Experimental Report

Proposal: **9-13-547** **Council:** 4/2014

Title: The adsorption of protein containing polymer-stabilised nanodiscs at the solid-liquid interface

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: DMPC:DMPG Nanodiscs containing ZipA
Silicon blocks coated with Octadecyltrichlorosilane
DOPC /DGS-NTA Lipid vessicles

Instrument	Req. Days	All. Days	From	To
D17 He3 Spin Filter	4	3	04/09/2014	07/09/2014

Abstract:

Nanostructured lipid aggregates offer enormous potential as tools for membrane structural biology and biophysics. Poly(Styrene-Maleic Acid) stabilized lipid nanodiscs can be easily prepared, without detergent, at low cost and can solubilize membrane proteins. In this proposal we wish to study the adsorption of polymer-stabilised lipid nanodiscs containing a membrane protein at a lipid
monolayer containing a lipid with a His-tag chelating ligand on a solid-liquid interface. We wish to determine whether the protein containing discs can be close packed in a single orientation at the interface to assist in future structure determination & potentially bulk 3D crystalisation. We will use ZipA, a membrane protein involved in cell division, with a single transmembrane helix (in collaboration with Prof Dafforn's group in Birmingham) for this proof-of-principle experiment.

The purpose of this experiment was to assess the interaction of membrane protein containing nanodiscs (MP-nanodiscs) upon lipid monolayers at the solid-liquid interface. We have been able to express and purify nanodiscs containing the membrane protein, ZipA, containing a His-tag on the extracellular part of the membrane protein. These systems are therefore small segments of phospholipid bilayer with an encapsulated membrane protein, stabilized by a polystyrene-co-maleic acid polymer belt.[1] Here we attempted to align a monolayer of MP-nanodiscs upon functionalized lipid monolayers containing the His-tag-chelating lipid DOGS-NTA-Ni (DOGS). Lipid monolayers containing 20 mol % of DOGS with a bulk diluting lipid (DOPC) were deposited onto an octadecyltrichlorosilane monolayer at the silicon-water interface. All layers were pre-characterised before the injection of the nanodisc solutions. Figure 1 shows neutron reflectometry measurements for the pre-characterisation of these layers, along with their corresponding scattering length density (SLD) profiles. The OTS layers produced are in good agreement with the literature in terms of area per molecule (APM) and thickness. The lipid monolayers had a good coverage and appropriate APM values.

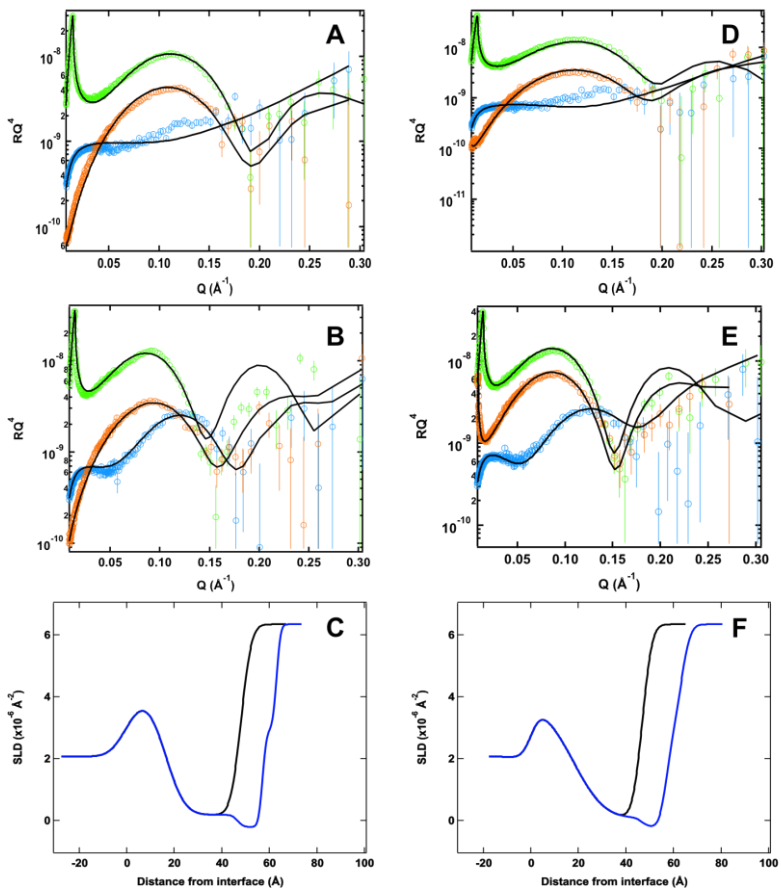

Fig 1- A-C) neutron reflectometry profiles for OTS (A) and DOPC monolayer (B) along with the corresponding scattering length density profile(C). D-E) neutron reflectometry profile for OTS (D), 20 mol % DOGS monolayer (E) and the corresponding scattering length density profile(F). D_2O contrast is shown in green, H_2O in blue and either SMW or 4MW in orange.

However, unfortunately, upon nanodisc injection no adsorption of the MP-nanodiscs to the surface was observed. This was demonstrated by identical reflectometry profiles to those found during the pre-characterisation of the supporting layers. Figure 2 shows neutron reflectometry profiles in a D_2O contrast for the lipid monolayers with (blue circles) and without (orange line) nanodiscs present. It is clear that there is no difference in the reflectometry profiles. If the membrane protein containing nanodiscs had adsorbed to this surface then we would have expected to see a large fringe present at ca. Q= 0.1-0.2 $Å^{-1}$, which would indicate a nanodisc layer on top of the lipid monolayer.

At this stage we are unsure as to why this is the case given that we have consistently previously observed adsorption of nanodiscs without protein to lipid interfaces. We think that possibly the large extracellular region of the membrane protein, may cause steric hindrance for adsorbing discs, which may heavily influence the lack of adsorption. This in turn will inhibit electrostatic interactions between lipid headgroups of the nanodisc system with headgroups within the lipid monolayer. Large steric hindrance will also inhibit polymer surface interactions, which we know heavily influences the adsorption of the nanodiscs. We

are therefore currently undertaking preliminary investigations for nanodiscs containing other membrane proteins and nanodiscs with a differing polymer stabilized belt. We hope that, in the future, this will enable us to adsorb protein-containing nanodiscs at a lipid interface which will enable novel ways to study membrane protein structure and have implications for biosensing.

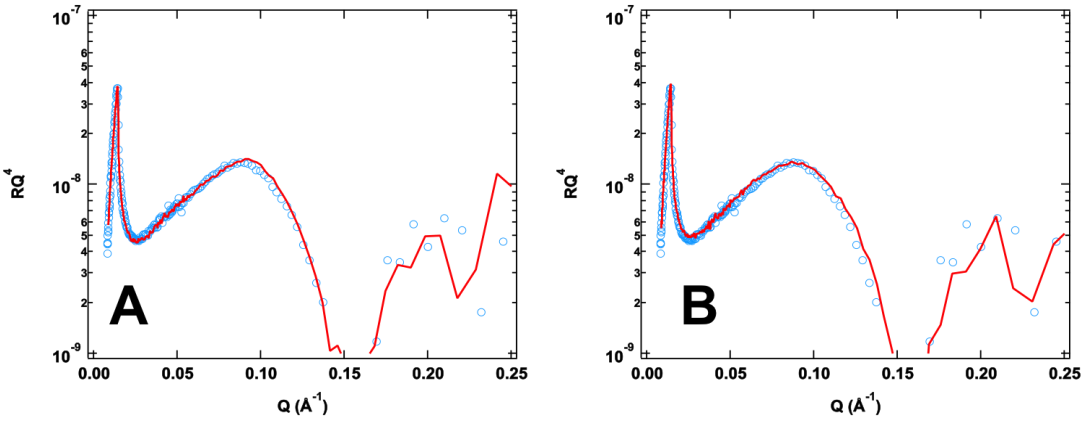

Fig 2- Neutron reflectometry profiles for the injection of membrane protein containing nanodiscs on a DOPC monolayer (A) and a 20 mol % DOGS monolayer (B). The blue circles represent the data shown in figure 1 without membrane protein present, whilst the red line corresponds to the reflectivity after nanodisc injection.

References:

(1) Jamshad, Grimard, Idini, Knowles, Dowle, Schofield, Sridhar, Lin, Finka, Wheatley, Thomas, Palmer, Overduin, Govaerts, Ruysschaert, Edler, Dafforn, *Nano Research* **2014**, 1; Jamshad, Lin, Knowles, Parslow, Harris, Wheatley, Poyner, Bill, Thomas, Overduin, Dafforn, *Biochem Soc Trans* **2011**, *39*, 813.