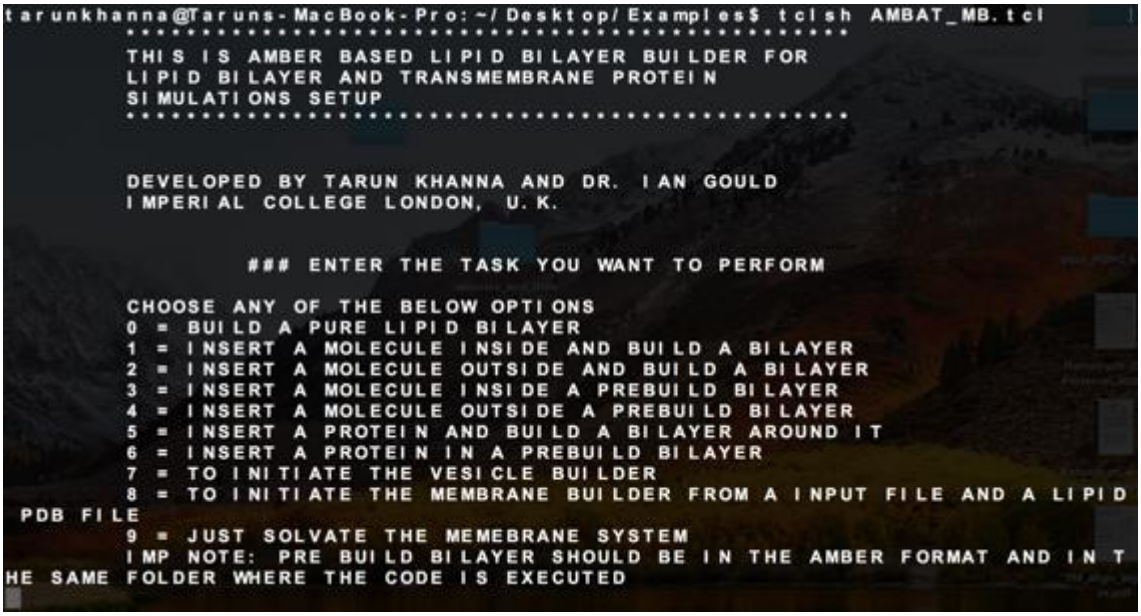
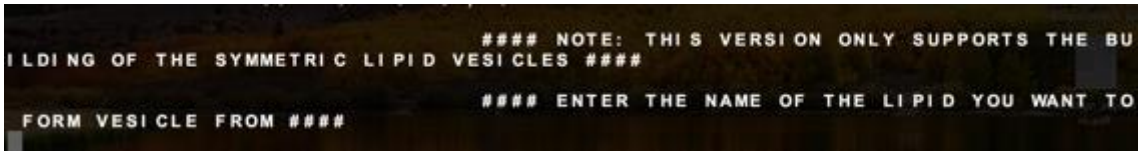
1. Dry POPC vesicle

In this example we form a dry POPC vesicle of radius 50.0 A.

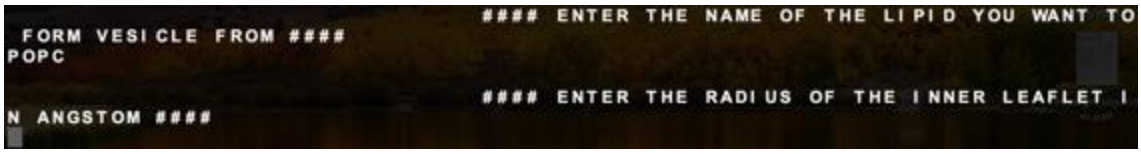
(a) On executing AMBAT choose **option '7'** to enter the vesicle builder suite.



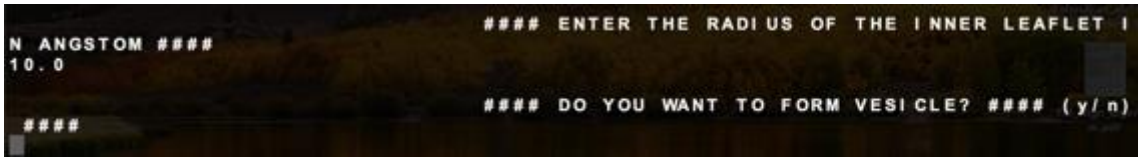
(b) Next, select the lipid you want to form the vesicles from, **type in POPC (uppercase)**



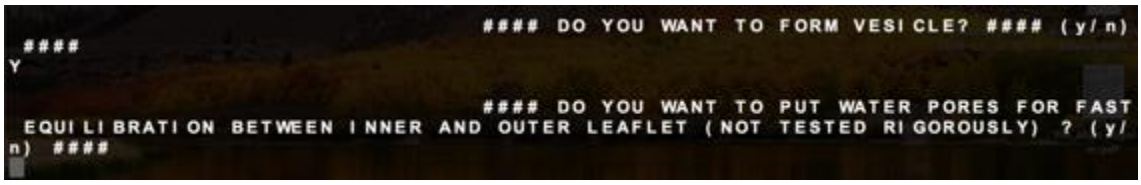
(c) Next, enter the radius of the inner leaflet. The radius of outer leaflet is defined as inner leaflet + expected bilayer thickness. And the size of the vesicle is the average of the two radii. For this example, **type in 10**



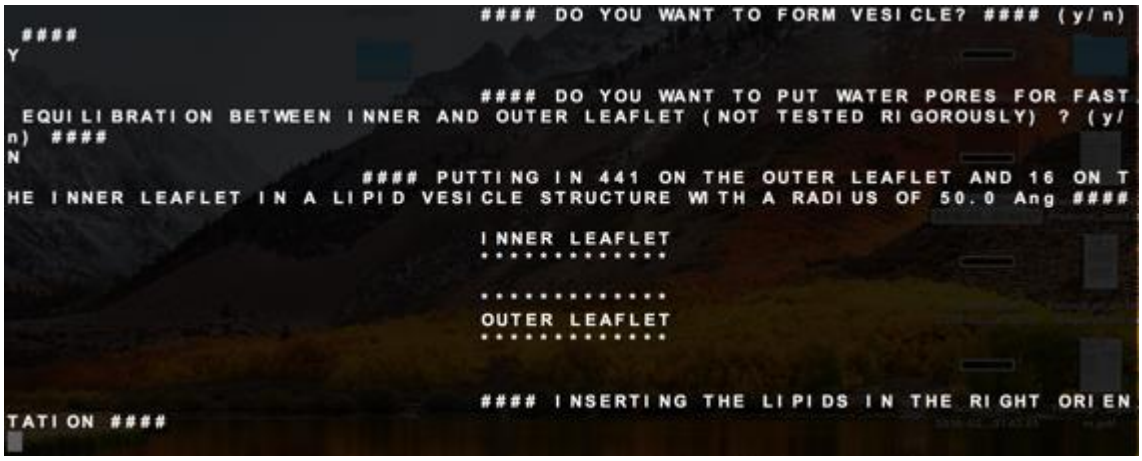
(d) Next, AMBAT will ask if you want to form vesicles or DIBs. **Type in 'Y'** to form vesicle.



(e) AMBAT allows the user to put artificial water pores in the system (similar to Martini vescicle builder of CHARMM-GUI). For this example, we choose to build a simple vesicle, so **type in 'N'**. (Point 2 builds the vesicle with water pores).



(f) Next, AMBAT will start building a POPC vesicle of 50 A size. (Execution should take less than a minute).



(g) After building the vesicle, AMBAT allows the user to solvate it. For forming dry vesicle**, type in 'N'.** (Point 2 forms a solvated vescicle).

(h) The final output from AMBAT is the description of all the files formed. In this example, box dimension will be printed out at the end along with a PDB named 'lipids_wo.pdb'.

```
                                #### DO YOU WANT TO PUT THE SOLVATING WATER?
 (y/n) ####
N
                                ***** lipids_wo.pdb IS THE FINAL DRY VESICLE
 *****

                           #### IMPORTANT NOTE : BOX DIMENSIONS OF THE SYSTEM I
S   112.741 112.527 112.886     ####

********************************************************************************
*************
                                #### DESCRIPTION OF THE FILES ####
                                (NOTE: SOME FILES MAY NOT BE GENERATED DEPEN
DING UPON THE OPTION YOU HAVE CHOSEN)

lipids_no.pdb : UNSOLVATED PDB OF PURE LIPID BILAYER
1_final_struct.pdb : UNSOLVATED PDB OF LIPID BILAYER WITH INSERTED COMPONENT
lip.pdb: SOLVATED PURE LIPID BILAYER
reff.pdb: FINAL SOLVATED PDB OF THE SYSTEM
mol.prmtop: FINAL AMBER PRMTOP FILE
mol.inpcrd: FINAL AMBER COORDINATE FILE
dir1: PROFILE OF THE PROTEIN INSIDE THE BILAYER
lipids_wo.pdb: FINAL DRY VESSICLE OR DIBs
sol_lipid.pdb: FINAL SOLVATED VESICLE OR DIBs
********************************************************************************
**************
```

2. Wet Vesicles with artificial water pores

(a) In this example we form a wet vesicle with artificial water pores. So, **type in 'y'** when AMBAT asks for building water pores ((e) in example 1).

```
                                #### DO YOU WANT TO FORM VESICLE? #### (y/n)
 ####
Y

                                #### DO YOU WANT TO PUT WATER PORES FOR FAST
 EQUILIBRATION BETWEEN INNER AND OUTER LEAFLET (NOT TESTED RIGOROUSLY) ? (y/
n) ####
```

(b) AMBAT will start building a POPC vesicle with 6 artificial water pores along the positive and negative x,y,z directions. (might take close to a minute).

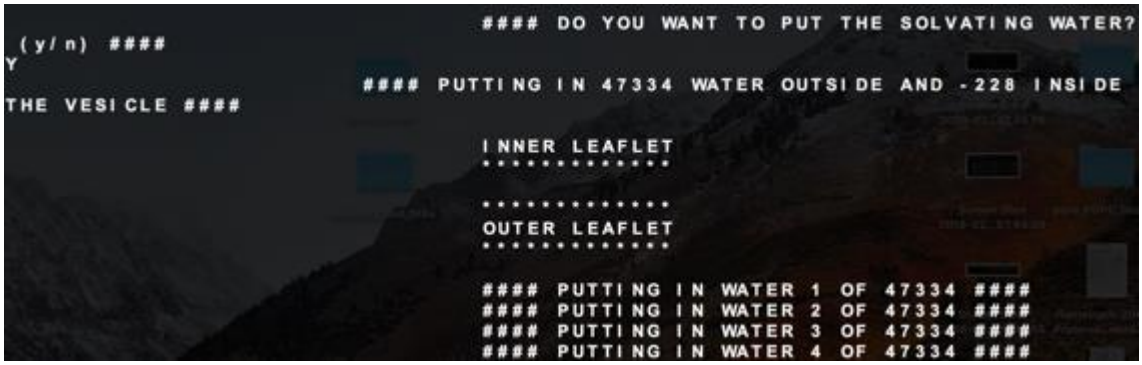
```
                                #### DO YOU WANT TO PUT WATER PORES FOR FAST
 EQUILIBRATION BETWEEN INNER AND OUTER LEAFLET (NOT TESTED RIGOROUSLY) ? (y/
n) ####
Y
                           #### PUTTING IN 484 ON THE OUTER LEAFLET AND 25 ON T
HE INNER LEAFLET IN A LIPID VESICLE STRUCTURE WITH A RADIUS OF 51.7 Ang ####
                                INNER LEAFLET
                                ************

                                ************
                                OUTER LEAFLET
                                ************


                                #### INSERTING THE LIPIDS IN THE RIGHT ORIEN
TATION ####
```

(c) Next, when asked for solvation, you can choose to form a dry vesicle with pores or solvate the system. For this example, type in 'Y' to solvate the above formed vesicle.

(d) AMBAT will now solvate the vesicle keeping the density of water less than the liquid water density. (might take a minute or 2).



(e) Finally, you will have a solvate vesicle ('sol_lipid.pdb').

3. Formation of DIBs

Imp note: Although AMBAT allows to form initial DIBs structure but we haven't found any working equilibration procedure. Most of the DIBs are found to be unstable after 100ns of MD simulations.

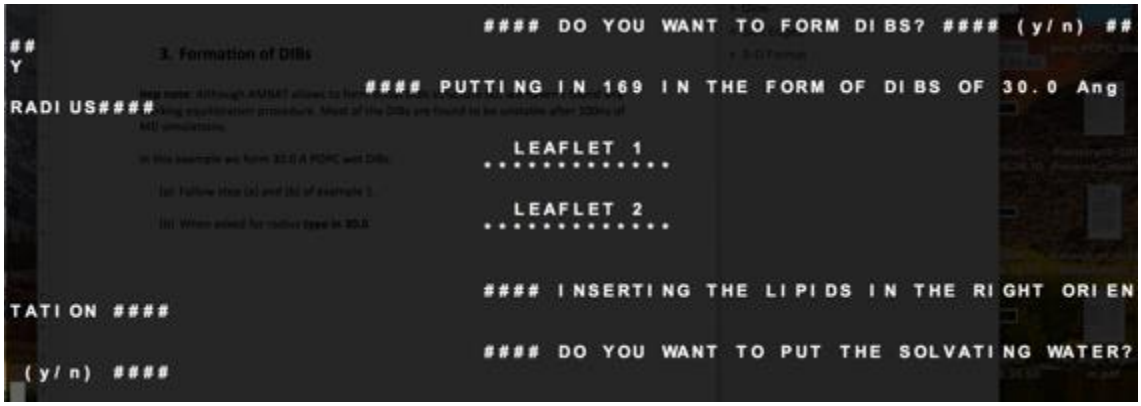
In this example we form 30.0 A POPC wet DIBs.

(a) Follow step (a) and (b) of example 1.

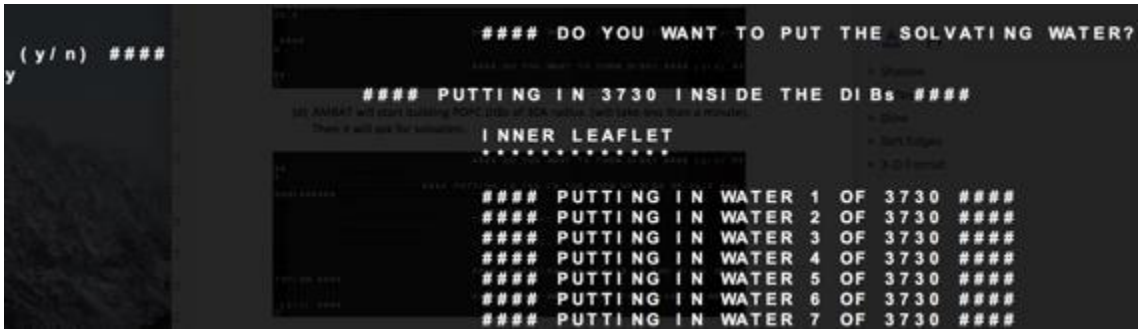
(b) When asked for radius **type in 30.0**

(c) Next **type in 'N'** for vesicle and **'Y'** for DIBs

(d) AMBAT will start building POPC DIBs of 30A radius. (will take less than a minute). Then it will ask for solvation.



(e) **Type in 'Y'** for solvation. AMBAT will start solvating the DIBs.



(f) Finally (after less than a minute), you will have a partially solvated DIBs PDB (sol_lipid.pdb). Partial, because you still need to add an organic solvent outside the DIBs. In present version AMBAT only adds water.