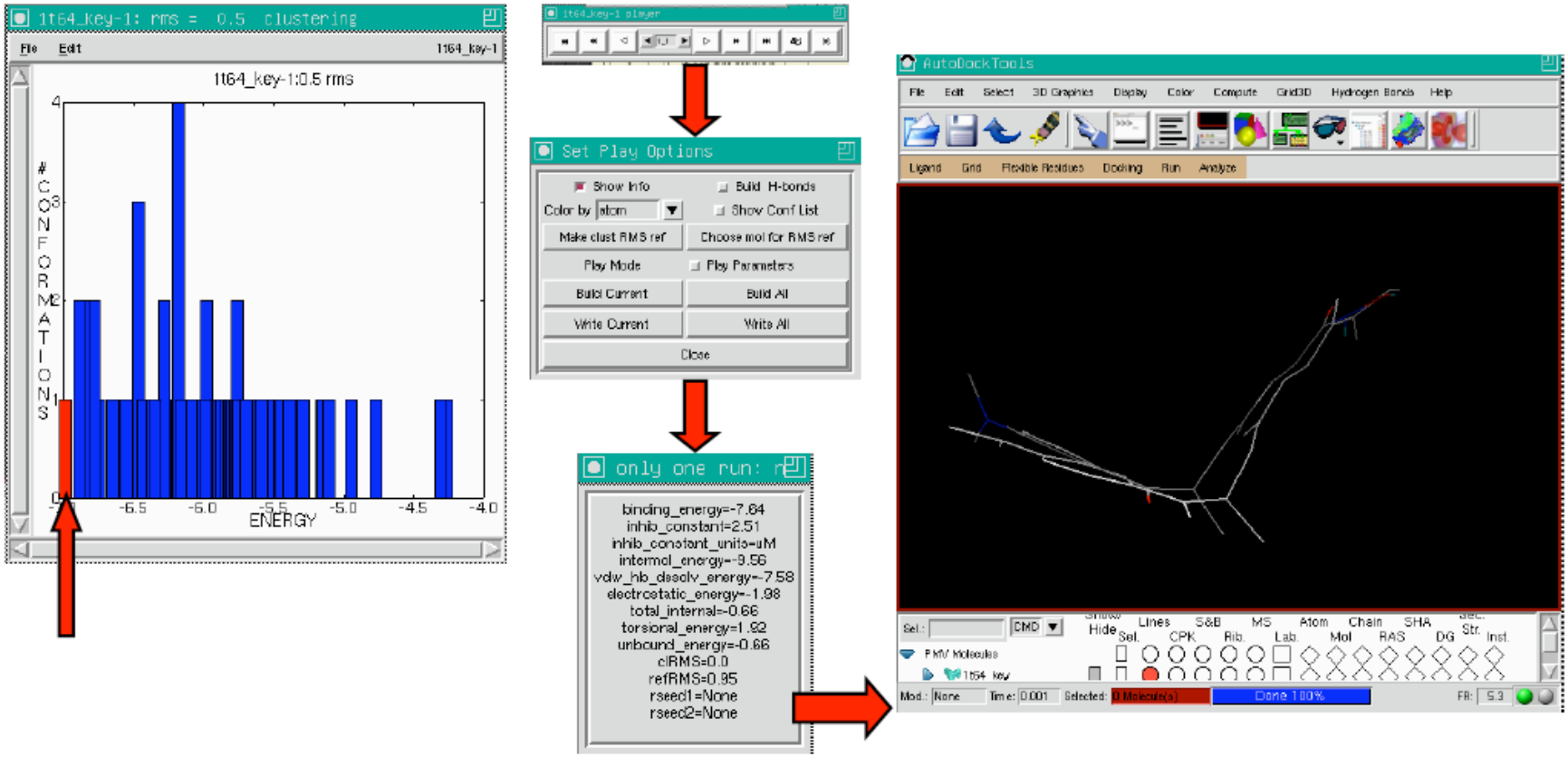
First we will examine the AutoDock clustering that we read in from **1t64.dlg** file. Next we will make new clusters at different rms tolerance values.

1.3.3.1. Displaying the Autodock clustering.

Analyze → Clustering → Show…

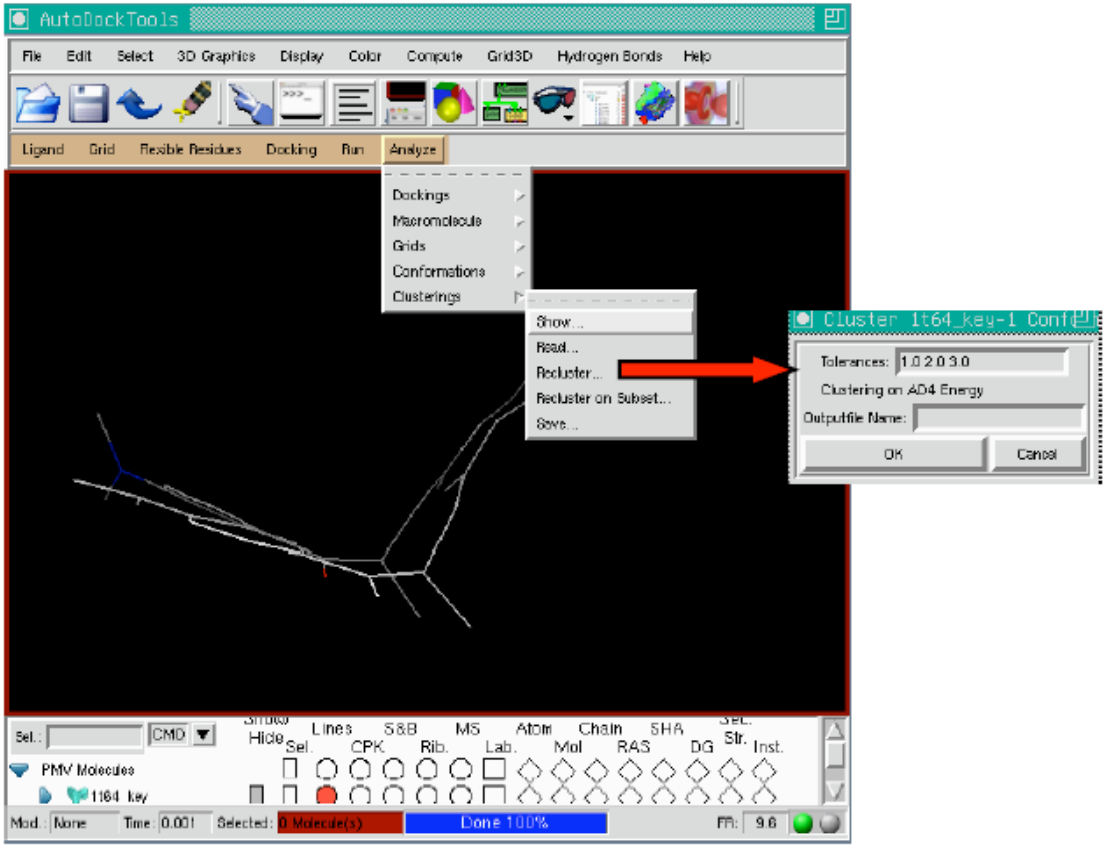


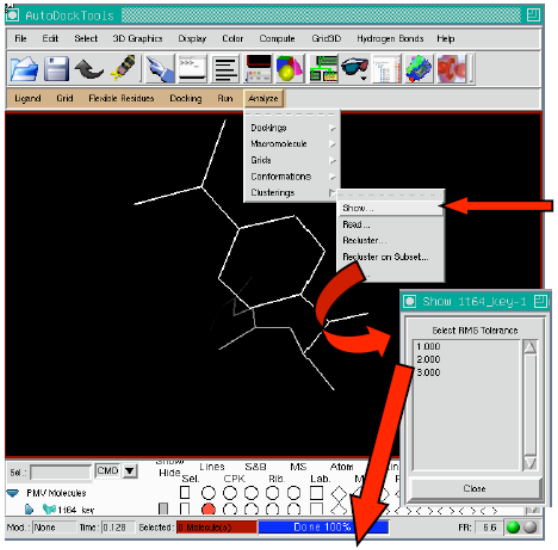


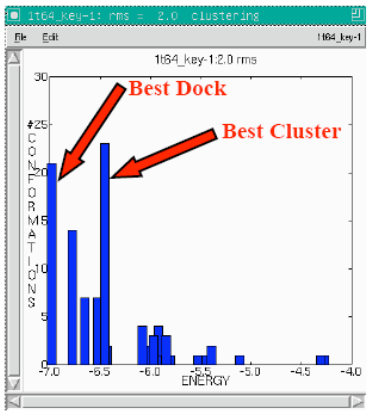

1t64_key-1: rms = 0.5 clustering
File Edit
1t64_key-1
1t64_key-1:0.5 rms
CONFORMATIONS
ENERGY
-6.5
-6.0
-5.5
-5.0
-4.5
-4.0
Set Play Options
Show Info
Build H-bonds
Color by atom
Show Conf List
Make clust RMS ref
Choose mol for RMS ref
Play Mode
Play Parameters
Build Current
Build All
Write Current
Write All
Close
only one run: r
binding_energy=-7.84
inhib_constant=2.51
inhib_constant_units=uM
intermol_energy=-9.56
vdw_hb_desolv_energy=-7.58
electrostatic_energy=-1.98
total_internal=-0.66
torsional_energy=1.92
unbound_energy=-0.66
cRMS=0.0
refRMS=0.95
rseed1=None
rseed2=None
AutoDockTools
File Edit Select 3D Graphics Display Color Compute Grid3D Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyze
Hide Lines S&B MS Atom Chain SHA
Sel. CPK Rib. Lab. Mol RAS DG Str. Inst.
PMV Molecules
1t64_key
Mod.: None Time: 0.001 Selected: 0 Molecule(s) Done 100%

1.3.3.2. Changing the clustering.

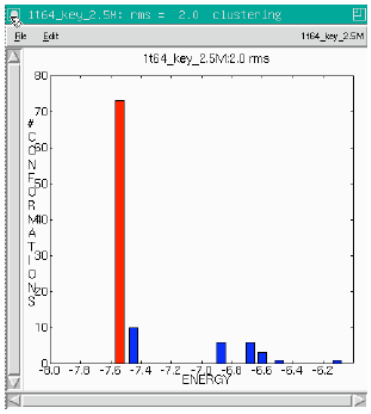
Analyze → Clustering → Recluster…



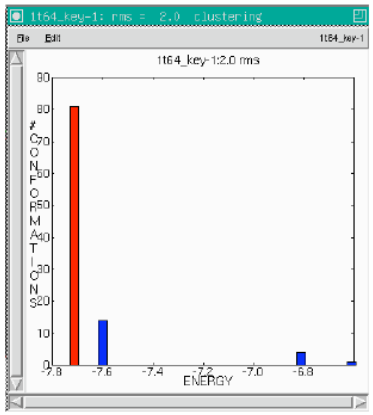




ga_num_eval = 250k
11m 49s



ga_num_eval = 2.5M
1h 43m

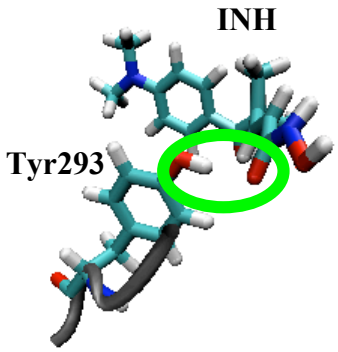


ga_num_eval = 25M
17h 3m

The system seems that reach the convergence with ga_num_eval = 2.5M

2. Flexible docking (the new feature in autodock4).

Inspecting the 1t64 complex it is possible to find that Tyr293 hydroxyl group is involved in a hydrogen bond with the carbonyl oxygen of the INH molecule. What if we allow the flexibility of Tyr293 in the docking?



2.1. Preparing the files.

Start ADT and follow this procedure:

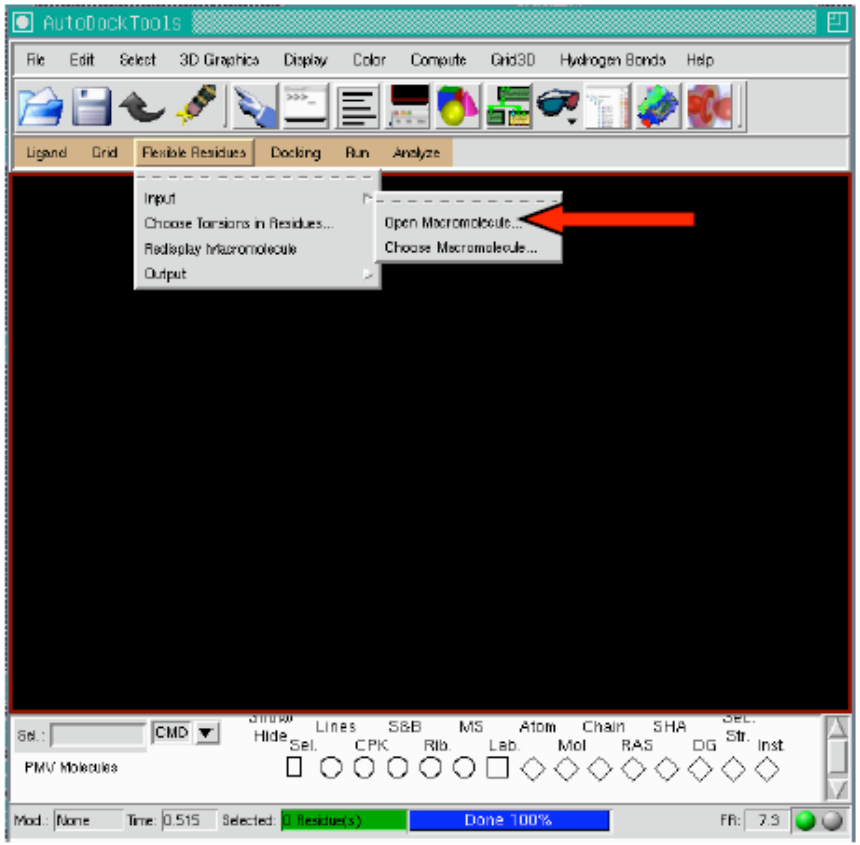
Flexible Residues →Input → Open Macromolecule… (*Select* 1t6a_lock.pdbqt)

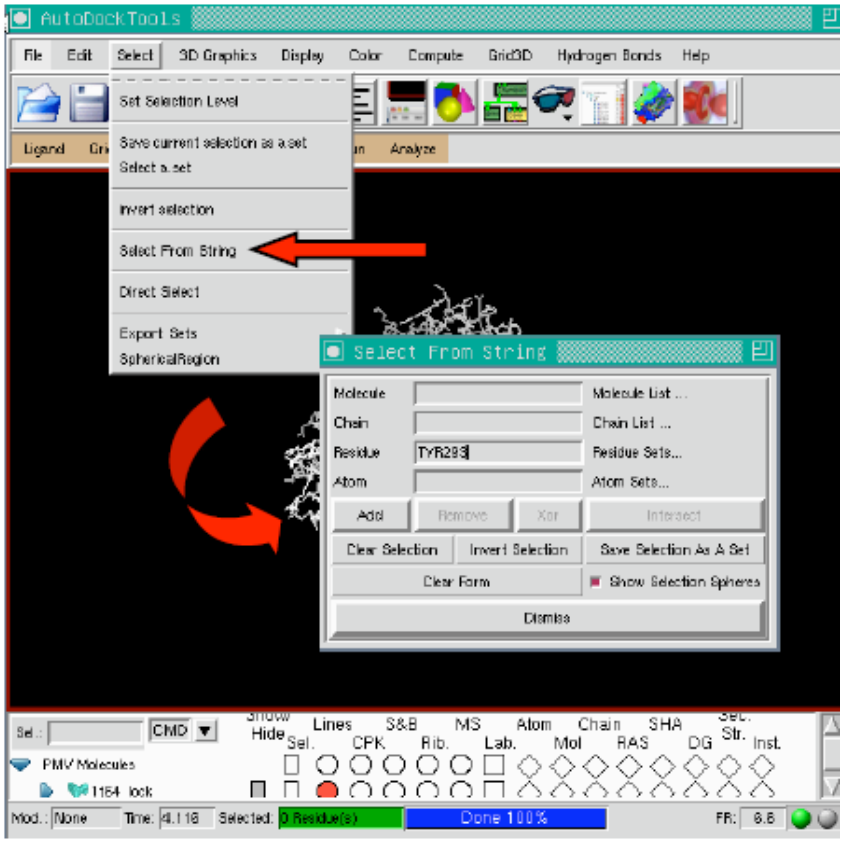
Select → Select From String (*write* TYR293) → Add

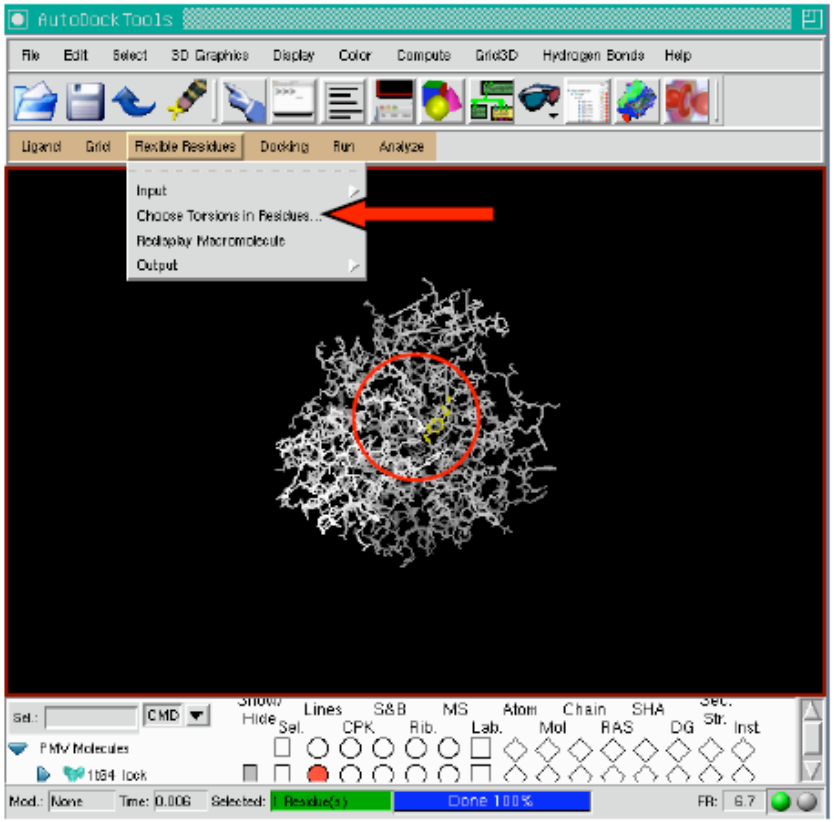
Flexible Residue → Choose Torsions in Residue…

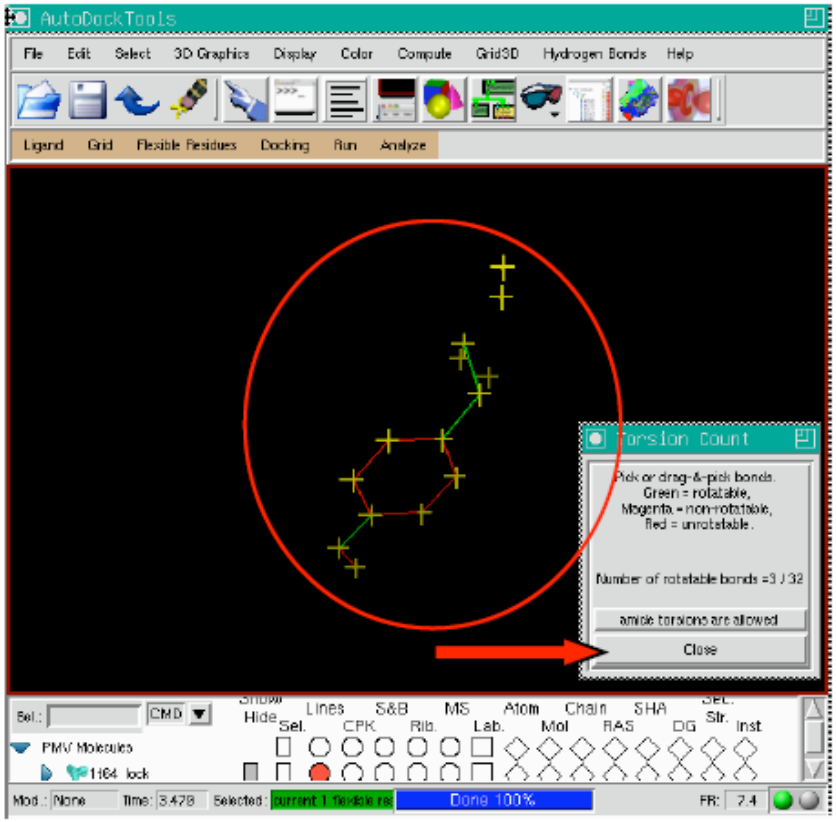
Flexible Residue → Output → Save Flexible PDBQT…(*write* 1t64_lock.flex.pdbqt) → Save

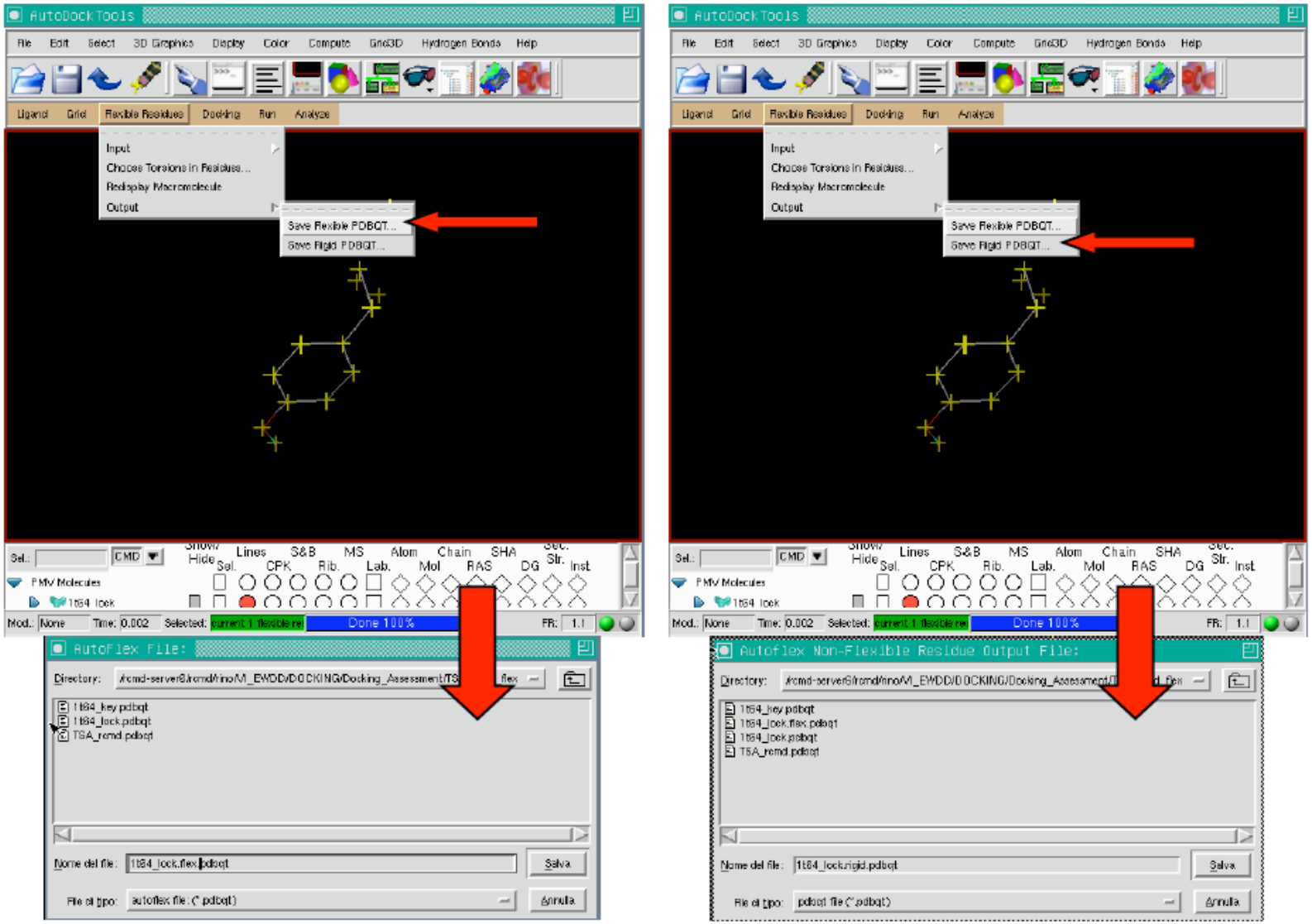
Flexible Residue → Output → Save Rigid PDBQT (*write* 1t64_lock.rigid.pdbqt) → Save


AutoDockTools
File Edit Select 3D Graphics Display Color Compute Grid3D Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyze
Input
Choose Torsions in Residues...
Redisplay Macromolecule
Output
Open Macromolecule...
Choose Macromolecule...
Sel.: CMD
PMV Molecules
Show Hide Sel. Lines CPK S&B Rib. MS Lab. Atom Mol Chain RAS SHA DG SEL. Str. Inst.
Mod.: None Time: 0.515 Selected: 0 Residue(s) Done 100% FR: 7.3


AutoDockTools
File Edit Select 3D Graphics Display Color Compute Grid3D Hydrogen Bonds Help
Ligand Analyze
Set Selection Level
Save current selection as a set
Select a set
Invert selection
Select From String
Direct Select
Export Sets
SphericalRegion
Select From String
Molecule
Chain
Residue TYR293
Atom
Molecule List ...
Chain List ...
Residue Sets...
Atom Sets...
Add Remove Xor Intersect
Clear Selection Invert Selection Save Selection As A Set
Clear Form
Show Selection Spheres
Dismiss
Sel.: CMD
PMV Molecules
1164 lock
Show Hide Sel. Lines CPK S&B Rib. MS Lab. Atom Mol Chain RAS SHA DG SEL. Str. Inst.
Mod.: None Time: 4.116 Selected: 0 Residue(s) Done 100% FR: 6.6


AutoDockTools
File Edit Select 3D Graphics Display Color Compute GridSD Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyze
Input
Choose Torsions in Residues...
Redisplay Macromolecule
Output
Sel.: CMD
Show/ Hide Lines S&B MS Atom Chain SHA Sec. Str.
Sel. CPK Rib. Lab. Mol RAS DG Inst.
PMV Molecules
1tb4 lock
Mod.: None Time: 0.006 Selected: 1 Residue(s) Done 100% FR: 6.7


AutoDockTools
File Edit Select 3D Graphics Display Color Compute GridSD Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyze
Torsion Count
Pick or drag-&-pick bonds.
Green = rotatable,
Magenta = non-rotatable,
Red = unrotatable.
Number of rotatable bonds =3 / 32
amide torsions are allowed
Close
Sel.: CMD
Show/ Hide Lines S&B MS Atom Chain SHA Sec. Str.
Sel. CPK Rib. Lab. Mol RAS DG Inst.
PMV Molecules
1tb4 lock
Mod.: None Time: 3.478 Selected: current 1 rotatable ro Done 100% FR: 7.4


AutoDockTools
File Edit Select 3D Graphics Display Color Compute Grid3D Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyse
Input
Choose Torsions in Residues...
Redisplay Macromolecule
Output
Save Flexible PDBQT...
Save Rigid PDBQT...
AutoFlex File:
Directory:
1t84_key.pdbqt
1t84_lock.pdbqt
TSA_remd.pdbqt
Nome del file: 1t84_lock.flex.pdbqt
Salva
File di tipo: autoflex file (*.pdbqt)
Annulla
AutoDockTools
File Edit Select 3D Graphics Display Color Compute Grid3D Hydrogen Bonds Help
Ligand Grid Flexible Residues Docking Run Analyse
Input
Choose Torsions in Residues...
Redisplay Macromolecule
Output
Save Flexible PDBQT...
Save Rigid PDBQT...
Autoflex Non-Flexible Residue Output File:
Directory:
1t84_key.pdbqt
1t84_lock.flex.pdbqt
1t84_lock.pdbqt
TSA_remd.pdbqt
Nome del file: 1t84_lock.rigid.pdbqt
Salva
File di tipo: pdbqt file (*.pdbqt)
Annulla

2.2. Preparing the parameter files for AutoGrid (gpf) and AutoDock (dpf) and running the AutoGrid and AutoDock programs.

To do this you can sections from 1.2.4 through 1.2.5.2 if you prefer the graphical way using ADT.

3.3. Results

The run at ga_num_eval = 2.5M has converged and these are the graphical and clustering results: