Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Materials

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This document contains 10 pages, 1 table and 7 figures.

Simulation details of unidirectional butanol penetration into the bilayer

Here, the same membrane with 360 lipids as preceding simulations were employed, and 120 butanol molecules were placed into the solvent on one side of the membrane. To ensure the penetration of butanol into the bilayer was unidirectional, the PLUMED plugin was used to apply a restraining potential (a wall) to prevent butanol molecules from crossing the water layer and interacting with the other bilayer leaflet. The collective variables were defined by the *z*-distance between the center of mass of all lipid phosphorous atoms and each butanol. During the simulation, an upper wall will be applied when the value of the collective variable is greater than 5 nm with the force constant (KAPPA) equal to 150.

Simulation analysis

Definition of intercalated and free butanol. Butanol molecules are divided into three groups based on the minimum distance between a butanol molecule and the membrane: a) intercalated butanol inside the membrane (< 4.5 Å); b) accumulated butanol layer around the membrane (>= 4.5 Å and <=7 Å); c) free butanol (> 7 Å). Then two parameters are defined: butanol (intercalated ones) to lipid ratio, and butanol concentration (*v/v*) in the bulk. The latter is calculated based on the numbers of water and butanol molecules with the minimum distance from lipids larger than 7 Å.

Area per lipid. Here, we chose the conventional approach to calculate the area per lipid, in which the total area of the simulation box was divided by the number of lipids in one monolayer and the area of the butanol molecules was neglected. The calculation can be simply represented as:

$$< A_{lipid} > = \frac{2 < XY >}{N_{lipid}}$$

where $< A_{lipid} >$ is area per lipid, $< XY >$ is the area of simulation box, and N_{lipid} is the number of lipids in the system.

2D-thickness map. GridMAT-MD was employed to measure the effect of butanol on the membrane thickness. The phosphorous atoms in the lipid head group served as the reference atom, upon which a grid was used to determine their locations and to calculate the local thicknesses. Total bilayer thicknesses were taken by averaging data from the last 20 ns every 200 ps.

The angular distributions. The orientations of the head group (P-N vector in POPE and P-C vector in POPG) and the angular distributions of the vectors between the first and last carbons of the hydrocarbon chains were calculated with respect to the bilayer.

Deuterium order parameters. The order parameter of lipid tails indicates the orientation of the carbon chain, which is calculated using GROMACS as the following equation:

$$S_z = \frac{3}{2} < cos^2\theta_z > \ - \frac{1}{2}$$

where θ_z is the angle between the *z*-axis of the simulation box (membrane normal) and the carbon−deuterium vector of the *i*th methylene group in the lipid tails. Order parameters vary between 1 and –0.5, indicating a fully ordered distribution along the normal direction to a fully ordered distribution perpendicular to the normal direction. In general, order decreases from the interface region to the bilayer center.

Lateral diffusion coefficients. GROMACS was used to measure the lateral diffusion coefficient of lipids, which is determined by linear regression of the mean square displacement (MSD) of the phosphorus atom of the headgroup based on the 80-100 ns trajectories. An error estimate is given to show the difference of the diffusion coefficients over the two halves of the fit interval (2-15 ns).

Mechanical properties. The membrane buckling method was used for the measurement of the membrane bending modulus K_C. Firstly, a buckled lipid bilayer was generated by using anisotropic pressure coupling, with a pressure of 6 bar in the *x* direction until a strain of 0.15 was achieved. Then, a 100-ns simulation was performed by fixing the box length in *x* and *y* directions, and the *xx* component of the stress tensor was used to derive the force exerted by the membrane in the *x* direction, which is related to K_C. The last 50 ns was used for analyses.

Table S1. Summary of the three systems used to simulate the effects of raising the intercalated butanol:lipid ratio, with regards to the total number of butanol molecules added with each iteration, the average butanol concentration in the bulk and butanol:lipid ratio across the 80-100 ns trajectory.

Iteration	Num. (butanol)			Butanol concentration (*v/v* %)			Butanol:Lipid ratio		
	BOL1	BOL2	BOL3	BOL1	BOL2	BOL3	BOL1	BOL2	BOL3
1	87	172	248	0.37±0.11	0.56±0.13	0.65±0.16	0.21±0.01	0.43±0.01	0.63±0.01
2	176	279	453	0.59±0.11	0.77±0.14	1.16±0.26	0.43±0.01	0.70±0.01	1.15±0.02
3	248	398	640	0.70±0.15	1.02±0.18	1.60±0.25	0.62±0.01	1.01±0.01	1.61±0.02
4	308	502	810	1.00±0.14	1.37±0.17	1.99±0.19	0.74±0.01	1.20±0.02	1.93±0.03
5	372	589	926	1.15±0.16	1.40±0.14	1.98±0.20	0.90±0.02	1.42±0.02	2.23±0.03
6	432	658	/	1.09±0.12	1.45±0.15	/	1.04±0.01	1.58±0.02	/
7	478	714	/	1.30±0.11	1.74±0.16	/	1.12±0.02	1.68±0.02	/
8	507	763	/	1.33±0.14	1.77±0.17	/	1.21±0.02	1.80±0.02	/
9	543	803	/	1.37±0.17	1.90±0.23	/	1.31±0.02	1.89±0.04	/
10	579	838	/	1.53±0.13	2.07±0.15	/	1.38±0.02	1.92±0.03	/
11	607	/	/	1.49±0.13	/	/	1.44±0.02	/	/
12	635	/	/	1.59±0.12	/	/	1.49±0.02	/	/
13	669	/	/	1.57±0.16	/	/	1.59±0.02	/	/
14	706	/	/	1.63±0.14	/	/	1.68±0.02	/	/
15	713	/	/	1.67±0.18	/	/	1.67±0.03	/	/

Supplementary figures

A

Butanol (v/v %)	Heating (°C)	Cooling (°C)	ΔT_m	T_m Hysteresis
0	22.11	20.15	N.A.	-1.96
0.25	20.88	18.77	-1.23	-2.11
0.5	20.11	17.89	-2.00	-2.22
1	18.37	15.92	-3.74	-2.45
2	15.83	13.07	-6.28	-2.76
4	12.11	9.07	-10.00	-3.04

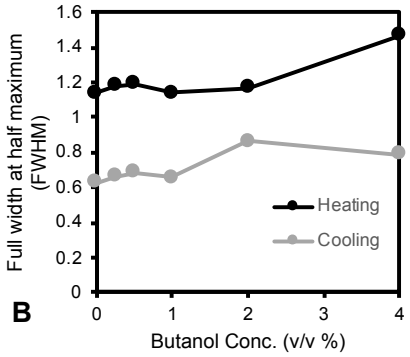

Figure S1 The reduction of the main transition temperature of lipid bilayer in the presence of butanol: (A) DSC results obtained at rates of 1 °C/min of vesicles with different butanol concentrations; (B) full width at half maximum.

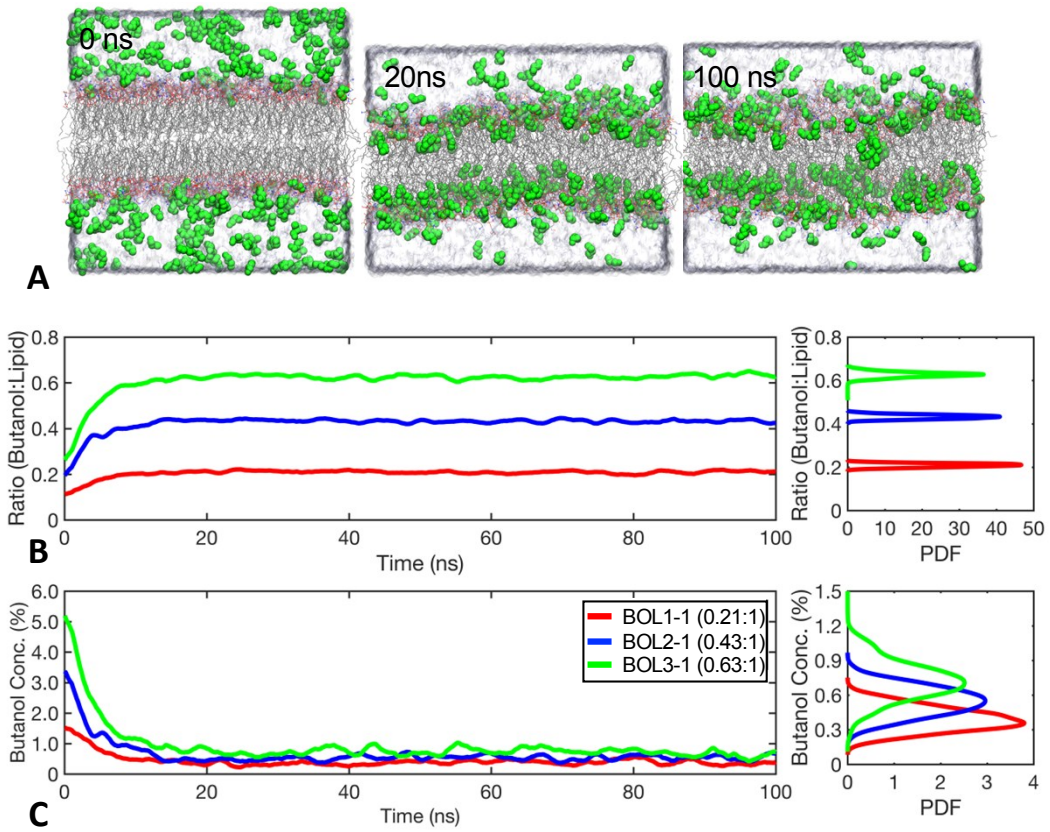

Figure S2 The partitioning of butanol into membrane. (A) Snapshots at different simulation times for BOL3-1 (butanol:lipid ratio: 0.63:1). Solvent is shown as transparent surfaces, bilayers as lines with carbon atoms in gray, and butanol as green spheres. Dynamic balance between butanol inside the bilayer and in the bulk in the three first iterations: (B) butanol to lipid ratio along the simulation time (left panel) and the probability distribution profiles of the last 50 ns; (C) butanol concentration (*v/v*, %) along the simulation time (left panel) and the probability distribution profiles of the last 50 ns.

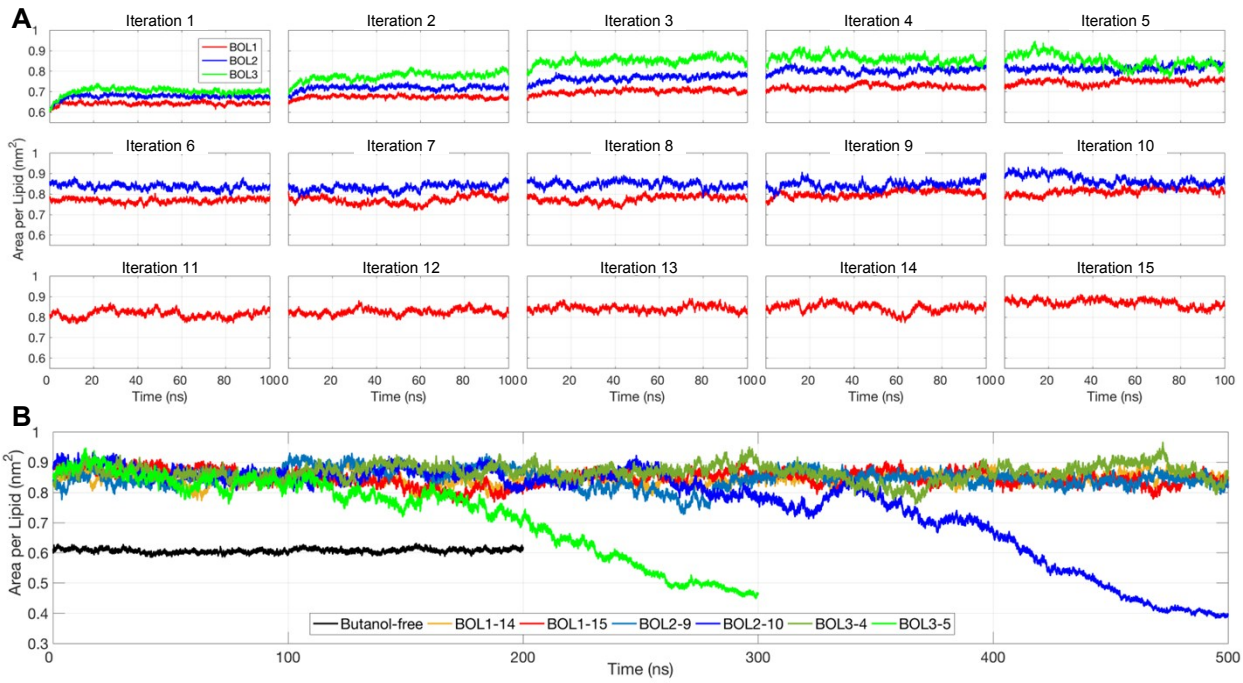

Figure S3 Comparison of area per lipid among all systems: (A) area per lipid of each system along the first 100 ns; (B) area per lipid along a longer simulation time frame.

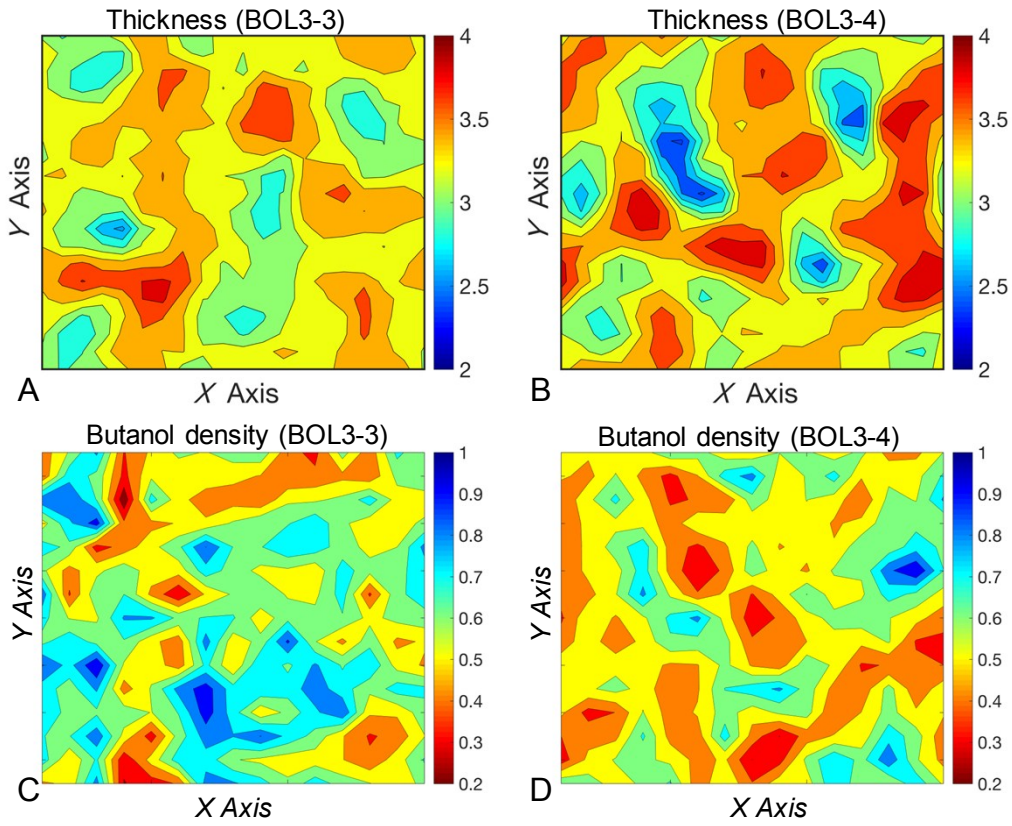

Figure S4 Comparison of the 2D-thickness map and the 2D-butanol density profile of BOL3-3 and BOL3-4 during the last 90-100 ns.

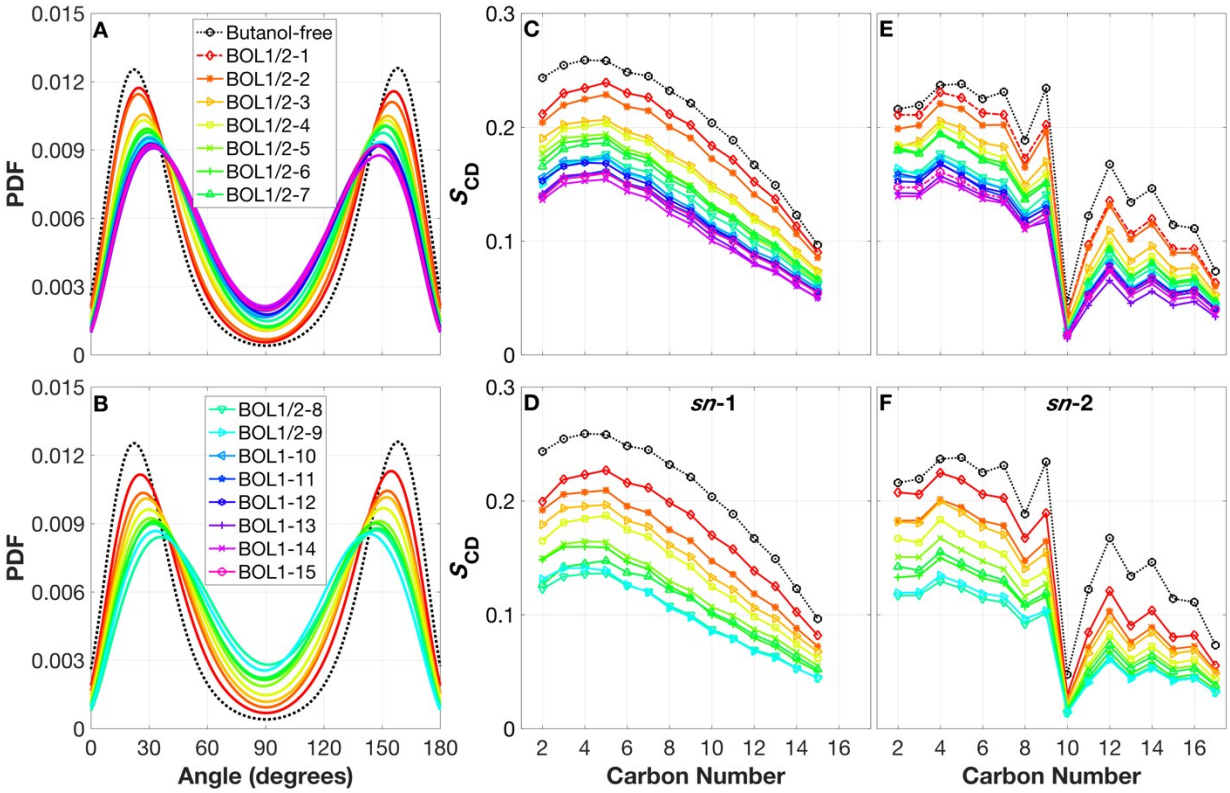

Figure S5 Orientation and ordering of lipid tails for BOL1 and BOL2 compared with the butanol-free system. For caption, see Figure 6(D-F).

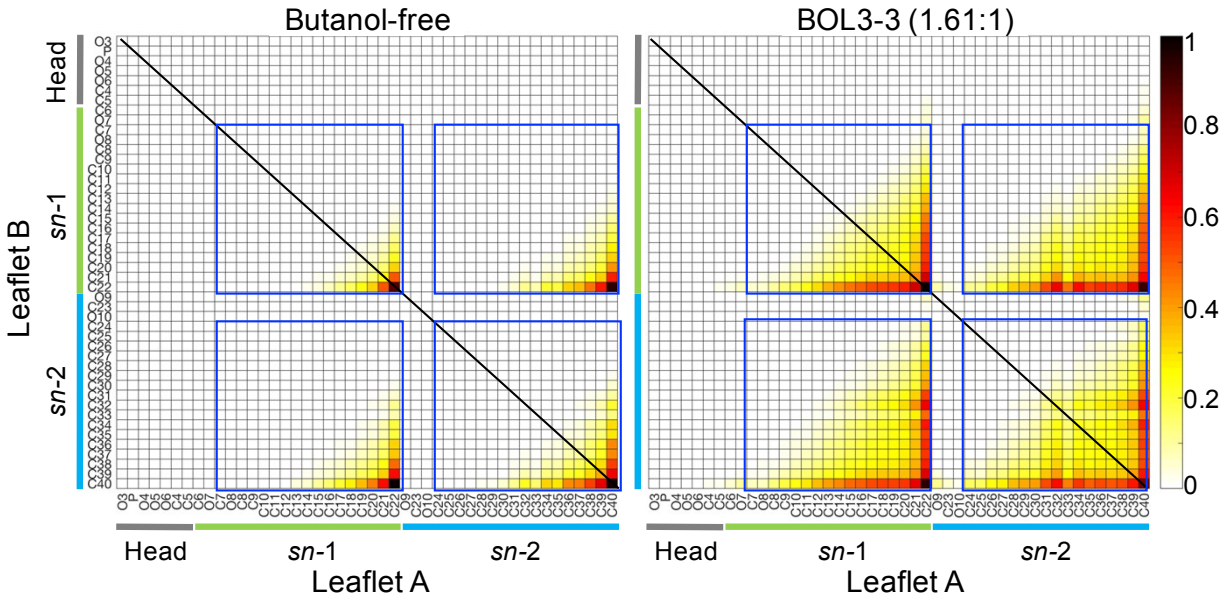

Figure S6 Atom-atom contact maps between the heavy atoms of POPE/POPG lipids in the two leaflets. Frequency distributions of inter-leaflet contacts, as predicted by simulations, between lipid atoms of (A) butanol-free and (B) BOL3-3 (1.61:1).

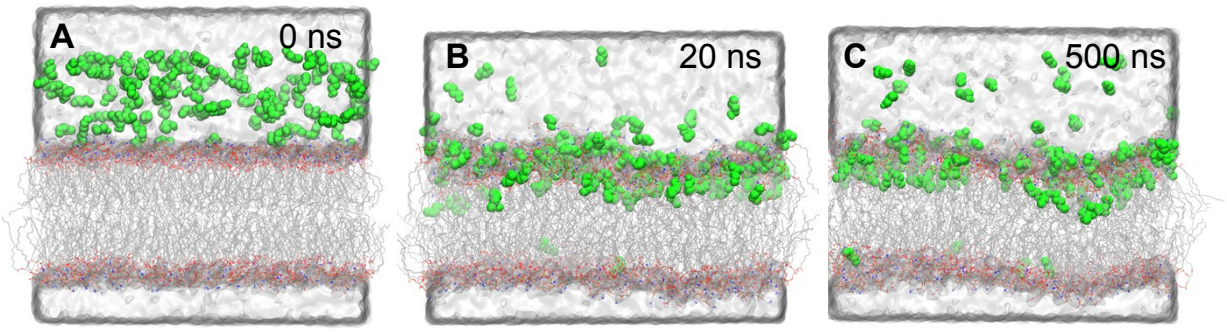

Figure S7 Unidirectional butanol penetration into the bilayer. Snapshots at (A) 0 ns, (B) 20 ns and (C) 500 ns.