Supplementary Material (ESI) for Soft Matter

Supporting information.

Darya V. Pyrkova[a], Natalya K. Tarasova[a,b], Timothy V. Pyrkov[a], Nikolay A. Krylov[a,c], Roman G. Efremov[*a]

Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

1 Geometrical parameters of the lipid bilayers under study

Table S1 Equilibrium macroscopic averages for lipid bilayers under study: MD simulation and experimental data

system	A_L (Å^2)[a]			$<D_{PP}>$(Å)[b]		
	MD simulation[c]	experiment	for ideal mixture[e]	MD simulation[d]	experiment	for ideal mixture[e]
DOPC	71.7	72.5±1[38,60]		35.8	36.1±0.8[38,59-61]	
DOPC90	66.4		70.7	37.9		36.0
DOPC80	66.1		69.6	37.8		36.2
DOPC70	65.6		68.6	37.8		36.3
DOPC50	64.2		66.6	37.7		36.7
DOPC30	63.9		64.5	37.2		37.1
DOPC20[f]	61.9		63.5	37.9		37.2
DOPC10	61.9		62.4	37.6		37.4
DPPC	61.4	63.5±0.5[49,59,60]		37.6	38.1±0.3[59-61]	

[aa] A_L – average area per lipid molecule. [b] $<D_{P-P}>$ – average bilayer thickness (distance between the planes determined by phosphorous atoms of lipids in different monolayers). [c] Standard deviation is equal to 0.1 Å^2. [d] Standard deviation is equal to 0.1 Å. [e] The values obtained assuming linear dependence of bilayer properties on the content of each lipid type. [f] Data for the bilayer DOPC20 are averaged based on two independent MD simulations..

2 Hydrophobic/hydrophilic organization of the water/bilayer interface in one- and two-component lipid bilayers

Table S2 Average fractions of hydrophobic (P_{pho}), neutral (P_{neu}), and hydrophilic (P_{phi}) surface area for studied systems

system	P_{pho}[a]	P_{neu}[a]	P_{phi}[a]
DOPC	0.22	0.47	0.31
DOPC90	0.18	0.49	0.33
DOPC80	0.18	0.49	0.33
DOPC 70	0.18	0.48	0.34
DOPC 50	0.17	0.49	0.34
DOPC 30	0.17	0.49	0.35
DOPC 20[b]	0.15	0.49	0.36
DOPC10	0.15	0.50	0.35
DPPC	0.14	0.50	0.36

[a] Maximal standard deviation is equal to 0.02. [b] Data for the bilayer DOPC20 are averaged based on two independent MD runs.

3 Lipid clustering observed in MD simulations is not an artifact originating from the starting configuration of the bilayer systems

In order to avoid possible influence of the starting geometry of a bilayer on the lateral heterogeneity parameters extracted from the final part of MD trajectory, cluster analysis was also done immediately after heating the system to the target temperature (325 K). The data were obtained for the bilayers DOPC20, DOPC50, and DOPC80. As a result, it was found that parameters of the geometrical clusters in the beginning and at the end of the simulations are sufficiently different. Thus, no clusters of three and more lipids were detected in the former case. On the contrary, size distributions of clustered lipids near the equilibrium (after 10 ns of MD collection run) reveal maxima corresponding to three molecules per cluster. Such a picture is observed for all studied bilayers. Therefore, we assume that the MD-trajectory length and the simulation protocol permit rather efficient sampling of the configurational space. It is good enough to remove inevitable non-equilibrium characteristics of the system introduced at the initial stages of bilayer assembling.

Another question arises: Might it be happen that the lateral organization of lipid bilayers reported in this study is just a random picture, which does not depend on the physical nature of microscopic interactions in water-lipid systems? To address this issue, we compared MD-data on 2D organization of lipids on the surface with those generated by random placement of lipids. The bilayer surface was covered with a hexagonal grid in such a way that each grid cell contained maximum one lipid (see Methods). Then, the number of neighbors was calculated for each occupied cell. As seen in Fig. S1, the corresponding distributions obtained from MD (DOPC70) and random data are sufficiently different. In fact, analysis performed with the Mann-Whitney *U*-test (1) at the 5% significance level indicates that the data are described by different distributions. By other words, lateral surface organization of lipids in the bilayers under study is not random. Instead, they are defined by exact physical interactions between the membrane components.

Table S3 Average number of H-bonds formed by different groups of lipids

System	clust_po[a]	clust_gl[b]	not_cl_po[c]	not_cl_gl[d]	incheng_po[e]	incheng_gl[f]
DOPC	1.66 ±0.06[g]	0.96 ± 0.06	1.92 ± 0.07	1.11 ± 0.07	1.85 ± 0.07	1.02 ± 0.07
DOPC90	1.64 ± 0.06	0.85 ± 0.06	1.91 ± 0.08	1.10 ± 0.08	1.86 ± 0.07	0.98 ± 0.07
DOPC80	1.62 ± 0.06	0.83 ± 0.06	1.88 ± 0.08	1.09 ± 0.07	1.83 ± 0.06	1.00 ± 0.05
DOPC70	1.63 ± 0.06	0.82 ±0.06	2.01 ± 0.08	1.10 ± 0.08	1.89 ± 0.07	0.99 ± 0.07
DOPC50	1.61 ± 0.06	0.83 ± 0.06	1.87 ± 0.07	0.97 ± 0.07	1.69 ± 0.06	0.88 ± 0.06
DOPC30	1.65 ± 0.06	0.80 ± 0.03	1.89 ± 0.07	0.95 ± 0.04	1.82 ±0.03	0.91 ±0.03
DOPC20[f]	1.62 ± 0.03	0.81 ± 0.02	1.85 ± 0.05	0.95 ± 0.03	1.75 ± 0.04	0.88 ± 0.02
DOPC10	1.59 ± 0.04	0.78 ± 0.03	1.86 ± 0.04	0.95 ±0.04	1.78 ± 0.03	0.89 ± 0.03
DPPC	1.39 ± 0.06	0.67 ± 0.03	1.77 ± 0.04	0.93 ± 0.06	1.77 ± 0.03	0.91 ± 0.03

[a] Phosphate oxygen for lipids in geometrical clusters; [b] glycerol oxygen for lipids in geometrical clusters; [c] phosphate oxygen for lipids staying in clusters less than 10% of time (group :non clustered"); [d] glycerol oxygen for lipids staying in clusters less than 10% of time (group "non clustered"); [e] phosphate oxygen for lipids in transient clustes (group "interchanging"); [f] glycerol oxygen for lipids in transient clusters (group "interchanging"); [g] The analysis was done based on the last nanosecond of MD trajectory.

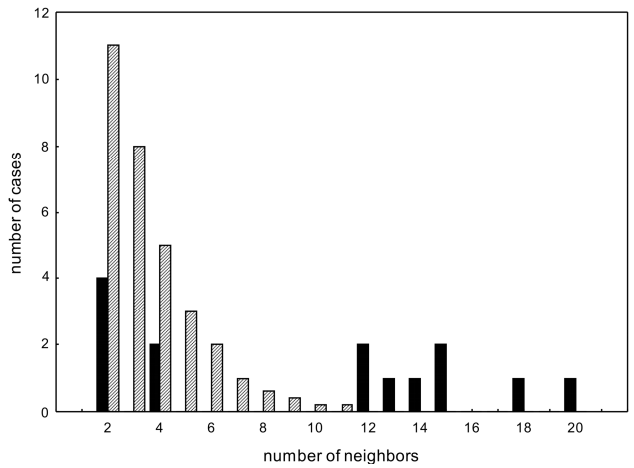

Fig. S1 Distribution of lipid neighbors on a hexagonal grid covering surface of the DOPC70 bilayer. The distributions are obtained *via* MD simulation (black bars) and random placement of lipids on the grid (hatched bars).

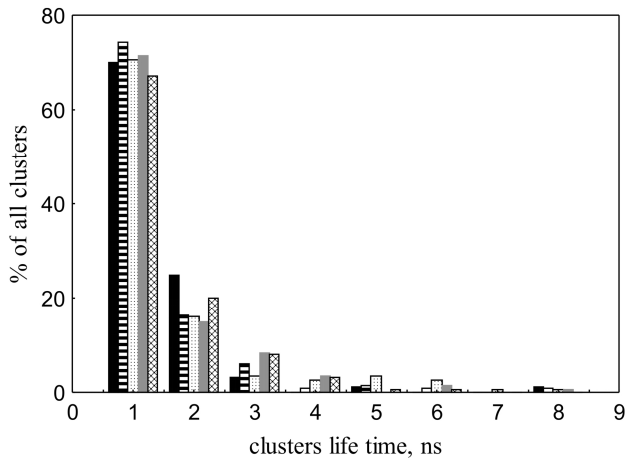

Fig. S2 Distribution of geometrical clusters over their life time in the course of MD simulation. The data are given for the following lipid bilayers: DOPC (black), DOPC80 (horizontal hatching), DOPC50 (dotted hatching), DOPC20 (gray), DPPC (rombic net hatching).