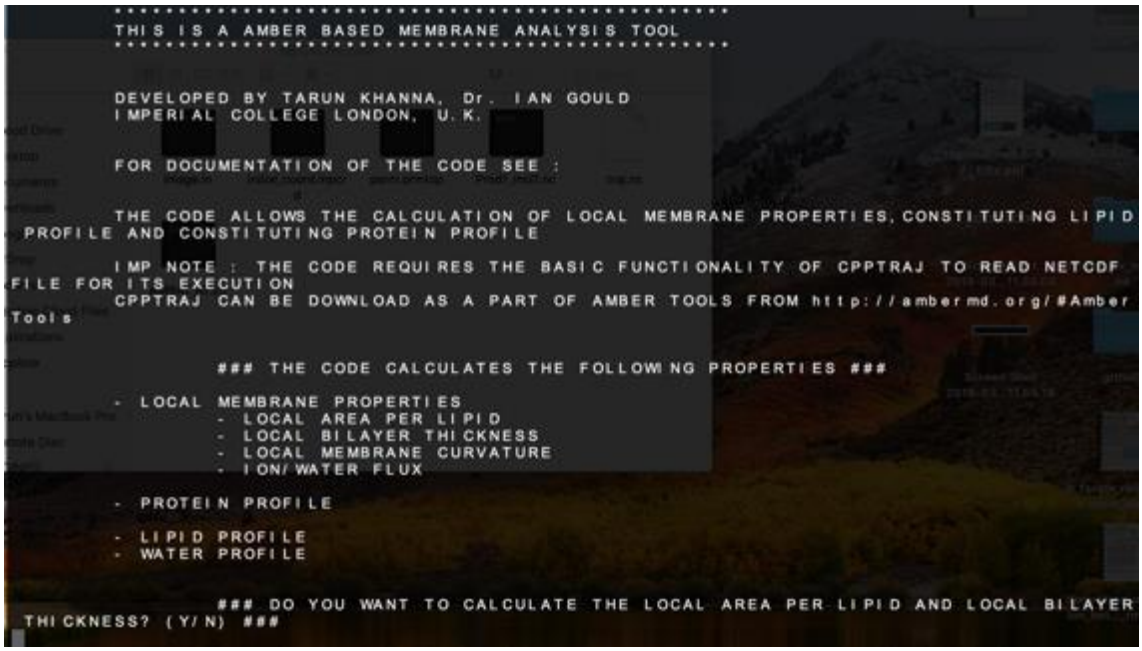
Short Instruction for running AMBAT-Analysis Tool

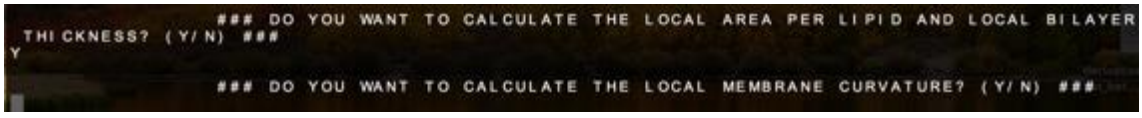
For these instructions we use last 10 frames of an AMBER based MD trajectory (traj.nc). 'parm.prmtop' and 'initial_coord.inpcrd' are the parameters and initial coordinates of the system respectively.

1. AMBAT_AT (Analysis Tool) requires the source code or the path to the source code for execution.

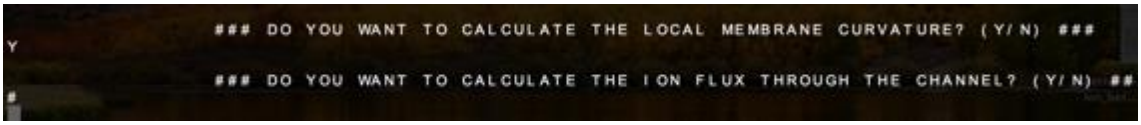
   Upon execution, AMBAT_AT will present the user with an interactive command line interface, which summarises the functionalities of the code. First of these functionalities is the calculation of local bilayer thickness and local area per lipid.



2. Type in Y or N. For this tutorial, type in 'Y' (case insensitive) for the calculation of local bilayer thickness and local area per lipid. Next functionality is local membrane curvature.

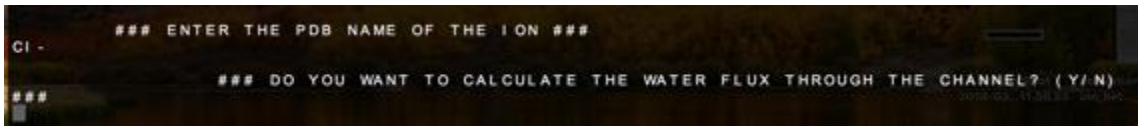


3. Type in 'Y' for the calculation of local membrane curvature. Next functionality is ion flux.



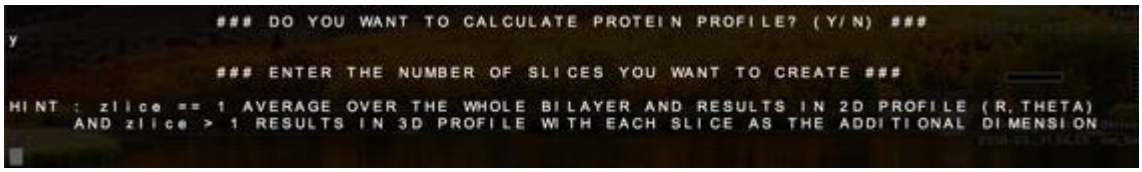
4. Type in ‘Y’ for the calculation of ion flux. Next AMBAT_AT will ask for the PDB name of the ion to identify its coordinates and parameters.

Enter the ion PDB name, ‘Cl-‘ (Chloride ions) for this example. Next functionality of AMBAT_AT is water flux.

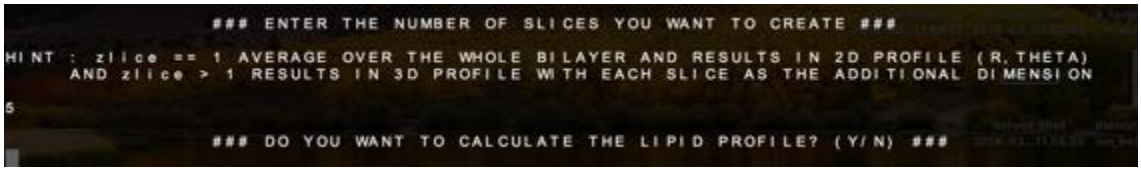


5. Type in ‘y’ for the calculation of water flux through the protein channel. Next functionality of AMBAT_AT is protein profile.

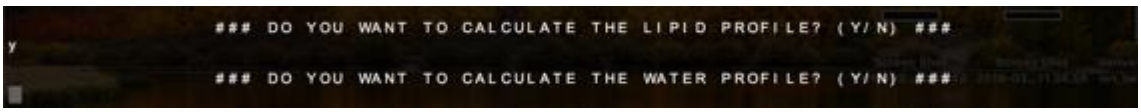
6. Type in ‘Y’ to calculate the 1D, 2D and 3D profiles of protein. For 3D profile AMBAT_AT asks for the number of slices along the bilayer normal between the lower and the upper leaflet.



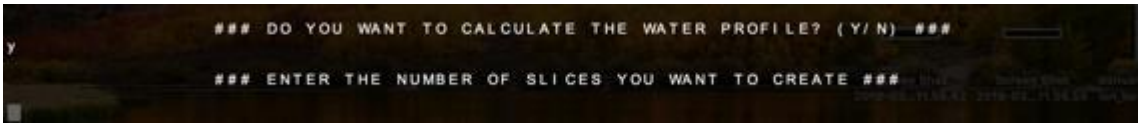
Enter desired number of slices. For this example, type in ‘5’ for 5 slices. Next, AMBAT_AT will ask for the calculation of lipid profile. Note for asymmetric bilayers AMBAT will create a separate lipid profile for each of the constituting lipid. For this example of symmetric bilayer, lipid profile will be generated only for POPC lipid.



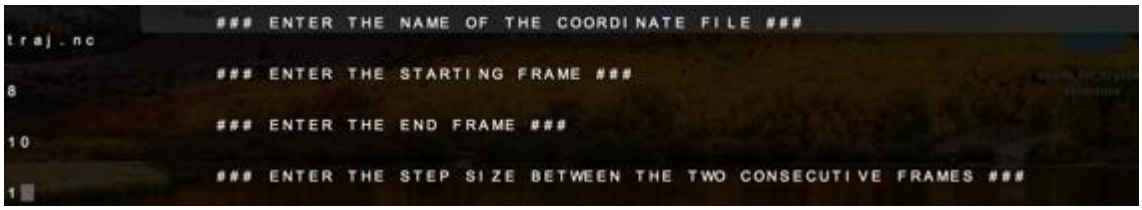
7. Type in ‘Y’ for the calculation of lipid profile. Finally, AMBAT_AT allows the user to calculate water profile for the slices along the bilayer normal (from upper leaflet to lower leaflet).



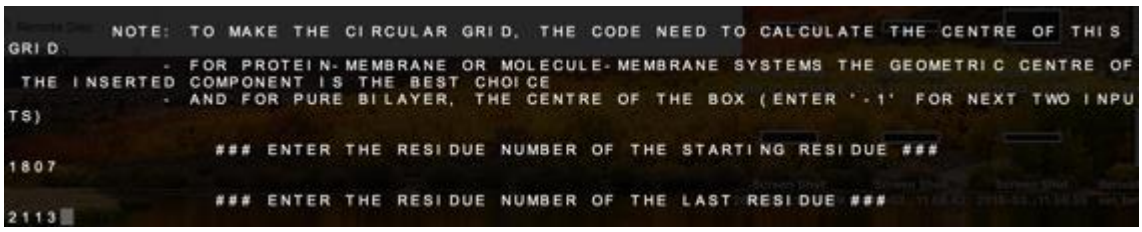
8. Type in ‘Y’ for the calculation of water profile. Next, like 3D protein profile, AMBAT_AT will ask for the number of slices.



Enter desired number of slices. For this example type ‘5’ for 5 slices. Now, AMBAT_AT will ask for the parameters file, trajectory file and the frames over which you want to do the calculations.
Type in ‘parm.prmtop’ for parameters, ‘traj.nc’ for AMBER trajectory (coordinate file). Next, type the starting frame, end frame and frame step. For this example, type ‘8’ for start frame, ‘10’ for end frame and ‘1’ for frame step

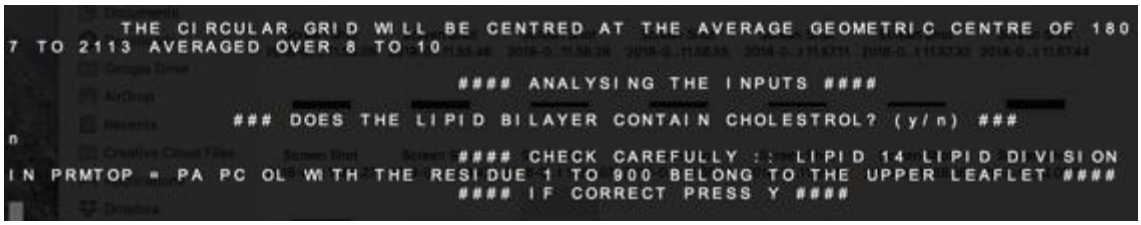


9. Next, AMBAT_AT will ask information about the inserted component. Enter the first and last residue id for the inserted component. For this example, enter ‘1807’ as the starting residue and ‘2113’ as the end residue of the inserted polypeptide. (For pure bilayer systems ‘-1’ is the input for both these entries)



10. Next, AMBAT_AT will ask if the bilayer contains Cholesterol. If your system contains Cholesterol type ‘Y’ otherwise type in ‘N’. For this example, Type in ‘N’.

11. Based on the input trajectory and parameter file, AMBAT_AT will try to determine the lipid type and its division among the upper and the lower leaflet. (If AMBAT_AT determine the wrong division user can manually provide the information). Type in 'Y' for this example.



12. Next comes the grid input. This step determines the speed of the code. For this example, we form a cylindrical grid starting from 10.0 A radius with the smallest arc of radius 10A and 36^0. Type in '10.0' for radius of the mesh, '10.0' for starting position and '36.0' for the angle of the circular mesh.

13. Next, AMBAT_AT will calculate the properties based on the input. As this example contain a medium size system (around 83,000 atoms), it will take around 15 min for the code to calculate all the local membrane properties and all the component profiles.

Output

For plotting you can use two python scripts:
"contrast_map_AMBAT_AT.py" for contrast maps and
"line_plots_AMBAT_AT.py" for line plots.

Syntax:

python contrast_map_AMBAT_AT.py $filename $column 3D (optional, used for 3D protein profiles)

python line_plot_AMBAT_AT.py $filename $column1 $column2 $column3 (optional, used for YXX plot)

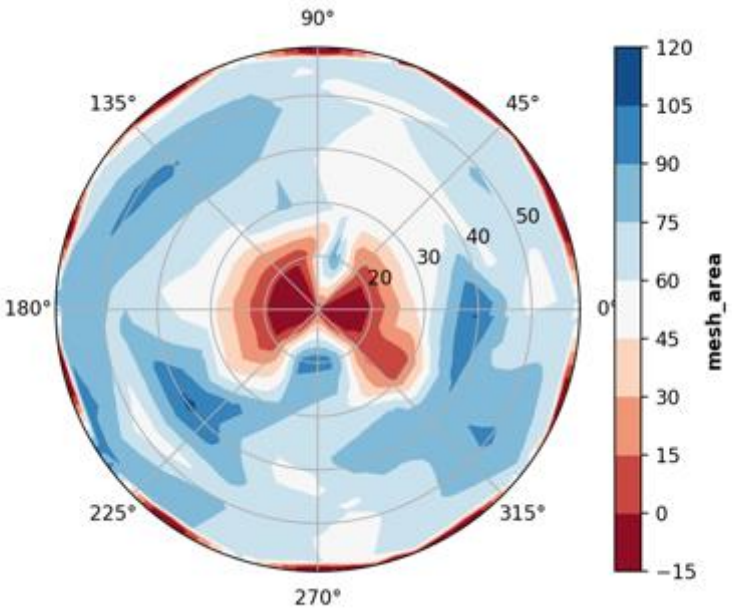
The files generated after execution:

(a) mesh_area.txt

Contains 12 columns:

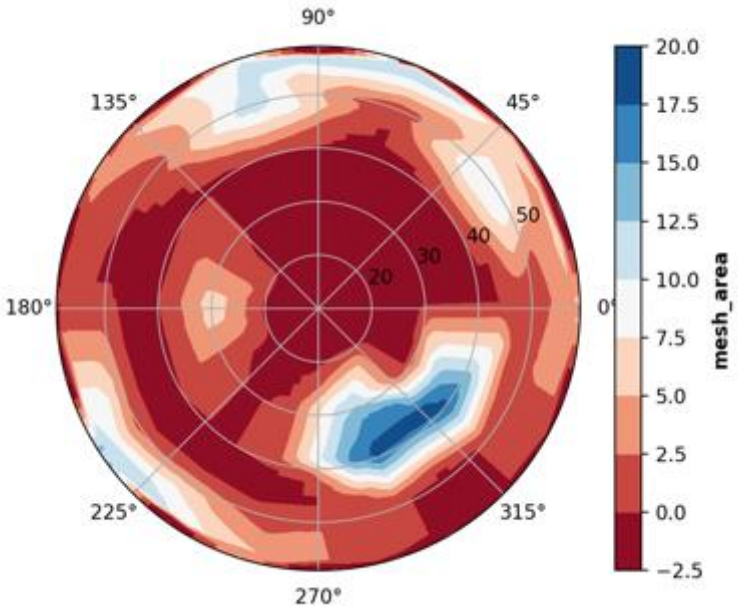
1. Radius
2. Theta
3. Area per lipid upper leaflet (APL_UL)
4. Standard deviation APL_UL
5. Area per lipid lower leaflet (APL_LL)
6. Standard deviation APL_LL
7. Number of lipids upper leaflet (Num_UL)
8. Standard deviation Num_UL
9. Number of lipids lower leaflet (Num_LL)
10. Standard deviation Num_LL
11. Average area per lipid (Avg_APL)
12. Standard deviation Avg_APL

For plotting this graph use:
python contrast_map_AMBAT_AT.py mesh_area.txt 3



Local area per lipid upper leaflet

For plotting this use:
python contrast_map_AMBAT_AT.py mesh_area.txt 4



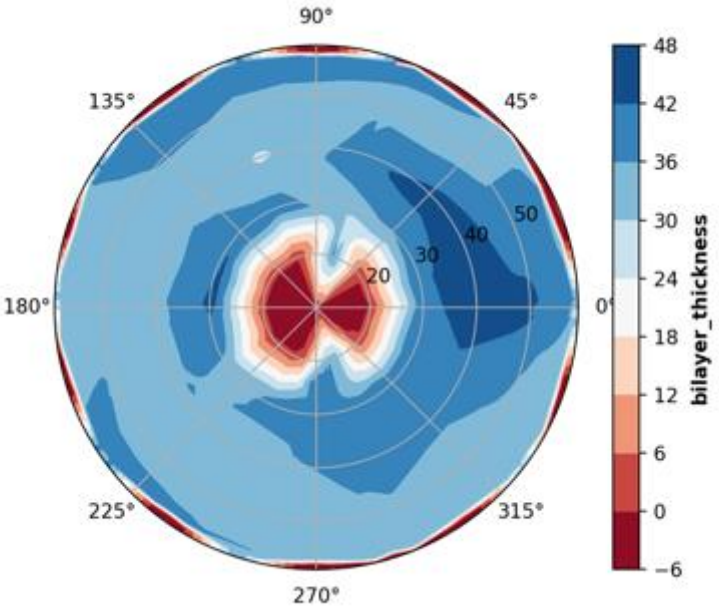
Standard deviation Local area per lipid upper leaflet

(b) bilayer_thickness.txt

Contains 4 columns:

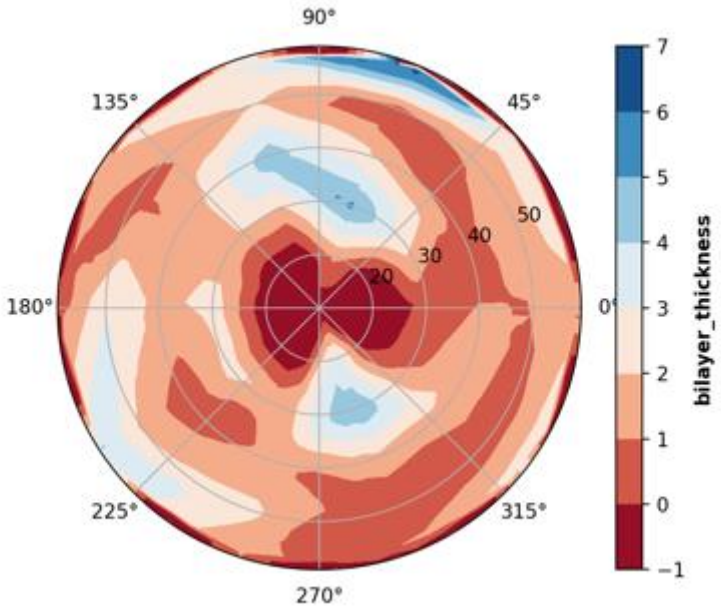
1. Radius
2. Theta
3. Bilayer thickness
4. Standard deviation bilayer thickness

For plotting this use:
python contrast_map_AMBAT_AT.py bilayer_thickness.txt 3



Bilayer thickness

For plotting this use:
python contrast_map_AMBAT_AT.py bilayer_thickness.txt 4



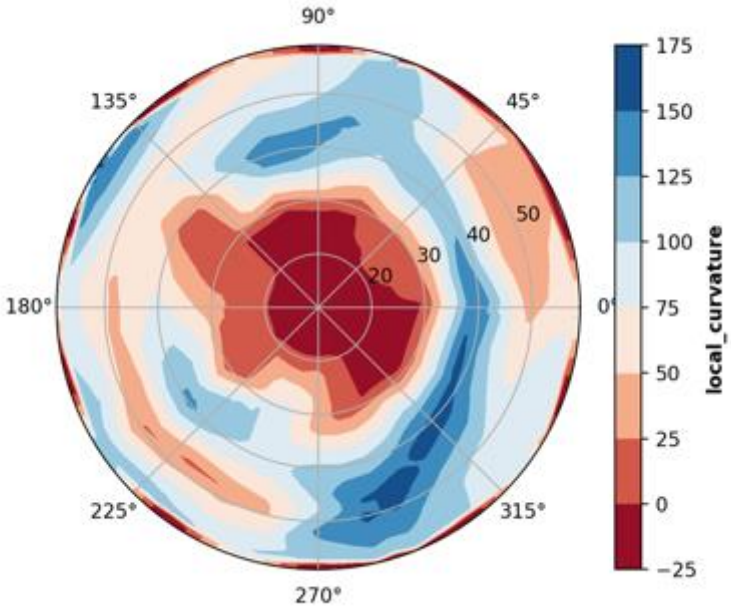
Standard deviation Bilayer thickness

(c) local_curvature.txt

Contains 4 columns for:

1. radius
2. theta
3. local curvature upper leaflet (°)
4. local curvature lower leaflet (°)

For plotting this use:
python contrast_map_AMBAT_AT.py local_curvature.txt 3

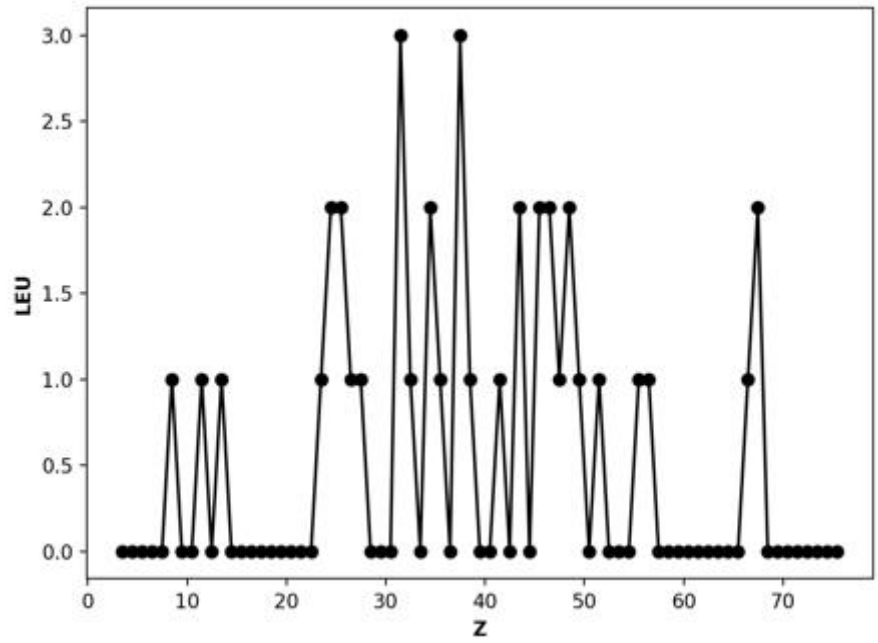


Local curvature (°) upper leaflet

5. amino_acid_dis.txt (1D profile)

Distribution of each amino acid along the bilayer normal. Contains 21 columns, first column for Z dimension and rest for 20 amino acids.

For plotting this use:
python line_plot_AMBAT_AT.py amino_acid_dis.txt 1 3

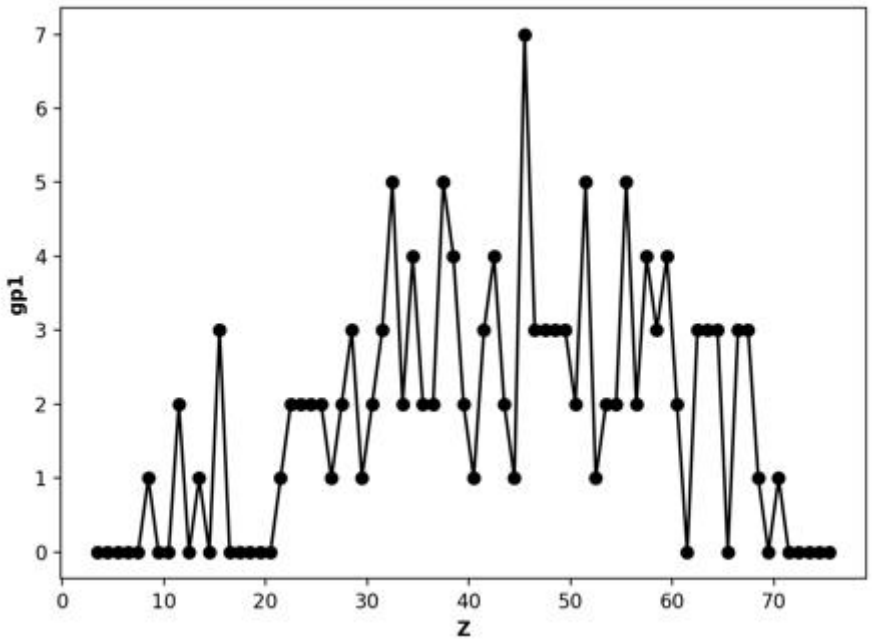


Distribution of Leucine along the bilayer normal

6. amino_acid_group_dis.txt (1D profile)

Distribution of each amino acid group along the bilayer normal. Contains 5 columns for each amino acid group.

For plotting this use:
python line_plot_AMBAT_AT.py amino_acid_group_dis.txt 1 2

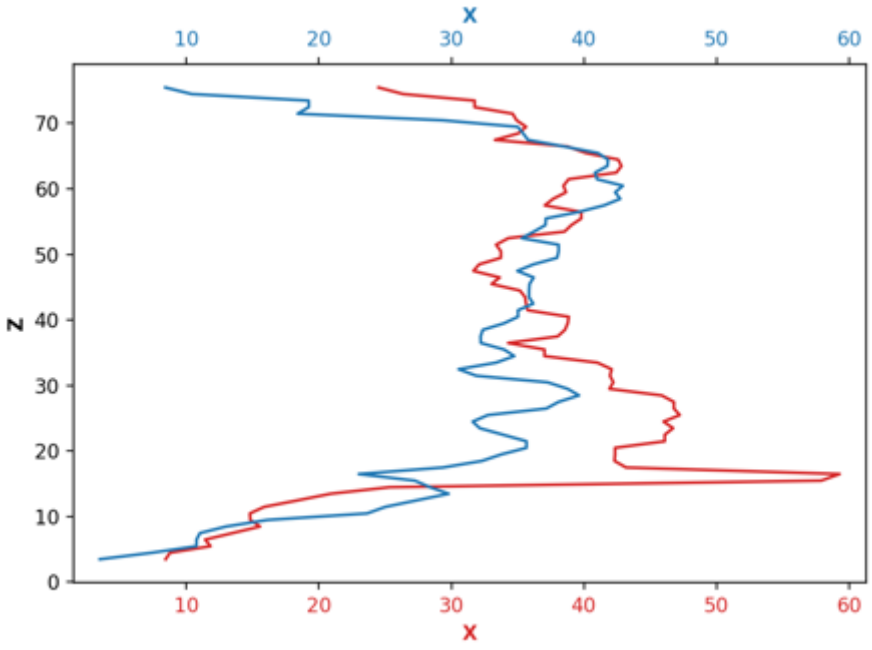


Distribution of hydrophobic amino acids (gp1) along the bilayer normal

7. protein_distribution.txt (2D profile)

Extension of the whole protein along the bilayer normal. Contains 3 columns for Z, X and Y coordinates.

For plotting this use:
python line_plot_AMBAT_AT.py protein_distribution.txt 1 2 3



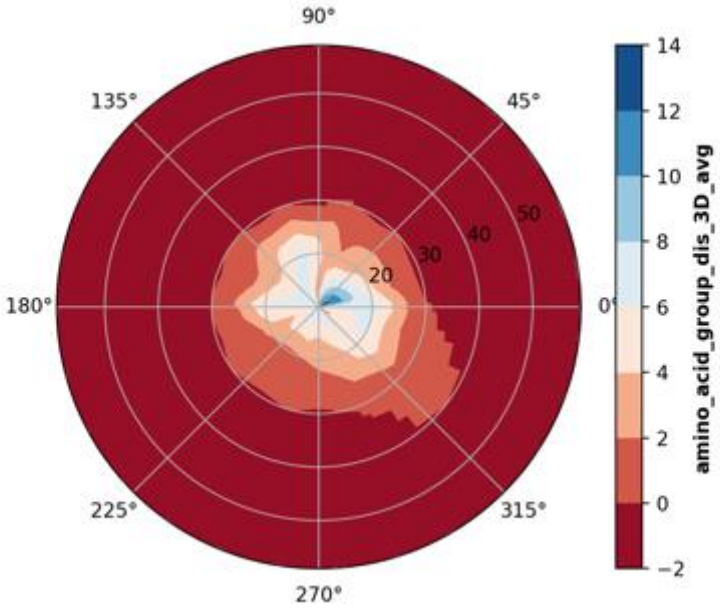
Extension of the protein along the bilayer normal (Z)

8. amino_acid_group_dis_3D_avg.txt (2D profile)

Contains 4 columns:

1. radius
2. theta
3. number of amino acids along bilayer thickness
4. standard deviation number of amino acids along bilayer thickness

For plotting this use:
python contrast_map_AMBAT_AT.py amino_acid_group_dis_3D_avg.txt 3



Amino acid distribution along the bilayer normal on a circular mesh

9. amino_acid_group_dis_3D.txt

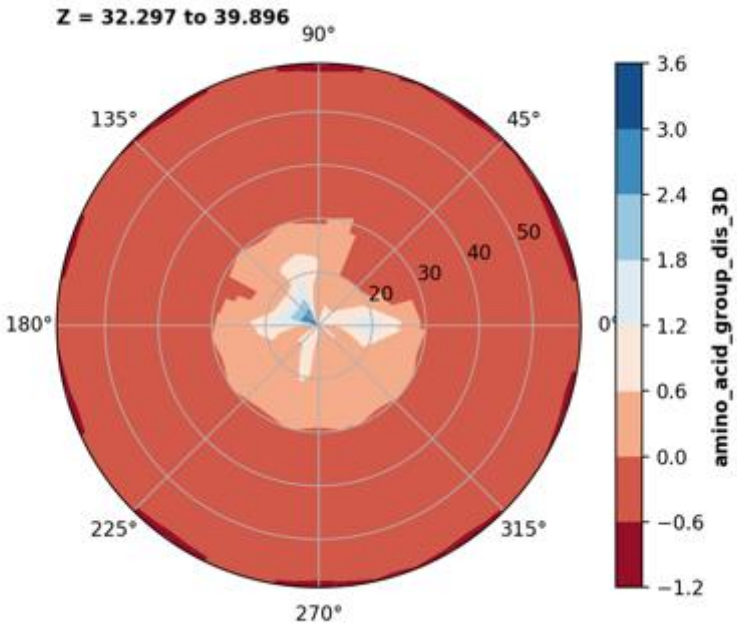
Contains 13 columns:

1. radius
2. theta
3. Z (slice position)
4. Group 1
5. Standard deviation group 1
6. Group 2
7. Standard deviation group 2
8. Group 3
9. Standard deviation group 3
10. Group 4

11. Standard deviation group 4
12. Group 5
13. Standard deviation group 5

For plotting this use:
python contrast_map_AMBAT_AT.py amino_acid_group_dis_3D.txt 4 3D

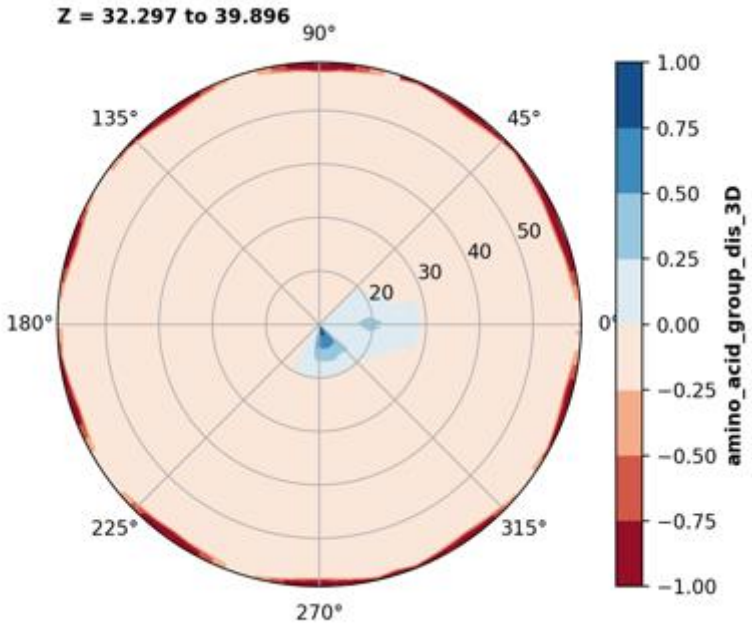
And select slice 2 as the input when asked



Distribution of hydrophobic amino acids along the bilayer normal. (Z = 32.297 to 39.896 slice)

For plotting this use:
python contrast_map_AMBAT_AT.py amino_acid_group_dis_3D.txt 6 3D

And select slice 2 as the input when asked



Distribution of hydrophilic amino acids along the bilayer normal. (Z = 32.297 to 39.896 slice)

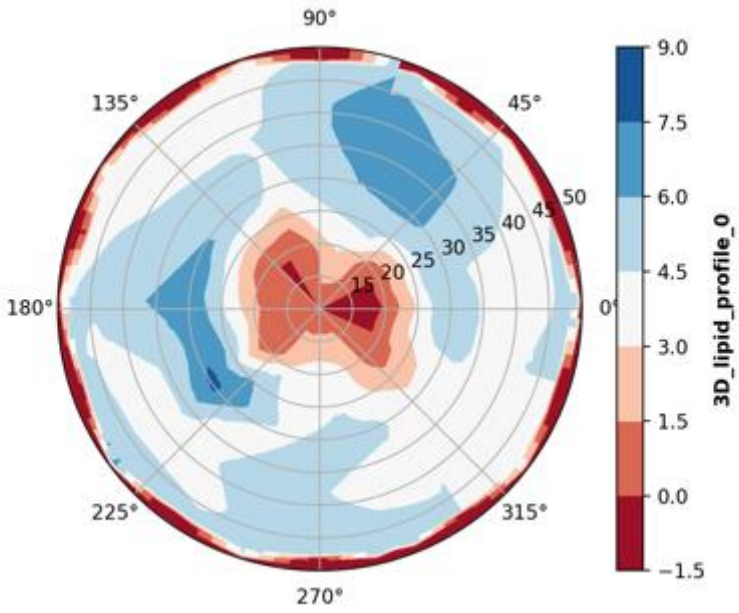
10. 3D_lipid_profile_0.txt

Lipid profile of POPC. Contain 6 columns:

1. radius
2. theta
3. lipid profile upper leaflet
4. standard deviation lipid profile upper leaflet
5. lipid profile lower leaflet
6. standard deviation lipid profile lower leaflet

For plotting this use:

1. python contrast_map_AMBAT_AT. 3D_lipid_profile_0.txt 3



Upper leaflet distribution of POPC

2. number_water.txt and number_Cl-.txt

Contain 2 columns:

1. MD frame
2. Number of water molecules/ions in a 20 A cube at the centre of the protein.

3. number_water-ent.txt and number_Cl--ent.txt

Contain 2 columns:

1. MD frame
2. Number of waters molecules/ions entering the 20 A cube at the centre of the protein.

4. number_water-lea.txt and number_Cl--lea.txt

Contain 2 columns:

1. MD frame
2. Number of waters molecules/ions leaving the 20 A cube at the centre of the protein.

5. In the folder videos ‘videos_files_wp’: z_$slice_pos.txt

Contains 4 columns:

1. Radius

2. Theta
3. Slice position
4. Number of water molecules

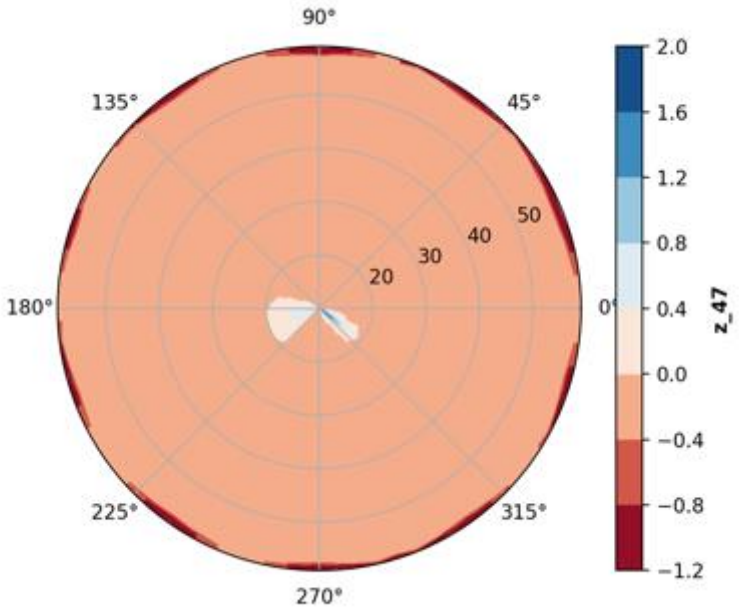
And file named ‘frame_$slice_pos.$frame.txt contain waters molecules in $slice_pos slice for frame $frame.

Contains 4 columns:

1. Radius
2. Theta
3. Slice position
4. Number of water molecules

For plotting this use:

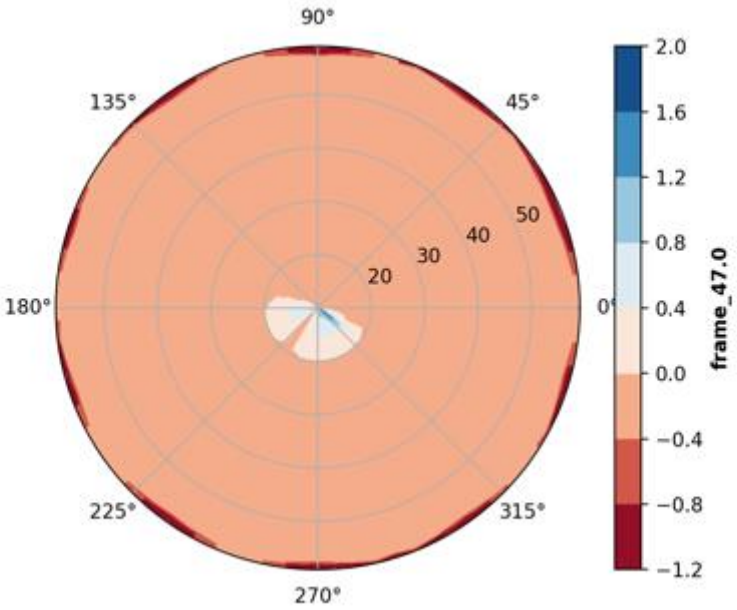
1. python contrast_map_AMBAT_AT. z_47.txt 4



Number of water molecules in Z=47 slice (centre of the bilayer)

For plotting this use:

1. python contrast_map_AMBAT_AT. frame_z_47.0.txt 4



Number of water molecules in Z=47 slice for frame 0 (centre of the bilayer)

2. Folder ‘video_files_blt’ contain local bilayer thickness per frame which can be plotted and converted into contrast map by using open software like ffmpeg.

3. Folder ‘video_files_cur’ contain local membrane curvature per frame which can be plotted and converted into contrast map by using open software like ffmpeg.

4. Folder ‘video_files_lp’ contain lipid profile for each constituting lipid per frame which can be plotted and converted into contrast map by using open software like ffmpeg.

5. For zslice = 1 for protein profile, another folder ‘’ is generated which contain protein profile per frame. The files can be plotted and converted into contrast map by using open software like ffmpeg. (Not generated for this example)