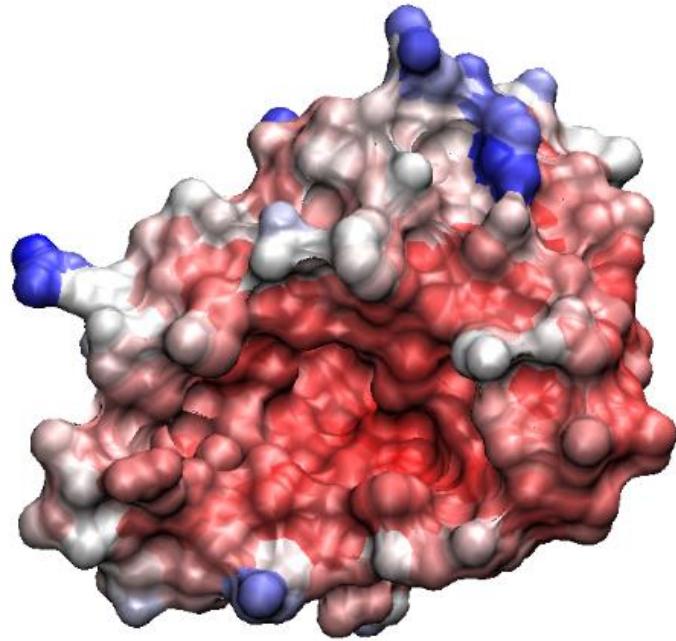
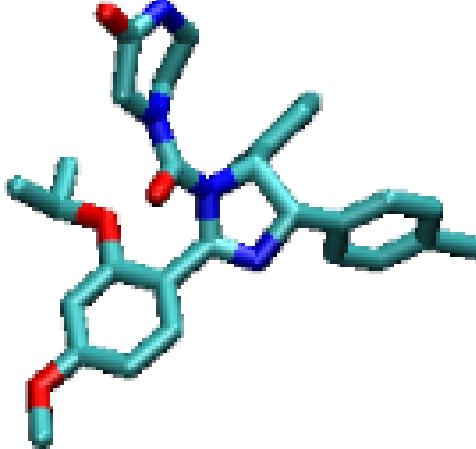




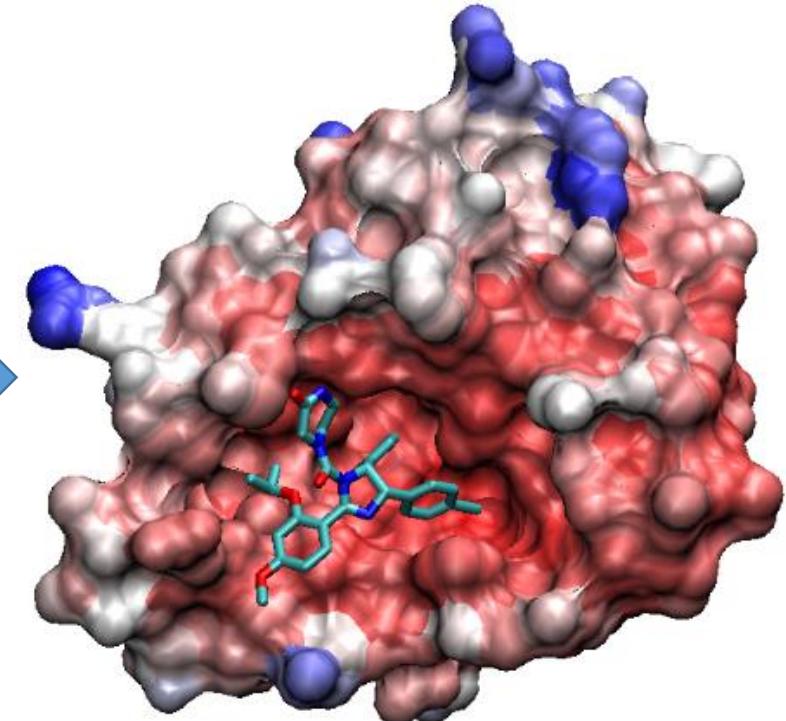
MOLECULAR DOCKING TUTORIAL USING AUTODOCK 4



Target Receptor



Ligand



Molecular Docking

Pre-requisite for Protein-Ligand docking using AutoDock

AutoDock 4.2

<https://autodock.scripps.edu/download-autodock4/>

After registration, you will get keys.

MGL Tools (AutoDockTools)

<https://ccsb.scripps.edu/mgltools/downloads/>

Youtube Video regarding installation of AutoDock

<https://www.youtube.com/watch?v=bjhcPjn7-60>

ChemSketch

<https://www.acdlabs.com/resources/freeware/chemsketch/>

DeepView - Swiss-PdbViewer

<https://spdbv.vital-it.ch/disclaim.html>

VMD (for visualization)

<https://www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=VMD>

Avogadro

<https://sourceforge.net/projects/avogadro/files/latest/download>

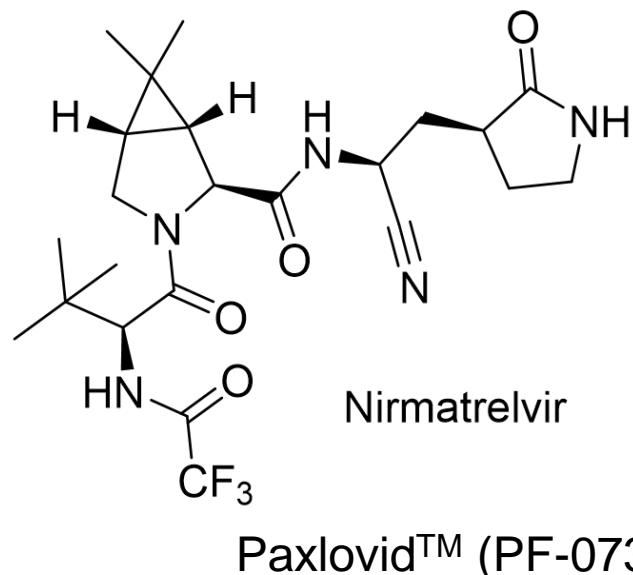
Steps involved in Molecular Docking

- 1) Choose the protein target and ligand molecule
- 2) Carry out protein and ligand preparation
- 3) Select the docking site
- 4) If ligand contains metal, preparation of parameters
- 5) Carry out ligand-protein docking using docking tools
- 6) Different ligand poses generated
- 7) Analysis of results

Choosing protein target

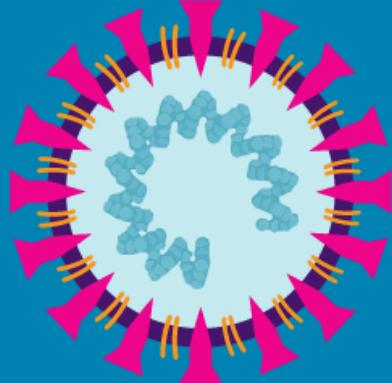
DOCKING OF ANTIVIRAL DRUGS AGAINST SARS-CoV-2-3CL

The coronavirus 3-chymotrypsin-like protease (3CL pro) or main protease, Mpro, is a key protein in the virus life cycle and a major drug target. Based on crystal structures of SARSCoV2 Mpro complexed with PF-07321332 inhibitors Pdb id: 7VH8), we will first validate the ligand docking using in build ligand. Molecular mechanism of antiviral drug action is shown in next slides.

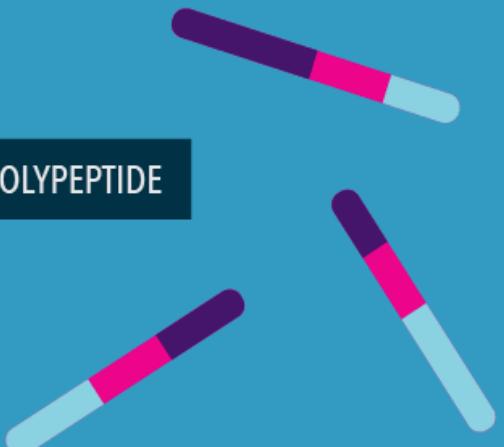


The SARS-CoV-2 virus is a single strand of RNA wrapped in a protein envelope. The RNA contains the genetic instructions the virus needs to make copies of itself.

CORONAVIRUS

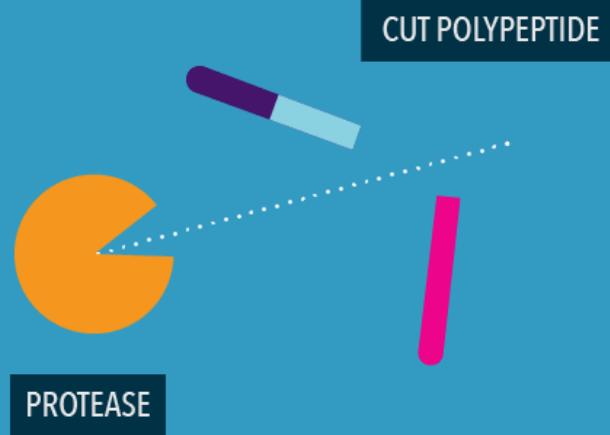


POLYPEPTIDE



When the virus invades a human cell, its' RNA is translated into a polypeptide, a long protein chain that includes several enzymes necessary to continue replication.

Before these enzymes can start working, they need to be separated from each other. The protease enzyme acts like a pair of scissors, cutting the polypeptide up into the different enzymes, that then become functional.



Protease inhibitors can interfere with this critical cutting step. These drugs are designed to tightly bind to the protease enzyme, blocking its ability to cut.

The virus then can't make copies of itself and further infect its host.

Repairing the PDB file

From the available experimental data for 7VH8, we will use chain A for docking. However, few sidechains atoms are missing. We will repair the protein structure using Swiss pdb viewer for chain A of 7VH8.

First create a new directory:

Ex. C:\Users\stemskillslab\Documents\SARSCOV23CL\

Now transfer the downloaded file to new directory.

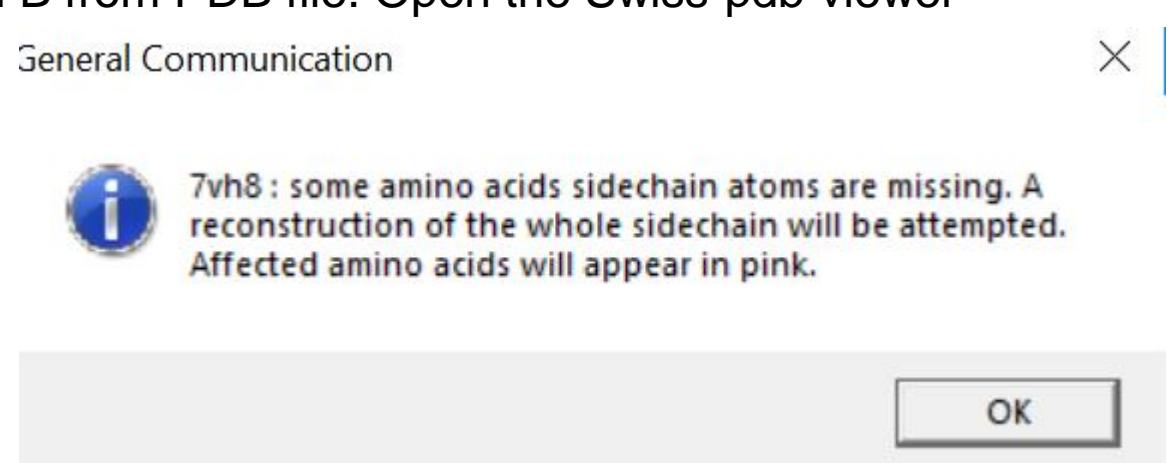
Method 1

Now, we will use Swiss-pdb viewer to save only chain B from PDB file. Open the Swiss-pdb viewer

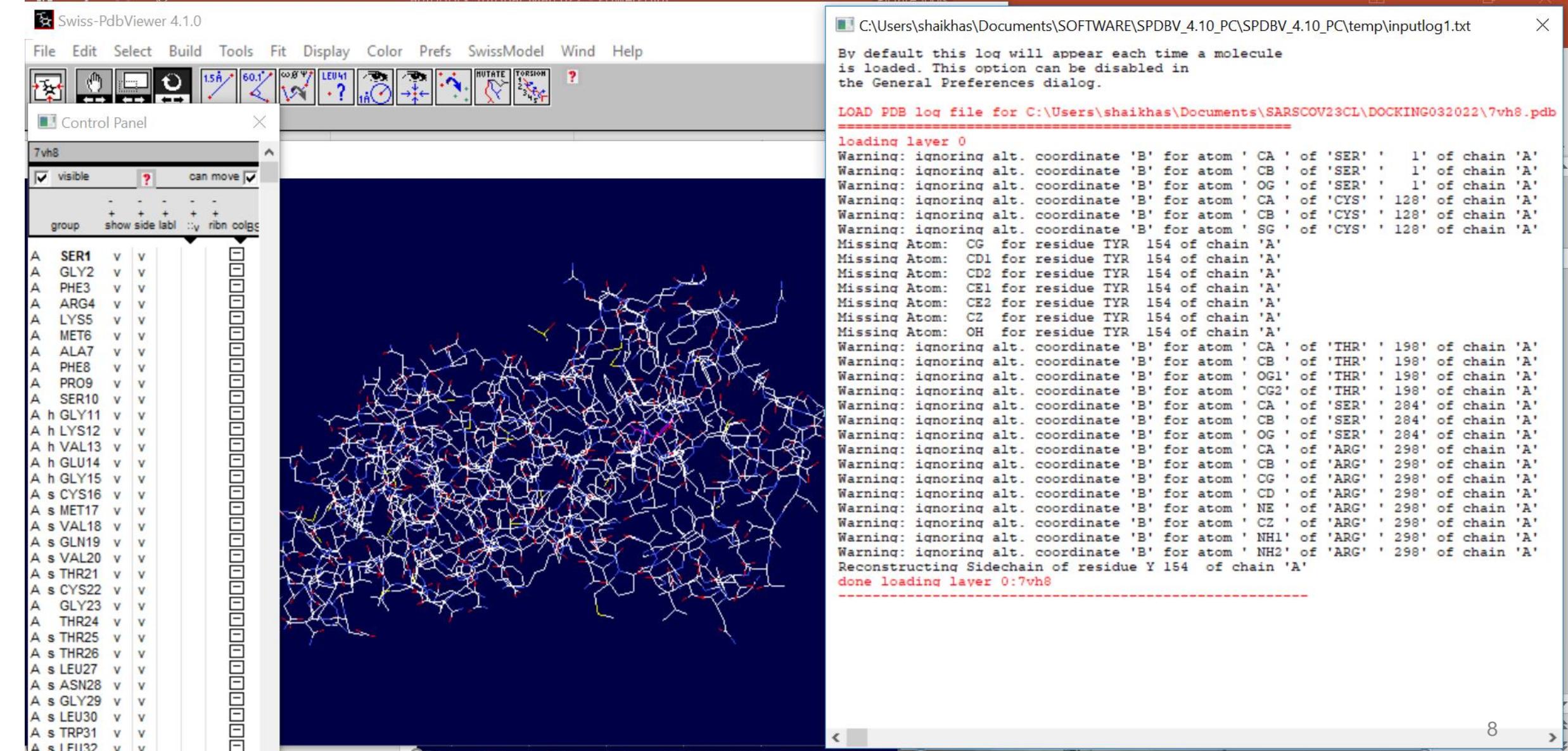
File > Open PDB file..> 7vh8.pdb

Warning message will appear

Click OK



Once protein is displayed, you can see that all missing sidechain atoms were added. Also, if there are more than two conformations for particular side, only dominant conformation will be kept. Modified residues were shown with magenta/pink color as shown below.



Selecting the chain A

Now, open the control panel

Wind > Control Panel

Control panel will open as shown in adjacent picture

File > Save > Selected Residues Of Current Layer...

7vh8-swiss.pdb and save

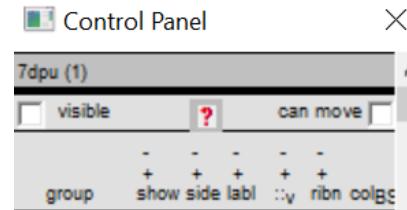
Scroll down and only 4WI401 by clicking on 4WI residue

File > Save > Selected Residues Of Current Layer...

pfiwer.pdb and save

Scroll down in control panel, and unselect any ligand which you do not want to use in docking. Use control tab and click on ligand which you do not want to use in docking. For example we do not want DMS and H2S ligand of chain A to be included in docking as shown below

A	II	SER301	v	v
A		GLY302	v	v
A		VAL303	v	v
A		THR304	v	v
A		PHE305	v	v
A		GLN306	v	v
A		4WI401	v	v
A		DMS402	v	v
A		H2S403	v	v



To select Chain A, click on A under group tab

A	h	ARG298	v	v
A	h	GLN299	v	v
A	h	CYS300	v	v
A	h	SER301	v	v
A		GLY302	v	v
A		VAL303	v	v
A		THR304	v	v
A		PHE305	v	v
A		GLN306	v	v
A		4WI401	v	v
A		DMS402	v	v
A		H2S403	v	v



group	show side	labl	by	ribn	colrg
A	SER1	v	v		
A	GLY2	v	v		
A	PHE3	v	v		
A	ARG4	v	v		
A	LYS5	v	v		
A	MET6	v	v		
A	ALA7	v	v		
A	PHE8	v	v		
A	PRO9	v	v		
A	SER10	v	v		
A	h GLY11	v	v		
A	h LYS12	v	v		
A	h VAL13	v	v		
A	h GLU14	v	v		
A	GLY15	v	v		
A	s CYS16	v	v		
A	s MET17	v	v		
A	s VAL18	v	v		
A	s GLN19	v	v		
A	s VAL20	v	v		
A	s THR21	v	v		
A	s CYS22	v	v		
A	GLY23	v	v		
A	THR24	v	v		
A	s THR25	v	v		
A	s THR26	v	v		
A	s LEU27	v	v		
A	s ASN28	v	v		
A	s GLY29	v	v		
A	s LEU30	v	v		
A	s TRP31	v	v		
A	s LEU32	v	v		
A	ASP33	v	v		
A	ASP34	v	v		
A	s VAL35	v	v		
A	s VAL36	v	v		
A	s TYR37	v	v		
A	s CYS38	v	v		
A	s PRO39	v	v		

Adding Hydrogen and repairing tautomeric form

In order to add all hydrogens and search tautomer's for GLN, ASN, and HIS, we will use MolProbity Program

<http://molprobity.biochem.duke.edu/>

Upload 7vh8-swiss.pdb

The screenshot shows the MolProbity upload confirmation page. On the left is the MolProbity logo (a stylized molecule icon). In the center, the text "Uploaded PDB file as 7dpuB_clean.pdb" is displayed. On the right is the Duke Biochemistry logo. Below this, a message states "Your file from local disk was uploaded as 7dpuB_clean.pdb." To the right of the message is a 3D ribbon model of a protein structure against a black background. At the bottom left is a "Continue >" button.

Your file from local disk was uploaded as 7dpuB_clean.pdb.

- 1 chain(s) is/are present [1 unique chain(s)]
- A total of 306 residues are present.
- Protein mainchain and sidechains are present.
- No explicit hydrogen atoms are included.
- 0 PDBv2.3 atoms were found. Proceeding assuming PDBv3 formatted file.

Continue >

Then Continue and finally Add Hydrogens.



Add hydrogens

Select a model to add H to:

7dpuB_clean.pdb

File (modified) uploaded by user

Select a method of adding H:

Asn/Gln/His flips

Add missing H, optimize H-bond networks, check for flipped Asn, Gln, His ([Reduce -build](#))

Advanced options:

- Make Flipkin kinemages illustrating any Asn, Gln, or His flips
 Use atom-renaming version of Reduce for output (**must** be refined before deposition)

No flips

Add missing H, optimize H-bond networks, leave other atoms alone ([Reduce -nobuild9999](#))

Select x-H bond-length:

Electron-cloud x-H

Use electron-cloud x-H bond lengths and vdW radii. Ideal for most cases, especially X-ray crystal structures.

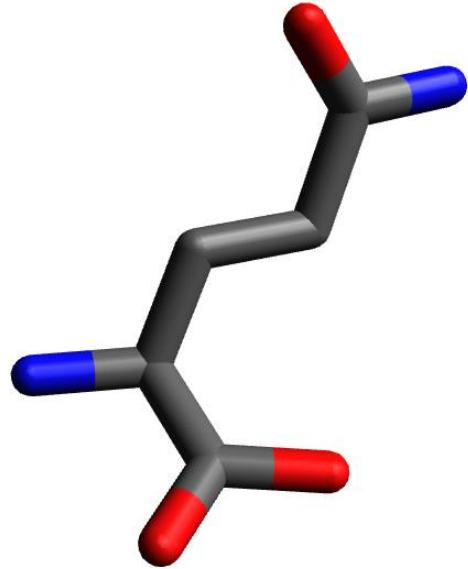
Nuclear x-H

Use nuclear x-H bond lengths and vdW radii. Ideal for NMR, neutron diffraction, etc.

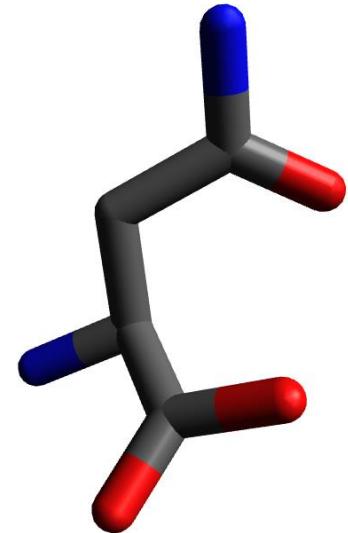
[Start adding H >](#)

Click Start Adding H and then Continue and save the resultant file as 7dpuBHFn.pdb

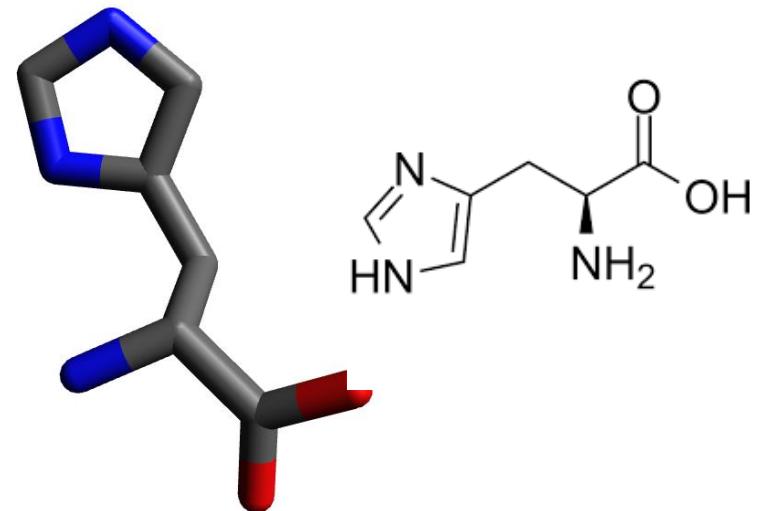
7v8h-swissHF.pdb we will use for docking.



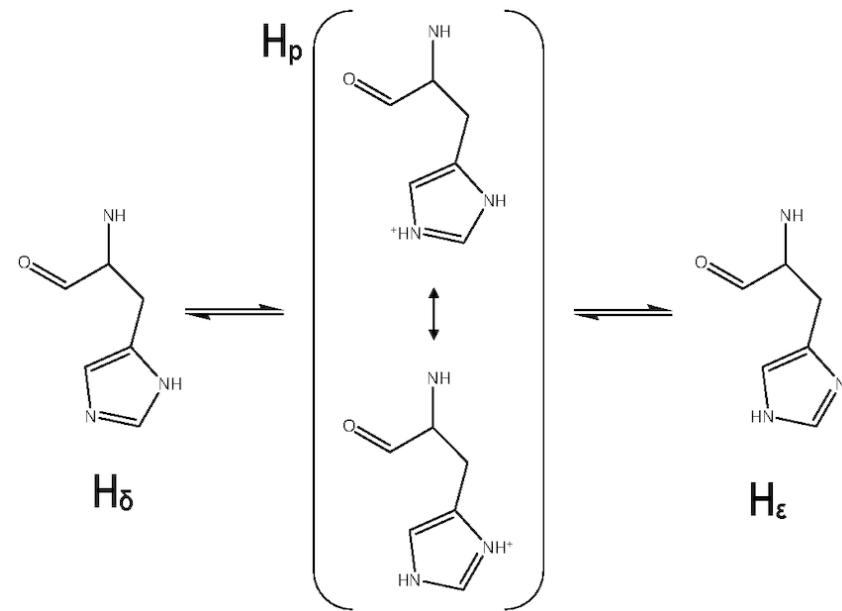
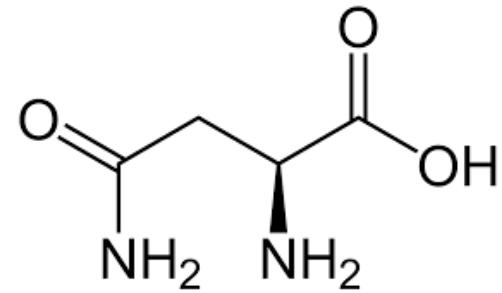
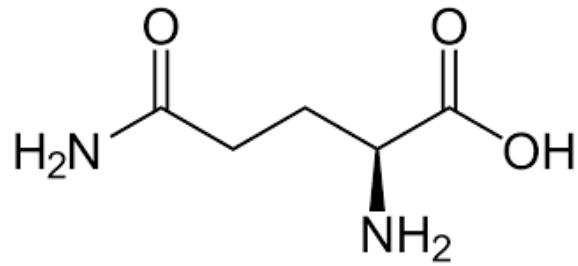
Glutamine (ASN)



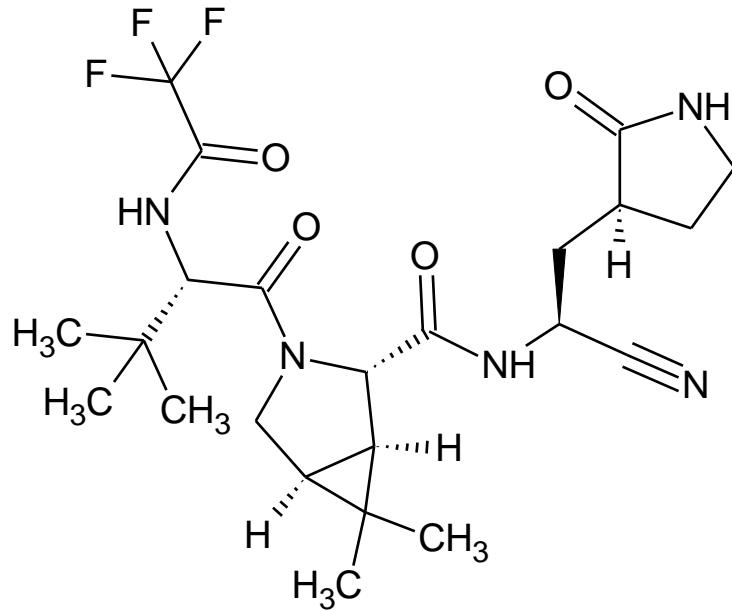
Asparagine (ASP)



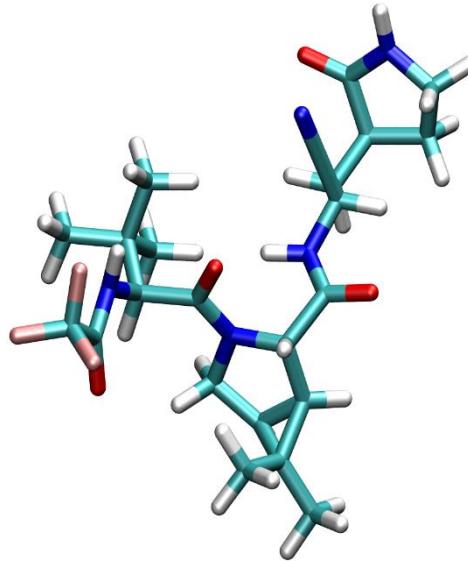
Histidine (HIS)



Researchers are studying protease inhibitors for their potential to stop SARS-CoV-2 , the virus that causes Covid-19, from replicating.



PF-07321332 (PAXLOVID)

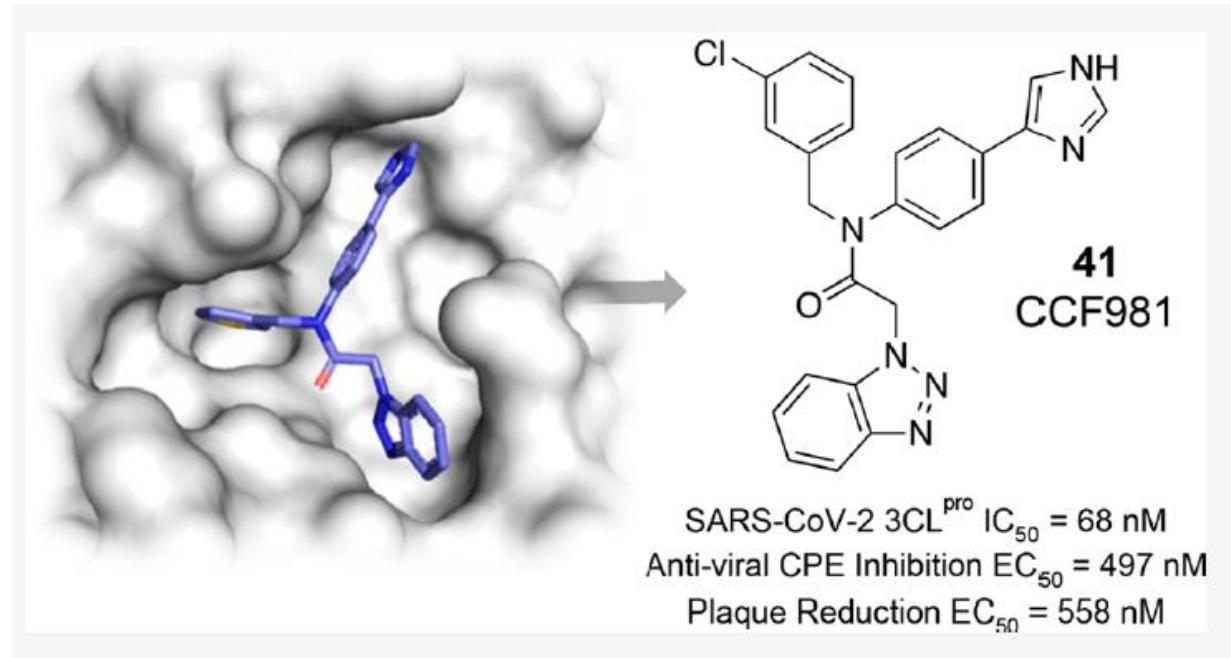


You can sketch the structure in chemSketch or download the pdb file from following link

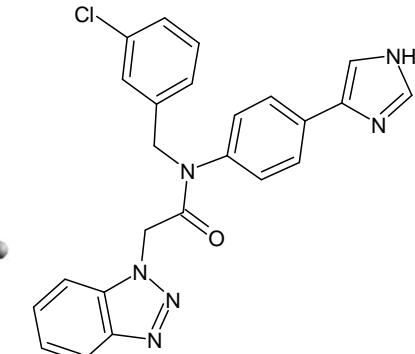
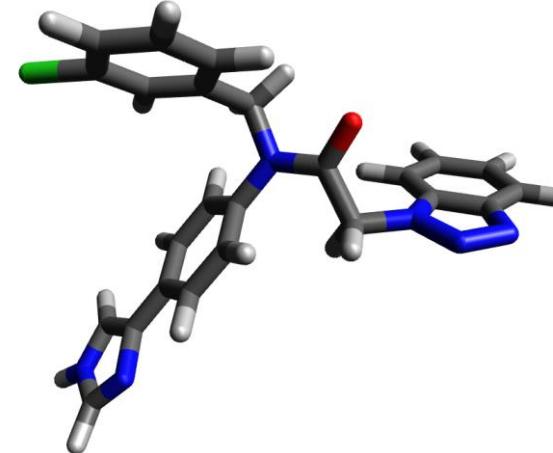
<https://go.drugbank.com/drugs/DB16691>

FDA Authorizes First Oral Antiviral for Treatment of COVID-19. It is found to inhibit 3CLpro enzyme.

Exercise: Researchers are studying protease inhibitors for their potential to stop SARS-CoV-2 , the virus that causes Covid-19, from replicating.



You can sketch the structure in Avogadro or chemSketch



Avogadro

<https://avogadro.cc/>

Activity of CCF981 was found to be in nano molar and comparable with Remdesivir

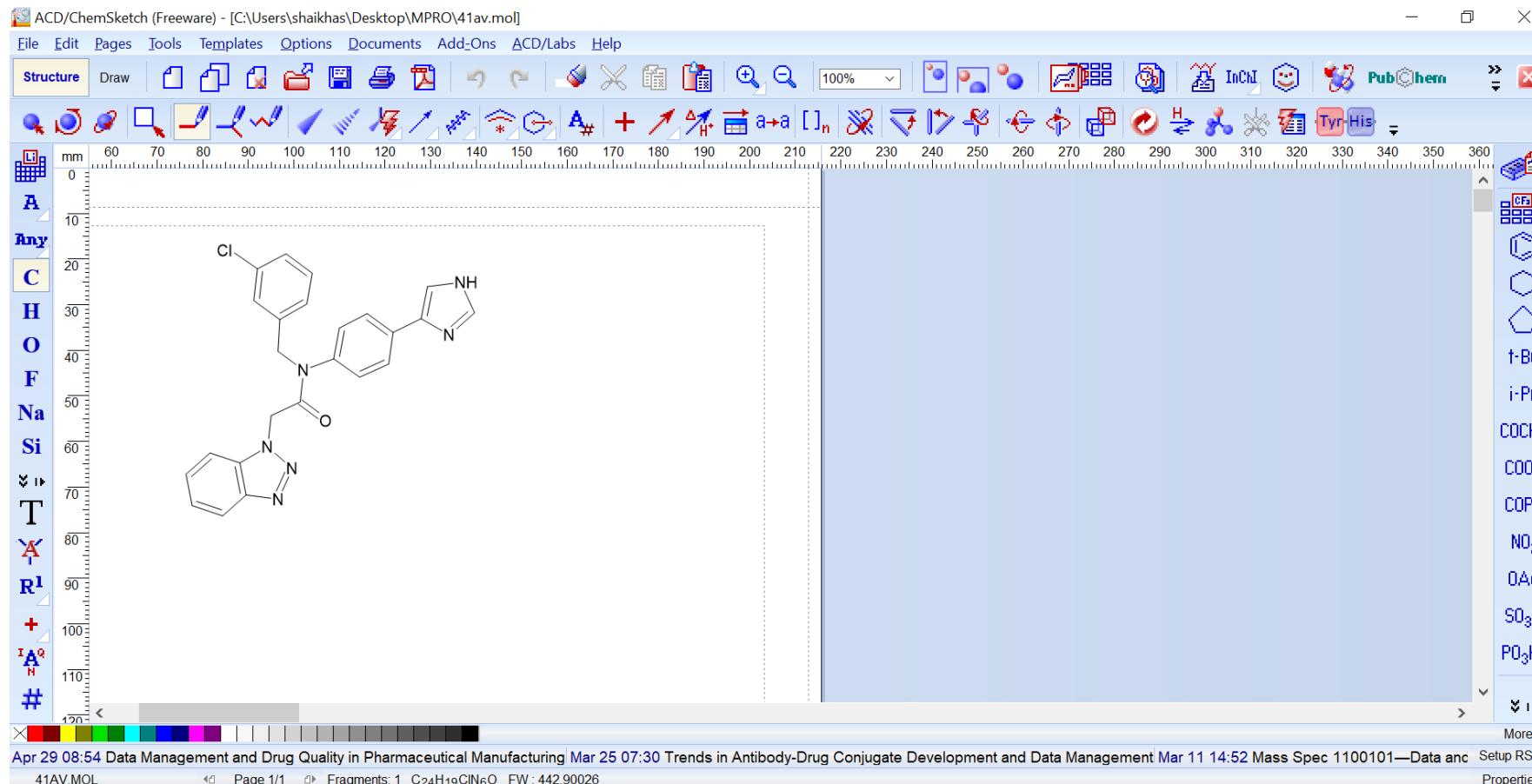
<https://doi.org/10.1021/acs.jmedchem.1c00598>

Chem Sketch

<https://www.acdlabs.com/resources/freeware/chemsketch/index.php>

Ligand Preparation

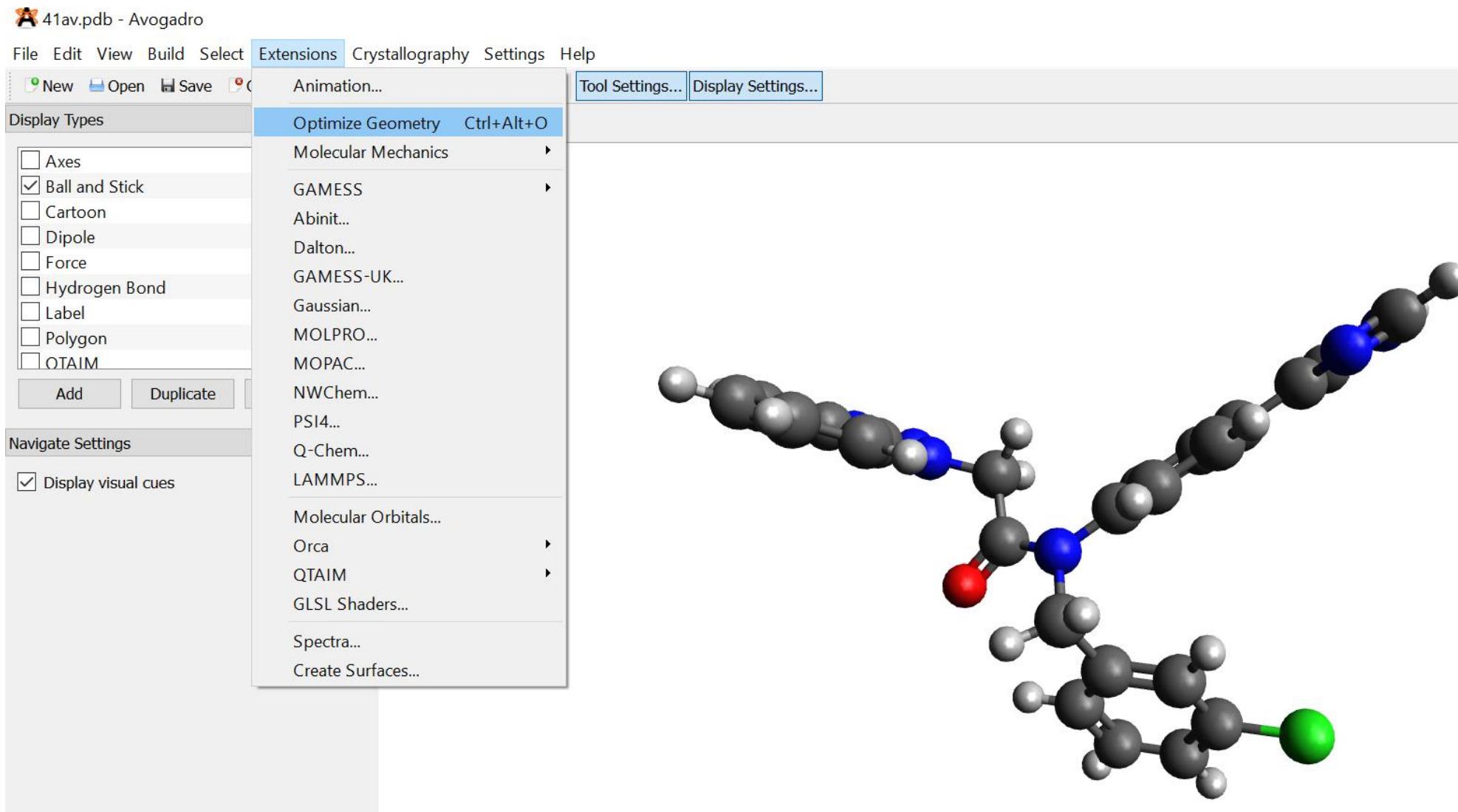
CHEM SKETCH



we can draw structure of ligand in ChemSketch. Then Tools > Clean Structure followed by Tools > 3D Structure Optimization. After that save it as 41av.mol. Then open this 41av.mol file in Avogadro.

In Avogadro, you can minimize the structure.
Extension > Optimize Geometry
Optimize 3-4 times in save it as 41min.pdb

In can also directly build the structure in Avogadro as shown in Hands-on session. The minimize it and save it as a pdb file



AutoDockTools

Now open the AutoDockTools. Once open, we can set the working directory as shown below

click File in the top left corner of AutoDockTools GUI

File > Preferences >

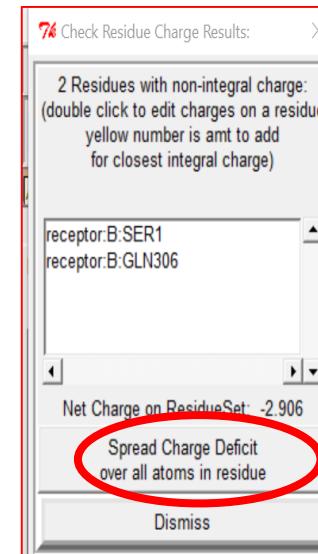
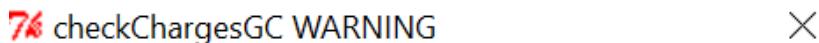
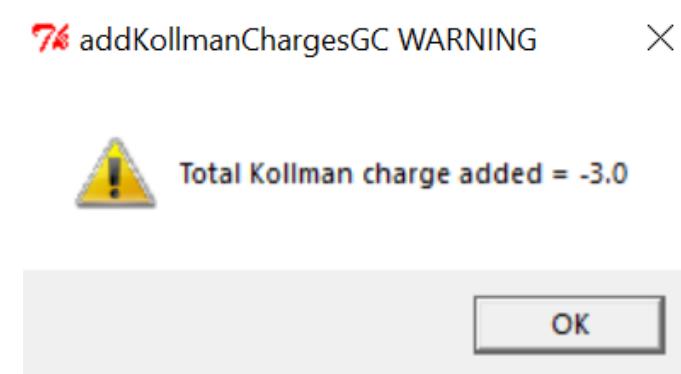
In Startup Directory For ex. ". C:\Users\stemskillslab\Documents\SARSCOV23CL\". And click Set and then Dismiss

Open the pdb 7v8hFH.pdb file in AutoDock Tools

File > Read Molecule > 7v8h-swissFH.pdb

Edit > Charges > Add Kollman Charges

Edit > Charges > Check Totals on Residues



Sometimes we get message as shown below

Click on Spread Charge deficit over all atoms in residue and then click Dismiss

Now show the protein in lines by going to Dashboard and clicking oval shape box under L

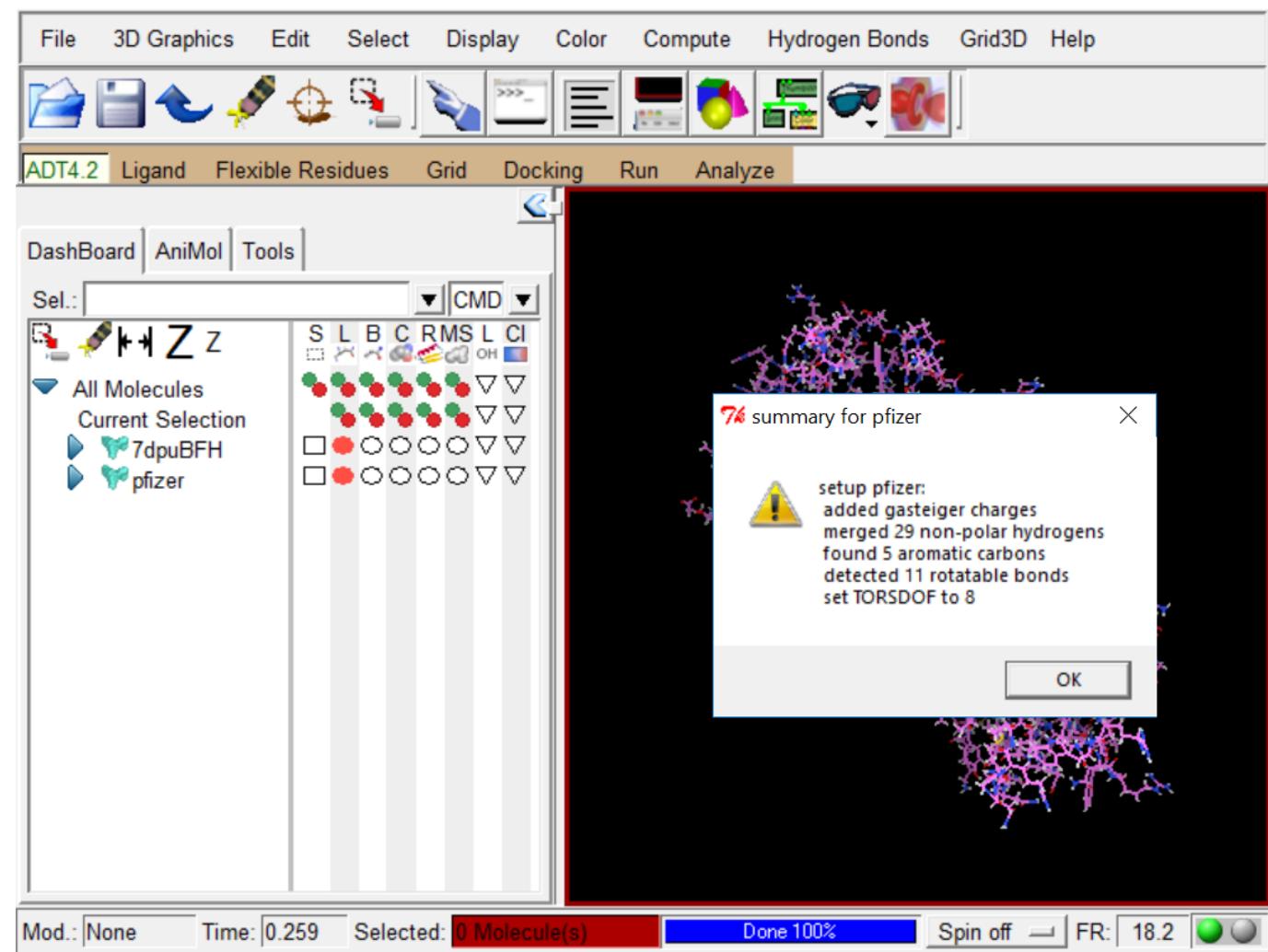
Now in AuodockTools, we will open ligand file.

Ligand > Input > Open ... > pfizer.pdb (before that change pdbqt files to pdb).

After opening you will receive message as shown in Figure.

Program added Gasteiger charges, found 29 aromatic carbons, detected 11 rotatable bonds. Set TOTSDOF to 8.

Click ok

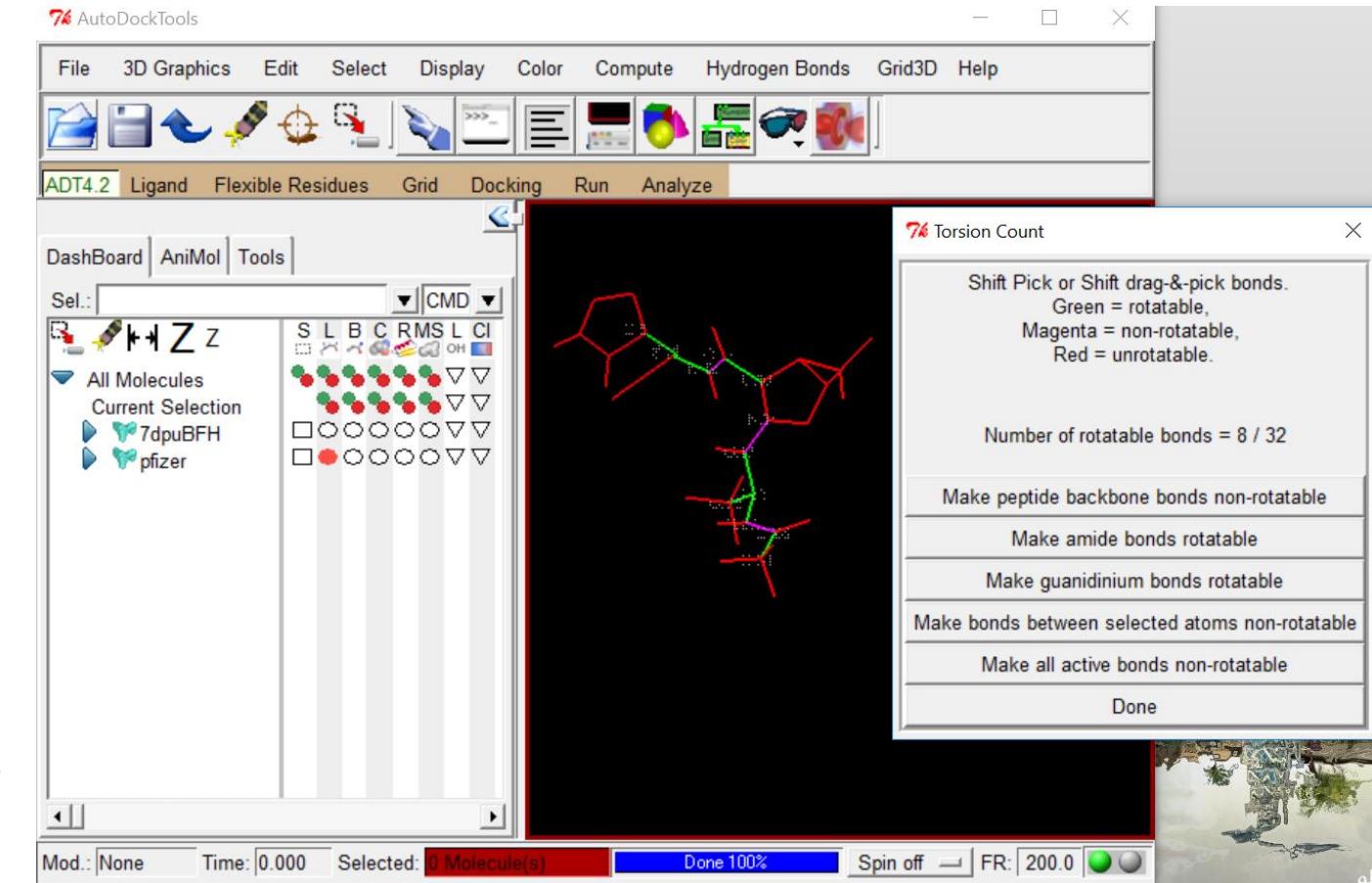


Ligand > Torsion Tree > Detect Root...

Ligand > Torsion Tree > Choose Torsions...

Bonds that are currently active are colored green, bonds that cannot be rotated are colored red while bonds that could be rotated but are currently marked as inactive are colored purple. In AutoDock only single bonds which are not in cycles and not to leaves can be rotated. ADT determines which bonds could be rotated.

You set which of these are to be rotatable by inactivating the others in the viewer.



In our ligand out of * bonds, * bonds are rotatable which are shown in green color.

Click Done

Ligand > Output > Save as PDBQT... > nutlin3a.pdbqt

```
ATOM    1  O3   NUT A 201    -23.667  5.839 -16.568  1.00 18.16      O
ATOM    2  C25  NUT A 201    -23.015  6.538 -15.757  1.00 16.08      C
ATOM    3  N2   NUT A 201    -21.695  6.402 -15.710  1.00 16.62      N
ATOM    4  C29  NUT A 201    -21.006  5.564 -16.697  1.00 18.26      C
ATOM    5  C28  NUT A 201    -19.624  6.037 -17.066  1.00 21.25      C
ATOM    6  O2   NUT A 201    -19.027  5.438 -17.957  1.00 26.40      O
ATOM    7  N3   NUT A 201    -19.096  7.088 -16.423  1.00 19.48      N
```

Pdb file

```
REMARK 6 active torsions:
REMARK status: ('A' for Active; 'I' for Inactive)
REMARK     I between atoms: C25_2 and N2_3
REMARK     I between atoms: C25_2 and N1_10
REMARK 1 A between atoms: C10_11 and C9_12
REMARK 2 A between atoms: C5_15 and O1_16
REMARK 3 A between atoms: O1_16 and C6_17
REMARK 4 A between atoms: C1_21 and O_22
REMARK 5 A between atoms: C18_24 and C19_25
REMARK 6 A between atoms: C11_32 and C12_34
ROOT
ATOM    1  O3   NUT A 201    -23.667  5.839 -16.568  1.00 18.16
ATOM    2  C25  NUT A 201    -23.015  6.538 -15.757  1.00 16.08
ATOM    3  N2   NUT A 201    -21.695  6.402 -15.710  1.00 16.62
ATOM    4  C29  NUT A 201    -21.006  5.564 -16.697  1.00 18.26
ATOM    5  C28  NUT A 201    -19.624  6.037 -17.066  1.00 21.25
ATOM    6  O2   NUT A 201    -19.027  5.438 -17.957  1.00 26.40
ATOM    7  N3   NUT A 201    -19.096  7.088 -16.423  1.00 19.48
```

Charge and atom type are present in pdbqt files which are absent in pdb file

Pdbqt file

```
-0.247 OA
0.329 C
-0.291 N
0.196 C
0.274 C
-0.268 OA
-0.234 NA
```

Preparing a Macromolecule

Now we will prepare our protein for docking

Grid > Macromolecule > Choose ...

Select 7v8h-swissFH and click on select molecule

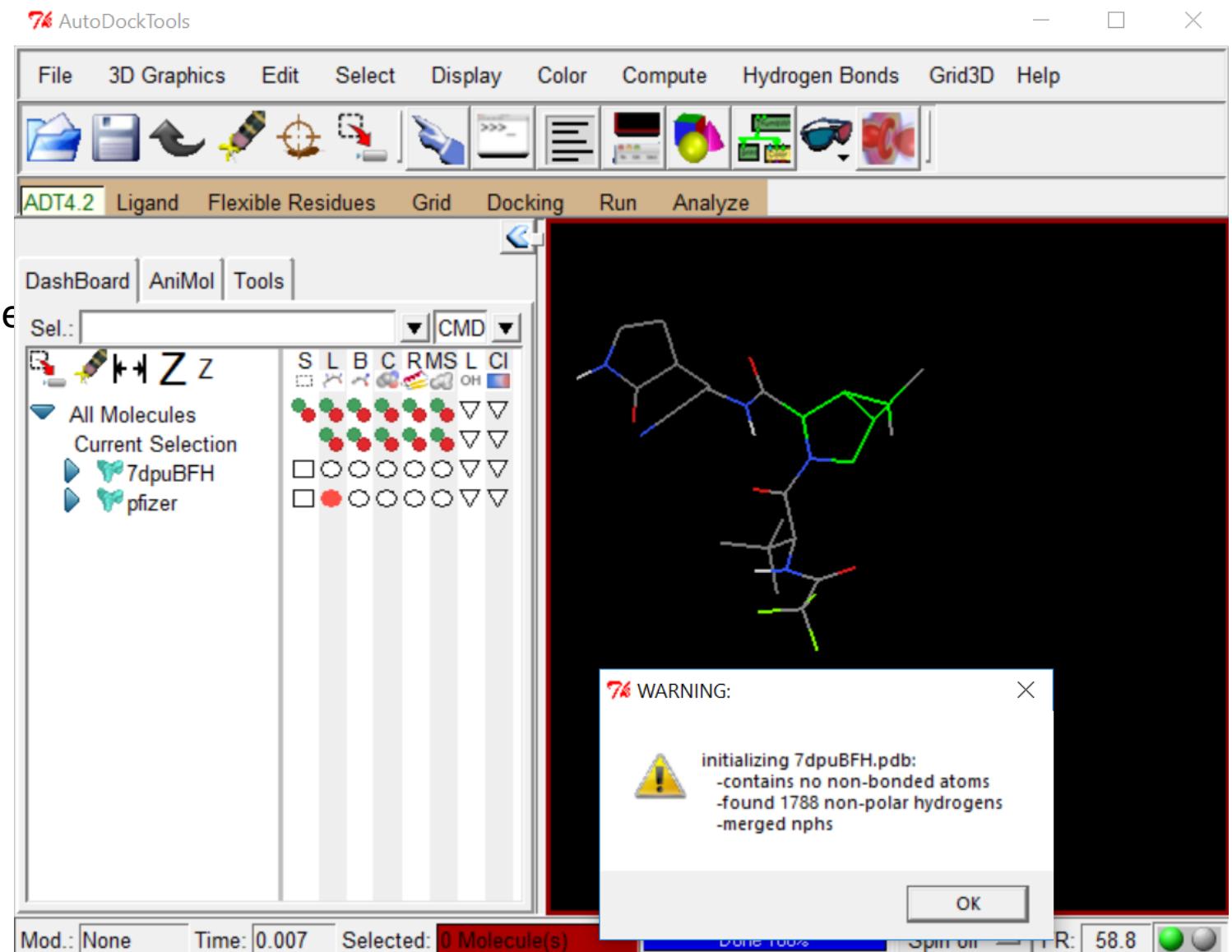
It will show WARNING as shown besides.

Initiallizing 7v8h-swissFH.pdb:

- contains no non-bonded atoms
- found 1788 non-polar hydrogens
- merged npbs

Click OK

Type in 7v8h.pdbqt, and save



ADT checks that the molecule has charges. If not, it adds Gasteiger charges to each atom. Remember that all hydrogens must be added to the macromolecule before it is chosen. If the molecule already had charges, ADT would ask if you want to preserve the input charges instead of adding Gasteiger charges.

Setting the Search Space

Grid > Grid Box...

In present case, we will set Grid Box around bound ligand.

Make sure, ligand is occupied from all side. Adjust the box using x, y, z center.

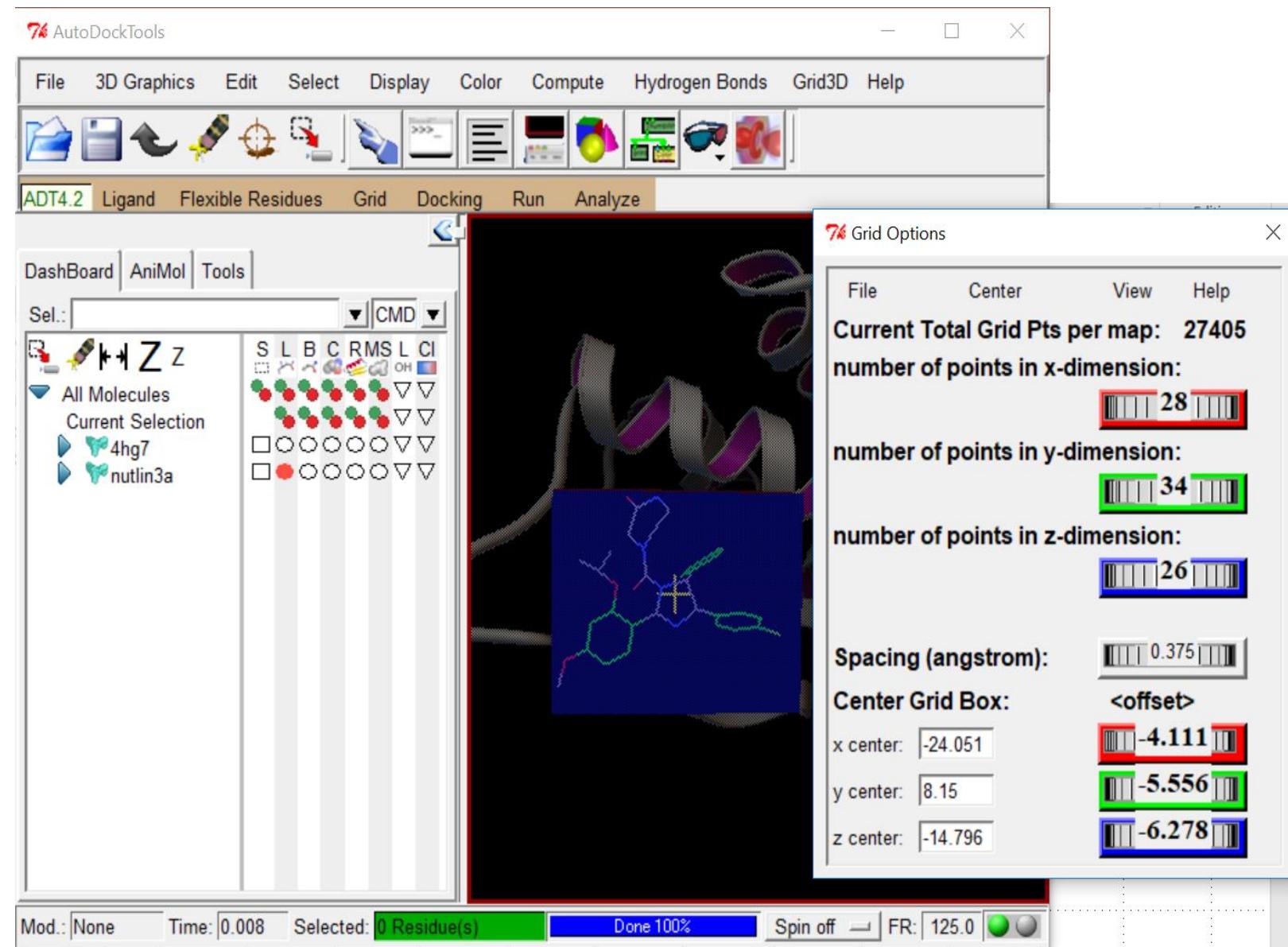
Increase the box length by sliding x-, y-, z- dimension:

Grid Options > File > Close saving current

FYI: GRID Options > Center

→ Center menu contains 4 shortcuts for setting the center of the grid box:

- Pick an atom,
- Center on ligand,
- Center on macromolecule
- On a named atom.



For representation purpose

Setting up the AutoGrid Box

➤ Macromolecule atoms in the rigid part Center:

- center of ligand;
- center of macromolecule;
- a picked atom; or
- typed-in x-, y- and z-coordinates.

➤ Grid point spacing:

default is 0.375\AA (from 0.2\AA to 1.0\AA :).

➤ Number of grid points in each dimension:

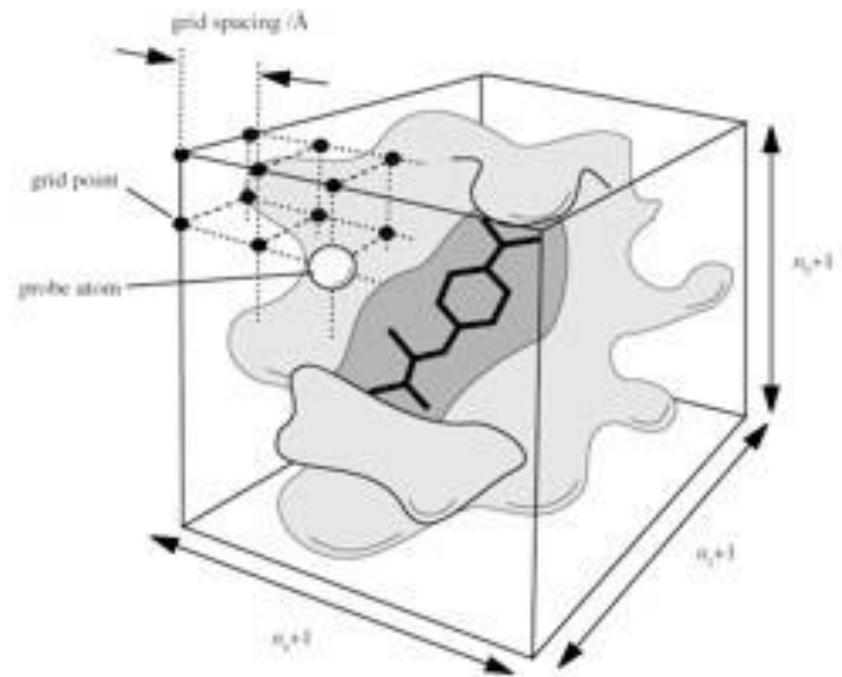
only give even numbers (from $2 \times 2 \times 2$ to $126 \times 126 \times 126$).

AutoGrid adds one point to each dimension.

➤ Grid Maps depend on the orientation of the macromolecule.

Grid Maps

AutoDock requires pre-calculated grid maps, one for each atom type present in the ligand being docked. The probe's energy at each grid point is determined by the set of parameters supplied for that particular atom type, and is the summation over all atoms of the macromolecule, within a non-bonded cutoff radius, of all pairwise interactions.



Preparing the AutoGrid Parameter File

Grid > Set Map Types > Choose Ligand

Choose Ligand> pfizer and then click Select Ligand

Grid > Output > Save GPF...

type-in ligand.gpf and then save it

Starting AutoGrid 4

Run > Run AutoGrid

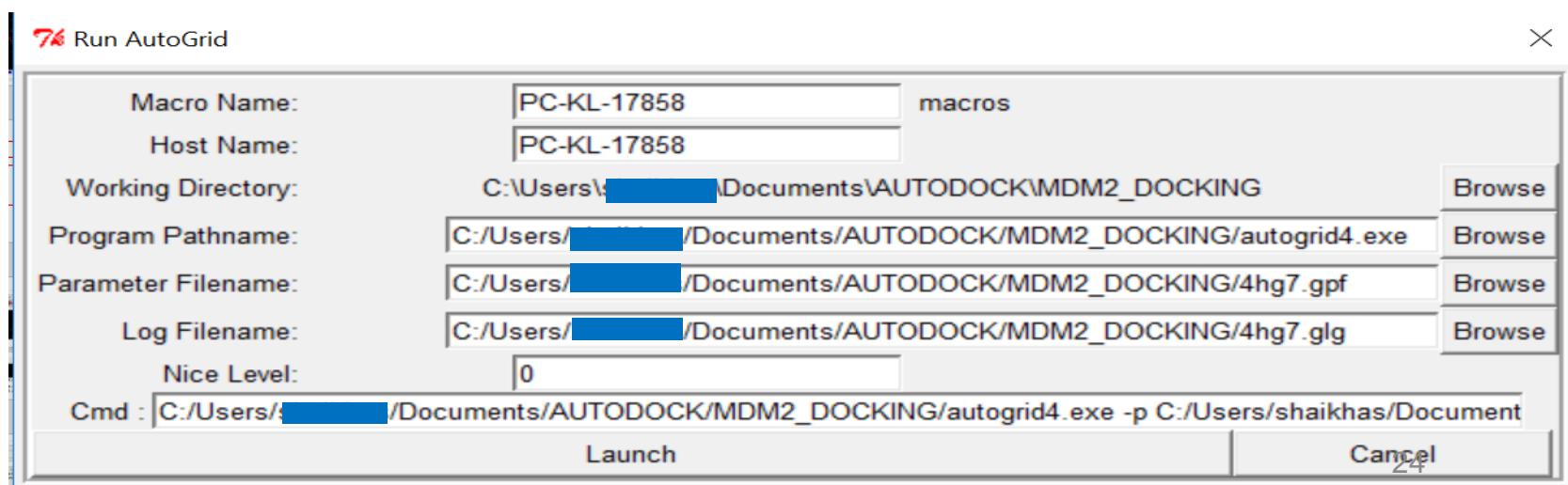
Using Browse window, select
Program Filename autogrid4.exe and
parameter file ligand.gpf

And then Launch the program.
Autodock4.exe and autodock4.grid
can be found in a directory where
you installed AutoDock

Sample grid parameter file

```
hpts 50 50 50          # num.grid points in xyz
gridfld receptor.maps.fld # grid_data_file
spacing 0.375           # spacing(A)
receptor_types A C H HD N NA OA SA # receptor atom types
ligand_types A C Cl NA OA N HD # ligand atom types
receptor receptor.pdbqt # macromolecule
gridcenter -22.15 12.97 16.12 # xyz-coordinates or auto
smooth 0.5             # store minimum energy w/in
rad(A)                 # atom-specific affinity map
map receptor.A.map      # atom-specific affinity map
map receptor.C.map      # atom-specific affinity map
map receptor.Cl.map     # atom-specific affinity map
map receptor.NA.map     # atom-specific affinity map
map receptor.OA.map     # atom-specific affinity map
map receptor.N.map      # atom-specific affinity map
map receptor.HD.map     # atom-specific affinity map
elecmap receptor.e.map   # electrostatic potential map
dsolvmap receptor.d.map # desolvation potential map
dielectric -0.1465       # <0, AD4 distance-
dep.diel;>0, constant
```

For demonstration purpose only



Preparing the AutoDock4 Parameter File

Docking > Macromolecule > Set Rigid Filename...

Select 7v8h-swissFH.pdbqt

Docking > Ligand > Choose..

Select pfizer.pdbqt

New window will appear as shown in picture .



Keep all options default and Accept it

Docking > Search Parameters > Genetic Algorithm...

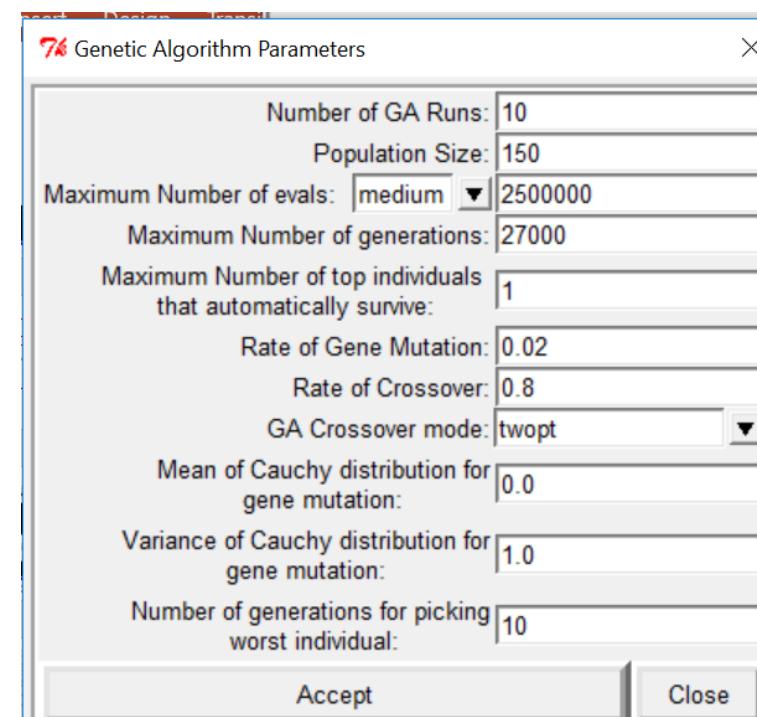
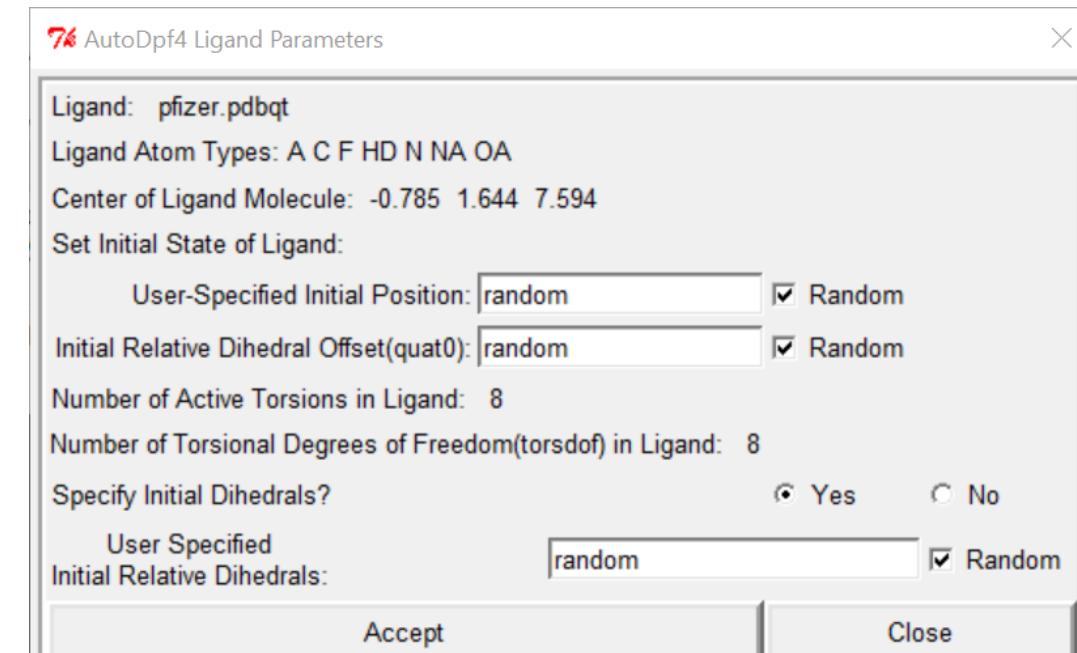
New window will appear as shown in picture .



Keep all options default and Accept it

Docking > Docking Parameters...

Accept default parameters. And then close it



Important Search Parameters

Genetic Algorithm & Lamarckian GA

Population size

ga_pop_size 300

Crossover rate

ga_crossover_rate 0.8

Mutation rate

ga_mutation_rate 0.02

Solis & Wets local search (LGA only)

sw_max_its 300

Termination criteria:

ga_num_evals 250000 # short

ga_num_evals 2500000 # medium

ga_num_evals 25000000 # long

ga_num_generations 27000

Parameters in GA

No. of Torsions in Ligand	ga_num_evals	ga_num_generations
0	25000 to 250000	27000
1-10	250000 to 25000000	27000
>10	>25000000	27000

For better results
ga_run 50 and **ga_pop_size 300**

Docking > Output > Lamackian GA(4.2)...

type-in 7v8h_receptor.dpf and save it.

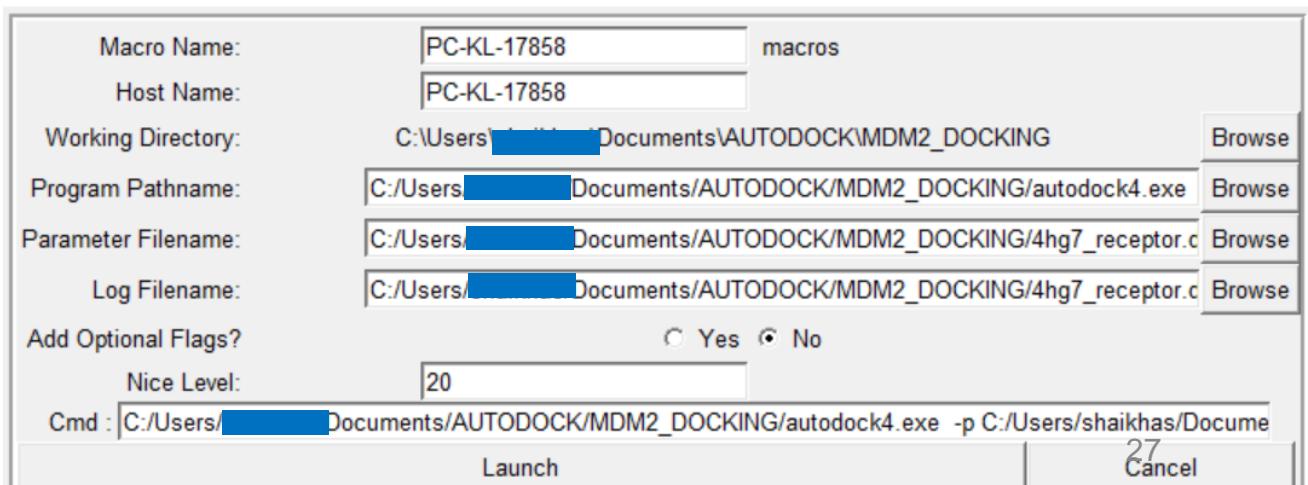
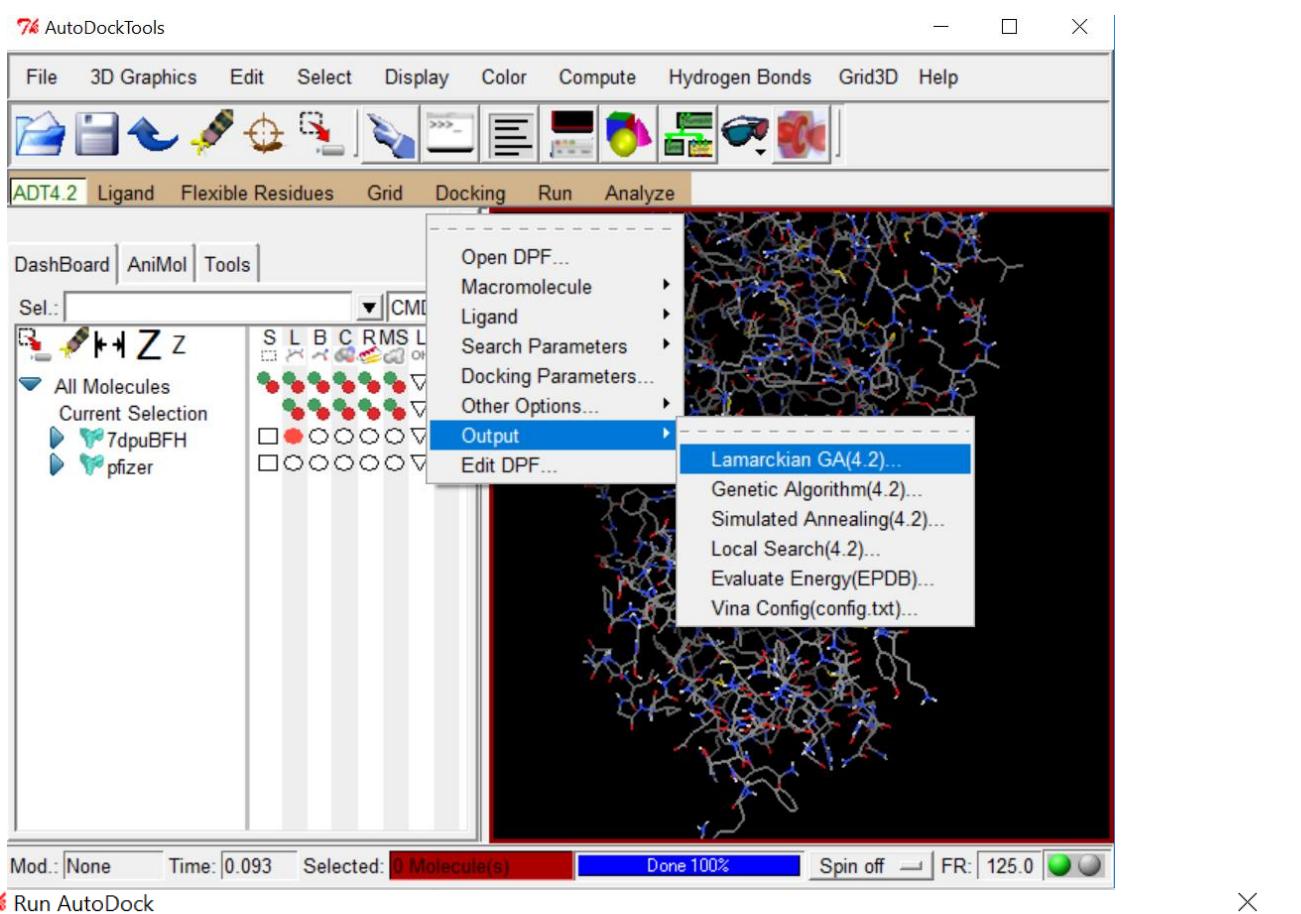
This will create docking parameter file.

Finally, run docking using following command

Run > Run AutoDock...

Using Browse window, select Program Filename
autodock4.exe and parameter file
7v8h_receptor.dpf

And then Launch the program. It will take some time to finish the job



Steps for analyzing docking results

Analyze > Dockings > Open...

Select 7dpu_receptor.dlg and click open.

A docking experiment is successful with respect to convergence if the majority of all available docking poses falls into the same cluster.

Analyze > Macromolecule > Open:

Protein will be loaded in graphical interface

Analyze > Conformations > Play, ranked by Energy

Button &: more information about the generated conformations

Button &

Show Info: Detailed information of values for the current docking pose:

Binding_energy: Binding energy between protein and ligand

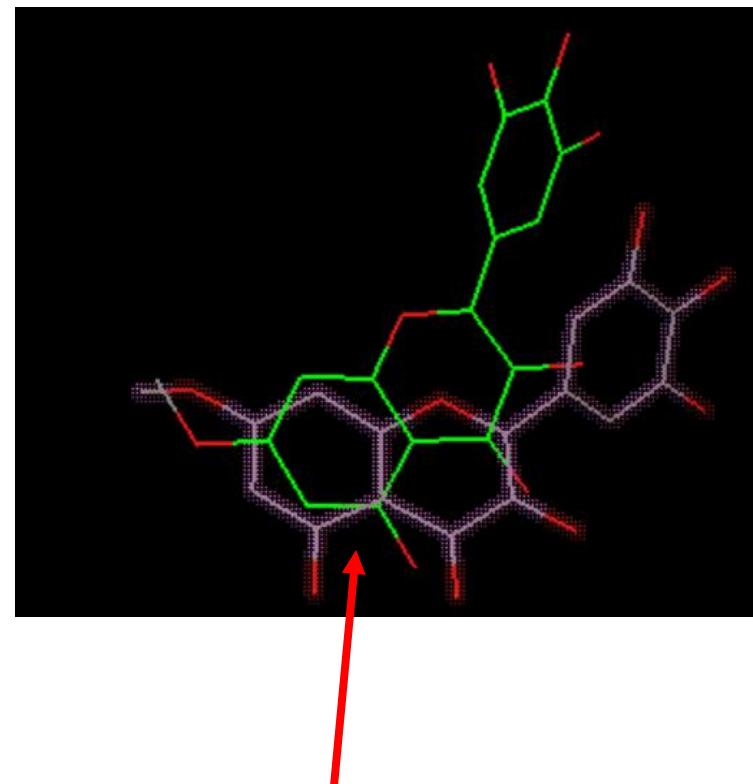
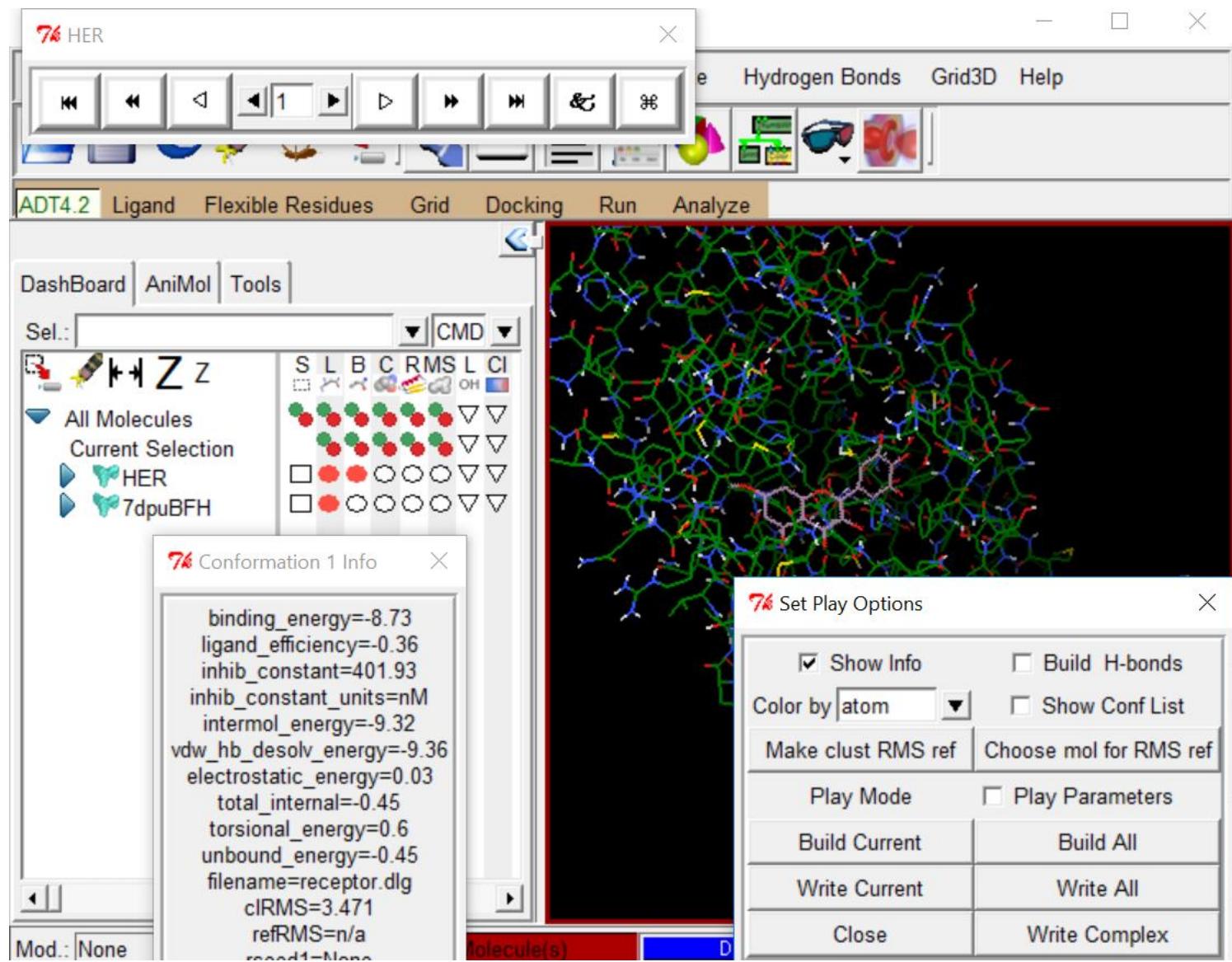
refRMS: RMSD value to the reference native binding pose

Inhibition_constant: Inhibition constant of the ligand to protein

Analyze > Conformations > Play, ranked by Energy

For representation purpose

Ligand > Open > HER.pdbqt



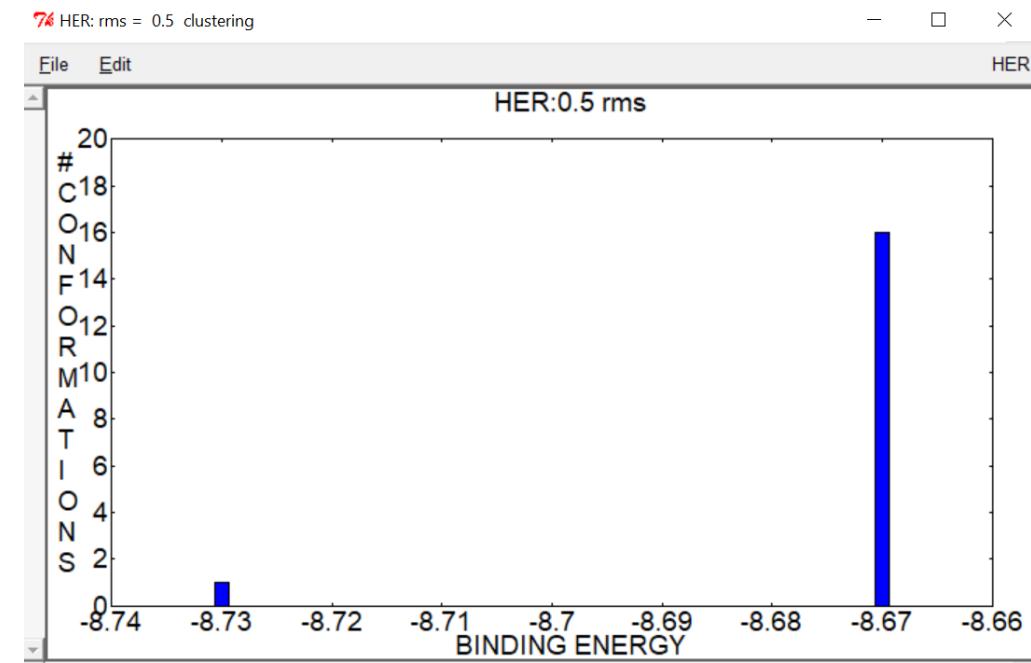
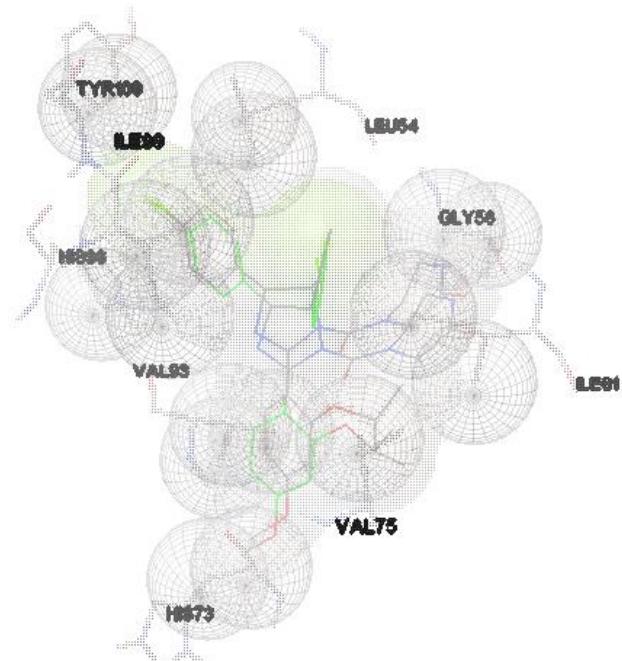
Comparison of crystal ligand conformation (green) and Conformation 1 (purple) of the docking results. RMSD is 3.47 Å. It indicates AutoDock can reproduce experimental binding structure. In present case, binding pose differs a bit with experimental pose.

Steps for analyzing docking results

Analyze > Clustering > Show...

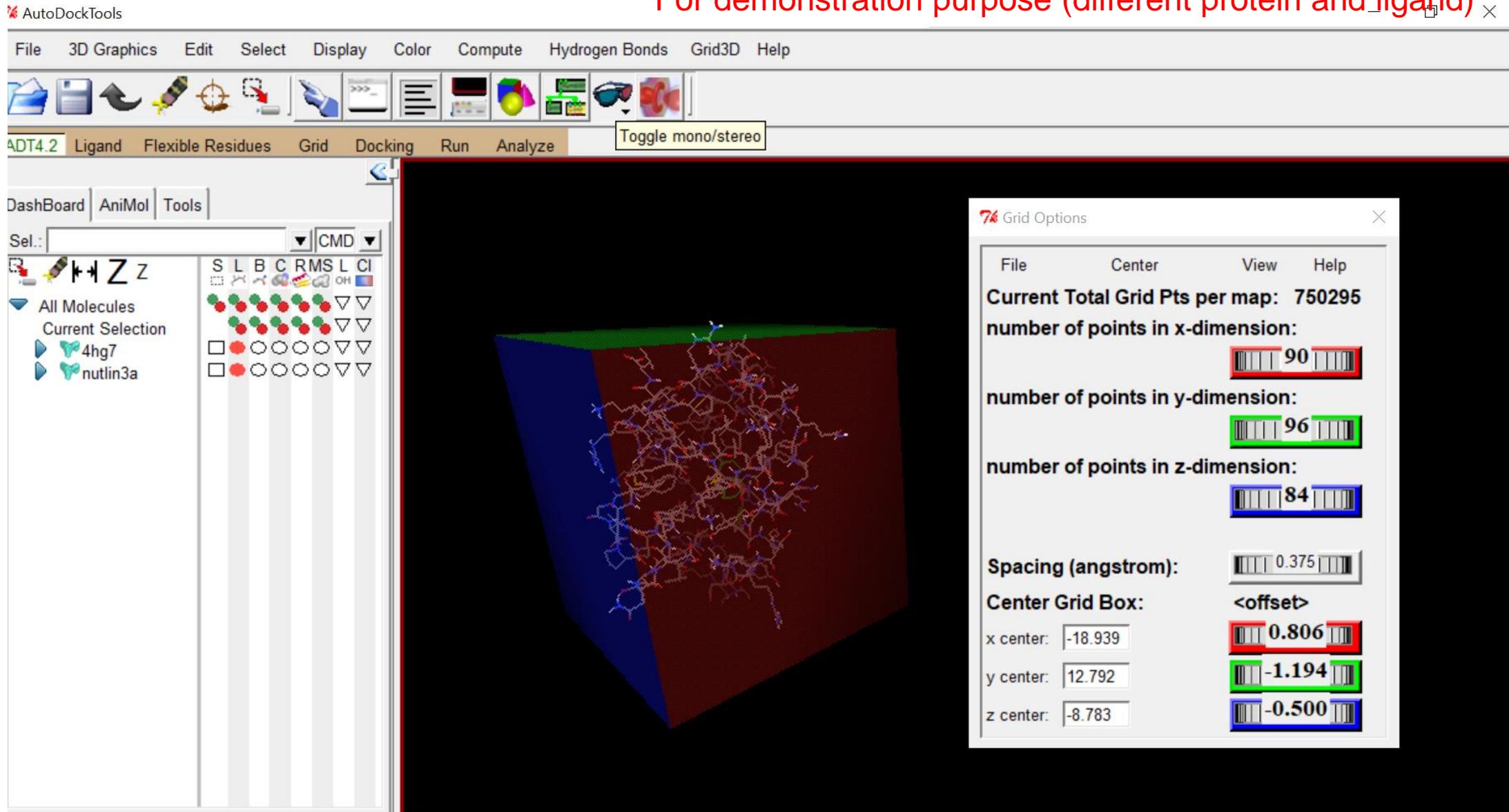
A docking experiment is successful with respect to convergence if the majority of all available docking poses falls into the same cluster.

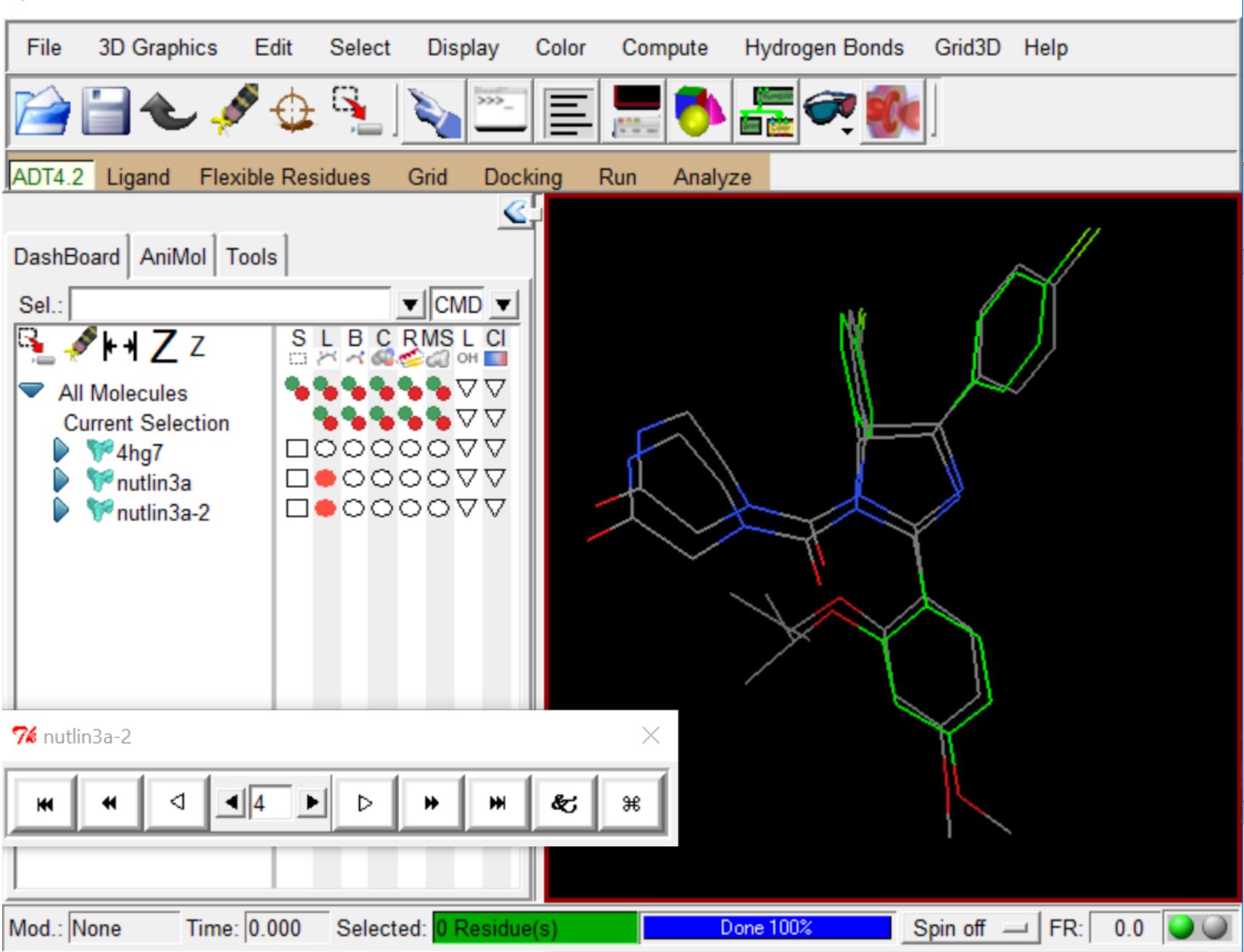
Analyze > Dockings > Show Interactions



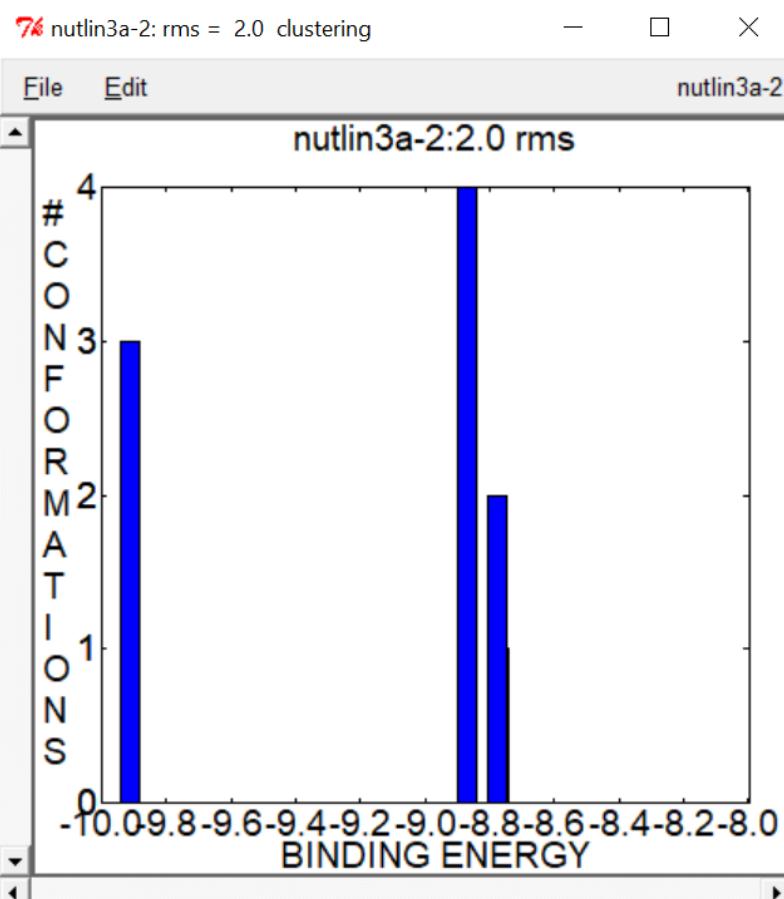
Docking using whole protein space:

For demonstration purpose (different protein and ligand)





For demonstration purpose



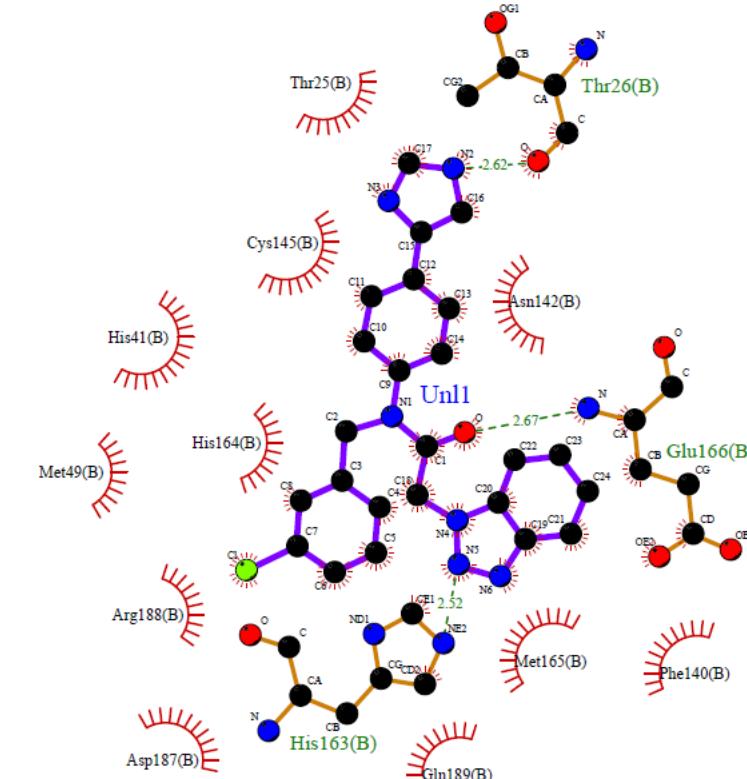
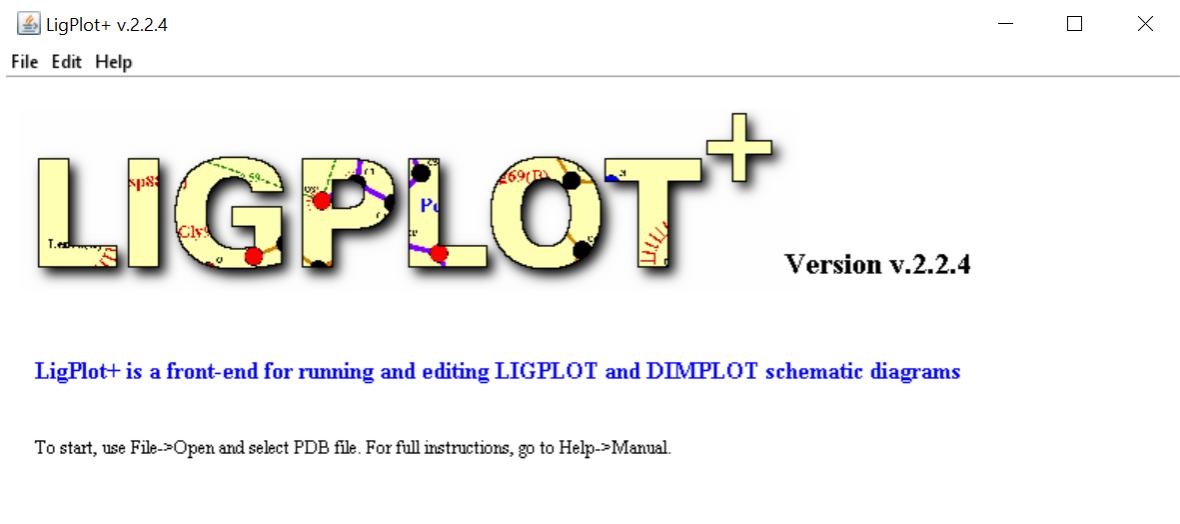
Conformation 4 is found to be similar as crystal structure

2D Interaction map using Lig Plot Plus

First you need to install Java Runtime Environment and then register for academic license for LigPlot⁺. Once you receive password, you can download LigPlot⁺ and install it.

<https://www.ebi.ac.uk/thornton-srv/software/LigPlus/install.html>

<https://www.java.com/en/download/>



7dpu_41_confl

Online Server to analyze protein-ligand complex

1) Protein-Ligand Interaction Profiler

<https://plip-tool.biotecltd.dresden.de/plip-web/plip/index>

Protein-Ligand Interaction Profiler

Welcome to the PLIP web tool!
Easy and fast identification of non-covalent interactions between biological macromolecules and their ligands.

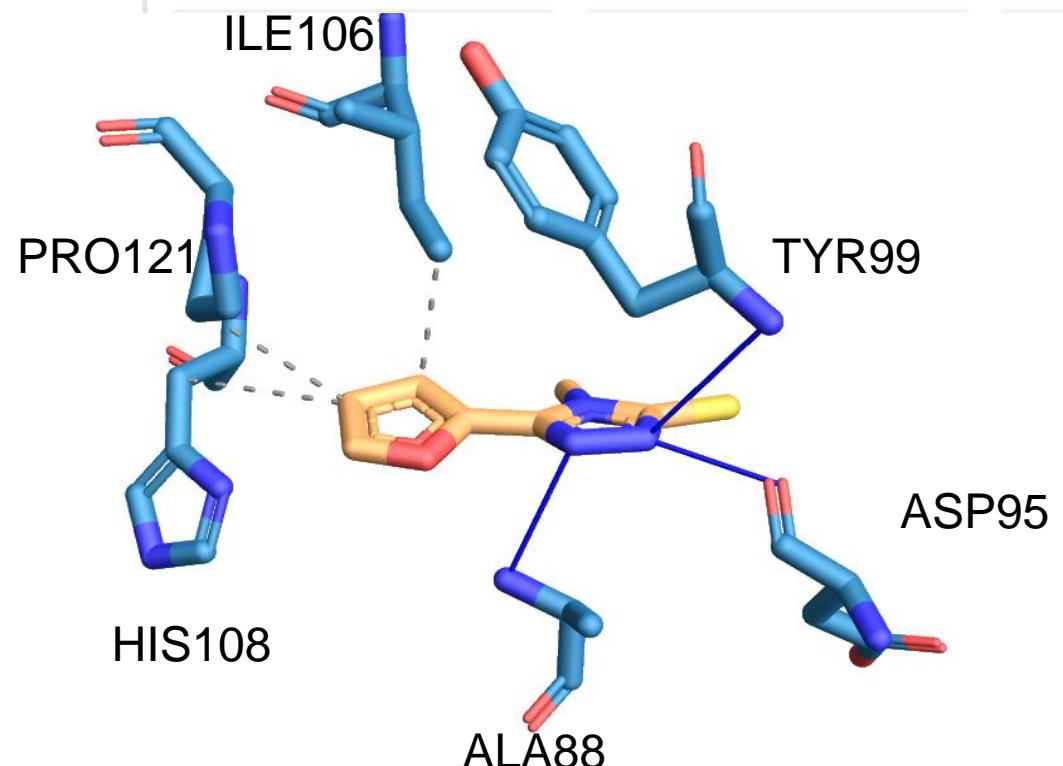
enter PDB ID (e.g. 1xdn)
Find PDB ID using our search tool

or

Drop PDB file here (max. 10 MB)
Choose file...

Advanced Options

ANALYZE



Hydrophobic Interaction

ILE106, PRO121 and HIS108

H-bond Interaction

ALA88, ASP95, TYR99

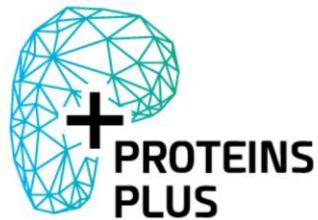
2) PROTEINS PLUS Server



Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG

ZBH - Center for Bioinformatics

Back



Waiting for www.google...

PDB-Code or search term:

① Upload Protein (PDB format): No file chosen
② Upload Ligand (SDF format): No file chosen

DoGSiteScorer

DoGSiteScorer is a grid-based method which uses a Difference of Gaussian filter to detect potential binding pockets - solely based on the 3D structure of the protein - and splits them into subpockets. [+](#)

Result

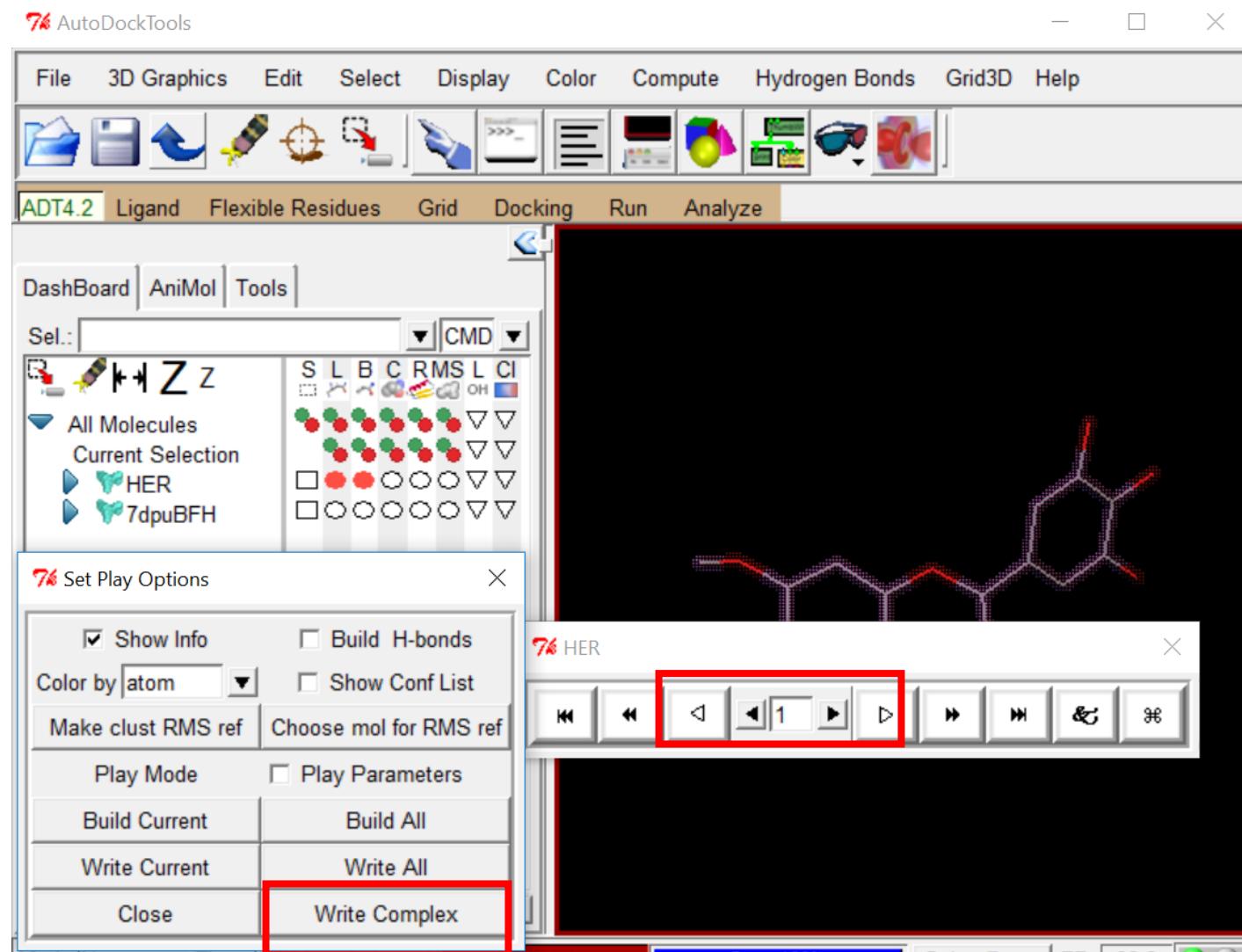
Click on the plus to see your selected parameters: [+](#)

Show 25 entries

Search:

		Name ↑	Volume Å ³ ↑	Surface Å ² ↑	Drug Score ↑	Simple Score ↑	Additional information ↑
◀		P_0	2390.34	2985.89	0.81	0.65	Click here to show/hide
◀		P_1	627.9	891.39	0.74	0.42	Click here to show/hide

Saving the docking results



Choose the conformation 1 of the ligand and save the protein-ligand complex using Write Complex.
Name the file as 7dpu_HER_conf1.pdbqt

Now, delete the protein and ligand from AutoDockTools.

Load the new complex

File > Read Molecule > 7dpu_HER_conf1.pdbqt

And then save it as a PDB file

File > Save > Write PDB

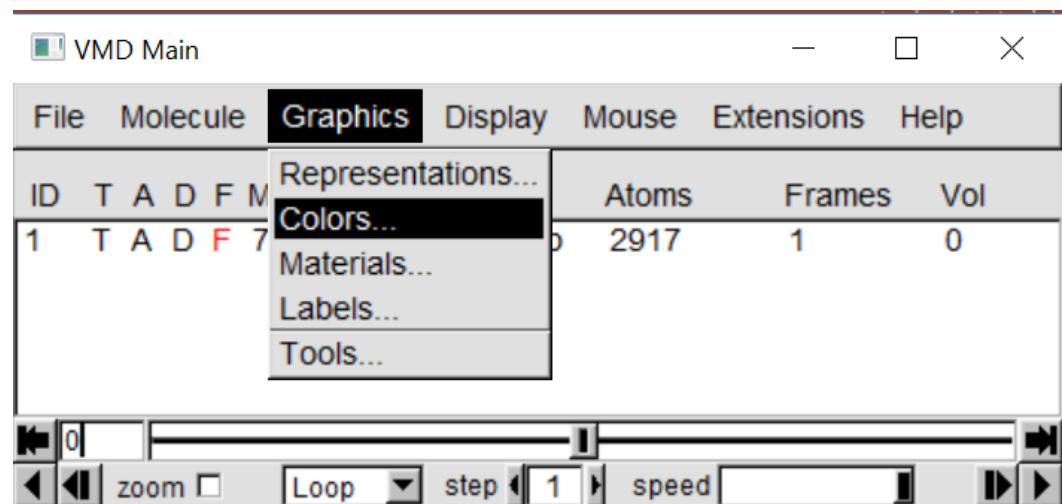
Save it as a 7dpu_HER_conf1.pdb

Analysis Using VMD

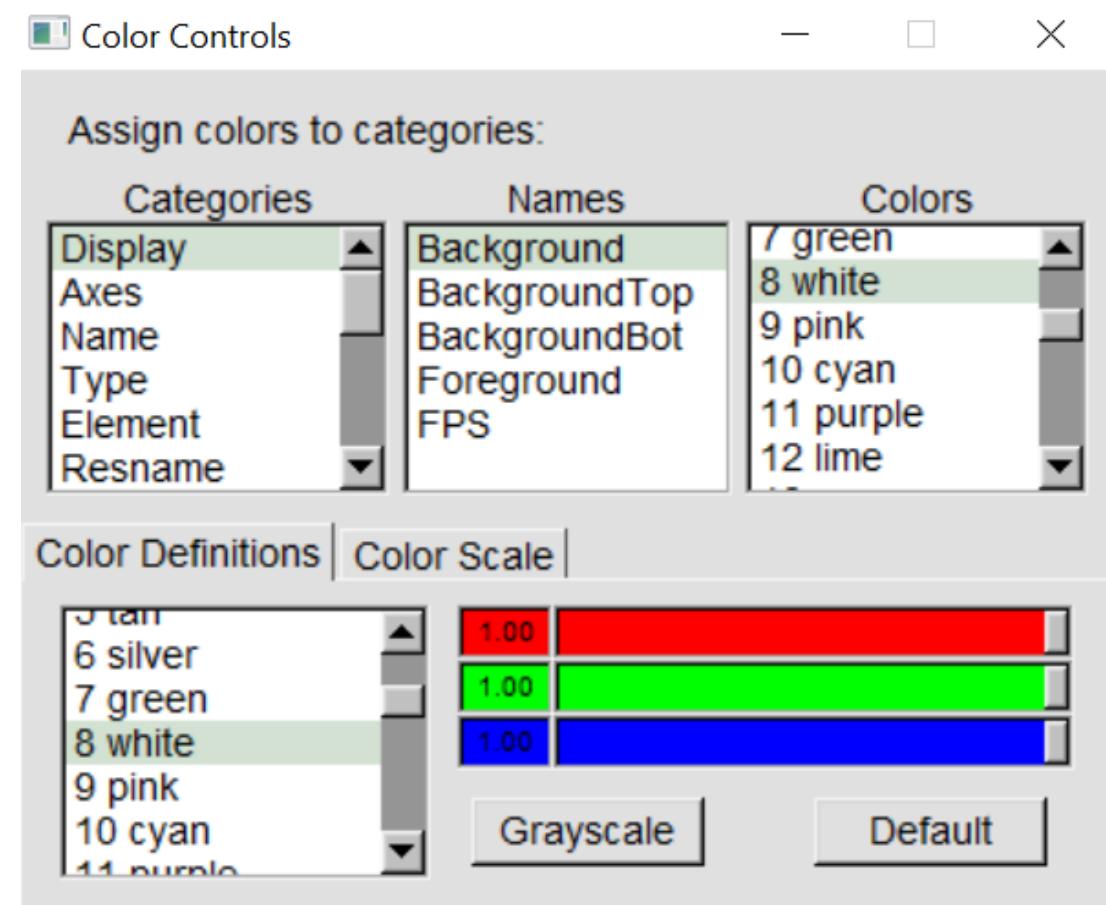
After opening VMD program, load the pdb file of our docked ligand-protein complex

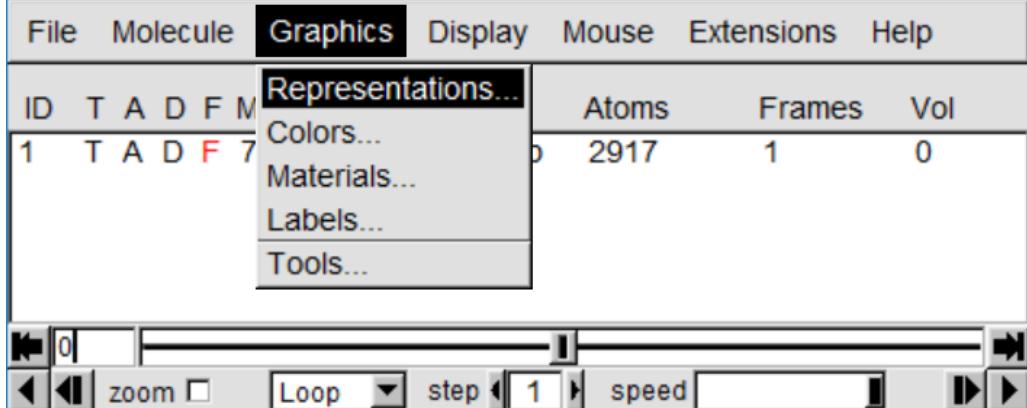
File > New Molecule > 7dpu_HER_conf1.pdb

Graphics > Colors...



Color Controls dialogue will appear. Change the background color to white. Then close the Color Controls window



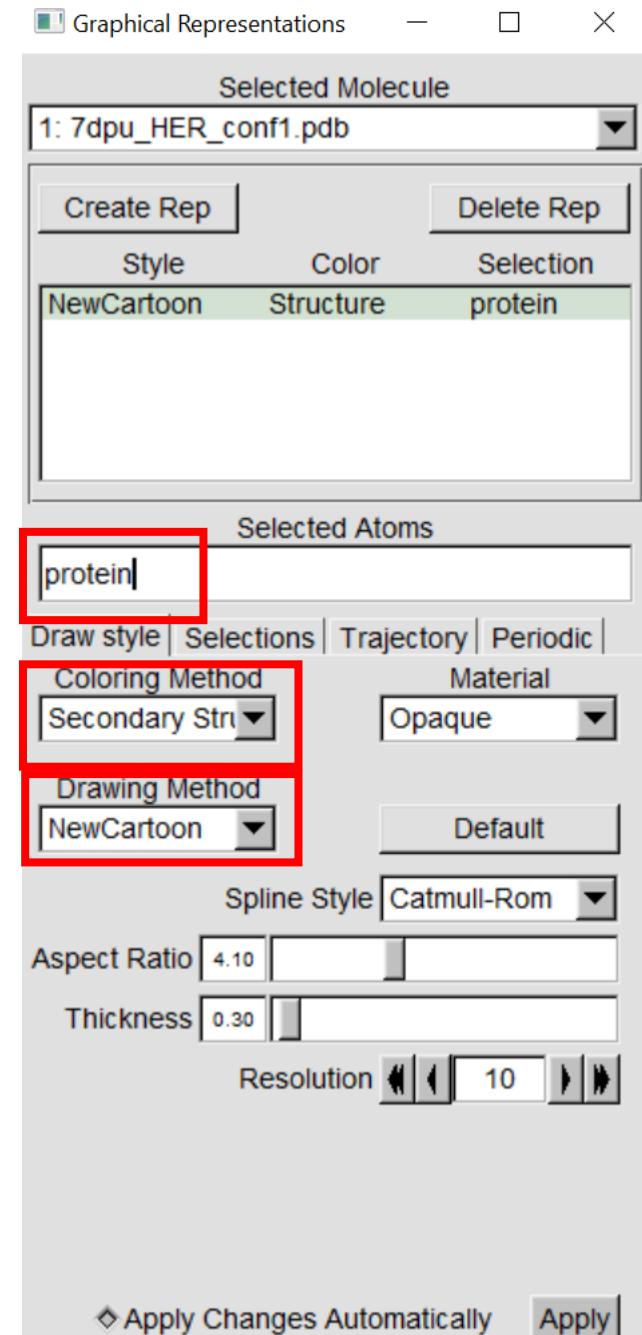
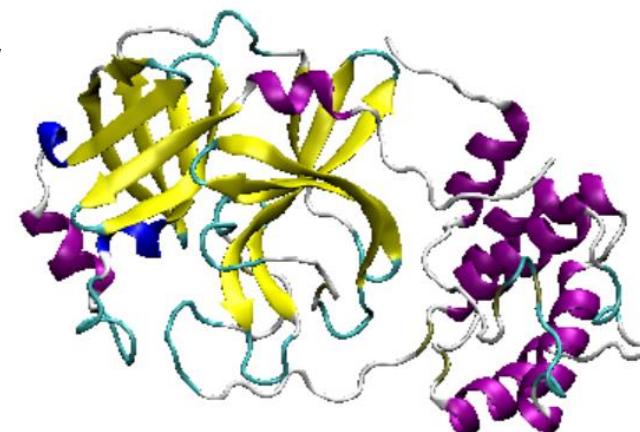


Graphical Representation window will open

In Selected Atoms, type **protein** and hit the enter key.

Change the Drawing Method **NewCartoon** and Coloring Method **Secondary Structure**

Protein will appear as shown below



Analyzing the active site

Check the 3 character name of ligand in pdb file (open it in WordPad and check the name)

ATOM	2889	OE1	GLN	B	306	3.323	27.266	22.236	1.00	53.42	7dpu	O
ATOM	2890	NE2	GLN	B	306	5.393	28.012	22.689	1.00	60.14	7dpu	N
ATOM	2891	HN	GLN	B	306	5.075	22.304	23.118	1.00	35.10	7dpu	H
ATOM	2892	1HE2	GLN	B	306	6.107	27.928	23.161	1.00	60.14	7dpu	H
ATOM	2893	2HE2	GLN	B	306	5.343	28.628	22.090	1.00	60.14	7dpu	H
TER	2894		GLN	B	306							
HETATM	1	C7	HER	B	401	-24.051	9.456	14.479	1.00	17.83	7dpu	C
HETATM	2	C8	HER	B	401	-24.736	10.115	12.287	1.00	22.31	7dpu	C
HETATM	3	C10	HER	B	401	-24.670	7.725	12.862	1.00	20.21	7dpu	C

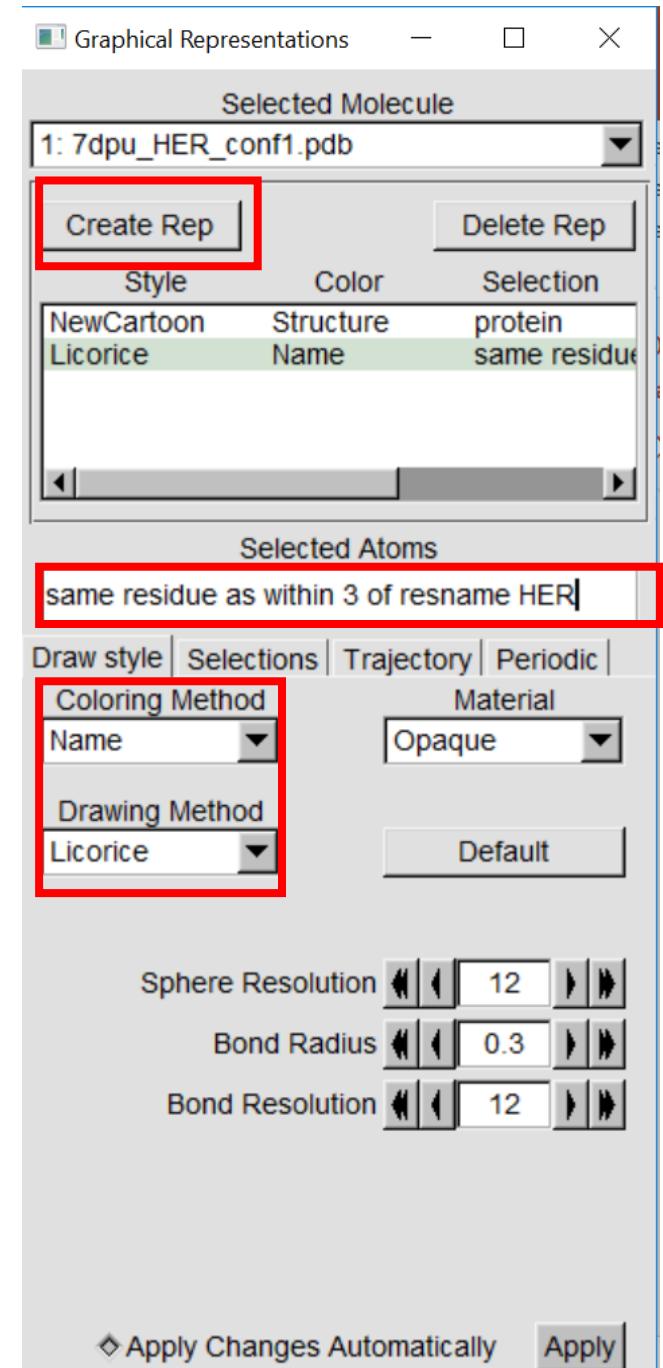
In Graphical Representations window, click on Create Rep.

In Selected Atoms type **same residue as within 3 of resname HER**

Coloring Method : Name

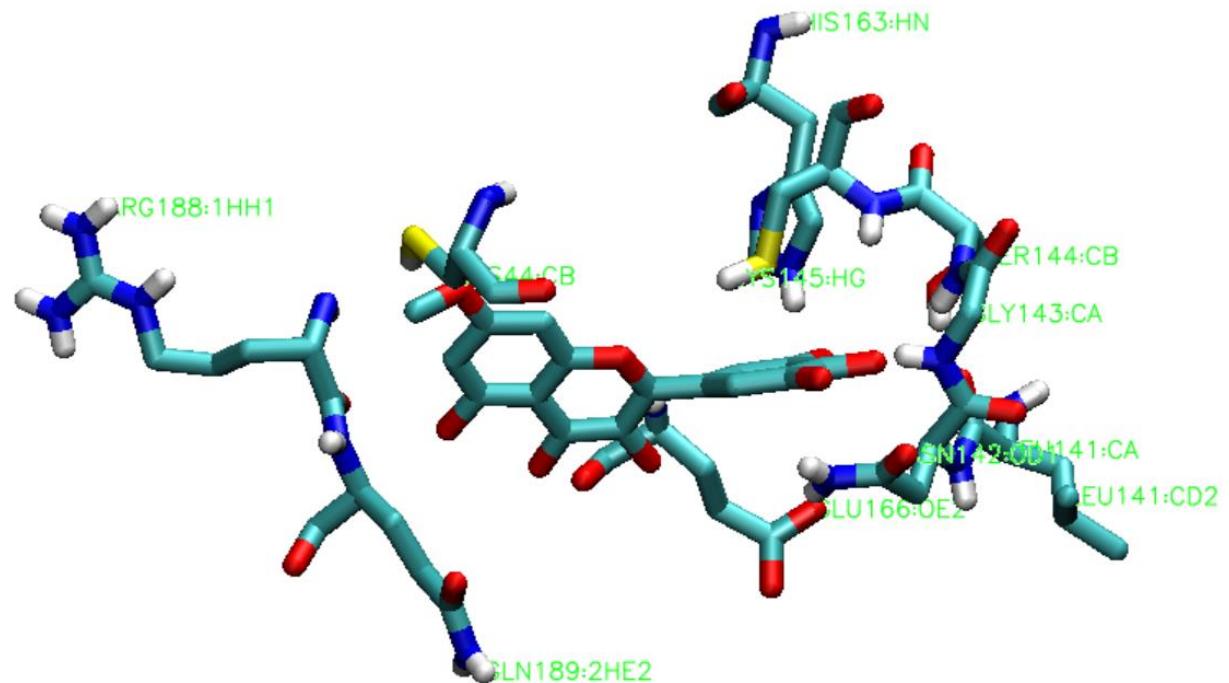
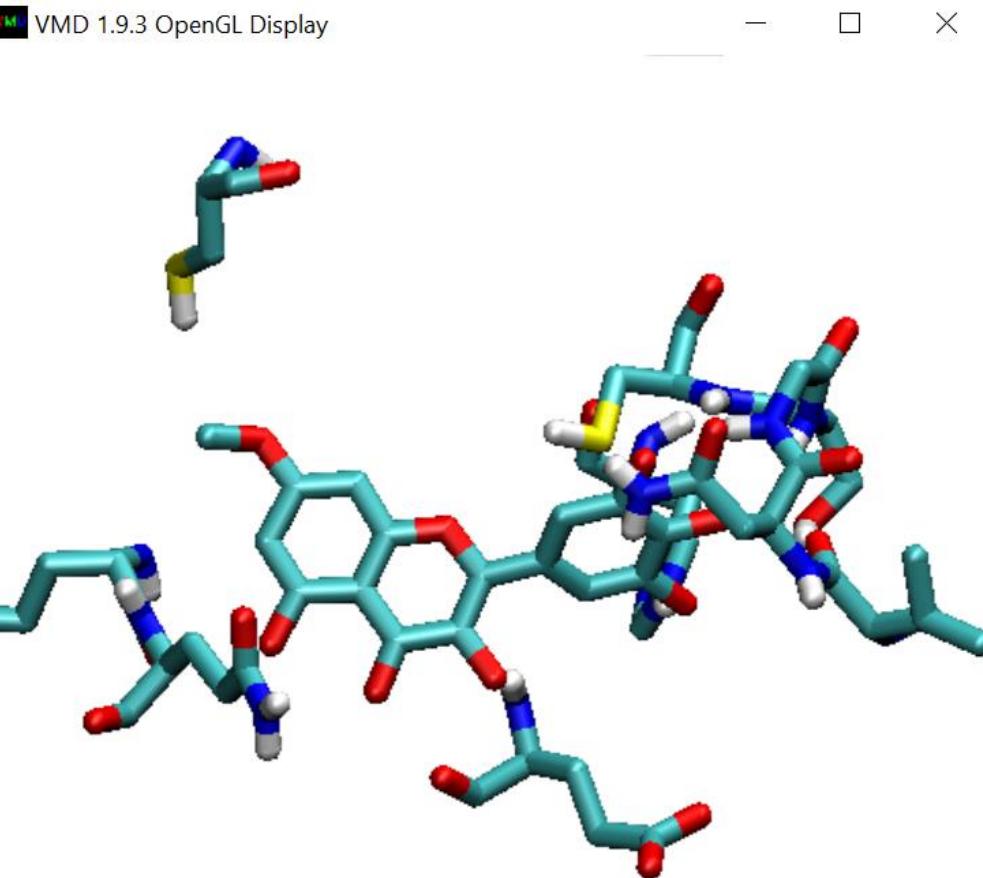
Drawing Method: Licorice

Hide the protein by double clicking on NewCartoon



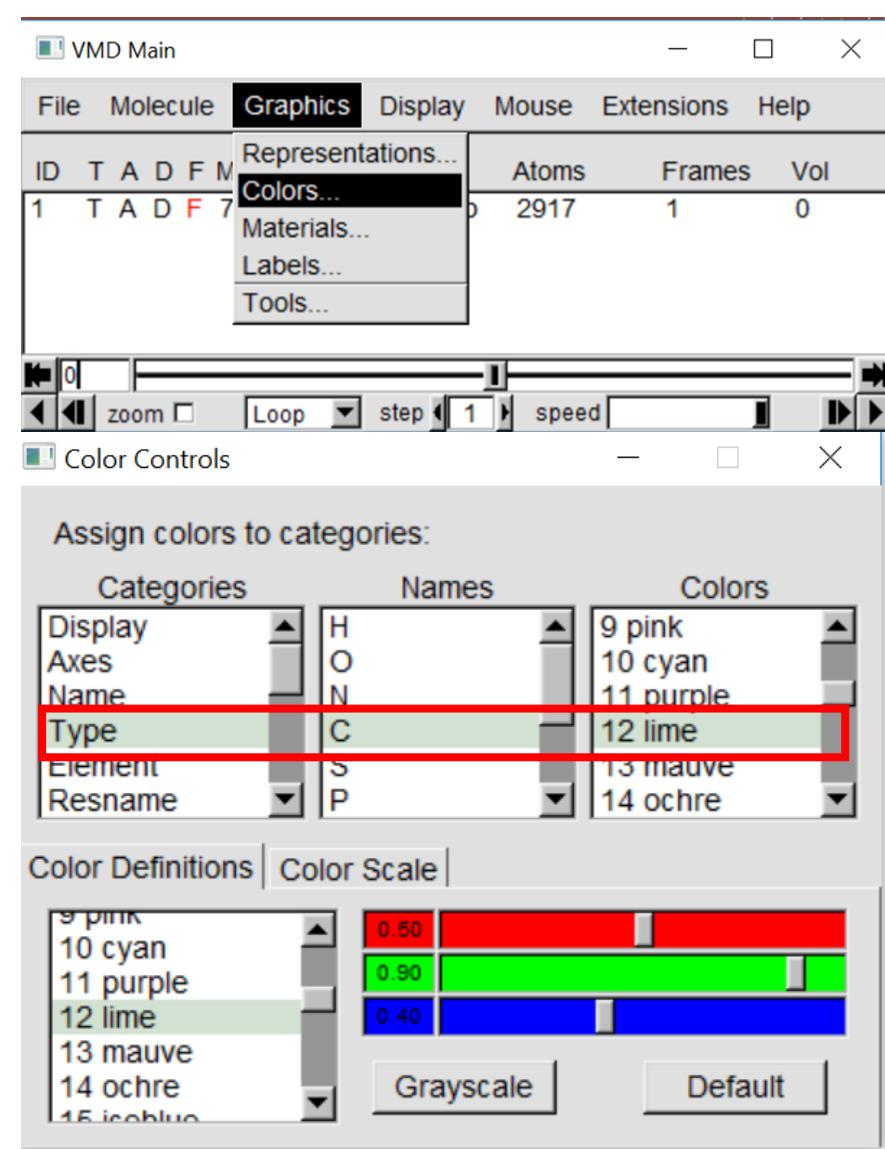
Selecting active site residues

VMD 1.9.3 OpenGL Display



Active site will look like as shown above. Now to find out active site residues, press 1 and click on residues in graphical window.

CYS44, LEU141, GLY143, SER144, CYS145, ASN142, HIS163, GLU166, ARG188, GLN189.
These are the residues within 3 Å of the ligand.



Now, we will change the color of C in the ligand as lime color.

Graphics > Colors.. > Type C > lime

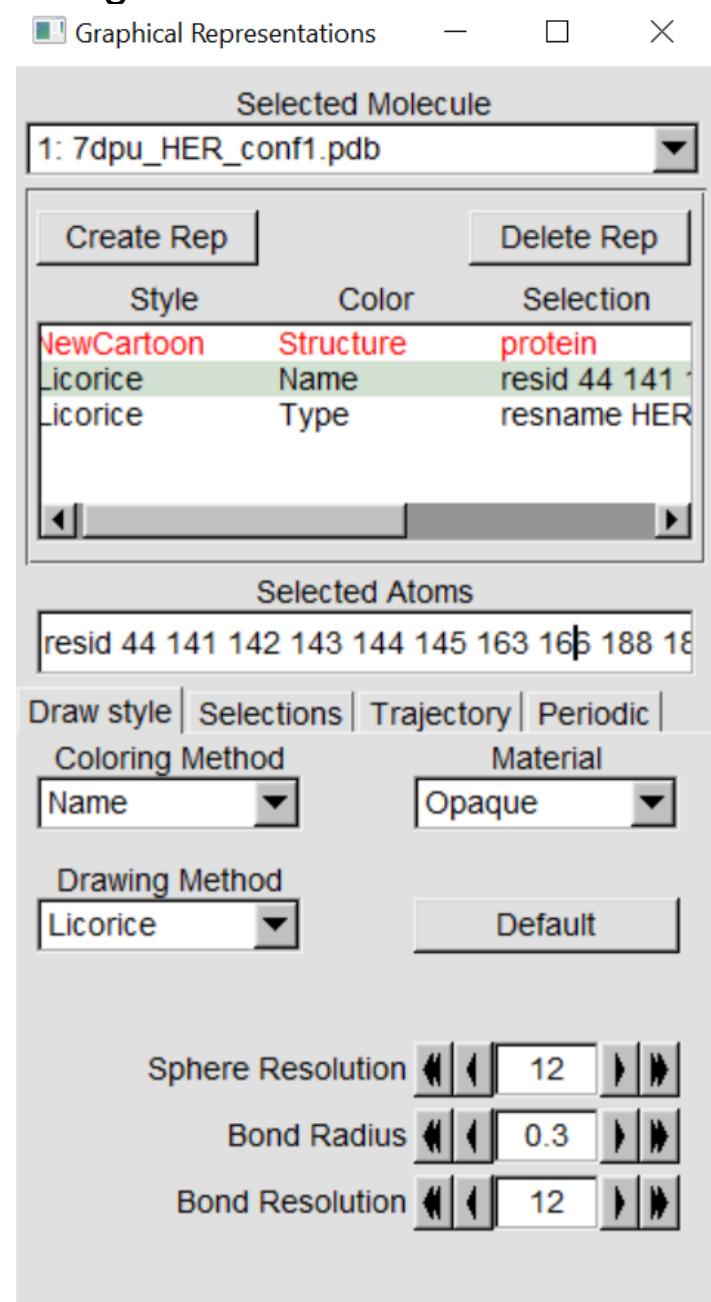
Graphics > Representations...

Create Rep and type the residue id's in it.

resid 44 141 142 143 144 145 163
166 188 189

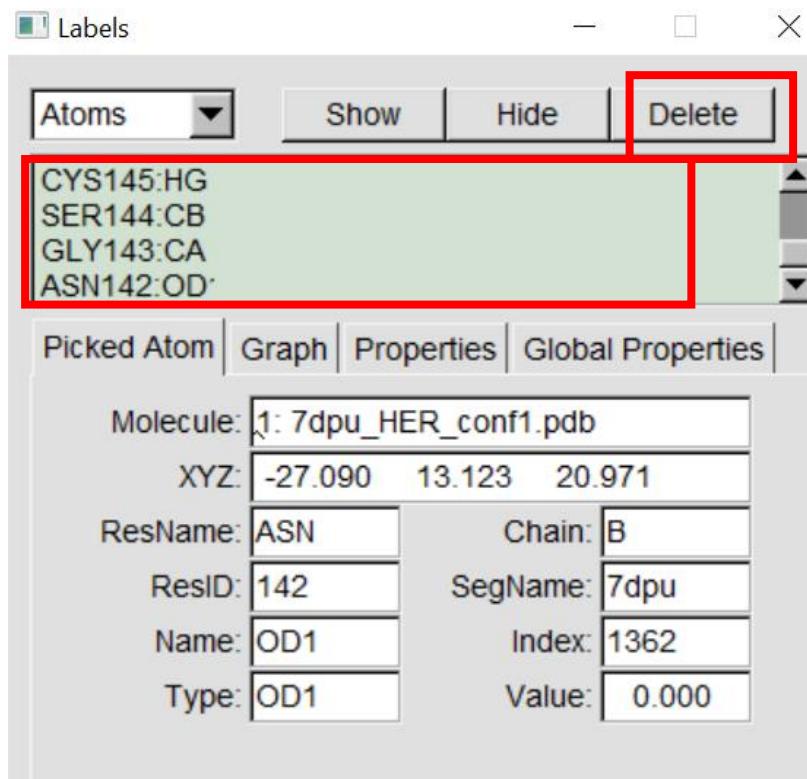
Rename “same residue as within 3 of resname HER” as “resname HER”

And change coloring method and drawing method it as shown in adjacent window



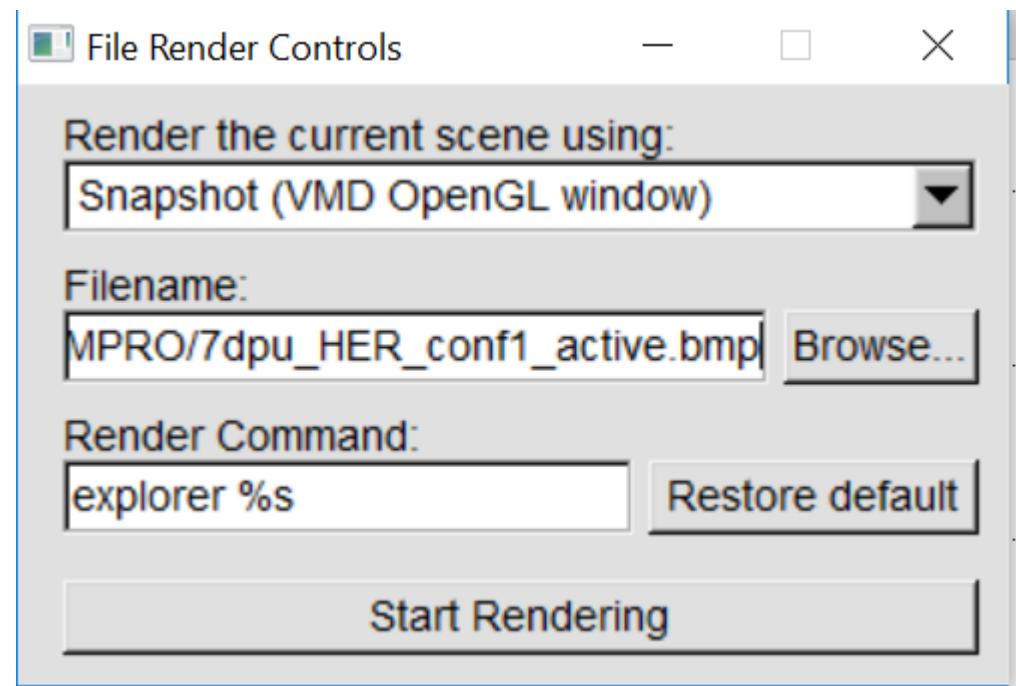
Now delete the labels

Graphics > Labels... > Select all atoms by clicking atom and while pressing Ctrl, select all the atoms. Once selected delete it



Then, orient the molecule in such a that interactions such as hydrogen bond with ligand is clear.
The we can save the image.

File > Render



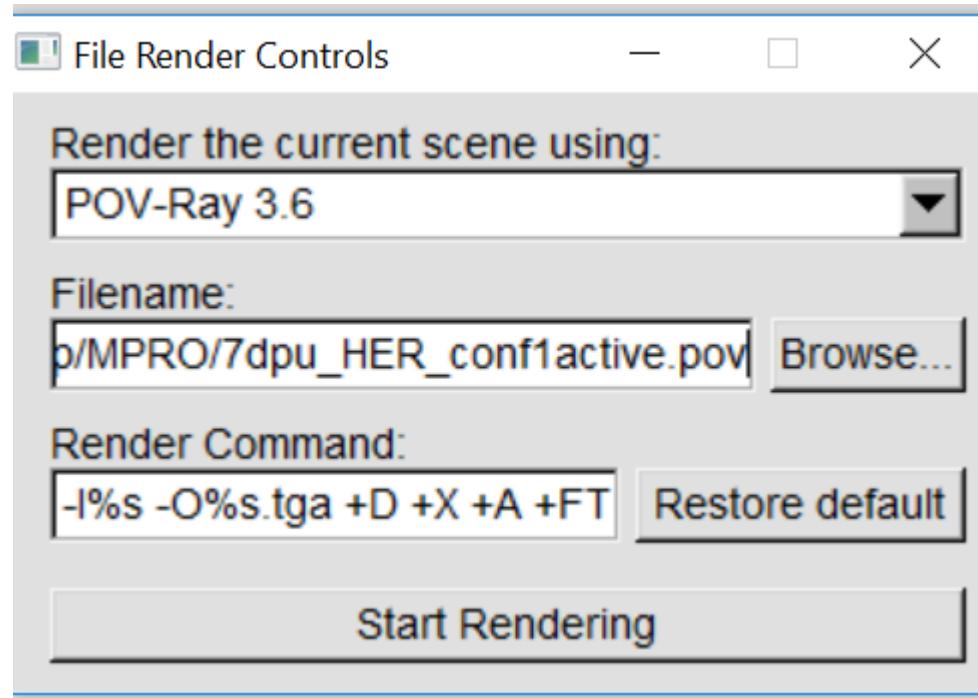
Browse to desired location where you want to save bmp file.
And then click Start Rendering

In order to create high resolution image, we can use POV Ray program. You can download Pov Ray program from following link

<https://www.povray.org/download/>

File > Render

Setting as shown below

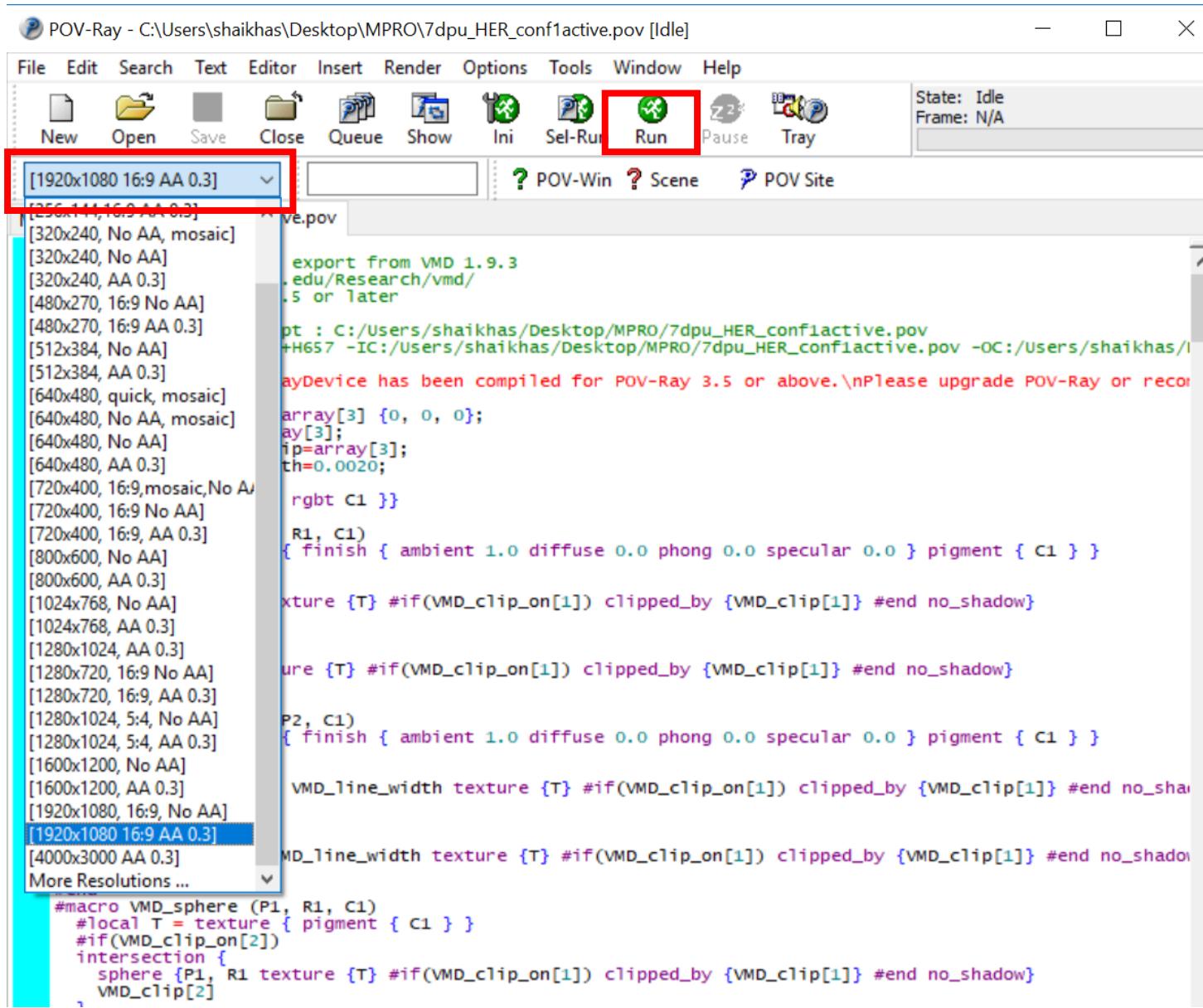


And click Start Rendering. Save the file at desired location as 7dpu_HER_conf1active.pov

Now, open Pov Ray

PovRay : File > Open File > 7dpu_HER_conf1active.pov

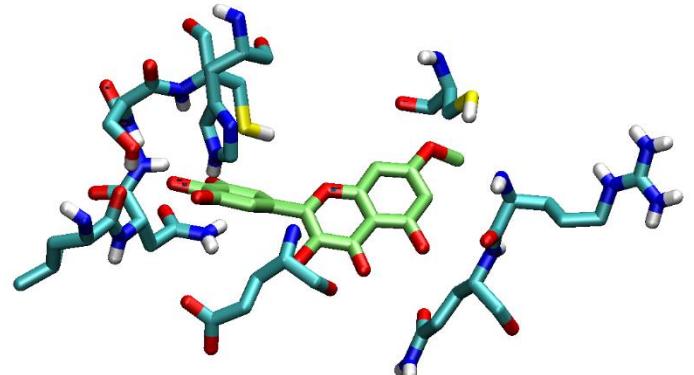
Select the resolution in Resolution window. For example, in present case we selected [1920 x 1080 16:9 AA 0.3]



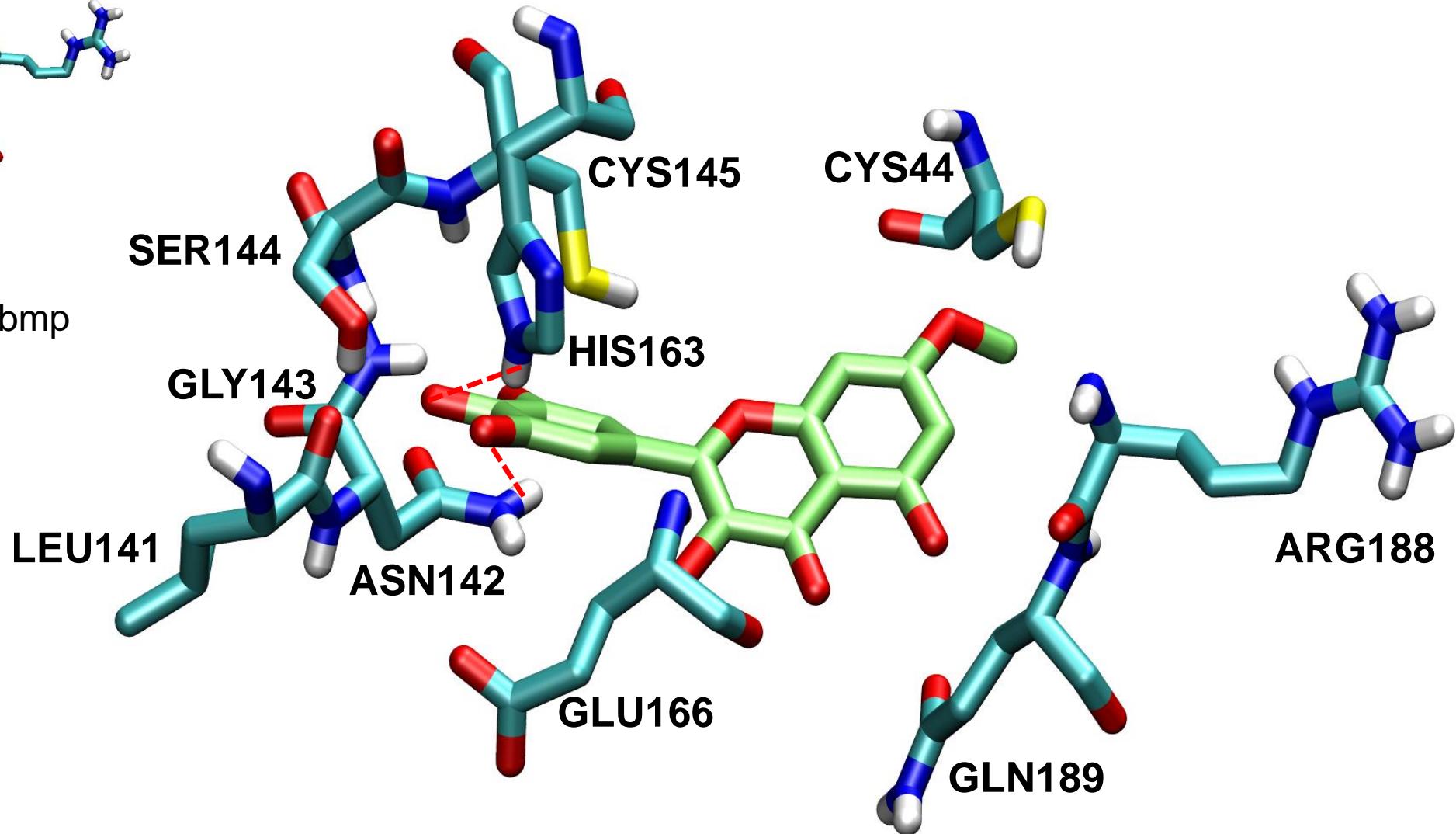
And then click Run.

It will create 7dpu_HER_conf1active.png file.

This file, we can insert it in power point.

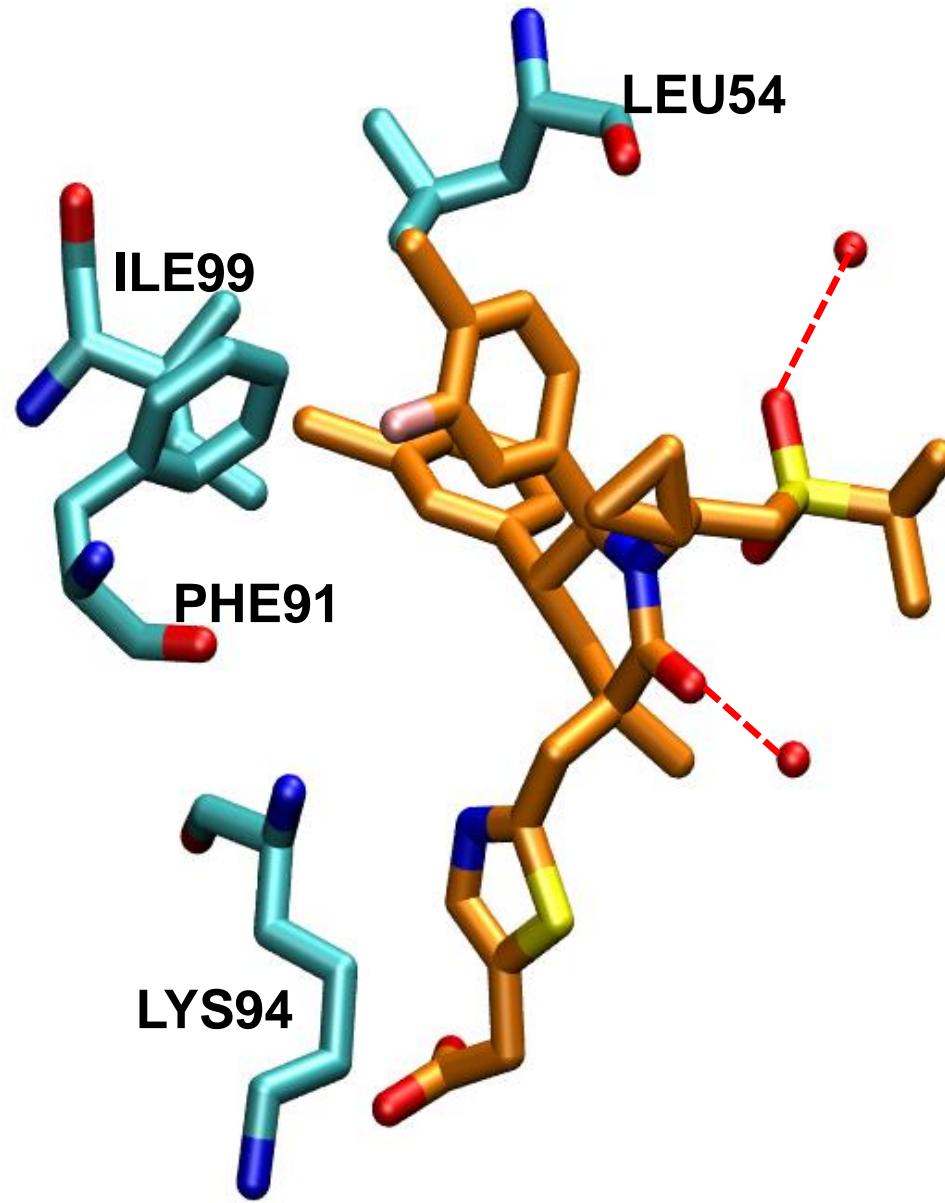
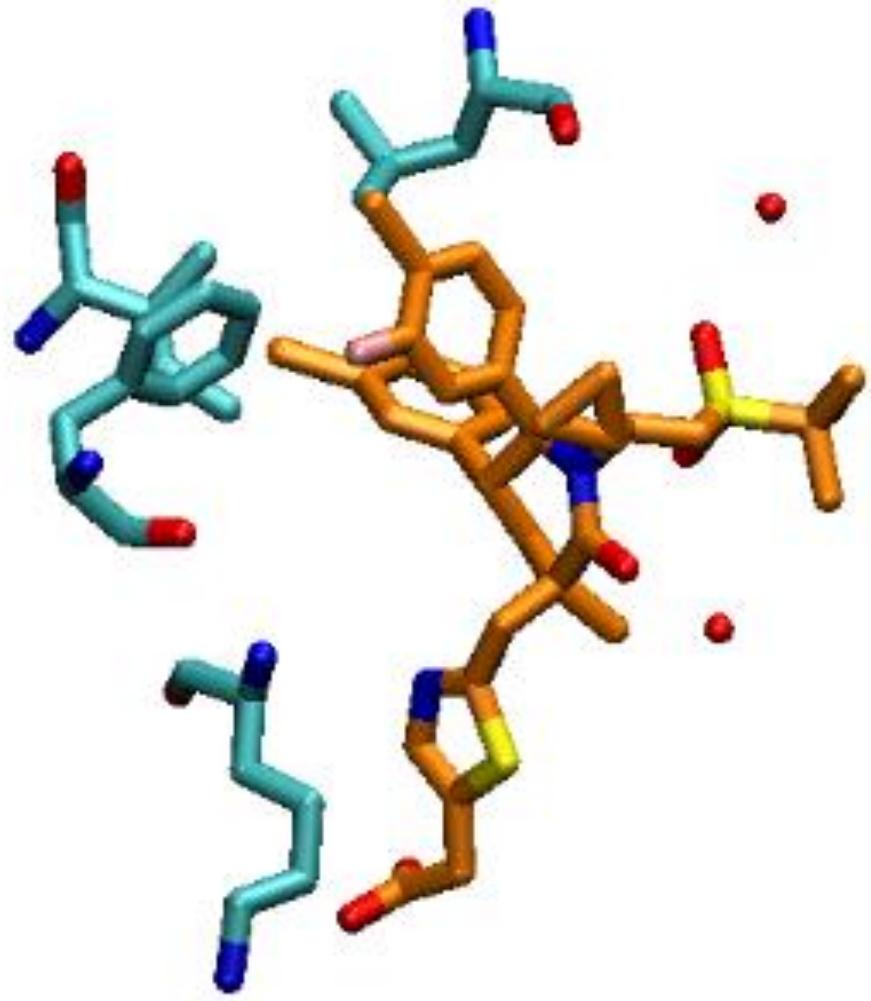


7dpu_HER_conf1_active.bmp



7dpu_HER_conf1active.png

Some other example



Kollman Charges

The Kollman charges are template values for each amino acid that were derived from the corresponding electrostatic potential using quantum mechanics. It is also good practice to use Kollman charges for protein and gasteiger charges for ligands.

Gasteiger Charges (or Marsili Charges)

The Gasteiger(-Marsili) charges are determined on basis of electronegativity equilibration, which can be computed by AutoDockTools for the given molecular system i.e. your ligand or co-factors in case you don't have any partial charges at hand. It assumes an overall net neutral state for the respective molecular system. Thus, check the protonation states of the amino acids and the ligand.

- iterative: redistribution of charges based on electronegativity

$$Q_i = \sum q_i^{<\alpha>}$$

$$q_i^{<\alpha>} = \left(\frac{1}{2} \right)^{\alpha} \sum_{v \in i} \left[\sum_{\mu \in j} \frac{\chi_{j,\mu}^{<\alpha>} - \chi_v^{<\alpha>}}{\chi_v^+} + \sum_{k \in k} \frac{\chi_{k,i}^{<\alpha>} - \chi_v^{<\alpha>}}{\chi_{k,i}^+} \right]$$

contribution to the atomic charge on the
a-th step of iteration of charge; j are
neighbors with higher electroneg.; k are
less-electronegative neighbors

$$\chi_{iv} = a_{iv} + b_{iv} Q_i + c_{iv} Q_i^2$$

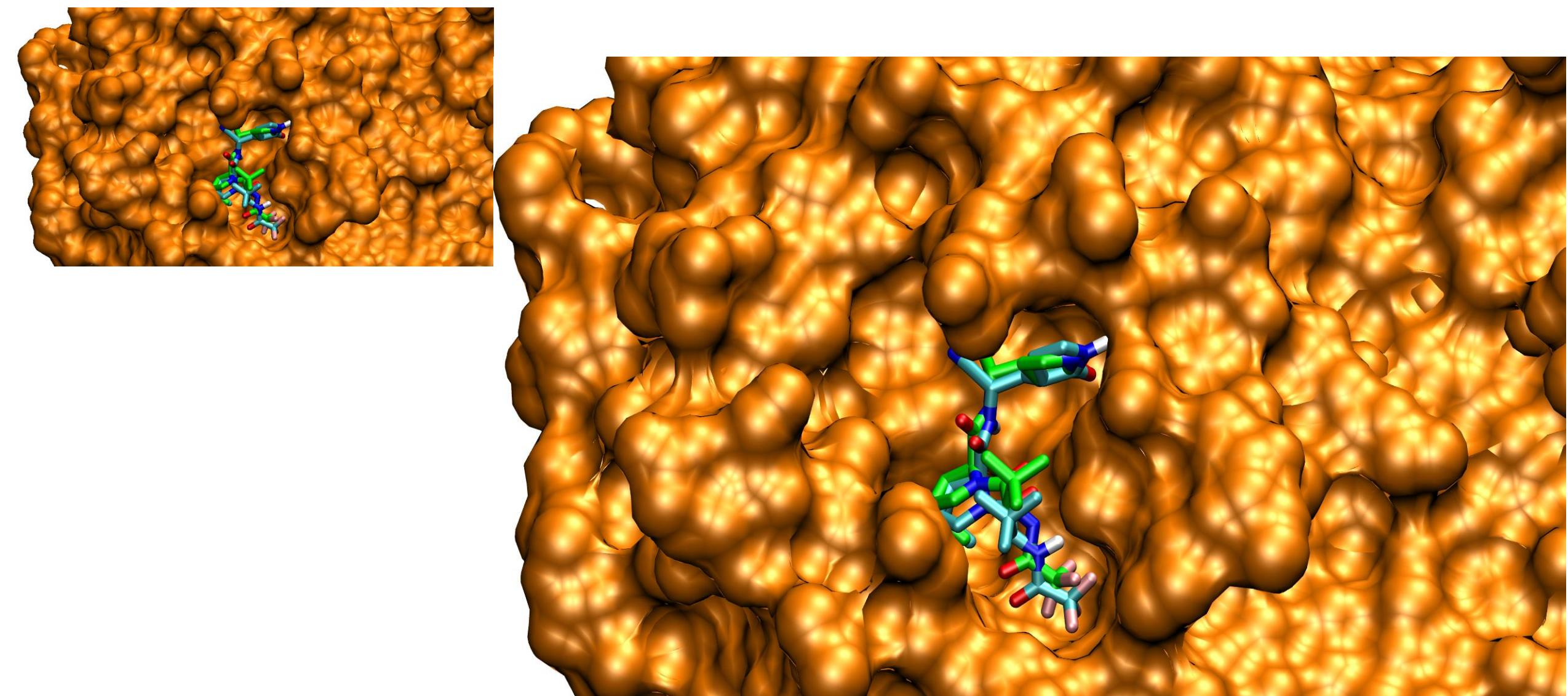
electronegativity of v'th orbital on atom i

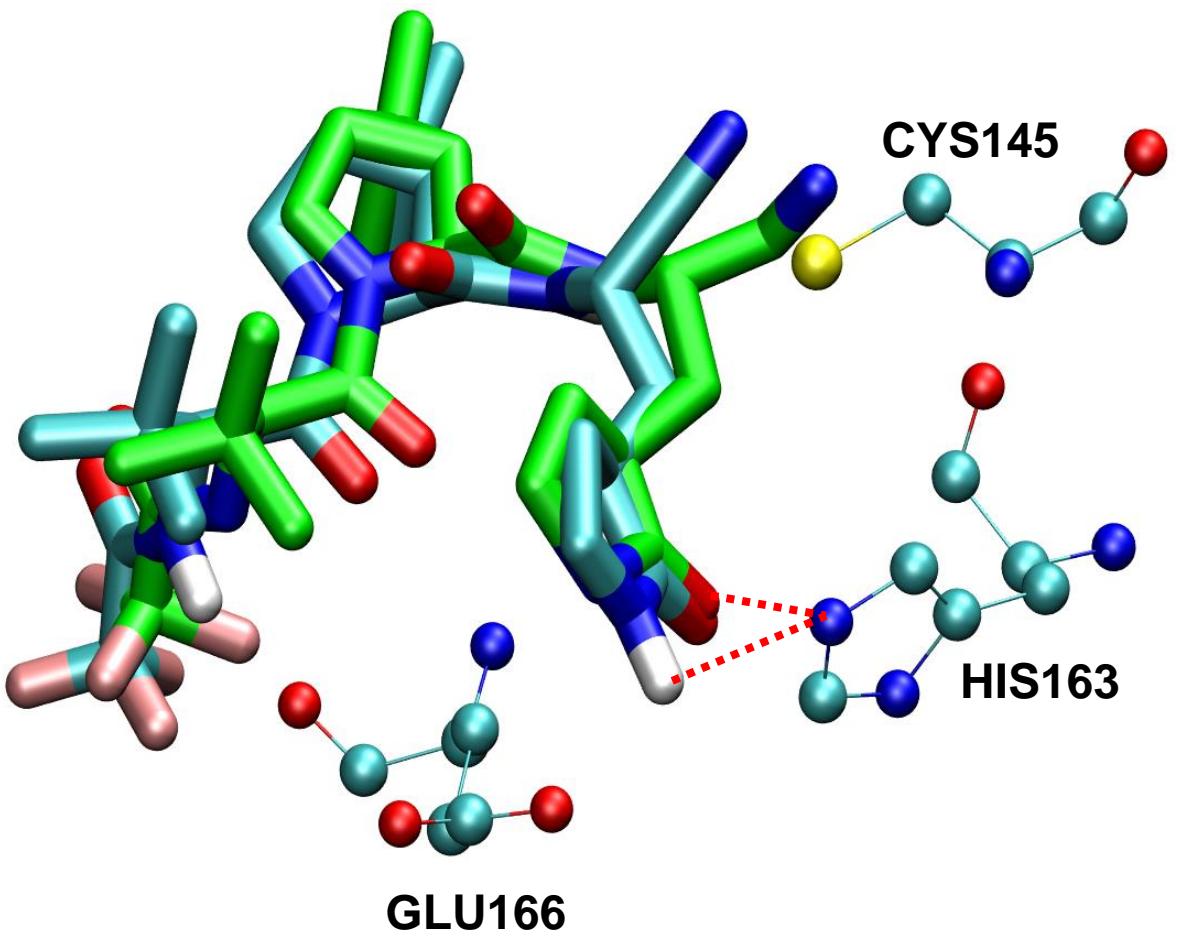
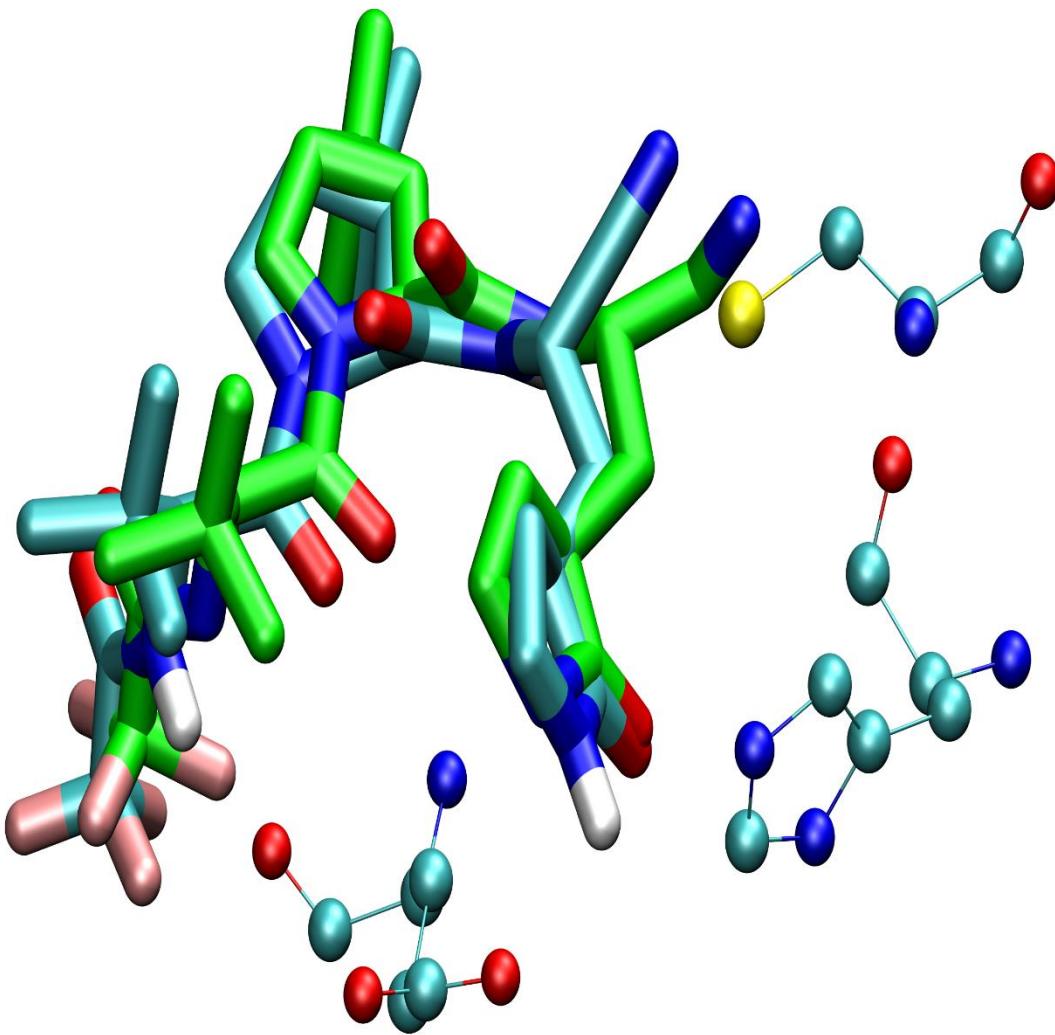
$$a_{iv} = \frac{I_{iv}^0 + E_{iv}^0}{2}$$

$$b_{iv} = \frac{I_{iv}^0 + E_{iv}^+ - E_{iv}^0}{4}$$

$$b_{iv} = \frac{I_{iv}^+ - I_{iv}^0 + E_{iv}^+ - E_{iv}^0}{4}$$

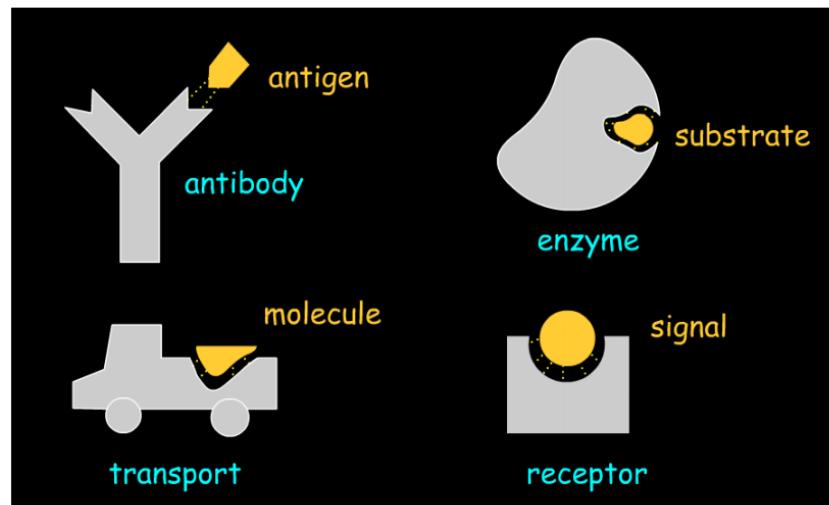
I: ionization potentials, E: electron affinities, 0=neutral atoms, + =positive ions





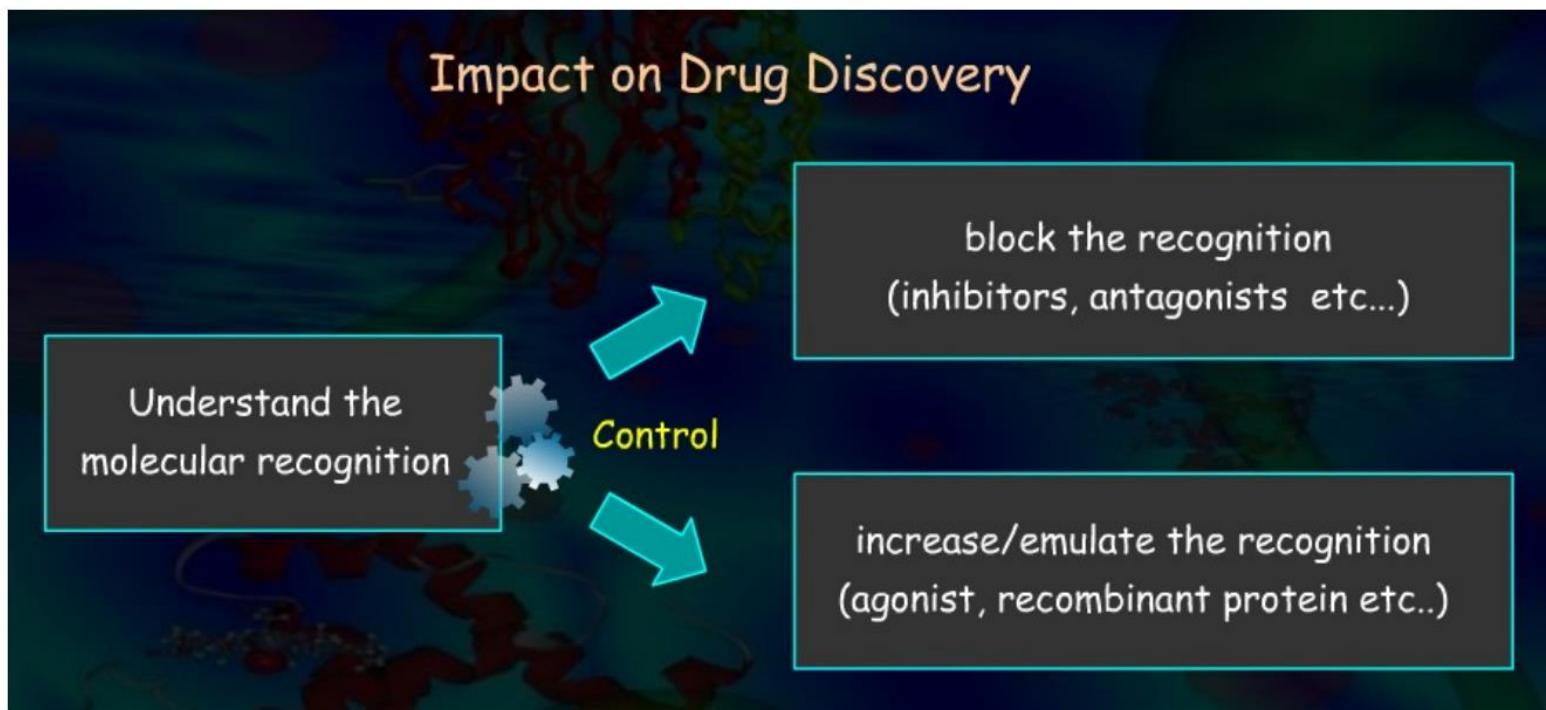
Molecular Recognition, Molecular Docking

- Molecular recognition is the ability of biomolecules to recognize other biomolecules and selectively interact with them in order to promote fundamental biological events such as transcription, translation, signal transduction, transport, regulation, enzymatic catalysis, viral and bacterial infection and immune response.
- Molecular docking is the process that involves placing molecules in appropriate configurations to interact with a receptor. Molecular docking is a natural process which occurs within seconds in a cell.
- In molecular modeling the term “molecular docking” refers to the study of how two or more molecular structures fit together



Understanding Molecular Recognition

- Understanding the principles of molecular recognition at the molecular level is essential to a good understanding of molecular function and biological process
- Knowledge of the mechanical features of a biological signal can be used to **design novel therapeutic agents**

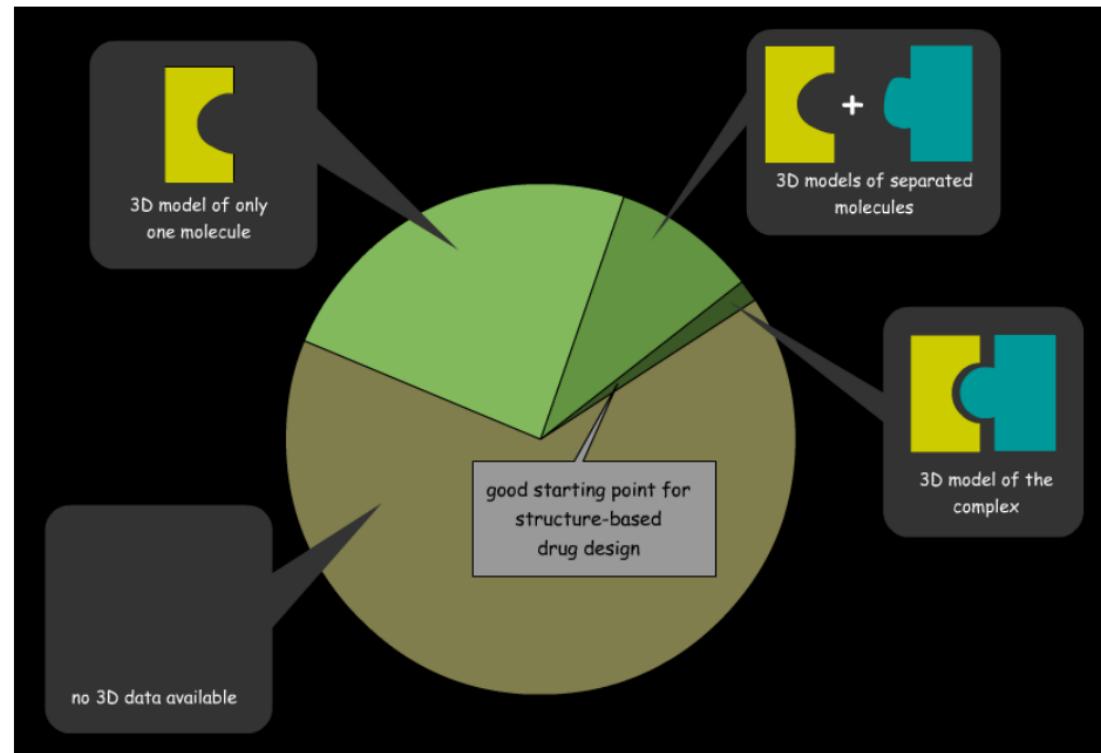


Experimental Methods to Study Molecular Docking

- Experimental techniques for study molecular recognition include **X-ray crystallography, NMR, electron microscopy**, site directed mutagenesis, co-immuno-precipitation etc...
- They allow us to experimentally solve the detailed **3-dimensional structures of biomolecules in their association form** which is a necessary step in identifying crucial residues, study the strength of interaction forces, their energetics, understand how molecular structures fit together, and investigate mechanisms of action

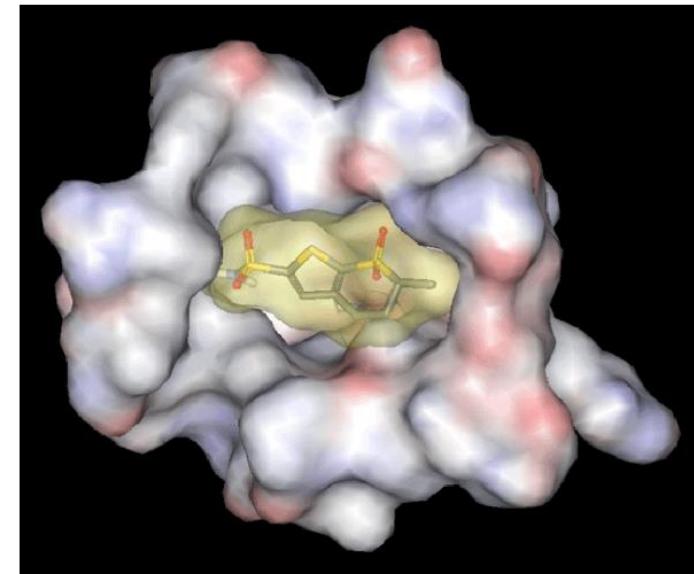
A Bottleneck in Drug Discovery

- Due to the limitations of current experimental methods, 3D structures of complexes are rarely available. But knowledge of the separated molecules in 3D is only weakly informative if we do not know how to assemble them.



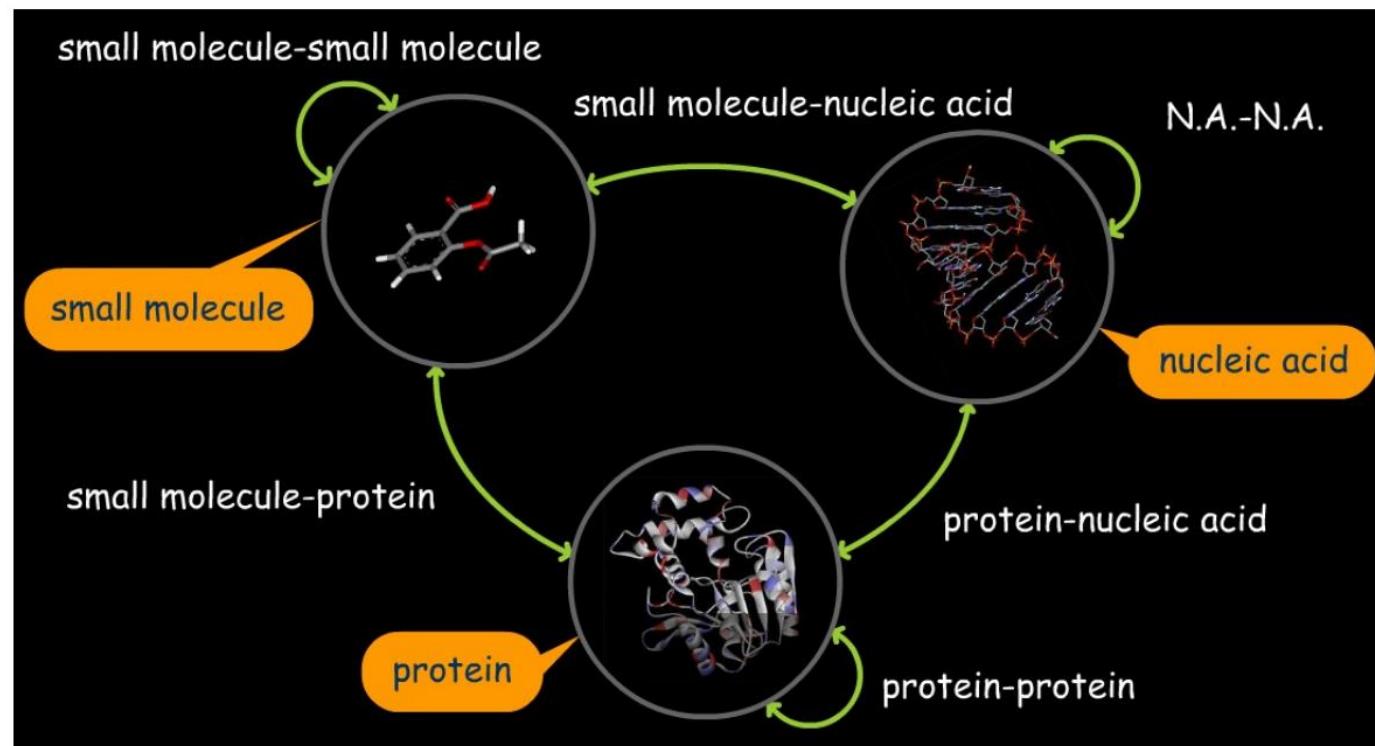
Triggering the Computational Docking Discipline

- The difficulties in obtaining experimentally structural data of macromolecular complexes have triggered the development of computational predictive methods
- Computational docking (also called *in silico* molecular docking or just docking) is a computational science aiming at predicting the optimal binding orientation and conformation of interacting molecules in space, and to estimate the stability of their complex
- Molecular docking predicts whether or not the two molecules interact, the binding affinity and the 3D structure of the complex
- Computational docking is an essential component in modern drug discovery. Over the last few decades, it has been routinely and successfully applied in most pharmaceutical and biotech companies for a large number of applications.



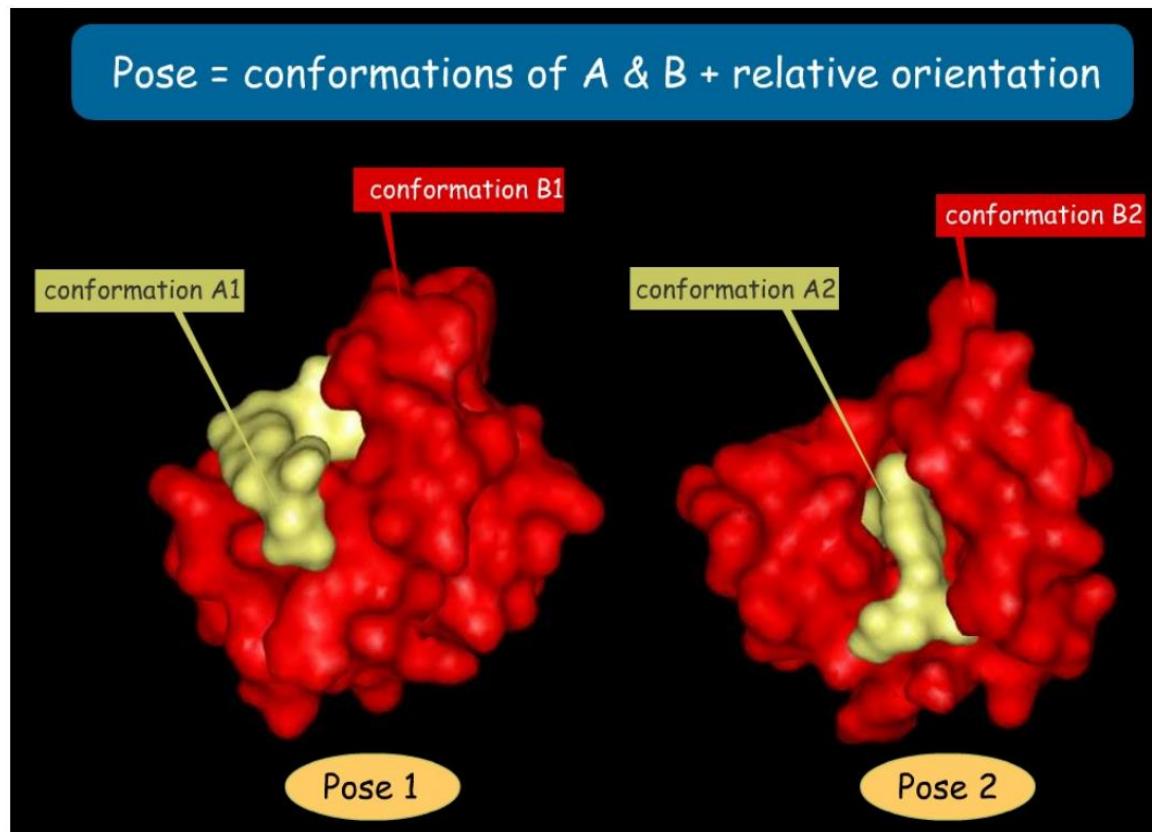
Docking Classification

- Molecular docking classifies biomolecules into three categories: small molecules (also called 'ligands'), proteins, and nucleic acids
- The most important types of docking systems are: **protein-ligand**, **protein-protein** and **nucleic acid-protein**
- The interactions between a small molecule and a protein are by far much better understood than those between a protein and a nucleic acid



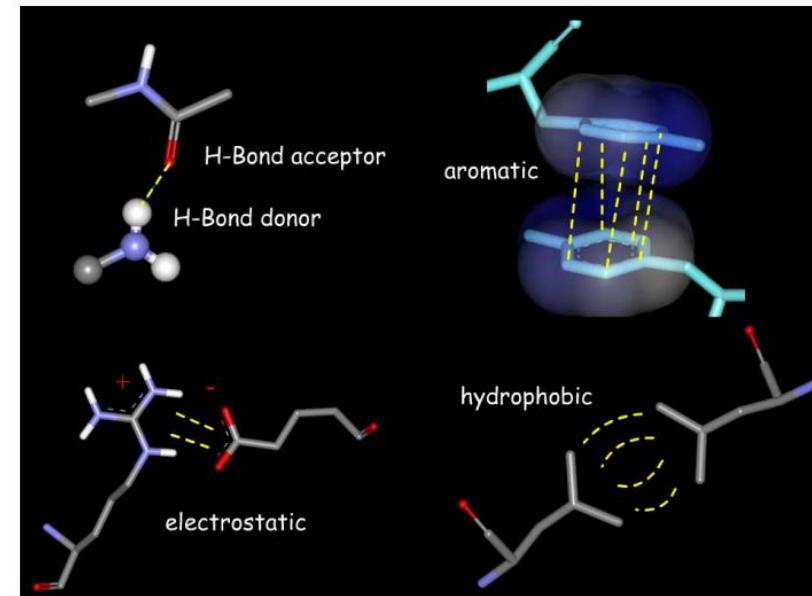
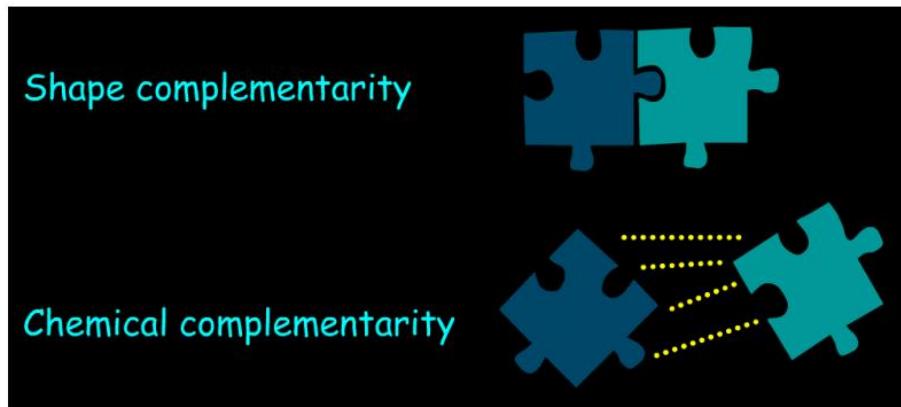
Definition of the "Pose"

- A "pose" is a term widely adopted for describing the geometry of a particular complex (also called "binding mode")
- It refers to a precise configuration which is characterized not only by the **relative orientation** of the docked molecules but also their respective **conformations**



Molecular Complementarity in Computational Docking

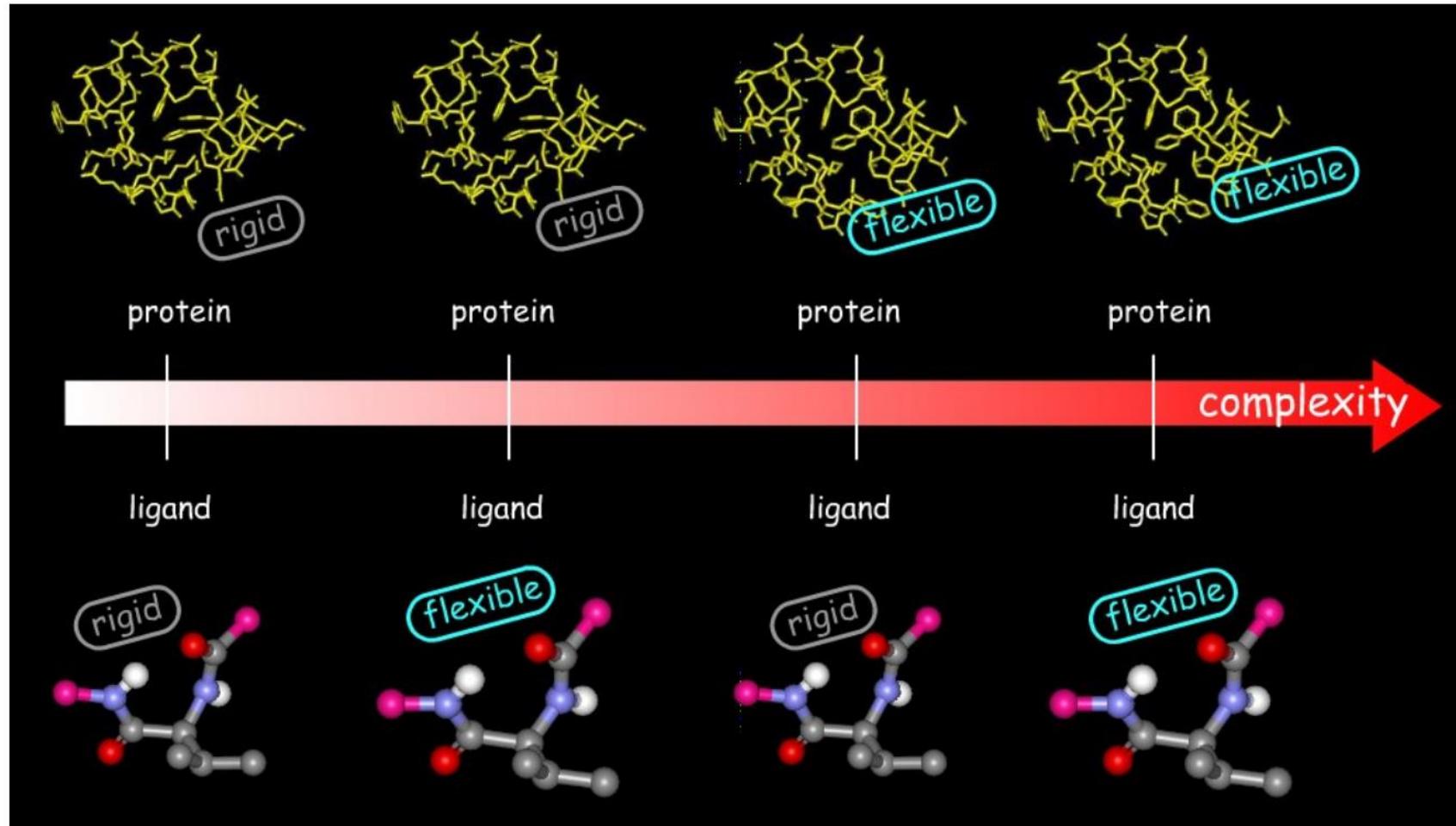
- Computational docking exploit the concept of molecular complementarity. The structures interact like a hand in a glove, where both the shape and the physico-chemical properties of the structures contribute to the fit.
- **Shape complementarity** is the primary criterion for evaluating the fit in the computational docking of two candidate structures
- In addition to shape compatibility, **chemical and physico-chemical complementarity** are also important criteria in the docking between candidate structures



Molecular Flexibility in Protein-Ligand Docking

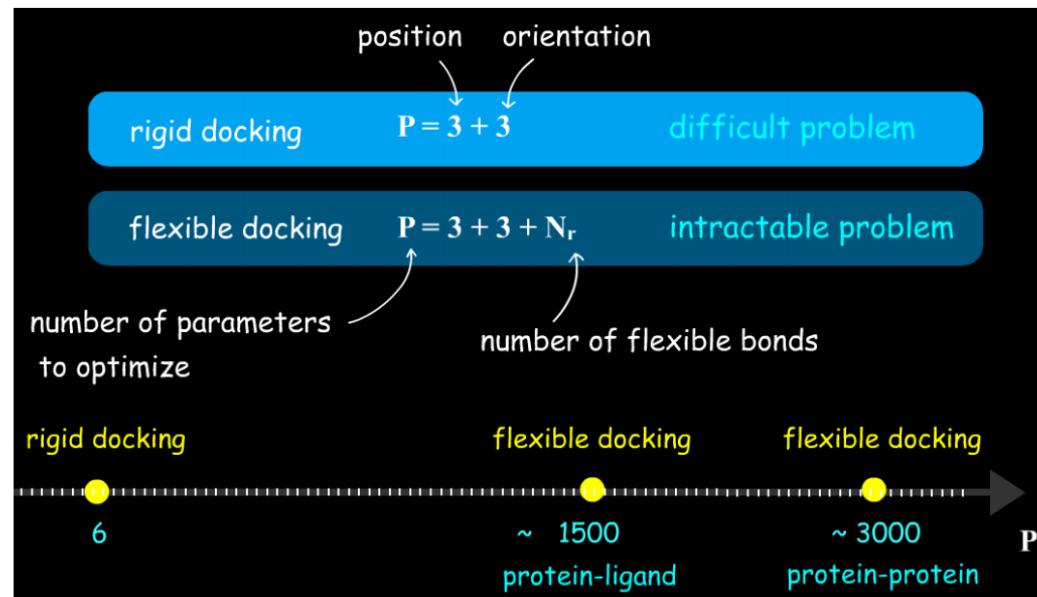
- The mutual adaptation of a ligand with its receptor is crucial to understanding ligand binding and protein function
- One of the major challenges in molecular docking is how to account for this adaptation in docking calculations
- The docking problem can be classified according to the way flexibility is modeled. In ascending order of complexity:
 1. **Rigid body docking** ignores the flexibility of the molecules and treats them like rigid objects
 2. **Rigid receptor - flexible ligand docking**: only the ligand is treated as flexible, receptor is rigid
 3. **Flexible receptor - flexible ligand docking**: both protein and ligand are treated as flexible.

Molecular Flexibility in Protein-Ligand Docking



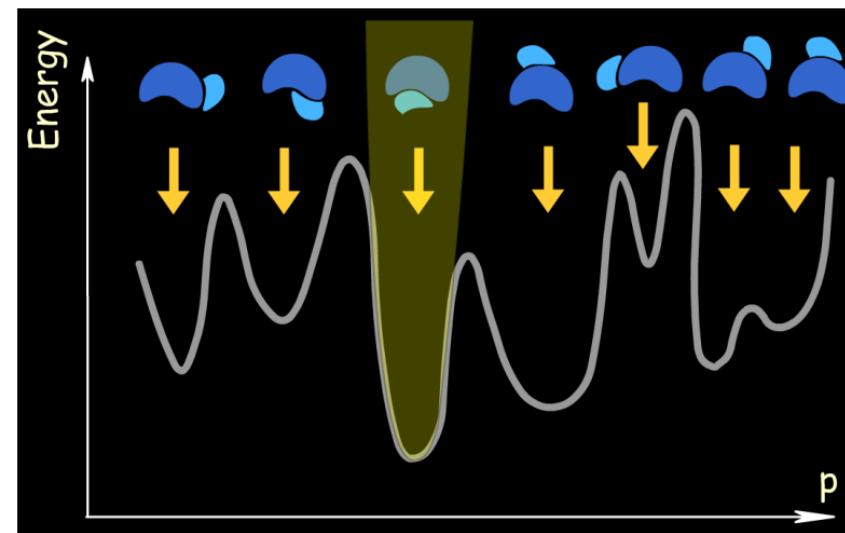
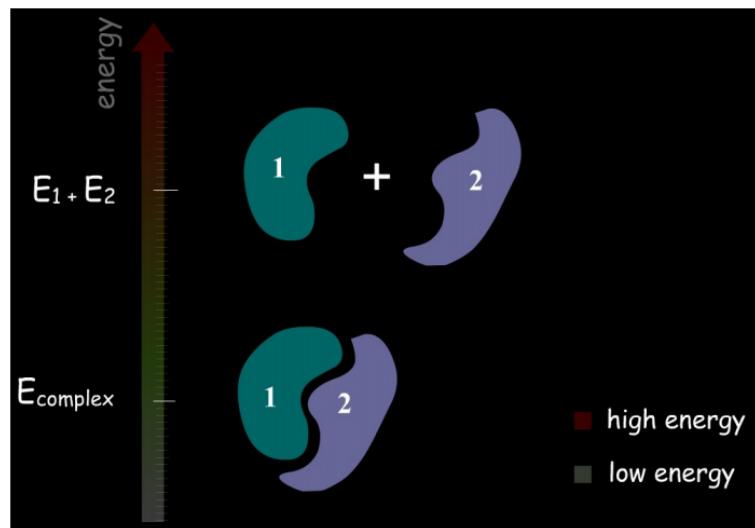
Degrees of Freedom in Flexible Docking

- The rigid-body docking approaches are often not sufficient to predict the structure of a protein complex from the separate unbound structures
- The incorporation of molecular flexibility into docking algorithms requires to add **conformational degrees of freedom** to translations and rotations
- Approximation algorithms need to be introduced to reduce the dimensionality of the problem and produce acceptable results within a reasonable computing time



Energy Dictates Molecular Associations

- The process of "self-assembly" is dictated by forces that are energy based: the complex has a lower potential energy than its constituent parts, and this keeps the parts together
- The goal of computational docking is to find the 3D configuration of the complex that minimizes the energy

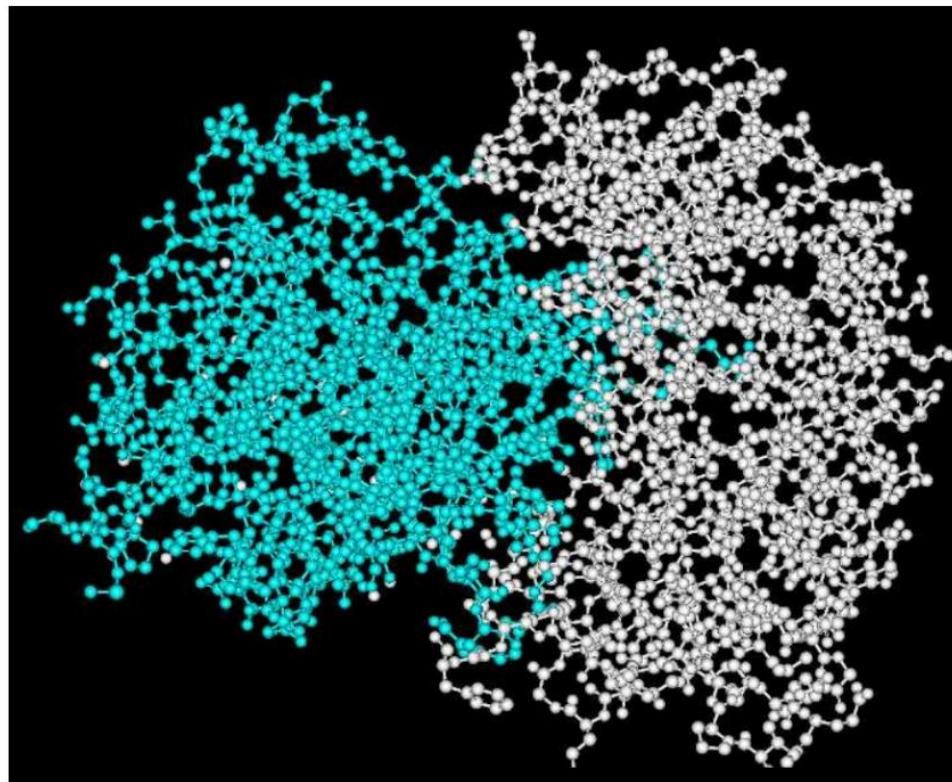


Three Components of Docking Software

- Docking software can be categorised based on the following criteria:
 1. **Molecular representation** - a way to represent structures and properties (atomic, surface, grid representation)
 2. **Scoring method** - a method to assess the quality of docked complexes (force field, knowledge-based approach, ...)
 3. **Searching algorithm** - an efficient search algorithm that decides which poses to generate (exhaustive search, Monte Carlo, genetic algorithms, simulated annealing, tabu search)

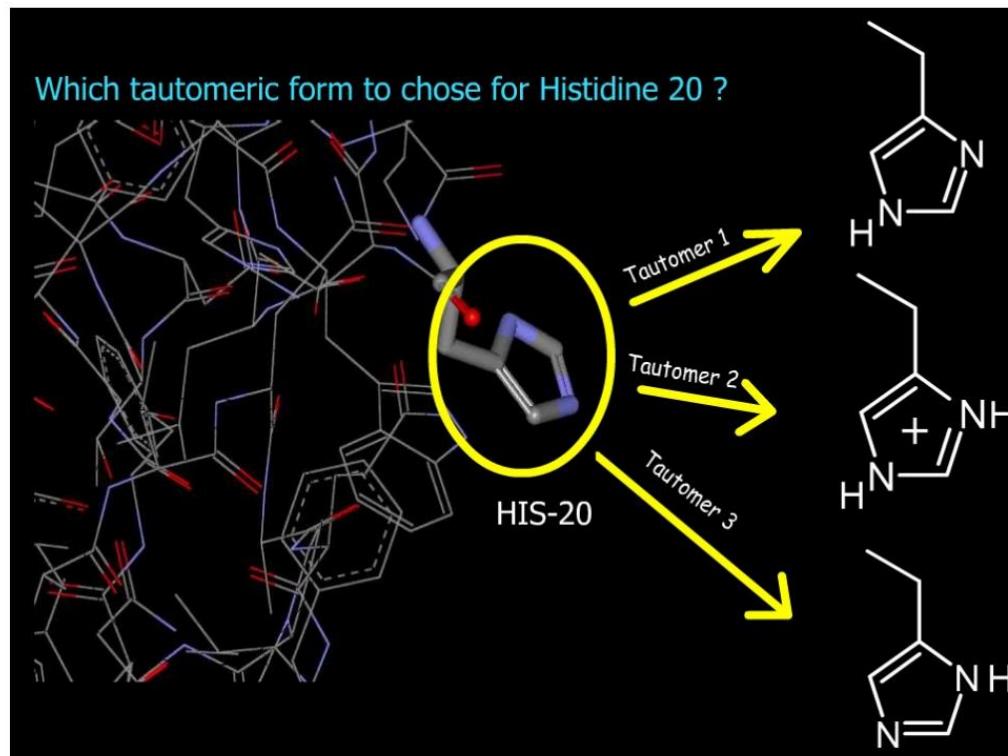
Atomic Representation

- In atomic representation, each atom is characterized by its coordinates and atom-type



Protein Preparation

- The preparation of the protein calls for great care. Important decisions include the choice of the **tautomeric forms of histidine residues**, the **protonation states of amino-acids** and conformations of some residues; their incorrect assignments may lead to docking errors.

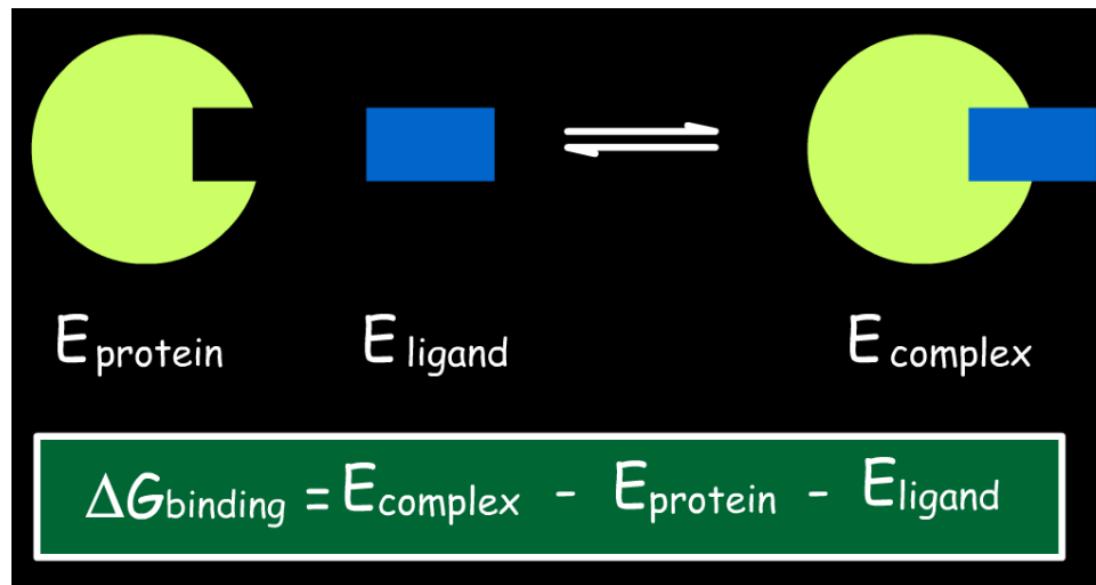


Small Molecule Preparation

- Before generating and docking the 3D structures of a library of ligands, it is important to "clean up" the 2D structures being used by removing any counter ions, salts, or water molecules that might be part of the registered structure
- All reactive or otherwise undesirable compounds must also be removed
- Possibly generate all optical isomers (enantiomers), cis/trans isomers, tautomers, and protonation states of the structures
- For most docking programs the tautomeric and protonation state of the ligands to be docked is defined by the user; in general the structure considered to be dominant at a neutral pH is generated; here also, incorrect assignments may lead to docking errors

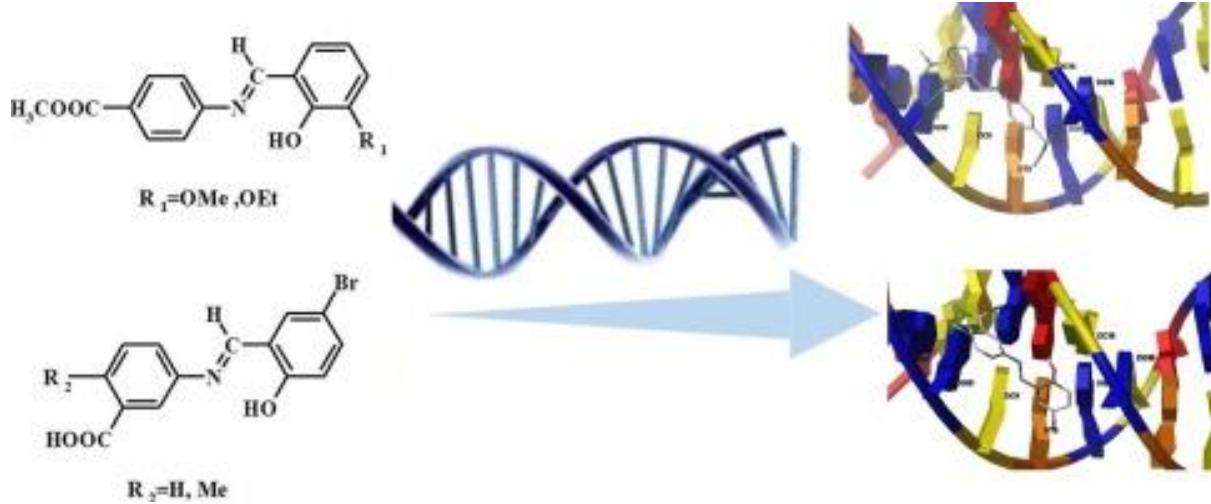
Calculation of the Binding Energies

- The binding energy $\Delta G_{\text{binding}}$ is the energy required to separate a complex into separate parts (protein and ligand). It is defined as the difference between the energy of the associated (bound) form (E_{complex}) and that of the separated (unbound) molecules (E_{protein} and E_{ligand}).
- A complex has a lower potential energy than its constituent parts. This is what keeps them together.



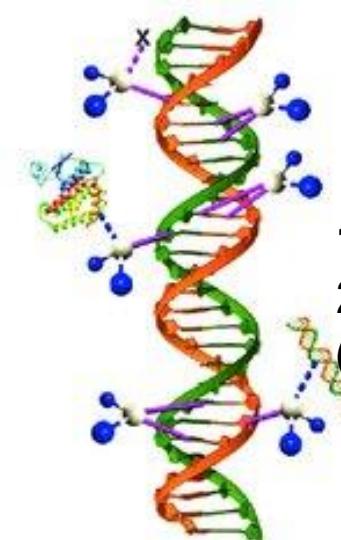
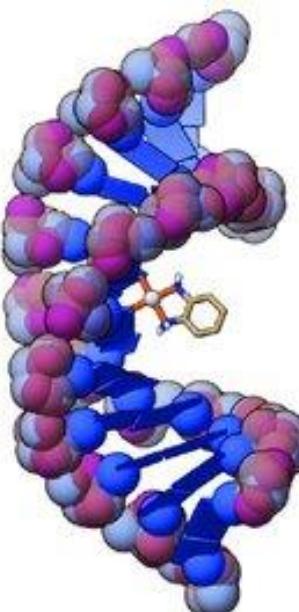
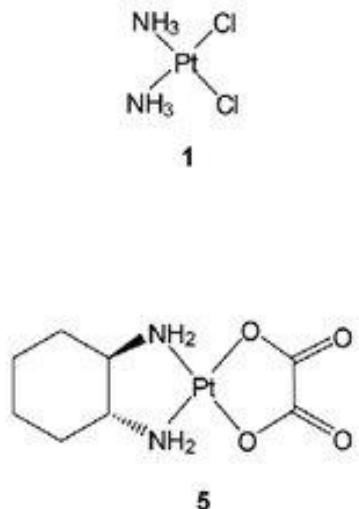
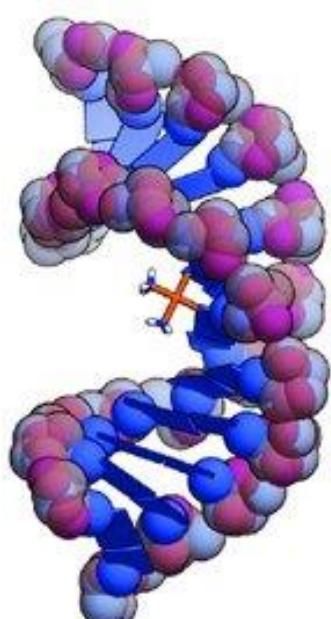
Limitations in Computational Docking

- Computational docking has emerged recently as a new discipline. Despite the important achievements that have been obtained, substantial progress remains to be made to exploit the full potential of this approach
- Current challenges of docking methods are:
 - Trade off between efficiency and accuracy
 - More effective scoring functions
 - Better model of flexibility



Organic-Biomolecule

16-17 July,
2022
(Sat, Sun)



19, 20 July,
2022
(Tue, Wed)

Inorganic-Biomolecule

Practical Session

Docking of Organic and Inorganic ligands with Biomolecules