

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'**

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

- (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.

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

### ENTER THE NUMBER OF DIFFERENT LIPIDS IN UPPER LEAFLET
1
(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 UPPER LEAFLET
### ENTER THE NUMBER OF DIFFERENT LIPIDS IN LOWER LEAFLET
```

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

```

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

```

- (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).

```

# ENTER THE NAME OF THE PDB CONTATING THE MOLECULE
MOL.pdb

### PUTING THE MOLECULE AT THE CENTRE OF THE BILAYER ###

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

WHICH VERSION DO YOU WANT TO EXECUTE? (VERSION 3.0 IS THE LATEST ONE)

```

- (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).

```

##### BOTH LIPID BILAYER AND INSERSION PART
OF THE CODE WILL BE EXECUTED #####
##### DO YOU WANT TO FORM A NON-RANDOM GRID BASED ON SIMPLE == AND != RULES? #####

```

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

```

****PUTTING THE LIPIDS INSIDE ****
*****
**** PDB :: POPC_A.pdb ****
**** USING VERSION 3.0 ****
**** REMOVING THE OVERLAPS IN BOTH LAYERS ACCORDING TO LIPID GROWTH ALGORITHM ****
**** REMOVING THE OVERLAPS IN UPPER LAYER ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****
**** READING PDB POPC_A.pdb ****

```

```

**** INSERTING A PROTEIN WITH PDB NAME MOL.pdb INSIDE THE LIPID BILAYER ****
**** CHECKING RESIDUE 1 ****
**** CHECKING RESIDUE 2 ****
**** CHECKING RESIDUE 3 ****
**** CHECKING RESIDUE 4 ****

```

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

```

**** CHECKING THE FINAL COMPOSITION OF LIPIDS ****

**** CHECK CAREFULLY ****
#### LIPID COMPOSITION BEFORE AND AFTER PROTEIN INSERTION ####
(L)  LIPID          INITIAL(UL)      FINAL(UL)      INITIAL(LL)
PC          64          49 64          53

```

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

```

          LIPID          INITIAL(UL)      FINAL(UL)      INITIAL(LL)
FINAL(UL)  PC          64          49 64          53
9
##### DO YOU WANT TO SOLVATE THE SYSTEM
? (REQUIRES AMBERTOOLS) (Y/N) #####

```

- (i) Next, AMBAT will ask if the system contains 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 file

```

##### DOES THE SYSTEM CONTAINS MORE THAN 100,000 RESIDUES (EXCLUDING WATER)? (Y/N) #####
n
##### ENTER THE NAME OF THE PARAMETER FILES (EACH SEPARATED BY A SPACE) #####
ff99SB lipid14 gaff
##### IS THERE ANY ADDITIONAL PARAMETER FILE? (frcmod file) (y/n) #####
y
##### ENTER THE NAME OF THE FILE (EACH SEPARATED BY SPACE) #####
MOL.frcmod
##### IS THERE ANY .LIB OR .OFF STRUCTURE FILES YOU WANT TO ADD? (Y/N) #####
y
##### ENTER THE NAME OF THE FILE (EACH SEPARATED BY SPACE) #####
MOL.lib
##### IS THERE ANY .prepin STRUCTURE FILES YOU WANT TO ADD? (Y/N) #####
n

```

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

```

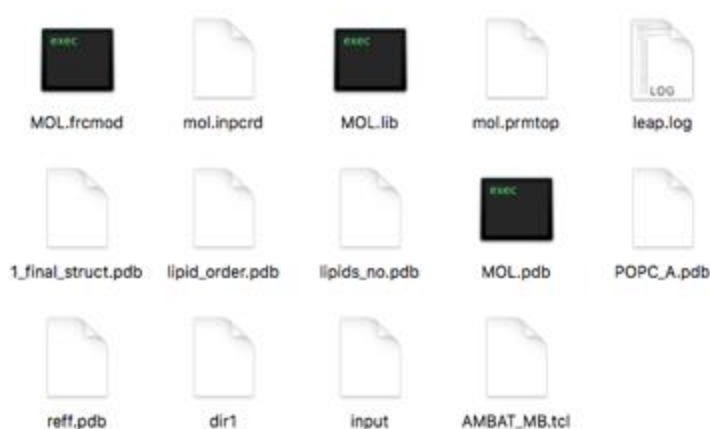
##### IS THERE ANY .prepin STRUCTURE FILES YOU WANT TO ADD? (Y/N) #####
n
##### ENTER THE THICKNESS OF THE WATER LAYER IN ANGSTOMS YOU WANT TO ADD #####
10

```

- (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.

```
STEP 5 :: REMOVING THE ERROR IN THE PDB FILE INTRODUCED BY LEAP
STEP 6 :: REPLACING THE PDB FILE WITH THE MODIFIED LIPID FILE
STEP 7 :: GETTING THE BOX DIMENSIONS
STEP 8 :: FORMING THE FINAL LEAP FILE TO GET THE FINAL PARAMETERS AND THE COORDINATES
##### DO YOU WANT TO MANUALLY ENTER THE
NUMBER OF IONS ( Y/ N ) #####
```

- (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.

```
tarunkhanna@Taruns-MacBook-Pro: ~/Desktop/Examples$ tclsh AMBAT_MB.tcl
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(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**.

```
64          ### ENTER THE NUMBER OF POPC LIPIDS IN LOWER LEAFLET
### ENTER THE NAME OF THE PRE BUILT BILAYER
```

(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**

```
### ENTER THE NAME OF THE PRE BUILT BILAYER
lipids_no.pdb
# NO PROBLEM WITH THE TEMPERATURE FACTOR VALUE
AND OCCUPANCY VALUE IN THE PDB
# NO CHAIN ID FOUND
# ENTER THE NAME OF THE PDB CONTATING THE MOLECULE
```

- (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.

```
# ENTER THE NAME OF THE PDB CONTATING THE MOLECULE
MOL.pdb

### HOW OUT YOU WANT THE MOLECULE FROM THE TOP LEAFLET? ###
```

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