Bond orientation properties in lipid molecules of membranes: molecular dynamics simulations

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Abstract. Atomistic molecular dynamics simulations have been carried out for 16 different fully hydrated phosphatidylcholine lipid bilayers, having 16 or 18 carbon atoms in fully saturated \textit{sn}−1 chain and from 18 to 22 carbon atoms in \textit{sn}−2 chain with different degree of unsaturation, with the purpose to investigate the effect of unsaturation on physical properties of lipid bilayers. Special attention has been paid to profiles of C-C and C-H bond order parameters of lipid molecules and the orientational fluctuations of these bond vectors. It was shown that the study of anisotropy degree of bond orientations probability distributions allows distinguishing extended regions with different types of angular fluctuations of bonds in a membrane formed by lipid molecules with unsaturated chains.

1. Introduction
One of the most important molecular systems in nature is a biomembrane. Biomembranes play a role of barriers separating the interior of a cell from the outside environment and are in control of the movement of various compounds into and out of cells. Biomembranes, built mostly by lipid molecules arranged in a bilayer, are very complex heterogeneous systems, which are characterized by anisotropy of structural and physical properties, knowledge of which is a key element of our general understanding of membrane functioning. A typical biological membrane contains many species of lipids, phosphatidylcholine (PC) being the most abundant one. The commonly occurring fatty acid (FA) chains of lipids may contain 1 – 6 carbon-carbon double bonds of the \textit{cis}-configuration in different positions. In most cases, at least half of the FA chains are unsaturated. Unsaturated FA tails of lipids are of great importance for the structure and function of animal and plant membranes [1–16]. The double bonds of polyunsaturated (PU) chains are, as a rule, methylene-interrupted. Throughout this paper, the shorthand nomenclature, i.e., the notation of \textit{M}: \textit{k}(n−\textit{j})\textit{cis} for describing the structure of each hydrocarbon chain of lipids will be used. Here the number \textit{M} specifies the total number of carbon atoms in the chain, the number \textit{k} specifies the number of double bonds, \textit{j} denotes the number of carbons between the chain terminal CH\textsubscript{3} group and the nearest double bond, assuming that all the other double bonds are methylene-interrupted, whereas \textit{cis} refers to the conformation around the double bonds. For brevity, the fragment \textit{(n−j)\textit{cis}} in the notation is frequently omitted.
Although the history of study of FA composition of different membranes in relation to the tentative role of lipid unsaturation is comparatively long [1–16], full understanding of the effects of lipid unsaturation on various physical properties of membranes at the molecular level, affecting their functioning, is not yet achieved. In order to investigate the role of lipid tails unsaturation we have carried out molecular dynamics (MD) simulations of 16 hydrated liquid crystalline phase PC bilayers composed of phospholipids with sn-1 saturated and sn-2 unsaturated chains. The following PC molecules were considered in the simulations:

- 1-stearoyl-2-oleoyl-sn-glycero-3-PC (18:0/18:1(n-9)cis PC),
- 1-stearoyl-2-linoleoyl-sn-glycero-3-PC (18:0/18:2(n-6)cis PC),
- 1-stearoyl-2-linolenoyl-sn-glycero-3-PC (18:0/18:3(n-3)cis PC),
- 1-stearoyl-2-octadecatetraenoyl-sn-glycero-3-PC (18:0/18:4(n-3)cis PC),
- 1-stearoyl-2-arachidonoyl-sn-glycero-3-PC (18:0/20:4(n-6)cis PC),
- 1-stearoyl-2-eicosapentaenoyl-sn-glycero-3-PC (18:0/20:5(n-3)cis PC),
- 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-PC (18:0/22:6(n-3)cis PC),
- 1-palmitoyl-2-oleoyl-sn-glycero-3-PC (16:0/18:1(n-9)cis PC),
- 1-palmitoyl-2-linoleoyl-sn-glycero-3-PC (16:0/18:2(n-6)cis PC),
- 1-palmitoyl-2-linolenoyl-sn-glycero-3-PC (16:0/18:3(n-3)cis PC),
- 1-palmitoyl-2-octadecatetraenoyl-sn-glycero-3-PC (16:0/18:4(n-3)cis PC),
- 1-palmitoyl-2-arachidonoyl-sn-glycero-3-PC (16:0/20:4(n-6)cis PC),
- 1-palmitoyl-2-eicosapentaenoyl-sn-glycero-3-PC (16:0/20:5(n-3)cis PC),
- 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-PC (16:0/22:6(n-3)cis PC).

2. Models and methods
Each simulated bilayer was composed of 128 lipids of one of 16 studied types, with 64 lipids in each leaflet. All hydrogen atoms were explicitly included in the computations. Each bilayer was hydrated by 3840 water molecules, which corresponds to condition of full hydration with 30 water molecules per lipid. The system was put into rectangular periodic cell, with the Z-axis parallel to the bilayer normal. The size of the box was varying during the simulations under condition of semianizotropic NPT-ensemble with two degrees of freedom: one in Z-direction and another in XY-direction, so that the box sizes in X and Y direction were equal at each time moment. The pressure was set to 1 bar and the temperature to 303 K.

The force field for lipids was based on the CHARMM27 parameter set for lipids [17] with modifications described in paper [18], which include scaling of the 1-4 electrostatic interactions by factor 0.83, and recalculated charges of the lipid headgroup on the bases of ab-initio computations. For bilayer composed of 14:0/14:0 PC lipids, modifications introduced in paper [18] provide perfect agreement with experimental data for the area per lipid, as well as with the X-ray structure factor and NMR order parameters. For lipids considered in this work, we use the same partial charges as in ref. [18] in the lipid headgroup including esters, while for tails we used charges adopted from the original CHARMM27 force field, with scaling of 1-4 electrostatic interactions by factor 0.83. All intramolecular bond and angle parameters, as well as Lennard-Jones interactions, were taken as in the original CHARMM27 force field [17]. Water molecules were described by the flexible SPC model [19]. Use of this water model, in connection with modified CHARMM27 force field, was verified in paper [18].

The double time step algorithm [20] was used to treat separately fast forces (covalent bonds, angles, torsions, collision Lennard-Jones forces within 5 Å distance) with time step 0.25 fs, and longer range forces with time step 2.5 fs. The long-range electrostatic interactions were treated by the Ewald summation method [21]. The reciprocal part of the Ewald sum was cut at the condition that the remaining terms do not contribute more than on 0.0001 level of the total.
value. The $\alpha$ parameter of Ewald sum was set to $\alpha = 2.6 r_{\text{cut}}$, and cutoff distance $r_{\text{cut}} = 14 \text{ Å}$ was optimized for computational performance according to ref. [22]. The dispersion correction from the Lennard-Jones interactions outside the cutoff distance was included into pressure [21].

In the starting configuration, the lipids were set parallel to each other, organized in a regular manner in two layers, and water molecules were distributed outside the bilayer. The systems were simulated 1 ns under constant volume and then 1 ns under constant pressure and isotropic cell fluctuations. The obtained configurations were considered as starting points for longer simulations with independent cell fluctuations in $Z$ and $XY$ directions. The time reversible Nose-Hoover constant temperature – constant-pressure algorithm [23] was implemented, with the thermostat and barostat relaxation time 30 fs and 1 ps respectively. All the systems were simulated during 100 ns. The first 20 ns of the simulations was considered as equilibration and disregarded in further analysis. Atom coordinates were saved each 1 ps in trajectories for further analysis. The simulation software was MDynaMix package v.5.2 [24].

3. Results and discussion

3.1. Area per lipid

The average area per lipid $A_{pl}$ is one of the most fundamental parameters of a lipid bilayer. The area monitoring in computer simulations is one of the most common ways to determine whether the bilayer system has reached equilibrium. Moreover, the average area per lipid defined in zero-tension computer simulations is a parameter which is most often used to define the quality of the force field, though more reliable validation of a force field can be done by comparison of simulated and experimental structure factors of the system [25].

From the observation of the time evolution of the simulated areas per lipid and calculations of block averages a conclusion was made that 20 ns of equilibration time is enough for all considered bilayer systems. Average values of the area per lipid computed during the 80 ns production stage, as well as compressibility modulus of the bilayers computed from the fluctuations of the total areas as $K_A = k_B T A / \langle (A - \langle A \rangle)^2 \rangle$ (where $A$ being the total area of the bilayer patch) are listed in Table 1. Some of available experimental average lipid areas are also given in Table 1. We should note that there is a wide spread of experimental data concerning some of the bilayers. Many experimental structural methods were reviewed in works [26,27], and considerable quantitative uncertainty was demonstrated in structural results for lipid bilayers, including the value of area $A_{pl}$ in the fluid phase.

A rather weak trend of the simulation and experimental results is that the average area is slightly increasing with the degree of unsaturation. Still, bilayers with highest degree of unsaturation, 16:0/22:6 PC and 18:0/22:6 PC show somewhat smaller $A_{pl}$ than those of the nearest less unsaturated bilayers. The reason for the decrease of $A_{pl}$ values of molecules 16:0/22:6 PC and 18:0/22:6 PC can possibly become more clear after analysis of packing arrangement of different chains. It is also worth to note the tendency that bilayers with higher average area per lipid have higher relative area fluctuations which render to their lower compressibility modulus.

3.2. Bond order parameters

The traditional measure for the orientational mobility of C-H bonds in lipid bilayers is the C-H bond order parameter which is available from $^2H$ NMR experiments [31–33]; it is defined as:

$$S_{CH} = (3\langle \cos^2 \theta_{CH} \rangle - 1)/2$$

where $\theta_{CH}$ is the angle between the C-H bond vector (C-D bond in $^2H$ NMR) and the bilayer normal. The order parameter can change from $S_{\text{min}} = -0.5$ (the bonds are always oriented parallel to the bilayer plane) to $S_{\text{max}} = 1$ (the bonds are always parallel to the bilayer normal). Strictly speaking, it is impossible to establish the sign of the C-D bond order parameter from
Table 1. Average areas per lipid, $A_{pl}$, and bilayer compressibility modulus, $K_A$, obtained for mixed-chain liquid-crystalline phase unsaturated phosphatidylcholine bilayers by MD simulations of the present work; $T = 303$ K.

| Lipid                  | $A_{pl}$ $(\pm \Delta)^a$, $nm^2$, Sim | $A_{pl}$, $nm^2$, Exp | $K_A$, mN/m |
|------------------------|----------------------------------------|-----------------------|-------------|
| 16:0/18:1(n-9)cis PC   | 0.634 $(\pm 0.002)$                    | 0.63 $^{[28]}b$       | 458         |
| 16:0/18:2(n-6)cis PC   | 0.636 $(\pm 0.003)$                    | 0.66 $^{[28]}b$       | 349         |
| 16:0/18:3(n-3)cis PC   | 0.632 $(\pm 0.002)$                    |                       | 359         |
| 16:0/18:4(n-3)cis PC   | 0.647 $(\pm 0.003)$                    |                       | 312         |
| 16:0/18:5(n-3)cis PC   | 0.646 $(\pm 0.004)$                    |                       | 274         |
| 16:0/20:4(n-6)cis PC   | 0.652 $(\pm 0.003)$                    | 0.68 $^{[28]}b$       | 339         |
| 16:0/20:5(n-3)cis PC   | 0.656 $(\pm 0.003)$                    |                       | 337         |
| 16:0/22:6(n-3)cis PC   | 0.643 $(\pm 0.002)$                    | 0.70 $^{[28]}b$       | 447         |
| 18:0/18:1(n-9)cis PC   | 0.627 $(\pm 0.002)$                    | 0.614 $^{[29]}c$; 0.666 $^{[30]}d$ | 440         |
| 18:0/18:2(n-6)cis PC   | 0.625 $(\pm 0.002)$                    | 0.673 $^{[30]}d$      | 432         |
| 18:0/18:3(n-3)cis PC   | 0.637 $(\pm 0.002)$                    | 0.666 $^{[30]}d$      | 366         |
| 18:0/18:4(n-3)cis PC   | 0.646 $(\pm 0.003)$                    |                       | 381         |
| 18:0/18:5(n-3)cis PC   | 0.653 $(\pm 0.003)$                    |                       | 339         |
| 18:0/20:4(n-6)cis PC   | 0.646 $(\pm 0.003)$                    | 0.706 $^{[30]}d$      | 428         |
| 18:0/20:5(n-3)cis PC   | 0.653 $(\pm 0.002)$                    | 0.691 $^{[30]}d$      | 387         |
| 18:0/22:6(n-3)cis PC   | 0.637 $(\pm 0.004)$                    | 0.692 $^{[29]}c$; 0.716 $^{[30]}d$ | 323         |

$a$ statistical error $\Delta$ for 20-100 ns is evaluated from the variance of 10 ns block averages;  
$b$ Langmuir film balance, 297K;  
$c$ $^2$H NMR and X-ray;  
$d$ $^2$H and $^{31}$P NMR

The $^2$H NMR experiments, that is why experimental values are usually given as absolute values $|S_{CD}|$ $^{[31]}$. Evidently, computer simulations can provide sign of the order parameters directly.

The C-H bond order parameters, calculated in our MD simulations, are presented in Fig. 1, 2 in the form of $-S_{CH}$, both for saturated and unsaturated chains. The plots demonstrate general features of C-H order parameters which agree with results from previous experimental (NMR) and computer simulation works. For the saturated chains the order parameter profile exhibits a small drop at the 3-rd carbon position, then reaches a plateau, and then drops gradually to the chain end. Behaviour of unsaturated chains is clearly different. Already a chain with a single double bond (18:1) has a clear drop in the order parameter for carbons at and near position of the double bond. The region of small order parameters is increasing with increase of the number of double bonds in the tail, and for polyunsaturated chains with 4 – 6 double bonds most of order parameters are below 0.1 and often even below 0.05. A clear tendency is that the more unsaturated $sn$-2 chain the smaller average of the absolute values of order parameters $|S_{CH}|$.

Another important result of our MD simulations is that C-H bond order parameters of both signs (plus and minus) are not uncommon, they coexist in polyunsaturated chains of PC lipids. Would the corresponding $^2$H NMR-data be available, they could be seen in these ranges as positive $|S_{CD}|$ values, as it is indicated by dashed lines in Figs. 1, 2. Generally speaking, this fact gives raise to a question how the correct signs of the bond order parameters of a chain of complicated chemical structure (e.g., a polyunsaturated chain) can be established when deuterium NMR data ($|S_{CD}|$) only are available; a computer modelling can possibly help in such cases.

Computer simulations allow to determine order parameters for other bonds for which experimental data are unavailable. The $S_{CC}$ values of the consecutive carbon-carbon bonds can give additional insight into ordering properties of lipid chains. The $S_{CC}$ bond order parameters calculated in our MD simulations are presented in Figs. 3, 4).
Figure 1. Order parameter $-S_{CH}$ as a function of the carbon atom number of the lipid hydrocarbon chains of PC bilayers with $sn$-1 chain 16:0 as determined from the MD simulations. The arrows indicate the double bonds. Dashed line shows the ranges where simulated MD-data (solid lines) and possible $^2$H NMR-data could be different due to the sign uncertainty of NMR data.

The $S_{CC}$ values in all the saturated and unsaturated chains are seen to vary non-monotonously. All the $S_{CC}$ profiles are zigzag-shaped and exhibit a trend of decreasing values toward the chain end. A difference in the behaviour of saturated and unsaturated tails is also well seen. The zigzag-shaped $S_{CC}$ profiles of the saturated chains are characterized by a systematic, strictly alternating rise and fall of successive $S_{CC}$ values ("odd-even effect", [34]). The order parameter $S_{CC}$ profiles of unsaturated chains are also zigzag-shaped, but differ significantly in size and shape from those in the saturated chains. The $S_{CC}$ order parameters of single C-C bonds next to the double bonds C=C in all unsaturated $sn$-2 chains are significantly, several times, lower than that of the double bond C=C and of the corresponding single bond C-C in the saturated chains $sn$-1. The substitution of double C=C bonds for a single C-C bonds affects
Figure 2. Order parameter $-S_{CH}$ as a function of the carbon atom number of the lipid hydrocarbon chains in PC bilayers with $sn$-1 chain 18:0 as determined from the MD simulations. The arrows indicate the double bonds. Dashed line shows the ranges where simulated MD-data (solid lines) and possible $^2$H NMR-data could be different due to the sign uncertainty of NMR data.

the C-C order parameters resulting in a sharp decline in the $S_{CC}$ of the single bonds adjacent to the double bonds, and in an increase in the $S_{CC}$ of the double bonds.

3.3. Bond orientation distribution functions and anisotropy coefficient

The order parameters for primarily C-H, but also for C-C bonds are routinely computed in many works on computer simulations of lipid membranes. They however do not provide full information about the ordering and orientation of the bonds. The order parameter defined by eq. 1 is an inherently integral quantity depending both on ordering of the bonds and their orientation. In a general case (except extreme cases $S = -0.5$ and $S = 1$) it is not possible to distinguish between these two contribution. For example, order parameter zero can
Figure 3. Order parameter $S_{CC}$ as a function of the $C-C$ bond number of the lipid hydrocarbon chains in PC bilayers with sn-1 chain 16:0 as determined from the MD simulations. The arrows indicate the double bonds.

be observed both in the case of completely isotropic distribution of the bond direction, and for perfectly ordered bonds in the direction which forms with the bilayer normal the so-called "magic angle" for which $3 \cdot \cos^2 \theta - 1 = 0$. Evidently, for distinguishing effects of "inherent ordering" and orientation one needs to know the distribution of angle between the bond and the bilayer normal [35].

In order to compute the orientational bond distribution, the range $0 \leq \theta \leq \pi$ was divided into 60 equal bins, and histograms of hits into each bin $n_i$ were collected. From these histograms, the angular distributions were determined as $\rho(\theta) = n_i / n_{tot}$, where $n_{tot}$ is the total number of frames in the MD-trajectory multiplied by the number of lipids. For further analysis it is appropriate to divide the obtained distributions by $\sin \theta$, accounting for the solid angle area corresponding to the polar angle $\theta$, and consider functions $\rho(\theta) / \sin \theta$. For the completely isotropic distribution of bonds $\rho(\theta) / \sin \theta$ function is constant.

We have computed such distributions for all C-H and C-C bonds in the tails of the simulated
Figure 4. Order parameter $S_{CC}$ as a function of the $C-C$ bond number of the lipid hydrocarbon chains in PC bilayers with sn-1 chain 18:0 as determined from the MD simulations. The arrows indicate the double bonds.

bilayers. These distributions provide a very detailed picture of bond ordering in lipid bilayers. The obtained distributions were of varying types, differing by a sharpness of the maximum, asymmetry, presence of plateau or other features, being dependent on the type of the bond, presence of double bonds in the chain, position along the chain. In order to characterize different bonds on equal footing, we propose to introduce a new parameter called "anisotropy coefficient" which describes relative sharpness of the distribution for each bond:

$$D_A = \frac{[\rho(\theta)/\sin \theta]_{\text{max}} - [\rho(\theta)/\sin \theta]_{\text{min}}}{[\rho(\theta)/\sin \theta]_M}$$

(2)

where $[\rho(\theta)/\sin \theta]_{\text{max}}$ is the maximum value of $\rho(\theta)/\sin \theta$ for the given bond, $[\rho(\theta)/\sin \theta]_{\text{min}}$ is its minimum value while $[\rho(\theta)/\sin \theta]_M$ is the maximum value of all $[\rho(\theta)/\sin \theta]_{\text{max}}$ taken over all bonds of the given type in the both chains of the lipid. Evidently, $0 \leq D_A \leq 1$, and zero values of $D_A$ corresponds to a uniform (isotropic) distribution with $[\rho(\theta)/\sin \theta]_{\text{max}} = [\rho(\theta)/\sin \theta]_{\text{min}}$. 
Figure 5. Anisotropy coefficients for C-H bonds of 16:0/18:1(n-9)cis PC bilayer (a) and of 18:0/22:6(n-3)cis PC bilayer (b).

Figure 6. Characterization of orientational ordering of C-H and C-C bonds in a 18:0/22:6(n-3)cis PC bilayer. Lines of same colour show bonds with similar ordering behaviour.

The introduced anisotropy coefficient describes how well the bonds are orientationally ordered (relative to a bond with the maximum ordering) irrespectively to the ordering direction. The $D_A$ coefficients for C-H bonds of 16:0/18:1(n-9)cis PC and 18:0/22:6(n-3)cis PC bilayers are plotted in Fig 5.

The profiles of the anisotropy coefficient for unsaturated lipid chains in Fig. 5 are clearly different from the respective order parameter profiles in Fig 1 and 2. While C-H order parameters at carbons of double bonds are small, the anisotropy coefficients of the same bonds are rather high, indicating that the small C-H order parameter at the double bonds is essentially orientational and not ordering effect.

We can also (somewhat arbitrary) to call orientational distribution of a bond quasi-isotropic if its anisotropy coefficient $D_A < 0.25$ and denote by letter $qI$. Bonds with $0.25 < D_A < 0.368$ we shall call quasi-anisotropic ($qA$) and with $D_A > 0.368$ anisotropic ($A$). The number 0.368 in the above definition corresponds to the inverse value of $e$ number ($1/e \approx 0.368$). Quasi-anisotropic and anisotropic bonds have typically a preferred orientation (at which the distribution reaches maximum), therefore for such bonds we complement notation $qA$ or $A$ by an index expressing the preferred angle in degrees. An example of characterization of all C-H and C-C bonds in tails of 18:0/22:6(n-3)cis PC bilayer according to this scheme is given in Fig. 6. We believe that such combined classification of bonds creates possibilities for deeper insight into ordering and orientational effects in the membrane interior.
4. Conclusions
We presented here results of molecular dynamics simulations of 16 lipid bilayers differing by degree of tail unsaturation and the number of carbons in the lipid tails. The analysis was concentrated on the orientational ordering behaviour of different lipids. Besides of computations of the conventional "order parameter" we suggested to characterize the bonds according to a newly introduced "anisotropy coefficient" computed from the angular distributions between the bond and the bilayer normal. We have demonstrated that such analysis allows one to get deeper insight into ordering and orientational behaviour of various bonds and to separate these two effects. In the forthcoming publications, we are planning to present a detailed analysis of orientational order of all 16 simulated bilayers according to the scheme presented here, and discuss possible biological implications.

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