Experimental Report

Proposal: **9-13-449** **Council:** 4/2012

Title: Structure of floating DPPC bilayerin presence of long-chain alcohols

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **BALGAVY Pavol**

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Samples:
dodecanol
octanol
tetradecanol
hexadecanol
octadecanol
dipalmitoylphosphatidylcholine
hexanol
decanol

Instrument	Req. Days	All. Days	From	To
D17	4	2	04/11/2012	06/11/2012

Abstract:

Primary aliphatic alcohols (CnOH, n is the number of alkyl carbons) are general anesthetics, they also display a wide range of toxic effects. In pharmacy, they are used as penetration enhancers in transdermal drug delivery. Their potency in most biological activities depends quasi-parabolically on alkyl length with a maximum at n~10-12. CnOHs partition in the lipid bilayer of biomembranes, changing its physical properties anisotropically depending on the concentration and chain length n. This leads to conformational changes in membrane proteins coupled to structural and dynamical changes of bilayer resulting in biological effects. The neutron reflectometry experiments proposed will determine how CnOH alcohols affect the bilayer structure. As a model membrane system, bilayers prepared from dipalmitoylphosphatidylcholine (DPPC) and freely floating above another bilayer adsorbed on a smooth solid substrate will be used. Final aims of reflectometry experiments will be determination of CnOH effects on the floating bilayer thickness and roughness, on the DPPC and CnOH interface area and on the hydration of bilayer polar regions.

Title: Structure of floating DPPC bilayer in presence of long-chain alcohols.

Experiment: 9-13-449 Dates: 04/11/2012 – 06/11/2012 Instrument: D17

Team: M. Belička, G. Fragneto, N. Kučerka, P. Westh, P. Balgavý

Local contact: G. Fragneto

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Primary aliphatic alcohols (CnOH, n is the number of alkyl carbons) are general anesthetics, they also display a wide range of toxic effects. In pharmacy they are used as penetration enhancers in transdermal drug delivery. Their potency in most biological activities depends quasi-parabolically on alkyl length with a maximum at n ~ 10 – 12. C*n*OHs partition in the lipid bilayer of biomembranes, changing its physical properties anisotropically depending on the concentration and chain length n. This leads to conformational changes in membrane proteins coupled to structural and dynamical changes of bilayer structure. As a model membrane system, bilayers prepared from dipalmitoylphosphatidylcholine (DPPC) and freely floating above another bilayer adsorbed on a smooth solid substrate will be used. Final aims of reflectometry experiments will be determination of CnOH effects on the floating bilayer thickness and roughness, on the DPPC and CnOH interface area on the hydration of bilayer polar regions.

Introduction

The cut-off effect observed in anesthetic potency of alcohols, but also other biologically active substances, does not correspond with behavior of their partition coefficient (Franks and Lieb, 1986), therefore it is not caused by any anomaly in partition equilibria. In previous experiments (Klacsová et al., 2011) it was found using the technique of small-angle neutron scattering that alcohols with different alkyl chain length influence the structure parameters – the lateral area per a lipid molecule and the bilayer thickness – in a different way. There are two principle ways how general anesthetics interact with biological systems, through direct contact with membrane proteins or through the change of physical and mechanical properties of lipid bilayers, which can influence the lipid membrane proteins as well. Another differences between the impact of shorter and longer alcohols intercalation on lipid bilayers were found in densitometry study of Aagaard et al. (Aagaard et al., 2006) where shorter alcohols tend to increase their volumes by the intercalation into lipid bilayers from aqueous environment, whereas alcohols with medium or longer alkyl chain lengths tend to decrease their molecular volume. The mentioned results led us to conclusion that the mentioned results are the consequences of different kind of interactions shorter and longer are exposed to. And thus, the cut-off effect might be correlated with the position of intercalated molecules inside the membrane.

Preliminary Report of Specular Neutron Reflectometry Results

SNR measurements were performed on the high flux D17 reflectometer with horizontal scattering geometry in time-of-flight mode using an interval of neutron wavelengths between 2 and 18 Å with incident angles 0.8° and 3.2°, what covered q range from 0.005 to 0.2 Å. The samples were measured in three different SLDs of water solvent at gradually increased temperature from 25 °C to 55 °C (below the main phase transition of diC22:0PC and below and above the main phase transition of DPPC). The direct experimental data obtained from the reflectometer were treated using LAMP software package, through which they were normalized and the corresponding resolution was determined.

For the data evaluation the scattering density profile structure model of Kučerka et al. (Kučerka et al., 2008; Heberle et al., 2012) was used as it describes the internal structure of lipid bilayer in a more detailed way (in the form of functional groups of lipid headgroups) in comparison to previous experiments. To translate the bilayer profile structure the kinematical approximation was chosen, but it was found that there were difficulties in the rate of coverage determination. Thus Parratt's recursion formalism was applied to obtain the model specular neutron reflectivity.

At the first step of the data evaluation only the reflectivity data of a supported diC22:0PC bilayer at 25 °C was successfully fitted with the applied model. The main reason of this complication is the high number of free model parameters with different common correlation rates. The results are shown on the Figures below. As it can be seen we did not find any water layer between the SiO_2 surface and the supported bilayer, thus the results suggest that the bilayer is in a direct contact with the silicon block. Further we found that the rate of silicon block coverage was above 90%. To incorporate the fact of an asymmetrical character of the supported bilayer we fitted the hydration/coverage of the lipid leaflets individually. We found the minor differences between the leaflet in the contact with the silicon block and the leaflet exposed to bulk water. This led us to the possibility of asymmetrical headgroup components distributions with regard to the hydrocarbon core borders, which was successfully applied.

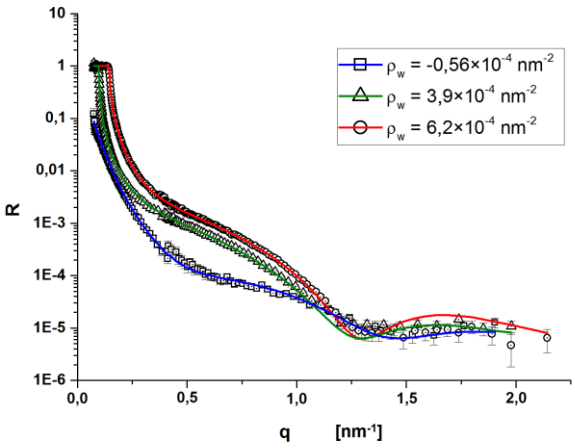

Fig. 1: The normalized reflectivity curves of a supported diC22:0PC bilayer on a silicon block with a hydrophilic surface. The bilayer was measured in three different contrasts: pure H_2O (squares, blue line), mixture of H_2O/D_2O (triangles, green line) and pure D_2O (circles, red line). The lines represent the best obtained fit by the applied model.

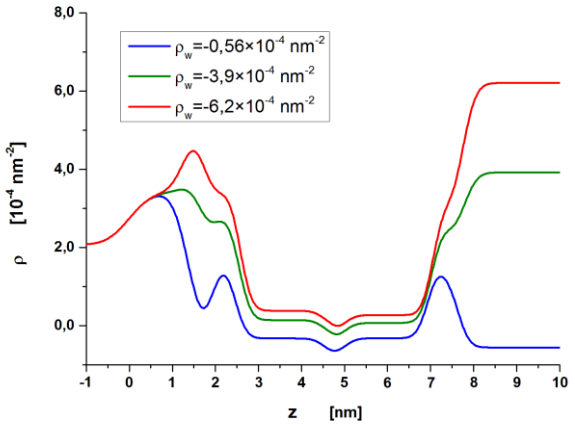

Fig. 2: The scattering length density profiles on the bilayer normal of a supported diC22:0PC on a silicon block. The lines represent the bilayer model in three different contrasts and corresponds to the fitting lines in Fig. 1.

References

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