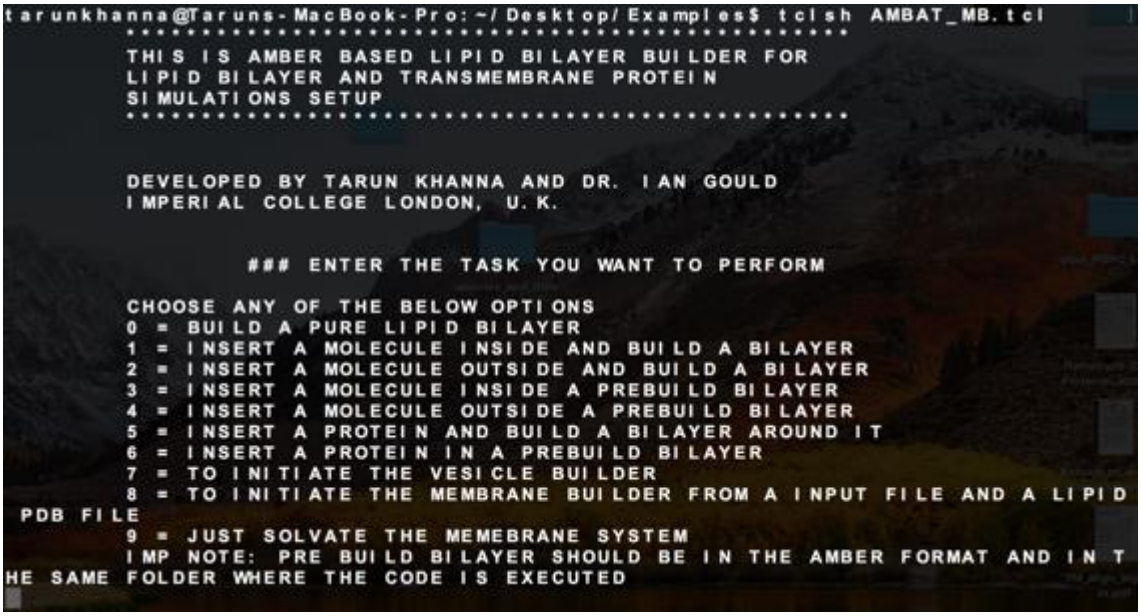
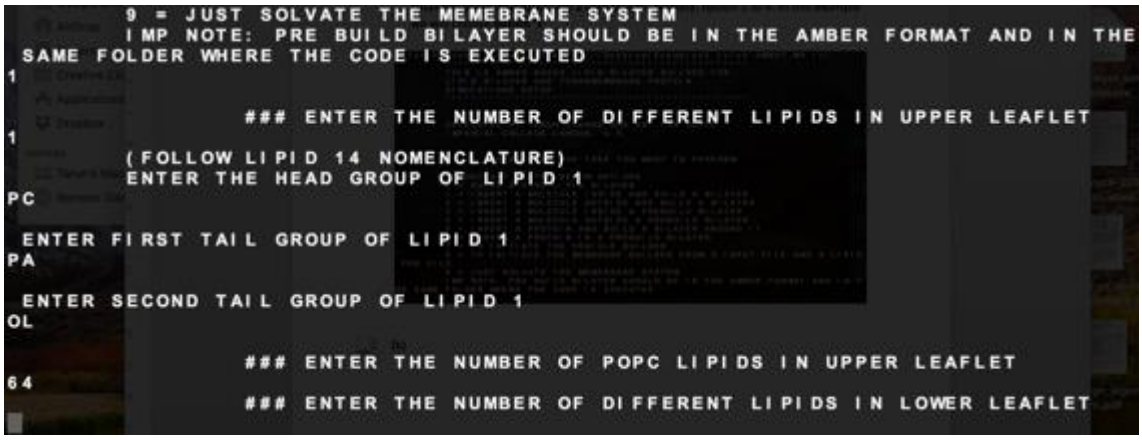
1. Lipid bilayer and small molecule(s)

In this example we insert a small molecule PDB (MOL.pdb) inside a pure POPC bilayer and solvate the final system.

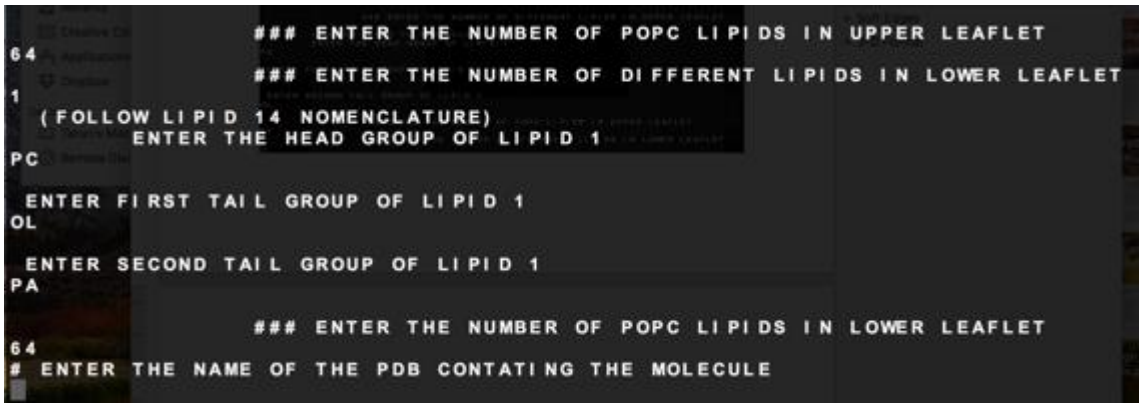
(a) On executing AMBAT, you will notice AMBAT allows multiple options to insert a small molecule inside/outside a lipid bilayer. Namely, option 1 to 4. In this example we will choose **option '1'**



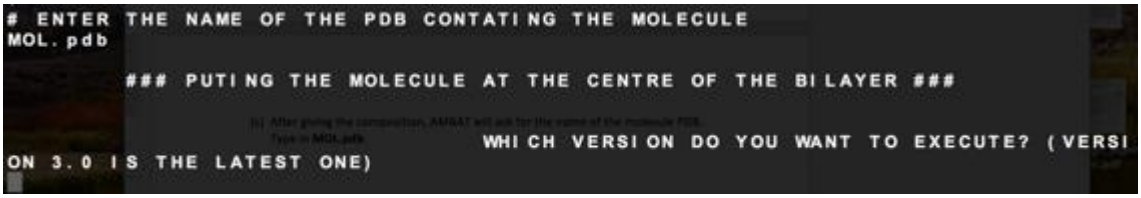
(b) Next, AMBAT will ask for the composition of the outer leaflet and then inner leaflet. Refer pure lipid bilayer example to know more about the format of this input. In short, AMBAT reads lipids similar to lipid 14 force field. It first ask for number of different lipids (1 in this case), head (PC), tail1(PA), tail2(OL) and finally number of lipids (64 in this case). Repeat the same for the lower leaflet.



(c) After giving the composition, AMBAT will ask for the name of the molecule PDB. Type in **MOL.pdb**.

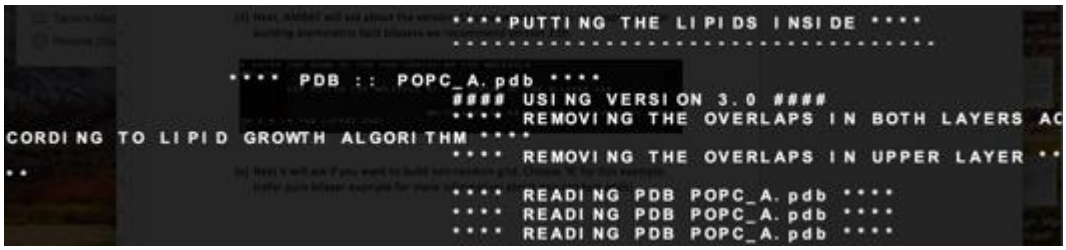


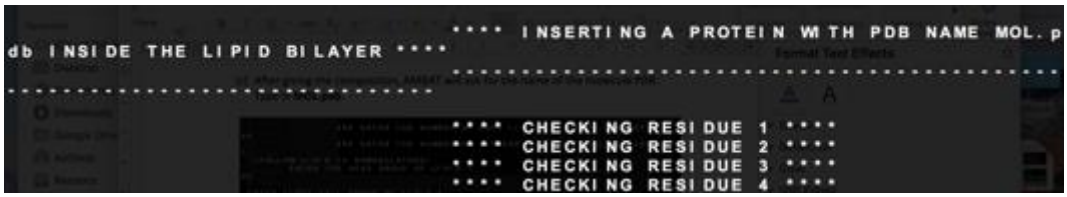
(d) Next, AMBAT will ask about the version. Choose **version 3.0** for this example. (For building asymmetric lipid bilayers we recommend version 2.0).



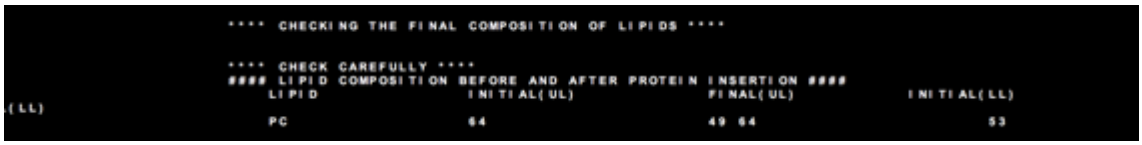
(e) Next it will ask if you want to build non-random grid. Choose 'N' for this example. (refer pure bilayer example for more information about non-random grids).

(f) Next it will execute the code to build a pure POPC bilayer and then insert MOL.pdb inside that bilayer.

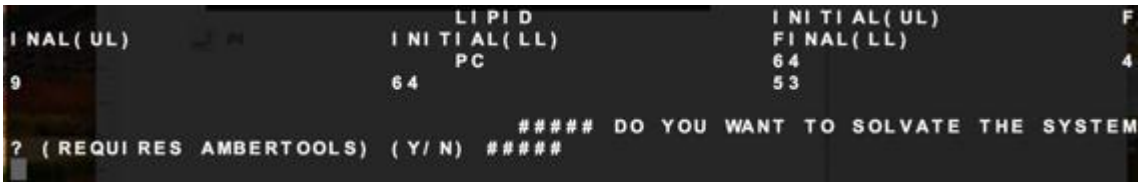




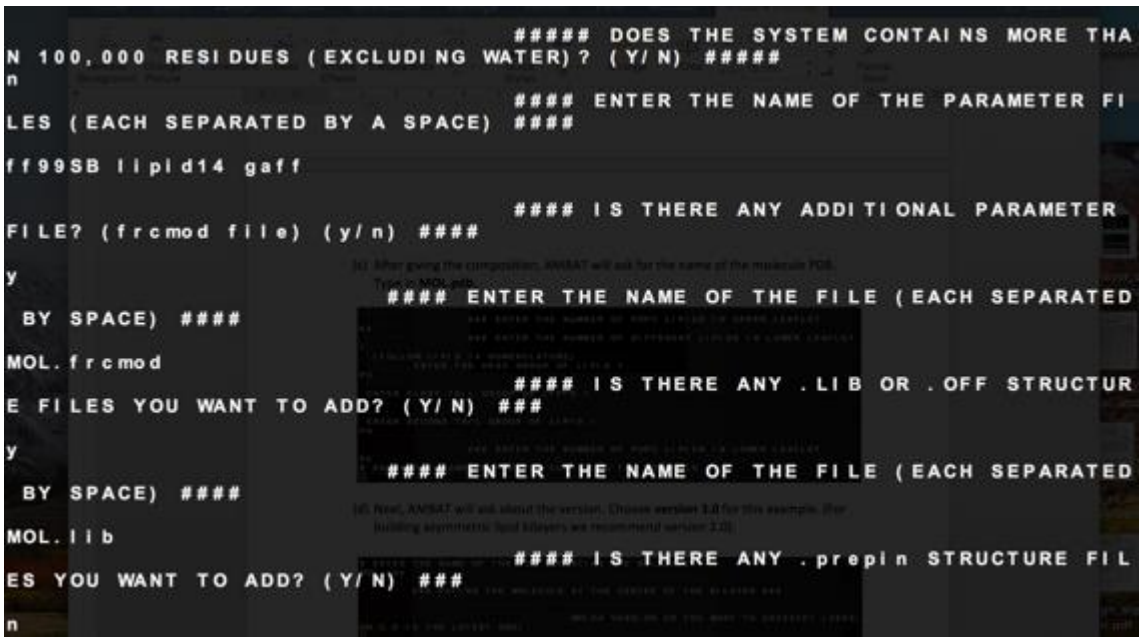
(g) After building the bilayer and molecule system, AMBAT will print out the final composition of the bilayer.



(h) Next it will ask for solvation, **type in 'y'** for solvating the system

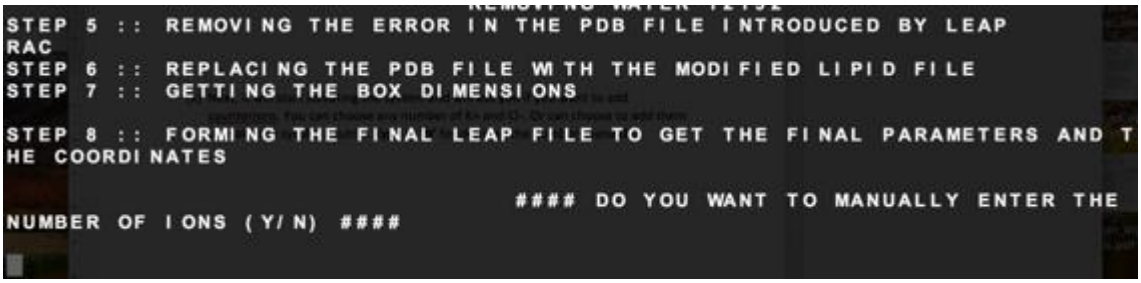


(i) Next, AMBAT will ask if the system contain more than 100,000 atoms, which in this case is not. So, **type in 'N'** for next input. Then, it will ask about the parameters of the system, starting from standard AMBER force fields (type in **ff99SB lipid14 gaff**), then additional parameter files (for this example a frcmod file, type in **'y'** and then **MOL.frcmod**), a .lib or. off file (type in **'y'** and then **MOL.lib**. Type in '**n'** for .prepin fil

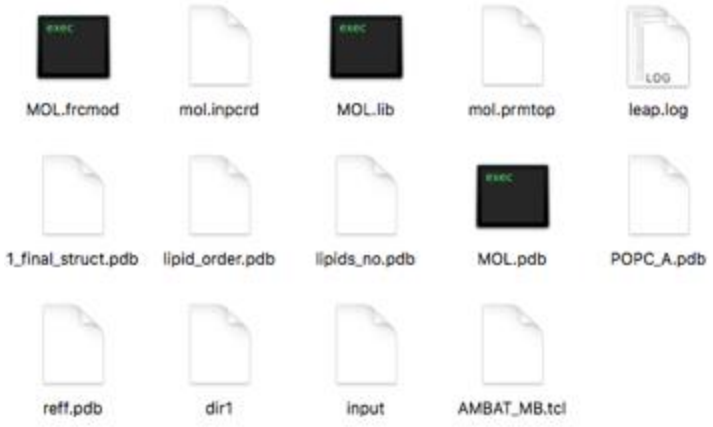


(j) Next, AMBAT will ask for the thickness of the water layer. **Type in 10**

(k) Next, it will start solvating the system and will ask you if you want to add counter ions. You can choose any number of K+ and Cl-. Or can choose to add them to make the system neutral. **Type in 'N'** for defining the number manually.



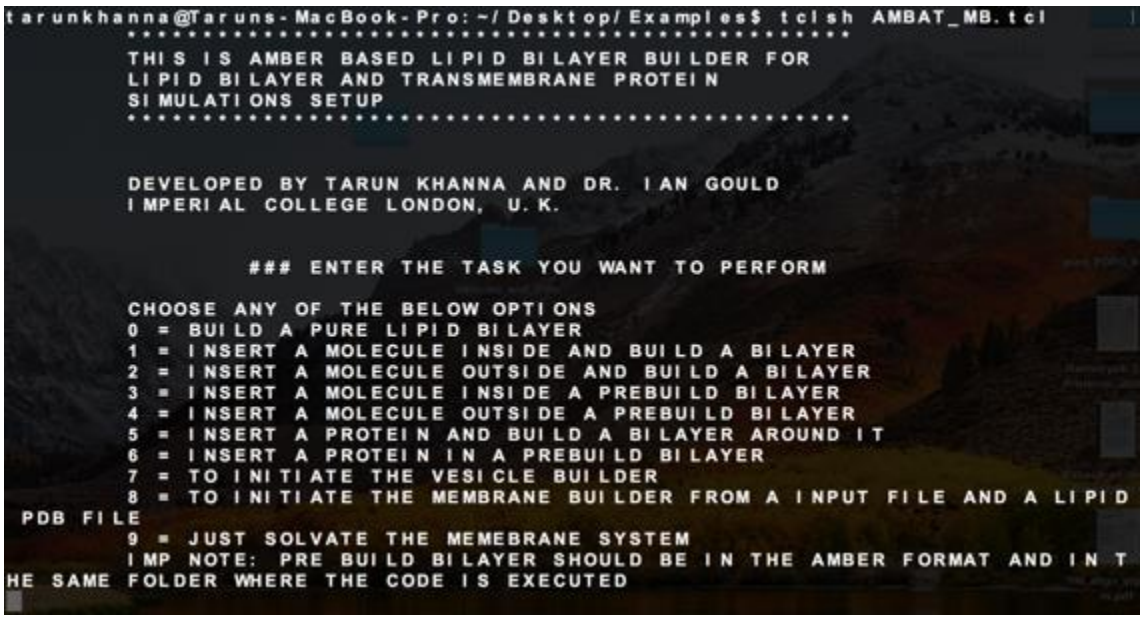
(l) Finally, AMBAT will print out the description of all the files formed.



2. Inserting a molecule outside a pre-formed lipid bilayer

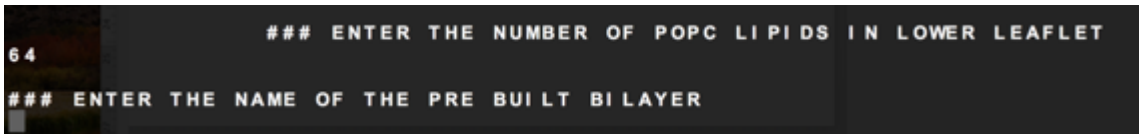
In this example we demonstrate option '4' of AMBAT. Inserting molecule MOL.pdb outside a pre-formed bilayer in the above example ('lipids_no.pdb').

(a) Select **option '4'** after executing AMBAT script.



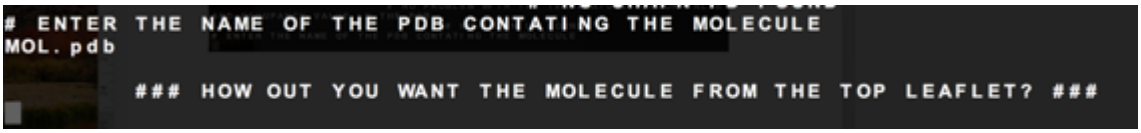
(b) Next follow the step (b) of example 1. Note: Input should match the composition of the pre-formed PDB. And this preformed PDB could be from any source, but should be in lipid 14 format.

(c) Next, Type in the name of the pre-formed PDB, **lipids_no.pdb**.



(d) AMBAT will do a few checks on this pre-formed PDB and change it according to the format AMBAT can read. Next, it will ask for the name of the file containing the molecule. Type in **MOL.pdb**

(e) Next, AMBAT will ask for the position of the molecule from the upper leaflet. Type in **10.0.** Which will put the molecule 10A from the upper leaflet. And select version 3.0 for building the bilayer system.



(f) AMBAT will then use the pre-formed bilayer (lipids_no.pdb) and insert MOL.pdb 10.0 A from the upper leaflet.