1. Forming pure lipid bilayer (Option ‘0’)

(a) TCL (tickle) being an interpreted language requires the path to the source code for execution. ‘tclsh’ is the prefix for the execution of a tickle script.

```
tarunkhanna@dyn1238-107:~/Desktop/Exampl es$ tclsh AMBAT_MB.tcl
```

(b) On execution, AMBAT gives user several options as shown below. For building pure bilayer **choose option ‘0’**

```
tarunkhanna@dyn1238-107:~/Desktop/Examples$ tclsh AMBAT_MB.tcl
        ********************************************
        THIS IS AMBER BASED LIPID BILAYER BUILDER FOR
        LIPID BILAYER AND TRANSMEMBRANE PROTEIN
        SIMULATIONS SETUP
        ********************************************


        DEVELOPED BY TARUN KHANNA AND DR. IAN GOULD
        IMPERIAL COLLEGE LONDON, U.K.


                ### ENTER THE TASK YOU WANT TO PERFORM

        CHOOSE ANY OF THE BELOW OPTIONS
        0 = BUILD A PURE LIPID BILAYER
        1 = INSERT A MOLECULE INSIDE AND BUILD A BILAYER
        2 = INSERT A MOLECULE OUTSIDE AND BUILD A BILAYER
        3 = INSERT A MOLECULE INSIDE A PREBUILD BILAYER
        4 = INSERT A MOLECULE OUTSIDE A PREBUILD BILAYER
        5 = INSERT A PROTEIN AND BUILD A BILAYER AROUND IT
        6 = INSERT A PROTEIN IN A PREBUILD BILAYER
        7 = TO INITIATE THE VESICLE BUILDER
        8 = TO INITIATE THE MEMBRANE BUILDER FROM A INPUT FILE AND A LIPID PDB FILE
        9 = JUST SOLVATE THE MEMEBRANE SYSTEM
        IMP NOTE: PRE BUILD BILAYER SHOULD BE IN THE AMBER FORMAT AND IN THE SAME FOLDER
WHERE THE CODE IS EXECUTED
```

(c) On choosing option ‘0’, It asks for the composition of the lipid bilayer, starting from the upper leaflet.

(i) Starting from number of different lipids in a leaflet. Let’s say we want to make a symmetric lipid bilayer. **Type ‘1’ for that**.

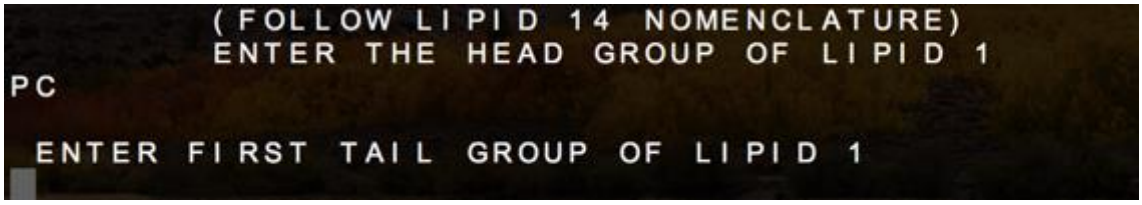
```
        IMP NOTE: PRE BUILD BILAYER SHOULD BE IN THE AMBER FORMAT AND IN THE SAME FOLDER
 WHERE THE CODE IS EXECUTED
0

                ### ENTER THE NUMBER OF DIFFERENT LIPIDS IN UPPER LEAFLET
```

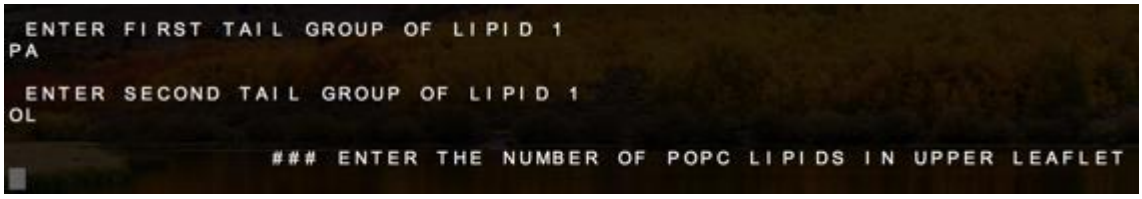
(ii) On typing ‘1’ it will ask for the type of lipid, which follows lipid 14 nomenclature of lipids (division into head and tail). Let’s make a POPC lipid, where head group residue is represented as ‘PC’. **Type in ‘PC’** (uppercase)

```
                ### ENTER THE NUMBER OF DIFFERENT LIPIDS IN UPPER LEAFLET
1
        (FOLLOW LIPID 14 NOMENCLATURE)
        ENTER THE HEAD GROUP OF LIPID 1
```

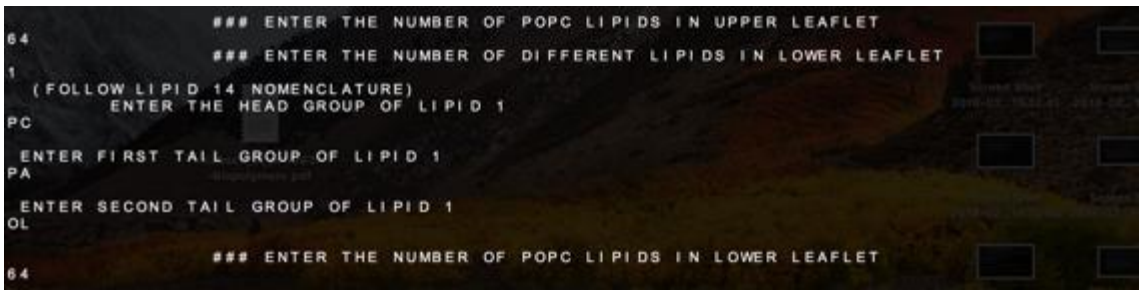
(iii) Next it will ask for the tail groups (one at a time, as phospholipids in lipid 14 usually composed of 2 tails), for POPC lipid14 nomenclature for 16:0 tail is 'PA' and 18:1 tail is 'OL'. **Type in 'PA' for tail 1** and **'OL' for tail 2**. (order doesn't matter)



(iv) Next it will ask for the number of POPC lipids in the upper leaflet. For this example, we **choose 64**.

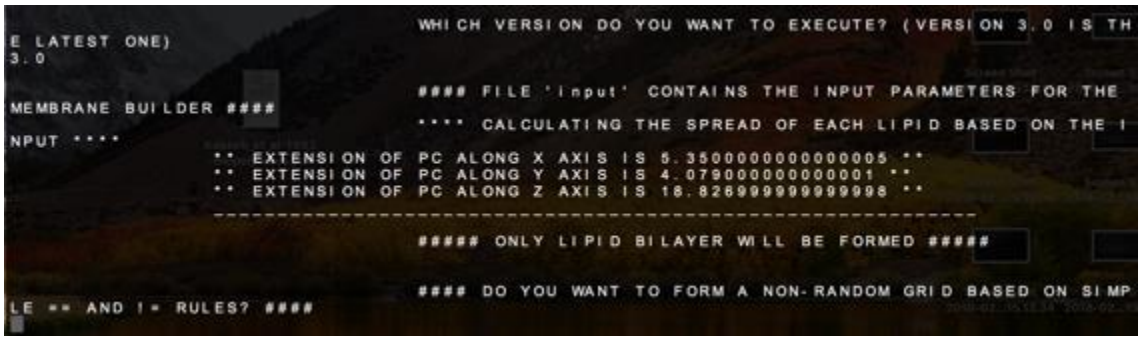


(d) Next it will ask for same information for the lower leaflet. Note: AMBAT builds both upper and lower leaflet independently.



(e) On providing the composition, AMBAT asks for the version you want to run for building the bilayer. For symmetric bilayer we recommend latest version 3.0 and for asymmetric bilayer we recommend version 2.0. For this example, we **choose version 3.0**

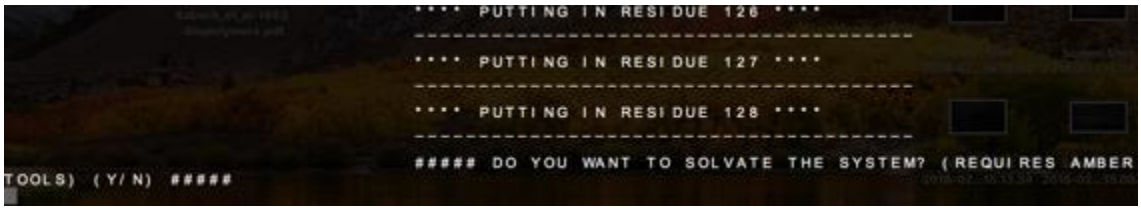
(f) AMBAT now execute a function to calculate the extension of the constituting lipids in the x,y and z direction (useful for some kind of simulation setups, not for this example). And then AMBAT will ask if you want to build a non-random grid. **Type 'n'** to form a random grid. (non-random grid is explained latter in point (3))



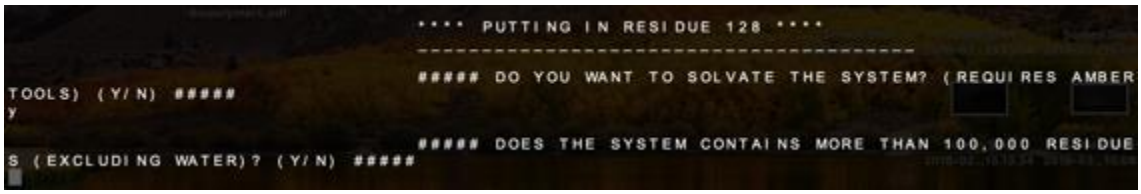
(g) This will execute the membrane builder to form a POPC bilayer composed of 128 lipids. (Execution should take few seconds….)

(h) Till this point, AMBAT is independent of any third-party software's. To solvate the above formed lipid bilayer, you need AmberTools installed on your machine. If that's not the case then you can **give 'n'** (no) to solvation and it will terminate the code and

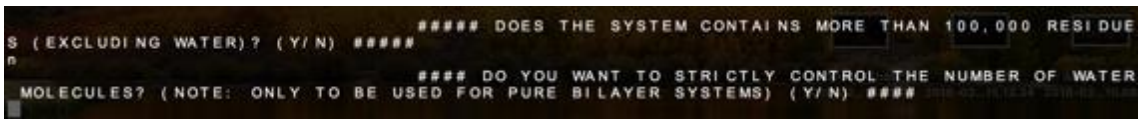
give you an unsolvated pure POPC bilayer as an output (file named 'lipids_no.pdb', sorry for this kind of naming !!!). (With detailed description of the files generated by AMBAT printed out at the end).



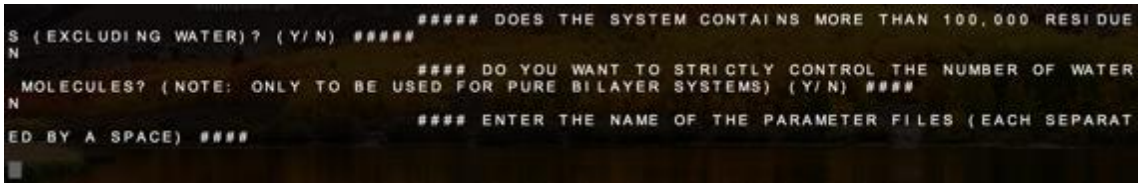
IF you do have AmberTools installed, then type 'y'.



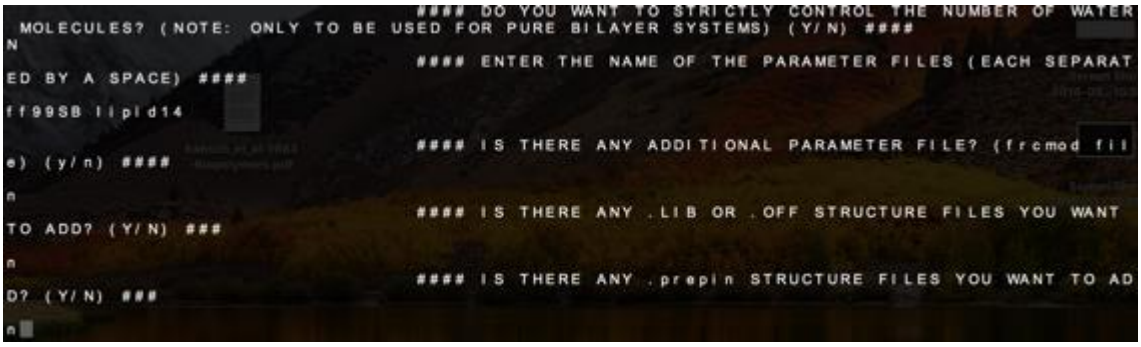
Next option is useful for huge biological system like Photosystem II, which are above the limit of the PDB files which can be handled by leap. (Can be ignored for most of the cases). So, **Type in 'N'**. Control over number of water ('Y') is demonstrated below in (2).



AMBAT have two solvation methods, one with a strict control over the number of waters and other with a strict control over the water thickness. For this example, lets control the water thickness so **type in 'n'**

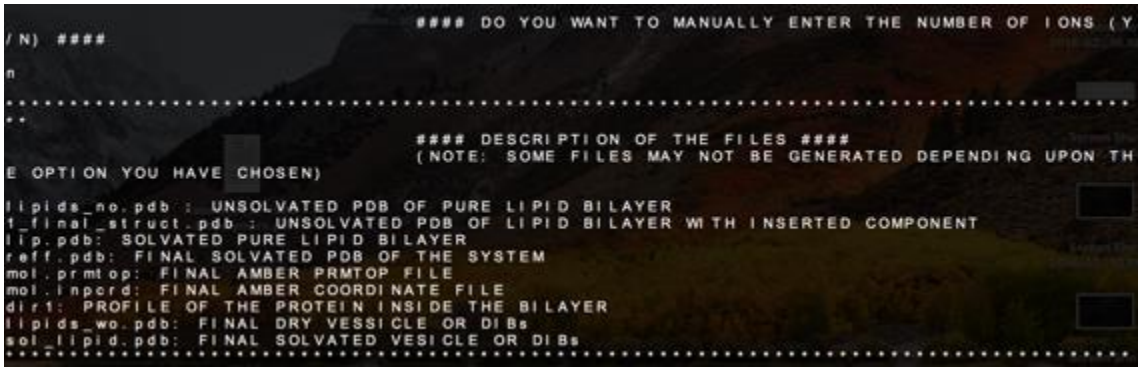


Next, AMBAT will ask for the name of the AMBER force fields (each separated by space) and frcmod, lib, or prepin files (if any).

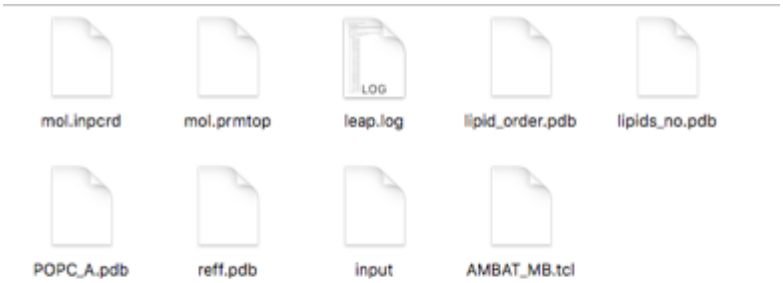


Next, it will ask for the thickness of the water layer. Type in any number you want. For this example, **let's say 10.0**.

Then it will solvate the system based on the input. Last thing it will ask is the number of ions you want to add. You can add any number or choose system neutrality as the option for the ions. For system neutrality **type 'N'**

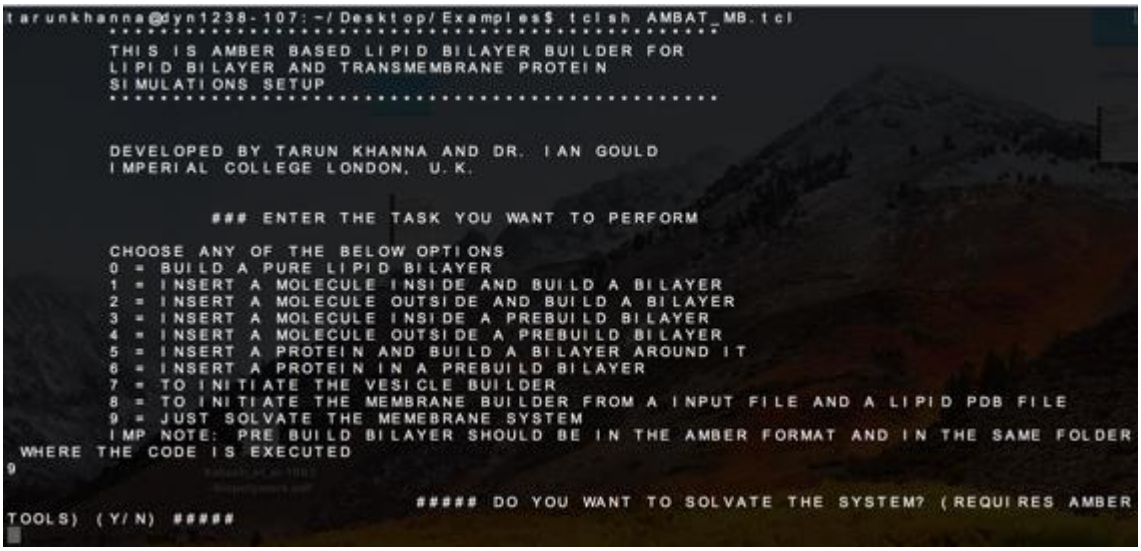


Finally, a prmtop and inpcrd file will appear in the folder where you executed AMBAT, which can be used to start an AMBER MD simulation.

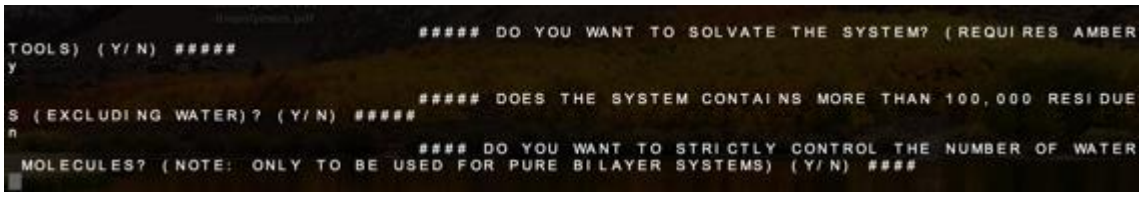


2. Solvating a pre-formed lipid bilayer (build through from AMBAT) (Option '9')

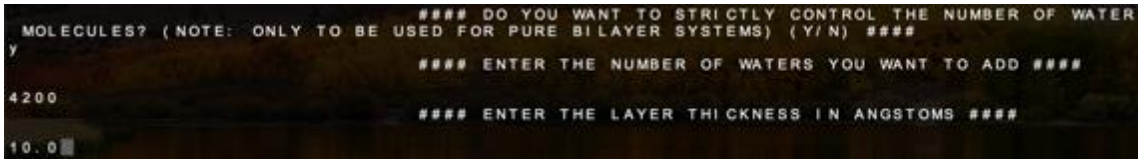
One of the point which was mentioned in point (f) of 1 is that you can get unsolvated bilayers from AMBAT. These unsolvated bilayers along with the 'input' file for forming them can be used as an input to just execute the solvation branch of AMBAT. **Option '9'** is the input for doing that.



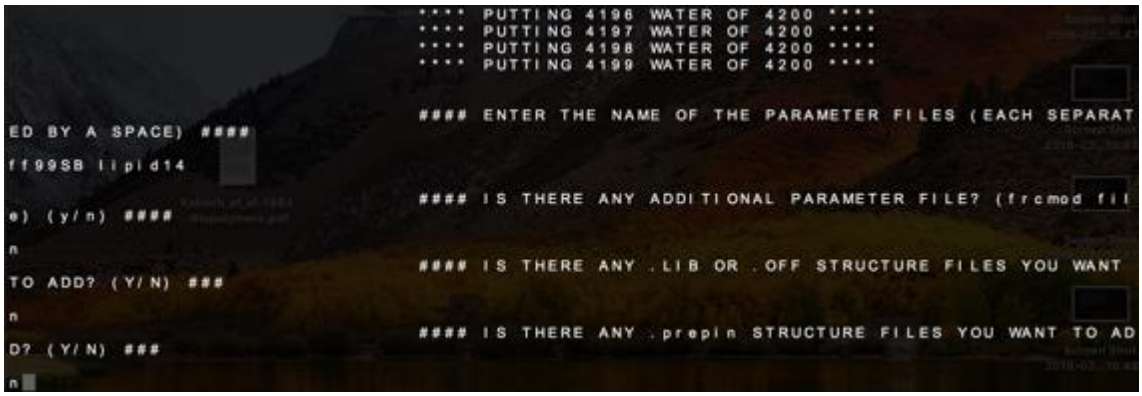
Now let's choose option of controlling the number of water. So, **type in 'Y'**.



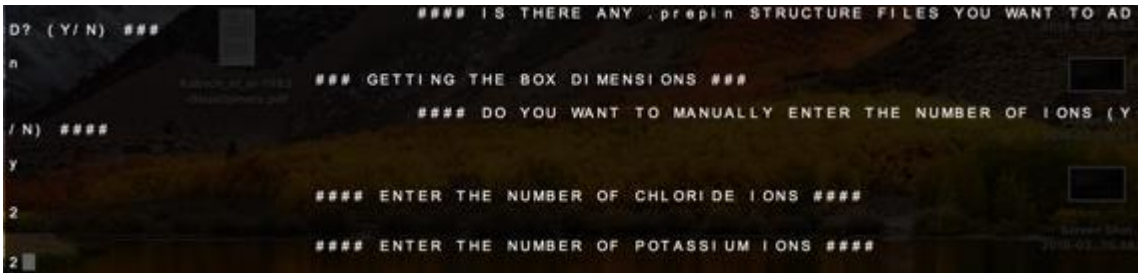
Next, it will ask for the number of water molecules and the water layer thickness. **Type in 4200 (or any number) and 10.0** (or any number) respectively. Now, AMBAT will try to distribute 4200 water molecules in 10 A distance from upper and lower leaflet.



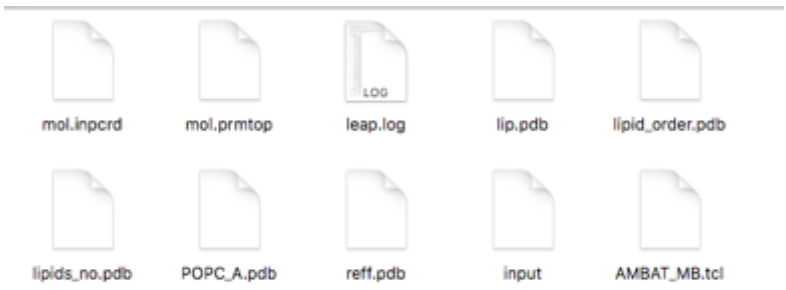
Next, it will ask for the parameter files.



Next, it will ask for the number of ions. Let's choose **2 K+ and 2 Cl-** ions.



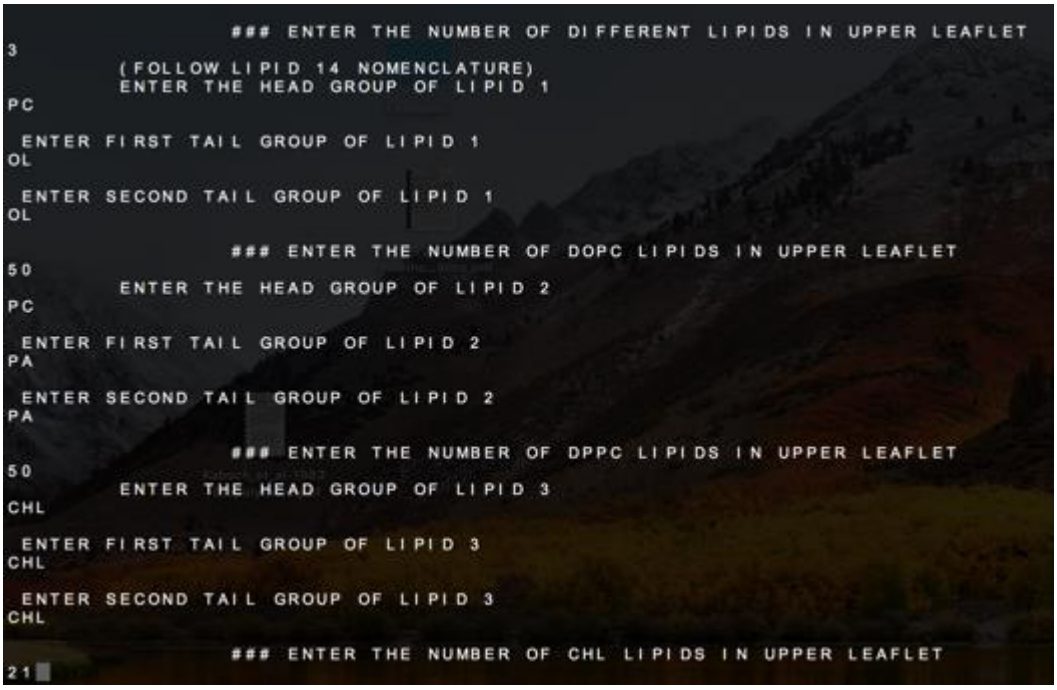
Finally, it will generate a prmtop and inpcrd file along with other files (will be described at the end of the execution).



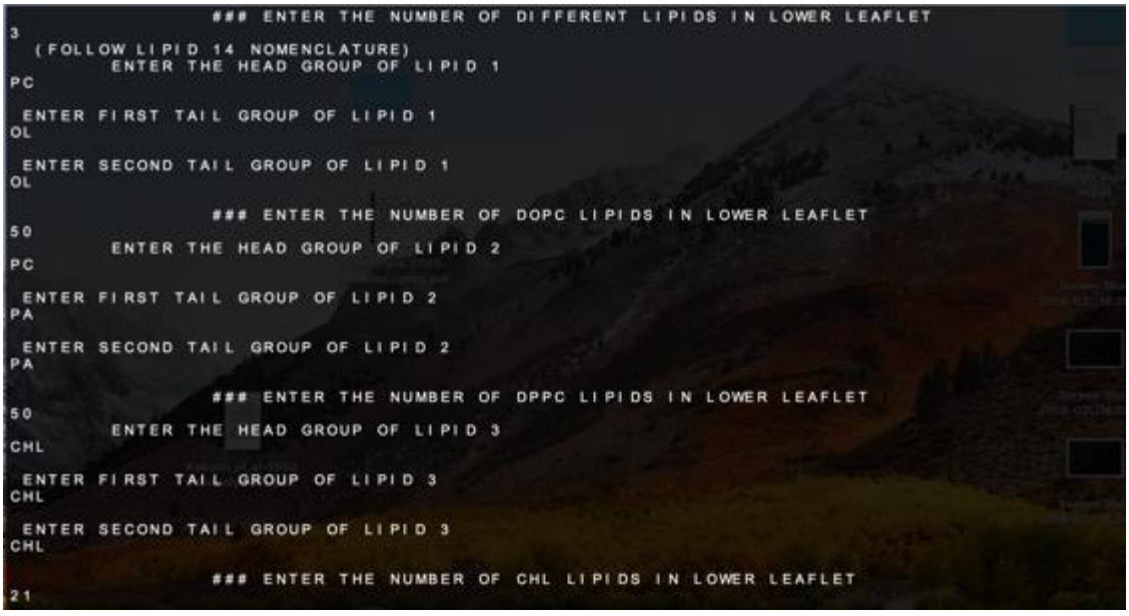
3. Asymmetric bilayer and Non-random grid

In this example we form an asymmetric lipid bilayer of 121 lipids per leaflet composed of 2:2:1 DPPC, DOPC and Cholesterol. And apply the non-random grid algorithm of AMBAT to form the initial lipid bilayer close to the equilibrium as well.

Various inputs for Upper leaflet:



Various inputs for lower leaflets:



Next for asymmetric bilayer we use **version 2.0** and form a non-random grid with **1 rule** of **PC==CHL 1** (cholesterol molecules preferentially located on the DOPC side of the bilayer).

The non-random grid algorithm in AMBAT weighs each grid with respect to the user defined rules. The cumulative score each leaflet shows the quality of grid with respect to those rules. (Score between 0 and number of lipids per leaflet).

The rest of the steps are similar to point 1 (from (h)).

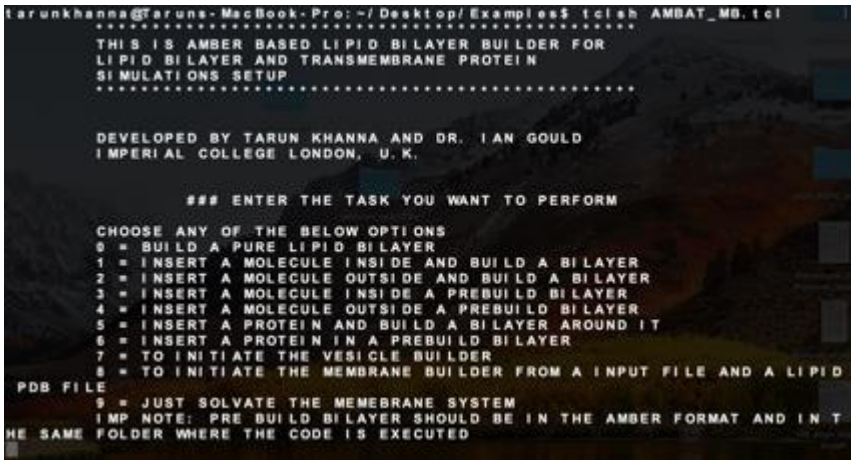
4. Lipids not there in lipid14

There is another option in AMBAT, option ‘8’ which allows the user to give their own lipids as inputs. For example, for forming a lipid bilayer, mimicking thylakoid membrane, user need to provide the constituting Galactolipids PDB’s and modify the input file as:

```
{ LIPIDS_PDB: DGDG.pdb MGDG.pdb PG.pdb SQDG.pdb }
{ LIPID_PDB_NAME: DGD LMG LHG SQD}
{ HEAD_TAIL_LINKAGE: 01G 08 08 048}
{ LIPID_COMPOSITION_UL: 87 102 27 40 }
{ LIPID_COMPOSITION_LL: 87 102 27 40 }
{ BOX_DIMENSIONS: 144.0 144.0 18.0}
{ HOLE_DIMENSIONS: }
{ PROTEIN_PDB: }
{ PROTEIN_SHIFT: }
{ LIPID_BILAYER_METHOD: 1 36.0 }
{ HOLE_METHOD: 1 }
{ LIPID14_DIVISION: }
{ CODE_EXECUTION: 0 }
{ VERSION: 2.0 DGD LMG LHG SQD 4 }
```

Here user have full control over the head_tail_linkage (we recommend glycerol linkage)

And then run AMBAT with **option ‘8’** as input:



Next AMBAT will be executed as usual and form a bilayer composed of Galactolipids (at present not there in lipid 16)