

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/Examples$ 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
.....
          POPC 100% 0.0000  AMBAT_01.tcl  AMBAT_01.tcl
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 PREBUILT BILAYER
4 = INSERT A MOLECULE OUTSIDE A PREBUILT BILAYER
5 = INSERT A PROTEIN AND BUILD A BILAYER AROUND IT
6 = INSERT A PROTEIN IN A PREBUILT 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 MEMBRANE 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)

```
( FOLLOW LIPID 14 NOMENCLATURE )
ENTER THE HEAD GROUP OF LIPID 1
PC
ENTER FIRST TAIL GROUP OF LIPID 1
```

```
ENTER FIRST TAIL GROUP OF LIPID 1
PA
ENTER SECOND TAIL GROUP OF LIPID 1
```

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

```
ENTER FIRST TAIL GROUP OF LIPID 1
PA
ENTER SECOND TAIL GROUP OF LIPID 1
OL
### ENTER THE NUMBER OF POPC LIPIDS IN UPPER LEAFLET
```

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

```
64 ### ENTER THE NUMBER OF POPC LIPIDS IN UPPER LEAFLET
1 ### ENTER THE NUMBER OF DIFFERENT LIPIDS IN LOWER LEAFLET
(FOLLOW LIPID 14 NOMENCLATURE)
ENTER THE HEAD GROUP OF LIPID 1
PC
ENTER FIRST TAIL GROUP OF LIPID 1
PA
ENTER SECOND TAIL GROUP OF LIPID 1
OL
64 ### ENTER THE NUMBER OF POPC LIPIDS IN LOWER LEAFLET
```

- (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**

```

64          ### ENTER THE NUMBER OF POPC LIPIDS IN LOWER LEAFLET
          WHICH VERSION DO YOU WANT TO EXECUTE? (VERSION 3.0 IS TH
E LATEST ONE)

```

- (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))

```

E LATEST ONE)
3.0
MEMBRANE BUILDER ####
INPUT ****
** EXTENSION OF PC ALONG X AXIS IS 5.3500000000000005 **
** EXTENSION OF PC ALONG Y AXIS IS 4.0790000000000001 **
** EXTENSION OF PC ALONG Z AXIS IS 18.826999999999998 **
-----
##### ONLY LIPID BILAYER WILL BE FORMED #####
LE == AND I = RULES? ####
#### DO YOU WANT TO FORM A NON-RANDOM GRID BASED ON SIMP

```

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

```

-----
##### ONLY LIPID BILAYER WILL BE FORMED #####
#### DO YOU WANT TO FORM A NON-RANDOM GRID BASED ON SIMP
LE == AND I = RULES? ####
NO
**** FORMING A LIPID BILAYER ****
**** BUILDING THE UPPER LAYER ACCORDING TO METHOD1 ****
**** BUILDING THE LOWER LAYER ACCORDING TO METHOD1 ****
-----
****PUTTING THE LIPIDS INSIDE ****
-----
**** PDB :: POPC_A.pdb ****
#### USING VERSION 3.0 ####
**** REMOVING THE OVERLAPS IN BOTH LAYERS ACCORDING TO L
LIPID GROWTH ALGORITHM ****
**** REMOVING THE OVERLAPS IN UPPER LAYER ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****

```

- (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).

```

**** PUTTING IN RESIDUE 126 ****
-----
**** PUTTING IN RESIDUE 127 ****
-----
**** PUTTING IN RESIDUE 128 ****
-----
##### DO YOU WANT TO SOLVATE THE SYSTEM? (REQUIRES AMBER
TOOLS) (Y/N) #####

```

```

*****
**
***** DESCRIPTION OF THE FILES *****
(NOTE: SOME FILES MAY NOT BE GENERATED DEPENDING UPON TH
E OPTION YOU HAVE CHOSEN)
lipids_no.pdb : UNSOLVATED PDB OF PURE LIPID BILAYER
t_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 VESICLE OR DIBs
sol_lipid.pdb: FINAL SOLVATED VESICLE OR DIBs
*****

```

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

```

**** PUTTING IN RESIDUE 128 ****
-----
##### DO YOU WANT TO SOLVATE THE SYSTEM? (REQUIRES AMBER
TOOLS) (Y/N) #####
y
##### DOES THE SYSTEM CONTAINS MORE THAN 100,000 RESIDUE
S (EXCLUDING WATER)? (Y/N) #####

```

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).

```

##### DOES THE SYSTEM CONTAINS MORE THAN 100,000 RESIDUE
S (EXCLUDING WATER)? (Y/N) #####
N
##### DO YOU WANT TO STRICTLY CONTROL THE NUMBER OF WATER
MOLECULES? (NOTE: ONLY TO BE USED FOR PURE BILAYER SYSTEMS) (Y/N) #####

```

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'**

```

##### DOES THE SYSTEM CONTAINS MORE THAN 100,000 RESIDUE
S (EXCLUDING WATER)? (Y/N) #####
N
##### DO YOU WANT TO STRICTLY CONTROL THE NUMBER OF WATER
MOLECULES? (NOTE: ONLY TO BE USED FOR PURE BILAYER SYSTEMS) (Y/N) #####
N
##### ENTER THE NAME OF THE PARAMETER FILES (EACH SEPARAT
ED BY A SPACE) #####

```

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

```

##### DO YOU WANT TO STRICTLY CONTROL THE NUMBER OF WATER
MOLECULES? (NOTE: ONLY TO BE USED FOR PURE BILAYER SYSTEMS) (Y/N) ####
n

##### ENTER THE NAME OF THE PARAMETER FILES (EACH SEPARAT
ED BY A SPACE) ####
ff99SB lipid14

##### IS THERE ANY ADDITIONAL PARAMETER FILE? (frcmod fil
e) (y/n) ####
n

##### IS THERE ANY .LIB OR .OFF STRUCTURE FILES YOU WANT
TO ADD? (Y/N) ###
n

##### IS THERE ANY .prepin STRUCTURE FILES YOU WANT TO AD
D? (Y/N) ###
n

```

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**.

```

##### IS THERE ANY .prepin STRUCTURE FILES YOU WANT TO AD
D? (Y/N) ###
n

##### ENTER THE THICKNESS OF THE WATER LAYER IN ANGSTOMS
YOU WANT TO ADD ####
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'**

```

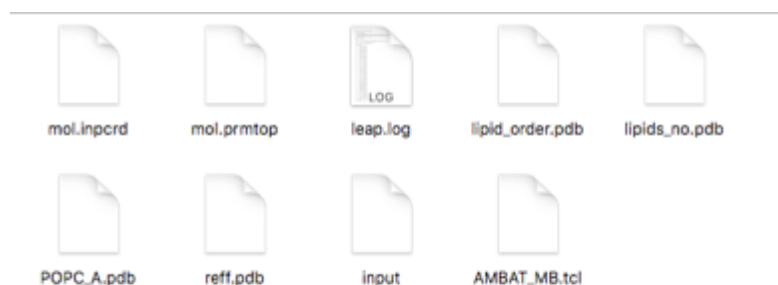
##### DO YOU WANT TO MANUALLY ENTER THE NUMBER OF IONS (Y
/N) ####
n

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

lipids_no.pdb : UNSOLVATED PDB OF PURE LIPID BILAYER
t_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
dirt: PROFILE OF THE PROTEIN INSIDE THE BILAYER
lipids_wo.pdb: FINAL DRY VESICLE OR DIBs
sol_lipid.pdb: FINAL SOLVATED VESICLE OR DIBs
*****

```

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.

```
tarunkhanna@syn1238-107: ~/Desktop/Examples$ telnet 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 PREBUILT BILAYER
4 = INSERT A MOLECULE OUTSIDE A PREBUILT BILAYER
5 = INSERT A PROTEIN AND BUILD A BILAYER AROUND IT
6 = INSERT A PROTEIN IN A PREBUILT 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 MEMBRANE SYSTEM
IMP NOTE: PRE BUILD BILAYER SHOULD BE IN THE AMBER FORMAT AND IN THE SAME FOLDER
WHERE THE CODE IS EXECUTED
9
***** DO YOU WANT TO SOLVATE THE SYSTEM? (REQUIRES AMBER
TOOLS) (Y/N) *****
```

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

```
***** DO YOU WANT TO SOLVATE THE SYSTEM? (REQUIRES AMBER
TOOLS) (Y/N) *****
y
***** DOES THE SYSTEM CONTAINS MORE THAN 100,000 RESIDUE
S (EXCLUDING WATER)? (Y/N) *****
n
***** DO YOU WANT TO STRICTLY CONTROL THE NUMBER OF WATER
MOLECULES? (NOTE: ONLY TO BE USED FOR PURE BILAYER SYSTEMS) (Y/N) *****
```

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.


```

##### DO YOU WANT TO STRICTLY CONTROL THE NUMBER OF WATER
MOLECULES? (NOTE: ONLY TO BE USED FOR PURE BILAYER SYSTEMS) (Y/N) ####
y
##### ENTER THE NUMBER OF WATERS YOU WANT TO ADD #####
4200
##### ENTER THE LAYER THICKNESS IN ANGSTOMS #####
10.0

```

Next, it will ask for the parameter files.

```

**** PUTTING 4196 WATER OF 4200 ****
**** PUTTING 4197 WATER OF 4200 ****
**** PUTTING 4198 WATER OF 4200 ****
**** PUTTING 4199 WATER OF 4200 ****

ED BY A SPACE) #####
ff99SB lipid14
##### ENTER THE NAME OF THE PARAMETER FILES (EACH SEPARAT
##### IS THERE ANY ADDITIONAL PARAMETER FILE? (trc.mod fil
e) (y/n) #####
n
##### IS THERE ANY .LIB OR .OFF STRUCTURE FILES YOU WANT
TO ADD? (Y/N) ###
n
##### IS THERE ANY .prepin STRUCTURE FILES YOU WANT TO AD
D? (Y/N) ###
n

```

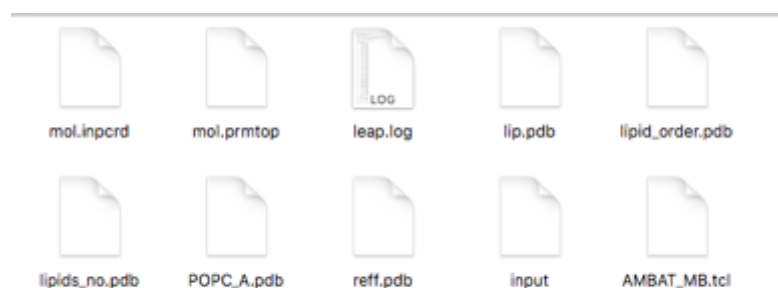
Next, it will ask for the number of ions. Let's choose **2 K+** and **2 Cl-** ions.

```

D? (Y/N) ###
n
##### GETTING THE BOX DIMENSIONS ###
##### DO YOU WANT TO MANUALLY ENTER THE NUMBER OF IONS (Y
/N) #####
y
##### ENTER THE NUMBER OF CHLORIDE IONS #####
2
##### ENTER THE NUMBER OF POTASSIUM IONS #####
2

```

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:

```
      ### ENTER THE NUMBER OF DIFFERENT LIPIDS IN UPPER LEAFLET
3      ( 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
OL
      ### ENTER THE NUMBER OF DOPC LIPIDS IN UPPER LEAFLET
50     ENTER THE HEAD GROUP OF LIPID 2
PC
      ENTER FIRST TAIL GROUP OF LIPID 2
PA
      ENTER SECOND TAIL GROUP OF LIPID 2
PA
      ### ENTER THE NUMBER OF DPPC LIPIDS IN UPPER LEAFLET
50     ENTER THE HEAD GROUP OF LIPID 3
CHL
      ENTER FIRST TAIL GROUP OF LIPID 3
CHL
      ENTER SECOND TAIL GROUP OF LIPID 3
CHL
      ### ENTER THE NUMBER OF CHL LIPIDS IN UPPER LEAFLET
21
```

Various inputs for lower leaflets:

```
      ### ENTER THE NUMBER OF DIFFERENT LIPIDS IN LOWER LEAFLET
3      ( 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
OL
      ### ENTER THE NUMBER OF DOPC LIPIDS IN LOWER LEAFLET
50     ENTER THE HEAD GROUP OF LIPID 2
PC
      ENTER FIRST TAIL GROUP OF LIPID 2
PA
      ENTER SECOND TAIL GROUP OF LIPID 2
PA
      ### ENTER THE NUMBER OF DPPC LIPIDS IN LOWER LEAFLET
50     ENTER THE HEAD GROUP OF LIPID 3
CHL
      ENTER FIRST TAIL GROUP OF LIPID 3
CHL
      ENTER SECOND TAIL GROUP OF LIPID 3
CHL
      ### ENTER THE NUMBER OF CHL LIPIDS IN LOWER LEAFLET
21
```


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).

```

E LATEST ONE)
2.0

WHICH VERSION DO YOU WANT TO EXECUTE? (VERSION 3.0 IS TH

MEMBRANE BUILDER #####
**** FILE 'input' CONTAINS THE INPUT PARAMETERS FOR THE
**** CALCULATING THE SPREAD OF EACH LIPID BASED ON THE I
NPUT ****
** EXTENSION OF PC ALONG X AXIS IS 5.5500000000000001 **
** EXTENSION OF PC ALONG Y AXIS IS 6.021 **
** EXTENSION OF PC ALONG Z AXIS IS 19.735 **
-----
** EXTENSION OF PC0 ALONG X AXIS IS 4.556 **
** EXTENSION OF PC0 ALONG Y AXIS IS 5.191 **
** EXTENSION OF PC0 ALONG Z AXIS IS 17.341 **
-----
** EXTENSION OF CHL ALONG X AXIS IS 2.819 **
** EXTENSION OF CHL ALONG Y AXIS IS 3.3419999999999996 **
** EXTENSION OF CHL ALONG Z AXIS IS 18.518 **
-----
##### ONLY LIPID BILAYER WILL BE FORMED #####

LE == AND I= RULES? (Y/N)###
Y
##### DO YOU WANT TO FORM A NON-RANDOM GRID BASED ON SIMP
ENTER THE RULES AS SIMPLE EQUAL AND NON-EQUAL STATEMENTS
LIKE :::: $lip1==/!= $lip2 $grid
##### ENTER THE NUMBERS OF RULES YOU WANT TO DEFINE #####
1
##### ENTER RULE 1 #####
PC==CHL 1

```

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).

```

-----
##### ONLY LIPID BILAYER WILL BE FORMED #####

LE == AND I= RULES? (Y/N)###
Y
##### DO YOU WANT TO FORM A NON-RANDOM GRID BASED ON SIMP
ENTER THE RULES AS SIMPLE EQUAL AND NON-EQUAL STATEMENTS
LIKE :::: $lip1==/!= $lip2 $grid
##### ENTER THE NUMBERS OF RULES YOU WANT TO DEFINE #####
1
##### ENTER RULE 1 #####
PC==CHL 1
##### CUMULATIVE SCORE UPPER LEAFLET = 78.01111111111115 #####
##### CUMULATIVE SCORE LOWER LEAFLET 78.26111111111113 #####
##### USING VERSION 2.0 #####
**** REMOVING THE OVERLAPS IN BOTH LAYERS ACCORDING TO L
LIPID GROWTH ALGORITHM ****
**** REMOVING THE OVERLAPS IN UPPER LAYER ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****
**** READING PDB DPPC_A.pdb ****

```

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

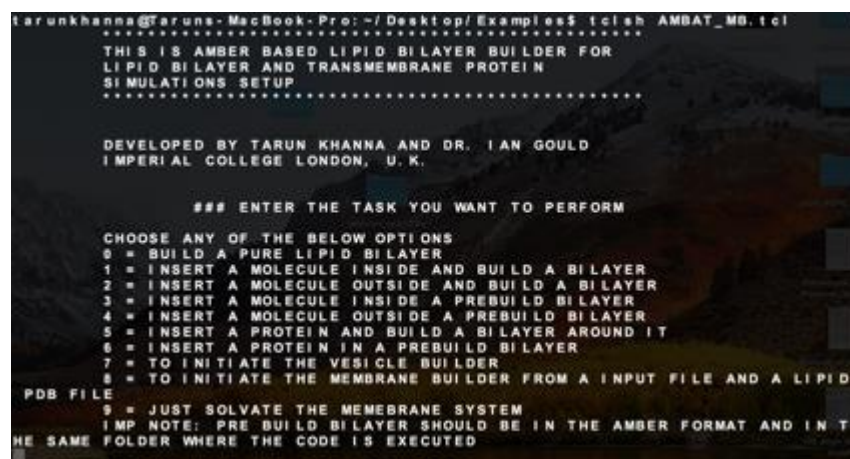
4. Lipids not there in lipid14

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:



```
tarunkhanna@Taruns-MacBook-Pro: ~/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
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### 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 PREBUILT BILAYER
4 = INSERT A MOLECULE OUTSIDE A PREBUILT BILAYER
5 = INSERT A PROTEIN AND BUILD A BILAYER AROUND IT
6 = INSERT A PROTEIN IN A PREBUILT 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 T
HE SAME FOLDER WHERE THE CODE IS EXECUTED
```

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



```
***** FORMING A LIPID BILAYER *****
***** BUILDING THE UPPER LAYER ACCORDING TO METHOD1 *****
***** BUILDING THE LOWER LAYER ACCORDING TO METHOD1 *****
*****PUTTING THE LIPIDS INSIDE *****
***** PDB :: DGDG.pdb *****
***** PDB :: MGDG.pdb *****
***** PDB :: PG.pdb *****
***** PDB :: SQDG.pdb *****
***** USING VERSION 2.0 *****
***** REMOVING THE OVERLAPS IN BOTH LAYERS *****
***** REMOVING THE OVERLAPS IN UPPER LAYER *****
```