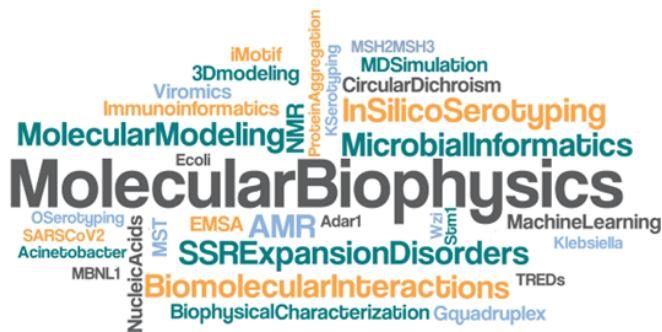


Documentation

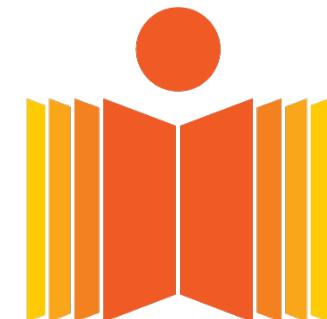
MDTAP: Molecular Dynamics Trajectory Analysis of Permeation A tool to analyze permeation events across membrane proteins

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భారతీయ సౌంకేతిక విజ్ఞాన సంస్థ హైదరాబాద్
भारतीय प्रौद्योगिकी संस्थान हैदराबाद
Indian Institute of Technology Hyderabad

Welcome to MDTAP !!

MDTAP is an MD (molecular dynamics) analysis software that captures and quantifies permeation events across proteins and nucleic acid channels. It allows the user to define a molecule of interest and track its permeation across the channel using the PDB structures or CHARMM or NAMD DCD files generated from MD trajectories. Using the MD trajectories, a methodology is developed here to detect the permeation events across the channel irrespective of their shape, size, and the type of solute molecules permeating. This tool is beneficial in analyzing and calculating the solute/solvent permeations in an automated fashion.

Prerequisites:

1. Linux/Mac-based system
2. Gnuplot
3. Fortran 90

Getting started:

Download and unpack the MDTAP scripts to any folder.

unzip MDTAP-main.zip OR gzip MDTAP-main.zip

To run/access MDTAP from any folder, follow the instructions given below:

1. Go to the MDTAP scripts folder after unpacking/unzipping

```
cd MDTAP-main/MDTAP-scripts/
```

2. Change the permission of all the scripts in the MDTAP folder

```
chmod 777 *.sh
```

3. Open the `~/.bashrc` file to export the path to the scripts and create an alias by pasting the following lines

```
export MDTAPPATH=/path/to/folder/MDTAP-main/MDTAP-scripts
```

```
alias mdtap='/path/to/folder/MDTAP-main/MDTAP-scripts/mdtap.sh'
```

4. Save the `bashrc` file and run it by executing the following command

```
source ~/.bashrc
```

After installing MDTAP, it can be run using the following command to call MDTAP and follow the on-screen instructions to analyze the MD trajectories.

```
mdtap
```

How to use MDTAP for the analysis of MD trajectories?

The following section describes the usage of the MDTAP tool. It demonstrates the interface of the software, the inputs required by each module, and the corresponding outputs generated by the various modules.

Demonstration of the usage of MDTAP and the outputs generated:

```
[testuser ~]$ mdtap
=====
Molecular Dynamics Trajectory Analysis of Permeation (MDTAP)
=====
Format of MD trajectories:
 1. PDB
 2. CHARMM/NAMD DCD
Enter an option (1 or 2) and press [ENTER]: 1

Modules:
 A. Rename PDBs
 B. Analyze Permeation
 C. Quit
Enter an option (A, B, or C) and press [ENTER]: B

Submodules under analyze permeation:
 1. Z-density profile
 2. XY-area profile
 3. Rate of change of molecules
 4. Permeation
 5. Net flux and Permeability coefficient (Pd)
 6. Residence time
 7. Track molecule
 8. Diffusion entry/exit
 9. Distance calculation
Enter an option (1, 2, or .... 9) and press [ENTER]:
```

Table 1. MDTAP interface with the various modules available. After installation of the MDTAP software, it can be invoked by typing "mdtap". Note that the Analyze Permeation module has nine submodules under it. The user can access the submodules of Analyze Permeation by entering options 1-9.

The next page briefly describes each submodule's functionalities under the “Analyze Permeation” module (APM), as well as the inputs required by each module.

Functionalities of the modules

APM Number	Module Name	Function
1	Z-density profile	Identifies the variation in the population of permeating molecules along the channel axis (<i>viz.</i> , Z-axis)
2	XY-area profile	Identifies the aerial and spatial distribution of the molecule of interest projected to the XY-plane
3	Rate of change of molecules	Calculates the number of molecules present inside the channel with respect to time
4	Permeation	Identifies and lists molecules that undergo complete and incomplete permeation through the protein
5	Net flux and permeability coefficient (P_d)	Provides the net flux and the diffusion permeability coefficient (P_d) of the molecule of interest across the channel
6	Residence time	Calculates the time (in ps) that the permeating molecule resides in the conduction path
7	Track molecule	Tracks the path followed by the permeating molecule along the pore axis (Z-axis) as it passes through the channel
8	Diffusion entry/exit	Calculates the number of molecules that enter or exit through a diffusion plane defined by three or more amino acids
9	Distance calculation	Calculates the distance between electronegative atoms of the molecule(s) of interest and the channel residues, and reports atoms that are within a distance of 3.5 Å

Inputs required by each module

Required inputs	Sub-modules under APM								
	1	2	3	4	5	6	7	8	9
Molecule of interest	✓	✓	✓	✓	✓		✓	✓	
Address/path of the PDBs	✓	✓	✓	✓	✓		✓	✓	✓
Start PDB	✓	✓	✓	✓	✓		✓	✓	✓
End PDB	✓	✓	✓	✓	✓		✓	✓	✓
PDBs to skip	✓	✓	✓	✓	✓		✓	✓	✓
Time difference between PDBs	✓	✓	✓	✓	✓		✓	✓	
Chain ID	✓	✓	✓	✓	✓		✓	✓	✓
Seg ID	✓	✓	✓	✓	✓		✓	✓	✓
Output folder name	✓	✓	✓	✓	✓		✓	✓	✓
Channel limits	✓	✓	✓	✓	✓		✓		✓
Molar volume of molecule					✓				
Minimum frequency/occurrence						✓			
Number of influx molecules							✓		
Number of efflux molecules								✓	
Time frame								✓	
Input file with atom IDs									✓
3 or more amino acid residues									✓

Note:

- Avoid the use of ":", "step_", ".pdb", ".dcd", or "." in the name of the input folder (*viz.*, the folder that contains the input PDBs/DCDs) and the names of the output folders given in each module during the analysis.
- Be cautious while choosing the intervals (skip) between the trajectories (avoid skipping too many PDBs/DCD frames during the analysis).
- The examples given in the following sections are with respect to PDBs as an input but MDTAP also supports CHARMM or NAMD DCDs.

```
=====
A. Rename PDBs
-----
This module renames the PDB files to step_i.pdb (1<=i<=n), which is compatible
with other modules. It renames the PDB files present in the path (address)
defined by the user. Note that the original files are renamed directly.
=====
Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):
.../MDTAP/testPDBs
The output files are stored in ..../MDTAP/testPDBs
1. Continue 2. Back 3. Quit
Enter an option (1, 2, or 3) and press [ENTER]: 3
```

Table 2. The "Rename PDBs" module is used to rename the user's PDB trajectories to make them compatible with other modules. The renamed PDBs are stored in the path defined by the user.

```
=====
B. 1. Z-density profile
-----
This module calculates the number of molecules present along the protein axis (Z-axis). The user-defined space is divided into slices of 1 Å thickness, and the number of molecules in each slice is calculated.
=====
```

Enter the molecule of interest (e.g. OH2 TIP for water oxygens) and press [ENTER]:

OH2 TIP

Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):

PDBs

Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]:

1

Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]:

200

Enter the PDBs to skip and press [ENTER]:

1

Enter the difference in time (in picoseconds) between each PDB and [ENTER]:

(e.g. If the PDBs are generated at every 10ps interval, then enter '10')

1000

Enter chain ID and press [ENTER] (Note: if chain ID is absent in the PDB files, just press [ENTER]):

Enter seg ID and press [ENTER] (Note: if seg ID is absent in the PDB files, just press [ENTER]):

Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten):

1-Z-density_profile

Recommended dimensions (Xmax Xmin Ymax Ymin Zmax Zmin) for the channel are: 69.070 17.260 66.210 4.400

96.220 -0.930

Enter the channel limits (Xmax Xmin Ymax Ymin Zmax Zmin) and press [ENTER]:

69.070 17.260 66.210 4.400 96.220 -0.930

The output files are stored in 1-Z-density_profile at PDBs/1-Z-density_profile

1. Continue 2. Back 3. Quit

Enter an option (1, 2, or 3) and press [ENTER]: **3**

Output file name: Z-densityprofile-##.dat

This file contains the variation in the number of OH2 TIP within the channel along the Z-axis.

Column 1: Sections along Z-axis (Å)

Column 2: Number of OH2 TIP

96-95 33

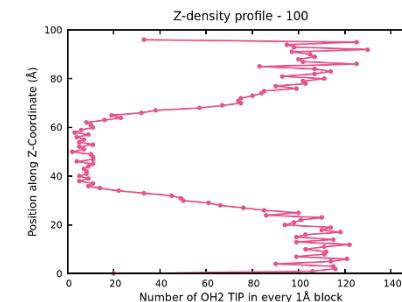
95-94 125

:

2-1 116

1-0 106

Output file name: Z-densityprofile-##.png



Output file name: Z-densityprofile-mean_sd.png

Mean Z-density profile with standard deviation

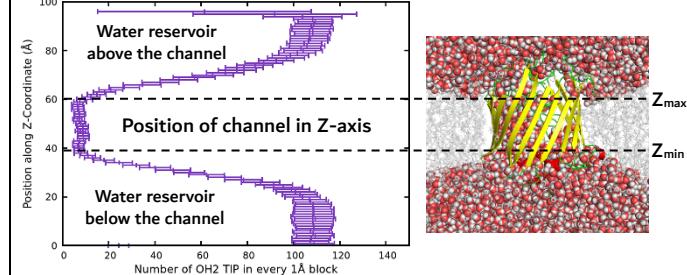


Table 3. The outputs generated by the "Z-density profile" module give an idea of the channel's position in the Z-axis. The example output shown (Z-densityprofile-mean_sd.png) is the front view of the channel. It spans between ~40-60 Å along the Z-axis. These Z-axis values can be used in the subsequent modules to analyze the molecules of interest within the channel. ## indicates that it is a representative example, and the number of files created is equal to the number of input PDBs analyzed. Note that the chain and seg IDs correspond only to the channel (protein) residues.

```
=====
B. 2. XY-area profile
=====
```

This module identifies the distribution of molecules in the user-defined space. The extremities in the X and Y directions are used to calculate the accessible area for the molecule of interest.

Enter the molecule of interest (e.g. OH2 TIP for water oxygens) and press [ENTER]:

OH2 TIP

Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):

PDBs

Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]:

1

Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]:

200

Enter the PDBs to skip and press [ENTER]:

1

Enter the difference in time (in picoseconds) between each PDB and [ENTER]:

(e.g. If the PDBs are generated at every 10ps interval, then enter '10')

1000

Enter chain ID and press [ENTER] (Note: if chain ID is absent in the PDB files, just press [ENTER]):

Enter seg ID and press [ENTER] (Note: if seg ID is absent in the PDB files, just press [ENTER]):

Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten):

2-XY-area_profile

Recommended dimensions (Xmax Xmin Ymax Ymin Zmax Zmin) for the channel are: 69.070 17.260 66.210 4.400

89.110 18.460

Enter the channel limits (Xmax Xmin Ymax Ymin Zmax Zmin) and press [ENTER]:

69.070 17.260 66.210 4.400 60.000 40.000

The output files are stored in 2-XY-area_profile at PDBs/2-XY-area_profile

1. Continue 2. Back 3. Quit

Enter an option (1, 2, or 3) and press [ENTER]: **3**

Output file name: XY-areaprofile.dat

This file contains the area accessible by OH2 TIP with respect to time.

Column 1: Time (*1000ps)

Column 2: Area (A^2)

1 2319.653

2 2533.405

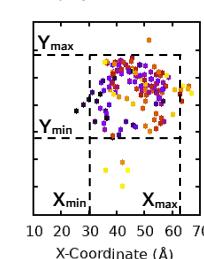
:

200 1714.586

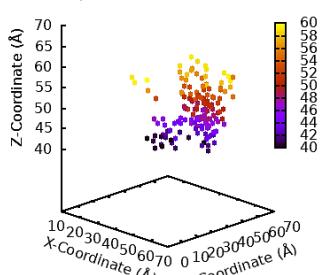
The mean area accessible by OH2 TIP is 1968.26 A^2 , and the standard deviation is 405.045

Output file name: XY-plot-##.png

XY-projection of OH2 TIP



Spatial distribution of OH2 TIP



Output file name: XY-density-Z-##.png

Density of OH2 TIP between 44-43 \AA (Z-axis)

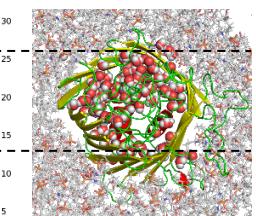
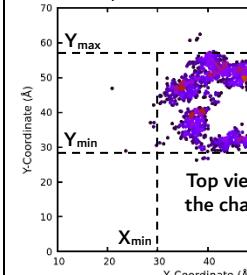


Table 4. The outputs generated by the "XY-area profile" module give an idea of the XY distribution of the molecule of interest with respect to time within the channel. Note that the Z-axis limits ($Z_{\text{max}} = 60.000$ and $Z_{\text{min}} = 40.000$) are entered according to the outputs that were generated in the "Z-density profile" module. The XY values seen from the outputs of this module can be used in the subsequent modules to perform the calculations exactly for the channel dimensions. **##** indicates that it is a representative example, and the number of files created is equal to the number of input PDBs analyzed. The chain and seg IDs correspond only to the channel (protein) residues.

=====
 B. 3. Rate of change of molecules

 This module calculates the number of water molecules present within the protein or in any user-defined area with respect to time.
 =====

Enter the molecule of interest (e.g. OH2 TIP for water oxygens) and press [ENTER]:

OH2 TIP

Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):

PDBs

Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]:

1

Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]:

200

Enter the PDBs to skip and press [ENTER]:

1

Enter the difference in time (in picoseconds) between each PDB and [ENTER]:

(e.g. If the PDBs are generated at every 10ps interval, then enter '10')

1000

Enter chain ID and press [ENTER] (Note: if chain ID is absent in the PDB files, just press [ENTER]):

Enter seg ID and press [ENTER] (Note: if seg ID is absent in the PDB files, just press [ENTER]):

Enter the molar volume of the molecule and press [ENTER]:

(e.g the molar volume of Water = 18.07cm^3, Sodium = 23.78cm^3, and Chlorine = 22.40cm^3. Note: The user is free to enter any value specific to the molecule of interest apart from the examples given.)

18.07

Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten):

3-Rate_of_change_of_molecules

Recommended dimensions (Xmax Xmin Ymax Ymin Zmax Zmin) for the channel are: 69.070 17.260 66.210 4.400 89.110 18.460

Enter the channel limits (Xmax Xmin Ymax Ymin Zmax Zmin) and press [ENTER]:

69.070 17.260 66.210 4.400 60.000 40.000

The output files are stored in 3-Rate_of_change_of_molecules at PDBs/3-Rate_of_change_of_molecules

1. Continue 2. Back 3. Quit

Enter an option (1, 2, or 3) and press [ENTER]: **3**

Output file name: Rate_output.dat

This file contains the number of OH2 TIP present within the user-defined space with respect to time.

Column 1: Time (*1000ps)

Column 2: Number of OH2 TIP

Column 3: Volume occupied by OH2 TIP (*10⁻²³ cm³)

The mean number of OH2 TIP is 149.731

1	153	458.95
2	148	443.95
3	146	437.95
:	:	:
198	144	431.95
199	152	455.95
200	151	452.95

Output file name: Rate_number.png

The mean number of water molecules is ~150

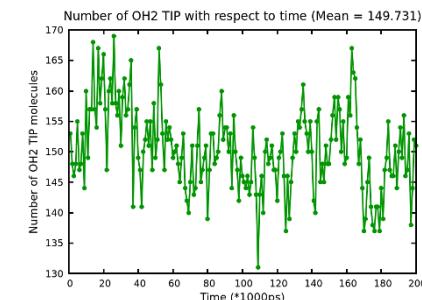


Table 5. The outputs generated by the "Rate of change of molecules" module show the rate of change in the number and volume of the molecule of interest within the channel with respect to time. Note that the Z-axis limits ($Z_{\max} = 60.000$ and $Z_{\min} = 40.000$) are entered according to the outputs that were generated in the "Z-density profile" module. The chain and seg IDs correspond only to the channel (protein) residues.

<p>B. 4. Permeation</p> <p>This module captures the molecules that permeate through the channel across either direction and lists the atom numbers of the permeating and non-permeating molecules.</p> <p>Enter the molecule of interest (e.g. OH2 TIP for water oxygens) and press [ENTER]: OH2 TIP</p> <p>Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]): PDBs</p> <p>Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]: 1</p> <p>Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]: 20</p> <p>Enter the PDBs to skip and press [ENTER]: 1</p> <p>Enter the difference in time (in picoseconds) between each PDB and [ENTER]: (e.g. If the PDBs are generated at every 10ps interval, then enter '10') 1000</p> <p>Enter chain ID and press [ENTER] (Note: if chain ID is absent in the PDB files, just press [ENTER]):</p> <p>Enter seg ID and press [ENTER] (Note: if seg ID is absent in the PDB files, just press [ENTER]):</p> <p>Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten): 4-Permeation</p> <p>Recommended dimensions (Xmax Xmin Ymax Ymin Zmax Zmin) for the channel are: 69.070 17.260 66.210 4.400 89.110 18.460</p> <p>Enter the channel limits (Xmax Xmin Ymax Ymin Zmax Zmin) and press [ENTER]: 70.000 20.000 60.000 15.000 65.000 35.000</p> <p>Enter the minimum frequency that should be considered for the calculation and press [ENTER]: 20</p> <p>The output files are stored in 4-Permeation at PDBs/4-Permeation</p> <p>The user can verify the PDBs generated by this module and further track the path traced by the atom numbers listed using the 'Track molecule' (APM #7)</p> <p>1. Continue 2. Back 3. Quit</p> <p>Enter an option (1, 2, or 3) and press [ENTER]: 3</p>	<p>Output file name: Permeation-dir1.dat</p> <p>This file contains the list of atom numbers of the molecules that are permeating across the channel in direction 1.</p> <p>33263 35978 36941 37400 : :</p> <p>Output file name: Permeation-dir2.dat</p> <p>This file contains the list of atom numbers of the molecules that are permeating across the channel in direction 2.</p> <p>32954 33320 33416 33704 : :</p> <p>Output file name: True_permeation.dat</p> <p>This file contains the list of atom numbers of the molecules that do not permeate across the channel.</p> <p>32057 32057 32270 32270 : :</p>
<p>Table 6. The outputs generated by the "Permeation" module list the atom numbers of the permeating molecules in both directions (direction 1 and direction 2) and the atom numbers of the molecules that do not permeate across the channel. In addition to this, .pdb files for every permeating and non-permeating module are generated for the user to visualize the localization of each atom within the channel during the simulation time. Note that the X, Y, and Z dimensions are selected after generating outputs from modules 1 and 2 and are appropriately relaxed to capture all the permeating events. The chain and seg IDs correspond only to the channel (protein) residues.</p>	

B. 5. Net flux and Permeability coefficient (Pd)

This module calculates the net flux and permeability coefficient Pd of the permeating molecules.

Note: Outputs from the permeation module provide atom numbers of molecules that completely permeate (influx/efflux). This gives an idea of the number of molecules permeating in each direction and can be used as inputs for this module.

Enter the number of influx molecules and press [ENTER]:

17

Enter the number of efflux molecules and press [ENTER]:

21

Enter the time frame (ns) over which the above influx/efflux is to be calculated and press [ENTER]:

200

Since the given membrane channel is bidirectional (i.e. both influx and efflux are greater than zero), the diffusion permeability coefficient will be calculated for both directions.

The diffusion permeability coefficient is $0.283 \times 10^{-14} \text{ cm}^3/\text{s}$

The net flux is 0.020 molecules/ns

1. Continue 2. Back 3. Quit

Enter an option (1, 2, or 3) and press [ENTER]: **3**

Table 7. The "Net flux and Permeability coefficient (P_d)" module calculates the diffusion permeability coefficient (cm³/s) and the net flux (molecules/ns) of the molecule of interest within the channel in the given time frame. The user can get an idea about the number of influx and efflux molecules across the channel based on the outputs of the "Permeation" module that lists the atom numbers of the permeating molecules in each direction.

=====

B. 6. Residence time

This module takes atom numbers (as defined in the PDB files) in the form of a text file as an input and captures its initial and final PDB files in the specific region and calculates time spent in that region.

=====

Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):

PDBs

Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]:

1

Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]:

200

Enter the PDBs to skip and press [ENTER]:

1

Enter the difference in time (in picoseconds) between each PDB and [ENTER]:
(e.g. If the PDBs are generated at every 10ps interval, then enter '10')

1000

Enter chain ID and press [ENTER] (Note: if chain ID is absent in the PDB files, just press [ENTER]):

Enter seg ID and press [ENTER] (Note: if seg ID is absent in the PDB files, just press [ENTER]):

Enter the name of the input file having the atom IDs (Note: the input file should be stored in the folder where the PDBs are present) and press [ENTER]:

input.txt

Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten):

6-Residence_time

Recommended dimensions (Xmax Xmin Ymax Ymin Zmax Zmin) for the channel are: 69.070 17.260 66.210 4.400
89.110 18.460

Enter the channel limits (Xmax Xmin Ymax Ymin Zmax Zmin) and press [ENTER]:

69.070 17.260 66.210 4.400 60.000 40.000

The output files are stored in 6-Residence_time at PDBs/6-Residence_time

Enter an option (1, 2, or 3) and press [ENTER]: 3

Output file name: Residence_time.dat

This file contains the residence time of the provided atom numbers.

Column 1: Atom number

Column 2: Residence time (*1000ps)

33263	74
33923	36
34055	94
:	:
39938	35
40220	64

Table 8. The outputs generated by the "Residence Time" module provide the time that the molecule of interest resides within the channel in picoseconds. In the example output shown, the atom numbers of the molecule of interest given in column 1 were given as an input (input.txt) to calculate how long they reside within the channel. Note that the Z-axis limits ($Z_{\text{max}} = 60.000$ and $Z_{\text{min}} = 40.000$) are entered according to the outputs that were generated in the "Z-density profile" module. The chain and seg IDs correspond only to the channel (protein) residues.

=====

B. 7. Track molecule

=====

This module takes atom numbers (as defined in the PDB files) in the form of a text file as an input and captures the molecule's Z-coordinate position (\AA) with respect to time and generates a plot. This gives an idea of the migration of the molecule with respect to time.

=====

Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):

PDBs

Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]:

1

Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]:

200

Enter the PDBs to skip and press [ENTER]:

1

Enter the difference in time (in picoseconds) between each PDB and [ENTER]:
(e.g. If the PDBs are generated at every 10ps interval, then enter '10')

1000

Enter the name of the input file having the atom IDs (Note: the input file should be stored in the folder where the PDBs are present) and press [ENTER]:

input.txt

Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten):

7-Track_molecule

The output files are stored in 7-Track_molecule at PDBs/7-Track_molecule

Enter an option (1, 2, or 3) and press [ENTER]: 3

Output file name: Track-##.dat

This file contains the Z-coordinates (\AA) with respect to time.

Column 1: Time (*1000ps)

Column 2: Z-coordinate (\AA)

1 93.6800

2 78.0400

3 5.9400

:

198 4.3200

199 22.6500

200 94.9100

Output file name: Track-##.png

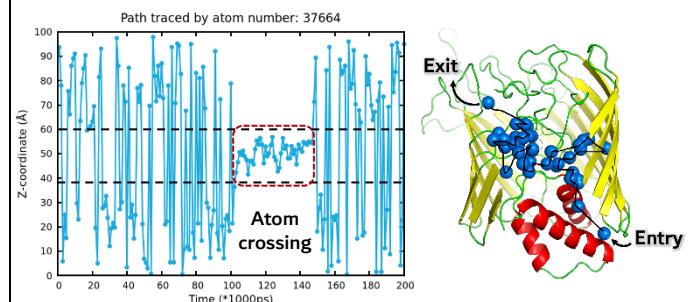
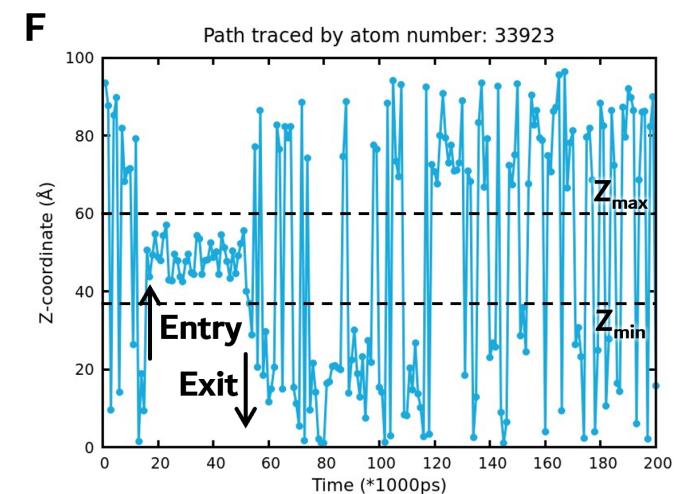
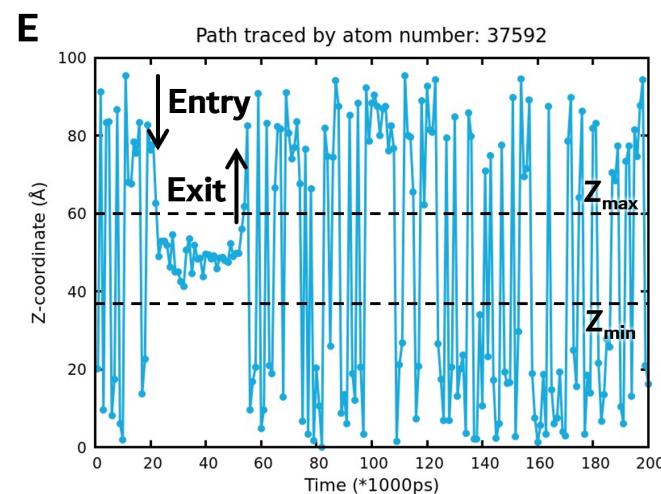
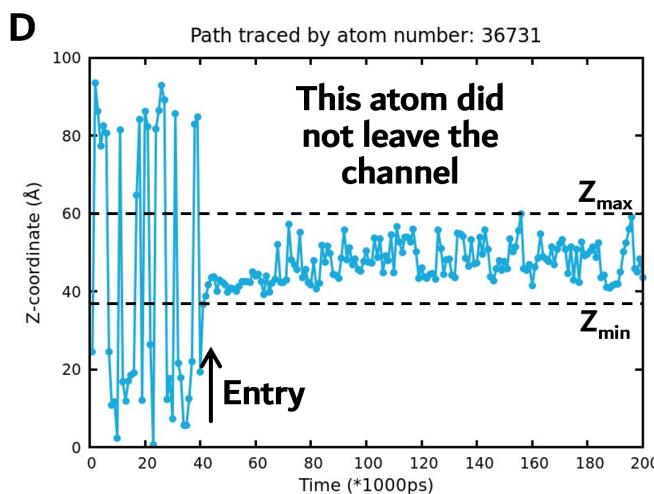
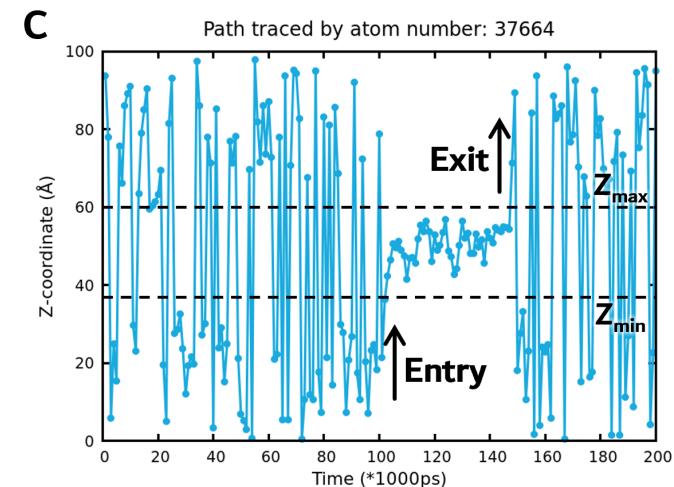
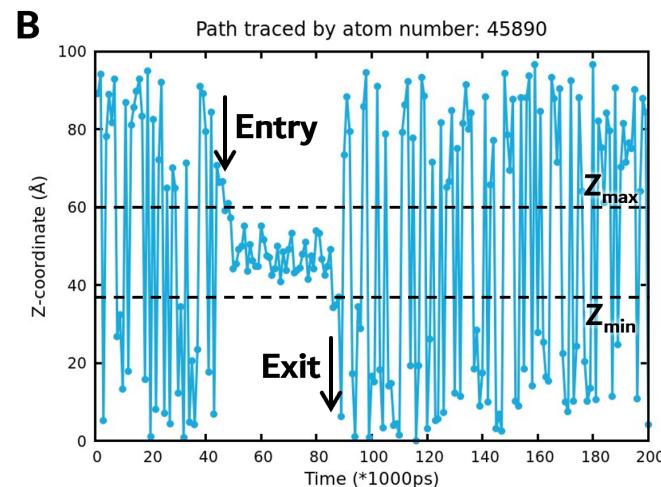
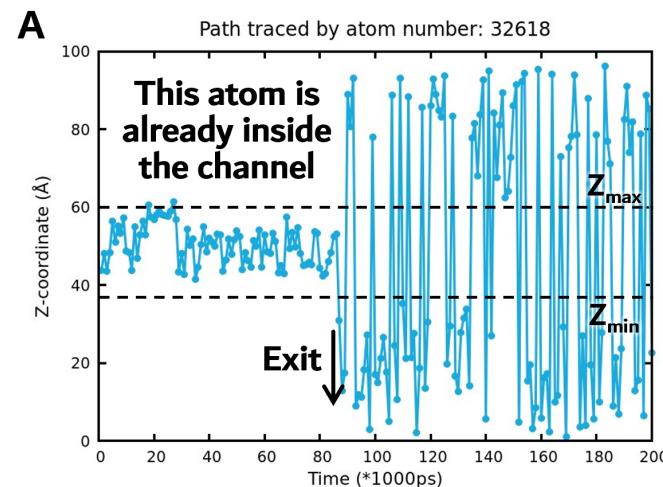


Table 9. The outputs generated by the "Track molecule" module show the path traced by the molecule of interest within the channel over a given time interval. A file with the atom numbers that need to be tracked is given as input to this module. In the example output shown (Track-##.png), it can be seen that the traced molecule enters the channel from the bottom at around 100 ns and completely permeates by around 150 ns (as the position of the channel is known to be between ~40-60 \AA along the Z-axis from the results of the "Z-density profile" module). The fluctuation in the graph results from the periodic boundary condition during MD simulations, which makes it appear as though the molecule has crossed the channel instantly when it really hasn't. ## indicates that it is a representative example, and the number of files created is equal to the number of atom numbers provided in the input file.

A few examples of plots generated by this module are explained on the next page.

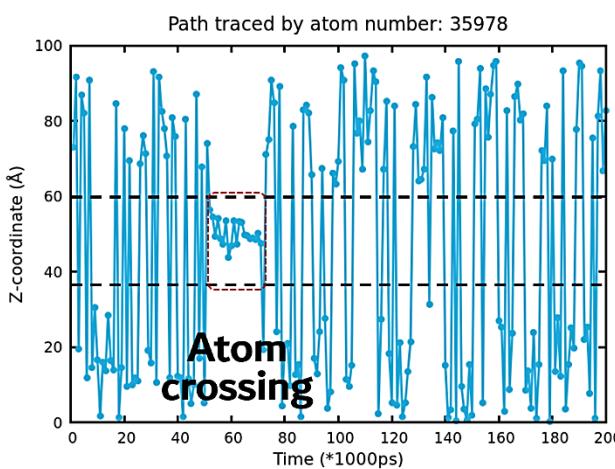
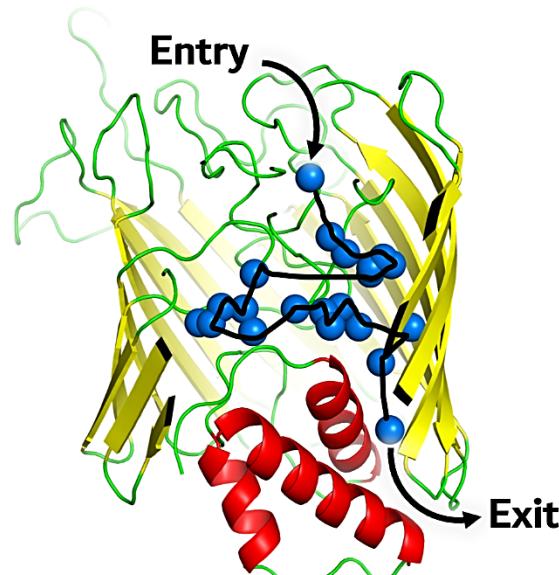
As a permeating molecule enters the channel, it can trace various paths, as shown below:

Among the following plots, B and C are permeation in directions 1 and 2, respectively. A, D, E, and F are examples of non-permeating molecules in the channel.

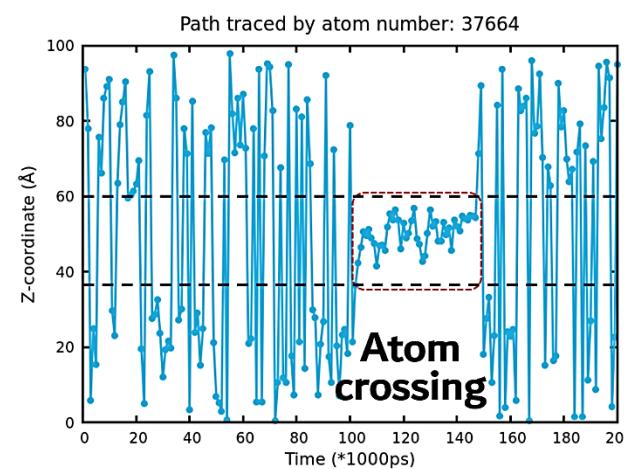
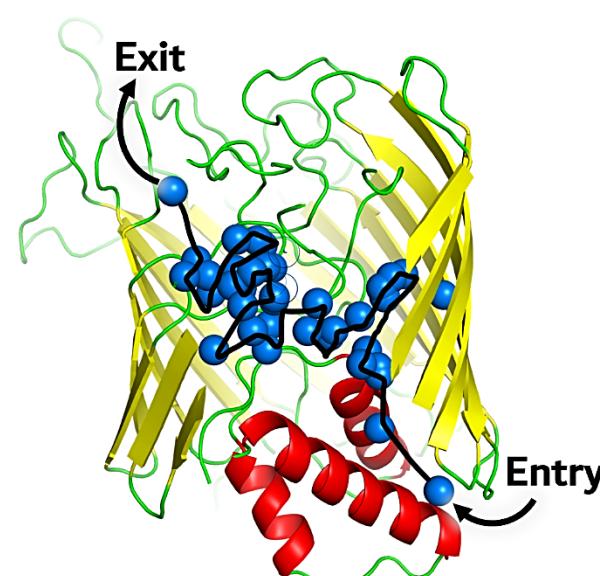


Here are a few examples of how the “Permeation” module outputs can be correlated to the plots generated by the “Track molecule” module.

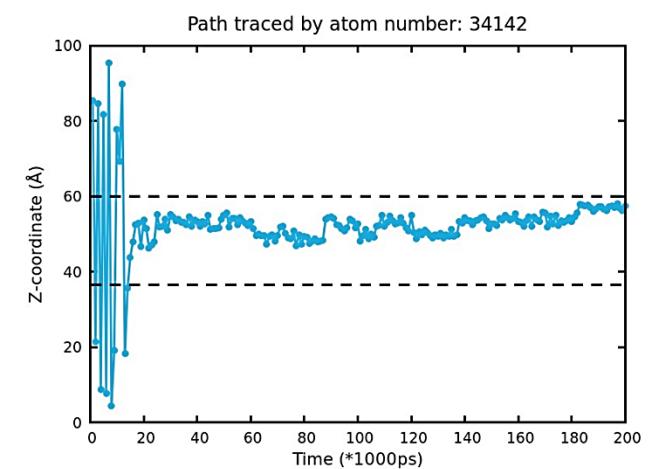
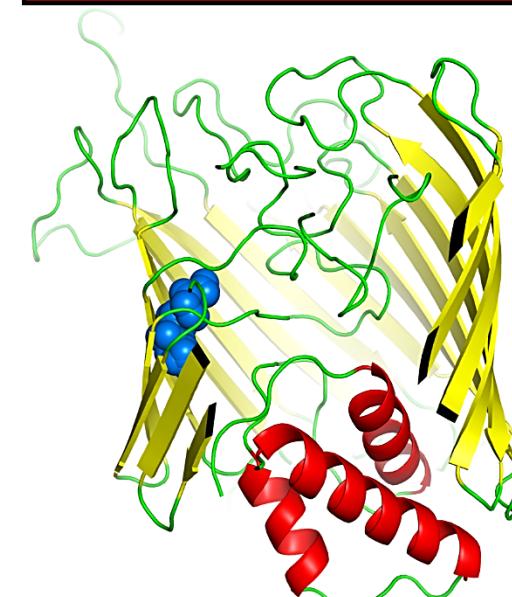
A Permeation direction 1



B Permeation direction 2



C Non-permeating



=====

B. 8. Diffusion entry/exit

This module calculates the number of solvent molecules that enter or exit through an entry point defined by three amino acid residues.

=====

Enter the molecule of interest (e.g. OH2 TIP for water oxygens) and press [ENTER]:

OH2 TIP

Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):

PDBs

Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]:

1

Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]:

200

Enter the PDBs to skip and press [ENTER]:

1

Enter chain ID and press [ENTER] (Note: if chain ID is absent in the PDB files, just press [ENTER]):

Enter seg ID and press [ENTER] (Note: if seg ID is absent in the PDB files, just press [ENTER]):

Enter 3 amino acid residue numbers (e.g. GLN 102 TYR 380 PHE 427) and press [ENTER]:

GLN 102 TYR 380 PHE 427

Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten):

8-Diffusion

The output files are stored in 8-Diffusion at PDBs/8-Diffusion

Enter an option (1, 2, or 3) and press [ENTER]: **3**

Output file name: Diffusion_direction1.dat

This file contains the atom number(s) of OH2 TIP that diffuse through the user-defined amino acid entry/exit points (GLN 102 TYR 380 PHE 427) in direction 1

35900

37400

44228

55982

Output file name: Diffusion_direction2.dat

This file contains the atom number(s) of OH2 TIP that diffuse through the user-defined amino acid entry/exit points (GLN 102 TYR 380 PHE 427) in direction 2

53408

60509

Table 10. The outputs generated by the "Diffusion entry/exit" module provide the list of atom numbers of the molecule of interest that diffuse through a plane defined by three amino acid residues. Two output files are created, one for each direction of diffusion of the molecule of interest. Note that the chain and seg IDs correspond only to the channel (protein) residues.

=====

B. 9. Distance calculation

This module calculates the distance between the electronegative atoms of the molecule(s) of interest and the channel residues, and reports atoms that are within 3.5 Å of each other

=====

Enter the residue name of the molecule of interest (e.g. TIP for water) and press [ENTER]:

TIP

Enter the address/path of the PDBs and press [ENTER] (Note: if the PDB files are present in the working directory, type '.' and press [ENTER]):

PDBs

Enter the start PDB (e.g. if step_10.pdb is the starting PDB, then enter '10') and press [ENTER]:

1

Enter the end PDB (e.g. if step_500.pdb is the final PDB, then enter '500') and press [ENTER]:

200

Enter the PDBs to skip and press [ENTER]:

1

Enter chain ID and press [ENTER] (Note: chain ID is considered only for the channel. If absent in the PDB files, just press [ENTER]):

Enter seg ID and press [ENTER] (Note: seg ID is considered only for the channel. If absent in the PDB files, just press [ENTER]):

Enter the output folder name and press [ENTER] (Note: if the folder already exists, it will be overwritten):

9-Distance

Recommended dimensions (Xmax Xmin Ymax Ymin Zmax Zmin) for the channel are: 69.070 17.260 66.210 4.400
89.110 18.460

Enter the channel limits (Xmax Xmin Ymax Ymin Zmax Zmin) and press [ENTER]:

69.070 17.260 66.210 4.400 60.000 40.000

The output files are stored in 9-Distance at PDBs/9-Distance

1. Continue 2. Back 3. Quit

Enter an option (1, 2, or 3) and press [ENTER]: **3**

Output file name: Distance.dat

This file contains the details of the atoms that are in close proximity (< 3.5 Å) with each other.

Column 1: Distance (Å), Column 2: Atom name of permeating molecule, Column 3: Atom number of permeating molecule, Column 4: Residue name of permeating molecule, Column 5: Residue number of permeating molecule, Column 6: Atom name of channel residue, Column 7: Atom number of channel residue, Column 8: Residue name of channel residue, Column 9: Residue number of channel residue

2.620, OH2, 39881, TIP, 3288, O, 1971, ASN, 153
2.585, OH2, 44705, TIP, 4896, O, 399, SER, 49
2.685, OH2, 53612, TIP, 7865, O, 2381, TYR, 179
2.674, OH2, 69071, TIP, 3018, O, 443, THR, 52
2.749, OH2, 72806, TIP, 4263, N, 111, ASP, 32

Table 11. The outputs generated by the "Distance calculation" module provides the list of electronegative atoms of the molecule(s) of interest and the channel residues that are within a distance of 3.5 Å of each other.

Analyzing the MD trajectories using input files

To use the various MDTAP modules to analyze the MD trajectories, the user can either manually enter the details as prompted by the software (refer to pages 8-14 and 17-18) or provide an input file directly. After downloading and unpacking the files from GitHub, the user can find a folder named “inputs”. The “inputs” folder contains a sample input for each module in MDTAP that uses the trajectories given in a folder named “testPDBs”. The inputs provided are named according to their module numbers. For example, a sample input for the Permeation module is named B4.in, which indicates that it is the 4th sub-module under the Analyze Permeation module (B).

To use these input files, first ensure that the present working directory is inside the “inputs” folder and execute the following command:

mdtap < B4.in

If the user wishes to run the calculations in the background, an ampersand (&) at the end.

mdtap < B4.in &

To save the values displayed on the screen during the calculation, it can be written in a file using the following command. This is especially useful for the “Permeation” module.

mdtap < B4.in > B4.out &

Description of the input file:

The following example is the file (B4.in) that can be given as input for the “Permeation” module. Note that the description of each line is mentioned on the right side after the hash (#).

```
1 # PDB as the MD trajectory format
B # Selecting Analyze Permeation module
4 # Selecting Permeation sub-module
OH2 TIP # Molecule of interest
./testPDBs # Address/path to PDB files
1 # Start PDB
25 # End PDB
1 # PDBs to skip
1000 # Time difference between each PDB (ps)
# Chain ID (Absent in the test case)
# Seg ID (Absent in the test case)
# Output folder name
# Channel limits
# Minimum frequency of occurrence
# Exitting the module
```

4-Permeation
69.070 17.260 66.210 4.400 60.000 40.000

5
3