Electronic supplementary information (ESI) for

Translocation of a hydroxyl functionalized carbon dot across lipid bilayer: an all-atom molecular dynamics simulation study

Shakkira Erimban, Snehasis Daschakraborty*

Department of Chemistry, Indian Institute of Technology Patna, Bihar 801106, India.

AUTHOR INFORMATION

Corresponding Author

*snehasis@iitp.ac.in

S1. Tables and Figures

Table S1. Average number of different types of H-bonds in the system at 300K

z (nm)	water···water	lipid···water	CD···water	CD···lipid
3.68	3.17	8.89	88.67	0.27
3.58	3.18	8.71	87.54	1.18
3.48	3.18	8.80	87.88	0.95
3.38	3.17	8.85	88.07	0.38
3.29	3.18	8.71	87.04	1.1
3.18	3.18	8.71	82.93	2.97
3.09	3.17	8.85	87.28	0.68
2.90	3.18	8.68	86.64	0.55
2.94	3.18	8.73	85.29	1.74
2.84	3.18	8.78	84.45	1.86
2.74	3.18	8.73	84.24	2.86
2.68	3.18	8.75	83.88	2.43
2.59	3.17	8.84	82.22	2.97
2.48	3.17	8.84	80.00	5.17
2.40	3.17	8.88	79.99	4.72
2.33	3.18	8.75	75.92	7.53
2.24	3.18	8.83	75.14	7.05
2.13	3.17	8.86	75.27	8.29
2.05	3.17	8.87	77.34	6.43
1.93	3.18	8.84	72.70	8.65
1.85	3.18	8.77	70.72	10.66
1.69	3.18	8.80	70.53	7.74
1.61	3.18	8.85	64.58	12.84
1.44	3.17	9.25	71.01	8.54
1.37	3.18	8.86	58.35	9.53
1.22	3.17	9.04	66.07	8.83
1.27	3.18	8.90	59.30	11.02
0.96	3.18	8.90	49.26	9.81
0.94	3.17	9.13	55.23	8.79
0.87	3.18	9.06	50.92	14.16
0.62	3.18	8.92	51.95	11.26
0.72	3.18	8.88	45.28	7.99
0.63	3.18	9.09	53.09	11.3
0.58	3.18	8.89	45.17	17.46
0.4	3.18	9.01	49.40	8.44
0.43	3.18	9.05	46.53	12.48
0.27	3.18	9.11	46.61	9.62
0.19	3.18	9.06	42.64	0
0.07	3.18	9.09	50.14	9.42

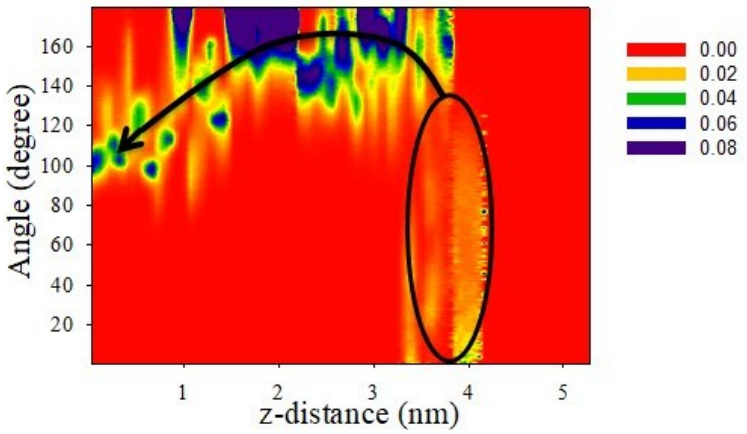

Figure S1. Two-dimensional probability density distribution of the distance (between the center of mass of the CD and the lipid bilayer center) and the angle (between the normal of the middle layer of the CD and z-axis). The black lines indicate the two-dimensional path (distance-angle) of the CD's permeation across the lipid bilayer.

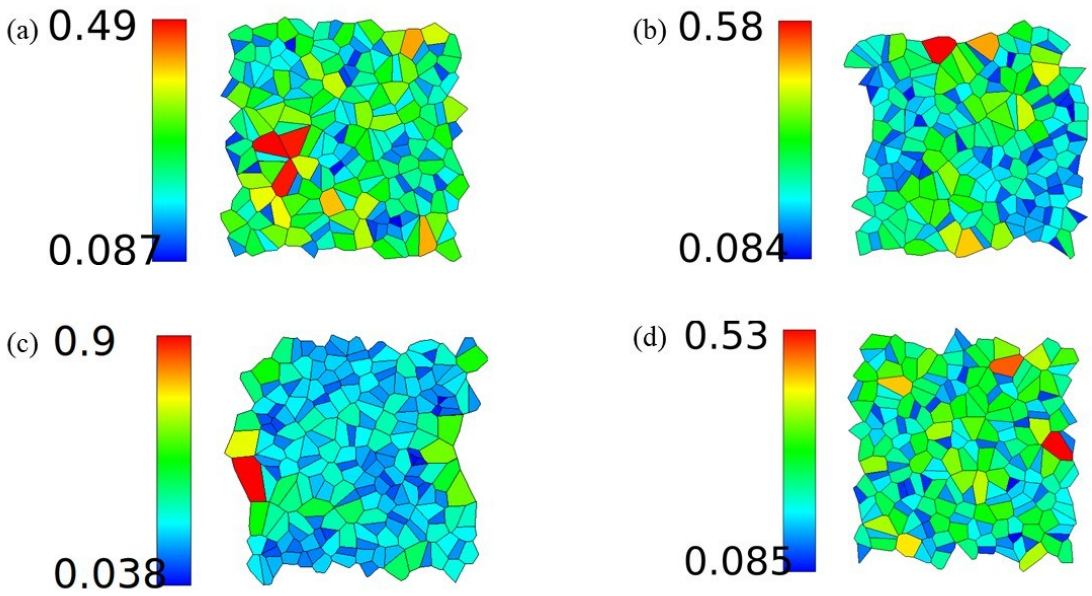

Figure S2. Area per lipid represented as Voronoi diagrams at different positions of CD (a) z= 3.88 nm (b) z = 2.08 nm (c) z = 0.00 nm from the bilayer center and for the (d) pure lipid bilayer in the absence of CD. All the plots are obtained from a short 5ns trajectory simulation run. The area is calculated by choosing key atoms of the lipid using APL@VORO analysis tool[1]. This software determines the cross-sectional areas of different molecules by projecting the coordinates of key atoms onto the XY plane and calculating the Voronoi diagram from

them. Three key atoms, the carbon atom of two carbonyl groups and one carbon of the glycerol moiety of the lipid, are chosen for the construction of the Voronoi cells.

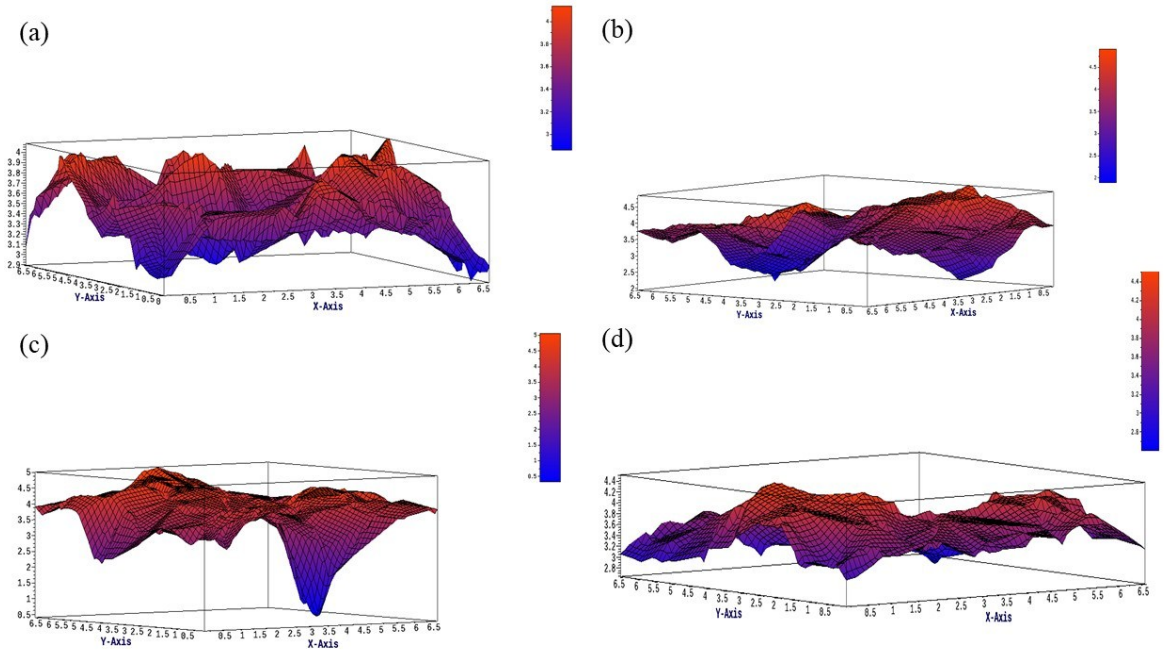

Figure S3. 2D Bilayer thickness at different positions of CD (a) z= 3.88 nm (b) z = 2.08 nm (c) z = 0.00 nm from the bilayer centre and for the (d) pure lipid bilayer in the absence of CD. Thickness is shown along z-axis All the plots are obtained from a short 5ns trajectory simulation run. Thickness is calculated by choosing the phosphate group of the lipid using APL@VORO analysis tool[1]. We considered the phosphate group of the lipid for the calculation.

S2. Checking Convergence of PMF

We have checked the convergence of PMF by investigating the time development of PMF shown in Figures S4a-b. We see that a continuous decrease of the PMF particularly for 300 K temperature even up to 100 ns simulation time. In order to check the convergence more clearly, we have now extended simulations by 20 ns for each of the samples having different z-distances. The PMF, calculated for 50-120 ns time interval, is shown in the revised Fig. S4 of the SI. Cleary the new PMF profile almost overlaps on the PMF calculated for 50-100 ns. Therefore, the convergence of the PMF is attained by 100 ns simulation. Furthermore, the PMF value at z=0 distance is plotted, in Figure S4c, against the maximum time at both 300 K and 320 K temperatures. Clearly, the convergence of PMF is observed by 100 ns and 90 ns for 300 K and 320 K temperatures, respectively.

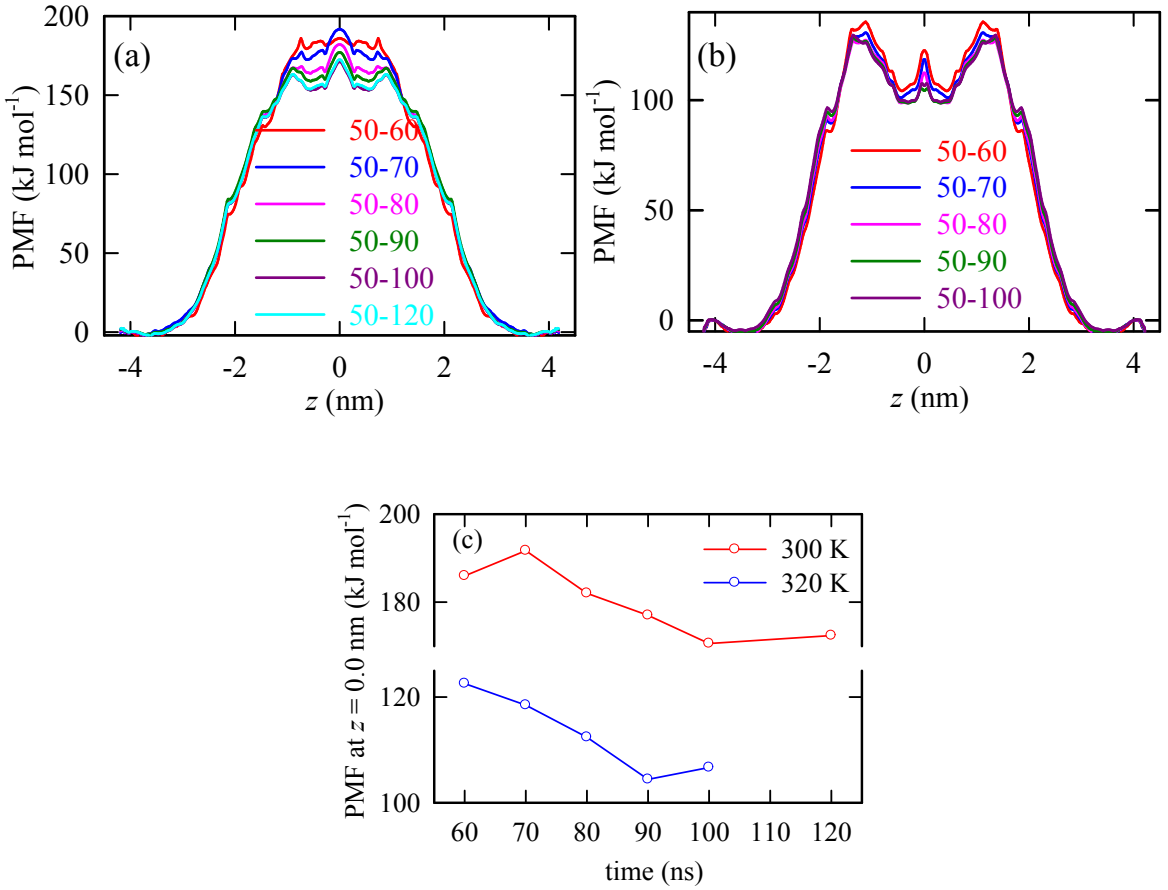

Figure S4. Time development profile of free energy by block analysis of the 50 ns long trajectory showing the convergence of PMF for both temperatures (a)300 K and (b) 320 K. (c) clearly shows the convergence of PMF values at bilayer center (z = 0.0 nm) with time for both the temperatures.

S3. Convergence of pull-force and pull-distance

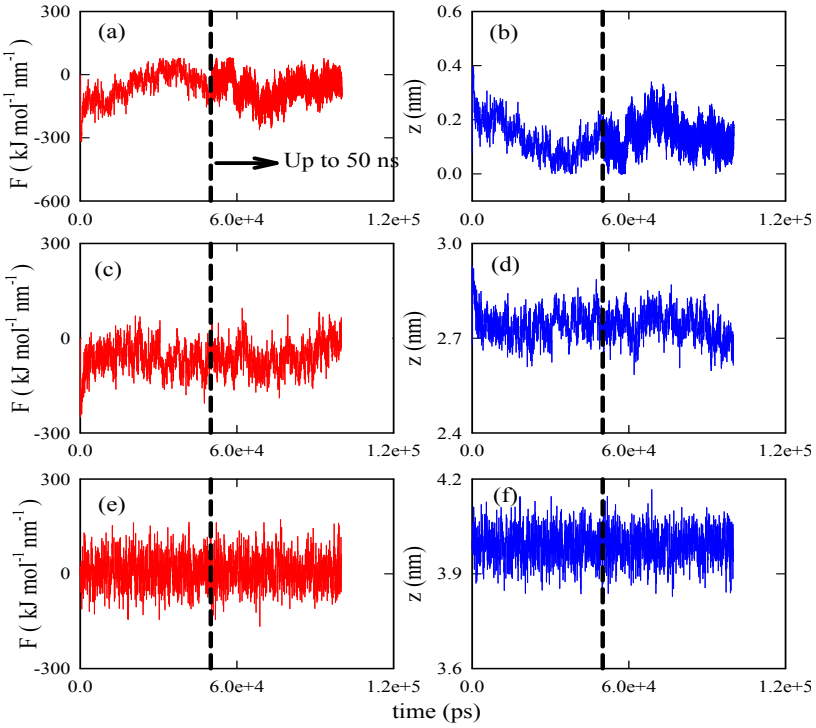

Figure S5. Convergence of pull force (F) and pull distance (z) after 50 ns equilibration run at three characteristic positions of the CD: z = 0.18 nm ((a) and (b)), z =2.88 nm ((c) and (d)) and z = 4.00 nm ((e) and (f)).

S4. Asymmetry of Lipid Bilayer

Asymmetry of the leaflets and counting the flip-flop events

Lipid flip flop occurrences during the permeation process of nanoparticles across the bilayer are previously reported.[2] A lipid flip flop is featured by a full rotation or reorientation of the lipid molecules along the bilayer normal from one leaflet to the other, causing asymmetry of the system. The water pore formed during the permeation aids the otherwise high energy required process. In our system, we have checked for such occurrences by calculating the number of lipid molecules in both the leaflets over time as shown in Figure S6. The calculations are all done based on the z distance of the lipid molecules featuring the upper and lower leaflets. The specific positions of the head groups are taken from the electron density profiles for the respective systems. Significant asymmetry is observed for some of the nearby positions of the CD. However, from some of the snapshots captured over different time gaps, no flip flop events are noticed. We have identified that as the CD penetrates through the bilayer it drags some of the lipid molecules along with it, but these dragged lipid molecules do not further add to the other leaflet. This introduces an asymmetry in the bilayer system. The favorable interaction of hydrophilic surface functionalization of CD with the hydrophilic head groups drives this phenomenon. For one of the positions of CD (z = 1.44 nm) where we observed the maximum water pore formation one out of the 15 dragged lipid molecules gets added to the upper leaflet. Otherwise, no such occurrences are detected in the system.

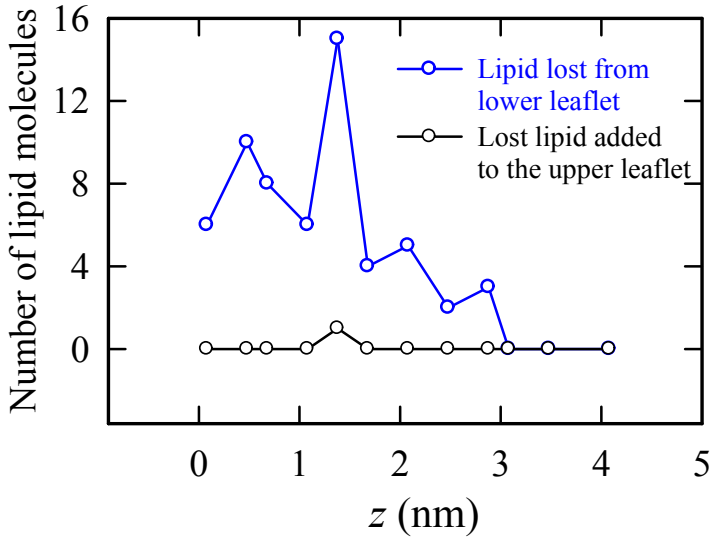

Figure S6. Asymmetry of the lipid bilayer for different positions of the CD. The number of lipid molecules lost from the lower leaflet (through which the CD is penetrated) (blue) and the number of lost lipids added to the upper leaflet for different positions of the CD (black).

Calculation of Lateral Pressure Profile

The lateral pressure profiles of the lipid bilayer in the presence of CD are calculated for four characteristic restraint positions of the CD using the GROMACS-LS[3], a modified version of the GROMACS package.[4] The calculations are done from a separate 20 ns simulation run at each of the positions. The calculation uses trajectory files (in *.trr formats, which store both the velocity and positions, unlike the *.xtc trajectory files which store only the positions). The massive file sizes (trajectory files in *.trr format) make it difficult to perform longer simulation for all the restraint positions of the CD. However, as reported previously, 20 ns simulation trajectory is reasonably good to obtain a characteristic pressure profile. (For more information, see the link http://mdstress.org/files/5914/4657/7530/Local_stress.pdf.) Here the velocities and positions are recorded after a duration of 10 ps for faster calculations.

The program gives an output file of stress tensor components as a function of the z bilayer dimension, the bilayer normal. From the stress components, the lateral (P_L) and normal (P_N) components of the pressure profile are then obtained using Eqs. 1 and 2, respectively.

$$P_L = -0.5 \times (\sigma_{xx} + \sigma_{yy}) \quad (1)$$

$$P_N = -\sigma_{zz} \quad (2)$$

The difference between P_L and P_N gives the z-dependent lateral pressure $\pi(z)$.

$$\pi(z) = P_L(z) - P_N(z) \quad (3)$$

The lateral pressure profiles $\pi(z)$ are plotted in Figure S7. We significant deviations of the lateral pressure profile from the typical profile obtained for pure lipid membrane without the presence of CD.[5, 6] Symmetric profiles are obtained when the CD is at the bilayer center (z = 0.00 nm). For all other positions, the lateral pressure profiles are somewhat asymmetrical. However, different characteristic regions are clearly seen in all the profiles. More specifically, large positive peaks are observed clearly for the bilayer at all the positions of the CD on approaching the head group region. This defines the specific water-head group interface region. This stems from the net repulsive forces acting on the head group region of a lipid bilayer which includes hydration, steric and electrostatic forces. Further, in the interface of head group and tail group, deep troughs of negative pressure profiles are observed. It is well known that the negative values of pressure correspond to the net intermolecular attractive forces acting on the bilayer so as to reduce the overall bilayer area. Maximum negative values are obtained in this region when the CD is at the bilayer center as shown in Figure S7a. In the central part of the bilayer, three characteristic peaks are obtained corresponding to the hydrophobic core of the bilayer. It is previously observed for the pure

phospholipid bilayer that the interior hydrophobic core gives positive peaks with three characteristic regions all arising from the repulsive forces acting between the closely packed tail groups of the lipid. However, in the presence of CD, the pressure drops to negative values at all the distances. This shows the influence of CD on the bilayer even when the CD is not fully penetrated inside the bilayer.

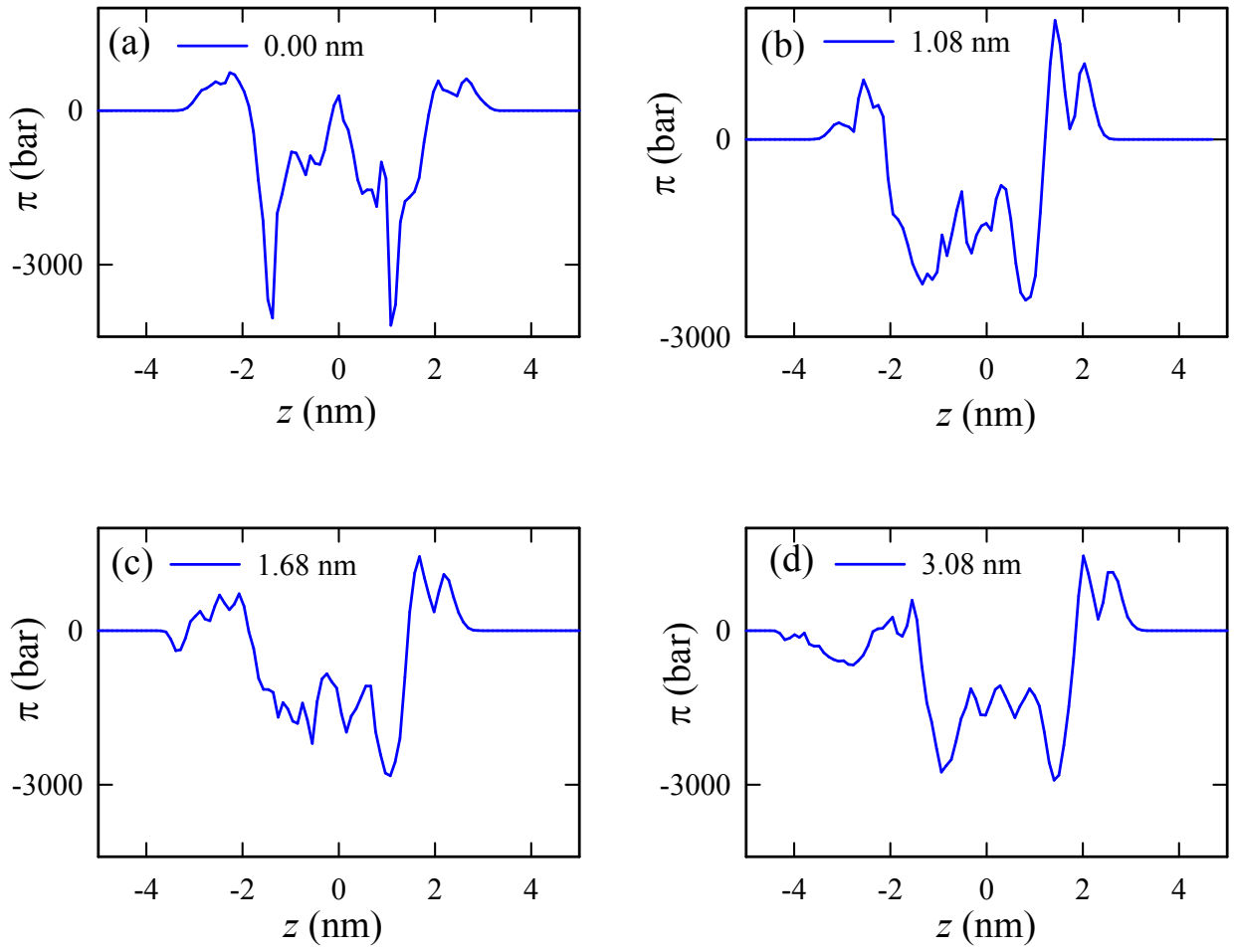

Figure S7. Lateral pressure profiles (π (*z*)) for the lipid bilayer at four restraint z-distance of the CD from the bilayer center (a) 0.00 nm, (b) 1.08 nm, (c)1.68 nm and (d) 3.08 nm respectively.

S5. Sample mdp file in our simulation

The following is a sample *.mdp file used for the simulation runs.

```
;       File 'mdout.mdp' was generated
;       By user: snehasis (1002)
;       On host: node4
;       At date: Fri Apr  5 16:19:08 2019
;
;       Created by:
;                    :-) GROMACS - gmx grompp, 2019 (-:
;
;       Executable:   /apps/codes/gromacs/2016//bin/gmx_mpi
;       Data prefix:  /apps/codes/gromacs/2016/
;       Working dir:  /home/snehasis/Shakkira/carbondot/Allatomalone/equilib
;       Command line:
;         gmx_mpi grompp -f grompp.mdp -c confout.gro -p topol.top -n index.ndx -maxwarn
1

; VARIOUS PREPROCESSING OPTIONS
; Preprocessor information: use cpp syntax.
; e.g.: -I/home/joe/doe -I/home/mary/roe
include             =
; e.g.: -DPOSRES -DFLEXIBLE (note these variable names are case sensitive)
define              =

; RUN CONTROL PARAMETERS
integrator          = md
; Start time and timestep in ps
tinit               = 0
dt                  = 0.002
nsteps              = 50000000
; For exact run continuation or redoing part of a run
init-step           = 0
; Part index is updated automatically on checkpointing (keeps files separate)
simulation-part     = 1
; mode for center of mass motion removal
comm_mode           = linear
; number of steps for center of mass motion removal
nstcomm             = 100
; group(s) for center of mass motion removal
comm-grps           =


; OUTPUT CONTROL OPTIONS
; Output frequency for coords (x), velocities (v) and forces (f)
nstxout             = 0
nstvout             = 0
nstfout             = 125
; Output frequency for energies to log file and energy file
nstlog              = 1000
```

```
nstcalcenergy           = 125
nstenergy               = 125
; Output frequency and precision for .xtc file
nstxout-compressed      = 125
compressed-x-precision  = 10000
; This selects the subset of atoms for the compressed
; trajectory file. You can select multiple groups. By
; default, all atoms will be written.
compressed-x-grps       =
; Selection of energy groups
energygrps              =

; NEIGHBORSEARCHING PARAMETERS
; cut-off scheme (Verlet: particle based cut-offs, group: using charge groups)
cutoff-scheme           = Verlet
; nblist update frequency
nstlist                 = 10
; ns algorithm (simple or grid)
ns_type                 = grid
; Periodic boundary conditions: xyz, no, xy
pbc                     = xyz
periodic-molecules      = no
; Allowed energy error due to the Verlet buffer in kJ/mol/ps per atom,
; a value of -1 means: use rlist
verlet-buffer-tolerance  = 0.005
; nblist cut-off
rlist                   = 1.0
; long-range cut-off for switched potentials

; OPTIONS FOR ELECTROSTATICS AND VDW
; Method for doing electrostatics
coulombtype             = pme
coulomb-modifier        = Potential-shift-Verlet
rcoulomb-switch         = 0
rcoulomb                = 1.0
; Relative dielectric constant for the medium and the reaction field
epsilon-r               = 1
epsilon-rf              = 0
; Method for doing Van der Waals
vdwtype                 = cut-off
vdw-modifier            = Potential-shift-Verlet
; cut-off lengths
rvdw-switch             = 0
rvdw                    = 1.0
; Apply long range dispersion corrections for Energy and Pressure
DispCorr                = EnerPres
; Extension of the potential lookup tables beyond the cut-off
table-extension         = 1
; Separate tables between energy group pairs
energygrp-table         =
```

```
; Spacing for the PME/PPPM FFT grid
fourierspacing          = 0.1
; FFT grid size, when a value is 0 fourierspacing will be used
fourier-nx              = 0
fourier-ny              = 0
fourier-nz              = 0
; EWALD/PME/PPPM parameters
pme_order               = 6
ewald_rtol              = 1e-5
ewald-rtol-lj           = 0.001
lj-pme-comb-rule        = Geometric
ewald-geometry          = 3d
epsilon-surface         = 0
implicit-solvent        = no

; OPTIONS FOR WEAK COUPLING ALGORITHMS
; Temperature coupling
Tcoupl                  = nose-hoover
nsttcouple              = -1
nh-chain-length         = 10
print-nose-hoover-chain-variables = no
; Groups to couple separately
tc-grps                 = popc sol DOT
; Time constant (ps) and reference temperature (K)
tau_t                   = 0.4 0.4 0.4
ref_t                   = 300 300 300
; pressure coupling
Pcoupl                  = berendsen
Pcoupltype              = semiisotropic
nstpcouple              = -1
; Time constant (ps), compressibility (1/bar) and reference P (bar)
tau_p                   = 1.0
compressibility         = 4.5E-5  4.5E-5
ref_p                   = 1.0  1.0
; Scaling of reference coordinates, No, All or COM
refcoord-scaling        = No


; GENERATE VELOCITIES FOR STARTUP RUN
gen_vel                 = yes
gen-temp                = 300
gen-seed                = -1

; OPTIONS FOR BONDS
constraints             = allbonds
; Type of constraint algorithm
constraint_algorithm    = lincs
; Do not constrain the start configuration
continuation            = no
; Use successive overrelaxation to reduce the number of shake iterations
```

```
Shake-SOR               = no
; Relative tolerance of shake
shake-tol               = 0.0001
; Highest order in the expansion of the constraint coupling matrix
lincs-order             = 4
; Number of iterations in the final step of LINCS. 1 is fine for
; normal simulations, but use 2 to conserve energy in NVE runs.
; For energy minimization with constraints it should be 4 to 8.
lincs-iter              = 1
; Lincs will write a warning to the stderr if in one step a bond
; rotates over more degrees than
lincs-warnangle         = 30
; Convert harmonic bonds to morse potentials
morse                   = no

; ENERGY GROUP EXCLUSIONS
; Pairs of energy groups for which all non-bonded interactions are excluded
energygrp-excl          =

; AWH biasing
awh                     = no

; ENFORCED ROTATION
; Enforced rotation: No or Yes
rotation                = no

; Group to display and/or manipulate in interactive MD session
IMD-group               =

; NMR refinement stuff
; Distance restraints type: No, Simple or Ensemble
disre                   = No
; Force weighting of pairs in one distance restraint: Conservative or Equal
disre-weighting         = Conservative
; Use sqrt of the time averaged times the instantaneous violation
disre-mixed             = no
disre-fc                = 1000
disre-tau               = 0
; Output frequency for pair distances to energy file
nstdisreout             = 100
; Orientation restraints: No or Yes
orire                   = no
; Orientation restraints force constant and tau for time averaging
orire-fc                = 0
orire-tau               = 0
orire-fitgrp            =
; Output frequency for trace(SD) and S to energy file
nstorireout             = 100
```

```
;Pull code
pull                = yes
pull_ngroups        = 2
pull_ncoords        = 1
pull_group1_name    = GR1 (CD)
pull_group2_name    = GR2 (Lipid)
pull_coord1_type    = umbrella     ; harmonic biasing force
pull_coord1_geometry  = distance   ; simple distance increase
pull_coord1_groups  = 1 2
pull_coord1_dim     = N N Y
pull_coord1_rate    = 0.00
pull_coord1_k       = 1000         ; kJ mol^-1 nm^-2
pull_coord1_start   = yes          ; define initial COM distance > 0
```

REFERENCE

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6. G. Shahane, W. Ding, M. Palaiokostas and M. Orsi, *J. Mol. Model.*, 2019, **25**, 76.