1) **Download the protein structure from Protein Data Bank : 1u19.pdb**

Crystal Structure of Bovine Rhodopsin

2) **Download orient.tar.gz**

http://www.ks.uiuc.edu/Research/vmd/script_library/scripts/orient/orient.tar.gz
tar -xvzf orient.tar.gr

3) **La101psx.tar.gz**

http://www.ks.uiuc.edu/Research/vmd/script_library/scripts/orient/la101psx.tar.gz

4) **Download VMD**

https://www.ks.uiuc.edu/Development/Download/download.cgi?UserID=&AccessCode=&ArchiveID=1475
Username: poonamvishwakarma
Password:poonam001

```
tar -xvzf
vmd-1.9.3.bin.LINUXAMD64-CUDA8-OptiX4-OSPRay111p1.opengl.tar.gz
cd vmd-1.9.3/
./configure LINUXAMD64
cd src
sudo make install
vmd
```

5) **Installation of Chimera software**

% chmod +x chimera-1.3-linux.exe

% ./chimera-1.3-linux.exe

/home/bren/opt/chimera/bin/chimera

6) **Open the structure in VMD**

7) **Open VMD TK command line**

VMD→Extensions →TK

8) **Align the molecule to principal axis**

Align the protein in proper orientation

```
package require Orient
namespace import Orient::orient
set sel [atomselect top "all"]
set I [draw principalaxes $sel]
set A [orient $sel [lindex $I 2] {0 0 1}]
$sel move $A
set I [draw principalaxes $sel]
set A [orient $sel [lindex $I 1] {0 1 0}]
$sel move $A
set I [draw principalaxes $sel]
```

Save pdb coordinate as all
save the protein
Protein1.pdb

9) Generate the protein PSF file

`Extensions → Modeling → AutoPSFbuilder`

10) Now in your working directory there is two file present i.e.

1) protein.pdb
2) protein.psf

11) Solvating the Protein

```
download solvate
tar -xvzf solvate_1.0.tgz
cd solvate_1.0
cc -ansi -O -o solvate solvate.c -lm
Run solvate to solvate the protein
solvate -t 5 -n 8 -w  protein solprotein
```

12) Combine the PDB file with rest of the system

```
set sol_infile solprotein.pdb
set protein_inbase protein
set outbase protein_solv_raw
package require psfgen
resetpsf
topology top_all27_prot_lipid.rtf
```

```
segment SOLV {
      auto none
      first NONE
      last NONE
      pdb $sol_infile
      }
coordpdb $sol_infile SOLV
readpsf ${protein_inbase}.psf
coordpdb ${protein_inbase}.pdb
writepdb ${outbase}.pdb
writepsf ${outbase}.psf

mol new protein_solv_raw.psf
mol addfile protein_solv_raw.pdb
set all [atomselect top all]
$all moveby [vecinvert [measure center $all]]
display resetview

set solv [atomselect top "segname SOLV"]
$solv set beta 1
set seltext "segname SOLV and same residue as  ((z < -20) or
(z>20))"
set sel [atomselect top $seltext]
$sel set beta 0
set badwater [atomselect top "name OH2 and beta > 0"]
set seglist [$badwater get segid]
set reslist [$badwater get resid]

mol delete all
package require psfgen
resetpsf
topology top_all27_prot_lipid.rtf
readpsf protein_solv_raw.psf
coordpdb protein_solv_raw.pdb
foreach segid $seglist resid $reslist {
      delatom $segid $resid
}
writepdb protein_solv.pdb
writepsf protein_solv.psf
```

13) Generating the lipid bilayer

Extensions → Modeling → Membrane Builder

14) Alignment of Membrane and Protein

```
set popc [atomselect top all]
set proteinmol [mol new protein_solv.psf]
mol addfile protein_solv.pdb
set protein [atomselect $proteinmol all]
$popc moveby [vecinvert [measure center $popc weight mass]]
$popc writepdb popc_TEMP.pdb
set vest [atomselect $proteinmol "protein and resid 116 to
130"]
$protein moveby [vecinvert [measure center $vest weight mass]]
display resetview
$protein move [transaxis z -25]
$protein writepdb protein_TEMP.pdb

mol delete all
package require psfgen
resetpsf
readpsf POPC.psf
coordpdb popc_TEMP.pdb
readpsf protein_solv.psf
coordpdb protein_TEMP.pdb
writepsf protein_popc_raw.psf
writepdb protein_popc_raw.pdb
```

15) Combination of Membrane and Protein

```
mol delete all
mol new protein_popc_raw.psf
mol addfile protein_popc_raw.pdb
set POPC "resname POPC"
set all [atomselect top all]
$all set beta 0
set seltext1 "$POPC and same residue as  (name P1 and z>0 and
abs(x)<15 and abs(y)<15)"
set seltext2 "$POPC and same residue as  (name P1 and z<0 and
abs(x)<10 and abs(y)<10)"
set seltext3 "$POPC and same residue as (within 0.6 of
protein)"
set sel1 [atomselect top $seltext1]
```

```
set sel2 [atomselect top $seltext2]
set sel3 [atomselect top $seltext3]
$sel1 set beta 1
$sel2 set beta 1
$sel3 set beta 1
set badlipid [atomselect top "name P1 and beta > 0"]
set seglistlipid [$badlipid get segid]
set reslistlipid [$badlipid get resid]
set seltext4 "(water and not segname WCA WCB WCC WCD WF SOLV)
and same residue as within 3 of  ((same residue as (name P1 and
beta>0)) or protein)"
set seltext5 "segname SOLV and same residue as  within 3 of
lipids"
set sel4 [atomselect top $seltext4]
set sel5 [atomselect top $seltext5]
$sel4 set beta 1
$sel5 set beta 1
set badwater [atomselect top "name OH2 and beta > 0"]
set seglistwater [$badwater get segid]
set reslistwater [$badwater get resid]

mol delete all
resetpsf
readpsf protein_popc_raw.psf
coordpdb protein_popc_raw.pdb
foreach segid $seglistlipid resid $reslistlipid {
delatom $segid $resid
}
foreach segid $seglistwater resid $reslistwater {
delatom $segid $resid
}
writepsf protein_popc.psf
writepdb protein_popc.pdb
```

16) Solvation and Ionization

```
mol delete all
mol new protein_popc.psf
mol addfile protein_popc.pdb
set water [atomselect top water]
measure minmax $water
```

```
package require solvate
solvate protein_popc.psf  protein_popc.pdb -o
protein_popc_water_TEMP -b 1.5  -minmax {{-45 -45 -47} {45 46
49}}

set all [atomselect top all]
$all set beta 0
set seltext "segid WT1 to WT99 and same residue as abs(z) < 25"
set sel [atomselect top $seltext]
$sel set beta 1
set badwater [atomselect top "name OH2 and beta > 0"]
set seglist [$badwater get segid]
set reslist [$badwater get resid]

mol delete all
package require psfgen
resetpsf
readpsf protein_popc_water_TEMP.psf
coordpdb protein_popc_water_TEMP.pdb
foreach segid $seglist resid $reslist {
     delatom $segid $resid
}
writepdb protein_popcw.pdb
writepsf protein_popcw.psf
```

17) Ionized the system

Load files protein_popcw.psf and protein_popcw.pdb.

17) open the kcsa_popcwimineq-01 file in gedit

Perform some changes

```
set everyone [atomselect top all]
measure minmax $everyone
```

This analyzes all atoms in the system and gives you the minimum and maximum values of x, y and z coordinates of the entire protein-water system,For example, if minimum and maximum values of x are returned as 10.44 and 51.12, respectively, x-coordinate of the center of the box would be 51.12+10.44 2 = 30.78

18) Running the system in Amber

16) open the terminal

17) source the terminal as

```
source /usr/local/amber16/amber.sh
```

18) charmmlipid2amber.py used to convert whole membrane system into the Lipid14 formatted PDB file

```
charmmlipid2amber.py -i 5do7_final_system.pdb -c
    charmmlipid2amber.csv -o out.pdb
```

19) Estimate Periodic Box Size , VMD script here to measure the periodic box dimensions

```
1)sh vmd_box_dims.sh -i out.pdb -s water
2)result- 112.262001037597654, 114.537002563476564,
142.99000167846680
```

20) start tleap in the terminal

```
1)type tleap
2)> source leaprc.lipid14
3)> source oldff/leaprc.ff14SB
4)> loadamberparams frcmod.ionsjc_tip3p
5)> pdb = loadpdb out.pdb
6)> set pdb box {112.262001037597654, 114.34800338745117,
142.56600189208985}

7)> desc  pdb
8)> charge pdb
9)> addions pdb k+ 0
10)> check pdb
11)saveAmberParm pdb 5do7.prmtop 5do7.inpcrd
```

21)save the pdb file from prmtop and inpcrd file

```
1)ambpdb -p 5do7.prmtop -c 5do7.inpcrd > amber.pdb
```

2)open the structure in chimera and see wether it is properly inserted in lipid bilayer or not.

22)now run the 1) minimization 2)heating 3) equilibrium 4)production run

min1.in

```
gpcr_test: initial    minimization
solvent + ions
&cntrl
imin = 1,
maxcyc     = 1000,
ncyc = 500,
```

```
ntb   = 1,
ntr   = 1,
cut   = 8.0,
/
Hold the solute fixed
50.0
RES 715 1062
END
END
```

Min2.in

```
gpcr_test: initial minimization of the whole system
&cntrl
imin = 1,
maxcyc     = 3000,
ncyc = 1500,
ntb   = 1,
ntr   = 0,
cut   = 8.0,
/
```

md1.in

```
gpcr_test: heating phase
&cntrl
imin = 0,
irest      = 0,
ntx   = 1,
ntb   = 1,
cut   = 8,
ntr     = 1,
ntc   = 2,
ntf   = 2,
tempi      = 10.0,
temp0      = 300.0,
ntt   = 3,
gamma_ln = 1.0,
nstlim = 30000, dt = 0.002,
ntpr = 5000, ntwx = 5000, ntwr = 5000, ig=-1,
/
Keep the solute fixed with weak restraints
```

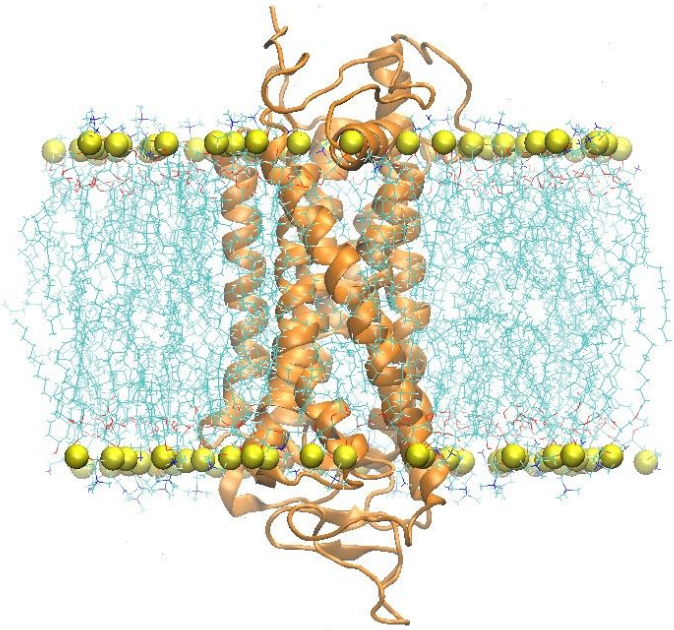
```
10.0
RES 715 1062
END
END
```

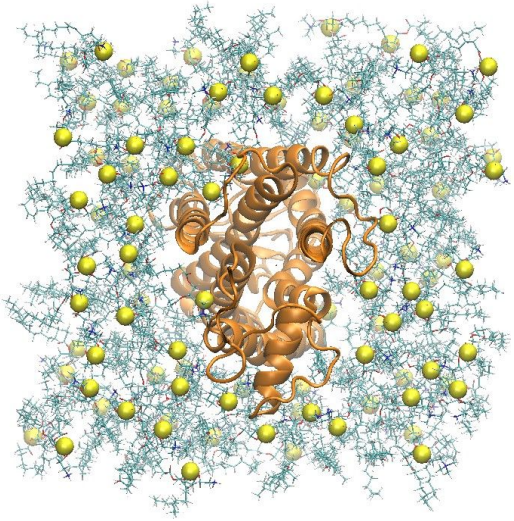
md2.in

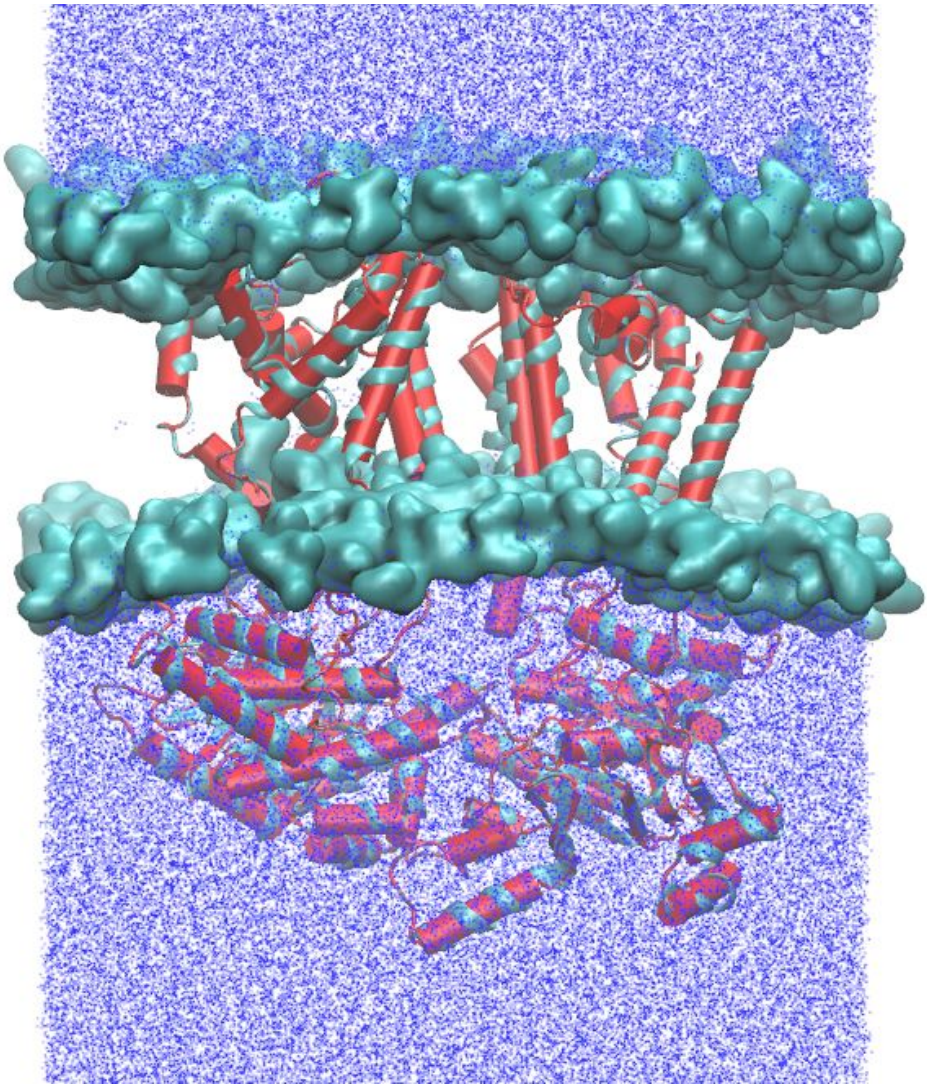
```
gpcr_test: equilibration phase
&cntrl
imin = 0, irest = 1, ntx = 5,
ntb = 2, pres0 = 1.0, ntp = 1,
taup = 2.0,
cut = 8.0, ntr = 0,
ntc = 2, ntf = 2,
temp0 = 300.0,
ntt = 3, gamma_ln = 1.0,
nstlim = 50000, dt = 0.002,
ntpr = 5000, ntwx = 5000, ntwr = 5000, ig=-1,
/
```

md3.in

```
gpcr: production phase
&cntrl
imin = 0, irest = 1, ntx = 5,
ntb = 2, pres0 = 1.0, ntp = 1,
taup = 1.0,
cut = 8.0, ntr = 0,
ntc = 2, ntf = 2,
tempi = 300.0, temp0 = 300.0,
ntt = 3, gamma_ln = 0.5,
nstlim = 5000000, dt = 0.002,
ntpr = 5000, ntwx = 5000, ntwr = 5000, ig=-1,
/
```





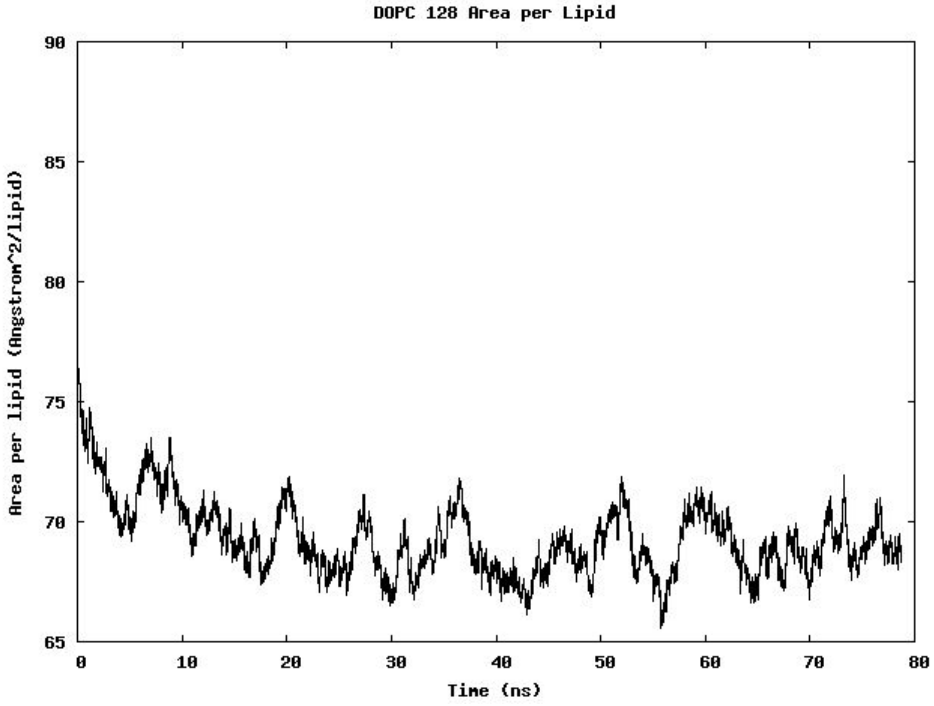


Analysis on membrane protein

1) **Area per Lipid**

   Area per lipid is the average area that a single phospholipid occupies in an interface and is usually reported in Angstroms squared. It can be calculated with:

   ```
   Area per lipid = (box X dimension) * (box Y dimension) /
   (number of phospholipids per layer)
   ```



2) Steered Molecular Dynamics