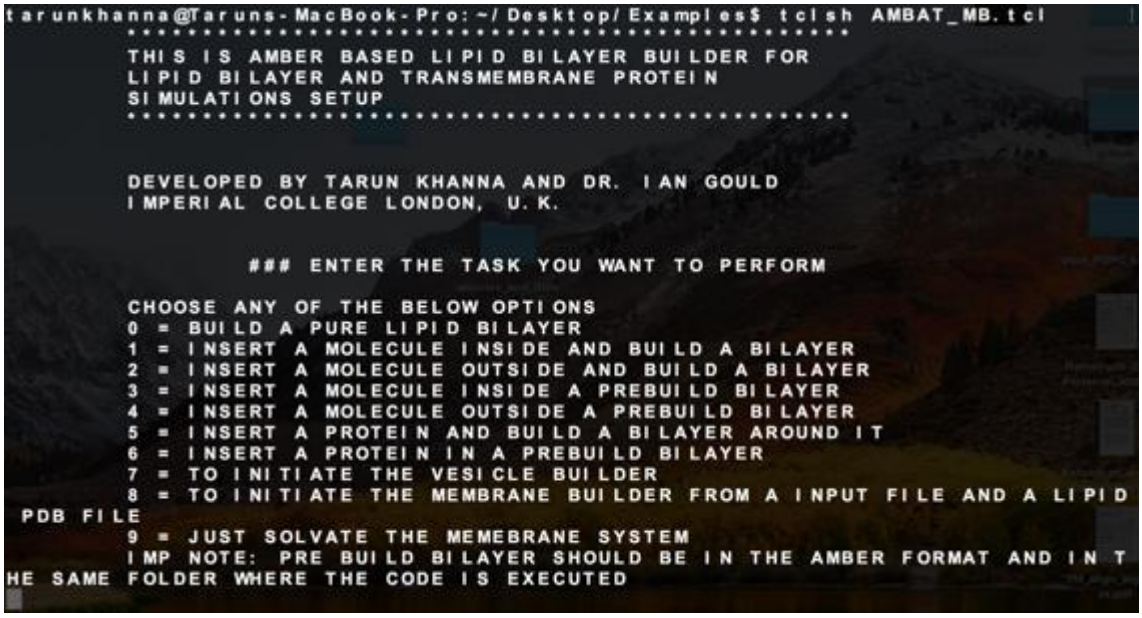
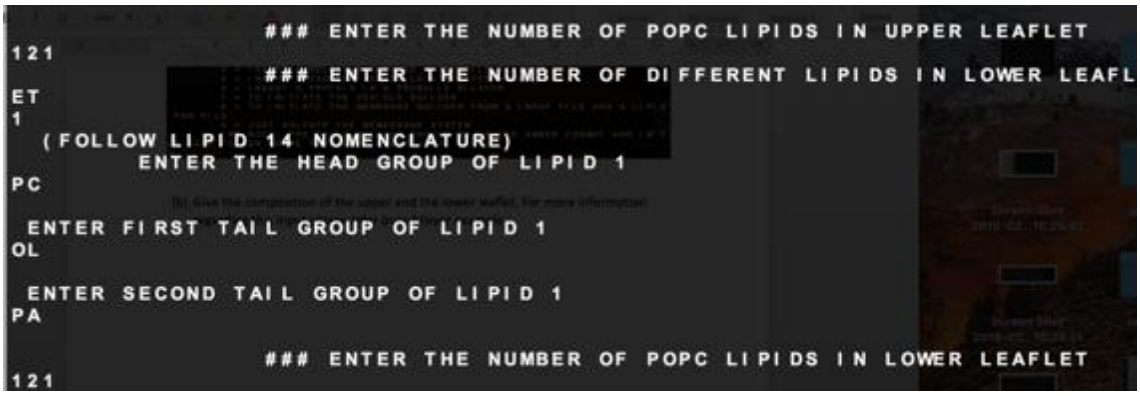
1. Lipid bilayer and protein (from OPM database)

Option 5 and 6 of AMBAT applies to protein insertion. In this example we insert a medium size polypeptide (CXCR4) in a 242 POPC lipid bilayer. CXCR4 PDB (4rws) is downloaded from OPM database hence, its already in the right orientation with respect to the bilayer. AMBAT is fully compatible with PDB's downloaded from OPM database.

(a) Choose Option **'5'** after executing AMBAT code.



(b) Give the composition of the upper and the lower leaflet. For more information regarding this input please refer pure bilayer example.



```
        9 = JUST SOLVATE THE MEMEBRANE SYSTEM
        IMP NOTE: PRE BUILD BILAYER SHOULD BE IN THE AMBER FORMAT AND I
N THE SAME FOLDER WHERE THE CODE IS EXECUTED
5

                ### ENTER THE NUMBER OF DIFFERENT LIPIDS IN UPPER LEAFL
ET
1
        (FOLLOW LIPID 14 NOMENCLATURE)
        ENTER THE HEAD GROUP OF LIPID 1
PC

 ENTER FIRST TAIL GROUP OF LIPID 1
OL

 ENTER SECOND TAIL GROUP OF LIPID 1
PA

                ### ENTER THE NUMBER OF POPC LIPIDS IN UPPER LEAFLET
121
```

(c) AMBAT will then enter the protein insertion suite of AMBAT. Input the name of the protein PDB. Type in '**4rws.pdb'**

```
        **************************************************
        THIS CODE HELPS TO ORIENT THE MEMBRANE PROTEIN IN
        RIGHT ORIENTATION
        **************************************************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        IMPERIAL COLLEGE LONDON, U.K.

                        #### ENTER THE NAME OF THE PDB ####
                            (MAKE SURE TO REMOVE THE REMARK SECTION OF
THE PDB)
```
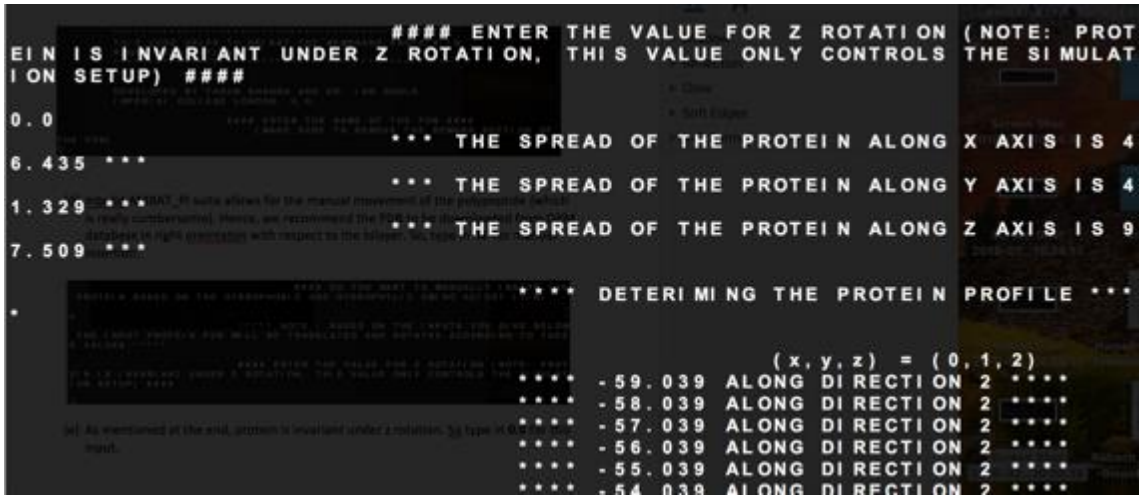

(d) Inbuild AMBAT_PI suite allows for the manual movement of the polypeptide (which is really cumbersome). Hence, we recommend the PDB to be downloaded from OPM database in right oreintaton with respect to the bilayer. So, type in **'N**' for manual insertion.

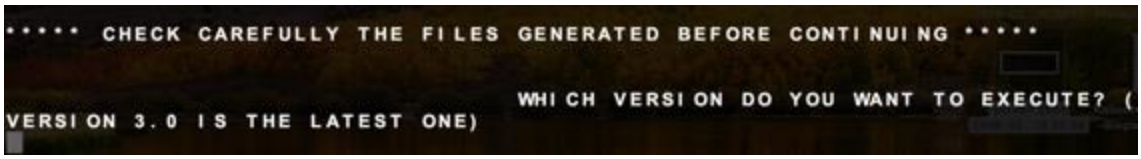
```
                                #### DO YOU WANT TO MANUALLY INSERT THE
 PROTEIN BASED ON THE HYDROPHOBIC AND HYDROPHYLIC AMINO ACIDS? (Y/N)

n
                            ***** NOTE : BASED ON THE INPUTS YOU GIVE BELOW
 THE INPUT PROTEIN PDB WILL BE TRANSLATED AND ROTATED ACCORDING TO THES
E VALUES *****

                            #### ENTER THE VALUE FOR Z ROTATION (NOTE: PROT
EIN IS INVARIANT UNDER Z ROTATION, THIS VALUE ONLY CONTROLS THE SIMULAT
ION SETUP) ####
```

(e) As mentioned at the end, protein is invariant under z rotation. So, type in **0.0** for this input.

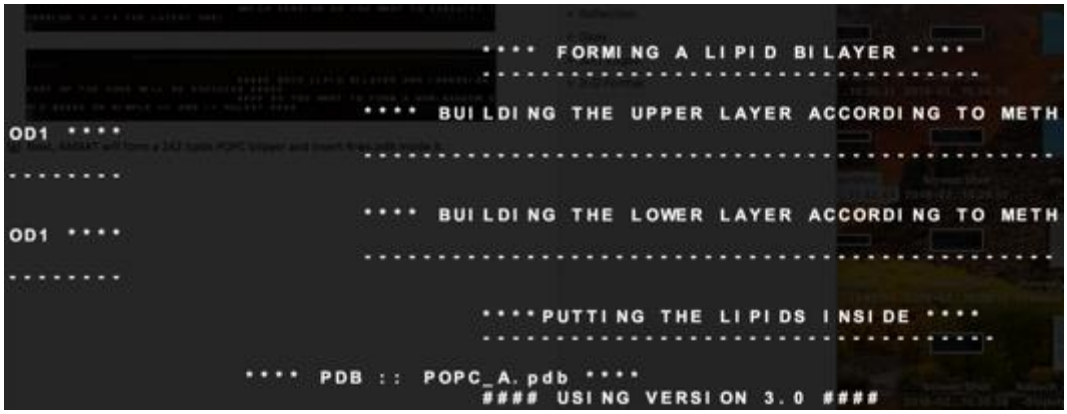


(f) There are few important files which will be generated at the end of this step "pro_ori.pdb" which shows the orientation of the polypeptide with respect to the bilayer (only placement along z dimension is important) , "ac_nature"; which contains 6 columns for the density of various classes of amino acids along the bilayer normal. Column 2 and 3 represents the most important class of hydrophobic and hydrophilic amino acids respectively ((-36.0 to 0) is the bilayer interior) and "ref1_no.pdb" which will be used further in the code for insertion into the bilayer

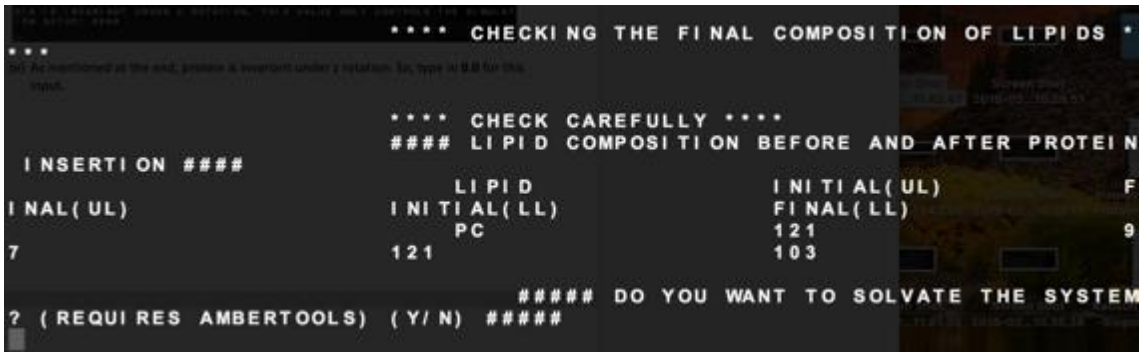
For version, as the bilayer is symmetric select 3.0 and for non-random grid type 'N' (non-random grid is explained further in pure bilayer example).



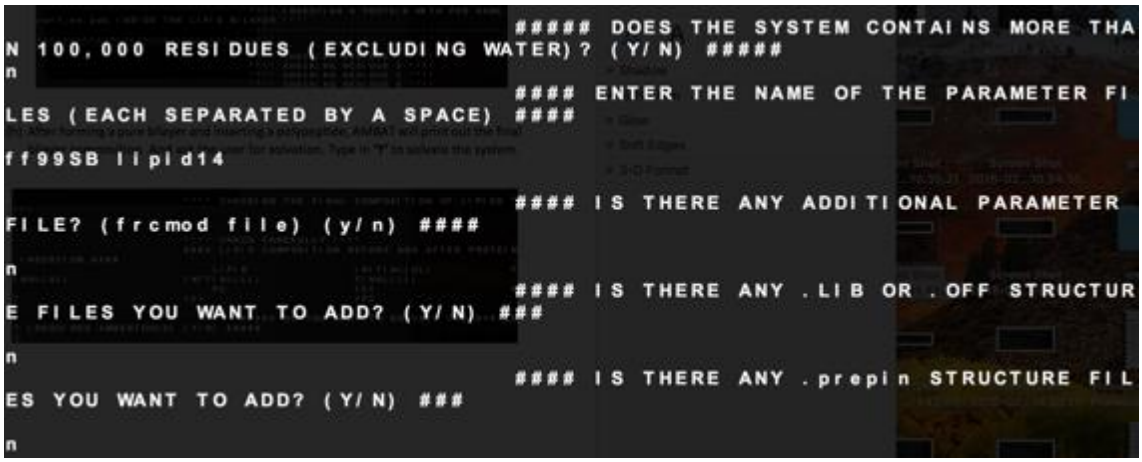
(g) Next, AMBAT will form a 242 lipids POPC bilayer and insert 4rws.pdb inside it.



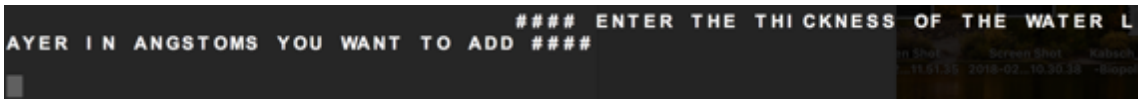
(h) After forming a pure bilayer and inserting a polypeptide, AMBAT will print out the final bilayer composition. And ask the user for solvation. Type in **'Y'** to solvate the system.



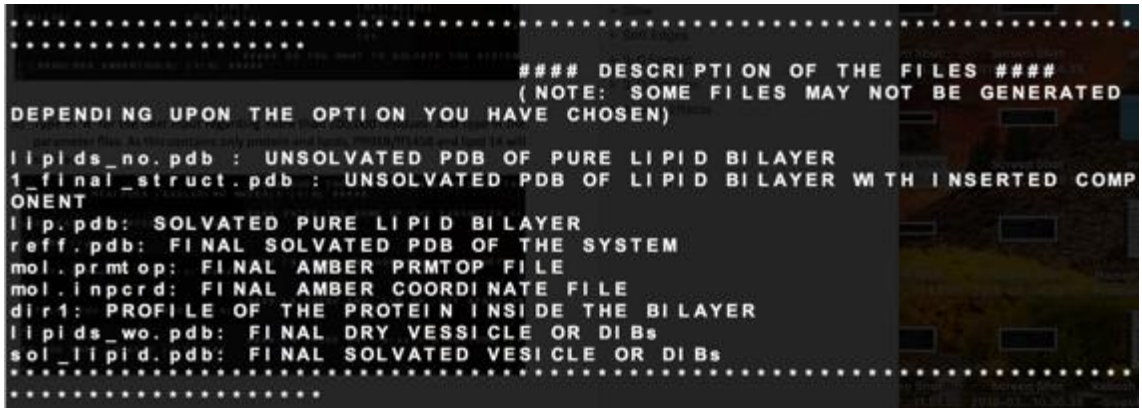
(i) Type in '**n'** for the next input regarding more than 100,000 residues. And type in the parameter files. As this contains only protein and lipids, ff99SB/ff14SB and lipid 14 will suffice.

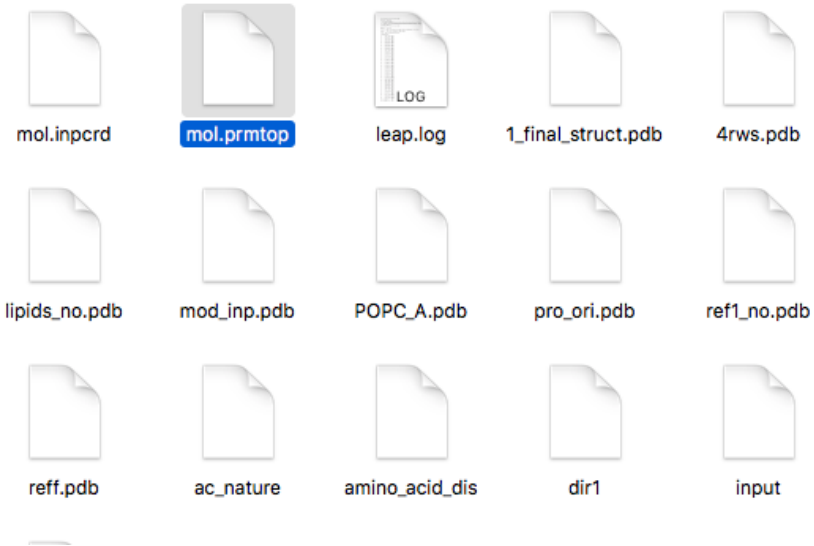


(j) Next, AMBAT will ask for water thickness. Type in **'10.0'.**



(k) Finally, AMBAT will print out the description of various files formed. And prmtop and inpcrd files for running MD simulation.





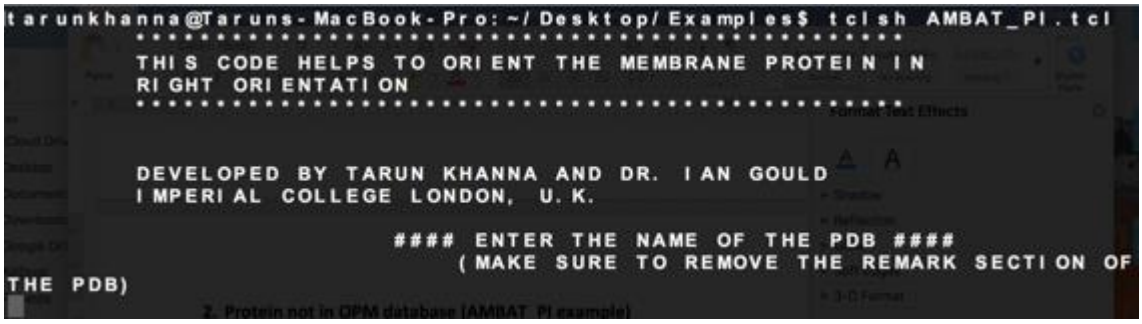
2. Protein not in OPM database (AMBAT_PI example)

In this example we would like to show the functioning of AMBAT_PI code for the proteins which are not there in OPM database or in some rare cases like 2n28 (virus protein U) wrongly identified.

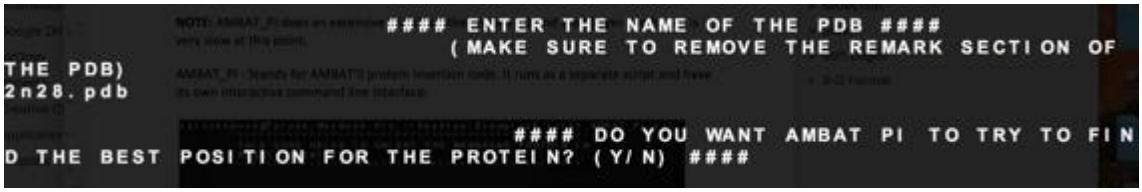
NOTE: AMBAT_PI does an extensive search on the regions around the bilayer and hence is very slow at this point.

AMBAT_PI - Stands for AMBAT'S protein insertion code. It runs as a separate script and have its own interactive command line interface.

Enter the name of the PDB you want to orient. For this example, we use **2n28.pdb** (downloaded from RCSB database, with remarks section removed).



Next, if you have an option to either just put the protein at the centre of the box (N) or use AMBAT_PI to position it (y). Type 'Y' to try AMBAT_PI



As the speed is a real problem with this code, (after a wait of around 15-20 min) you will have the final structure 'ref1_no.pdb' and its representative orientation ("pro_ori.pdb") in the execution folder.