Steps to be followed-

Starting Autodock

Open directory where MGL tool is installed
(home/MGLtool/bin) and run 'adt' in terminal
It opens AutoDock Tool GUI.

There are **two menu** sets in ADT. The **upper** one is for **visualization** of a structure in different styles and patterns. The second **below** menu is for **docking**. It gives option to open and set properties for a ligand and then create environment for protein- ligand docking. From here we can generates docking results also and visualize ligand docking in protein molecule.

Protein – Ligand Docking in AutoDock

For this you should have a protein molecule 3d-structure file (PDB file) and a ligand 3d-structure file. (PDB or mol2 format

Part - A

Open Protein Molecule in viewer

1. (File -> Read Molecule) **Eg.1hkb.pdb** (human brain hexokinase)

Browse and open protein structure file

Visualize Protein Structure

2. Use various option from top menu and visualize protein structure.

Preparing PDB file

PDB files are not perfect every time when it is opened very first time in ADT. There are some potential problems such as missing atoms, chain breaks, added water or other hetero atoms etc. You have to correct these problems from PDB file by adding or removing required elements.

i) Removing 'Water' molecule or hetero atoms from PDB-
For this, goto
(a) Select -> Select from string
This opens a box contains four option to select molecule, chain, residue or atom.
Enter '**HOH***' in residue box and click on '**Add**'. It select all water molecules of protein structure.
You can enter other residue name(s) for their selection. For multiple selection enter name and number of all residues separated with column containing no spaces.
(example: ARG1,TYR39,PRO12)
You can choose other option too, to select content at their level.
After selection of water molecules in structure, go to
ii) Edit -> Delete -> Delete Selected Atoms
It will ask for confirmation. **Continue** to it.

Adding 'Hydrogen' to set noBond Order to molecule

iii) Edit -> Hydrogen -> Add
It will ask for add hydrogen, choose **All Hydrogens** with **noBondOrder** and click **OK.**

Save as PDB file

To keep original file safe, save this modified protein structure to a new pdb file.
iv) File -> Save -> WritePDB
Select write options and click to OK.

Part – B

Preparing Ligand file

Now this is time to open ligand molecule into ADT and prepare it for docking. For this go to
i) Ligand -> Input -> Open
Browse and open ligand molecule.
This ligand should have torsional bonds and partial atomic charges with it so can associate with a better position in protein. So there is a rigid part in ligand known as 'root' which keeps hold torsional bonds and flexible parts known as branches emanate from root, which shows torsions.
A ligand must have both polar and non-polar hydrogens. When you open a ligand file in ADT, it automatically **computes Gasteiger charges (partial charges)** and rotatable bonds, and if no charges present, ADT tries to add charges in ligand and also adds a TORSDOF (Torsion Degree of Freedom) value.
This ligand file is written in PDBQT format where Q and T stand for partial charges and torsion angles. Means this file has additional information of partial charges and torsional angles.

Detect Root and Torsions for ligand

For this, go to
ii)Ligand -> Torsion Tree -> Detect Root
ADT detects for the finest root in ligand and show a ball shaped root marker.
To show/hide this root marker
iii)Ligand -> Torsion Tree -> Show/Hide Root Marker

Choose torsions in ligand

To view and choose torsional bonds in ligand, go to
iv)Ligand -> Torsoin Tree -> Choose Torsions
A 'Torsion Count box' will appear showing information about number of rotatable bonds. There are three types of bonds represented by three different colors-

- **Green** – Rotatable bonds*
- **Magenta** – Non-Rotatable bonds*
- **Red** – Unrotatable bonds

(*These rotatable and non-rotatable bonds can be converted into each other by clicking on them while holding 'Shift' key.)

So choose desired rotatable/torsional bonds in ligand and click on '**done**' on Torsion Count box.
To select only a number of torsional bonds, go to
v) Ligand -> Torsion Tree -> Set Number of Torsions

Checking partial atomic charges on ligand

For this, go to
vi) Edit -> Charges -> Compute Gasteiger

Save ligand to file
vii) Ligand -> Output -> Save as PDBQT
Write ligand name with extension 'pdbqt' added (example: ligand.pdbqt) and save it to default directory.

Part – C

Preparing Flexible Residues

This is the flexible part of protein (macromolecule) residues i.e. their sidechains, which shows moving property at the time of docking with ligand. These residues must be known, situated at pocket site in protein. Rest portion of protein is set to be rigid part.
To select these residues firstly protein molecule is selected-

i) Flexible Residues -> Input -> Choose Macromolecule

Select Protein molecule name and click on Select Molecule.

To select residues in protein, go to

ii) Select -> Select from String

Add name and number of these residues in Residue box (for example: ARG1) and click on '**ADD**' and click on '**Dismiss**'

Select torsions for these residues

iii) Flexible Residues -> Choose Torsions in Currently Selected Residues

This will hide whole protein molecule except selected residues and a box will rise up asking for pick bonds. Select desired torsional bonds from these residues and click on '**Close**'.
To display protein molecule again

iv)Flexible Residues -> Redisplay Macromolecule

Save Flexible & Rigid PDBQT files

Flexible Residues
i) Flexible Residues -> Output -> Save Flexible PDBQT
Enter a name (example – **protein_flex.pdbqt**) and save it to default directory.
Rigid Residues
ii) Flexible Residues -> Output -> Save Rigid PDBQT
Enter a name (example – **protein_rigid.pdbqt**) and save it to default directory.

Now this protein molecule's work is finished in this session, so delete this protein by

iii)Edit -> Delete -> Delete Molecule

Select this protein name and click on '**Delete Molecule**'.

Part – D

Generating Grid Parameter File (GPF)

A grid parameter file is that which computes records about the receptor. These records are in form of various maps such as locations, electrostatic, potential energy etc. This GPF file tells autodock these information of receptor that where will the docking be performed.

Steps to generating GPF file are as follow-

i) Grid -> Macromolecule -> Open

Browse and open that '**protein_rigid.pdbqt**' file. This will open rigid part of receptor protein molecule.

Now ligand and flexible residue file will be selected. For this, go to

ii) Grid -> Set Map types -> Choose Ligand

This will open select molecule box, select ligand molecule from there.

iii) Grid -> Set Map types -> Open FlexRes

Browse and open '**protein_flex.pdbqt**' file. This will open flexible residue part of receptor protein molecule.

Setting up grid box

A grid box is to be set where the docking will occur.This grid box is a minimum volume containing only pocket portion of receptor i.e. flexible residue part.
For this, go to

i)Grid -> Grid Box

This will open a Grid Option Box and a box will also appear in visualizer. This Grid Option Box contains two sections, the above one is to set volume of this grid box in X,Y and Z axis. The below section is to move this grid box in X,Y and Z axis.
Set up this grid box according to your docking area and after that -

ii)Grid Option Box -> File -> Close saving current

Now this grid is saved as a file. For this, go to

iii)Grid -> Output -> Save GPF

Save gpf file in default directory with a name following '.gpf' extension (example: protein.gpf)

Now this is time to run this GPF file so that grid log file and required maps files can be generated. For this, go to

iv)Run -> Run AutoGrid

This will open Run AutoGrid box. Here we set up parameters to run autogrid.

i) **Program Pathname** – Browse and select path of autogrid bin file.

ii) **Parameter file name** – Browse default directory and open GPF file.

iii) **Log filename** – When you browse for GPF file, after that it automatically sets path and log filename follwing extension '.glg'.

iv) **Set Nice Level to 20** and Launch AutoGrid.

After a few seconds, this process will complete telling whether successful or not.

Part – E

Generating Docking parameter File (DPF)

A DPF file is that which contains records of all selected parameters and desired map file's information that will help **autodock** to compute position of ligand into receptor protein molecule. Means it is used to perform final protein-ligand docking.

Following steps are taken for docking-

i) Set rigid part of receptor molecule
Docking -> Macromolecule -> Set Rigid Filename

ii) Set flexible residues of receptor
Docking -> Macromolecule -> Set Flexible Residue Filename

iii) Set ligand
Ligand -> Choose (Select ligand and click on select ligand)

iv) Set energy evolution parameters
Search Parameter -> Genetic Algorithm
Set Maximum number of evals to short, so the process can be completed in less time.

v) **Docking Parameters -> Accept**

vi) Save Docking parameter file (DPF)
Output -> Lamarckian GA
Browse the default directory and save '**ligand.dpf**' file.

After set up all parameters for receptor and ligand, finally docking will be performed.
For this, go to

Run -> Run AutoDock

This will open Run AutoDock box. Here we set up parameters to run autodock.

i) **Program Pathname** – Browse and select path of autodock bin file.
ii) **Parameter file name** – Browse default directory and open DPF file.
iii) **Log filename** – When you browse for DPF file, after that it automatically sets path and log filename follwing extension '.dlg'.
iv) **Set Nice Level to 20** and Launch AutoDock.

After a few seconds, this process will complete telling whether successful or not.
This will also create a Docking Log File which contains final results of protein-ligand docking.

Part –F

Visualization of Protein-Ligand Docking
Now we can visualize performed docking in ADT visualizer.
For this, go to

i) **Analyze -> Docking -> Open**
Browse to default directory and open docking log file (**ligand.dlg**)
Macromolecule -> Choose (or can Open)
Select '**protein_rigid**' file.
Confirmation -> Play
Click on 'next' or 'play' button on player to view various position of ligand in docking. These numbers tell ranking of ligand docking confirmation.