# Experimental report

03/02/2016

**Proposal:** 9-12-398 **Council:** 10/2014

**Title:** Lipid-polymer nanodiscs stabilizedwith amine-based polymers

**Research area:** Soft condensed matter

**This proposal is a new proposal**


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**Samples:** DMPC, poly(styrene-alt-maleic acid): ((C16H14Na2O8)n) D2O/H2O, phosphate buffer, NaCl
DMPC, poly(styrene-alt-maleimide) ((C17H23N2O2)n(CH3COO)n), D2O/H2O, acetate buffer, NaCl
dDMPC, poly(styrene-alt-maleimide) ((C17H23N2O2)n(CH3COO)n), D2O/H2O, acetatee buffer, NaCl

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D33 | 3 | 0 | | |
| D22 | 3 | 0 | | |
| D11 | 3 | 2 | 01/07/2015 | 03/07/2015 |


**Abstract:**

Polymer stabilized lipid nanodiscs offer enormous potential as tools for enabling membrane protein structural studies and biophysics. Previously we have studied nanodisc formation using a poly(styrene-alt-maleic acid) (PSMA) copolymer, which forms discs above pH 8. However this anionic polymer is sensitive to Ca2+ ions (common in biological solutions) and some membrane proteins prefer lower pH environments. Thus here we propose to study lipid nanodiscs stabilized using a new amine-based polymer, poly(styrene-alt-maleimide) (PSMI). Preliminary experiments demonstrate that this polymer also forms discs, which are stable at lower pH values. Recent synthesis in our group has produced PSMA using RAFT polymerisation with well-defined molecular weights. PSMI can be generated directly from this material giving PSMI with identical polymer lengths. The length of the maleimide chain on the PSMI will also be varied from propyl, to ethyl to methyl. Thus here we wish to characterise and compare PSMA-stabilized discs to our new PSMI-stabilized discs to determine whether the structures are equivalent and how the variation in polymer structure affects the discs.

## INTRODUCTION and BACKGROUND

Phospholipid bilayer nanodiscs are a patch of lipid bilayer, with the hydrophobic disc edges surrounded and stabilized by a polymer[1] or protein[2] belt. Polymer stabilized lipid nanodiscs can be used to extract membrane proteins from cell membranes, without use of detergent and while retaining the native lipid composition around the protein in the disc. These nanodiscs therefore offer enormous potential as tools for enabling membrane protein structural studies and biophysics. Previously nanodiscs have been made with poly(styrene-alt-maleic acid) (PSMA) copolymer, which is soluble, and thus forms discs above pH 8. However some membrane proteins prefer lower pH environments, so to enable this, we have used a new commercially available amine-based polymer, poly(styrene-alt-maleimide) (PSMI), which is soluble within the pH range from 3 to 6.5. Recent synthesis in our group has produced PSMA using RAFT polymerisation with several molecular weights and with a fixed Styrene to Maleic anhydride ratio of 2:1. In this experiment therefore we have compared the size and structures of aggregates formed between PSMI polymers and a range of lipids, and compared these to similar systems in which the molecular weight of the PSMA was varied. Deuterated and hydrogenated polymers and lipids were used to highlight the lipid and polymer regions of the structures.

## EXPERIMENTAL DETAIL AND RESULTS

Phospholipid nanodiscs were prepared using dimyristoyl-*sn*-glycero-3-phosphocholine (DMPG, purity ≥ 99%) dimyristoylphosphatidylcholine (DMPC, purity ≥ 99%), monoolein (purity≥ 99%), all from Sigma Aldrich, deuterated dimyristoylphosphatidylcholine (d-DMPC, purity ≥ 99%), and deuterated 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (d-DMPG, purity ≥ 99%) purchased from Avanti Polar Lipids. PSMI from Cray Valley with two styrene:maleimide molar ratios was used (SMI1000 ratio 1:1, and SMI2000, ratio 2:1). RAFT synthesis methods were used to prepare hydrogeneous PSMA with molar ratio 2:1 styrene:maleic acid, at molecular weights of 6kDa and 11kDa, and the corresponding polymers with deuterated styrene. The experiment was carried out on D11, a SANS instrument. The positions of the detector were fixed at 1.20, 7.99 and 20m for the high, middle and low q angles respectively. Data was reduced and combined using standard procedures, and a suitable background subtracted before data analysis.

To prepare nanodisc samples solutions of final concentration of 1.5wt% polymer and 5mg/ml lipid were prepared. When polymer is added to the lipid the solutions rapidly clear, indicating formation of nanoscale structures. In order to get rid of polymer left in solution, samples made with the commercial copolymers (SMI1000 and 2000) were purified using gel filtration. Figure 1 reports the gel filtration pattern from the elution of samples made by adding the polymer to a phospholipid suspension of hDMPC with final concentration of 1.5%wt and 5mg/mL respectively. The elution trace for a similar solution prepared using the copolymer SMA2000P (copolymer of Styrene:Maleic anhydride ratio 2:1 and MW 7KDa) is also shown in Figure 1 (dotted line). The first peak is in this pattern is established to be that corresponding to nanodiscs and it superimposes with the first peak of the samples made with SMI1000 and hDMPC (continuous line). The sample made with SMI2000 (dashed line) is different and the sample was then divided in 3 parts in order to understand the structure of each component.

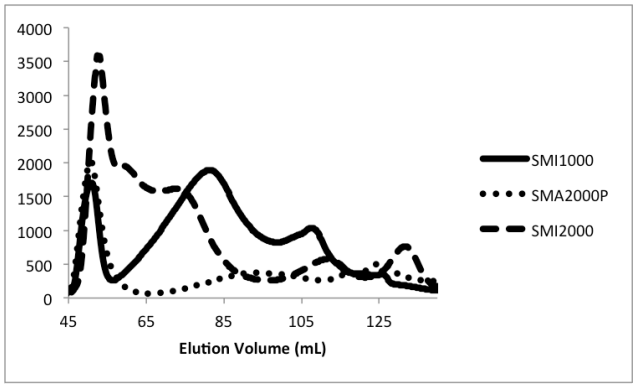


**Figure 1: gel filtration curve for samples made with SMI 1000, 2000 and SMA_2000P**

Figure 2 reports the scattering patterns for all the 3 fractions collected.

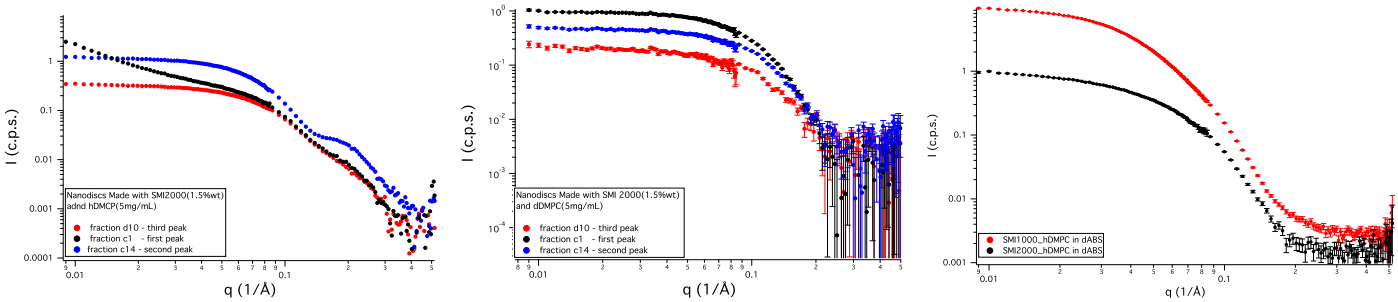


**Figure 2: Scattering pattern for the 3 fractions collected for the samples made with SMI2000 and hDMPC in dPBS (left) and with dDMCP in hPBS (middle). (right) Comparison of scattering patterns for nanodiscs made with SMI1000 and 2000 with hDMPC in dPBS. The final concentrations were 1.5%wt and 5mg/mL for the polymer and DMPC respectively**

From the gel filtration scattering patterns, even though we need to fit data yet, the first peak seems to be the closest peak to that expected for nanodisc formation. The final pattern in Figure 2 compares the scattering patterns for nanodiscs made with SMI1000 and 2000. This suggests that the nanodiscs have similar size, although the SMI1000 sample may be more concentrated.

Since the cell membrane is composed of multiple phospholipids, the structure of composites prepared using different phospholipid compositions were tested with SMI2000. The results are reported in figure 3 below. A first fit has been done for those patterns using a model of concentric cylinders, where the outer cylinder models the polymer belt encircling a core of phospholipid tails, with separate layers at top and bottom corresponding to the lipid headgroup regions. The fitting of these patterns is however still being refined using the multiple contrast data recorded.

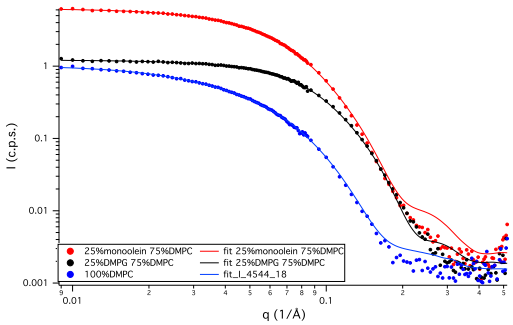


**Figure 3: Nanodiscs made in deuterated acetate buffer (dABS, 50mM with 0.2M NaCl.). The graph shows scattering data (dots) for discs prepared using different composition of phospholipids with the same polymer, SMI2000. Solid lines are initial fits to the model described in the text. Fitting of this data is ongoing.**

Samples made with polymers made via RAFT polymerisation were also prepared, where the length of the polymer was changed from 6kDa to 11kDa. In figure 4 scattering patterns for polymer alone are reported: deuterated polymer in hPBS (left) and hydrogenated polymer dPBS (right). Initial fits suggest that these structures resemble hydrogel spheres, with a porous internal structure and lower density polymer chains at the particle surfaces.

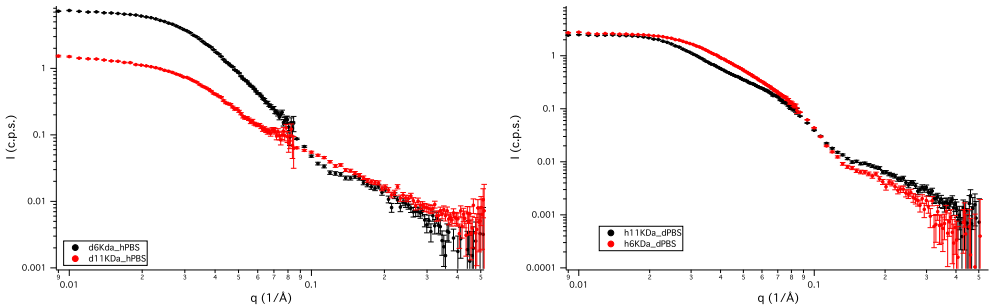


**Figure 4: Scattering pattern for the deuterated (left graph) and hydrogenated polymers(right graph) in hPBS and dPBS respectively**

In figure 5 scattering patterns for supramolecular structures made with different lipid contrasts and deuterated RAFT polymer are reported. It is currently unclear what these structures are, but it appears that they are not nanodiscs, so fitting is continuing to determine the structures formed in these solutions.

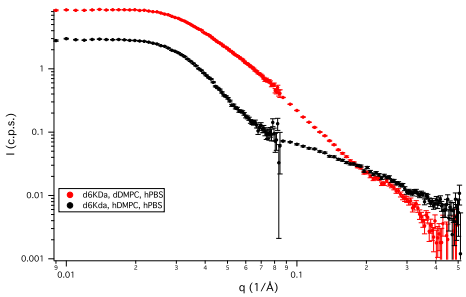


**Figure 5: Scattering pattern for supramolecular structure made from deuterated 6KDa polymer with different contrast: dDMPC in hPBS (red) and hDMPC in hPBS (black)**

## CONCLUSION AND FUTURE WORK

Even though data fitting is not yet complete, the composite polymer lipid structures as well as the structures of the polymer alone in solution seem to change with different polymer chain length. For future work we would be interested to characterise these systems by making polymers having the same molecular weight but with different architecture of the polymer, using a higher hydrophobic to hydrophilic ratio, creating tri-block copolymers or changing the hydrophobic/hydrophilic balance by functionalising the polymer by copolymerising the styrene with p-methylstyrene or by decreasing the acid moiety to one unit by using acrylic acid instead of maleic acid to copolymerise styrene. Such species will give us new insights into the polymer characteristics required for disc formation, and those which cause alteration of the lipid polymer composite materials into different structures in solution. Although DLS can suggest the presence of structures in the expected nanodisc size range in our samples, only SANS can give the structural resolution to determine the relative locations of polymer and lipid in these samples, and to confirm the formation of discs or other uniform aggregates in these solutions.

## References:

1. (a) Jamshad, M.; Grimard, V.; Idini, I.; Knowles, T. J.; Dowle, M. R.; Schofield, N.; Sridhar, P.; Lin, Y.; Finka, R.; Wheatley, M.; Thomas, O. R. T.; Palmer, R. E.; Overduin, M.; Govaerts, C.; Ruysschaert, J.-M.; Edler, K. J.; Dafforn, T., Structural analysis of a nanoparticle containing a lipid bilayer used for detergent-free extraction of membrane proteins. *Nano Research* **2015,** *8* (3), 774-789; (b) Knowles, T. J.; Finka, R.; Smith, C.; Lin, Y.-P.; Dafforn, T.; Overduin, M., Membrane Proteins Solubilized Intact in Lipid Containing Nanoparticles Bounded by Styrene Maleic Acid Copolymer. *J. Am. Chem. Soc.* **2009,** *131* (22), 7484–7485.

2. Bayburt, T. H.; Grinkova, Y. V.; Sligar, S. G., Self-assembly of discoidal phospholipid bilayer nanoparticles with membrane scaffold proteins. *Nano Lett.* **2002,** *2* (8), 853-856.