Experimental report

04/09/2017

Proposal: 9-12-457 **Council:** 4/2016

Title: Direct observation of the kineticsof lipid exchange in polymer stabilised nanodiscs with and without proteins

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DMPC/DMPG/DODAB in Chloroform
poly(styrene-co-maleic acid) + d-DMPC
poly(styrene-co-maleamide) + d-DMPC

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	3	4	20/09/2016	23/09/2016
			08/10/2016	09/10/2016

Abstract:

The use of nanodiscs to contain and solubilise phospholipid bilayers and membrane proteins is becoming more widely used. This proposal aims to directly observe the kinetics of lipid exchange between polymer stabilised nanodiscs and a monolayer of lipids at the air water interface. Previous neutron reflectometry measurements have demonstrated that this exchange occurs rapidly; faster than the time required to measure a full reflectivity profile. Although SANS data has also demonstrated that exchange occurs, for technical reasons we have been unable to successfully measure exchange kinetics using that technique. Here we propose to use the unique high flux, low-Q mode of FIGARO to directly measure the rate of change in scattering as deuterated lipids move between nanodiscs and the interfacial monolayer. We will investigate this as a function of temperature, lipid and the polymer used to encapsulate the lipids. We will also directly observe whether exchange is also seen for nanodiscs made from natural E.coli lipids and containing a membrane protein. This may potentially allow manipulation of protein function via control of their surrounding lipid environment

Experiment number: 9-12-457

Experiment dates: 20/09/2016 – 23/09/2016 and 08/10/2016 – 09/10/2016

Title: Direct observation of the kinetics of lipid exchange in polymer stabilised nanodiscs

Abstract: Polymer stabilised nanodiscs comprised of a phospholipid bilayer wrapped with a poly(styrene-co-maleic acid) (SMA) polymer have been shown to spontaneously exchange with exogenous sources of lipids (Hazell et al., 2016). This experiment aimed to measure the kinetics of lipid exchange between nanodiscs (formed using three different polymers: the commercially available SMA2000p, an in house RAFT-synthesised SMA and a poly(styrene-co-maleimide) (SMI) variant) and a Langmuir monolayer at the air-liquid interface. Following observation of kinetics, structural changes occurring at the interface due to the presence of nanodiscs in the subphase were measured.

Experiment description: This experiment aims to investigate the kinetics of lipid exchange between polymer stabilised phospholipid nanodiscs and a Langmuir monolayer at the air liquid interface. At very low *q*, the specular reflected intensity is independent of interfacial structure and purely dependant on the scattering excess, defined product of the total scattering length density of molecules at the interface and the layer thickness, allowing surface processes to be monitored in real time without needing to collect a full q-range for structural analysis that would otherwise sacrifice time resolution.

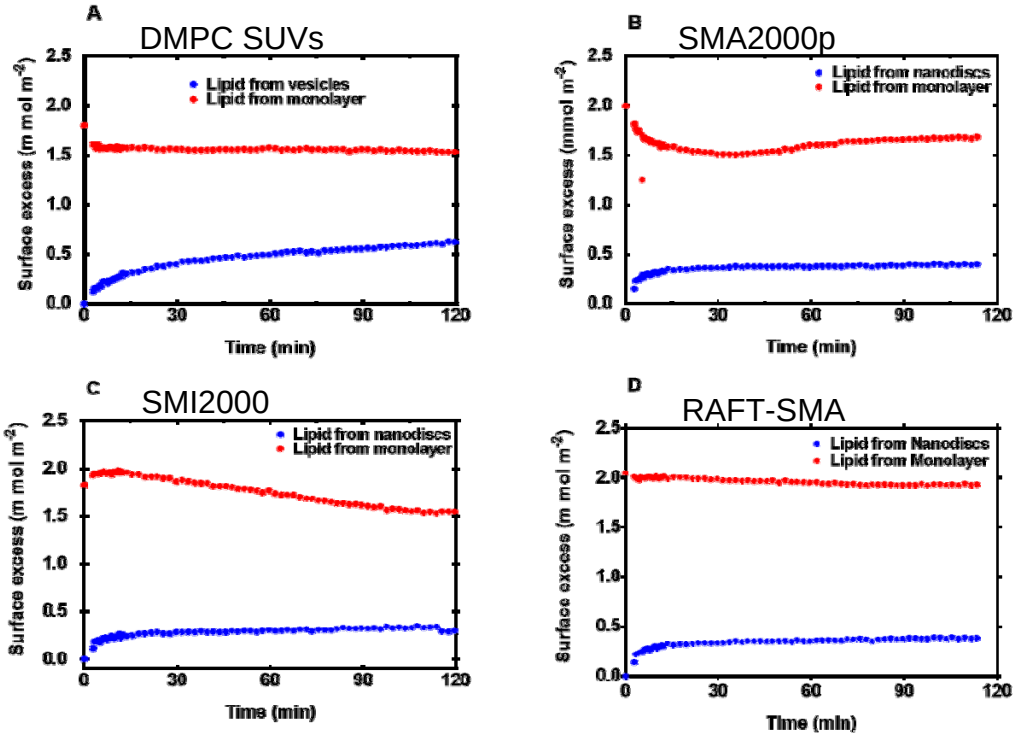

Figure 1 Kinetic data showing surface excess of lipid present at the interface that originated at the monolayer (red) and that which originated within polymer stabilised nanodiscs (blue).

Either hDMPC or d_{54}DMPC was spread to a surface pressure of 10 mN.m^{-1} on an air contrast matched water (ACMW) subphase containing 50 mM sodium phosphate, pH 8 and 200 mM NaCl. A full q-range measurement was performed to determine the interfacial structure prior to the introduction of nanodiscs. To hDMPC monolayers, nanodiscs containind dDMPC were injected into the subphase and low-

q reflectivity recorded at time intervals of 2 min for 120 minutes. Full q-range data was collected after to determine the interfacial structural changes occurring after the introduction of nanodiscs. By collecting data on dDMPC monolayers exchanging lipids with hDMPC containing nanodiscs, and the reverse scenario (hDMPC monolayer exchanging with dDMPC containing nanodiscs), we have been able to calculate the time-resolved surface excess of hydrogenated and deuterated DMPC at the interface.

Initially, lipid exchange was measured between SUV's and a Langmuir monolayer as a baseline to compare lipid exchange in nanodiscs. This shows a rapid initial transfer of lipids from the monolayer to vesicles, following a more gradual transfer of lipids from the vesicles to the Langmuir monolayer. In comparison, SMA2000p nanodiscs show a slower but more stoichiometric initial exchange. After 30 minutes, no more lipid is transferred from the nanodiscs to the interface and the system is assumed to be at equilibrium. SMI nanodiscs initially deposit lipid at the interface with a slower and more gradual exchange of lipids from the monolayer to nanodiscs. RAFT-SMA nanodiscs however, show only monodirectional exchange. While no lipid is exchanged from the monolayer into nanodiscs, the nanodiscs appear to lose lipids to the monolayer at a similar rate to the other two nanodisc systems.

Fits of the structural data (Figure 2) indicate that lipid exchange is not due to nanodisc adsorption to the interface, and the data can be fitted assuming the only structural difference is an increase or decrease in the SLD of the DMPC tails. This corresponds to an exchange of lipids from the nanodiscs to the monolayer.

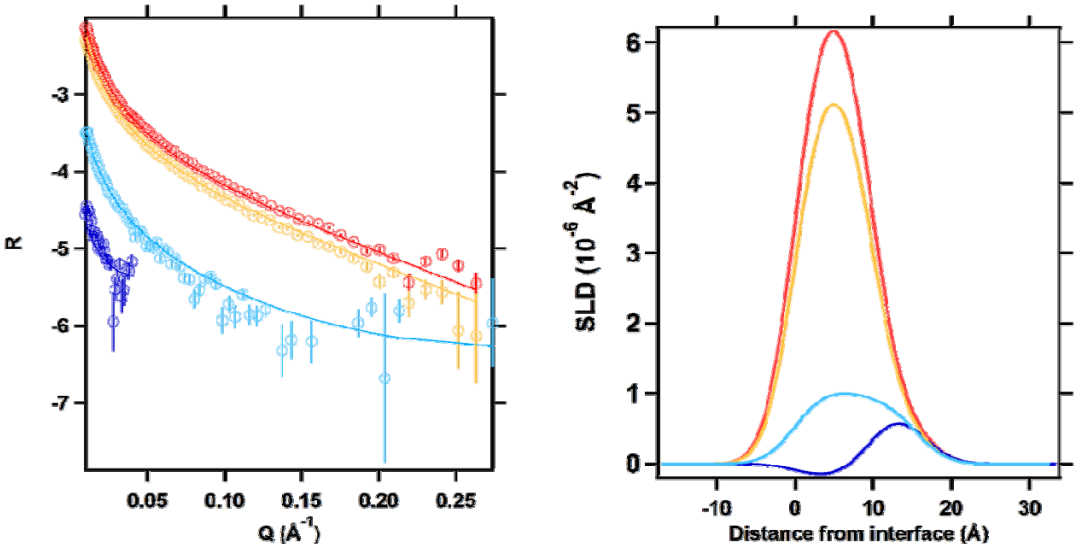

Figure 2 Reflectivity from DMPC monolayers at the air-liquid interface on an ACMW subphase before (red, dark blue) and after (orange, light blue) the introduction of SMA2000p nanodiscs into the subphase, and corresponding fits (solid lines) and SLD profile. The changes to the reflectivity profile can be accounted for by fitting a changing to the SLD of the phospholipid tail region, without any further structural changes. These data indicate that lipid exchange does not occur due to nanodisc adsorption at the interface.

Conclusion: These results show the kinetics of lipid exchange between polymer stabilised nanodiscs and a Langmuir monolayer are polymer dependant, and in all cases differ to that observed when exchanging lipids with SUV's. These data are being prepared for publication while further work is ongoing to measure the occurrence of lipid exchange in polymer stabilised nanodiscs in bulk solution.

References:

Hazell, G., Arnold, T., Barker, R.D., Clifton, L.A., Steinke, N-J., Tognoloni, C., and Edler, K.J. (2016). Evidence of Lipid Exchange in Styrene Maleic Acid Lipid Particle (SMALP) Nanodisc Systems. Langmuir *32*, 11845–11853.