

VIII.1 VALIDASI METODE DOCKING (3E9S.PDB)

8.1.1. Preparasi Protein dan *ref_ligand*

Langkah-langkah:

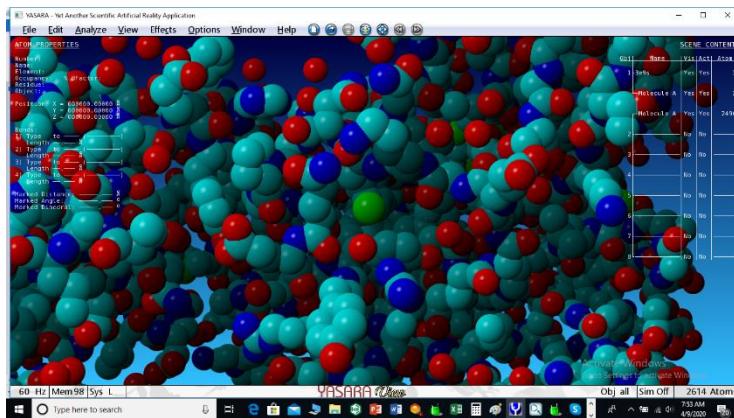
Buat folder Validasi 3E9S di Hardisk D

8.1.1.1. Buka link berikut: <https://www.rcsb.org/structure/3E9S>

8.1.1.2. Download file 3E9S.PDB Lihat gambar berikut ini:



8.1.1.3. Buka YASARA (klik shortcut YASARA di desktop). Load file 3E9S.PDB ke YASARA (YASARA | File > Load > PDB File ... cari direktori tempat menyimpan file tersebut, klik "OK")



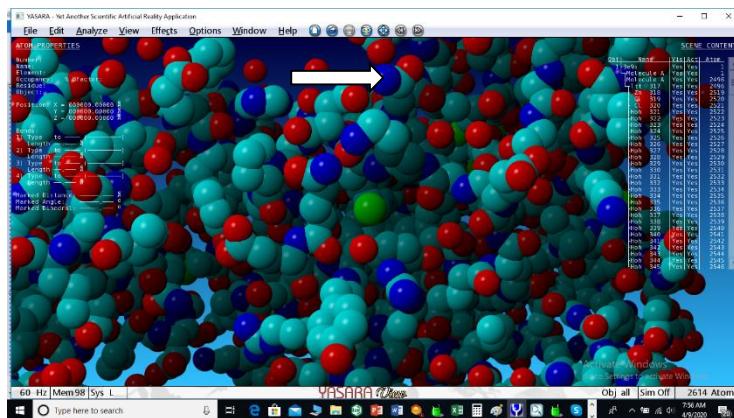
Catatan:

Berbeda dengan aplikasi visualiasi lain seperti MOE dan PyMOL, di YASARA molekul akan mengalami perpindahan koordinat 3D jika digeser atau diubah posisinya secara visual.

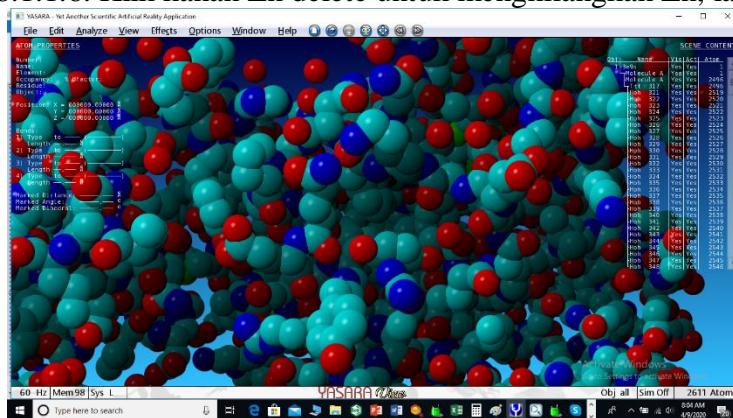
8.1.1.4. Hapus bagian dari system yang tidak diperlukan dalam *protocol docking* (yang dibutuhkan hanya satu protein, termasuk air jika esensial, dan satu ligan).

8.1.1.5. Klik 3e9s dibawah name

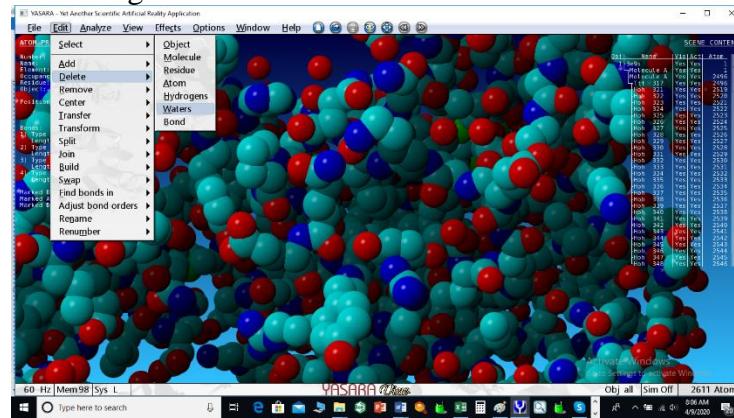
Klik molecule A bagian bawah akan tampak

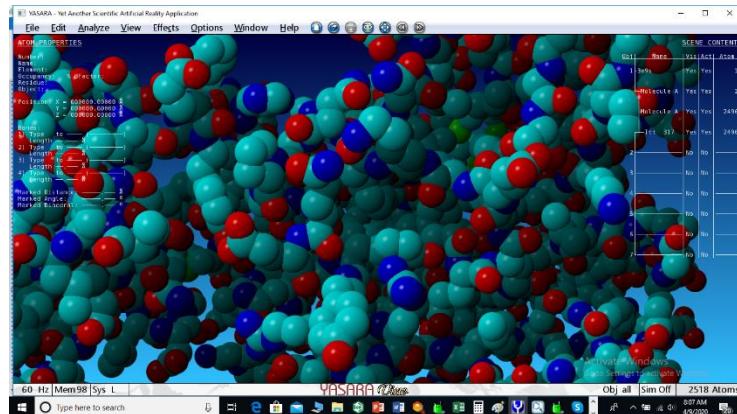


8.1.1.6. Klik kanan Zn delete untuk menghilangkan Zn, lakukan juga untuk Cl secara bertahap

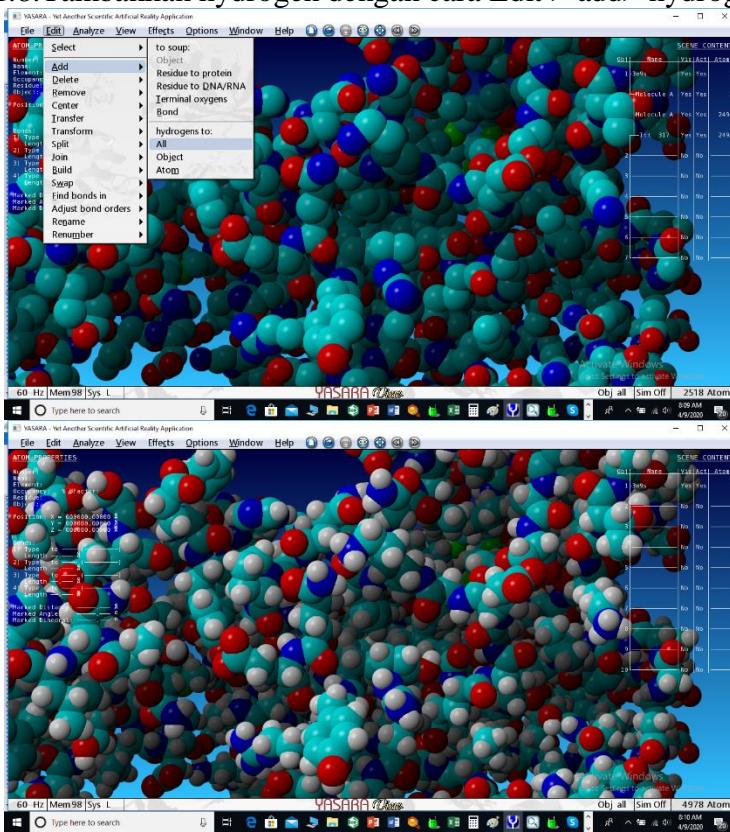


8.1.1.7. Hilangkan air dengan cara Edit > delete water

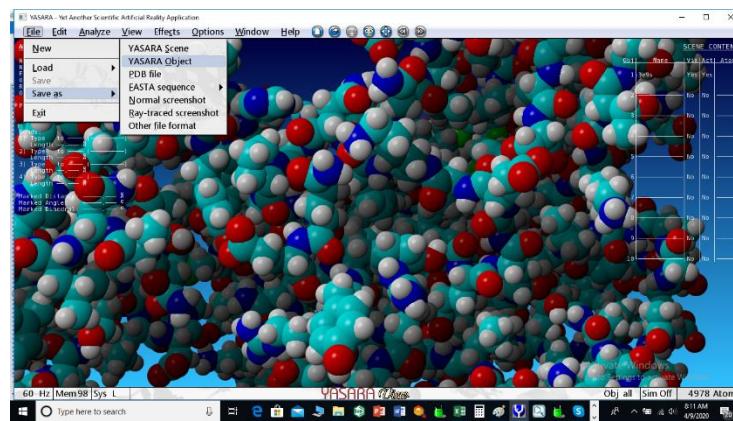




8.1.1.8.Tambahkan hydrogen dengan cara Edit > add> hydrogen > All



8.1.1.9.Simpan sebagai 3E9S.Yob dengan cara File > Save as > Yasara Obejct



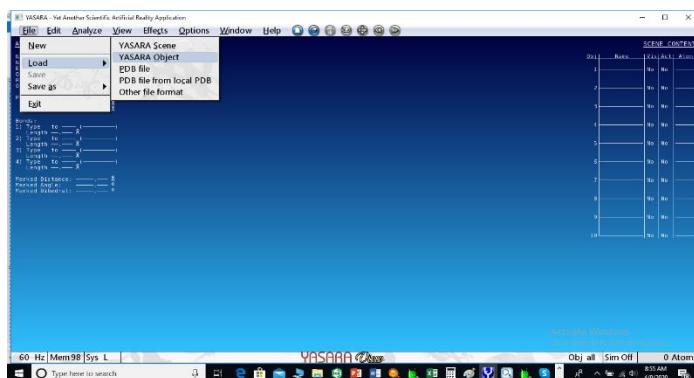
8.1.1.10. Simpan sebagai 3E9S.Yob dalam folder Validasi 3E9S.PDB seperti berikut

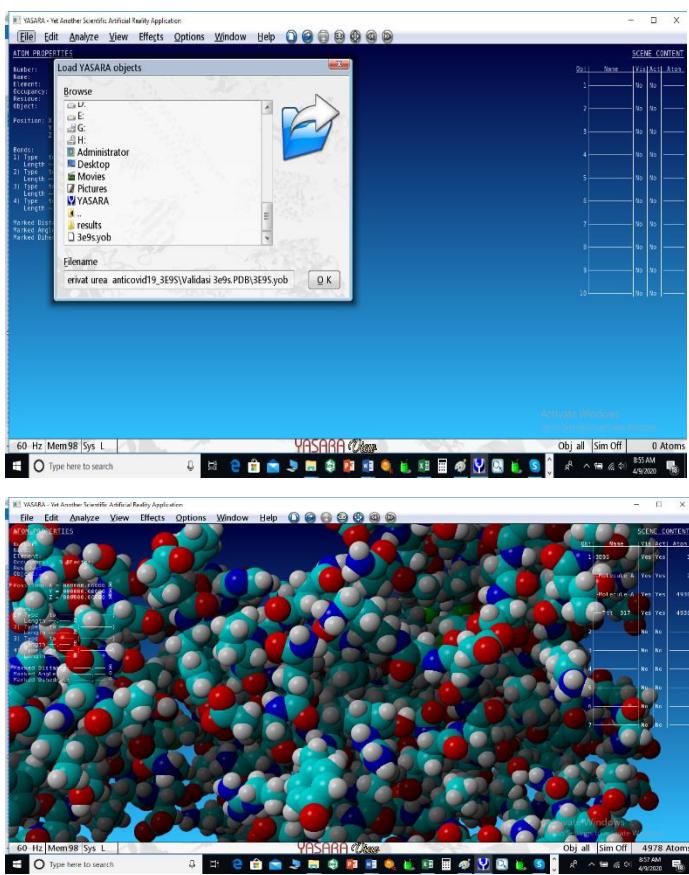


8.1.1.11. Preparasi Protein

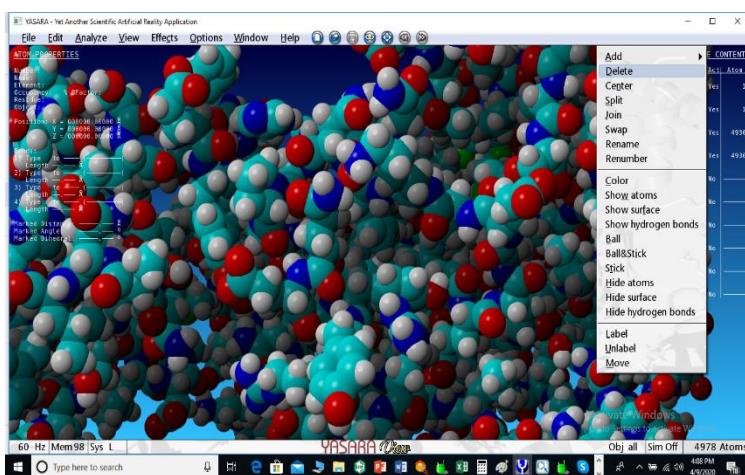
Buka YASARA dengan double klik

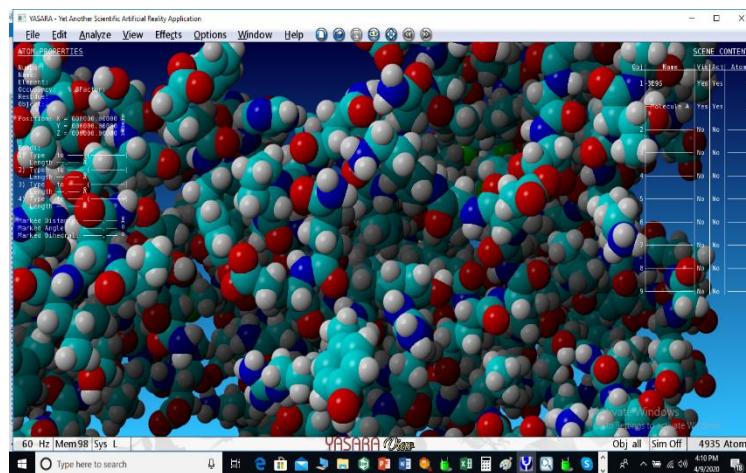
Load File 3E9S. Yob dengan cara





8.1.1.12 .Hilangkan ligand native Ttt dengan klik kanan pada ttt delete OK



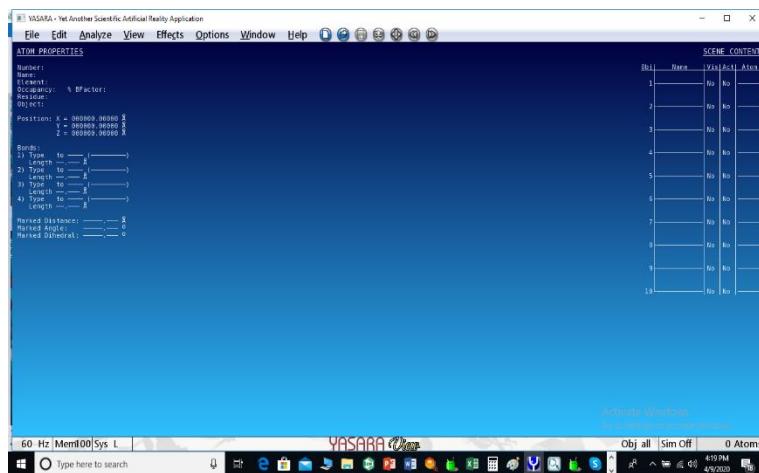


8.1.1.13. Simpan sebagai protein.mol2 pada folder Validasi 3E9S dengan cara seperti tampilan berikut

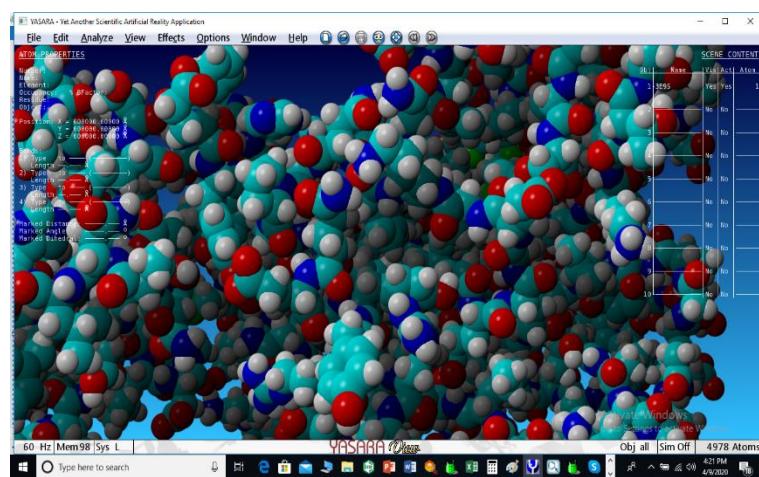
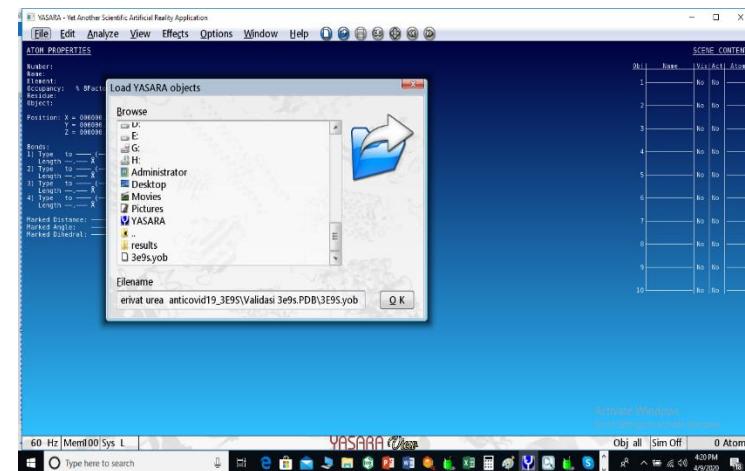
Preparasi protein.mol2 selesai

8.1.1.14. Preparasi ref_ligand.mol2

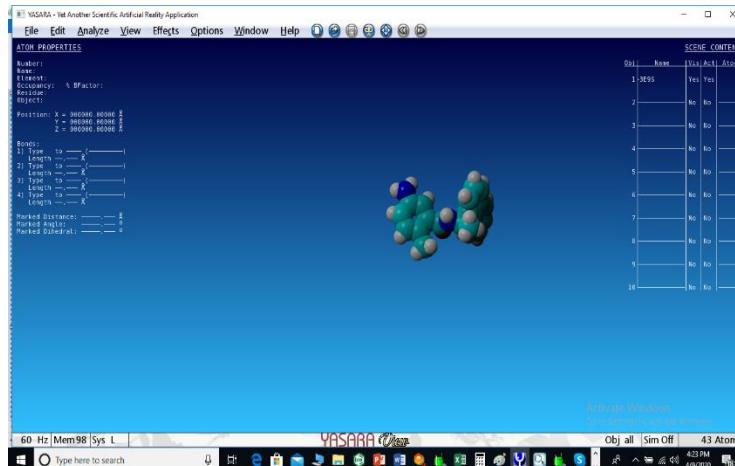
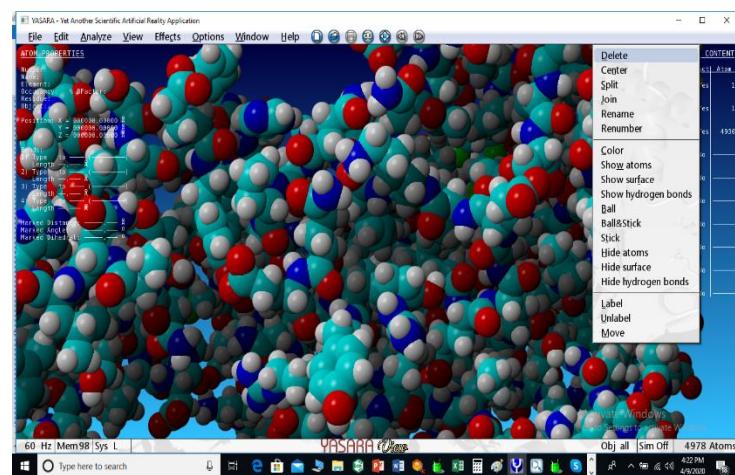
Buka layar baru dengan cara
File > New YES



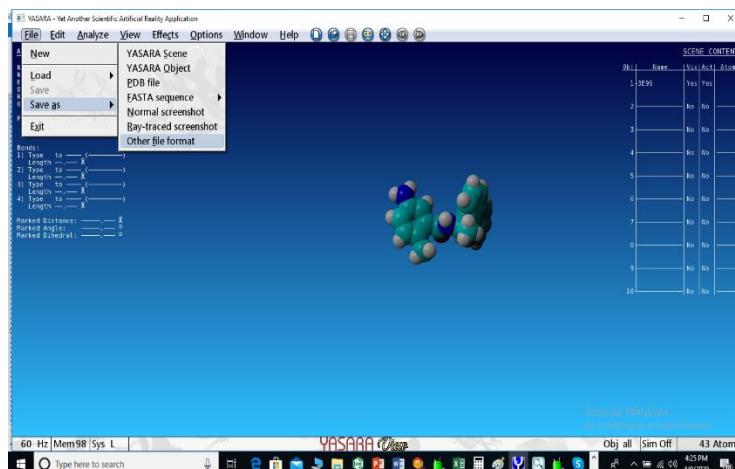
Load file 3e9s.yob

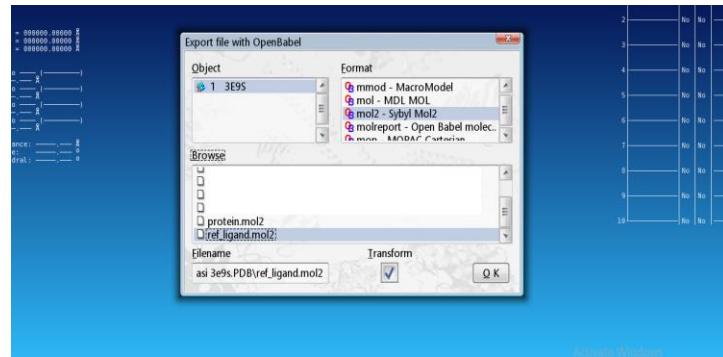


8.1.1.15. Klik kanan molecule A bagian atas > klik delete



8.1.1.16. Simpan sebagai ref_ligand.mol2 di folder Validasi 3E9S dengan cara berikut : File >save as > other file format> klik 3E9S pada object > klik Mol2-Sybyl Mol2 pada format dan tempatkan pada folder Validasi 3E9S lalu ganti * dengan ref_ligand.mol2





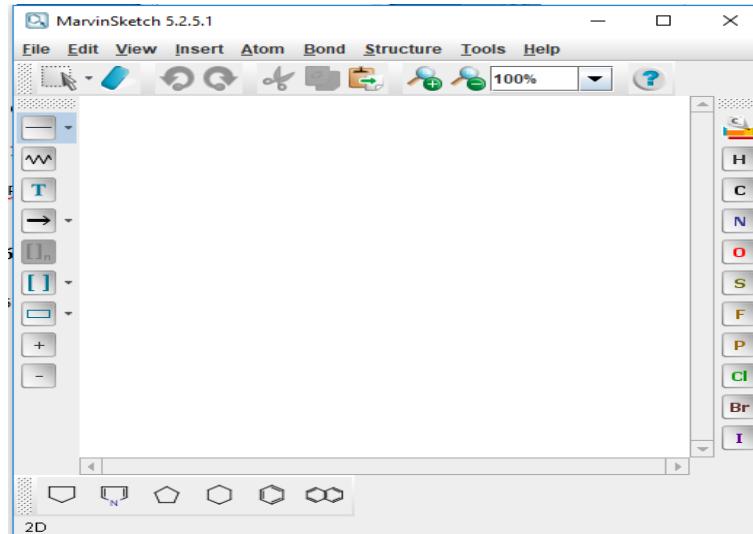
OK

Preparasi protein.mol2 dan ref_ligand.mol2 sudah selesai YASARA dapat ditutup.

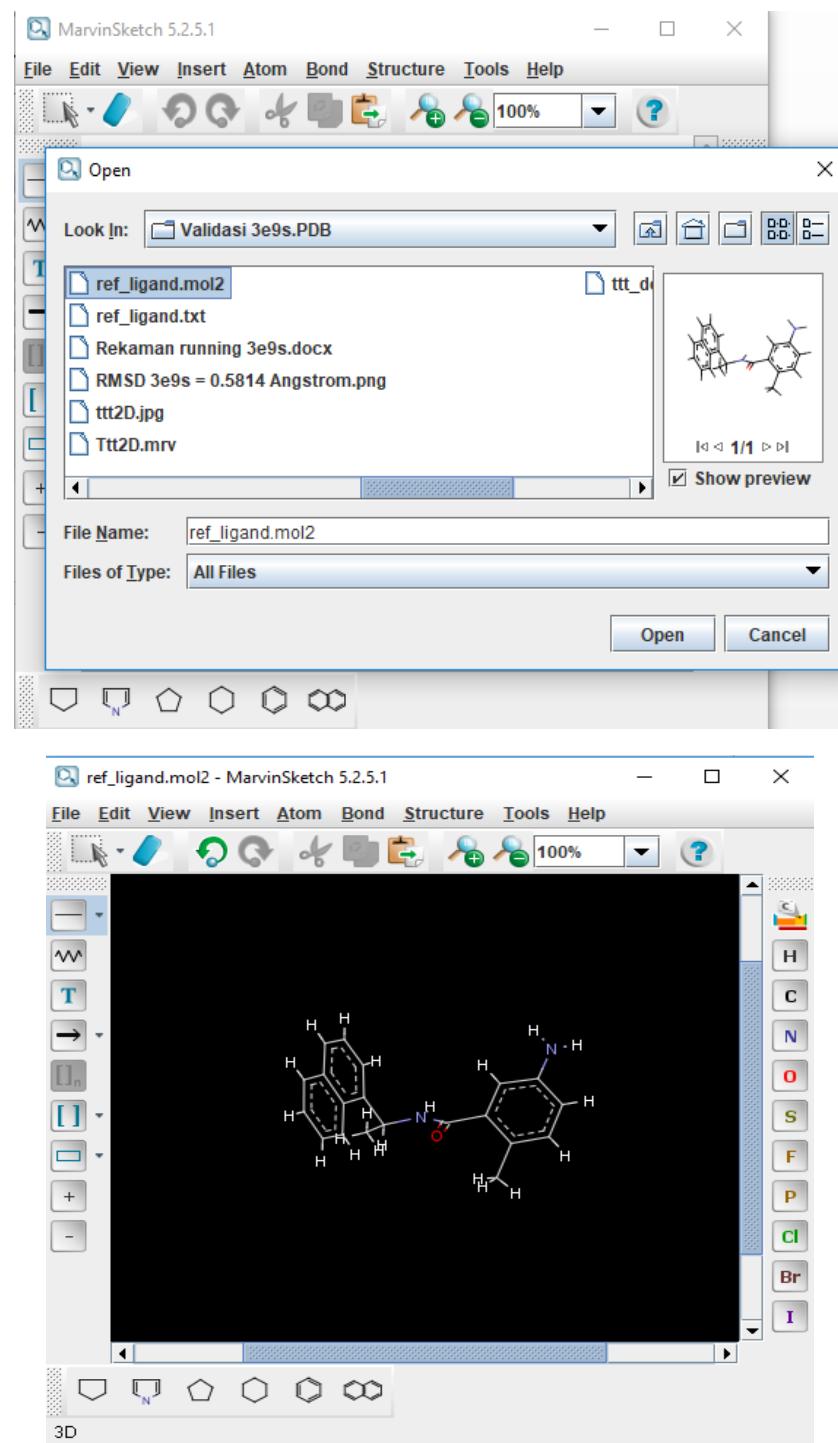
Perhatikan disitu ada file protein yang sudah dibuat sebelumnya

8.1.2. Preparasi ligand

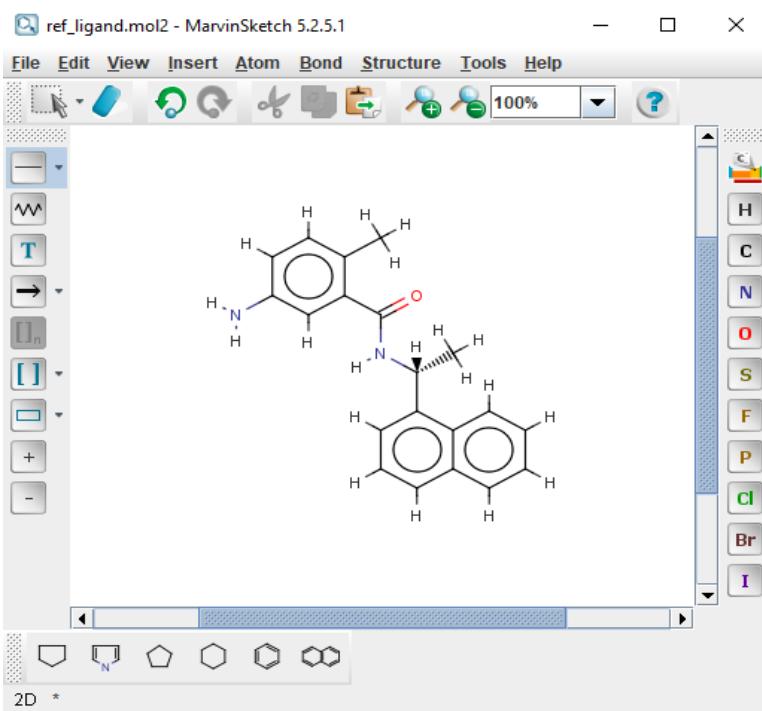
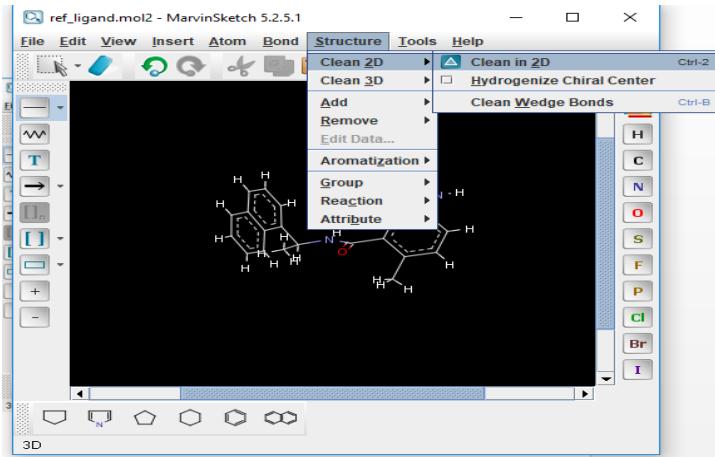
8.1.2.1. Buka MarvinSketch dengan double klik



8.1.2.2. Load file ref_ligand.mol2 yang sudah dibuat sebelumnya dengan cara berikut

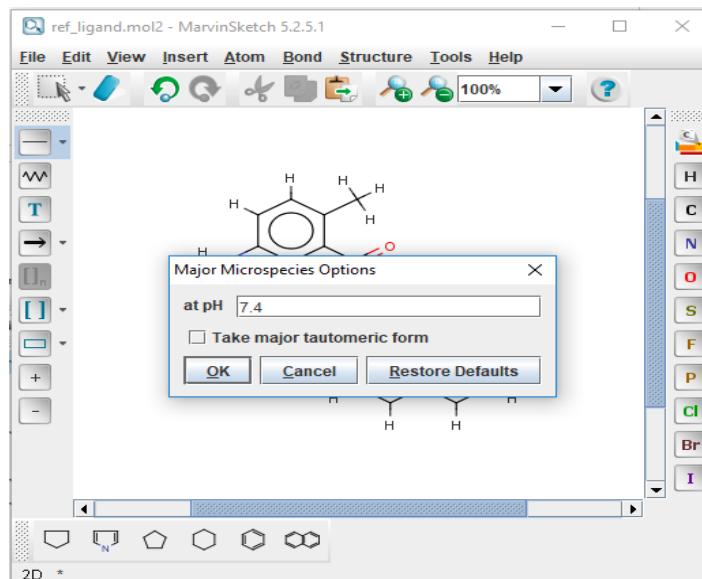
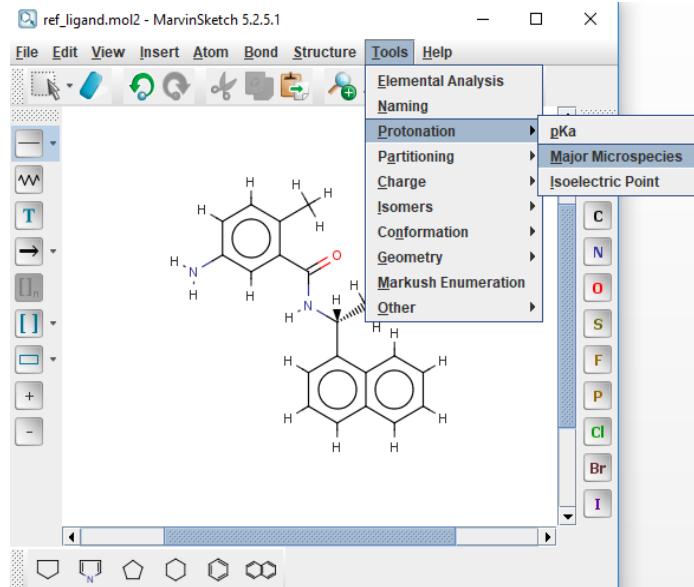


8.1.2.3. Rapikan struktur dengan cara klik structure > clean in 2D

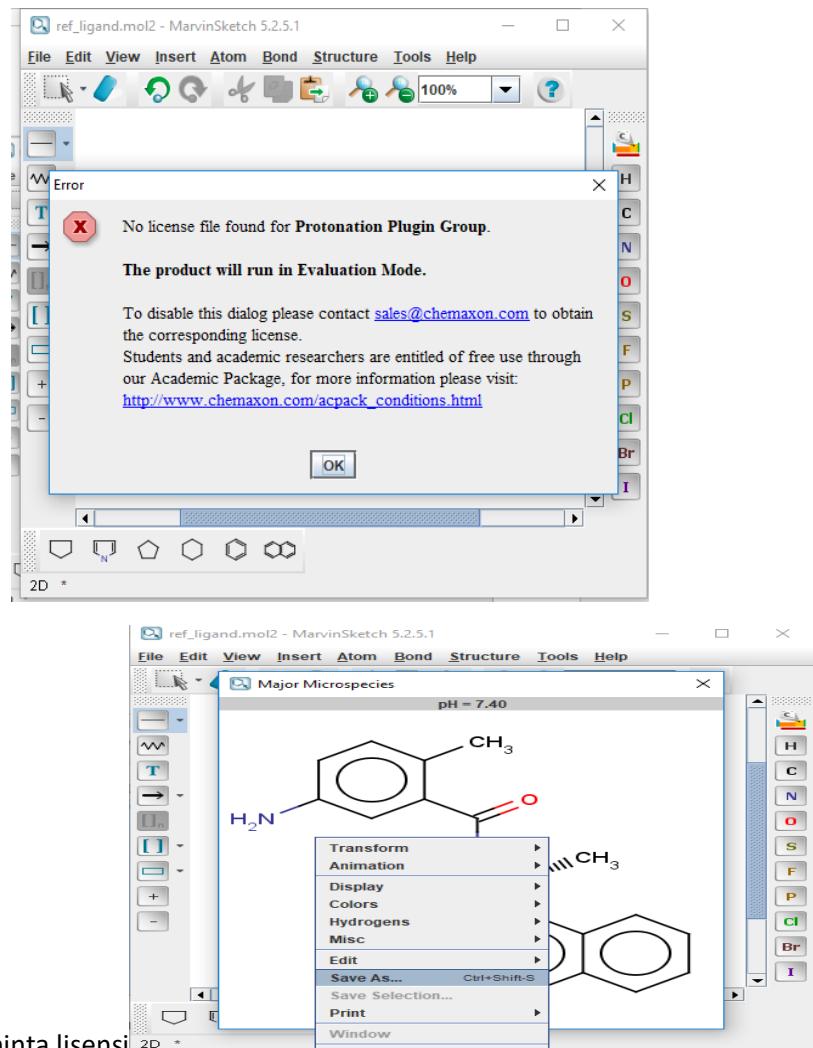


8.1.2.4. Molekul dikondisikan pada pH = 7.4 dan simpan sebagai

ligand 2D.mrv dengan cara



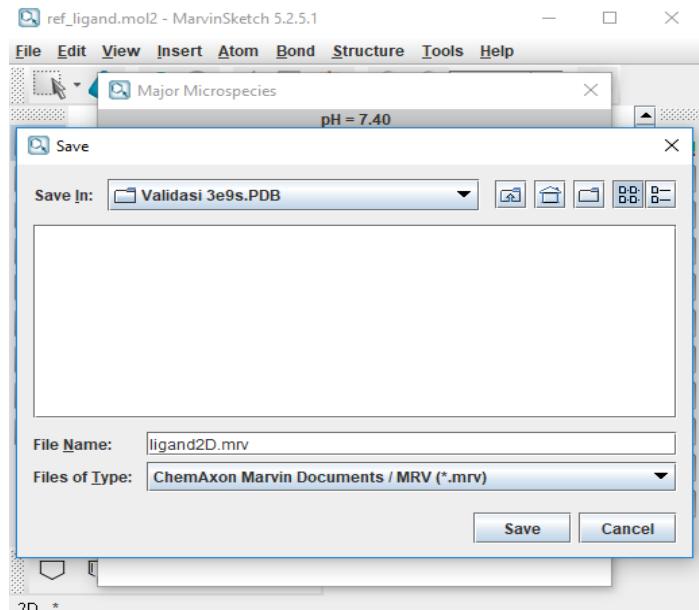
OK



Klik OK jika minta lisensi

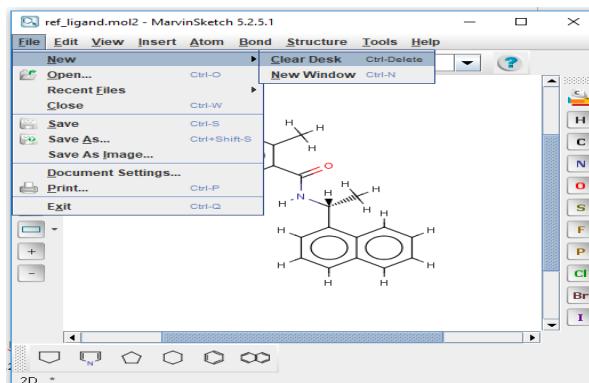
Klik kanan pada jendela baru lalu pilih Save as

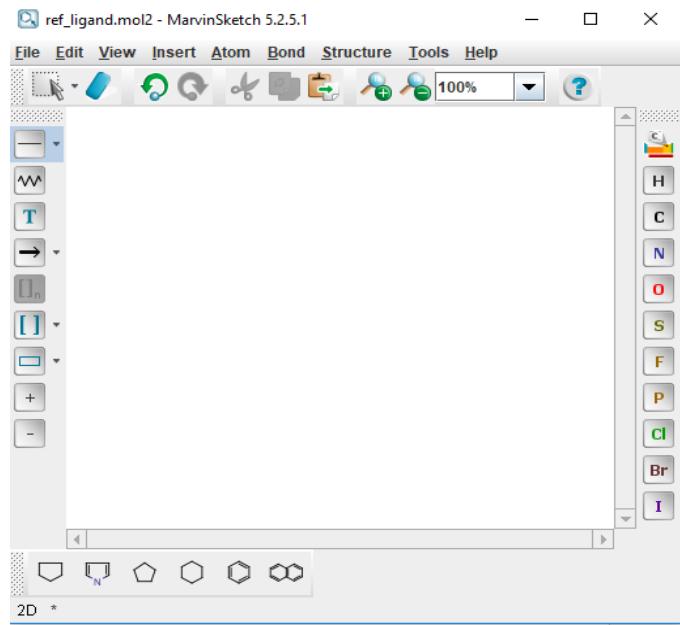
8.1.2.5. Simpan sebagai ligand2D.mrv pada folder Validasi 3E9S



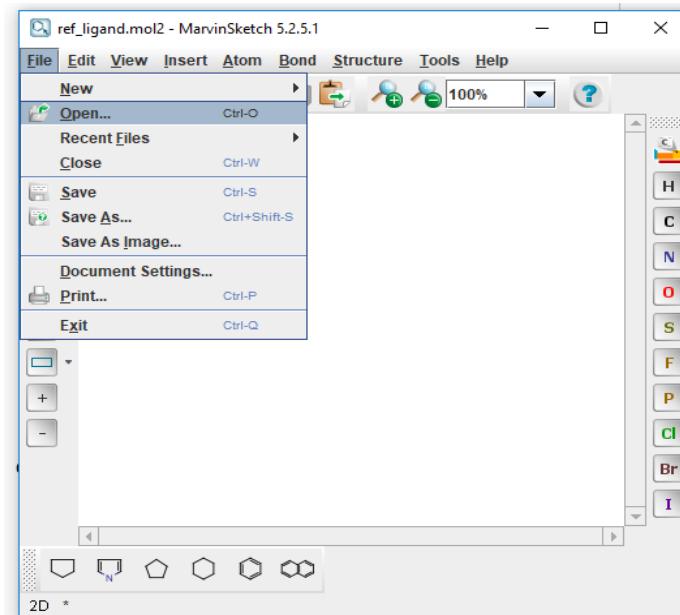
Klik Save

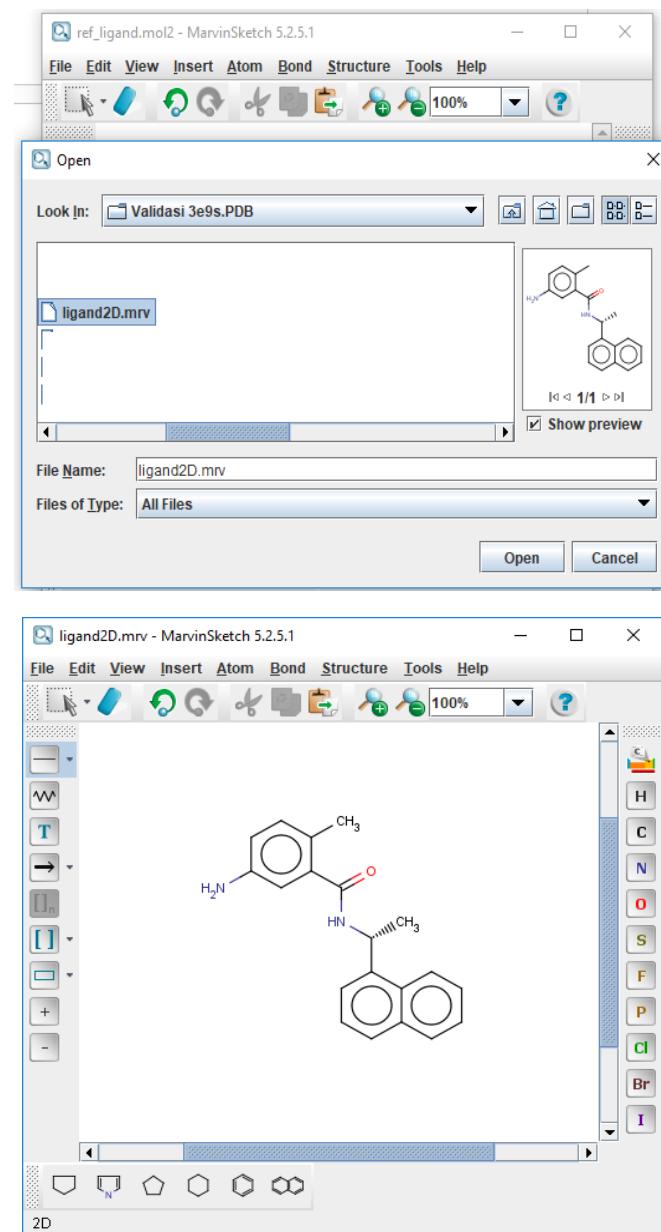
8.1.2.6. Bersihkan layar marvinSketch dengan cara File > New > Clear desk

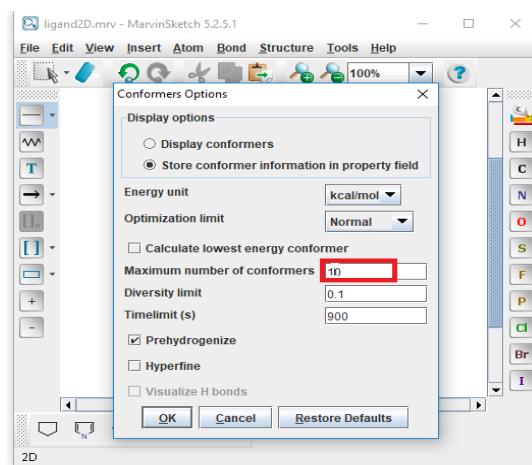
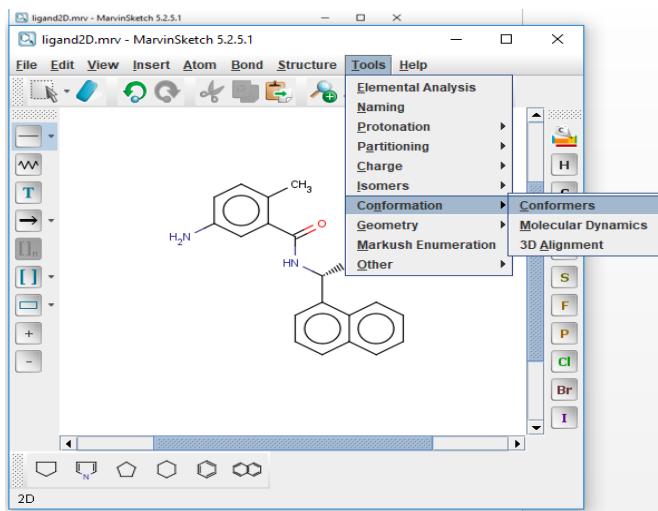




8.1.2.7. Load ligand2D.mrv yang sudah tersimpan sebelumnya

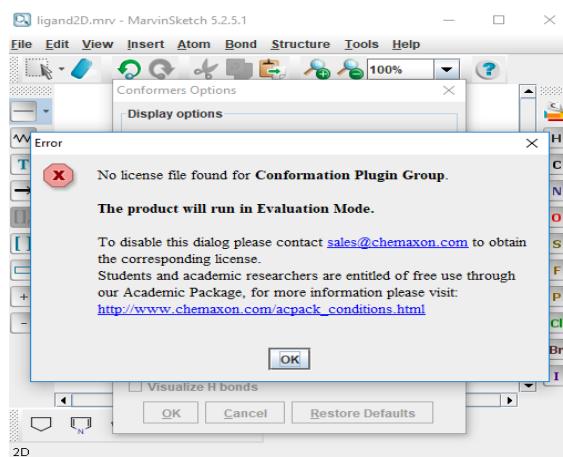




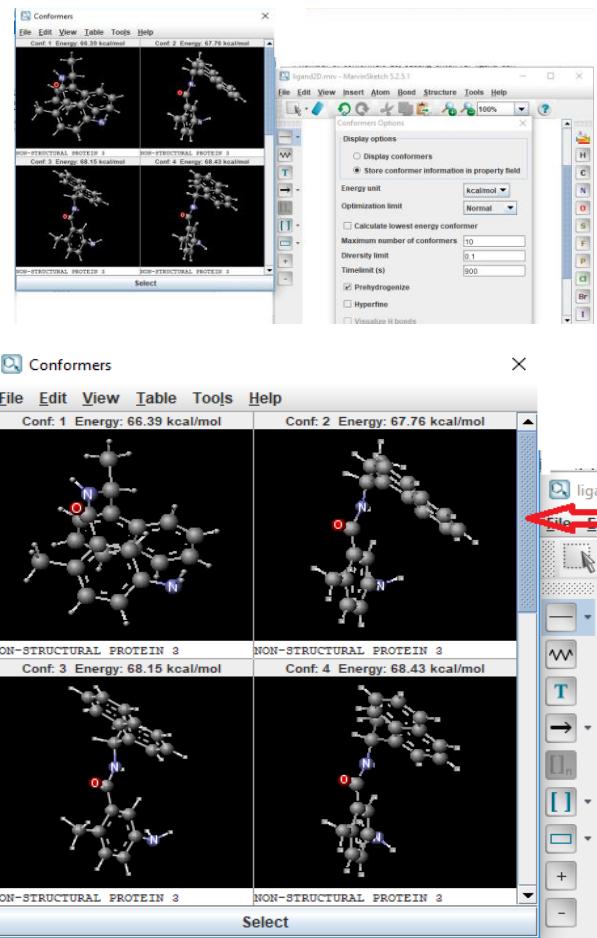


Untuk ref_ligand dari 3E9S.PDB validasinya kita menggunakan maximum number of conformers 10, sedang untuk ref_ligand dari 6LU7.PDB kita menggunakan maximum number of conformers 20. Defaultnya adalah 10 tetapi jika dengan conformer 10 belum tercapai validitasnya, maka dapat kita naikkan maximum number of conformers menjadi 20, 25 dan seterusnya.

8.1.2.8. Kita klik OK sehingga akan terlihat tampilan seperti berikut



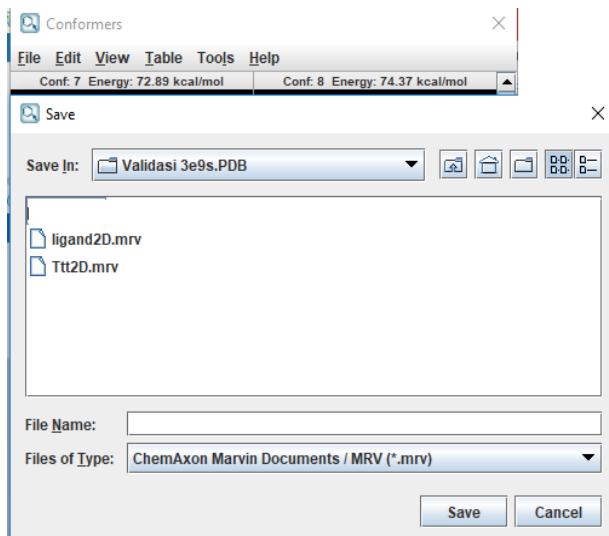
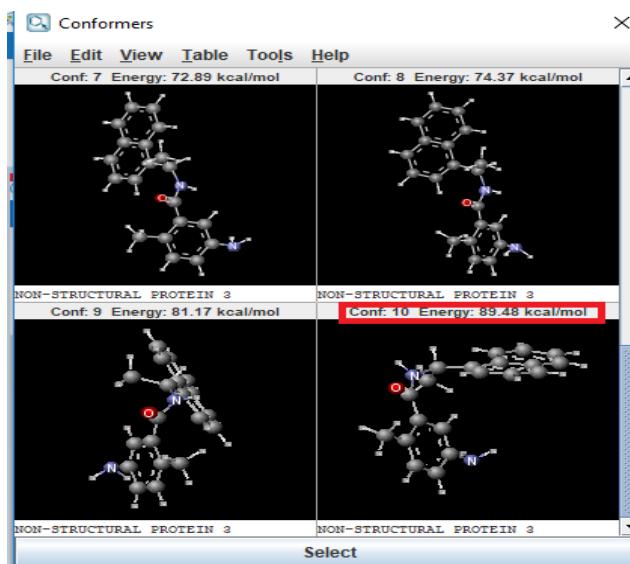
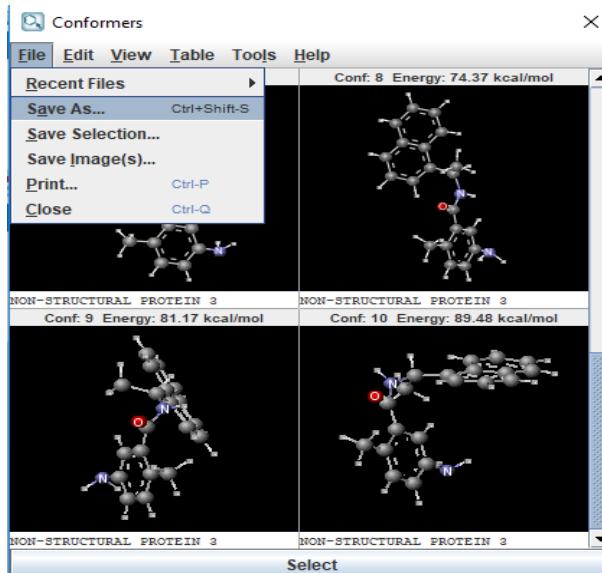
Klik OK



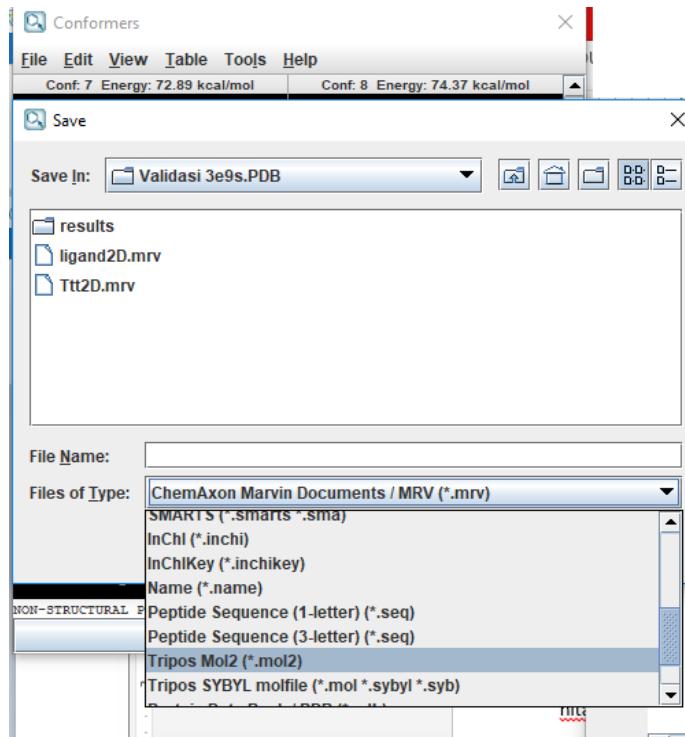
Terlihat ada 10 conformer jika cursor diletakkan sesuai anak panah merah dalam gambar dan digesarkan ke bawah.

8.1.2.9. Simpan ke 10 conformer di folder Validasi3E9S sebagai ligand.mol2 dengan cara sebagai berikut:

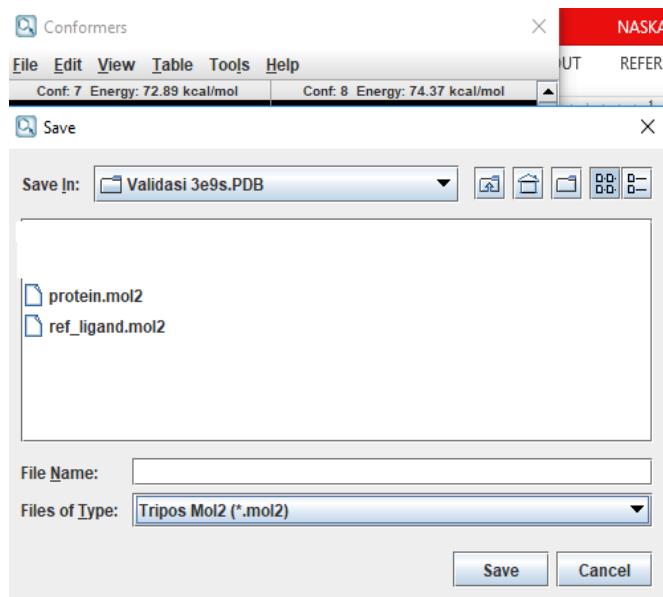
File > Save as



Di layar terlihat File of Type mrv, JANGAN DI SAVE, tetapi klik segitiga hitam dan geser ke bawah, cari type file Tripos.mol2 seperti berikut

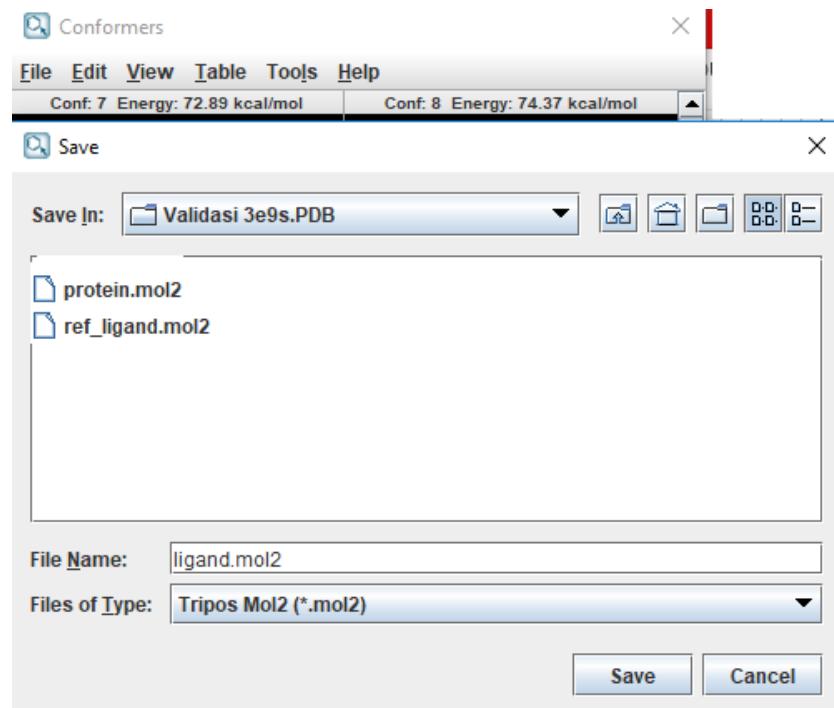


Klik Tripos.Mol2

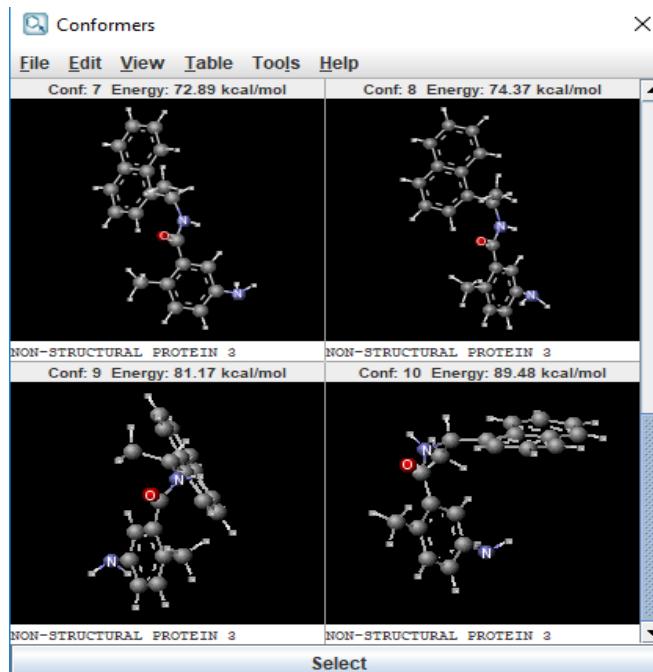


Ketik di File name : ligand.mol2

Di Folder Validasi 3e9s.PDB terlihat sudah ada file protein.mol2 dan ref_ligand.mol2 yang sudah kita buat sebelumnya.



Sekarang klik Save



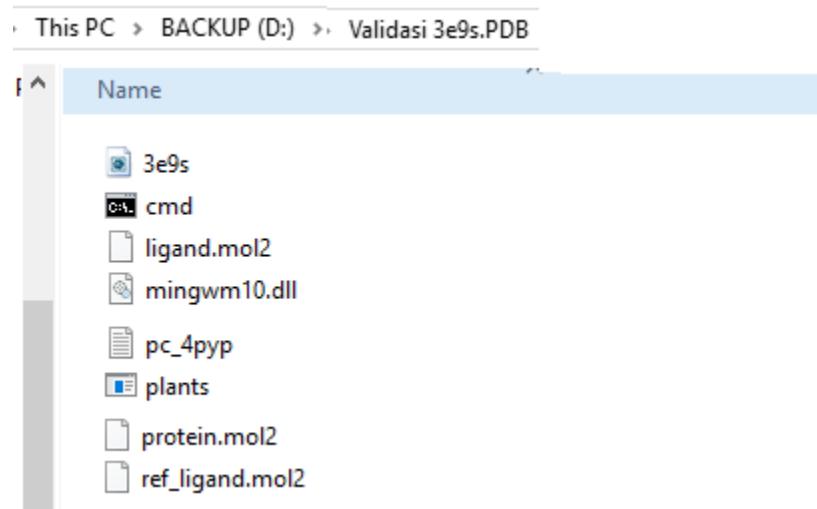
Preparasi ligand.mol2 sudah selesai. MarvinSketch dapat ditutup.

8.1.3. Running molecular docking

Periksa folder Validasi 3E9S.PDB, yang harus ada adalah file:

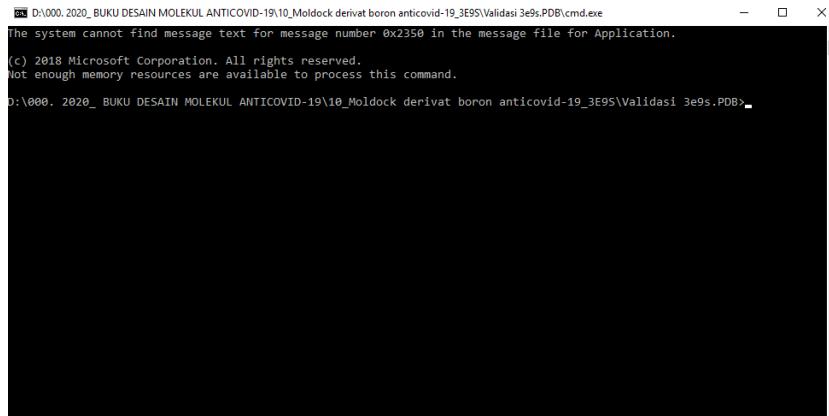
cmd.exe
plants.exe
mingwm10.dll
pc_4pyp.txt
protein.mol2
ref_ligand.mol2
ligand.mol2

Jika ada yang belum tersedia seperti cmd.exe, plants.exe ,mingwm10.dll , pc_4pyp.txt dapat dikopi dari CD yang menyertai buku ini.



Jika file yang diperlukan sudah lengkap lakukan

Double klik cmd pada folder Validasi 3E9S.PDB maka akan tampil



Perhatikan folder terakhir Validasi 3e9e.PDB >

Ketik plants --mode bind ref_ligand.mol2 5 protein.mol2 ENTER

```
\Validasi 3e9s.PDB>plants --mode bind ref_ligand.mol2 5 protein.mol2

      PLANTS
      Protein-Ligand ANT System
      version 1.1

author: Oliver Korb
scientific contributors: T.E. Exner, T. Stuetzle
contact: Oliver.Korb@uni-konstanz.de

run PLANTS: PLANTS --mode screen yourconfigfile

Calculated bindingsite definition (from 1 ligands):
bindingsite_center -31.0613 21.8543 30.3307
bindingsite_radius 10.4627

PLANTS warning: Unknown atom type S.O2 requested.
PLANTS warning: VDW radius of unknown atom type S.O2 requested.
PLANTS warning: VDW radius of unknown atom type S.O2 requested.

PLANTSactiveSiteResidues.mol2 written...
PLANTSactiveSite.mol2 written...

D:\000. 2020_BUKU DESAIN MOLEKUL ANTICOVID-19\10_Moldock derivat boron anticovid-19_3E9S
\Validasi 3e9s.PDB>
```

Abaikan PLANTS warning jika molekul atau ref_ligand tidak mengandung S.O2

Buka pc_4pyp.txt



pc_4pyp - Notepad

File Edit Format View Help

```
# scoring function and search settings
scoring_function chemplp
search_speed speed1

# input
protein_file protein.mol2
ligand_file ligand.mol2

# output
output_dir results

# write single mol2 files (e.g. for RMSD calculation)
write_multi_mol2 0

# binding site definition
bindingsite_center 586.746 -29.9896 207.583
bindingsite_radius 14.4181

# cluster algorithm
cluster_structures 10
cluster_rmsd 2.0
```

Ganti

bindingsite_center 586.746 -29.9896 207.583

bindingsite_radius 14.4181 dari 4_pyp.tvt dengan

bindingsite_center -31.0613 21.8543 30.3307

bindingsite_radius 10.4627 dari 3e9s diatas sehingga menjadi

```
pc_4pyp - Notepad
File Edit Format View Help
# scoring function and search settings
scoring_function chemplp
search_speed speed1

# input
protein_file protein.mol2
ligand_file ligand.mol2

# output
output_dir results

# write single mol2 files (e.g. for RMSD calculation)
write_multi_mol2 0

# binding site definition
bindingsite_center -31.0613 21.8543 30.3307
bindingsite_radius 10.4627

# cluster algorithm
cluster_structures 10
cluster_rmsd 2.0
```

Simpan Save as sebagai pc_3e9s.txt

Kembali ke cmd

Ketik plants –mode screen pc_3e9s.txt ENTER

D:\Validasi 3e9s>plants --mode screen pc_3e9s.txt

PLANTS

Protein-Ligand ANT System

version 1.1

author: Oliver Korb

scientific contributors: T.E. Exner, T. Stuetzle

contact: Oliver.Korb@uni-konstanz.de

run PLANTS: PLANTS --mode screen yourconfigfile

PLANTS warning: Unknown atom type S.O2 requested.

PLANTS warning: VDW radius of unknown atom type S.O2 requested.

PLANTS warning: VDW radius of unknown atom type S.O2 requested.

PLANTS info: CHO hydrogen found 315 HD2 HIS

PLANTS info: CHO hydrogen found 317 HE1 HIS

PLANTS info: CHO hydrogen found 774 HD2 HIS

PLANTS info: CHO hydrogen found 776 HE1 HIS

PLANTS info: CHO hydrogen found 821 HD2 HIS

PLANTS info: CHO hydrogen found 823 HE1 HIS

PLANTS info: CHO hydrogen found 1190 HD2 HIS

PLANTS info: CHO hydrogen found 1192 HE1 HIS

PLANTS info: CHO hydrogen found 1440 HD2 HIS

PLANTS info: CHO hydrogen found 1442 HE1 HIS

PLANTS info: CHO hydrogen found 2724 HD2 HIS

PLANTS info: CHO hydrogen found 2726 HE1 HIS

PLANTS info: CHO hydrogen found 2796 HD2 HIS

PLANTS info: CHO hydrogen found 2798 HE1 HIS

PLANTS info: CHO hydrogen found 3052 HD2 HIS

PLANTS info: CHO hydrogen found 3054 HE1 HIS

PLANTS info: CHO hydrogen found 4251 HD2 HIS

PLANTS info: CHO hydrogen found 4253 HE1 HIS

PLANTS info: CHO hydrogen found 4303 HD2 HIS

PLANTS info: CHO hydrogen found 4305 HE1 HIS

PLANTS info: CHO hydrogen found 4526 HD2 HIS

PLANTS info: CHO hydrogen found 4528 HE1 HIS

virtual screening progress: 1 of 1

current ligand: ligand.mol2 (entry 1)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -111.47

current ligand: ligand.mol2 (entry 2)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf
optimization finished after 0.00s
best score: -110.56

current ligand: ligand.mol2 (entry 3)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -111.05

current ligand: ligand.mol2 (entry 4)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -110.51

current ligand: ligand.mol2 (entry 5)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -109.99

current ligand: ligand.mol2 (entry 6)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -110.13

current ligand: ligand.mol2 (entry 7)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -111.06

current ligand: ligand.mol2 (entry 8)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -111.07

current ligand: ligand.mol2 (entry 9)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -110.76

current ligand: ligand.mol2 (entry 10)

LIGAND DOFs: 11

PROTEIN DOFs: 4

Simplex dimension: 15

Simplex dimension: 15

iterations : 290

starting optimization ...

problem dimension: 15

ATOMS / s: inf

EVAL / s: inf

optimization finished after 0.00s

best score: -109.74

total virtual screening time: 0.00s

Ligands skipped due to errors: 0

Setelah Ligands skipped due to errors: 0

Ketik cd results ENTER

D:\Validasi 3E9S.PDB>cd results

Lalu ketik more bestranking.csv ENTER

D:\ Validasi 3E9S.PDB \results>more bestranking.csv

Akan muncul di layar seperti berikut

TOTAL_SCORE,SCORE_RB_PEN,SCORE_NORM_HEVATOMS,SCORE_NORM_CRT_HEVATOMS,SCORE_NO
RM_WEIGHT,SCORE_NORM_CRT_WEIGHT,SCORE_RB_PEN_NORM_CRT_HEVATOMS,SCORE_NORM_CO
NTACT,EVAL,TIME

NON-STRUCTURAL PROTEIN 3_entry_00001_conf_01,-111.466,-101.466,-4.84634,-39.1952,-0.366206,-
16.5705,-35.6788,-4.45863,2465643,0

NON-STRUCTURAL PROTEIN 3_entry_00002_conf_01,-110.561,-100.561,-4.807,-38.877,-0.363233,-
16.436,-35.3606,-4.42244,2514226,0

NON-STRUCTURAL PROTEIN 3_entry_00003_conf_01,-111.048,-101.048,-4.82816,-39.0481,-0.364832,-
16.5084,-35.5318,-4.4419,2415642,0

NON-STRUCTURAL PROTEIN 3_entry_00004_conf_01,-110.509,-100.509,-4.80476,-38.8588,-0.363064,-
16.4283,-35.3425,-4.42038,2513830,0

NON-STRUCTURAL PROTEIN 3_entry_00005_conf_01,-109.992,-99.9923,-4.78228,-38.677,-0.361365,-
16.3515,-35.1607,-4.39969,2575064,0

NON-STRUCTURAL PROTEIN 3_entry_00006_conf_01,-110.134,-100.134,-4.78844,-38.7269,-0.361831,-
16.3725,-35.2105,-4.40536,2495995,0

NON-STRUCTURAL PROTEIN 3_entry_00007_conf_01,-111.064,-101.064,-4.82887,-39.0538,-0.364886,-
16.5108,-35.5375,-4.44256,2515467,0

NON-STRUCTURAL PROTEIN 3_entry_00008_conf_01,-111.069,-101.069,-4.82908,-39.0556,-0.364902,-
16.5115,-35.5393,-4.44276,2552712,0

NON-STRUCTURAL PROTEIN 3_entry_00009_conf_01,-110.761,-100.761,-4.81569,-38.9473,-0.36389,-
16.4657,-35.431,-4.43044,2556745,0

NON-STRUCTURAL PROTEIN 3_entry_00010_conf_01,-109.735,-99.7353,-4.7711,-38.5866,-0.360521,-
16.3133,-35.0703,-4.5723,2425928,0

D:\Validasi 3E9S.PDB\results>

Cari skor terstabil (paling minus), dalam contoh yang diberi latar kuning.

Tiap tiap laptop atau PC dapat memberi hasil yang berbeda tentang skor terstabil tergantung spesifikasi laptop atau PC yang digunakan untuk running molecular docking.

Buka folder Validasi 3E9S.PDB maka akan terlihat folder baru **results** yang berisi hasil rekaman running docking.

Buka folder results dan cari file conformer terstabil tersebut

Name	Date modified	Type
bestranking	3/18/2020 5:52 PM	Microsoft Excel C...
constraints	3/18/2020 5:52 PM	Microsoft Excel C...
correspondingNames	3/18/2020 5:52 PM	Microsoft Excel C...
features	3/18/2020 5:52 PM	Microsoft Excel C...
ligand	3/18/2020 5:52 PM	Text Document
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_01.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_01_protein.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_02.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_02_protein.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_03.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_03_protein.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_04.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_04_protein.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_05.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_05_protein.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_06.mol2	3/18/2020 5:48 PM	MOL2 File
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_06_protein.mol2	3/18/2020 5:48 PM	MOL2 File

Dalam contoh ini terlihat file yang ditandai dengan kotak merah, kopi file tersebut dan paste kan di samping ref_ligand.mol2 berada atau di folder Validasi 3E9S.PDB

results	
3e9s	PLANTSactiveSiteResidues.mol2
3e9s.sce	protein.mol2
3e9s.yob	ref_ligand.mol2
bindingsite.def	pc_3e9s
cmd	pc_4pyp
ligand.mol2	plants
ligand2D	PLANTS-6880.pid
mingwm10.dll	PLANTSactiveSite.mol2
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_01.mol2	

File bestranking dalam folder results berisi resume hasil molecular docking yang dapat dibuka dengan excel

TOTAL_SCORE	SCORE_RB_PEN	SCORE_NC	SCORE_NC
NON-STRUCTURAL PROTEIN 3_entry_00001_conf_01	-111.466	-101.466	-4.84634
NON-STRUCTURAL PROTEIN 3_entry_00002_conf_01	-110.561	-100.561	-4.807
NON-STRUCTURAL PROTEIN 3_entry_00003_conf_01	-111.048	-101.048	-4.82816
NON-STRUCTURAL PROTEIN 3_entry_00004_conf_01	-110.509	-100.509	-4.80476
NON-STRUCTURAL PROTEIN 3_entry_00005_conf_01	-109.992	-99.9923	-4.78228
NON-STRUCTURAL PROTEIN 3_entry_00006_conf_01	-110.134	-100.134	-4.78844
NON-STRUCTURAL PROTEIN 3_entry_00007_conf_01	-111.064	-101.064	-4.82887
NON-STRUCTURAL PROTEIN 3_entry_00008_conf_01	-111.069	-101.069	-4.82908
NON-STRUCTURAL PROTEIN 3_entry_00009_conf_01	-110.761	-100.761	-4.81569
NON-STRUCTURAL PROTEIN 3_entry_00010_conf_01	-109.735	-99.7353	-4.7711

Kolom pertama score adalah sebagai score docking, berlatar kuning adalah yang terstabil conformer nya atau paling minus.

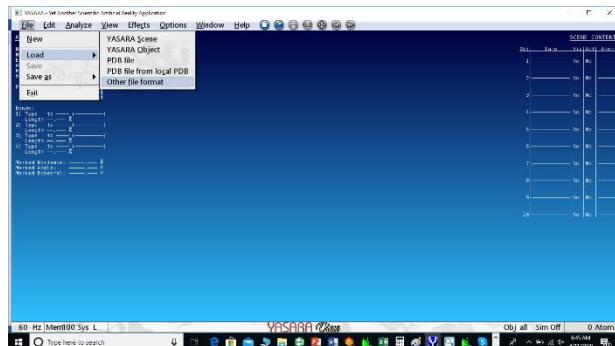
Proses docking sudah selesai.

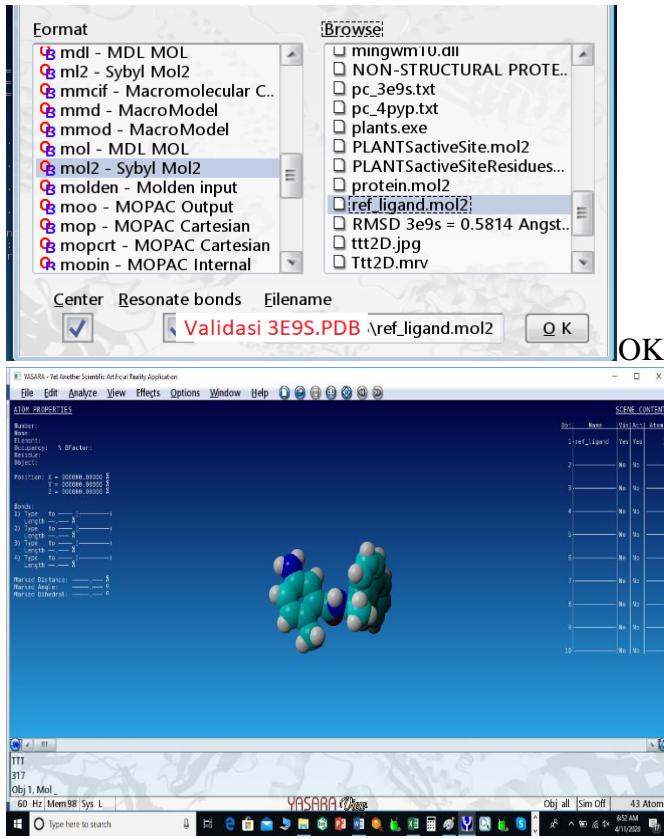
Menghitung RMSD atau Validasi RMSD

1. Buka YASARA

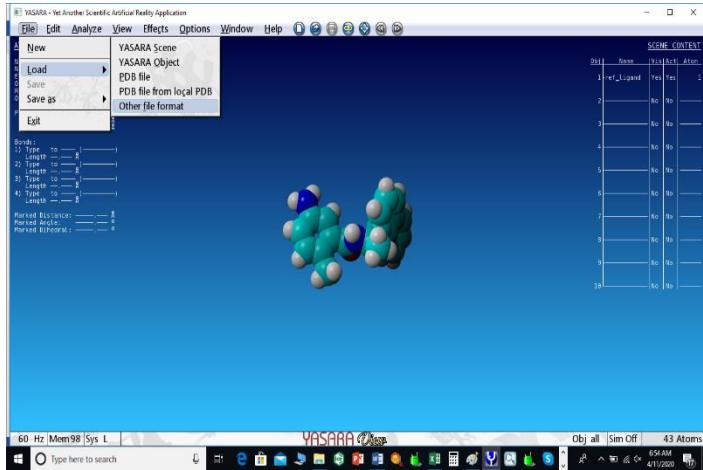


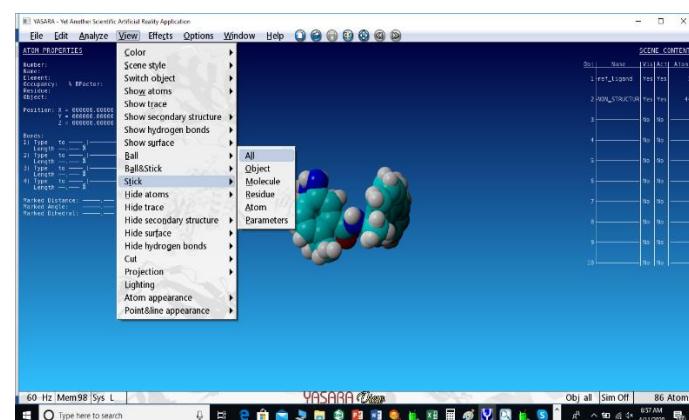
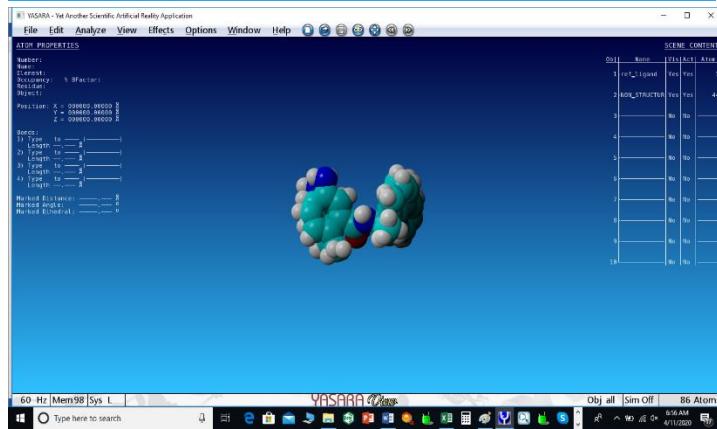
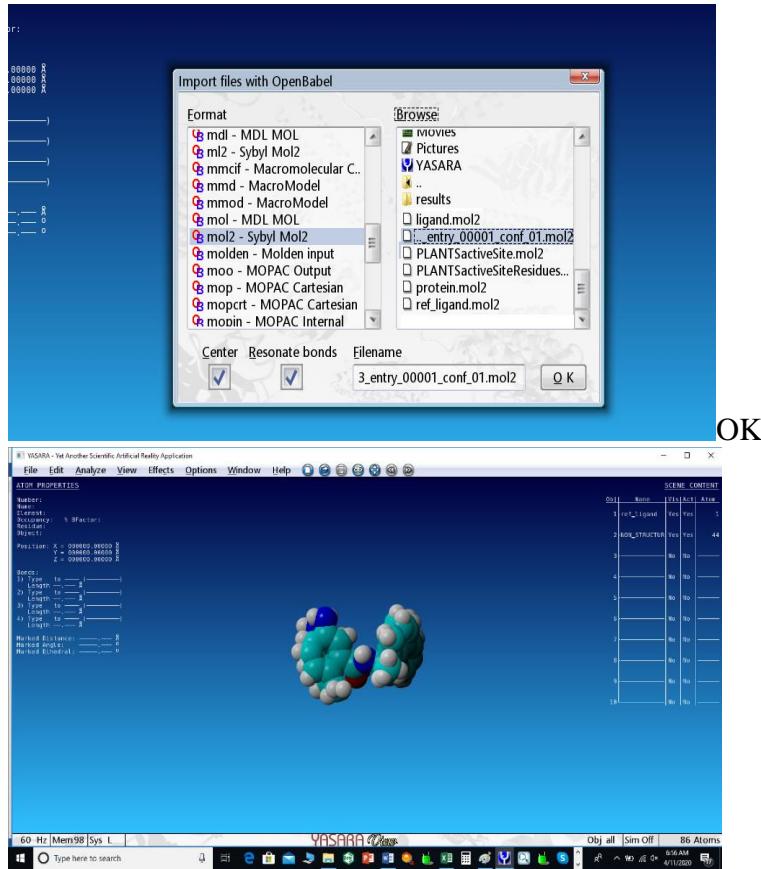
2. Load ref_ligand.mol2 di folder validasi 3E9S.PDB File > load >



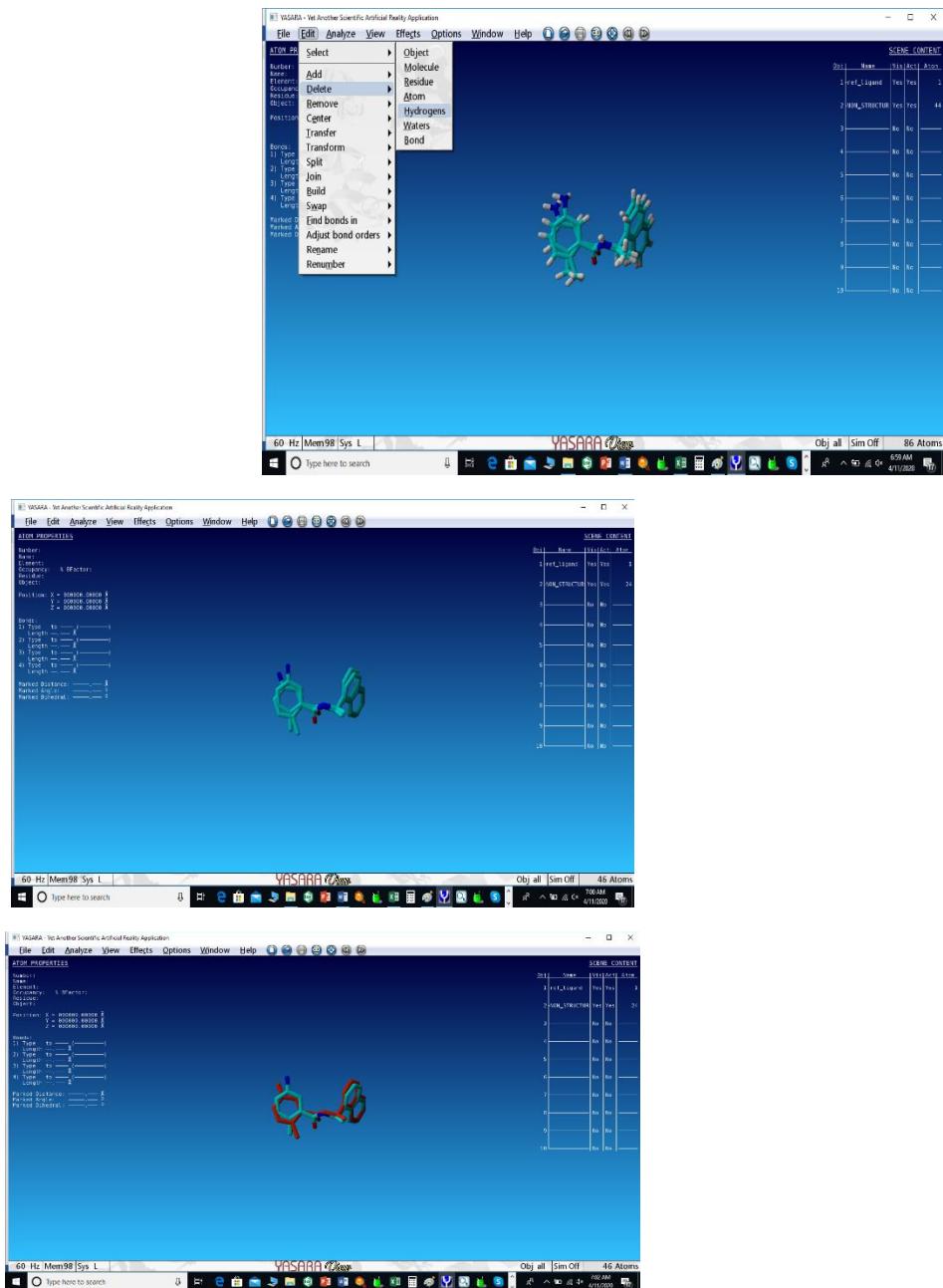


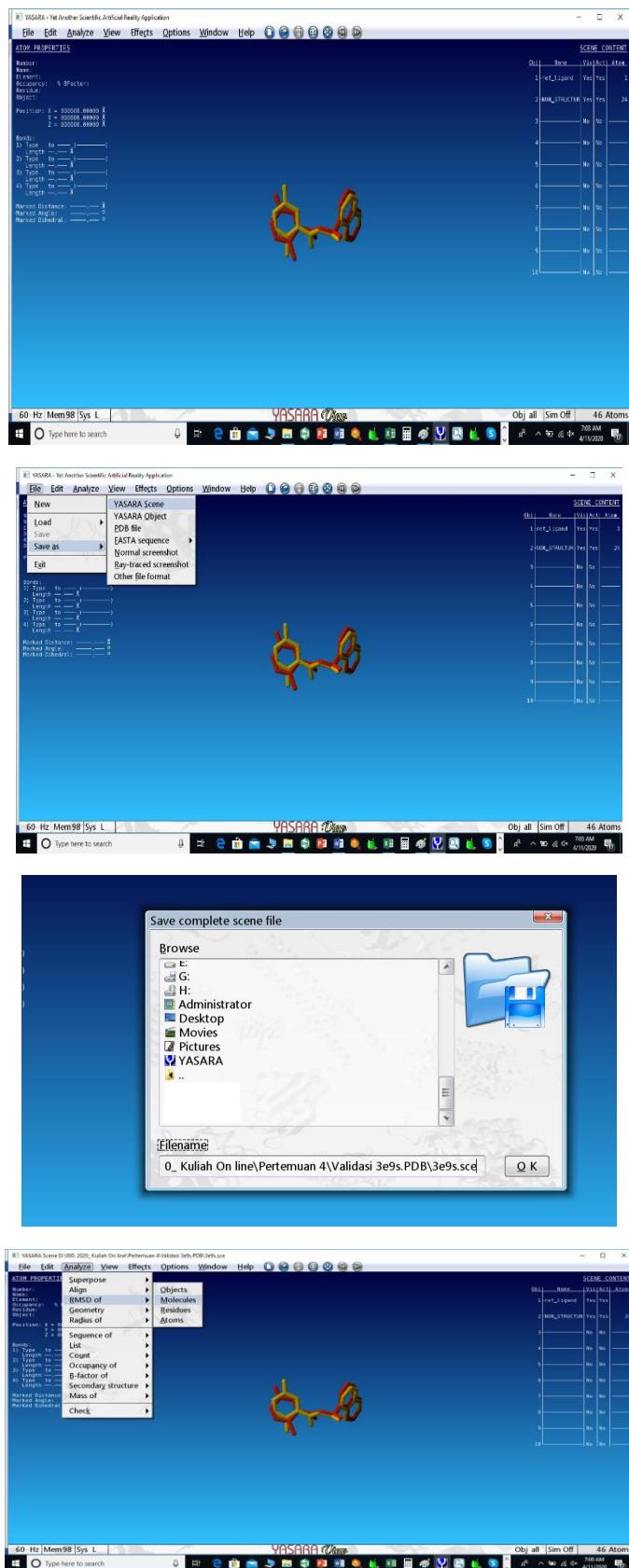
3. Load conformer terstabil

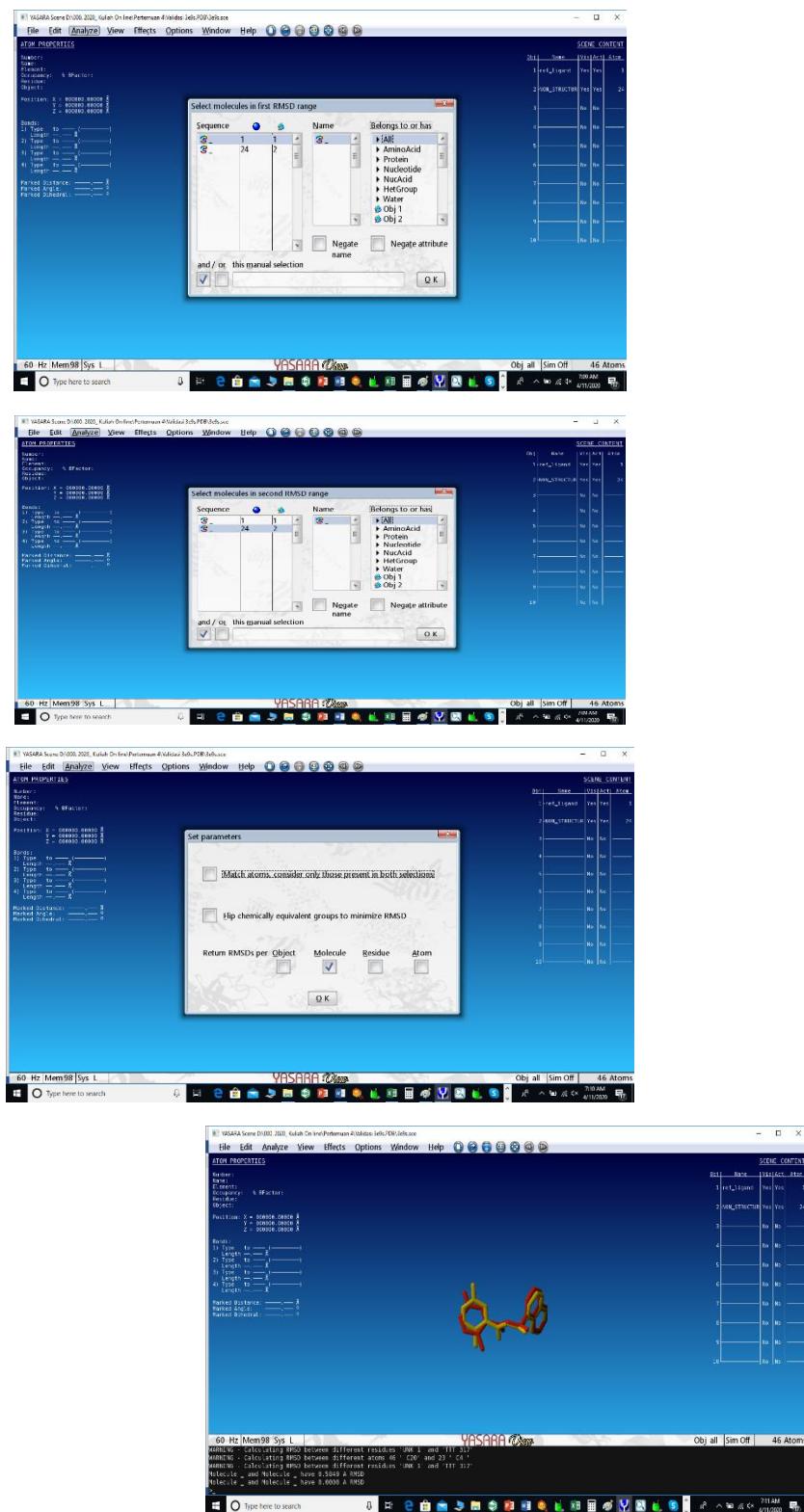


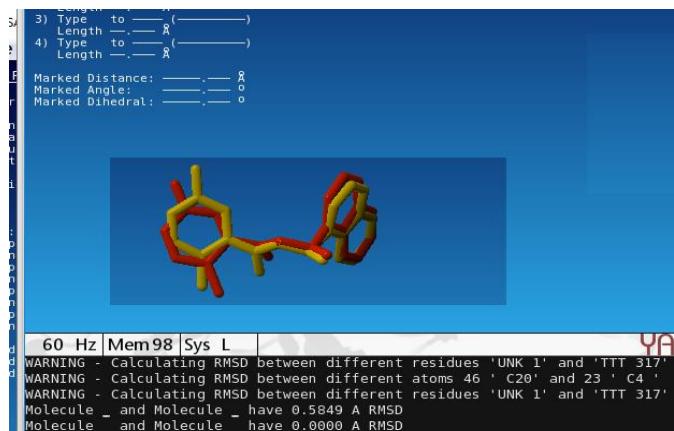


Hilangkan hydrogen, karena di PDB tanpa hidrogen









Terlihat di kiri bawah layar adalah nilai RMSD = 0.5849 Å

Berarti metode valid