Experimental report

08/02/2017

Proposal: 9-12-458 **Council:** 4/2016

Title: Polymer interactions with phospholipid monolayers: Understanding formation of polymer-stabilized nanodiscs

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DMPC with poly(d8-styrene-alt-maleic acid)
DMPG with poly(d8-styrene-alt-maleic acid)
DPPC with poly(d8-styrene-alt-maleic acid)

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	2	2	06/07/2016	08/07/2016

Abstract:

Polymer stabilized phospholipid nanodiscs are gaining rapid interest as a detergent free method of extracting membrane proteins from cell membranes and maintaining them in an active state in solution. Although the structure of the discs is established, with the polymer belt wrapping the exposed hydrophobic tails of a ~10nm disc of lipid bilayer, it is not known how this structure forms from the initially intact lipid membrane. Formation speed of the discs depends on polymer molecular weight and composition but can be as fast as 20s. In this experiment we aim to use the unique high flux, low Q mode of FIGARO to follow the kinetics of adsorption and/or penetration of deuterated polymer to lipid monolayers at the air water interface, as well as to characterise the final structure of the lipid/polymer layer. We will vary the surface pressure and composition of the lipid membrane, and the polymer MW and hydrophobic/hydrophilic balance. These experiments will access timescales inaccessible on other neutron reflectometers, to improve our understanding of how polymer-stabilized nanodiscs form, which will improve their future application and uptake in membrane protein biology.

Introduction

Polymer stabilized lipid nanodiscs are self-assembled structures made of phospholipids embedded around the phospholipids tail by a polymer. This system offers enormous potential as tools for enabling membrane protein structural studies & biophysics. Polymer stabilized lipid bilayer discs are easily made by adding polymer to a suspension of lipids or disrupted cell membranes, with discs forming within ~5 minutes. It is not clear how the polymer forms discs from these systems, since in vesicles or cell membranes the headgroups are exposed to solution, and the soluble polymer, but in the final discs, the polymer is wrapped around the tail regions of the lipid bilayer. In this experiment a study into the polymer-lipid interactions at the first stages of forming nanodiscs was carried out making use of a model system of a lipid monolayer on a Langmuir trough, with the polymer injected underneath. The high-flux, low-Q mode was used on FIGARO to follow polymer adsorption into the monolayer, followed by standard reflectivity curve measurements to characterize the final state.

Experimental Section

For the experiment a 45mL Langmuir through was used. The area of the trough could be changed between 70 and 97cm^2 via the movable barriers. For the experiment lipids were dissolved in chloroform with a concentration of 0.5mg/mL. This solution was spread on the surface of 50mM phosphate buffer with 0.2M NaCl in the Langmuir trough and pressure was controlled by the moving barriers of the trough and set initially at 25mN/m^2, holding a constant area. Two different lipids compositions were used: pure DMPC and a mixture of DMPC:DMPG with a percentage in weight of 80:20 respectively. The subphase was either prepared in air-contrast matched water (ACMW) or in D_2O, and the lipid monolayer was either prepared from deuterated or hydrogenated DMPC and DMPG. The contrasts used and lipids compositions measured are reported in table 1. The nanodisc forming polymer, poly(styrene-maleic acid) (SMA) was previously synthesised via RAFT polymerisation, using deuterated styrene to give a partially deuterated polymer with a well defined molecular weight (6.1kDa) and a 2:1 styrene:maleic acid molar ratio. The polymer was injected under the lipid monolayer using a long needle, to give a calculated final solution concentration of polymer (see Table 1) and the adsorption of the polymer to the interface monitored with time using both neutron reflection and surface pressure in the Langmuir trough. The reflectivity was followed until the surface pressure changes were complete. We had initially planned to run higher polymer concentrations in solution, however the surface pressure did not stabilize at higher concentrations, presumably due to disc formation and lipid exchange with subphase discs complicating the adsorption. Therefore lower polymer concentrations were used, resulting in longer total adsorption times. This restricted the number of measurements which could be made during the awarded time. A reflectivity pattern for monolayer characterisation was run before and after the polymer injection in each case.

Table 1: Summary of the contrasts run during the experiment.

Lipid composition	Polymer concentration	Subphase contrast
d DMPC	0.01%wt	ACMW
hDMPC		ACMW
dDMPC		D_2O
d (DMPC:DMPG)	0.01%wt	ACMW
h (DMPC:DMPG)		ACMW
d (DMPC:DMPG)		D_2O
d DMPC	0.1%wt	ACMW
h DMPC		ACMW

The 2 contrasts run on ACMW were analysed to obtain the composition of the surface adsorbed layer, which allows calculation of the polymer to phospholipid ratio on the surface for the 2 different composition of phospholipids in the monolayer.

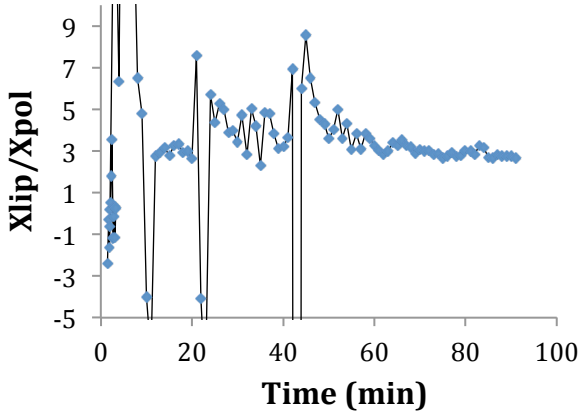

Figure 1: Molar ratio of lipids to monomers of the polymer, x_{lip}/x_{pol}, for the DMPC monolayer interacting with SMA (0.1%wt final concentration in solution).

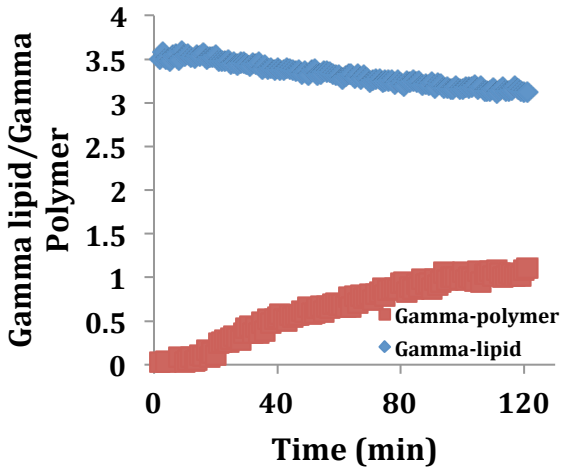

Figure 2:Surface excess of lipids and polymer plotted against time for the DMPC monolayer interacting with SMA (0.01%wt final concentration in solution)

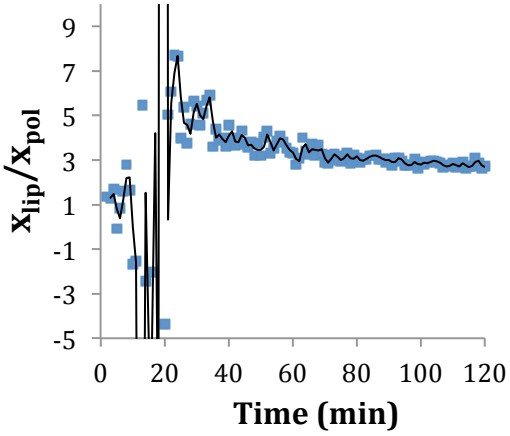

Figure 3: ratio of surface excess of lipids to polymer for the DMPC monolayer interacting with SMA (0.01%wt final concentration in solution)

Table 2: Summary of the results for different lipid compositions and polymer concentrations in the subphase.

Lipid composition	Polymer concetration	x_{lip}/x_{pol}
DMPC	0.1%wt	3
DMPC	0.01%wt	3
DMPG	0.01%wt	5

As reported in Table 2 using a higher concentration of the polymer in the subphase solution does not change the extent of polymer to lipid interaction, but it changes drastically the kinetic of the process. The adsorption of the polymer to the DMPC monolayer is shown for the two different polymer concentrations in Figure 1 and 3, demonstrating the much more rapid interaction at higher concentration, as expected.

An additional run on D_2O allowed us to co-refine the sets of scattering patterns taken before and after the polymer injection in order to obtain structural information from the system. Fitting of this reflectivity data are shown in Figure 4

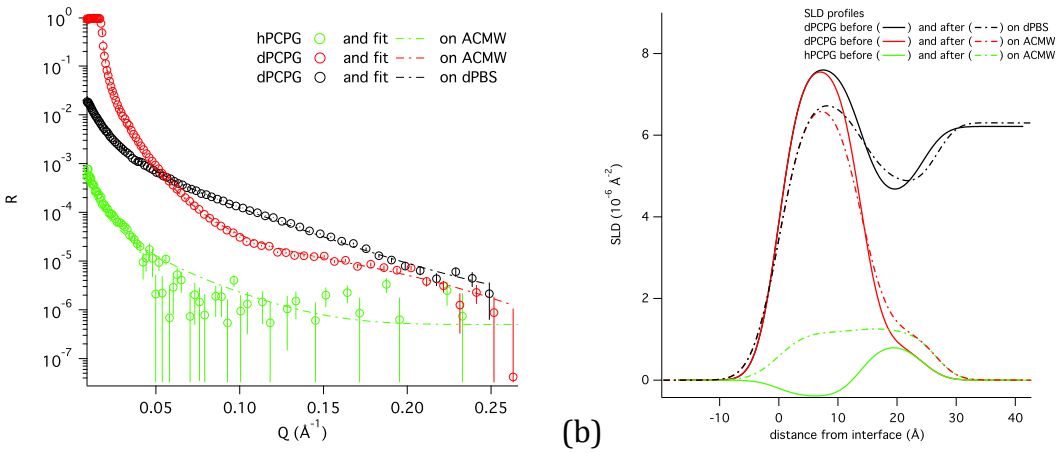

Figure 4: (a) scattering pattern for deuterated DMPC:DMPG on ACMW (black), D_2O (red) and hydrogenated DMPC:DMPG on ACMW (green) after the polymer injection (final solution concentration 0.01wt%). (b) Scattering length density profile for the system before (line) and after (dotted line) the polymer injection. All the measurements were carried out on phosphate buffer, 50mM, NaCl 0.2M.

An increase in the scattering length of the hydrogenated tails is shown after the polymer is injected and has adsorbed to the monolayer. This is probably due to the insertion of the polymer in the phospholipid monolayer, with the deuterated styrene inserted into the hydrophobic tails. As the SLD of the deuterated polymer is close to that of the deuterated tail, the SLD of the tails for the deuterated lipid monolayer after the polymer injection does not change drastically as for the hydrogenated phospholipids tail. A small change in the SLD of the headgroup region is also consistent with adsorption of the polymer into this region of the monolayer. However no separate layer of adsorbed polymer is found below the lipid monolayer suggesting the polymer is entirely segregated into the lipid layer.

Conclusion

Distinct differences between polymer adsorption to a zwitterionic DMPC monolayer and a negatively charged mixed DMPC:DMPG monolayer were found. This effect is probably due to the different electrostatic charge on the surface of lipid monolayers, causing the interaction of the polymer with the monolayer to change with the changing the nature of the head groups. The interaction of polymer, though, does not change with different polymer concentration. Further work will involve the study of the injection of the polymer beneath a monolayer using either a higher MW of the polymer or, in order to confirm that the difference between DMPC and DMPG is an electrostatic effect, with an high concentration of salt or using mixtures with positively charged lipids. A publication is currently in preparation, using these initial successful results.