Experimental report

11/02/2017

Proposal:	**9-13-651**	**Council:**	4/2016
Title:	Influence of static electric fieldon fast flip-flop dynamics in model bilayer systems		
Research area:	Soft condensed matter		

This proposal is a new proposal

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Samples: 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine
1-palmitoyl-d31-2-oleoyl-sn-glycero-3-phosphocholine

Instrument	Requested days	Allocated days	From	To
FIGARO	2	0		
D17	2	2	15/12/2016	17/12/2016

Abstract:

The phospholipid bilayer is the basic structural motif of most biological membranes. As such, many biological processes occur within or in the proximity of the cell membrane, and therefore, interest in the properties and behavior of lipids in membranes is considerable. For example, it is found that in nature the lipid distribution across the inner and outer leaflet of cell membranes is asymmetric [1] and this asymmetry plays a prominent role in processes like cell fusion, activation of the coagulation processes and the recognition and removal of apoptotic cells by macrophages. Therefore there is great interest in studying the factors determining lipid movement across membranes, process far from being understood and characterized. During previous Neutron Reflectometry (NR) experiments we discovered that the structure of an asymmetric reconstituted lipid bilayer spontaneously relaxes, on a subsecond time-scale, if the lipids are in the fluid phase i.e. in biological relevant conditions. We propose now to modify the time scale of this process by application of an external electric field to a solid supported membrane in order to capture structural signature of the flip-flop process.

The experiment 9-13-651 was performed on December 2016 on the time-of-flight neutron reflectometer D17.

The electrochemistry cell available on D17 was used in its standard 2-electrodes configuration. The working electrode was a Titanium-coated surface and a titanium-coated plate was used as counter electrode. The electrode material was chosen to avoid any chemical reaction with typical salts used in biological buffers. Silicon substrates were coated by the ILL multilayer lab and the Ti layer was characterized BEFORE and AFTER the measurements to assure that application of the DC voltage didn't oxidize or reduce the titanium.

A sample composed by DMPS and d54DMPC (1:1 by mol) was deposited by vesicle fusion method. The obtained bilayer was characterized prior any application of electric potential and resulted perfectly symmetric in composition.

Voltage was applied between working and counter electrode in 0.1 mV steps going from 0 mV to 800 mV and then from 800 mV to -800mV. Since this was the first experiment some tries were needed to optimize the working conditions properly.

NR measurements were synchronized to the applied potential ramps. In order to allow measurements of fast changes the measurements were performed by exploiting a fully divergent neutron beam and data reduced using the newly developed coherent re-binning method.

Unfortunately, because of problems with the sample environment and more precisely with the control of the sample temperature the measurement time was reduce to 1 day. Recovery beamtime is scheduled for the 23/02/2017 i.e. after the next proposal deadline.

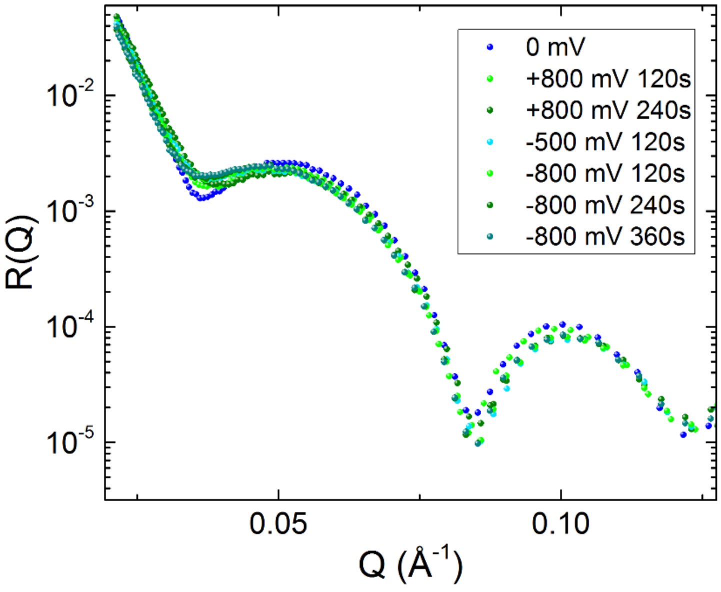

Figure 1. Neutron reflectivity measurements collected for a 1.2° incidence angle at different applied DC voltage and for different time. It is clearly visible that the NR curve indicating the starting fully mixed DMSP:d54DMPC bilayer is changed upon application of the voltage. The first minimum shifts to higher Q values when positive voltage is applied and then to lower Q for negative potential values. Such changes are in agreement with segregation of the zwitterionic and polar component of the bilayer in the inner and outer leaflets.

During this 1 day beamtime we could successfully measure changes induced by changes in applied voltage on the structure of the deposited 1:1 DMPS:d54DMPC bilayer as shown in Figure 1. The observed changes indicate that the changes in the surface charge (working electrode) promote attraction or repulsion of the DMPS molecules that migrate from one leaflet of the bilayer to the other. Such migration is balanced by a counter-migration of DMPC molecules (zwitterionic but globally neutral) in order to maintain the bilayer intact.

A full analysis of the curves will be performed together with the analysis of the data collected in the recovery beamtime.