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The need to revisit lipid areas

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Abstract. We have studied the structural properties of lipid bilayers made up of monounsaturated phosphatidylcholines (i.e. diCn:1PC, where n=14, 16, 18, 20, 22 and 24). High-resolution x-ray scattering data were analyzed in conjunction with contrast varied neutron scattering data, using the recently developed technique by Kučerka et al. [Biophys. J. **95,** 2356 (2008)]. Analyses of the data show that with increasing n lipid bilayers do not thicken in a linear fashion, as is often assumed, but quadratically, and that lipid area assumes a maximum value for n~18 bilayers. More importantly, compared to previous data our results strongly suggest that lipid areas are smaller by about 10%. This observation highlights the need to revisit lipid areas as they are extensively used in molecular dynamics simulations and for calibrating their force fields.

1. Introduction

The complex dynamics exhibited by biological membranes - characteristic of amphiphilic systems - are highly dependent on the membrane's various structural parameters. It should therefore not come as a surprise that accurate structural data regarding membrane components are important in determining specific biomembrane functions. One such datum is a lipid's lateral area, which is commonly understood to influence lipid-lipid and lipid-protein interactions, and which plays a central role in the outcome of molecular dynamics (MD) simulations.

Despite their importance, published lipid areas have been relatively scarce, and for the most part, inconsistent [1]. Noteworthy are the discrepancies between lipid areas as determined by x-ray and neutron scattering - arguably two of the most widely used experimental techniques in structural biology [2]. On the other hand, these inconsistencies have also been highlighted by the disparate results arising from MD simulations using different force fields. For example, MD simulations based on CHARMM potentials are performed at non-zero surface tension in order to agree with x-ray scattering data [3], while GROMOS potentials do not seem to require this additional "tweaking" [4].

Since the MD force fields are considered to be "well tuned" if they are able to reproduce experimental data, there is clearly much work that needs to be done in order to reconcile simulations and experiment.

Recently, we have developed a model for calculating scattering density profiles (SDP) from the simultaneous analyses of x-ray and neutron scattering data [5]. By appropriately parsing a lipid molecule and simultaneously analyzing the different "contrast" data (i.e. x-ray and different deuteration neutron scattering data), a more precise bilayer structure can be determined.

2. Results and Discussion

The SDP model is graphically shown in Figure 1. Briefly, the component groups in the SDP model are chosen on the basis that each group has the same functional form for all of the different contrast conditions. For example, carbonyl and glycerol groups are described by a single Gaussian (CG), the phosphate and part of choline (CH_2CH_2N) by another Gaussian (PCN), and the remaining choline ($3xCH_3$) by yet another Gaussian ($CholCH_3$). In effect, three Gaussians are used to describe the lipid headgroup. The error function represents the total hydrocarbon region (i.e. CH_2, CH and CH_3 groups). The CH and CH_3 groups are each described by a single Gaussian, which are then subtracted from the error function to obtain the CH_2 distribution. The water distribution is not defined by any particular function, rather it is calculated based on the "complementarity" requirement, whereby all of the probabilities add up to one. In this way, the model satisfies spatial conservation while capturing all of the features of the different SDPs [5].

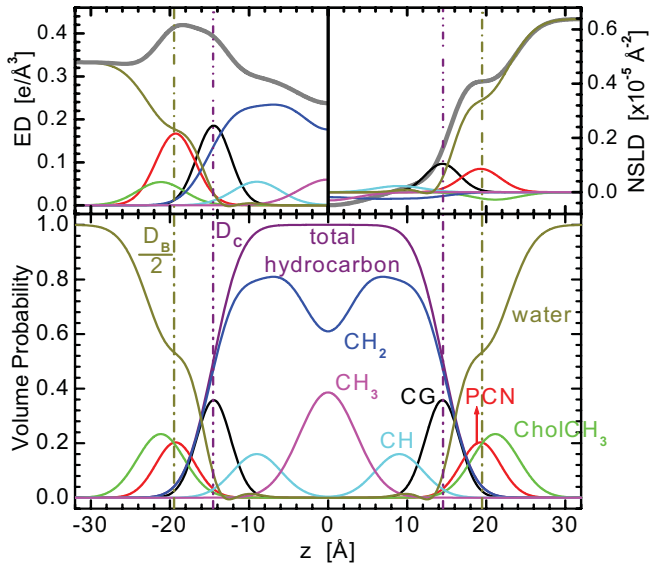

Figure 1: SDP model representation of a diC18:1PC bilayer. The top left panel shows electron densities, while the top right panel depicts neutron scattering length densities of the various component distributions, including the total scattering density (thick gray lines). The bottom panel shows volume probability distributions, where the total probability is equal to 1 at each point across the bilayer.

From Figure 1 it is obvious that the neutron and x-ray techniques are sensitive to different parts of the bilayer. For example, in the case of x-rays, the electron dense phosphate groups contrast very well with the low electron dense hydrocarbon region. Thus, x-ray data are well suited for the refinement of the lipid headgroups and hydrocarbon chains. On the other hand, the high neutron scattering length density of D_2O, permits neutron scattering to accurately determine the total bilayer thickness and consequently, lipid area.

Bilayers made up of the diCn:1PC, where n=14, 16, 18, 20, 22 and 24, were prepared in the form of unilamellar vesicles (ULVs) of diameter ~600 Å [6]. High-resolution small-angle x-ray scattering (SAXS) data were obtained over a q range [$4\pi/\lambda\sin(\theta/2)$, where λ is the wavelength and θ is the scattering angle] from 0.06 $Å^{-1}$ to 0.65 $Å^{-1}$. Small-angle neutron scattering (SANS) data were collected

on the same lipids, but dispersed in 100% D_2O. Additional contrasts of 70% and 50% D_2O were used for n=14, 18 and 22 bilayers.

One of the important structural parameters when considering the hydrophobic matching of lipids and proteins is the hydrocarbon chain thickness, D_C. The SDP model defines D_C at the center of the error function, i.e. the hydrocarbon chain distribution (see Figure 1). Another important parameter is the total bilayer thickness (D_B) that we define as the Gibbs dividing surface for the water region (i.e. $D_B/2$) (please see Ref. [5] for details). As was mentioned, D_B is robustly determined by neutron scattering and leads to the determination of lipid area (A) - assuming that lipid volume is already known [7].

The pioneering work of Lewis and Engelman [8] studied three of these lipids (n=18, 22, and 24) using SAXS and pauci-lamellar vesicles (PLVs). When comparing to their data, we find our D_B values to be ~2 Å larger, while following the similar trend, and our lipid areas are smaller by as much as 6 $Å^2$. A recent x-ray study [9] has supported the Lewis and Engelman lipid area results for n=18 and 22 bilayers, while neutron scattering data have suggested much smaller areas [10, 11]. This inconsistency between SANS and SAXS data was thought to be the result of rudimentary models used to analyze SANS data. However, it was recently realized [5] that SAXS is better applied in determining the internal structure of lipid bilayers, but that it can underestimate D_B and A by as much as 10%, a task possibly more appropriate for SANS. However, by combining both techniques in one analysis the various bilayer parameters can be better determined.

Figure 2 shows the dependency of the various structural parameters as a function of hydrocarbon chain length. Both thicknesses (i.e. D_C and D_B) increase almost linearly with n, exhibiting a small, but not negligible quadratic behavior. Perhaps a more surprising result is how lipid area changes as a function of n (Figure 2). First it increases and then it decreases, deviating from the accepted monotonic behavior with a maximum near n~18. Although surprising at first, this behavior is consistent with the effect of the double bond position. For n=14 to 18 lipids the double bond is at the 9-cis position, while for n=20, 22 and 24 lipids the double bond is at position 11-cis, 13-cis and 15-cis, respectively. Coarse grained bilayer simulations reproduced this trend and qualitatively predicted the effects of double bond position on lipid area (S. J. Marrink, personal communication). As was discussed by Karlovská et al. [12], increasing the hydrocarbon chain length results in increased van der Waals attraction resulting in an ordering of the hydrocarbon chains, and thus reducing area per lipid. However, lipid chain disorder also depends on the position of the double bond, having the most effect when the double bond is located in the middle of the hydrocarbon chain [13]. Importantly, this indicates that lipid area, the result of a fine balance between intrabilayer forces, is a good indicator of lateral interactions within the bilayer.

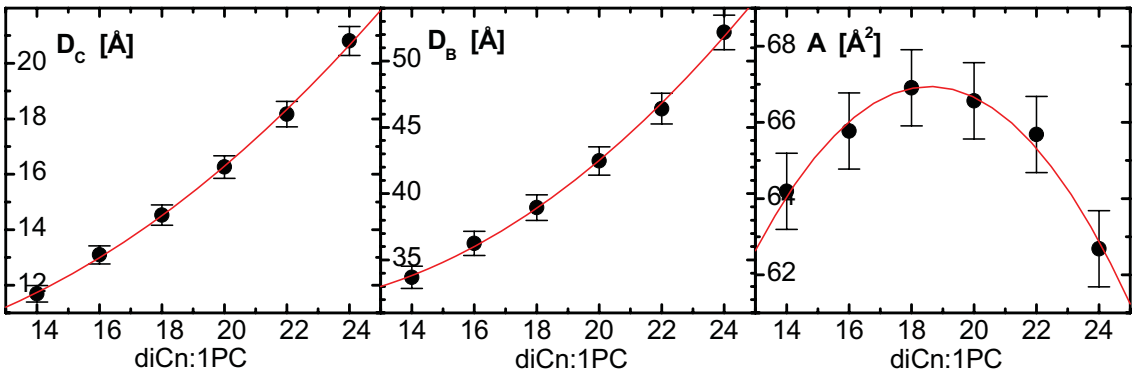

Figure 2: Bilayer structural parameters obtained by the simultaneous analysis of x-ray and neutron scattering data. Structural parameters D_c, D_B and A are plotted as a function of chain length, n.

3. Conclusions

In conclusion, recent developments in structural biophysics have allowed for a more precise determination of biomembrane properties. Through the simultaneous analysis of high resolution x-ray and neutron scattering data, we are able to robustly obtain bilayer structural parameters for the various di-monounsaturated phospholipids. Our results show a quadratic behavior of bilayer thicknesses as a function of n, and an area per lipid maximum near n=18 hydrocarbon chains. The present results can be used to better understand biomembrane-protein interactions, which are well known to depend on bilayer thickness and area per lipid. Perhaps more importantly, these results should serve as the foundation for fine-tuning the force field parameters of MD simulations, for which accurately known lipid areas are central. We encourage the MD simulators to compare their simulations results not only against x-ray scattering data, but to also include neutron data that are more sensitive to D_B and consequently A.

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