Note: in VMD Tk console you cannot copy-paste

<prepare psf file for the protein>

```
extensions > Tk console > cd ../folder_for_membrane
file > new > load NAME_final(_aligned).pdb
extensions > modelling > automatic PSF builder > I'm feeling lucky
```

<build the membrane>

```
extensions > Tk console > cd ../folder_for_membrane > pwd
extensions > modelling > membrane builder:
                              POPE
                              x = y = 100
                              path_to_folder/pope
                              charmm36
```

<orient the membrane>

```
set pope [atomselect top all]
$pope moveby [vecinvert [measure center $pope]]
$pope writepdb pope_TEMP.pdb
```

<load protein, move it into the membrane with vecinvert, then orient along its axes with orient package>

```
set gadcmol [mol new gadCWT_final_autopsf.psf]
mol addfile gadCWT_final_autopsf.pdb
set gadc [atomselect $gadcmol all]

$gadc moveby [vecinvert [measure center $gadc]]

display resetview

lappend auto_path /Users/nina/Downloads/la1.0
lappend auto_path /Users/nina/Downloads/orient

package require Orient
namespace import Orient::orient

set I [draw principalaxes $gadc]
set A [orient $gadc [lindex $I 2] {0 0 1}]
$gadc move $A
set I [draw principalaxes $gadc]
set A [orient $gadc [lindex $I 1] {0 1 0}]
$gadc move $A
set I [draw principalaxes $gadc]
```

<now make the tilt from z axis as defined in OPM database; as it's not strictly specified how to rotate along x and y axes, so do it by comparing with OPM data/ structure; in transaxis you can define x or y axis, and + or - angles>

```
$gadc move [transaxis y -10]
$gadc writepdb gadc_TEMP.pdb
```

VISUALIZATION:
protein > VDW > ResType
resname POPE and name P > VDW > Name

now the white part is supposed to be in the membrane, and the colored (charged and polar amino acids) outside

you can also use specified transmembrane residues (bottom of page in OPM) to help you with orienting the protein

<note: POPE membrane seemed a bit thicker compared to the one showed in OPM>

<make pdb and psf files for oriented membrane and protein>

```
mol delete all
package require psfgen
resetpsf
readpsf pope.psf
coordpdb pope_TEMP.pdb
readpsf gadCWT_final_autopsf.psf (za MUT: gadCmut02_final_aligned_autopsf.psf)
coordpdb gadc_TEMP.pdb   (za MUT: gadCmut02_final_aligned_autopsf.pdb)
writepsf gadc_pope_raw.psf
writepdb gadc_pope_raw.pdb
```

<remove lipids and water that overlap with the protein>

```
mol delete all
mol new gadc_pope_raw.psf
mol addfile gadc_pope_raw.pdb
set POPE "resname POPE"
set all [atomselect top all]
$all set beta 0
```

<in tutorial they do 3 selections because the width of the protein is asymmetric in upper and in the lower layer of membrane; if your protein is symmetric (mine was around 40-50A all around), no need to do separate selection for z<>0; I'm adjusting abs(x) i abs(y) for my protein; also, POPE has P, and POPC used in the manual has P1 atom, so take care of that; normally seltext2 overrides seltext1, but it doesn't have to be the case if protein has a pore/central channel, which mine does so I'm keeping seltext1>

```
set seltext1 "$POPE and same residue as (name P and abs(x)<15 and abs(y)<15)"
set seltext2 "$POPE and same residue as (within 0.6 of protein)"
set sel1 [atomselect top $seltext1]
```

```
set sel2 [atomselect top $seltext2]
$sel1 set beta 1
$sel2 set beta 1
set badlipid [atomselect top “name P and beta > 0”]
set seglistlipid [$badlipid get segid]
set reslistlipid [$badlipid get resid]

<select waters that overlap with protein because membrane is hydrated from the
start>

set seltext4 “(water and not segname WCA WCB WCC WCD WF SOLV) and same residue
      as within 3 of ((same residue as (name P 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]

<now delete the “bad” lipids and waters selected in previous steps>

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



<solvatation>

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

package require solvate
solvate gadc_pope.psf gadc_pope.pdb -o gadc_pope_water_TEMP -b 1.5 -minmax {{-x
      -y -z} {x y z}}

<replace x, y and z with numbers from system measuring above + leave extra 10-15
A of space for the protein>

mol delete all
mol new gadc_pope_water_TEMP.psf
mol addfile gadc_pope_water_TEMP.pdb
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 gadc_pope_water_TEMP.psf
coordpsb gadc_pope_water_TEMP.pdb
foreach segid $seglist resid $reslist {delatom $segid $resid}
writepdb gadc_popew.pdb
writepsf gadc_popew.psf
```

<adding ions>

```
mol delete all
mol new gadc_popew.psf
mol addfile gadc_popew.pdb
Extensions > Modelling > Add Ions:
                              Input PSF: gadc_popew.psf
                              Input PDB: gadc_popew.pdb
                              Output prefix: gadc_popewi
                              Salt: NaCl
                              Ion placement: Only neutralize system with NaCl
                              Min distance from solute: 5 A
                              Min distance between ions: 5 A
                              Segment name of places ions: ION
                              > Autoionize
```

<load files wi and check that everything's OK>