

Original article:

A SIMPLE CLICK BY CLICK PROTOCOL TO PERFORM DOCKING: AUTODOCK 4.2 MADE EASY FOR NON-BIOINFORMATIANS

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ABSTRACT

Recently, bioinformatics has advanced to the level that it allows almost accurate prediction of molecular interactions that hold together a protein and a ligand in the bound state. For instance, the program AutoDock has been developed to provide a procedure for predicting the interaction of small molecules with macromolecular targets which can easily separate compounds with micromolar and nanomolar binding constants from those with millimolar binding constants and can often rank molecules with finer differences in affinity. AutoDock can be used to screen a variety of possible compounds, searching for new compounds with specific binding properties or testing a range of modifications of an existing compound. The present work is a detailed outline of the protocol to use AutoDock in a more user-friendly manner. The first step is to retrieve required Ligand and Target.pdb files from major databases. The second step is preparing PDBQT format files for Target and Ligand (Target.pdbqt, Ligand.pdbqt) and Grid and Docking Parameter file (a.gpf and a.dpf) using AutoDock 4.2. The third step is to perform molecular docking using Cygwin and finally the results are analyzed. With due confidence, this is our humble claim that a researcher **with no previous background in bioinformatics research** would be able to perform molecular docking using AutoDock 4.2 program by following stepwise guidelines given in this article.

Keywords: computer aided docking, free offline docking; non-bioinformaticians, AutoDock, drug discovery, enzyme-ligand interaction

INTRODUCTION

Computer-aided docking is an important tool for gaining understanding of the binding interactions between a ligand (small molecule) and its target receptor (enzyme) (Anderson, 2003; Schneider, 2010) and has emerged as a reliable, cost-effective and time-saving technique for the discovery of lead compounds (Walters et al., 1998; Schneider and Böhm, 2002; Waszkowycz et al., 2001). In recent years, the virtual screening approach for docking small mol-

ecules into a known protein structure is a powerful tool for drug design and has become an integral part of the drug discovery process. Computational tools like AutoDock offer the advantage of delivering new drug candidates more quickly and at a lower cost (Gilbert, 2004; Warren et al., 2006). AutoDock is an excellent non-commercial docking program that is widely used. Further, it employs a stochastic Lamarckian genetic algorithm for computing ligand conformations and simultaneously minimiz-

ing its scoring function which approximates the thermodynamic stability of the ligand bound to the target protein (Morris et al., 1998, 2009). The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process. Theoretically the application of AutoDock in virtual screening is constrained only by the chemical compounds features that can be calculated and the relation between these features and the target (Lazarova, 2008). But the problem arises in practical implementa-

tion of AutoDock in virtual screening of compounds which requires several considerations. Thus, this paper provides an easier protocol for the use of AutoDock for molecular docking purposes and will hopefully help in practically implementing AutoDock and AutoDock tools for the virtual screening purposes. To make it easier to understand, an example of experiment of the docking of Imipenem-hydrolyzing enzyme beta-lactamase SME-1 with Imipenem as ligand was made using AutoDock 4.2/ADT.

REQUIREMENTS

1) Windows XP or Windows 7

Freely available software's for **non-commercial** uses:

2) MGL tools

<http://mgltools.scripps.edu/downloads>

3) Cygwin

<http://www.cygwin.com/install.html>

(Click setup-x86.exe for 32-bits version while setup-x86_64.exe for 64-bits version)

4) Discovery Studio Visualizer

<http://accelrys.com/products/discovery-studio/visualization-download.php>

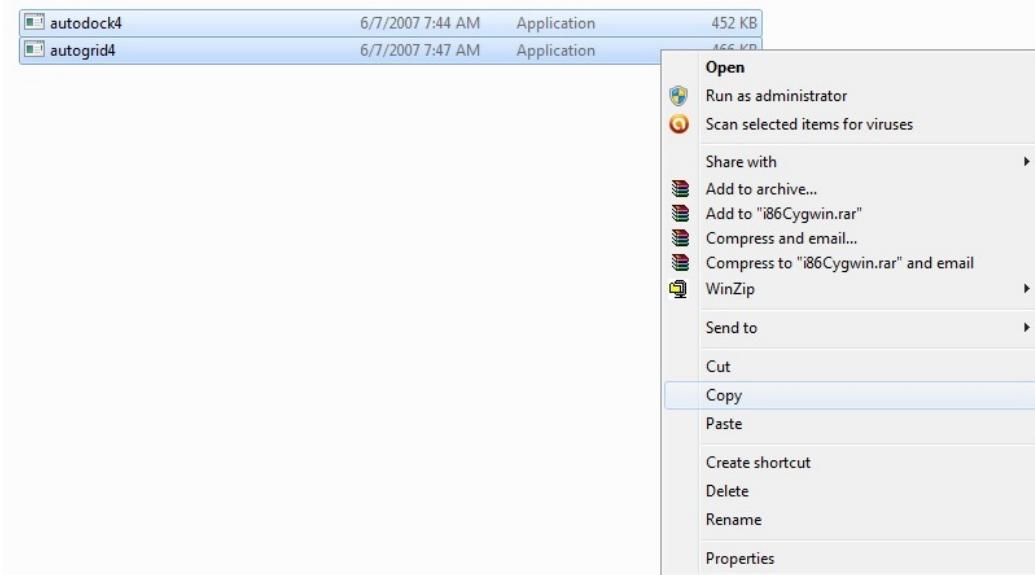
5) Binary files

<http://autodock.scripps.edu/downloads/autodock-registration/autodock-4.0.1-and-autogrid-4.0.0>

The screenshot shows the AutoDock 4.0.1 and AutoGrid 4.0.0 download page. The left sidebar has a navigation menu with links like Home, Downloads, Resources, FAQs & Help, Forum, Contact, and a 'How do I get started with' section. The main content area is titled 'AutoDock 4.0.1 and AutoGrid 4.0.0' and lists binary files for different platforms. A red arrow points to the 'Windows / Cygwin' entry, which is 310 KB in size. The right sidebar contains news items and a calendar for February 2013.

All	
	■ Source, all binaries, examples (38 MB) md5sum cd38263e9c0cd7e407681ced94ce5355
	■ Linux: RedHat (32-bit) (198 KB) md5sum 159e3a7195df4a96999cd075b0c99ff9
	■ Linux: Itanium (IA64) (390 KB) md5sum 326e29497f868b70213c7c82b1e343b0
	■ Mac OS X 10.4 (Intel) (208 KB) md5sum 8dddbecc6aeb8fa542ae469705ffc3db
	■ Mac OS X 10.4 (PowerPC) (232 KB) md5sum 64eb759a38120c0e65f2f42ef3c4312b
	■ Windows / Cygwin (310 KB) md5sum 81bbb8b6de372cf4e10210aec95edb56
	■ SGI IRIX 6.5 (1.4 MB) md5sum c5f3f1a14f79885327e8f05877c3f99f
	■ Sun Solaris (Sparc) (287 KB) md5sum c4ac8775b8d09037e49d21f3f9001ac3
	■ Source code (1.9 MB) md5sum da0e9c67ab539964a734e4de6840506a

- Download and Extract autodocksuite-4.0.1-i86Cygwin.tar
- Copy autodock4.exe and autogrid4.exe



- Paste in My computer\ C drive\ Cygwin\ bin

6) Java

<http://www.java.com/en/download/index.jsp>

METHODS

1) Retrieving Required Ligand and Target .pdb files from major databases:

1.1 Retrieving Target.pdb files from major protein databases

<http://www.rcsb.org/pdb/home/home.do>

A screenshot of the RCSB PDB website. The header features the RCSB PDB logo and navigation links for PDB-101, MyPDB, Home, and various policies. The main search bar has an arrow pointing to it. Below the search bar is a section titled 'Biological Macromolecular Resource' with a 'Full Description' link. A 'Featured Molecules' section highlights the 'Proton-Gated Urea Channel' with a molecular model and a brief description. To the right, there are sections for 'New Structures', 'New Features', and 'RCSB PDB News'. A sidebar on the left provides links for PDB-101, MyPDB, and general site navigation.

- Type the query protein or enzyme (Imipenem-hydrolyzing beta-lactamase SME-1)
- Select enzyme (Imipenem-hydrolyzing beta-lactamase SME-1)

STRUCTURE OF THE IMIPENEM-HYDROLYZING BETA-LACTAMASE SME-1

Authors: Sougakoff, W., L'Hermite, G., Billy, I., Guillet, V., Naas, T., Nordman, P., Jarlier, V., Delettre, J.

Release: 2001-01-26 **Classification:** Hydrolase

Experiment: X-RAY DIFFRACTION with resolution of 2.13 Å **Residue Count:** 534

Compound: 1 Polymer [Hide Polymer Details | Display for All Results]

Molecule: CARBAPENEM-HYDROLYSING BETA-LACTAMASE SME-1

Polymer: 1 **Type:** protein **Length:** 267

Chains: A, B

EC#: 3.5.2.6

Details: APO FORM

Organism: *Serratia marcescens*

UniProtKB: Protein Feature View | Search PDB | Q54488

Citation: Structure of the Imipenem-Hydrolyzing Class A Beta-Lactamase Sme-1 from *Serratia Marcescens*. (2002) Acta Crystallogr., Sect.D 58: 267

PubMed Abstract: The structure of the beta-lactamase SME-1 from *Serratia marcescens*, a class A enzyme characterized by its significant activity against imipenem, has been determined to 2.13 Å resolution. The overall structure of SME-1 is similar to that of other class A beta-lactamases. In the active-site cavity, most of the residues found in SME-1 are conserved among class A beta-lactamases, except at positions 104, 105 and 237, where a tyrosine, a histidine and a serine are found, respectively, and at position 238, which is occupied by a cysteine forming a disulfide bridge with the other cysteine residue located at position 69. The crucial role played by this disulfide bridge in SME-1 was confirmed by site-directed mutagenesis of Cys69 to Ala, which resulted in a mutant unable to confer resistance to imipenem and all other beta-lactam antibiotics tested. Another striking structural feature found in SME-1 was the short distance separating the side chains of the active serine residue at position 70 and the strictly conserved glutamate at position 166, which is

- Select download files
- Click PDB file (gz) and download it

STRUCTURE OF THE IMIPENEM-HYDROLYZING BETA-LACTAMASE SME-1

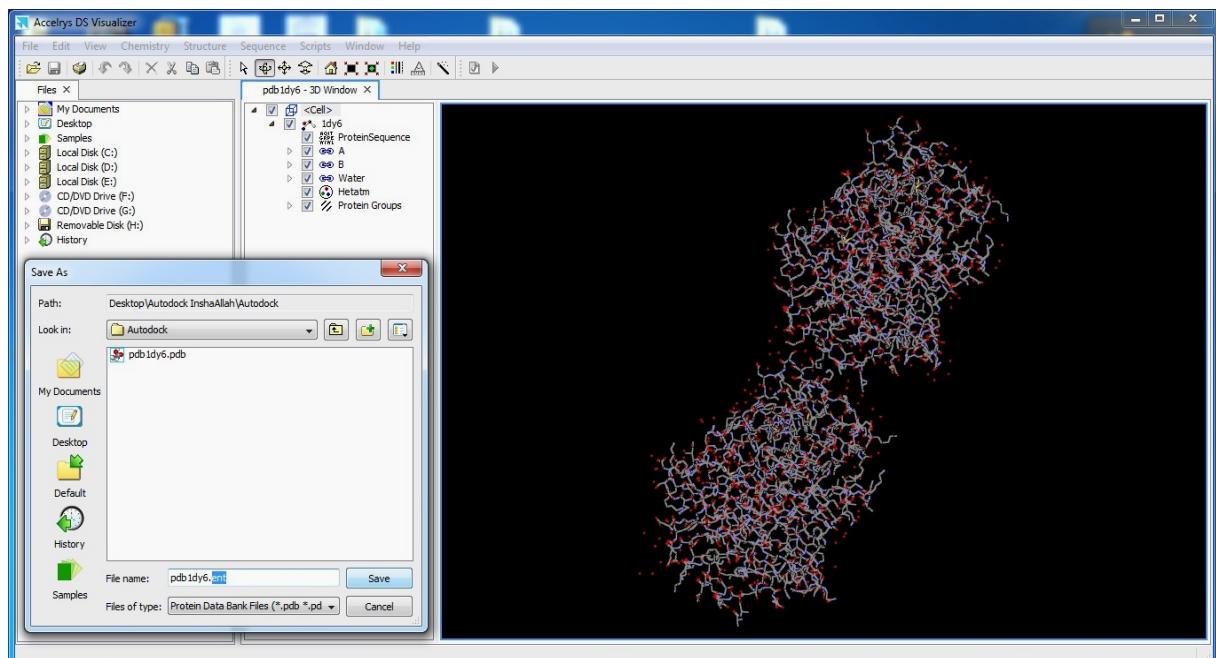
DOI: 10.2210/pdb1dy6/pdb

Primary Citation: Structure of the imipenem-hydrolyzing class A beta-lactamase SME-1 from *Serratia marcescens*. Sougakoff, W., L'Hermite, G., Billy, I., Pernot, L., Guillet, V., Naas, T., Nordmann, P., Jarlier, V., Delettre, J. (2002) Acta Crystallogr., Sect.D 58: 267

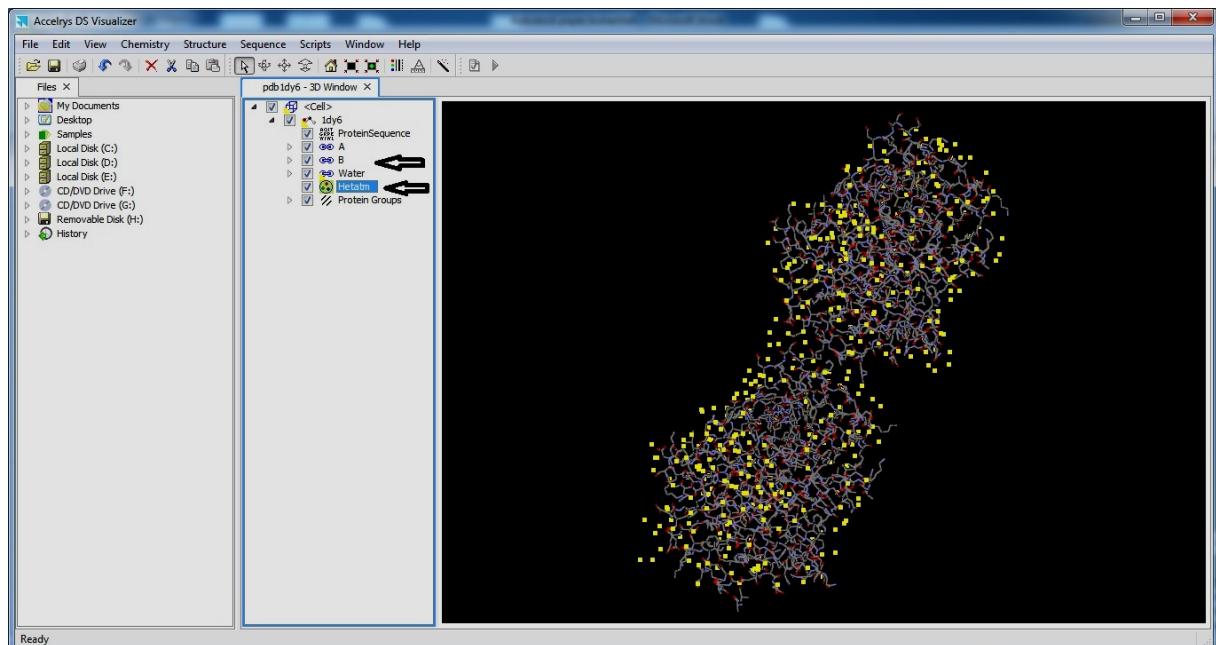
PubMed: 11807251

PubMed Abstract: The structure of the beta-lactamase SME-1 from *Serratia marcescens*, a class A enzyme characterized by its significant activity against imipenem, has been determined to 2.13 Å resolution. The overall structure of SME-1 is similar to that of other class A beta-lactamases. In the active-site cavity, most of the residues found in SME-1 are conserved among class A beta-lactamases, except at positions 104, 105 and 237, where a tyrosine, a histidine and a serine are found, respectively, and at position 238, which is occupied by a cysteine forming a disulfide bridge with the other cysteine residue located at position 69. The crucial role played by this disulfide bridge in SME-1 was confirmed by site-directed mutagenesis of Cys69 to Ala, which resulted in a mutant unable to confer resistance to imipenem and all other beta-lactam antibiotics tested. Another striking structural feature found in SME-1 was the short distance separating the side chains of the active serine residue at position 70 and the strictly conserved glutamate at position 166, which is

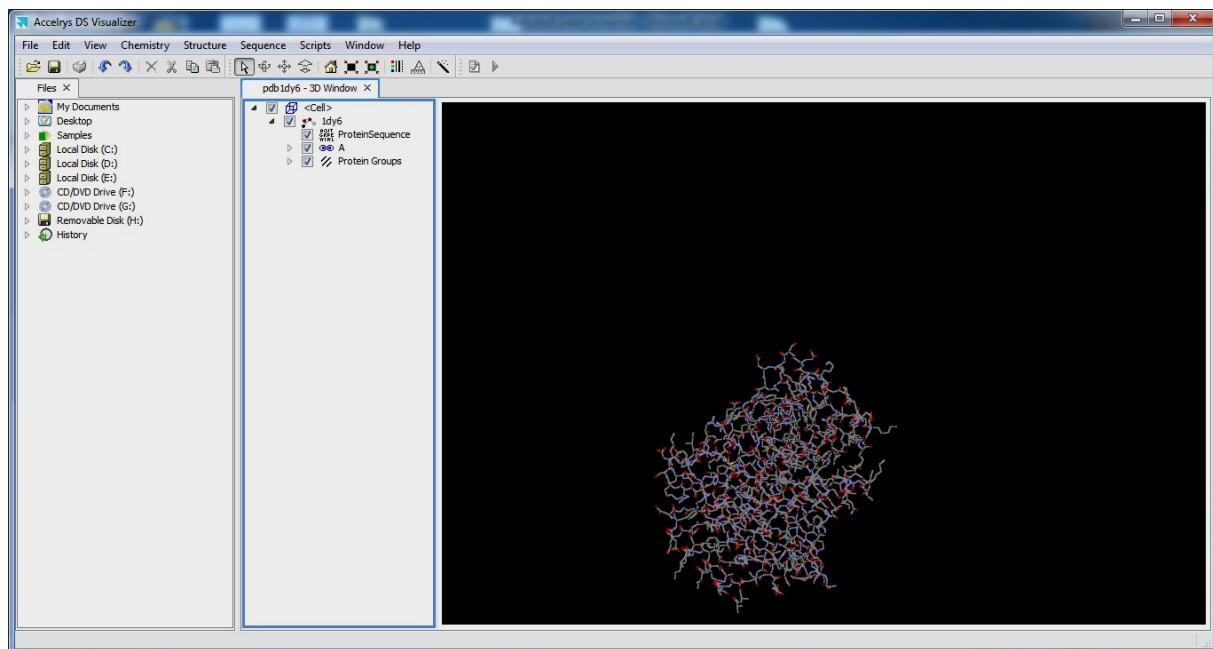
- Open it in Discovery Studio Visualiser
- Save as .pdb format



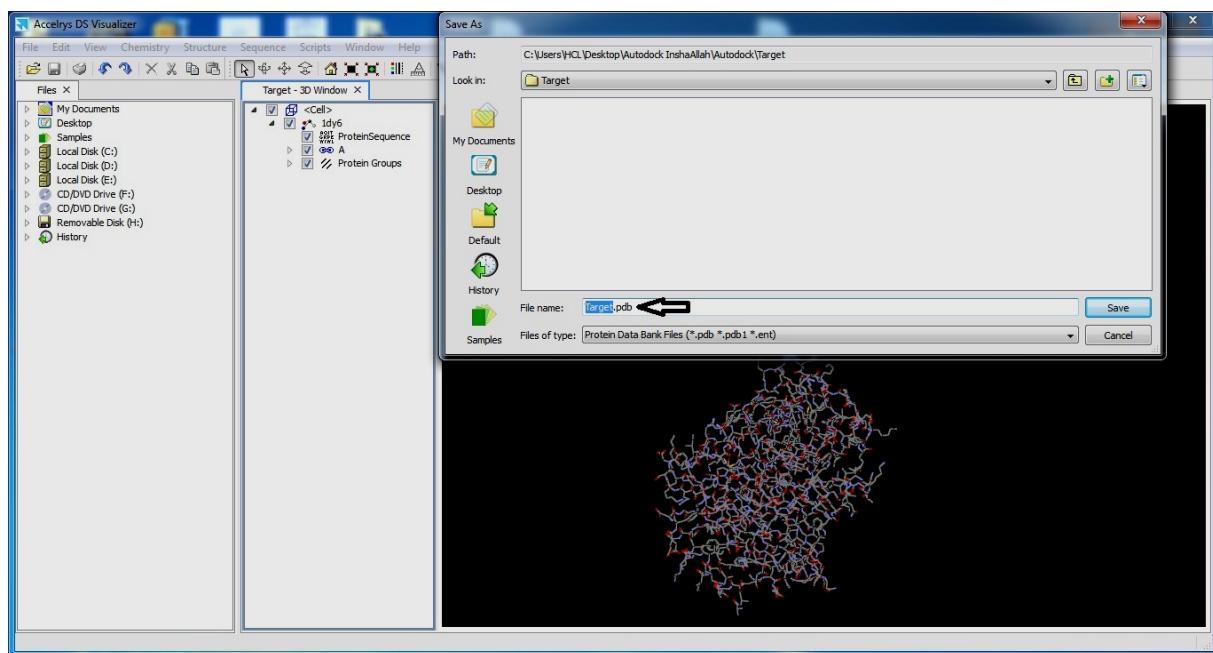
- Press Control+H
- Select Het atm and Delete



- Select B chain and Delete
- (As both A and B chain are similar and Imipenem can bind to anyone of the two chains)



➤ Save as Target.pdb



1.2 Retrieving Ligand.pdb files from major ligand databases

<http://www.drugbank.ca/> or
<http://pubchem.ncbi.nlm.nih.gov/>

➤ Search your Ligand (Imipenem)

- Click on Ligand (Imipenem)

- Click 3D image
- Open SDF
- Save 3D SDF

Imipenem - Compound Summary (CID 104838)

Also known as: Tienamycin, Imipenide, N-Formimidoylthienamycin, Primaxin, 64221-86-9, Imipenem anhydrous, Imipenem (INN), Prestwick_844, MK 0787

Molecular Formula: C₂₂H₁₇N₃O₄S Molecular Weight: 299.34608 InChIKey: ZSKVGTGRCRGINV-ZXFLCMHBSA-N

Semsynthetic thienamycin that has a wide spectrum of antibacterial activity against gram-negative and gram-positive aerobic and anaerobic bacteria, including many multiresistant strains. It is stable to beta-lactamases. Clinical studies have demonstrated high efficacy in the infections of various body systems. Its effectiveness is enhanced when it is administered in combination with CILASTATIN, a renal dipeptidase inhibitor. From: MeSH

Table of Contents Show subcontent titles

- Identification
- Related Records
- Pharmacology
- Biomedical Effects and Toxicity
- Literature
- Patents
- Biomolecular Interactions and Pathways
- Biological Test Results
- Classification
- Chemical and Physical Properties

Expand all sub-sections

2D Structure **3D Conformer** ←

Links and Related Information

- 2D SDF: Display
- 2D SDF: Save
- 3D SDF: Display
- 3D SDF: Save

XLogP 3.1A: 0.1
 H-Bond Donor: 3
 H-Bond Acceptor: 6

BioActivity Data Links

This Compound with Similar Compounds with Similar Conformers

Related Compounds

- Same, Connectivity (12)
- Same, Stereochemistry (2)
- Same, Isotopes (11)
- Similar Compounds (216)
- Similar Conformers (89) View

Related Substances

- All (114)
- Same Structure (54)
- Mixture (60)

- Open 3D SDF file of Ligand in Discovery Studio visualiser
- Right Click to ‘show structure in 3D window’

Accelrys DS Visualizer

File Edit View Chemistry Structure Sequence Scripts Window Help

Files X

CID_104838 (1) - Table Browser X

Structure	Name	Index	PUBCHEM_COMPO	PUBCHEM_CONF0	PUBCHEM_CONF0F	PUBCHEM_MMFF94	PUBCHEM_EFFECTI	PUBCHEM_PHARMA	PUBCHEM_HEAVY_	PUBCHEM_A
1	in4838	1	in4838	0.8	49	1,1,32	6.4	1,2,acetoxy	20	3

Show structure in 3D Window ←

Copy Ctrl+C

Sort...

Select Columns...

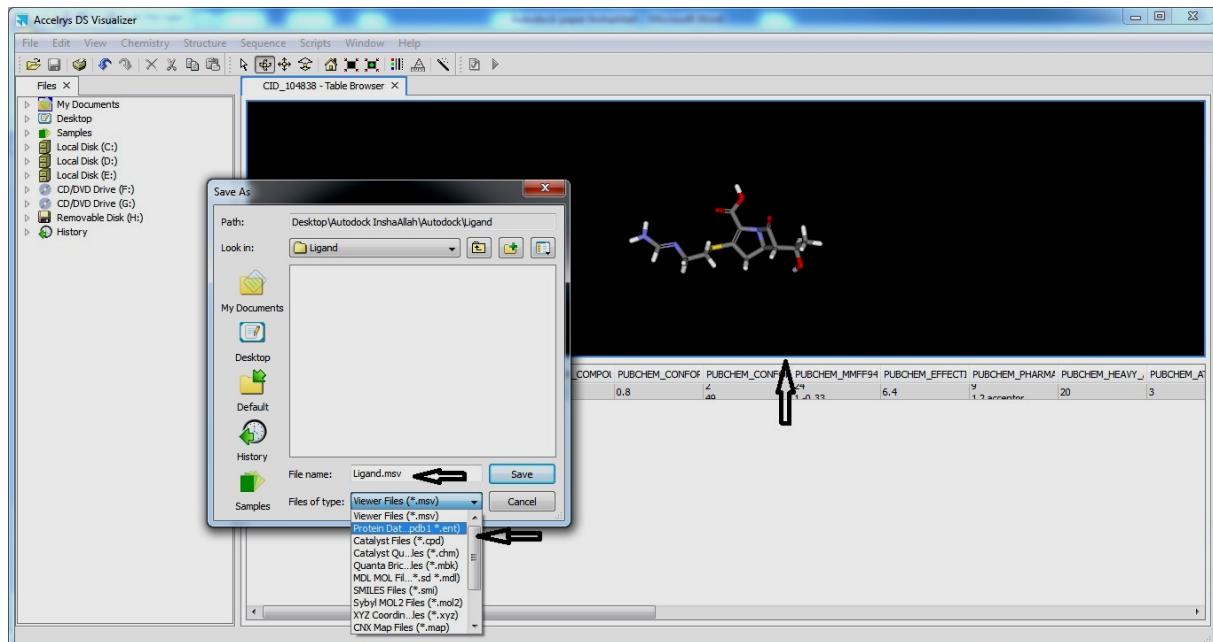
Show Chemistry

Hide Chemistry

Group By...

Represent By...

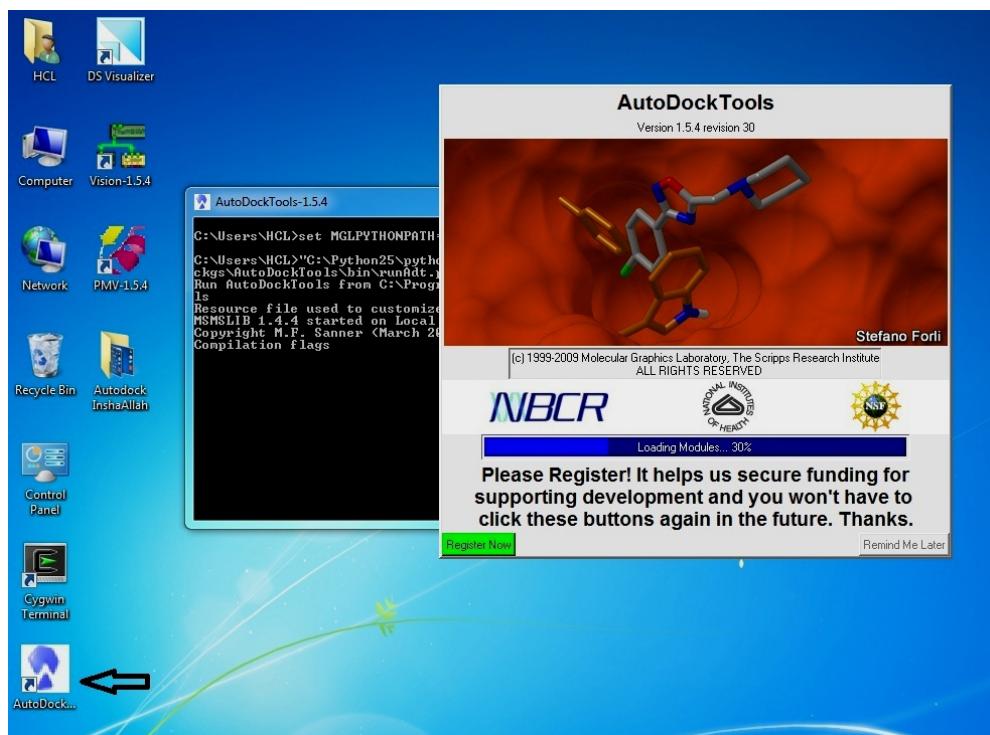
- Click on 3D image and Save as Ligand.pdb file



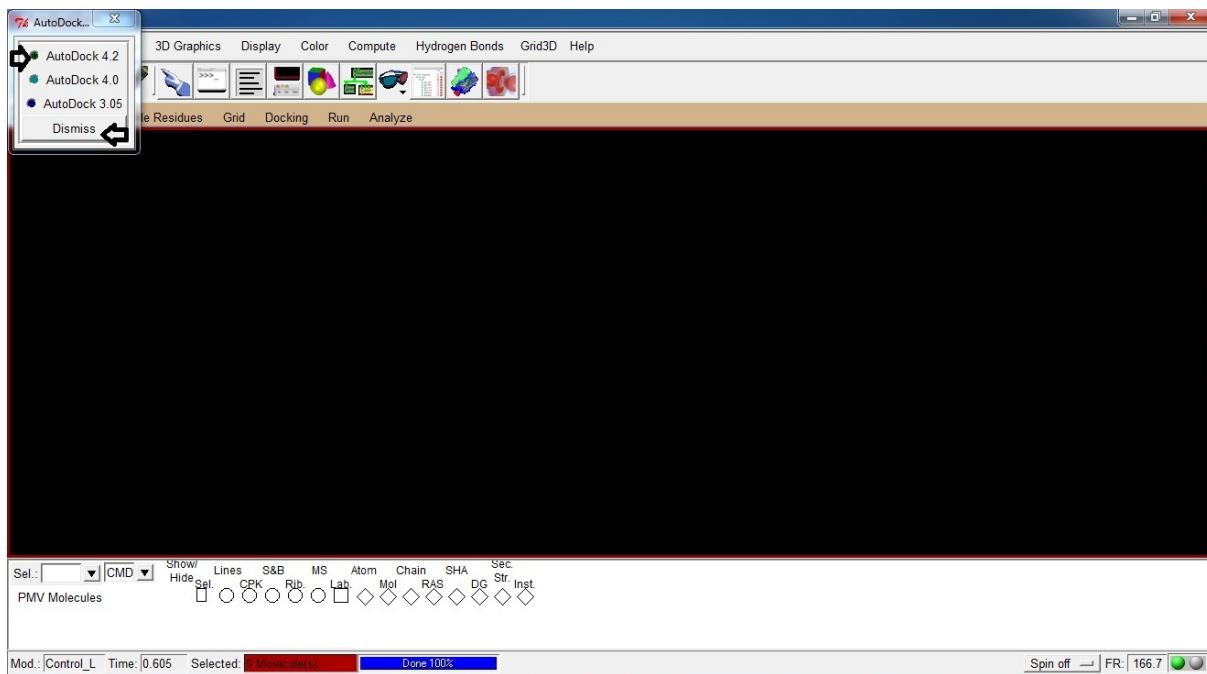
(*Note: AutoDock accepts files only in .pdb format. So, Ligand and Target must be converted into .pdb format)

2) Preparing PDBQT format for Target and ligand (Target.pdbqt, Ligand.pdbqt), Grid and Docking Parameter file (a.gpf and a.dpf) using AutoDock 4.2

- Open AutoDock present on desktop
- (*Created after successful installation of MGL Tools)

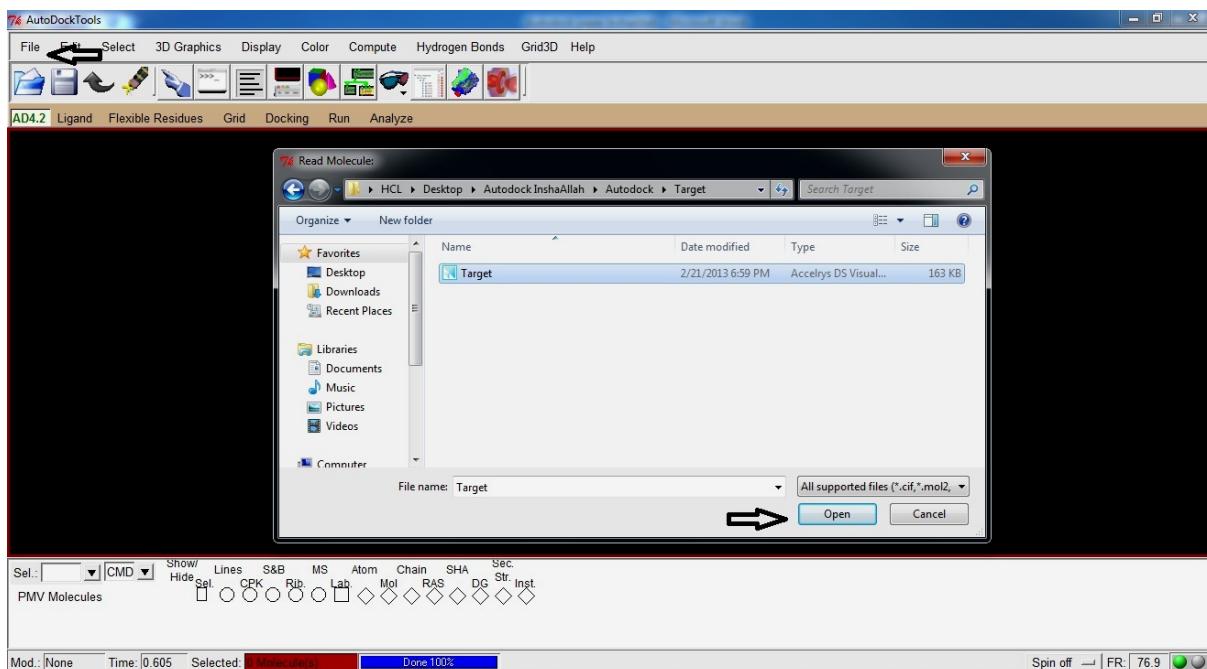


- Select AutoDock 4.2
- Dismiss

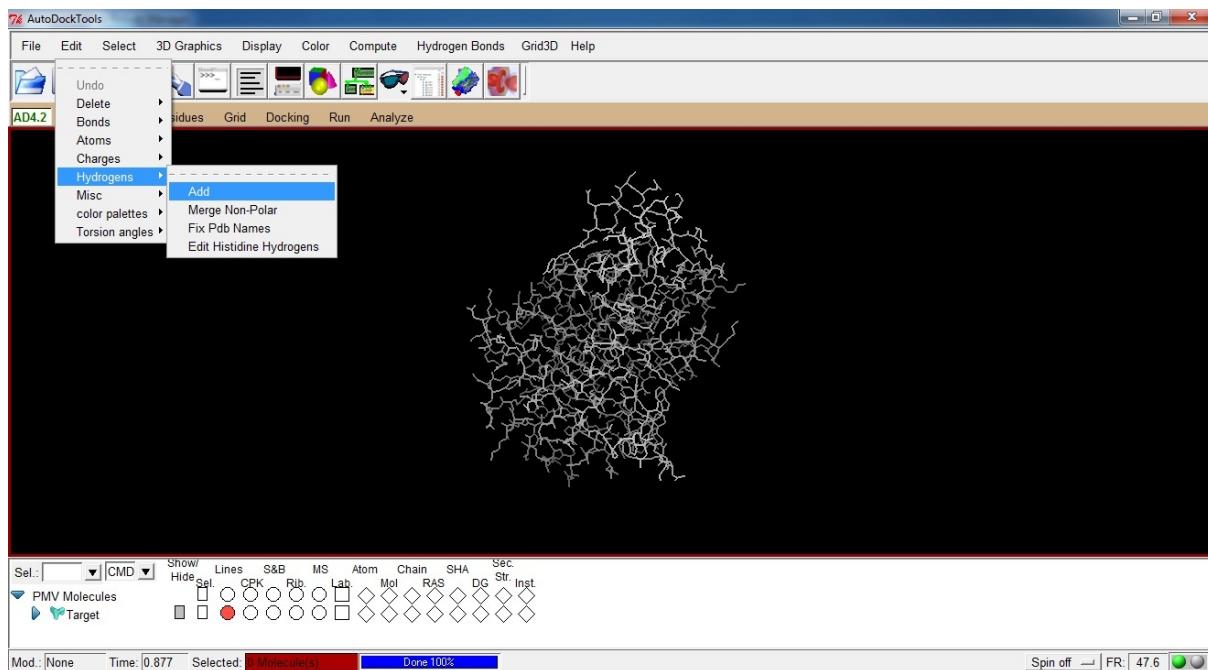


2.1 Preparation of Target.pdbqt file

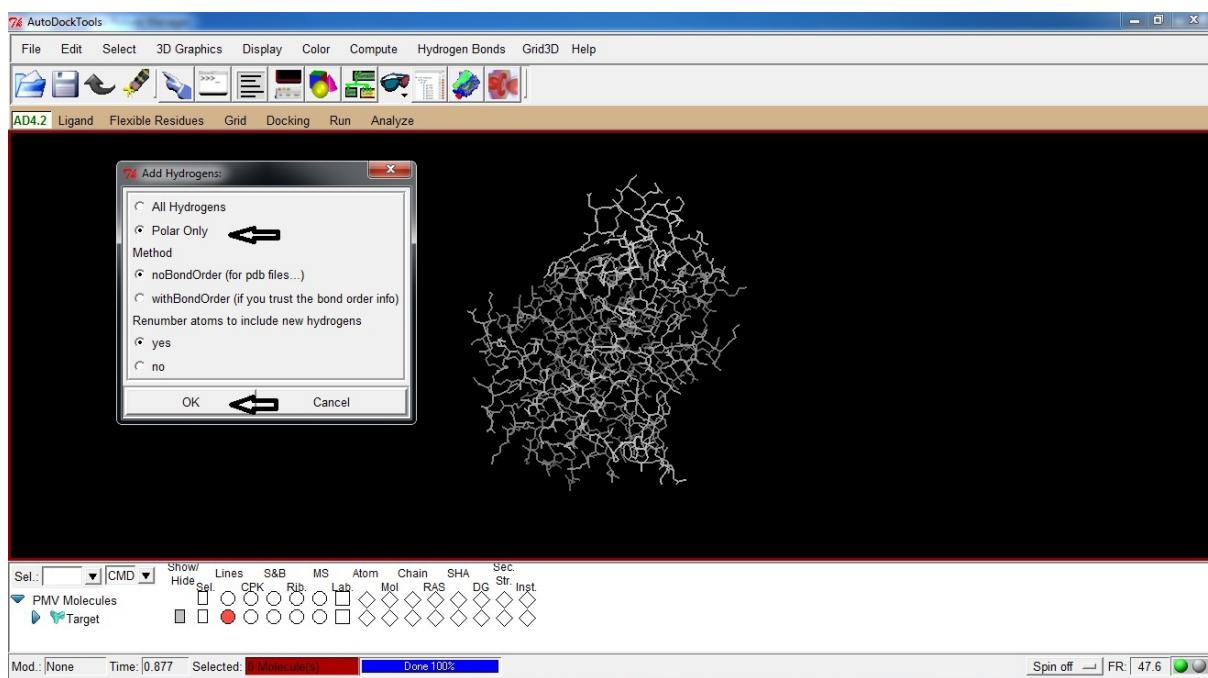
- Open File
- Read Molecule
- Select and Open Target.pdb (*Created in first step)



- Target molecule will appear on screen
- Click on Edit
- Click on Hydrogens
- Click on Add

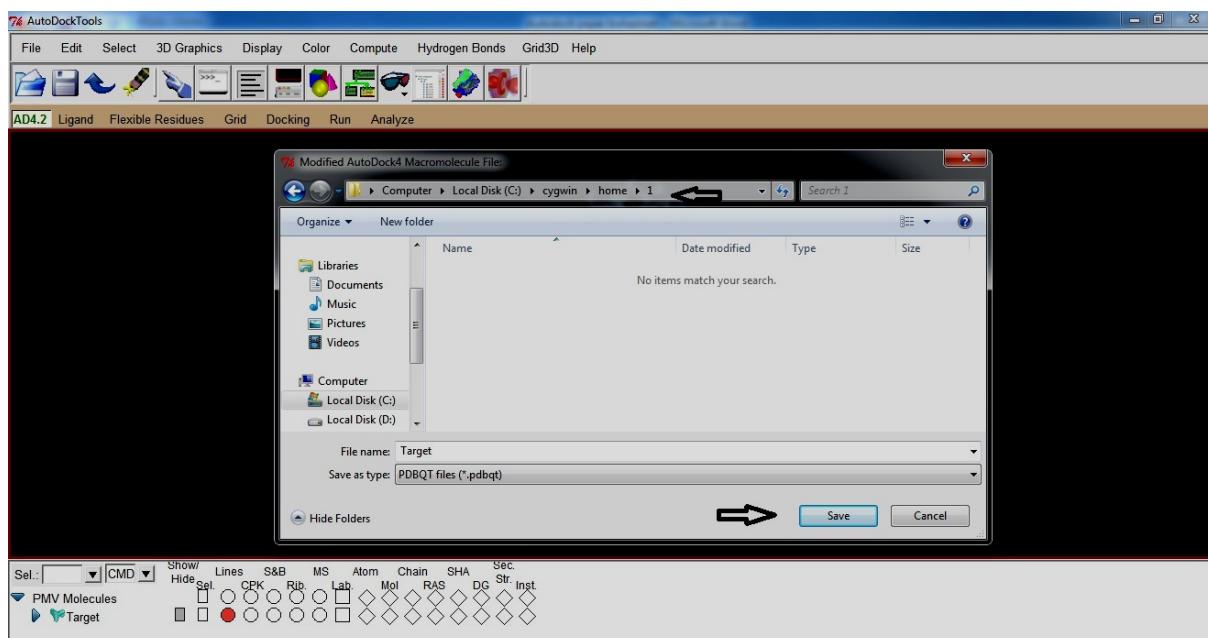


- Click Polar Only
- Click OK

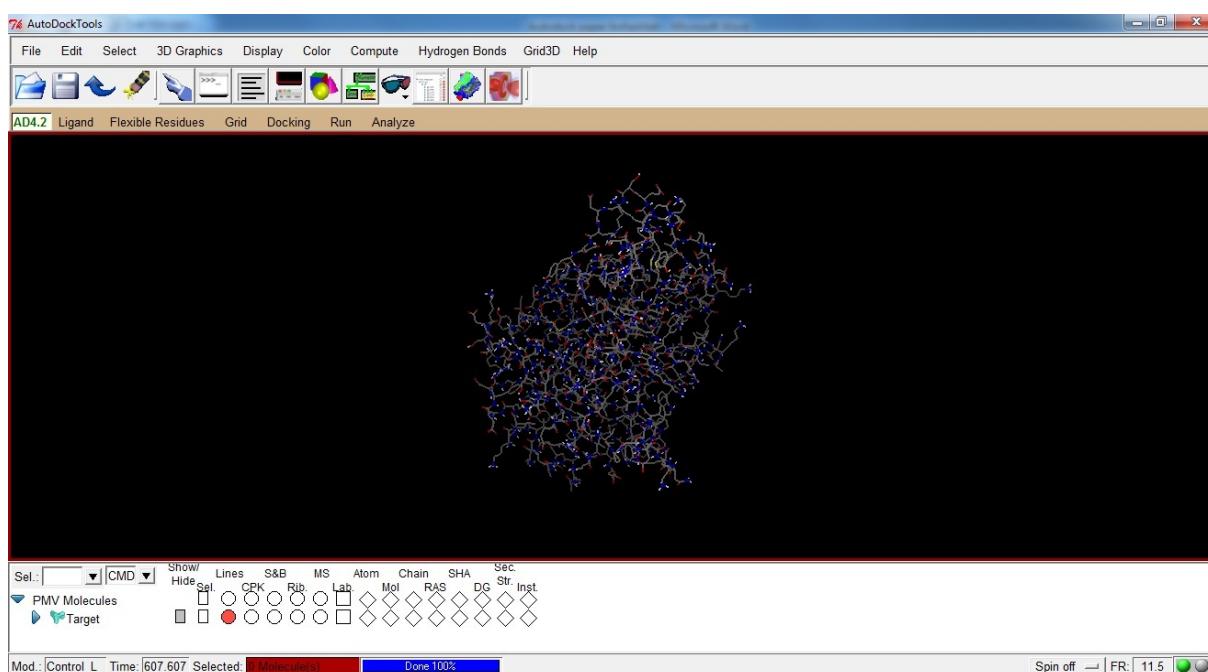


- Again Edit
- Click Charges
- Add Kollman Charges
- Click OK
- Open Grid
- Click on Macromolecules
- Click on Choose

- Click Target
- Click Select Molecule
- Click OK

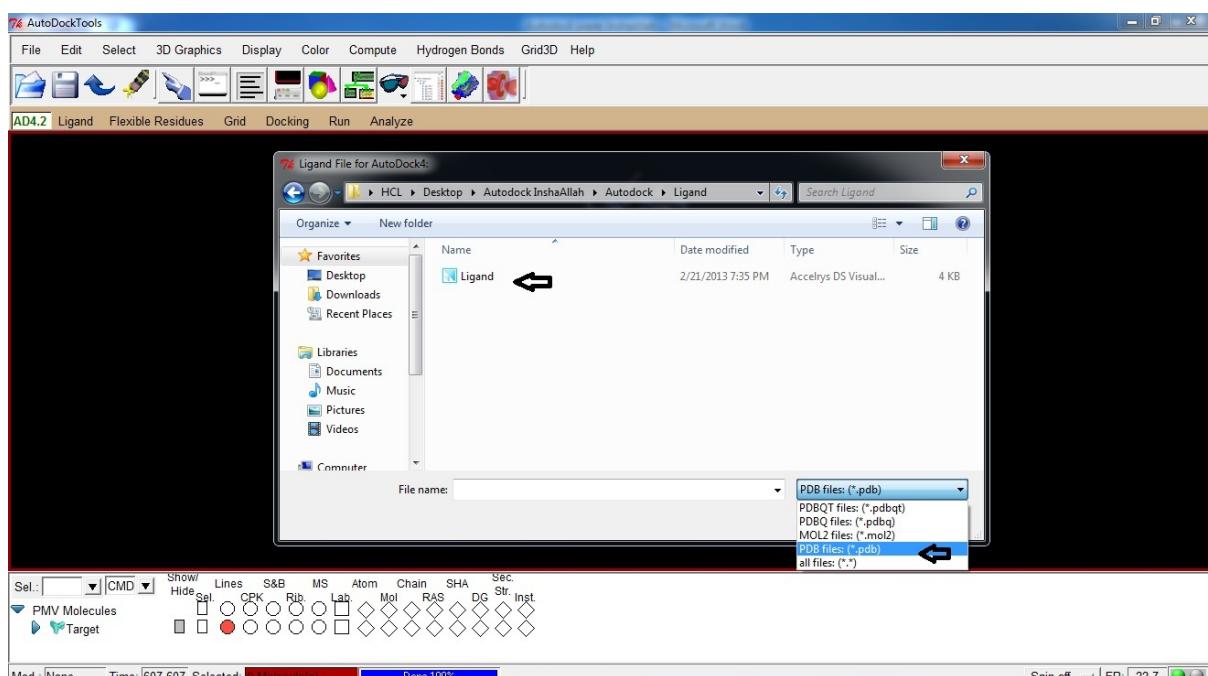


- Open My computer
 - Open C drive
 - Open Cygwin
 - Open home
 - Create new folder and rename it as 1 (or any other shortname)
 - Save Target in Folder 1
- (*In short: save Target.pdbqt in C:\Cygwin\home\1 and after saving macromolecule gets coloured)

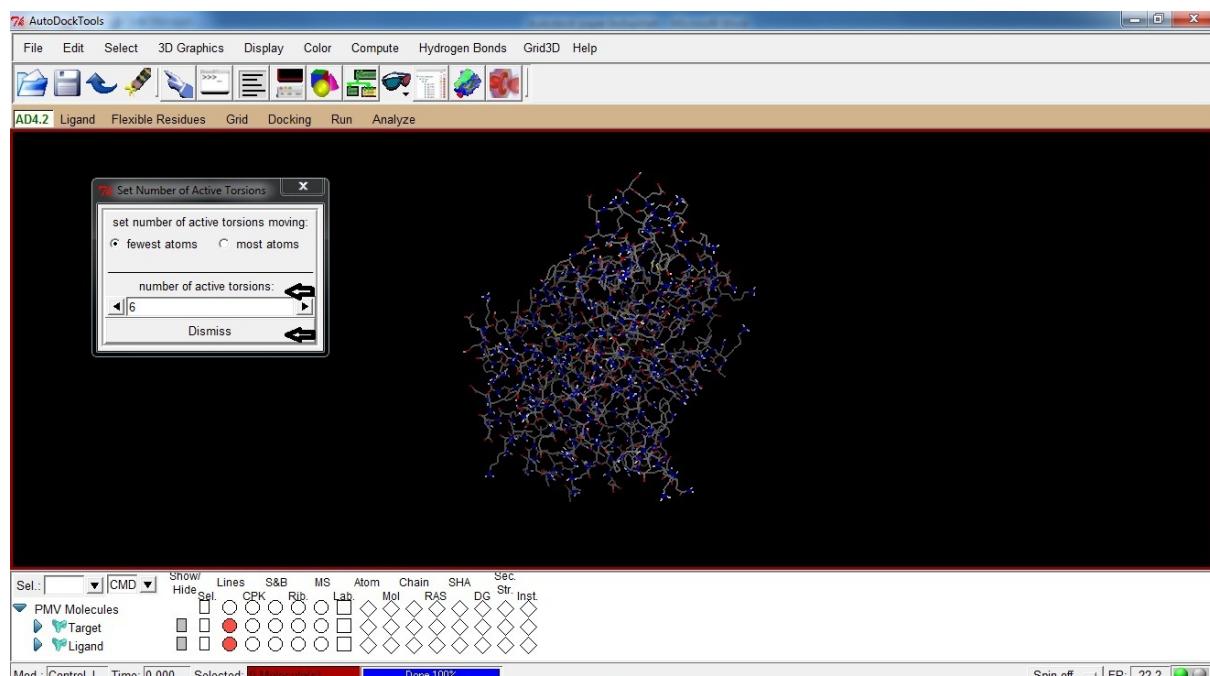


2.2 Preparation of Ligand.pdbqt file

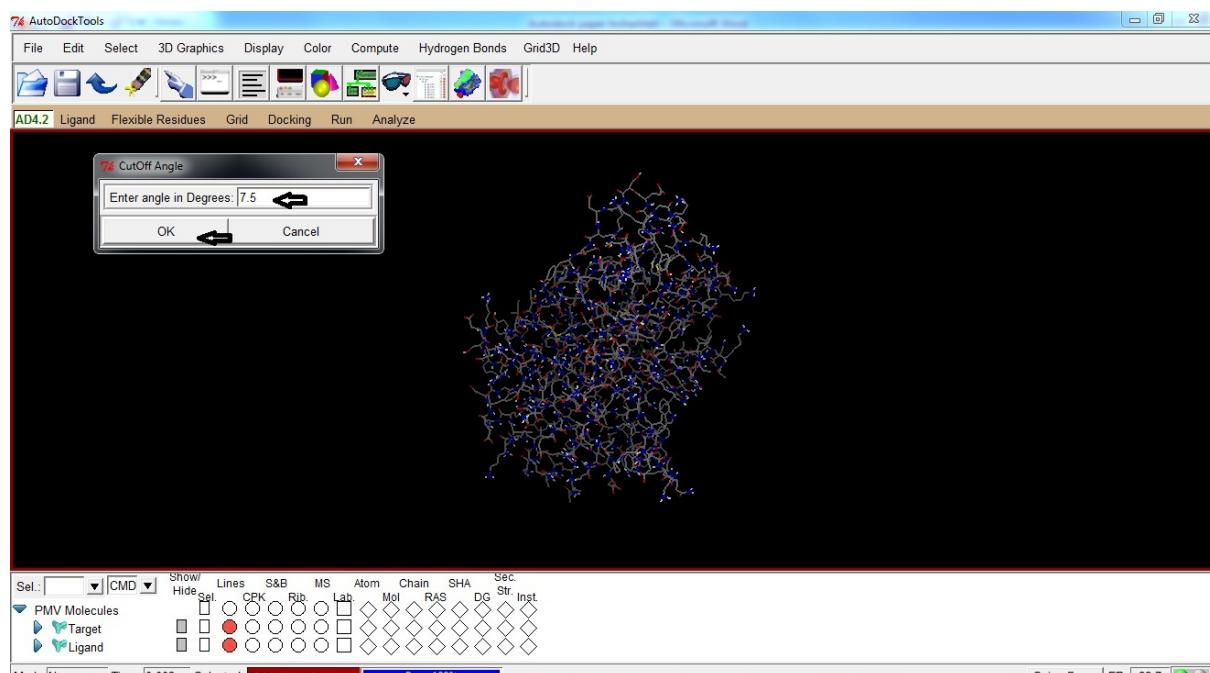
- Open Ligand
- Click Input
- Click Open
- Change format from .pdbqt to .pdb



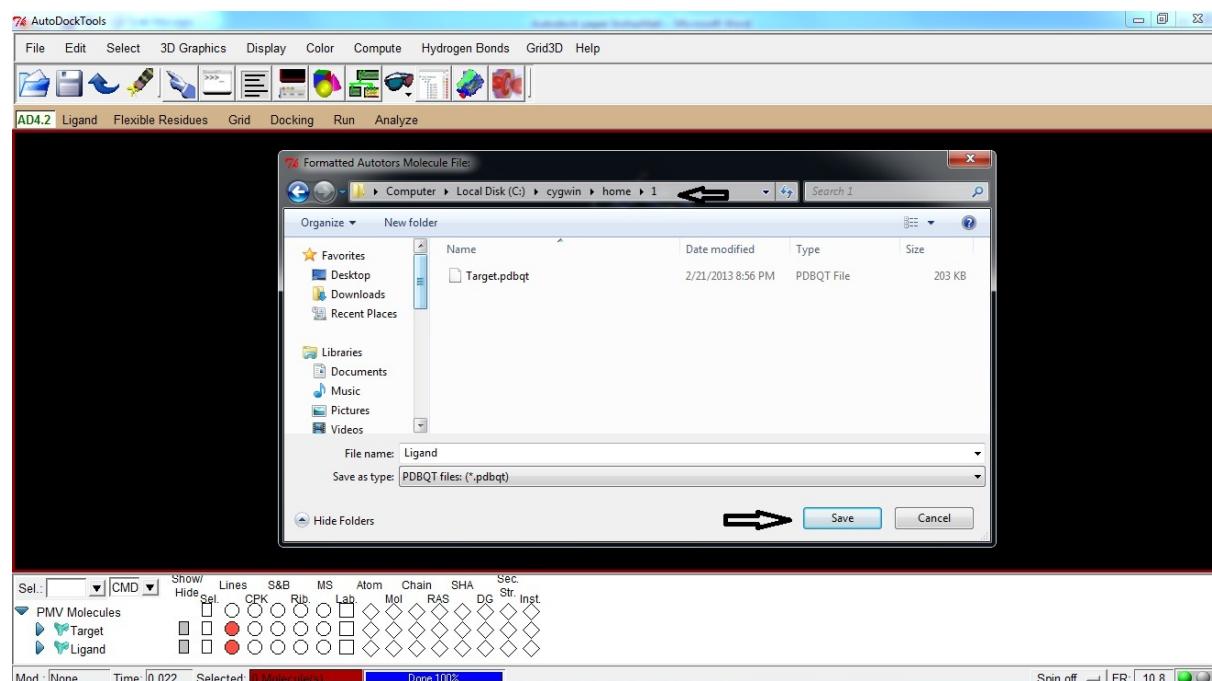
- Select Ligand
- Click Open
- Click OK
- Again Open Ligand
- Click Torsion Tree
- Click Detect Root
- Again Open Ligand
- Click Torsion Tree
- Click Set Number of Torsions
- Set number of active torsions between 1 to 6
- Click Dismiss



- Again Open Ligand
- Click Aromatic Carbons
- Click Aromaticity criterion
- Click OK (* If 'Enter angle in Degrees: 7.5')

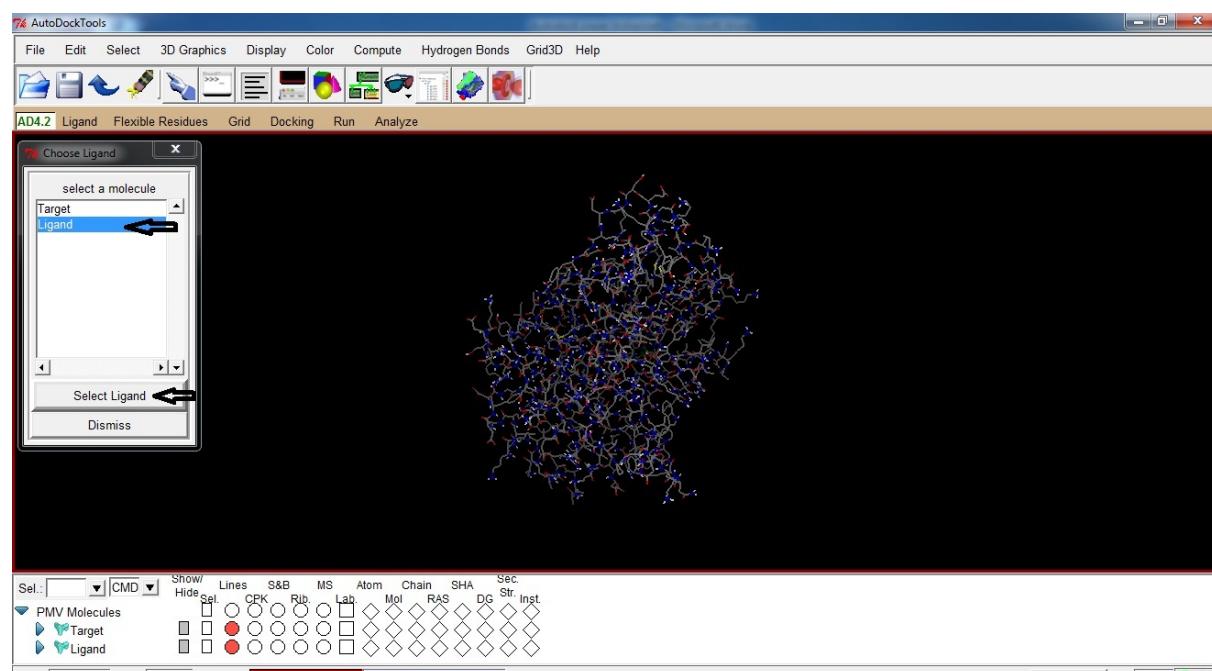


- Again Open Ligand
- Click Output
- Click Save as PDBQT
- Save Ligand file in C:\Cygwin\home\1
 (* In the same folder and in same way as Target.pdbqt file)



2.3 Preparation of Grid Parameter File (a.gpf)

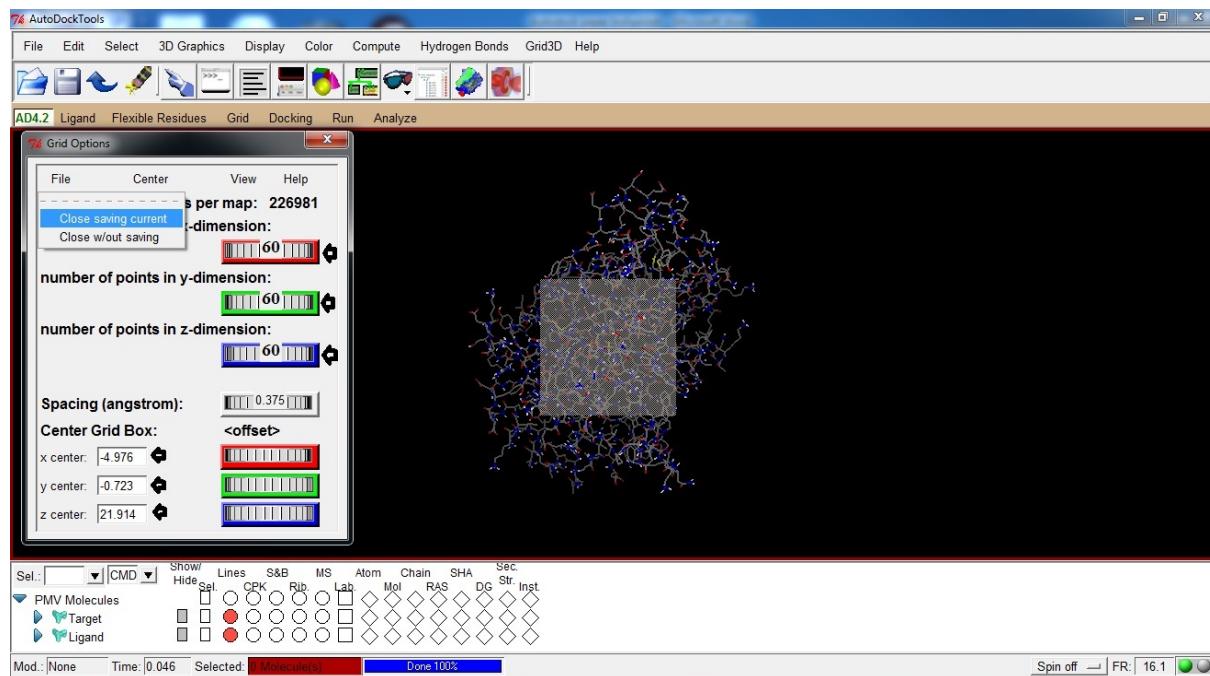
- Open Grid
- Click Set Map Types
- Click Choose Ligand
- Click Ligand
- Click Select Ligand



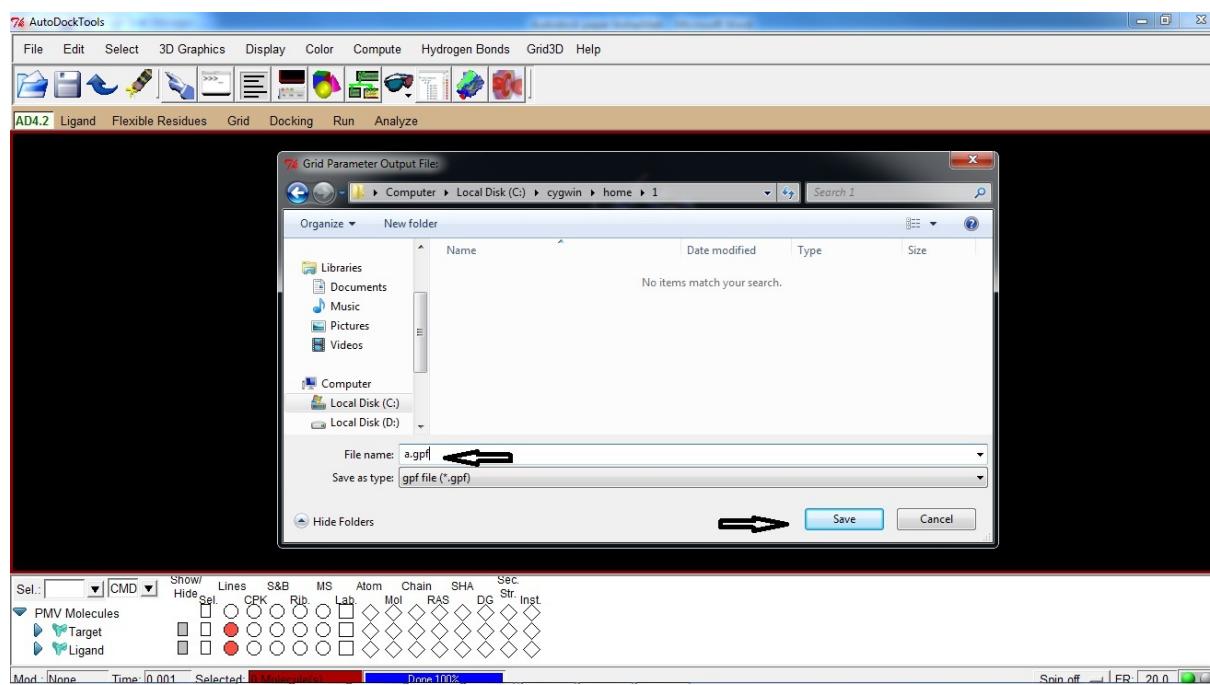
- Again Open Grid
- Click Grid Box

(*We have used X,Y,Z dimension as 60x60x60. Further X,Y,Zcenter (Center Grid Box) can be changed according to the requirements but we are taking them as Default)

- Click File
- Click Close saving current

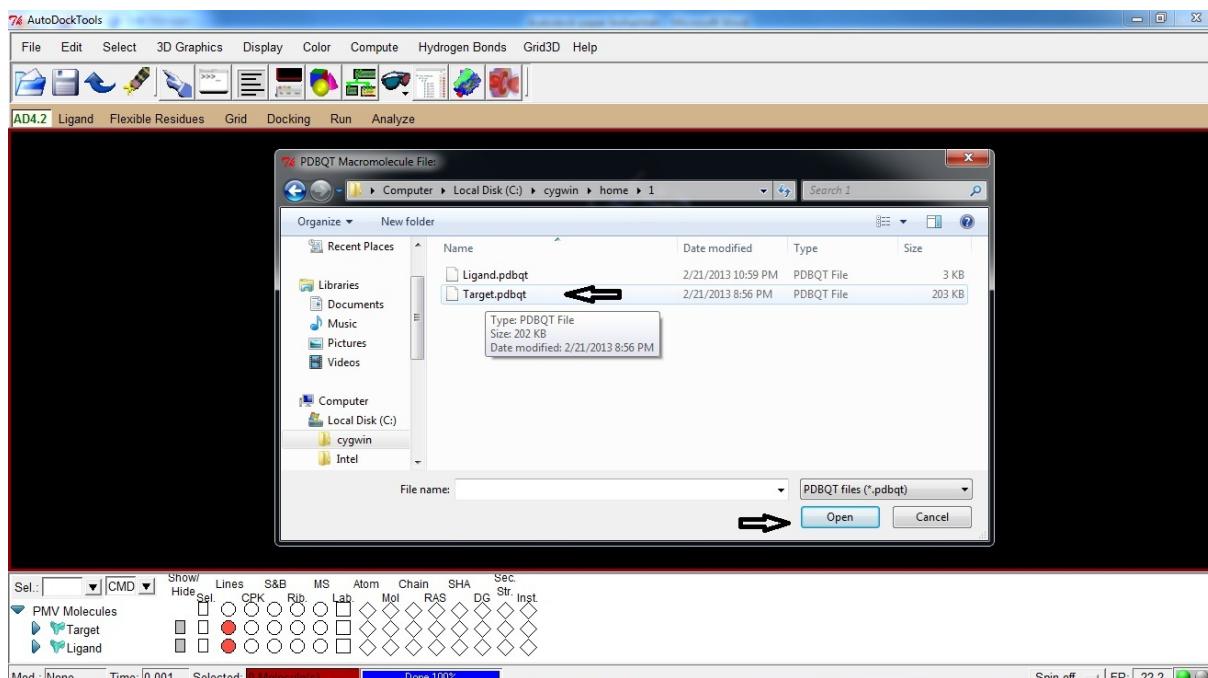


- Again Open Grid
- Click Output
- Click Save GPF
- Name the File name as a.gpf
- Save a.gpf file (.gpf format) in C:\Cygwin\home\1 (* In the same file where Target and Ligand .pdbqt files were saved)

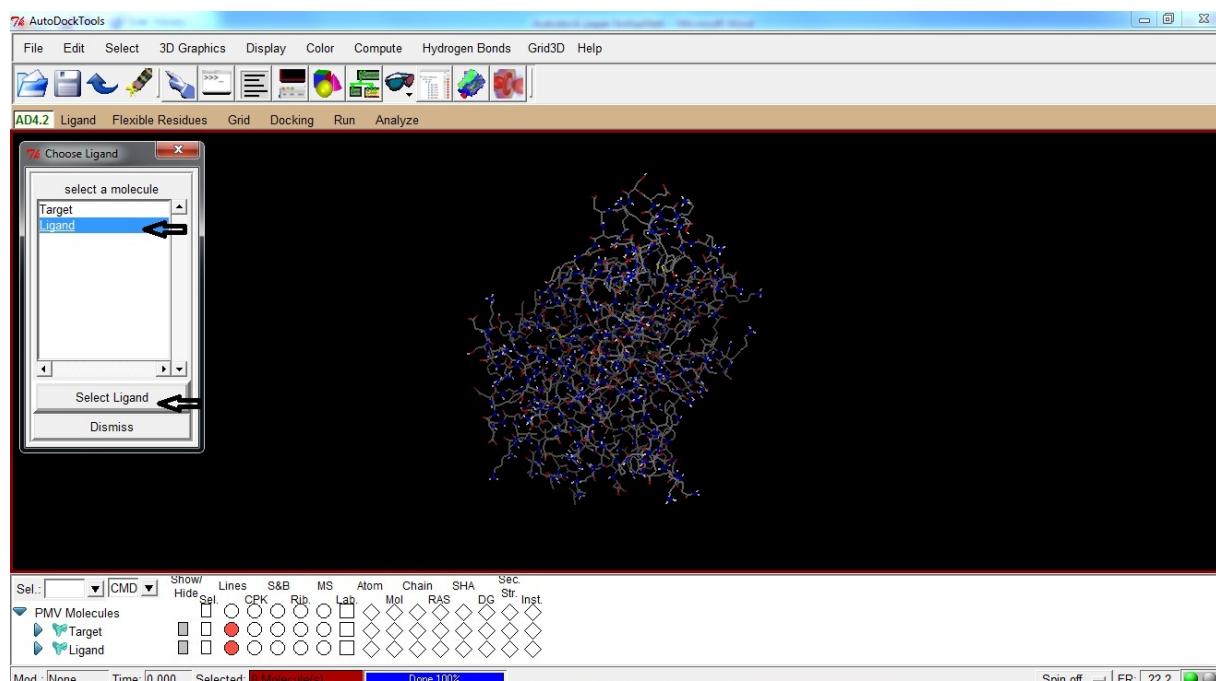


2.4 Preparation of Docking Parameter File (*a.dpf*)

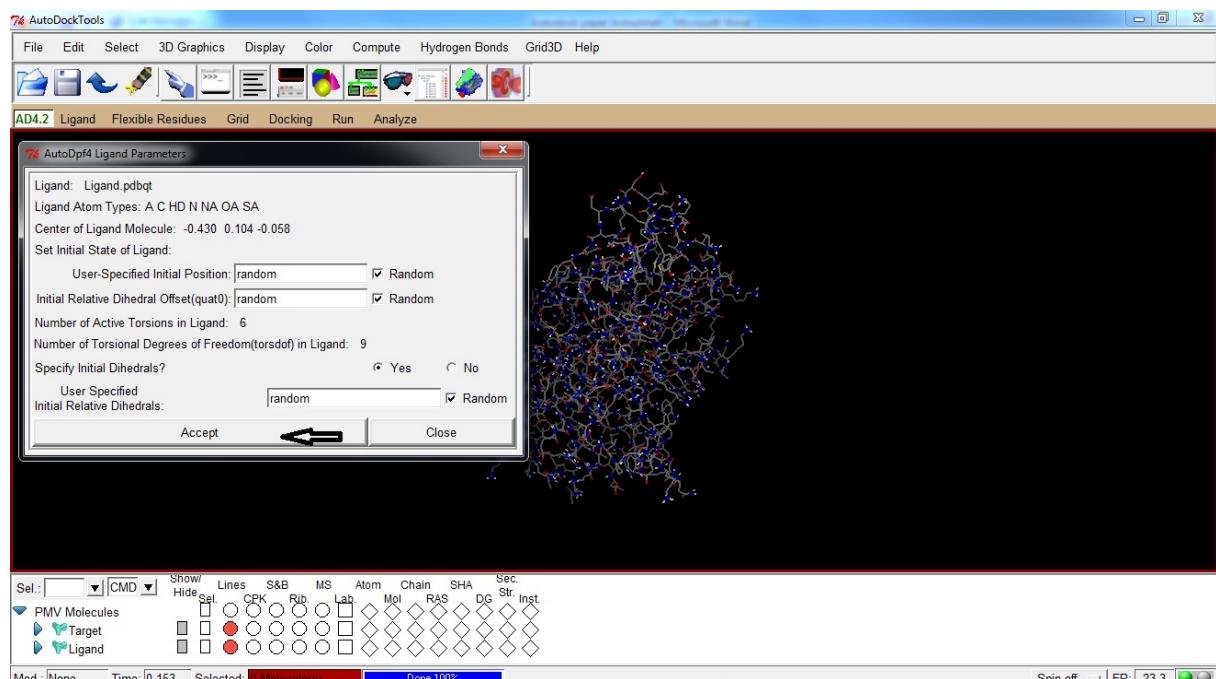
- Open Docking
- Click Macromolecules
- Click Set Rigid Filename
- Go to C:\ Cygwin\ home\ 1
- Select Target.pdbqt
- Click Open



- Again Docking
- Click Ligand
- Click Choose
- Click Ligand
- Click Select Ligand

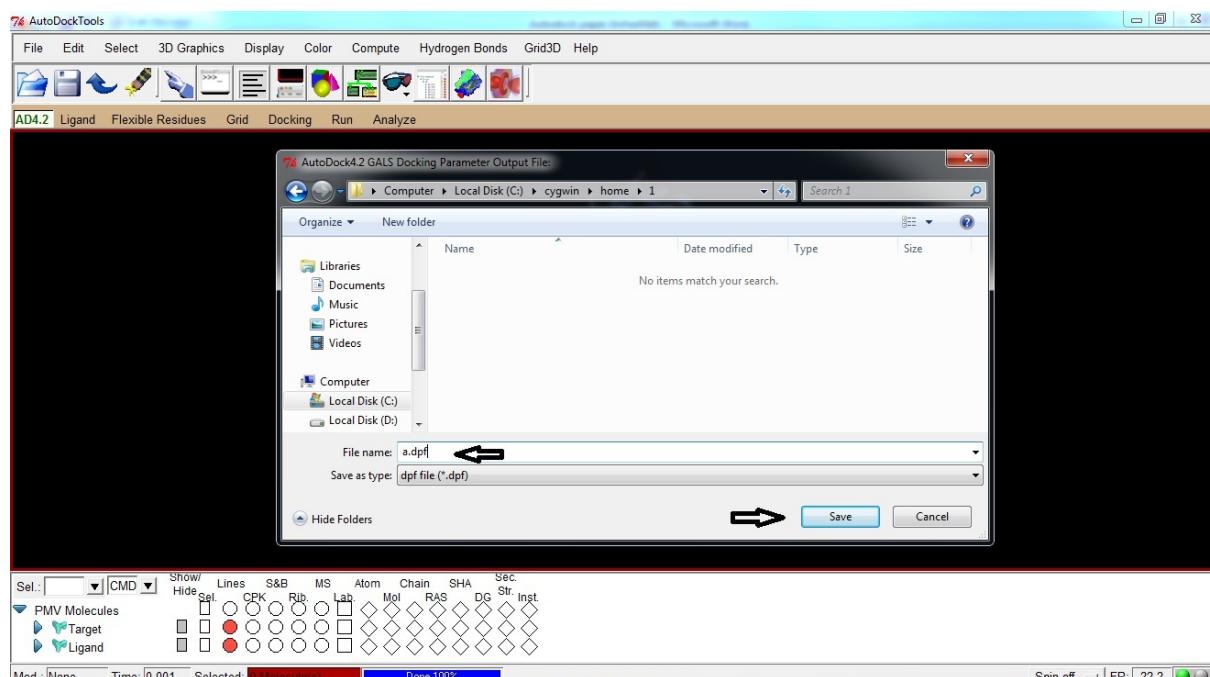


➤ Click Accept

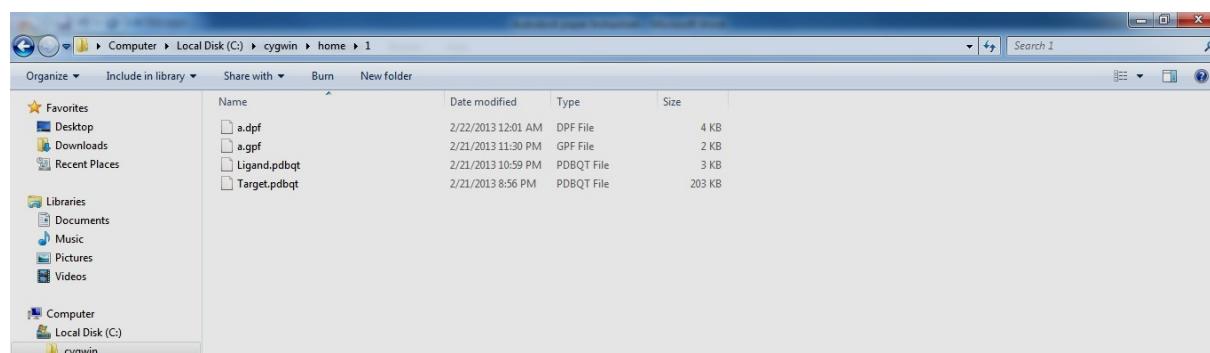


- Again Docking
- Click Search Parameters
- Click Genetic Algorithm
- Click Accept (*Using Default but we can change no. of GA runs)
- Again Docking
- Click Docking parameters
- Click Accept (*Using Default)
- Again docking

- Click Output
 - Click LamarkianGA(4.2)
 - Name the File name as a.dpf
 - Save a.dpf file (.dpf format) in C:\Cygwin\home\1
- (* In the same file where Target and Ligand .pdbqtand a.gpf files were saved)



At last four files Target.pdbqt, Ligand.pdbqt, a.gpf and a.dpf are present in the C:\ Cygwin\home\1



3) Using Cygwin for Molecular Docking

Open Cygwin (*By clicking icon on the desktop)

Use these commands highlighted in brown font color by copy and paste in Cygwin and press enter after each command:

(cd..)cd<space>..

(ls)ls<space>

(cd 1) cd<space>1(or foldername)<space>

(ls)ls<space>

(autogrid4.exe -p a.gpf -l a.glg &)

autogrid(tab)<space>-p<space>a.gpf<space>-l<space>a.glg&

```
[HCL@HCL-PC ~]$ cd ..  
[HCL@HCL-PC /home]$ ls  
1 HCL  
[HCL@HCL-PC /home]$ cd 1  
[HCL@HCL-PC /home/1]$ ls  
a.dpf a.gpf Ligand.pdbqt Target.pdbqt  
[HCL@HCL-PC /home/1]$ autogrid4.exe -p a.gpf -l a.glg &
```

(tail -f a.glg &) tail<space>-f<space>a.glg<space>&

```
[HCL@HCL-PC ~]$ cd ..  
[HCL@HCL-PC /home]$ ls  
1 HCL  
[HCL@HCL-PC /home]$ cd 1  
[HCL@HCL-PC /home/1]$ ls  
a.dpf a.gpf Ligand.pdbqt Target.pdbqt  
[HCL@HCL-PC /home/1]$ autogrid4.exe -p a.gpf -l a.glg &  
[1] 2924  
[HCL@HCL-PC /home/1]$  
autogrid4: Successful Completion.  
tail -f a.glg &  
[2] 3952  
[1] Done autogrid4.exe -p a.gpf -l a.glg  
[HCL@HCL-PC /home/1]$  
7 HD -0.60 1.11e+05  
8 e -35.88 2.82e+01 Electrostatic Potential  
9 d 0.00 1.42e+00 Desolvation Potential  
  
* Note: Every pairwise-atomic interaction was clamped at 100000.00  
  
autogrid4: Successful Completion.  
Real= 18.76s, CPU= 18.60s, System= 0.12s  
[HCL@HCL-PC /home/1]$
```

(autodock4.exe -p a.dpf -l a.dlg &)
autodock(tab)<space>-p<space>a.dpf<space>-l<space>a.dlg&
(tail -f a.dlg &) tail<space>-f<space>a.dlg<space>&

```

/home/1
.00 27 20.789 95.1%      2.62s Real= 0.87, CPU= 0.87, System= 0
.00 28 21.164 96.7%      1.75s Real= 0.87, CPU= 0.87, System= 0
.00 29 21.539 98.4%      0.86s Real= 0.86, CPU= 0.84, System= 0
.02

autogrid4: Successful Completion.
.00 30 21.914 100.0%     0.00s Real= 0.84, CPU= 0.84, System= 0

Grid   Atom   Minimum   Maximum
Map    Type    Energy    Energy
          (kcal/mol) (kcal/mol)

1      A       -0.90    2.01e+05
2      C       -1.01    2.01e+05
3      NA      -1.49    2.00e+05
4      OA      -1.73    2.00e+05
5      N       -1.04    2.00e+05
6      SA      -1.32    2.04e+05
7      HD      -0.94    1.11e+05
8      e       -35.92   3.44e+01   Electrostatic Potential
9      d       0.00     1.42e+00   Desolvation Potential

* Note: Every pairwise-atomic interaction was clamped at 100000.00

autogrid4: Successful Completion.
Real= 57.92s, CPU= 57.72s, System= 0.14s
[1]- Done           autogrid4.exe -p a.gpf -l a.glg
HCL@HCL-PC /home/1
$ autodock4.exe -p a.dpf -l a.dlg &
[3] 1404
HCL@HCL-PC /home/1
$ Beginning Lamarckian Genetic Algorithm (LGA), with a maximum of 2500000
energy evaluations.

Generation: 100 Oldest's energy: -5.810 Lowest energy: -5.810 Num.evals.: 52944 Timing: Real= 0.03s, CPU= 0.03s, System= 0.00s
Generation: 200 Oldest's energy: -6.860 Lowest energy: -6.860 Num.evals.: 106816 Timing: Real= 0.03s, CPU= 0.03s, System= 0.00s
Generation: 300 Oldest's energy: -7.114 Lowest energy: -7.114 Num.evals.: 159635 Timing: Real= 0.03s, CPU= 0.03s, System= 0.00s
Generation: 400 Oldest's energy: -7.322 Lowest energy: -7.322 Num.evals.: 214300 Timing: Real= 0.03s, CPU= 0.03s, System= 0.00s
Generation: 500 Oldest's energy: -7.322 Lowest energy: -7.322 Num.evals.: 268271 Timing: Real= 0.02s, CPU= 0.02s, System= 0.00s
Generation: 600 Oldest's energy: -7.447 Lowest energy: -7.447 Num.evals.: 321871 Timing: Real= 0.03s, CPU= 0.03s, System= 0.00s
Generation: 700 Oldest's energy: -7.789 Lowest energy: -7.789 Num.evals.: 374365 Timing: Real= 0.03s, CPU= 0.03s, System= 0.00s
Generation: 800 Oldest's energy: -7.801 Lowest energy: -7.801 Num.evals.: 428654 Timing: Real= 0.03s, CPU= 0.03s, System= 0.00s

```

(After Successful Completion)

```

/home/1
ATOM 7 C12 MOL 1 -10.084 2.427 30.015 -0.19 +0.11 +0.213 30.307
ATOM 8 C14 MOL 1 -11.534 3.879 28.721 -0.18 +0.05 +0.105 30.307
ATOM 9 C15 MOL 1 -12.461 4.590 29.376 -0.22 +0.00 +0.008 30.307
ATOM 10 C18 MOL 1 -11.937 7.206 29.811 -0.23 +0.02 +0.101 30.307
ATOM 11 C19 MOL 1 -12.317 8.666 29.617 -0.31 +0.03 +0.160 30.307
ATOM 12 C17 MOL 1 -10.815 4.247 27.507 -0.30 +0.14 +0.189 30.307
ATOM 13 O5 MOL 1 -11.344 4.521 26.440 -0.50 -0.39 -0.645 30.307
ATOM 14 O4 MOL 1 -9.484 4.239 27.742 -0.30 -1.03 -0.772 30.307
ATOM 15 H32 MOL 1 -9.163 3.350 28.006 +0.11 +0.20 +0.167 30.307
ATOM 16 N7 MOL 1 -12.271 8.978 28.186 -0.38 -0.02 -0.284 30.307
ATOM 17 C20 MOL 1 -11.419 8.305 27.501 -0.34 +0.02 +0.193 30.307
ATOM 18 N8 MOL 1 -11.086 8.308 26.174 -0.23 +0.21 -0.394 30.307
ATOM 19 H37 MOL 1 -10.965 9.176 25.666 -0.27 -0.27 +0.156 30.307
ATOM 20 H36 MOL 1 -10.952 7.444 25.658 -0.16 -0.10 +0.156 30.307
ATOM 21 C13 MOL 1 -10.732 0.791 31.889 -0.22 +0.04 +0.128 30.307
ATOM 22 C16 MOL 1 -12.092 0.104 31.947 -0.25 +0.01 +0.040 30.307
ATOM 23 O2 MOL 1 -9.858 0.025 31.061 -0.49 -0.18 -0.395 30.307
ATOM 24 H29 MOL 1 -9.475 -0.677 31.612 -0.33 -0.00 +0.210 30.307
TER
ENDMDL

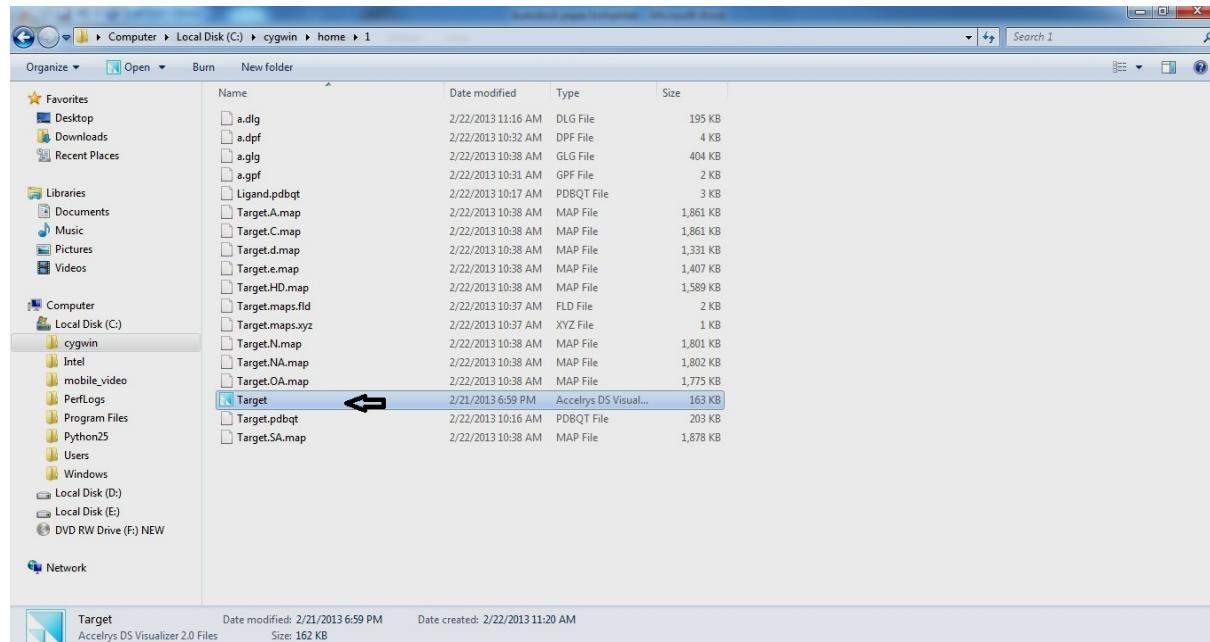
AVSFLD: # AVS field file
AVSFLD: #
AVSFLD: # Created by AutoDock
AVSFLD: #
AVSFLD: ndim=2          # number of dimensions in the field
AVSFLD: nspace=1         # number of physical coordinates
AVSFLD: veclen=7          # vector size
AVSFLD: dim1=24          # atoms
AVSFLD: dim2=2          # conformations
AVSFLD: data=Real        # data type (byte,integer,Real,double)
AVSFLD: field=uniform    # field coordinate layout
AVSFLD: label= x y z vdW Elec q RMS
AVSFLD: variable 1 file = a.dlg.pdb filetype = ascii offset = 5 stride = 12
AVSFLD: variable 2 file = a.dlg.pdb filetype = ascii offset = 6 stride = 12
AVSFLD: variable 3 file = a.dlg.pdb filetype = ascii offset = 7 stride = 12
AVSFLD: variable 4 file = a.dlg.pdb filetype = ascii offset = 8 stride = 12
AVSFLD: variable 5 file = a.dlg.pdb filetype = ascii offset = 9 stride = 12
AVSFLD: variable 6 file = a.dlg.pdb filetype = ascii offset = 10 stride = 12
AVSFLD: variable 7 file = a.dlg.pdb filetype = ascii offset = 11 stride = 12
AVSFLD: # end of file

>>> Closing the docking parameter file (DPF)...
This docking finished at:           11:16 51" a.m., 02/22/2013

autodock4: Successful Completion on "HCL-PC"
Real= 38m 02.70s, CPU= 37m 58.21s, System= 0.19s

```

Copy Target.pdb file in C:\Cygwin\ home\1



Copy and Paste the following commands in Cygwin Window and press enter after each command:

```
(grep '^DOCKED' a.dlg | cut -c9- >a.pdbqt)
(cut -c-66 a.pdbqt> a.pdb)
(catTarget.pdb a.pdb | grep -v '^END  ' | grep -v '^END$' > complex.pdb)
```

```
/home/1
ATOM   20  H36 MOL   1    -10.952   7.444  25.658 -0.16 -0.10   +0.156 30.307
ATOM   21  C13 MOL   1    -10.732   0.791  31.889 -0.22 +0.04   +0.128 30.307
ATOM   22  C16 MOL   1    -12.092   0.104  31.947 -0.25 +0.01   +0.040 30.307
ATOM   23  O2  MOL   1    -9.858   0.025  31.061 -0.49 -0.18   -0.395 30.307
ATOM   24  H29 MOL   1    -9.475  -0.677  31.612 -0.33 -0.00   +0.210 30.307
TER
ENDDMDL

AVSFLD: # AVS field file
AVSFLD: #
AVSFLD: # Created by AutoDock
AVSFLD: #
AVSFLD: ndim=2          # number of dimensions in the field
AVSFLD: nspace=1          # number of physical coordinates
AVSFLD: veclen=7          # vector size
AVSFLD: dim1=24          # atoms
AVSFLD: dim2=2          # conformations
AVSFLD: data=Real         # data type (byte,integer,Real,double)
AVSFLD: field=uniform     # field coordinate layout
AVSFLD: label=x y z vdw Elec q RMS
AVSFLD: variable 1 file = a.dlg.pdb filetype = ascii offset = 5 stride = 12
AVSFLD: variable 2 file = a.dlg.pdb filetype = ascii offset = 6 stride = 12
AVSFLD: variable 3 file = a.dlg.pdb filetype = ascii offset = 7 stride = 12
AVSFLD: variable 4 file = a.dlg.pdb filetype = ascii offset = 8 stride = 12
AVSFLD: variable 5 file = a.dlg.pdb filetype = ascii offset = 9 stride = 12
AVSFLD: variable 6 file = a.dlg.pdb filetype = ascii offset = 10 stride = 12
AVSFLD: variable 7 file = a.dlg.pdb filetype = ascii offset = 11 stride = 12
AVSFLD: # end of file

>>> Closing the docking parameter file (DPF)...
This docking finished at:           11:16 51" a.m., 02/22/2013

autodock4: Successful Completion on "HCL-PC"
Real= 38m 02.70s, CPU= 37m 58.21s, System= 0.19s

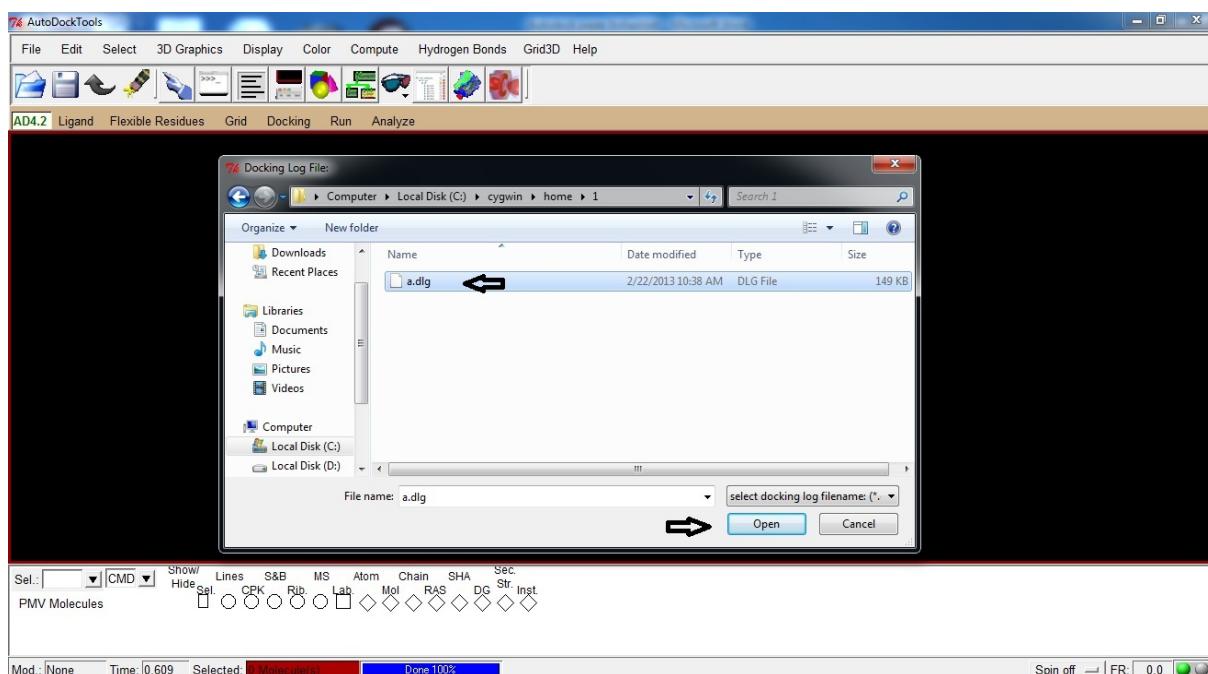
[3]- Done                      autodock4.exe -p a.adpf -l a.dlg
HCL@HCL-PC /home/1
$ grep '^DOCKED' a.dlg | cut -c9- > a.pdbqt
HCL@HCL-PC /home/1
$ cut -c-66 a.pdbqt > a.pdb
HCL@HCL-PC /home/1
$ cat Target.pdb a.pdb | grep -v '^END  ' | grep -v '^END$' > complex.pdb
HCL@HCL-PC /home/1
$
```

- Close Cygwin Window
- Click OK

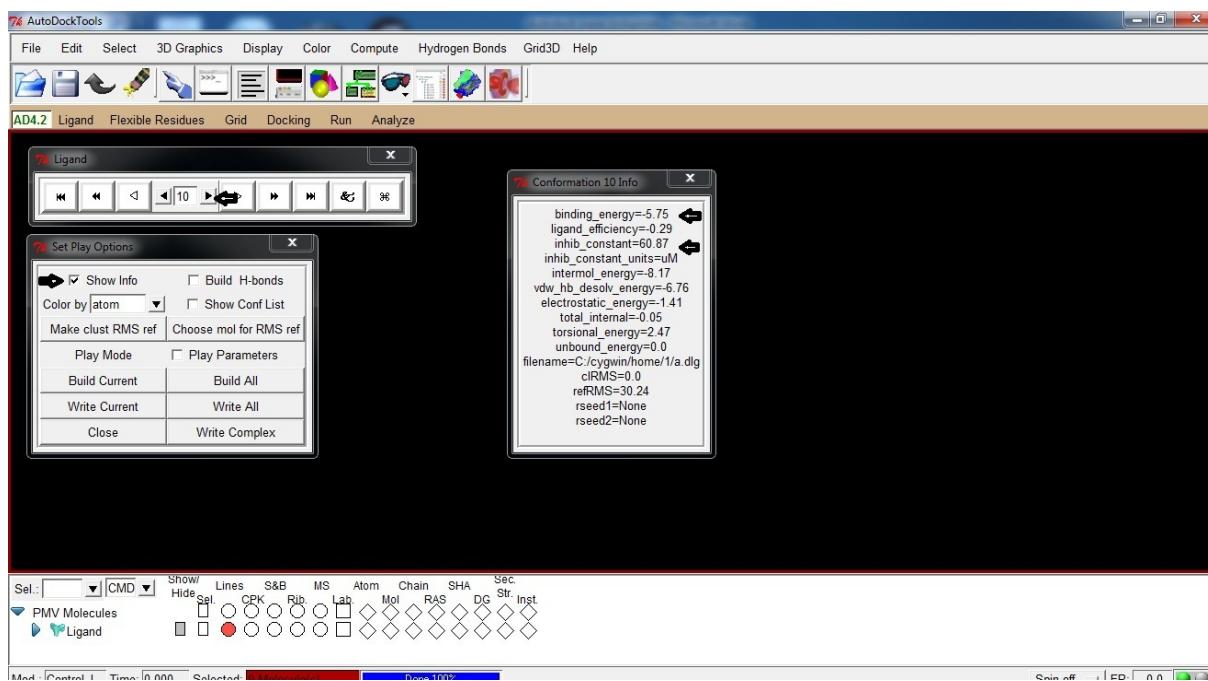
4) Analyzing results and Retrieving Ligand-Enzyme interaction complex .pdb

4.1 Analyzing Results

- Open AutoDock
- Click Analyze
- Click Docking
- Click Open
- Select a.dlg
- Click Open

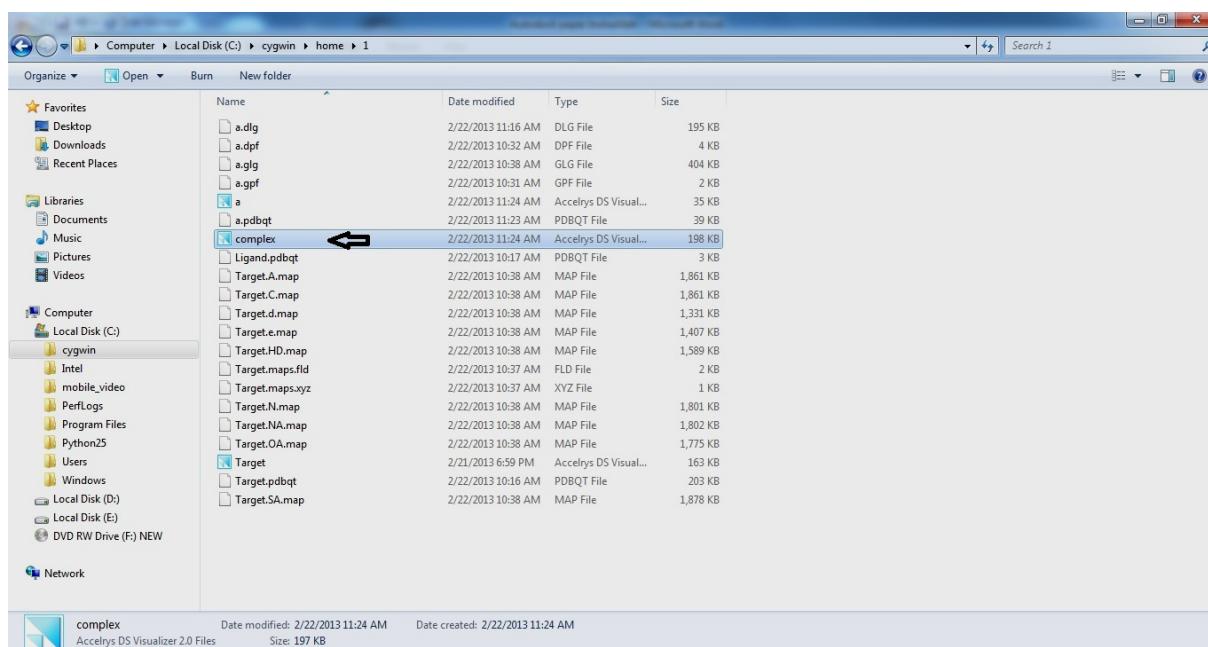


- Click OK
 - Again Analyze
 - Click Conformations
 - Click Play
 - Click &
 - Click show information
 - Click this sign to observe each conformation from 1 to 10
- Note the confirmation showing best down binding energy and inhibition constant
(*In our case 10 conformation was best with binding energy (ΔG) as -5.75 and inhibition constant (Ki) as 60.87 μM)



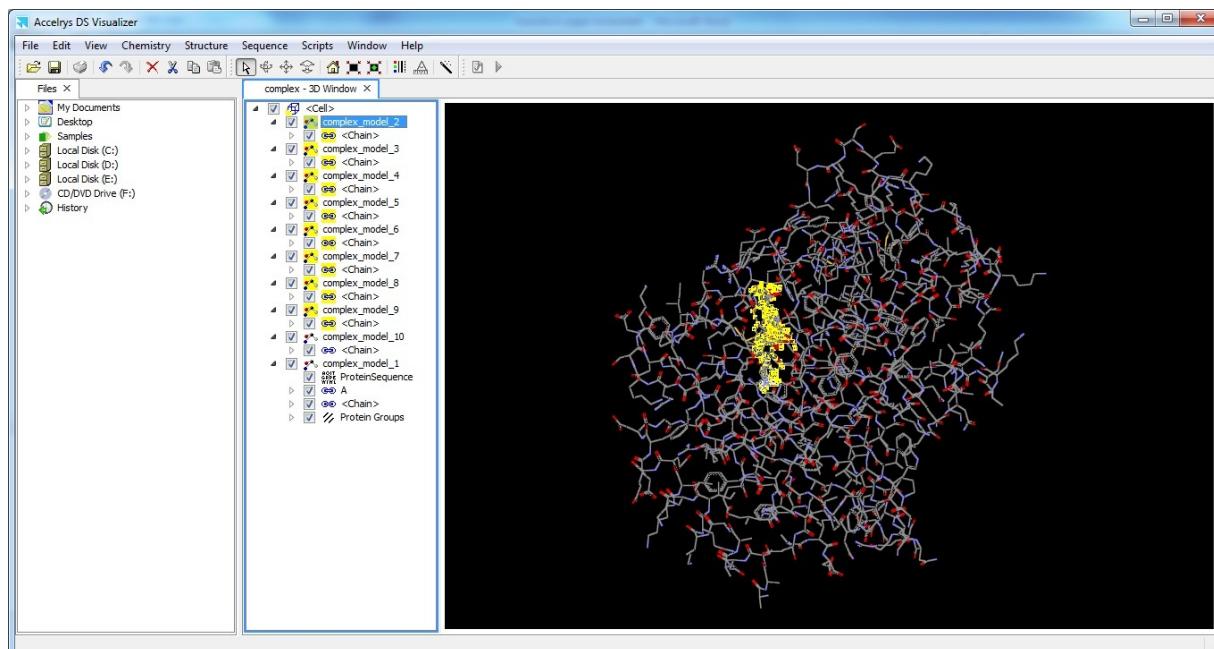
4.2 Retrieving Ligand-Enzyme interaction complex .pdb

- Open C drive
- Open Cygwin
- Open home
- Open 1
- Open complex.pdb in Discovery Studio Visualizer

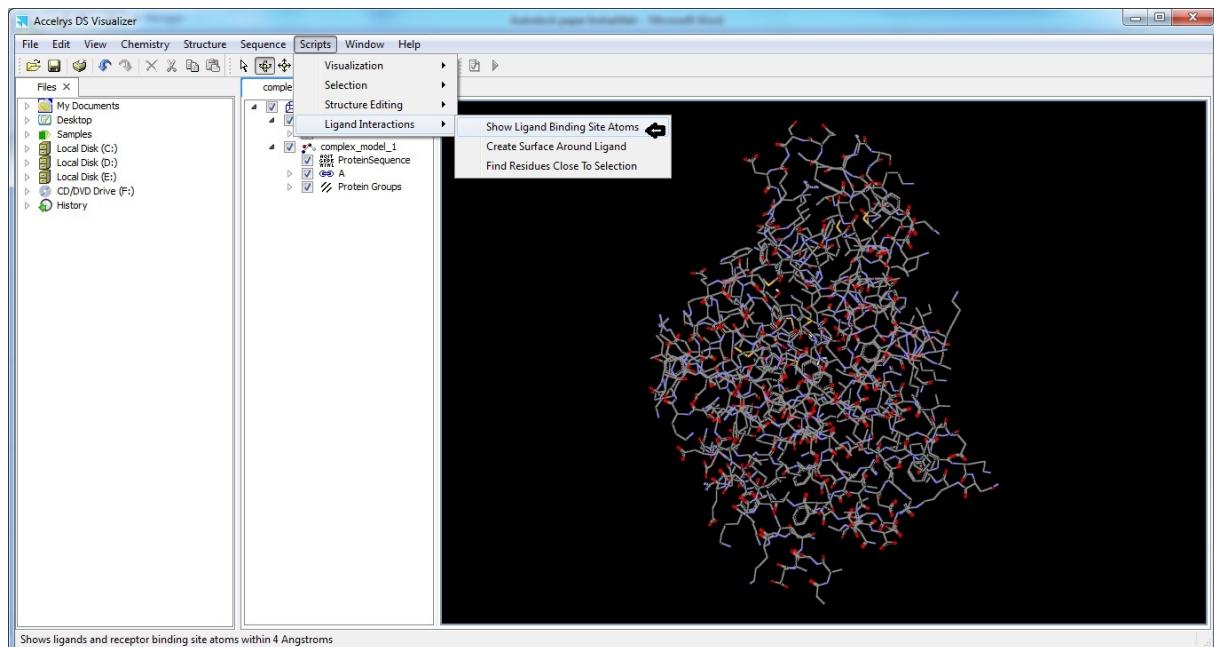


Select all other complexes and delete them except the best

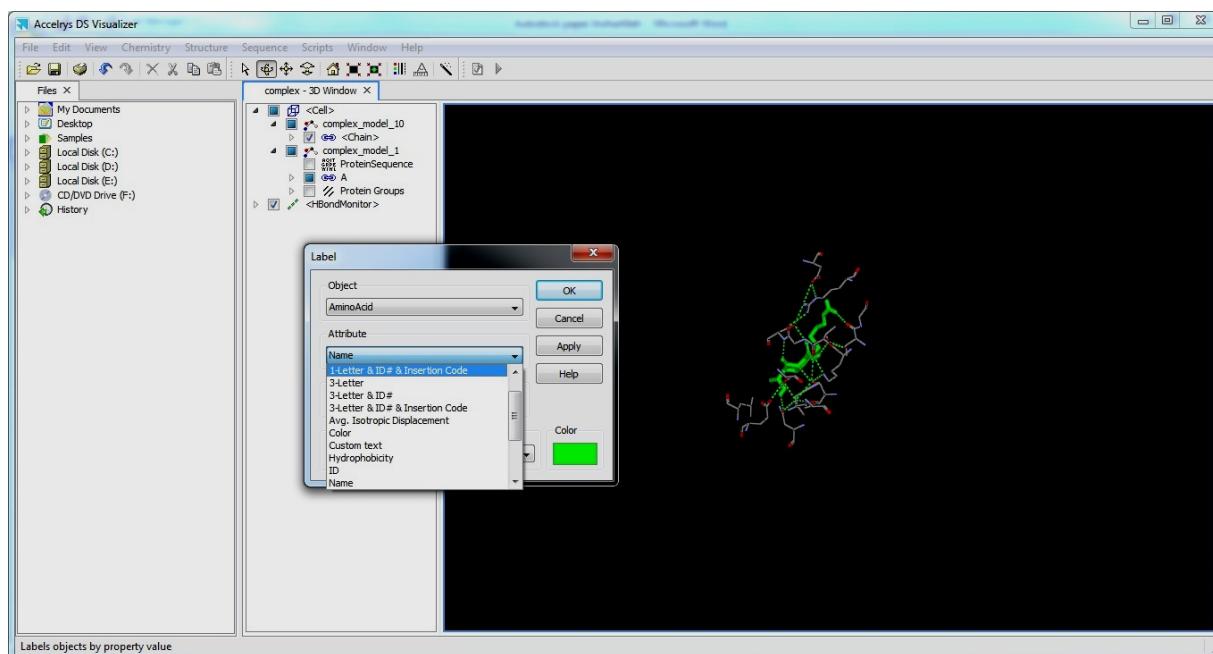
(*In our case Complex model 10 was best as conformation 10 was showing best results in our case).



- Click Scripts
- Click Ligand Interactions
- Click Show Ligand Binding Site Atoms



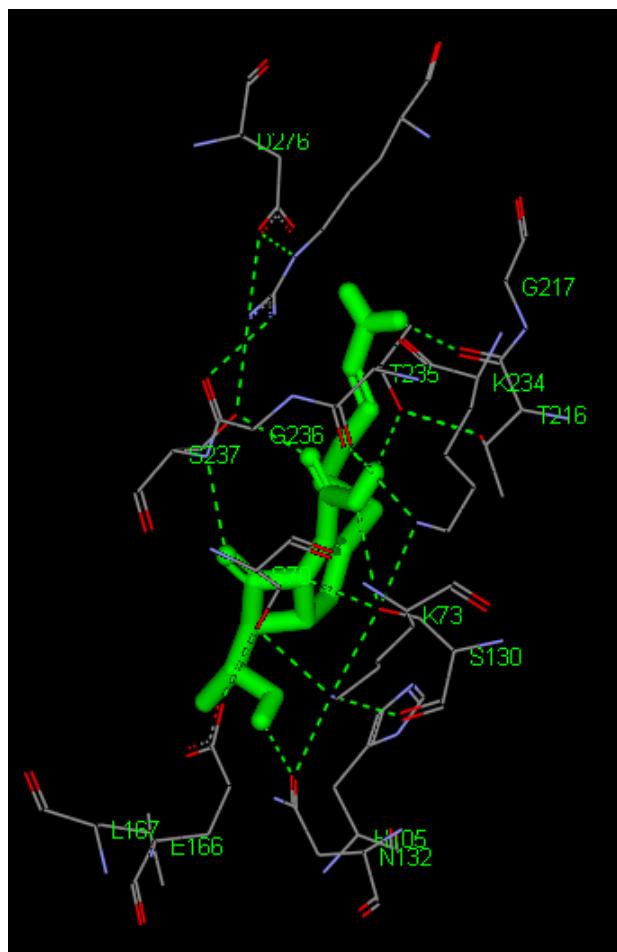
- Right Click on Complex
- Click Label
- Select Object: AminoAcid
- Select Attributes: 1 Letter & ID insertion code
- Click OK



➤ Save as Image files

CONCLUSION

AutoDock is a popular non-commercial docking program that docks a ligand to its target protein and performs well (accurate and computationally fast). In this paper we propose an easier user-friendly docking protocol for docking ligands with target protein that utilizes AutoDock and Cygwin for docking operations. Our protocol provides a detailed outline and advice for use of AutoDock, AutoDock Tools, its graphical interface and to analyze interaction complexes using computational docking. The example of a docking experiment between Imipenem-hydrolyzing beta-lactamase SME-1 (an enzyme) and Imipenem (a ligand) using AutoDock 4.2/ADT has been given. Our sincere aim is to spread knowledge and make scientific research accessible to researchers who could not afford to buy software or pay high subscription fees of online docking servers. With due confidence, this is our humble claim that a researcher with no previous background in bioinformatics research would be able to perform molecular docking using AutoDock 4.2 program by following stepwise guidelines given in this article.



ACKNOWLEDGEMENTS

The authors are thankful to all the scientists of this world who possess a burning desire to share their knowledge and skills with the entire world free of charge and solely for the benefit of mankind and expect its reward from Allah alone. We extend sincere thanks to the inventors of ‘AutoDock’.

REFERENCES

- Anderson AC. The process of structure-based drug design. *Chem Biol* 2003;10: 787–97.
- Gilbert D. Software review: bioinformatics software resources. *Brief Bioinform* 2004; 5:300-4.
- Lazarova M. Virtual screening-models, methods and software systems. International Scientific Conference Computer Science 2008;55-60.
- Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J Comput Chem* 1998;19: 1639–62.
- Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS et al. AutoDock 4 and AutoDockTools 4: automated docking with selective receptor flexibility. *J Comput Chem* 2009;30:2785–91.
- Schneider G. Virtual screening: an endless staircase? *Nat Rev Drug Discov* 2010;9: 273–6.
- Schneider G, Böhm H. Virtual screening and fast automated docking methods: combinatorial chemistry. *Drug Discov Today* 2002;7:64-70.
- Walters W, Stahl M, Murcko M. Virtual screening - An overview. *Drug Discov Today* 1998;3:160-78.
- Warren G, Andrews C, Capelli A, Clarke B, LaLonde J, Lambert MH et al. A critical assessment of docking programs and scoring functions. *J Med Chem* 2006;49:5912-31.
- Waszkowycz B, Perkins T, Sykes R, Li J. Large-scale virtual screening for discovering leads in the postgenomic Era. *IBM Systems J* 2001;40:360-76.