Experimental Report

Proposal: **8-02-698** **Council:** 4/2014

Title: Cell Membrane Mimetic Asymmetric Supported Lipid Bilayers.

This proposal is a new proposal

Researh Area: Other...

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Samples: C42H51NO8PD31

Instrument	Req. Days	All. Days	From	To
D17	4	3	01/12/2014	04/12/2014

Abstract:

Cell membranes are characterized by a complex lipid composition and asymmetric distribution of lipids (lipids facing the outside of the cell are different from the lipids that are facing the inside of the cell). We developed a model system that captures these features. It is based on supported lipid bilayers (SLBs) on TiO2. They contain the canonical high-melting, low-melting, cholesterol lipid mixture commonly used to model cell membranes, plus phosphatidyl serine (PS), which strongly interacts with TiO2 and is therefore distributed asymmetrically. The strong PS-TiO2 interaction mimics the cytoskeleton-cell membrane interactions that occur in the cell. The goal of this proposal is to quantify lipid composition and distribution in these asymmetric cell membrane mimics by neutron reflectometry.

Proposal 8-02-698: Cell Membrane-Mimetic Asymmetric Supported Lipid Bilayers.

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Introduction: Membranes of cells are asymmetric with respect to the distribution of lipids between the two bilayer leaflets. Lipids such as phosphatidyl serine (PS) are found on the inner leaflet of the membrane, while the outer leaflet consists predominantly of phosphatidyl cholines (PCs) and sphingomyelins.[1,2] Both leaflets contain cholesterol. The canonical high-melting, low-melting, cholesterol lipid mixture commonly used to model cell membranes[3] actually reflects the outer leaflet composition.

This asymmetric distribution of lipids has been difficult to model: in biological system, it is maintained through the action of ATP-dependent lipid transporters, flipases.[2] In artificial systems, an appropriate component of the free energy is required to oppose the entropy of mixing for maintaining the asymmetry. Alternatively, metastable asymmetric systems can be constructed by preparing the individual leaflets separately and merging them later. It turns out that in the case of solid-supported lipid bilayers (SLBs), the interactions between the lipids and the substrates are sufficient to induce and maintain lipid asymmetry. In particular, two-component PS-containing SLBs on TiO2 are asymmetric with respect to the distribution of the lipid, which is found in excess in the leaflet facing the substrate.[4]

The aim of this proposal was to investigate lipid distribution in membrane-mimetic SLBs composed of the canonical high-melting, low-melting PCs + cholesterol mixture that included POPS, on TiO2. Four mixtures with nominally identical compositions (26 : 26 : 18 : 30 POPC, DPPC, POPS, cholesterol) were prepared, with one deuterated component at a time (Table 1, rows 1 – 4). A significant amount of work has been previously performed on these mixtures by fluorescence microscopy and AFM to insure conditions for bilayer formation.[5]

Despite the previous thorough characterization, two of these (2 and 4) did not yield bilayers for reasons that are not entirely clear. The remainder of the time was used to perform an experiment on a 1 : 1 POPC : dPOPS mixture (Table 1, row 5) which was brought as a back-up.

Substrates, TiO2-coated Si blocks, and the bilayers, were characterized in four contrasts each. This was needed because of the complexity of the system: three layers comprising the substrate and four layers comprising the lipids.

Experiments with the POPC DPPC POPS cholesterol mixture were performed at 60 C, a condition we found required for forming these bilayers. POPC : POPS SLB formed at room temperature.

Fits and conclusions. In accordance with our previous work (proposals 8-02-519, 8-02-560), TiO2 substrates were fitted with a three-layer model, Si/SiO2/TiO2-1/TiO2-2. The results are shown in Table 2. The SLD of TiO2-1 layer is expected to be 2.37 (anatase), but was found to vary from block to block despite the fact that all blocks were coated at the same time and handled identically (Table 1). Fixing this parameter considerably reduced the quality of the fits. The block used in experiments 2 and 5 was the same, but was cleaned the second time prior to experiment 5. Substrate parameters were kept fixed during the fitting of the SLBs. SLBs were fit with a four-layer model (headgroups (h), chains (c), c, h). An example of one such fit is shown in Figure 1. The fit requires further improvement, but leads to a reasonable scattering length density profile (Figure 2) and molecular volume parameters.

Table 1: Experiments performed in this proposal.

#	Liposomes	Result
1	POPC dDPPC POPS cholesterol	SLB
2	dPOPC DPPC POPS cholesterol	No SLB
3	POPC DPPC dPOPS cholesterol	SLB
4	POPC DPPC POPS dcholesterol	No SLB
5	POPC dPOPS 1 : 1	SLB

The SLD profile (Figure 2) shows a pronounced asymmetry with respect to the distribution of the deuterated PS. Consistent with our previous work and fluorescence data, there is a considerable excess of this lipid in the leaflet facing the substrate: 63% vs. 19% in the outer leaflet. The total fraction of PS in the SLB was 40%, compared to 18% in the starting liposomes. These numbers, however, depend on the

Table 2: Substrate parameters. Thickness (d) and roughness (r) in Å, SLD: $\times 10^{-6}$ Å^{-2}. The same block (re-cleaned) was used in experiments 1 and 5 used. Parameter was kept constant during fits are italicized.

Substrate		1	2	3	4	5
r backgr.		9	15	13	29	12
SiO2	d	30	29	24	34	29
	SLD	*3.41*	*3.41*	*3.41*	*3.41*	3.41
	% slvn	*0*	*0*	*0*	*0*	0
	r	3	5	4	4	3
TiO2-1	d	150	152	154	148	150
	SLD	2.57	2.58	2.47	2.59	2.64
	% slvn	*0*	*0*	*0*	*0*	0
	r	5	5	8	2	4
TiO2-2	d	8	7	10	11	6
	SLD	4.24	3.91	3.76	3.22	4.16
	% slvn	35	30	40	43	55
	r	10	14	10	4	5

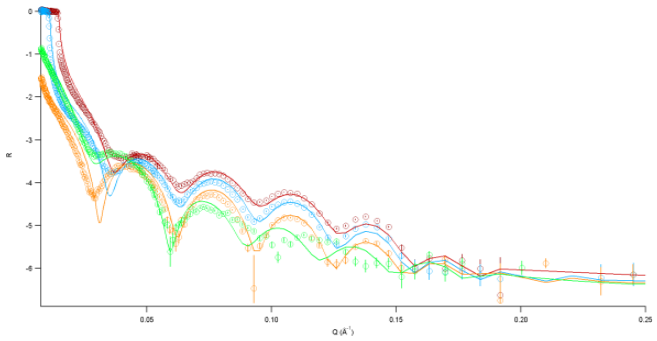

Figure 1: Initial fit of the reflectivity obtained from sample 3 (deuterated PS). Red: D2), blue: 4.5- orange: 2.07 – matched water, green: H2O.

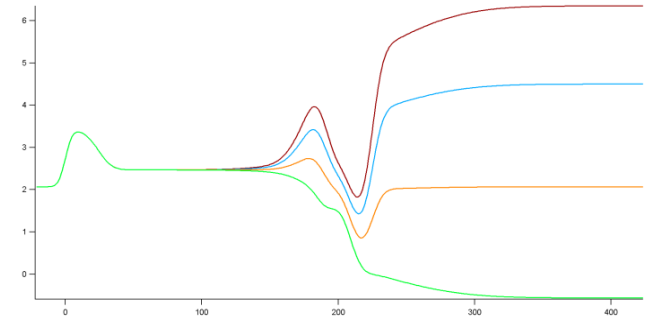

Figure 2: The scattering length density profile corresponding to the fit shown in Figure 1. Note the considerable asymmetry: higher sld next to the substrate translates into excess of PS next to the substrate.

exact lipid composition of the SLB, which is not known. Liposome composition was used in the calculations.

Fitting of the results obtained in experiment 1 also lead to an asymmetric SLD profile, with excess of dDPPC in the outer leaflet of the SLB: 29% in the outer leaflet vs. 6% in the inner leaflet. The total fraction of dDPPC in the SLB was 18% compared to 26% in the liposomes. These numbers are subject to change as the fits are improved.

In experiment 5, we found that the inner leaflet contained 72% PS, while the outer leaflet - 13%. The total fraction of PS was 58% compared to the ~ 50% in the starting liposomes. This is consistent with our previous results and completes the previous study.

In summary, we were able to quantify the distribution of two out of four components of the membrane-mimetic, cholesterol and PS containing SLBs, and confirm the asymmetry in the distribution of POPPS and DPPC.

Further work: In our opinion, the next step is to repeat this experiment with the same lipid mixture (POPC DPPC POPS cholesterol 26 : 26 : 18 : 30), with four deuterations, but on both TiO2 and SiO2 surfaces, because it is important to allow the same liposomes to interact with the two different surfaces to compare the transfer of lipids from liposomes to SLBs on different surfaces under identical conditions. The other two compositions (experiments 2 and 4) also need to be completed to be certain of the total composition of the SLBs. The knowledge of the total SLB composition is needed for reliably calculating the fraction of each component, without relying on the starting composition of the liposomes, which is likely to be different than the SLB composition.

References:

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(5) Zhu, L., PhD thesis. University of the Basque Country and CIC biomaGUNE, 2013.