Using MolAICal: 3D drug design in the pocket of GCGR by artificial intelligence and *de novo* method

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1. Introduction

In this tutorial, the standard protocol of MolAICal (https://doi.org/10.1093/bib/bbaa161) is introduced for the drug design of glucagon receptor (GCGR) by artificial intelligence and *de novo* method. It will help the pharmacologist, chemists and other scientists design rational drugs according to three-dimensional active pocket of proteins.

2. Materials

2.1. Software requirement

1) MolAICal: https://molaical.github.io
2) UCSF Chimera: https://www.cgl.ucsf.edu/chimera/

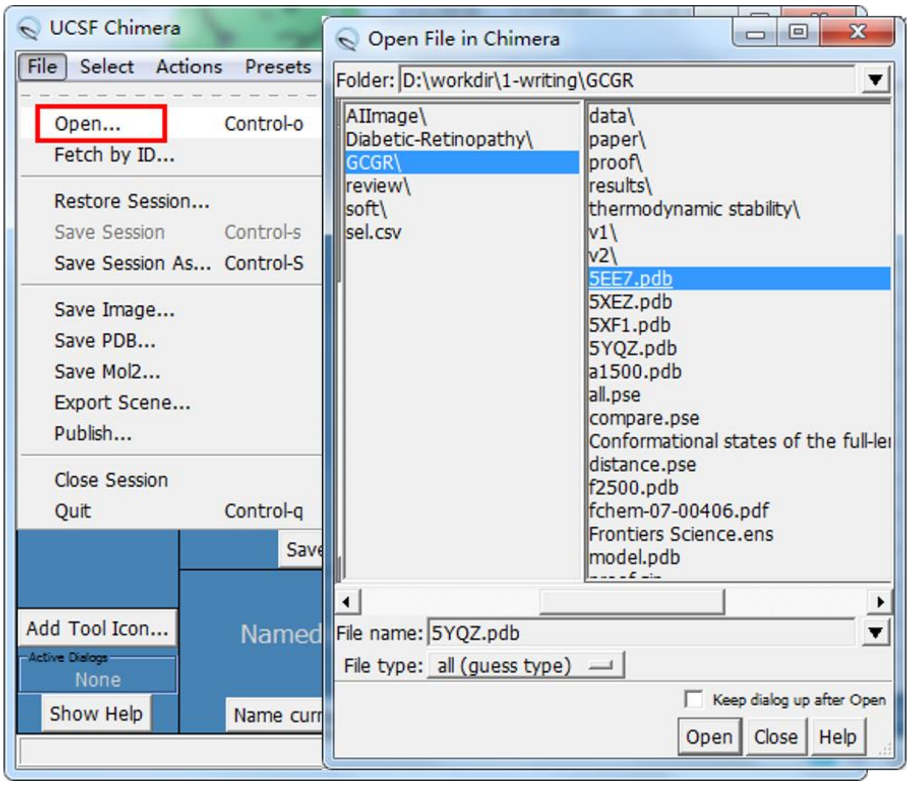
2.2. Protocol files

All the necessary tutorial files are downloaded from:
https://github.com/MolAICal/tutorials/tree/master/001-AIGrow

3. Procedure

3.1. Dealing with receptor

1. Download the human glucagon receptor (GCGR) PDB ID: 5EE7:
   http://www.rcsb.org/structure/5EE7
2. Start UCSF Chimera: File→Open, load the PDB file 5EE7.pdb in your directory (see Figure 1).



Folder: D:\workdir\1-writing\GCGR

File name: 5YQZ.pdb

File type: all (guess type)

Figure 1. Loading a molecule.

3. Select and delete the chosen molecules.
   Select the no useful ligand: Select→Residue→OLA (see Figure 2).

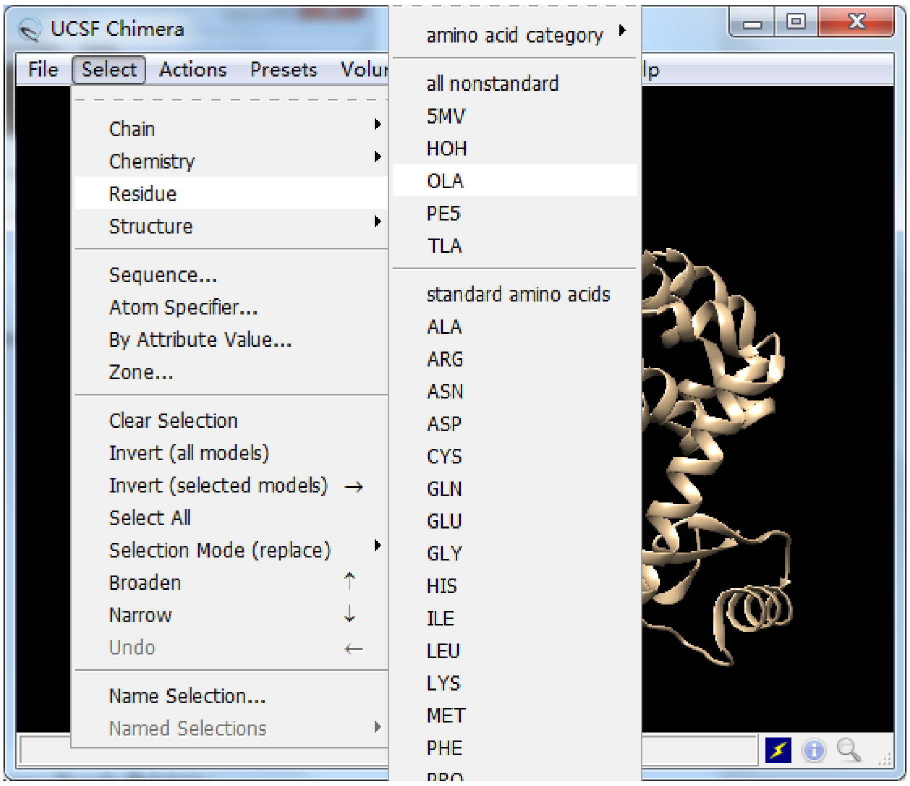


Figure 2. Selecting a molecule.

Delete no useful ligand: Actions→Atoms/Bonds→delete (see Figure 3).

We only show the simple example, you need delete HOH, PE5, TLA, *etc*. in the same way.

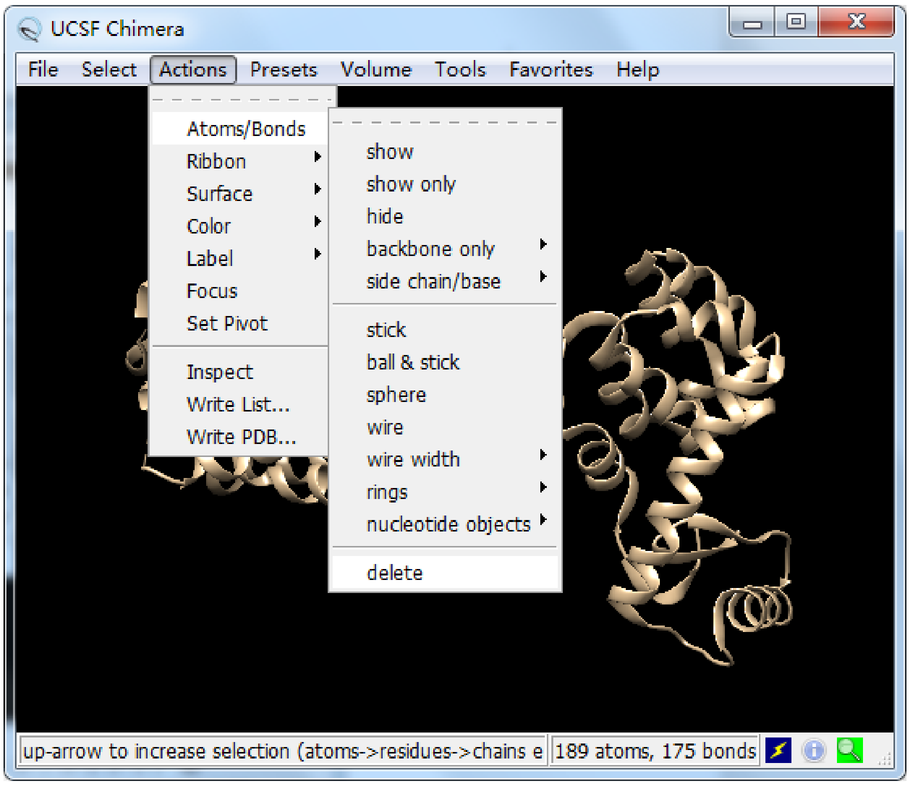


Figure 3. Deleting the selected molecule.

4. Add hydrogen. Tools→Structure Editing→AddH (see Figure 4).

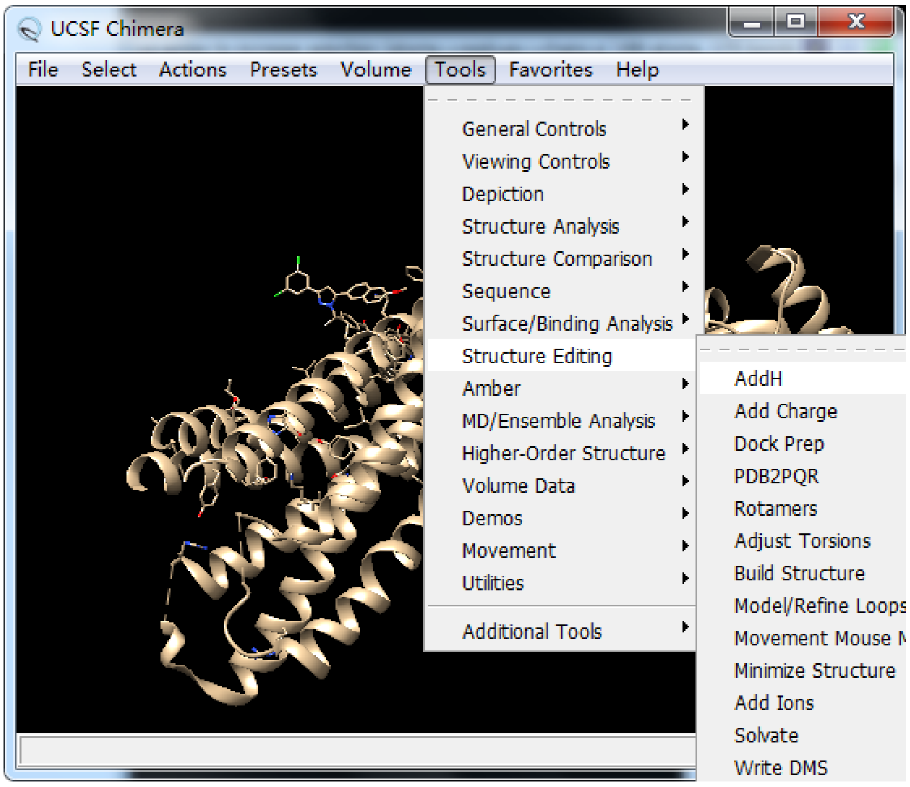
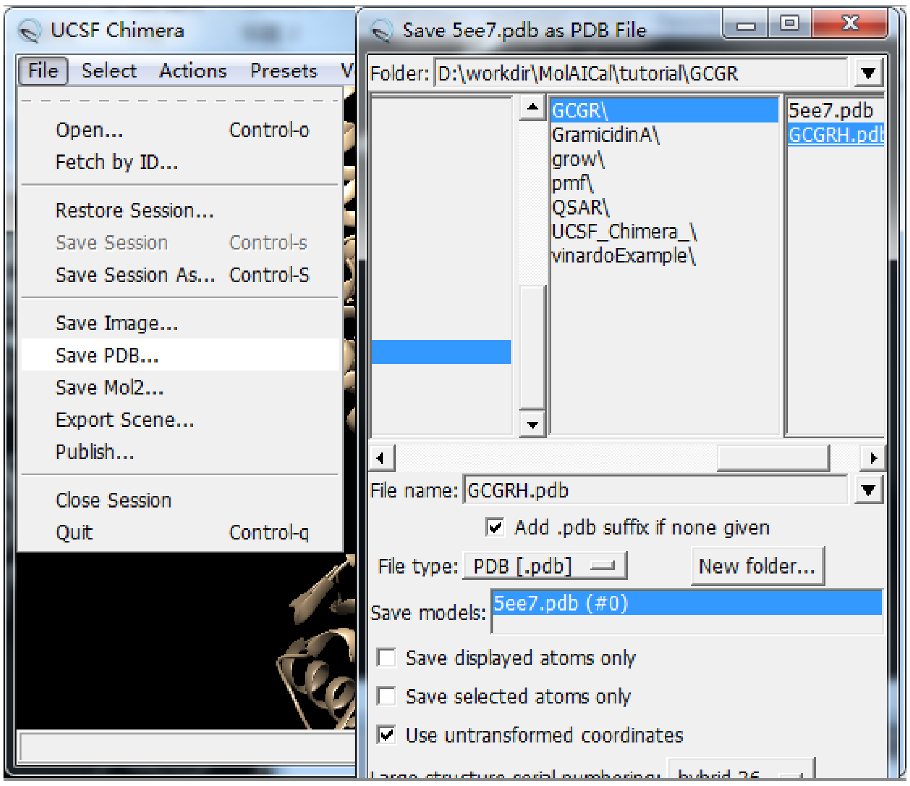


Figure 4. Adding hydrogen.

Notice: because MolAICal deals with receptors by the Amber force field, please delete hydrogen atoms before adding hydrogen by UCSF Chimera. If the receptor had the redundant hydrogen, please delete it, then added it. The detail procedures: firstly, select hydrogen: Select→Chemistry→element→H, then delete selection: Actions→Atoms/Bonds→delete (see Figure 3). Here, the crystal GCGR has no redundant hydrogen atoms, so this step is omitted.

5. Save the coordinate: GCGRH.pdb. Saving GCGRH.pdb can be as a checkpoint in the process of drug design. You can start this step to deal with GCGR directly (see Figure 5).


UCSF Chimera

File Select Actions Presets V

Open... Control-o
Fetch by ID...
Restore Session...
Save Session Control-s
Save Session As... Control-S
Save Image...
Save PDB...
Save Mol2...
Export Scene...
Publish...
Close Session
Quit Control-q

Save 5ee7.pdb as PDB File

Folder: D:\workdir\MolAICal\tutorial\GCGR

GCGR\
GramicidinA\
grow\
pmf\
QSAR\
UCSF_Chimera_\
vinardoExample\

5ee7.pdb
GCGRH.pdb

File name: GCGRH.pdb

[✓] Add .pdb suffix if none given

File type: PDB [.pdb]

New folder...

Save models: 5ee7.pdb (#0)

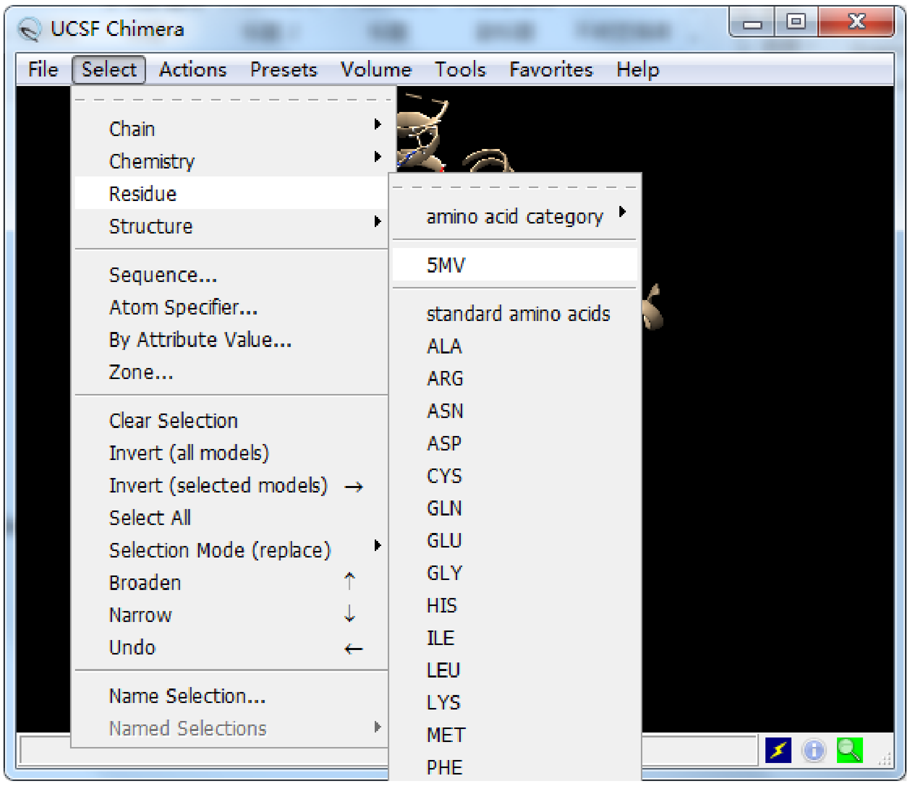
[ ] Save displayed atoms only

[ ] Save selected atoms only

[✓] Use untransformed coordinates

Figure 5. Saving the coordinates of receptor and ligand as PDB file.

6. Selecting ligand 5MV and deleting it (see Figure 6 and 7). Save the coordinates of apo-state GCGR (unliganded bound): GCGRNoLigand.pdb (see Figure 8).


UCSF Chimera

File Select Actions Presets Volume Tools Favorites Help

Chain
Chemistry
Residue
Structure
Sequence...
Atom Specifier...
By Attribute Value...
Zone...
Clear Selection
Invert (all models)
Invert (selected models) →
Select All
Selection Mode (replace)
Broaden ↑
Narrow ↓
Undo ←
Name Selection...
Named Selections

amino acid category
5MV
standard amino acids
ALA
ARG
ASN
ASP
CYS
GLN
GLU
GLY
HIS
ILE
LEU
LYS
MET
PHE

Figure 6. Selecting ligand 5MV.

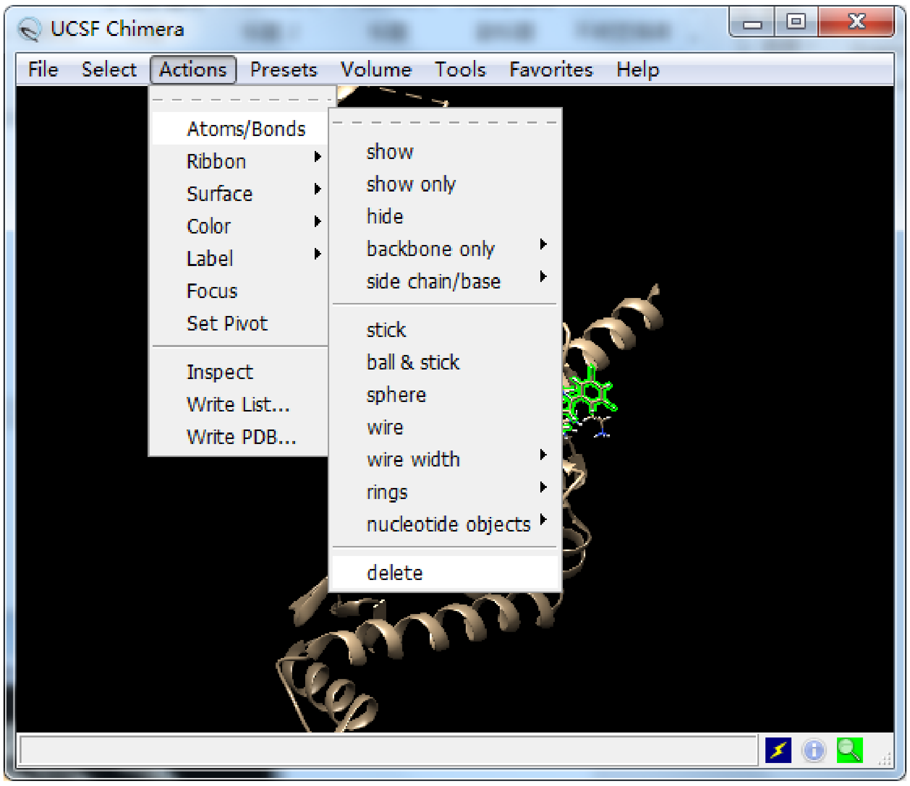
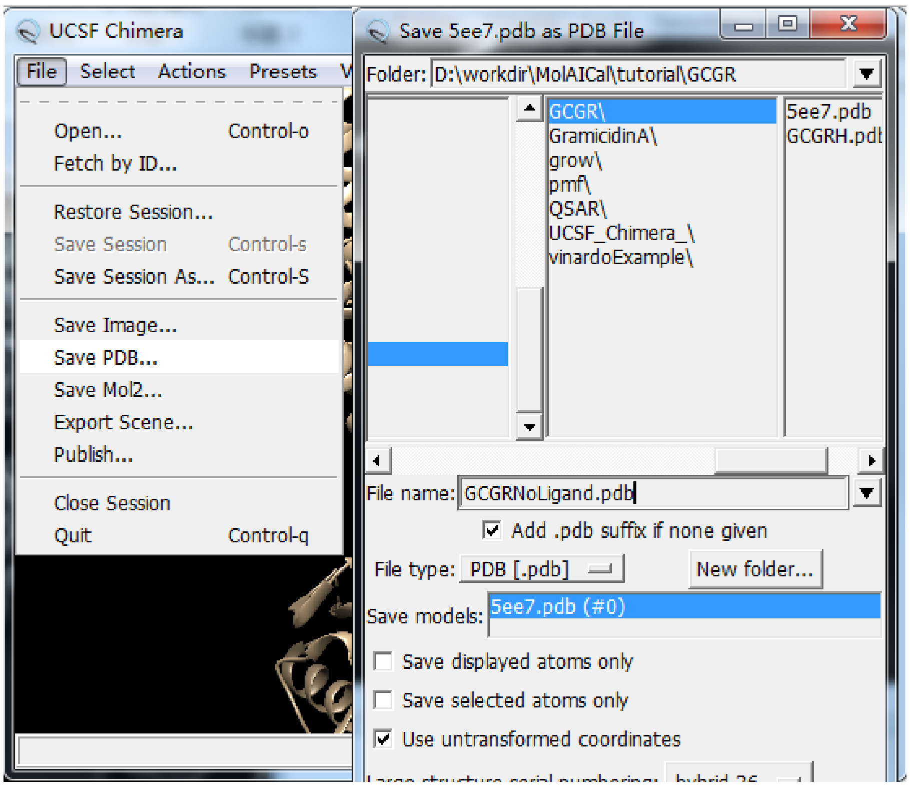


Figure 7. Deleting ligand 5MV.



Folder: D:\workdir\MolAICal\tutorial\GCGR

File name: GCGRNoLigand.pdb

☑ Add .pdb suffix if none given

File type: PDB [.pdb]

Save models: 5ee7.pdb (#0)

☐ Save displayed atoms only

☐ Save selected atoms only

☑ Use untransformed coordinates

Figure 8. Saving receptor coordinates as PDB file.

3.2. Dealing with ligand

1. Close session (File→Close Session) and reload the GCGRH.pdb, then select with ligand 5MV (see Figure 9 and 10):

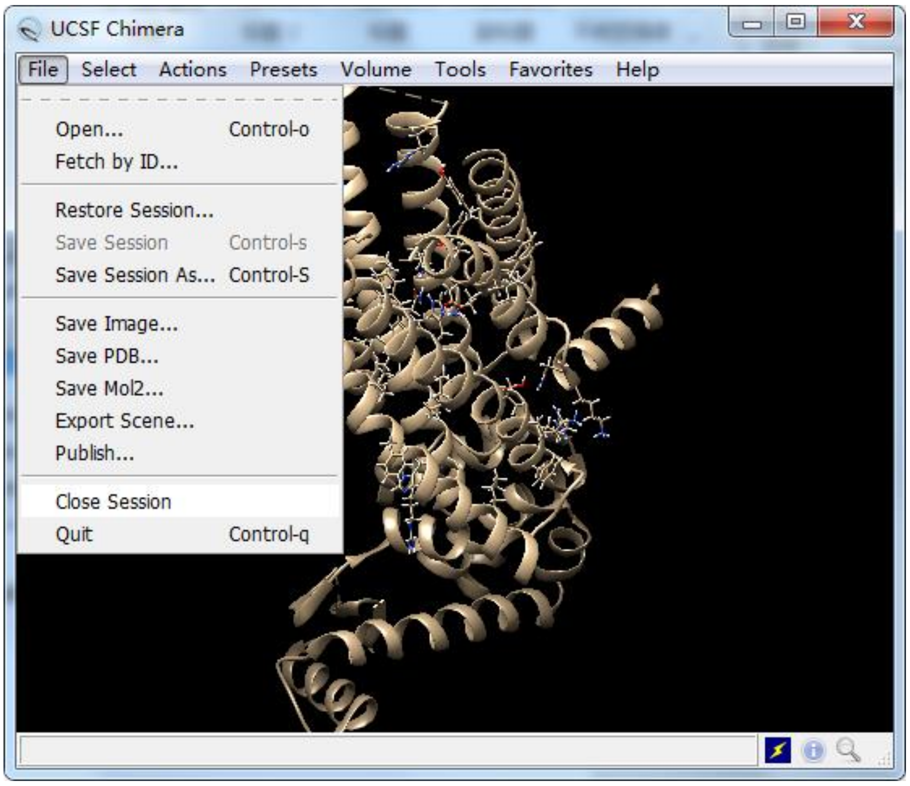
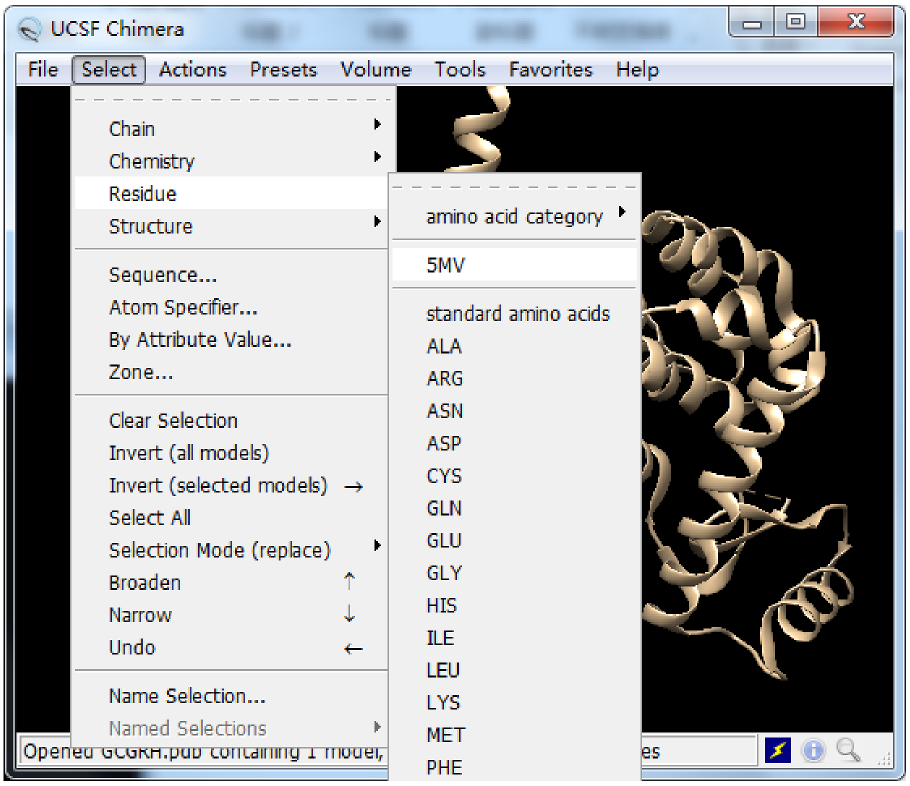


Figure 9. Closing session.



Opened GCGRH.pdb containing 1 model, ... es

Figure 10. Selecting ligand 5MV.

2. Invert (select models): invert select ligand 5MV (see Figure 11):

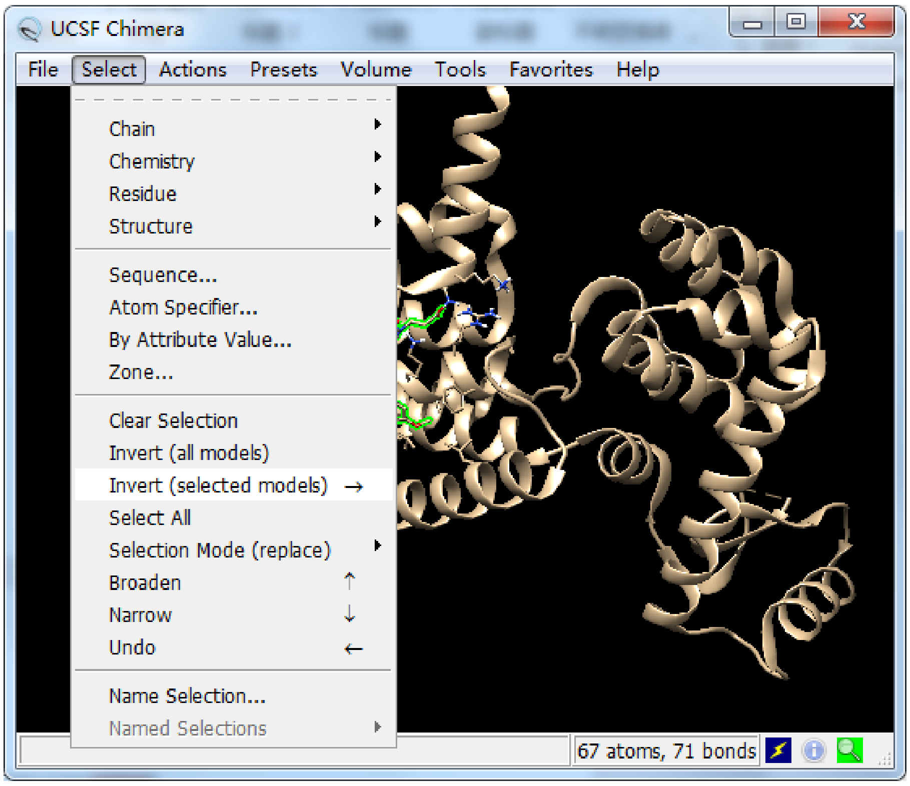


Figure 11. Inverting selected ligand 5MV.

3. Then delete invert selection (see Figure 12):

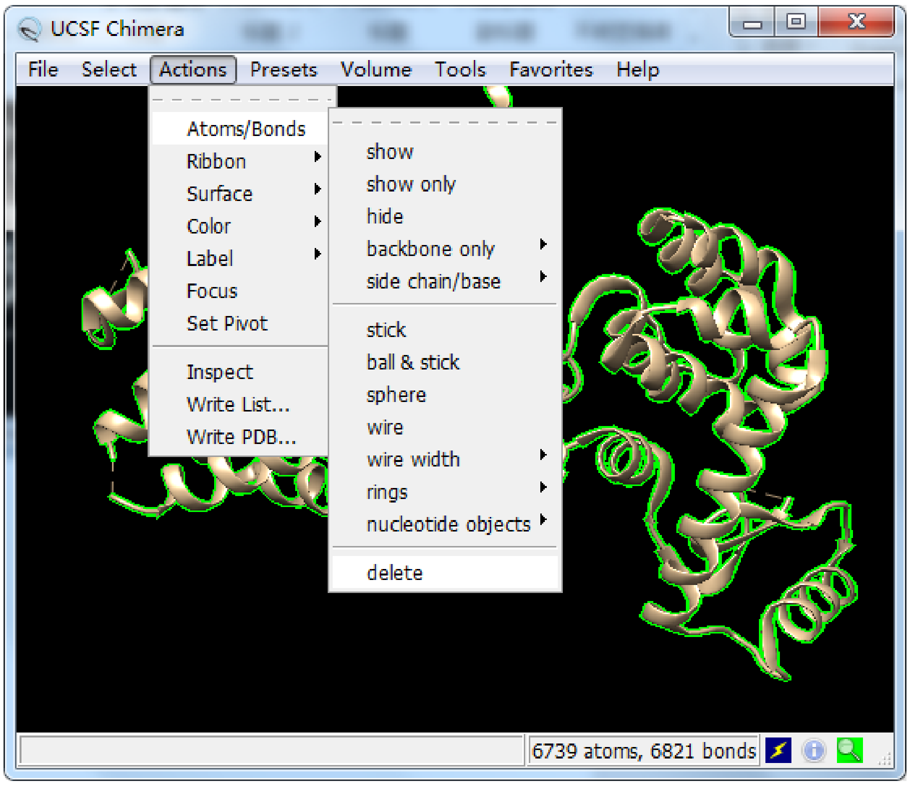
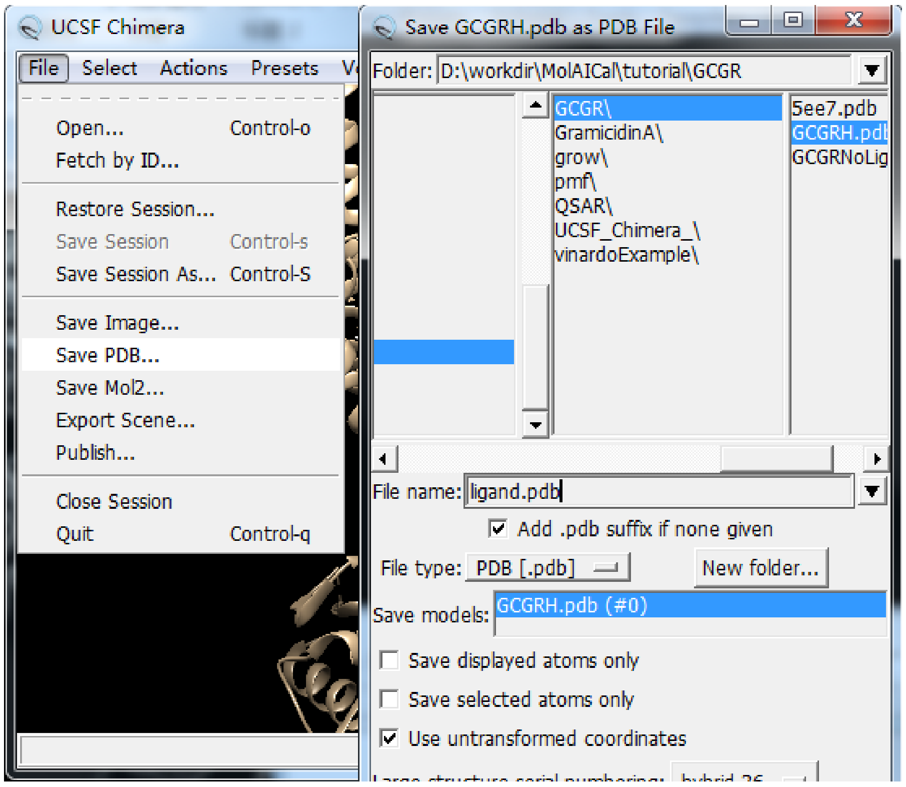


Figure 12. Deleting receptor.

4. Then save ligand to ligand.pdb. It can be split into different fragments which can be chosen as the start growth fragment in the pocket of GCGR (see Figure 13).



Folder: D:\workdir\MolAICal\tutorial\GCGR

GCGR\
GramicidinA\
grow\
pmf\
QSAR\
UCSF_Chimera_\
vinardoExample\

5ee7.pdb
GCGRH.pdb
GCGRNoLig

File name: ligand.pdb

☑ Add .pdb suffix if none given

File type: PDB [.pdb]

Save models: GCGRH.pdb (#0)

☐ Save displayed atoms only

☐ Save selected atoms only

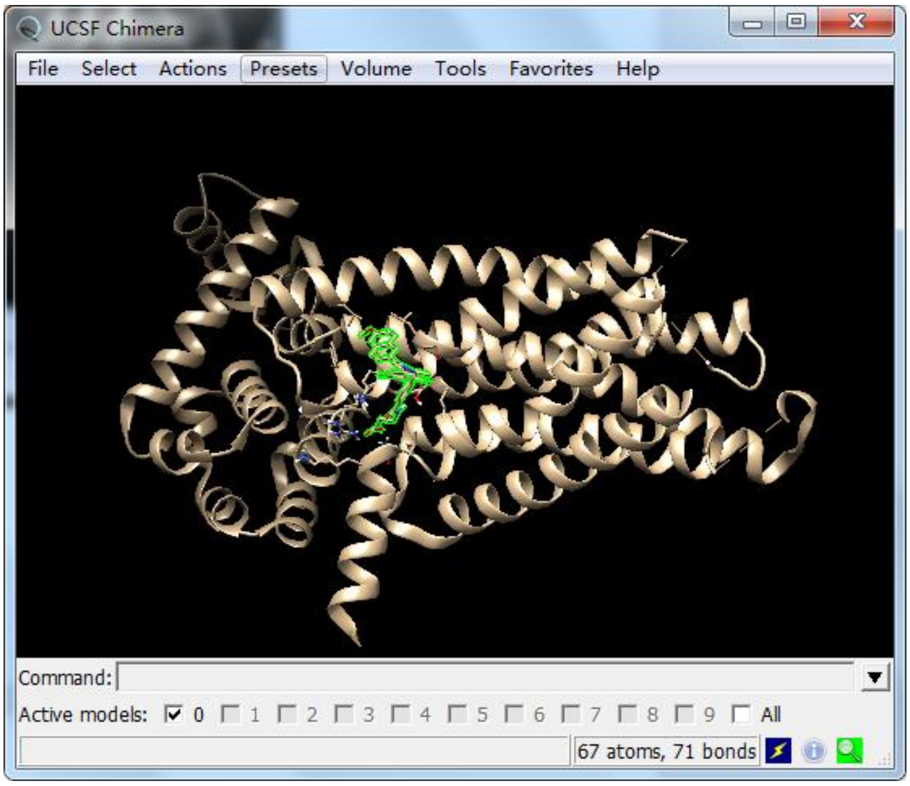
☑ Use untransformed coordinates

Figure 13. Saving ligand coordinate.

3.3. Calculating center of growth box

Calculating the geometry center of growth box. (Tip: If no ligand, you can choose the key residues reported in the experiment.)

1. Close session (File→Close Session) and reload the GCGRH.pdb, then select the ligand (see Figure 14):



Active models: ☑ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ All

67 atoms, 71 bonds

Figure 14. Selecting ligand coordinates.

2. Tools→Structure Analysis→Distance (see Figure 15):

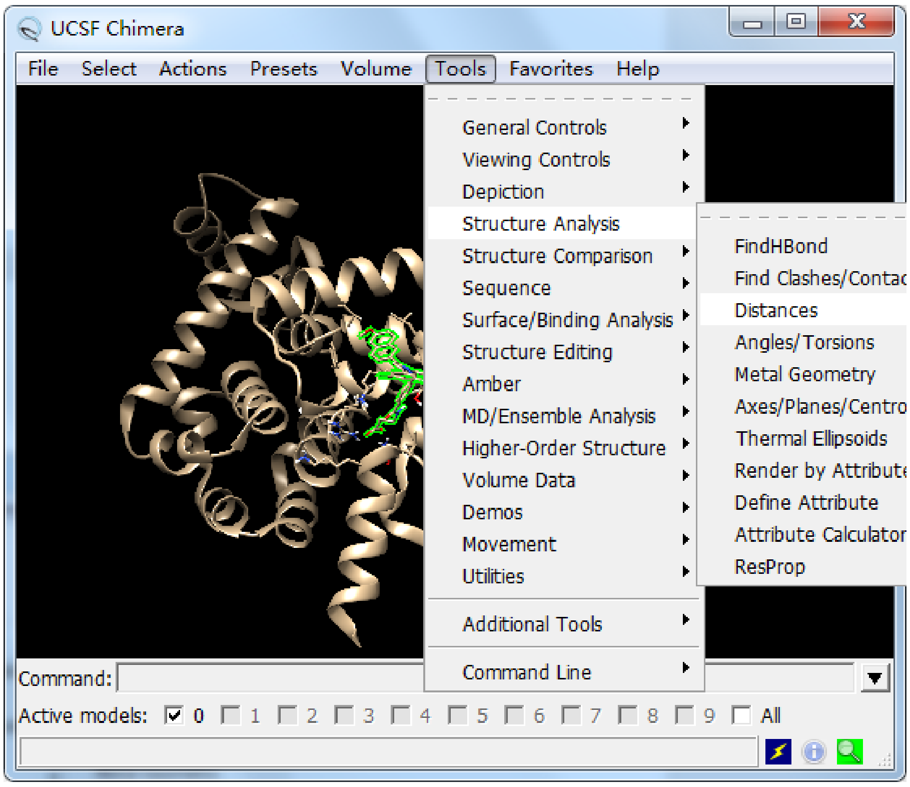


Figure 15. Selecting distance tool.

3. Click Axes/Planes/Centroids, then click "Define centroid" (see Figure 16):

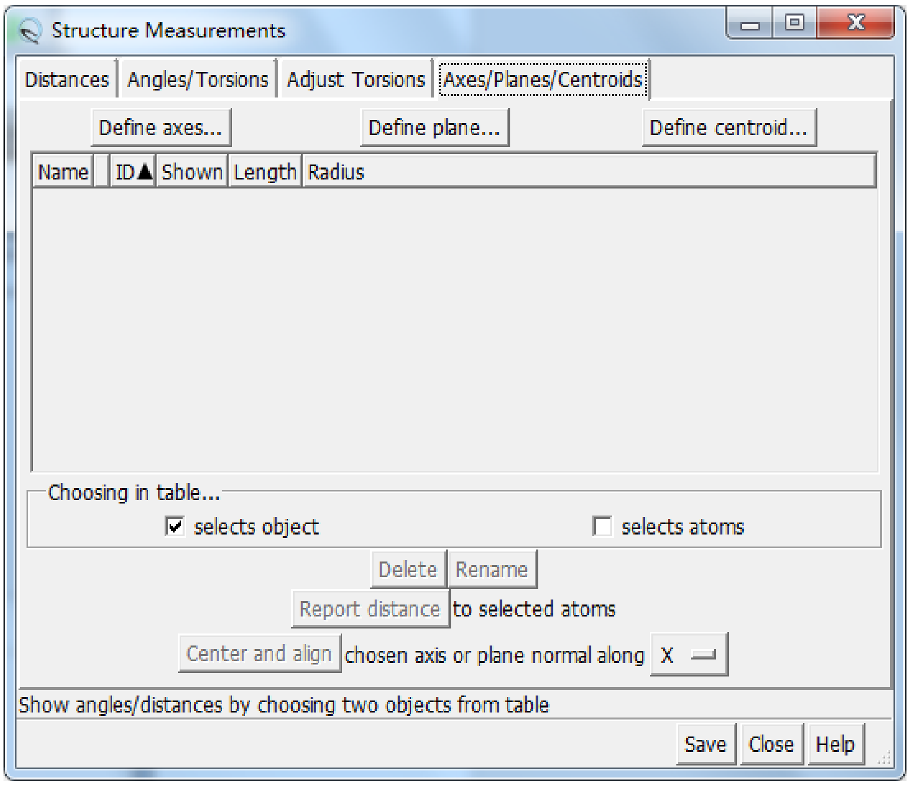
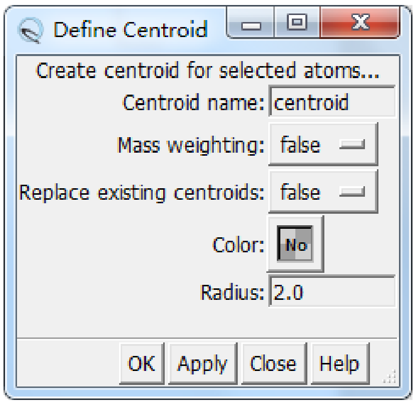


Figure 16. Define centroid.

4. Click "OK" in the pop-up (see Figure 17).



Define Centroid

Create centroid for selected atoms...

Centroid name: centroid

Mass weighting: false

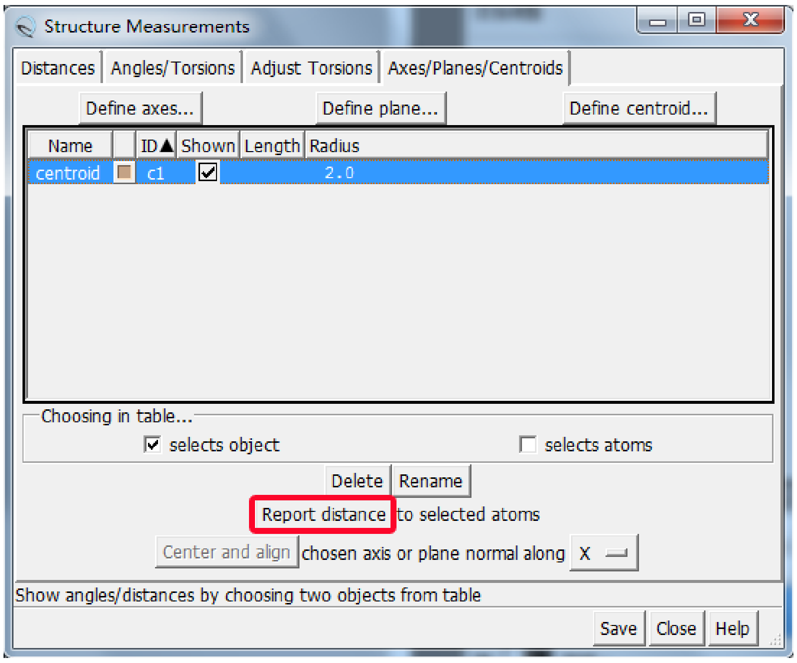
Replace existing centroids: false

Color: No

Radius: 2.0

Figure 17. Define the centroid box.

5. Select the defined centroid (blue part) and click "Report distance" (see Figure 18)



Structure Measurements

Name		ID▲	Shown	Length	Radius
centroid	▨	c1	☑		2.0

Choosing in table...

☑ selects object ☐ selects atoms

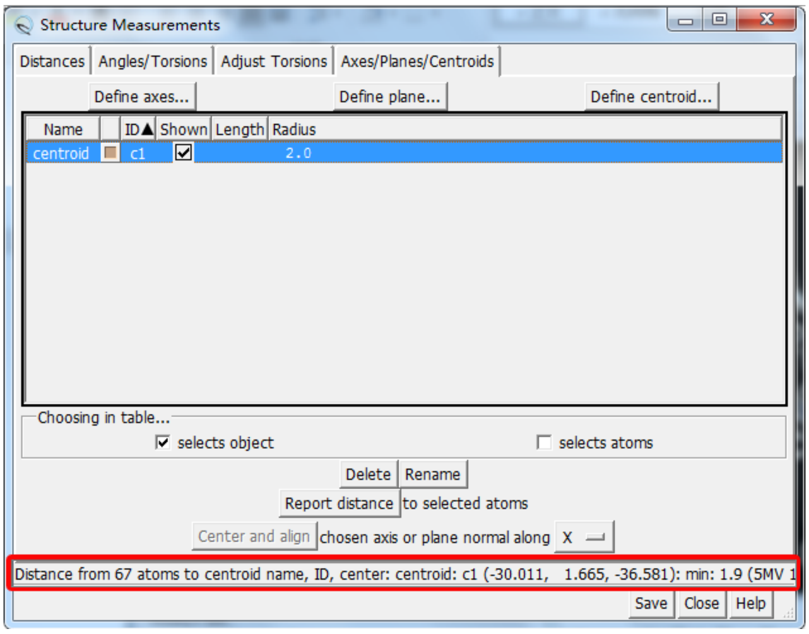
Report distance to selected atoms

Center and align chosen axis or plane normal along X

Show angles/distances by choosing two objects from table

Figure 18. Report distance.

6. Then you can see the center centroid (x, y, z): -30.011, 1.665, -36.581 (see Figure 19):

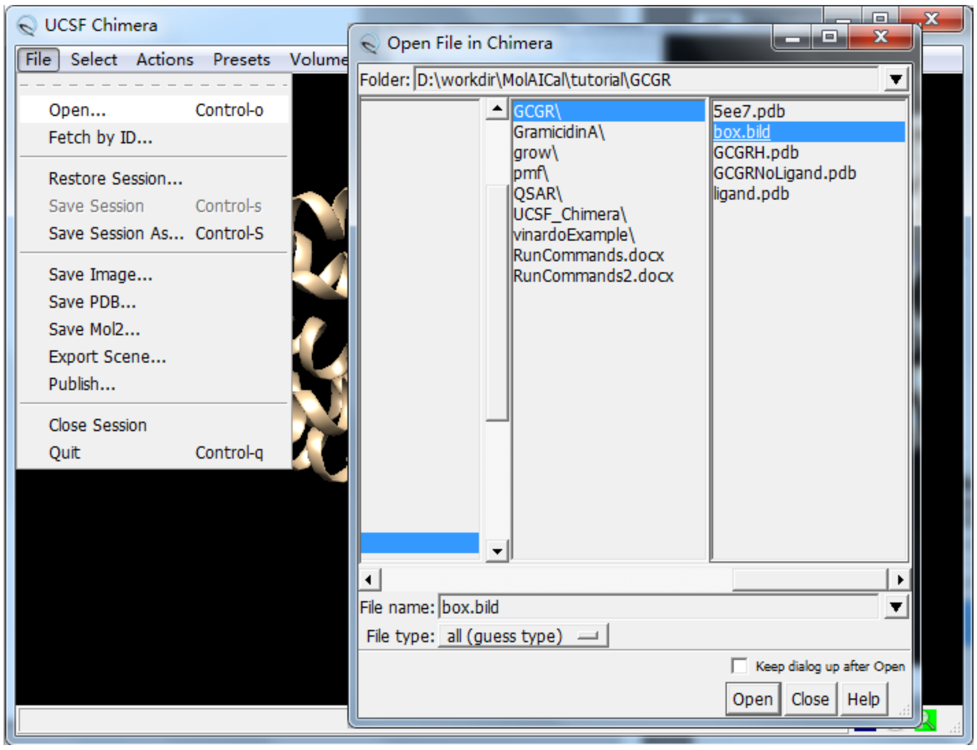


Name		ID▲	Shown	Length	Radius
centroid		c1	✓		2.0

Distance from 67 atoms to centroid name, ID, center: centroid: c1 (-30.011, 1.665, -36.581): min: 1.9 (5MV 1

Figure 19. Showing coordinate values of centroid box.

7. Calculating the final box size. You can try X, Y, Z, lengths of 30, 30, 30. Generate the "box.bild" by using command of MolAICal as below (note: **the double quotes are necessary for X, Y, Z coordinates. The interval distance among X, Y, Z coordinates and box lengths should be one space**.):
   1) molaical.exe -tool box -i "-30.011 1.665 -36.581" -l "30.0 30.0 30.0" -o "D:\001-AIGrow\box.bild"
   2) File→open, then open "**box.bild**" (see Figure 20), and check whether the generated box is suitable (see Figure 21).



Folder: D:\workdir\MolAICal\tutorial\GCGR

File name: box.bild

File type: all (guess type)

Figure 20. Opening box.bild.

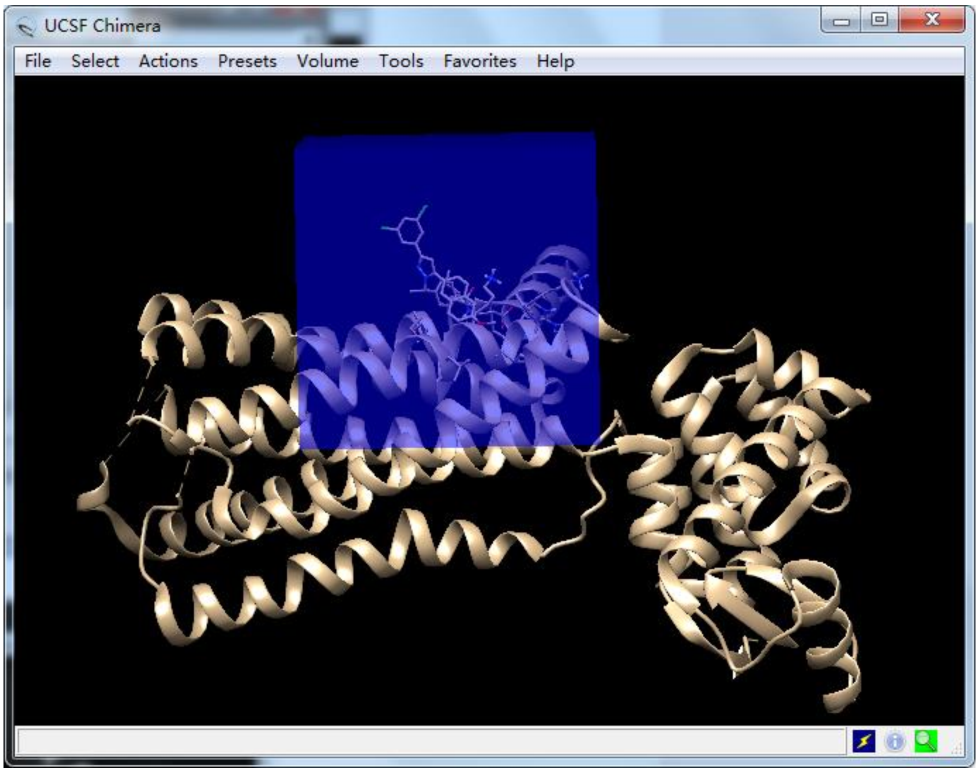


Figure 21. Showing the box in the pocket of GCGR.

The box size of 30, 30, 30 is suitable, so the final center parameter is -30.011, 1.665, -36.581 and the final box lengths of X, Y, Z are 30.0, 30.0, 30.0.

3.4. Making the initial growth fragment

Here, two options are introduced. You can choose anyone of options for de novo drug design. We recommend option 1 if the crystal structure of ligand is determined in the pocket of the receptor.

Option 1. Fragment extracted from the crystal structure of ligand

In this option, the initial fragment is extracted from the crystal structure of ligand in the pocket of GCGR.

1. Make the initial growth fragment by UCSF Chimera. Open “ligand.pdb” saved above. You chose atom via mouse and keyboard:
   1) Ctrl + left key of mouse: it can select one atom at a time.
   2) Ctrl + Shift + left key of mouse: it can select many atoms.

   In this tutorial, we selected intimal atoms as the initial growth fragment by dragging the Ctrl + left key of mouse (see Figure 22).

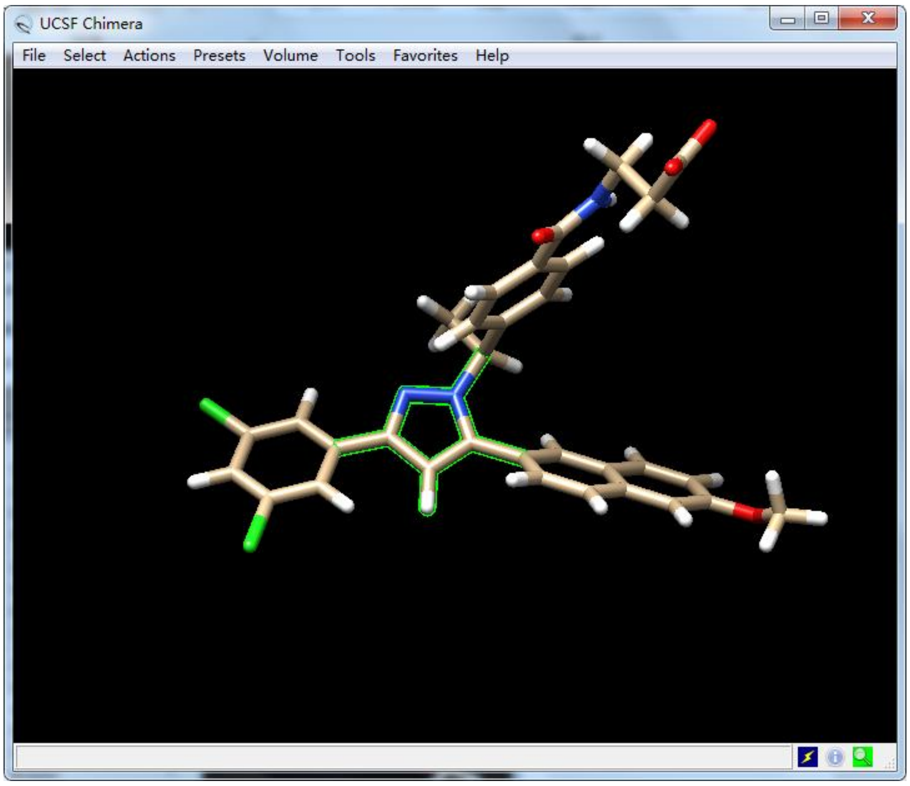


Figure 22. Selecting initial growth part.

2. Invert (selected models) as shown in Figure 23:

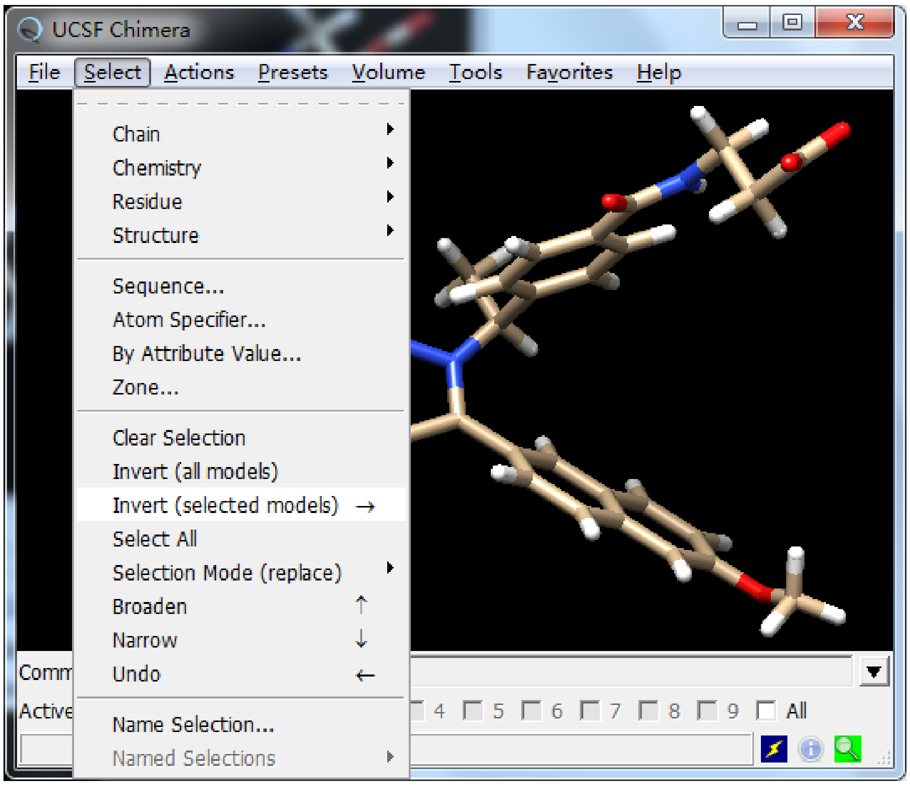


Figure 23. Invert selecting the initial growth part.

3. Then delete the invert selected part: Actions→Atoms/Bonds→delete.

Finally, we choose the below molecule as the starting fragment (see Figure 24):

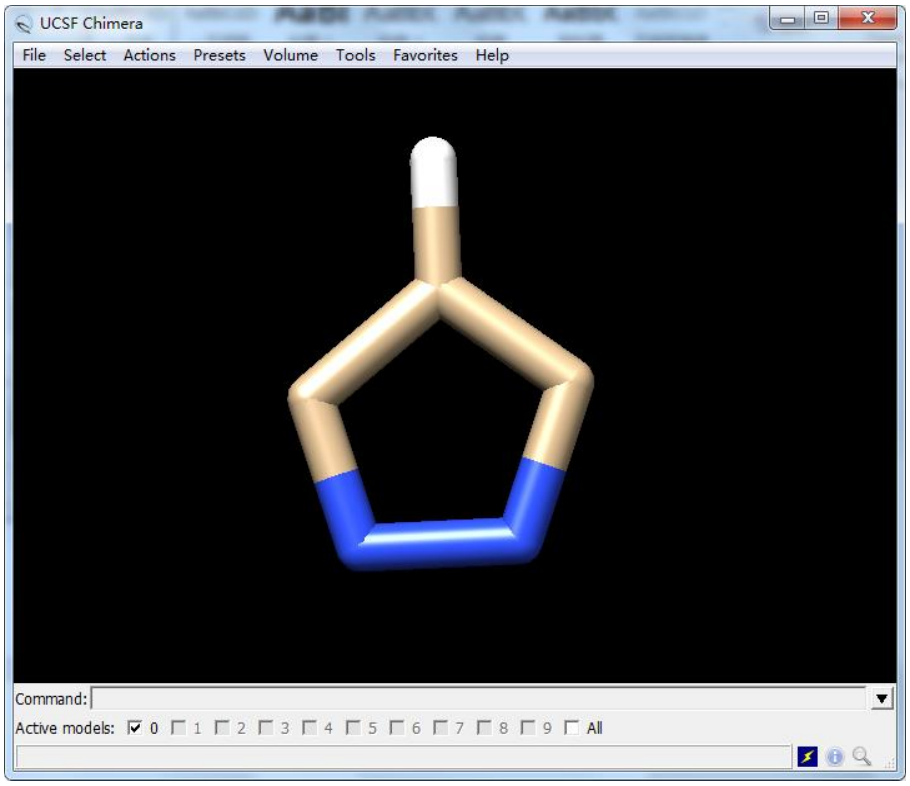
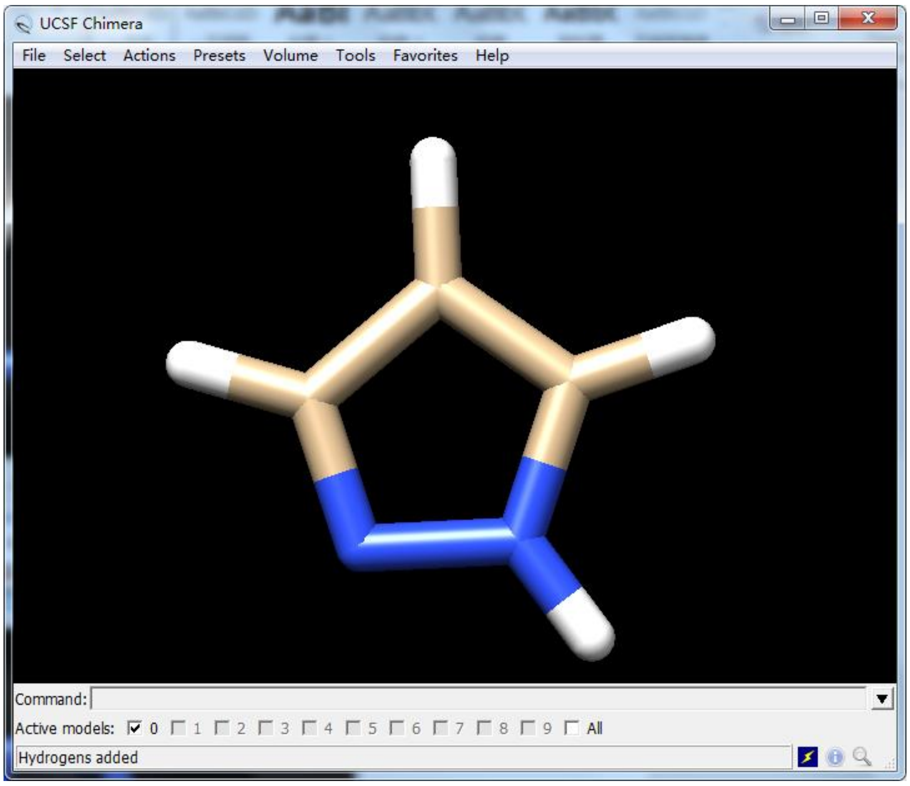

UCSF Chimera
File Select Actions Presets Volume Tools Favorites Help
Command:
Active models: 0 1 2 3 4 5 6 7 8 9 All

Figure 24. Initial growth fragment.

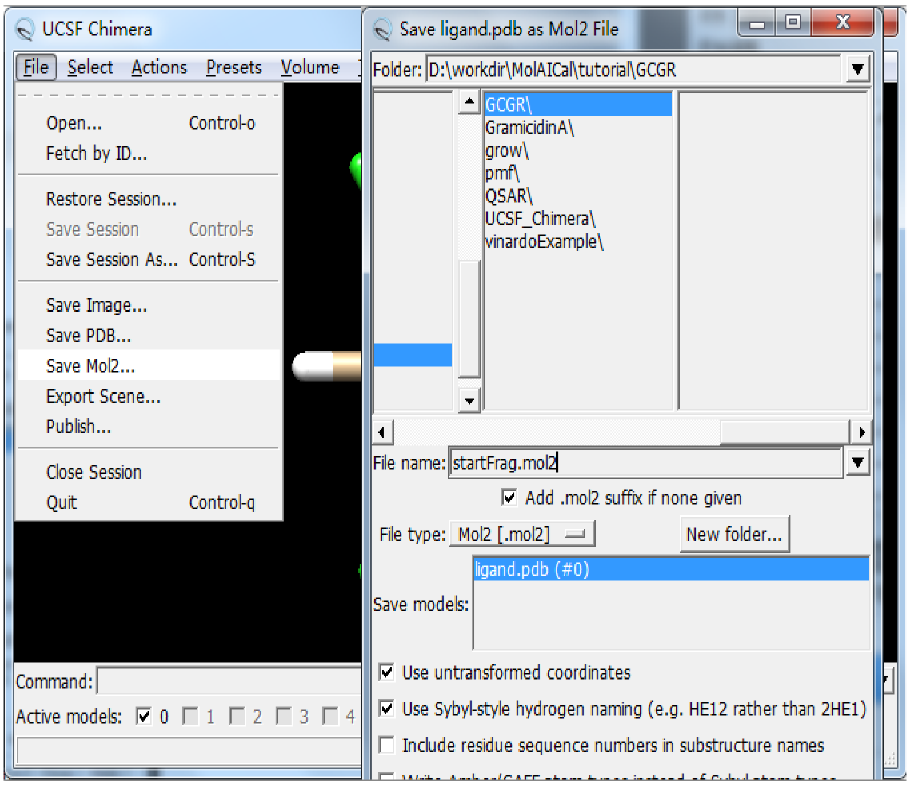
4. To let the molecule grow in the right way, the initial fragment part should add hydrogen, so "Tools→Structure Editing→AddH" is performed (see Figure 25).


UCSF Chimera
File Select Actions Presets Volume Tools Favorites Help
Command:
Active models: 0 1 2 3 4 5 6 7 8 9 All

Hydrogens added

Figure 25. Adding hydrogen in the initial growth fragment.

5. Then, save this fragment as Sybyl Mol2 format named "startFrag.mol2" (see Figure 26).



Folder: D:\workdir\MolAICal\tutorial\GCGR

File name: startFrag.mol2

☑ Add .mol2 suffix if none given

File type: Mol2 [.mol2]

Save models: ligand.pdb (#0)

☑ Use untransformed coordinates

☑ Use Sybyl-style hydrogen naming (e.g. HE12 rather than 2HE1)

☐ Include residue sequence numbers in substructure names

Active models: ☑ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Figure 26. Saving coordinate of the initial growth fragment.

Option 2. No crystal structure of ligand in the pocket.
If there is no crystal structure of ligand in the pocket of receptor, you can choose an atom of the key residues according to the literature reported or your experience by the same procedure of option 1. And make this atom as the independent file and give an initial fragment with SMILES format. MolAICal will search the best position of SMILES format fragment in the pocket of GCGR automatically.

Set “growMethod” to randomFrag. Then add parameters “startAtomPosition” and “startSmiFrag”. The “startAtomPosition” contains one atom that is from your selected residues. The “startSmiFrag” is your appointed initial fragment with SMILES format. The parameter instance is as below:

---

growMethod	randomFrag
startFragFile	D:/GCGR/genstartFrag.mol2
startAtomPosition	D:/GCGR/resPosition.pdb
startSmiFrag	C

Notice: the startSmiFrag decides the initial seed. If the value of “startSmiFrag” is Canonical SMILES string, the seed file which is assigned by parameter “startFragFile” will be generated according to this Canonical SMILES string. If the value of “startSmiFrag” is null, the user must supply their made 3D initial seed file with mol2 format in the part of parameter “startFragFile”. Then, MolAICal can search the suitable position of the user-defined fragment in the receptor pocket, automatically.

Here, the **option 1** is chosen for drug design.

3.5. Running the de novo drug design

There are two options: Option 1 is running de novo drug design by AI model and classical programming. Option 2 is running de novo drug design by pure classical programming. To save the beginner's time, you can try any below option for your learning.

Option 1. De novo drug design by AI model and classical programming.

Set "libStyle" to AIFrag. The instance is as below:

```
----------------------------------------------------------------------------------------------------------------
# define read library way: mol2, SMILES, AIFrag
libStyle                            AIFrag
----------------------------------------------------------------------------------------------------------------
```

Note: if it shows the err information: "*Warning: Atom 9 C.3 overlaps with protein!*" and "*Warning: some atoms overlap with protein.*" You can minimize the receptor-ligand complex by MD simulations tools or UCSF Chimera, etc. Only the win64 or linux64 version of MolAICal can perform in this option.

Change console to the directory "001-AIGrow" according to the specific position of tutorial material.

```
#> cd 001-AIGrow
```

Finally, run the following command in the background:

For Linux:

```
#> molaical.exe -denovo grow -i InputParFileAI.dat >& denovo.log &
```

For Windows (using PowerShell):

```
#> molaical.exe -denovo grow -i InputParFileAI.dat
```

If you want to run it background, you can run below command:

```
#> powershell -windowstyle hidden -command "molaical.exe -denovo grow -i InputParFileAI.dat"
```

Option 2. De novo drug design by classical programming

Set "libStyle" to mol2. The MolAICal will use the user-defined libraries. The instance is as below:

```
----------------------------------------------------------------------------------------------------------------
# define read library way: mol2, SMILES, AIFrag
libStyle                            mol2
----------------------------------------------------------------------------------------------------------------
```

Note: if it shows the err information: "*Warning: Atom 9 C.3 overlaps with protein!*" and "*Warning: some atoms overlap with protein.*" You can minimize the receptor-ligand complex by MD simulations tools or UCSF Chimera, etc. Any version of MolAICal can perform in this option.

MolAICal can invoke many CPU cores for de novo drug design by JAVA parallel stream, you should define the parameter "coreNum" according to your computer in the input configure file.

Change console to the directory "001-AIGrow" according to the specific position of tutorial material.

```
#> cd 001-AIGrow
```

Finally, run the following command in the background:

For Linux:

```
#> molaical.exe -denovo grow -i InputParFileCP.dat >& denovo.log &
```

For Windows (using PowerShell):

```
#> molaical.exe -denovo grow -i InputParFileCP.dat
```

If you want to run it background, you can run below command:

```
#> powershell -windowstyle hidden -command "molaical.exe -denovo grow -i InputParFileCP.dat"
```

4. Results

You can find the directory "results" when the program finishes. In this tutorial, the generated cycle is set to 30. It needs about 1~2 day by using 30 CPU cores. If you try to see the results, please open the "results" directory of 001-AIGrow. Then you can find a file named "AstatisticsFile.dat" which contains the drug design information. It is just an example that does not contain all results. You can find information in the "AstatisticsFile.dat" as blow:

```
-------------------------------------------------------------------------------------------------------------------
ID   Name   Cluster   Affinity(kcal/mol) Formula   InChIKey   Synthetic_Accessibility
1   lig_1.mol2 [1]   -3.27   C9H13N6O14S2   BEQXRJFDXZCPLY-GRQBKTHUSA-O   77.41
2   lig_2.mol2 [1]   -7.67   C13H13N11O9S   IQOZHKOALGXOOC-WVXRZKCLSA-O   75.31
…………….
-------------------------------------------------------------------------------------------------------------------
```

The "Affinity" is the binding score. The "Cluster" represents the k-means cluster results. You can pick up the representative ligands for your research. Synthetic_Accessibility represents a score that ranges from 0 to 100. The value 100 is maximal synthetic accessibility which means this compound is most easily synthesizable. For example, load "GCGRNoLigand.pdb", "ligand.mol2" and "lig_2.mol2" by UCSF Chimera (see Figure 27). The ligand colored with red is "lig_2.mol2". It has grown the analog of GCGR crystal ligand named "ligand.mol2".

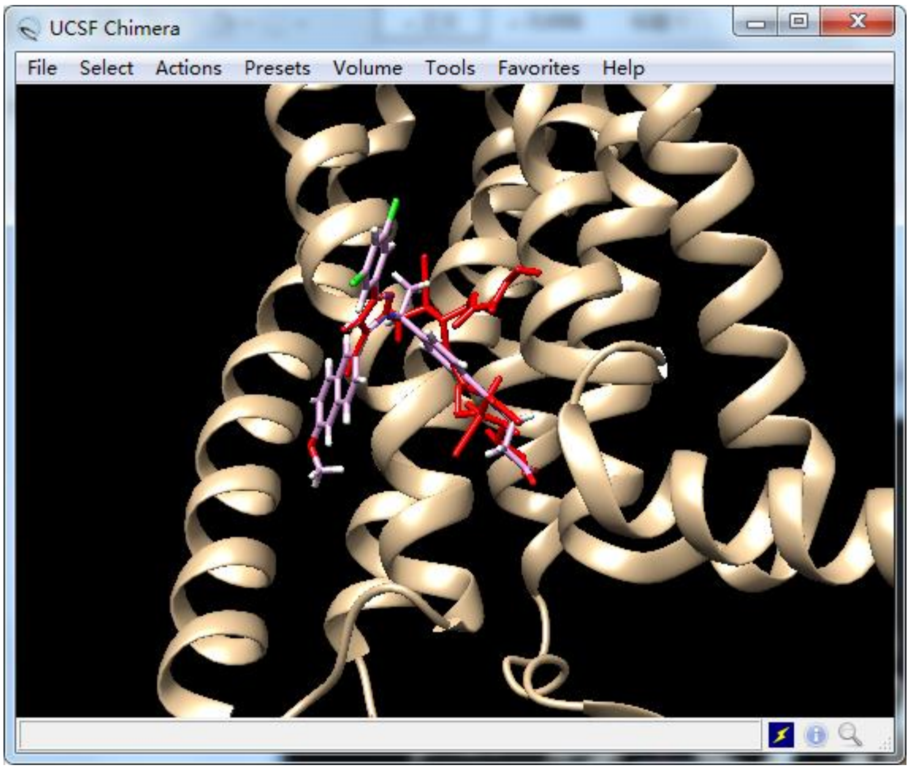
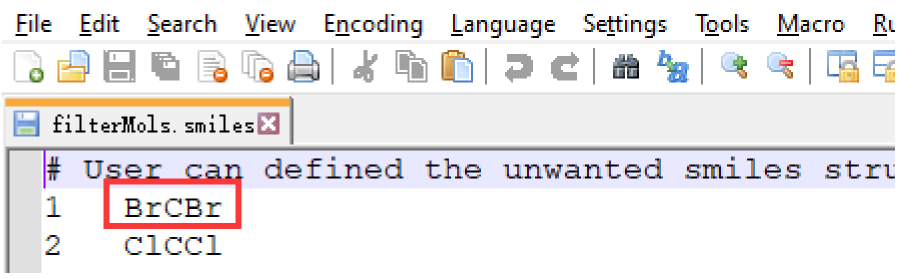


Figure 27. Results of drug design.

Notice: You may find weird hydrogen on hydroxyl (OH group). It may be due to the weird fragments generated by the deep learning model. **This hydrogen is not considered in the binding score. You can delete the hydrogen by UCSF Chimera, and then add hydrogen again.** To evaluate the binding ability of these ligands precisely, molecular dynamics simulation and MM/GBSA are recommended.

Tricks: if users do not wish to get the generated molecules that contain the unwanted fragments (e.g. weird fragments, *etc*), users can set the SMILES strings of unwanted fragments in "MolAICal-xxx\filterMols\filterMols.smiles". One unwanted fragment is put in an independent line of "MolAICal-xxx\filterMols\filterMols.smiles" like below:


File Edit Search View Encoding Language Settings Tools Macro Ru
filterMols.smiles

```
# User can defined the unwanted smiles stru
1   BrCBr
2   ClCCl
```

Where "MolAICal-xxx" is the downloaded version of MolAICal. The SMILES strings are in OpenSMILES (https://github.com/opensmiles/OpenSMILES). Users can convert mol2 to OpenSMILES by MolAICal (For more information, please check the manual of MolAICal):

```
#> molaical.exe -tool mol2tosmi -i "D:/mol2tosmi/zinc_98180786.mol2"
```