Experimental Report

Proposal: **9-13-468** **Council:** 4/2012

Title: Lipid Exchange in Polymer Stabilized Lipid Nanodiscs with Varying Curvature

This proposal is resubmission of: 9-13-433

Researh Area: Soft condensed matter

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Samples: h-DMPC, h-DMPG, styrene-maleic acid copolymer, D2O, H2O
d-DMPC, d-monoolein, styrene-maleic acid copolymer, D2O, H2O
h-DMPC, h-monoolein, styrene-maleic acid copolymer, D2O, H2O
d-DMPC, d-DMPG, styrene-maleic acid copolymer, D2O, H2O

Instrument	Req. Days	All. Days	From	To
D33	0	2	03/04/2013	05/04/2013

Abstract:

Styrene-Maleic Acid Lipid Particles (SMALPs) are stable nano-aggregates of polymer and lipid which self-assemble into monodisperse discs in solution. They have been shown to solubilize membrane proteins which gives them potential to assist in membrane protein structure determination. Our recent studies of these discs suggest the disc curvature can be modified by the lipid mixtures used. Since curvature affects membrane protein structures and function we therefore wish to explore the properties of our nanodiscs by studying lipid exchange between discs of selected curvature, induced either by lipid charge or by altering tail volumes. Lipid exchange will be probed by following changes in scattered intensity with time when solutions containing h-lipid discs and d-lipid discs are mixed. Following exchange rates at different temperatures will allow the thermodynamics of lipid exchange in this polymer stabilized system to be compared with studies by others on protein stabilized nanodiscs and liposomes. This work will form the basis of future structure/function studies on proteins in lipid nanodiscs with controlled curvature.

The aim of this experiment was to investigate the dynamic properties of phospholipids embedded in phospholipid nanodiscs stabilized via a poly(styrene-maleic acid) belt[1] and to compare our results with previous experiments performed on similar nanodiscs stabilised by a protein belt[2] in order to highlight differences and similarities between these two structures due to the differences in the belt composition.

Phospholipid nanodiscs were prepared using dimyristoyl-*sn*-glycero-3-phosphocholine (DMPG, purity ≥ 99%) dimyristoylphosphatidylcholine (DMPC, purity ≥ 99%), both 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. The stabilizing polymer was poly(styrene-co-maleic acid, SMA) supplied by Malvern Cosmeceutics, with an average Mw of 9.5kDa. Nanodisc samples analysed in this experiment were prepared using either only DMPC or a mixed composition of DMPC and DMPG with different proportions. Both lipids were used in deuterated and non-deuterated form, with a non-deuterated poly(styrene-maleic acid) copolymer. To determine the lipid exchange rate, discs containing h-lipid and d-lipid at the same composition were mixed and the change in intensity, as the lipids exchanged within the discs, was monitored. Contrasts used were lipid mixtures in buffers prepared in 100% D_2O, 100%H_2O or 50%D_2O/50%H_2O solvents. The buffer used was 50mM phosphate buffer with 200mM NaCl prepared in 100% D_2O or 100% ultrapure water.

The experiments were carried out by mixing an equivalent volume of deuterated discs in either hydrogenated deuterated solvent and hydrogenated discs in hydrogenated or deuterated solvent, depending on the desired final solution contrast. The two solutions were mixed directly in the cell by agitating briefly before putting the cell in the temperature controlled rack on the beam line. The time-resolved experiment was started as fast as possible after the safety inspection was completed. The total count/second was measured in 10s patterns and the count rate for the solvent alone was subtracted before further calculations were made. Experiments were carried out at 25°C, 35°C and 45°C.

The normalised contrast was calculated using the following formula:

$$\frac{\boldsymbol{\Delta\rho(t)}}{\boldsymbol{\Delta\rho(0)}} = \frac{\left[\boldsymbol{I(t)^{\frac{1}{2}}}\right] - \left[\boldsymbol{I(\infty)^{\frac{1}{2}}}\right]}{\left[\boldsymbol{I(0)^{\frac{1}{2}}}\right] - \left[\boldsymbol{I(\infty)^{\frac{1}{2}}}\right]}$$

where: I(t) is the count rate at the time t after mixing;
I(0) is the count rate at the first data point;
I(∞) is the count rate after a sufficient period of time to allow complete mixing of the lipids in the discs in the two solutions.

Results

Our experimental results, compared with the work done previously using protein stabilised nanodiscs,[3] showed a similar lipid exchange behaviour, but with a much faster decay, as all the exchange processes were complete within the first ten minutes. This more rapid lipid exchange behaviour might be related to the different interaction of the polymer belt with the lipid core compared to the protein-lipid interaction. Data reported in figure 1 and 2 belong to a sample composed of the initial two solutions: A) 80wt% dDMPC and 20wt% dDMPG and non deuterated copolymer added, to an equal amount of solution B) composed of 80wt% non deuterated DMPC plus 20wt% non deuterated DMPG with non deuterated polymer. The data was plotted as the $\ln(\Delta\rho(t)/\Delta\rho(0))$ vs time. This experiment was performed at the temperatures of 25°C, 30°C and

45°C in order to obtain the Arrhenius constant and activation energy for lipid exchange. The

Arrhenius plot in figure 2 shows a good linear relationship between temperature and mixing rate constant.

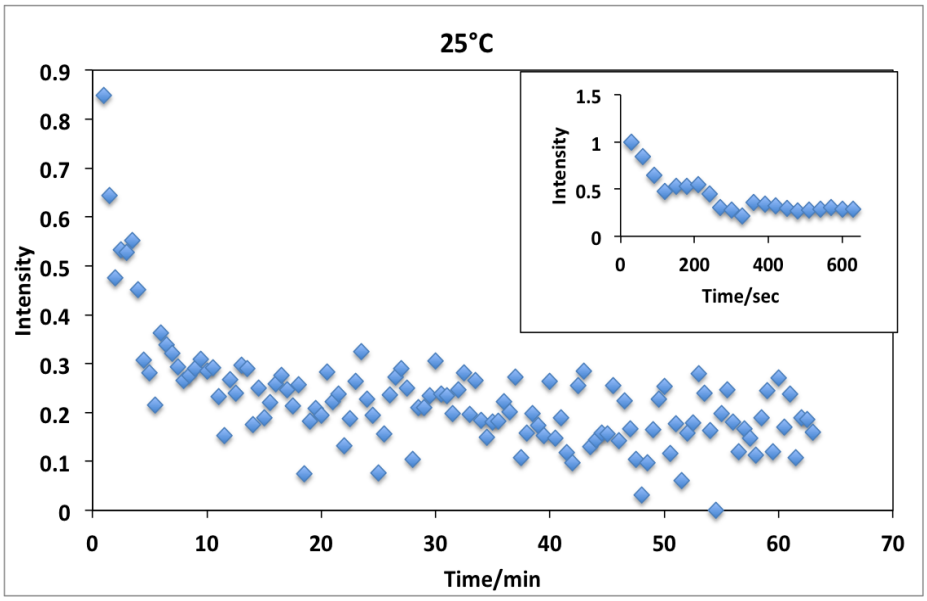

Figure 1: Contrast decay after mixing D-discs and H-discs. D-discs were composed of 80%dDMPC, 20%dDMPG, h-polymer in 60% D_2O phosphate buffer. H-discs were composed of 80%hDMPC, 20%hDMPC, h-polymer in 100% H_2O phosphate buffer.

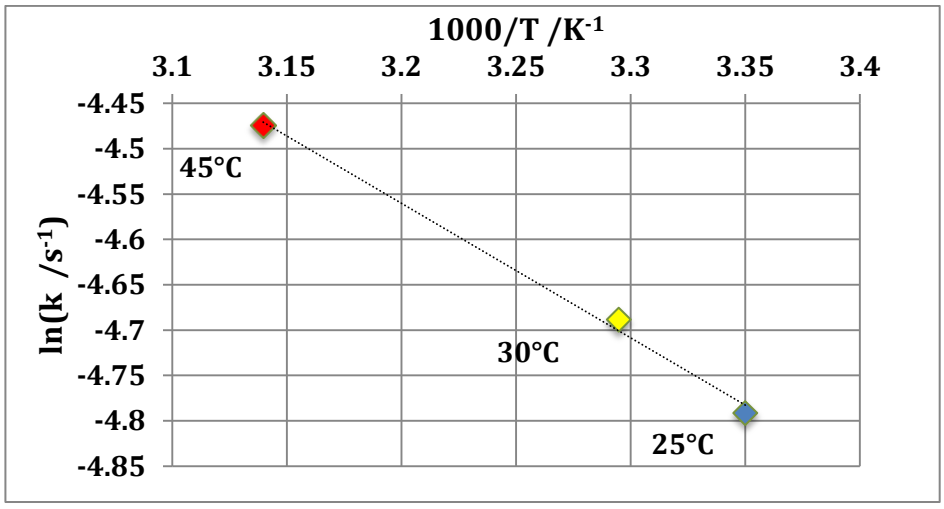

Figure 2 Arrhenius plot of rate constant of interparticle lipid exchange with 1/T. The decay curves were fitted by a linear function to determine the decay constants K at three different temperatures-

Future Work Further data analysis is continuing in order to understand the role of lipids composition in the disc on the rate of lipid exchange and give a more complete and detailed understanding of this new system.

References:

1. 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. Nakano, M.; Fukuda, M.; Kudo, T.; Miyazaki, M.; Wada, Y.; Matsuzaki, N.; Endo, H.; Handa, T., Static and Dynamic Properties of Phospholipid Bilayer Nanodiscs. *J. Am. Chem. Soc.* **2009,** *131* (23), 8308-8312.

3. Miyazaki, M.; Tajima, Y.; Handa, T.; Nakano, M., Static and Dynamic Characterization of Nanodiscs with Apolipoprotein A-I and Its Model Peptide. *J. Phys. Chem. B* **2010,** *114* (38), 12376–12382.