

## 1. Dry POPC vesicle

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

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

```
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
*****

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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 MEMBRANE SYSTEM
IMP NOTE: PRE BUILT BILAYER SHOULD BE IN THE AMBER FORMAT AND IN THE
SAME FOLDER WHERE THE CODE IS EXECUTED
```

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

```
***** NOTE: THIS VERSION ONLY SUPPORTS THE BUILDING OF THE SYMMETRIC LIPID VESICLES *****
### ENTER THE NAME OF THE LIPID YOU WANT TO FORM VESICLE FROM ###
```

(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**

```
### ENTER THE NAME OF THE LIPID YOU WANT TO FORM VESICLE FROM ###
POPC
### ENTER THE RADIUS OF THE INNER LEAFLET IN ANGSTROM ###
```

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

```
### ENTER THE RADIUS OF THE INNER LEAFLET IN ANGSTROM ###
10.0
### DO YOU WANT TO FORM VESICLE? ### (y/n)
```

- (e) AMBAT allows the user to put artificial water pores in the system (similar to Martini vesicle 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).

```
##### 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) #####
```

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

```
##### 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) #####
N
##### PUTTING IN 441 ON THE OUTER LEAFLET AND 16 ON T
HE INNER LEAFLET IN A LIPID VESICLE STRUCTURE WITH A RADIUS OF 50.0 Ang #####
INNER LEAFLET
*****
*****
OUTER LEAFLET
*****
##### INSERTING THE LIPIDS IN THE RIGHT ORIEN
TATION #####
```

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

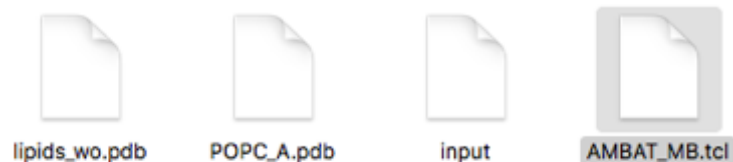
```
##### PUTTING IN 441 ON THE OUTER LEAFLET AND 16 ON T
HE INNER LEAFLET IN A LIPID VESICLE STRUCTURE WITH A RADIUS OF 50.0 Ang #####
INNER LEAFLET
*****
*****
OUTER LEAFLET
*****
##### INSERTING THE LIPIDS IN THE RIGHT ORIEN
TATION #####
##### DO YOU WANT TO PUT THE SOLVATING WATER?
(y/n) #####
```

- (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 VESICLE 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 ORIE
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.

```
##### PUTTING IN WATER 117 OF 122 #####
##### PUTTING IN WATER 118 OF 122 #####
##### PUTTING IN WATER 119 OF 122 #####
##### PUTTING IN WATER 120 OF 122 #####
##### PUTTING IN WATER 121 OF 122 #####
##### PUTTING IN WATER 122 OF 122 #####
##### DO YOU WANT TO PUT THE SOLVATING WATER?
(y/n) #####
```

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

```
##### DO YOU WANT TO PUT THE SOLVATING WATER?
Y
THE VESICLE #####
##### PUTTING IN 47334 WATER OUTSIDE AND -228 INSIDE
INNER LEAFLET
*****
OUTER LEAFLET
*****
##### PUTTING IN WATER 1 OF 47334 #####
##### PUTTING IN WATER 2 OF 47334 #####
##### PUTTING IN WATER 3 OF 47334 #####
##### PUTTING IN WATER 4 OF 47334 #####
```

- (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**

```
##### NOTE: THIS VERSION ONLY SUPPORTS THE BUILDING OF THE SYMMETRIC LIPID VESICLES #####
##### ENTER THE NAME OF THE LIPID YOU WANT TO FORM VESICLE FROM #####
POPC
##### ENTER THE RADIUS OF THE INNER LEAFLET IN ANGSTROM #####
N
```

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

```
##### ENTER THE RADIUS OF THE INNER LEAFLET IN ANGSTROM #####
30.0
##### DO YOU WANT TO FORM VESICLE? ##### (y/n)
N
##### DO YOU WANT TO FORM DIBS? ##### (y/n)
Y
```

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

```
##### DO YOU WANT TO FORM DIBS? ##### (y/n)
Y
3. Formation of DIBs
##### PUTTING IN 169 IN THE FORM OF DIBS OF 30.0 Ang
RADIUS#####
LEAFLET 1
.....
LEAFLET 2
.....
##### INSERTING THE LIPIDS IN THE RIGHT ORIENTATION #####
(y/n) #####
Y
##### DO YOU WANT TO PUT THE SOLVATING WATER?
Y
```

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

```
(y/n) ###
y

##### DO YOU WANT TO PUT THE SOLVATING WATER?
#####
##### PUTTING IN 3730 INSIDE THE DIBs #####
#####
##### INNER LEAFLET #####
#####
##### PUTTING IN WATER 1 OF 3730 #####
##### PUTTING IN WATER 2 OF 3730 #####
##### PUTTING IN WATER 3 OF 3730 #####
##### PUTTING IN WATER 4 OF 3730 #####
##### PUTTING IN WATER 5 OF 3730 #####
##### PUTTING IN WATER 6 OF 3730 #####
##### PUTTING IN WATER 7 OF 3730 #####
```

(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.

