Experimental report

05/03/2020

Proposal: 9-13-822 **Council:** 10/2018

Title: Hydrocarbon Chain-Mediated Transleaflet Coupling in Asymmetric Lipid Vesicles

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Georg PABST

Experimental team: Haden SCOTT
Moritz Paul Karl FREWEIN
Lisa MARX
Enrico Federico SEMERARO

Local contacts: Lionel PORCAR
Yuri GERELLI

Samples: POPC
DPPC_d71
SOPC
PMPC
MSPC
SMPC

Instrument	Requested days	Allocated days	From	To
D22	2	2	25/06/2019	27/06/2019

Abstract:

We propose to study the influence of hydrocarbon chain composition on transleaflet coupling in large unilamellar vesicles (size ~ 100 nm) with an asymmetric distribution of phospholipids. The asymmetric unilammelar vesicles (aLUVs) will be differently contrasted by using different combinations of deuterated phosphatidylcholines and D2O/H2O solvent. In particular we propose SANS experiments on combinations of di16:0 PC_d71 (DPPC_d71) with mixed chain lipids of increasing difference in chain length, i.e., 18:0-18:1 PC (SOPC), 16:0-18:1 PC (POPC), 16:0-14:0 PC (PMPC), 14:0-18:0 PC (MSPC) and 18:0-14:0 PC (SMPC). Data jointly analyzed with SAXS data to obtain leaflet specific structural information. This will allow us to interrogate whether or not the physicochemical state of one leaflet influences (couples to) the properties of the other.

Introduction

Large unilamellar vesicles are a popular model system for biological membranes to study properties of lipids and other membrane components in detail. They are particularly well suited for scattering studies, since the overall vesicle shape has only negligible contributions in the q-range related to the trans-bilayer structure. Recent advances in sample preparation have facilitated the production of vesicles with two compositionally different (asymmetric) bilayer leaflets. As most membranes in nature show such an asymmetry [1], it is particularly interesting to investigate its effects on the overall bilayer structure. In fact, several groups have already reported experiments or simulations in which they found the individual leaflets influencing each others structure, dynamics or melting behavior [2]. The aim of this experiment was to study if such a coupling can be induced by hydrocarbon-chain interdigitation effects in the fluid phase of asymmetric vesicles, produced by the method of cyclodextrin-mediated lipid exchange [3].

Description of the Experiment

We used our experiment to probe the structure of symmetric and asymmetric liposomes containing chain-deuterated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (di16:0 PC, DPPCd62) and a variety of mixed-chain lipids: 16:0-18:1 PC (POPC), 18:0-18:1 PC, 14:0-18:0 PC, 16:0-14:0 PC and 18:0-14:0 PC. We used quartz cells with an optical path length of 1 mm and approximately 15 mg lipid per ml H_2O/D_2O. Experiments were conducted at sample to detector distances from 2 m to 17 m, whereby the long distance measurements provides us information about vesicle size (~ 100 nm) and polydispersity, and the short distance gives insight into the trans-bilayer structure, which this study was focused on. To stay well over the phase transition temperature of DPPC, the samples were heated to 50 °C, using a circulating bath, and equilibrated for 10 min before measurement.

Results and Outlook

As D22 is well-tried for experiments with liposomes, our measurements worked without complications. The data shows clear differences between symmetric and asymmetric vesicles (see Fig 1). In particular, we observed a lift-off at q-values around 0.1 $Å^{-1}$, which turned out to be directly related to the contrast between inner and outer leaflet. Further characterization of the overall composition of our samples using gas chromatography, we are able to calculate the asymmetry in leaflet-specific composition directly from SANS.

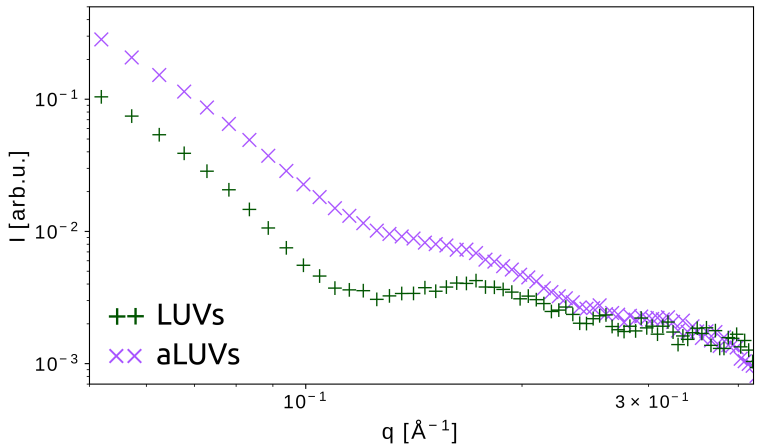

Fig 1: SANS-data recorded at a sample-to-detector-distance 2 m of compositionally symmetric (LUVs) and asymmetric vesicles (aLUVs) composed of DPPCd62 and POPC. The inset shows possible corresponding trans-bilayer scattering length density profiles.

Beside the asymmetry, the main output of the data is the bilayer thickness. As the lipid head region in membranes is partially hydrated however, the head group shows little contrast to the solvent and

preliminary analysis showed that parameters describing the head groups are highly correlated among each other as well as with the area per lipid. Also, the data contains little information about interdigitation states, as the terminal methyl groups show little contrast to the the main hydrocarbon chain region. Therefore, we plan to complement our measurements with SANS-measurements of methyl-group deuterated lipids as well as small-angle X-ray scattering data, which are highly sensitive to the position of the phosphate and also contain some information about the methyl-group localization. Fig 2 shows a possible model fit [4] for 2 contrasts of an asymmetric system.

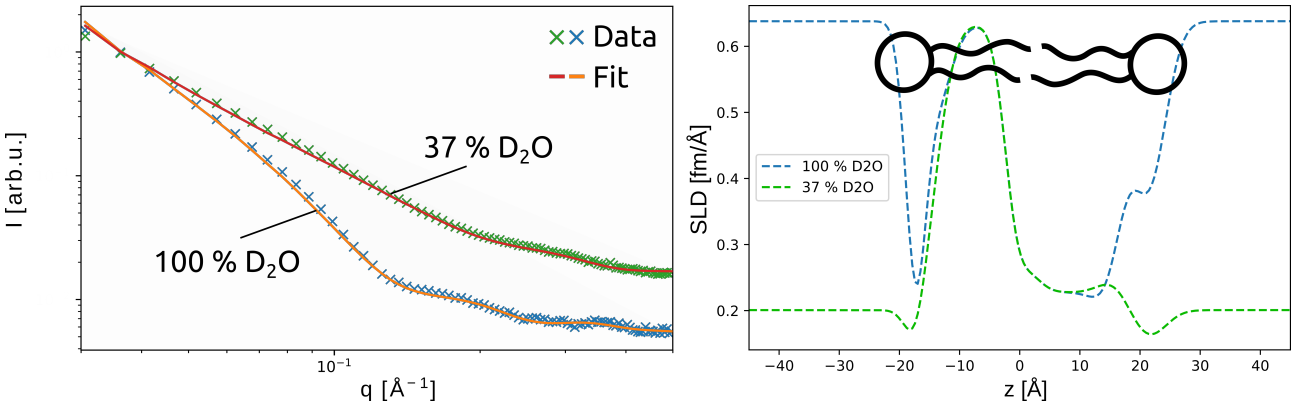

Fig 2: SANS-data of asymmetric vesicles containing DPPCd62 and 18:0-14:0 PC suspended in different H2O/D2O mixtures and one possible model-fit from joint-analysis of both measurements (left). The right subfigure shows SLD-profiles corresponding to the fit. One can see that at 37 % D2O the outer leaflet is approximately contrast-matched, facilitating the explicit examination of the inner leaflet.

References

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