SUPPORTING INFORMATION

Cholesterol-like Condensing Effect of Perfluoroalkyl Substances on a Phospholipid Bilayer

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Table S1: Summary of different simulations in the main text. Four different sets of simulations were performed for different purposes: (1) Test whether PFAS molecules can penetrate into the lipid membrane. The entire 30 ns simulations were used to see the evolution of penetrated PFAS number; (2) Analyze the membrane properties with inserted PFAS; The configurations in the last 5 ns were used to analyze the structure of the membrane. (3) Analyze the membrane properties with inserted cholesterol as a comparison. The configurations in the last 5 ns were used to analyze the structure of the membrane. (4) Perform a free energy analysis through umbrella sampling. The lipid membrane was first relaxed for 30 ns. Then, a PFAS molecule was placed above the membrane to perform the free energy analysis through the umbrella sampling process. The entire umbrella sampling process was 60 ns.

Target	PFAS number	POPC number	Cholesterol number	Initial configurations	Simulation times	Data for analysis
Test whether PFAS molecules can penetrate into lipid membrane	26	274	0	PFAS molecules are placed above (below) the upper (lower) leaflet	30 ns	0-30 ns
Analyze the membrane properties with inserted PFAS	14, 26, 36, 46	274	0	PFAS molecules are evenly pre-inserted in the lower and upper leaflets	30 ns	25-30 ns
Analyze the membrane properties with inserted cholesterol as a comparison	0	274	46	Cholesterol molecules are evenly pre-inserted in the lower and upper leaflets	30 ns	25-30 ns
Perform free energy analysis	1	274	0	The PFAS molecule is placed above the upper leaflet	30 ns pre-relax + 60 ns production run	60 ns

1. Convergence of free energy analysis during umbrella sampling.

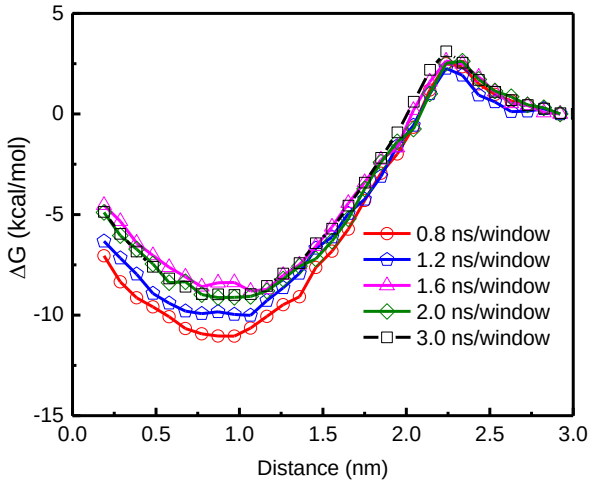

Figure S1: To check the convergence of free energy, we performed 5 independent sets of umbrella sampling processes with different time spans. As shown in the figure, the free energy profile is already converged at simulation with 1.6 ns relaxation time in each window. The different curves in the figure represent simulations with 0.8 ns, 1.2 ns, 1.6 ns, 2.0 ns and 3.0 ns per window, respectively. The corresponding total simulation time spans are 24 ns, 36 ns, 48 ns, 60 ns and 90 ns, respectively.

2. Confirmation of the computational time.

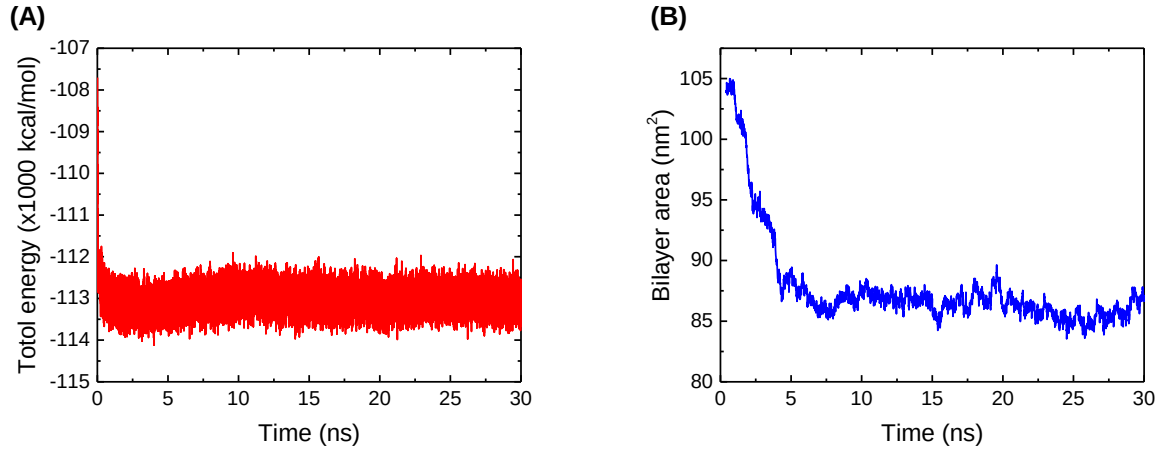

Figure S2: Evolutions of the total energy (A) and the area of lipid bilayer (B) of POPC bilayer embedded with PFOA molecules at the molar ratio of 14 mol% during the relaxation process. It can be seen from these figures that 30 ns in simulations is long enough to relax the bilayer.

3. Effect of initial distance during free penetration process.

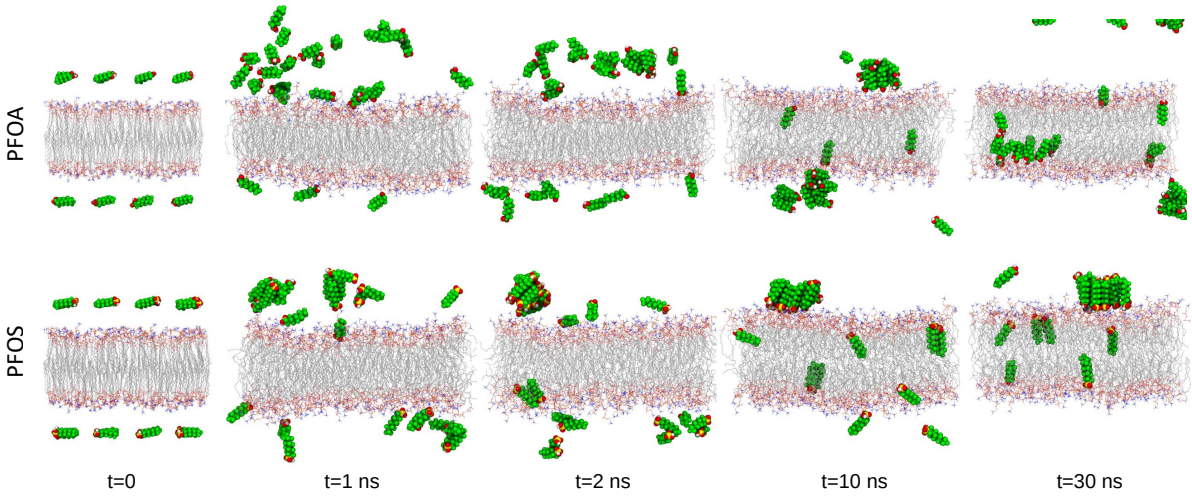

Figure S3: To test the influence of initial distance between a PFAS molecules and the lipid membrane during the penetration process, we increase the their initial distance to the value of 2 nm for PFOA and PFOS. As given in these figures, the PFAS molecules still can spontaneously penetrated into the lipid membrane during a time period of 30 ns.

4. Effect of the bilayer size.

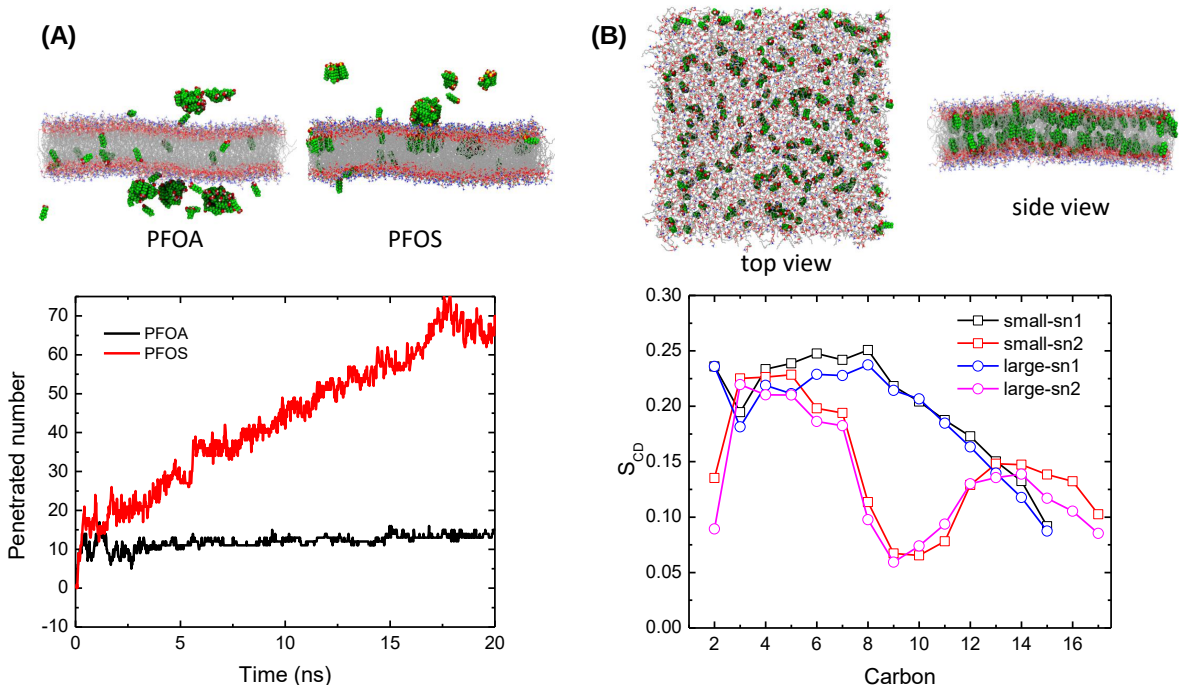

Figure S4: (A) To test the size effect of lipid bilayer during free penetration, we enlarged the system in Fig.2.A of the main text at both the lateral directions. The lipid bilayer and number of PFAS molecules shown in the snapshots here are four times larger than the one in the main text. Compared to PFOA, the PFOS molecules still have a larger probability to penetrate into the lipid bilayer. (B) To test the size effect of lipid bilayer embedded with PFAS molecules, we built a lipid bilayer with PFOA molecules that four times larger than the ones in Fig.4 of the main text. The molar ratio of PFOA here is 14 mol%. As confirmed in the figures, the order parameters of the larger system here are almost the same as those of the smaller system in the main text. As confirmed by these results, a lipid bilayer with 274 POPC is large enough to investigate the penetration of PFAS molecules and their effect on the bilayer proprieties.

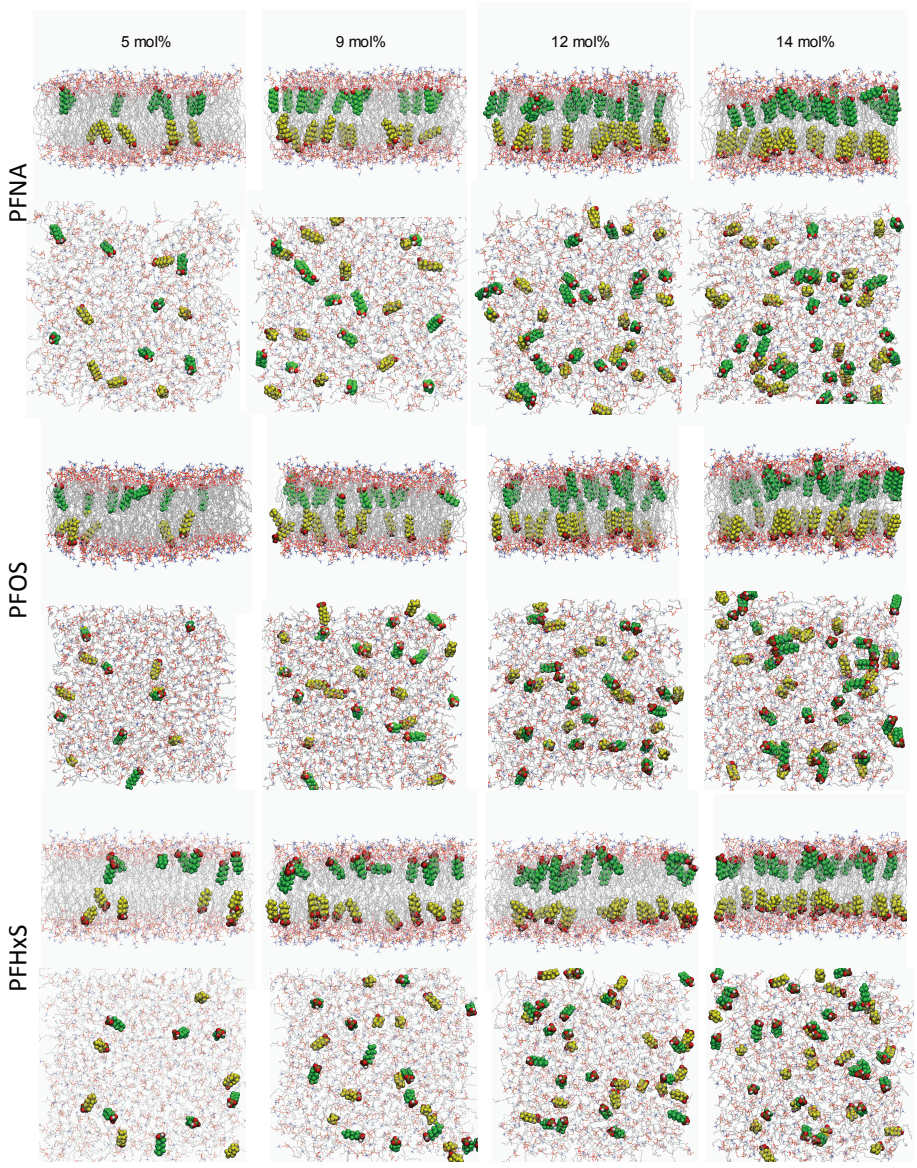

Figure S5: Snapshots of lipid membrane embedded with PFNA, PFOS and PFHxS molecules of different molar ratios.

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!rtf file for PFOA built by MATCH
  22       0
MASS     128 O2D1   15.99900 O
MASS     123 C302   12.01100 C
MASS     129 O311   15.99900 O
MASS     122 C2O2   12.01100 C
MASS     127 HGP1   1.008000 H
MASS     124 C312   12.01100 C
MASS     125 FGA2   18.99800 F
MASS     126 FGA3   18.99800 F

AUTO ANGLES DIHE

RESI   PFOA   0.000000
GROUP
ATOM C      C302     0.340000
ATOM C1     C312     0.420000
ATOM C2     C312     0.340000
ATOM C3     C312     0.340000
ATOM C4     C312     0.340000
ATOM C5     C312     0.340000
ATOM C6     C312     0.300000
ATOM C7     C2O2     0.840000
ATOM F      FGA3    -0.140000
ATOM F1     FGA3    -0.140000
ATOM F2     FGA3    -0.140000
ATOM F3     FGA2    -0.170000
ATOM F4     FGA2    -0.170000
ATOM F5     FGA2    -0.170000
ATOM F6     FGA2    -0.170000
ATOM F7     FGA2    -0.170000
ATOM F8     FGA2    -0.170000
ATOM F9     FGA2    -0.170000
ATOM F10    FGA2    -0.170000
ATOM F11    FGA2    -0.170000
ATOM F12    FGA2    -0.170000
ATOM F13    FGA2    -0.170000
ATOM F14    FGA2    -0.170000
ATOM O      O2D1    -0.630000
ATOM O1     O311    -0.590000
ATOM H      HGP1     0.420000
BOND C      C1
BOND C1     C2
BOND C2     C3
BOND C3     C4
BOND C4     C5
BOND C5     C6
BOND C7     C6
BOND C      F
BOND C      F1
BOND C      F2
BOND C1     F3
BOND C1     F4
BOND C2     F5
BOND C2     F6
BOND C3     F7
BOND C3     F8
BOND C4     F9
BOND C4     F10
BOND C5     F11
BOND C5     F12
BOND C6     F13
BOND C6     F14
BOND C7     O
BOND C7     O1
BOND H      O1
IMPR C7     C6     O      O1
PATCH  FIRST  NONE  LAST  NONE

--------------------------------------------------------
!prm file of PFOA built by MATCH
BONDS
C302     C312     250.00       1.5200
C312     C312     198.00       1.4500
C2O2     C312     200.00       1.5220
C302     FGA3     265.00       1.3400
C312     FGA2     349.00       1.3530
C2O2     O2D1     750.00       1.2200
C2O2     O311     230.00       1.4000
HGP1     O311     545.00       0.9600

ANGLES
FGA3     C302     C312     42.00        112.00
C302     C312     C312     75.70        110.10
C302     C312     FGA2     50.00        115.00
FGA2     C312     C312     50.00        115.00
C312     C312     C312     45.80        120.00
C312     C312     C2O2     52.00        108.00
O2D1     C2O2     C312     70.00        125.00
O311     C2O2     C312     55.00        110.50
C2O2     C312     FGA2     50.00        115.00
FGA3     C302     FGA3     118.00       107.00
FGA2     C312     FGA2     150.00       107.00
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O311    C2O2    O2D1    50.00       123.00
C2O2    O311    HGP1    55.00       115.00

DIHEDRALS
FGA3    C302    C312    C312    0.2500      3       0.00
FGA3    C302    C312    FGA2    0.2500      3       0.00
C302    C312    C312    C312    0.9000      2       0.00
C302    C312    C312    C312    0.7000      3       0.00
C302    C312    C312    C312    0.1200      4       0.00
C302    C312    C312    C312    0.4000      1       0.00
C302    C312    C312    FGA2    0.3000      2       0.00
FGA2    C312    C312    C312    0.3000      2       0.00
FGA2    C312    C312    FGA2    0.3000      2       0.00
C312    C312    C312    C312    0.2000      3       0.00
C312    C312    C312    C2O2    0.2000      3       0.00
FGA2    C312    C312    C2O2    0.3000      2       0.00
C312    C312    C2O2    O2D1    0.0500      6       180.00
C312    C312    C2O2    O311    0.0500      6       180.00
O2D1    C2O2    C312    FGA2    0.0000      3       0.00
HGP1    O311    C2O2    C312    2.0500      2       180.00
O311    C2O2    C312    FGA2    0.1000      3       0.00
HGP1    O311    C2O2    O2D1    2.0500      2       180.00

IMPROPER
C2O2    C312    O2D1    O311    65.0000     0       0.00

NONBONDED
C302    0.0000      -0.0200     2.3000
C312    0.0000      -0.0420     2.0500
C2O2    0.0000      -0.0980     1.7000
FGA3    0.0000      -0.0970     1.6000
FGA2    0.0000      -0.1050     1.6300
O2D1    0.0000      -0.1200     1.7000
O311    0.0000      -0.1921     1.7650
HGP1    0.0000      -0.0460     0.2245

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!rtf file for PFOS built by MATCH
  22     0
MASS    129 S3O2    32.06000 S
MASS    124 FGA2    18.99800 F
MASS    125 FGA3    18.99800 F
MASS    123 C312    12.01100 C
MASS    126 HGP1    1.008000 H
MASS    127 O2P1    15.99900 O
MASS    128 O311    15.99900 O
MASS    122 C302    12.01100 C

AUTO ANGLES DIHE

RESI   PFOS   0.000000
GROUP
ATOM C      C302    0.340000
ATOM C1     C312    0.420000
ATOM C2     C312    0.340000
ATOM C3     C312    0.340000
ATOM C4     C312    0.340000
ATOM C5     C312    0.340000
ATOM C6     C312    0.340000
ATOM C7     C312    0.560000
ATOM S      S3O2    0.540000
ATOM F      FGA3   -0.140000
ATOM F1     FGA3   -0.140000
ATOM F2     FGA3   -0.140000
ATOM F3     FGA2   -0.170000
ATOM F4     FGA2   -0.170000
ATOM F5     FGA2   -0.170000
ATOM F6     FGA2   -0.170000
ATOM F7     FGA2   -0.170000
ATOM F8     FGA2   -0.170000
ATOM F9     FGA2   -0.170000
ATOM F10    FGA2   -0.170000
ATOM F11    FGA2   -0.170000
ATOM F12    FGA2   -0.170000
ATOM F13    FGA2   -0.170000
ATOM F14    FGA2   -0.170000
ATOM F15    FGA2   -0.170000
ATOM F16    FGA2   -0.170000
ATOM O      O2P1   -0.420000
ATOM O1     O311   -0.340000
ATOM O2     O2P1   -0.420000
ATOM H      HGP1    0.420000
BOND C      C1
BOND C1     C2
BOND C2     C3
BOND C3     C4
BOND C4     C5
BOND C5     C6
BOND C6     C7
BOND C7     S
BOND C      F
BOND C      F1
BOND C      F2
BOND C1     F3
BOND C1     F4
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BOND C2    F5
BOND C2    F6
BOND C3    F7
BOND C3    F8
BOND C4    F9
BOND C4    F10
BOND C5    F11
BOND C5    F12
BOND C6    F13
BOND C6    F14
BOND C7    F15
BOND C7    F16
BOND O     S
BOND O1    S
BOND O2    S
BOND H     O1
PATCH FIRST NONE LAST NONE


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!prm file of PFOS built by MATCH
BONDS
C302    C312    250.00      1.5200
C312    C312    198.00      1.4500
C312    S3O2    185.00      1.7900
C302    FGA3    265.00      1.3400
C312    FGA2    349.00      1.3530
O2P1    S3O2    630.00      1.4400
O311    S3O2    235.00      1.6400
HGP1    O311    545.00      0.9600

ANGLES
FGA3    C302    C312    42.00       112.00
C302    C312    C312    75.70       110.10
C302    C312    FGA2    50.00       115.00
FGA2    C312    C312    50.00       115.00
C312    C312    C312    45.80       120.00
C312    C312    S3O2    45.00       105.00
FGA2    C312    S3O2    50.00       115.00
C312    S3O2    O2P1    75.00       107.50
C312    S3O2    O311    90.10       90.00
FGA3    C302    FGA3    118.00      107.00
FGA2    C312    FGA2    150.00      107.00
O2P1    S3O2    O311    90.00       109.00
O2P1    S3O2    O2P1    85.00       121.00
HGP1    O311    S3O2    42.30       113.20

DIHEDRALS
FGA3    C302    C312    C312    0.2500      3       0.00
FGA3    C302    C312    FGA2    0.2500      3       0.00
C302    C312    C312    C312    0.9000      2       0.00
C302    C312    C312    C312    0.7000      3       0.00
C302    C312    C312    C312    0.1200      4       0.00
C302    C312    C312    C312    0.4000      1       0.00
C302    C312    C312    FGA2    0.3000      2       0.00
FGA2    C312    C312    C312    0.3000      2       0.00
FGA2    C312    C312    FGA2    0.3000      2       0.00
C312    C312    C312    C312    0.2000      3       0.00
C312    C312    C312    S3O2    0.0770      3       0.00
FGA2    C312    C312    S3O2    0.3000      2       0.00
C312    C312    S3O2    O2P1    0.1800      3       0.00
C312    C312    S3O2    O311    0.4000      3       0.00
C312    C312    S3O2    O311    0.1000      2       0.00
FGA2    C312    S3O2    O2P1    0.0000      3       0.00
FGA2    C312    S3O2    O311    0.1000      3       0.00
C312    S3O2    O311    HGP1    1.6000      2       0.00
C312    S3O2    O311    HGP1    0.2000      1       180.00
O2P1    S3O2    O311    HGP1    0.2000      3       0.00

IMPROPER

NONBONDED
C302    0.0000      -0.0200     2.3000
C312    0.0000      -0.0420     2.0500
S3O2    0.0000      -0.3500     2.0000
FGA3    0.0000      -0.0970     1.6000
FGA2    0.0000      -0.1050     1.6300
O2P1    0.0000      -0.1200     1.7000
O311    0.0000      -0.1921     1.7650
HGP1    0.0000      -0.0460     0.2245
```